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

Jingjing Guo¹, Miriam Glendell², Jeroen Meersmans³, Frédérique Kirkels¹, Jack J Middelburg¹, Francien Peterse¹

¹Department of Earth Sciences, Utrecht University, 3584 CB Utrecht, the Netherlands
²The James Hutton Institute, Aberdeen, AB15 8QH, UK
³TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium

Correspondence to: Jingjing Guo (j.guo@uu.nl)

In preparation for submission to: Biogeosciences

Abstract. Soils represent the largest reservoir of organic carbon (OC) on land. Upon mobilisation, this OC is either returned to the atmosphere as carbon dioxide (CO₂), or transported and ultimately locked into (marine) sediments, where it will act as a long-term sink of atmospheric CO₂. These fluxes of soil OC are, however, difficult to evaluate, mostly due to the lack of a soil-specific tracer. In this study, a suite of branched glycerol dialkyl glycerol tetraethers (brGDGTs), which are membrane lipids of soil bacteria, is tested as specific tracers for soil OC from source (soils under arable land, ley, grassland and woodland) to sink (Lake Loe Pool sediments) considering a small catchment located in southwest England (i.e. Carminowe Creek draining into Lake Loe Pool). The analysis of brGDGTs in catchment soils reveals that their distribution is not significantly different across different land use types (p > 0.05), and thus does not allow tracing land use-specific soil contributions to Lake Loe Pool sediments. Furthermore, the significantly higher contribution of 6-methyl brGDGT isomers in creek sediments (isomerization ratio (IR) = 0.48 ± 0.10; mean ± s.d., standard deviation; p < 0.05) compared to that in catchment soils (IR = 0.28 ± 0.11) indicates that the initial soil signal is substantially altered by brGDGT produced in situ. Similarly, the riverine brGDGT signal appears to be overwritten by lacustrine brGDGTs in the lake sedimentary record, indicated by remarkably lower Methylation of Branched Tetraethers (MBT⁵ME = 0.46 ± 0.02 in creek bed sediment and 0.38 ± 0.01 in lake core sediment; p < 0.05) and higher Degree of Cyclisation (DC = 0.23 ± 0.02 in creek bed sediment and 0.32 ± 0.08 in lake core sediment). Thus, in this small catchment, brGDGTs do not allow us to trace soil OC transport. Nevertheless, the downcore changes in the degree of cyclisation and the abundance of isoprenoid GDGTs produced by methanogens in the Lake Loe Pool sediment do reflect local environmental conditions over the past 100 years, and have recorded the eutrophication history of the lake.

1 Introduction

Globally, around 1500–2000 Pg of carbon is stored in soils in the form of organic matter, which is about two times the amount of carbon in the atmosphere and three times the amount of carbon in vegetation (Janzen, 2004; Smith, 2008). Soil organic carbon (OC) plays an important role in the global carbon cycle, as subtle alterations in the soil OC reservoir may affect the concentration of atmospheric CO₂ and thus influence climate change (Davidson and Janssens, 2006). Atmospheric CO₂ that is fixed by plants through photosynthesis will be stored into soil OC pool, part of which will be transferred to streams and rivers. Upon fluvial discharge, soil OC is buried and locked into the marine or lacustrine sediment, where it will act as a long-term carbon sink. However, instead of a passive pipeline in the carbon cycle, rivers actually represent a dynamic channel, where part of the soil OC is respired back to the atmosphere, and another part may be stored in river bed or lake sediments before reaching the ocean (Cole et al., 2007; Battin et al., 2009; Aufdenkampe et al., 2011). Hence, it is hard
to determine the exact amount of soil OC that is transported to the ocean, as the dynamic processes that soil OC undergoes during transport, such as degradation and sequestration, are elusive. This is mostly due to the lack of a specific tracer to distinguish soil OC from the total pool of OC that is also comprised of plant-derived OC, aquatic produced OC, and fossil OC from rock erosion (Blair et al., 2004; Aufdenkampe et al., 2011).

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

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

These brGDGTs have not only been found in soils, but also in coastal marine sediments, where they have been used as the terrestrial end-member in the Branched and Isoprenoid Tetraether (BIT) index that determines the relative contribution of fluvially supplied soil organic matter to marine sediments, where the latter is represented by amounts of the isoprenoid GDGT crenarchaeol (Hopmans et al., 2004). For example, the relative abundance of brGDGTs in a marine sediment core from the Bay of Biscay revealed the early re-activation of European rivers after the last deglaciation (Ménot et al., 2006). Furthermore, brGDGTs stored in continental margin sediments are assumed to represent an integrated climate signal of the nearby land, and have been used as such to generate temperature records of deglacial tropical Africa (Weijers et al., 2007b), and Pliocene North-Western Europe (Dearing Crampton-Flood et al., 2018).
Recently, however, brGDGTs have also been found to be produced in aquatic systems such as coastal marine areas (Peterse et al., 2009b; Sinninghe Damsté, 2016), rivers (Kim et al., 2012; Zell et al., 2013, 2014) and lakes (Sinninghe Damsté et al., 2009a; Tierney and Russell, 2009; Loomis et al., 2011, 2014; Schoon et al., 2013; Weber et al., 2015, 2018), which complicates the interpretation of brGDGT-based proxy records. A contribution of in situ produced brGDGTs in lakes or on the continental shelf may bias BIT index values towards a more terrestrial signal (e.g. Sinninghe Damsté et al., 2009; De Jonge et al., 2015). Aquatic production in coastal marine areas became first apparent upon comparison of brGDGTs in Svalbard fjord sediments and nearby soils. Whereas the brGDGT signal in the fjord sediments was dominated by compounds containing cyclopentane moieties, soils where characterized by brGDGTs without cyclisation (Peterse et al., 2009b). These substantially different brGDGT signatures in combination with the increasing concentration of brGDGTs towards the open ocean then pointed towards a contribution of in situ produced brGDGTs to the fjord sediments. Similarly, brGDGT distributions in lake sediments were found to differ from those in soils surrounding the lake (Sinninghe Damsté et al., 2009; Tierney and Russell, 2009), and generated temperature estimates that severely underestimated actual MAT, mostly due to a high relative abundance of hexamethylated brGDGTs (e.g. Tierney et al., 2010; Loomis et al., 2014; Weber et al., 2015).

Finally, the presence of brGDGTs with a polar headgroup still attached in suspended particulate matter (SPM) of several large rivers (Zhang et al., 2012; Zell et al., 2013; De Jonge et al., 2014a) provided strong evidence for aquatic production, as these headgroups are thought to be lost within days after cell death (e.g. Harvey et al., 1986). Notably, these and subsequent studies proposed ways to recognize in situ production of brGDGTs in aquatic environments. For example, a high degree of cyclisation is an indicator of brGDGT production in coastal marine zones (Peterse et al., 2009b; Sinninghe Damsté, 2016), for which Sinninghe Damsté (2016) proposed that a weighed number of rings in tetramethylated brGDGTs, quantified as \( \text{#rings}_{\text{tetra}} > 0.7 \), indicates a purely marine source of brGDGTs in continental margin sediments. In rivers, aquatic brGDGTs appear to be characterized by a relatively high contribution of 6-methyl brGDGT isomers, and can be quantified using the IR (De Jonge et al., 2014a).

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

2 Methods

2.1 Study site and sampling

An overview of the study area and sampling sites is given by Glendell et al. (2018). Briefly, the Carminowe Creek catchment is located in Cornwall in southwest England (50°14’ N, 5°16’ W), covers an area of around 4.8 km\(^2\) and varies in elevation from 0 to 80 m above sea level (Fig. 2). It is divided into two subcatchments (‘north’ and ‘south’). The two streams converge around 100 m before their joint outlet, and then flow into a natural freshwater lake Loe Pool (50 ha), which is separated from the Atlantic Ocean by a natural shingle barrier. The mean annual temperature (MAT) and mean annual precipitation (MAP) in this area are approximately 11 °C and 1000 mm year\(^{-1}\), respectively. The land use in this studied catchment is dominated
by arable land and temporary grasslands (ley), which are under rotation. The steeper hillslopes are under permanent grassland, and riparian woodland covers the areas near the creek. For this study, 74 surface soil samples (0–15 cm) were collected along 14 hillslope transects, including 31 arable land sites, 14 permanent grassland sites, 24 temporary grassland (ley) sites and 5 woodland sites (Fig. 2). Riverbed sediments were collected at three locations along each of the two tributaries (upstream, midstream and downstream), and one more at the joint outlet. A 50 cm long sediment core was taken in the lake, about 150 m away from the joint outlet. The lake core has been dated by the activity of Caesium-137 (\(^{137}\text{Cs}\)), and it covers the last 100 years (Glendell et al., 2018).

### 2.2 Bulk soil properties

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

### 2.3 GDGT extraction and analysis

In total, 74 soil samples, 7 creek bed sediment and 25 lake core sediment samples were analysed for GDGTs. First, 5–7 g of the soils or 3–5 g of the sediments were freeze dried and homogenized, after which they were extracted three times with dichloromethane (DCM) : MeOH (9 : 1, v/v) using an accelerated solvent extractor (ASE 350, Dionex\textsuperscript{TM}) at 100 °C and 7.7 × 10⁴ Pa to obtain a total lipid extract (TLE). After addition of a known amount of C\(_{46}\) GDGT internal standard (Huguet et al., 2006), the TLEs were dried under a N\(_2\) stream, and then separated into apolar and polar fractions by passing them over an activated Al\(_2\)O\(_3\) column using hexane : DCM (9 : 1, v/v) and DCM : MeOH (1 : 1, v/v) respectively. The polar fraction, which contains the GDGTs, was evaporated to dryness under a gentle N\(_2\) stream. After this, the samples were prepared for further analysis by re-dissolving them in a hexane : isopropanol (99 : 1, v/v) mixture, and filtration through a 0.45 μm polytetrafluoroethylene (PTFE) filter.

The GDGTs were analysed on an Agilent 1260 Infinity ultra high performance liquid chromatography (UHPLC) coupled to an Agilent 6130 single quadrupole mass spectrometer (MS) with settings according to Hopmans et al. (2016). The GDGTs were separated over two silica Waters Acquity UPLC BEH Hilic columns (1.7 μm, 2.1 mm × 150 mm) preceded by a guard column with the same packing. GDGTs were eluted isocratically at a flow rate of 0.2 ml min\(^{-1}\) using 82% A and 18% B for 25 min, followed by a linear gradient to 70% A and 30% B for 25 min, where A = hexane and B = hexane : isopropanol (9 : 1, v/v). Sample injection volumes were 10 μL. Ionization of the GDGTs was achieved by atmospheric pressure chemical ionization with the following source settings: gas temperature 200 °C, vaporizer temperature 400 °C, N\(_2\) flow 6 L min\(^{-1}\), capillary voltage 3500 V, nebulizer pressure 25 psi and a corona current of 5.0 μA. By scanning the [M+H]\(^+\) ions (protonated mass) in selected ion monitoring (SIM) mode, the target compounds were detected at m/z 1302 (GDGT-Ia), 1292 (crenarchaeol), 1050 (brGDGT–IIa), 1048 (brGDGT–IIb), 1046 (brGDGT–IIc), 1036 (brGDGT–Ia), 1034 (brGDGT–Ib), 1032 (brGDGT–Ic), 1022 (brGDGT–Ia), 1020 (brGDGT–Ib), 1018 (brGDGT–Ic), with m/z 744 for the internal standard. Quantitation was achieved by peak area integration of the [M+H]\(^+\) ions in Chemstation software B.04.03.

### 2.4 GDGT proxy calculations

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

\[
BIT = \frac{[\text{IIa}]+[\text{IIIa}]+[\text{Ia}]+[\text{Ia}']}{[\text{IIa}]+[\text{IIa}]+[\text{Ia}]+[\text{Ia}']+[\text{crenarchaeol}]} \tag{1}
\]
The degree of methylation (MBT<sub>SME</sub>) and relative abundances of tetra-, penta-, and hexamethylated brGDGTs were calculated following De Jonge et al. (2014b) and Sinninghe Damsté et al. (2016):

\[ MBT<sub>SME</sub>' = \frac{[ia]+[ib]+[ic]}{[ia]+[ib]+[ic]+[ia]+[ib]+[ic]+[ia]'} \] (2)

%<sub>tetra</sub> = Σ[tetramethylated brGDGTs] = [ia] + [ib] + [ic] \] (3)

%<sub>penta</sub> = Σ[pentamethylated brGDGTs] = [IIa] + [IIb] + [IIc] + [IIa'] + [IIb'] + [IIc'] \] (4)

%<sub>hexa</sub> = Σ[hexamethylated brGDGTs] = [IIIa] + [IIIb] + [IIIC] + [IIIda] + [IIIb'] + [IIIC'] \] (5)

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

\[ DC = \frac{[ib]+2+[ic]+[ib']+[ib']}{[ia]+[ib]+[ic]+[ia]+[ib]+[ic]+[ia]} \] (6)

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

\[ IR = \frac{[ia]+[ib]+[ic]+[ia]+[ib]+[ic]}{[ia]+[ia]+[ia]+[ib]+[ib]+[ib]} \] (7)

2.5 Statistical analysis and data visualization

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

3 Results

3.1 BrGDGTs in soils

Most of the brGDGTs were present in all soils. Only brGDGT–IIIc and brGDGT–IIIc’ were always below the detection limit (peak height > 3x baseline), and brGDGT–IIc’ was below the detection limit in 13 of the soils (three in arable land, four in grassland and six in ley). The brGDGTs were dominated by pentamethylated (49.4 ± 3.0%, mean ± s.d., standard deviation), followed by tetramethylated (39.7 ± 4.9%) and then hexamethylated brGDGTs (10.9 ± 2.6%; Table 1). The concentration of brGDGTs ranged between 0.1 and 1.7 \( \mu \)g g<sup>−1</sup> soil, with average of 0.2 ± 0.1 \( \mu \)g g<sup>−1</sup> soil in arable land, 0.6 ± 0.4 \( \mu \)g g<sup>−1</sup> soil in grassland, and 0.4 ± 0.3 \( \mu \)g g<sup>−1</sup> soil in ley (i.e. the temporary grassland). However, the concentration of brGDGTs in woodland was 3.0 ± 1.0 \( \mu \)g g<sup>−1</sup> soil, which was significantly higher than that in other land use types (0.4 ± 0.3 \( \mu \)g g<sup>−1</sup> soil; \( p < 0.05 \); Fig. 3a). The C-normalized concentration of brGDGTs in catchment soils ranged between 2.8 to 49.8 \( \mu \)g g<sup>−1</sup> C, 8.1 ± 3.6 \( \mu \)g g<sup>−1</sup> C in arable land, 11.2 ± 6.7 \( \mu \)g g<sup>−1</sup> C in grassland, 10.5 ± 4.8 \( \mu \)g g<sup>−1</sup> C in ley, and 37.6 ± 11.0 \( \mu \)g g<sup>−1</sup> C in woodland (Fig. 3a; Table 1). The trend of the concentration of brGDGTs along the soil transects was not obvious.
BIT index values ranged from 0.57 to 1.00 among land use types (Fig. 3b), with an average value of 0.96 ± 0.03 in woodland, 0.90 ± 0.12 in ley, 0.88 ± 0.14 in grassland and 0.83 ± 0.09 in arable land (without significant differences, \( p > 0.05 \)). However, the BIT values increased from hillslope to downslope along several transects in north catchment, while the BIT values show no clear trends in south catchment (Fig. A1). The MBT<sub>5ME</sub> ranged from 0.37 to 0.71 and was mostly similar between all land use types (0.48 ± 0.04; \( p > 0.05 \); Fig. 3c; Table 1). The degree of cyclisation between land use types was similar (DC = 0.23 ± 0.13; Fig. 3d; Table 1; \( p > 0.05 \)), likewise, the IR ranged from 0.10 to 0.60 (0.28 ± 0.01 on average; Fig. 3e; Table 1; \( p > 0.05 \)), without clear trend along the soil transects. However, four transects in the north catchment have on average significantly higher IR values (> 0.36) than the other transects in the catchment (0.24 ± 0.09; \( p < 0.05 \); Fig. A1). In general, the IR increases with increasing soil pH in the catchment (\( r^2 = 0.36, p < 0.001 \)).

3.2 BrGDGTs in creek bed sediments

All brGDGT compounds were detected in creek bed sediments, except for in the upstream site from north catchment, where brGDGT–IIIc' was below detection limit. The brGDGTs in creek bed sediments were dominated by pentamethylated brGDGTs (45.0 ± 0.7%), followed by tetramethylated brGDGTs (30.1 ± 4.5%), and hexamethylated brGDGTs (24.9 ± 4.7%) (Table 1). The C-normalized concentration of brGDGTs in creek bed sediments was 34.7 ± 17.4 μg g<sup>−1</sup> C on average (Fig. 3a; Table 1), where the concentration increased from 32.7 μg g<sup>−1</sup> C to 57.0 μg g<sup>−1</sup> C downstream in north catchment, and from 14.3 μg g<sup>−1</sup> C to 25.2 μg g<sup>−1</sup> C downstream in south catchment, reaching a maximum value of 59.3 μg g<sup>−1</sup> C at the outlet (Fig. 5a). The concentration of brGDGTs in creek bed sediments was higher than that in soils under any land use types, except for woodland (9.6 ± 4.9 μg g<sup>−1</sup> C; Fig. 3a; Table 1).

The BIT values for creek sediments were on average 0.90 ± 0.06 (Fig. 3b; Table 1). The MBT<sub>5ME</sub> was relatively constant between 0.44 and 0.49, with an average of 0.46 ± 0.02. The DC ranged from 0.21 to 0.25 in the creek sediments with an average of 0.23 ± 0.02 (Fig. 3e; Table 1). The IR was relatively invariable with an average of 0.48 ± 0.10 (Fig. 3e; Table 1).

The brGDGT-based proxies for creek bed sediments were similar to those for soils, except for the IR, which was higher than that in soils under any land use types (0.28 ± 0.11; Fig. 3; Table 1).

3.3 BrGDGTs in Lake Loe Pool sediment core

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

The BIT values for the lake sediment core were rather uniform, varying between 0.95 and 0.97 (Fig. 3b). Similarly, the values of MBT<sub>5ME</sub> along the lake core ranged only between 0.36 and 0.39. The MBT<sub>5ME</sub> of 0.37 for the lake surface sediment was significantly lower than that in creek bed sediment (0.46 ± 0.02; \( p < 0.05 \); Fig. 3c; Fig. 5b). Conversely, the DC in the lake surface sediment was 0.39, which was significantly higher than that in creek bed sediment (0.23 ± 0.02; \( p < 0.05 \);
mean air temperature is (Peterse et al., 2012; Weijers et al., 2007a). However, in the small (ca. 4.8 km$^2$) Carminowe Creek catchment, the annual mean air temperature is practically the same for all soils. Similarly, the range in soil pH is relatively small among different land use types (from 5.4 ± 0.3 in woodland to 6.6 ± 0.1 in arable land; Table 1), which makes it difficult to separate brGDGTs in terrestrial environments have a relatively long turnover time (ca. 18 years in soils (Weijers et al., 2010), and up to 40 years in peat (Huguet et al., 2017)), especially when compared to the cropland rotation time. Taken together, these factors may contribute to the relatively similar brGDGT signal in all soils in the Carminowe catchment, further limiting the variation in brGDGT signals in catchment soils.

### 4 Discussion

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

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

Indeed, previous work has also shown that brGDGT distributions are not primarily affected by land use. For example, brGDGTs in soils along an altitudinal transect in the Ethiopian highlands revealed that brGDGTs mainly reflect the decrease in temperature with increasing elevation, regardless of drastic changes in land use along the transect (Jaeschke et al., 2018).

However, other studies report that vegetation cover does exert a great influence on brGDGT signatures in soils from Minnesota and Ohio, USA (Weijers et al., 2011), around Lake Rotsee, Switzerland (Naerher et al., 2014), in the Tibetan Plateau (Liang et al., 2019), and paddy and upland soils from subtropical (China and Italy) and tropical (Indonesia, Philippines and Vietnam) areas (Mueller-Niggemann et al., 2016). The explanations for the similar distribution of brGDGTs under different land use types in the Carminowe Creek catchment could be the rotation and ploughing in land use in combination with the turnover time of brGDGTs. Although the soil bacterial community composition is generally different across distinct land use types (Fierer and Jackson, 2006; Steenwerth et al., 2003), the regular rotation (generally less than 5 years) of arable land and temporary grassland (ley) in the catchment (Glendell et al., 2018) may create a mixed bacterial community under all vegetation types. Beyond vegetation, regular ploughing as applied across the Carminowe catchment soils (arable land and ley) is recognized to have a more dominant, long-last effect on microbial communities (Drenovsky et al., 2010). Moreover, brGDGTs in terrestrial environments have a relatively long turnover time (ca. 18 years in soils (Weijers et al., 2010), and up to 40 years in peat (Huguet et al., 2017)), especially when compared to the cropland rotation time. Taken together, these factors may contribute to the relatively similar brGDGT signal in all soils in the Carminowe catchment, further limiting the variation in brGDGT signals in catchment soils.

Some spatial trends are visible in spite of the overall comparable brGDGT signals across the catchment (Fig. A1), which may be explained by variations in other environmental factors than land use or vegetation. Mean air temperature and soil pH have been shown to be the main factors controlling the distribution of brGDGTs in soils worldwide (Weijers et al., 2007a; Peterse et al., 2012; De Jonge et al., 2014b). However, in the small (ca. 4.8 km$^2$) Carminowe Creek catchment, the annual mean air temperature is practically the same for all soils. Similarly, the range in soil pH is relatively small among different land use types (from 5.4 ± 0.3 in woodland to 6.6 ± 0.1 in arable land; Table 1), which makes it difficult to separate brGDGT
The higher IR in the creek bed sediments which separates the creek sediments from both the soils and lake sediments on PC2 that is associated with the IR (Fig. 3). Furthermore, brGDGTs in creek sediments have a higher IR value (i.e. 0.48 ± 0.04) than soils (30.1 ± 1.7% and 10.9 ± 0.6%, respectively; Table 1). This is likely due to the relatively minor range and variation in soil pH (from 5.4 ± 0.6 to 6.6 ± 0.1). Nevertheless, the soils with high IR values in the north catchment also have pH values > 6.0 with an average value of 6.6 ± 0.1.

4.2 Tracing brGDGTs from soils to creek bed sediments

Based on the similar brGDGT signatures for soils under different land use types, these compounds cannot be used to trace back the exact source of the soil OC after mobilisation and transport throughout the catchment. However, the concentration and general soil signature of the brGDGTs can be compared with those in creek bed sediments to trace the transfer of OC from the soils into the creeks. The C-normalized concentration of brGDGTs in the creek sediments is higher than that in most of the soils (34.7 ± 17.4 μg g⁻¹ C and 9.6 ± 4.9 μg g⁻¹ C respectively), except for those in the woodland soils at the riverbanks (37.6 ± 11.0 μg g⁻¹ C; Table 1). Thus, purely based on the concentration, this suggests that brGDGTs in the creek would be primarily derived from the woodland, which also appeared to be the main source of n-alkanes in creek bed sediment (Glendell et al., 2018). However, when looking at the relative distribution of the brGDGTs, the percentage of hexamethylated brGDGTs in creek sediments is higher than that in soils (24.9 ± 1.8% and 10.9 ± 0.3%, respectively), whereas the percentage of tetramethylated brGDGTs is lower than in soils (30.1 ± 1.7% and 39.7 ± 0.6%, respectively; Table 1). Furthermore, brGDGTs in creek sediments have a significantly higher IR (i.e. 0.48 ± 0.04) than soils under any of the land use types (0.28 ± 0.01 on average in the catchment; p < 0.05; Fig. 3e; Table 1). This is clearly reflected in the PCA, which separates the creek sediments from both the soils and lake sediments on PC2 that is associated with the IR (Fig. 4e). The higher IR in the creek bed sediments can be explained by a contribution of aquatically (i.e. in situ) produced 6-methyl...
brGDGTs. Similar contributions of 6-methyl brGDGTs, and thus higher IR, were also observed in suspended particulate matters from the Yenisei River (De Jonge et al., 2014a), and upstream of the Iron Gates in the Danube River, where the higher IR was coupled to in-river production facilitated by the lower flow velocity and decreased turbidity of the river water (Freymond et al., 2017). Hence, the significantly higher IR in combination with the higher C-normalized concentrations of brGDGTs in the Carminowe creek sediments suggests that the brGDGT signal is mainly aquatic.

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

\[
IR_{\text{creek}} = \frac{[6-\text{me creek}]}{[5-\text{me creek}] + [6-\text{me creek}]} = \frac{[6-\text{me woodland}] + [6-\text{me in situ}]}{[5-\text{me creek}] + [6-\text{me woodland}] + [6-\text{me in situ}]} \tag{8}
\]

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

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

4.3 Sources of brGDGTs in the sediments of Lake Loe Pool

In theory, rivers would transport soil-derived OC together with any aquatic OC produced along the way. Once discharged, in 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. However, the soil brGDGT signal cannot be recognized in the sediments from Loe Pool since it is already lost upon entering the Carminowe creek. Indeed, the PCA of the relative distributions of brGDGTs indicates that lake sediments plot completely separated from both the soils and creek sediments, mostly due to a higher relative abundance of GDGT–IIIa (Fig. 4a, b). As a result, the MBT S₃ME is significantly lower in Loe Pool sediments (0.38 ± 0.00) compared to in the creek bed.
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 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; $p < 0.05$; Fig. 3d; Table 1). The distinct brGDGT signature of the lake sediments suggests that brGDGTs in the lake again are significantly altered compared to those in the soils and creek sediments. This implies that the riverine brGDGT signal is either replaced or overwritten in the lake.

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

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

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

The C-normalized concentration of brGDGTs starts to increase around 23 cm, reaching a maximum concentration of 48.0 $\mu$g g$^{-1}$ C at 11 cm depth (Fig. 6b). The increased brGDGT concentrations coincide with an increase in the degree of cyclisation (Fig. 6c), which generally responds to a change in pH, where more cyclopentane moieties correspond to a higher pH (Weijers et al., 2007a; Schoon et al., 2013). According to historical records, agriculture and anthropogenic perturbations such as mining and urban pollution intensified in the 1960s (~ 26 cm depth), which increased the input of soil and nutrients into Lake Loe Pool (Coard et al., 1983), and resulted in eutrophication (i.e. blooms of cyanobacteria and algae) since at least 1986 (~ 23 cm depth) (O’Sullivan, 1992; Flory and Hawley, 1994). Earlier studies have also recognized an increased use of farmyard manures and septic tanks at this time in the nitrogen isotopic composition of the lake sediments, and have detected higher inputs of terrestrial organic material resulting from intensified farming practices and a higher erosion rate during the 1960s to 1980s based on ratios of aquatic- and terrestrial-derived plant waxes (Glendell et al., 2018). Thus, the high brGDGT
concentrations and DC in the sediments likely reflect the eutrophic conditions of the lake resulting from the increased nutrient input to the lake (Coard et al., 1983). The DC has then recorded the increase in lake water pH associated with eutrophication, whereas brGDGT concentrations express increased aquatic production. Due to remediation measures taken by the local government in 1996 (~12 cm depth), the eutrophication has reduced over the past twenty years (Glendell et al., 2018). The partial recovery of the lake has likely resulted in a return to lower lake water pH, as manifested in the decrease in the DC from ~10 cm depth upwards (Fig. 6c).

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

5 Conclusions

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

Comparison of the soil-derived brGDGT signals to that of creek bed sediments reveals that the soil brGDGT signal is almost completely overprinted by aquatically produced brGDGTs, indicated by a substantially higher fractional abundance of 6-methyl brGDGTs in the creek. Upon discharge into the lake, the creek brGDGT signal is replaced by and/or mixed with a lacustrine in situ produced brGDGT signal, which is characterized by a relatively higher DC and lower MBT5Me, as well as a specifically high fractional abundance brGDGT-IIIa. Despite regular ploughing of the land, the absence of a profound rainy season and limited relief likely limits the degree of soil mobilisation necessary to transfer the soil-derived brGDGT signal to the lake sediments in the modern system. Still, downcore variations in GDGT distributions in the sediments of Loe Pool do reflect local environmental conditions over the past 100 years. The degree of cyclisation of brGDGTs as well as the ratio of isoprenoidal GDGT-0 and crenarchaeol produced by Archaea trace the historical record of lake eutrophication induced by increased nutrient input from intensified agricultural activity in the catchment during the 1960s to 1980s, and its recovery after measures taken by the owner since 1996. Our study shows that GDGTs in sedimentary archives are good recorders of past environmental and land management (e.g. agricultural intensification, increased fertilizer use) change, although the ability of brGDGTs to trace soil OC along a soil-aquatic continuum requires a higher degree of soil mobilisation.
Data availability

All data are available in the Supplementary Information.

Author Contribution

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 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.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

This study was supported financially by NWO Veni grant #863.13.016 to Francien Peterse. Desmond Eefting and Klaas Nierop (UU) are acknowledged for technical support. Dr. R. Sparkes and anonymous reviewers are thanked for their comments, which helped to improve this manuscript.

References


Figure 1: Molecular structures of 5-methyl and 6-methyl branched GDGTs, GDGT-0 and crenarchaeol. The 6-methyl brGDGTs are represented by apostrophe. The structures of penta- and hexamethylated brGDGTs with cyclopentane moiety(ies) IIb', IIc', IIb', IIIc' are tentative.
Figure 2: Map of the Carminowe Creek catchment in southwest England showing land use types, 14 soil transects (labelled T1-14), creek bed and lake core sediment sampling locations. The coloured circles and stars indicate soil samples under different land use types and creek bed sediments along the streams, respectively. Adjusted from Glendell et al. (2018).
Figure 3: Box plots displaying (a) the C-normalized concentration of brGDGTs, and brGDGT-based proxies: (b) BIT index (branched and isoprenoid tetraether ratio), (c) MBT'_{5Me} (methylation of 5-methyl branched tetraethers), (d) DC (degree of cyclisation) and (e) IR (isomerization ratio). The triangles represent the average values, the bold line indicates the median (50th percentile), bottom and top of the box indicate first quartile (25th percentile) and third quartile (75th percentile) respectively, whiskers cover the smallest and largest value within 1.5 times of the interquartile range (i.e. the distance between the top and bottom of the box). Any data points outside the whiskers are considered as outliers. Different letters indicate differences between samples: A and B for differences between catchment soils and aquatic sediments, a and b for soils under different vegetation types, and m and n for creek bed and lake core sediments ($p < 0.05$).
Figure 4: PCA based on the relative abundances of 13 major brGDGTs. Figure (a) shows the distribution of 13 brGDGTs (brGDGT-IIIc and brGDGT-IIIc' are excluded as they are below the detection limit) along the first two PCs, roman numerals and English alphabet represent the compounds shown in Fig. 1. Figure (b) shows sampling sites loading scores on the first two PCs and 95% confidence interval ellipses surrounding the mean point of different groups of land use: arable land (n = 31), grassland (n = 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 (degree of cyclisation). Figure (d) and (e) show cross plots of PC2 with MBT'SME (methylation of 5-methyl branched tetraethers) and IR (isomerization ratio) respectively. The linear correlation was calculated excluding creek and lake sediment.
Figure 5: Spatial variability of (a) C-normalized concentration of brGDGTs and (b) MBT’\textsubscript{SME} (methylation of 5-methyl branched tetraethers) and DC (degree of cyclisation) in downstream direction of both substreams in the Carminowe Creek catchment.
Figure 6: Lake sediment core profiles of (a) $^{137}$Cs to date, (b) C-normalized concentration of brGDGTs, (c) DC (degree of cyclisation) and (d) ratio between GDGT-0 and crenarchaeol. The red dashed line indicates the year of 1963.
Appendix Fig.1: Spatial variability of the (a) BIT (branched and isoprenoid tetraether ratio) and (b) IR (isomerization ratio) along 14 soil transects in the Carminowe Creek catchment. The coloured circles show the concentrations and proxy values. Tx indicates soil transects discussed in the text. The background colours indicate different land use types. Adjusted from Glendell et al. (2018).
<table>
<thead>
<tr>
<th>Land use (n)</th>
<th>C% *</th>
<th>pH</th>
<th>Conc. (µg g⁻¹ soil)</th>
<th>Conc. (µg g⁻¹ C)</th>
<th>BIT</th>
<th>MBT'5ME</th>
<th>%tetra</th>
<th>%penta</th>
<th>%hexa</th>
<th>DC</th>
<th>IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>arable (31)</td>
<td>2.9 ± 0.5</td>
<td>6.6 ± 0.4</td>
<td>0.2 ± 0.1</td>
<td>8.1 ± 3.6</td>
<td>0.83 ± 0.09</td>
<td>0.50 ± 0.02</td>
<td>40.1 ± 3.1</td>
<td>49.7 ± 1.5</td>
<td>10.2 ± 1.8</td>
<td>0.25 ± 0.11</td>
<td>0.31 ± 0.10</td>
</tr>
<tr>
<td>grass (14)</td>
<td>5.6 ± 1.2</td>
<td>6.0 ± 0.5</td>
<td>0.6 ± 0.4</td>
<td>11.2 ± 6.7</td>
<td>0.88 ± 0.14</td>
<td>0.48 ± 0.04</td>
<td>39.8 ± 4.0</td>
<td>49.4 ± 2.3</td>
<td>10.8 ± 2.1</td>
<td>0.21 ± 0.14</td>
<td>0.26 ± 0.12</td>
</tr>
<tr>
<td>ley (24)</td>
<td>3.6 ± 0.9</td>
<td>6.0 ± 0.3</td>
<td>0.4 ± 0.3</td>
<td>10.5 ± 4.8</td>
<td>0.90 ± 0.12</td>
<td>0.46 ± 0.04</td>
<td>37.8 ± 3.7</td>
<td>50.2 ± 2.0</td>
<td>12.0 ± 2.9</td>
<td>0.23 ± 0.14</td>
<td>0.27 ± 0.13</td>
</tr>
<tr>
<td>woodland (5)</td>
<td>8.2 ± 2.1</td>
<td>5.4 ± 0.7</td>
<td>3.0 ± 1.0</td>
<td>37.6 ± 11.0</td>
<td>0.96 ± 0.03</td>
<td>0.50 ± 0.12</td>
<td>45.4 ± 13.0</td>
<td>44.4 ± 8.3</td>
<td>10.3 ± 4.8</td>
<td>0.22 ± 0.12</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>all soils (74)</td>
<td>4.0 ± 1.8</td>
<td>6.2 ± 0.5</td>
<td>0.6 ± 0.8</td>
<td>11.5 ± 8.9</td>
<td>0.87 ± 0.12</td>
<td>0.48 ± 0.04</td>
<td>39.7 ± 4.9</td>
<td>49.4 ± 3.0</td>
<td>10.9 ± 2.6</td>
<td>0.23 ± 0.13</td>
<td>0.28 ± 0.11</td>
</tr>
<tr>
<td>creek (7)</td>
<td>2.3 ± 0.8</td>
<td>7.1 ± 0.2</td>
<td>0.8 ± 0.4</td>
<td>34.7 ± 17.4</td>
<td>0.90 ± 0.06</td>
<td>0.46 ± 0.02</td>
<td>30.1 ± 4.5</td>
<td>45.0 ± 0.7</td>
<td>24.9 ± 4.7</td>
<td>0.23 ± 0.02</td>
<td>0.48 ± 0.10</td>
</tr>
<tr>
<td>Lake (25)</td>
<td>7.5 ± 1.0</td>
<td>5.7 ± 0.2</td>
<td>2.6 ± 0.7</td>
<td>34.0 ± 8.7</td>
<td>0.96 ± 0.01</td>
<td>0.38 ± 0.01</td>
<td>28.9 ± 0.7</td>
<td>50.2 ± 1.8</td>
<td>21.0 ± 1.4</td>
<td>0.32 ± 0.08</td>
<td>0.32 ± 0.01</td>
</tr>
</tbody>
</table>

*From Glenell 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).

<table>
<thead>
<tr>
<th>BIT</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>T8</th>
<th>North catchment</th>
<th>South catchment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T9</td>
<td>T10</td>
</tr>
<tr>
<td>hilltop</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>0.65</td>
<td>0.66</td>
<td>0.77</td>
<td>0.97</td>
<td>0.99</td>
<td>0.97</td>
<td>0.77</td>
<td>0.58</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>S2</td>
<td>0.57</td>
<td>0.66</td>
<td>0.86</td>
<td>0.99</td>
<td>0.99</td>
<td>0.93</td>
<td>0.65</td>
<td>0.59</td>
<td>0.91</td>
<td>0.63</td>
</tr>
<tr>
<td>S3</td>
<td>0.73</td>
<td>0.77</td>
<td>0.87</td>
<td>1.00</td>
<td>1.00</td>
<td>0.94</td>
<td>0.82</td>
<td>0.80</td>
<td>0.98</td>
<td>0.90</td>
</tr>
<tr>
<td>S4</td>
<td>0.88</td>
<td>0.83</td>
<td>0.96</td>
<td>0.99</td>
<td>-</td>
<td>0.96</td>
<td>0.70</td>
<td>0.85</td>
<td>1.00</td>
<td>0.92</td>
</tr>
<tr>
<td>S5</td>
<td>0.95</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.97</td>
<td>0.76</td>
<td>0.98</td>
<td>1.00</td>
<td>0.91</td>
</tr>
<tr>
<td>S6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.96</td>
<td>0.94</td>
<td>0.97</td>
<td>1.00</td>
<td>0.93</td>
</tr>
<tr>
<td>S7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.81</td>
<td>-</td>
<td>0.95</td>
<td>-</td>
</tr>
<tr>
<td>downslope</td>
<td>S8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.92</td>
</tr>
</tbody>
</table>
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 Al₂O₃ 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 normalize 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/CO2 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$_{86}$ 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:


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-diabilic acid), a common membrane-spanning lipid of Acidobacteria subdivisions 1 and 3. Applied and Environmental Microbiology 77, 4147-4154.


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


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.
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 they 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.
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).

<table>
<thead>
<tr>
<th>Transect</th>
<th>North Catchment</th>
<th>South Catchment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BIT</td>
<td>North Catchment</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>hilltop</td>
<td>S1</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>S5</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>S6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S7</td>
<td>-</td>
</tr>
<tr>
<td>downslope</td>
<td>S8</td>
<td>-</td>
</tr>
</tbody>
</table>

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 ± 0.1 °C vs the locally historical recorded temperature of 10.9 ± 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.
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.

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


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.