

1 **Seasonal variability and sources of *in situ* brGDGT**
2 **production in a permanently stratified African crater lake**

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4 Loes van Bree^a, Francien Peterse^{a*}, Allix Baxter^a, Wannes De Crop^b, Sigrid van Grinsven^c,
5 Laura Villanueva^c, Dirk Verschuren^b, Jaap Sinninghe Damsté^{a,c}

6
7 ^a Utrecht University, Faculty of Geosciences, Department of Earth Sciences, Princetonlaan
8 8A, 3584 CB Utrecht, The Netherlands.

9 ^b Ghent University, Limnology Unit, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium.

10 ^c NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology
11 and Biogeochemistry, and Utrecht University, PO Box 59, 1790 AB Den Burg, The
12 Netherlands.

13
14 *Corresponding author. Utrecht University, Faculty of Geosciences, Department of Earth
15 Sciences, Princetonlaan 8A, 3584 CB Utrecht, The Netherlands. F.Peterse@uu.nl.

16
17 **Keywords:**

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24 **Highlights:**

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26 - BrGDGTs in the tropical African lake Chala are produced *in situ*
27 - Acidobacteria are not the dominant source of aquatic brGDGTs
28 - Stratification and mixing drive aquatic brGDGT production and their signature

29 **Abstract**

30 Lake sediments are important archives of continental climate history, and their lipid
31 biomarker content can be exploited to reconstruct paleoenvironmental conditions. Branched
32 glycerol dialkyl glycerol tetraethers (brGDGTs) are bacterial membrane lipids widely used in
33 paleoclimate studies to reconstruct past temperature. However, major gaps still exist in our
34 understanding of the environmental controls on *in situ* (i.e., aquatic) production in lake
35 systems. In Lake Chala, a permanently stratified tropical crater lake in East Africa, we
36 determined the concentrations and fractional abundances of individual brGDGTs along depth
37 profiles of suspended particulate matter collected monthly from September 2013 to January
38 2015, and in settling particles collected monthly at 35 m water depth from August 2010 to
39 January 2015, and compared these brGDGT distributions with those in surficial lake-bottom
40 sediments and catchment soils. We find that brGDGTs are primarily produced within the
41 water column, and that their concentrations and distributions vary greatly with depth and over
42 time. Comparison with concentration-depth profiles of the monthly distribution and
43 abundance of bacterial taxa, based on 16S rRNA gene amplicon sequencing and
44 quantification, indicates that Acidobacteria are likely not the main producers of brGDGTs in
45 Lake Chala. Shallowing of the oxic-anoxic boundary during seasonal episodes of strong
46 water-column stratification promoted production of specific brGDGTs in the anoxic zone.
47 BrGDGT distributions in the water column do not consistently relate with temperature, pH, or
48 dissolved-oxygen concentration, but do respond to transitions between episodes of strong
49 stratification and deep (but partial) lake mixing, as does the aquatic bacterial community.
50 Hence, the general link between brGDGT distributions and temperature in brGDGT-based
51 paleothermometry is more likely driven by a change in bacterial community composition than
52 by membrane adaptation of specific members of the bacterial community to changing
53 environmental conditions. Although temperature is not the principal driver of distributional
54 changes in aquatic brGDGTs in this system, at least not during the 17-month study period,
55 abundance-weighted and time-integrated averages of brGDGT fractional abundance in the 53-
56 month time series of settling particles reveal systematic variability over longer time scales that
57 indirectly relates to temperature. Thus, although we do not as yet fully understand the drivers
58 of modern-day brGDGT fluxes and distributions in Lake Chala, our data do support the
59 application of brGDGT paleothermometry to time-integrated archives such as sediments.

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61

62 1. Introduction

63 Lake sediments are important archives of continental climate history, especially in (sub-)
64 tropical regions where other long-term, high-resolution natural archives such as ice cores are
65 lacking. Lipid biomarkers preserved in those sediments can be used to examine present and
66 past environmental conditions, and often provide more specific information on those
67 conditions than bulk geochemical proxies (see Castañeda and Schouten, 2011 for a review).
68 For example, plant waxes stored in lake sediments are used to reconstruct past vegetation and
69 hydroclimate dynamics (e.g., Freeman and Pancost, 2013; Diefendorf and Freimuth, 2017),
70 while the presence and distribution of (iso-)loliolide, long-chain *n*-alk-1-enes or 1,15 *n*-alkyl
71 diols can be linked to shifts in algal community composition and/or primary productivity
72 (e.g., Volkman et al., 1998; Castañeda and Schouten, 2011; van Bree et al., 2018).

73 Temperature is probably the most important climate parameter to be reconstructed
74 quantitatively from lacustrine settings. Although temperature variation in the tropics is
75 relatively modest, even on glacial-interglacial timescales (2-4 °C at sea level; e.g. Loomis et
76 al., 2017; Chevalier et al., 2020), this has major impact on tropical continental rainfall through
77 its control on sea-surface evaporation and monsoon dynamics between the ocean and adjacent
78 continents. Therefore, no rainfall or moisture-balance reconstruction from the tropics can be
79 properly interpreted without knowing local/regional temperature history as reference frame.
80 However, despite more than a decade of substantial effort, generating long and continuous
81 quantitative temperature reconstructions from tropical regions remains challenging.

82 One promising proxy for continental paleothermometry is based on a suite of
83 membrane lipids supposedly derived from bacteria, namely the branched glycerol dialkyl
84 glycerol tetraethers (brGDGTs; Sinninghe Damsté et al., 2000). These consist of tetra- (I),
85 penta- (II) or hexamethylated (III) components, with none (suffix a), one (b) or two (c)
86 cyclopentyl moieties, and with methyl groups on the 5th (5-methyl) or 6th (6-methyl; indicated
87 with a prime notation) carbon position of their alkyl chain (Fig. 1; Sinninghe Damsté et al.,
88 2000; Weijers et al., 2006, De Jonge et al., 2013). The distribution of brGDGTs in modern
89 surface soils and peats shows empirical relationships with mean annual air temperature
90 (MAAT) and the pH of the soil or peat in which they are produced (Weijers et al., 2007b; De
91 Jonge et al., 2014a; Naafs et al., 2017a,b). Although the bacteria that produce brGDGTs are
92 still largely unknown (Sinninghe Damsté et al., 2018), this relationship has been commonly
93 used as proxy for continental air temperature in paleoclimate reconstructions. For example,
94 analysis of brGDGTs in loess soils, peats and marine sediments has produced

95 paleotemperature records across a wide range of geological ages (e.g., Weijers et al., 2007a;
96 Peterse et al., 2011; Naafs et al., 2017a; Zheng et al., 2017).

97 The application of this temperature proxy on lake-sediment records was initially based
98 on the premise that all sedimentary brGDGTs are derived from catchment soils and washed
99 into the lake by erosion. However, when brGDGT distributions in lake sediments were found
100 to differ substantially from those in soils surrounding the lake, it became clear that there must
101 also be an *in situ* source of brGDGTs contributing to the lake sediments (e.g., Tierney and
102 Russell, 2009; Tierney et al., 2009; Sinninghe Damsté et al., 2009; Loomis et al., 2011;
103 Schouten et al., 2013; Buckles et al., 2014; Colcord et al., 2015; Li et al., 2016). In addition,
104 brGDGT isomers of type IIIa with methyl branches at the 5th position on the one end and at
105 the 6th position on the other end (IIIa'') have so far been detected exclusively in lakes and not
106 in soils, providing further evidence for their *in situ* production (Weber et al., 2015, 2018).
107 Furthermore, lacustrine brGDGTs are significantly more ¹³C-depleted than those in nearby
108 soils, implying that at least some of their sources are distinct (Weber et al., 2015; 2018;
109 Colcord et al., 2017).

110 Water-column studies show that brGDGT concentrations generally increase below the
111 oxycline, suggesting that they are mainly produced in the anoxic portion of the hypolimnion
112 (Sinninghe Damsté et al., 2009; Bechtel et al., 2010; Blaga et al., 2011; Woltering et al., 2012;
113 Buckles et al., 2014; Loomis et al., 2014b; Miller et al., 2018). Further, brGDGT production
114 often varies seasonally (Sinninghe Damsté et al., 2009; Woltering et al., 2012; Buckles et al.,
115 2014), which may introduce a temperature bias towards the season(s) with high brGDGT
116 production (Loomis et al., 2014b; Miller et al., 2018). The contribution of aquatic brGDGTs,
117 especially that of IIIa, generally results in a substantial underestimation of present-day
118 temperature when the transfer function based on soil brGDGTs is used (Tierney et al., 2010),
119 which has stimulated the development of temperature calibrations based on lake sediments
120 (Tierney et al., 2010; Pearson et al., 2011; Sun et al., 2011; Loomis et al., 2012; Russell et al.,
121 2018). As in soils, the amount and distribution of brGDGTs in lake sediments seems to be
122 influenced mostly by temperature and lake-water pH (Tierney et al., 2010; Sun et al., 2011;
123 Loomis et al., 2014a), although a wide range of other factors, such as oxygen availability
124 (e.g., Tierney et al., 2012; Loomis et al., 2014a; Weber et al., 2018), light (Loomis et al.,
125 2014b), mixing regime (Loomis et al., 2014b), nutrients (Tierney et al., 2010; Loomis et al.,
126 2014a), alkalinity (Schoon et al., 2013), redox state (Weber et al., 2018) and conductivity
127 (Tierney et al., 2010), have also been suggested to influence the *in situ* production of
128 brGDGTs in lakes.

129 Temperature calibrations based on brGDGTs in soils and peats have substantially
130 improved following the identification and chromatographic separation of 5-methyl and 6-
131 methyl brGDGT isomers (De Jonge et al., 2014a; Naafs et al., 2017a, 2017b; Dearing
132 Crampton-Flood et al., 2020). Initial scanning of surficial bottom sediments from East African
133 lakes revealed that especially 6-methyl brGDGTs behave differently in lakes compared to
134 soils, suggesting that they are produced by different bacteria, or that brGDGT producers in
135 lakes respond differently to environmental changes than those in soils (Russell et al., 2018).
136 Separation of the 5-methyl and 6-methyl brGDGTs yields slightly better error statistics for the
137 East African lake calibration and lacks outliers such as are present in the calibration without
138 separation of these isomers, re-affirming the potential of brGDGTs for paleotemperature
139 reconstructions in lakes (Russell et al., 2018). Nevertheless, Weber et al. (2018) recently
140 showed that variations in brGDGT composition above and below the oxycline in Lake
141 Lugano (Switzerland) are linked to the occurrence of distinct bacterial groups that thrive in
142 the oxic and anoxic parts of the water column. In addition, the carbon isotopic composition of
143 brGDGTs in the sediments of Alpine lakes indicates that brGDGT producers are
144 differentiated according to lake trophic status. Together, this suggests that brGDGT signatures
145 in a lake sediment record may also be influenced by temperature-independent factors, such as
146 variations in community composition and primary production (Weber et al., 2018).

147 In this study we examined brGDGTs in suspended particulate matter (SPM) from the
148 water column of a permanently stratified lake (Lake Chala) in tropical Africa over a 17-month
149 period to further constrain the seasonal and depth distribution of different brGDGTs, to
150 identify their main producers, and to ascertain the sources of brGDGTs eventually stored in
151 lake sediments. To this effect the SPM brGDGT data were compared with measurements of
152 temperature, pH and dissolved oxygen (DO) obtained through concurrent water-column
153 monitoring, and with the composition and abundance of bacterial taxa in the SPM based on
154 16S rRNA gene amplicon sequencing and quantification. We also analyzed brGDGTs in
155 settling particles collected at monthly intervals over a 4.5-year (53-month) period, to reveal
156 possible long(er)-term trends in the seasonality of brGDGT production in this lake, which
157 may help elucidate its environmental drivers. Finally, comparison of the aquatic brGDGT
158 signature with that of soils surrounding the lake and the lake sediments itself was expected to
159 shed light on the significance of paleoclimate reconstructions based on brGDGTs in lake
160 sediments.

161

162

163 **2. Material and methods**

164 **2.1. Study system**

165 Lake Chala (locally “Challa”, after a nearby village) is a small (4.2 km²), deep (~90 m) and
166 permanently stratified (meromictic) crater lake, situated at ~880 m above sea level and
167 bridging the border of Kenya and Tanzania (3°19' S, 37°42' E) in the foothills of Mt.
168 Kilimanjaro. At this near-equatorial location, mean monthly air temperature (MMAT) varies
169 between 21-22 °C in July-August and 26-28 °C in January-February (Buckles et al., 2014;
170 Bodé et al., 2020). The tropical rain belt associated with latitudinal migration of the Inter-
171 Tropical Convergence Zone (ITCZ) passes across the region twice yearly, resulting in two
172 wet seasons and two dry seasons. Short rains occur from late October to December, and long
173 rains from March to mid-May. The principal dry season occurs during southern hemisphere
174 winter (June-September) and is characterized by lower air temperature and higher wind
175 speeds. The latter drive evaporative cooling which promotes deep convective mixing of the
176 water column of Lake Chala to ~40-60 m depth, while the deeper water remains permanently
177 stratified and anoxic (Wolff et al., 2011; Buckles et al., 2014; van Bree et al., 2018). A second
178 period of lesser mixing, to 25-30 m depth, occurs during the short dry season of January-
179 February. Primary productivity is highest during the principal dry season (June to October),
180 when nutrient-rich deep water is mixed upwards into the normally unproductive epilimnion
181 (Wolff et al., 2014; van Bree et al., 2018). The lake’s water balance is partly maintained by
182 rainfall on the lake surface and over the steep-sloping crater basin, occasionally supplemented
183 by high rainfall over the catchment of a small creek which breaches the north-western crater
184 rim (Buckles et al., 2014). As lake-surface evaporation (1700 mm yr⁻¹) greatly exceeds annual
185 rainfall (600 mm yr⁻¹), water balance is maintained by substantial subsurface inflow (Payne,
186 1970) of water that originates from percolation in or above the forest belt on Mt. Kilimanjaro
187 (Hemp, 2006; Bodé et al., 2020).

188

189 **2.2. Field observations and sample collection**

190 **2.2.1. Temperature, pH, and dissolved-oxygen monitoring of the water column**

191 Vertical profiles of temperature, dissolved oxygen (DO), conductivity (K25) and pH were
192 measured at 2-m intervals through the upper 50 m of the water column using a Hydrolab
193 Quanta® multi-sensor probe at a mid-lake position (Fig. 2), at monthly intervals between
194 September 2013 and January 2015 (van Bree et al., 2018). Additionally, water temperature
195 was measured by automatic temperature loggers, at 2-hourly intervals between September

196 2010 and January 2015, suspended at a selection of the following water depths: 2, 10, 20, 25,
197 30, 35, 40, 45, 50 and 85 m. The set of monitoring depths varied over time due to the
198 occasional malfunctioning and subsequent replacement of loggers. Due to loss of logger data
199 during retrieval, no water-column temperature information is available for the period between
200 7 January and 11 September 2012. The entire 53-month temperature record was corrected for
201 drift of individual loggers, using the Hydrolab profiles as reference. Periods of water-column
202 mixing and stratification were determined on the basis of the temperature-logger time series,
203 or estimated for the abovementioned hiatus period on the basis of mean monthly air
204 temperature (MMAT) data from Bodé et al. (2020); the latter represents a savanna site ~25
205 km to the west of Lake Chala and at similar elevation.

206

207 **2.2.2. Suspended particulate matter sampling**

208 Collection of the SPM profiles used in this study has been described by van Bree et al. (2018).
209 In short, 5 to 10 L of lake water was collected at 13 discrete depths, monthly between
210 September 2013 and January 2015. The samples were filtered on pre-combusted glass fiber
211 GF/F filters (142 mm diameter, Whatman), stored frozen, and freeze-dried prior to analysis.
212 The SPM was collected at or near the start of every month as discussed here, with the sample
213 taken at, for example, 07-09-2013 representing September 2013, and the sample taken at 30-
214 09-2013 representing October 2013 (see Table S.1).

215

216 **2.2.3. Sampling of settling particles**

217 A sediment trap (UWITEC, double-funneled, 86 mm diameter) suspended in 35 m water
218 depth at a mid-lake position (Fig. 2) was installed in November 2006, after which it was
219 emptied and redeployed at about monthly intervals (Table S.1). The collected material was
220 allowed to settle for two days, and stored frozen after decantation of excess water. Prior to
221 analysis, the samples were thawed, filtered over pre-weighed and pre-combusted (400 °C, 5 h)
222 glass fiber GF/F filters (110 mm diameter, Whatman), then frozen and freeze-dried. Bulk
223 mass flux was calculated for each month by using the dry weight of the collected particles, the
224 number of days covered and the surface area of the sediment trap (58 cm²), and is expressed
225 as mg m⁻² day⁻¹. This study focuses on the brGDGTs in settling particles representing the
226 period from September 2010 until January 2015 ($n = 53$; Table S.2).

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230 **2.2.4. Soil sampling**

231 Seven soil samples from the collection obtained by Buckles et al. (2014) were selected for
232 brGDGT analysis based on site dissimilarity, i.e. from different origins (lakeshore forest,
233 crater rim, savanna hinterland, small ravine; see Table S.3) as described in the original study.

234

235 **2.2.5. Lake sediment sampling**

236 Intact surficial lake-bottom sediment (2-5 cm depth) from 3 sites (CH10-06G: 3°19.049' S,
237 37°41.879' E; CH10-09G: 3°18.704' S, 37°41.448' E and CH10-10G: 3°18.575' S,
238 37°41.419' E; see Table S.4) forming a transect from close to the creek inlet towards the
239 middle of the lake (Buckles et al., 2014) was collected by gravity coring in January-February
240 2010, then freeze-dried and homogenized prior to extraction.

241

242 **2.3. Sample preparation and lipid extraction**

243 Sample preparation for SPM was described in detail by van Bree et al. (2018). For this study,
244 SPM was used from all depths for the months of November 2013 and August 2014, as well as
245 from 0, 10, 25, 35, 50, 60, 70 and 80 m depth for all other months (total $n = 146$). In short, the
246 freeze-dried filters were cut in small pieces and extracted using a modified Bligh-Dyer
247 method. Each extract was acid-hydrolyzed with 1.5 N HCl in methanol (MeOH). After a 2 h
248 reflux at 80 °C, the pH of the hydrolyzed extract was adjusted to 4-5 by addition of 1 N KOH
249 / MeOH (96%), and washed three times with dichloromethane (DCM). The combined organic
250 phases were passed over a Na₂SO₄ column and dried under N₂. The total lipid extract (TLE)
251 obtained was separated on an activated Al₂O₃ column into a-polar, neutral and polar fractions,
252 using respectively hexane:DCM (9:1, v/v), DCM and DCM:MeOH (1:1, v/v) as eluents. The
253 freeze-dried filters with sediment-trap material were cut in small pieces and extracted directly
254 by acid hydrolysis. The obtained TLE was further processed in similar manner as the SPM
255 TLE. The lake-sediment samples were also extracted and processed as the SPM.

256 A known amount of internal standard (99 ng C₄₆ GDGT; Huguet et al., 2006) was
257 added to the polar fraction of SPM, settling particles and sediments. All polar fractions of
258 SPM, sediment trap, surface sediments and soils were re-dissolved in hexane:isopropanol
259 (99:1, v/v), and then passed over a 0.45 µm PTFE filter.

260

261 **2.4. GDGT analysis and proxy calculation**

262 GDGT analysis was performed with an Agilent 1260 Infinity ultrahigh performance liquid
263 chromatography (UHPLC) coupled to an Agilent 6130 single quadrupole mass detector, either

264 at Utrecht University (most SPM, soil, surface sediments) or at the NIOZ (settling particles,
265 SPM at 0 m, except November 2013 and September 2014). The instruments at both
266 laboratories are tuned towards the same standards and follow the method of Hopmans et al.
267 (2016), in which separation is achieved by two silica Waters Acquity UPLC HEB Hilic ($\text{\O} 1.7$
268 μm) columns at $30\text{ }^{\circ}\text{C}$, preceded by a guard column with similar packing. Isocratic elution
269 was used for GDGT separation, starting with 82% A (hexane) and 18% B (hexane :
270 isopropanol, 9:1) for 25 min at a flow rate of 0.2 mL min^{-1} , followed by a linear gradient to
271 70% A and 30% B for 25 min. Injection volume was $10\text{ }\mu\text{L}$ for settling particles, sediment and
272 soils, and $20\text{ }\mu\text{L}$ for SPM. Ionization of the GDGTs was achieved by atmospheric pressure
273 chemical ionization with gas temperature of $200\text{ }^{\circ}\text{C}$, vaporizer temperature of $400\text{ }^{\circ}\text{C}$, N_2 flow
274 of 6 L min^{-1} , capillary voltage of 3500 V , nebulizer pressure of 25 psi and corona current of
275 $5.0\text{ }\mu\text{A}$ as source conditions.

276 GDGTs were identified by detecting the $[\text{M}+\text{H}]^+$ ions in selected ion monitoring
277 (SIM) mode for m/z 1018, 1020, 1022, 1032, 1034, 1036, 1046, 1048, 1050 (brGDGTs) and
278 744 (internal C_{46} GDGT standard). Peak area integration of the GDGTs was done with
279 Chemstation (SPM, soil, sediment) or Agilent Masshunter (settling particles, SPM at 0, 35, 60
280 and 70 m) software. For quantification, areas were compared to that of the internal standard,
281 assuming a comparable response of the mass spectrometer for all GDGTs. Fractional
282 abundances of brGDGTs were calculated by dividing the peak area of a specific brGDGT
283 divided by the peak areas of all measured brGDGTs.

284 The Roman numerals in the following equations refer to the molecular structures of
285 GDGTs as shown in Fig. 1, with 6-methyl brGDGTs distinguished by an accent, and square
286 brackets indicating the fractional abundances of the 15 different brGDGTs. The Cyclisation of
287 Branched Tetraethers (CBT') was defined by De Jonge et al. (2014b) as

288
289
$$\text{CBT}' = -\log \left\{ \frac{([\text{Ic}] + [\text{IIa}'] + [\text{IIb}'] + [\text{IIc}'] + [\text{IIIa}'] + [\text{IIIb}'] + [\text{IIIc}'])}{([\text{Ia}] + [\text{IIa}] + [\text{IIIa}])} \right\}$$

290 (1)

291 where $[\text{x}]$ refers to the fractional abundance of a specific brGDGT.

292
293 The isomerization ratio of the 6-methyl penta- and hexamethylated brGDGTs over 5-methyl
294 and 6-methyl brGDGTs ($\text{IR}_{6\text{ME}}$) was modified from De Jonge et al. (2014b) and Sinninghe
295 Damsté (2016), and calculated as

296

297 $IR_{6ME} = ([IIa'] + [IIb'] + [IIc'] + [IIIa'] + [IIIb'] + [IIIc']) / ([IIa] + [IIb] + [IIc] + [IIIa] + [IIIb]$
298 $+ [IIIc] + [IIa'] + [IIb'] + [IIc'] + [IIIa'] + [IIIb'] + [IIIc'])$ (2)

299

300 Mean annual air temperature (MAAT) was reconstructed with the stepwise-forward-selection
301 (SFS) calibration of the brGDGT distribution in the East African lakes dataset (Russell et al.,
302 2018):

303

304 $MAAT_{SFS} = 23.81 - 31.02*[IIIa] - 41.91*[IIb] - 51.59*[IIb'] - 24.70*[IIa] + 68.80*[Ib]$ (3)

305

306 Surface-water pH was reconstructed with the Russell et al. (2018) transfer function
307 determined for East African lakes:

308

309 $Surface\ water\ pH = 8.95 + 2.65*CBT'$ (4)

310

311 **2.5. Determination of 16S rRNA gene diversity and abundance**

312 DNA was extracted from 1/32 section of the SPM filters using the PowerSoil DNA extraction
313 kit (Mo Bio Laboratories, Carlsbad, CA, USA). The 16S rRNA gene amplicon sequencing
314 and analysis was performed with the general 16S rRNA archaeal and bacterial primer pair
315 515F and 806RB targeting the V4 region (Caporaso et al., 2012), as described in Besseling et
316 al. (2018). PCR products were gel-purified using the QIAquick Gel-Purification kit (Qiagen),
317 pooled and diluted. Sequencing was performed at the Utrecht Sequencing Facility (Utrecht,
318 the Netherlands) using an Illumina MiSeq 2x300 bp sequencing platform. The 16S rRNA
319 gene amplicon sequences were analyzed by an in-house pipeline including quality assessment
320 by FastQC (Andrews, 2010), assembly of the paired-end reads with PEAR (Zhang et al.,
321 2013), and taxonomic assignment (including picking of a representative set of sequences with
322 the 'longest' method; Caporaso et al., 2010) with BLAST (Altschul et al., 1990) by using the
323 Silva 128 release as reference database (<https://www.arb-silva.de/>). The 16S rRNA gene
324 copies were quantified using quantitative PCR (qPCR) with the same primer pair (515F,
325 806RB) as used for amplicon sequencing. The 25 μ l qPCR reaction mixture contained 1 U of
326 Pico Maxx high-fidelity DNA polymerase (Stratagene, Agilent Technologies, Santa Clara,
327 CA), 2.5 μ l of 10x Pico Maxx PCR buffer, 2.5 μ l of each dNTP at a concentration of 2.5 mM,
328 0.5 μ l BSA at a concentration of 20 mg ml⁻¹, 0.02 pmol/ μ l of primers, Invitrogen SYBR
329 Green® (optimized concentration) diluted 10,000 times, 0.5 μ l of 50 mM MgCl₂, and
330 ultrapure sterile water. The cycling conditions for the qPCR reaction were the following:

331 initial denaturation at 98 °C for 30 s, 45 cycles at 98 °C of 10 s each, pausing at 56 °C for 20 s
332 followed by a plate read, then at 72 °C for 30 s, and finally at 80 °C for 25 s. Specificity of the
333 reaction was tested with a gradient melting-temperature assay, from 55 °C to 95 °C with a 0.5
334 °C increment for 5 s. The qPCR reactions were performed in triplicate with standard curves
335 from 100 to 107 molecules per microliter. qPCR efficiency for the 16S rRNA quantification
336 was on average 95 % with $R^2 = 0.998$.

337

338 **2.6 Statistical analysis**

339 To assess variability in brGDGT distribution among (types of) samples we performed
340 principal component analysis (PCA) in the R-package FactoMineR (Lê et al., 2008). For
341 SPM, statistic analysis only used the fractional abundance of the most abundant brGDGTs,
342 i.e., Ia, Ib, IIa, IIa', IIb, IIb', IIIa and IIIa'. Water temperature and pH were also included in the
343 PCA, with pH between 50 and 90 m water depth assumed to be similar to the pH measured at
344 50 m depth. Although complete pH profiles from Lake Chala show that pH still decreases
345 slightly with depth below 50 m (~0.5 pH units; Wolff et al., 2014), this represents only a
346 quarter of the total pH depth gradient.

347 Concentrations of brGDGTs (ng L^{-1}) were correlated with the estimated abundance of
348 microbial groups to assign a possible source of the former. The abundance of specific
349 bacterial groups was estimated by multiplying their relative abundance as obtained by 16S
350 rRNA gene amplicon sequencing analysis with the absolute abundance of microorganisms in
351 a given sample based on qPCR. For simplicity it was assumed that each microbe contains a
352 single 16S rRNA copy in their genome; the abundance was accordingly expressed as 16S
353 rRNA gene copies L^{-1} . On the premise that potential brGDGT producers must be frequently
354 present in the water column, microbial species present in less than 10% of the SPM samples
355 were excluded from this comparison.

356

357 **3. Results**

358 **3.1. Seasonal mixing and stratification**

359 Surface-water temperature as measured by temperature loggers at 2 m depth, over the 29-
360 month period from September 2012 to January 2015, ranged between 22.8 °C during the
361 mixing-season in August 2013 and 27.6 °C during the period of strong stratification in April
362 2013 (Fig. 3). Temperatures at 10, 20 and 25 m depth, i.e. in lower epilimnetic and upper
363 hypolimnetic water, varied seasonally with minima during the period of shallow mixing (SM;

364 January-February) and towards the end of the period of deep mixing (DM; May to mid-
365 September). Seasonal temperature variation at 50 m depth, i.e. near the mixing limit, was
366 already strongly muted, and at 85 m depth water temperature remained stable at ~ 22.4 °C
367 (Fig. 3), indicating lack of mixing. Over the 4.5-year monitoring period from September 2010
368 to January 2015 also the upper water column of Lake Chala developed stratification generally
369 from September until April, with most strongly stratified conditions (i.e., greatest temperature
370 contrast between the surface and deep water) shortly after the annual peak in local air
371 temperature (February-March; Fig. 3).

372 During the 17-month period of lake monitoring between September 2013 and January
373 2015, the thickness of oxygenated upper part of the water column, as based on the depth to
374 anoxia (shallowest depth with < 0.2 mg L⁻¹ dissolved oxygen), varied between 17 m in
375 October-November 2013 and 44 m in October-November 2014 (Fig. 3). Depression of the
376 oxycline resulted from convection-driven oxygen injection, mainly towards the end of the
377 stratified period and throughout the principal mixing period. In contrast, the period of shallow
378 mixing in January-February had little impact on the depth of the oxycline (Fig. 3).

379

380 **3.2. Spatial and temporal distribution of brGDGTs in SPM**

381 BrGDGTs were detected in all SPM samples analyzed ($n = 143$, Fig. 4). However, the
382 abundance of brGDGTs with one or two cyclopentyl moieties (types b and c; Fig. 1) was
383 often too low for reliable quantification (i.e., peak height less than three times the noise level
384 of the baseline). Specifically, concentrations of brGDGTs IIIc and IIIc' were always below
385 detection limit; and brGDGTs Ic, IIC, IIC', IIIb and IIIb' were present in less than half of the
386 SPM samples and in very modest amounts, often around the detection limit. The IIIa'' isomer
387 (Weber et al., 2015), which has so far been detected only in lakes, was not detected at all in
388 our samples. Consequently, in the following analysis we focus on the eight brGDGTs that
389 were detected in at least 60% of the samples (i.e., Ia, Ib, IIa, IIa', IIB, IIB', IIIa and IIIa') unless
390 stated otherwise.

391 The total concentration of these eight brGDGTs in the water column (Σ brGDGTs)
392 varied between 0.2 and 24 ng L⁻¹ ($n = 143$), and generally increased with depth, especially in
393 the anoxic part of the water column (Fig. 4). The concentration-weighted mean fractional
394 abundances of the individual brGDGTs in SPM collected at all depths above the sediment trap
395 (0-35 m; SPM_{abovetrap}) and below it (40-90 m; SPM_{belowtrap}) over the 17-month sampling period
396 are shown in Fig. 5A. Pentamethylated (type II) brGDGTs were the most common overall,
397 with a summed fractional abundance ranging from 0.44 to 0.74 in the total dataset ($n = 143$)

398 and average values of 0.64 in $SPM_{\text{abovetrap}}$ ($n = 72$) and 0.72 in $SPM_{\text{belowtrap}}$ ($n = 71$). BrGDGT
399 Ila' was often dominant in the SPM of Lake Chala, with a fractional abundance ranging from
400 0.11 to 0.60, and average values for $SPM_{\text{abovetrap}}$ and $SPM_{\text{belowtrap}}$ of 0.56 and 0.57,
401 respectively (Fig. 5A). The tetramethylated (type I) brGDGTs amount to between 0.06 and
402 0.47 of the brGDGT fractional abundance ($n = 143$), with average values of 0.31 in
403 $SPM_{\text{abovetrap}}$ and 0.21 in $SPM_{\text{belowtrap}}$; brGDGT Ia is the second-most abundant of all
404 brGDGTs (Fig. 5A). Finally, the concentration of hexamethylated (type III) brGDGTs is
405 relatively variable with a combined fractional abundance between 0 and 0.25 ($n = 143$), where
406 brGDGT IIIa' is the most dominant compound. The hexamethylated brGDGTs have relatively
407 low concentration-weighted average fractional abundances of 0.05 in $SPM_{\text{abovetrap}}$ and 0.08 in
408 $SPM_{\text{belowtrap}}$. Overall the tetramethylated brGDGTs are relatively more common in the upper
409 water column (fractional abundances $SPM_{\text{abovetrap}} > SPM_{\text{belowtrap}}$), whereas penta- and
410 hexamethylated brGDGTs are relatively more abundant in the lower water column
411 ($SPM_{\text{abovetrap}} < SPM_{\text{belowtrap}}$).

412 In >86% of all SPM samples, 6-methyl brGDGTs were more abundant than 5-methyl
413 brGDGTs, which is reflected in an average IR_{6ME} of 0.74 (range 0.38 - 1.00). The 5-methyl
414 brGDGTs were relatively abundant only between November 2013 and August 2014, with a
415 maximal fractional abundance of 0.33 at 35 m in February 2014. Absolute concentrations of
416 5-methyl brGDGTs peaked at 8.0 ng L^{-1} at 60 m depth in April 2014. This is mainly the result
417 of brGDGT-IIb, which contributed 4.8 ng L^{-1} to this amount (Fig. 4). The concentration of 6-
418 methyl brGDGTs was typically highest in the non-mixing deepest part of the water column
419 (>60 m), and reaches 9.3 ng L^{-1} at 80 m in February 2014 (Fig. 4). However, averaged over
420 the sampling period the concentration-weighted fractional abundance of 6-methyl brGDGTs
421 was quite similar in shallow and deep water, with an $SPM_{\text{abovetrap}}$ value of 0.62 and
422 $SPM_{\text{belowtrap}}$ value of 0.66.

423 The concentrations of both ΣbrGDGT and individual brGDGTs were highest in the
424 anoxic part of the Chala water column under stratified conditions (Fig. 4). Importantly, depth-
425 integrated concentrations (i.e., averaged over the entire water column) were also highest
426 during the stratification periods, and lowest towards the end of the deep-mixing period in
427 2014 when the oxycline was maximally depressed. Towards the end of both 2013 and 2014,
428 brGDGT concentrations increased when stratification developed after the period of deep
429 mixing. However, the two stratification periods differed with regard to the fractional
430 abundance of individual brGDGTs. During stratification in 2013/2014, the brGDGT
431 assemblage mainly consisted of Ib and IIb at 35-60 m water depth, and Ila' and IIIa' at 60-80

432 m, whereas during stratification in late 2014 and early 2015, concentrations of Ib and Iib were
433 strongly reduced and notably high concentrations of Iia' and IIIa' were evident up to 25-35 m
434 water depth (Fig. 4).

435 The first three principal components (PCs) of a principle component analysis (PCA)
436 on the fractional abundances of the eight major brGDGTs in all SPM samples ($n = 143$)
437 together explain 83.5% of the observed variation in their distribution (Figs. 6A-B). PC1
438 explains 49.4% of the variance, has strong negative loadings for the 6-methyl brGDGTs Iia'
439 and IIIa', and strong positive loadings for the 5-methyl brGDGTs Ib, Iia, Iib and IIIa. PC2
440 explains 21.0% of the variance, and mainly shows strong negative loadings for Ia and Iib'.
441 PC3 explains 13.1% of the variance and shows a strong positive loading for Iib' and IIIa.

442

443 **3.3. BrGDGTs in settling particles**

444 The total brGDGT flux captured by the sediment trap at 35 m depth varied by two orders of
445 magnitude (between 84 and 5300 $\text{ng m}^{-2} \text{day}^{-1}$) over the 53-month period of sediment-trap
446 deployment (Fig. 7B). Total brGDGT flux is not related ($R^2 = 0.02$, $p = 0.91$) to the bulk flux
447 of settling particles (Fig. 7A), nor does its production (or sedimentation) appear to be
448 concentrated in a specific season. BrGDGT concentrations in the monthly collection of settled
449 particles were generally higher than in the snap-shot SPM samples, enabling quantification of
450 all studied brGDGTs except IIIc. Nevertheless, like in SPM, the fractional abundance of
451 brGDGTs Iic, IIIb and IIIc' was <0.02 at all times, and rarely >0.02 for Ic, Iic', IIIa and IIIb'
452 (Fig. 5A). BrGDGTs Iic, IIIb and IIIc' were also found in only 62-77% of the 53 trap samples,
453 whereas all other brGDGTs were found in at least 94% of these samples.

454 The distribution of brGDGTs shows large variation throughout the period of settling
455 particle collection (Figs. 7B-F). The majority of brGDGTs in settling particles were
456 pentamethylated, with a combined fractional abundance ranging between 0.46 and 0.61 ($n =$
457 53), similar to what was found for SPM. BrGDGT Iia' was again most often the dominant
458 compound (fluxes of up to 3200 $\text{ng m}^{-2} \text{day}^{-1}$ in November 2014; Fig. 5A), although at times
459 Iib (340 $\text{ng m}^{-2} \text{day}^{-1}$ in March 2014) and Iib' (530 $\text{ng m}^{-2} \text{day}^{-1}$ in August 2013) were also
460 abundant in the settling particle flux. The fractional abundance of tetramethylated brGDGTs
461 (mostly Ia, as in the SPM) ranged from 0.11 to 0.46, and hexamethylated brGDGTs (mostly
462 IIIa', as in the SPM) ranged from 0.07 to 0.28. Further, 6-methyl brGDGTs most often (41 out
463 of 53 months) comprised at least 80% of the total 5- and 6-methyl brGDGTs ($\text{IR}_{6\text{ME}} > 0.8$),
464 except in June 2011 and from November 2013 to September 2014 (Fig. 7C).

465 The first three PCs of the PCA on the fractional abundances of the eight brGDGTs
466 most common in settling particles (Ia, Ib, IIa, IIa', IIb, IIb', IIIa and IIIa', i.e. as in the SPM)
467 together explain 91.4% of the observed variation through time ($n = 53$; Fig. 6C-D). PC1
468 explains 57.5% of the variance and has strong negative loadings for the 6-methyl brGDGTs
469 IIa' and IIIa', and positive loadings for the 5-methyl brGDGTs Ia, Ib, IIa, IIb and IIIa. PC2
470 explains 23.7% of the total variance and has strong negative loadings for Ia and IIb', and
471 positive loadings for especially IIa, IIb and IIIa. PC3 explains 10.2% of the total variance and
472 has a strong positive loading for Ia. Thus, variation in individual brGDGT distributions is
473 overall similar in SPM and settling particles (cf. Figs. 6A-B and 6C-D).

474 The combined PCA on all 143 SPM and 53 sediment-trap samples (Fig. 6E) indicates
475 that variation in brGDGT distributions is mainly structured by the relative abundance of 5-
476 and 6-methyl brGDGTs (PC1 explains 48.6% of the total variance), and by a different
477 behavior of brGDGTs Ia and IIb' from the six other brGDGTs (PC2 explains 22.4% of the
478 total variance). As expected, the brGDGT distribution in settling particles is most similar to
479 that in SPM from the upper water column, i.e. sampled at depths situated above the sediment
480 trap (Fig. 6E).

481

482 **3.4. BrGDGTs in catchment soils**

483 BrGDGTs in soils surrounding Lake Chala ($n = 7$) are predominantly tetramethylated
484 (fractional abundance 0.48-0.84), followed by pentamethylated (0.15–0.44) and
485 hexamethylated (0.01–0.09) compounds (Fig. 5B). Tetramethylated brGDGTs as well as
486 compounds IIa', IIb' and IIIa' were present in all analyzed soils. Several penta- and
487 hexamethylated brGDGTs were below detection limit in two (IIa, IIIb), three (IIb), four (IIa',
488 IIIb'), five (IIIa) or all seven (IIIc, IIIc') soil samples, and the fractional abundance of IIc, IIc',
489 IIIb and IIIb' was usually <0.02 (Fig. 5A). The IR_{6ME} of Chala soils ranges between 0.52 and
490 0.90, with an average value of 0.73 (Fig. 7C). Variation in brGDGT distributions among soils
491 is explained mainly by their location in- or outside of the crater basin (hinterland savanna,
492 ravine, crater rim or lakeshore forest), in line with results of earlier analyses that did not
493 differentiate between 5- and 6-methyl brGDGTs (Buckles et al., 2014). The brGDGT
494 distributions in soils differ substantially from those in SPM and settling particles, mainly
495 because of higher fractional abundances of brGDGTs Ia, Ib and IIa, and correspondingly
496 lower proportions of 6-methyl brGDGTs (Fig. 5A). When soil brGDGT distributions are
497 added to the PCA of water column brGDGTs as passive samples, all soils plot in the third

498 quadrant of positive PC1 and negative PC2 values, distinct from the lake SPM and settling
499 particles (Fig. 6E).

500

501 **3.5. BrGDGTs in surficial lake-bottom sediments**

502 All brGDGTs except IIIc and IIIc' were detected in recently deposited lake-bottom sediments
503 ($n = 3$), although the fractional abundances of Ic, Iic, Iic', IIIb and IIIb' are always <0.02 (Fig.
504 5A). The distribution of individual brGDGTs is highly similar among the three analyzed
505 samples (Figs. 5B and 6E), with fractional abundances of 0.47-0.48 for penta-, 0.40-0.41 for
506 tetra-, and 0.12 for hexamethylated brGDGTs, and Ia (0.31) and Iia' (0.27) being the
507 dominant compounds. IR_{6ME} is ~ 0.67 (Fig. 7C). The brGDGT distribution in these lake
508 sediments falls within the range of those found in SPM and settling particles (Fig. 5B),
509 however with only positive PC1 scores (Fig. 6E).

510

511 **3.6. Microbial diversity and abundance in the water column of Lake Chala**

512 The diversity and abundance of prokaryotes in the water column of Lake Chala was
513 determined by analysis of all collected SPM samples ($n = 216$), using Q-PCR and 16S rRNA
514 gene amplicon sequencing. Proteobacteria formed the most important group of microbes but
515 Acidobacteria, Actinobacteria, Chlorobi, Chloroflexi, Cyanobacteria, Firmicutes,
516 Bacteroidetes, Planctomycetes, Parcubacteria, and Verrucomicrobia, were also present in
517 varying relative amounts. The relative abundance of Acidobacteria reached a maximum of 4%
518 but on average represented only 0.1% of the prokaryotic population (Table S.5). Among the
519 Acidobacteria, sequences closely affiliated to Blastocatellia (subdivision (SD) 4), as well as
520 those closely related to SD 21 and SD 6 dominated throughout the water column (Table S.5).

521 The absolute concentrations of the eight most common brGDGTs in the Lake Chala
522 SPM are linked to the spatiotemporal distribution of specific bacterial groups based on 16S
523 rRNA gene abundance estimates (Fig. 8). For Acidobacteria, a suspected phylum of brGDGT
524 producers (Sinninghe Damsté et al, 2011), the highest degree of correlation was found
525 between Acidobacteria SD 21 and brGDGTs Ib ($R^2 = 0.23$, $p < 0.001$, $n = 132$) and Iib ($R^2 =$
526 0.22 , $p < 0.001$, $n = 117$). However, most correlations between individual brGDGTs and
527 Acidobacteria SDs are weak ($R^2 < 0.15$; Fig. 8A). Outside the phylum Acidobacteria, modest
528 positive correlations ($R^2 \geq 0.2$) were found between at least one of the eight major brGDGTs
529 and the 16S rRNA gene abundance of 12 individual bacterial taxa (Fig. 8B). The highest
530 positive correlation was found between brGDGT Iia and an uncultured bacterium of the
531 phylum Aminicenantes ($R^2 = 0.40$, $p < 0.001$, $n = 125$).

532 4. Discussion

533 4.1. An aquatic origin of brGDGTs in Lake Chala

534 BrGDGTs in lakes can originate from both terrestrial and aquatic sources, and hence a mixed
535 signal can be expected. There are several indications that the SPM of Lake Chala primarily
536 contains brGDGTs produced within the water column rather than being washed in with
537 eroding catchment soils. Firstly, at all times in the seasonal cycle brGDGT concentrations in
538 the SPM show an order-of-magnitude increase with depth (Fig. 4). Based on data from a
539 limited number of SPM profiles, it was previously thought that this pattern mainly originated
540 because of favorable conditions for organic preservation in the anoxic lower water column of
541 Lake Chala (Sinninghe Damsté et al., 2009; Buckles et al., 2014). Such a presumed stable
542 brGDGT reservoir might be formed when slowly sinking organic particles become neutrally
543 buoyant in the cooler hypolimnion and consequently accumulate over time, combined with a
544 lack of processes (such as grazing and aggregation) to remove these particles from the water
545 column (Sinninghe Damsté et al., 2009; Buckles et al., 2014). However, since our data also
546 show significant variation in the distribution of individual brGDGTs between different depth
547 intervals within the anoxic portion of the water column (Fig. 4), the concept of a static
548 hypolimnetic brGDGT reservoir is untenable. Secondly, the depth-integrated total brGDGT
549 concentration in SPM is lower at the end of the mixing season (and start of the ensuing
550 stratification) than during peak stratification conditions (Fig. 4), arguing against the notion
551 that upwelling during the mixing season merely disperses deep-water brGDGTs throughout
552 the water column. Thirdly, the distribution and abundance of individual brGDGTs changes
553 not only with depth but also through time (Fig. 4). Especially the changes in the lower water
554 column are remarkable given the fact that the maximum mixing depth between September
555 2013 and January 2015 was limited to ~45 m (Fig. 3; van Bree et al., 2018). For example, the
556 total brGDGT concentration at 80 m depth fluctuated between 0.98 ng L⁻¹ (February 2014)
557 and 16 ng L⁻¹ (October 2014), and the different temporal trends of the individual brGDGTs
558 result in variations in the degrees of cyclization, methylation, and 5- or 6-methyl positioning
559 within the brGDGTs. Finally, the contrast between brGDGT distributions in soils and in SPM
560 from either the oxygenated or anoxic parts of the water column (which largely correspond
561 with the zones above and below the sediment trap; Fig. 5A-B), strongly suggests that high
562 deep-water brGDGT concentrations do not result primarily from the accumulation of soil-
563 derived brGDGTs preserved in anoxic conditions, but from *in situ* production, especially
564 below the oxycline. Our combined evidence indicates that over the studied 17-month interval,

565 (almost) all brGDGTs in the SPM of the water column of Lake Chala have an aquatic source
566 while terrestrial input is negligible. This result corroborates the findings of Buckles et al.
567 (2014), and is also consistent with the general lack of terrestrial biomarkers, such as long-
568 chain *n*-alkanes, in the SPM of Lake Chala during this same time interval (van Bree et al.,
569 2018).

570

571 **4.2. Spatiotemporal variation in brGDGT distributions**

572 There is large variation in the fractional abundances of brGDGTs in the water column of Lake
573 Chala over time. Under stratified conditions from November 2013 to August 2014, the
574 expanded anoxic zone is characterized by high fractional abundances of brGDGTs Ib and Iib,
575 which both peak in abundance at 60 m depth (Fig. 4). Although seemingly similar
576 environmental conditions occurred at the end of 2014, when after the end of seasonal deep
577 mixing the oxycline moved upwards again, the concentrations of Ib and Iib do not return to
578 their earlier levels. Instead, concentrations of Iia' and IIIa' rapidly increase at this time.
579 Hence, it appears that deeper mixing promotes either the production of Ib+Iib (5-methyl
580 brGDGTs with rings), as observed in late 2013 and early 2014, or of Iia'+IIIa' (6-methyl
581 brGDGTs with no rings but additional methylation), as observed in late 2014. This temporal
582 variation in brGDGT composition is captured by PC1 in the PCA, which clearly separates 5-
583 and 6-methyl brGDGTs (PC1 49.4%; Fig. 6A). Moreover, the associated alternation of
584 brGDGTs with and without cyclopentyl moieties is reflected by PC2 (Fig. 6A-B). Notably,
585 where 5-methyl brGDGTs mostly occur between 35-60 m depth, 6-methyl brGDGTs
586 generally reside in the lowermost portion of the water column (60-80 m; Fig. 4). Also 5- and
587 6-methyl brGDGTs with cyclopentyl moieties (Ib and Iib vs Iib') occur in different parts of
588 the water column (Fig. 4). Although the concentrations of Iib' in SPM are overall quite low,
589 this depth segregation suggests that the incorporation of cyclopentyl moieties into 5-methyl
590 and 6-methyl brGDGTs is driven by different factors.

591

592 **4.3. Membrane plasticity vs community changes in aquatic brGDGT producers**

593 It is generally assumed that brGDGT producers adjust the molecular structure of their
594 membrane lipids in response to environmental changes, and in fact, these membrane
595 adaptations are at the heart of brGDGT-based paleoenvironmental proxies (e.g. Weijers et al.,
596 2007b). In general, the distribution of individual brGDGTs in the SPM of Lake Chala is in
597 line with the ambient environmental conditions. Overall dominance of the 6-methyl brGDGTs
598 (Fig. 5) may be associated with the relatively high pH of the lake (8.2-9.3 at the surface;

599 Wolff et al., 2014), given that 6-methyl brGDGTs are predominantly produced under high-pH
600 conditions in soils (De Jonge et al., 2014a) as well as river (De Jonge et al., 2014b) and lake
601 (Russell et al., 2018) water. Also the relatively low abundance of hexamethylated (both 5- and
602 6-methyl) brGDGTs is characteristic for warm lakes (Tierney et al., 2010; Loomis et al.,
603 2014a; Russell et al., 2018). However, in Lake Chala there is no straightforward link between
604 brGDGT distributions and the seasonal cycle of environmental conditions, as illustrated by
605 the distinct brGDGT distributions in the two episodes of strong stratification during our 17-
606 month study period, and by the apparently different drivers of cyclization in 5-methyl versus
607 6-methyl brGDGTs (see section 4.2). The fact that temporal variation in the fractional
608 abundance of aquatically produced brGDGTs is not easily linked to the seasonal cycle in
609 either temperature, dissolved oxygen distribution, or pH suggests that the brGDGT molecular
610 structure is not primarily governed by membrane adaptation to changing abiotic conditions.
611 Instead, they may result from variation in the composition of the lake's bacterial community,
612 which, if the different bacterial taxa produce different brGDGTs, will result in different
613 brGDGT assemblages at different times. This was also observed in the deep and meromictic
614 Lake Lugano (Switzerland), where compositional changes in brGDGTs with depth are
615 strongly related to bacterial community changes across the oxycline (Weber et al., 2018).

616 The producers of aquatic brGDGTs in Lake Chala can potentially be identified by
617 comparing the depth distribution and temporal variation of individual brGDGTs with 16S
618 rRNA gene data obtained from the same SPM samples, following the approach of Weber et
619 al. (2018) and Sollai et al. (2019). The only mesophilic bacteria currently known to produce
620 the assumed precursor lipids for brGDGTs, namely *iso*-diabolic acid and its 5- and 6-
621 methylated derivatives, are Acidobacteria (Sinninghe Damsté et al., 2011a, 2018). However,
622 their presence has only been demonstrated in soil-derived aerobic Acidobacterial strains
623 belonging to SDs 1, 3, 4, and 6, while strains of SDs 8, 10 and 23 do not contain these lipids.
624 Ether-bound *iso*-diabolic acid and its derivatives occur only in high abundance in SD4
625 (Sinninghe Damsté et al., 2011a, 2018). Small amounts of ether-bound *iso*-diabolic acid and
626 its derivatives, including brGDGT Ia, have been detected in two Acidobacteria SD1 species
627 isolated from soil. So far, only SD4 species have been shown to produce 5-methyl *iso*-
628 diabolic acid derivatives, whereas the other SDs formed 6-methyl *iso*-diabolic acids. This
629 suggested that the position of methylation of *iso*-diabolic acid may be controlled by
630 phylogenetic affiliations within the Acidobacteria and thus may not be a direct response to
631 ambient environmental conditions (Sinninghe Damsté et al., 2018). Only little is known about
632 the occurrence and diversity of Acidobacteria in lakes (e.g., Zimmermann et al., 2012;

633 Parvenova et al., 2016; Preheim et al., 2016), but the concentrations of some individual
634 brGDGTs in Lake Lugano at one instance during the seasonal cycle (in contrast to the
635 prolonged monthly sampling realized here) showed a strong empirical correlation ($R^2 > 0.56$)
636 with the abundance of certain Acidobacteria SDs (i.e. 3, 5, 6, 8, 15, 17, 21; Weber et al.,
637 2018).

638 In our data from Lake Chala, individual brGDGTs are only modestly correlated with
639 Acidobacteria SDs (Fig. 8A). Surprisingly, whereas in Lake Chala the concentration of
640 brGDGTs Ib and Iib correlates with the abundance of SD21 Acidobacteria, in Lake Lugano
641 this same SD correlates instead with the concentrations of brGDGTs Iia, IIIa and IIIa'' (Weber
642 et al., 2018). The weaker correlation observed in Lake Chala may be partly due to the method
643 of analysis; in contrast to the studies of Weber et al. (2018) and Sollai et al. (2019) we
644 determined the sum of core and intact polar lipid-derived individual brGDGTs rather than the
645 intact polar lipids separately. Intact polar lipids are generally considered to be better markers
646 for 'live' bacteria because the polar head group is thought to be lost quickly after cell death
647 (White et al., 1979; Harvey et al., 1986). However, given the overall low abundance of
648 Acidobacterial 16S rRNA sequences in Lake Chala SPM (on average 0.2% of total
649 prokaryotes, with values up to 6%), the omnipresence and high concentrations of brGDGTs
650 (Fig. 4), and the mostly weak correlation between them, it seems unlikely that Acidobacteria
651 are the predominant producers of brGDGTs in Lake Chala.

652 To investigate alternative bacterial sources for the brGDGTs, we also correlated their
653 individual concentrations with those of the bacterial taxa identified in the SPM (Fig. 8B).
654 Although empirical co-occurrence of brGDGTs and microbial taxa alone does not suffice to
655 reveal the exact source organism(s) of those brGDGTs, the detected phyla might either
656 contain brGDGT-producing organisms or be associated with similar habitats. For example,
657 correlation of brGDGT Ib and Iib with the Actinobacteria phylum may be indicative of the
658 depth habitat or growth season of the organism producing these specific brGDGTs. Our broad
659 brGDGT-16S rRNA data comparison clusters different groups of brGDGTs, and can broadly
660 be defined as Ib+Iib, non-cyclic 5-methyl brGDGTs, and 6-methyl brGDGTs, which all relate
661 to a different bacterial composition (Fig. 8B). The clustering is consistent with the
662 spatiotemporal alternation of brGDGTs Ib+Iib and Iia'+IIIa' observed in the water column of
663 Lake Chala (Fig. 4), and suggests that these different brGDGTs (i.e., 5-methyl vs 6-methyl,
664 and cyclic vs non-cyclic brGDGTs) may be produced by different (groups of) bacteria.
665 Despite our extensive SPM dataset, it is at this stage not possible to determine exactly which
666 aquatic bacteria produce the brGDGTs in Lake Chala but the higher correlations with

667 bacterial taxa other than Acidobacteria suggests that an exclusive origin of lacustrine
668 brGDGTs by Acidobacteria is deemed unlikely, in agreement with earlier work (Weber et al.,
669 2018).

670

671 **4.4. Congruence between brGDGT distributions in SPM and settling particles**

672 It was previously noted that Lake Chala SPM is deprived of terrestrial biomarkers (van Bree
673 et al., 2018), whereas they do occur in the lake's bottom sediments (e.g., Sinninghe Damsté et
674 al., 2011b). Since the brGDGTs associated with SPM appear to be produced within the water
675 column, a possible contribution of soil-derived brGDGTs to the lake sediments may be
676 recognized in settling particles collected monthly in the sediment trap at 35 m, which
677 represents a depth- and time-integrated signal of the upper water column (although the trap is
678 located above the predominant zone of aquatic brGDGT production; Fig. 4). The 53-month
679 sediment-trap record fully encompasses our 17-month SPM time series and thus enables
680 direct comparison between the datasets. Notably, the brGDGT distribution in sediment-trap
681 material is highly similar to that in the SPM, both during the overlapping time period and
682 averaged over the 53-month interval, and dissimilar from the brGDGT distribution in
683 catchment soils (Figs. 5A and 6E). It thus appears that also the vast majority of brGDGTs in
684 particles settling through the water column has an aquatic origin, and consequently can be
685 expected to show the same temporal trends as the brGDGTs in SPM. Indeed, the observed
686 alternation in the summed fractional abundances of brGDGTs Ib+IIb versus Ila'+IIIa' in SPM
687 can also be recognized in the settling particles (Fig. 7D-E). Onset of upper water-column
688 stratification is marked by the relative increase of one of these two groups: the fractional
689 abundance of Ila'+IIIa' increased sharply at the onset of stratification in late 2010, 2012 and
690 2014, whereas those of Ib+IIb increased in late 2011 and 2013. The similar behavior of
691 brGDGTs in SPM and settling particles is also reflected in the PCA including both sample
692 series (Fig. 6E), where PC1 separates 5- and 6-methyl brGDGTs resulting from the temporal
693 alternation of Ib+IIb and Ila'+IIIa'. Although it is not clear which environmental variable
694 controls the predominance of either group in any one year, this pattern supports our
695 suggestion that changing brGDGT distributions in Lake Chala primarily reflect distinct
696 seasonal and inter-annual variation in the composition of its aquatic microbial community
697 rather than a physiological response in a compositionally stable resident community.

698 Notwithstanding their apparently similar dynamics, the sediment-trap and SPM time
699 series are not fully equivalent. The brGDGTs in settling particles are supposedly produced in
700 the upper 35 m of the water column, and indeed mostly plot with the SPM_{abovetrap} samples in

701 the PCA (Fig. 6E). However, for the overlapping 17-month period the time-integrated
702 brGDGT distribution in sediment-trap material appears more similar to the abundance-
703 weighted, depth- and time-integrated brGDGT signal in SPM from the mostly anoxic water
704 column below the sediment trap (> 35 m) than to that of the more oxic upper water column ($<$
705 35 m; Fig. 5A-B). This suggests that a substantial contribution of brGDGTs to the sediment
706 trap may occur during periods when the oxycline is situated well above 35 m. In addition,
707 material from deeper water layers can also reach the sediment trap during mixing episodes
708 (reaching down to *ca* 40 - 45 m in 2014, and to as much as 60 m in other years; Verschuren et
709 al., 2009).

710 Temporal variation in brGDGT concentrations in the Lake Chala SPM appears to
711 respond mainly to the seasonal cycle of stratification and mixing (Fig. 4). This pattern is not
712 reflected in the 4.5-year sediment-trap record, where fluxes of both settling particles as a
713 whole and brGDGTs do not show a clear, recurring annual pattern (Fig. 7A-B). Substantial
714 temporal variation in the total flux and distribution of brGDGTs in Lake Chala has been
715 reported previously, based on analysis of settling particles collected monthly between
716 November 2006 and August 2010 (Sinninghe Damsté et al., 2009; Buckles et al., 2014). This
717 contrasts with findings from sediment-trap studies in north-temperate lakes (e.g. Loomis et
718 al., 2014b; Miller et al., 2018), where brGDGT distributions in settling particles remain
719 relatively stable despite large seasonal variation in their fluxes. Aside from the large temporal
720 variation in brGDGT distributions in Lake Chala, the whole of our 17-month SPM sampling
721 period, and the period from September 2013 to September 2014 in particular, stand out in the
722 53-month sediment-trap record due to the relatively high flux of 5-methyl brGDGTs with
723 rings (Ib+IIb), and relatively low flux of 6-methyl brGDGTs (IIa'+IIIa') (Fig. 7D-E). The
724 relatively large contribution of 5-methyl brGDGTs during this period is reflected in low IR_{6ME}
725 values (< 0.7), otherwise uncommon in the entire 53-month time series (Fig. 7C). Notably,
726 this 13-month period of low IR_{6ME} is also characterized by the near-absence of terrestrial plant
727 biomarkers in the SPM (van Bree et al., 2018), which may indicate that this distinct brGDGT
728 signature is representative of a primarily aquatic source of the brGDGTs. Indeed, any
729 contribution of soil-derived brGDGTs would be revealed by increased abundance of
730 brGDGT-Ia, the dominant brGDGT in Chala catchment soils (Buckles et al., 2014; Fig. 5A).
731 However, the fractional abundance of brGDGT-Ia remains relatively stable over the entire 53-
732 month study period (Fig. 7F), implying that the vast majority of the brGDGTs in the
733 sediment-trap record have an aquatic origin. The caveat is that due to the mid-lake position of
734 the sediment trap and steeply sloping crater walls, we cannot exclude the possibility for soil

735 material to be deposited on the lake floor without reaching the sediment trap. This scenario
736 may also explain the contrasting occurrence of terrestrial biomarkers in surficial bottom
737 sediments and the water column (i.e., SPM) of Lake Chala.

738

739 **4.5 Discrepancy between brGDGT signatures in the water-column, soils, and sediments**

740 The brGDGT distribution in bottom sediments of Lake Chala clearly differs from those in the
741 SPM and settling particles, even when distributions in the latter are integrated over time and
742 weighted-averaged (Fig. 7C-F). For example, the full range of IR_{6ME} values is very wide in
743 SPM (0.35-1.00) and settling particles (0.45-1.00) whereas the three sediment samples have
744 near-identical IR_{6ME} values (~0.67) that are substantially lower than the overall weighted-
745 average IR_{6ME} of the SPM and the settling particles (Fig. 7C). In other words, over 88% ($n =$
746 17) or 96.2% ($n = 53$) of brGDGTs II and III in SPM and settling particles belong to the 6-
747 methyl variety (Fig. 7B), whereas this is only 67% in the bottom sediment. Also the brGDGT
748 fractional abundances in the sediments are different from those in the water column, and in
749 particular those of the 5-methyl brGDGTs without cyclopentane moieties (Ia, IIa, IIIa; Figs. 5
750 and 7). Interestingly, the sedimentary brGDGT signature is also clearly different from that of
751 the catchment soils (Figs. 5 and 7). Although this may be the result of mixed aquatic and soil-
752 derived brGDGTs, this cannot explain the fractional abundances of in particular brGDGT-IIa
753 and IIb in the sediment, which are higher than those in both the water column and the soils
754 (Fig. 5A). These differences in brGDGT signatures in the soils, water column and the
755 sediment suggest that additional brGDGT production may take place within the bottom
756 sediments, as suggested previously (Buckles et al., 2014). Hence, the final brGDGT signal
757 that is stored in Lake Chala sediments is influenced by i) seasonal changes and substantial
758 inter-annual variability in aquatic brGDGT production in the water column, ii) production
759 within the sediments, and iii) varying proportions of aquatic and terrestrial brGDGTs over
760 time, although the evidence for a soil contribution to Lake Chala sedimentary GDGTs
761 remains weak.

762

763 **4.6. Implications for brGDGT-based paleoclimate reconstruction**

764 In order to use brGDGTs extracted from lake sediments for paleoclimate reconstruction, we
765 need to understand how the environmental parameters of interest (primarily temperature and
766 pH) are reflected by the signal that is finally exported to and preserved in the sedimentary
767 record. An earlier study of brGDGTs in time series of settling particles from Lake Chala
768 (Buckles et al., 2014) indicated that mean annual air temperature (MAAT) was

769 underestimated by ~11-13 °C (estimates were 14±5 °C and 13±6 °C, using the East African
770 Lake (EAL) calibrations of Tierney et al. (2010) and Loomis et al. (2012), respectively).
771 Furthermore, maxima and minima in reconstructed temperatures from the time series of
772 settling particles lagged changes in air temperature by up to 5-6 months. Buckles et al. (2014)
773 attributed these offsets to a shifted ratio of aquatic versus soil-derived brGDGTs, and also
774 noted that the brGDGTs in Lake Chala may have a different relationship with temperature
775 than those in the EAL calibrations. Moreover, the co-elution of 5- and 6-methyl brGDGTs in
776 their analysis may have contributed to the observed offset. Application of the improved
777 chromatography method (Hopmans et al., 2016) in the current study allows us to use the most
778 recent temperature calibration based on stepwise forward selection of 5- and 6-methyl
779 brGDGTs in the EAL dataset (MAAT_{SFS}; Russell et al., 2018). Application of this transfer
780 function to our sediment-trap record generates MAAT estimates between 18.5 and 25.2 °C (on
781 average 22.1±1.7 °C), and a flux-weighted overall average of 22.8 °C (Fig. 7G).
782 Underestimation of the observed local MAAT (24.5 °C) is thereby reduced to about 2 °C, i.e.
783 in the range of the calibration error of 2.1 °C.

784 Nevertheless, seasonal variation in reconstructed temperature based on settling
785 particles does not seem very consistent (Fig. 7G). Whereas highest air (and surface-water)
786 temperature occurs during the period of strong water-column stratification, brGDGT-based
787 temperature inferences peak during the periods of stratification in 2010-2011, 2013-2014 and
788 at the end of 2014, but also during the periods of deep mixing in 2011 and 2012 (Fig. 7G). As
789 the temporal variation in brGDGT distributions in the water column of Lake Chala appears to
790 be linked to microbial community changes that are at best only indirectly related to
791 temperature, this may be one reason why brGDGT signatures do not clearly track measured
792 MMAT (Fig. 7G). On the other hand, the modestly higher temperature of the epilimnion
793 compared to that of the lower water column (up to ~4 °C, depending on the season) is
794 reflected in a higher average reconstructed temperature for SPM_{abovetrap} (23.4 °C, $n = 72$) than
795 SPM_{belowtrap} (21.5 °C, $n = 71$, $p < 0.01$; Fig. 7G). Similarly, the decrease of lake-water pH with
796 depth (Wolff et al., 2014; van Bree et al., 2018) is reflected by higher reconstructed pH for the
797 lake surface water (on average 8.5 at 0 m) than that of deeper water layers (on average 8.1 at
798 80 m; $p < 0.01$). Also the elevated surface-water pH that may be expected to occur during
799 peak primary productivity in the mixing season appears to be recorded by brGDGTs in
800 settling particles (8.1 during mixing ($n = 16$) versus 7.7 during stratification ($n = 37$; $p <$
801 0.02), even though the 53-month time series of brGDGT-inferred pH does not follow clear
802 seasonal trends (Fig. 7H). Especially during the interval from September 2013 to September

803 2014 that is characterized by lower IR_{6ME} values, the inferred pH is nearly constant (as is,
804 incidentally, the observed pH: Fig. 7H). This low IR_{6ME} is the net result of the relative
805 increase in cyclopentane moieties (Ib+IIb, Fig. 7F) and decrease in the degree of
806 isomerization (more 5-methyl brGDGTs, Fig. 7C), whereas they are both positively related to
807 pH at least in global soils (Weijers et al., 2007; De Jonge et al., 2014). The opposite trends in
808 the degrees of cyclisation and isomerization of brGDGTs in settling particles of Lake Chala
809 also may explain the generally weak relationship between bottom-sediment brGDGT
810 distribution and surface-water pH in the EAL dataset (Tierney et al., 2010; Loomis et al.,
811 2014; Russell et al., 2018), and supports the suggestion made by these authors that an
812 environmental variable other than pH is responsible for changes in brGDGT signatures in
813 lakes.

814 Notably, average MAAT and pH values inferred from the brGDGTs in surficial
815 bottom sediments are yet again different from those based on brGDGTs produced in the water
816 column and in catchment soils (Fig. 7G, H). The distinct signature of the sedimentary
817 brGDGTs (Fig. 5A) suggests that besides an aquatic source and a potential, but unlikely, soil
818 contribution, those brGDGTs are partly produced within the sediment. However, although
819 brGDGT signals in lake sediments are generally, but not systematically, characterized by
820 larger proportions of hexamethylated brGDGTs and compounds with cyclopentane moieties
821 than those in catchment soils (Tierney and Russel, 2009; Sinninghe Damsté et al., 2009;
822 Tierney et al., 2010; Loomis et al., 2014b; Miller et al., 2018; Guo et al., 2020), it is still not
823 possible to determine the exact contributions of the different potential brGDGT sources to the
824 brGDGT signature stored in the sediments of a lake. This is in contrast to rivers and the
825 coastal marine environment, where *in situ* brGDGT production can be quantified on the basis
826 of the relative abundance of 6-methyl brGDGTs (De Jonge et al., 2014b) or the weighed
827 number of rings in the tetramethylated brGDGTs (Sinninghe Damsté, 2016). Regardless, the
828 values of MAAT (21.9 °C) and pH (9.1) inferred from sedimentary brGDGTs in Lake Chala,
829 as generated using the most recent EAL calibration (Russell et al., 2018), are within
830 reasonable range of measured MAAT (24.5 °C) and surface water pH (9.0). This result is
831 consistent with the belief that brGDGTs in lake sediments carry truthful environmental
832 information (e.g., Tierney et al., 2010; Russell et al., 2018), albeit indirectly, and suggests that
833 the available brGDGT-temperature calibrations already take the *in-situ* sedimentary
834 production into account. If this principle holds over longer timescales, it implies that we can
835 use brGDGTs in lake sediments for paleoclimate reconstructions, even without fully
836 understanding the mechanism that determines their signature. Nevertheless, given the lack of

837 an obvious link between brGDGT composition (or flux) in the 53-month record of settling
838 particles and observed MMAT indicates that sediment samples used for temperature
839 reconstruction must integrate over multiple years.

840

841 **5. Conclusions**

842 BrGDGTs in the water column of Lake Chala are primarily produced *in situ*. The amounts
843 and distributions of individual aquatic brGDGT compounds are highly variable with depth
844 and over time, and do not consistently relate to ambient temperature, pH or oxygen but still
845 appear to respond to the seasonal alternation of water-column mixing and stratification, which
846 is under climatic control. The aquatic brGDGT assemblage is alternatively dominated by the
847 compounds Ib+IIb and IIa'+IIIa', with each pair linked to the occurrence of different bacterial
848 taxa, other than, or besides the Acidobacteria. Hence, temporal changes in brGDGT
849 assemblages are likely due to the sequential occurrence of different groups of aquatic bacteria
850 producing different types of brGDGTs, rather than by membrane adaptation within one group.
851 BrGDGTs in settling particles reveal substantial inter-annual variation in the bacterial
852 community of this tropical lake, superimposed on seasonal variation. Although the brGDGT
853 distributions in SPM and settling particles from Lake Chala cannot be directly linked to local
854 variation in air or water temperature, temporally-integrated and flux-weighted brGDGT
855 compositions do produce reasonable temperature and surface-water pH estimates when using
856 the new EAL calibration of Russell et al. (2018). Regardless, the distinct brGDGT signature
857 of surficial bottom sediments suggests that part of the sedimentary brGDGT pool is produced
858 within the sediment itself. It thus remains crucial to discover the producers of brGDGTs, and
859 the general drivers of brGDGT production, in lakes so that the uncertainties in lacustrine
860 paleothermometry can be further constrained.

861

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863

864 **Data availability**

865

866 All data are available in the supplementary tables at PANGAEA:

867 <https://doi.pangaea.de/10.1594/PANGAEA.922776> (Tables S.1 and S.5)

868 <https://doi.pangaea.de/10.1594/PANGAEA.922780> (Table S.2)

869 <https://doi.pangaea.de/10.1594/PANGAEA.922781> (Table S.3)

870 <https://doi.pangaea.de/10.1594/PANGAEA.922783> (Table S.4)

871

872

873 **Author contribution**

874 LvB, FP, DV, and JSD designed the study, LvB and AB carried out brGDGT analysis, WDC
875 interpreted water column properties, SvG and LV carried out 16S rRNA analysis. LvB and FP
876 took lead in writing with contributions of all co-authors.

877

878

879 **Competing interests**

880 The authors declare that they have no conflict of interest.

881

882

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1172

1173 **Figure captions**

1174 Figure 1. Molecular structures of brGDGTs, consisting of two ether-linked dialkyl chains with
1175 zero to two additional methyl branches (I, II and III) and zero to two cyclopentyl moieties
1176 (suffixes a, b and c). The 6-methyl isomers are denoted with a prime. Compounds indicated in
1177 bold are the eight most common brGDGTs encountered in Lake Chala, and focused upon in
1178 data presentation and discussion.

1179

1180 Figure 2. A: Location of Lake Chala in East Africa, on the border of Kenya and Tanzania, and
1181 B: The bathymetry of Lake Chala situated within its steep-sided crater catchment (outer bold
1182 full line), with sampling locations of suspended particulate matter (SPM; black square),
1183 settling particles (sediment trap; open triangle), surficial lake-bottom sediments (grey
1184 triangles), and terrestrial soils (open circles). Bathymetry adapted from Moernaut et al.
1185 (2010).

1186

1187 Figure 3. Temperature (°C) variation within the water column of Lake Chala between
1188 September 2010 and January 2015, based on automatic loggers suspended at 2, 10, 20, 25, 50
1189 and 80 m depth (as available), in relation to the Bodé et al. (2020) time series of mean
1190 monthly air temperature (MMAT; stippled line) at a savanna site ~25 km west of Lake Chala.
1191 The dark blue line shows the position of the oxycline between September 2013 and December
1192 2015, based on the shallowest depth with dissolved oxygen concentration $<0.2 \text{ mg L}^{-1}$ as
1193 measured by monthly water-column profiling (van Bree et al., 2018). Grey shading highlights
1194 the seasonal periods of upper water-column stratification (S) and deep mixing (DM). Due to
1195 the hiatus in temperature logging data, timing of the start and end of the deep-mixing period
1196 in 2012 was inferred from the MMAT trend.

1197

1198 Figure 4. Depth-interpolated concentrations (in ng L^{-1}) of the summed and eight most
1199 common individual brGDGT compounds in SPM collected at eight depth intervals
1200 (occasionally 13) between 0 and 80 m in Lake Chala, at approximately monthly intervals
1201 between September 2013 and January 2015. Also indicated is the varying position of the
1202 oxycline (bold stippled line) in relation to the static position of the sediment trap at 35 m
1203 depth (thin dashed line), which separates the $\text{SPM}_{\text{abovetrap}}$ and $\text{SPM}_{\text{belowtrap}}$ zones. Grey
1204 background shading indicates the seasonal periods of upper water-column stratification (S)
1205 and deep mixing (DM), as in Fig.3.

1206 Figure 5. Average distribution of brGDGTs in the various sets of samples analyzed in this
1207 study. A: Temporally-integrated, concentration- or flux-weighted average fractional
1208 abundances of individual brGDGT compounds in SPM from above (light blue) and below
1209 (dark blue) the sediment trap, and settling particles trapped over the 17-month period of SPM
1210 sampling (Sept-2013 to Jan-2015; light green) and over the longer 53-month period starting
1211 three years earlier (Sept-2010 to Jan-2015, dark green), compared with average fractional
1212 abundances of the same brGDGTs in surficial lake-bottom sediments (orange) and catchment
1213 soils (red). B: Proportion of tetra-, penta- and hexamethyl brGDGTs in SPM from above
1214 (light blue circles) and below (dark blue circles) the sediment trap, and in settling particles
1215 (green squares), lake sediments (orange diamonds) and soils (red triangles) plotted over
1216 corresponding data from the surficial bottom sediments of 65 East African Lakes (grey
1217 triangles; Russell et al., 2018).

1218

1219 Figure 6. Principal component analysis (PCA) of the fractional abundances of the eight major
1220 brGDGTs in SPM ($n = 143$) and settling particles ($n = 53$) from Lake Chala. A-B: PC1 vs
1221 PC2 (A) and PC2 vs PC3 (B) of the SPM samples, with black vectors indicating the PCA
1222 scores of individual brGDGTs, and blue vectors showing the PCA scores of environmental
1223 variables added passively. Temperature and pH are measured (0-50 m depth, van Bree et al.,
1224 2018) and assumed constant from 50 m to 80 m depth. C-D: PC1 vs PC2 (C) and PC2 vs PC3
1225 (D) of the settling particles, with black vectors indicating the PCA scores of individual
1226 brGDGTs, and blue vector showing the PCA score of the total bulk settling flux added
1227 passively. E: Combined PCA of the fractional abundances of the (mainly aquatic) brGDGTs
1228 in all SPM (blue circles) and settling-particle (green squares) samples, with distinction
1229 between SPM from above (light blue) and below (dark blue) the sediment trap. The PCA
1230 scores of lake sediments (orange diamonds) and soils (red triangles) were added passively.

1231 Figure 7. Time series of settling-particle data from Lake Chala, based on 53 months of
1232 sediment-trap deployment between August 2010 and January 2015. A: Temporal variation in
1233 total bulk dry flux ($\text{mg m}^{-2} \text{day}^{-1}$). B: Total brGDGT flux ($\text{ng m}^{-2} \text{day}^{-1}$) with indication of the
1234 proportions of tetra-methylated (green), 5-methyl (purple) and 6-methyl (brown) brGDGTs.
1235 C: Fraction of 6-methyl penta- and hexamethylated brGDGTs ($\text{IR}_{6\text{ME}}$). D-F: Fractional
1236 abundances of brGDGTs Ib+IIb (D), IIa'+IIIa' (E) and Ia (F). G: Reconstructed mean annual
1237 air temperature (MAAT), using the EAL calibration of Russell et al. (2018). Also indicated
1238 are MMAT (red dashed line) and MAAT (black dashed line), both from Bodé et al. (2020). H:

1239 Reconstructed surface-water pH, using the EAL calibration of Russell et al. (2018), and pH
1240 measured at the surface (0 m) during the period September 2013 to January 2015 (van Bree et
1241 al., 2018). The right-hand panels show boxplots indicating median, interquartile, minimum,
1242 maximum and outlier values of the bulk and brGDGT fluxes and proxies (A-B), suspended-
1243 particulate data (SPM in C-H; $n = 143$), settling-particle data (ST in C-H; $n = 53$), lake-
1244 sediment data (SED in C-H; $n = 3$) and catchment soil data (SOIL in C-H; $n = 7$). These box
1245 plots are superimposed with flux- or abundance-weighted average values of the same for
1246 $SPM_{\text{abovetrap}}$ (light blue circle), $SPM_{\text{belowtrap}}$ (dark blue circle), settling particles trapped over the
1247 17-month period of SPM sampling (September 2013 to January 2015, crossed green square)
1248 or over the 53-month period starting three years earlier (September 2010 to January 2015,
1249 green square), lake sediments (orange diamond) and soils (red triangle). Grey background
1250 shading highlights the seasonal periods of upper water-column stratification (S) and deep
1251 mixing (DM).

1252

1253 Figure 8. Correlation matrix (R^2 ; represented by shades of blue) between the absolute
1254 concentration of the eight major brGDGTs in Lake Chala SPM (ng L^{-1}) and estimated 16S
1255 rRNA gene abundances (copies L^{-1} , see details in text). A: Acidobacteria SD 6, 18, 19 and 21,
1256 and the sum of all Acidobacteria. The Acidobacterial SD OTUs are present in at least 10% of
1257 the SPM samples measured for both brGDGTs and gene abundances. Only SDs that correlate
1258 with at least one brGDGT with an $R^2 \geq 0.05$ are shown. B: The 14 taxa of bacteria displaying
1259 highest correlation with individual brGDGTs, divided in clusters of highest correlation with Ib
1260 and Iib or with the other brGDGTs. The bacterial OTUs are present in at least 10% of the
1261 SPM samples measured for both biomarkers and gene abundances. Only SDs that correlate
1262 with at least one brGDGTs with $R^2 \geq 0.2$ are shown.