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

Alison J Blyth a*, Catherine Jex b,c, Andy Baker b, Stuart Kahn c, Stefan
Schouten d,e

a WA-OIGC, Department of Chemistry, Chemistry and Resources Precinct,
Curtin University, GPO Box U1987, Perth, WA 6845, Australia
b Connected Waters Initiative Research Centre, University of New South
Wales, Sydney 2052, Australia
c Water Research Centre, School of Civil and Environmental Engineering,
University of New South Wales, Australia
d Department of Marine Organic Biogeochemistry, NIOZ Royal Netherlands
Institute for Sea Research, ‘t Horntje, Texel, The Netherlands
e Department of Earth Sciences, Faculty of Geosciences, Utrecht University,
Utrecht, The Netherlands

*Corresponding author. Tel.: +61(0)892669388; fax: +61(0)892662300.
E mail address: alison.blyth@curtin.edu.au (A.J. Blyth).
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 higher 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 cave microenvironment also acts to produce site-specific differences.
1. Introduction

Understanding past changes in our terrestrial environment, and especially identifying local and regional changes in continental temperature and the associated environmental response, is vital in understanding how our 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, for example, stable oxygen isotopes reflecting rainfall and fluctuations in global climate systems, (e.g. McDermott, 2004; Lachniet, 2009); and vegetation, for example, 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 & Watson 2009). Recent work has demonstrated that glycerol dialkyl glycerol tetraethers (GDGTs), compounds whose structure and composition in sedimentary records are known to 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 GDGTs (Fig. 1) derived from...
aquatic archaea (e.g. TEX_{86} (TetraEther indeX of tetraethers consisting of 86 carbon atoms), Schouten et al., 2002) and one using branched 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, TEX_{86} has been applied to aquatic, in particular marine, settings, whilst the branched 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 branched and isoprenoid 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 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 temperatures. If they are soil derived, then they should be based on modern surface or soil temperatures. At present, the published calibration equations are based on surface air temperature as the 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 temperatures can be obtained. Additionally, our 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.,
Branched GDGTs formed a relatively minor component, in contrast to the distribution seen 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 isoprenoid GDGTs, while the soil was dominated by bacterially derived branched GDGTs. Additionally, the internal composition of the isoprenoid and branched compound 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 present in soils recovered from above five caves in the UK and Australia, with a surface mean annual air temperature (MAT) range of 9 – 16 °C, and a 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; Wombyan Caves Reserve (New South Wales, Australia), a highly developed karst system formed in the high purity Wombeyan Marble unit in the Great Dividing Range, south-west 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 is taken from the sample set analysed in 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 previously 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, where the speleothems are known to have a
maximum age of 100 years dating from the mine abandonment, the samples were thin and actively forming at collection. The sample from Wombeyan encompasses the last 40 years.

At each site a minimum of two soil samples were taken. Where contrasting vegetation or soil regimes were present over the cave (e.g. at Lower Balls Mine (LBM), where both woodland and agricultural grassland are present, and Pooles Cavern, where there is both a natural soil and soil developed over lime waste), a sample was taken from each regime. At all sites, 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.

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)/methanol (MeOH), at high temperature (100 °C) and pressure (7.6 x 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$_2$, rediluted in DCM and separated into non-polar and polar fractions over activated Al$_2$O$_3$, eluted 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$_2$O$_3$ 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 (ø 4 mm).

Soil pH was measured at NIOZ (LBM and Poole’s Cavern), and UNSW (Wombyean 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 the previous speleothem analyses, and used the same analytical method as Blyth & 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). HPLC-APCI-MS used an Agilent 1100 series LC with a Prevail Cyano column (2.1 x 150 mm, 3 µm; Alltech) maintained at 30 °C. GDGTs were eluted using a changing mixture of hexane and propanol as follows: 99% 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 above 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):

Branched and Isoprenoid Tetraether index (Hopmans et al., 2004)

\[ \text{BIT} = \frac{(III + II+ I)}{(Cren + III+ II+ I)} \] \[1\]

TetraEther indeX of tetraethers consisting of 86 carbon atoms (Schouten et al., 2002)

\[ \text{TEX}_{86} = \frac{(2 + 3 + Cren’)}{(1 + 2 + 3 + Cren’)} \] \[2\]

Methylation of branched tetraethers (Weijers et al., 2007)

\[ \text{MBT} = \frac{(I + Ib + Ic)}{(I + Ib + Ic + II + IIb + IIc + III + IIIb + IIIc)} \] \[3\]
Cyclisation of branched tetraethers (Weijers et al., 2007)

CBT = -Log[(\(I_b + II_b\))/(I + II)] [4]

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

DC = \((I_b + I_c + II_b + II_c)/(2 \times I + 2 \times II)\) [5]

pH from CBT (Weijers et al., 2007)

Calculated pH = \((3.33 - CBT)/0.38\) [6]

3. Results and discussion

3.1. GDGT composition

All samples, with the exception of speleothem LBM-S3, contained archaean GDGTs 0, 1, 2, 3, crenarchaeol and the regio isomer of crenarchaeol. LBM-S3 contained all of the above except for the regio isomer, which was below the detection limit. For the branched GDGTs, speleothem LBM-S3 was removed from the data set due to compound abundance being below detection limits. 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 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 branched GDGTs (I, II, III). Crenarchaeol is indicative of
Thaumarcheota, while GDGT 0 (also known in the literature 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 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 to 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 (r² 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 branched 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 branched 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 isoprenoid / branched 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 branched 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
was the lowest measured.

3.3. Variation in relative composition of isoprenoid tetraethers

To investigate the variation in compound relative abundance in the
isoprenoid GDGTs, two measures were considered, TEX$_{86}$, and a principal
components analysis (PCA) of the full compound distribution. For
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 archeal 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 a good
correlation between TEX$_{86}$ and surface MAT (Fig 4.b; $r^2$ 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. 4.c; 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 isoprenoid 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). Figure 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 under estimated the temperature by > 4 °C. Collectively, the distribution of the isoprenoid compounds indicate 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 branched 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 cluster together on PC-2 and 3. On PC-1 there is some separation between the Wellington soils, and the Wombeyan soils, the latter of which cluster with Pooles-soil-1. However, they all have negative scores compared with the speleothems. Only the LBM soils cluster
differently, having positive scores on PC1 and 3, and slightly negative on PC-2. The speleothems are distinct from the soils (with the exception of the soils from LBM), being largely positive on PC-1. However, they show 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 branched GDGTs) and at Gaden Cave, Wellington, Wel-G-1 had a distinctly higher MBT than related soils. At Poole’s Cavern, the speleothem was 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, r² 0.75, p < 0.0001; speleothems, r² 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 is 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, 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 perforce 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 is not fully representative of longer term variations 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
above 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 the 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 branched 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 show 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 isoprenoid GDGT
composition similar to that for the Wellington soils, but the branched 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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References


Table and figure captions

Table 1.
Location and environmental details for samples.

Table 2.
Relative abundance of isoprenoid GDGTs, normalised to total isoprenoid GDGTs and total isoprenoid GDGTs excluding GDGT 0 (speleothem samples are marked in italics).

**Fig. 1.** Structures for the isoprenoid and branched GDGTs.

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

**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) TEX$_{86}$ in speleothem and soil samples; b) relationship between TEX$_{86}$ and surface MAT; and c) the relationship between TEX86 and surface MAP in the speleothems and soils respectively.

**Fig. 5.** a) PCA scores plot for isoprenoid 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.

**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

**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 levels, 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.

Fig 10. Scatter plots comparing average speleothem and soil GDGT parameters for each site. a) BIT; b) TEX86; c) CBT (triangle represents Poole’s Cavern which has been excluded from this regression due to the abnormal drip-water pH); d) MBT.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Type</th>
<th>Location</th>
<th>Soil pH</th>
<th>Drip water pH</th>
<th>Surface MAT °C</th>
<th>MAP mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE-1a</td>
<td>Stalagmite</td>
<td>Pooles Cavern, England</td>
<td>-</td>
<td>11.7 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC-soil-1</td>
<td>Soil (top 10 cm)</td>
<td>Pooles, natural soil above cave, adjacent to lime spoil heap</td>
<td>6.4</td>
<td>-</td>
<td>9</td>
<td>1300</td>
</tr>
<tr>
<td>PC-soil-2</td>
<td>Soil (top 10 cm)</td>
<td>Pooles, soil from lime soil heap above cave</td>
<td>7.8</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBM-S2</td>
<td>Stalagmites</td>
<td>Lower Balls Mine (LBM), England</td>
<td>-</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBM-S3</td>
<td></td>
<td>LBM, thin soil under light woodland, over limestone. Outside upper entrance to mine</td>
<td>-</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBM-soil-1</td>
<td>Soil (top 10 cm)</td>
<td>LBM, soil under light woodland, over limestone. Low</td>
<td>7.6</td>
<td>-</td>
<td>10</td>
<td>995</td>
</tr>
<tr>
<td>LBM-soil-2</td>
<td>Soil (top 10 cm)</td>
<td>LBM, soil under agricultural grassland above mine, halfway between upper and lower entrances</td>
<td>7.5</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WM-4</td>
<td>Stalagmite</td>
<td>Wombeyan Caves, New South Wales, Australia</td>
<td>-</td>
<td>7.6 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB-soil-1a</td>
<td>Soil (0-2 cm)</td>
<td>Wombeyan, above caves, very thin soil under open woodland</td>
<td>8.0</td>
<td>-</td>
<td>13.7</td>
<td>804</td>
</tr>
<tr>
<td>WB-soil-1b</td>
<td>Soil (2-5 cm)</td>
<td>Wombeyan, above caves, very thin soil under open woodland</td>
<td>8.2</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB-soil-2a</td>
<td>Soil (0-2 cm)</td>
<td>Wellington, above Gaden Cave, Wellington, NSW, Australia</td>
<td>8.0</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB-soil-2b</td>
<td>Soil (2-5 cm)</td>
<td>Wellington, above Gaden Cave, Wellington, NSW, Australia</td>
<td>7.7 ± 0.5</td>
<td>-</td>
<td>16</td>
<td>617</td>
</tr>
<tr>
<td>Wel-C-1</td>
<td>Straw</td>
<td>Wellington, above Gaden Cave, Wellington, NSW, Australia</td>
<td>7.7 ± 0.5</td>
<td>-</td>
<td>16</td>
<td>617</td>
</tr>
<tr>
<td>Wel-C-2</td>
<td>Flowstone</td>
<td>Cathedral Cave, Wellington, NSW, Australia</td>
<td>7.7 ± 0.5</td>
<td>-</td>
<td>16</td>
<td>617</td>
</tr>
<tr>
<td>Wel-C-3</td>
<td>Flowstone on bottle</td>
<td>Cathedral Cave, Wellington, NSW, Australia</td>
<td>7.7 ± 0.5</td>
<td>-</td>
<td>16</td>
<td>617</td>
</tr>
<tr>
<td>Cat-soil-1</td>
<td>Soil (top 20 cm)</td>
<td>Wellington, above Gaden Cave, Wellington, NSW, Australia</td>
<td>7.5</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat-soil-2</td>
<td>Soil (top 20 cm)</td>
<td>Wellington, above Gaden Cave, Wellington, NSW, Australia</td>
<td>7.3</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wel-G-1</td>
<td>Straw</td>
<td>Wellington, above Gaden Cave, Wellington, NSW, Australia</td>
<td>7.3</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gad-soil-1</td>
<td>Soil (top 20 cm)</td>
<td>Wellington, above Gaden Cave, Wellington, NSW, Australia</td>
<td>7.3</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gad-soil-2</td>
<td>Soil (top 20 cm)</td>
<td>Wellington, above Gaden Cave, Wellington, NSW, Australia</td>
<td>7.8</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Isoprenoid GDGTs (%)</th>
<th>Isoprenoid GDGTs (%; GDGT 0 excluded)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GDGT 0</td>
<td>GDGT 1</td>
</tr>
<tr>
<td><strong>PE-1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC-soil-1</td>
<td>17.7</td>
<td>21.4</td>
</tr>
<tr>
<td>PC-soil-2</td>
<td>16.9</td>
<td>9.5</td>
</tr>
<tr>
<td><strong>LBM-S2</strong></td>
<td>24.4</td>
<td>12.6</td>
</tr>
<tr>
<td><strong>LBM-S3</strong></td>
<td>17.1</td>
<td>18.1</td>
</tr>
<tr>
<td>LBM-soil-1</td>
<td>88.5</td>
<td>1.2</td>
</tr>
<tr>
<td>LBM-soil-2</td>
<td>21.7</td>
<td>6.2</td>
</tr>
<tr>
<td><strong>WM-4</strong></td>
<td>12.8</td>
<td>14.9</td>
</tr>
<tr>
<td>WB-soil-1a</td>
<td>14.0</td>
<td>6.3</td>
</tr>
<tr>
<td>WB-soil-1b</td>
<td>10.2</td>
<td>5.7</td>
</tr>
<tr>
<td>WB-soil-2a</td>
<td>10.4</td>
<td>7.3</td>
</tr>
<tr>
<td>WB-soil-2b</td>
<td>9.5</td>
<td>7.0</td>
</tr>
<tr>
<td><strong>Wel-C-1</strong></td>
<td>8.9</td>
<td>11.0</td>
</tr>
<tr>
<td><strong>Wel-C-2</strong></td>
<td>8.0</td>
<td>9.6</td>
</tr>
<tr>
<td><strong>Wel-C-3</strong></td>
<td>8.8</td>
<td>9.9</td>
</tr>
<tr>
<td>Cat-soil-1</td>
<td>15.0</td>
<td>8.3</td>
</tr>
<tr>
<td><strong>Cat-soil-2</strong></td>
<td>22.1</td>
<td>4.7</td>
</tr>
<tr>
<td><strong>Wel-G-1</strong></td>
<td>6.9</td>
<td>7.3</td>
</tr>
<tr>
<td>Gad-soil-1</td>
<td>15.4</td>
<td>7.8</td>
</tr>
<tr>
<td><strong>Gad-soil-2</strong></td>
<td>13.0</td>
<td>7.2</td>
</tr>
</tbody>
</table>
Isoprenoid GDGTs:
GDGT 0: m/z 1302  GDGT 1: m/z 1300
GDGT 2: m/z 1298  GDGT 3: m/z 1296
Crenarchaeol: m/z 1292

Branched GDGTs:
GDGT I: m/z 1022  Ib: m/z 1020  Ic: m/z 1018
GDGT II: m/z 1036  IIb: m/z 1034  IIc: m/z 1032
GDGT III: m/z 1050  IIIb: m/z 1048  IIIc: m/z 1046
Fig. 4

(a) TEX86 values for various soils and speleothems.

(b) Scatter plot showing the relationship between surface MAT °C and TEX86. R² = 0.93 for speleothems and R² = 0.75 for soils.

(c) Scatter plot showing the relationship between MAP mm and TEX86. R² = 0.67 for speleothems and R² = 0.96 for soils.
Fig. 6

a

PC-3

PC-1

PC-2

b

c

- WM-4
+ WB soils
△ Pooles-1
△ Pooles soils
● LBM-2
○ LBM soils
◆ Wel-G-1
◇ Gad soils
■ Wel-C-1,2,3
□ Cat soils

- GDGT III (m/z 1050)
○ GDGT IIIb (m/z 1048)
○ GDGT IIIc (m/z 1046)
▲ GDGT II (m/z 1036)
△ GDGT IIb (m/z 1034)
△ GDGT IIc (m/z 1032)
■ GDGT I (m/z 1022)
□ GDGT I (m/z 1020)
■ GDGT Ic (m/z 1018)
\[ R^2 = 0.63 \]

\[ R^2 = 0.75 \]

\[ R^2 = 0.00 \]
Fig. 8

a

CBT

Soils
Speleothems

b

DC

Soils
Speleothems

WB Soil1a-ii
WB Soil1b-ii
WB Soil2a-ii
WB Soil2b-ii
PE-1
PC soil1b
PC soil2b
LBM soil1b
LBM soil2b
Wel-G-1
Wel-C-1
Wel-C-3
Cat Soil1ii
Cat Soil2ii
Fig. 10

(a) BIT (soils) vs. BIT (speleothems), $R^2 = 0.14$, $P = 0.53$

(b) TEX$_{86}$ (soils) vs. TEX$_{86}$ (speleothems), $R^2 = 0.89$, $P < 0.02$

(c) CBT (soils) vs. CBT (speleothems), $R^2 = 0.29$, $P = 0.47$

(d) MBT (soils) vs. MBT (speleothems), $R^2 = 0.52$, $P = 0.17$