Identification of novel 7-methyl and cyclopentanyl branched glycerol dialkyl glycerol
tetraethers in lake sediments

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ABSTRACT

Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are bacterial membrane lipids that are widely used as valuable paleoenvironmental proxies. The recently discovered 6-methyl brGDGTs improved the accuracy of the proxies for temperature “methylation branched tetraethers (MBT)” and soil pH “cyclization branched tetraethers (CBT)”. However, the calibration uncertainties are still substantial for brGDGT-derived proxies (e.g., 5 °C for MBT'_{5ME}). Here we report a series of novel 7-methyl brGDGT isomers that co-eluted with the known 5- and 6-methyl brGDGTs in commonly applied normal phase high performance liquid chromatography (HPLC). Using an optimized HPLC gradient the novel 7-methyl brGDGTs could be structurally characterized and quantified. Their mean relative abundance was in the range of 6% of the total brGDGTs in Chinese and Cameroon lake sediments. The 7-methyl brGDGT IIa₇ correlates with sediment pH ($R^2 = 0.44$, root-mean-square error = 0.26 pH unit), a result that motivates the re-analysis of brGDGTs in soils and sediments to further reassess brGDGT-based proxies and to determine the source of 7-methyl brGDGTs. In addition to the 7-methyl brGDGTs, we identified two novel pentamethylated brGDGTs based on the mass spectra of its ether-cleaved hydrocarbon products.

1. Introduction

Branched glycerol dialkyl glycerol tetraethers (brGDGTs), which contain 4 to 6 methyl groups and up to two cyclopentane rings, are ubiquitously found in soils, peats, lakes and marginal seas (e.g., Weijers et al., 2007; Sinninghe Damsté et al., 2011; Hu et al., 2012; Günther et al., 2014; De Jonge et al., 2015b). Although these branched membrane lipids are detected in some *Acidobacteria* (Weijers et al., 2009; Sinninghe Damsté et al., 2011), their sources in the environment remain unknown (Anderson et al., 2014; Buckles et al., 2014; Naeher et al., 2014; Zell et al., 2014; De Jonge et al., 2016). By using liquid chromatography–mass spectrometry (HPLC–MS) and high-field nuclear magnetic resonance spectroscopy, Sinninghe Damsté et al. (2000) first discovered a group of GDGTs with branched carbon skeletons in peats, lakes, and marine sediments. In the same year, Schouten et al. (2000) reported another type of brGDGTs with one cyclopentane ring in addition to methyl group branches. Later, Weijers et al. (2006) tentatively identified additional brGDGT isomers with 0–2 cyclopentane rings, increasing the number of groups of brGDGT isomers up to 9. A survey of globally distributed soils revealed that the relative amount of methyl-branched tetraethers (MBT) correlates with mean annual air temperature (MAT) while cyclized branched tetraethers (CBT) correlate with soil pH (Weijers et al., 2007). However, the environmental temperature reconstruction using these proxies results in considerable scatter (ca. 5 °C for MAT) (Schouten et al., 2013). Progress in the field was made by improving the HPLC separation of brGDGTs. After

Liu et al. (2012) reported a first 6-methyl brGDGT in marine sediments, De Jonge et al. (2013) unambiguously identified several novel 6-methyl brGDGTs from a Siberian peat core. These novel compounds co-eluted with previously known 5-methyl brGDGTs using conventional separation protocols. Becker et al. (2013) found novel brGDGT-III isomers (m/z 1050) in Aarhus Bay sediments by utilizing ultrahigh performance liquid chromatography–atmospheric pressure chemical ionization–tandem mass spectrometry (UHPLC–APCI-MS²), but the exact positions of the methyl groups were not clarified. Weber et al. (2015) identified a novel hexamethylated brGDGT-IIIa_{5/6} in a Swiss mountain lake. The $\delta^{13}\text{C}$ values of the alkyl chains indicated that it was likely produced in-situ in the lake’s water column or sediment. Samples from a diversity of environments such as loess-paleosol sequences (e.g., Zech et al., 2012), soils (e.g., Yang et al., 2015) and marine sediments (e.g., Sinninghe Damsté, 2016) could be successfully separated and investigated. Different types of LC columns used include, for example, two BEH amide columns (Becker et al., 2013) or two UHPLC silica columns in tandem (Yang et al., 2015). Hopmans et al. (2016) compared different HPLC protocols and came up with an improved chromatography using two UHPLC silica columns in series. Remarkably, GDGT-based proxies differed for MBT_{5Me} determination depending on the protocol used for separation (ca. 3 °C deviation when used for MAT reconstruction). With improvements in chromatography, the number of brGDGTs detected increased from 9 to 15, and several modified proxies containing 6-methyl brGDGTs were proposed for

reconstruction of soil pH and MAT (Ding et al., 2015; Xiao et al., 2015; Wang et al., 2016).

Numerous studies utilize the methylation pattern of brGDGTs for environmental correlations. Dang et al. (2016), for example, found a moisture control on 6-methyl brGDGT distributions in semi-arid and arid soils. Results of the analyses of sources and distributions of 6-methyl brGDGTs in coastal marine and lake sediments suggest that brGDGTs are universally produced in the water column (De Jonge et al., 2015a, 2015b, 2016; Sinninghe Damsté, 2016).

These results suggest that the adjustment of membrane lipids to temperature and pH are manifold and that still further methylation, cyclization, and isomerization patterns may exist. Their discovery may improve the quality of our calibrations for environmental parameters. Here we report a series of new 7-methyl isomers that eluted after 5- and 6-methyl brGDGTs which were characterized by a modified HPLC separation combined with fraction collection and identification of ether cleavage hydrocarbon products in extracts from Chinese lake sediments. Furthermore, pentamethylated brGDGTs with one cyclopentane ring (IIb, m/z 1034) were found to comprise two different structures in a single HPLC peak. A lake sediment from Baleng in Cameroon was used to test if 7-methyl brGDGTs can be found in other lakes.

2. Materials and methods

2.1. Sampling

In July 2014, a total of 102 surface lake sediments (0–5 cm) were collected from 37 Chinese lakes with a grab sampler. The Chinese lakes span a large longitude and latitude range (86°51'11"–132°31'33" E, 24°54'16"–49°18'17" N; for the exact sampling location see Supplementary Table 1), displaying a wide range of MAT (–0.7 to 17.5 °C) and mean annual precipitation (MAP, 77–1323 mm). The MAT and MAP values (1970–2000) were obtained from the WorldClim data set (Hijmans et al., 2005). The water pH of these lakes varies from 6.9 (Poyang Lake) to 10.2 (Tai Lake) with an average value of 8.3 suggesting that most lakes are alkaline. The lake sediment of Baleng was collected in November 2011 according to Garcin et al. (2012). After collection, all samples were stored at –20 °C until analysis.

2.2. brGDGTs purification and collection

Surface sediments (each ca. 6 g) were freeze-dried, extracted and concentrated according to Ding et al. (2015). All the samples were dissolved in 300 µL of a mixture of 84% *n*-hexane (A) and 16% ethyl acetate (B). A 10 µL portion of each sample was injected on an Agilent 1200 series HPLC system coupled to a Thermo Scientific Orbitrap Velos Pro ion trap mass spectrometer for the determination of individual brGDGT fractional abundance. Two Hypersil GOLD silica columns (150 mm × 2.1 mm; 1.9 µm; Thermo Fisher Scientific, USA) in series were used by means of a modified method based on the protocol of Yang et al. (2015). The HPLC was

operated at a constant flow rate of 0.2 mL/min. The initial solvent composition was held at 84% A and 16% B for 65 min, followed by an increase to 100% B within 20 min before re-equilibration at 84% A and 16% B for 30 min. Atmospheric pressure chemical ionization (APCI) settings were according to De Jonge et al. (2013), spectra were recorded in SIM mode in the mass range of m/z 1017–1051 (2 μ scans; 100 ms max injection time).

After the quantification of brGDGTs from all the lake sediments, 66% of each sample were taken, combined and concentrated to dryness under a stream of nitrogen. Two HPLC purification steps were conducted for further fraction collection. First, the combined sample was re-dissolved in ca. 5 mL of the HPLC solvent mixture consisting of 84% A and 16% B. Repeated injections of 100 μ L were performed on HPLC–MS with one Hypersil GOLD silica column. The HPLC was operated isocratically using 84% *n*-hexane (A) and 16% ethyl acetate (B) at a flow rate of 0.5 mL/min. The HPLC outlet was equipped with a 1:10 splitter (mass spectrometer: manual fraction collection) and connected to the APCI-MS. The brGDGT fractions were combined and concentrated under a stream of nitrogen.

Secondly, the pre-purified brGDGTs were re-dissolved in 200 μ L of a mixture of 84% A and 16% B for further purification. Aliquots of 10 μ L of this mixture were injected on two Hypersil GOLD silica columns in series. The HPLC–MS conditions were the same as in the quantification method described above. Two fractions were collected (fraction A from 45.2 to 47.0 min and fraction B from 49.7 to 53.0 min; Fig.

1A and B). For further quantitative HPLC–MS analysis, a 10% aliquot of each fraction was dried under a stream of nitrogen and re-dissolved in 250 μ L of 84% A and 16% B. 50 μ L of an 11.9 ppm (ng/ μ l) solution of the C₄₆ GDGT was added as an internal standard (Huguet et al., 2006). We did not achieve baseline separation of the novel brGDGT isomers that co-eluted with other brGDGTs of different mass. Nonetheless, these mixtures were sufficiently pure for detailed analysis of hydrocarbon moieties.

2.3. Ether cleavage of brGDGTs and analysis

The remaining 90% of two specific fractions (see Section 2.2; Fig. 1A and B) were submitted to ether cleavage using 57 wt% HI in H₂O followed by reduction to hydrocarbons using H₂/PtO₂ according to published procedures (Kaneko et al., 2011; De Jonge et al., 2013). The products were separated on a Thermo Trace 1310 gas chromatograph (Bremen, Germany) coupled with a Thermo TSQ 8000 electron impact (EI) triple quadrupole mass (GC–MS). The PTV injector was operated in splitless mode at an initial temperature of 70 °C. With injection, the injector was heated to 300 °C at a programmed rate of 720 °C/min and held at this temperature for 2.5 min. Separation was performed on a Thermo TG-5SilMS column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness). The GC oven temperature was held at 100 °C for 1 min and subsequently raised to 320 °C at a rate of 5 °C/min. This temperature was held for 3 min before cooling and re-equilibration. Data acquisition started at 10 min,

monitoring the mass range between 50 and 650 Da in EI+ (70 eV) mode. The MS transfer line and the ion source temperature were set to 300 °C.

3. Results and discussion

3.1. Identification of 7-methyl brGDGTs

The modified HPLC method allows the separation of new brGDGT isomers from the known 5- and 6-methyl brGDGTs with the same mass (Fig. 1). To identify the structure of the unknown compounds, the two fractions of brGDGTs from Chinese lake sediments were subjected to ether cleavage and the hydrocarbons generated were analyzed using GC–MS. To initially monitor the success of fractionation and ether cleavage we focused on fraction A (Fig. 1) that did not contain any novel brGDGTs. In this fraction we detected a mixture of the well-described lipids 5-methyl brGDGT (m/z 1050, IIIa₅), 5/6-methyl brGDGT (m/z 1050, IIIa_{5/6}) and 6-methyl brGDGT (m/z 1050, IIIa₆) (Supplementary Fig. S1). Thus this fraction was ideally suited for method verification. After cleavage of the ether bonds, five hydrocarbons arising from the brGDGTs could be detected (Fig. 2a–e). The most abundant two isomers were 6,13,16-trimethyloctacosane (b) and 5,13,16-trimethyloctacosane (c), and the other three minor compounds were 13,16-dimethyloctacosane (a), 6,13,16,24-tetramethyloctacosane (d) and 5,13,16,24-tetramethyloctacosane (e) (Fig. 2; fraction A). All hydrocarbons were identified according to their specific fragmentation patterns in EI mass spectra and according to their retention time (Fig.

2a–e). These data were in good agreement with Sinninghe Damsté et al. (2002), Liu et al. (2012) and De Jonge et al. (2013). However, 5,13,16,23-tetramethyloctacosane was not reported in previous studies, presumably due to its low concentration (Weber et al., 2015). The composition of methylated hydrocarbons released during ether cleavage thus reflects directly the composition of their parent brGDGTs, supporting the applicability of this approach for the identification of unknown tetraethers.

Fraction B (Fig. 1 and Table 1) mainly contained unknown brGDGT isomers (m/z 1050, 1048 and 1036) as well as known 5-methyl brGDGTs (m/z 1036, IIa₅; m/z 1034, IIb₅) and 6-methyl brGDGTs (m/z 1036, IIa₆; m/z 1034, IIb₆). Hydrocarbons derived from the ether cleavage were separated using GC–MS (Fig. 2; fraction B). The earlier eluting compounds were assigned as 13,16-dimethyloctacosane (a), 6,13,16-trimethyloctacosane (b) and 5,13,16-trimethyloctacosane (c), which are also present in fraction A. Mass spectra of hydrocarbons f–h, however, indicated the presence of a cyclopentane ring. While f is mono-methylated, both g and h contained an additional methyl group at position 5 and 6 of the alkyl chain, respectively. Compound f was hitherto only described as its methylthioether derivative (Schouten et al., 2000), but is here for the first time supported by a mass spectrum of the non-functionalized hydrocarbon (Fig. 2). In addition, we observed a new compound i which eluted before hydrocarbon b. Although the abundance of i was relatively small, its mass spectrum clearly showed differences from that of b and c, i.e., increased intensity of fragments at m/z 351 and 323 in i, but higher intensity of fragments at m/z

365 and 379 in b and c, respectively (Table 2). Such fragmentation pattern suggests that hydrocarbon i is methylated at C-7 of its alkyl chain rather than at C-5 or C-6. The comparison of the extracted ion chromatogram (m/z 351.3, red solid line in Fig. 2; fractions A and B) clearly confirms that the peak shoulder at retention time 34.2 min in Fig. 2 fraction B corresponds to a defined peak, which further proves the existence of a defined hydrocarbon with 7-methyl substitution. In contrast, the signal of the same ion trace at similar retention time in Fig. 2 fraction A does not have a defined shoulder.

The error (7 units) between the measured (2895) and calculated (2902) Kováts index of 7,13,16-trimethyloctacosane was comparable to that of 5,13,16-trimethyloctacosane (6 units) and lower than the error of 6,13,16-trimethyloctacosane (12 units) (Kissin et al., 1986), which further confirmed the structure of the newly identified carbon skeleton i (Table 1). This hydrocarbon thus clearly supports the presence of 7-methyl brGDGTs in lake sediments (Fig. 3). Furthermore, the elution times of 7-methyl brGDGTs in HPLC–MS analysis were in accordance with their structural properties (i.e., the elution order of IIIa is listed as IIIa₅, IIIa_{5/6}, IIIa₆ and finally IIIa₇ as shown in Supplementary Fig. S2; see also Weber et al., 2015).

LC–MS and GC–MS data provide semi-quantitative information about the respective brGDGTs and their hydrocarbon cleavage products. Assuming similar response factors for the different brGDGTs in LC–MS as well as for the hydrocarbons

in GC–MS analyses, our data allows an evaluation of the proportions of the respective lipids. The relative abundance of brGDGTs in fraction B is given in Table 1 and the relative abundance of the hydrocarbons generated by ether cleavage of fraction B in Table 3. Formally, three hydrocarbons could be generated by cleavage of two isomeric forms of IIIa₇ (a, i and 7,13,16,22-tetramethyloctacosane; Fig. 3). However, the latter hydrocarbon was not detected which may be due to the absence or low concentration of the corresponding isomer (IIIa₇*). Therefore, brGDGT-IIIa₇ generated two equivalents of hydrocarbon i. Hydrocarbon i accounts for 4.5% of total hydrocarbons released from fraction B, supporting the presence of a substantial proportion of the novel 7-methyl branched GDGTs. The similar retention time interval between structures IIIa₅, IIIa₆, and the novel compound during LC–MS analysis further supports the presence of two methyl branches in IIIa₇ as similarly reported for IIIa₅ and IIIa₆. Since we have not detected any other peaks eluting in between (like observed for the previously identified mixed compound IIIa_{5/6}) we exclude branching at other positions.

The ratios of carbon skeletons $i/(a + f)$ with respect to brGDGTs before ether cleavage (based on HPLC–MS peak integration) and their ether-cleavage products hydrocarbons i, a and f (based on GC–MS peak integration) were calculated to further test whether all three new isomers (Fig. 1; m/z 1050, 1048, 1036) were 7-methyl brGDGTs:

$$\text{Before ether cleavage: } i/(a + f) = (IIIa_7 \times 2 + IIIb_7 + IIa_7)/(IIa_5 + IIa_6 +$$

$$\text{IIa}_7 + \text{IIb}_5 + \text{IIb}_6 = 0.102$$

After ether cleavage: $i/(a + f) = 0.102$

The similar ratios observed before and after the ether cleavage are additional support for the 7-methyl brGDGTs structure of the new isomers (Fig. 3).

3.2. Identification of two pentamethylated brGDGTs' structural isomers

Besides novel 7-methyl compounds, we tentatively identified novel structural isomers of the cyclopentane-containing pentamethylated brGDGT isomers IIb (m/z 1034, IIb₅ and IIb₆). IIb₅ and IIb₆ would liberate hydrocarbons b/c and f. Previous studies treated IIb₅ as a single compound contributing to hydrocarbons c and f (e.g., Weijers et al., 2007). However, we found hydrocarbons g and h in fraction B that could only be related to IIb₅ and IIb₆. Therefore we assume that IIb₅ and IIb₆ both consist of two structural isomers (Fig. 4); IIb₅ contains brGDGTs with hydrocarbons c and f or g and a, while IIb₆ is composed of hydrocarbons b and f or h and a. Evidence that the sum of the relative abundance of hydrocarbons a and f (43.9%, another 3.8% contributed to one chain of IIa₇) is similar to the sum of b, c, g and h (47.8%: these four hydrocarbons have to be located in the second chain, besides a or f, of the brGDGTs) confirms our hypothesis.

3.3. Method evaluation

In order to evaluate our modified method, we analyzed one of the lake samples

from Bosten Lake (BSTH-D4) in comparison to the HPLC method reported by Hopmans et al. (2016). Retention times using our method were longer compared to the ones reported by Hopmans et al. (2016), while the sensitivity of both methods was comparable. Both methods allow the separation of 7-methyl brGDGTs, sufficient for quantification (Supplementary Fig. S2). However, only using our method, IIIa_{5/6} could be seen as a shoulder peak on IIIa₆. This isomer co-eluted with IIIa₆ using chromatography from Hopmans et al. (2016). For exact quantification, peak deconvolution algorithms could be applied to the chromatograms obtained with our method.

3.4. Survey of Chinese and Cameroon lake sediments

The average fractional abundance of brGDGTs from 102 samples in 37 Chinese lakes is shown in Supplementary Fig. S3. The 7-methyl brGDGTs were detected in 92 out of 102 lake sediments and comprised on average 6.2% of the total amount of brGDGTs. For those sediments where 7-methyl brGDGTs were not detected, the total abundance of brGDGTs was very low and only a few brGDGTs were above the limit of detection (LOD). In some lake sediments, the relative abundance of 7-methyl brGDGTs can reach 11% of total brGDGTs (e.g., DC-D2 from Dai lake and HLH-D7 from Hunlun lake). The pH of these two samples (8.5 for DC-D2 and 8.2 for HLH-D7) was higher than those of the other investigated lake sediments. In the analysis of the correlation between sediment pH and individual brGDGTs in Chinese lake sediments,

we found that IIa₇ shows the best correlation with sediment pH ($R^2 = 0.44$, root-mean-square error = 0.26 pH unit; Supplementary Fig. S4), suggesting that the carbon skeleton isomerization of branched tetraethers is an important strategy for brGDGTs-producing bacteria to adapt to ambient pH (Ding et al., 2015). According to our survey 7-methyl brGDGTs appear ubiquitous in lakes, some like IIa₇ even with a fractional abundance above 5%. For comparison, a global soil dataset lists only 6 out of 15 brGDGTs with a fractional abundance above 5% (IIIa₅, IIIa₆, IIa₅, IIa₆, Ia and Ib) (De Jonge et al., 2014). The novel 7-methyl brGDGTs should thus be considered when brGDGT-proxies are further developed. Penta- and tetramethylated brGDGTs (IIa, IIb, Ia and Ib) showed relatively high fractional abundances (> 10%) in our 102 Chinese lake sediment samples. 7-Me brGDGTs have not been encountered in a Siberian peat (De Jonge et al., 2013) or in a soil dataset (De Jonge et al., 2014), which can be either explained by their absence in soils or by the omission of the screening for these, then unknown, brGDGT isomers. Therefore, we suggest the careful reevaluation of brGDGTs in soils in order to clarify the exact source of 7-methyl brGDGTs in lakes such as those in our study.

To verify if 7-methyl brGDGTs are also found in other sediments than those of the lakes described above, we investigated a sediment sample from the Lake of Baleng in Cameroon (Fig. 5). The relative abundance of 7-methyl brGDGTs is comparable between the Lake of Baleng and the mean of the Chinese lakes (4.3% vs 6.2%), indicating that these compounds might be universally distributed and

significant. This calls for 7-methyl brGDGTs to be considered when establishing new brGDGT proxies.

4. Conclusions

We identified a novel class of brGDGTs with methylation at C-7 that contribute 6.2% of the total brGDGTs in Chinese lake sediments (IIIa₇ 1.4%, IIIb₇ 0.3% and IIa₇ 4.5% of total brGDGTs) and 4.3% in Lake of Baleng in Cameroon (IIIa₇ 1.4% and IIa₇ 2.9% of total brGDGTs). IIa₇ shows the best correlation with sediment pH in Chinese lake sediments. This finding has important implications for the improvement of existing brGDGT-based pH proxies such as CBT and IBT. Follow-up studies should test for the presence of these novel compounds and examine whether 7-methyl brGDGTs could be attributed to specific environmental conditions or microorganisms. In addition, we found that the known brGDGTs (*m/z* 1034) with one cyclopentane moiety, IIb₅ and IIb₆, are actually composed of two structural isomers that can further complicate previously defined brGDGT-based proxies. The mechanism upon which bacteria produce different brGDGT membrane lipid isomers is still needed to be further studied based on improved HPLC methods that allow individual quantification of tetraether compounds.

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References

- Anderson, V.J., Shanahan, T.M., Saylor, J.E., Horton, B.K., Mora, A.R., 2014. Sources of local and regional variability in the MBT'/CBT paleotemperature proxy: Insights from a modern elevation transect across the Eastern Cordillera of Colombia. *Organic Geochemistry* 69, 42–51.
- Becker, K.W., Lipp, J.S., Zhu, C., Liu, X.-L., Hinrichs, K.-U., 2013. An improved method for the analysis of archaeal and bacterial ether core lipids. *Organic Geochemistry* 61, 34–44.
- 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.

Dang, X., Yang, H., Naafs, B.D.A., Pancost, R.D., Xie, S., 2016. Evidence of moisture control on the methylation of branched glycerol dialkyl glycerol tetraethers in semi-arid and arid soils. *Geochimica et Cosmochimica Acta*.

De Jonge, C., Hopmans, E.C., Stadnitskaia, A., Rijpstra, W.I.C., Hofland, R., Tegelaar, E., Sinninghe Damsté, J.S., 2013. Identification of novel penta- and hexamethylated branched glycerol dialkyl glycerol tetraethers in peat using HPLC–MS², 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., 2014. Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in soils: Implications for palaeoclimate reconstruction. *Geochimica et Cosmochimica Acta* 141, 97–112.

De Jonge, C., Stadnitskaia, A., Cherkashov, G., Sinninghe Damsté, J.S., 2016. Branched glycerol dialkyl glycerol tetraethers and crenarchaeol record post-glacial sea level rise and shift in source of terrigenous brGDGTs in the Kara Sea (Arctic Ocean). *Organic Geochemistry* 92, 42–54.

De Jonge, C., Stadnitskaia, A., Fedotov, A., Sinninghe Damsté, J.S., 2015a. Impact of riverine suspended particulate matter on the branched glycerol dialkyl glycerol tetraether composition of lakes: The outflow of the Selenga River in Lake Baikal (Russia). *Organic Geochemistry* 83–84, 241–252.

- De Jonge, C., Stadnitskaia, A., Hopmans, E.C., Cherkashov, G., Fedotov, A., Streletskaia, I.D., Vasiliev, A.A., Sinninghe Damsté, J.S., 2015b. Drastic changes in the distribution of branched tetraether lipids in suspended matter and sediments from the Yenisei River and Kara Sea (Siberia): Implications for the use of brGDGT-based proxies in coastal marine sediments. *Geochimica et Cosmochimica Acta* 165, 200–225.
- Ding, S., Xu, Y., Wang, Y., He, Y., Hou, J., Chen, L., He, J.S., 2015. Distribution of branched glycerol dialkyl glycerol tetraethers in surface soils of the Qinghai–Tibetan Plateau: implications of brGDGTs-based proxies in cold and dry regions. *Biogeosciences* 12, 3141–3151.
- Günther, F., Thiele, A., Gleixner, G., Xu, B., Yao, T., Schouten, S., 2014. Distribution of bacterial and archaeal ether lipids in soils and surface sediments of Tibetan lakes: Implications for GDGT-based proxies in saline high mountain lakes. *Organic Geochemistry* 67, 19–30.
- Garcin, Y., Schwab, V.F., Gleixner, G., Kahmen, A., Todou, G., Séné, O., Onana, J.-M., Achoundong, G., Sachse, D., 2012. Hydrogen isotope ratios of lacustrine sedimentary *n*-alkanes as proxies of tropical African hydrology: Insights from a calibration transect across Cameroon. *Geochimica et Cosmochimica Acta* 79, 106–126.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. *International*

Journal of Climatology 25, 1965–1978.

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

Hu, J., Meyers, P.A., Chen, G., Peng, P.a., Yang, Q., 2012. Archaeal and bacterial glycerol dialkyl glycerol tetraethers in sediments from the Eastern Lau Spreading Center, South Pacific Ocean. *Organic Geochemistry* 43, 162–167.

Huguet, C., Hopmans, E.C., Febo-Ayala, W., Thompson, D.H., Sinninghe Damsté, J.S., Schouten, S., 2006. An improved method to determine the absolute abundance of glycerol dibiphytanyl glycerol tetraether lipids. *Organic Geochemistry* 37, 1036–1041.

Kaneko, M., Kitajima, F., Naraoka, H., 2011. Stable hydrogen isotope measurement of archaeal ether-bound hydrocarbons. *Organic Geochemistry* 42, 166–172.

Kissin, Y.V., Feulmer, G.P., Payne, W.B., 1986. Gas chromatographic analysis of polymethyl-substituted alkanes. *Journal of Chromatographic Science* 24, 164–169.

Liu, X.-L., Summons, R.E., Hinrichs, K.-U., 2012. Extending the known range of glycerol ether lipids in the environment: structural assignments based on tandem mass spectral fragmentation patterns. *Rapid Communications in Mass Spectrometry* 26, 2295–2302.

Naeher, S., Peterse, F., Smittenberg, R.H., Niemann, H., Zigah, P.K., Schubert, C.J., 2014. Sources of glycerol dialkyl glycerol tetraethers (GDGTs) in catchment

soils, water column and sediments of Lake Rotsee (Switzerland) – Implications for the application of GDGT-based proxies for lakes. *Organic Geochemistry* 66, 164–173.

Schouten, S., Hopmans, E.C., Pancost, R.D., Sinninghe Damsté, J.S., 2000.

Widespread occurrence of structurally diverse tetraether membrane lipids: Evidence for the ubiquitous presence of low-temperature relatives of hyperthermophiles. *Proceedings of the National Academy of Sciences* 97, 14421–14426.

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., 2000. Newly discovered non-isoprenoid glycerol dialkyl glycerol tetraether lipids in sediments. *Chemical Communications*, 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.

- Wang, H., Liu, W., Lu, H., 2016. Appraisal of branched glycerol dialkyl glycerol tetraether-based indices for north China. *Organic Geochemistry* 98, 118–130.
- 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 branched GDGT isomer in lake sediments: Evidence for lacustrine branched GDGT 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., Schouten, S., van den Donker, J.C., Hopmans, E.C., Sinninghe Damsté, J.S., 2007. 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., Damste, J.S.S., 2009. Constraints on the biological source(s) of the orphan branched tetraether membrane lipids. *Geomicrobiology Journal* 26, 402–414.
- Xiao, W., Xu, Y., Ding, S., Wang, Y., Zhang, X., Yang, H., Wang, G., Hou, J., 2015. Global calibration of a novel, branched GDGT-based soil pH proxy. *Organic Geochemistry* 89–90, 56–60.

- Yang, H., Lü, X., Ding, W., Lei, Y., Dang, X., Xie, S., 2015. The 6-methyl branched tetraethers significantly affect the performance of the methylation index (MBT') in soils from an altitudinal transect at Mount Shennongjia. *Organic Geochemistry* 82, 42 – 53.
- Zell, C., Kim, J.-H., Hollander, D., Lorenzoni, L., Baker, P., Silva, C.G., Nittrouer, C., Sinninghe Damsté, J.S., 2014. Sources and distributions of branched and isoprenoid tetraether lipids on the Amazon shelf and fan: Implications for the use of GDGT-based proxies in marine sediments. *Geochimica et Cosmochimica Acta* 139, 293–312.

Figure and Table captions

Fig. 1. HPLC–APCI-MS chromatograms of brGDGTs in Chinese lake sediments, including base peak chromatogram (BPC), m/z 1050, 1048, 1036 and 1034 extracted ion chromatograms. Numbers represent the 5- and 6-methylated isomers of brGDGTs, while the question mark represents the newly identified isomers.

Fig. 2. GC–MS chromatogram of hydrocarbons (a–i) generated by ether cleavage of the two brGDGT fraction A and fraction B. Both BPC and extracted ion chromatogram (m/z 351.3, red solid line) are shown in the chromatogram of fraction A and of fraction B. Mass spectra and hydrocarbon structures (a–i) are shown, with

preferred fragmentation indicated.

Fig. 3. Structures of newly identified 7-methyl brGDGTs (m/z 1050, 1048, 1036). All structures of novel compounds are tentatively identified because no pure standard compounds are available. IIIa₇* was not recognized due to absence or low concentration of 7,13,16,22-tetramethyloctacosane, IIIb₇ was identified by the similar ratio of $i/(a + f)$ before (HPLC–MS) and after (GC–MS) ether cleavage (Section 3.2).

Fig. 4. Ether cleavage products of brGDGTs-IIb isomers. The conventional IIb₅ and IIb₆ are suggested to be composed of two structural isomers (IIb₅* and IIb₆*). Labels (a, b, c, f, g and h) correspond to the hydrocarbons in Fig. 2.

Fig. 5. HPLC–APCI-MS chromatograms of brGDGTs from the sediment of Lake of Baleng in Cameroon, including BPC, m/z 1050 and 1036 extracted ion chromatograms

Table 1. Relative abundance of brGDGTs in fraction B (see Fig. 1) evaluated by integration of the respective ion traces during LC–MS analysis. Predicted hydrocarbons arising from ether cleavage are indicated in the last column.

Table 2. Carbon skeletons generated from 5- to 7-methyl brGDGTs, their measured and calculated Kováts indexes and diagnostic fragment ions in the mass spectra.

Table 3. Relative abundance of hydrocarbons generated by ether cleavage of fraction B (see Fig. 1).

brGDGT type	Relative abundance	m/z	Contribute to fragments
IIIa ₇	2.6%	1050	i
IIIb ₆	0.3%	1048	b,g
IIIb ₇	0.8%	1048	i
IIa ₅	17.7%	1036	c,a
IIa ₆	49.3%	1036	b,a
IIa ₇	3.8%	1036	i,a
IIb ₅	20.3%	1034	c,f (h,a)
IIb ₆	5.2%	1034	b,f (g,a)

brGDGT branched methyl position	Carbon skeleton	RI measured	RI calculated*	Diagnostic ions in mass spectrum
5	c	2905	2911	379, 351, 196, 211, 253, 267
6	b	2900	2912	365, 337, 196, 211, 253, 267
7	i	2895	2902	351, 323, 196, 211, 253, 267

* Using additivity principles for the calculation of relative retention factors (RRF) to obtain RI (calculated).

Carbon skeleton	Relative abundance
a	43.3%
i	4.5%
b	22.2%
c	12.2%
f	4.4%
g	1.8%
h	11.6%