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Invited review

Organic proxies in speleothems – New developments, advantages and limitations

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ABSTRACT

Research into organic matter in speleothems has progressed recently to encompass new analytical approaches and applications, which offer increased potential in areas such as palaeo-temperature reconstruction and high-resolution palaeo-environmental records from the Quaternary. Here we review three major areas of relevance for future work in the field – the origin, transport and transformation of the organic matter which is ultimately preserved in speleothems; the types of proxies currently available for use or in development, and their advantages and issues; and the recently developed prospect of high-resolution organic matter records derived from the analysis of organic/trace elements complexes. The continuing extension of work in these research areas offers excellent potential for organic speleothem proxies to grow as a valuable tool in palaeoenvironmental research.

1. Introduction

Our ability to understand and reconstruct past environments and climates depends on the availability of appropriate "archives" of information in the form of biological, chemical, and physical records preserved in the natural environment (e.g. McKay et al., 2016; Douglas et al., 2016). In a terrestrial context, possible archives include lake sediments (e.g. Morris et al., 2015; Zolitschka et al., 2015), peat cores (e.g. Swindles et al., 2012), loess/palaeosol sequences (e.g. Maxbauer et al., 2016), and speleothems (e.g. Fairchild and Baker, 2012; Wong and Breecker, 2015).

Speleothems are chemically precipitated deposits, usually formed of calcium carbonate, that grow within a cave environment as the result of degassing of cave waters (Fairchild and Baker, 2012). A wide variety of forms can develop, depending on the location of the deposit within the cave, the rate of water flow, and microbial involvement. However, the most commonly used in palaeoenvironmental studies are stalagmites and flowstones (Fig. 1.) Stalagmites grow upwards from a surface beneath drip-points, while flowstones develop under more rapidly flowing water films. Both types form in incremental layers which can be used to constrain any environmental proxy records that they contain (Baker et al., 2008a). Stalactites and soda straws which grow downwards in tubular form from the drip points are sometimes used in palaeoenvironmental work, but less commonly, as blockages in their drip-pathways can result in a less coherent laminated structure.

Speleothems are particularly useful as palaeoclimatic archives as they are highly amenable to chemical dating, potentially back to several million years (Dorale et al., 2004; Woodhead et al., 2006; Hellstrom, 2006; Hellstrom and Pickering, 2015). At the other end of the scale, manual or automated counting of growth laminations can allow an annual or subannual resolution (Meyer et al., 2006; Baker et al., 2008a; Smith et al., 2009). Their connection to the surrounding environment via their feed dripwater allows multiple chemical signals to be preserved, including organic material (OM) which can be derived from the overlying vegetation, soil, or vadose zone, or from within the cave environment. In this paper we follow the broadly defined concept of OM applied by Blyth et al. (2008) in a previous review of the field, including molecular and detrital material transported as dissolved or suspended components in dripwaters, or generated within the cave environment, but excluding identifiable fossil material such as pollen.

The study of OM in speleothems has expanded over the last twenty years, due to the potential for development of new types of

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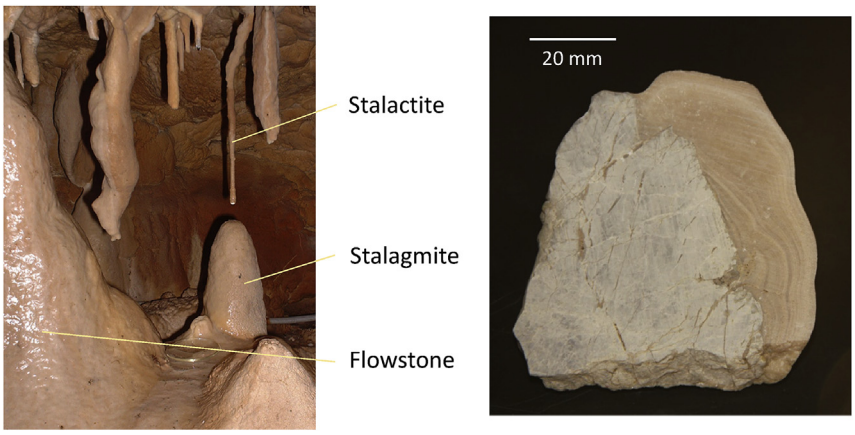

Fig. 1. These photographs show (left) the growth modes of different speleothem types within the cave, and (right) incremental growth lamination in a sectioned stalagmite that has grown vertically on the side of a block of limestone.

palaeoenvironmental record. The majority of speleothem records centre on inorganic proxies such as oxygen isotopes, which have led to a significant body of work, and particular utility in palaeohydrological and palaeoclimatic research (see McDermott (2004), Lachniet (2009), Fairchild and Baker (2012), and Wong and Breecker (2015) for reviews). Carefully targeted analysis of OM offers the opportunity to expand the range of chemical proxy records recovered, including those deriving directly from plant and microbial communities. This holds out the tantalising possibility of creating fully integrated multi-proxy records comprising of both climatic and ecological signals in a single well-dated sample. The amount of organic carbon preserved in speleothems is low, being estimated at 0.01–0.3% of total carbon (Blyth et al., 2013a; Li et al., 2014; Quiers et al., 2015). However, with increasing numbers of researchers working in this field across a range of scientific approaches, viable analytical techniques are becoming established. This review updates the Quaternary community on the current state of knowledge with respect to OM in speleothems, with particular consideration given to the potential sources of the organic signals; the range of techniques currently available for the analysis of preserved biomarker signatures; the potential use of trace element analysis as a high resolution proxy for OM input to a cave; and suggestions for the most necessary avenues of further research.

2. Sources of OM in caves

The two main sources of OM preserved in speleothems are the overlying environment (vegetation, soil, and possibly atmosphere) and microbial communities living within the cave (Yang et al., 2011; Perrette et al., 2013; Blyth et al., 2014). In most karst environments, the soil is likely to be the biggest store of organic carbon, but the contribution of this carbon in speleothems will depend on leaching from the soil, and chemical and biological alteration during transport (Fig. 2.). It is important to understand the relative contribution of surface, karst and in-cave sources of OM in any particular speleothem sample, as this may be central to achieving the best calibration of palaeoenvironmental proxies (Yang et al., 2011; Blyth et al., 2014).

2.1. Transport and alteration of surface derived OM

Soil-derived OM derives from the degradation of material such as plant litter and soil fauna. This OM can be transported in dissolved (DOM), colloidal or particulate form, with the different fractions operationally defined by their separation on filtration. DOM is rapidly adsorbed to mineral surfaces (Jardine et al., 1989; Qualls and Haynes, 1992), with differences in the extent and rate of adsorption depending on the chemical nature of the OM and the soil properties. Hydrophilic fractions (e.g. carboxylic acids and amino acids) are most likely to be labile, in contrast to hydrophobic fractions (e.g. *n*-alkanes or lignin and its derivatives). The long-term stability of OM in the soil is discussed by Lehmann and Kleber (2015). The conventional view that soil OM comprises stable, persistent, large molecular size 'humic substances' (for example, see Karavanova (2013)) has been replaced by an understanding that soil OM is a continuum of degrading compounds (Lehmann and Kleber, 2015). This soil continuum model (SCM) recognises that soil OM can be stabilised if it is protected from microbial access through sorption to mineral surfaces and formation of aggregates. Desorption from mineral surfaces or the destruction of soil aggregates releases the OM into the pool of material accessible for microbial breakdown. In the SCM, soil OM is therefore a function of the characteristics of the surface litter input characteristics and the soil mineralogy, as well as the temperature, moisture and the biota present. For speleothem scientists, the SCM suggests that whenever OM is being transported, microbial breakdown of it is likely to occur. This can occur beyond the soil zone, when the OM is being transported from soil to cave.

Soil OM may be leached into the vadose zone (here defined as excluding the soil zone). Experimental quantification of the amount of downward flux of OM (leached DOM) compared to other fluxes (such as biological uptake) has proven difficult (Ghani et al., 2010). Downward leaching of DOM requires the production of transportable (dissolved or colloidal) OM in the soil, and rainfall that exceeds field capacity, in order to generate infiltration to the unsaturated zone. Hydrophilic DOC fractions are more likely to be available for leaching. However, if water movement generates the detachment and transport of colloidal material, hydrophobic DOC fractions will also be leached. An example of this was observed in an Ethiopian speleothem (Blyth et al., 2007), where the *n*-alkane record showed a rapid change at the same time as anthropogenic vegetation change.

In the vadose zone, as discussed above, soil-derived OM is still available for biological and physiochemical processing. There have been relatively few studies of the processing of vadose zone and groundwater OM outside of a contaminated site context, but those that have been undertaken provide evidence of biodegradation and mineral adsorption (Shen et al., 2014). Considering rates of process, mineral adsorption is near-instantaneous (Qualls and Haynes,

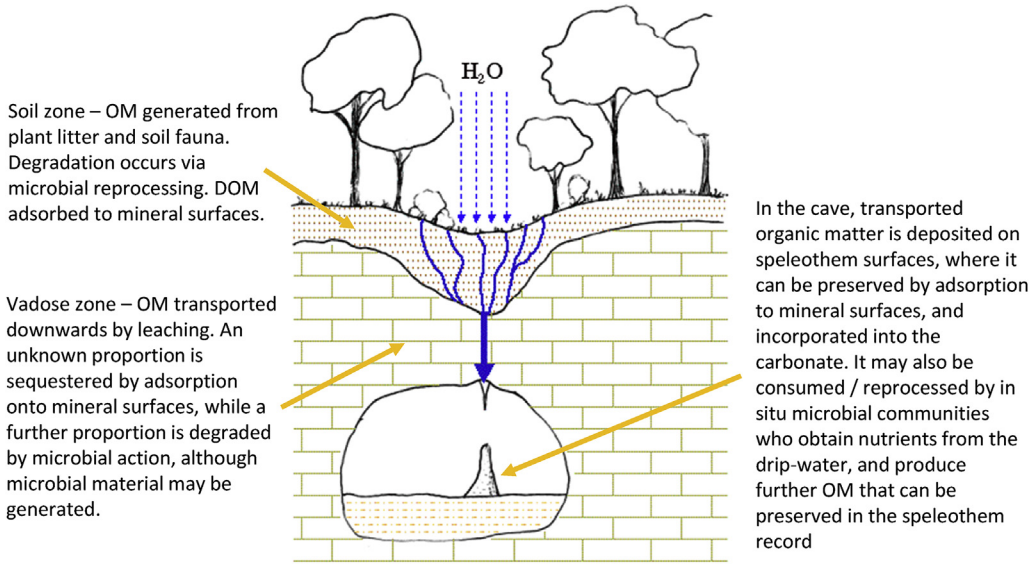

Fig. 2. A schematic diagram indicating the potential sources of OM in a karst system, and the main transformation stages during transport.

1992). Therefore, as long as adsorption sites are available, OM will be rapidly sequestered by mineral surfaces. Biological degradation will also occur, with the OM providing an energy source to *in situ* microbial communities. Biological processing, along with root respiration, will produce CO_2, and CO_2 produced by these two processes within the vadose zone is consistent with observed elevated concentrations of CO_2 there (the "ground-air carbon dioxide" hypothesis of Atkinson (1977), developed more recently as a general conceptual model of CO_2 behaviour in the vadose zone (the "Gibraltar model", Mattey et al. (2016)). Shen et al. (2014) postulate a vadose zone 'chromatography' in OM composition due to the biological processing and adsorption between soil source and the phreatic zone (delimited by the water table). The concept of vadose zone processing of OM is consistent with fluorescence analysis of speleothems, which has shown a decrease in fluorescence intensity (and therefore OM abundance), with depth of overlying bedrock (Baker et al., 1996) as well as a change in OM fluorescence properties compared to those of the overlying soil (Baker and Genty, 1999; van Beynen et al., 2000). Whether the depth below surface affects the compound classes of palaeoenvironmental interest in speleothems has not been explored. A useful conceptual framework for transport of OM from the soil to the cave is the idea of transportable (dissolved and colloidal) OM decreasing in concentration with the duration of flow from its source, due to abiotic and biotic processes (Fig. 3). OM will also change in character with duration of flow, with the most hydrophilic and least bioavailable material persisting for longest. The bedrock and the type of flow path control the rate and duration of infiltration, so are critically important in determining transport time, and the extent of contact with mineral surfaces. Caves where drip waters take years to decades to reach the cave might be expected to have relatively low concentrations of allochthonous OM, whereas caves with rapid infiltration should have higher abundances. For example, Lower Traligill Cave in Assynt is sited in Cambro-Ordivician dolomite with no primary porosity and dominated by fracture flow. This site records a range of allochthonous biomarkers showing a response to known environmental changes (Blyth et al., 2011). A range of allochthonous biomarkers was also detected in a sample from Mechara, Ethiopia (Blyth et al., 2007), but the mixed fracture and porous seepage system was reflected in the contrast between the speed of incorporation of the *n*-alkane signal (rapid, and hypothesised to be colloidally transported via fractures), and the smoothing and lags seen in the stable isotope and radiocarbon data (Blyth et al., 2007). Within an individual cave, a speleothem type with a rapid-flow component (e.g. a flowstone) is hypothesised to contain more allochthonous OM than a speleothem without such flow (e.g. a soda straw stalactite) and a greater proportion of colloidally-transported OM (e.g. lignin).

2.2. Microbial communities in caves as OM sources

Microbiological research continues to demonstrate the presence of diverse cave microbial communities (Barton and Northup, 2007; Epure et al., 2014) to the extent that cave microbial communities are now part of cave management (Northup, 2011). Jones and Bennett (2014) demonstrated that local and global diversity of microbial communities were partly determined by mineral properties such as buffering capacity and nutrient content of the rock, suggesting that there are microbial adaptions to specific rock types. Cave microbial and fungal community structures have been determined from next generation sequencing (Epure et al., 2014). An in-cave microbial source of OM in speleothems is likely, as microbes have been shown to be present in drip waters, on cave surfaces and in sediments (Blyth and Frisia, 2008; Yang et al., 2011; Blyth et al., 2014; Yun et al., 2015; Baker et al., 2016; Tomczyk-Zak and Zielenkiewicz, 2016). For example, Yun et al. (2015) present the first analyses of cave drip water bacterial communities from Heshang Cave, China, where they demonstrate a community structure which is dominated by oligotrophs which were utilising inorganic P present in the dripwater. At Wellington Cave, Australia, analysis of selected microbial membrane compounds has been performed for soil and speleothem samples (Blyth et al., 2014) and from drip waters during an artificial irrigation experiment (Baker et al., 2016). All three have different compositions, with the inference that the speleothem signal is derived predominantly from a microbial community present within the cave.

The OM preserved in the speleothem archive is therefore expected to contain both allochthonous OM, which will have been subject to varying degrees of reprocessing in the soil and vadose zone, and autochthonous microbial material. The relative importance of the two sources is likely to be unknown due uncertainty with respect to microbial contribution, but some generalisations can be proposed based on Fig. 3. Speleothems fed by water with a long residence time might be expected to have experienced a

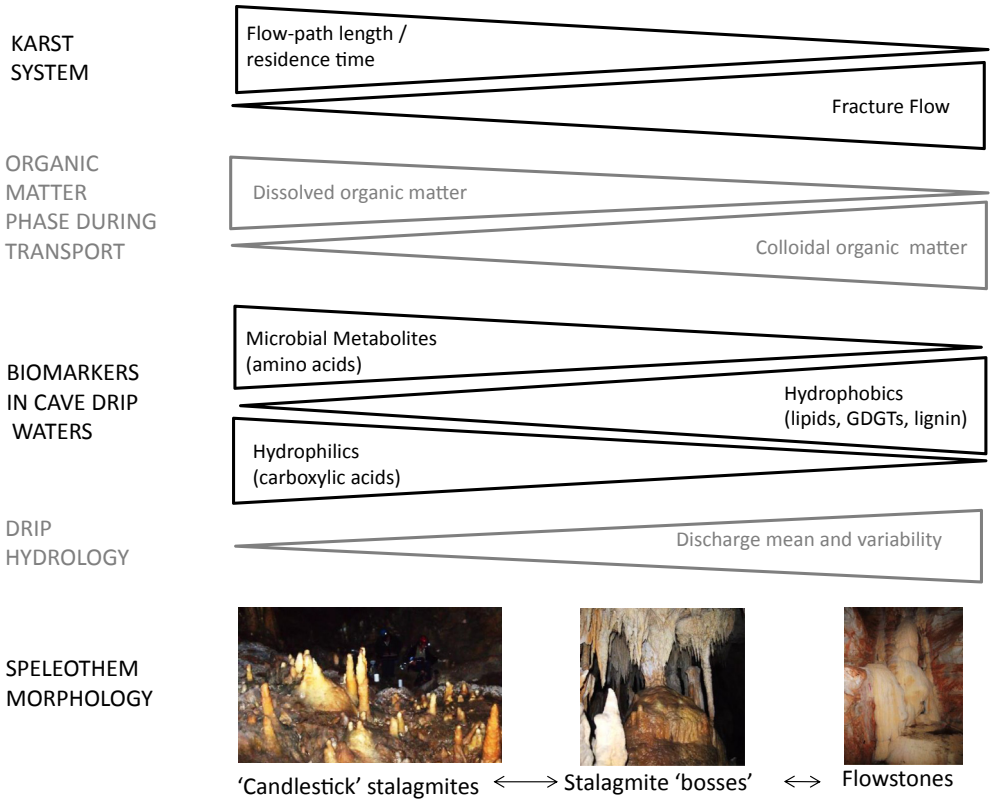

Fig. 3. Conceptual figure of the relationship between hydrology, speleothem morphology and organic matter character and composition. Arrows and horizontal bar thicknesses represent increasing and decreasing amounts.

greater amount of OM processing and sorption along the flow path, leading to a decreased concentration of allochthonous OM. The opposite would be expected for speleothems with a relatively short residence time. However, the increased processing of OM with residence time would likely result in an overall decrease in concentration of drip water organic carbon, which could then become limiting for autochthonous production. Evidence for a drip water limitation on the microbial OM is seen in results from Golgotha and Labyrinth Caves, SW Australia (Blyth, unpublished data). These caves formed in a Quaternary bedrock with high primary porosity, dominated by seepage flow. Speleothems from these caves are the only samples to-date from which microbial biomarkers could not be usefully measured. We hypothesise that this arises from reduced allochthonous input to the speleothem resulting in decreased external energy sources, which in turn negatively affects the microbial communities and reduces autochthonous input.

3. Analysis and interpretation of organic compounds preserved in speleothems as proxies for climate or environment

Once OM has been preserved in speleothems, our next challenge is to identify the most important compounds for the recovery of palaeoenvironmental signals. Research in this area has had two main themes: methodological studies to optimise clean extraction and analysis, and research developing useful proxies for environment, vegetation, and climate.

3.1. Recovery and analysis of lipid biomarkers from speleothem calcite

There are two broad approaches to measuring OM signals in speleothems: in-situ techniques such as fluorescence analysis (see Section 4), and extractive techniques that chemically separate the target organic components from the calcite. To date the latter approaches have been necessary to achieve detailed molecular characterisation of the OM, and to measure target molecules believed to be derived from a specific source (e.g. *n*-alkanes derived from plants). The historical obstacle to the application of extractive OM proxies in speleothems has been the sample size of calcite required to obtain a useable signal, with early studies using solvent extraction techniques on powdered subsamples of around 100 g calcite (Rousseau et al., 1995; Xie et al., 2003, 2005). Use of an acid digestion technique has substantially resolved this problem, releasing more of the preserved OM (Blyth et al., 2006; Huang et al., 2008) and allowing analysis of 1 g or less of calcite in some speleothems (e.g. Blyth et al., 2007; Rushdi et al., 2011). The acid digestion process also causes a partial acid hydrolysis of the organic material, and the initial digestion can be followed by boiling the solution under reflux to complete this process (Blyth et al., 2006). This has the advantage of maximising the release of potential compounds of interest from cell fragments and macro-molecular complexes. Wang et al. (2012) tested the acid concentration and reflux temperature of the procedure to investigate impact on recovery of fatty acids, and recommended use of 3 M hydrochloric acid and a reflux temperature of 130 °C, although whether this is also optimal for other compounds has not been established.

There are two main extractive approaches that can be taken once the acid solution has been obtained and hydrolysed as appropriate (Fig. 4). The first is to subject the solution to a manual liquid/liquid extraction with a non-miscible organic solvent (usually dichloromethane) in a separating funnel. This technique has had good success in recovering a range of common biomarker compounds including *n*-alkanes, fatty acids, sterols and glycerol dialkyl glycerol tetraethers (Blyth et al., 2006, 2011; Yang et al., 2011), and can be undertaken in a basic laboratory set-up with

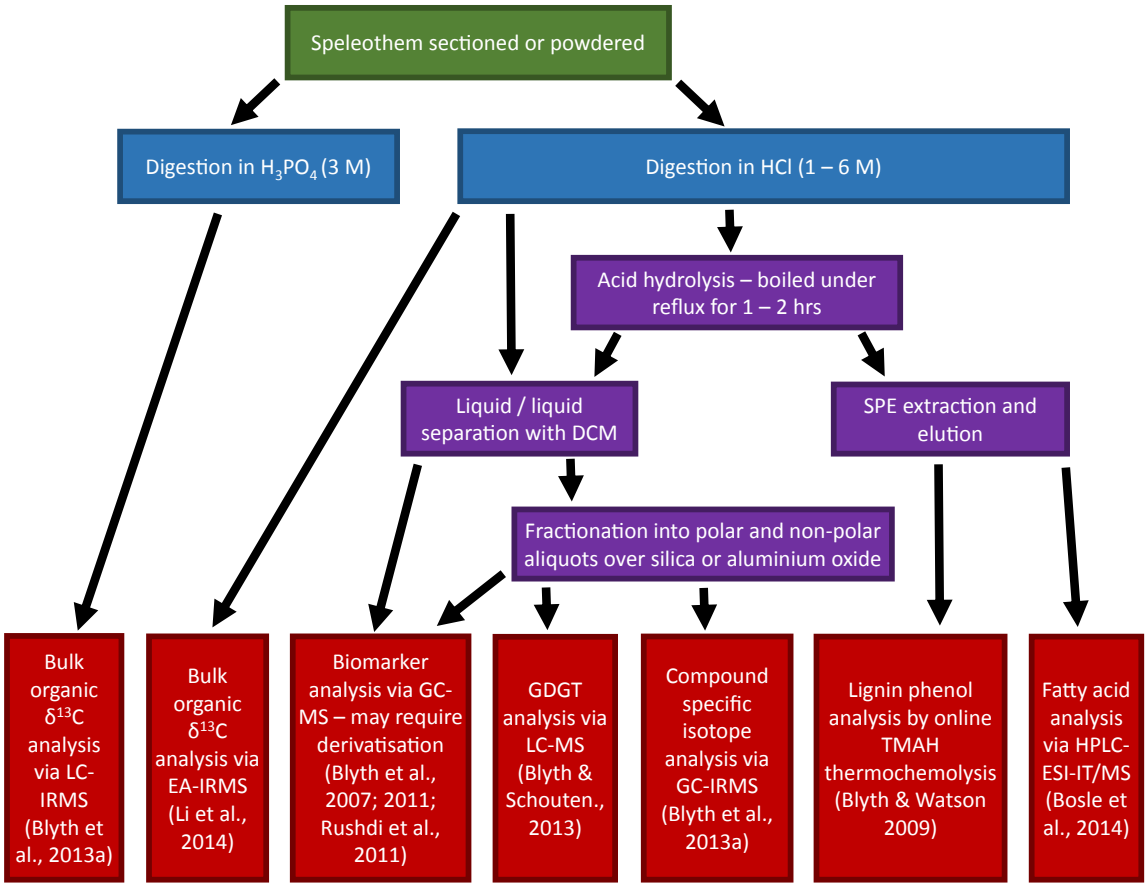

Fig. 4. A schematic diagram showing the main forms of extraction, processing, analytical techniques and applications for organic matter preserved in speleothems.

standard glassware. However, it is selective, in that only compounds amenable to extraction into the chosen solvent can be recovered, and the choice of solvents is limited by miscibility. In any extraction, an as yet unquantified proportion of the OM will be left in the residual acid solution, including larger molecules such as lignin. The second alternative is to pass the acid solution through a solid phase extraction column, and then recover the organic fractions via elution with a sequence of solvents (the choice of which will depend on the compounds of interest). This has been applied to the recovery of fatty acids up to a chain length of C_{20} (Bosle et al., 2014), as well as to lignin (Blyth and Watson, 2009). Where recovery of both DCM extractable and non-DCM extractable material is desired, the two techniques can be used in sequence with the solution being first subject to liquid/liquid extraction, and the acid residue then passed through an SPE column (Blyth et al., 2010). Solid phase extraction is likely to be amenable to smaller calcite sample sizes, and via choice of the column packing and elution solvents, allows for a more precise isolation of compounds of interest (Bosle et al., 2014). However, it has not been tested on a wide range of molecules of palaeoenvironmental interest in the speleothem context, and some problems have been observed in the recovery of longer chain-length aliphatic acids (Bosle et al., 2014).

Once target compounds have been extracted and isolated, they are analysed either by gas chromatography – mass spectrometry (GC-MS) or liquid chromatography – mass spectrometry (LC-MS) depending on the volatility of the molecules (De Hoffmann et al., 1996). Newer techniques such as Electrospray Ionisation – Fourier Transform – Ion Cyclotron Resonance – Mass Spectrometry (ESI-FT-ICR-MS) allow for finger-printing of complex organic mixtures (Kujawinski, 2002), and although not yet published in speleothems, early results in karst systems indicate considerable potential (Lechleitner et al., 2016).

Due to the low abundances of OM preserved in speleothems, it is important to be able to identify organic contamination, which may arise from storage and handling, especially if plastic wrappings are used, or from the laboratory process. The latter can be monitored via laboratory blanks, and appears to be minimised by use of an acid digestion method as the basis for compound extraction (Blyth et al., 2006). However, prior contamination of the samples cannot be controlled for so easily. Wynn and Brocks (2014) investigated this possibility with small sample sizes analysed by GC-MS, identifying phthalates, cholesterol and *n*-alkanes as potential contaminants. Phthalate contamination has long been known in speleothems (Blyth et al., 2006) and is unlikely to be misattributed to an environmental source. Cholesterol contamination has also previously been identified (Blyth et al., 2006), and should be excluded from environmental interpretation. Of more concern is the finding with regards to the *n*-alkanes, as these have previously been applied as environmental proxies (Xie et al., 2003; Blyth et al., 2007, 2011). However, the reported *n*-alkanes in both the samples and the blank of Wynn and Brocks (2014) show no odd over even preference, indicating that an oil-based source contaminated the exterior of the samples. This is different from the *n*-alkane sequences that have previously been interpreted in the palaeoenvironmental literature, where a marked odd over even preference indicated a predominantly vegetation-derived source (Blyth et al., 2007, 2011). We suggest that *n*-alkanes should be included in speleothem analyses, but attention should be paid to

laboratory cleanliness, thorough pre-cleaning of the samples including acid removal of the outer surface where sample size allows, and interpretation should be undertaken with care, with a carbon preference index being employed to identify any affected samples.

3.2. Proxies for the measurement of climatic parameters: direct measurement of temperature

The development of quantitative temperature proxies is an important goal in speleothem research, as incremental growth and ease of radiometric dating offers an excellent framework for understanding regional terrestrial climate. Various geochemical techniques offer potential in this area, including clumped isotope analysis and fluid inclusion analysis (for a comparison, see Meckler et al., 2015). In the field of organic geochemistry, measurement of structural variation in a group of compounds known as glycerol dialkyl glycerol tetraethers (GDGTs), which are derived from microbial membranes also shows promise (Blyth et al., 2013a,b). GDGTs are derived from certain groups of archaea and bacteria, and show a correlation between their core carbon structure (e.g. number of methyl branches, number and type of cyclic moieties) and the temperature and pH of their host environment (e.g. Schouten et al., 2002; Weijers et al., 2007), giving considerable potential in reconstructing climate (for a full review of GDGTs and their applications, see Schouten et al., 2013). The approach is widely applied in aquatic environments (e.g. Powers et al., 2004; Zink et al., 2010; Pearson et al., 2011), but has only been tested in speleothems in the last five years.

Research indicates that GDGTs are consistently recoverable from speleothems at measurable levels, with data from seventeen sites in Europe, Africa, China, and Australasia having been published to date (Yang et al., 2011; Blyth and Schouten, 2013). Quantified data from Heshang Cave in China found total concentrations of GDGTs in speleothems to range from 0.15 to 4.19 ng g^{-1} dry weight of calcite (Yang et al., 2011). This compared to 0.27–0.40 μg g^{-1} dry weight in the overlying soil, and 74.4–160.7 pg in particulate matter collected from the cave drip water (Yang et al., 2011). GDGTs can be divided into isoprenoid GDGTs, where the compounds of interest are derived from Thaumarchaeota (Schouten et al., 2013), and branched GDGTs which are believed to be derived from bacteria (Schouten et al., 2013). Soils generally have higher levels of branched GDGTs (Fig. 5) and this pattern might be expected in speleothems if the GDGTs preserved are derived from the soil zone. However, in many speleothems studied to date, isoprenoid GDGTs are more abundant, with high relative abundances of crenarchaeol (Fig. 5. Yang et al., 2011; Blyth and Schouten, 2013; Blyth et al., 2014). At Heshang Cave, dominance of crenarchaeol was also seen in dripwaters, and in samples taken from the surface of bedrock within the cave, but not the overlying soils (Yang et al., 2011). Differences have also been found between soils and speleothems in compound distribution within the isoprenoid and branched GDGT classes (Fig. 5; Blyth et al., 2014). It therefore seems likely that speleothem GDGT signals are derived from the cave system, either within the cave itself, or along the dripwater transport pathway. Evidence for an in-cave rather than drip-water source was recently demonstrated in an artificial irrigation experiment (Baker et al., 2016). This contention is also supported by microbiological evidence showing communities of low temperature crenarchaeota within caves (Chelius and Moore, 2004; Gonzalez et al., 2006; Barton et al., 2014).

GDGTs preserved within speleothems show a good relationship with temperature. The published calibrations (Blyth and Schouten, 2013) are based a dataset of thirty three speleothem samples from sixteen sites in Europe, Africa, and Australasia, and show

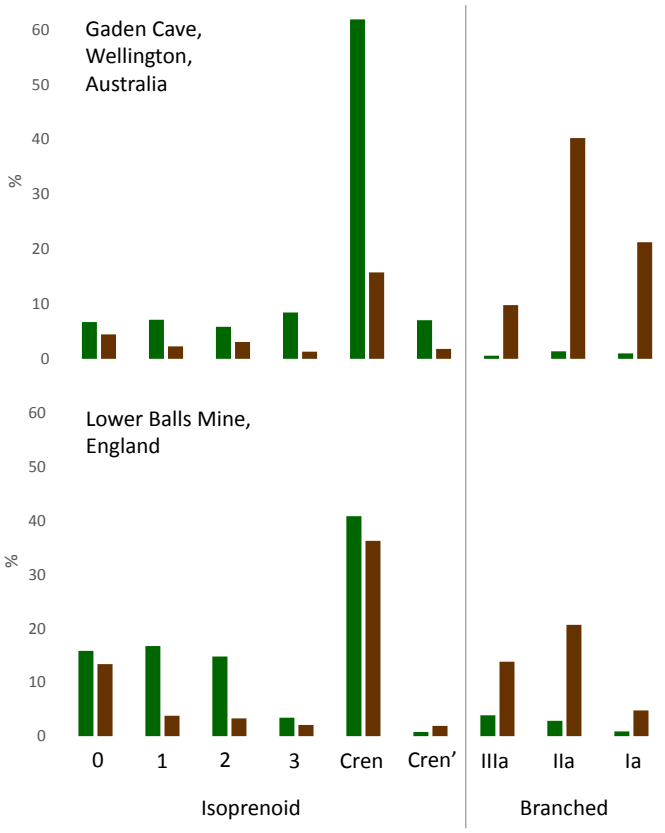

Fig. 5. Bar charts showing branched and isoprenoid GDGT distributions in speleothems (green) and soils (brown) from two sites in Australia and England. Data has been replotted from Blyth et al., 2014.

temperature correlations between surface air temperature and both isoprenoid GDGT proxies (TEX86, $r^2 = 0.78$, standard error of the estimate 2.3 °C), and branched GDGT proxies (MBT/CBT, $r^2 = 0.73$, standard error of the estimate 2.7 °C). A new preliminary calibration using a slightly expanded dataset (thirty-seven samples from eighteen sites) is shown in Fig. 6. The sample number still needs expanding, especially, given the evidence for an in-situ microbial source, with respect to sites where in-cave temperature measurements are available. Multi-proxy comparisons of GDGT-derived temperatures with techniques such as clumped isotope analysis or fluid inclusion analysis have not yet been published and

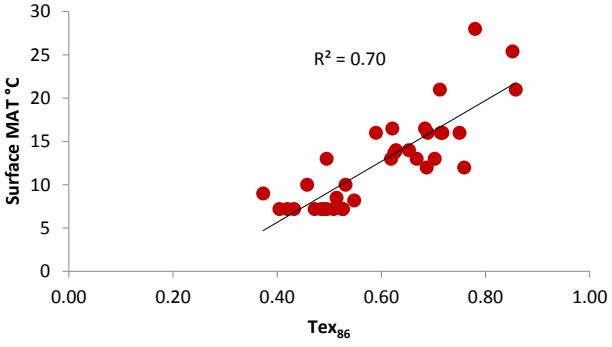

Fig. 6. A scatter graph showing the relationship between TEX_{68} and surface MAT in a newly expanded speleothem data set, consisting of data from Blyth and Schouten (2013), supplemented by three newly analysed samples, and two data points published by Yang et al. (2011). The r^2 value is lower than that in the Blyth and Schouten (2013) calibration, indicating the need for this sample set to be further increased.

would be a valuable addition to the field.

Heterogeneity of the GDGT signal within caves requires further research. Data from Blyth and Schouten (2013) showed a noticeable amount of scatter in the temperature signal between multiple speleothems from a single site, but in most cases the temperature range was within the error for the calibration. Relatively little is known about the controls on this scatter, although local variations in microbial community, microclimate, and OM input are all potential factors. The examination of additional branched GDGT isomers, which refined analytical protocols have identified in peat, soils and lake sediments (De Jonge et al., 2013, 2014; Weber et al., 2015), would also be beneficial in understanding the complexity of the GDGT signal and its relationship with cave microbiology and environment.

Aside from GDGTs, other organic temperature proxies in cave contexts are limited to an assessment of seasonal air temperature changes in fatty acid distributions in dripwaters (Li et al., 2011). Based on measurements from 2006 to 2008 at Heshang cave in China, this indicated that ratios of $nC_{16:1}/nC_{16:0}$ and $nC_{18:1}/nC_{18:0}$ fatty acids (i.e. straight carbon chain unsaturated acids with one double bond, considered as a ratio to the straight carbon chain saturated acid) had an inverse relationship to air temperature (as represented by "warm" and "cold" periods based on local measurements, but without direct quantitative calibration). More research is required to establish whether this can be developed as a proxy within speleothem records. In particular, it would be useful to know the source of these compounds (vadose zone or soil), the speed of water transport via the drip-pathway in order to calibrate the signal more precisely to the season of origin, and the extent to which the drip-water signal is preserved in the speleothems. The target fatty acids concerned are microbial compounds, and so it is entirely likely, based on the recent results from GDGT analyses (Baker et al., 2016) that an in-situ cave signal will be overlaid on the drip-water signal.

3.3. Biomarker and organic isotopic proxies for vegetation and soil conditions

Along with microbial signals from within the cave, or along the drip-pathway, there is clear evidence for preservation of allochthonous vegetation derived biomarkers in speleothems, in the form of longer chain *n*-alkanes (hydrocarbons shown to be derived from plant waxes by their carbon chain distribution, Xie et al., 2003; Blyth et al., 2007, 2011); plant derived sterols (Rousseau et al., 1995; Blyth et al., 2011); lignin phenols (Blyth and Watson, 2009; Blyth et al., 2010; Hitzemann and Hoffmann, 2016), and larger complex organic molecules inferred from the presence of fluorescent OM (Baker et al., 1996; Li et al., 2014) or directly measured by chromatographic techniques (Smailer and White, 2013; Rutlidge et al., 2015). Anthropogenic molecules such as polycyclic aromatic hydrocarbons (PAHs) have also been reported (Perrette et al., 2008, 2013).

3.3.1. Long chain n-alkanes

Of the plant-derived biomarkers preserved in speleothems, longer chain *n*-alkanes are the most studied, with carbon chain length ratios being used to interpret overlying vegetation in samples from the Last Glacial Maximum in China (Xie et al., 2003), a 100 year record in Ethiopia (Blyth et al., 2007), and from the last 2000 years in Scotland (Blyth et al., 2011). From a methodological perspective, *n*-alkanes, being simple non-polar aliphatic hydrocarbons, are easy to recover via solvent extraction from an acid digest, and highly amenable to analysis via gas chromatography. Alkanes derive from a wide range of sources including both plants and microbes, as well as potential sources of hydrocarbon contamination such as oils and plastics. However, as mentioned in Section 3.1, the number of carbon atoms in the chain varies with source. In particular, *n*-alkanes freshly derived from higher plant material are dominated by chain lengths between C_{21} and C_{37} (Bush and McInerney, 2013), and show a strong odd over even predominance – i.e. their compound distribution shows high abundances of compounds with an odd number of carbons, and a low abundance of those with an even number. Degraded *n*-alkanes from petroleum products and bacteria have no odd over even predominance (Marzi et al., 1993).

Interpretations of vegetation change from *n*-alkanes preserved in speleothems have been based on the ratios between with the abundances of C_{31} and C_{27} in a sample. C_{31} dominance has previously been associated with an increased input of grasses, while C_{27} or C_{29} dominance has been associated with woody plants (Xie et al., 2003; Blyth et al., 2007, 2011). In samples from Assynt in Scotland, C_{23} and C_{25} were also associated with sphagnum mosses (Blyth et al., 2011). These interpretations were based on previous organic geochemical studies and wide application of related proxies (e.g. Rieley et al., 1991a; Meyers, 1997; Marseille et al., 1999; Brincat et al., 2000; Pancost et al., 2002; Wiesenberg et al., 2004), and apparent relationships were found in speleothems with predicted climatic regime (Xie et al., 2003) or vegetation regimes cross-checked against other evidence (Blyth et al., 2007, 2011). However, recent studies of alkane chain length distributions in living plants, including a significant meta-analysis of published data (Bush and McInerney, 2013) has shown that the underlying assumption for use of chain lengths to interpret dominant vegetation type is not robust (Fig. 7, Bush and McInerney, 2013, 2015). The primary issue is that both gramminoids (grasses and related plants) and woody plants, especially woody angiosperms, show a very wide degree of variation within their longer chain *n*-alkane distributions (Bush and McInerney, 2013) which has not been fully accounted for in previous research. These findings do not change the interpretation of long chain *n*-alkane speleothem records where there has been a multiproxy approach (e.g. Blyth et al., 2007, 2011). They do highlight uncertainty in the use of this proxy. Whilst alkane ratios can still be used to infer periods of environmental effects on vegetation, considerable caution should be applied in the details of the interpretation.

3.3.2. Lignin phenols

If *n*-alkanes are not reliable indicators of vegetation type, then there is a need to identify alternative, more specific, plant biomarkers that can be preserved in speleothems. In soils and

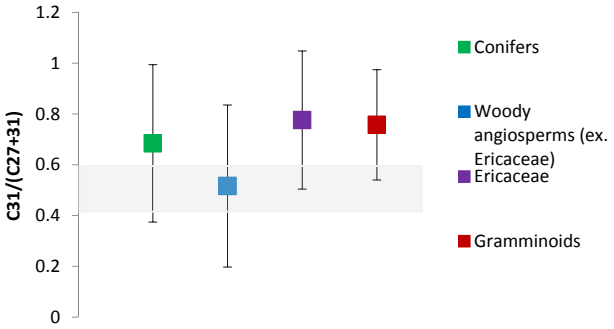

Fig. 7. The $C_{31}/(C_{27}+C_{31})$ ratios for major plant types in temperate environments, replotted from data published by Bush and McInerney (2013). Error bars are 1 s.d. The grey box shows the range of values for the same ratio calculated for the Tral-1 speleothem for which biomarker records were published in Blyth et al. (2011). It is clear that direct interpretation of vegetation type from *n*-alkane chain length dominance no longer has a robust evidence base.

sediments, terpenoids and their derivatives are often utilised in this niche (e.g. Jacob et al., 2007; Regnery et al., 2013; Garel et al., 2014), however, these have yet to be reported above the limit of detection in speleothems. The most promising avenue for future vegetation analysis therefore seems to be lignin phenols.

Lignin is a complex molecule derived from vascular plants which constitutes a significant component of OM in soils (Jex et al., 2014). It is therefore not surprising to find it preserved in speleothems. However, only limited work has been published in this context (Blyth and Watson, 2009; Blyth et al., 2010). Lignin is a complex biopolymer, so for analysis it is first broken into its constituent phenolic compounds by chemical means. In other types of sedimentary sample this has been most widely performed by cupric oxidation (CuO) (e.g. Goni and Montgomery, 2000; Kaiser and Benner, 2012; Duboc et al., 2014), which yields three main phenolic groups of environmental interest – vanillyl, syringyl, and cinnamyl (Hedges and Mann, 1979). The relative abundance of these groups show a close relationship with vegetation type, e.g. angiosperm, gymnosperm, woody, non-woody (Hedges and Mann, 1979), and these measures have been applied in a variety of environmental contexts (for a comprehensive review, see Jex et al., 2014). CuO is considered the most efficient form of digestion for bulk sediment samples with low organic abundances (Wysocki et al., 2008), and although studies using this technique have so far not been published in speleothems, unpublished preliminary data indicate the approach may have potential, especially if combined with high resolution mass spectrometry (Hitzemann and Hoffmann, 2016).

An alternative technique for the analysis of lignin derived phenols is thermochemolysis in the presence of tetramethylammonium hydroxide (TMAH) (Hatcher et al., 1995), which has the advantage of methylating functional groups on the phenolic compounds as part of the thermochemolysis process, allowing it to be performed in an online pyrolysis unit, with direct injection into a GC-MS for identification and quantification. The technique can be undertaken using small liquid samples of 5–10 μl, so allowing the extract recovered from the speleothem via solid phase extraction to be easily handled via redilution in solvent in a concentrated state, and analysis has been possible using calcite samples of 1 g (Blyth and Watson, 2009). However, a greater range of GC amenable compounds are produced by the thermochemolysis process than in CuO methods. These include products analogous to the V, S and C phenolic groups (guaiacyl, syringyl, and *p*-coumaryl respectively), but crucially, due to the breakdown and derivatisation process that occurs, it also includes superficially identical compounds derived from non-lignin sources such as tannins (Filley et al., 1999, 2006). The net result is that the vegetation index ratios which work well in the interpretation of CuO products are not equally applicable in TMAH analysis (Wysocki et al., 2008). Improvements can be achieved by using ^{13}C-labelled TMAH in the reaction (Filley et al., 1999), as this allows mass-spectrometric separation of compounds with natural methyl groups from those with methyl groups that have been added during the thermochemolysis reaction. However, this has yet to be published in speleothems. Thus, whilst studies using unlabelled TMAH have demonstrated that phenolic compounds are preserved in speleothems (Blyth and Watson, 2009), including samples dating back to 2.8 Ma (Blyth et al., 2010), and may show some relationship with known vegetation regime (Blyth et al., 2010), further methodological work is required to develop a robust vegetation proxy.

3.3.3. *Stable isotope proxies of OM*

Although stable isotopes of carbon and oxygen within speleothem calcite are widely applied in palaeoenvironmental studies (for reviews see McDermott, 2004; Lachniet, 2009; Wong and Breecker, 2015), measurement of stable isotope ratios in preserved OM are uncommon in this context, although they have been relatively more widely used in clastic cave sediments (e.g. Turney et al., 2001; Panno et al., 2004; Polk et al., 2007, 2013). Measurement of carbon isotopes within targeted organic compounds has the potential to provide an isotopic signal directly connected back to specific sources, whilst even bulk measurements may expand the amount of information recoverable about processes in the soil and vegetation, and so improve our interpretation of higher resolution calcite δ^{13}C records.

To obtain an isotopic signal explicitly linked to molecules derived from a specific source (e.g. vegetation) it is necessary to use compound specific isotope analysis (CSIA) (e.g. Rieley et al., 1991b; Chikaraishi and Naraoka, 2003). This is where an organic extract is first separated by gas or liquid chromatography, and then each compound peak is measured by isotope ratio mass spectrometry (Meier-Augenstein, 1999). CSIA of δ^{13}C has been attempted on plant derived *n*-alkanes in speleothems (Blyth et al., 2013b), but only on a very limited scale, as the available extracts were near the measurement limit of the technique and so robust characterisation of errors was not possible. Nevertheless, CSIA of OM in speleothems is clearly possible, if likely to be restricted to larger sub-samples or organic rich specimens. Future investigation should focus on establishing the limitations of the technique and a framework for interpretation.

The isotopic analysis of bulk OM in speleothems is simpler than CSIA, but has been rarely attempted, probably due to the difficulty in separating the OM from the acid digest without retaining acid salts, losing water-soluble portion of the sample, fractionating the signal or adding contaminatory carbon. Where acid salts remain within the sample, they may potentially damage some components of the Elemental Analyser, so not all laboratories will permit the work. Nonetheless, measurement of δ^{13}C in speleothem derived OM by elemental analysis has yielded promising results from samples initially composed of 2 g of calcite (Li et al., 2014). The analytical error of the technique has been calculated as 0.2–0.4‰, which is larger than the error for carbonate δ^{13}C measurements in the same sample (Hu et al., 2008; Li et al., 2014), but still useable for the creation of palaeoenvironmental records (Li et al., 2014).

An alternative method for measuring δ^{13}C was proposed by Blyth et al. (2013a), taking advantage of the flow-injection mode on a liquid-chromatography-isotope ratio mass spectrometer. The basic technique mixes the sample liquid flow with an acid and oxidant before reaction to convert the carbon to CO_2 for measurement (Blyth and Smith, 2015). The approach has the advantage of being able to measure both DIC (by using only the acid to create milder oxidation conditions which do not convert the organic carbon), or DOC (by purging the sample in advance to remove DIC, and then analysing with both the acid and oxidant). The technique also allows analysis of 0.2 g calcite at 0.011% TOC (equivalent to 23 μg of organic carbon in each subsample, Blyth et al., 2013a, 2013b). Errors for the LC-IRMS technique were also comparable to the EA approach at 0.2‰, although it is worth noting that the elemental analysis technique was cross-checked in two laboratories (Li et al., 2014), whereas the LC-IRMS technique has not yet been replicated on a second set of instrumentation. Therefore the errors of the former might be considered more robust at this stage.

4. High resolution and minimally destructive techniques

A limitation with the biomarker techniques discussed above is that they are destructive of the speleothem sample, and require sample sizes of calcite (0.2 g – around 20 g) that provide a much coarser temporal resolution than that obtainable with inorganic geochemical techniques. For example in Blyth et al. (2013b), δ^{13}C of

the calcite was obtained on 0.8 mg samples, while $\delta^{13}C$ of the bulk OM required 200 mg. Accordingly, improving the temporal resolution techniques for the analysis of OM in speleothems needs to be a priority for future research.

4.1. Fluorescence

Fluorescent OM contained within speleothems preserves paleoclimate and paleoenvironmental information on timescales from sub-annual to millennial (e.g. Shopov et al., 1994; Baker et al., 1998, 1999; Linge et al., 2009; Orland et al., 2014; Quiers et al., 2015). The fundamentals of this approach have been previously reviewed (e.g, McGarry and Baker, 2000; Blyth et al., 2008), but here we also consider how recent advances in freshwater and marine science, and environmental engineering can improve our understanding of fluorescent OM in cave contexts.

It is well recognised that a fraction of dissolved OM fluoresces when excited by higher energy, ultra-violet and visible light (Coble et al., 2014). Predominantly, it is aromatic groups within NOM that have weakly bound electrons that can be excited in the dissolved phase, emitting fluorescence in the longwave UV and blue-violet wavelengths (360–450 nm). These structures are associated with lignin-derived material (Aiken, 2014) and are often described as 'humic-like substances' as they have similar optical properties to humic and fulvic acid standards. The 'humic-like substance' nomenclature is misleading, as other fluorescent substances can also fluoresce in this region, and the analysis of model compounds (Barsotti et al., 2016) suggests that fluorescence in this wavelength region is the expected end-member of OM with three or more phenolic compounds. Fluorescence is also emitted at UV wavelengths (300–350 nm) from OM with one or two aromatic rings such as tryptophan and tyrosine-like molecules, associated with microbial activity (Hudson et al., 2008), as well as from other low molecular weight, aromatic compounds such as polycyclic aromatic hydrocarbons (PAHs) (Baker et al., 2014).

Fluorescence and absorbance spectroscopy are widely used techniques for the characterisation and quantification of DOM in surface water, marine and water engineering applications (for a comprehensive multidisciplinary study see Coble et al., 2014). Within the fluorescence emitted at 360–450 nm from 'humic substances', there is a general observation that NOM with a lower emission wavelength is more hydrophilic and less aromatic (Gabor et al., 2014; Ishii and Boyer, 2012). The existing paradigm is that biological processing of OM produces fluorescent DOM (fDOM) with a shorter wavelength than its precursor material. The total fluorescence intensity in this region in general correlates with DOC concentration (Cumberland and Baker, 2007), although this individual fluorescence – DOC regressions can vary between sites and over time (Tissier et al., 2013). The fluorescence intensity in the tryptophan-like fluorescence region at 350 nm correlates with the biochemical oxygen demand and microbial activity (Hudson et al., 2008). When fluorescence excitation – emission matrices are generated from a water sample and analysed by parallel factor analysis (PARAFAC), a minimum of three components to the fluorescence signal can be identified (Ishii and Boyer, 2012). These models typically separate the fluorescence at long-UV wavelength emission (360–450 nm) into two components (of higher and lower emission wavelength), and also model the fluorescence emitted at 350 nm. Although it is not known if these model components are physically meaningful (e.g. relate to actual chemical compounds), the separation of fluorescence in the 360–450 nm emission region into two components agrees with the general observation that aromaticity and hydrophilicity of NOM is related to fluorescence emission wavelength (McKnight et al., 2001; Baker et al., 2008b). The observed decrease in NOM fluorescence emission wavelength during transport from soil and cave drip water (Baker and Genty, 1999; van Beynan et al., 2000) fits into this conceptual framework.

Within speleothem research, only a limited number of studies include DOM excitation-emission matrix (EEM) fluorescence in their cave monitoring. The limited use of fluorescence DOM analyses in drip waters is particularly surprising given that it requires a small samples sizes (<4 ml) and no pretreatment, and can also be used in-situ. Recent exceptions include Hartland et al. (2012) and Rutlidge et al. (2014, 2015). Rutlidge et al. (2014, 2015) used PARAFAC to identify the three common fluorescence components (Ishii and Boyer, 2012) in drip water samples. Interpreting increases in component scores for the long-UV wavelength fluorescence components as a quantifiable measure of soil-derived DOM in the dripwater, this was used together with inorganic geochemical analyses to identify soil vs limestone water residence times in speleothem forming drip-waters. fDOM measurements can provide information on OM processing from soil to speleothem, including relative changes in DOC concentration and the hydrophobicity of the fluorescent fraction, which will help understand the wider biomolecular signals contained in speleothems.

Several recent studies have improved our understanding of OM fluorescence within speleothems. Smailer and White (2013) compared for the first time speleothem fluorescence properties with the chromatography of the extracted OM, and demonstrated a typical molecular weight of the fluorescent fraction of 4000–6000 Da, a range typical of humic-like substances. Analysing Holocene stalagmites, Quiers et al. (2015) derived a non-linear relationship between speleothem fluorescence intensity and total organic carbon analyses to create a surface map of speleothem organic carbon, and propose an alternative approach to speleothem TOC measurements. Paleoclimate records derived from speleothem fluorescence records continue to focus on annual-scale variability and the development of annual records of growth-rate (e.g. Baker et al., 2015; Driese et al., 2015) or changes in flow regime (Orland et al., 2012). Orland et al. (2012) characterised the fluorescent annual growth band pattern in a speleothem formed over the last 34 ka to infer changes in flow regime and the seasonality of precipitation. Driese et al. (2015) compared annual growth rates determined from 4796 fluorescent laminae to stable isotope data to obtain a Holocene climate record from the USA. Using annual fluorescent laminae, Baker et al. (2015) compiled a composite record of annual growth rate from five stalagmites to obtain a record of the North Atlantic Oscillation for the last 3 ka. These studies all show that OM fluorescence, whilst one of the older techniques available, is still one of the most robust approaches for applying OM studies in speleothems to Quaternary science.

4.2. Electron paramagnetic resonance

Electron Paramagnetic Resonance (EPR) is another spectroscopy technique that is capable of detecting distinct characteristic signals that may be considered fingerprints of OM sources (Perette et al., 2015). It has previously occasionally been used in speleothems for dating purposes (Ikeya, 1975), but is generally under-exploited in this context, especially with regard to OM. Perrette et al. (2015) have recently applied this technique to identifying presence of OM derived from different soil types within a stalagmite and flowstone, using the EPR lines of semiquinone-type radicals as fingerprints. The results were unexpected, showing a different soil signature in the two analysed samples. The flowstone signature was also much stronger than that in the speleothem, despite broadly similar TOC levels. After consideration of other possible mechanisms for this, Perrette et al. (2015) concluded that the difference in signal and strength and therefore free-radical concentration was most likely a function of the time the OM spent in the

transport pathway, with longer transport times allowing conformational changes in the parent humic substances. This is consistent with the hypothesis presented here in Section 2, Fig. 3. There is clearly promise in this technique both for tracing the soils contributing to different flow paths and for investigating changes in soil composition over time. However, to fully understand the signals received, and why two comparable speleothems may preserve signals from different soils requires a larger proof of concept sample set to be investigated.

4.3. Laser micropyrolysis gas-chromatography mass-spectrometry

Fluorescence analysis provides potentially subannual resolution, but its major limitation is in the degree of the molecular characterisation possible, for example in terms of detecting biomarkers. Complementary approaches offering increased chemical detail are therefore also required. One recently tested technique is laser micropyrolysis gas-chromatography mass-spectrometry (Blyth et al., 2015). This approach uses a laser to ablate OM, with the resultant gas being collected on-line and the injected into a GC-MS for separation and identification of the compounds. The approach has been used in a wide range of contexts (see Blyth et al., 2015 and references therein) but has not previously been applied to Quaternary science.

In the existing study, distinct compound signatures were seen between guano inclusions and other detrital inclusions, and dissolved OM believed to be of humic origin (Blyth et al., 2015). However, the data was not extensive enough to establish if these provide robust source signatures, and it is important to note that the approach was limited to organic inclusions and one sample with abnormally high organic abundances within the crystal matrix, as the available infra-red laser was unable to ablate standard calcite crystals. This means that the technique has yet to be tested on conventional speleothem laminae. Further research, especially that seeking an optimal laser system allowing both ablation of the calcite and collection of the OM is needed.

5. OM interactions with trace elements

A significant future option for high resolution analysis of OM in speleothems is the use of trace elements as a proxy for dripwater solution OM concentration and composition. This has major positive implications for the use of speleothem OM in Quaternary science, offering analyses at a far higher temporal resolution than possible with any other technique.

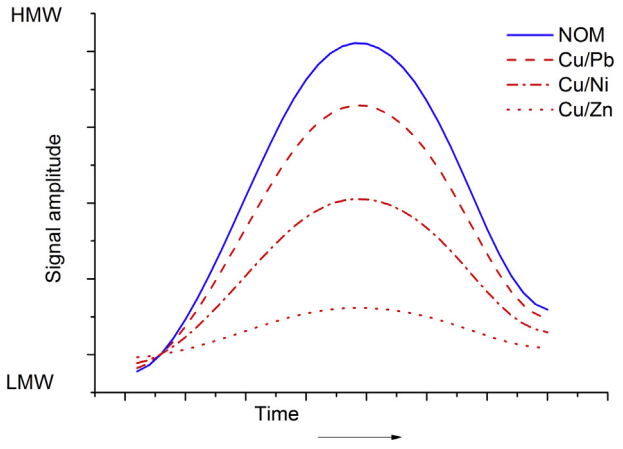

Fig. 8. Conceptual figure summarising the hypothetical response in trace metal pairs for a given fluctuation in NOM average molecular weight. Metals which show the greatest fractionation between NOM molecular weight fractions such as Cu and Pb are predicted to record the largest response to a transient high molecular weight (HMW) NOM pulse in dripwater which is superimposed on a ubiquitous LMW background NOM composition.

5.1. Early data from speleothems

The use of trace elements as proxies for OM in speleothems is rooted in work on the Grotta di Ernesto, Italy (Huang et al., 2001), which culminated with Borsato et al. (2007), who described the hierarchical association of trace element enrichments (Y > Zn, Cu and Pb > P and Br) on annual laminae resolvable either under transmitted light or UV (fluorescent) microscopy. Importantly, Borsato et al. (2007) demonstrated that a suite of elements show coherent, spatially discrete enrichments (at micron resolution) within speleothems. The driver of these enrichments was interpreted to be a direct pulse of colloidal/dissolved OM flushed from the soil zone by autumnal infiltration. This interpretation was corroborated by Hartland et al. (2012) who reported a temporal coincidence of elevated concentrations of transition metals, higher NOM concentrations and fluorescence intensities in dripwaters, indicative of simultaneous transmission of particulates, fine colloids and DOM.

Fairchild et al. (2010) applied highly detailed μ-XRF to stalagmites from Obir cave, Austrian Alps, and proposed that micron-scale enrichments in trace elements arise due to crystallographic, temperature or hydrological processes. This is based on the foundation of previous experimental and field evidence and is sensible in terms of the chemical affinity of the suites of elements encompassed by this interpretation. Of these mechanisms hydrologic and crystallographic factors are likely to have the most impact on the trace element signal originating from OM.

5.2. NOM-metal complexes in dripwaters

Several studies have shown that the first-row transition metals in cave waters from vanadium to zinc, have some degree of association with material in the colloidal size range (Hartland et al., 2011, 2012) as seen in water samples from other aquatic systems (Buffle, 1988; Stolpe et al., 2005). Colloidal material is defined as any particle with a dimension between 1 nm and 1 μm, and encompasses a range of organic and inorganic species (Lead and Wilkinson, 2006), often found combined as aggregates of OM, Fe oxides and aluminosilicates at larger particle diameters (Hartland et al., 2011). These studies unanimously point to colloidal and truly-dissolved (dimensions < ca. 1 nm) NOM as being the only viable phases yet identified in cave drip-waters capable of forming abundant complexes with transition metals (Hartland et al., 2011). Inorganic phases detected include iron and manganese oxides (Hartland et al., 2010), which are environmentally important and capable of binding trace metals (Lyvén et al., 2003). However, the concentrations of these elements in dripwaters are generally an order-of-magnitude too low to support the patterns of colloidal association observed (Simon et al., 2007; Toth, 1998).

Few studies have examined the relationship between the colloidal speciation of trace elements in dripwaters and variations in cave hydrology (Hartland et al., 2012), but work from shallow caves appears to confirm the inter-dependence between colloidal delivery of NOM-complexed trace metals and effective infiltration (Baldini et al., 2012; Hartland et al., 2012; Rutlidge et al., 2015).

As well as having hydrological relevance, metals carried by OM in dripwaters also retain information on the composition of the OM. Changes in the ratio of Cu:Ni, for example, may reflect variations in OM molecular weight. Hartland et al. (2012) observed that Cu:Ni data plotted on linear trends which were consistent with two distinct end-member compositions: experimental soil leachates

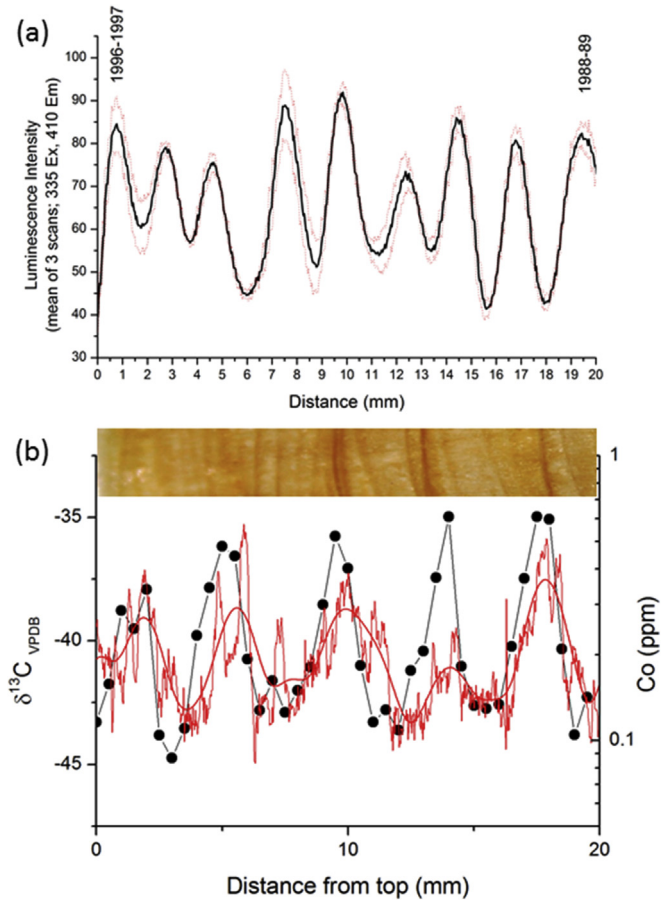

Fig. 9. Similar cyclical variations in humic-like fluorescence intensity (a; Baker et al., 1999), cobalt concentration (red line) and stable carbon isotopes ($\delta^{13}C$, black points) in hyperalkaline carbonates formed beneath the PE1 drip point in Pooles Cavern, Buxton, UK.

from the studied caves, and humic/fulvic acid Cu:Ni binding affinity ratios (Milne et al., 2003). The underlying mechanism for changes in the ratio of Cu:Ni is hypothesised to be the distribution of functional groups present within the respective NOM end-members, but this is yet to be fully tested. Indirect supporting evidence has been supplied by Rutlidge et al. (2015) who found significant correlations between DOM molecular weight (based on size exclusion chromatography) and Cu:Ni at one of their sites. In the entire dataset, DOM components were well correlated with Cu and Ni ($R_2 = 0.76$ and 0.80, respectively).

The results of the aforementioned studies lead to the hypothesis that transition metal ratios in speleothems (such as Cu:Ni) could be used to trace changes in the composition of NOM delivered in dripwaters. Only limited data on the molecular weight size distribution of NOM-metal complexes exists from caves (Hartland et al., 2011), but we can consult data from other circumneutral freshwater environments. To a first approximation, transmission of LMW hydrophilic NOM is predicted to coincide with an increase in Cd > Pb > Zn > Cr > Co > Ni > Cu, with the series reversed with transmission of HMW, hydrophobic NOM (Wu et al., 2004). Given the very low abundance of Cd in unpolluted systems Cd may be less susceptible to contamination, thus the trace metal pairing of Cu:Cd may provide the greatest sensitivity to the full range of NOM molecular weight distributions. Discussion to date has focused on Cu:Ni, however this is largely the result of analytical bias toward these more abundant elements. Given the preference of both Cu and Ni to complex with HMW NOM, and the poor correlation of these elements with lower molecular weight components (Rutlidge et al., 2015), Cu:Ni is likely to show a lower sensitivity to fluctuations in the proportions of averaged HMW and LMW natural OM end-members, and so Cu:Pb or Cu:Cd is advocated as a more appropriate proxy for further development (Fig. 8).

Finally, it is important to mention the oft-noted association of halogens (Br, I) with natural aqueous OM (Stolpe et al., 2005; Gilfedder et al., 2007, 2010). Organo-halogens may provide another tracer for OM in speleothem-forming systems. Again, progress in this area is hampered by a lack of direct speciation measurements on the target elements.

5.3. Signal capture in speleothems and experimental precipitates

The prospect of trace element proxies for OM in speleothems is clearly attractive on the basis of the wealth of high-resolution trace element data now available from techniques like laser-ablation (LA) ICP-MS (Jamieson et al., 2015), and micrometer-resolution synchrotron x-ray fluorescence (XRF) (Wynn et al., 2014). Such analyses are now becoming routine and provide a wealth of chemical data for reconstruction of environmental change. To provide this type of research with an organic dimension, we need to address the question of how NOM and complexed trace elements are captured by carbonates, which has been the subject of comparatively few studies (Chalmin et al., 2013). The literature on trace metal partitioning into minerals has almost entirely focused on inorganic systems (Prieto and Stoll, 2011). There is one study that we are aware of on the formation of ternary complexes at speleothem surfaces (Hartland et al., 2014). Ternary complexation can be viewed as co-adsorption of metal cations and organic molecules at the same mineral surface site (Eqn (1)).

$$\equiv SOH^{0}_{(s)} + L - M^{(l^- + m^+)}_{(aq)} + H^{+}_{(aq)} \quad \leftrightarrow \equiv S - L - M^{(m-l+1)}_{(s)} + H_2O_{(aq)} \tag{1}$$

where ≡S represents a generic, crystallographically bound metal cation, S, at the surface-water interface, and $L - M^{(L^- + M^+)}$ represents the organic acid-cation complex (Fein, 2002).

Ternary complexes correspond to a situation where aqueous complexes with high stabilities are adsorbed at a ratio of 1. Although no direct spectroscopic measurements have been made in speleothem-forming systems, evidence from experimental studies suggests that ternary complexes are not favoured (Lee et al., 2005). What can be said with certainty is that divalent metal cations such as Cu(II), Pb(II) and Zn(II) display a strong affinity for calcite surfaces, forming complexes (Chada et al., 2005; Godelitsas et al., 2003; Zachara et al., 1991) that are stable over long timeframes (Elzinga et al., 2006). These provide a sink for metals at the calcite-water surface.

The best evidence for non-ternary complexation of trace metals in speleothem-forming systems comes from apparent partition coefficients derived from a comparison of the metal:NOM ratio in dripwaters and speleothems (Hartland et al., 2014). This analysis used a speleothem (PC-08-1) and drip-site (PE-1) from the hyperalkaline Poole's Cavern cave system in England, which has been previously studied (Baker et al., 1999). The research showed that the ratio of metal-to-ligand (based on TOC measurements) in the stalagmite was much higher than in the drip-water for V, Ni and Cu, with the exception of Co which partitioned at a ratio of 0.9. This finding was consistent with the measured dissociation kinetics of NOM-Co in the PE1 dripwater (Hartland et al., 2011), which was much slower than other metals in this system: supporting the hypothesis that Co variations closely relate to adsorption of

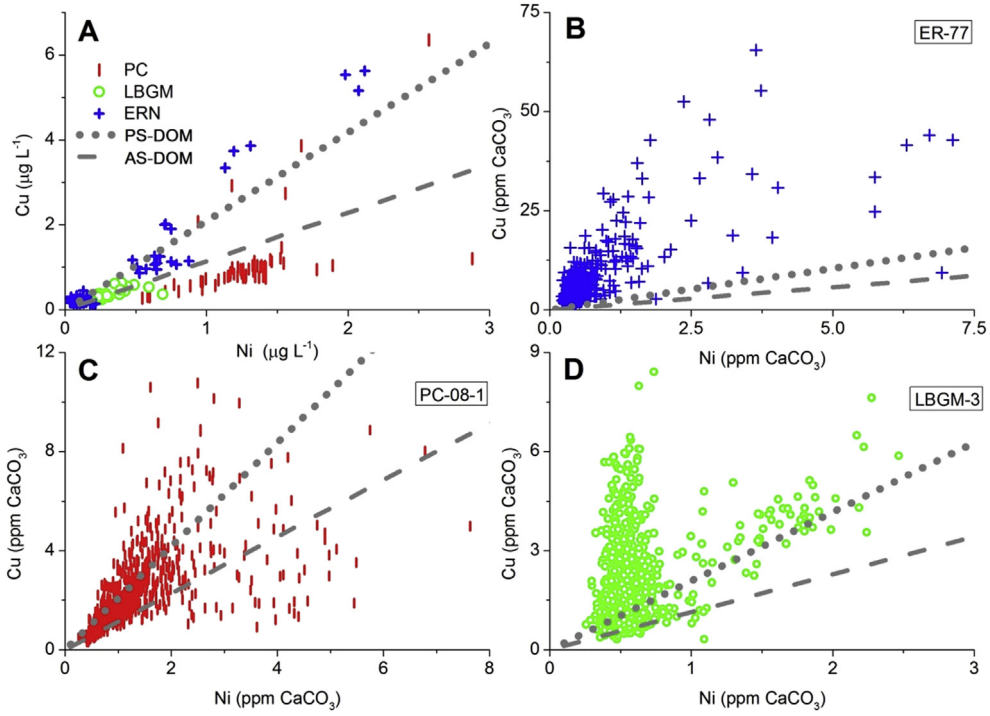

Fig. 10. Inferred competitive binding of Cu and Ni ions by NOM in cave waters from Pooles Cavern (PC), Lower Balls Green Mine (LBGM) and Grotta di Ernesto (ERN) (a; Hartland et al., 2012). Dripwater data plot between poorly-soluble (PS) and acid-soluble (AS) DOM end-members (equivalent to humic and fulvic terminology). In B-D, laser-ablation ICP-MS data for Cu and Ni in the conjugate stalagmites (ER-77 from Grotta di Ernesto (B), PC-08-1 from Poole's Cavern (C) and LBGM-3 from Lower Balls Green Mine (D)) are shown relative to AS-DOM and PS-DOM metal ratios (Data from Hartland PhD Thesis, 2011).

nominally-dissolved NOM in the speleothem (ternary complexes). When compared to the fluorescence signal from stalagmite PC-97-1 which grew from the same drip point (Baker et al., 1999), the cyclical variation in Co shows remarkable similarity to the fluorescence signal, and appears to corroborate the argument that Co fluctuations in the PC-08-1 stalagmite relate to kinetically-driven variations in the absorption of NOM (Fig. 9). The strong fractionation in stable isotopes associated with cycles of fluorescence and Co in the Poole's Cavern samples, highlight one additional point: that the adsorption and occlusion of NOM by speleothems is likely to show some dependency on growth rate, albeit accentuated by the very fast growth rates encountered in that setting.

Further evidence for metal partitioning of NOM-metal complexes between dripwaters and speleothems is provided in Fig. 10, in which the concentrations of Cu and Ni are shown in X–Y plots. The data in Fig. 4 demonstrate that the characteristic ratios of Cu:Ni of OM end-members identified in Hartland et al. (2012), are modified during speleothem formation, with a positive bias toward Cu. These results therefore indicate that partitioning will alter the ratio of NOM-metal complexes between drip-water and speleothem and that the ratios in speleothems must therefore be corrected before being interpreted as a proxy for OM composition. According to Hartland et al. (2014), the calculation of the solution composition with respect to a given metal can be obtained from Equation (2).

$$\left[M_{(aq)}\right] = \frac{\left(\frac{\left[M_{(s)}\right]}{\left[Ca_{(s)}\right]} \times \left[Ca_{(aq)}\right]\right)}{f_M} \quad (2)$$

where $[M_{(aq)}]$ and $[Ca_{(aq)}]$ are the aqueous total metal and calcium concentrations, $[M_{(s)}]$ and $[Ca_{(s)}]$ are the total metal and calcium concentrations in the carbonate, K_d is the inorganic distribution coefficient for M, and f_M is the fraction of metal able to dissociate and be incorporated in the carbonate. The term f_M effectively encompasses the 'free' metal (i.e. present in simple inorganic complexes) and the metal contained in labile NOM-metal complexes.

For systems where the proportion of truly free metal is close to zero, as may be the case for many cave waters (Hartland et al., 2011, 2012), the control on the incorporation of a given metal is governed almost completely by the rate of dissociation of NOM-metal complexes, which have been shown to follow first-order rate expressions (Hartland et al., 2011). Under these specific circumstances the original ratio of a trace metal pair in solution (hypothetically representing the average NOM molecular weight) may be approximated by Equation (3).

$$\frac{\left[M_{1(aq)}\right]}{\left[M_{2(aq)}\right]} = \frac{\left(\frac{\left[M_{1(s)}\right]}{\left[M_{2(s)}\right]} \times \left[Ca_{(aq)}\right]\right)}{\frac{K_{d1}}{K_{d2}}} \Big/ \frac{K_1}{K_2} \quad (3)$$

where M_1 and M_2 are the metal cations representing the HMW (usually Cu) and LMW (ideally Cd or Pb) endmembers, K_{d1} and K_{d2} are the corresponding inorganic partition coefficients and k_1 and k_2 are the corresponding NOM-metal dissociation rate constants.

In many cases, appropriate partition coefficient values for trace metals are not available in the literature (Day and Henderson, 2013). This represents a significant gap in our ability to interpret trace elements in speleothems as proxies of OM, or indeed more generally.

We are now approaching a point where trace metals may be feasibly utilised to derive information regarding organic ligand

Table 1
A summary table listing the main organic proxies available in speleothem work, along with their sample size and methodology, potential information available, limitations, and priority areas for future work.

Technique	Sampling method and analytical approach. (sample size of calcite in g)	Main forms of environmental information recoverable	Summary and current limitations	Priority directions for future work
Biomarkers − *n*-alkanes	Destructive, extracts analysed by GC-MS. (0.3–20 g)	Changes in overlying environment/vegetation	Although changes in response to environmental perturbations are apparent, it is clear that this proxy is no longer suitable for assessing vegetation type.	*n*-Alkanes are easily analysable via established methodologies. Focus now needs to be in understanding the interpretation of their response to environmental change
Biomarkers - lignin	Destructive, extracts processed by CuO or TMAH Thermochemolysis, and then GC-MS. (1 g approx. for TMAH; unknown for CuO)	Changes in overlying environment, probably via change in vegetation regime, although may also be affected by soil degradation and transport	Lignin phenol composition is known to link closely to vegetation type, although an as yet unquantified degree of processing is likely between the soil and speleothem. Additional work is needed to demonstrate whether coherent vegetation records can be preserved.	Proof of concept work on CuO methods in speleothem samples with known overlying vegetation, as this will give the clearest test of lignin as a vegetation proxy. Application of ^{13}C labelled TMAH to build on existing studies, and provide an alternative methodology for small calcite samples.
Biomarkers - GDGTs	Destructive, extracts analysed by LC-MS (5–20 g used in calibration work)	Quantitative or semi-quantitative temperature (cave or surface)	A linear calibration exists between speleothem GDGT proxies and measured surface air temperature, but scatter demonstrates influences by as yet unknown factors. Relatively large samples sizes have been used to date, limiting temporal resolution.	Expansion of the calibration data set, especially for samples with known cave temperatures. Investigation of variation in GDGT composition with in-cave variables such as location, speleothem morphology, and cave micro-environment.
Biomarkers - other	Destructive, extracts analysed by GC-MS or HPLC (0.3–20 g)	Probably links with overlying environmental change, possible links with temperature.	A range of other biomarkers include fatty acids, alcohols, ketones and sterols have been detected in speleothems, and in some cases shown to vary with known environmental changes. However, the most appropriate interpretation of these changes is not yet known.	Identification of the most appropriate compound classes for further study. Testing whether newer forms of instrumentation such as GC-GC-MS can help measure low abundance compounds such as terpenoids. Alternative ultra-high resolution instrumentation such as ESI-FT-ICR-MS offers the potential to significantly increase temporal resolution and analysable compound type.
Bulk organic $\delta^{13}C$ analysis	Destructive, extracts analysed by either LC-IRMS (0.2 g) or EA-IRMS (2 g)	Carbon isotope measurements from the bulk organic matter, mostly originally derived from the overlying soil, with possible additional contributions from OM in the vadose zone and in-cave.	Clear palaeoenvironmental records can be derived with this technique showing response to environmental perturbations, and strong promise to expand available isotopic records by pairing with data from the inorganic carbon $\delta^{13}C$. Precise interpretation of these records, and the variation in interpretation between sites is not yet established.	Further method development work in the LC-IRMS technique to verify any variation with instrumental parameters. Application in a much wider range of records to refine interpretation. Paired analysis with soil-derived biomarkers such as fluorescence or lignin phenols.
Compound-specific $\delta^{13}C$ analysis	Destructive, extracts analysed by GC-IRMS (approx. 20 g)	Carbon isotope measurements derived directly from specific parts of the ecosystem, e.g. vegetation (n-alkanes, lignin), soil and cave microbes (fatty acids, possibly GDGTs)	The underlying concept and methodology for this approach is well established from other environmental records. The limitation in speleothems is the low concentration of specific organic compounds, which provides an analytical limit to the detection of the minor isotope. The technique has only been published once in speleothems with ambiguous results and lacking robust errors.	Further proof of concept work in this context to constrain the necessary sample size, errors, and interpretation in relation to environmental parameters.
Fluorescence spectroscopy	Minimally destructive spectroscopy with potential for sub-annual resolution.	A predominantly soil-derived proxy, which gives a broad scale characterisation of the OM contained in the speleothem, and potential to identify climatic variations and changes in soil OM characteristics	Very well established technique. Main limitation is the limited molecular detail available.	The overall field would benefit from fluorescence characterisation being paired more regularly with other organic and inorganic proxies.

(*continued on next page*)

Table 1 (*continued*)

Technique	Sampling method and analytical approach. (sample size of calcite in g)	Main forms of environmental information recoverable	Summary and current limitations	Priority directions for future work
Electron Paramagnetic Resonance	Minimally destructive spectroscopy, with potential for annual resolution	Potential for identifying OM inputs derived from different soil types	New technique in speleothems, which needs to be applied in more samples to establish broad applicability. Knowing drip-water flow and transport pathway may be crucial in reliability.	Application of the technique in a broad range of samples from known soil regimes and drip-water transport pathways.
Laser pyrolysis	Destructive, in that the analytical rigs require 5 mm × 5 mm sample blocks. Ablated products analysed by GC-MS. Potential for annual or decadal resolution	May be able to characterise bulk OM signals at a high resolution	New technique, currently only applicable to solid organic inclusions, rather than OM preserved in the calcite crystal structure. Differences have been observed between inclusions, but firm interpretations of source are not yet possible.	Development of the technique with alternative laser sources to allow OM preserved in the calcite laminae to be analysed.
Trace element analysis	Partially destructive by LA-ICPMS (ablation can be undertaken on slabs, but some damage can occur). Sub-annual resolution possible.	Characterisation of changes in OM input via analysis of changes in complexed trace elements.	Analysis of trace elements in their own right is an established and growing field in speleothems. Using trace elements as a proxy for complexed OM with which they are transported from the overlying soil has high potential, and the main limitation is currently the relatively small amount of research in this field. Detailed molecular information is unlikely to be possible.	Calibration of the trace element ratios to the OM concentration and characteristics Experimentation to better understand partitioning during deposition into the calcite Experiments to extract and characterise the NOM from layers where trace elements are also measured to improve interpretation.

composition and will be useful in other locations in combination with careful, targeted analysis of dripwaters and speleothems. We suggest a few, key experiments are needed to cement the relationship between trace metals and NOM for their unambiguous interpretation in speleothems:

- Size exclusion based analysis of trace metals in cave waters along with NOM (e.g. by UV–Vis spectroscopy/fluorescence) to calibrate the metal ratio: molecular weight relation.
- Experimental measurements of metal partitioning from NOM complexes into carbonate.
- Extraction and characterisation of NOM from speleothems and analysis of trace metal ratios at the same intervals.

6. Conclusion

Recent research shows that the use of OM in speleothems is a growing field, with good potential for contributing to cave and Quaternary science, and it is now possible to identify what proxies are worth pursuing and where their advantages and limitations lie. Table 1 summarises the techniques currently available, their most appropriate uses and limitations, and the areas associated with each that we consider should be a priority for future research. We strongly encourage workers speleothem science to consider how analysis of OM in speleothems might add useful information to their research outcomes, with the aim of both stimulating this future research, and of increasing the geographically and environmentally broad data-sets the field needs to understand the full complexity of OM sources and preservation in karst systems and produce truly robust proxies that will add to our understanding of past and future environments.

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