



Contrasting distributions of glycerol dialkyl glycerol tetraethers (GDGTs) in speleothems and associated soils



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ABSTRACT

Glycerol dialkyl glycerol tetraethers (GDGTs) preserved in speleothems can form useful records of terrestrial palaeotemperature. However, understanding of the sources of these compounds in caves is limited, particularly whether or not they should be considered as an in situ signal derived from microbial communities in the cave or vadose zone, a transported soil signal, or a mixture of the two. We have analysed speleothem samples and related soils from five cave sites and demonstrate that clear differences were apparent between soils and speleothems in GDGT distributions. Speleothems were primarily, but not uniformly, dominated by crenarchaeol, reflected in the branched and isoprenoid tetraether (BIT) index values, and had a lower relative abundance of the crenarchaeol regioisomer than soils. The most distinct differences were in the bacterially derived branched GDGTs, where no relationship was seen between speleothems and soils for the cyclisation of branched tetraethers (CBT) index, with speleothems in four out of five caves showing a greater degree of cyclisation in GDGT structures than could be explained by measured pH values. Differences in speleothem GDGT composition between sites were also seen. We suggest that the speleothem GDGT record is distinct from the GDGT distribution produced in soils, and is primarily derived from in situ microbial communities within the cave or vadose zone. Variation within these communities or in the cave microenvironment also acts to produce site-specific differences.

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1. Introduction

Understanding past changes in the terrestrial environment, in particular identifying local and regional changes in continental temperature and the associated environmental response, is vital in understanding how the world will change in future. Speleothems (chemically precipitated cave deposits) are particularly well placed to provide such integrated terrestrial palaeoenvironmental records. They can be robustly dated and contain a wealth of chemical signals, reflecting climate, e.g. stable oxygen isotopes reflecting rainfall and fluctuations in global climate systems (e.g. McDermott, 2004; Lachniet, 2009), vegetation, e.g. stable carbon isotopes of both the calcite and organic matter (e.g. Genty et al., 2003; Blyth et al., 2013), lipid biomarkers (e.g. Xie et al., 2003; Blyth et al., 2007, 2011) and lignin (Blyth and Watson, 2009). Recent work has demonstrated that glycerol dialkyl glycerol tetraethers

(GDGTs), compounds whose structure and composition in sedimentary records relate to environmental parameters and, in particular, temperature (Schouten et al., 2013), are present in speleothems at recoverable levels (Yang et al., 2011; Blyth and Schouten, 2013). Two types of temperature proxy have been proposed using GDGTs, one using isoprenoid(i) GDGTs (Fig. 1) derived from aquatic archaea (e.g. TEX₈₆ (tetraether index of tetraethers consisting of 86 carbons; Schouten et al., 2002) and one using branched (br) GDGTs (Fig. 1) derived from bacteria in soils and other terrestrial environments (e.g. MBT/CBT (methylation of branched tetraethers, and cyclisation of branched tetraethers; Weijers et al., 2007). Generally, TEX₈₆ has been applied to aquatic, in particular marine, settings, whilst the br GDGTs have been associated with the terrestrial environment (reviewed by Schouten et al., 2013). For speleothems it has been shown that indices based on both br and i compound groups have a clear relationship with temperature (Blyth and Schouten, 2013). The use of a geographically diverse sample set to correlate speleothem GDGT composition with surface air temperature provided two

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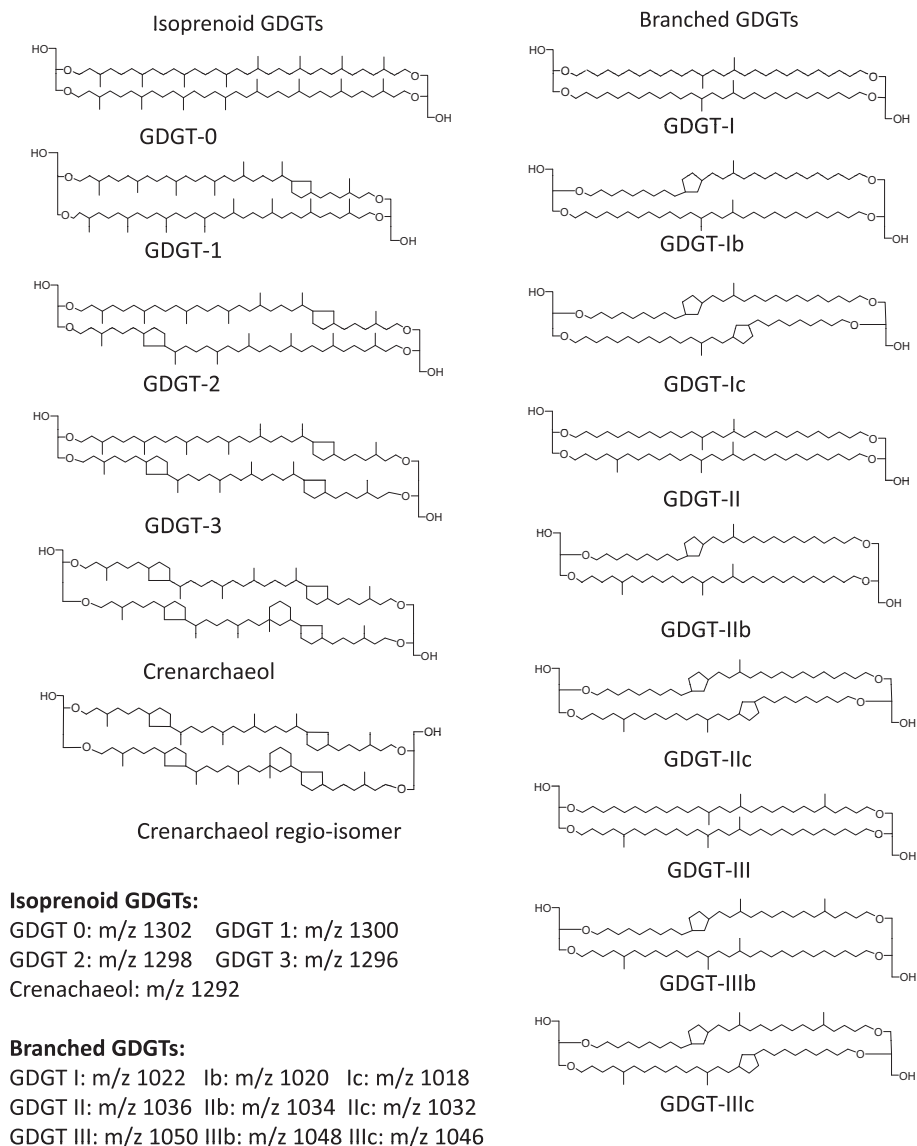


Fig. 1. Structures for the i and br GDGTs.

speleothem-specific calibration equations (Blyth and Schouten, 2013), one for TEX_{86} (r^2 0.78, standard error of estimate ± 2.3 °C) and one for MBT/CBT (r^2 0.73, standard error of estimate ± 2.7 °C). It is therefore clear that speleothems have the potential to provide GDGT based palaeotemperature records.

A complicating factor identified by both Yang et al. (2011) and Blyth and Schouten (2013), is the difficulty in identifying the source environment of the GDGTs, with potential contributions from both in situ input from microbial communities in the cave and within the vadose zone of the overlying bedrock, and allochthonous input transported from the soil via infiltrating groundwater. The issue is of importance because the source of the compounds dictates which modern temperature measurements should be used in future calibrations. If the compounds are primarily cave derived, then the optimal calibration should be based on measured cave temperature values. If they are soil derived, then they should be based on modern surface or soil temperature values. At present, the published calibration equations are based on surface air temperature as values were available for the largest data set, and mean annual surface temperature and cave air temperature are considered to form a reasonable if not perfect approximation. However, if the compounds could be shown to be primarily in situ cave

derived, then there would be a strong case for significantly expanding the data set of available sites where modern calcite and accurately measured cave temperature values can be obtained. Additionally, understanding of the more subtle relationships between the distributions of GDGTs and environmental parameters is constantly evolving as increasing numbers of studies are undertaken (e.g. Xie et al., 2012; Dirghangi et al., 2013; Huguet et al., 2013). Increasing understanding of GDGT production in cave and vadose zone environments and microenvironments should add to the sum of this knowledge, especially if later combined with appropriate microbiological research.

Clues about the origin of GDGTs in speleothems can be identified on the basis of the composition of the GDGT signal. Blyth and Schouten (2013) found that in most, but not all, samples, the speleothem GDGT signal was dominated by crenarchaeol, a specific biomarker lipid for Thaumarchaeota, whose presence in caves has been noted in DNA studies (Gonzalez et al., 2006). Br GDGTs formed a relatively minor component, in contrast to the distribution in most soils (Weijers et al., 2006; Schouten et al., 2013). Similarly, Yang et al. (2011) analysed soil, drip water and cave calcite samples from Heshang Cave in China and found the cave signal (including speleothems and surface cave bedrock samples) to be

dominated by archaeal i GDGTs, while the soil was dominated by bacterially derived br GDGTs. Additionally, the internal composition of the i and br groups differed markedly between the soils and the calcite, lending credence to the idea of predominantly in situ GDGT production. However, to test the hypothesis of cave derived GDGTs more fully, it is necessary to look at paired soil and calcite samples from a broader range of geographical locations.

Here we have analysed the GDGTs in soils from above five caves in the UK and Australia, with a surface mean annual air temperature (MAT) range of 9–16 °C and surface mean annual precipitation (MAP) range of 617–1300 mm (Pooles Cavern, UK; Lower Balls Mine, UK; Wombeyan Caves, New South Wales, Australia; Gaden and Cathedral Caves, Wellington cave system, New South Wales, Australia). At least one speleothem from each of these caves has been previously analysed and included in the Blyth and Schouten (2013) calibrations and the speleothems show a range of BIT (branched and isoprenoid tetraether index) values (0.05–0.69), indicating a varying degree of branched or isoprenoid compound dominance.

2. Material and method

2.1. Sites and samples

Table 1 lists the locations and environmental parameters for the five cave sites: Poole's Cavern (Derbyshire, UK) a shallow cave formed in Lower Carboniferous limestone, and overlain by woodland formed on abandoned lime kilns; Lower Balls Mine (Wiltshire, UK) a now abandoned limestone mine sunk into Middle Jurassic

Oolites and overlain by agricultural pasture (lower mine) and woodland (upper mine), with carbonaceous clayey soils; Wombeyan Caves Reserve (New South Wales, Australia), a highly developed karst system formed in the high purity Wombeyan Marble unit in the Great Dividing Range, southwest of Sydney; and two caves at Wellington Caves Reserve (New South Wales, Australia), formed in the mixed thinly bedded and massive limestones of the Early Devonian Garra Formation. Speleothem GDGT data for these sites are taken from the sample set analysed by Blyth and Schouten (2013) and these sites were chosen in part because the speleothems are some of the guaranteed youngest in the sample set, providing closest comparability with the newly collected soils. The sample from Poole's Cavern was taken from regrowth on a stalagmite boss sampled in the late 1990s. At Wellington the samples were recently formed drip-straws and flowstones formed on man-made artefacts, and at Lower Balls Mine (LBM), where the speleothems are known to have a maximum age of 100 yr dating from the mine abandonment, the samples were thin and actively forming at collection. The sample from Wombeyan encompasses the last 40 yr.

At each site, a minimum of two soil samples was taken. Where contrasting vegetation or soil regimes were present over the cave (e.g. at LBM, where both woodland and agricultural grassland were present, and Pooles Cavern, where there was both a natural soil and soil developed over lime waste), a sample was taken from each regime. At all sampling locations, the soil profile was thin and the sample encompassed the whole available depth before the sampler hit either bedrock or rubble. All soils were analysed in replicate to take account of natural small scale heterogeneity.

Table 1
Location and environmental details for samples.

Sample	Type	Location	Soil pH	Drip water pH ^b	Surface MAT °C ^c	MAP mm ^d
PE-1 ^a	Stalagmite	Pooles Cavern, England	–	11.7 ± 0.4		
PC-soil-1	Soil (top 10 cm)	Pooles, natural soil above cave, adjacent to lime spoil heap	6.4	–	9	1300
PC-soil-2	Soil (top 10 cm)	Pooles, soil from lime soil heap above cave	7.8	–		
LBM-S2	Stalagmites	Lower Balls Mine (LBM), England	–	8	10	
LBM-S3				8		
LBM-soil-1	Soil (top 10 cm)	LBM, thin soil under light woodland, over limestone. Outside upper entrance to mine	7.6	–		995
LBM-soil-2	Soil (top 10 cm)	LBM, soil under agricultural grassland above mine, halfway between upper and lower entrances	7.5	–		
WM-4	Stalagmite	Wombeyan Caves, New South Wales, Australia	–	7.6 ± 0.4	13.7	804
WB-soil-1a	Soil (0–2 cm)	Wombeyan, above caves, very thin soil under open woodland	8.0	–		
WB-soil-1b	Soil (2–5 cm)		8.2	–		
WB-soil-2a	Soil (0–2 cm)		–	–		
WB-soil-2b	Soil (2–5 cm)		8.0	–		
Wel-C-1	Straw	Cathedral Cave, Wellington, NSW, Australia	–	7.7 ± 0.5		
Wel-C-2	Flowstone			7.7 ± 0.5		
Wel-C-3	Flowstone on bottle			7.7 ± 0.5		
Cat-soil-1	Soil (top 20 cm)	Wellington, above Cathedral Cave, degraded box grass woodland, with bare soil and sparse tree cover	7.5	–	16	617
Cat-soil-2	Soil (top 20 cm)		7.3	–		
Wel-G-1	Straw	Gaden Cave, Wellington NSW, Australia	–	7.7 ± 0.5		
Gad-soil-1	Soil (top 20 cm)	Wellington, above Gaden Cave, box grass woodland, not degraded	7.3	–		
Gad-soil-2	Soil (top 20 cm)		7.8	–		

^a PC-1 in Blyth and Schouten (2013).

^b Drip water pH taken from: Poole's Cavern, Hartland et al. (2011); LBM, I. Fairchild personal communication; Wombeyan, McDonald et al. (2007); Wellington, Martin Andersen, Nerilee Edwards personal communication.

^c Surface MAT as reported by Blyth and Schouten (2013).

^d Surface mean annual rainfall: Poole's Cavern and LBM, Hartland et al. (2012); Wellington and Wombeyan data from the Australian Government Bureau of Meteorology.

2.2. Extraction

Speleothem samples were processed via acid digestion and liquid/liquid extraction, as described by Blyth and Schouten (2013). Soil samples were freeze-dried and aliquots of 1–10 g were crushed in a pestle and mortar. Samples from Pooles Cavern and LBM were extracted using 9:1 (*v/v*) dichloromethane (DCM)/MeOH, at high temperature (100 °C) and pressure (7.6×10^6 Pa) with a Dionex Accelerated Solvent Extractor (ASE) at NIOZ, while samples from Wombeyan and Wellington were extracted using a Dionex 150 ASE following the NIOZ methods at UNSW. The extracts were dried under N_2 , diluted in DCM and separated into non-polar and polar fractions over activated Al_2O_3 , eluting with DCM and 1:1 DCM/MeOH respectively. Samples Gad-soil-1 and Cat-soil-1 from above Gaden and Cathedral caves at Wellington were pre-filtered over dry $MgSO_4$ and cleaned cotton wool to remove excess particulates that otherwise blocked the Al_2O_3 column. The polar fraction was dried under N_2 , rediluted in 99:1 (*v/v*) hexane/propanol and filtered through a 0.45 μm PTFE filter (ϕ 4 mm).

Soil pH was measured at NIOZ (LBM and Poole's Cavern), and UNSW (Wombeyan and Wellington). Briefly, an aliquot of crushed dry soil was suspended in deionised water at a ratio of 1 g soil:2.5 ml water, agitated for 5 min and allowed to settle for 10 min. The pH was then measured using a calibrated probe (2 point calibration, standard solutions of pH 4 and 7) suspended in solution just above the surface of the soil. Measurements were performed in triplicate and averaged for each soil sample. WB-soil-2a was excluded from pH measurement due to lack of sample.

2.3. GDGT analysis

All analyses were undertaken at NIOZ in order to provide consistency with previous speleothem analyses using the same analytical method as Blyth and Schouten (2013). Polar fractions were analysed for GDGTs using high performance liquid chromatography-atmospheric pressure positive ion chemical ionization-mass spectrometry (HPLC-APCI-MS) following Schouten et al. (2007).

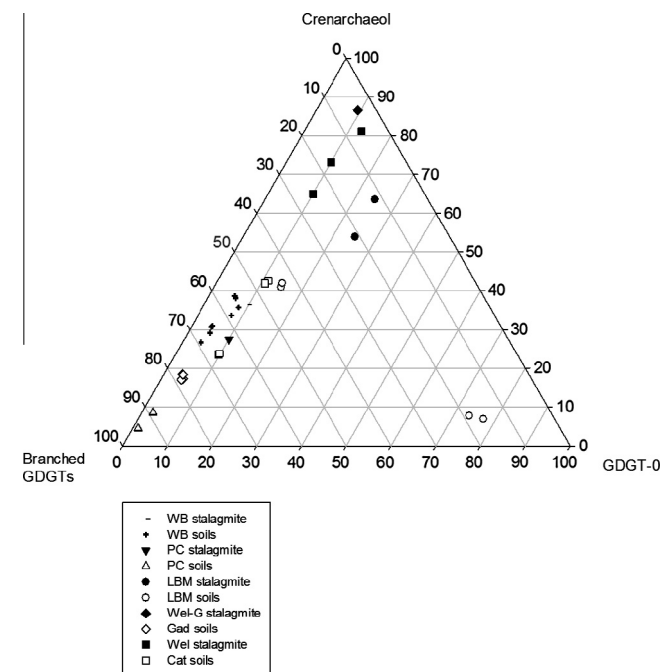


Fig. 2. Ternary plot of relative abundances of GDGT 0, crenarchaeol and summed branched GDGTs (GDGT I, II, III).

HPLC-APCI-MS used an Agilent 1100 series LC with a Prevail Cyano column (2.1×150 mm, 3 μm ; Alltech) at 30 °C. GDGTs were eluted using a changing mixture of hexane and propanol as follows: 99%

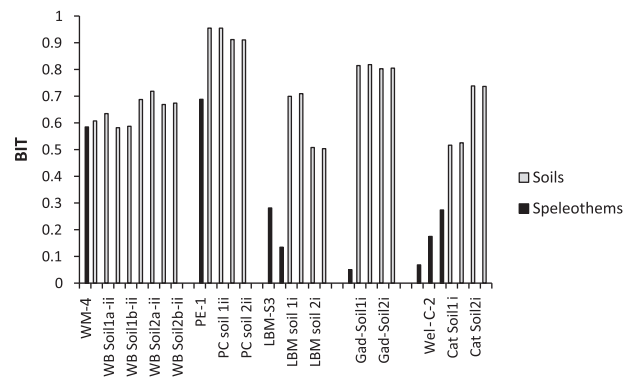


Fig. 3. BIT index for the speleothem and soil samples. No relationship is apparent between the soil and speleothem values for each site.

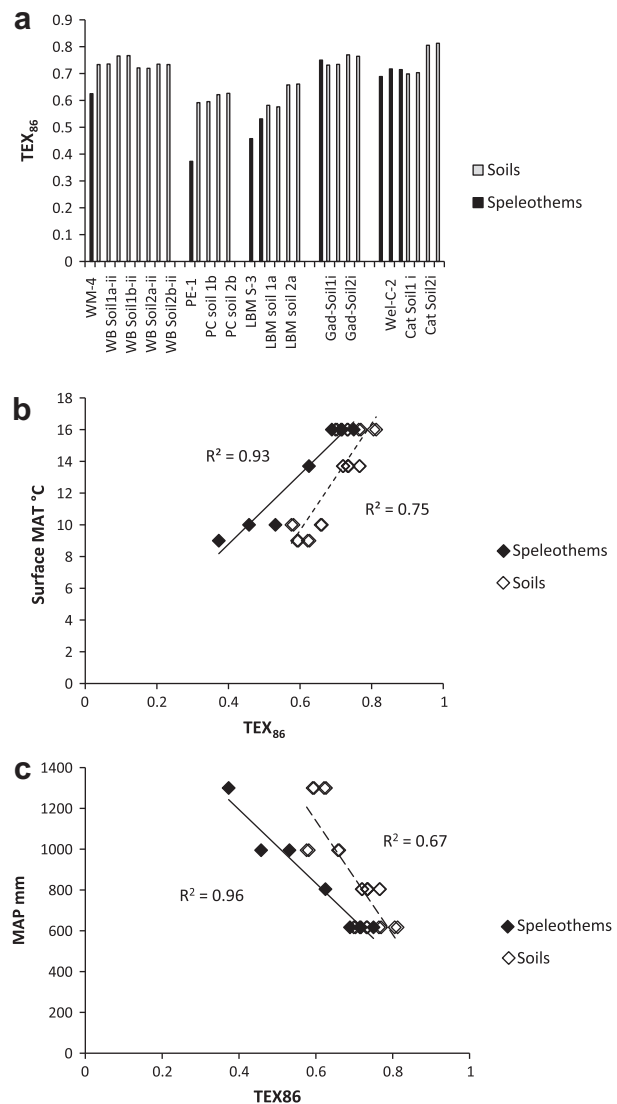


Fig. 4. (a) TEX₈₆ in speleothem and soil samples, (b) relationship between TEX₈₆ and surface MAT and (c) the relationship between TEX₈₆ and surface MAP in the speleothems and soils respectively.

hexane/1% propanol (5 min), then a linear gradient to 1.8% propanol in 45 min. Flow rate was 0.2 ml/min. Single ion monitoring was set to scan the $[M + H]^+$ ions of the GDGTs with a dwell time of 237 ms for each ion. Only peaks with areas > 5000 were considered as being above the limit of quantitation (c.f. Schouten et al., 2007).

The following ratios were calculated (cren = crenarchaeol; cren' = crenarchaeol regio isomer):

BIT index (Hopmans et al., 2004),

$$\text{BIT} = (\text{III} + \text{II} + \text{I}) / (\text{Cren} + \text{III} + \text{II} + \text{I}) \quad (1)$$

TEX₈₆ (Schouten et al., 2002),

$$\text{TEX}_{86} = (2 + 3 + \text{Cren}') / (1 + 2 + 3 + \text{Cren}') \quad (2)$$

MBT (Weijers et al., 2007),

$$\text{MBT} = (\text{I} + \text{Ib} + \text{Ic}) / (\text{I} + \text{Ib} + \text{Ic} + \text{II} + \text{IIb} + \text{IIc} + \text{III} + \text{IIIb} + \text{IIIc}) \quad (3)$$

CBT (Weijers et al., 2007),

$$\text{CBT} = -\text{Log}[(\text{Ib} + \text{IIb}) / (\text{I} + \text{II})] \quad (4)$$

Degree of cyclisation of branched tetraethers (closely related to CBT),

$$\text{DC} = (\text{Ib} + \text{Ic} + \text{IIb} + \text{IIc}) / (2 \times \text{I} + 2 \times \text{II}) \quad (5)$$

pH from CBT (Weijers et al., 2007)

$$\text{Calculated pH} = (3.33 - \text{CBT}) / 0.38 \quad (6)$$

3. Results and discussion

3.1. GDGT composition

All samples, with the exception of speleothem LBM-S3, contained archaeal GDGTs 0, 1, 2, 3, crenarchaeol and the regio isomer of crenarchaeol. LBM-S3 contained all the above except for the regio isomer, which was below detection limit. For the br GDGTs, speleothem LBM-S3 was removed from the data set due to compound abundance being below detection limit. All the other samples contained GDGT I, Ib, II, IIb, IIc and III. GDGT Ic occurred in all samples except for speleothems LBM-S2 and PE-1. GDGT IIIb was detected in all samples except speleothem Wel-G-1, while GDGT IIIc occurred in all speleothem and soil samples from Poole's

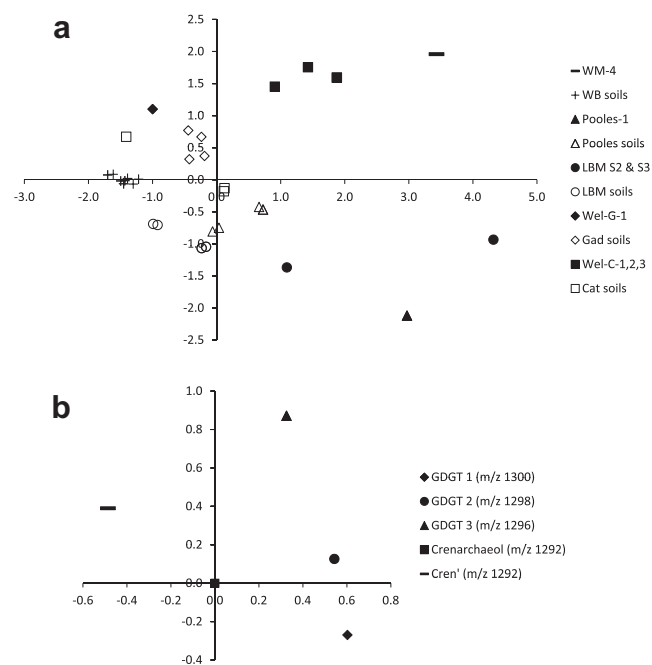


Fig. 5. (a) PCA scores plot for i GDGTs showing separation of samples on two components, (b) PCA plot showing loadings for isoprenoid compounds. This analysis was run with GDGT 0 excluded due to distorting methanogenic input to LBM soils.

Cavern and LBM in the UK, but was only seen in two Australian samples – a single soil replicate from Wombeyan (WB-soil-1bi) and speleothem Wel-C-2.

3.2. Variation in GDGT distribution between soils and speleothems

Fig. 2 shows a ternary plot of crenarchaeol, GDGT 0 and the combined br GDGTs (I, II, III). Crenarchaeol is indicative of Thaumarchaeota, while GDGT 0 (also known as caldarchaeol) can be derived from Euryarchaeota, including methanogenic archaea, Crenarchaeota and Thaumarchaeota. A ratio value of GDGT 0 to crenarchaeol > 2 has been proposed as a marker for methanogenic input (Blaga et al., 2009). In the majority of samples crenarchaeol

Table 2

Relative abundance of i GDGTs, normalised to total i GDGTs and to total i GDGTs excluding GDGT 0 (speleothem samples are marked in italics).

Sample	Isoprenoid GDGTs (%)						Isoprenoid GDGTs (%;GDGT 0 excluded)					
	GDGT 0	GDGT 1	GDGT 2	GDGT 3	Cren	Cren isomer	GDGT 1	GDGT 2	GDGT 3	Cren	Cren isomer	
<i>PE-1</i>	17.7	21.4	10.2	2.0	48.1	0.6	26.0	12.4	2.4	58.5	0.7	
PC-soil-1	16.9	9.5	7.6	3.9	59.8	2.4	11.4	9.1	4.7	71.9	2.9	
PC-soil-2	16.8	10.1	9.8	4.7	56.5	2.2	12.1	11.7	5.7	67.9	2.6	
<i>LBM-S2</i>	24.4	12.6	7.2	3.4	52.4	0.0	16.6	9.6	4.5	69.3	0.0	
<i>LBM-S3</i>	17.1	18.1	16.0	3.7	44.2	0.8	21.9	19.3	4.5	53.4	1.0	
LBM-soil-1	88.5	1.2	1.0	0.4	8.6	0.3	10.8	8.7	3.8	74.3	2.4	
LBM-soil-2	21.7	6.2	5.3	3.4	60.2	3.2	7.9	6.8	4.4	76.8	4.1	
WM-4	12.8	14.9	11.4	12.2	47.6	1.3	17.1	13.0	13.9	54.5	1.4	
WB-soil-1a	14.0	6.3	6.5	4.0	62.4	6.8	7.3	7.5	4.7	72.5	7.9	
WB-soil-1b	10.2	5.7	6.6	4.2	65.4	7.9	6.3	7.3	4.7	72.9	8.8	
WB-soil-2a	10.4	7.3	7.4	3.8	63.3	7.7	8.2	8.2	4.2	70.7	8.6	
WB-soil-2b	9.5	7.0	7.6	3.7	64.0	8.1	7.8	8.4	4.1	70.8	8.9	
<i>Wel-C-1</i>	8.9	11.0	10.7	11.9	55.7	1.8	12.1	11.8	13.1	61.1	2.0	
<i>Wel-C-2</i>	8.0	9.6	9.8	10.8	58.2	3.7	10.4	10.7	11.7	63.2	4.0	
<i>Wel-C-3</i>	8.8	9.9	10.1	12.1	56.6	2.6	10.9	11.0	13.3	62.0	2.9	
Cat-soil-1	15.0	8.3	10.9	4.1	57.2	4.5	9.8	12.8	4.8	67.4	5.2	
Cat-soil-2	22.1	4.7	7.7	4.3	53.3	7.9	6.0	9.8	5.6	68.4	10.1	
<i>Wel-G-1</i>	6.9	7.3	6.0	8.7	63.8	7.3	7.9	6.5	9.4	68.5	7.8	
Gad-soil-1	15.4	7.8	10.4	4.5	55.5	6.5	9.2	12.3	5.3	65.6	7.6	
Gad-soil-2	13.0	7.2	11.2	5.3	56.2	7.2	8.3	12.8	6.0	64.6	8.3	

was consistently the dominant isoprenoid compound. The only exception was LBM-soil-1 where there was a high relative abundance of GDGT 0. LBM-soil-1 had a GDGT 0/cren ratio of 9–11, in comparison with values of 0.1–0.5 for all the other soils as well as the speleothems. Similarly low values were reported in other speleothems (Blyth and Schouten, 2013). This confirmed that LBM-soil-1 was an outlier, with an abnormally high GDGT-0 input, presumably due to highly localised methanogenic activity. Yang et al. (2012) proposed an increase in GDGT 0 as a response to higher pH, but no relationship between measured pH and relative abundance of GDGT 0 was seen in the data here (r^2 0.00, data not shown), although it is worth noting the range of measured pH was relatively limited.

The BIT index was originally designed to compare the input of bacterially derived br GDGTs against crenarchaeol derived from Thaumarchaeota as a proxy for soil input to marine environments (Hopmans et al., 2004). Here, we use it as a measure to compare the distribution of GDGTs in soils with that in speleothems. At Poole's Cavern, LBM and both Wellington sites, the speleothem BIT values were clearly lower than those for the corresponding soils, indicating lower comparative abundances of the br tetraethers (Fig. 3). At Wombeyan, the difference was less marked, with WB-soil-1 in particular being very similar to the underlying speleothems. Recent studies have suggested that BIT values for soils

may be affected by both pH and moisture, with more alkaline soils and drier soils showing lower values (Dirghangi et al., 2013; Yang et al., 2012). This has also been reflected in a broader i/br GDGT index using all GDGTs (Xie et al., 2012); however, no meaningful relationship was seen with any measured environmental parameter to explain the variation in this limited data set (pH r^2 0.01, p 0.94; surface MAP r^2 0.16, p 0.05; surface MAT r^2 0.13, p 0.13; data not shown).

Interestingly, whilst br GDGTs were dominant in all the soils, the ternary plot and BIT values show that they also dominated in two speleothems – Pooles-1 and WM-4. The results suggest that, as indicated by the BIT results of Blyth and Schouten (2013), the crenarchaeol dominance seen by Yang et al., (2011) is site specific and that the relative proportion of the two groups of GDGTs in the speleothem bears no obvious relationship with that in the associated soils – e.g. the soil BIT values at Gaden Cave, Wellington were the second highest, whilst the underlying speleothem BIT value was the lowest measured.

3.3. Variation in relative composition of isoprenoid tetraethers

To investigate the variation in compound relative abundance in the i GDGTs, two measures were considered, TEX_{86} , and a principal components analysis (PCA) of the full compound distribution. For

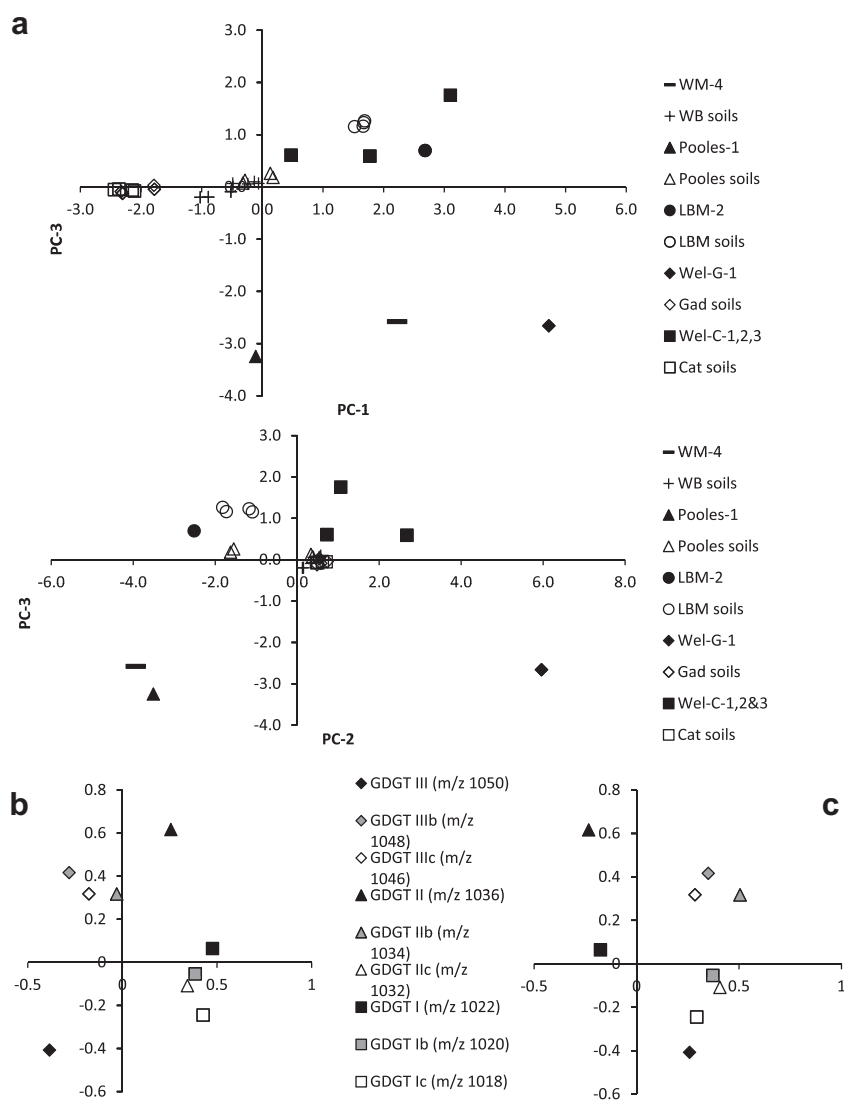


Fig. 6. (a) PCA scores plots for a 3 component model for branched GDGTs, (b) loadings plot of PC-1 vs. PC-3, (c) loadings plot for PC-2 vs. PC-3.

Wombeyan, Poole's Cavern and LBM, the speleothems showed a lower TEX_{86} value than the soils, while the samples from both Wellington caves were approximately in the same range as their associated soils (Fig. 4). The lower speleothem TEX_{86} values at Wombeyan, LBM and Poole's were primarily driven by a lower relative abundance of the crenarchaeol regio isomer (Table 2). A recent study of soil dwelling Thaumarchaeota showed that this isomer is produced in significant quantities in soils only where the I.1b subgroup of Thaumarchaeota are present (Sinninghe Damsté et al., 2012), suggesting that the difference seen here may reflect differences in the types of archaeal communities present in some caves. Future microbiological and genetic studies are required to confirm this. However, despite the differences, both the speleothem and soil sample sets showed good correlation between TEX_{86} and surface MAT (Fig. 4b; r^2 0.93, $p < 0.0001$ and r^2 0.75, $p < 0.0001$, respectively), the soil data set showing higher TEX_{86} values, particularly at lower temperature. Similar inverse correlations were seen between TEX_{86} and surface MAP (Fig. 4c; speleothems, r^2 0.96, $p < 0.0001$; soils, r^2 0.67, $p < 0.0001$); however, as there is a clear inverse relationship between temperature and rainfall at these sites, this would be expected, and cannot be used to further extrapolate the role of rainfall in GDGT distribution.

Two PCAs were run, one including all the i GDGTs, and one excluding GDGT 0 to avoid distortion from the LBM soil outliers for this compound. Both indicate that the variation within the data could be explained by a simple two component model (eigenvalues > 1) and in both cases the speleothems were separated from the soils. The loadings plots indicate that this is a result of differences in the relative abundances of the crenarchaeol regio isomer (PC-1) and of GDGTs 1, 2, and 3 (PC-2). Fig. 5 shows the PCA excluding GDGT-0. The soils generally cluster around the origin, with a tendency to score negatively on PC-1, while most of the speleothems score positively on PC-1, but are split into two groups by PC-2. The exception is Wel-G-1 which clusters with the soils from that site. The division of the speleothems in PC-2 is driven by GDGTs 1, 2 and 3, with PE-1 and the LBM speleothems having a higher relative abundance of GDGT-1 and a lower relative abundance of GDGT 3. This is not simply driven by the differences in MAT between the UK and Australian sites since, using the Blyth and Schouten (2013) calibration equations, LBM S-2 and S-3, WM-4, and all Wellington speleothems showed TEX_{86} derived temperatures within the error of the calibration (generally within 1 °C of measured), while PE-1 underestimated the temperature by > 4 °C. Collectively, the distribution of the isoprenoid compounds indicates that speleothems and soils were generally distinct, possibly due to the types of Thaumarchaeota in the microbial community, but that there was an overall response to temperature, with some variation between different cave sites.

3.4. Variation in relative composition of branched tetraethers

Fig. 6 shows the scores and loadings plots for a PCA based on the relative abundances of the br GDGTs. The variation is explained by a three component model (eigenvalues > 1) and, although the PCA did not show very distinct relationships between the compounds and groups of samples, it is clear from the loadings plots that certain compounds grouped consistently as might be expected (e.g. I and II; Ib and Ic; IIIb, and IIIc) and that some compounds did influence certain sample scores (e.g. the score for WM-4 appeared to have a consistent relationship with GDGT III). Some consistent trends can also be seen in the grouping of soils and speleothems. All the Australian soils and Pooles-soil-1 clustered together on PC-2 and 3. On PC-1 there was some separation between the Wellington soils, and the Wombeyan soils, the latter of which clustered with Pooles-soil-1. However, they all had negative scores compared with the speleothems. Only the LBM soils clustered

differently, having positive scores on PC1 and 3, and slightly negative on PC-2. The speleothems were distinct from the soils (with the exception of the soils from LBM), being largely positive on PC-1. However, they showed much greater scatter, indicating variable relationships with different compounds. As GDGT IIIc was only present at two sites, a second PCA was run with this compound removed, but the results were broadly the same.

To investigate the role of cyclisation and degree of methyl branching in distinguishing between samples, Figs. 7 and 8 show plots of the MBT index (the degree of methylation, believed to be influenced by pH and temperature; Weijers et al., 2007) and the CBT and DC ratios, depicting the degree of cyclization (influenced by pH). MBT, as defined by Peterse et al. (2012), excluding IIIb and IIIc, was calculated for the sample set but, as the resulting values were within 0.01 of MBT, we used the Weijers et al. (2007) equation to maintain consistency with Blyth and Schouten (2013).

The results show that the speleothems at LBM and Cathedral Cave, Wellington were within the same range of MBT values as their overlying soils, but that at Wombeyan Caves, the speleothem had a lower MBT (e.g. a greater relative abundance of br GDGTs) and at Gaden Cave, Wellington, Wel-G-1 had a distinctly higher MBT than related soils. At Poole's Cavern, the speleothem was

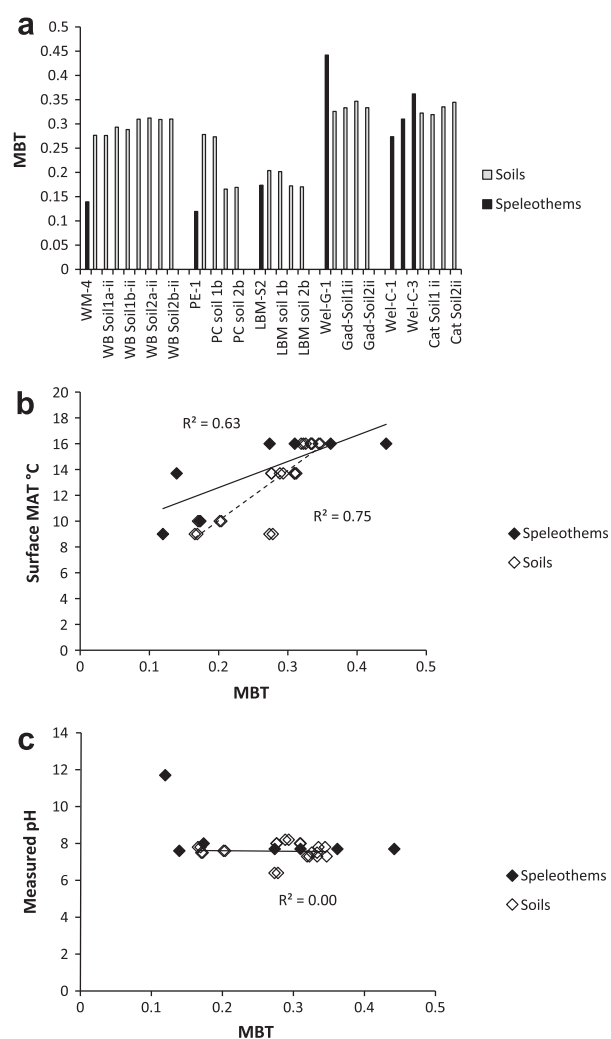


Fig. 7. (a) MBT for speleothem and soil samples, (b) relationships in the two groups between MBT and MAT, showing a stronger correlation for the soil data set, (c) relationship between MBT and pH showing no correlation. The regression line for the speleothems was not included as it was distorted by the abnormally high drip water value at Poole's Cavern.

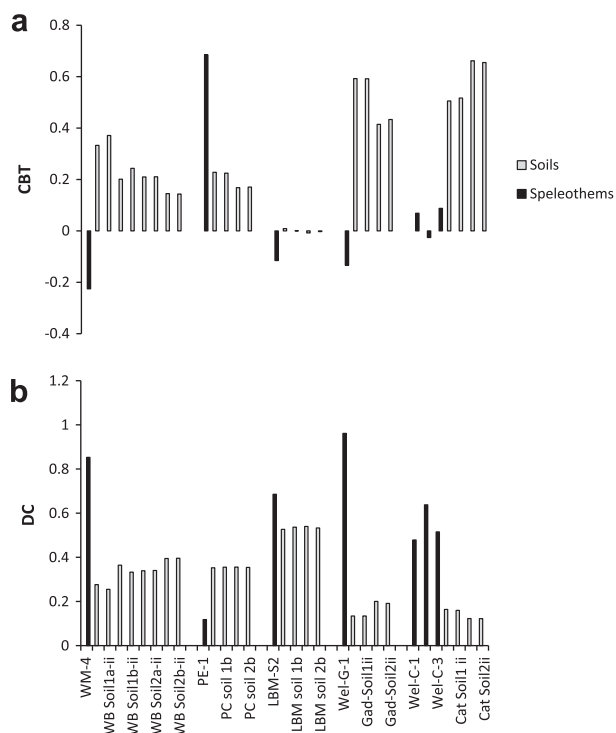


Fig. 8. (a) CBT for speleothem and soil samples, (b) DC for speleothem and soil samples. PE-1 shows an opposite response to the rest of the speleothem samples.

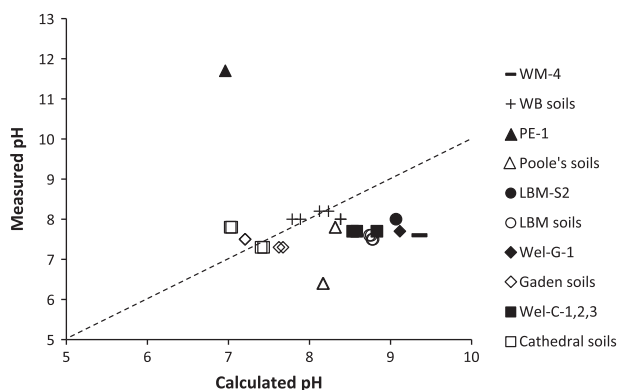


Fig. 9. Measured vs. calculated [Eq. (6)] pH, with the dotted line indicating 1:1. PE-1 forms a clear outlier, consistent with the relationship between CBT and pH breaking down at high pH, as observed for lakes (Schoon et al., 2013). Slight overestimation of pH in the other speleothems may result from the use of a soil calibrated equation.

broadly similar to Pooles-soil-2, but much lower than Pooles-soil-1. When correlated against environmental parameters, MBT in the soils showed a better relationship with MAT than the speleothems (soils, r^2 0.75, $p < 0.0001$; speleothems, r^2 0.63, p 0.03. Fig. 7b), while no relationship between MBT and pH was apparent (Fig. 7c.). Both groups had an inverse correlation with MAP, although as noted above, this was most likely due to the relationship between MAT and MAP at these sites.

The CBT and DC ratios of the speleothems were distinct from the soils at all sites (Fig. 8a and b). For Wombeyan, Wellington and LBM, the speleothems had a lower CBT/higher DC (i.e. more compounds with cyclopentane moieties) than their related soils. The reverse was the case for Poole's Cavern. Fig. 9 shows the calculated pH based on the CBT values (following Weijers et al., 2007), against measured pH for the soils and drip water. For the soils, all the Australian sites showed a good match between measured

and calculated pH, while Poole's Cavern and LBM soils had a higher calculated pH than the measured values. In the speleothems, the CBT proxy consistently overestimated pH, except for PE-1 from Poole's Cavern, where there is a very high drip water pH, which was substantially underestimated by the calculated value. The general overestimation of pH vs. drip water values may simply be due to the fact we were performing using a soil-derived equation (Weijers et al., 2007) to estimate pH in a speleothem context – a speleothem specific CBT–pH calibration needs to be developed in future to test this. Another possibility is that the drip water pH sampling was not fully representative of longer term variation in the cave water pH that might occur during speleothem formation. The finding from PE-1 is in line with work from lakes and soils, which found that at high pH levels > 7.5 – 8.5 , the relationship between CBT and pH breaks down (Xie et al., 2012; Schoon et al., 2013), possibly due to differences in the proton gradients within the cell membranes in high pH environments. Nonetheless, excluding Poole's Cavern, there were marked differences between the CBT and DC values of the soils on one hand and speleothems on the other, which were not reflected in the measured pH values. This was especially noticeable at the Wellington Caves sites, where the drip water and soil pH values were within error of each other, but the CBT and DC of the speleothems against the soils were very clearly distinct. This suggests that additional parameters, tending towards increasing the relative abundance of cyclic moieties within br GDGTs, act on the speleothem signal.

4. Conclusions

The results clearly show that there are substantial differences between GDGT distributions in soils and speleothems. Fig. 10 shows a summary graph plotting soils against speleothems for the major GDGT parameters. Some relationship is apparent in TEX_{86} and MBT, although in both cases the range of values in the speleothem samples is greater than that in the corresponding soils. Neither BIT or CBT shows any relationship between the two groups. In some cases, the results show similarities in the GDGT signals at a specific site. However, in no case does this extend across all the measured parameters (e.g. Wel-G-1 has an i GDGT composition similar to that for the Wellington soils, but the br GDGT composition is markedly different). We therefore conclude that there is clear evidence that the dominant sources of GDGTs in speleothems result from in situ production within either the cave or the overlying vadose zone and, whilst we do not rule out some soil derived input to the signal, this appears to be a minor component of the overall speleothem GDGT record. We suggest that the relationships between soils and speleothems (e.g. in TEX_{86} , and to a lesser extent MBT) are due to parallel response to the same environmental parameter, most likely temperature in this case, rather than a common GDGT source. To enhance understanding of the speleothem GDGT signal further, future work is indicated in three directions: further in-depth studies of specific sites to identify where in the cave/bedrock the primary source is located; combined geochemical and microbiological studies of modern cave environments to establish the degree of variation within and between cave sites and the relationship with environmental parameters; lastly, the collection of an increased modern speleothem sample set from sites with monitored cave temperatures in order to refine the speleothem TEX_{86} and MBT/CBT calibrations for use in palaeoenvironmental research.

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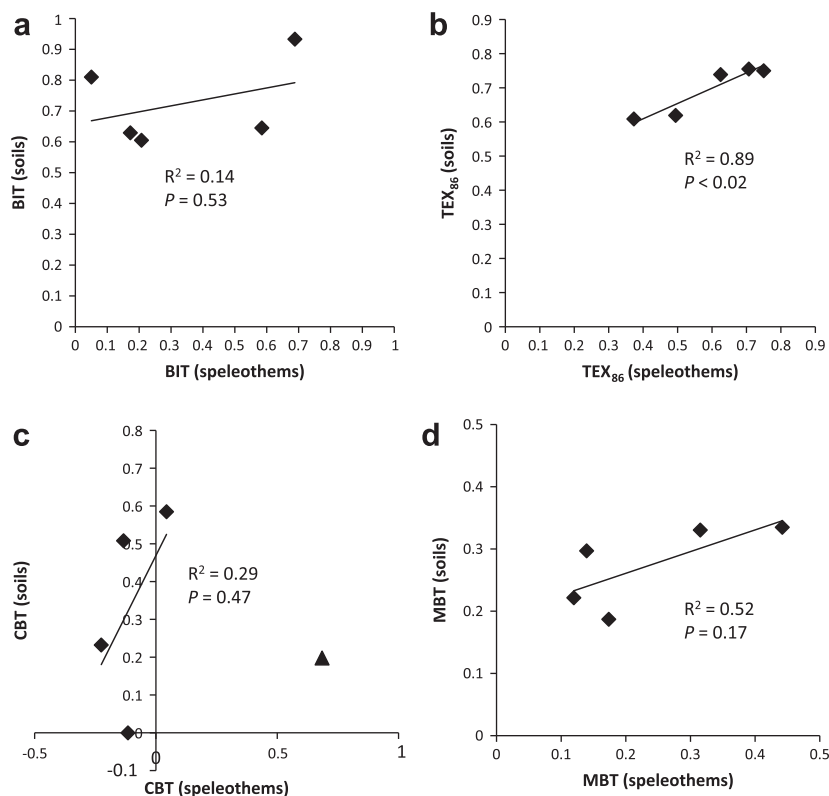


Fig. 10. Scatter plots comparing average speleothem and soil GDGT parameters for each site. (a) BIT, (b) TEX_{86} , (c) CBT (triangle represents Poole's Cavern which was excluded from the regression due to the abnormal drip-water pH), (d) MBT.

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References

- Blaga, C.I., Reichart, G.J., Heiri, O., Sinninghe Damsté, J.S., 2009. Tetraether membrane lipid distributions in water column particulate matter and sediments: a preliminary study. *Organic Geochemistry* 40, 1029–1031.
- Blyth, A.J., Watson, J.S., 2009. Thermochemolysis of organic matter preserved in stalagmites: a preliminary study. *Organic Geochemistry* 40, 1029–1031.
- Blyth, A.J., Schouten, S., 2013. Calibrating the glycerol dialkyl glycerol tetraether signal in speleothems. *Geochimica et Cosmochimica Acta* 109, 312–328.
- Blyth, A.J., Asrat, A., Baker, A., Gulliver, P., Leng, M., Genty, D., 2007. A new approach to detecting vegetation and land-use change: high resolution lipid biomarker records in stalagmites. *Quaternary Research* 68, 314–324.
- Blyth, A.J., Baker, A., Thomas, L.E., van Calsteren, P., 2011. A 2000-year lipid biomarker record preserved in a stalagmite from northwest Scotland. *Journal of Quaternary Science* 26, 326–334.
- Blyth, A.J., Smith, C.I., Drysdale, R.N., 2013. A new perspective on the $\delta^{13}\text{C}$ signal preserved in speleothems using LC-IRMS analysis of bulk organic matter and compound specific stable isotope analysis. *Quaternary Science Reviews* 75, 143–149.
- Dirghangi, S.S., Pagani, M., Hren, M.T., Tipple, B.J., 2013. Distribution of glycerol dialkyl glycerol tetraethers in soils from two environmental transects in the USA. *Organic Geochemistry* 59, 49–60.
- Genty, D., Blamart, D., Ouahdi, R., Gilmour, M., Baker, A., Jouzel, J., Van-Exter, S., 2003. Precise dating of Dansgaard-Oeschger climate oscillations in western Europe from stalagmite data. *Nature* 421, 833–837.
- Gonzalez, J.M., Portillo, M.C., Saiz-Jimenez, C., 2006. Metabolically active Crenarchaeota in Altamira Cave. *Naturwissenschaften* 93, 42–45.
- Hartland, A., Fairchild, I.J., Lead, J.R., Zhang, H., Baalousha, M., 2011. Size, speciation and lability of NOM-metal complexes in hyperalkaline cave dripwater. *Geochimica et Cosmochimica Acta* 75, 7533–7551.
- Hartland, A., Fairchild, I.J., Lead, J.R., Borsato, A., Baker, A., Frisia, S., Baalousha, M., 2012. From soil to cave: transport of trace metals by natural organic matter in karst dripwaters. *Chemical Geology* 304–305, 68–82.
- Hopmans, E.C., Weijers, J.W.H., Schefuß, E., Herfort, L., Sinninghe Damsté, J.S., Schouten, S., 2004. A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids. *Earth and Planetary Science Letters* 224, 107–116.
- Huguet, A., Fosse, C., Laggoun-Déforge, F., Delarue, F., Derenne, S., 2013. Effects of a short-term experimental microclimate warming on the abundance and distribution of branched GDGTs in a French Peatland. *Geochimica et Cosmochimica Acta* 105, 294–315.
- Lachniet, M.S., 2009. Climatic and environmental controls on speleothem oxygen-isotope values. *Quaternary Science Reviews* 28, 412–432.
- McDermott, F., 2004. Palaeo-climate reconstruction from stable isotope variations in speleothems: a review. *Quaternary Science Reviews* 23, 901–918.
- McDonald, J., Drysdale, R., Hill, D., Chisari, R., Wong, H., 2007. The hydrochemical response of cave drip waters to sub-annual and inter-annual climate variability, Wombeyan Caves, SE Australia. *Chemical Geology* 244, 605–623.
- Peterse, F., van der Meer, J., Schouten, S., Weijers, J.W.H., Fierer, N., Jackson, R.B., Kim, J.-K., Sinninghe Damsté, J.S., 2012. Revised calibration of the MBT-CBT paleotemperature proxy based on branched tetraether membrane lipids in surface soils. *Geochimica et Cosmochimica Acta* 96, 215–229.
- Schoon, P.L., de Kluijver, A., Middelburg, J.J., Downing, J.A., Sinninghe Damsté, J.S., Schouten, S., 2013. Influence of lake water pH and alkalinity on the distribution of core and intact polar branched glycerol dialkyl glycerol tetraethers (GDGTs) in lakes. *Organic Geochemistry* 60, 72–82.
- Schouten, S., Hopmans, E.C., Schefuß, E., Sinninghe Damsté, J.S., 2002. Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea water temperatures? *Earth and Planetary Science Letters* 204, 265–274.

- Schouten, S., Huguët, C., Hopmans, E.C., Sinninghe Damsté, J.S., 2007. Improved analytical methodology of the TEX₈₆ paleothermometry by high performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry. *Analytical Chemistry* 79, 2940–2944.
- Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: a review. *Organic Geochemistry* 54, 19–61.
- Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Jung, M.-Y., Kim, J.-G., Rhee, S.-K., Stieglmeier, M., Schleper, C., 2012. Intact and core glycerol dibiphytanyl glycerol tetraether lipids of Group I.1a and I.1b *Thaumarchaeota* in soil. *Applied and Environmental Microbiology* 78, 6866–6874.
- Weijers, J.W.H., Schouten, S., Spaargaren, O.C., Sinninghe Damsté, J.S., 2006. Occurrence and distribution of tetraether membrane lipids in soils: implications for the use of the TEX₈₆ proxy and the BIT index. *Organic Geochemistry* 37, 1680–1693.
- Weijers, J.W.H., Schouten, S., van den Donker, J.C., Hopmans, E.C., Sinninghe Damsté, J.S., 2007. Environmental controls on the bacterial tetraether membrane lipid distribution in soils. *Geochimica et Cosmochimica Acta* 71, 703–713.
- Xie, S., Yi, Y., Huang, J., Hu, C., Cai, Y., Collins, M., Baker, A., 2003. Lipid distribution in a subtropical southern China stalagmite as a record of soil ecosystem response to palaeoclimate change. *Quaternary Research* 60, 340–347.
- Xie, S., Pancost, R.D., Chen, L., Evershed, R.P., Yang, H., Zhang, K., Huang, J., Xu, Y., 2012. Microbial lipid records of highly alkaline deposits and enhanced aridity associated with significant uplift of the Tibetan Plateau in the Late Miocene. *Geology* 40, 291–294.
- Yang, H., Ding, W., Zhang, C.L., Wu, X., Ma, X., He, G., Huang, J., Xie, S., 2011. Occurrence of tetraether lipids in stalagmites: implications for sources and GDGT-based proxies. *Organic Geochemistry* 42, 108–115.
- Yang, H., Ding, W., Wang, J., Jin, C., He, G., Qin, Y., Xie, S., 2012. Soil pH impact on microbial tetraether lipids and terrestrial input index (BIT) in China. *Science China Earth Sciences* 55, 236–245.