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Molecular isotopic heterogeneity of fossil organic matter: implications for $\delta^{13}C_{biomass}$ and $\delta^{13}C_{palaeoatmosphere}$ proxies

Imogen Poole [a,b,*], Pim F. van Bergen [a], Johan Kool [a], Stefan Schouten [c], David J. Cantrill [d]

[a] *Faculty of Earth Sciences, Organic Geochemistry Group, University of Utrecht, P.O. Box 80021, 3508 TA Utrecht, The Netherlands*
[b] *Palaeontological Museum, Oslo University, P.O. Box 1172, Blindern, N-0318 Oslo, Norway*
[c] *Royal Netherlands Institute for Sea Research, Marine Biogeochemistry and Toxicology, P.O. Box 59, 1790 AB Den Burg, The Netherlands*
[d] *Swedish Museum of Natural History, Palaeobotany, P.O. Box 50007, Stockholm 104 05, Sweden*

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Abstract

The degree of isotopic variation in fossil organic matter renders bulk $\delta^{13}C$ signatures strongly influenced by molecular isotopic heterogeneity. For example, in fossil wood the relative abundance of less depleted ^{13}C moieties, i.e. preserved ^{13}C enriched polysaccharides versus the relatively ^{13}C depleted lignin moieties, can be seen to significantly bias $\delta^{13}C_{fossil\ wood}$ values. Moreover the variation in $\delta^{13}C$ values of specific compounds within fossil material are themselves highly variable and reflect the heterogeneity in isotopic values of different carbon atoms within individual compounds. For studies using $\delta^{13}C$ values of fossil plant material as proxies (e.g., for $\delta^{13}C_{palaeoatmosphere}$, $\delta^{13}C_{biomass}$) it is recommended that the biases introduced through molecular heterogeneity, preservation type and taxonomic status of the fossil material are determined initially. Biases inherent in the bulk signature can then be reduced, rendering this value more robust. Alternatively, compound specific stable carbon isotope measurements of individual moieties preserved through geological time might prove to be an alternative proxy for monitoring changes in the bulk $\delta^{13}C$ value of the plant and might reveal atmospherically induced trends.

1. Introduction

Stable carbon isotope ($\delta^{13}C$) data are an important prerequisite in global carbon models in order to constrain carbon flows from one reservoir to another and for understanding more fully terrestrial ecosystems in the past. Obtaining reliable $\delta^{13}C$ values for the atmosphere (i.e. CO_2) and biomass (i.e. the $\delta^{13}C$ signature of the bulk of organic matter, $\delta^{13}C_{biomass}$) reservoirs in the geological past is pivotal to many of these models. A terrigenously-based $\delta^{13}C$ signature derived from fossil plants may provide a more direct proxy for relative changes in the $\delta^{13}C$ values of the palaeoatmosphere ($\delta^{13}C_{palaeoatm}$; Gröcke et al., 1999) to document, for example, rapid and massive emissions of ^{13}C-depleted methane from gas hydrates (Hesselbo et al., 2000) or

* Corresponding author. Tel.: +31 30 2535068; fax: +31 30 2535030.
E-mail address: i.poole@geo.uu.nl (I. Poole).

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the drawdown of CO_2 during oceanic anoxic events (Prokoph et al., 2001). Reconstructing events primarily affecting the atmosphere from the marine fossil record (1) introduces a large time lag between the fossil record and the event, (2) dampens the signal due to the huge size of the oceanic carbon pool as compared with the atmosphere and (3) neglects changes in oceanic carbon chemistry and temperature.

One good source of terrigenous $\delta^{13}C$ values is that derived from fossil plant material. However, plant material is subject to intrinsic chemical variation with each moiety having its own $\delta^{13}C$ signature which in turn affects the bulk value measured for the fossil plant. Therefore, more discrete fossil entities, such as fossil wood, have been used recently to this end. The potential of fossil wood, and in particular charcoal, as a $\delta^{13}C_{palaeoatm}$ proxy has been recognised by a number of workers (e.g., Gröcke et al., 1999; Hesselbo et al., 2000). The ligno-cellulose in fossil wood provides a ubiquitous and abundant source of material through geological time complete with $\delta^{13}C$ signature. The bulk of wood however is composed of a ligno-cellulose complex constituting polysaccharides, cellulose and hemicellulose, and lignin. Their relative abundances can be assessed easily from pyrolysates based on their specific products (see below). Importantly, from a stable carbon isotope viewpoint, all three molecular entities have different $^{13}C/^{12}C$ ratios. Lignin is most depleted in ^{13}C whereas hemicellulose is less depleted (Benner et al., 1987; Spiker and Hatcher, 1987). Moreover these moieties have different preservation potentials. Although recalcitrant, often remaining morphologically recognisable for millions of years, wood will undergo decay. During burial and subsequent fossilisation, wood is subject to chemical taphonomy (van Bergen and Poole, 2002). It is well known that under natural anaerobic conditions the polysaccharides in wood are preferentially degraded compared with lignin (e.g., Benner et al., 1987; Hedges et al., 1985). Within the polysaccharide fraction hemicellulose degrades faster than cellulose, such that hemicellulose lasts for perhaps thousands of years whereas cellulose can survive millions of years. Lignin however can still be recognised after 10s, if not 100s, millions of years (van Bergen et al., 2004). This phenomenon has a significant effect on the stable carbon isotope composition of the bulk wood sample because the removal of polysaccharides results in the fossil material becoming more depleted in ^{13}C (cf. Fig. 1 in van Bergen and Poole, 2002). Furthermore, ligno-cellulose degradation by certain fungi (white rot fungi) may cause the reverse effect, viz. the selective removal of lignin over cellulose (Mulder et al., 1991). In such a case the change in stable carbon isotope composition would lead to relatively less depleted bulk wood values that might be erroneously construed as being caused by changes in $\delta^{13}C_{palaeoatm}$. This relative degree of removal, and thus the resulting chemical composition, is interrelated with the sedimentary environment (van Bergen et al., 1994) and thus preservation type of the fossil. Hence, determining the chemical composition of the fossils is pivotal for unbiased data interpretation.

The issue is further confounded by the fact that $^{13}C/^{12}C$ ratios in wood vary due to a number of intrinsic aspects, including growth habit and taxonomy. The habitat the tree grows in may affect the $^{13}C/^{12}C$ contents of the tree through water availability and/or CO_2 used. Reduced water availability can lead to less depleted $\delta^{13}C$ values because of lower stomatal conductance. Regarding the isotope composition of the CO_2 pool, trees growing as understorey species in dense forests will be influenced by more ^{13}C depleted respired CO_2 which will cause the $\delta^{13}C$ of the CO_2 used by the plant to be more ^{13}C depleted when compared with $\delta^{13}C$ values of normal atmospheric CO_2. With respect to taxonomy, Stuiver and Braziunas (1987) documented the differences between the bulk $\delta^{13}C$ signature of the wood range of angiosperms and conifers as being ca. -25 to $-27‰$ and -23 to $-24‰$, respectively. Therefore, the relationship between the $\delta^{13}C$ value of the original wood and the fossil is interdependent on habitat, geological age, type- and degree of chemical preservation and taxon. Thus, it can no longer be assumed that $\delta^{13}C_{fossil\ wood}$ values simply reflect the $\delta^{13}C$ values of the original biomass at that point in Earth history. This paper outlines a new approach whereby more reliable estimates of $\delta^{13}C_{biomass}$ from fossil plants material, using wood as our example, can be obtained, which thus provides a terrestrially derived signature which can be used to obtain $\delta^{13}C_{palaeoatm}$ values through geological time.

The concept underlying the approach lies in plant fractionation in favour of ^{12}C from the atmosphere which affects the relative $^{13}C/^{12}C$ ratio locked up within the organic entities metabolised from the photosynthetic end product (i.e. glucose; Farquhar et al., 1989). Assuming a typical pi/pa ratio of 0.7 for modern plants (Polley et al., 1993) this can be expressed as

$$\delta^{13}C_a = \delta^{13}C_p + 20.22 \quad \text{(I)}$$

where $\delta^{13}C_a$ is the isotopic composition of the atmosphere under investigation and $\delta^{13}C_p$ is the isotopic composition of the plant (i.e. biomass). For fossil plant material the following relationships have been proposed: According to Arens et al. (2000) the inverse prediction solution to reconstruct the $\delta^{13}C$ value of the palaeoatmosphere under which it was fixed is determined by the equation

$$\delta^{13}C_a = \frac{\delta^{13}C_p + 18.67}{1.10} \quad \text{(II)}$$

This was based on fossil plants. Alternatively, Gröcke (2002) suggested a pi/pa of 0.6 based on empirical fossil wood data leading to the following expression using Farquahar's equation:

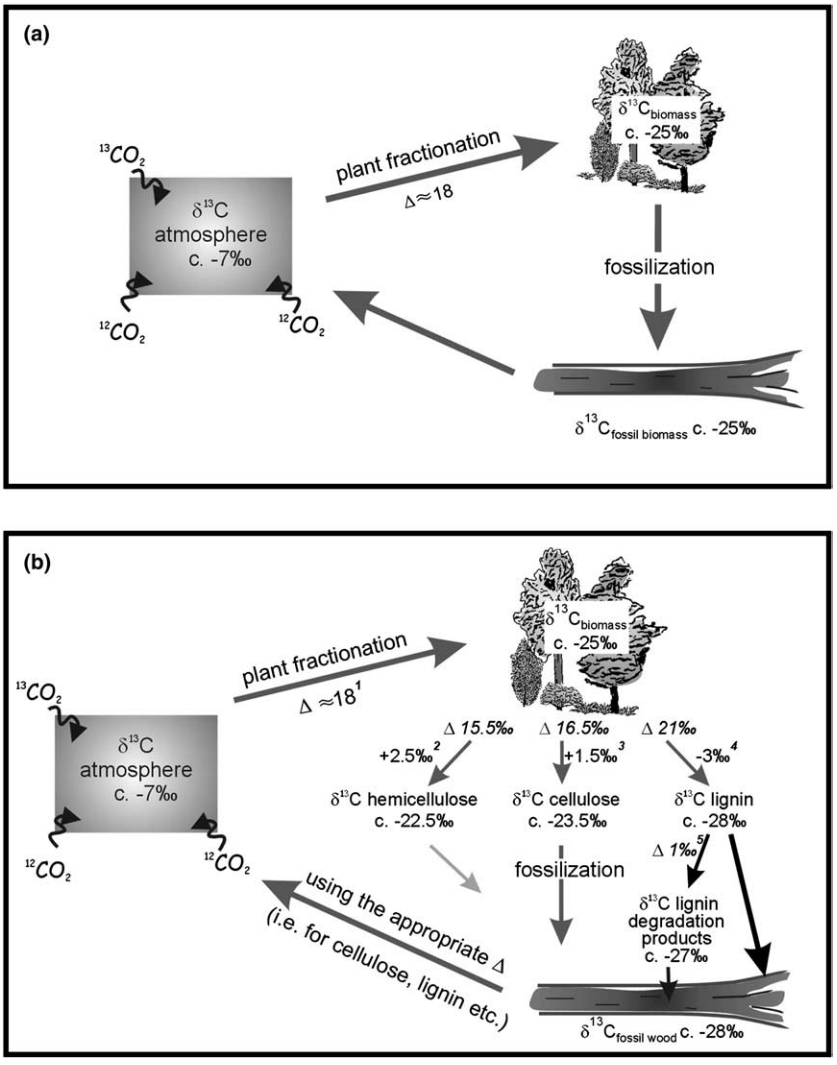

Fig. 1. (a) Conceptual outline of the relationship for stable carbon isotope composition of atmosphere, biomass and fossil biomass. (b) Detailed outline of the applied fractionation factors (Δ) with reference to fossil wood material as given in this study. [1]Average derived from Arens et al. (2000), Gröcke, 2002 and references cited therein; [2]Benner et al., 1987; [3]Leavitt and Long (1986), Sass-Klaassen et al. (2004); [4]Spiker and Hatcher (1987); [5]Galimov (1985).

$$\delta^{13}C_a = \delta^{13}C_p + 17.33 \quad \text{(III)}$$

Importantly, Arens et al. (2000) state that the substrate, uniquely derived from terrestrial vascular plants, must be identified and isolated and that the isotopic composition of the isolated substrate reflects the isotopic composition of the whole plant tissue faithfully and with low variability.

In Fig. 1(a) this idea is exemplified using a preindustrial $\delta^{13}C_{atmosphere}$ value of $-7‰$ and an average Δ value of 18‰, thus resulting in a bulk plant biomass $\delta^{13}C$ signature of ca. $-25‰$. In time, following fossilisation, the bulk $\delta^{13}C$ signature of the resistant organic material ($\delta^{13}C_{fossil\ wood}$) can be measured and, using the assumption that there has been no change in plant fractionation over time, one can arrive at a $\delta^{13}C$ signature for the palaeoatmosphere. In practice, however, the concept is more complex [Fig. 1(b)]. The $\delta^{13}C_{biomass}$ signature of the plant depends upon the fossilised organ under investigation. In the case of wood we are concerned with a ligno-cellulose complex with fractions which have their own isotopic signatures [i.e. hemicellulose, cellulose and lignin with values ca. $-22.5‰$, $-23.5‰$ and $-28‰$, respectively, assuming a bulk wood value of $-25‰$; Fig. 1(b)] and average fractionation factors [Δ, Fig. 1(b)] which differ from that for the bulk plant [Fig. 1(a),(b)]. It is the relative contribution of these different chemical

fractions to the fossilised wood remains that determine its overall $\delta^{13}C$ signature.

In summary, the older the material the greater the taphonomic processes at work on the individual moieties, and thus, the more likely the fossil is to be lignin dominated. Therefore, by understanding the fossil material, its $\delta^{13}C$ value, applying the appropriate fractionation factor [Fig. 1(b)], very different values for $\delta^{13}C_{palaeoatm}$ can result. Here representative samples from wood floras through the Late Cretaceous and Cainozoic were examined both for their molecular and isotopic composition to determine the effect of preservation state, chemical composition and taxonomic status on $\delta^{13}C_{biomass}$ values derived from fossil wood and consequent implications for determining and interpreting $\delta^{13}C_{palaeoatm}$.

2. Materials and methods

2.1. Samples

Fossil wood, identified as originating from C_3 trees, representing four different preservation types was used. Mummified material originated from a submerged, archaeological site (approximately 6000 BP) off the south coast of England (prefixed by A; Whitcombe, 1995); Miocene brown coal, Germany (Az, As); the Pliocene-Miocene Madre de Dios Formation (Campbell et al., 2001) in Peru (PU); Oligocene-early Miocene Balfour Formation (B) and the early Oligocene Little Rapid River Formation (LRR), in Tasmania (Hill, 2001); and the Miocene-Pliocene Meyer Desert Formation, Sirius Group (S.; McKelvey et al., 1991) West Antarctica. Permineralised material originated from Antarctica: Silicified material from the Coniacian Williams Point Beds on Livingston Island, Antarctica (P; Rees and Smellie, 1989); calcified and charcoalified material from Santonian-Campanian Santa Marta Formation (Crame et al., 1991) on James Ross Island (D.), Maastrichtian Lopez de Bertodano Formation (DJ.1056; Rinaldi et al., 1978), Paleocene Sobral Formation (DJ.1059; Macellari, 1988) and Eocene La Meseta Formation (DJ.1057; Elliot and Trautman, 1982) on Seymour Island.

2.2. Off-line pyrolysis

Permineralised material was demineralised using standard HCl/HF techniques (e.g., Wellman and Axe, 1999) and checked for any organic contamination (e.g., bore holes containing remains of shells, cuticle fragments etc, fungal presence within the wood itself). Powdered organic residues (i.e. wood representing several growth rings weighing approximately 200 mg) were cleaned using organic solvents (3× MeOH and 3× DCM). Extracted residues were subjected to bulk isotope ratio mass spectrometry (IRMS) and off-line pyrolysis (1 h at 300 °C) For detailed information on the off-line pyrolysis method the reader is referred to Poole and van Bergen (2002).

2.3. On-line pyrolysis

Small quantities of powdered, solvent-extracted archaeological and mummified Miocene brown coal material were examined using on-line flash pyrolysis-gas chromatography–mass spectrometry (Py-GC–MS) to obtain detailed molecular information on the degree of preserved polysaccharides and lignin (c.f. van Bergen and Poole, 2002). For a detailed description of the pyrolysis method used here, the reader is referred to van Bergen et al. (2000). Identification of the various pyrolysis products (e.g., levoglucosan derived from cellulose, guaiacol and syringol products derived from lignin) was based on mass spectral data and retention time comparisons with reference samples and data reported in the literature (e.g., Saiz-Jimenez and de Leeuw, 1986; Pouwels et al., 1987, 1989; van Bergen et al., 1996, 2000; Stankiewicz et al., 1997).

2.4. Molecular and isotopic analyses

The compounds released during off-line pyrolysis were subsequently cold-trapped (DCM/MeOH 1:1 v/v; 0 °C) and derivatised using BSTFA with 1% TMCS (1 h at 80 °C). Molecular analysis was undertaken using a Hewlett–Packard 5890 GC–MS and the compounds identified using mass spectral data reported in the literature (Poole and van Bergen, 2002). Briefly, each sample (1 μl) was injected on-column on to a CP-Sil 5CB capillary column (50 m × 0.32 mm, 0.12 μm film thickness) with helium as carrier gas (3 ml min^{-1}). The GC oven was temperature programmed as follows: isothermal at 40 °C for 5 min, then rising to 300 at 4 °C min^{-1}, with an isothermal period of 10 min. Compounds were identified using a Fisons Instruments VG platform II mass spectrometer (Manchester, UK) operating at 70 eV, scanning the range *m/z* 50–650 with a cycle time of 0.65 s. Compound specific stable carbon isotope values were measured using a ThermoFinnigan Delta^{PLUS} XL GC-combustion-IRMS with similar chromatographic conditions as the GC/MS analysis. Values represent averages of two analyses with analytical errors typically <0.8‰ based on standards. Corrections for the TMS groups were determined from derivatised myo-inisitol with an underivitised $\delta^{13}C$ value of −26.7‰.

3. Results and discussion

A large number of fossil wood specimens have been examined both in terms of molecular and stable carbon

isotope composition in order to evaluate the effects of taxonomy, preservation state and chemical composition on bulk wood $\delta^{13}C$ values.

3.1. Taxonomic status and $\delta^{13}C$

Molecular and isotope analysis of a number of Miocene brown coal conifer and angiosperm woods (Fig. 2) indicate that the taxonomic differences in isotope composition between modern conifers and angiosperms (e.g., Stuiver and Braziunas, 1987; Fig. 3) are retained in fossil material (cf. van Bergen and Poole, 2002). Figure 2 illustrates the differences between these taxonomic groupings in mummified Miocene brown coal woods derived from the same horizon. The $\delta^{13}C_{fossil\ wood}$ signatures of both the angiosperm [Fig. 2(a)] and conifer [Fig. 2(b)] are in accordance with those noted for modern material (Fig. 4). However, the difference in $\delta^{13}C_{fossil\ wood}$ signature of the Miocene specimens is further exacerbated by the difference in the relative abundance of cellulose preserved as evidenced by the presence of the pyrolysis product levoglucosan. The relative abundance of levoglucosan in the angiosperm material [Fig. 2(a)] has biased the $\delta^{13}C_{fossil\ wood}$

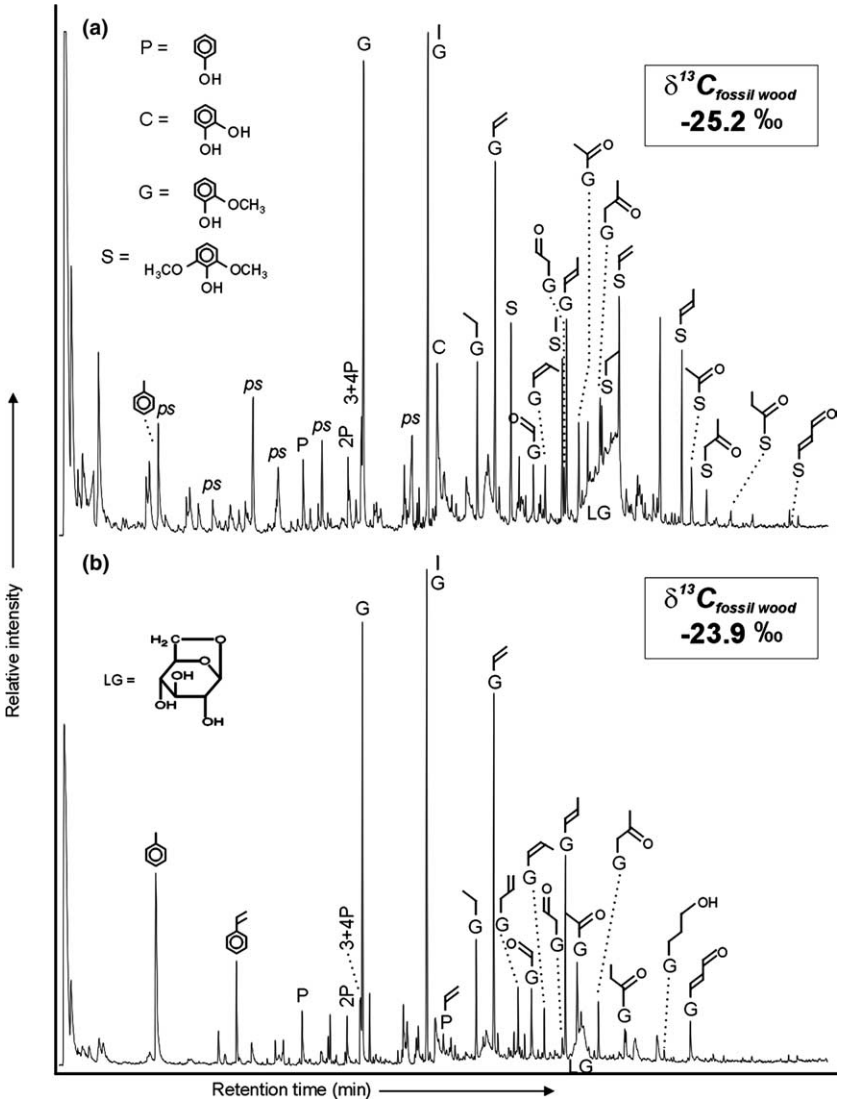

Fig. 2. Gas chromatograms of on-line pyrolysates (Curie temperature 610 °C) of mummified wood specimens from brown coal deposits in Germany (a) angiosperm (As1) and (b) conifer (Az1) showing the effects of taxon on the $\delta^{13}C_{fossil\ wood}$ values. PS polysaccharide pyrolysis products; 2P 2-methylphenol, 3 + 4P coeluting 3-, 4-methylphenols, LG levoglucosan, P phenol, C catechol, G guaiacol, S syringol. Side chains indicated are attached at carbon number 4 *para* to the OH group.

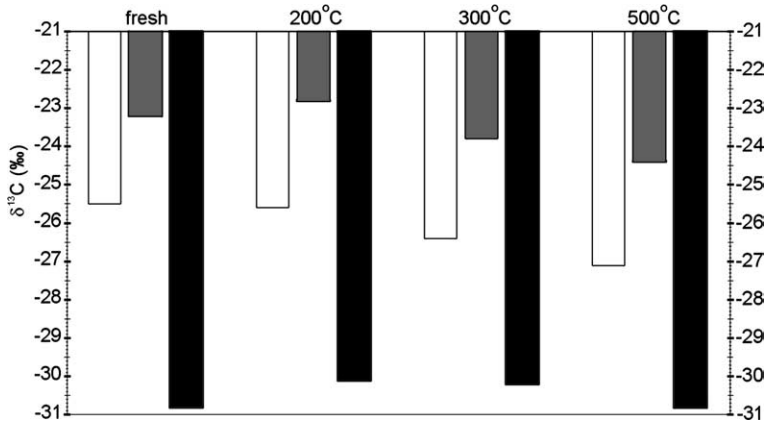

Fig. 3. Stable carbon isotope variation induced by heating to different temperatures compared with unheated values for oak (*Quercus*) open bar, pine (*Pinus*) grey bar and Miocene-Pliocene mummified material (PU1) solid bar. For additional information see text.

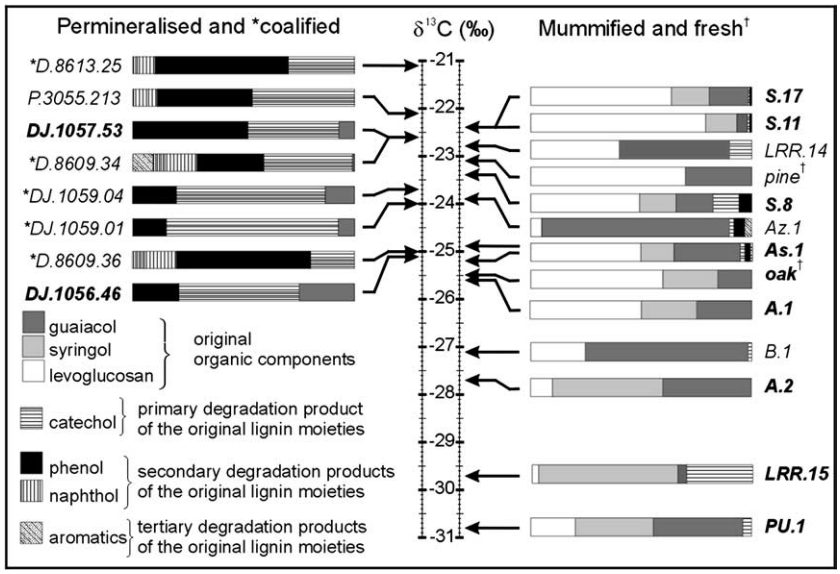

Fig. 4. Relative abundance of the organic moieties released by pyrolysis from modern and fossil wood. Angiosperm sample numbers given in bold to distinguish them from the remaining conifer samples.

signature in favour of the isotopically heavy levoglucosan (see also below) and thereby reducing the taxonomic distinction. In other Miocene mummified specimens (not illustrated) where levoglucosan is in similar abundance the taxonomic distinction becomes greater. This illustrates the fundamental importance of determining the taxonomic signal expressed in the $\delta^{13}C_{fossil\ wood}$ signature.

3.2. Preservation state and $\delta^{13}C$

Preservation state appears to be interrelated to organic moiety preservation in fossil material, which in turn affects the $\delta^{13}C$ signature of the fossil. Pyrolysis of the fossil wood specimens revealed that the quantity and quality of the original organic compound preservation was highest in the mummified specimens studied (Figs. 2, 4–6), relative to the other preservation types, as organic moieties originally present in modern wood dominated [i.e. cellulose pyrolysis products such as levoglucosan, and lignin building blocks such as guaiacyl (G) and/or syringyl (S) units; Fig. 2, 5]. The bulk isotopic compositions of the mummified specimens show large isotopic variability (ca. 9‰).

Permineralised material (e.g., calcified) yielded no polysaccharide products, but showed original lignin moieties and primary degradation products [C; Fig. 7(a)] indicative of reduced quality of original organic

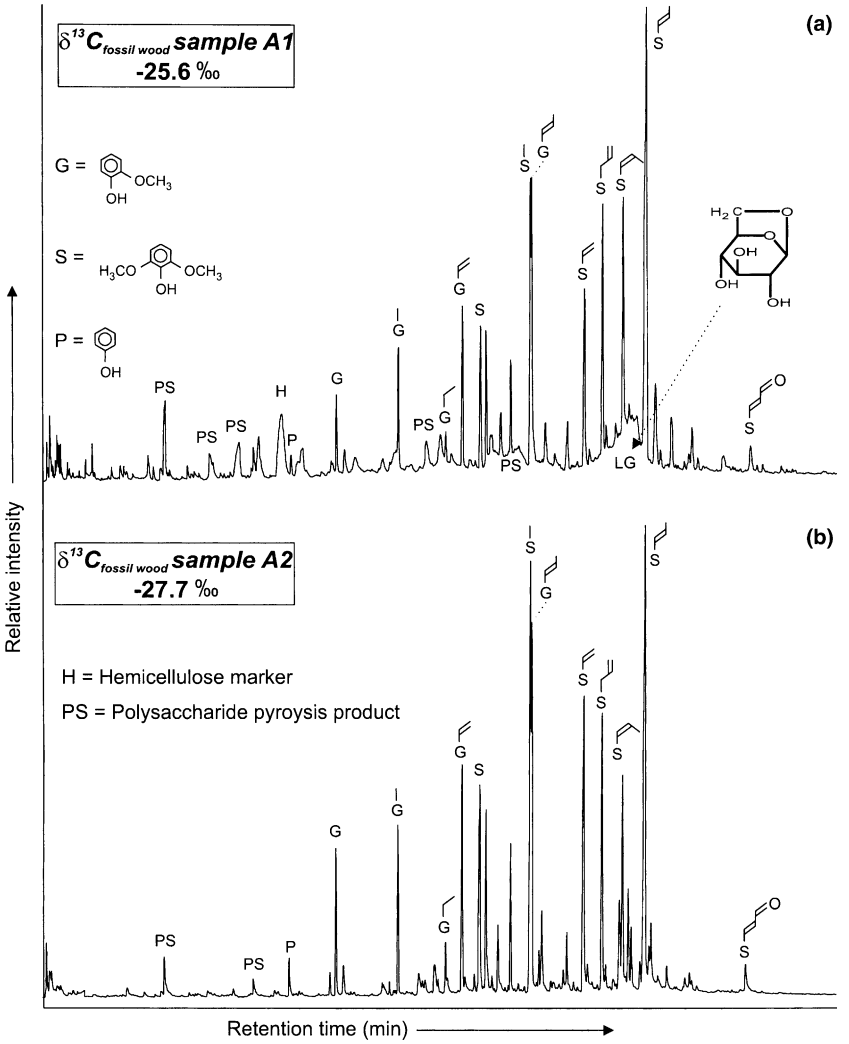

Fig. 5. Gas chromatograms of on-line pyrolysates (Curie temperature 610 °C) of mummified archaeological wood samples (A1 and A2) showing the effect of chemical composition on $\delta^{13}C_{fossil\ wood}$ values. For additional information see caption for Fig. 2.

preservation when compared with the mummified material. Interestingly, the silicified material from the Coniacian of Antarctica (P.3055.213) is also dominated by degradation products, including naphthols, rather than original lignin moieties (Fig. 4). This material is preserved in volcanic tuff, and therefore, the abundant presence of naphthols may indicate a heating effect prior to, or during, preservation as naphthols are known to be produced upon pyrolysis of thermally altered wood samples. The bulk isotopic compositions of the specimens show less isotopic variability (ca. 3‰) than that seen for mummified wood.

Strongly coalified and charcoalified material revealed a drastically modified chemical composition based on the absence of polysaccharides and the abundant presence of lignin degradation products such as catechols, phenols and naphthols (cf. Hatcher and Clifford, 1997 and references therein; Fig. 4). The thermal effect on wood during charcoalification further complicates the issue related to the isotopic composition. To date, the effect of heat induced isotope changes on plant remains has received little attention (Jones et al., 1993; Turekian et al., 1996; Schleser, 1999; Beuning and Scott, 2001). Some studies have suggested that thermally altered, charred/charcoalified plant remains can be used to determine stable carbon isotope changes related to palaeoclimate without considering these effects (e.g., Hesselbo et al., 2000). However, charring (heating) of whole plant material has been shown to lead to residues less depleted in ^{13}C in the order of about 1‰ (Turekian et al., 1996; Beuning and Scott, 2001). Turekian et al. (1996) interpreted this relative enrichment as evidence of a normal

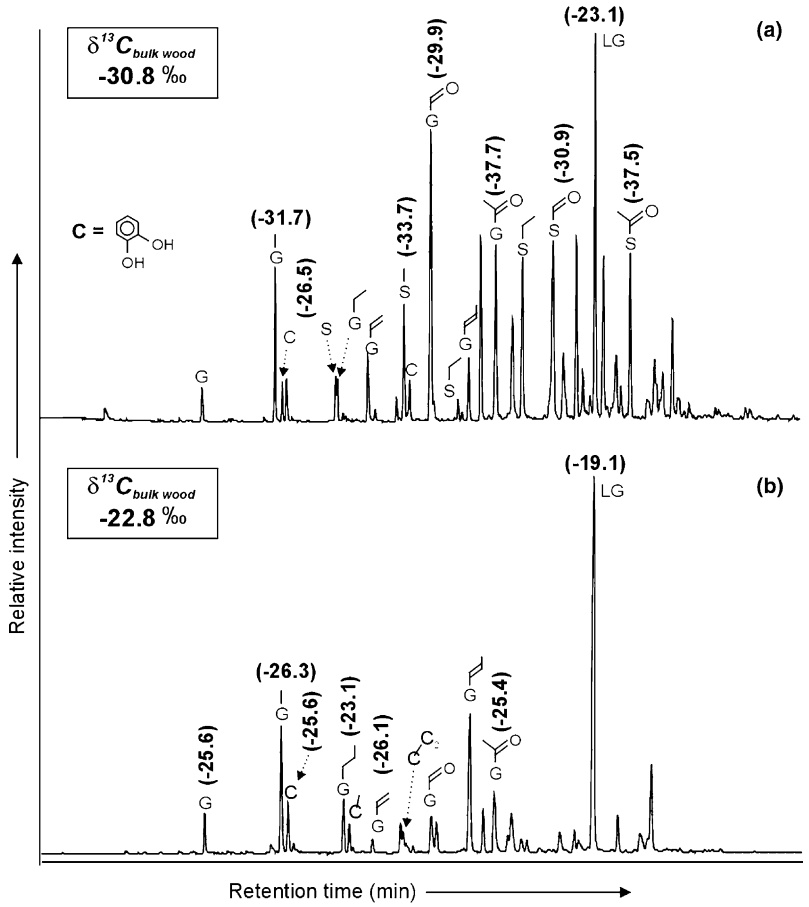

Fig. 6. Off-line pyrolysate products, analysed as BSTFA derivatised compounds, of mummified, (a) PU1 and (b) LRR14 fossil wood showing evidence of polysaccharides (LG) and lignin (G and S) units characteristic of well preserved ligno-cellulose and the degradation products (C). For additional information see caption for Fig. 2 and text.

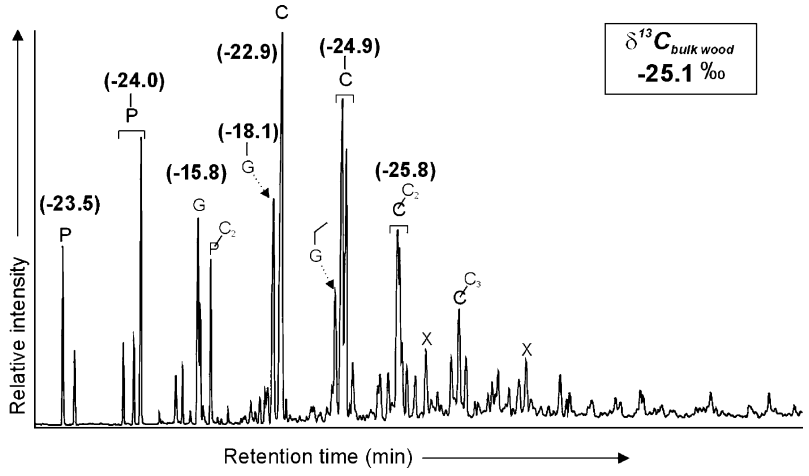

Fig. 7. Off-line pyrolysate products of permineralised, DJ.1056.46, fossil wood showing evidence of degraded lignin based on the abundance of the degradation products (C, P) and the relatively small amounts of G. For additional information see caption for Fig. 2 and text.

kinetic isotope effect caused by the preferential breaking of ^{12}C bonds leading to ^{13}C depleted gas and a less depleted residue. This interpretation emphasises the fact that the carbon in thermally altered fossil material does not constitute a closed system as certain organic moieties are preferentially lost as volatiles during the charring of modern plant material (e.g., Poole et al., 2002).

Experimental charring under an N_2 atmosphere of modern angiosperm (oak) and gymnosperm (pine) wood specimens also revealed stable carbon changes of up to 2‰ in the residues produced. The experiments yielded residues less depleted in ^{13}C when heated to 200 °C, but when heated to higher temperatures the residues became more ^{13}C depleted (Fig. 3). These data indicate preferential loss of ^{13}C enriched material during hotter (>200 °C) treatments. As cellulose and polysaccharides are in general less depleted in ^{13}C when compared with lignin (Benner et al., 1987) and polysaccharides are more thermally labile compared with lignin, these results imply the preferential loss of polysaccharide moieties from the ligno-cellulose complex. Molecular analyses of the gasses released during such heating experiments have also revealed that polysaccharide pyrolysis products are more abundant at lower temperatures compared with lignin products (Poole and van Bergen, 2002). Schleser (1999) noted that the carbon isotope signature of wood, during decay recreated in water at 180 °C over periods of minutes to months, underwent a discrimination against ^{13}C during an initial phase (to a maximum of ≈1‰) followed by a subsequent increase in $^{13}C/^{12}C$ ratio up to the initial value (−25.5‰). They also explained this as the presumed preferential decomposition of the cellulose relative to the lignin. Jones et al. (1993) found that with experimentally charred wood there was a slight shift in isotopic composition by an average amount of +0.2‰ when wood was heated to 300 °C. From 300–500 °C the shift changed to −0.4‰ and −0.8‰ at 600 °C relative to the starting material. Again, this fractionation was explained by the volatilisation of the isotopically heavier cellulose derived carbon leaving the residue enriched in isotopically lighter lignin-derived carbon.

The isotope data for the charred fossilised wood material (Fig. 3) do not show the same phenomenon but reveal less depleted values. This may be explained by the preferential breaking of ^{12}C bonds, resulting in a relatively ^{13}C enriched residue. At higher temperatures, i.e. 300 °C and 500 °C this effect appears to decrease, which is entirely consistent with the fact that higher temperatures reduce kinetic isotope effects as cracking of bonds is less discriminant (Turekian et al., 1996).

3.3. Molecular composition and $\delta^{13}C$

On-line flash pyrolysis data for two archaeological, oak wood samples (approximately 6000 years old) revealed a distinct difference in the relative amount of the polysaccharides preserved (Fig. 5). The pyrolysate of sample A1 showed a relative abundance of polysaccharides [Fig. 5(a)] almost identical to that of on-line pyrolysates of modern oak wood samples (cf. van Bergen et al., 2000) indicating the presence of a well-preserved lignin-cellulose complex. In contrast, the pyrolysate of sample A2 [Fig. 5(b)] revealed that selective removal of both the hemicellulose and cellulose moieties had taken place. The bulk stable carbon isotope composition of these two specimens is also distinctly different in that the well-preserved specimen, sample A1, is less depleted in ^{13}C (−25.6‰) when compared with the degraded specimen, sample A2, (−27.7‰). Thus, from the molecular pyrolysis data it is clear that the amount of polysaccharides preserved in the two archaeological oak wood sub samples is one of the main factors determining the bulk carbon isotope signature.

The bulk isotopic compositions of the mummified specimens show large variability (ca. 9‰; Fig. 4). The differences in mummified $\delta^{13}C_{\text{fossil wood}}$ values relative to fresh wood (Fig. 4) can be explained by relative abundances of the moieties present. For example, the Miocene-Pliocene angiosperm from Peru [PU.1, Figs. 4 and 6(a)] has a high relative abundance of original ^{13}C-depleted lignin moieties (i.e. S and G), and a low abundance of degradation products (i.e. catechol) and relatively ^{13}C-enriched polysaccharides (i.e. levoglucosan, LG). This explains the ^{13}C depleted $\delta^{13}C_{\text{fossil wood}}$ value relative to that of modern wood samples (i.e. oak and pine; Fig. 4). Similarly, the Oligocene Tasmanian mummified specimen LRR.15 has relatively low amounts of LG and is therefore ^{13}C depleted $\delta^{13}C_{\text{fossil wood}}$ [Fig. 6(b)]. In contrast, the Miocene-Pliocene mummified Antarctic angiosperm specimens (samples S.17, S.11 and S.8) and the Oligocene Tasmanian mummified specimen LRR.14 yield less depleted ^{13}C values of $\delta^{13}C_{\text{fossil wood}}$ that can be related to the high relative amounts of ^{13}C enriched sugars, with differences in their relative amounts probably accounting for the slight variation in the $\delta^{13}C_{\text{fossil wood}}$ values (Fig. 4). The higher than expected polysaccharide preservation in some of the older specimens, e.g., LRR.14, must be related to the degradation mechanism. A different kind of fungal degradation could explain this, with lignin being preferentially degraded over cellulose (Mulder et al., 1991). However, it should be noted that the specimen yielded no visible evidence of fungal remains. The intermediate $\delta^{13}C_{\text{fossil wood}}$ value of the Oligocene Tasmanian mummified specimen B.1 can be related to the moderate abundance of lignin moieties relative to LG. In summary, $\delta^{13}C_{\text{fossil wood}}$ values of mummified specimens studied can be readily explained in term of the relative contribution of the moieties preserved (i.e. lignin versus cellulose).

The $\delta^{13}C_{\text{fossil wood}}$ values of permineralised (Fig. 4) and coalified specimens are less depleted in ^{13}C relative

to most mummified lignin-rich specimens and similar to cellulose-rich mummified specimens. However, this enrichment cannot be related to the presence of sugars since levoglucosan has been preferentially removed and only lignin and degradation products therefrom remain (Figs. 4 and 7). Rather, isotopic analysis of the pyrolysis products of permineralised and coalified specimens suggest that their bulk values can be related to the relative abundance of few, relatively ^{13}C enriched (ca. −11‰) G moieties and abundant ^{13}C depleted lignin degradation products (Fig. 4). Indeed, with the exception of the coalified specimen D.8609, the specimens containing higher amounts of secondary, phenols, and tertiary, aromatics, degradation products are less ^{13}C depleted when compared with specimens containing the original lignin product guaiacol and the primary degradation products catechols. This is in agreement with previous studies that documented ^{13}C enrichments of bulk organic matter during coalification (e.g., Whiticar, 1996). However, preliminary studies suggest that the isotopic shifts occurring during thermal alteration cannot be accounted for by chemical modifications alone and are still more complicated than first envisaged (Poole et al., 2002).

3.4. Compound specific $\delta^{13}C$ variation

Tracking compound specific values of individual moieties, such as the original lignin building block guaiacol, back through geological time was believed to aid the evaluation of shifts in $\delta^{13}C_{compound}$ to relative changes in $\delta^{13}C_{biomass}$, thereby eliminating the chemical taphonomical problems associated with using bulk organic matter. The compound specific data for off-line pyrolysis products showed that the simple two-component variability alone, polysaccharides versus lignin (see Section 3.3) is an oversimplification of the isotopic variation caused by chemical preservation in mummified specimens. The Pliocene-Miocene mummified Peruvian specimen [PU.1; Fig. 6(a)] yielded $\delta^{13}C$ values ranging from −37.7‰ for S and G derivatives to −23.1‰ for levoglucosan (LG). Similarly, for the Oligocene mummified specimen [Fig. 6(b)] $\delta^{13}C$ values ranged from −26.3‰ for G moieties to −19.1‰ for LG. The ^{13}C values for the cellulose-derived levoglucosan compared with the ^{13}C depleted values for the lignin-derived pyrolysis products is in accordance with values of bulk lignin and cellulose measurements (cf. Benner et al., 1987; Spiker and Hatcher, 1987) of modern wood, confirming that the off-line pyrolysis method used here yields products that represent $\delta^{13}C$ values of their original polymer building blocks. When comparing the values of the various G and S derivatives a large difference of 7‰ is noted between the formyl and acetyl members in both G and S. The presence of the beta carbon leads to significantly more depleted $\delta^{13}C$ values. This suggests that there is a large intra-molecular isotopic heterogeneity within the lignin building units themselves. Thus, not only the relative preservation of lignin versus cellulose but also the preservation state of lignin itself, i.e. degree of side chain oxidation or the extent of side chain degradation, may significantly affect $\delta^{13}C_{fossil\ wood}$.

The compound specific analyses of the permineralised specimens also revealed large differences between compounds, for example up to 17‰ for the permineralised specimen DJ.1057.53 (Fig. 8). Both the primary- (catechols) and secondary (phenols) degradation products yielded similar values (i.e. between −22‰ and −26‰) which are relatively similar in range to the bulk values (Fig. 8). Intriguingly and in stark contrast, the guaiacol derivatives are now significantly ^{13}C-enriched compared with the other products by up to 11‰ (Figs. 7 and 8), a phenomenon not previously observed. To date, we have observed this phenomenon in all permineralised samples studied that yielded both catechols and guaiacols (but note that not all permineralised specimens necessarily yield guaiacols).

It has been suggested that the conversion of guaiacols to catechols, i.e. demethylation of the mexthoxy

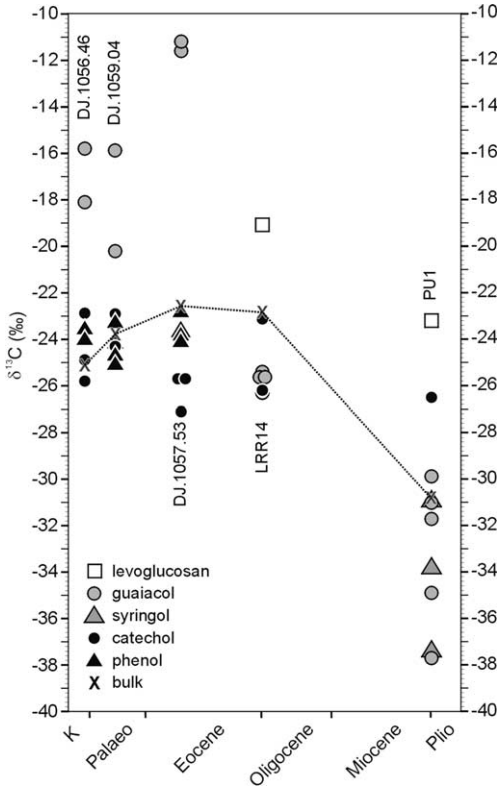

Fig. 8. Stable carbon isotope heterogeneity amongst different compounds within five representative fossil woods. Variation in measured $\delta^{13}C_{compound}$ values is indicated but in many cases smaller than the marker. For additional information see caption for Fig. 2 and text.

group (e.g., Hatcher and Clifford, 1997), leads to less ^{13}C depleted catechols, rendering the remaining guaiacols ^{13}C depleted (Galimov, 1985). Enriched catechols relative to the guaiacols are observed in the PU1 sample (Figs. 4 and 6). However, in the older permineralised samples the values of the guaiacols are significantly ^{13}C enriched relative to the catechols and thus this conversion alone cannot account for the discrepancies in the $\delta^{13}C$ values. Thus, whatever the cause for the relatively lower ^{13}C depletion, guaiacols appear to be relatively unreliable as a proxy for $\delta^{13}C_{biomass}$ when derived from permineralised specimens that contain no original ligno-cellulose and thus have undergone drastic chemical alteration.

3.5. Implications for $\delta^{13}C_{biomass}$ of the original plant and $\delta^{13}C_{palaeoatmosphere}$

Measurement of $\delta^{13}C$ has been used to determine changes in $\delta^{13}C_{palaeoatm}$ (Gröcke et al., 1999; Hesselbo et al., 2000) whereby the bulk $\delta^{13}C$ values were considered to represent the $\delta^{13}C_{biomass}$ of the original plant. However, the data presented above show that the fossils have often undergone chemical changes, causing a non-directional off set between bulk $\delta^{13}C$ measured and the original $\delta^{13}C_{biomass}$ when the plant was living. Thus, appropriate correction factors will need to be applied, depending on taxon, preservation type and molecular composition, to determine the actual $\delta^{13}C_{biomass}$ of the original plant material, which in turn can be used to calculate $\delta^{13}C_{palaeoatm}$.

To illustrate this approach, the molecular and isotopic data obtained, and the concept shown in Fig. 1, will be used to evaluate $\delta^{13}C_{fossil\ biomass}$ and $\delta^{13}C_{palaeoatm}$ for some of the specimens studied (PU1, LRR14 and DJ.1056.46). Based on the various relationships between $\delta^{13}C_{plant}$ and $\delta^{13}C_{atmosphere}$ presented (see equations I, II and III) we have arbitrarily used an average fractionation factor (Δ) of 18‰ (Fig. 1). In addition, specific fractionation factors for the various compound classes have been assigned (i.e. $\Delta_{hemicellulose}$ 15.5, $\Delta_{cellulose}$ 16.5 and Δ_{lignin} 21) knowing that the difference between lignin and cellulose is approximately 5‰ and an off set between cellulose and bulk wood is approximately 1.5‰ to less ^{13}C depleted values (Loader et al., 2003; Sass-Klaassen et al., 2004).

If applying only the average Δ of 18‰ to determine the $\delta^{13}C_{palaeoatm}$ without considering the chemical composition, and assuming the measured $\delta^{13}C$ value of the fossil equals $\delta^{13}C_{fossil\ biomass}$, the following values would be obtained: $\delta^{13}C_{palaeoatm}$ (PU1) −12.8‰, $\delta^{13}C_{palaeoatm}$ (LRR14) −4.8‰, $\delta^{13}C_{palaeoatm}$ (DJ.1056.46) −7.1‰. However, the chemical composition is known and thus corrections can be made.

For the Peruvian Pliocene-Miocene angiosperm specimen (PU1, $\delta^{13}C_{fossil\ wood}$−30.8‰), off-line pyrolysis data show a chemical composition based on lignin [Figs. 4 and 6,(a)]. Thus a correction factor (CF) for the original biomass of −3‰ and a Δ_{lignin} of 21‰ for the palaeoatmosphere can be used. These yield $\delta^{13}C_{biomass}$ of −27.8‰ and a $\delta^{13}C_{palaeoatm}$ of −9.8‰. This latter value is still depleted relative to the preindustrial $\delta^{13}C$ atmosphere value but not as strongly depleted as when uncorrected. A possible explanation for this depleted value could be related to this specimen being derived from sediments representing a fossil tropical lowland flora. If this fossil grew in the subcanopy it would have used partially respired CO_2, explaining the more ^{13}C depleted-biomass and thus calculated, $\delta^{13}C_{palaeoatm}$ values.

With respect to the mummified Oligocene conifer specimen, LRR14 ($\delta^{13}C_{fossil\ wood}$−22.8‰), it could in general be assumed that specimens of this age would be dominated by lignin. If one could calculate the $\delta^{13}C$ based on lignin the $\delta^{13}C_{fossil\ biomass}$ would be −19.8‰, with a $\delta^{13}C_{palaeoatm}$ of −1.8‰. However, the pyrolysis data have shown that this specimen is dominated by cellulose derived products [Fig. 6(b)] so a CF of +1.5‰ and a Δ of 16.5‰ need to be applied. Using these corrections $\delta^{13}C_{biomass}$ would be −24.3‰ and $\delta^{13}C_{palaeoatm}$ −6.3‰. A comparison between the corrected data $\delta^{13}C_{biomass}$ from both these two specimens, PU1 and LRR14, shows an off set of about 3.5‰ whereas the uncorrected bulk values differed by 8‰. The latter could have been interpreted as a large change in $\delta^{13}C_{palaeoatm}$. The corrected biomass values are more in line with the differences observed between the levoglucosan (−23.1‰ and −19.1‰; Fig. 4), implying that the corrections would allow more meaningful $\delta^{13}C_{palaeoatm}$ value calculations.

The final example is a piece of calcified wood (DJ.1056.46) from the Maastrichtian of Antarctica ($\delta^{13}C_{fossil\ wood}$−25.1‰). If lignin is assumed to dominate this specimen, then the calculated $\delta^{13}C_{biomass}$ and $\delta^{13}C_{palaeoatm}$ would be −22.1‰ and −4.1‰, respectively. However pyrolysis data have shown that this specimen is dominated by lignin degradation products [Fig. 7(a)] so a CF of +2‰ (i.e. +3‰ for lignin plus a −1‰ shift because of demethylation, Galimov, 1985) and a Δ of 20‰ (i.e. 21–1‰; Fig. 1(b)) need to be applied, resulting in a $\delta^{13}C_{biomass}$ of −23.1‰ and $\delta^{13}C_{palaeoatm}$ −5.1‰, a difference of 1‰ for both biomass and palaeoatmospheric values.

The $\delta^{13}C_{fossil\ wood}$ values have been shown to be useful for deriving $\delta^{13}C_{palaeoatm}$ values. However, when the material under study is not the same taxon, this taxonomic shift needs to be corrected for, since bulk isotopic values derived from angiosperm material cannot be compared directly with those derived from conifer material. Therefore further insight is needed into the degree of variation between different angiosperms and conifers. Correction for taxon will normalise the data such that relative shifts in $\delta^{13}C_{palaeoatm}$ become more meaningful.

The shifts in the corrected bulk values mirror those observed in the $\delta^{13}C$ signatures of the levoglucosan in the mummified samples (Fig. 8), indicating that the $\delta^{13}C$ signature of specific compounds might provide a more reliable proxy for $\delta^{13}C_{palaeoatm}$ without the need to correct in particular polysaccharide-derived and G and S values from geologically young samples (i.e. Late Tertiary through to modern). For fossil material from which the cellulose fraction has been preferentially lost (i.e. geologically older and/or permineralised specimens), the polysaccharides are ineffective for monitoring changes in $\delta^{13}C_{palaeoatm}$ values. Therefore it had been hoped that for relatively old and/or permineralised material specific G members would provide an ideal compound on which to focus. However, the same specific derivative would have to be compared as variations between different derivatives can be as much as 8‰ (Fig. 8). Interestingly, the values for the characteristic lignin marker guaiacol in relatively old material (e.g., DJ.1057.53) become extremely heavy (up to −10.5‰; Fig. 8). However, in pre-Miocene samples the values of the guaiacols are significantly ^{13}C enriched relative to the catechols and thus the demethylation leading to catechols, as suggested by Galimov (1985), cannot alone account for the discrepancies in the $\delta^{13}C$ values, suggesting that the guaiacols are relatively unreliable as a proxy for $\delta^{13}C_{palaeoatm}$. The degradation products catechol and phenol may provide a more reliable proxy for the $\delta^{13}C_{palaeoatm}$ values with respect to older specimens. Indeed, they have a much narrower range of isotopic variation. However, catechols are the primary degradation products of guaiacol whereas the phenols are secondary degradation products, and fractionation processes involved in the transformation from guaiacol to catechols and from catechols to phenols are currently unknown. Before these specific compounds can be used as proxies for $\delta^{13}C_{palaeoatm}$ a greater understanding of the isotopic fractionation patterns of lignin degradation on a molecular level is required.

4. Conclusions

This study has investigated the feasibility of using the $\delta^{13}C$ biomass signature of fossil material as a proxy for $\delta^{13}C_{palaeoatm}$ through considering the bias engendered through taxonomic status, preservation type and chemical taphonomy. Even though we have focussed on fossil wood which, due to its ubiquity and abundance through geological time, exhibits great potential for such studies, these ideas are applicable to all fossil material. Ignoring taxonomy and the effects of preservation type and chemical taphonomy on the bulk isotopic signature, errors in the $\delta^{13}C_{biomass}$ values will be introduced which in turn affect calculated $\delta^{13}C_{palaeoatm}$ values. Chemical heterogeneity can be determined from detailed molecular analyses. Such analyses indicate that the bulk values of fossilised wood are biased towards specific preserved ligno-cellulose moieties (i.e. mainly sugars and degraded lignin, or mainly lignin but no sugars). Moreover the dramatic variations in the $\delta^{13}C$ values between chemically very similar lignin building blocks will themselves affect the bulk value upon selective degradation of the resistant lignin, resulting in ^{13}C enrichment. This chemical heterogeneity is interlinked with the type of preservation and the taxonomic status of the fossil under investigation. Thus in order to overcome these biases studies ideally should focus either on the same taxon with the same type of preservation, or correct for these biases, to determine any relative shift in $\delta^{13}C_{palaeoatm}$. Moreover the degree of chemical preservation (whether or not similarly preserved) of the ligno-cellulose moieties within the fossil needs to be determined in order to obtain a more accurate $\delta^{13}C_{biomass}$ value from which $\delta^{13}C_{palaeoatm}$ can be derived.

Alternatively, future studies could focus on specific organic compound classes and their relative shifts in $\delta^{13}C$ signature. For subfossil material, cellulose (as shown by others e.g., Lücke et al., 1999; Spiker and Hatcher, 1987) or its pyrolysis product levoglucosan, or alternatively specific guaiacol or syringol products derived from lignin might prove more reliable when trying to determine shifts in $\delta^{13}C_{paleoatm}$. However, with respect to the latter compound classes, ideally the same specific derivative should be used to overcome large variations exhibited by different derivatives. For pre-Quaternary fossil material cellulose is generally preferentially lost and thus renders sugars ineffective for reconstructing changes in $\delta^{13}C_{palaeoatm}$ values far back in geological time. The ^{13}C enrichment of the original lignin moiety guaiacol in these older samples is not yet fully understood and thus perhaps the relative shifts in the isotopic signature of its primary degradation product, catechol, may be more reliable.

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