



Distributions of 5- and 6-methyl branched glycerol dialkyl glycerol tetraethers (brGDGTs) in East African lake sediment: Effects of temperature, pH, and new lacustrine paleotemperature calibrations

James M. Russell^{a,*}, Ellen C. Hopmans^b, Shannon E. Loomis^{a,c}, Jie Liang^a, Jaap S. Sinninghe Damsté^{b,d,*}

^a Brown University, Department of Earth, Environmental, and Planetary Sciences, 324 Brook St., Box 1846, Providence, RI 02912, USA

^b NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, and Utrecht University, PO Box 59, 1790 AB Den Burg, Texel, Netherlands

^c University of Texas at Austin, Jackson School of Geosciences, 1 University Station, Stop C1160, Austin, TX 78712, USA

^d University of Utrecht, Faculty of Geosciences, P.O. Box 80.021, 3508 TA Utrecht, Netherlands

ARTICLE INFO

Article history:

Received 14 November 2017

Received in revised form 9 December 2017

Accepted 11 December 2017

Available online 15 December 2017

Keywords:

GDGT

Tetraether

Branched GDGT

Temperature reconstruction

Lake

Africa

Tropics

Paleolimnology

Calibration

ABSTRACT

The distribution of branched glycerol dialkyl glycerol tetraethers (brGDGTs) in soils, peats, and lake sediments has been shown to correlate with mean annual air temperature (MAAT) and has provided valuable new climate reconstructions. Here we use an improved chromatographic method to quantify the fractional abundances of 5- and 6-methyl isomers in surface sediments from 65 East African lakes spanning temperatures 1.6–26.8 °C, and investigate the relationships between these fractional abundances and temperature, lake pH, and other environmental variables. We find that temperature exerts a strong control on brGDGT distributions, including the relative abundances of 5- and 6-methyl isomers, whereas other environmental variables, including lake pH, are weakly correlated to the fractional abundances of the brGDGTs. The distributions of brGDGTs in our lake sediments differ from those of soils and peats, leading to temperature offsets if soil- and peat-based brGDGT temperature calibrations are applied. We develop new calibrations for MAAT for use in lake sediment based upon the MBT'5Me and Index 1 ratios, as well as a multivariate regression of brGDGT fractional abundances on temperature using stepwise forward selection. We obtain root mean square errors (RMSE) between ~2.1 and 2.5 °C for these calibrations, highlighting the potential for brGDGTs to provide precise temperature reconstructions using lake sediment cores. Calibrations for lake pH perform more poorly, likely due to weak correlations between pH and brGDGT distributions in East African lakes. These results indicate that quantification of 5- and 6-methyl isomers separately in lake sediment can improve paleoclimatic reconstructions.

© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Paleoclimate reconstructions provide fundamental insight into the mechanisms governing long-term climate change, and constitute the sole test-bed to investigate the ability of global climate models (GCMs) to simulate climate under boundary conditions that differ strongly from the present (Braconnot et al., 2012; Otto-Bliesner et al., 2014). Reconstructions of terrestrial climate are crucial to this endeavor, but are challenging due to a lack prox-

* Corresponding authors at: Brown University, Department of Earth, Environmental, and Planetary Sciences, 324 Brook St., Box 1846, Providence, RI, 02912, USA (James Russell) and NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, and Utrecht University, PO Box 59, 1790 AB Den Burg, Texel, Netherlands (J.S. Sinninghe Damsté).

E-mail addresses: James.Russell@Brown.edu (J.M. Russell), Jaap.Damste@nioz.nl (J.S. Sinninghe Damsté).

ies for past temperature that have sufficient sensitivity and precision to provide robust tests of GCM simulations. This is particularly the case in the tropics, where traditional temperature proxies such as tree-rings and ice core datasets are rarely available. In recent years, the development and application of temperature proxies based on isoprenoidal and branched glycerol dialkyl glycerol tetraethers (iGDGTs and brGDGTs, respectively; e.g., Schouten et al., 2002, 2013; Weijers et al., 2007a, 2007b; Tierney et al., 2010), have begun to address this issue by providing continuous continental temperature reconstructions from the tropics (Powers et al., 2005; Tierney et al., 2008; Bendle et al., 2010; Berke et al., 2012; Loomis et al., 2012, 2015, 2017; Sinninghe Damsté et al., 2012). Of these, the brGDGTs have perhaps the most promise, as they have been used to reconstruct temperature in many different geological archives, including lake sediments (Loomis et al., 2017),

river fan deposits (Weijers et al., 2007a), loess (Peterse et al., 2011), and peat (Zheng et al., 2017).

BrGDGTs are membrane-spanning lipids produced by bacteria, and contain straight alkyl core chains with four to six methyl groups and one or two cyclopentyl moieties formed by internal cyclization involving a methyl group (Sinninghe Damsté et al., 2000, 2011; Schouten et al., 2013). These bacteria are thought to alter the composition of their lipid membrane to adjust its fluidity in response to different environments, in part by varying the degree of methylation and cyclization of their brGDGTs. A survey of global soils showed that changes in the fractional abundances of a set of nine brGDGTs, expressed as the degree of Methylation and Cyclization of Branched Tetraethers (MBT and CBT, respectively) are correlated with mean annual air temperature (MAAT) and soil pH (Weijers et al., 2007b). Acidobacteria are suspected precursor organisms for the production of brGDGT since they produce the specific building block *iso*-diabolic acid, although predominantly in an ester- and not in an ether-bound form (Sinninghe Damsté et al., 2011, 2014). BrGDGTs were initially thought to be produced only in soil and peat environments, where acidobacteria often comprise a substantial fraction of the bacterial community (Weijers et al., 2009), and continental temperature reconstructions have been developed using marine sediment cores assuming that sedimentary brGDGTs were derived from soil erosion (Weijers et al., 2007a; Bendle et al., 2010). However, subsequent work has shown that the fractional abundances of brGDGTs in aquatic environments, and in particular lakes, can differ strongly from catchment soils, often precluding the use of soil-based brGDGT calibrations to lake sediment cores and indicating an in situ source for brGDGTs in lakes (Tierney and Russell, 2009; Tierney et al., 2010; Zink et al., 2010; Buckles et al., 2014; Loomis et al., 2011, 2012, 2014a, 2014b; Pearson et al., 2011; Li et al., 2016). There are now multiple calibrations for temperature based upon the fractional abundances of brGDGTs in lake sediments which have shown promise in reconstructing past temperature change (Blaga et al., 2010; Pearson et al., 2011; Sun et al., 2011; Loomis et al., 2012; Foster et al., 2016; Vandergoes et al., 2016). This work has been particularly successful in tropical Africa, where lacustrine brGDGT calibrations have relatively low errors of prediction (Loomis et al., 2012), and where the proxy has been successfully applied to multiple sites and to test GCM simulations of the Last Glacial Maximum (e.g., Loomis et al., 2017).

The structure of brGDGTs was initially elucidated using NMR spectroscopy applied to brGDGTs isolated from peat, and subsequent work identified the nine brGDGTs that comprise many existing temperature calibrations (Sinninghe Damsté et al., 2000; Weijers et al., 2006). More recently, however, De Jonge et al. (2013) improved the chromatographic separation of brGDGTs and identified a new set of brGDGT isomers that differ in the position of one of the methyl group on the branched alkyl chains of the GDGTs. This led to the recognition of two isomers of the major pentamethylated brGDGT, including one compound with the methyl group at position 5 (compound IIa, originally identified by Sinninghe Damsté et al., 2000), and a second with the alkyl group at position 6, termed IIa'. Similar 5-methyl and 6-methyl isomers were identified for the corresponding mono- and bicyclic brGDGTs (IIb and IIb', and IIc and IIc', respectively) as well as the hexamethylated brGDGTs (IIIa and IIIa', IIIb and IIIb', IIIc and IIIc'). Chromatographic separation and quantification of the 5- and 6-methyl isomers has been shown to greatly improve the error statistics of temperature and pH calibrations based upon brGDGTs in soils through removal of the interference of the 6-methyl compounds from 5-methyl brGDGT fractional abundances (De Jonge et al., 2014a). Moreover, De Jonge et al. (2014a) found that the fractional abundances of the 6-methyl brGDGT correlate strongly with each other, suggesting a common biological source in soils. Weber et al.

(2015) analyzed sediment and watershed soils from a Swiss lake and documented the presence of a novel “mixed” hexamethylated brGDGT possessing a 5,13,16- and a 6,13,16-trimethyloctacosanyl moiety. This compound was only detected in the lake and not in soils of the surrounding watershed, providing further evidence for in situ production of brGDGTs in lakes and suggesting that bacteria in different environments may produce distinct brGDGTs. This raises the possibility that separation of the 5- and 6-methyl isomers could produce more ‘universal’ brGDGT calibrations applicable to soil, lacustrine, and other sediment samples.

To date, there is little work examining the distribution and environmental controls on the abundances of the 6-methyl brGDGTs in lacustrine environments. Here we analyze a set of East African lake surface sediments, quantify both the 5-methyl and 6-methyl brGDGTs, evaluate the environmental factors that control the isomerization of the 5- and 6-methyl brGDGTs, and test and develop calibrations of the fractional abundances of the 5-methyl and 6-methyl brGDGTs for temperature and pH.

2. Methods

2.1. Geographic setting and sample and data collection

We analyzed surface sediment samples from 65 lakes in tropical East Africa (Fig. 1), mainly from lakes in Kenya and western Uganda. These lakes span a mean annual air temperature gradient of 1.6–26.8 °C, surface water pH gradient of 3.8–9.6, and range in depth from 0.3 to 180 m. The 65 samples are a subset of the 110 lake sediment samples previously analyzed by Loomis et al. (2012, 2014b) to determine the environmental controls on the fractional abundances of brGDGTs using a chromatographic method that did not allow the separation of 5- and 6-methyl isomers. Only 65 of these samples were measured because the others contained insufficient material for further analysis.

Complete information about the environmental setting of these samples is available in Loomis et al. (2014b) and in the

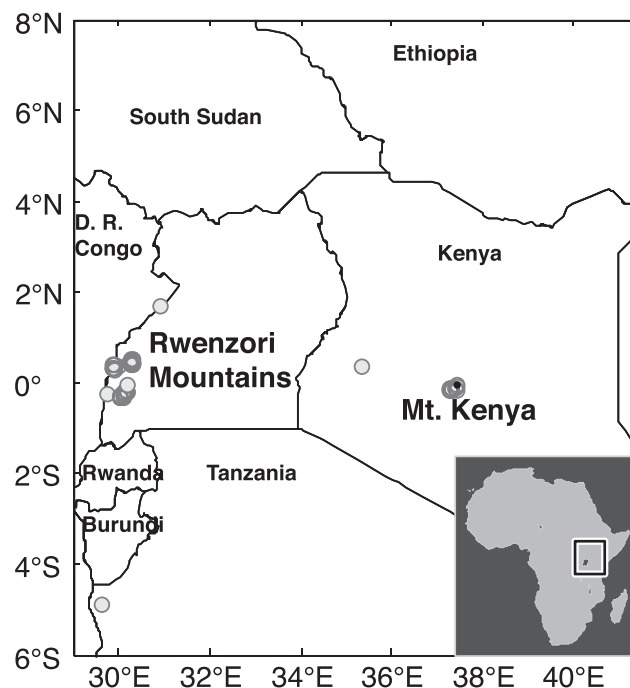


Fig. 1. Map showing the location of the 65 sample sites used in this study in gray circles. Heavier gray circles indicate multiple adjacent lakes. The inset at lower left shows the location of the study area.

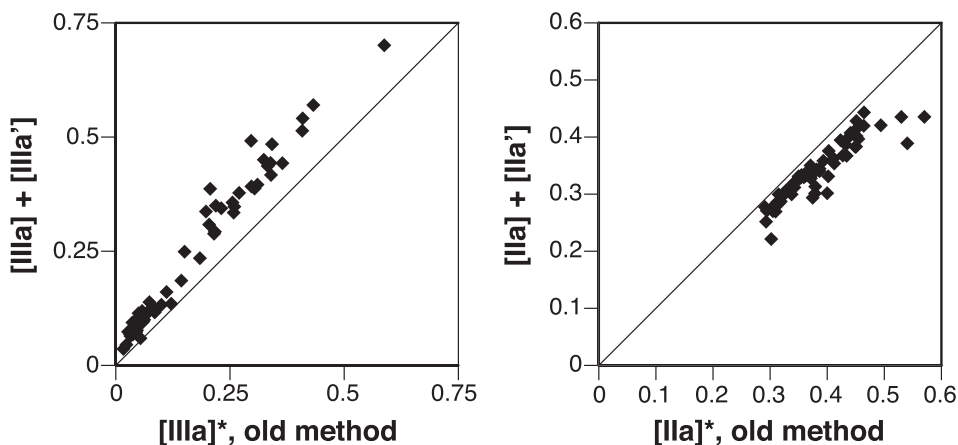


Fig. 2. Plot of the fractional abundances of IIIa^{*} and IIa^{*} measured using a Preveil Cyano column (old method, x-axis) that did not separate 5- and 6-methyl brGDGT isomers vs the summed abundances of the 5- and 6-methyl isomers of brGDGTs measured in this study using the new method (y-axis). The majority of the samples fall off the 1:1 line indicating their abundances as measured using the old method were affected by the presence of 6-methyl isomers.

Supplementary Material of this paper. Briefly, limnological data (water column temperatures, pH, dissolved oxygen content, and conductivity) were measured over multiple field expeditions conducted in different seasons and/or obtained from the literature (Loomis et al., 2014b). MAAT values for most sites were calculated from regional lapse rates (i.e., temperature changes as a function of elevation). Lapse rates were determined from measured air temperatures at different elevations in Uganda and Kenya (Eggermont et al., 2009; Loomis et al., 2017). Locally monitored temperatures were substituted for elevation-dependent temperatures where such data were available, generally at large lakes (Lakes Tanganyika, Albert).

2.2. Sample preparation and analysis

Samples were previously processed by Loomis et al. (2014b), who extracted lipids from ~ 1 cm³ freeze-dried and homogenized samples using a Dionex 350 Accelerated Solvent Extractor (ASE) using dichloromethane (DCM):methanol (MeOH) (1:1, v:v). The total lipid extract was separated over an Al₂O₃ column using hexane:DCM (9:1, v:v) and DCM:MeOH (1:1, v:v) as eluents to isolate the apolar and polar fractions, respectively. The polar fractions of these samples were re-dissolved and filtered prior to analysis for the present study. Polar fractions were analyzed at the Royal Netherlands Institute for Sea Research to determine their core lipid distribution by atmospheric pressure chemical ionization/high performance liquid chromatography–mass spectrometry (APCI/HPLC–MS) using the identical chromatographic and equipment configuration as described by Hopmans et al. (2016). Analyses were performed using selective ion monitoring (SIM) mode to track *m/z* 1302, 1300, 1298, 1296, 1292, 1050, 1048, 1046, 1036, 1034, 1032, 1022, 1020, 1018, and 744. Peak areas were integrated manually.

2.3. Statistical methods and proxy calculations

We calculated a range of ratios and regressions to evaluate the response of different GDGTs to environmental gradients in East African lakes, based upon previous work in global soils by De Jonge et al. (2014a) and our previous analyses of brGDGTs in East African lakes (Tierney et al., 2010; Loomis et al., 2012, 2014b). Using data from global soils, Weijers et al. (2007b) defined the MBT index to quantify the abundances of tetramethylated brGDGTs (Ia–Ic) relative to tetramethylated, pentamethylated (IIa–IIc), and hexamethylated (IIIa–IIIc) compounds. De Jonge

et al. (2014a) subsequently redefined the index to include both the 5-methyl and 6-methyl isomers as:

$$\text{MBT}' = (\text{Ia} + \text{Ib} + \text{Ic}) / (\text{Ia} + \text{Ib} + \text{Ic} + \text{IIa} + \text{IIa}' + \text{IIb} + \text{IIb}' + \text{IIc} + \text{IIc}' + \text{IIIa} + \text{IIIa}' + \text{IIIb} + \text{IIIb}' + \text{IIIc} + \text{IIIc}') \quad (1)$$

De Jonge et al. (2014a) also found that removal of the 6-methyl isomers could improve temperature calibrations, and defined a new MBT index using only the 5-methyl isomers as:

$$\text{MBT}'_{\text{5ME}} = (\text{Ia} + \text{Ib} + \text{Ic}) / (\text{Ia} + \text{Ib} + \text{Ic} + \text{IIa} + \text{IIb} + \text{IIc} + \text{IIIa} + \text{IIIb} + \text{IIIc}) \quad (2)$$

De Jonge et al. (2014a) defined a new CBT index for the cyclization of branched GDGTs as:

$$\text{CBT}' = -\log(\text{Ic} + \text{IIa}' + \text{IIc}' + \text{IIIa}' + \text{IIIb}' + \text{IIIc}') / (\text{Ia} + \text{IIa} + \text{IIIa}) \quad (3)$$

and a new CBT index based only upon 5-methyl isomers as

$$\text{CBT}'_{\text{5ME}} = -\log[(\text{Ib} + \text{IIb}) / (\text{Ia} + \text{IIa})] \quad (4)$$

The isomerization ratio (IR) (De Jonge et al., 2014b) quantifies the fractional abundance of 6-methyl and 5-methyl isomers as:

$$\text{IR} = (\text{IIa}' + \text{IIb}' + \text{IIc}' + \text{IIIa}' + \text{IIIb}' + \text{IIIc}') / (\text{IIa} + \text{IIa}' + \text{IIb} + \text{IIb}' + \text{IIc} + \text{IIc}' + \text{IIIa} + \text{IIIa}' + \text{IIIb} + \text{IIIb}' + \text{IIIc} + \text{IIIc}') \quad (5)$$

Lastly, De Jonge et al. (2014a) developed a calibration for MAAT based upon Index 1, calculated as:

$$\text{Index 1} = \log[(\text{Ia} + \text{Ib} + \text{Ic} + \text{IIa}' + \text{IIIa}') / (\text{Ic} + \text{IIa} + \text{IIc} + \text{IIIa} + \text{IIIa}')] \quad (6)$$

We calculated correlation coefficients (*r* values) and *p*-values for all combinations of environmental and brGDGT data. In the present study, we focussed on relationships of the brGDGTs with MAAT, water column depth (Depth), lake surface area (SA), conductivity (Cond), and average bottom and surface water pH and dissolved oxygen content (BW pH, SW pH, BW DO, SW DO, respectively) from the 65 lakes. Lake surface and bottom water temperature were not included in our analyses due to their very strong correlations with MAAT (*r*² = 0.98, *p* < 0.0001 for each). Furthermore, there are fewer data available to constrain lake temperatures, as many of the sites have very few and sporadic lake temperature profiles (Eggermont et al., 2009). We therefore use MAAT for temperature calibrations. To determine the variability

in the brGDGT data that can be explained by each of the environmental variables, we performed a principal component analysis (PCA) and redundancy analysis (RDA), a direct gradient analysis that analyzes the linear relationships between response variables (brGDGTs) and explanatory environmental variables (ter Braak, 1994). Missing environmental data were replaced with the means of each environmental variable from the East African lake dataset, in order to retain all 65 sites without affecting the correlation matrix and therefore the PCA and RDA analyses. This resulted in six substitutions, including three in SA, two in SW DO, one in BW DO. Because many of the environmental variables in our calibration data are correlated to each other, we also calculated the variance explained by each environmental variable after accounting for the effects of the other variables using forward selection in RDA (Dray et al., 2006; Borcard et al., 2011). Stepwise forward selection builds a linear regression model starting with the environmental variable with strongest correlation (r^2) to the brGDGT data, and then sequentially adds additional variables based upon the significance of the F -statistic as determined through Monte-Carlo permutation tests (499 simulations). Variable selection and addition stops when addition of new variables does not explain a significant fraction of the remaining variance as determined through permutation testing. Permutation testing also allows calculation of the variance in the brGDGT data explained by each variable after accounting for the influences of the other variables (Borcard et al., 2011).

We analyzed the fit of previously published brGDGT calibrations for temperature based upon soil and peat samples measured using the new method (De Jonge et al., 2014a; Naafs et al., 2017a, 2017b) by applying these calibrations to our lake data and calculating correlation coefficients (r^2), root mean square errors (RMSE), and average offsets between estimated and observed temperatures. We also developed new calibrations for MAAT and pH based upon MBT', MBT'_{SME}, Index 1, CBT', and CBT'_{SME} by regressing these indices from our lake sediment data on observed MAAT and lake water pH (SW and BW), and compare the calibrations' error statistics (RMSE, r^2 , etc.) to those from calibrations based on global soils, peat, and previously published East African lake calibrations. For the latter, we recalculated indices and calibrations from Loomis et al. (2012, 2014b) using only the 65 sites measured in the present study. We developed a multiple linear regression to predict MAAT from the fractional abundances of brGDGTs using stepwise forward selection (SFS) regression. This technique calculates a multiple linear regression of the brGDGT data that best predicts MAAT by sequentially regressing individual brGDGTs on MAAT, calculating the significance of adding each additional brGDGT using an F -test to avoid model overfitting (Hocking, 1976). Statistical analysis and modeling of our data were performed in Matlab, RDA analyses were performed in R with the vegan package (Borcard et al., 2011), and stepwise forward selection in RDA was performed in Canoco (ter Braak and Smilauer, 2002).

3. Results

The improved chromatographic procedure of Hopmans et al. (2016; hereafter termed the “new method”) for analysis of GDGTs achieved full separation of 5- and 6-methyl brGDGTs in our lake surface sediments. Some samples, however, had concentrations of cyclic and especially bicyclic compounds that were too low to be reliably integrated and quantified. This is particularly the case for the 5- and 6-methyl isomers IIc, IIIb, and IIIc; abundances of which were too low to be determined reliably for between 26% (IIc) and 95% (IIIc) of the samples. Our statistical analyses and calibrations, therefore, use fractional abundances calculated from the

sum of only the major brGDGTs (Ia, Ib, Ic, IIa, IIa', IIb, IIb', IIIa, IIIa'), except where otherwise noted.

6-Methyl brGDGTs are abundant in many of our surface sediments, averaging 24% and ranging as high as 54% of the major brGDGTs (Fig. 3A). Pentamethylated brGDGTs constitute the largest fraction (48.5%) of the major brGDGTs in the East African lake sediments, followed by tetramethylated (38.9%) and hexamethylated (11.6%) compounds (Fig. 3A). PCA on the correlations of the fractional abundances of the major brGDGTs in the East African lakes yields three principal components that cumulatively explain 89% of the variance in the brGDGT data (Fig. 4). The first principal component, which explains 57% of the variance, has negative loadings from brGDGT IIIa and IIa and positive loadings from Ia, Ib, Ic, IIa', and IIb', whereas the second principal component (19% of variance) has strong positive loading from IIb and negative loading from IIa' and IIIa'. The third principal component (14% of the variance) has negative loadings from Ia and positive loading from Ic, IIb, and IIIa'. The first two RDA axes explained 80.2% of the variance in the brGDGT data; almost all of which (93.6%) is explained by axis 1 (Fig. 4B, Table 2). RDA axes 2–7 each explained 5% or less of the brGDGT variance and will not be discussed. The fractional abundances of IIIa and IIa have strong positive scores on RDA axis 1, whereas Ia, Ib, IIa', and, to a lesser extent, IIb' show negative scores on axis 1. Many of these same GDGTs show strong correlations to MAAT and surface water pH (Figs. 5 and 6), discussed below.

4. Discussion

4.1. Fractional abundances of 5- and 6-methyl brGDGTs in lakes vs soils

Comparing our results to the fractional abundances determined by Loomis et al. (2012, 2014b), incomplete separation of the 5- and 6-methyl isomer clearly affected the fractional abundances previously determined using chromatographic separation with a Prevail cyano column (Weijers et al., 2007b; hereafter the “old method”; Fig. 2). The sum of the fractional abundances of IIIa and IIIa' determined with the new method is higher in almost all of our samples than the abundances of IIIa* (hereafter we designate the abundance of compounds determined with the old method with an asterisk). This is so because the old method achieved partial separation of 5- and 6-methyl isomers, resulting in a chromatographic peak from which the tail was cut during integration, reducing the overall fractional abundances of non-cyclic hexamethylated brGDGTs (see also De Jonge et al., 2014a). Our results differ from a similar analysis of global soil samples (De Jonge et al., 2014a), in which the majority of samples had nearly the same abundances of IIIa + IIIa' measured with the new method as the non-cyclic hexamethylated brGDGTs measured with the old method. This difference likely stems from the higher average fractional abundance of IIIa and IIIa' in lake sediments, which facilitated the definition of partly separated peaks during integration. In contrast, the sum of the fractional abundances of IIa and IIa' in our new data is lower than the abundance of the non-cyclic pentamethylated brGDGTs determined by the old method (IIa*, Fig. 2). This has two reasons: (i) the sum of total brGDGT abundance in our dataset obtained using the new method now includes IIIa', which constitutes a non-negligible fraction (average 6%) of the total brGDGT abundances, and therefore reduces the fractional abundances of the other brGDGTs, and (ii) in the old method IIa and IIa' coelute and hence they were quantified as the sum of the two, whereas the new method, although it separates the two isomers, does not lead to detection of “more” non-cyclic pentamethylated brGDGTs.

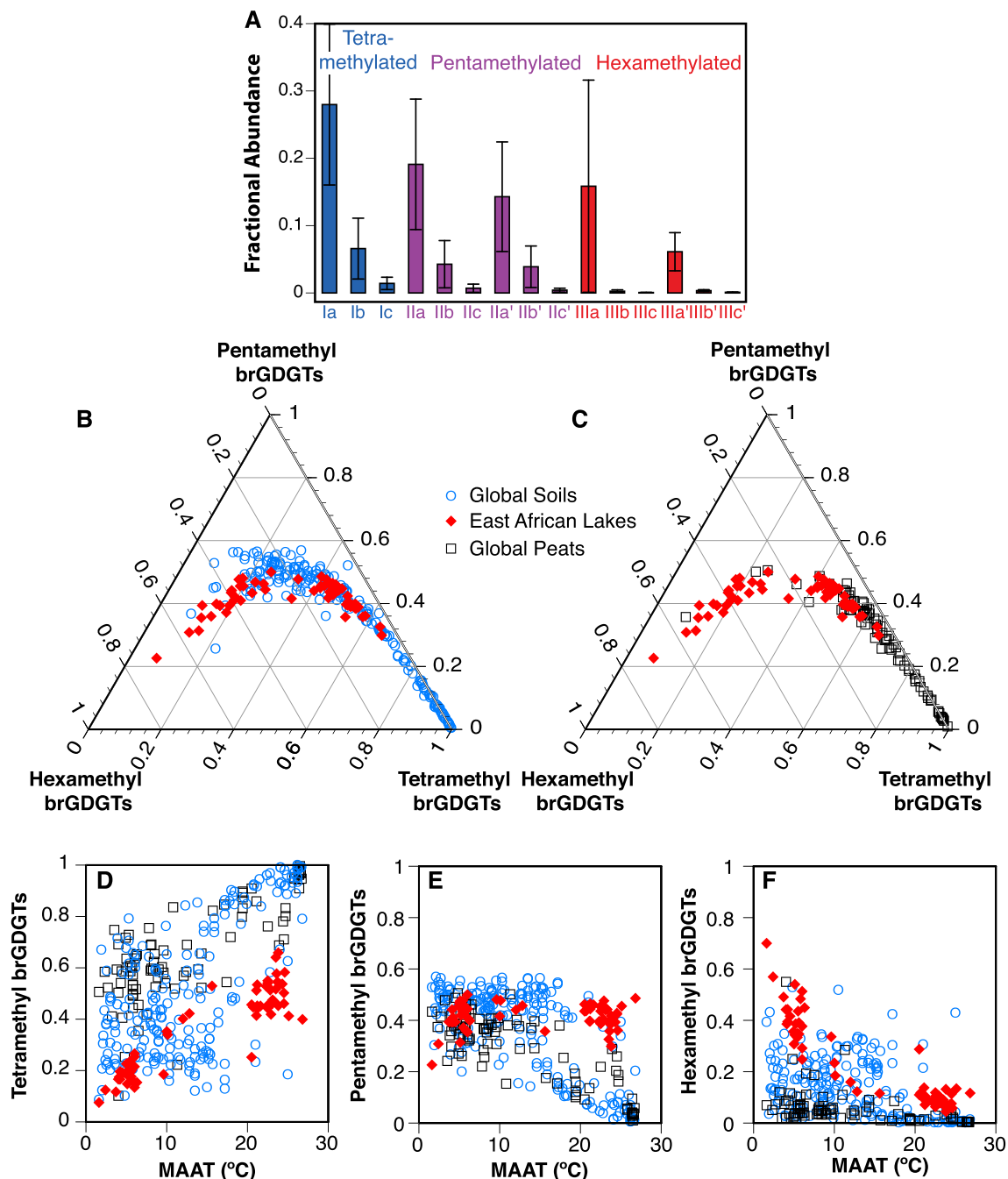


Fig. 3. (A) Fractional abundances of the individual brGDGTs in the East African lakes. The fractional abundances of summed tetramethylated, pentamethylated, and hexamethylated brGDGTs in East African lakes and global soils (from De Jonge et al., 2014a) spanning the same temperature range as our lakes (1.6–26.8 °C) are plotted in panel B. Panel C is the same as B, but for tropical peats (from Naafs et al., 2017a). Panels D–F show the fractional abundances of tetramethylated (D), pentamethylated (E), and hexamethylated (F) brGDGTs in East African lakes, soils, and peats relative to temperature.

Similar observations were made for the mono- and bicyclic penta- and hexamethylated brGDGTs. Because (i) the brGDGT fractional abundances determined through separation using the new method consistently differ from those obtained using the old method, (ii) the sum of the 5- and 6-methyl isomers often differs from the abundance of the un-separated compounds determined using the old method, and (iii) IIIa is important to many MAAT inference models and differs strongly between these methods, we strongly recommend against applying calibrations determined with the old method to data measured with the new method.

The fractional abundance of 6-methyl brGDGTs in our dataset (24%) is identical to that of the global soil dataset of De Jonge

et al. (2014a), yet the fractional abundances differ notably in the two datasets in other ways. Fig. 3B–3F compares the fractional abundances of tetra, penta- and hexamethylated brGDGTs in the global soil dataset (De Jonge et al., 2014a), the global peat dataset (Naafs et al., 2017a), and our East African lake sediment samples after removing samples from the soil and peat dataset that lie outside the range of MAATs (i.e., 1.6–26.8 °C) of our East African lake sediments. The relative abundances of tetra, penta- and hexamethylated brGDGTs in our lake sediments (Fig. 3) differ from those in soils (De Jonge et al., 2014a) over the same temperature range. In particular, the East African lake sediments have less abundant tetramethylated and more abundant hexamethylated brGDGTs

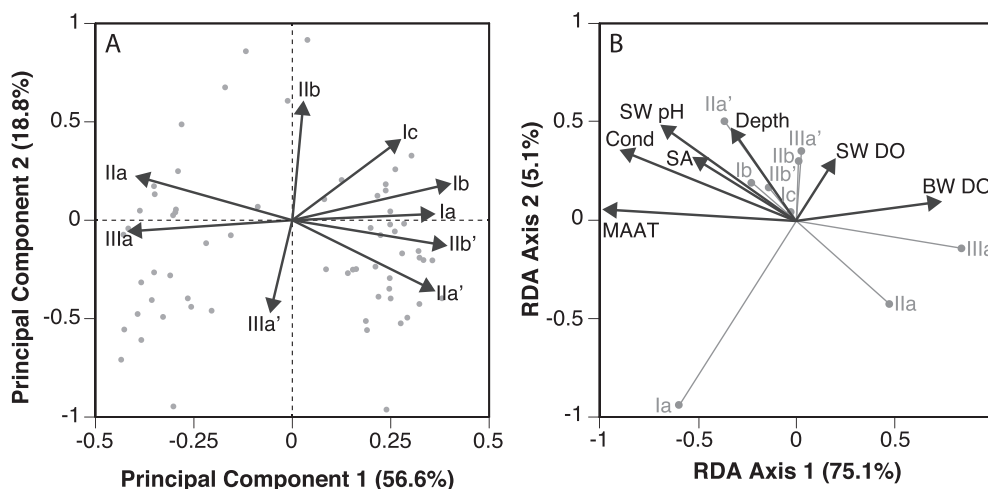


Fig. 4. Panel A: results of principal component analysis showing the major modes of variation of the fractional abundances of the brGDGTs. Gray dots indicate axis scores of lake sediments, whereas black vectors indicate axis scores of brGDGTs. Panel B: results of direct gradient analysis (RDA) of the environmental and brGDGT data. Gray vectors ending in dots show scores of brGDGTs, whereas black vectors show scores of environmental data. MAAT is mean annual air temperature, Cond is conductivity, SW pH is surface water pH, SA is surface area, SW DO is surface water dissolved oxygen, and BW DO is bottom water dissolved oxygen.

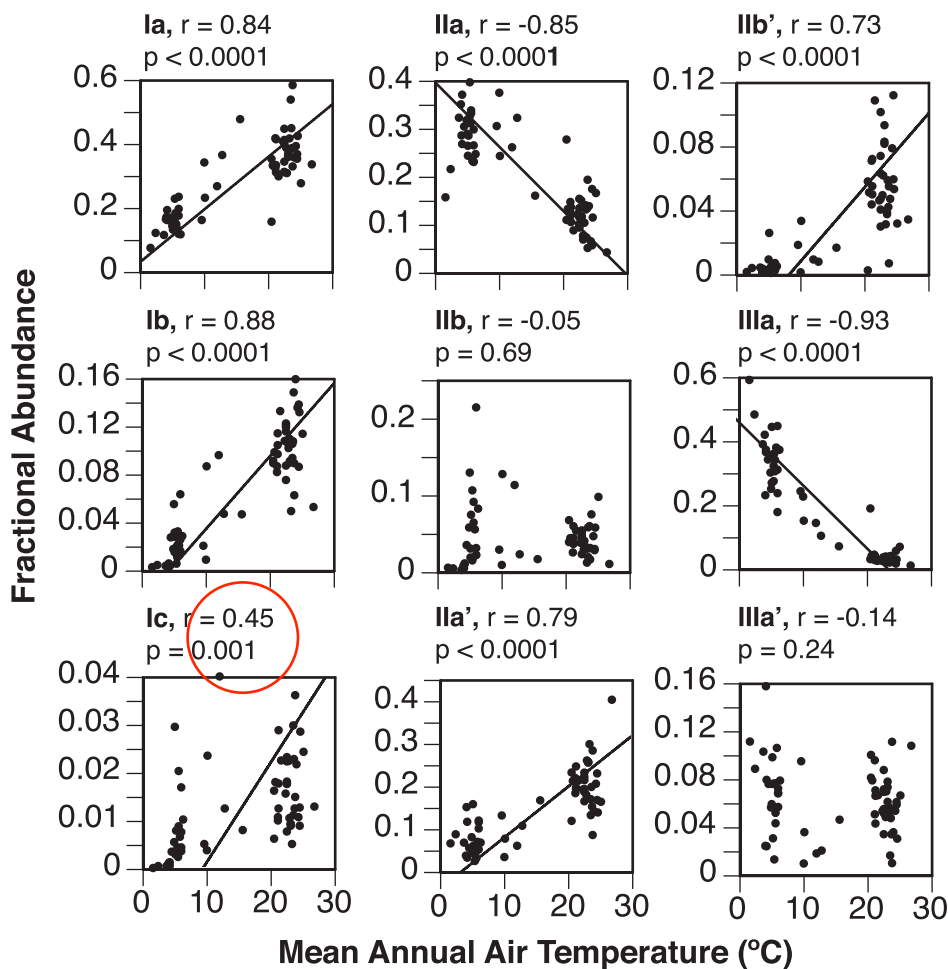


Fig. 5. Fractional abundances of the major branched GDGTs plotted against mean annual air temperature. The correlation coefficient (*r* value) and significance (*p*-value) are given for each plot. Linear regression lines are shown for all correlations significant at the 99% confidence level or higher.

than the soils (Fig. 3B). This occurs even though the soil dataset has a lower average temperature than the lake dataset (12.7 vs 15.2 °C), which should favor higher relative abundances of penta- and

hexamethylated brGDGTs. We observe even larger differences between the brGDGTs in East African lakes and global peats (Naafs et al., 2017a) within the 1.6–26.8 °C temperature range

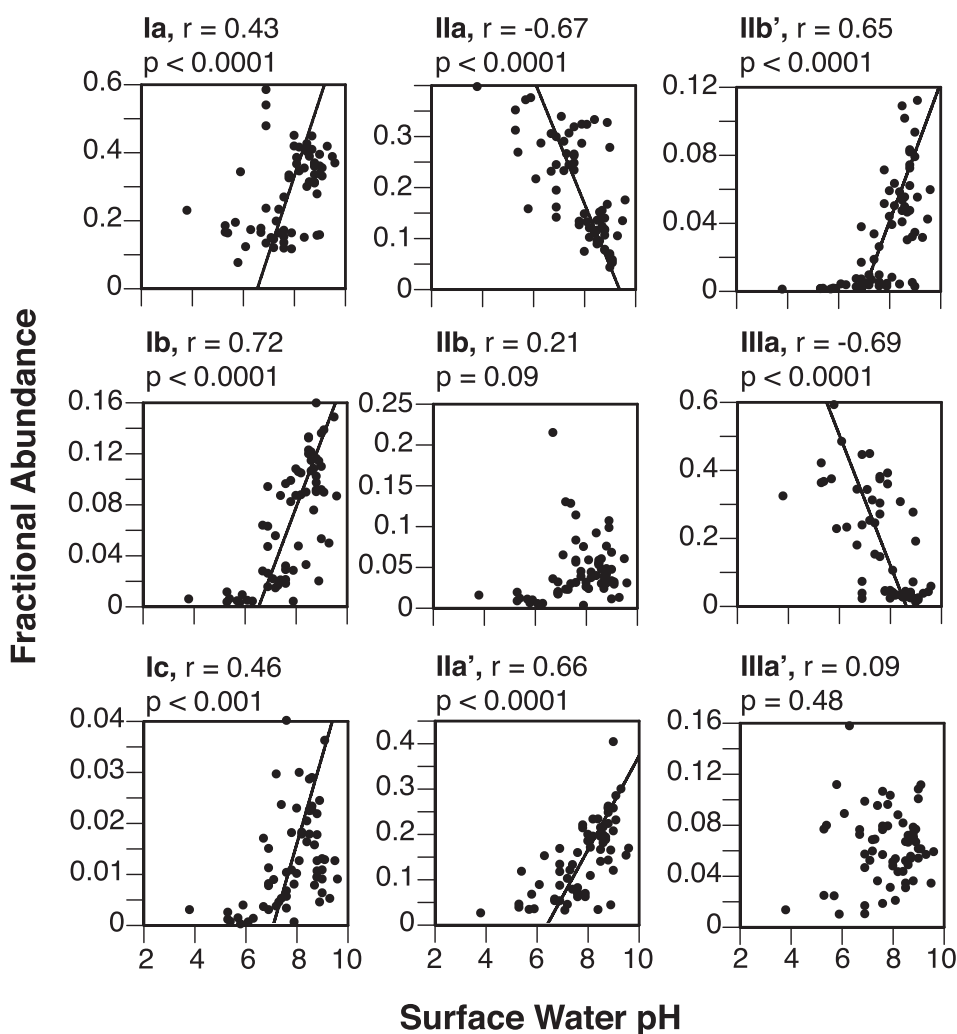


Fig. 6. Fractional abundances of branched GDGTs plotted against lake surface water pH. The correlation coefficient (r value) and significance (p -value) are given for each plot. Linear regression lines are shown for all correlations significant at the 99% confidence level or higher.

(Fig. 3C), even though the peat dataset also has a lower average temperature than the lake dataset (12.3 vs 15.2 °C). Analyses of brGDGT distributions measured with the old method have consistently shown that lake sediments often contain less tetramethylated and more hexamethylated compounds than soils in their catchments (Loomis et al., 2011, 2014a; Buckles et al., 2014), and recent analyses with the new method show similar differences in offshore coastal sediments relative to sediments from river mouths in Indonesia (Sinninghe Damsté, 2016).

Differences in the fractional abundances of brGDGTs in our East African tropical lake sediments from those in tropical soils and peats could be partly related to the pH conditions in these different datasets. Lower pH values favor increased abundances of tetramethylated brGDGTs (Weijers et al., 2007b). The peats analyzed by Naafs et al. (2017a) are on average more acidic than the soils (4.8 vs 6.2), the soils are more acidic than our lakes (6.2 vs 7.8), and the fractional abundance of the tetramethylated brGDGTs increases from lakes to soils to peats. However, the environmental differences alone cannot fully explain the differences in brGDGT distributions in these datasets, as the fractional abundances of tetra-, penta- and hexamethylated brGDGTs show different responses to temperature in lakes relative to soils and peats (Fig. 3D–F). The abundance of tetramethyl brGDGTs increases with temperature in soils, lakes, and peat (Fig. 3D). Pentamethylated brGDGTs generally decrease with temperature in soils and peats,

but show little change in our East African lake samples (Fig. 3E). In contrast, hexamethylated brGDGTs decrease with temperature in the East African lakes, but show little change in soils and peats (Fig. 3F). We infer that these differences must stem from different bacterial floras that produce the brGDGTs and different relationships between brGDGT production and temperature in lakes, soils, and peats.

4.2. Environmental controls on 5- and 6-methyl brGDGTs in lake sediments

PCA of the fractional abundances of the major brGDGTs in the East African lakes indicate many correlations among the brGDGTs that are similar to those in global soils (De Jonge et al., 2014a), such as opposing PC1 scores of tetramethylated brGDGTs vs IIIa. However, other aspects of the variance structure of the African lake and global soil brGDGT data differ. In the soil samples, 6-methyl compounds grouped together on PC1, whereas in our lake data the variance in IIa' and IIb' is orthogonal to that of IIIa'. The IIIa' and IIb' are orthogonal to their 5-methyl counterparts, IIIa and IIb, suggesting that different environmental parameters control the fractional abundances of the 5- and 6-methyl isomers of these brGDGTs. In contrast, IIa and IIa' have strong, opposite loadings on both PC 1 and PC2, suggesting opposing responses of these compounds to the same environmental factors.

Previous work has shown that environmental temperature and pH exert strong influences on the fractional abundances of brGDGTs (Weijers et al., 2007b; Tierney et al., 2010), findings that have recently been extended to the relative abundances of the 5- and 6-methyl isomers (De Jonge et al., 2014a; Sinnighe Damsté, 2016; Naafs et al., 2017a). In the East African lake data, the fractional abundances of most of the major brGDGTs are significantly correlated with MAAT (Fig. 5). IIIa and IIa are strongly negatively correlated to temperature, whereas Ia and Ib are strongly positively correlated. IIb', IIa' and Ic are less strongly but still significantly ($p < 0.001$) correlated, whereas IIb and IIIa' are not significantly correlated to MAAT. Previous work has found correlations between the fractional abundances of the brGDGTs and MAAT in East African lakes (Loomis et al., 2014b), indicating separation of the 5- and 6-methyl isomers has not dramatically changed these responses. De Jonge et al. (2014a) also observed positive correlations between MAAT and the fractional abundances of Ia, Ib, and Ic, and negative correlations to IIIa and IIa, indicating similar responses to temperature in both soil and lacustrine environments. However, de Jonge et al. (2014a) observed no correlation between MAAT and the abundances of the 6-methyl isomers except for IIa', which was weakly negatively correlated to MAAT. This contrasts with our data, in which the abundance of IIa', IIb', and the IR ($r^2 = 0.74$, $p < 0.0001$) are positively correlated to MAAT.

The fractional abundances of the brGDGTs in global soils are generally more strongly correlated with soil pH than with MAAT. This is particularly the case for the 6-methyl isomers, the abundances of which are all positively correlated with soil pH (De Jonge et al., 2014a). The fractional abundances of many of the brGDGTs in the East African lakes are correlated to SW pH (Fig. 6); however, the pH of the East African lakes is correlated to exactly the same subset of compounds in exactly the same direction that are correlated to MAAT (cf. Figs. 5 and 6; Table 1). The absolute r values of the correlations between the brGDGT abundances and pH are in all cases lower than with MAAT, in contrast to global soil data, but pH is still significantly ($p < 0.0001$ or 0.001) positively correlated to Ia, IIb', Ib, Ic, and IIa' and negative correlated to IIIa and IIa. An identical pattern emerges with BW pH, but with still weaker correlations. Indeed, examining the correlations between the brGDGT data and all the environmental data, the fractional abundances of brGDGTs are generally correlated to SW and BW pH, BW DO, conductivity and, to some extent depth

if those same brGDGTs are correlated to MAAT, but with lower r^2 values than with MAAT (Table 1). Conductivity, SW pH, BW pH, and depth are correlated to each brGDGT in the same direction as MAAT, whereas the correlations with BW DO are opposite. Surface area and SW DO are generally not significantly correlated to the brGDGT fractional abundances. These patterns of correlation are identical to those of the environmental parameters themselves: SW pH, BW pH, conductivity, and depth are all significantly ($p < 0.001$) positively correlated to MAAT, BW DO is significantly ($p < 0.001$) negatively correlated, and SW DO and SA are not significantly correlated. This suggests that many of the correlations between the brGDGT data and the environmental data are influenced by correlations among the environmental variables (e.g., Loomis et al., 2014b).

The fractional abundances of IIIa and IIa have strong positive scores on RDA axis 1, whereas Ia, Ib, IIa', and, to a lesser extent, IIb' show negative scores. This is similar to the structure of the correlations of these brGDGTs with MAAT. MAAT has the strongest negative score on axis 1, and conductivity, SW pH, and SA also have negative scores, whereas BW DO has the strongest positive score. Variance partitioning using RDA/stepwise forward selection indicates that all environmental variables except SW DO can explain a considerable fraction of the variance in the brGDGT data if the effects of the other variables are not considered (i.e. the marginal effects; ter Braak and Smilauer, 2002). However, when considering all environmental variables, only MAAT, conductivity, SW pH, and depth contribute significant ($p < 0.05$) fractions of the variance in the brGDGT data. Of these, MAAT contributes far more strongly than the others, explaining 82% of the variance in the brGDGT data that can be explained by the environmental variables. Stepwise forward selection in RDA can produce overly liberal estimates of the variance associated with the first factor included in the model (Borcard et al., 2011) – in this case MAAT; however, manual construction of stepwise models using all of the other environmental variables first, followed by MAAT, shows that MAAT explains almost as much or more variance than the other variables after forcing each of them into the model.

These results demonstrate that temperature explains the majority of the variance in the East African lake brGDGT data. These results are surprising, given the importance of pH to brGDGT distributions in soils (Weijers et al., 2007b; Peterse et al., 2012; De Jonge et al., 2014a), coastal marine sediments (Sinnighe Damsté,

Table 1
Pairwise correlations of environmental data and brGDGT fractional abundances.

		Ia	Ib	Ic	IIa	IIb	IIa'	IIb'	IIIa	IIIa'
MAAT	r	0.8425	0.8766	0.4465	-0.8449	-0.0505	0.7944	0.7292	-0.9282	-0.1465
°C	p -value	<0.0001	<0.0001	0.0012	<0.0001	0.6895	<0.0001	<0.0001	<0.0001	0.2443
SW pH	r	0.4331	0.7198	0.4647	-0.6695	0.2106	0.6601	0.6539	-0.6897	0.0886
	p -value	0.0003	<0.0001	0.0007	<0.0001	0.0922	<0.0001	<0.0001	<0.0001	0.4827
BW pH	r	0.3013	0.5616	0.4286	-0.5117	0.1591	0.4854	0.4194	-0.5019	0.0546
	p -value	0.0147	<0.0001	0.0019	<0.0001	0.2056	<0.0001	0.0020	<0.0001	0.666
BW DO	r	-0.7005	-0.6792	-0.2233	0.5705	0.2347	-0.5722	-0.6853	0.7046	0.289
mg/L	p -value	<0.0001	<0.0001	0.1191	<0.0001	0.0598	<0.0001	<0.0001	<0.0001	0.0195
SW DO	r	-0.2705	-0.134	-0.0945	0.0366	0.0411	-0.0793	-0.0946	0.2402	0.1921
mg/L	p -value	0.0293	0.2873	0.514	0.772	0.745	0.5299	0.5049	0.054	0.1253
Cond.	r	0.4371	0.6835	0.2625	-0.7210	-0.0104	0.7346	0.6726	-0.6532	0.1379
µS/cm	p -value	0.0003	<0.0001	0.0655	<0.0001	0.9344	<0.0001	<0.0001	<0.0001	0.2733
Depth	r	0.1742	0.4214	0.2459	-0.3927	0.0411	0.4361	0.3935	-0.3824	0.1114
(m)	p -value	0.1651	0.0005	0.0852	0.0012	0.7452	0.0003	0.0039	0.0017	0.3771
Surface	r	0.0489	0.0586	0.3321	-0.2192	-0.0539	0.2716	0.0575	-0.1349	0.239
Area (m ²)	p -value	0.6988	0.6429	0.0185	0.0794	0.6698	0.0286	0.6854	0.2841	0.0552
	p -value	0.6988	0.6429	0.0185	0.0794	0.6698	0.0286	0.6854	0.2841	0.0552
SW Temp	r	0.8349	0.8613	0.4373	-0.8140	-0.1062	0.7739	0.7196	-0.9019	-0.181
°C	p -value	<0.0001	<0.0001	0.0015	<0.0001	0.3997	<0.0001	<0.0001	<0.0001	0.149
	p -value	<0.0001	<0.0001	0.0015	<0.0001	0.3997	<0.0001	<0.0001	<0.0001	0.149
BW Temp	r	0.8221	0.8636	0.4508	-0.8189	-0.0818	0.7838	0.7102	-0.9062	-0.151
°C	p -value	<0.0001	<0.0001	0.0010	<0.0001	0.517	<0.0001	<0.0001	<0.0001	0.2173

Table 2
RDA results. Columns 1 and 2 give the scores of each environmental variable on RDA axes 1 and 2. Marginal effects are the percentage of the variance in the brGDGTs that can be explained by the environmental variables for each environmental variable without accounting for effects from the other environmental variables. Conditional effects are the percentage of the variance in the brGDGTs that can be explained by the environmental variables for each environmental variable after accounting for effects from the other environmental variables using permutation testing.

	RDA axis 1 Score	RDA axis 2 Score	Marginal effects, % Variance	Conditional effects, % Variance
MAAT	−0.995	0.053	82.3	82.3
Cond	−0.907	0.358	50.0	6.5
SW pH	−0.703	0.483	48.4	4.8
Depth	−0.346	0.476	16.1	3.2
SA	−0.542	0.32	29.0	1.6
BW DO	0.193	0.097	46.8	1.6
SW DO	0.737	0.313	3.2	0.0

2016), and at least some lake settings (Tyler et al., 2010; Schoon et al., 2013), and given the large pH gradient observed among the East African lakes. Bacteria are thought to vary their membrane lipid compositions in response to pH in order to regulate proton transport across cell membranes (Booth, 1985). Most bacteria regulate their internal pH to near neutrality, and make their cell membrane more and more rigid at higher environmental pH to regulate proton loss to their surroundings. Our results could suggest that the stress placed upon membrane composition by environmental pH variations in soils is much higher than in lakes – i.e. proton transport and/or regulation of proton loss is more easily accomplished in aqueous environments – or that bacteria in aquatic environments regulate proton loss across their cell membranes via mechanisms other than alteration of the brGDGTs. However, these hypotheses would not explain the abundance of cyclic brGDGTs in alkaline marine sediments, which are thought to derive from in situ production (Peterse et al., 2009; Sinninghe Damsté, 2016).

It is also possible that this pattern reflects differences in the degree to which the calibration data reflects temperature and pH of the environments in which the brGDGTs are produced in the East African lakes and the global soil samples. Temperatures used in the soil calibrations are generally derived from nearby weather station data, whereas soil pH is determined directly from the soil samples (Weijers et al., 2007b). The pH therefore more directly measures the environmental conditions experienced by the bacteria that produce brGDGTs. Indeed, soil temperature can vary considerably from air temperatures measured in weather stations due to shading and other processes that alter the relationship between soil and air temperature. The MAAT data in the East African lake calibration is determined from local monitoring of air and lake temperatures at many sites, which formed the basis for an elevation-based model of temperature (Eggermont et al., 2009; Tierney et al., 2010). Temperature lapse rates are very uniform over tropical Africa (Loomis et al., 2017), and there is little to no temperature seasonality, in contrast to global temperature data. As noted above, measured surface and bottom lake temperatures are strongly correlated to MAAT in our dataset, attesting to the validity of our elevation-based MAAT model. In contrast, the pH data for the East African lakes is based on sporadic measurements. Lake surface and bottom water pH are more weakly correlated than surface and bottom temperatures, and it is unclear where in these lakes most of the brGDGTs are produced, creating uncertainty in the ‘target’ pH. Thus, it is possible that the soil pH calibration data is of better quality than soil temperature, and vice versa for lakes, elevating the uncertainty in the brGDGT–soil temperature and brGDGT–lake pH relationships. Alternatively, the weak influence of pH on brGDGT distributions could signify different sources of brGDGTs. **Some unknown fraction of the brGDGTs in East African lake sediment may derive from soils.** The temperature of East African soils is generally similar to that of the lakes, whereas the pH can differ substantially and is generally more acidic (Loomis et al., 2011). It is therefore possible that mixtures of soil- vs

lake-derived brGDGTs might obscure correlations with pH more than temperature. We will return to this issue in Section 4.3.

Despite the uncertainties with respect to pH, our results demonstrate that temperature is an important control on brGDGT distributions in East African lake sediment, and provide insight into the value of separating 5- and 6-methyl isomers in East African lake sediment samples for climate reconstruction. Previous analyses showed that brGDGT IIIa* was more strongly correlated with temperature than any other brGDGT (Tierney et al., 2010; Loomis et al., 2012, 2014b) however, there was a potential error introduced in this relationship by coelution of brGDGTs IIIa and IIIa'. Indeed, the orthogonal relationship between IIIa and IIIa' (Fig. 4) and insignificant correlation between IIIa' and temperature (Fig. 5) indicates that separating IIIa from its 6-methyl isomer could indeed improve temperature reconstructions. Perhaps more importantly, in our dataset derived using the old method, IIa* was only weakly correlated to MAAT (Loomis et al., 2014b). Separation of IIa and IIa' indicates strong negative correlation between IIa and MAAT and positive correlation between IIa' and MAAT, indicating opposing and competing influences that may have masked the response of IIa* to MAAT. Taken together these results suggest that separation of 5- and 6-methyl isomers could improve environmental calibrations based upon brGDGTs.

4.3. brGDGT calibrations for temperature and pH

We tested the application of previously published temperature and pH calibrations that are based on methods that achieve separation of 5- and 6-methyl brGDGTs to our East African lake dataset. These calibrations are based upon globally distributed soils (De Jonge et al., 2014a; Naafs et al., 2017b) and peats (Naafs et al., 2017a). These include calibrations of MBT_{5Me} to temperature based upon peats (Naafs et al., 2017a) and soils (De Jonge et al., 2014a; Naafs et al., 2017b); calibration of Index 1 to MAAT in soils (De Jonge et al., 2014a); the MAT_{mrs}, which is a multivariate regression of the fractional abundances of the major brGDGTs (Ia, IIa, and IIIa) to MAAT (De Jonge et al., 2014a); and the MAT_{mr}, a multivariate regression of the brGDGTs with the strongest correlation to MAAT in the global soil data (De Jonge et al., 2014a). As discussed above, we use MAAT values for the lakes, despite the fact that lake temperatures should regulate the distribution of brGDGTs produced in situ. We do so because lake temperatures are measured sporadically, whereas the MAAT datasets that form our lapse rate model (Eggermont et al., 2009; Loomis et al., 2012, 2017) are more complete. MAAT and lake temperatures are strongly correlated ($r^2 = 0.98$, $p < 0.0001$), indicating that MAAT can serve as proxy for lake temperature in our study sites.

All of these calibrations capture the general pattern of cold vs warm lakes in our dataset, with r^2 values ranging from 0.78 for MAT_{mrs} to 0.92 for calibrations based on MBT_{5Me} and Index 1 (Fig. 7, Table 3). However, the peat- and soil-based calibrations generally have low accuracy, highlighted by RMSEs that range from

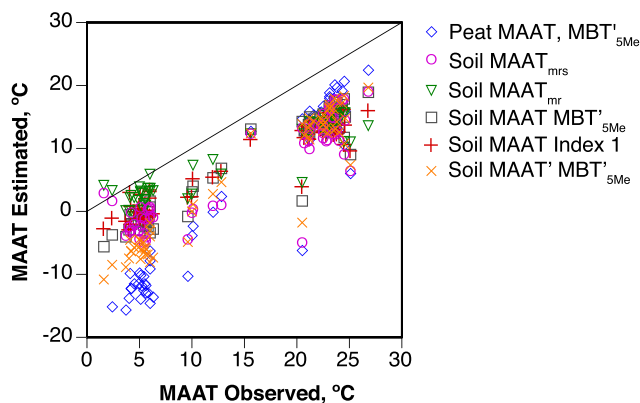


Fig. 7. Results of applying soil- and peat-based temperature calibrations based upon brGDGTs measured using the new method in globally distributed soil and peat samples to our East African lake dataset. Peat MAAT MBT'_{5Me} uses an equation from Naafs et al. (2017a) for samples with IR values <0.5; Soil MAAT_{mrs}, Soil MAAT_{mr}, Soil MAAT MBT'_{5Me}, and Soil MAAT Index 1 use equations from De Jonge et al. (2014a), and Soil MAAT' MBT'_{5Me} uses an equation from Naafs et al. (2017b). All calibrations produce relatively large offsets from observed temperatures (1:1 line).

7.6 to 13.2 °C (Table 3). Much of this error is a product of large biases toward colder average temperatures predicted by these calibrations, with average offsets from the MAATs observed at our sites that range from −6.6 °C (for MAAT_{mr}, De Jonge et al., 2014a) to −11.8 °C (for the peat-based MBT'_{5Me} calibration; Naafs et al., 2017a). Many of the calibrations also have strong trends in the residuals; for instance, the MAAT_{mr} calibration shows larger offsets at the warmest sites, whereas the peat-based MBT'_{5Me} calibration produces the largest offsets at the coldest sites. These errors and biases are not surprising given the large differences in the distributions of the brGDGTs between lakes, soils, and peats. The relatively high r^2 values suggest that these calibrations could capture general trends in temperature in sediment cores from the East African lakes, i.e. they could record the timing of intervals of warming and cooling. However, the large biases and trends in residuals also indicate that both the average value and amplitude of past temperature changes must be treated with caution, at the very least, when applying soil- and/or peat-based brGDGT calibrations to East African lake sediments. We do not recommend applying these calibrations to lake sediments unless it can be demonstrated that the lake brGDGT distributions resemble those of soils of the watershed of the lake.

Given the high RMSE and significant offsets of estimated MAATs from soil- and peat-based brGDGT calibrations applied to our lake sediments, we developed several new lacustrine brGDGT calibrations to temperature. To test the utility of existing calibration types, we regressed MBT', MBT' and CBT', MBT'_{5Me}, and Index 1 on MAAT (Fig. 8). The calibration

$$\text{MAAT} = -1.21 + 32.42 \times \text{MBT}'_{5\text{Me}} \quad (7)$$

has a RMSE of 2.44 °C ($r^2 = 0.92$, $p < 0.0001$), and the calibration

$$\text{MAAT} = 12.22 + 18.79 \times \text{Index1} \quad (8)$$

has a RMSE of 2.47 °C ($r^2 = 0.92$, $p < 0.0001$).

As expected, these new calibrations have substantially better fit to the East African MAAT values than the global soil- and peat-based brGDGT calibrations, most likely due to the large differences between lacustrine and soil brGDGT distributions that bias reconstructed MAAT values. The East African lake calibrations also have a lower RMSE than the soil- and peat-based calibrations, which average 4.7 °C. The causes of this improvement in model performance are unclear, but could be due to the stronger correlations between brGDGTs and MAAT in the East African lakes than in soils and peat, which are also strongly influenced by pH; more accurate MAAT calibration data; more variability in the lacustrine brGDGT data, which are not dominated by Ia and therefore have a larger dynamic range; and/or the use of a regional rather than global calibration training set, which could reduce the variability in secondary environmental variables that could influence the fractional abundance of brGDGTs (e.g., Loomis et al., 2014b).

We also developed a new calibration using stepwise forward selection (SFS), which builds a multivariate linear regression of brGDGT abundances on MAAT, adding the one brGDGT at a time that improves the calibration most at each step. The addition of variables ends when the addition no longer decreases the regression error (Hocking, 1976). This calibration has been shown to significantly improve the error statistics of lacustrine brGDGT calibrations developed using old method data (Pearson et al., 2011; Loomis et al., 2012) as well as temperature reconstructions (Loomis et al., 2012, 2014b, 2017). The SFS calibration

$$\begin{aligned} \text{MAAT} = & 23.81 - 31.02 \times \text{IIIa} - 41.91 \times \text{IIb} - 51.59 \times \text{IIb}' \\ & - 24.70 \times \text{IIa} + 68.80 \times \text{Ib} \end{aligned} \quad (9)$$

has a lower RMSE of 2.14 °C and higher correlation coefficient ($r^2 = 0.94$, $p < 0.0001$) than the calibrations based upon Index1 and MBT'_{5Me}.

We also attempted other multivariate linear regressions of the brGDGT data on MAAT. We regressed all the brGDGTs that were significantly ($p < 0.0001$) correlated to MAAT, which included Ia, Ib, IIa, IIa', IIb', IIIa. This approach is similar to the MAAT_{mr} model of De Jonge et al. (2014a), but resulted in a slightly higher RMSE (2.21 °C) than the SFS model. However, F -statistics revealed that the coefficients on several of the brGDGTs in this model (IIa, IIb') were not significant ($p > 0.1$), indicating model overfitting. Because the SFS model includes brGDGT (IIb) whose fractional abundance is not correlated to temperature, we also developed a linear regression using the same brGDGTs as the SFS model but excluding IIb. This model performed more poorly than the SFS (RMSE = 2.50 °C, $r^2 = 0.92$). This indicates that, although IIb is uncorrelated to MAAT, it does improve the calibration to MAAT, perhaps by removing the

Table 3

Results of temperature calibrations, including RMSE, r^2 , and average temperature offsets, calculated as the average of the estimated minus observed temperatures for each sample.

	RMSE	r^2	Average T offset	Citation
Peat MAAT MBT' _{5Me}	13.17	0.92	−11.8	Naafs et al. (2017a)
Soil MAAT MBT' _{5Me}	8.25	0.92	−7.8	De Jonge et al. (2014b)
Soil MAAT Index 1	8.41	0.92	−7.8	De Jonge et al. (2014b)
Soil MAAT _{mr}	7.63	0.9	−6.6	De Jonge et al. (2014b)
Soil MAAT _{mrs}	9.54	0.81	−8.8	De Jonge et al. (2014b)
Soil MAAT' MBT' _{5Me}	10.40	0.92	−9.9	Naafs et al. (2017b)
New EA Lake MBT' _{5Me}	2.43	0.92	0	This study
New EA Lake Index 1	2.47	0.92	0	This study
New EA Lake SFS	2.14	0.94	0	This study

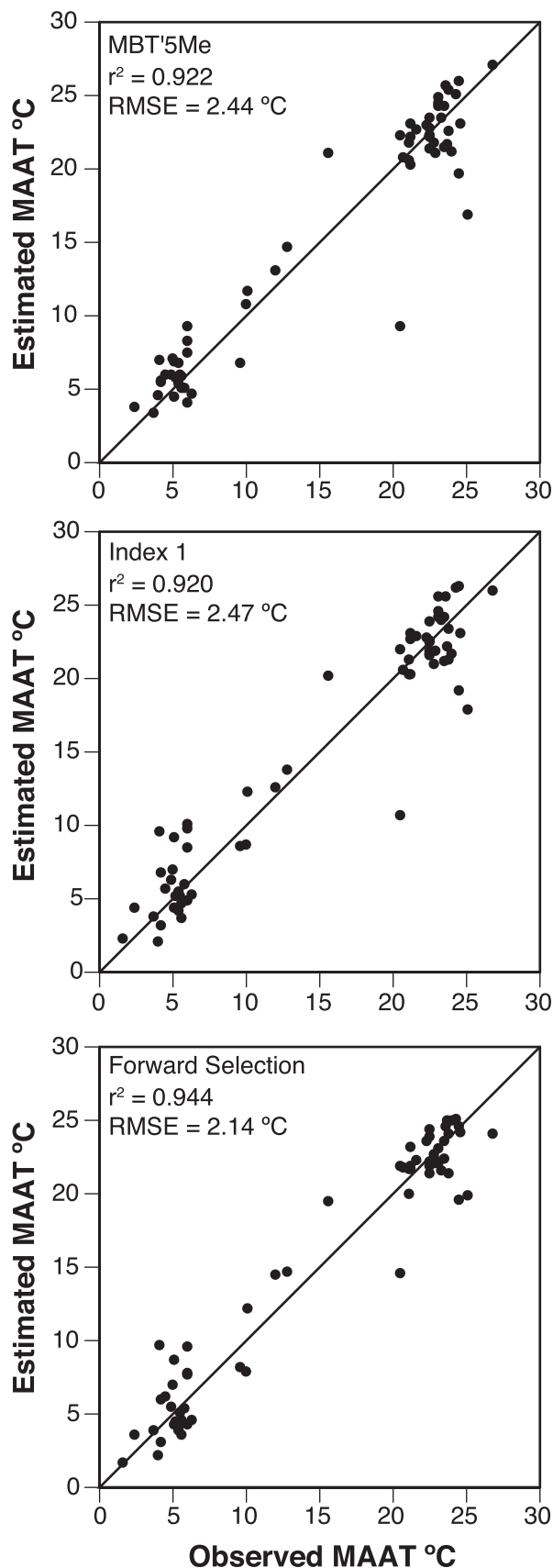


Fig. 8. New lacustrine brGDGT calibrations for mean annual air temperature based upon MBT'_{5Me}, Index I (De Jonge et al., 2014a), and stepwise forward selection (Eqs. 7–9). RMSE and r^2 values are given for each calibration.

effects of secondary environmental variables that are correlated to MAAT (Loomis et al., 2014b).

Separation of the 5- and 6-methyl brGDGT isomers substantially improved the error statistics of MAAT calibrations in the global soil dataset (De Jonge et al., 2014a). To assess whether this is the case in the East African lakes, we recalculated models for MAAT using brGDGT data measured using the old method from Loomis et al. (2012) in only the 65 lakes analyzed in the present study (recalculation of the models was necessary because Loomis et al. measured more lakes than the present study). The MAAT model based upon MBT and CBT values measured using the old method have a higher RMSE than our new calibration based upon MBT'_{5Me} (Eq. 7) determined using the new method (RMSE of 3.38 °C vs 2.44 °C for old and new method, respectively). Interestingly, the MBT'_{5Me} model also has a lower error than MAAT models based upon MBT' (RMSE = 3.51, $r^2 = 0.84$) and MBT' and CBT' (RMSE = 3.33, $r^2 = 0.86$).

In the global soil dataset, fractional abundances of the 6-methyl isomers are strongly correlated to pH, and De Jonge et al. (2014a) showed that separation of these isomers using MBT'_{5Me} improved MAAT reconstructions partially by removing the pH effects on the degree of methylation. Although it is possible that there are similar effects in our East African lake data, our RDA and variance partitioning tests suggest that pH does not strongly influence brGDGT distributions in our dataset. Indeed, including CBT' in MBT'-based MAAT calibrations does not substantially improve the error statistics of a calibration using MBT' alone. Rather, we note that many brGDGTs have higher correlations (r^2) to MAAT when measured using the new method. This includes not only the fractional abundances of brGDGTs that have 5- and 6-methyl isomers (such as IIa and IIa', and IIIa and IIIa'), but also Ia and Ib, which do not and whose fractional abundance is only indirectly affected by the improved quantification of brGDGTs in the new method. We therefore surmise that the improved model performance using the new method is due to improved quantification of the fractional abundances of the brGDGTs as a whole, rather than solely due to separation of the isomers.

In contrast to MBT-based calibrations, our new SFS model for MAAT has nearly identical error statistics to the SFS model Loomis et al. (2012) developed using old method data (RMSE = 2.1 °C for both models). We note, however, that several of the largest East African lakes, namely Lakes Albert, Edward, Malawi, and Tanganyika had strong negative outlying temperatures (too cold) in the SFS calibration of Loomis et al. (2012). This is no longer the case using our new SFS calibration. The old SFS calibration included several brGDGTs whose abundance was affected by the partial coelution of 5- and 6-methyl isomers, including IIIa, IIa, and IIc, and apparently separation of the 5- and 6-methyl isomers improved temperature estimation in these large lakes. Although the relative abundance of 6-methyl isomers is not correlated to lake size in our dataset, two of the three large lakes (Albert and Tanganyika) have the highest IR values of the lakes in our dataset (0.90 and 0.82, respectively). Whatever the cause, this suggests that although the new chromatographic method may not substantially improve RMSE values from SFS-based temperature reconstructions in most lakes, it may broaden the application of brGDGT-based temperature reconstructions to the largest lakes, which are of large interest from a palaeoclimatic point of view. This would be valuable, as it would avoid comparisons of temperature reconstructions based upon different methods such as brGDGTs and TEX₈₆ (e.g., Loomis et al., 2017).

Most of the temperature calibrations converge on a RMSE of ~2–2.5 °C. This is even the case for the stepwise forward

selection model developed using old method data, which, using 110 lakes, achieved an RMSE of 2.1 °C, nearly identical to the error of our SFS calibration using new method data. It is not clear how, or whether, these errors could be further reduced. The similarity in these RMSEs, despite differences in the measurement and modeling technique, could derive from multiple sources including: (i) variability inherent in the response of brGDGT distributions to temperature; (ii) differences in the temperatures of air, surface water, bottom water, and/or shallow sediments relative to brGDGT production in these different environment; (iii) and/or mixtures of GDGTs derived from soils and in situ production within lakes. The inherent variability of brGDGT responses cannot be quantified; however, admixture of soil-derived brGDGTs is most likely, given the relatively large differences in brGDGT responses to temperature suggested by our data (Fig. 7) vs the relatively small differences in temperature between air, surface water, bottom water, and shallow sediments in these lakes.

Given the similar RMSE values for the different models, validation experiments are required to determine which models perform best in different environmental settings and applications. Previous work using the old method showed that the SFS model produced the most reliable results when applied to sediment cores from East African lakes (Loomis et al., 2012); however, the SFS calibration may be affected by the correlation of multiple environmental variables, such as pH and dissolved oxygen, to MAAT (Loomis et al., 2014b). Because these variables may exhibit different correlations to MAAT in other regions, we suggest that calibrations based on MBT_{5Me} or Index 1 may be more appropriate in tropical lakes outside East Africa. In addition, we urge caution in applying these calibrations to lakes with strong seasonal changes in temperature since seasonal biases in brGDGT production could strongly influence reconstructed temperatures (Buckles et al., 2014; Loomis et al., 2014a).

We also tested whether the East African lake brGDGT data could be used to develop calibrations for lake pH by regressing CBT' and CBT'_{5Me} on lake pH. It is unclear whether brGDGTs are produced primarily in surface or bottom water (e.g., Buckles et al., 2014), so we regressed CBT on both surface water and bottom water pH. All the regressions produced significant ($p < 0.0001$) correlations. In contrast to the findings from our calibrations to MAAT, separation of 5- and 6-methyl isomers did not improve the goodness of fit of models to pH. We obtained r^2 values of 0.52 and 0.30 for regression of CBT'_{5Me} on SW and BW pH vs r^2 values of 0.57 and 0.33 for regression of CBT' on SW and BW pH. Notably, both CBT'_{5Me} and CBT' have substantially higher correlation with SW pH than they do with BW pH, similar to results determined using the old method (Tierney et al., 2010; Loomis et al., 2014b), implicating production of brGDGT in the surface mixed layer. Our best calibration:

$$\text{Surface water pH} = 8.95 + 2.65 * \text{CBT}' \quad (10)$$

had an RMSE of 0.80 and an r^2 of 0.57.

In general, the error statistics (such as r^2 values) are worse for our pH calibrations than our MAAT calibrations. This is not surprising given our findings that temperature influences a much larger fraction of the variance in brGDGT distributions than pH (Table 2), and previous work indicating that the degree of cyclization of the brGDGTs in the surface sediment of East African lakes is only weakly correlated with pH (Tierney et al., 2010; Loomis et al., 2014b). In studies of Lake Challa, East Africa, Buckles et al. (2014) observed large seasonal changes in the CBT of both core and intact polar GDGTs in settling organic matter in the lake water column that could not be explained by fluctuations in either lake water or soil pH. Together these results suggest the degree of cyclization of brGDGTs in these lakes may be controlled by variables other

than pH. As noted above, this could reflect brGDGT contributions from soils that experienced similar temperatures but different pH values than the lakes. However, we think it is unlikely that this is a major effect on the lacustrine brGDGT distributions and calibrations. First, and foremost, our results demonstrate that soil-derived brGDGTs have a very different distribution than lakes, which leads to strong, negative cold biases when applying soil-based brGDGT calibrations (Fig. 7). We therefore do not necessarily expect that the similar temperatures in soils and lakes should lead to less 'contamination' and error in MAAT calibrations than pH calibrations. Secondly, the soils in our sampling region are generally more acidic than the lakes (Loomis et al., 2011). Mixing soil-derived brGDGTs, which have distributions that are biased toward cold (higher degree of methylation) and acidic (lower degree of cyclization) values, should therefore cause individual lakes to be negatively offset in both brGDGT-estimated MAAT and pH. The residuals in MAAT and pH calibrations should therefore be correlated. The correlation between the residuals of our 'best' model for MAAT based upon SFS and our CBT' model for pH are significantly correlated ($p < 0.01$), but only very weakly so, with an r^2 of 0.11, indicating only a small fraction of shared variance. Together with the large differences in brGDGT distributions between lakes and soils (Section 4.1), this suggests that **the majority of the brGDGTs in the East African lakes are produced in situ, as suggested by previous studies (Tierney et al., 2010; Buckles et al., 2014; Loomis et al., 2014b), and that these lake-derived brGDGTs are only weakly influenced by pH.** Whatever the case may be, these results indicate that reconstructions of lake pH must be interpreted with caution.

5. Conclusions

Application of new chromatographic separation methods to surface sediments from East Africa lakes demonstrates that 6-methyl brGDGT isomers can be highly abundant. Temperature exerts a dominant control on the distributions of brGDGTs in the lake sediments for all 5-methyl and some 6-methyl isomers. This contrasts with soils in which the abundances of 6-methyl isomers are strongly correlated to pH, suggesting that the bacteria producing brGDGTs respond differently to temperature and chemical gradients in soils vs lakes. Due to large differences in the distributions of brGDGTs in our tropical lake sediment vs tropical soils and peats, previously published temperature calibrations from soils and peats exhibit large offsets and errors when applied to lake sediments. However, temperature calibrations based upon MBT'_{5Me}, Index 1, and stepwise forward selection of the fractional abundances of the brGDGTs using our samples have low errors (RMSE of 2.14–2.47 °C), highlighting their excellent potential for temperature reconstructions using lake sediment cores. Separation of the 6-methyl isomers improves the RMSE of temperature calibrations based upon MBT indices in lake sediments. Separation does not improve the error statistics of temperature calibrations based upon stepwise forward selection, but does remove significant outliers that were present in calibrations that did not separate 5- and 6-methyl isomers. We suggest that these improvements reflect an improved ability to quantify brGDGT abundances using the new chromatographic methods, rather than environmental biases introduced by the 6-methyl brGDGTs.

Acknowledgements

We thank J. Ossebaar and A. Mets for their help with the UHPLC-APCI-MS analysis. This work was partially supported by grants from the National Science Foundation to James Russell and by a University of Texas at Austin postdoctoral fellowship to

Shannon Loomis. The work at NIOZ was supported by funding from the Netherlands Earth System Science Center (NESSC) through a gravitation grant (NWO 024.002.001) from the Dutch Ministry for Education, Culture and Science to JSSD. We thank David Naafs and three anonymous reviewers for helpful comments on an earlier version of this paper.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.orggeochem.2017.12.003>.

Associate Editor—Philip Meyers

References

- Bendle, J., Weijers, J.W.H., Maslin, M., Sinninghe Damsté, J.S., Schouten, S., Hopmans, E.C., Boot, C., Pancost, R.D., 2010. Major changes in glacial and Holocene terrestrial temperatures and sources of organic carbon recorded in the Amazon Fan by tetraether lipids. *Geochemistry, Geophysics, Geosystems* 11, Q12007.
- Berke, M.A., Johnson, T.C., Werne, J.P., Schouten, S., Sinninghe Damsté, J.S., 2012. A Mid-Holocene thermal maximum at the end of the African humid period. *Earth and Planetary Science Letters* 351–352, 95–104.
- Blaga, C.I., Reichart, G.J., Schouten, S., Lotter, A.F., Werne, J.P., Kosten, S., Mazzeo, N., Lacerot, G., Sinninghe Damsté, J.S., 2010. Branched glycerol dialkyl glycerol tetraethers in lake sediments: can they be used as temperature and pH proxies? *Organic Geochemistry* 41, 1225–1234.
- Booth, I.R., 1985. Regulation of cytoplasmic pH in bacteria. *Microbiological Reviews* 49, 359–378.
- Borcard, D., François, G., Legendre, P., 2011. *Numerical Ecology* with R. Springer.
- Braconnot, P., Harrison, S.P., Kageyama, M., Bartlein, P.J., Masson-Delmotte, V., Abe-Ouchi, A., Otto-Bliesner, B., Zhao, Y., 2012. Evaluation of climate models using palaeoclimatic data. *Nature Climate Change* 2, 417–424.
- Buckles, L.K., Weijers, J.W.H., Verschuren, D., Sinninghe Damsté, J.S., 2014. Sources of core and intact branched tetraether membrane lipids in the lacustrine environment: anatomy of Lake Challa and its catchment, equatorial East Africa. *Geochimica et Cosmochimica Acta* 140, 106–126.
- De Jonge, C., Hopmans, E.C., Stadnitskaia, A., Rijpstra, W.I.C., Hofland, R., Tegelaar, E. W., Sinninghe Damsté, J.S., 2013. Identification of novel penta- and hexamethylated branched glycerol dialkyl glycerol tetraethers in peat using HPLC–MS, GC–MS, and GC–SMB–MS. *Organic Geochemistry* 54, 78–82.
- De Jonge, C., Hopmans, E.C., Zell, C.I., Kim, J.-H., Schouten, S., Sinninghe Damsté, J.S., 2014a. Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in soils: implications for paleoclimate reconstruction. *Geochimica et Cosmochimica Acta* 141, 97–112.
- De Jonge, C., Stadnitskaia, A., Hopmans, E.C., Cherkashov, G., Fedotov, A., Sinninghe Damsté, J.S., 2014b. In situ produced branched glycerol dialkyl glycerol tetraethers in suspended particulate matter from the Yenisei River, Eastern Siberia. *Geochimica et Cosmochimica Acta* 125, 476–491.
- Dray, S., Legendre, P., Peres-Neto, P.R., 2006. Spatial modeling: a comprehensive framework for principal coordinate analysis of neighbor matrices (PCNM). *Ecol Model* 196, 483–493.
- Eggermont, H.R., Heiri, O., Russell, J.M., Vuille, M., Audenaert, L., Verschuren, D., 2009. Paleotemperature reconstruction in tropical Africa using fossil Chironomidae (Insecta: Diptera). *Journal of Paleolimnology*. <https://doi.org/10.1007/s10933-009-9339-2>.
- Foster, L.C., Pearson, E.J., Juggins, S., Hodgson, D.A., Saunders, K.M., Verleyen, E., Roberts, S.J., 2016. Development of a regional glycerol dialkyl glycerol tetraether (GDGT)-temperature calibration for Antarctic and sub-Antarctic lakes. *Earth and Planetary Science Letters* 433, 370–379.
- Hocking, R.R., 1976. The analysis and selection of variables in linear-regression. *Biometrics* 32, 1–49.
- Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2016. The effect of improved chromatography on GDGT-based palaeoproxies. *Organic Geochemistry* 93, 1–6.
- Li, J., Pancost, R.D., Naafs, B.D.A., Huan, Y., Cheng, Z., Xie, S., 2016. Distribution of glycerol dialkyl glycerol tetraether (GDGT) lipids in a hypersaline lake system. *Organic Geochemistry* 99, 113–124.
- Loomis, S.E., Russell, J.M., Ladd, B., Eggermont, H.R., Street-Perrott, F.A., Sinninghe Damsté, J.S., 2012. Calibration and application of the branched GDGT proxy on East African lake sediments. *Earth and Planetary Science Letters* 357–358, 277–288.
- Loomis, S.E., Russell, J.M., Ladd, B., Eggermont, H.R., Street-Perrott, F.A., Sinninghe Damsté, J.S., 2011. Distribution of branched GDGTs in soils and lake sediments from Western Uganda: implications for a lacustrine paleothermometer. *Organic Geochemistry* 42, 739–751.
- Loomis, S.E., Russell, J.M., Heuroux, A.M., D'Andrea, W.J., Sinninghe Damsté, J.S., 2014a. Seasonal variability of branched glycerol dialkyl glycerol tetraethers (brGDGTs) in a temperate lake system. *Geochimica et Cosmochimica Acta* 144, 173–187.
- Loomis, S.E., Russell, J.M., Sinninghe Damsté, J.S., Verschuren, D., Eggermont, H.R., 2014b. Environmental controls on branched GDGTs in East African lakes: temperature, nutrients, chemistry, and morphometry. *Organic Geochemistry* 66, 25–37.
- Loomis, S.E., Russell, J.M., Lamb, H.F., 2015. Northeast African temperature variability since the late Pleistocene. *Palaeogeography, Palaeoclimatology, Palaeoecology* 423, 80–90.
- Loomis, S.E., Russell, J.M., Verschuren, D., Morrill, C., de Cort, G., Sinninghe Damsté, J.S., Olago, D.O., Eggermont, H.R., Street-Perrott, F.A., Kelly, M.A., 2017. The tropical lapse rate steepened during the Last Glacial Maximum. *Science Advances* 3, e1600815.
- Naafs, B.D.A., Inglis, G.N., Zheng, Y., Amesbury, M.J., Biester, H., Bindler, R., Blewett, J., Burrows, M.A., Castillo Torres, D.D., Chambers, F.M., Cohen, A.D., Evershed, R. P., Feakins, S.J., 2017a. Introducing global peat-specific temperature and pH calibrations based upon brGDGT bacterial lipids. *Geochimica et Cosmochimica Acta* 208, 285–301.
- Naafs, B.D.A., Gallego-Sala, A.V., Inglis, G.N., Pancost, R.D., 2017b. Refining the global branched glycerol dialkyl glycerol tetraether (brGDGT) soil temperature calibration. *Organic Geochemistry* 106, 48–56.
- Otto-Bliesner, B.L., Russell, J.M., Clark, P.U., Liu, Z., Overpeck, J.T., Konecky, B.L., DeMenocal, P., Nicholson, S.E., He, F., Lu, Z., 2014. Coherent changes of southeastern equatorial and northern African rainfall during the last deglaciation. *Science* 346, 1223–1227.
- Pearson, E.J., Juggins, S., Talbot, H.M., Weckström, J., Rosén, P., Ryves, D.B., Roberts, S. J., Schmidt, R., 2011. A lacustrine GDGT-temperature calibration from the Scandinavian Arctic to Antarctica: renewed potential for the application of GDGT-paleothermometry in lakes. *Geochimica et Cosmochimica Acta* 75, 6225–6238.
- Peterse, F., Kim, J.-H., Schouten, S., Kristense, D.K., Koc, N., Sinninghe Damsté, J.S., 2009. Constraints on the application of the MBT/CBT palaeothermometer at high latitude environments (Svalbard, Norway). *Organic Geochemistry* 40, 692–699.
- Peterse, F., Prins, M.A., Beets, C.J., Troelstra, S.R., Zheng, H., Gu, Z., Schouten, S., Sinninghe Damsté, J.S., 2011. Decoupled warming and monsoon precipitation in East Asia over the last deglaciation. *Earth and Planetary Science Letters* 301, 256–264.
- Peterse, F., van der Meer, J., Schouten, S., Weijers, J.W.H., Fierer, N., Jackson, R.B., Kim, J.-K., Sinninghe Damsté, J.S., 2012. Revised calibration of the MBT-CBT paleotemperature proxy based on branched tetraether membrane lipids in surface soils. *Geochimica et Cosmochimica Acta* 96, 215–229.
- Powers, L.A., Johnson, T.C., Werne, J.P., Castaneda, I.S., Hopmans, E.C., Sinninghe Damsté, J.S., Schouten, S., 2005. Large temperature variability in the southern African tropics since the last glacial maximum. *Geophysical Research Letters* 32, L08706.
- Schoon, P.L., de Kluijver, A., Middelburg, J.J., Downing, J.A., Sinninghe Damsté, J.S., Schouten, S., 2013. Influence of lake water pH and alkalinity on the distribution of core and intact polar branched glycerol dialkyl glycerol tetraethers (GDGTs) in lakes. *Organic Geochemistry* 60, 72–82.
- Schouten, S., Hopmans, E.C., Schefuß, E., Sinninghe Damsté, J.S., 2002. Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea-surface temperatures? *Earth and Planetary Science Letters* 204, 265–274.
- Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: a review. *Organic Geochemistry* 54, 19–61.
- Sinninghe Damsté, J.S., 2016. Spatial heterogeneity of sources of branched tetraethers in shelf systems: the geochemistry of tetraethers in the Berau River delta (Kalimantan, Indonesia). *Geochimica et Cosmochimica Acta* 186, 13–31.
- Sinninghe Damsté, J.S., Hopmans, E.C., Pancost, R.D., Schouten, S., Geenevasen, J.A.J., 2000. Newly discovered non-isoprenoid dialkyl diglycerol tetraether lipids in sediments. *Journal of the Chemical Society. Chemical Communications* 73, 1683–1684.
- Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Weijers, J.W.H., Foesel, B.U., Overmann, J., Dedysh, S.N., 2011. 13,16-Dimethyl octacosanedioic acid (*iso*-diabolic acid), a common membrane-spanning lipid of *Acidobacteria* subdivisions 1 and 3. *Applied and Environmental Microbiology* 77, 4147–4154.
- Sinninghe Damsté, J.S., Ossebaer, J., Schouten, S., Verschuren, D., 2012. Distribution of tetraether lipids in the 25-kyr sedimentary record of Lake Challa: extracting reliable TEX₈₆ and MBT/CBT palaeotemperatures from an equatorial African lake. *Quaternary Science Reviews* 50, 43–54.
- Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Foesel, B.U., Wüst, P.K., Overmann, J., Stott, M.B., 2014. Ether- and ester-bound *iso*-diabolic acid and other lipids in members of *Acidobacteria* subdivision 4. *Applied and Environmental Microbiology* 80, 5207–5218.
- Sun, Q., Chu, G., Liu, M., Xie, M., Li, S., Ling, Y., Wang, X., Shi, L., Jia, G., Lü, H., 2011. Distributions and temperature dependence of branched glycerol dialkyl glycerol tetraethers in recent lacustrine sediments from China and Nepal. *Journal of Geophysical Research* 116. <https://doi.org/10.1029/2010JG001365>.
- ter Braak, C.J.F., 1994. Canonical community ordination. Part I: basic theory and linear methods. *Ecology* 75, 127–140.
- ter Braak, C.J.F., Smilauer, P., 2002. *CANOCO reference manual and CanoDraw for Windows User's guide*. Biometrics, Ithaca, NY, USA.
- Tierney, J.E., Russell, J.M., Huang, Y., Sinninghe Damsté, J.S., Hopmans, E.C., Cohen, A. S., 2008. Northern hemisphere controls on tropical southeast African climate during the last 60,000 years. *Science* 332, 252–255.

- Tierney, J.E., Russell, J.M., 2009. Distributions of branched GDGTs in a tropical lake system: implications for lacustrine application of the MBT/CBT paleoproxy. *Organic Geochemistry*. <https://doi.org/10.1016/j.orggeochem.2009.04.014>.
- Tierney, J.E., Russell, J.M., Eggermont, H.E., Hopmans, E.C., Verschuren, D., Sinninghe Damsté, J.S., 2010. Environmental controls on branched tetraether lipid distributions in tropical East African lake sediments. *Geochimica et Cosmochimica Acta* 74, 4902–4918.
- Tyler, J.J., Nederbragt, A.J., Jones, V.J., Thurow, J.W., 2010. Assessing past temperature and soil pH estimates from bacterial tetraether membrane lipids: evidence from the recent lake sediments of Lochnagar, Scotland. *Journal of Geophysical Research* 115. <https://doi.org/10.1029/2009JG001109>.
- Vandergoes, M.J., Bauersachs, T., Newnham, R.M., Rees, A.B.H., Schwark, L., 2016. A refined paleotemperature calibration for New Zealand limnic environments using differentiation of branched glycerol dialkyl glycerol tetraether (brGDGT) sources. *Journal of Quaternary Science* 31, 823–835.
- Weber, Y., De Jonge, C., Rijpstra, W.I.C., Hopmans, E.C., Stadnitskaia, A., Schubert, C.J., Lehmann, M.F., Sinninghe Damsté, J.S., Niemann, H., 2015. Identification and carbon isotope composition of a novel GDGT isomer in lake sediments: evidence for lacustrine brGDGT production? *Geochimica et Cosmochimica Acta* 154, 118–129.
- Weijers, J.W.H., Schouten, S., Hopmans, E.C., Geenevasen, J.A.J., David, O.R.P., Coleman, J.M., Pancost, R.D., Sinninghe Damsté, J.S., 2006. Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. *Environmental Microbiology* 8, 648–657.
- Weijers, J.W.H., Schefuß, E., Schouten, S., Sinninghe Damsté, J.S., 2007a. Coupled thermal and hydrological evolution of tropical Africa over the last deglaciation. *Science* 315. <https://doi.org/10.1126/science.1138131>.
- Weijers, J.W.H., Schouten, S., van den Donker, J.C., Hopmans, E.C., Sinninghe Damsté, J.S., 2007b. Environmental controls on bacterial tetraether membrane lipid distribution in soils. *Geochimica et Cosmochimica Acta* 71, 703–713.
- Weijers, J.W.H., Panoto, E., van Bleijswijk, J., Schouten, S., Rijpstra, W.I.C., Balk, M., Stams, A.J.M., Sinninghe Damsté, J.S., 2009. Constraints on the biological source (s) of the orphan branched tetraether membrane lipids. *Geomicrobiology Journal* 26, 402–414.
- Zheng, Y., Pancost, R.D., Liu, X., Wang, Z., Naafs, B.D.A., Xie, X., Liu, Z., Yu, X., Yang, H., 2017. Atmospheric connections with the North Atlantic enhanced the deglacial warming in northeast China. *Geology* 45, 1031–1034.
- Zink, K.-G., Vandergoes, M.J., Mangelsdorf, K., Dieffenbacher-Krall, A.C., Schwark, L., 2010. Application of bacterial glycerol dialkyl glycerol tetraethers (GDGTs) to develop modern and past temperature estimates from New Zealand lakes. *Organic Geochemistry* 41, 1060–1066.