

Dear editor,

Please find enclosed our revised manuscript titled Assessing branched tetraether lipids as tracers of soil organic carbon transport through the Carminowe Creek catchment (southwest England). We thank the reviewers for their comments and we have followed most of their suggestions, as you can read in the replies posted in the online forum and enclosed here. Below we list the major changes that we have made to the manuscript. All these changes are highlighted in our revised manuscript enclosed herewith. We hope that you find this revised version suitable for publication in Biogeosciences.

On behalf of all co-authors,

Jingjing Guo

- We have followed most of the suggestions for textual changes throughout the revised manuscript.
- In the introduction, we have elaborated on the description of the elusive producer of brGDGTs, and explained the implications for the interpretation of brGDGT-based proxies. We have also clarified and expanded the explanation of *in situ* production of brGDGTs in aquatic environments and added appropriate references.
- In discussion part, we have specified the changes in the BIT index and the IR along the soil transects, and marked them in the appendix figure. Furthermore, a table with original data of the BIT index was added into the appendix. Finally, we have added the references that were suggested by the reviewers, specifically on the river transport of brGDGTs (Kim et al., 2012; Kim et al., 2015) and the turnover time of terrestrial brGDGTs (Huguet et al., 2017).
- Figures: the color of box plot (Figure 3) was changed to make different land types easier to distinguish. The mean point of different land types in PCA plot (Figure 4b) was added.
- Additionally, we discovered that we accidentally reported the incorrect IR value in section 4.2, where we use this ratio to calculate the amount of river *in situ* brGDGT production. We have corrected this value in the revised version. Importantly, this has no influence on the interpretation of the data and our conclusions.

# 1 Assessing branched tetraether lipids as tracers of soil organic carbon 2 transport through the Carminowe Creek catchment (southwest 3 England)

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11 **Abstract.** Soils represent the largest reservoir of organic carbon (OC) on land. Upon mobilisation, this OC is either returned  
12 to the atmosphere as carbon dioxide (CO<sub>2</sub>), or transported and ultimately locked into (marine) sediments, where it will act as  
13 a long-term sink of atmospheric CO<sub>2</sub>. These fluxes of soil OC are, however, **difficult to evaluate**, mostly due to the lack of a  
14 soil-specific tracer. In this study, a suite of branched glycerol dialkyl glycerol tetraethers (brGDGTs), which are membrane  
15 lipids of soil bacteria, is tested as specific tracers for soil OC from source (soils under arable land, ley, grassland and  
16 woodland) to sink (Lake Loe Pool sediments) considering a small catchment located in southwest England (i.e. Carminowe  
17 Creek draining into Lake Loe Pool). The analysis of brGDGTs in catchment soils reveals that their distribution is not  
18 significantly different across different land use types ( $p > 0.05$ ), and thus does not allow tracing land use-specific soil  
19 contributions to Lake Loe Pool sediments. Furthermore, the significantly higher contribution of 6-methyl brGDGT isomers  
20 in creek sediments (isomerization ratio (IR) =  $0.48 \pm 0.10$ ; mean  $\pm$  s.d., standard deviation;  $p < 0.05$ ) compared to that in  
21 catchment soils (IR =  $0.28 \pm 0.11$ ) indicates that the initial soil signal is substantially altered by brGDGT produced *in situ*.  
22 Similarly, the riverine brGDGT signal appears to be overwritten by lacustrine brGDGTs in the lake sedimentary record,  
23 indicated by remarkably lower Methylation of Branched Tetraethers (MBT'<sub>SME</sub> =  $0.46 \pm 0.02$  in creek bed sediment and  $0.38$   
24  $\pm 0.01$  in lake core sediment;  $p < 0.05$ ) and higher Degree of Cyclisation (DC =  $0.23 \pm 0.02$  in creek bed sediment and  $0.32 \pm$   
25  $0.08$  in lake core sediment). Thus, in this small catchment, brGDGTs do not allow us to trace soil OC transport.  
26 Nevertheless, the downcore changes in the degree of cyclisation and the abundance of isoprenoid GDGTs produced by  
27 methanogens in the Lake Loe Pool sediment do reflect local environmental conditions over the past 100 years, and have  
28 recorded the eutrophication history of the lake.

## 29 1 Introduction

30 Globally, around 1500–2000 Pg of carbon is stored in soils in the form of organic matter, which is about two times the  
31 amount of carbon in the atmosphere and three times the amount of carbon in vegetation (Janzen, 2004; Smith, 2008). Soil  
32 organic carbon (OC) plays an important role in the global carbon cycle, as subtle alterations in the soil OC reservoir may  
33 affect the concentration of atmospheric CO<sub>2</sub> and thus influence climate change (Davidson and Janssens, 2006). Atmospheric  
34 CO<sub>2</sub> that is fixed by plants through photosynthesis will be stored into soil OC pool, part of which will be transferred to  
35 streams and rivers. Upon fluvial discharge, soil OC is buried and locked into the marine or lacustrine sediment, where it will  
36 act as a long-term carbon sink. However, instead of a passive pipeline in the carbon cycle, rivers actually represent a  
37 dynamic channel, where part of the soil OC is respired back to the atmosphere, and another part may be stored in river bed or  
38 lake sediments before reaching the ocean (Cole et al., 2007; Battin et al., 2009; Aufdenkampe et al., 2011). Hence, it is hard

39 to determine the exact amount of soil OC that is transported to the ocean, as the dynamic processes that soil OC undergoes  
40 during transport, such as degradation and sequestration, are elusive. This is mostly due to the lack of a specific tracer to  
41 distinguish soil OC from the total pool of OC that is also comprised of plant-derived OC, aquatic produced OC, and fossil  
42 OC from rock erosion (Blair et al., 2004; Aufdenkampe et al., 2011).

43 To circumvent this problem, lipid biomarkers can be used to trace a specific part of the total OC pool in complex natural  
44 environmental systems (Brassell and Eglinton, 1986; Wakeham and Lee, 1993). For example, odd-numbered long chain *n*-  
45 alkanes derived from epicuticular plant waxes are widely used to detect the contribution of terrestrial OC to river-dominated  
46 marine sediments (Eglinton and Hamilton, 1967; Hedges et al., 1997; Fernandes and Sicre, 2000; Glendell et al., 2018).  
47 Similarly, lignin, an abundant biopolymer in vascular plants (Hedges et al., 1997), has been used to trace OC transport along  
48 the terrestrial-aquatic continuum by e.g., in the Mississippi River (Goñi et al., 1997; Bianchi et al., 2004), the Amazon River  
49 (Hedges et al., 1986, 2000; Feng et al., 2016), and Arctic rivers (Feng et al., 2013). However, these biomarkers are derived  
50 from vegetation, which, although land-derived, is not fully representative of soil OC. Thus, in order to specifically trace and  
51 quantify the pool of soil OC, another biomarker is needed.

52 Branched glycerol dialkyl glycerol tetraethers (brGDGTs; Fig. 1) are membrane spanning tetraether lipids synthesized by  
53 heterotrophic bacteria that thrive in soils and peats all over the world (Weijers et al., 2006a, 2007a; Naafs et al., 2017a).  
54 Although the exact producers of these lipids are still unknown, after the detection of a brGDGT and the presumed brGDGT  
55 precursor lipid *iso*-diabolic acid in Acidobacterial cultures (Sinninghe Damsté et al., 2011, 2014, 2018), it was assumed that  
56 this members of the phylum are the main source organisms of brGDGTs in soils. However, a biological source outside the  
57 phylum of Acidobacteria cannot be excluded (Sinninghe Damsté et al., 2018). The occurrence and relative distribution of  
58 brGDGTs in a global set of modern surface soils showed that they can have 4 to 6 methyl groups attached to their alkyl  
59 backbone, where the degree of branching increases in soils from colder areas. Furthermore, brGDGTs respond to changes in  
60 soil pH by forming up to 2 cyclopentane moieties following internal cyclisation, where a higher number of cyclopentane  
61 moieties corresponds to a higher soil pH (Weijers et al., 2007a). Initially, a combination of two proxies, the Methylation of  
62 Branched Tetraethers (MBT) index and Cyclisation of Branched Tetraethers (CBT) index, was proposed as a proxy to  
63 reconstruct the mean air temperature (MAT) and pH of a soil (Weijers et al., 2007a; Peterse et al., 2012). After the  
64 identification of novel brGDGT isomers that possess a methyl group at the  $\alpha$  and/or  $\omega$  6 position rather than at position 5  
65 (Fig. 1) and the improvement of the chromatography method used for brGDGT analysis, a modified temperature proxy, the  
66 MBT<sub>SME</sub> was developed (De Jonge et al., 2013, 2014b). Furthermore, the relative abundance of 6-methyl brGDGT isomers,  
67 quantified as the Isomerization Ratio (IR), appeared to also relate to soil pH (De Jonge et al., 2014b). Indeed, the analysis of  
68 brGDGTs in peat profiles and loess-paleosol sequences has resulted in long-term continental paleotemperature records for  
69 various areas, e.g. in deglacial central China (Peterse et al., 2011) and northeast China (Zheng et al., 2017), and western  
70 Europe during the early Eocene (Inglis et al., 2017).

71 These brGDGTs have not only been found in soils, but also in coastal marine sediments, where they have been used as the  
72 terrestrial end-member in the Branched and Isoprenoid Tetraether (BIT) index that determines the relative contribution of  
73 fluvially supplied soil organic matter to marine sediments, where the latter is represented by amounts of the isoprenoid  
74 GDGT crenarchaeol (Hopmans et al., 2004). For example, the relative abundance of brGDGTs in a marine sediment core  
75 from the Bay of Biscay revealed the early re-activation of European rivers after the last deglaciation (Ménot et al., 2006).  
76 Furthermore, brGDGTs stored in continental margin sediments are assumed to represent an integrated climate signal of the  
77 nearby land, and have been used as such to generate temperature records of deglacial tropical Africa (Weijers et al., 2007b),  
78 and Pliocene North-Western Europe (Dearing Crampton-Flood et al., 2018).

79 Recently, however, brGDGTs have also been found to be produced in aquatic systems such as coastal marine areas (Peterse  
80 et al., 2009b; Sinninghe Damsté, 2016), rivers (Kim et al., 2012; Zell et al., 2013, 2014) and lakes (Sinninghe Damsté et al.,  
81 2009a; Tierney and Russell, 2009; Loomis et al., 2011, 2014; Schoon et al., 2013; Weber et al., 2015, 2018), which  
82 complicates the interpretation of brGDGT-based proxy records. A contribution of *in situ* produced brGDGTs in lakes or on  
83 the continental shelf may bias BIT index values towards a more terrestrial signal (e.g. Sinninghe Damsté et al., 2009; De  
84 Jonge et al., 2015). Aquatic production in coastal marine areas became first apparent upon comparison of brGDGTs in  
85 Svalbard fjord sediments and nearby soils. Whereas the brGDGT signal in the fjord sediments was dominated by compounds  
86 containing cyclopentane moieties, soils were characterized by brGDGTs without cyclisation (Peterse et al., 2009b). These  
87 substantially different brGDGT signatures in combination with the increasing concentration of brGDGTs towards the open  
88 ocean then pointed towards a contribution of *in situ* produced brGDGTs to the fjord sediments. Similarly, brGDGT  
89 distributions in lake sediments were found to differ from those in soils surrounding the lake (Sinninghe Damsté et al., 2009;  
90 Tierney and Russell, 2009), and generated temperature estimates that severely underestimated actual MAT, mostly due to a  
91 high relative abundance of hexamethylated brGDGTs (e.g. Tierney et al., 2010; Loomis et al., 2014; Weber et al., 2015).  
92 Finally, the presence of brGDGTs with a polar headgroup still attached in suspended particulate matter (SPM) of several  
93 large rivers (Zhang et al., 2012; Zell et al., 2013; De Jonge et al., 2014a) provided strong evidence for aquatic production, as  
94 these headgroups are thought to be lost within days after cell death (e.g. Harvey et al., 1986). Notably, these and subsequent  
95 studies proposed ways to recognize *in situ* production of brGDGTs in aquatic environments. For example, a high degree of  
96 cyclisation is an indicator of brGDGT production in coastal marine zones (Peterse et al., 2009b; Sinninghe Damsté, 2016),  
97 for which Sinninghe Damsté (2016) proposed that a weighed number of rings in tetramethylated brGDGTs, quantified as  
98  $\#rings_{tetra} > 0.7$  indicates a purely marine source of brGDGTs in continental margin sediments. In rivers, aquatic brGDGTs  
99 appear to be characterized by a relatively high contribution of 6-methyl brGDGT isomers, and can be quantified using the IR  
100 (De Jonge et al., 2014a).

101 Here we test brGDGTs as tracers for soil OC in Carminowe Creek catchment, a small catchment in southwest England.  
102 Previously, an attempt was made to follow OC transport from soil (source) to Lake Loe Pool, the final sink of this  
103 catchment, using a combination of stable isotopes of bulk soil OC and plant leaf wax *n*-alkanes as fingerprints for the  
104 different vegetation types present in the catchment (i.e. arable land, grassland, ley and woodland) (Glendell et al., 2018).  
105 Although most land use types had a distinct *n*-alkane fingerprint, OC derived from arable land and temporary grassland (ley)  
106 could not be distinguished (Glendell et al., 2018). Hence, by assuming a primary soil source of the brGDGTs, their analysis  
107 in the same samples may contribute to tracing soil OC from different land use types during transport in Carminowe Creek.  
108 Moreover, changes in GDGT distributions in a 50 cm long sediment core from Loe Pool may be used to infer changes in soil  
109 OC transport dynamics in the catchment over the past century, and potentially couple them to climate or anthropogenic  
110 activity related events in the catchment area.

## 111 2 Methods

### 112 2.1 Study site and sampling

113 An overview of the study area and sampling sites is given by Glendell et al. (2018). Briefly, the Carminowe Creek catchment  
114 is located in Cornwall in southwest England (50°14' N, 5°16' W), covers an area of around 4.8 km<sup>2</sup> and varies in elevation  
115 from 0 to 80 m above sea level (Fig. 2). It is divided into two subcatchments ('north' and 'south'). The two streams converge  
116 around 100 m before their joint outlet, and then flow into a natural freshwater lake Loe Pool (50 ha), which is separated from  
117 the Atlantic Ocean by a natural shingle barrier. The mean annual temperature (MAT) and mean annual precipitation (MAP)  
118 in this area are approximately 11 °C and 1000 mm year<sup>-1</sup>, respectively. The land use in this studied catchment is dominated

119 by arable land and temporary grasslands (ley), which are under rotation. The steeper hillslopes are under permanent  
120 grassland, and riparian woodland covers the areas near the creek. For this study, 74 surface soil samples (0–15 cm) were  
121 collected along 14 hillslope transects, including 31 arable land sites, 14 permanent grassland sites, 24 temporary grassland  
122 (ley) sites and 5 woodland sites (Fig. 2). Riverbed sediments were collected at three locations along each of the two  
123 tributaries (upstream, midstream and downstream), and one more at the joint outlet. A 50 cm long sediment core was taken  
124 in the lake, about 150 m away from the joint outlet. The lake core has been dated by the activity of Caesium-137 (<sup>137</sup>Cs), and  
125 it covers the last 100 years (Glendell et al., 2018).

## 126 2.2 Bulk soil properties

127 Total carbon contents were reported by Glendell et al. (2018). Soil pH was measured in this study using a pH meter in a soil  
128 to water ratio of 1:5 (w:v) after shaking for two hours.

## 129 2.3 GDGT extraction and analysis

130 In total, 74 soil samples, 7 creek bed sediment and 25 lake core sediment samples were analysed for GDGTs. First, 5–7 g of  
131 the soils or 3–5 g of the sediments were freeze dried and homogenized, after which they were extracted three times with  
132 dichloromethane (DCM) : MeOH (9 : 1, v/v) using an accelerated solvent extractor (ASE 350, Dionex™) at 100 °C and 7.7  
133 × 10<sup>6</sup> Pa to obtain a total lipid extract (TLE). After addition of a known amount of C<sub>46</sub> GDGT internal standard (Huguet et  
134 al., 2006), the TLEs were dried under a N<sub>2</sub> stream, and then separated into apolar and polar fractions by passing them over  
135 an activated Al<sub>2</sub>O<sub>3</sub> column using hexane : DCM (9 : 1, v/v) and DCM : MeOH (1 : 1, v/v) respectively. The polar fraction,  
136 which contains the GDGTs, was evaporated to dryness under a gentle N<sub>2</sub> stream. After this, the samples were prepared for  
137 further analysis by re-dissolving them in a hexane : isopropanol (99 : 1, v/v) mixture, and filtration through a 0.45 μm  
138 polytetrafluoroethylene (PTFE) filter.

139 The GDGTs were analysed on an Agilent 1260 Infinity ultra high performance liquid chromatography (UHPLC) coupled to  
140 an Agilent 6130 single quadrupole mass spectrometer (MS) with settings according to Hopmans et al. (2016). The GDGTs  
141 were separated over two silica Waters Acquity UPLC BEH Hilic columns (1.7 μm, 2.1 mm x 150 mm) preceded by a guard  
142 column with the same packing. GDGTs were eluted isocratically at a flow rate of 0.2 ml min<sup>-1</sup> using 82% A and 18% B for  
143 25 min, followed by a linear gradient to 70% A and 30% B for 25 min, where A = hexane and B = hexane : isopropanol (9 :  
144 1, v/v). Sample injection volumes were 10 μL. Ionization of the GDGTs was achieved by atmospheric pressure chemical  
145 ionization with the following source settings: gas temperature 200 °C, vaporizer temperature 400 °C, N<sub>2</sub> flow 6 L min<sup>-1</sup>,  
146 capillary voltage 3500 V, nebulizer pressure 25 psi and a corona current of 5.0 μA. By scanning the [M+H]<sup>+</sup> ions (protonated  
147 mass) in selected ion monitoring (SIM) mode, the target compounds were detected at *m/z* 1302 (GDGT-0), 1292  
148 (crenarchaeol), 1050 (brGDGT-IIIa), 1048 (brGDGT-IIIb), 1046 (brGDGT-IIIc), 1036 (brGDGT-IIa), 1034 (brGDGT-  
149 IIb), 1032 (brGDGT-IIc), 1022 (brGDGT-Ia), 1020 (brGDGT-Ib), 1018 (brGDGT-Ic), with *m/z* 744 for the internal  
150 standard. Quantitation was achieved by peak area integration of the [M+H]<sup>+</sup> ions in Chemstation software B.04.03.

## 151 2.4 GDGT proxy calculations

152 The roman numerals in following equations refer to the molecular structures of GDGTs in Fig.1. The ratios below were  
153 calculated based on the fractional abundances (indicated by using square brackets) of GDGTs. The BIT index was calculated  
154 according to Hopmans et al. (2004), and modified to also include 6-methyl brGDGTs:

$$155 \text{ BIT} = \frac{[Ia] + [IIa] + [IIIa] + [IIa'] + [IIIa']}{[Ia] + [IIa] + [IIIa] + [IIa'] + [IIIa'] + [\text{crenarchaeol}]} \quad (1)$$

156 The degree of methylation ( $MBT'_{5ME}$ ) and relative abundances of tetra-, penta-, and hexamethylated brGDGTs were  
157 calculated following De Jonge et al. (2014b) and Sinninghe Damsté et al. (2016):

$$158 \quad MBT'_{5Me} = \frac{[Ia] + [Ib] + [Ic]}{[Ia] + [Ib] + [Ic] + [IIa] + [IIb] + [IIc] + [IIIa]} \quad (2)$$

$$159 \quad \%tetra = \sum[tetramethylated \ brGDGTs] = [Ia] + [Ib] + [Ic] \quad (3)$$

$$160 \quad \%penta = \sum[pentamethylated \ brGDGTs] = [IIa] + [IIb] + [IIc] + [IIa'] + [IIb'] + [IIc'] \quad (4)$$

$$161 \quad \%hexa = \sum[hexamethylated \ brGDGTs] = [IIIa] + [IIIb] + [IIIc] + [IIIa'] + [IIIb'] + [IIIc'] \quad (5)$$

162 Furthermore, the degree of cyclisation (DC) was calculated according to Baxter et al. (2019):

$$163 \quad DC = \frac{[Ib] + 2*[Ic] + [IIb] + [IIb']}{[Ia] + [Ib] + [Ic] + [IIa] + [IIa'] + [IIb] + [IIb']} \quad (6)$$

164 The isomerization ratio (IR) is the ratio between penta- and hexamethylated 6-methyl brGDGTs and the total amount of both  
165 5- and 6-methyl penta- and hexamethylated brGDGTs (De Jonge et al., 2014a):

$$166 \quad IR = \frac{[IIa'] + [IIb'] + [IIc'] + [IIIa'] + [IIIb'] + [IIIc']}{[IIa] + [IIa'] + [IIb] + [IIb'] + [IIc] + [IIc'] + [IIIa] + [IIIa'] + [IIIb] + [IIIb'] + [IIIc] + [IIIc']} \quad (7)$$

## 167 2.5 Statistical analysis and data visualization

168 The statistical analysis and data visualization were undertaken in R programming (version 3.5.2) (R Core Team, 2018).  
169 Differences in the concentration of brGDGTs and brGDGT-based proxies between different land use types (i.e. arable land,  
170 grassland, ley and woodland), creek bed and lake core sediments were examined by one-way nested ANOVA under  
171 generalized linear model (GLM) followed by post-hoc analysis (Tukey HSD (honest significant difference) test), and were  
172 performed with package 'car', 'carData' and 'agricolae'. Differences were considered to be significant at level of  $p < 0.05$ .  
173 **To show how close our sample** mean is to the population mean, standard deviation is used (mean  $\pm$  s.d.). To examine  
174 whether brGDGT signatures could distinguish soil OC derived from different land use types, principal component analysis  
175 (PCA) was performed with package 'FactoMineR' and 'factoextra'. The box plot and scatter plots were carried out with  
176 package 'ggplot2'.

## 177 3 Results

### 178 3.1 BrGDGTs in soils

179 Most of the brGDGTs were present in all soils. Only brGDGT-IIIc and brGDGT-IIIc' were always below the detection limit  
180 (peak height  $> 3x$  baseline), and brGDGT-IIc' was below the detection limit in 13 of the soils (three in arable land, four in  
181 grassland and six in ley). The brGDGTs were dominated by pentamethylated ( $49.4 \pm 3.0\%$ , mean  $\pm$  s.d., standard deviation),  
182 followed by tetramethylated ( $39.7 \pm 4.9\%$ ) and then hexamethylated brGDGTs ( $10.9 \pm 2.6\%$ ; Table 1). The concentration of  
183 brGDGTs ranged between 0.1 and  $1.7 \mu\text{g g}^{-1}$  soil, with average of  $0.2 \pm 0.1 \mu\text{g g}^{-1}$  soil in arable land,  $0.6 \pm 0.4 \mu\text{g g}^{-1}$  soil in  
184 grassland, and  $0.4 \pm 0.3 \mu\text{g g}^{-1}$  soil in ley (i.e. the temporary grassland). However, the concentration of brGDGTs in  
185 woodland was  $3.0 \pm 1.0 \mu\text{g g}^{-1}$  soil, which was significantly higher than that in other land use types ( $0.4 \pm 0.3 \mu\text{g g}^{-1}$  soil;  $p <$   
186  $0.05$ ; Fig. 3a). The C-normalized concentration of brGDGTs in catchment soils ranged between 2.8 to  $49.8 \mu\text{g g}^{-1}$  C,  $8.1 \pm$   
187  $3.6 \mu\text{g g}^{-1}$  C in arable land,  $11.2 \pm 6.7 \mu\text{g g}^{-1}$  C in grassland,  $10.5 \pm 4.8 \mu\text{g g}^{-1}$  C in ley, and  $37.6 \pm 11.0 \mu\text{g g}^{-1}$  C in woodland  
188 (Fig. 3a; Table 1). The trend of the concentration of brGDGTs along the soil transects was not obvious.

189 BIT index values ranged from 0.57 to 1.00 among land use types (Fig. 3b), with an average value of  $0.96 \pm 0.03$  in  
190 woodland,  $0.90 \pm 0.12$  in ley,  $0.88 \pm 0.14$  in grassland and  $0.83 \pm 0.09$  in arable land (without significant differences,  $p >$   
191  $0.05$ ). However, the BIT values increased from hillslope to downslope along several transects in north catchment, while the  
192 BIT values show no clear trends in south catchment (Fig. A1). The  $MBT'_{5ME}$  ranged from 0.37 to 0.71 and was mostly  
193 similar between all land use types ( $0.48 \pm 0.04$ ;  $p > 0.05$ ; Fig. 3c; Table 1). The degree of cyclisation between land use types  
194 was similar ( $DC = 0.23 \pm 0.13$ ; Fig. 3d; Table 1;  $p > 0.05$ ), likewise, the IR ranged from 0.10 to 0.60 ( $0.28 \pm 0.01$  on  
195 average; Fig. 3e; Table 1;  $p > 0.05$ ), without clear trend along the soil transects. However, four transects in the north  
196 catchment have on average significantly higher IR values ( $> 0.36$ ) than the other transects in the catchment ( $0.24 \pm 0.09$ ;  $p <$   
197  $0.05$ ; Fig. A1). In general, the IR increases with increasing soil pH in the catchment ( $r^2 = 0.36$ ,  $p < 0.001$ ).

### 198 **3.2 BrGDGTs in creek bed sediments**

199 All brGDGT compounds were detected in creek bed sediments, except for in the upstream site from north catchment, where  
200 brGDGT–IIIc' was below detection limit. The brGDGTs in creek bed sediments were dominated by pentamethylated  
201 brGDGTs ( $45.0 \pm 0.7\%$ ), followed by tetramethylated brGDGTs ( $30.1 \pm 4.5\%$ ), and hexamethylated brGDGTs ( $24.9 \pm$   
202  $4.7\%$ ) (Table 1). The C-normalized concentration of brGDGTs in creek bed sediments was  $34.7 \pm 17.4 \mu\text{g g}^{-1} \text{C}$  on average  
203 (Fig. 3a; Table 1), where the concentration increased from  $32.7 \mu\text{g g}^{-1} \text{C}$  to  $57.0 \mu\text{g g}^{-1} \text{C}$  downstream in north catchment,  
204 and from  $14.3 \mu\text{g g}^{-1} \text{C}$  to  $25.2 \mu\text{g g}^{-1} \text{C}$  downstream in south catchment, reaching a maximum value of  $59.3 \mu\text{g g}^{-1} \text{C}$  at the  
205 outlet (Fig. 5a). The concentration of brGDGTs in creek bed sediments was higher than that in soils under any land use  
206 types, except for woodland ( $9.6 \pm 4.9 \mu\text{g g}^{-1} \text{C}$ ; Fig. 3a; Table 1).

207 The BIT values for creek sediments were on average  $0.90 \pm 0.06$  (Fig. 3b; Table 1). The  $MBT'_{5ME}$  was relatively constant  
208 between 0.44 and 0.49, with an average of  $0.46 \pm 0.02$ . The DC ranged from 0.21 to 0.25 in the creek sediments with an  
209 average of  $0.23 \pm 0.02$  (Fig. 3e; Table 1). The IR was relatively invariable with an average of  $0.48 \pm 0.10$  (Fig. 3e; Table 1).  
210 The brGDGT-based proxies for creek bed sediments were similar to those for soils, except for the IR, which was higher than  
211 that in soils under any land use types ( $0.28 \pm 0.11$ ; Fig. 3; Table 1).

### 212 **3.3 BrGDGTs in Lake Loe Pool sediment core**

213 All brGDGTs were detected in the lake sediment core, except at 20 cm depth, where brGDGT–IIIc' was below the detection  
214 limit. The brGDGTs in the lake sediments were mainly dominated by pentamethylated brGDGTs ( $50.2 \pm 1.8\%$ ), followed by  
215 tetramethylated brGDGTs ( $28.9 \pm 0.7\%$ ), and hexamethylated brGDGTs ( $21.0 \pm 1.4\%$ ; Table 1). The amount of brGDGTs in  
216 lake core sediment ranged from  $19.9$  to  $48.0 \mu\text{g g}^{-1} \text{C}$  (Fig. 3a; Table 1). The brGDGT concentration in the surface sediment  
217 (0–2 cm), of  $37.7 \mu\text{g g}^{-1} \text{C}$ , which was about 1.6 times lower than that in the creek sediment at the outlet (Fig. 5a), increased  
218 to a maximum of  $48.0 \mu\text{g g}^{-1} \text{C}$  around 11 cm depth, and then decreased to a minimum of  $19.9 \mu\text{g g}^{-1} \text{C}$  at 23 cm depth (Fig.  
219 6b). The concentration of GDGT-0 ranged between  $9.0 \mu\text{g g}^{-1} \text{C}$  and  $27.1 \mu\text{g g}^{-1} \text{C}$  with an average of  $17.4 \pm 6.0 \mu\text{g g}^{-1} \text{C}$ ,  
220 concentration of crenarchaeol ranged from  $0.6 \mu\text{g g}^{-1} \text{C}$  to  $1.4 \mu\text{g g}^{-1} \text{C}$  with an average of  $1.0 \pm 0.2 \mu\text{g g}^{-1} \text{C}$  in the lake  
221 sediment core. In general, the concentration of brGDGTs in lake core ( $34.0 \pm 8.7 \mu\text{g g}^{-1} \text{C}$ ; Table 1) was similar with that in  
222 river and in woodland, while it was significantly higher than the brGDGTs in soils except for the woodland ( $9.6 \pm 4.9 \mu\text{g g}^{-1}$   
223  $\text{C}$ ;  $p < 0.05$ ; Fig. 3a; Table 1).

224 The BIT values for the lake sediment core were rather uniform, varying between 0.95 and 0.97 (Fig.3b). Similarly, the  
225 values of  $MBT'_{5ME}$  along the lake core ranged only between 0.36 and 0.39. The  $MBT'_{5ME}$  of 0.37 for the lake surface  
226 sediment was significantly lower than that in creek bed sediment ( $0.46 \pm 0.02$ ;  $p < 0.05$ ; Fig 3c; Fig. 5b). Conversely, the DC  
227 in the lake surface sediment was 0.39, which was significantly higher than that in creek bed sediment ( $0.23 \pm 0.02$ ;  $p < 0.05$ ;

228 Fig. 3d; Fig. 5b). The average value of DC for the lake core sediments was  $0.32 \pm 0.08$ . The DC increased from the surface  
229 to a maximum value (0.44) at around 10 cm depth, and then decreased with slight fluctuations to 0.22 at 43 cm depth (Fig.  
230 6c). The IR was constant downcore ( $0.32 \pm 0.01$  on average; Fig. 3e; Table 1) and was significantly lower than that in creek  
231 bed sediment ( $p < 0.05$ ; Fig. 3e).

## 232 4 Discussion

### 233 4.1 Spatial variation of brGDGT signals in catchment soils

234 Spatial variations in the relative distribution of brGDGTs in all catchment soils were first evaluated by performing **principal**  
235 **component analysis (PCA)** using the fractional abundances of the 13 major brGDGTs detected. The first two principal  
236 components (PCs) explain 65.2% of the variance in the dataset. PC1 describes 49.5% of the variance, and separates acyclic  
237 brGDGT-Ia and brGDGT-IIa from all the other brGDGTs (Fig. 4a). In line with this observation, PC1 has a strong positive  
238 relationship with the degree of cyclisation of brGDGTs in the soils ( $r^2 = 0.97$ ; Fig. 4c). PC2 describes another 15.7% of the  
239 variance, and separates tetramethylated brGDGTs as well as most of the 6-methyl brGDGTs from the majority of the 5-  
240 methyl penta- and hexamethylated brGDGTs. As a result, PC2 is negatively correlated with MBT'<sub>SME</sub> ( $r^2 = 0.49$ ; Fig. 4d) as  
241 well as the IR ( $r^2 = 0.58$ ; Fig. 4e) in soils. Despite the clear relation of the first two PCs with the degree of cyclisation and the  
242 degree of methylation, respectively, the position of the soils in the PCA diagram reveals that different land use types are  
243 largely overlapping (Fig. 4b). Indeed, the brGDGTs proxies for different land use types are not significantly different ( $p >$   
244  $0.05$ ; Fig. 3), making it difficult to distinguish the provenance of soil OC solely based on brGDGT signatures.

245 Indeed, previous work has also shown that brGDGT distributions are not primarily affected by land use. For example,  
246 brGDGTs in soils along an altitudinal transect in the Ethiopian highlands revealed that brGDGTs mainly reflect the decrease  
247 in temperature with increasing elevation, regardless of drastic changes in land use along the transect (Jaeschke et al., 2018).  
248 However, other studies report that vegetation cover does exert a great influence on brGDGT signatures in soils from  
249 Minnesota and Ohio, USA (Weijers et al., 2011), around Lake Rotsee, Switzerland (Naeher et al., 2014), in the Tibetan  
250 Plateau (Liang et al., 2019), and paddy and upland soils from subtropical (China and Italy) and tropical (Indonesia,  
251 Philippines and Vietnam) areas (Mueller-Niggemann et al., 2016). The explanations for the similar distribution of brGDGTs  
252 under different land use types in the Carminowe Creek catchment could be the rotation and ploughing in land use in  
253 combination with the turnover time of brGDGTs. Although the soil bacterial community composition is generally different  
254 across distinct land use types (Fierer and Jackson, 2006; Steenwerth et al., 2003), the regular rotation (generally less than 5  
255 years) of arable land and temporary grassland (ley) in the catchment (Glendell et al., 2018) may create a mixed bacterial  
256 community under all vegetation types. Beyond vegetation, regular ploughing as applied across the Carminowe catchment  
257 soils (arable land and ley) is recognized to have a more dominant, long-last effect on microbial communities (Drenovsky et  
258 al., 2010). **Moreover, brGDGTs in terrestrial environments have a relatively long turnover time (ca. 18 years in soils**  
259 **(Weijers et al., 2010), and up to 40 years in peat (Huguet et al., 2017)),** especially when compared to the cropland rotation  
260 time. Taken together, these factors may contribute to the relatively similar brGDGT signal in all soils in the Carminowe  
261 catchment, further limiting the variation in brGDGT signals in catchment soils.

262 Some spatial trends are visible in spite of the overall comparable brGDGT signals across the catchment (Fig. A1), which  
263 may be explained by variations in other environmental factors than land use or vegetation. Mean air temperature and soil pH  
264 have been shown to be the main factors controlling the distribution of brGDGTs in soils worldwide (Weijers et al., 2007a;  
265 Peterse et al., 2012; De Jonge et al., 2014b). However, in the small (ca. 4.8 km<sup>2</sup>) Carminowe Creek catchment, the annual  
266 mean air temperature is practically the same for all soils. Similarly, the range in soil pH is relatively small among different  
267 land use types (from  $5.4 \pm 0.3$  in woodland to  $6.6 \pm 0.1$  in arable land; Table 1), which makes it difficult to separate brGDGT



268 signals based on these parameters. Additionally, the soil water content (SWC) has been shown to affect the distribution and  
269 abundance of brGDGTs in soils, either directly by changing the microbial community, or indirectly by altering soil  
270 temperature, soil pH, or soil oxygen content (Dirghangi et al., 2013; Menges et al., 2014; Dang et al., 2016). The SWC  
271 positively correlates with the abundance of brGDGTs in soils from Qinghai-Tibetan Plateau (Wang et al., 2013), as well as  
272 in soils along an aridity transect in the USA (Dirghangi et al., 2013). Moreover, the degree of methylation of 6-methylated  
273 brGDGTs is sensitive to the SWC, especially in semi-arid and arid regions (Dang et al., 2016). Although MAP is also the  
274 same for the whole catchment, the subtle altitudinal differences in this small creek catchment (i.e. 0-80 m above sea level)  
275 may result in an increase in SWC from hilltop to downslope. This would introduce just enough variability in SWC to explain  
276 some of the trends in brGDGT signals along hillslope transects. In the north catchment, the BIT index values gradually  
277 increase from the presumably better aerated soils at the hilltops towards the wetter soils closer to the creek (Fig. A1). **The**  
278 **increase is > 0.3 for Transects 1 and 8, but also Transects 2, 3, and 7 show an increase in BIT values downslope, albeit to a**  
279 **smaller degree (0.17, 0.19, and 0.04, respectively; Table A1).** The change in BIT index values is driven by both an increase  
280 in the amount of brGDGTs and a slight decrease in crenarchaeol concentrations **with the presumed increase in SWC**  
281 **downslope**, similar to previous findings (Dirghangi et al., 2013; Wang et al., 2013; Menges et al., 2014). The trend in BIT is  
282 likely enhanced by the (minor) change in soil pH along Transects 1 and 8 **(from 6.2 to 6.1 along Transect-1 and from 6.6 to**  
283 **5.7 along Transect-8)**, which may influence the BIT index as a result of the generally positive relation of crenarchaeol  
284 concentrations and a negative relation of brGDGT concentrations with increasing soil pH (Weijers et al., 2006b; Peterse et  
285 al., 2010). Nevertheless, these trends in the BIT index are visible in **five of the transects and only occur in the north part of**  
286 **the catchment.**

287 Interestingly, the IR is also significantly higher in soils along four transects in north catchment **(all > 0.36 averagely for**  
288 **Transects 1, 2, 7, and 8)** compared to the average IR value **for the rest of the transects in the entire catchment** ( $0.24 \pm 0.09$ ;  $p$   
289  $< 0.05$ ). The majority of the sites with higher IR are in cropland, except for those in the Transect-1, which is under grassland  
290 (Fig. A2). Although a relative increase in 6-methyl brGDGTs has been linked to higher soil pH in the global soil dataset (De  
291 Jonge et al., 2014b), this relation is not so strong in the soils **from the Carminowe creek catchment** ( $r^2 = 0.36$ ,  $p < 0.001$ ),  
292 likely due to the relatively minor range and variation in soil pH (from  $5.4 \pm 0.3$  to  $6.6 \pm 0.1$ ). Nevertheless, the soils with  
293 high IR values in the north catchment also have pH values  $> 6.0$  with an average value of  $6.6 \pm 0.1$ .

#### 294 **4.2 Tracing brGDGTs from soils to creek bed sediments**

295 Based on the similar brGDGT signatures for soils under different land use types, these compounds cannot be used to trace  
296 back the exact source of the soil OC after mobilisation and transport throughout the catchment. However, the concentration  
297 and general soil signature of the brGDGTs can be compared with those in creek bed sediments to trace the transfer of OC  
298 from the soils into the creeks. The C-normalized concentration of brGDGTs in the creek sediments is higher than that in  
299 most of the soils ( $34.7 \pm 17.4 \mu\text{g g}^{-1} \text{C}$  and  $9.6 \pm 4.9 \mu\text{g g}^{-1} \text{C}$  respectively), except for those in the woodland soils at the  
300 riverbanks ( $37.6 \pm 11.0 \mu\text{g g}^{-1} \text{C}$ ; Table 1). Thus, purely based on the concentration, this suggests that brGDGTs in the creek  
301 would be primarily derived from the woodland, which also appeared to be the main source of *n*-alkanes in creek bed  
302 sediment (Glendell et al., 2018). However, when looking at the relative distribution of the brGDGTs, the percentage of  
303 hexamethylated brGDGTs in creek sediments is higher than that in soils ( $24.9 \pm 1.8\%$  and  $10.9 \pm 0.3\%$ , respectively),  
304 whereas the percentage of tetramethylated brGDGTs is lower than in soils ( $30.1 \pm 1.7\%$  and  $39.7 \pm 0.6\%$ , respectively; Table  
305 1). Furthermore, brGDGTs in creek sediments have a significantly higher IR (i.e.  $0.48 \pm 0.04$ ) than soils under any of the  
306 land use types ( $0.28 \pm 0.01$  on average in the catchment;  $p < 0.05$ ; Fig. 3e; Table 1). This is clearly reflected in the PCA,  
307 which separates the creek sediments from both the soils and lake sediments on PC2 that is associated with the IR (Fig. 4e).  
308 The higher IR in the creek bed sediments can be explained by a contribution of aquatically (i.e. *in situ*) produced 6-methyl

309 brGDGTs. Similar contributions of 6-methyl brGDGTs, and thus higher IR, were also observed in suspended particulate  
310 matters from the Yenisei River (De Jonge et al., 2014a), and upstream of the Iron Gates in the Danube River, where the  
311 higher IR was coupled to in-river production facilitated by the lower flow velocity and decreased turbidity of the river water  
312 (Freymond et al., 2017). Hence, the significantly higher IR in combination with the higher C-normalized concentrations of  
313 brGDGTs in the Carminowe creek sediments suggests that the brGDGT signal is mainly aquatic.

314 In attempt to further prove the riverine *in situ* production of brGDGTs, we roughly estimate the minimum amount of 6-  
315 methyl brGDGTs that needs to be produced in the creek in order to reach the higher IR. We hereby assume that the  
316 brGDGTs derived from woodland soils are completely transferred into creek without any degradation. Thus, the  
317 concentration of 6-methyl brGDGTs in the creek sediments [ $6\text{-me}_{\text{creek}}$ ] resembles the sum of the average concentration of 6-  
318 methyl brGDGTs in woodland soils [ $6\text{-me}_{\text{woodland}}$ ] and those produced *in situ* [ $6\text{-me}_{\text{in situ}}$ ]. The minimum amount of 6-methyl  
319 brGDGTs produced *in situ* can then be calculated using the brGDGT-concentration-weighted IR for creek sediments ( $IR_{\text{creek}}$   
320 = 0.47) and the following equation (Eq. 8).

$$321 \quad IR_{\text{creek}} = \frac{[6\text{-me}_{\text{creek}}]}{[5\text{-me}_{\text{creek}}] + [6\text{-me}_{\text{creek}}]} = \frac{[6\text{-me}_{\text{woodland}}] + [6\text{-me}_{\text{in situ}}]}{[5\text{-me}_{\text{creek}}] + [6\text{-me}_{\text{woodland}}] + [6\text{-me}_{\text{in situ}}]} \quad (8)$$

322 Solving this equation results in a minimum amount of  $7.4 \mu\text{g g}^{-1} \text{ C}$  6-methyl brGDGTs that needs to be additionally  
323 produced in the creek to reach the higher IR. This accounts for 65% of the total amount of 6-methyl brGDGTs in the creek  
324 bed sediment that we measured. Considering a mixture of all soils rather than only woodland as source for soil-derived  
325 brGDGTs in the creek results in the *in situ* production of  $9.3 \mu\text{g g}^{-1} \text{ C}$  6-methyl brGDGTs, corresponding to 81% of the 6-  
326 methyl brGDGT pool in the creek bed sediments. This implies that the initial soil brGDGT signal is rapidly overprinted by a  
327 riverine *in situ* signal upon entering the creek. Only the IR for the downstream site in the northern creek approaches that of  
328 the adjacent soil ( $IR = 0.30$  in the creek bed sediment and  $0.38 \pm 0.07$  for Transect-7; Fig. A2), and may be explained by its  
329 use as arable land (Fig. 5a), which involves regular ploughing and subsequent soil mobilisation and implies a temporary,  
330 local overprint.

331 The absence of a clearly recognizable soil brGDGT signal in the creek bed sediments may be further explained by the  
332 relatively limited input of soil material into the creek. So far, river systems that have shown to transport a soil-derived  
333 brGDGT signal are either characterized by a distinct rainy season (e.g. the Congo River (Weijers et al., 2007b; Hemingway  
334 et al., 2017) or the Amazon River (Kim et al., 2012)), or have experienced a recent episode of extreme rainfall (e.g. the  
335 Danube River, >100 mm in 3 days causing a 100-year flood event, (Freymond et al., 2017) or the Rhône River, with heavy  
336 rainfall during sampling (Kim et al., 2015)). The Carminowe creek area does not have a clear rainy season, and is further  
337 characterized by its limited relief. Hence, the relatively minor input of soil-derived brGDGTs seems to be easily overprinted  
338 by riverine *in situ* production. Alternatively, the soil-derived brGDGTs could be preferentially degraded in an aquatic  
339 environment as a result of priming effect (Bianchi, 2011), which would lead to a signature that is dominated by brGDGTs  
340 that are produced *in situ*.

#### 341 4.3 Sources of brGDGTs in the sediments of Lake Loe Pool

342 In theory, rivers would transport soil-derived OC together with any aquatic OC produced along the way. Once discharged, in  
343 this case into a lake, the OC would settle and then be buried into the sediments where it would act as a long-term sink of OC.  
344 However, the soil brGDGT signal cannot be recognized in the sediments from Loe Pool since it is already lost upon entering  
345 the Carminowe creek. Indeed, the PCA of the relative distributions of brGDGTs indicates that lake sediments plot  
346 completely separated from both the soils and creek sediments, mostly due to a higher relative abundance of GDGT-IIIa (Fig.  
347 4a, b). As a result, the  $MBT_{5ME}$  is significantly lower in Loe Pool sediments ( $0.38 \pm 0.00$ ) compared to in the creek bed

348 sediments ( $0.46 \pm 0.01$ ;  $p < 0.05$ ) and soils ( $0.48 \pm 0.01$ ;  $p < 0.05$ ; Fig. 5b; Table 1). Furthermore, the DC is significantly  
349 higher in lake sediments than in both soil and creek bed sediments ( $0.32 \pm 0.02$ ,  $0.23 \pm 0.01$  and  $0.23 \pm 0.01$ , respectively;  $p$   
350  $< 0.05$ ; Fig. 3d; Table 1). The distinct brGDGT signature of the lake sediments suggests that brGDGTs in the lake again are  
351 significantly altered compared to those in the soils and creek sediments. This implies that the riverine brGDGT signal is  
352 either replaced or overwritten in the lake.

353 Lacustrine *in situ* production of brGDGTs has been reported in other studies (Sinninghe Damsté et al., 2009; Tierney and  
354 Russell, 2009; Buckles et al., 2014; Loomis et al., 2011, 2014a; Weber et al., 2015, 2018; Miller et al., 2018). However,  
355 there are no generally recognized indicators (yet) to identify lacustrine brGDGT production, although several studies  
356 reported a “cold bias” while attempting to reconstruct the mean air temperature (MAT) based on brGDGTs in lake sediments  
357 using a soil-based transfer function (Tierney et al., 2010). In a study on East African lakes, this cold bias was linked to a  
358 large *in situ* contribution of brGDGT-IIIa (Tierney et al., 2010), similar to in Loe Pool. However, the East African lake  
359 dataset was generated using the ‘old’ chromatography method that does not separate 5-methyl and 6-methyl brGDGTs. A  
360 recent study that has re-analysed the East African Lake dataset indicates that the presumed contribution of GDGT-IIIa  
361 mainly consists of brGDGT-IIIa' (Russell et al., 2018), which is less prominent in lake Loe Pool. Although the identity of  
362 brGDGT-producer(s) in lakes still remain(s) elusive, a recent study from the stratified Lake Lugano (Switzerland) showed  
363 that the majority of the brGDGTs are produced in the lower, anoxic part of the water column rather than in the sediment  
364 (Weber et al., 2018). Furthermore, the combination of brGDGT analysis with molecular biological methods revealed that  
365 brGDGTs appeared to be produced by multiple groups of bacteria thriving under different redox regimes in this stratified  
366 lake. Specifically, brGDGT-IIIa occurred in the entire water column and continuously increased with depth, whereas  
367 brGDGT-IIIa' was mainly produced in the upper, oxygenated part of water column (Weber et al., 2018). Extrapolating the  
368 ecological niches of brGDGT production in Lake Lugano to Loe Pool we can speculate that brGDGT-IIIa, which is  
369 dominating the brGDGT signal in the Loe Pool sediments, is mostly produced in the lake during summer, when the  
370 eutrophic state of the lake may seasonally cause the anoxic conditions favourable for its (i.e. brGDGT-IIIa) production.  
371 However, our dataset does not allow to further pinpoint the time and depth of lacustrine brGDGT production, or whether  
372 brGDGTs are solely produced in the water column of Loe Pool or also in the lake sediment.

#### 373 4.4 Reconstructing local environmental changes based on GDGTs in Loe Pool lake sediments

374 Downcore variations in the brGDGT distribution of Lake Loe Pool sediments may provide information on past  
375 environmental changes in the catchment, in spite of the lacustrine *in situ* production in Lake Loe Pool. The 50 cm deep  
376 sediment core covers about the last 100 years based on  $^{137}\text{Cs}$  activity (Glendell et al., 2018). The peak activity correlated  
377 with bomb testing in the 1960s was detected at 26 cm depth (Fig. 6a), which can thus be linked to 1963 (Glendell et al.,  
378 2018).

379 The C-normalized concentration of brGDGTs starts to increase around 23 cm, reaching a maximum concentration of  $48.0 \mu\text{g}$   
380  $\text{g}^{-1} \text{C}$  at 11 cm depth (Fig. 6b). The increased brGDGT concentrations coincide with an increase in the degree of cyclisation  
381 (Fig. 6c), which generally responds to a change in pH, where more cyclopentane moieties correspond to a higher pH  
382 (Weijers et al., 2007a; Schoon et al., 2013). According to historical records, agriculture and anthropogenic perturbations  
383 such as mining and urban pollution intensified in the 1960s (~ 26 cm depth), which increased the input of soil and nutrients  
384 into Lake Loe Pool (Coard et al., 1983), and resulted in eutrophication (i.e. blooms of cyanobacteria and algae) since at least  
385 1986 (~ 23 cm depth) (O'Sullivan, 1992; Flory and Hawley, 1994). Earlier studies have also recognized an increased use of  
386 farmyard manures and septic tanks at this time in the nitrogen isotopic composition of the lake sediments, and have detected  
387 higher inputs of terrestrial organic material resulting from intensified farming practices and a higher erosion rate during the  
388 1960s to 1980s based on ratios of aquatic- and terrestrial-derived plant waxes (Glendell et al., 2018). Thus, the high brGDGT

389 concentrations and DC in the sediments likely reflect the eutrophic conditions of the lake resulting from the increased  
390 nutrient input to the lake (Coard et al., 1983). The DC has then recorded the increase in lake water pH associated with  
391 eutrophication, whereas brGDGT concentrations express increased aquatic production. Due to remediation measures taken  
392 by the local government in 1996 (~ 12 cm depth), the eutrophication has reduced over the past twenty years (Glendell et al.,  
393 2018). The partial recovery of the lake has likely resulted in a return to lower lake water pH, as manifested in the decrease in  
394 the DC from ~ 10 cm depth upwards (Fig. 6c).

395 The process of eutrophication and subsequent recovery can also be recognized in the ratio between GDGT-0 and  
396 crenarchaeol, which are isoprenoidal GDGTs produced by Archaea. Crenarchaeol is produced by ammonia oxidizing  
397 Thaumarchaeota (Sinninghe Damsté et al., 2002) in aquatic environments (Schouten et al., 2000; Powers et al., 2004) and to  
398 a lesser extent also in soils (Weijers et al., 2006a), whereas GDGT-0 is a membrane lipid that occurs in all major groups of  
399 Archaea, but is indicative of methanogens and thus anaerobic conditions, with a typical ratio of GDGT-0 and crenarchaeol >  
400 2 (Blaga et al., 2009). The ratio of GDGT-0/crenarchaeol in the sediments of Loe Pool is > 2 throughout the entire core, and  
401 ranges between 10.9 and 24.3, indicating that at least the bottom waters of the lake have been (seasonally) anoxic over the  
402 past 100 years (Fig. 6d), **although the isoGDGTs may potentially be produced in deeper sediments**. The ratio reaches its  
403 maximum at 16 cm depth, suggesting that eutrophic conditions and bottom water anoxia were most severe around this time.  
404 The recovery of the lake after the remediation measures is again reflected in the return to pre-1960 values **at ~ 10 cm depth**  
405 (Fig. 6d).

## 406 5 Conclusions

407 In this study, brGDGTs were tested as a tracer for the transport of soil OC from different vegetation and land use types from  
408 source (soil) to sink (lake Loe Pool) in the Carminowe Creek catchment with the aim to reconstruct the provenance of the  
409 soil OC in lake Loe Pool sediments over time. Unfortunately, brGDGT signatures in the catchment soils are not distinct for  
410 land use types, indicating that other environmental parameters have a larger influence on the distribution of brGDGTs in  
411 these soils. Although temperature and precipitation can be considered equal for all soils due to the small size of the  
412 catchment, changes in BIT index values and the relative contribution of 6-methyl brGDGTs along a part of the hilltop  
413 transects indicate that soil water content (SWC) **may** exert a control on brGDGT signals, assuming that SWC increases  
414 downslope. The regular rotation of cropland in this catchment and the relative long turnover time of brGDGTs in soils could  
415 be another reason to explain the limited spatial variation in brGDGT signals.

416 Comparison of the soil-derived brGDGT signals to that of creek bed sediments reveals that the soil brGDGT signal is **almost**  
417 **completely overprinted** by aquatically produced brGDGTs, indicated by a substantially higher fractional abundance of 6-  
418 methyl brGDGTs in the creek. Upon discharge into the lake, the creek brGDGT signal **is replaced by and/or mixed with** a  
419 lacustrine *in situ* produced brGDGT signal, which is characterized by a relatively higher DC and lower MBT'<sub>5ME</sub>, as well as a  
420 specifically high fractional abundance brGDGT-IIIa. Despite regular ploughing of the land, the absence of a profound rainy  
421 season and limited relief likely limits the degree of soil mobilisation necessary to transfer the soil-derived brGDGT signal to  
422 the lake sediments in the modern system. Still, downcore variations in GDGT distributions in the sediments of Loe Pool do  
423 reflect local environmental conditions over the past 100 years. The degree of cyclisation of brGDGTs as well as the ratio of  
424 isoprenoidal GDGT-0 and crenarchaeol produced by Archaea trace the historical record of lake eutrophication induced by  
425 increased nutrient input from intensified agricultural activity in the catchment during the 1960s to 1980s, and its recovery  
426 after measures taken by the owner since 1996. Our study shows that GDGTs in sedimentary archives are good recorders of  
427 past environmental and **land management (e.g. agricultural intensification, increased fertilizer use)** change, although the  
428 ability of brGDGTs to trace soil OC along a soil-aquatic continuum requires a higher degree of soil mobilisation.

429 **Data availability**

430 All data are available in the Supplementary Information.

431 **Author Contribution**

432 J.M., F.K., and F.P designed the study, M.G. and J.M. collected the sample material. J.G. conducted the biomarker analysis  
433 and interpreted the data under supervision of F.P. and J.J.M, J.G. and F.P wrote the paper with input from all co-authors.

434 **Competing interests**

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

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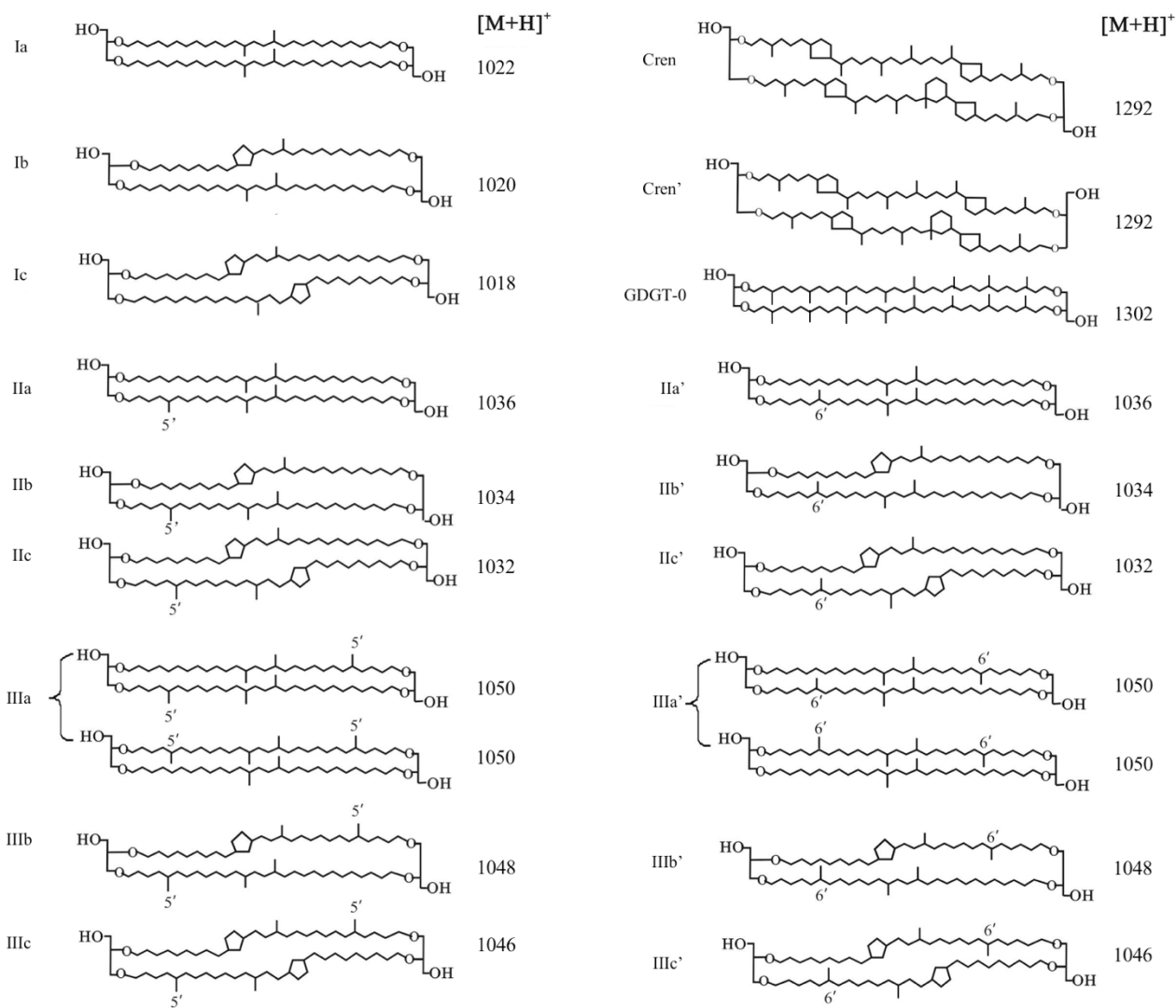
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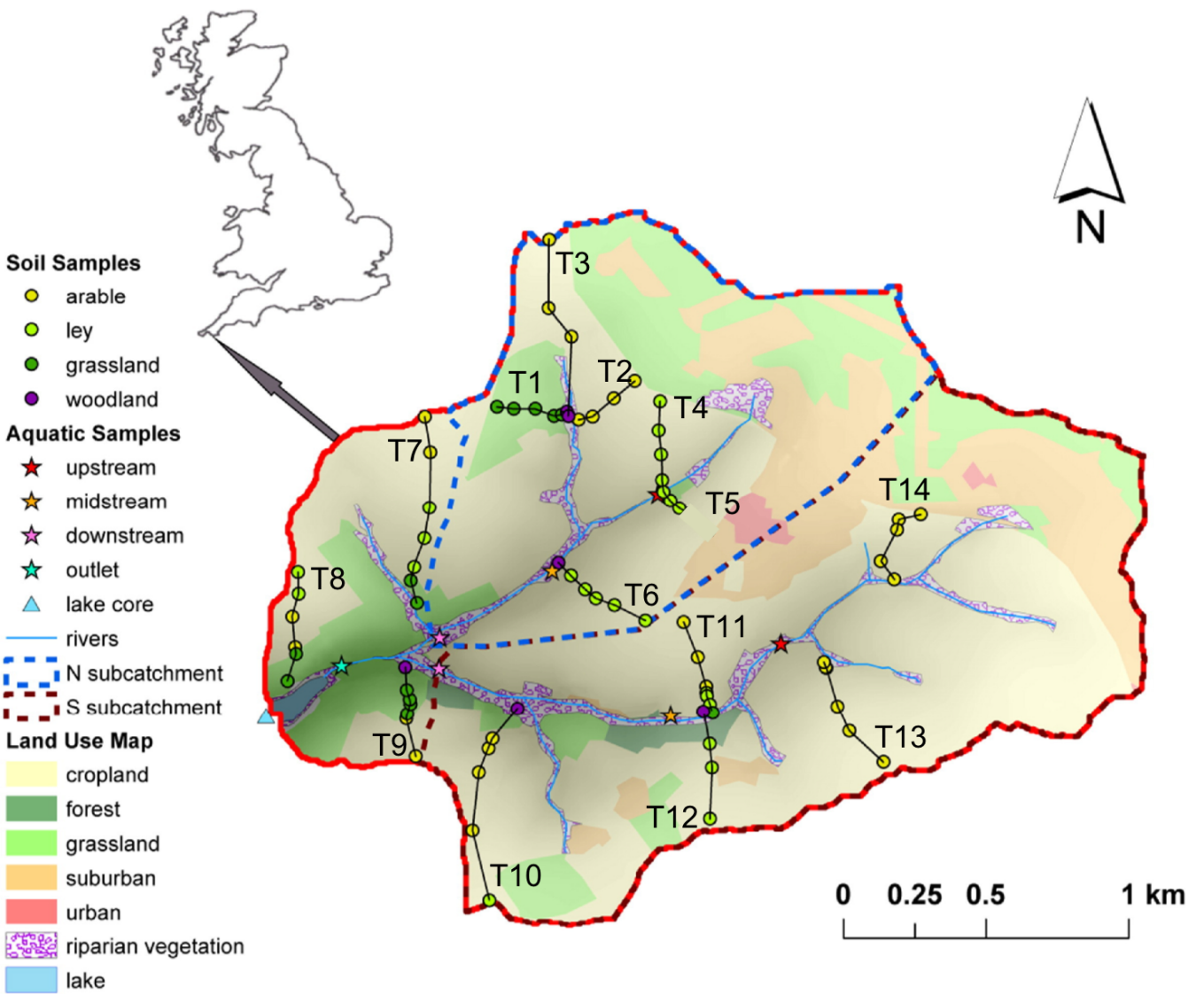
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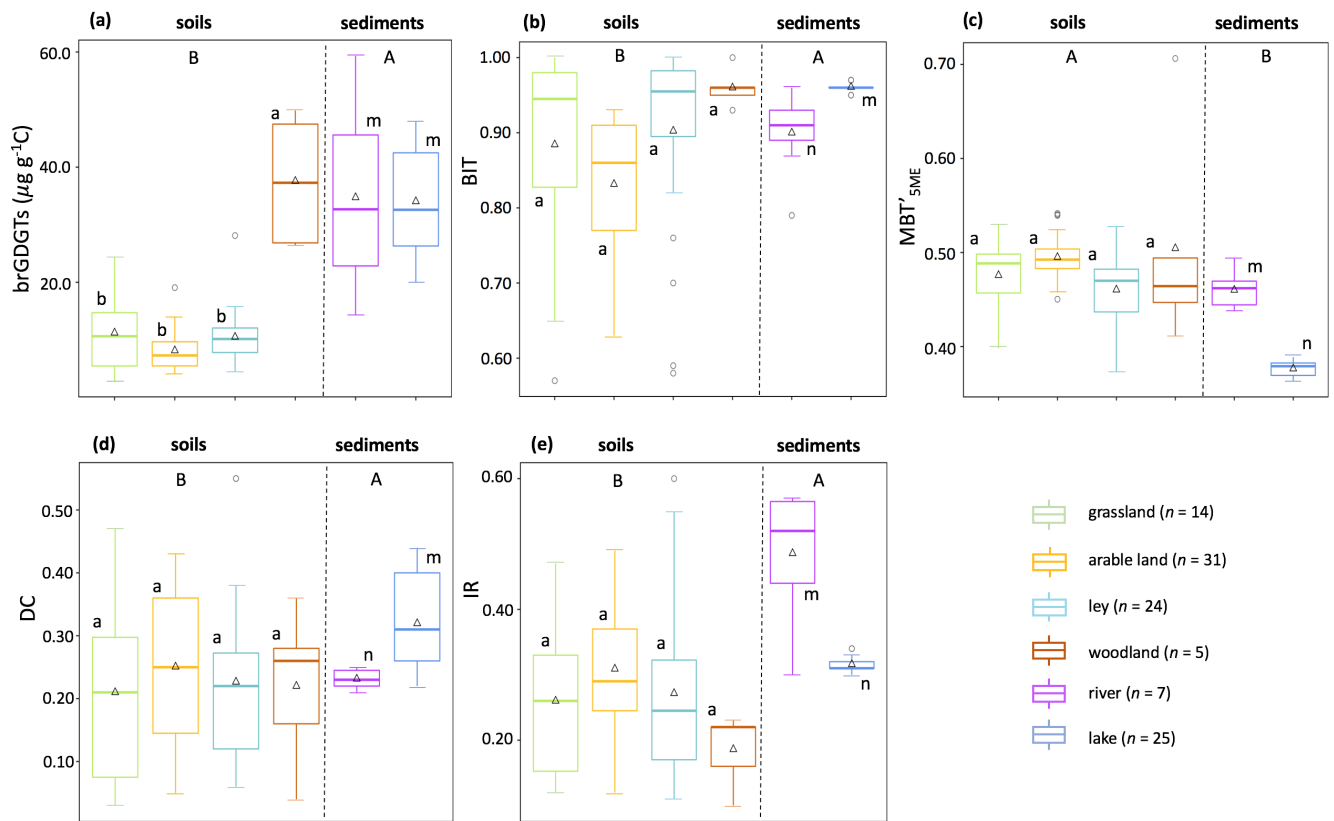
682

683 **Figure 1: Molecular structures of 5-methyl and 6-methyl branched GDGTs, GDGT-0 and crenarchaeol. The 6-methyl brGDGTs**  
 684 **are represented by apostrophe. The structures of penta- and hexamethylated brGDGTs with cyclopentane moiety(ies) IIb', IIc',**  
 685 **IIIb', IIIc' are tentative.**



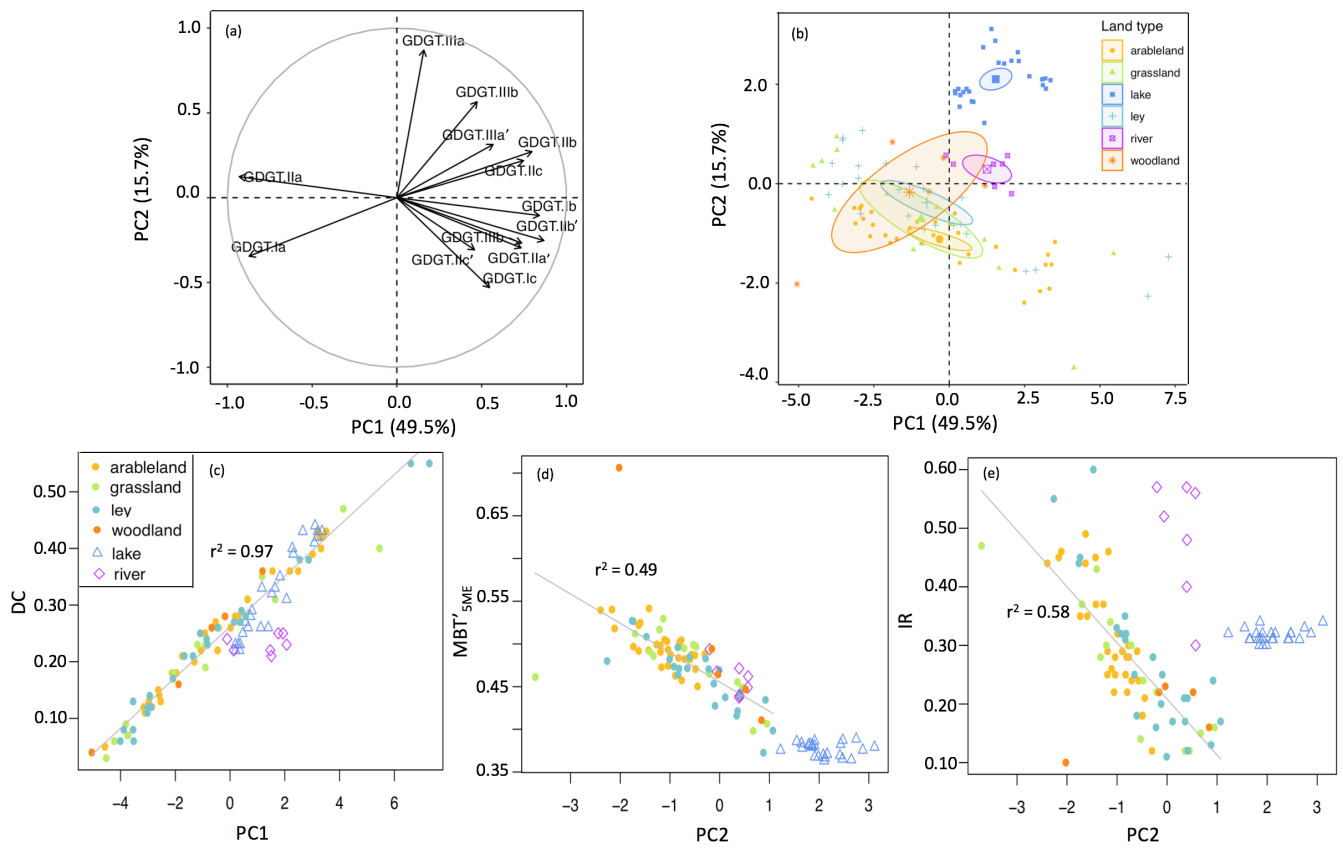
686

687 **Figure 2: Map of the Carminowe Creek catchment in southwest England showing land use types, 14 soil transects (labelled T1-14),**  
 688 **creek bed and lake core sediment sampling locations. The coloured circles and stars indicate soil samples under different land use**  
 689 **types and creek bed sediments along the streams, respectively. Adjusted from Glendell et al. (2018).**



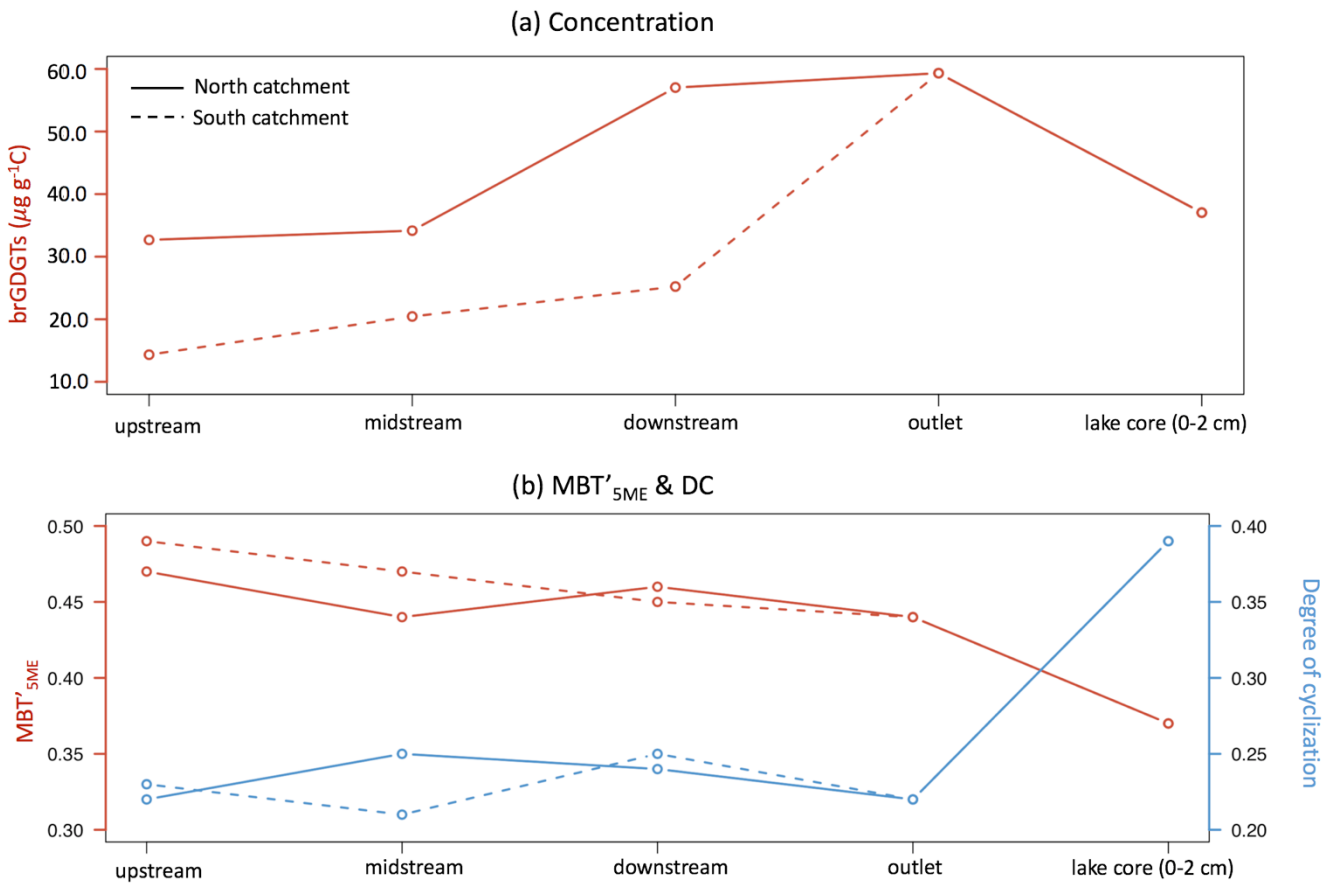
690

691 **Figure 3:** Box plots displaying (a) the C-normalized concentration of brGDGTs, and brGDGT-based proxies: (b) BIT index  
 692 (branched and isoprenoid tetraether ratio), (c)  $\text{MBT}'_{5\text{ME}}$  (methylation of 5-methyl branched tetraethers), (d) DC (degree of  
 693 cyclisation) and (e) IR (isomerization ratio). The triangles represent the average values, the bold line indicates the median (50<sup>th</sup>  
 694 percentile), bottom and top of the box indicate first quartile (25<sup>th</sup> percentile) and third quartile (75<sup>th</sup> percentile) respectively,  
 695 whiskers cover the smallest and largest value within 1.5 times of the interquartile range (i.e. the distance between the top and  
 696 bottom of the box). Any data points outside the whiskers are considered as outliers. Different letters indicate differences between  
 697 samples: A and B for differences between catchment soils and aquatic sediments, a and b for soils under different vegetation types,  
 698 and m and n for creek bed and lake core sediments ( $p < 0.05$ ).



699

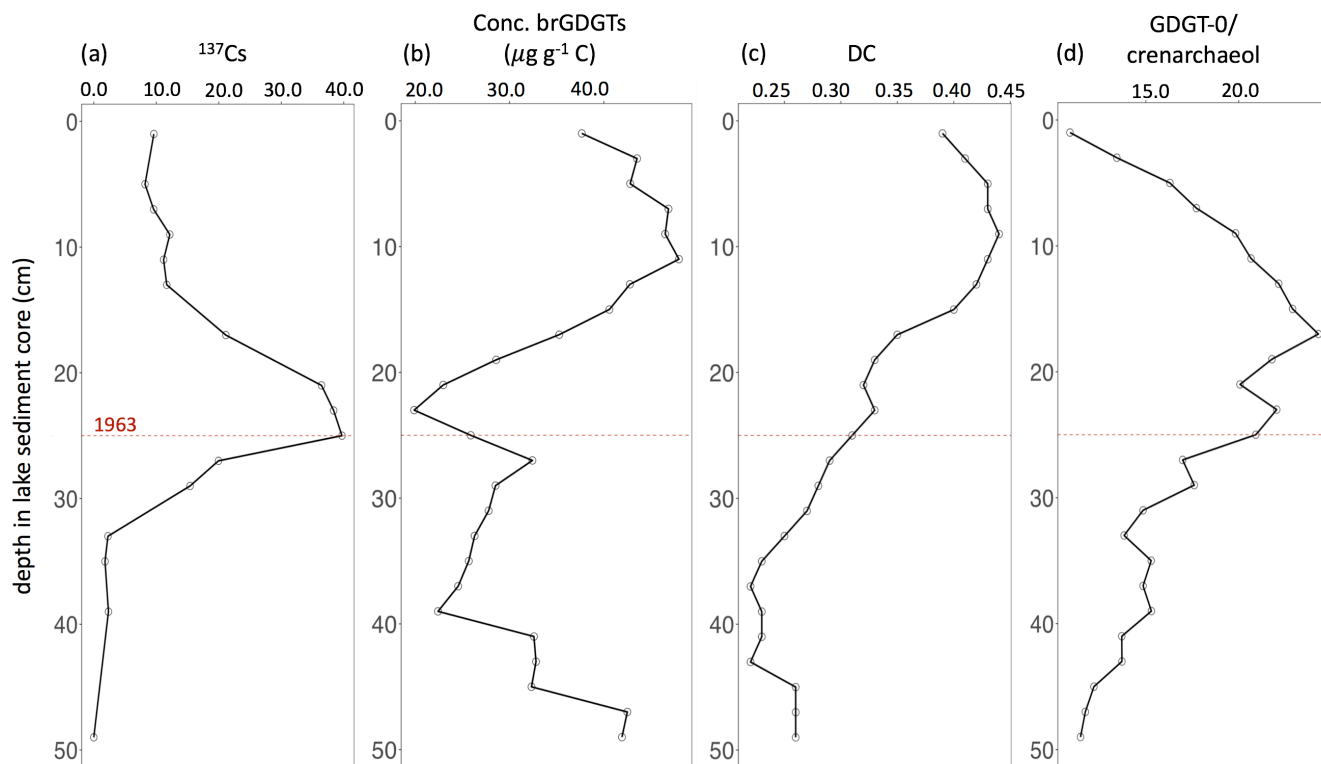
700 **Figure 4:** PCA based on the relative abundances of 13 major brGDGTs. Figure (a) shows the distribution of 13 brGDGTs  
 701 (brGDGT-IIIc and brGDGT-IIIc' are excluded as they are below the detection limit) along the first two PCs, roman numerals and  
 702 English alphabet represent the compounds shown in Fig. 1. Figure (b) shows sampling sites loading scores on the first two PCs and  
 703 95% confidence interval ellipses surrounding the mean point of different groups of land use: arable land ( $n = 31$ ), grassland ( $n =$   
 704  $14$ ), ley ( $n = 24$ ) and woodland ( $n = 5$ ), and creek ( $n = 7$ ) and lake ( $n = 25$ ). Figure (c) shows cross plots between PC1 and DC  
 705 (degree of cyclisation). Figure (d) and (e) show cross plots of PC2 with MBT'₅ME (methylation of 5-methyl branched tetraethers)  
 706 and IR (isomerization ratio) respectively. The linear correlation was calculated excluding creek and lake sediment.



707

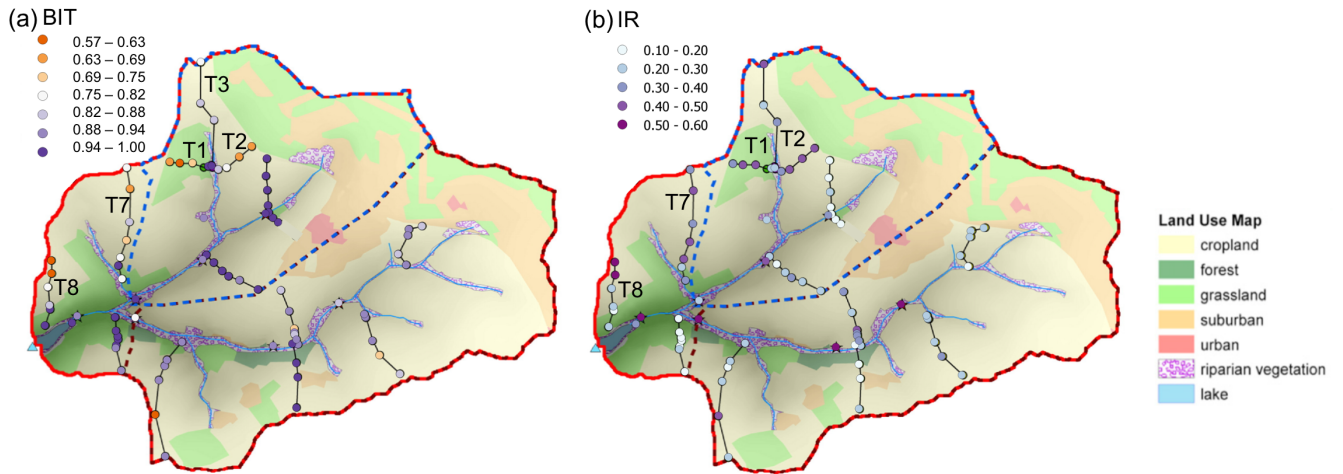
708 **Figure 5: Spatial variability of (a) C-normalized concentration of brGDGTs and (b) MBT'<sub>5ME</sub> (methylation of 5-methyl branched**  
 709 **tetraethers) and DC (degree of cyclisation) in downstream direction of both substreams in the Carminowe Creek catchment.**





710

711 **Figure 6: Lake sediment core profiles of (a)  $^{137}\text{Cs}$  to date, (b) C-normalized concentration of brGDGTs, (c) DC (degree of**  
 712 **cyclisation) and (d) ratio between GDGT-0 and crenarchaeol. The red dashed line indicates the year of 1963.**



713


714 **Appendix Fig.1:** Spatial variability of the (a) BIT (branched and isoprenoid tetraether ratio) and (b) IR (isomerization ratio) along  
 715 14 soil transects in the Carminowe Creek catchment. The coloured circles show the concentrations and proxy values. Tx indicates  
 716 soil transects discussed in the text. The background colours indicate different land use types. Adjusted from Glendell et al. (2018).

**Table 1. C% (carbon content), pH values, average concentrations of brGDGTs and brGDGT-based proxies under different land use types. BIT (branched and isoprenoid tetraether ratio), MBT<sub>5ME</sub> (methylation of 5-methyl branched tetraethers), %tetra (percentage of tetramethylated brGDGTs), %penta (percentage of pentamethylated brGDGTs), %hexa (percentage of hexamethylated brGDGTs), DC (degree of cyclisation), IR (isomerization ratio) (mean  $\pm$  standard deviation, s.d.).**

| Land use (n)   | C% *          | pH            | Conc.<br>( $\mu\text{g g}^{-1}$ soil) | Conc.<br>( $\mu\text{g g}^{-1}$ C) | BIT             | MBT <sub>5ME</sub> | %tetra          | %penta         | %hexa          | DC              | IR              |
|----------------|---------------|---------------|---------------------------------------|------------------------------------|-----------------|--------------------|-----------------|----------------|----------------|-----------------|-----------------|
| arable (31)    | 2.9 $\pm$ 0.5 | 6.6 $\pm$ 0.4 | 0.2 $\pm$ 0.1                         | 8.1 $\pm$ 3.6                      | 0.83 $\pm$ 0.09 | 0.50 $\pm$ 0.02    | 40.1 $\pm$ 3.1  | 49.7 $\pm$ 1.5 | 10.2 $\pm$ 1.8 | 0.25 $\pm$ 0.11 | 0.31 $\pm$ 0.10 |
| grass (14)     | 5.6 $\pm$ 1.2 | 6.0 $\pm$ 0.5 | 0.6 $\pm$ 0.4                         | 11.2 $\pm$ 6.7                     | 0.88 $\pm$ 0.14 | 0.48 $\pm$ 0.04    | 39.8 $\pm$ 4.0  | 49.4 $\pm$ 2.3 | 10.8 $\pm$ 2.1 | 0.21 $\pm$ 0.14 | 0.26 $\pm$ 0.12 |
| ley (24)       | 3.6 $\pm$ 0.9 | 6.0 $\pm$ 0.3 | 0.4 $\pm$ 0.3                         | 10.5 $\pm$ 4.8                     | 0.90 $\pm$ 0.12 | 0.46 $\pm$ 0.04    | 37.8 $\pm$ 3.7  | 50.2 $\pm$ 2.0 | 12.0 $\pm$ 2.9 | 0.23 $\pm$ 0.14 | 0.27 $\pm$ 0.13 |
| woodland (5)   | 8.2 $\pm$ 2.1 | 5.4 $\pm$ 0.7 | 3.0 $\pm$ 1.0                         | 37.6 $\pm$ 11.0                    | 0.96 $\pm$ 0.03 | 0.50 $\pm$ 0.12    | 45.4 $\pm$ 13.0 | 44.4 $\pm$ 8.3 | 10.3 $\pm$ 4.8 | 0.22 $\pm$ 0.12 | 0.19 $\pm$ 0.05 |
| all soils (74) | 4.0 $\pm$ 1.8 | 6.2 $\pm$ 0.5 | 0.6 $\pm$ 0.8                         | 11.5 $\pm$ 8.9                     | 0.87 $\pm$ 0.12 | 0.48 $\pm$ 0.04    | 39.7 $\pm$ 4.9  | 49.4 $\pm$ 3.0 | 10.9 $\pm$ 2.6 | 0.23 $\pm$ 0.13 | 0.28 $\pm$ 0.11 |
| creek (7)      | 2.3 $\pm$ 0.8 | 7.1 $\pm$ 0.2 | 0.8 $\pm$ 0.4                         | 34.7 $\pm$ 17.4                    | 0.90 $\pm$ 0.06 | 0.46 $\pm$ 0.02    | 30.1 $\pm$ 4.5  | 45.0 $\pm$ 0.7 | 24.9 $\pm$ 4.7 | 0.23 $\pm$ 0.02 | 0.48 $\pm$ 0.10 |
| Lake (25)      | 7.5 $\pm$ 1.0 | 5.7 $\pm$ 0.2 | 2.6 $\pm$ 0.7                         | 34.0 $\pm$ 8.7                     | 0.96 $\pm$ 0.01 | 0.38 $\pm$ 0.01    | 28.9 $\pm$ 0.7  | 50.2 $\pm$ 1.8 | 21.0 $\pm$ 1.4 | 0.32 $\pm$ 0.08 | 0.32 $\pm$ 0.01 |

717 \*From Glendell et al. (2018)

**Appendix Table 1.** BIT values along 14 transects (Tx indicates the transect number, and Sx indicates the sample point, where 1 represents the hilltop and subsequent numbers are further downslope).

|                                                                                              |           | North catchment |             |             |      |      |      |             |             | South catchment |      |      |      |      |      |     |
|----------------------------------------------------------------------------------------------|-----------|-----------------|-------------|-------------|------|------|------|-------------|-------------|-----------------|------|------|------|------|------|-----|
|                                                                                              |           | BIT             | T1          | T2          | T3   | T4   | T5   | T6          | T7          | T8              | T9   | T10  | T11  | T12  | T13  | T14 |
| hilltop<br> | S1        | <b>0.65</b>     | <i>0.66</i> | <i>0.77</i> | 0.97 | 0.99 | 0.97 | <i>0.77</i> | <b>0.58</b> | 0.92            | 0.92 | 0.84 | 0.95 | 0.84 | 0.86 |     |
|                                                                                              | S2        | <b>0.57</b>     | <i>0.66</i> | <i>0.86</i> | 0.99 | 0.99 | 0.93 | <i>0.65</i> | <b>0.59</b> | 0.91            | 0.63 | -    | -    | 0.73 | 0.88 |     |
|                                                                                              | S3        | <b>0.73</b>     | <i>0.77</i> | <i>0.87</i> | 1.00 | 1.00 | 0.94 | <i>0.82</i> | <b>0.80</b> | 0.98            | 0.90 | 0.92 | 0.98 | 0.91 | 0.88 |     |
|                                                                                              | S4        | <b>0.88</b>     | <i>0.83</i> | <i>0.96</i> | 0.99 | -    | 0.96 | <i>0.70</i> | <b>0.85</b> | 1.00            | 0.92 | 0.72 | 0.98 | 0.92 | 0.86 |     |
|                                                                                              | S5        | <b>0.95</b>     | -           | -           | -    | -    | 0.97 | <i>0.76</i> | <b>0.98</b> | 1.00            | 0.91 | 0.91 | 1.00 | 0.91 | 0.93 |     |
|                                                                                              | S6        | -               | -           | -           | -    | -    | 0.96 | <i>0.94</i> | <b>0.97</b> | 1.00            | 0.93 | 0.85 | -    | 0.90 | -    |     |
|                                                                                              | S7        | -               | -           | -           | -    | -    | -    | <i>0.81</i> | -           | 0.95            | -    | 0.92 | -    | -    | -    |     |
|                                                                                              | downslope | S8              | -           | -           | -    | -    | -    | -           | -           | -               | -    | -    | 0.92 | -    | -    | -   |

Dear editor,

We are pleased to hear that our manuscript is accepted for public review. We hereby upload our final manuscript for publication on the online forum of Biogeosciences Discussions. In this version, we have incorporated the feedback provided by one of the initial referees, as indicated below. Please see our replies in italics.

On behalf of all co-authors,

Jingjing Guo

Reviewer #2

In this work Guo et al. conduct a thorough analysis on brGDGT distribution in the Carminowe Creek catchment to assess its application for tracing soil organic matter transportation. Data is well presented with comprehensive discussion. However, in the main text there is substantial amount of discussion about the application of GDGT records for past deposition environment which is not related to the title specified objective of tracing soil OM transportation. Please consider reorganizing the text or modifying the title to reflect the overall objectives of work.

*Reply: As the title indicates, the primary focus of our manuscript is indeed on tracing soil OC, and not the application of GDGTs as proxies in downcore records. In the current manuscript, the application of GDGT proxies in the paleo-domain are only limited to a few examples to indicate how GDGTs can be used once stored in a sedimentary archive. Since the GDGT signature may be altered during mobilisation, transport, and deposition, this has implications for the interpretation of paleorecords based on their occurrence in sediments. Hence, we believe this makes mentioning their function as paleo-environmental proxies worthwhile. At this stage we choose to keep the title of our work and the content of the introduction as is.*

Specific Comments:

Line 40-42, are there any numbers of estimated proportion of each OC pool from the literatures? That will show how significant is the soil OC to the total carbon pool.

*Reply: We assume that with 'total carbon pool' the reviewer means the proportion of plant-derived, aquatic produced, and fossil OC from rock erosion that together make up the total pool of OC transported by rivers. The contributions of each of those separate OC pools will depend on the catchment area and the processes that take place during transport. For the Carminowe Creek catchment that we studied, these data are not available. In part this is due to the lack of specific tracers for each of those OC pools, which is one of the motivations for our study.*

Line 49-50, Is soil OC mainly composed of humus from plants? Living soil microbe contributes very small amount of OC. This is not a strong argument why we need another biomarker for tracing soil OC.

*Reply: Soil OC has a mixture of sources, among which plant material and microbes. Even though plant material may contribute more to the soil OC pool than the microbial community, plant biomarkers can also be transported directly from their source-plant by wind, thereby bypassing the soil. Hence, plant biomarkers in any system will represent a variety of transport pathways. In contrast, brGDGTs are derived from bacterial that thrive in soils, and as such have a clear soil origin, despite their relatively minor contribution to the total soil OC pool.*

Line 57, what does the ‘internal cyclisation’ mean?

*Reply: internal cyclisation is the process that describes the formation of cyclopentane moieties in the alkyl backbone of the branched GDGT (Weijers et al., 2006).*

Line 98-99, is this a conclusion from Glendell et al., 2018?

*Reply: Yes, this is correct. We have added Glendell et al. as a reference at the end of this sentence.*

Line 99-100, what is the reasoning behind this speculation? What makes you to expect that brGDGTs could reflect different land types?

*Reply: The brGDGTs know a large structural diversity, which relates to several environmental parameters (pH, temperature, soil moisture availability). These parameters may also be influenced by different vegetation types, which will consequently affect the brGDGT signal.*

Line 110, in this studied catchment...

*Reply: We have changed this.*

Line 126-127, please specify column length and diameter.

*Reply: We have used about 3cm of activated Al<sub>2</sub>O<sub>3</sub> column in a Pasteur pipette according to the general practice in Organic Geochemistry labs worldwide.*

Line 133, delete silica.

*Reply: The silica refers to the packing of the HPLC columns that we have used and is crucial information as it determines the elution behaviour of the GDGTs. We have left this sentence as is.*

Line 178, what is C-normalized concentration? Total organic carbon?

*Reply: This refers to total carbon (inorganic and organic).*

Line 195, brGDGTs concentrations of soil and lake sediment are normalized to both sediment/soil and total C, but there is only C-normalized for creek sediment, why?

*Reply: We focus on C-normalized brGDGT concentrations to enable a fair comparison between the three environments (soil, river, lake), as the mineral fraction, and thus the weight, of the samples is highly variable and would bias sediment weight-based brGDGT concentrations. We only include weight-normalized brGDGT concentrations for the soils in order to compare our data with those from the literature.*

Line 248, is this mixed bacterial community the brGDGTs producing bacteria? The assumption of ‘mixed bacterial community’ does not sound convincing to me. It should be the physical and chemical parameters, such as temperature, pH and redox, that directly affect the brGDGTs synthesis. These parameters may or may not change with land use or vegetation types. It is the same logic for the discussion of soil water content in the following paragraph. SWC does not directly influence the brGDGTs production, but the oxygen/CO<sub>2</sub> content that determined by SWC do.

*Reply: As of yet it is not known which bacteria produce brGDGTs, although brGDGT-Ia has been found in two species of Acidobacteria (Sinninghe Damsté et al., 2011). Acidobacteria have also been found to produce brGDGT precursor lipids (Sinninghe Damsté et al., 2014, 2018), and are currently thought of the most likely producers.*

*As a result of the orphan status of the brGDGTs, it is also unknown what drives the changes in the molecular structure. There is ongoing debate whether changes are a result of membrane adaptation as a response to changing environmental parameters (as suggested by the reviewer), or of a change in the microbial community (which respond to the same environmental parameters), where different species produce distinct brGDGTs.*

*We realize that this information had not made it to the introduction, so we have added it to the submitted version.*

Line 290-291, It will be better to just speculate based on the n-alkanes data that woodland could be a source of brGDGTs in creek sediment. Concentration can not indicate source.

*Reply: We are not so sure what the reviewer means here, as n-alkanes and brGDGTs have different sources (vegetation vs soil bacterial), and can thus not be directly compared.*

Line 302-303, totally agree, further evidence is the relatively higher pH of creek. As cited in Line 280 that IR is positively correlated to pH (De Jonge et al., 2014a).

*Reply: Thanks.*

Line 344, linked to a large in situ contribution of...

*Reply: We have changed this.*

Line 384-388, if this is the case, then you can also calculate the Methane Index (Zhang et al., 2011) to indicate anoxic lake history.

*Reply: The Methane Index is most commonly used in marine environments to assess the potential influence of methanotrophic archaea on the TEX<sub>86</sub> sea surface proxy based on isoprenoidal GDGTs. Since this proxy is designed for the marine environment and is based on marine archaea, it does not necessarily function in a similar fashion in freshwater systems. In lacustrine settings, the ratio between GDGT-0 and crenarchaeol (Blaga et al., 2009) is more often used to indicate the presence and potential influence of methane, as we have also done in our study. Just for your information, the Methane Index in the lake sediment core is 0.30-0.35, indeed indicating the presence of methane in Lake Loe Pool.*

Figure 3. the color coding for arable land and woodland is not easy to recognize. Please consider changing to more contrast colors.

*Reply: we have changed this.*

#### References:

Blaga et al., 2009. Tetraether membrane lipid distributions in water-column particulate matter and sediments: a study of 47 European lakes along a north-south transect. *Journal of Paleolimnology* 41, 523-540.

Weijers et al., 2006. Membrane lipids in mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. *Environmental Microbiology* 8, 648-657.

Sinninghe Damsté et al., 2011. 13,16-Dimethyl octacosanedioic acid (*iso*-diablic acid), a common membrane-spanning lipid of *Acidobacteria* subdivisions 1 and 3. *Applied and Environmental Microbiology* 77, 4147-4154.

Sinninghe Damsté et al., 2014. Ether- and ester-bound *iso*-diabolic acid and other lipids in members of *Acidobacteria* subdivision 4. *Applied and Environmental Microbiology* 80, 5207-5218.

Sinninghe Damsté et al., 2018. An overview of the occurrence of ether- and ester-linked *iso*-diabolic acid membrane lipids in microbial cultures of the *Acidobacteria*: Implications for brGDGT paleoproxies for temperature and pH. *Organic Geochemistry* 124, 63-76.



We would like to thank this reviewer for their feedback on our manuscript. Below we indicate how we will address their comments in our revised version. Our replies are in italics.

Anonymous Referee #3

Review of “Assessing branched tetraether lipids as tracers of soil organic carbon transport through the Carminowe catchment (southwest England)” by J. Guo. Biogeosciences Discussions, 2020.

The authors of this paper aimed to use brGDGTs as soil OC tracers in a small catchment located in southwest England and compared the concentration and distribution of these lipids in soils under different land use, riverbed and lake sediments. They showed that the relative abundance of brGDGTs does not significantly differ between soils under different land use and that brGDGTs in the riverbed and land sediments are mainly produced in situ (in the water column and/or sediment). Therefore, they cannot be used as soil OC tracers in this specific catchment. The analysis of brGDGTs and isoGDGTs along a lacustrine sediment core covering the last 100 yrs additionally showed that the distribution of these lipids (the degree of cyclisation of brGDGTs and the ratio of isoGDGT-0 vs. crenarchaeol) is roughly consistent with eutrophication changes over this period of time.

This study is of interest, as it is comprehensive and one of the few comparing extensively 5- and 6-methyl brGDGT distribution in soils under different land use, river and lake sediments. The paper is well-written and easy to read, and to my mind deserves publication in Biogeosciences after some revisions. The authors should sometimes be more moderate in their assertions and should avoid overinterpreting the data.

The following comments should help in improving the manuscript:

Line 13: Here, the authors mention the fact some tracers are required to quantify the fluxes of soil OC. Nevertheless, brGDGTs would be more qualitative than quantitative tracers. Therefore, this sentence should be modified.

*Reply: We agree with the reviewer, and we will change this.*

Lines 52-53: Here, I would directly say that brGDGTs are ubiquitous lipids, present in terrestrial and aquatic environments, and thus not necessarily specific soil tracers.

*Reply: We chose to follow a chronological order for our introduction, and thus first introduce the discovery of brGDGTs, followed by the development of brGDGT-based proxies, additional production in different aquatic environments (i.e. coastal marine area, rivers and lakes), and the implications of mixed sources for their use as proxies. We prefer to leave this as is.*

Line 54-55: This sentence should be rephrased, as only some of the brGDGT producers may belong to the phylum Acidobacteria. As brGDGTs were detected in various settings, it seems unlikely that they are produced by the same microorganisms everywhere.

*Reply: We will clarify this.*

Lines 77-93: It should be clearly mentioned somewhere that BIT index can be largely biased by in situ production of brGDGTs in aquatic settings (which was not taken into account in the initial hypothesis by Hopmans et al. 2004) and therefore should be applied with caution in coastal and lacustrine settings.

*Reply: We will emphasize this directly after introducing aquatic brGDGT production.*

Lines 82-90: These two studies are restrictive and specific. Other examples of studies dealing with brGDGT in situ production should be mentioned here (Miller et al., 2018, *Climate of the Past*; Loomis et al., 2014, *GCA*; Buckles et al., 2014; *Biogeosciences* etc.). Please also mention that in situ production of more cyclized but also more methylated brGDGTs is generally observed in aquatic vs. terrestrial settings.

*Reply: We agree that there are many more studies that show aquatic brGDGT production than the two that are mentioned in this comment. Please note that we already listed a large number of studies on aquatic brGDGT production in lines 77-79. Our selection includes those studies that were either first in suggesting that in situ production takes place in a certain aquatic environment, provided direct evidence for in situ production, or propose (quantitative) ways to identify the aquatic contribution. We do note, however, that in situ production in lakes is not further clarified in our manuscript. One reason for this is that there is no consistent trend among lakes that enables the identification of in situ brGDGT production, in contrast to production in rivers (more 6-methyl brGDGTs) or in coastal marine environments (higher degree of cyclisation). We will add this information to the introduction of our revised manuscript and add the appropriate references.*

Lines 100-102: In order to trace soil OC with brGDGTs, these lipids should be mainly derived from soils, with only reduced in situ production. Such an assumption should be clearly specified.

*Reply: We will add this.*

Lines 160: Were some samples analysed in replicates?

*Reply: No, we did not analyze samples in replicates.*

Lines 172-181: IsoGDGT-0 concentrations are only reported for the lacustrine sediments. What about the soils and the riverine sediments?

*Reply: We only reported the concentration of isoprenoid GDGTs for the lacustrine sediments as we only discuss them for this environment as part of the GDGT-0/crenarchaeol ratio (section 4.4). Concentration data for GDGT-0 in the other environments will be added to the supplementary table in the excel file.*

Line 227: principal component analysis instead of principle component analysis

*Reply: Thanks. We will correct this.*

Line 236: In Fig. 4b, a lot of samples are outside the circles (the 3 groups of soils) and do not overlap. This should be acknowledged.

*Reply: There are several ways to display these results. We here followed the approach of Glendell et al. (2018), who previously studied the same set of samples. The circles in Fig. 4b represent the 95% confidence interval around the mean point of the group (the enlarged symbol inside the ellipse), which is the reason why there are multiple points that plot outside the ellipse. We will clarify this in the figure caption.*

Line 251: Regarding the turnover of brGDGTs in soils, please also refer to the publication by Huguet et al. (2017, GCA), with turnover times between 8 and 41 years in the same range as Weijers et al. (2010).


*Reply: We will add this reference.*

Lines 269-270: please specify the 2 transects along which large spatial variations in BIT are observed. T1 and T2? All the discussion about spatial variations in BIT and soil moisture remains very speculative. How can you explain that these variations occur only along 2 transects? What about the other transects? Are there any in situ measurements of soil moisture available to strengthen the argumentation? Or measurements in the lab (after having dried the soil samples)?

*Reply: The BIT index values gradually increase from the hilltop downwards along Transect-1 and Transect-8. As can be seen in the table below, Transect-1 and Transect-8 show the largest change in BIT index values (>0.3). Transect-2, Transect-3 and Transect-7 also show an increase from hilltop downslope, albeit to a smaller degree (0.17, 0.19 and 0.04 increase, respectively). The other three transects (Transect-4, Transect-5 and Transect-6) in north catchment have stable BIT values, and the BIT values in south catchment do not show an obvious trend at all. Also based on the comments of Dr. Sparkes, we will clarify our discussion on the BIT index in a revised version.*

*Unfortunately, the soil water content was not analyzed.*

Table1. BIT values along 14 transects (Tx indicates the transect number, and Sx indicates the sample point, where 1 represents the hilltop and subsequent numbers are further downslope).

|                                                                                   |    | North catchment |      |      |      |      |      |      |             | South catchment |      |      |      |      |      |     |
|-----------------------------------------------------------------------------------|----|-----------------|------|------|------|------|------|------|-------------|-----------------|------|------|------|------|------|-----|
|                                                                                   |    | BIT             | T1   | T2   | T3   | T4   | T5   | T6   | T7          | T8              | T9   | T10  | T11  | T12  | T13  | T14 |
| hilltop                                                                           | S1 | <b>0.65</b>     | 0.66 | 0.77 | 0.97 | 0.99 | 0.97 | 0.77 | <b>0.58</b> | 0.92            | 0.92 | 0.84 | 0.95 | 0.84 | 0.86 |     |
|  | S2 | <b>0.57</b>     | 0.66 | 0.86 | 0.99 | 0.99 | 0.93 | 0.65 | <b>0.59</b> | 0.91            | 0.63 | -    | -    | 0.73 | 0.88 |     |
|                                                                                   | S3 | <b>0.73</b>     | 0.77 | 0.87 | 1.00 | 1.00 | 0.94 | 0.82 | <b>0.80</b> | 0.98            | 0.90 | 0.92 | 0.98 | 0.91 | 0.88 |     |
|                                                                                   | S4 | <b>0.88</b>     | 0.83 | 0.96 | 0.99 | -    | 0.96 | 0.70 | <b>0.85</b> | 1.00            | 0.92 | 0.72 | 0.98 | 0.92 | 0.86 |     |
|                                                                                   | S5 | <b>0.95</b>     | -    | -    | -    | -    | 0.97 | 0.76 | <b>0.98</b> | 1.00            | 0.91 | 0.91 | 1.00 | 0.91 | 0.93 |     |
|                                                                                   | S6 | -               | -    | -    | -    | -    | 0.96 | 0.94 | <b>0.97</b> | 1.00            | 0.93 | 0.85 | -    | 0.90 | -    |     |
|                                                                                   | S7 | -               | -    | -    | -    | -    | -    | 0.81 | -           | 0.95            | -    | 0.92 | -    | -    | -    |     |
| downslope                                                                         | S8 | -               | -    | -    | -    | -    | -    | -    | -           | -               | -    | 0.92 | -    | -    | -    |     |

Line 277: similarly, please specify the 4 transects along which large spatial variations in IR index are observed.

*Reply: We will add the specifications.*

Lines 281: Is the relationship between the relative abundance of 6-methyl brGDGTs and pH given for all the soils of the catchments or only those of the 4 transects previously mentioned?

*Reply: The reported relationship between the relative abundance of 6-methyl brGDGTs and pH is for all the soils in the study catchment. We will specify this in the manuscript.*

Lines 281-283: similarly, please specify to which soils correspond the different pH values (those of the 4 transects, the total dataset etc).

*Reply: We will further specify this.*

Lines 321-322: In addition to Congo, brGDGTs are also mainly derived from soils in other large riverine systems such as the Amazon (Kim et al., 2012, GCA) or Rhône river (Kim et al., 2015, Frontiers in Earth Science).

*Reply: We will add these studies.*

Line 326: why would brGDGTs would be degraded more rapidly in soils than in aquatic settings? This sentence should be removed as it appears too speculative.

*Reply: The line that the reviewer refers to is on purpose phrased as a potential explanation for our results, and thus meant to be speculative. Note that we do not compare brGDGT degradation in soils vs an aquatic setting, but the degradation of soil-derived vs aquatic brGDGTs in the same aquatic environment. One process that could explain this process is priming. We will add this explanation and appropriate references (e.g. Bianchi, 2011) to the revised version.*

Line 348-349: as said above, the identity of the brGDGT producers remains elusive in soils as well.

*Reply: Yes, we agree with the referee, although so far there are more clues on the producer(s) of brGDGTs in soils than there are for aquatic systems.*

Lines 352-358: I do not see the interest of this part of this discussion on the ecological niches of brGDGTs producers in Loe Pool as it is totally speculative and has no direct link with the main aim of the paper (using brGDGTs as soil OC tracers).

*Reply: In this section it becomes clear that the brGDGTs in the lake sediment are not derived from soils, but are most likely produced in the lake itself. Since we can, therefore, not use the brGDGTs as tracer for soil OC, we instead use this section to further explore the environmental significance of their signature stored in the lake sediments. For this, it is important to understand the depth and season of brGDGT production in Lake Loe Pool, for which we compare our dataset with the latest insights on brGDGT production in lakes in general, i.e. the ecological niches identified in Lake Lugano (Weber et al., 2018).*

Lines 359-389: in this section about local environmental changes, what about reconstruction of past temperature/pH variations with brGDGT-based indices? It would be complementary to the discussion about the lake eutrophication.

*Reply: We agree with the reviewer that records of past temperature and pH variations would be a valuable addition to the discussion. However, the aquatic source of the brGDGTs in the lake sediments disqualifies the use of the transfer functions from e.g. De Jonge et al., 2014 or Naafs et al., 2017, that are based on soils. We did apply the transfer functions in the latest lake calibration (Russell et al., 2018), however, the calibration dataset only includes lake sediments from tropical east Africa, and results in reconstructed temperatures that are too high ( $13.7 \pm 0.1$  °C vs the locally historical recorded temperature of  $10.9 \pm 0.6$  °C (average of 1978 to 2018, UK metoffice)). It thus seems that both the global soil calibration and the tropical lake calibration are not appropriate for the brGDGTs in this temperate lake, and therefore decided to not include these records in our manuscript.*

Lines 385-387: The authors should also mention the in situ production of isoGDGTs in deep lacustrine sediments, as it could bias the signal recorded in the sediments.

*Reply: We will add that isoGDGTs may potentially be produced in deeper sediments, although we are not aware of a study that has shown this and we can add as a reference. Given the resemblance of the trends in GDGT proxies with that of the eutrophication history of the lake, we also assume that the contribution of a deep-sediment-producer will be minor.*

Lines 395-397: I would rephrase this sentence. There is no direct evidence that soil moisture exerts a control on brGDGT distribution here and the variations in BIT were observed along 2 transects only.

*Reply: As we mentioned above, the trend in BIT values is evident in five out of eight transects in the north catchment, although the increase is relatively small in three of them. Based on the influence of soil water content reported in the literature (e.g. Dirghangi et al., 2013; Menges et al., 2014), and the supposedly lower ground water table at the hilltop compared to the soils downslope, we will leave this interpretation as is.*

Line 401: Please replace “replaced” by “mixed”, as the soil brGDGT signal is not replaced by the aquatic brGDGT signal, the two signals are mixed in the sediment.

*Reply: We will change this sentence.*

Lines 407-410: please be more moderate here, as the interpretation based on brGDGTs is purely qualitative and complementary to previous data. I would rather say that the trends derived from GDGT data are roughly consistent with the historical record of lake eutrophication.

*Reply: We will change this accordingly.*

Lines 411: this sentence should be modified, as in the case of the Carminowe Creek catchment, this study clearly showed that brGDGTs do not record land management change and that in situ production dominates in the riverine system.

*Reply: Note that we here refer to GDGTs in general, not just the brGDGTs. The land management that we mention refers to the increased use of manure and septic tanks and intensified agriculture that caused the eutrophication of the lake, and the subsequent restoration efforts that are reflected in the GDGT proxy records from the lake core (Fig. 6). The conclusion that brGDGTs in the lake sediments are produced within the lake is already clearly mentioned in line 402-403.*

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Bianchi, T. S.: The role of terrestrially derived organic carbon in the coastal ocean: A changing paradigm and

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- Dirghangi, S. S., Pagani, M., Hren, M. T. and Tipple, B. J.: Distribution of glycerol dialkyl glycerol tetraethers in soils from two environmental transects in the USA, *Org. Geochem.*, 59, 49–60, doi:10.1016/j.orggeochem.2013.03.009, 2013.
- De Jonge, C., Hopmans, E. C., Zell, C. I., Kim, J.-H., Schouten, S. and Sinninghe Damsté, J. S.: Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in soils: Implications for palaeoclimate reconstruction, *Geochim. Cosmochim. Acta*, 141, 97–112, doi:10.1016/j.gca.2014.06.013, 2014.
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- Naafs, B. D. A., Gallego-Sala, A. V., Inglis, G. N. and Pancost, R. D.: Refining the global branched glycerol dialkyl glycerol tetraether (brGDGT) soil temperature calibration, *Org. Geochem.*, 106, 48–56, doi:10.1016/j.orggeochem.2017.01.009, 2017.
- Russell, J. M., Hopmans, E. C., Loomis, S. E., Liang, J. and Sinninghe Damsté, J. S.: Distributions of 5- and 6-methyl branched glycerol dialkyl glycerol tetraethers (brGDGTs) in East African lake sediment: Effects of temperature, pH, and new lacustrine paleotemperature calibrations, *Org. Geochem.*, 117, 56–69, doi:10.1016/j.orggeochem.2017.12.003, 2018.
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We would like to thank Robert Sparkes for his kind and encouraging words. We appreciate his comments and indicate how we will address them in a revised version of our manuscript below. Our replies are in italics.

Dr. R. Sparkes

Guo and colleagues have carried out an in-depth study of GDGT mobilisation, transport and production in single catchment, Loe Pool Lake, in the UK. They attempt to use branched GDGTs as tracers for soil mobilisation from different parts of the catchment, but conclude that this is not possible using the available samples. Instead they use data from the creek and lake to reconstruct catchment-wide changes through the last century.

This is an exemplary study, demonstrating how to carry out a thorough investigation of GDGT data. The authors should be applauded for their careful application of laboratory, analytical and statistical techniques. I recommend that this paper is accepted for publication.

Minor comments are limited to typographical and grammatical changes:

Line 166: The word ‘close’ is repeated

*Reply: We will delete the repeated ‘close’.*

Line 256: Extra space ( Weijers

*Reply: We will delete the space.*

Line 268 – 271: These sentences do not make it clear the direction of the trend. “BIT values gradually increase > 0.4” lacks context – do they increase or decrease with altitude? Also, be specific about whether crenarchaeol is decreasing up or down the transect

*Reply: The BIT index values gradually decrease with altitude, i.e. they are lowest on the hilltop. The change in BIT index values is driven by both an increase in the amount of brGDGTs and a slight decrease in the amount of crenarchaeol from the hilltop down the transect. We will specify the trend of the BIT values along different transects in our revised manuscript.*

Line 277: “Interestingly, also . . .” is grammatically odd

*Reply: We will change this to “Interestingly, the IR is also...”.*

Line 315: suggest replacing ‘occupied’ with ‘accounted for’ or similar words



*Reply: We will replace this.*