Carbon isotope alteration during the thermal maturation of non-flowering plant species representative of those found within the geological record

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Rationale: The carbon isotope ($\delta^{13}C$) composition of fossil plant material is routinely used as a proxy of past climate and environment change. However, palaeoclimate interpretation requires assumptions about the stability of $\delta^{13}C$ in plant material during decomposition and incorporation into sediments. Previous work on modern angiosperm species show $\delta^{13}C$ changes of several per mille during simulated decomposition experiments. However, no such tests have been undertaken on non-flowering plants, which are found extensively within geological record. These plants have distinctly different cellulose to lignin ratios than their angiosperm counterparts, potentially creating hitherto unknown variations in the original to fossil $\delta^{13}C$ signatures.

Methods: To test the extent of $\delta^{13}C$ change during decomposition we have subjected a number of plants representing more basal, non-flowering plant lineages (cycads, ferns, horsetails and dawn redwood), to artificial decay using a hydrothermal maturation technique at two temperatures over periods of up to 273 hours. Subsamples were extracted every 12-16 hours and analysed for $\delta^{13}C$ and %C by a Carlo Erba 1500, a VG TripleTrap and Optima dual-inlet mass spectrometer.

Results: %C values increased for all samples through the maturation process at both temperatures with the largest increases observed within the first 24 hours. Decreases in $\delta^{13}C$ values were observed for all plants at 300ºC and for two of the species at the lower temperature (200ºC). The maximum shift in $\delta^{13}C$ related to experimental decomposition was –0.90‰ (horsetail) indicating a preferential loss of ^{13}C during thermal maturation.

Conclusions: The reduction in the $\delta^{13}C$ value potentially suggests a preferential loss of isotopically heavier cellulose in relation to the isotopically lighter lignin component during maturation. The isotopic offset observed here (<0.9‰) means that palaeoclimatic interpretation of $\delta^{13}C$ from non-flowering plant material within the geological record remains robust, but only where interpretations are based on variations in $\delta^{13}C$ greater than 1‰.

Keywords: Carbon isotopes, palaeoclimate reconstruction, vascular plants, diagenesis, Palaeozoic

INTRODUCTION

Palaeoenvironmental reconstruction using the carbon isotope composition ($\delta^{13}C$) of plant organic matter has the potential to provide information on vegetation composition, local water stress and changes in atmospheric CO_2 concentrations.[1–3] Such reconstructions can be undertaken over a diverse temporal range, from relatively modern reconstruction using tree rings[2,4] through to palaeo-reconstructions using fossil wood from the early Palaeozoic[5]; although few studies attempt climatic reconstructions on deeply buried geological materials such as coal and graphite, due to difficulties of isotope analysis. The main obstacle to the application of $\delta^{13}C$ as a tool for palaeoenvironmental and palaeoclimatic reconstructions is that the bulk isotope composition of plant remains is changed during sedimentary processes, including diagenesis and fossilisation. For example hemicellulose and cellulose (enriched in ^{13}C) decay differently to lignin (^{13}C depleted), potentially altering the original $\delta^{13}C$ in the process.[6–8] These changes are caused by the effects of pressure, heat and time. Consequently, understanding the extent of diageneticially mediated isotope fractionation is essential if changes in palaeo-CO_2 levels and chemical characterisation of past ecologies are to be undertaken from deposits derived from non-flowering plant material.

Previous studies on the diagenesis of plant remains

To investigate the impact of plant tissue diagenesis and fossilisation on the final $\delta^{13}C$ of the organic matter, several studies have simulated diagenesis using a combination of increased heat and pressure. For example, Schleser et al.,[9] simulated thermolysis of wood in sealed glass vessels at 180°C in air with the presence of liquid water to simulate decomposition. They found that during the experiment $\delta^{13}C$ initially decreased by about –1‰ (~7 hours) then gradually returned to the initial starting value, or gave slightly more positive $\delta^{13}C$ values than fresh wood. Maximal deviations from the original unheated counterpart at 7 h were: –1‰ oak, –0.7‰ sequoia, –0.6‰ pine and –0.5‰ beech (Table 1), these shifts in $\delta^{13}C$ were thought to be due to 30-60% cellulose decomposition.[9] The change in $\delta^{13}C$ to more negative values was therefore explained by the preferential loss of cellulose and relative enrichment in isotopically light lignin upon diagenesis. In another study, the effect of temperature on the $\delta^{13}C$ of hard and softwood has been studied to understand black carbon (BC) formation which mainly occurs during forest fires.[10] Birch and pine woods were heated at 150, 340 and 480°C in an oven flushed with argon to create an oxygen free atmosphere.[10] Both woods showed a shift of 0.3‰ to more positive $\delta^{13}C$ at 150°C, but became progressively more negative by –0.5 to –0.8‰ at 340°C and –0.6 to –1.1‰ at 480°C compared to their untreated counterparts (Table 1).[10] More recently the carbonization of wood was examined by wrapping eucalyptus, oak and pine in aluminium foil packets and heating at 200, 400, 600 and 800°C in a muffle furnace at atmospheric pressure.[11] The charred woods showed a systematic decrease in $\delta^{13}C$ with increasing temperature, shifts in $\delta^{13}C$ were reported in the range of –0.2 to –1.6‰ for eucalyptus, –0.1 to –1.4‰ for oak and 0.0 to –1.7‰ in pine

(Table 1).[11] Similarly isotope fractionations of up to –2‰ have been observed during anaerobic carbonization of plants utilized by prehistoric peoples such as *Zea mays* and *Pachyrhizus erosus* heated at 275°C (5 hours).[12] In combination, these studies suggest that carbon isotope fractionations during burial and carbonization are limited to shifts of between 0.3 and 2‰,[7,9,11] and that plant organic matter preserved in the geological record retains its $\delta^{13}C$ signature to within 2‰. They conclude that $\delta^{13}C$ can be used to indicate palaeo-vegetation sources and reconstruct past CO_2 variations within this uncertainty.[13]

Study	Reaction procedure	Species	Raw $\delta^{13}C$ (‰)	Max change in $\delta^{13}C$ (‰)
This study	Hydrothermal maturation at 300ºC for <237h	Tree fern *(Alsophila spinulosa)*	-31.6	-0.6
		Horsetail *(Equisetum arvense)*	-24.4	-0.9
		Cycad *(Cycas revoluta)*	-26.0	-0.6
		Dawn Redwood *(Metasequoia glyptostroboides)*	-27.1	-0.6
Schleser et al., [9]	Hydrothermal maturation at 180ºC for <3000h	Oak *(Quercus robur)*	-	-1.0
		Beech *(Fagus sylvatica)*	-	-0.5
		Pine *(Pinus sylvestris)*	-	-0.6
		Sequoia *(Sequoiadendron giganteum)*	-25.5	-0.7
Turney et al., [11]	Carbonisation at 200ºC and 400ºC for 4h	Eucalyptus *(Eucalyptus spp,)*	-26.5	-0.2 (200ºC) -1.3 (400ºC)
		Oak *(Quercus robur)*	-25.6	-0.1 (200ºC) -1.0 (400ºC)
		Pine *(Pinus radiata)*	-25.1	0.0 (200ºC) -1.1 (400ºC)
Czimczik et al., [10]	Carbonisation at 150ºC and 340ºC for 15h	Scots pine *(Pinus sylvestris)*	-29.4	0.3 (150ºC) -0.8 (340ºC)
		Birch *(Betula pendula)*	-28.2	0.3 (150ºC) -0.5 (340ºC)

Table 1: Data from previous thermal degradation experiments on angiosperms alongside data from our experiments on non-flowering plant species, including the species used and the change in $\delta^{13}C$ from the untreated sample.

However, the above studies have focused mainly on modern flowering plants (angiosperms) which developed approximately 160 Ma years ago in the Mesozoic and a few gymnosperms including eucalyptus, pine and sequoia. Prior to the dominance of angiosperms, non-flowering plants including free-sporing vascular plants (ferns, horsetails and lycopods) and gymnosperms were more common and their remains constitute a significant portion of the terrestrial geological record.[3] These non-flowering plants tend to have far higher lignin content (30-50%) than angiosperms (~20%).[14,15] Due to the different $\delta^{13}C$ of lignin and cellulose[9] there is still uncertainty about the relative impact of plant tissue diagenesis on

$\delta^{13}C$ in these non-flowering plant species, which have a far higher lignin content. Here, we report a series of thermal maturation experiments, at two temperatures, over 273 hours, on plants which are the direct decedents of these high lignin content species. By using modern non-flowering plants as a proxy we are able to better evaluate the impact of diagenesis and fossilisation on the $\delta^{13}C$ of non-flowering plant remains, which make up a significant part of the geological record in many regions.

METHODS

Fresh samples of: 1) Cyatheaceae, *Alsophila spinulosa* (Wall ex Hook, tree fern) fronds, 2) Cupressaceae, *Metasequoia glyptostroboides* (dawn redwood) leaves, and 3) Cycadaceae, *Cycas revoluta* (Cycad) fronds; were harvested on 18th-19th October 2006, at the Fairy Lake Botanic Gardens, Shenzhen China. Multiple leaves or fronds were harvested from the same plant to create a bulk sample for that individual. Whole plant samples of: 4) *Equisetum arvense* (horsetail), were collected on 4th June 2007 in the UK. In the laboratory all samples were rinsed with 18 MΩ distilled water (Millipore, Merck Millipore Suite 21, Building 6, Croxley Green, Watford UK) before freeze drying. Samples were ground to a fine powder using a freezer mill (SPEX CertiPrep 6850, 2 Dalston Gdns, Stanmore, Middlesex UK) and stored in a vacuum desiccator in the presence of P_2O_5, experimental work was undertaken soon afterwards (late 2007).

For each thermal maturation experiment borosilicate glass tubing was sealed at one end with a natural gas/oxygen flame prior to addition of 5 mg of dry crushed vegetation and 20 μL water. Water was added to simulate natural burial conditions; the maximum amount of water which could be added to each vessel was calculated using the ideal gas equation of state[16] taking into account the number of moles of water, pressure, volume of vessel and temperature. The vessel was attached to a vacuum line maintained at a pressure of 10^{-1} torr with an FNF Neuberger (Avenue Two, Station Lane Industrial Estate, Witney, Oxfordshire UK) rotary pump. Before opening up the vessel to the vacuum, the contents were frozen with liquid nitrogen. The air was evacuated and then following three freeze-pump-thaw cycles the cell sealed off by heating the connection to the vacuum.[17] The reaction vessels were then heated at either 200 or 300°C in triplicate (each temperature and time interval had 3 repeats) in a preheated gas chromatograph oven (Varian Model 3700, now Agilent Technologies, Edinburgh Park, 4-5 Lochside Ave, Edinburgh UK). These temperatures were chosen to mimic average geological temperatures and extend the temperature range of previous experiments undertaken in the presence of water.[9] The reaction vessels were removed from the oven every 12-16 hours up to and including 273 hours to give approximately 22 time points for each experiment. Upon removal from the oven the vessels were allowed to cool to room temperature prior to extraction of the charred plant remains with a spatula to clean vial washed with solvent to remove re-condensed volatile organic carbon (VOC), air dried and transfer to clean glass vial prior to analysis for $\delta^{13}C$ and %C.

$^{13}C/^{12}C$ measurements were performed on fresh (hour 0) and heated (hour 9, 24, 33… up to and including hour 273) vegetation samples (3 of each temperature and time interval). The

samples were weighed into tin capsules and placed into a Carlo Erba 1500 elemental analyser (now Thermo Scientific, Waltham, MA, USA) furnace at 1020°C under a continuous flow of helium carrier gas. An exothermal flash oxidation of the tin and the gases produced were further oxidised by chromium and cobaltous oxide in the lower part of the furnace[18]. After removal of excess oxygen and water (by passage through hot copper and magnesium perchlorate), the remaining N_2 and CO_2 then passed through a GC column and past a thermal conductivity detector. This generated an electrical signal proportional to the concentrations of N_2 and CO_2 present in the helium stream, producing %N and %C data for the sample[18]. The helium stream then carried the CO_2 through a trap at −90°C (for complete removal of water), before reaching the VG Triple-Trap (IsoPrime, Cheadle Hulme, UK) held at −196°C. Here the CO_2 was frozen, allowing any N_2 and He to vent to the atmosphere. The TripleTrap was then evacuated before the CO_2 trap was warmed, allowing the sample CO_2 to expand into the inlet of the isotope ratio VG Optima mass spectrometer (IsoPrime, Cheadle Hulme, UK)[18]. The Optima mass spectrometer has triple collectors allowing simultaneous monitoring of CO_2 ion beams at m/z 44, 45 and 46; and a dual-inlet allowing rapid comparison of sample CO_2 with a reference CO_2. The 45/44 ratios were converted into $^{13}C/^{12}C$ ratios after correction for common ion effects ('Craig' correction)[18]. $\delta^{13}C$ values were calculated to the VPDB (Vienna Pee Dee Belemnite) scale using a in house laboratory standard (BROC2, a sample of *Brassica oleracea* (broccoli) grown in Nottingham University field trials at Sutton Bonington, UK and a secondary low %C standard SOILB, an international soil standard from LECO corporation, St. Joseph, MI, USA), BROC2 was calibrated against NBS-19 and NBS-22 (held and distributed by the International Atomic Energy Agency, Vienna, Austria). Where undertaken, replicate measurements of well-mixed individual samples indicated a precision of $\pm$ <0.1‰ (1 SD). Percent carbon (%C) was measured on the same instrument and replicate measurements indicated a precision of $\pm$ 1% (1SD).

RESULTS AND DISCUSSION

Carbon percentage

Pre-experiment %C ranged between 34% (*Equisetum arvense*) and 45% (*Metasequoia glyptostroboides*) (Figure 1). The extended thermal maturation of these plant materials resulted in a %C increase for all samples at both reaction temperatures (Figure 1),[9,10] although %C increases were always slightly higher (between 12% and 28%) at 300ºC than at 200ºC (between 10% and 20%, Figure 1). Maximum change in %C was observed within the first 24 hours of the experiment, after which carbon content only increased slightly or plateaued at both temperatures (Figure 1). The rapid increase in %C highlights an initial loss of non-carbon components including gaseous and non-gaseous hydrocarbons[10] from within the plant structure. Further, more gradual increases in %C were potentially related to the preferential loss of cellulose which has lower organic carbon percentage in comparison to the more resilient lignin.[10]

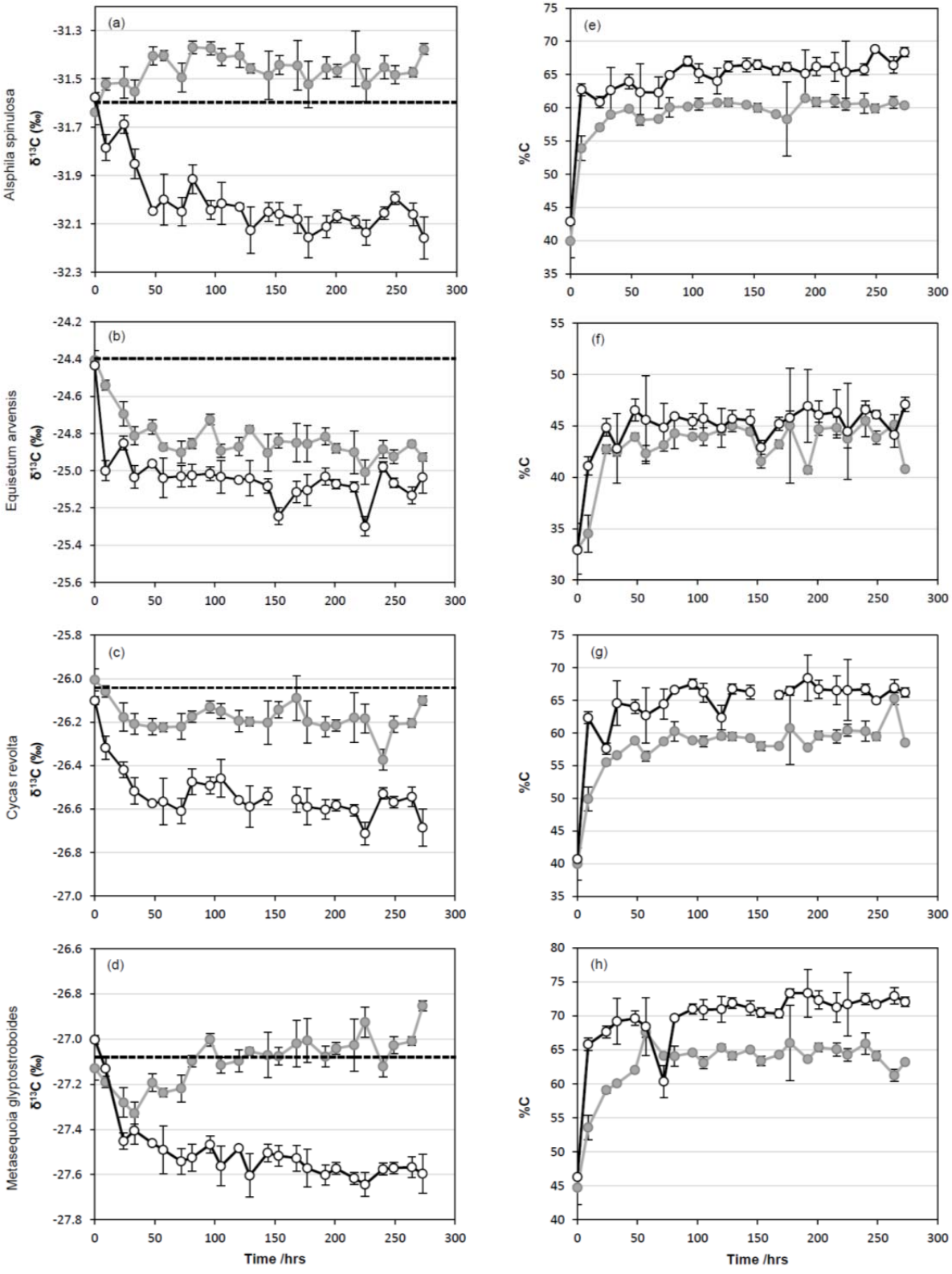

Figure 1: Evolution of δ13C and %C during the 273 hour thermal maturation experiment at both 200ºC (grey circles) and 300ºC (white circles) for Alsphila spinulosa (a and e), Equisetum arvensis (b and f), Cycas revolta (c and g) and Metasequoia glyptostroboides (d and h).

Stable isotope composition

The initial $\delta^{13}C$ values of the plant materials ranged between –24.4‰ (*Equisetum arvense*) and –31.6‰ (*Alsophila spinulosa*, Table 2). Heating at 200ºC produced small but significant reductions in $\delta^{13}C$ value in *Cycas revoluta* (–0.30‰) and *Equisetum arvense* (–0.61‰), characterised initially by a rapid $\delta^{13}C$ depletion by 50 hours and then a more gradual decrease throughout the experiment to 273 hours (Figure 2). However, *Alsophila spinulosa* (+0.20‰) and *Metasequoia glyptostroboides* (+0.25‰) both show an overall increase in $\delta^{13}C$ value during the experiment. In the *Alsophila spinulosa* sample this increase in $\delta^{13}C$ value occurs rapidly, with $\delta^{13}C$ plateauing 0.2‰ higher than initial value after 50 hours (Figure 2). In contrast, the *Metasequoia glyptostroboides* sample has an initial decrease in $\delta^{13}C$ value with a maximum negative isotope shift of –0.21‰ after 33 hours, at which point $\delta^{13}C$ begins to increase, finally becoming more negative than the initial value after 120 hours and remaining so until 273 hours (Figure 2).

Thermal maturation of samples at the higher temperature (300ºC) led to a reduction in $\delta^{13}C$ value in all cases (Table 2), following a similar pattern to the isotopic evolution of *Cycas revoluta* and *Equisetum arvense* at 200ºC (Figure 2). Maximum reductions in $\delta^{13}C$ were observed in *Equisetum arvense* (–0.90‰) and *Metasequoia glyptostroboides* (–0.64‰) (Table 2). Similar magnitude reductions in $\delta^{13}C$ value during thermal maturation have been identified previously in angiosperm samples,[6,9] attributed to cellulose loss. The reduction in isotopically heavy cellulose means that sample $\delta^{13}C$ gradually approaches the lower $\delta^{13}C$ composition of the remaining lignin component.[6]

When considering experiments at both temperatures the majority of samples show a decrease in $\delta^{13}C$ during sample heating and maturation of upto –0.90‰, similar to results of Benner et al.,[6] and Spiker and Hatcher.[7] and the initial phase of isotope change identified by Schleser et al.,[9]. Only one of our non-angiosperm species (*Metasequoia glyptostroboides*) exhibits isotopic fractionation behaviour similar to the second stage described by Schleser et al.,[9], where after an initial decrease in $\delta^{13}C$, values begin to increase totally obscuring the initial decrease. This secondary positive isotope shift may be due to the breakdown of lignin, where isotopically lighter components including methoxyl-groups are preferentially lost.[9] The fact we only show this characteristic isotope decline followed by increase in one sample and at the lower temperature (close to the 180ºC used by Schleser et al.,[9]) suggests that the non-angiosperm samples tested here behave slightly differently during diagenesis than the wood samples (angiosperms) used in previous experiments, but appear to replicate the overall isotopic fractionation range displayed by angiosperm samples from similar experiments.

	Raw $\delta^{13}C$ (‰)	Error (1stdv)	Max $\delta^{13}C$ change at 200ºC (‰)	Error (1stdv)	Max $\delta^{13}C$ change at 300ºC (‰)	Error (1stdv)	Max difference from raw $\delta^{13}C$ (‰)
Alsophila spinulosa (Tree fern)	-31.6	0.05	-31.4	0.11	-32.2	0.09	0.56
Equisetum arvense (Horsetail)	-24.4	0.03	-25.0	0.03	-25.3	0.04	0.90
Cycas revoluta (Cycad)	-26.0	0.02	-26.4	0.04	-26.7	0.10	0.61
Metasequoia glyptostroboides (Dawn Redwood)	-27.1	0.05	-26.9	0.01	-27.6	0.08	0.64

Table 2: Data from this thermal maturation experiment showing the change in $\delta^{13}C$ (at 200ºC and 300ºC) from the untreated value, highlighting the maximum $\delta^{13}C$ change recorded for each species.

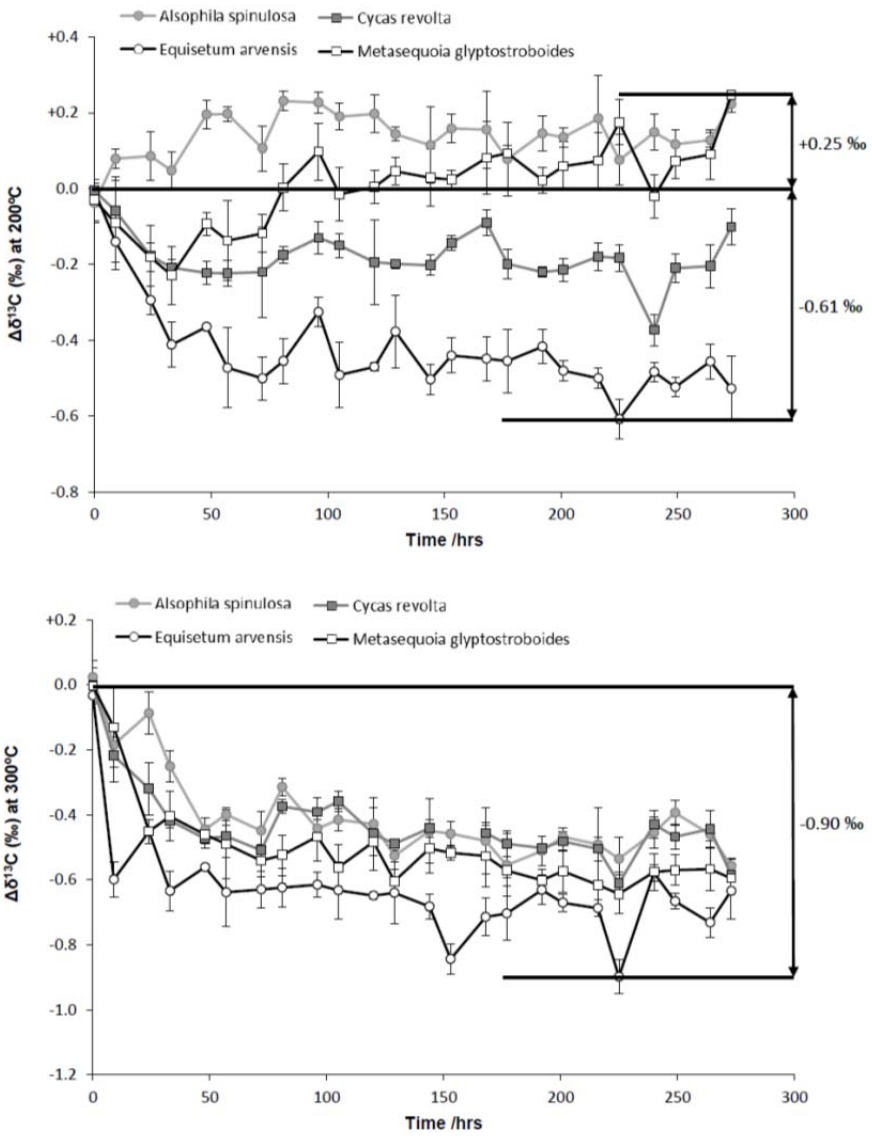

Figure 2: Change in $\delta^{13}C$ ($\Delta^{13}C$) from the initial $\delta^{13}C$ value of the plant material at both 200ºC (top panel) and 300ºC (bottom panel). At 300ºC all species show a rapid reduction in $\delta^{13}C$ by 50 hours followed by a more gradual decrease. At 200ºC one species (*Alsphila spinulosa*) exhibits an immediate rise in $\delta^{13}C$, the other three species have an initial decrease in isotope values at which point values increase above the starting value for *Metasequoia glyptostroboides* but plateau or continue to decrease in *Cycas revolta* and *Equisetum arvensis*.

CONCLUSION

The thermal maturation of four different, non-flowering plants at 200ºC and 300ºC demonstrates a $\delta^{13}C$ value decrease of up to –0.90‰, likely related to the preferential loss and decomposition of isotopically heavier cellulose in relation to lighter lignin. This process occurs rapidly, with the majority of $\delta^{13}C$ change occurring within the first 50 hours of degradation. This carbon isotope shift occurs uniformly across all species at the higher temperature but at 200ºC two of the species exhibit a slight (< +0.25‰) but significant rise in $\delta^{13}C$ values. Importantly, our experiment shows that the extent of $\delta^{13}C$ fractionation in ancient non-angiosperm species is similar to fractionations which are known to occur during the decomposition of modern plant material. Palaeoclimate reconstruction from plants commonly found in the early geological record should therefore only need to consider a relatively small isotope offset (<1‰) related to sedimentation and degradation of non-angiosperm plant material in the early geological record.

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References

[1] T. T. Nguyen Tu, J. Kvaček, D. Uličný, H. Bocherens, A. Mariotti, J. Broutin. Isotope reconstruction of plant palaeoecology. Case study of Cenomanian floras from Bohemia. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **2002**, *183*, 43.

[2] D. McCarroll, N. Loader. Stable isotopes in tree rings. *Quat. Sci. Rev.* **2004**, *23*, 771.

[3] N. Arens, A. Jahren, R. Amundson. Can C3 plants faithfully record the carbon isotopic composition of atmospheric carbon dioxide? *Paleobiology* **2000**, *26*, 137.

[4] K. S. Treydte, D. C. Frank, M. Saurer, G. Helle, G. H. Schleser, J. Esper. Impact of climate and CO2 on a millennium-long tree-ring carbon isotope record. *Geochim. Cosmochim. Acta* **2009**, *73*, 4635.

[5] D. R. Gröcke. Carbon-isotope analyses of fossil plants as a chemostratigraphic and palaeoenvironmental tool. *Lethaia* **1998**, *31*, 1.

[6] R. Benner, M. L. Fogel, E. K. Sprague, R. E. Hodson. Depletion of 13C in lignin and its implication for stable carbon isotope studies. *Nature* **1987**, *329*, 708.

[7] E. C. Spiker, P. G. Hatcher. The effects of early diagenesis on the chemical and stable carbon isotopic composition of wood. *Geochim. Cosmochim. Acta* **1987**, *51*, 1385.

[8] P. F. Van Bergen, I. Poole. Stable carbon isotopes of wood: A clue to palaeoclimate? *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **2002**, *182*, 31.

[9] G. H. Schleser, J. Frielingsdorf, A. Blair. Carbon isotope behaviour in wood and cellulose during artificial aging. *Chem. Geol.* **1999**, *158*, 121.

[10] C. I. Czimczik, C. M. Preston, M. W. I. Schmidt, R. A. Werner, E.-D. Schulze. Effects of charring on mass, organic carbon, and stable carbon isotope composition of wood. *Org. Geochem.* **2002**, *33*, 1207.

[11] C. S. M. Turney, D. Wheeler, A. R. Chivas. Carbon isotope fractionation in wood during carbonization. *Geochim. Cosmochim. Acta* **2006**, *70*, 960.

[12] M. J. DeNiro, C. a. Hastorf. Alteration of and ratios of plant matter during the initial stages of diagenesis: Studies utilizing archaeological specimens from Peru. *Geochim. Cosmochim. Acta* **1985**, *49*, 97.

[13] G. H. Schleser, G. Helle, A. Lücke, H. Vos. Isotope signals as climate proxies: the role of transfer functions in the study of terrestrial archives. *Quat. Sci. Rev.* **1999**, *18*, 927.

[14] J. M. Robinson. Lignin , land plants , and fungi : Biological evolution affecting Phanerozoic oxygen balance Lignin , land plants , and fungi : Biological evolution affecting Phanerozoic oxygen balance. *Geology* **1990**, *15*, 607.

[15] E. Novaes, M. Kirst, V. Chiang, H. Winter-Sederoff, R. Sederoff. Lignin and biomass: a negative correlation for wood formation and lignin content in trees. *Plant Physiol.* **2010**, *154*, 555.

[16] P. . Atkins. *Physical Chemistry*. W.H. Freeman And Company, New York, **1986**.

[17] C. H. Vane, G. D. Abbott. Proxies for land plant biomass: Closed system pyrolysis of some methoxyphenols. *Org. Geochem.* **1999**, *30*, 1535.

[18] E. R. Hurrell, P. A. Barker, M. J. Leng, C. H. Vane, P. Wynn, C. P. Kendrick, D. Verschuren, F. Alayne Street-Perrott. Developing a methodology for carbon isotope analysis of lacustrine diatoms. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 1567.