

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 ($\delta^{13}\text{C}$) 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 $\delta^{13}\text{C}$ in plant material during decomposition and incorporation into sediments. Previous work on modern angiosperm species show $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ signatures.

Methods: To test the extent of $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values were observed for all plants at 300°C and for two of the species at the lower temperature (200°C). The maximum shift in $\delta^{13}\text{C}$ related to experimental decomposition was -0.90% (horsetail) indicating a preferential loss of ^{13}C during thermal maturation.

Conclusions: The reduction in the $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ from non-flowering plant material within the geological record remains robust, but only where interpretations are based on variations in $\delta^{13}\text{C}$ greater than 1‰.

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

1 INTRODUCTION

2 Palaeoenvironmental reconstruction using the carbon isotope composition ($\delta^{13}\text{C}$) of plant
3 organic matter has the potential to provide information on vegetation composition, local
4 water stress and changes in atmospheric CO_2 concentrations.^[1-3] Such reconstructions can be
5 undertaken over a diverse temporal range, from relatively modern reconstruction using tree
6 rings^[2,4] through to palaeo-reconstructions using fossil wood from the early Palaeozoic^[5];
7 although few studies attempt climatic reconstructions on deeply buried geological materials
8 such as coal and graphite, due to difficulties of isotope analysis. The main obstacle to the
9 application of $\delta^{13}\text{C}$ as a tool for palaeoenvironmental and palaeoclimatic reconstructions is
10 that the bulk isotope composition of plant remains is changed during sedimentary processes,
11 including diagenesis and fossilisation. For example hemicellulose and cellulose (enriched in
12 ^{13}C) decay differently to lignin (^{13}C depleted), potentially altering the original $\delta^{13}\text{C}$ in the
13 process.^[6-8] These changes are caused by the effects of pressure, heat and time.
14 Consequently, understanding the extent of diagenetically mediated isotope fractionation is
15 essential if changes in palaeo- CO_2 levels and chemical characterisation of past ecologies are
16 to be undertaken from deposits derived from non-flowering plant material.

17 Previous studies on the diagenesis of plant remains

18 To investigate the impact of plant tissue diagenesis and fossilisation on the final $\delta^{13}\text{C}$ of the
19 organic matter, several studies have simulated diagenesis using a combination of increased
20 heat and pressure. For example, Schleser et al.,^[9] simulated thermolysis of wood in sealed
21 glass vessels at 180°C in air with the presence of liquid water to simulate decomposition.
22 They found that during the experiment $\delta^{13}\text{C}$ initially decreased by about -1‰ (~ 7 hours) then
23 gradually returned to the initial starting value, or gave slightly more positive $\delta^{13}\text{C}$ values than
24 fresh wood. Maximal deviations from the original unheated counterpart at 7 h were: -1‰
25 oak, -0.7‰ sequoia, -0.6‰ pine and -0.5‰ beech (Table 1), these shifts in $\delta^{13}\text{C}$ were
26 thought to be due to 30-60% cellulose decomposition.^[9] The change in $\delta^{13}\text{C}$ to more negative
27 values was therefore explained by the preferential loss of cellulose and relative enrichment in
28 isotopically light lignin upon diagenesis. In another study, the effect of temperature on the
29 $\delta^{13}\text{C}$ of hard and softwood has been studied to understand black carbon (BC) formation
30 which mainly occurs during forest fires.^[10] Birch and pine woods were heated at 150, 340 and
31 480°C in an oven flushed with argon to create an oxygen free atmosphere.^[10] Both woods
32 showed a shift of 0.3‰ to more positive $\delta^{13}\text{C}$ at 150°C , but became progressively more
33 negative by -0.5 to -0.8‰ at 340°C and -0.6 to -1.1‰ at 480°C compared to their untreated
34 counterparts (Table 1).^[10] More recently the carbonization of wood was examined by
35 wrapping eucalyptus, oak and pine in aluminium foil packets and heating at 200, 400, 600
36 and 800°C in a muffle furnace at atmospheric pressure.^[11] The charred woods showed a
37 systematic decrease in $\delta^{13}\text{C}$ with increasing temperature, shifts in $\delta^{13}\text{C}$ were reported in the
38 range of -0.2 to -1.6‰ for eucalyptus, -0.1 to -1.4‰ for oak and 0.0 to -1.7‰ in pine

39 (Table 1).^[11] Similarly isotope fractionations of up to -2‰ have been observed during
 40 anaerobic carbonization of plants utilized by prehistoric peoples such as *Zea mays* and
 41 *Pachyrhizus erosus* heated at 275°C (5 hours).^[12] In combination, these studies suggest that
 42 carbon isotope fractionations during burial and carbonization are limited to shifts of between
 43 0.3 and 2‰ ,^[7,9,11] and that plant organic matter preserved in the geological record retains its
 44 $\delta^{13}\text{C}$ signature to within 2‰ . They conclude that $\delta^{13}\text{C}$ can be used to indicate palaeo-
 45 vegetation sources and reconstruct past CO_2 variations within this uncertainty.^[13]

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

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

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

57 $\delta^{13}\text{C}$ in these non-flowering plant species, which have a far higher lignin content. Here, we
58 report a series of thermal maturation experiments, at two temperatures, over 273 hours, on
59 plants which are the direct decedents of these high lignin content species. By using modern
60 non-flowering plants as a proxy we are able to better evaluate the impact of diagenesis and
61 fossilisation on the $\delta^{13}\text{C}$ of non-flowering plant remains, which make up a significant part of
62 the geological record in many regions.

63 METHODS

64 Fresh samples of: 1) Cyatheaceae, *Alsophila spinulosa* (Wall ex Hook, tree fern) fronds, 2)
65 Cupressaceae, *Metasequoia glyptostroboides* (dawn redwood) leaves, and 3) Cycadaceae,
66 *Cycas revoluta* (Cycad) fronds; were harvested on 18th-19th October 2006, at the Fairy Lake
67 Botanic Gardens, Shenzhen China. Multiple leaves or fronds were harvested from the same
68 plant to create a bulk sample for that individual. Whole plant samples of: 4) *Equisetum*
69 *arvense* (horsetail), were collected on 4th June 2007 in the UK. In the laboratory all samples
70 were rinsed with 18 M Ω distilled water (Millipore, Merck Millipore Suite 21, Building 6,
71 Croxley Green, Watford UK) before freeze drying. Samples were ground to a fine powder
72 using a freezer mill (SPEX CertiPrep 6850, 2 Dalston Gdns, Stanmore, Middlesex UK) and
73 stored in a vacuum desiccator in the presence of P₂O₅, experimental work was undertaken
74 soon afterwards (late 2007).

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

95 $^{13}\text{C}/^{12}\text{C}$ measurements were performed on fresh (hour 0) and heated (hour 9, 24, 33... up to
96 and including hour 273) vegetation samples (3 of each temperature and time interval). The

97 samples were weighed into tin capsules and placed into a Carlo Erba 1500 elemental analyser
98 (now Thermo Scientific, Waltham, MA, USA) furnace at 1020°C under a continuous flow of
99 helium carrier gas. An exothermal flash oxidation of the tin and the gases produced were
100 further oxidised by chromium and cobaltous oxide in the lower part of the furnace^[18]. After
101 removal of excess oxygen and water (by passage through hot copper and magnesium
102 perchlorate), the remaining N₂ and CO₂ then passed through a GC column and past a thermal
103 conductivity detector. This generated an electrical signal proportional to the concentrations of
104 N₂ and CO₂ present in the helium stream, producing %N and %C data for the sample^[18]. The
105 helium stream then carried the CO₂ through a trap at -90°C (for complete removal of water),
106 before reaching the VG Triple-Trap (IsoPrime, Cheadle Hulme, UK) held at -196°C. Here
107 the CO₂ was frozen, allowing any N₂ and He to vent to the atmosphere. The TripleTrap was
108 then evacuated before the CO₂ trap was warmed, allowing the sample CO₂ to expand into the
109 inlet of the isotope ratio VG Optima mass spectrometer (IsoPrime, Cheadle Hulme, UK)^[18].
110 The Optima mass spectrometer has triple collectors allowing simultaneous monitoring of CO₂
111 ion beams at m/z 44, 45 and 46; and a dual-inlet allowing rapid comparison of sample CO₂
112 with a reference CO₂. The 45/44 ratios were converted into ¹³C/¹²C ratios after correction for
113 common ion effects ('Craig' correction)^[18]. δ¹³C values were calculated to the VPDB
114 (Vienna Pee Dee Belemnite) scale using a in house laboratory standard (BROC2, a sample of
115 *Brassica oleracea* (broccoli) grown in Nottingham University field trials at Sutton
116 Bonington, UK and a secondary low %C standard SOILB, an international soil standard from
117 LECO corporation, St. Joseph, MI, USA), BROC2 was calibrated against NBS-19 and
118 NBS-22 (held and distributed by the International Atomic Energy Agency, Vienna, Austria).
119 Where undertaken, replicate measurements of well-mixed individual samples indicated a
120 precision of ± <0.1‰ (1 SD). Percent carbon (%C) was measured on the same instrument and
121 replicate measurements indicated a precision of ± 1% (1SD).

122 **RESULTS AND DISCUSSION**

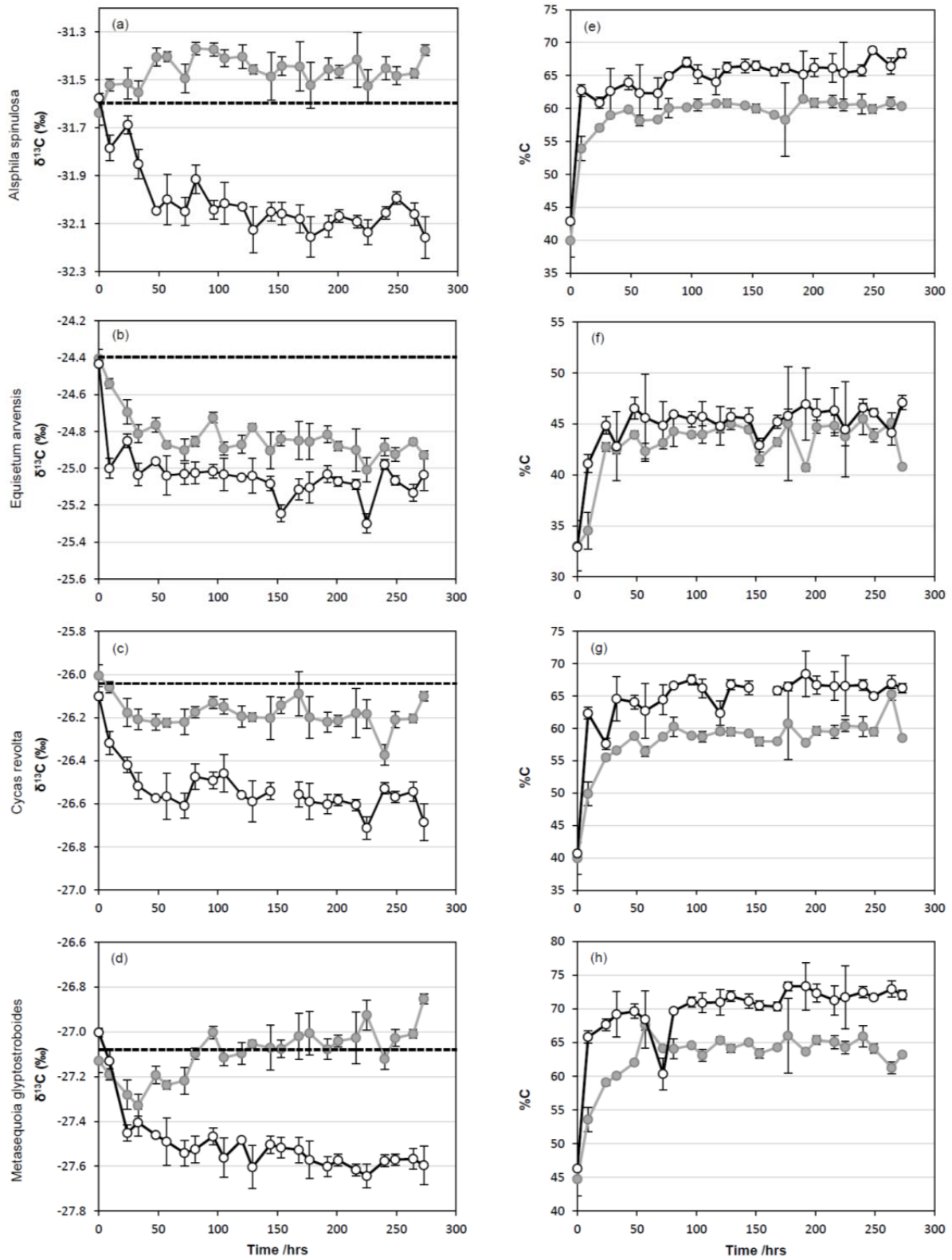
123 **Carbon percentage**

124 Pre-experiment %C ranged between 34% (*Equisetum arvense*) and 45% (*Metasequoia*
125 *glyptostroboides*) (Figure 1). The extended thermal maturation of these plant materials
126 resulted in a %C increase for all samples at both reaction temperatures (Figure 1),^[9,10]
127 although %C increases were always slightly higher (between 12% and 28%) at 300°C than at
128 200°C (between 10% and 20%, Figure 1). Maximum change in %C was observed within the
129 first 24 hours of the experiment, after which carbon content only increased slightly or
130 plateaued at both temperatures (Figure 1). The rapid increase in %C highlights an initial loss
131 of non-carbon components including gaseous and non-gaseous hydrocarbons^[10] from within
132 the plant structure. Further, more gradual increases in %C were potentially related to the
133 preferential loss of cellulose which has lower organic carbon percentage in comparison to the
134 more resilient lignin.^[10]

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158 Figure 1: Evolution of $\delta^{13}\text{C}$ and %C during the 273 hour thermal maturation experiment at both
 159 200°C (grey circles) and 300°C (white circles) for *Alsphila spinulosa* (a and e), *Equisetum arvensis* (b
 160 and f), *Cycas revolta* (c and g) and *Metasequoia glyptostroboides* (d and h).

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163 **Stable isotope composition**

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

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

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

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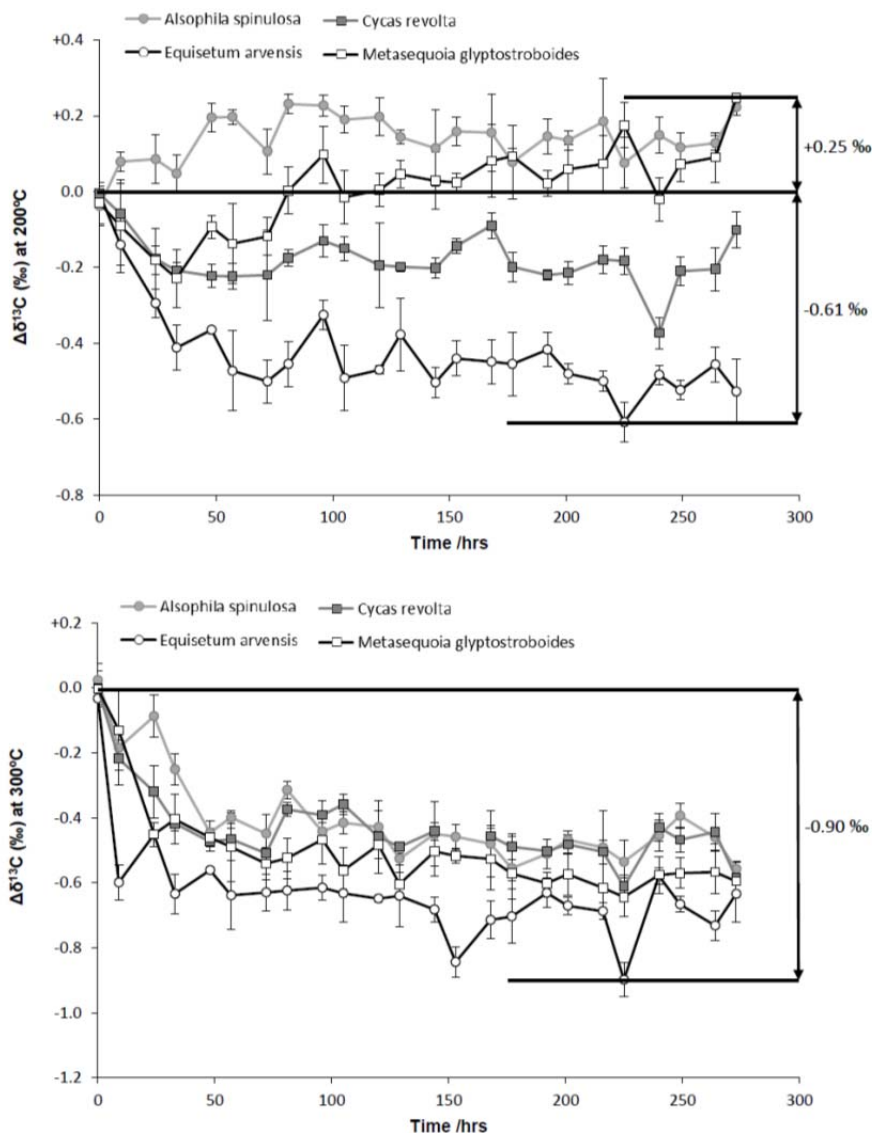
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	Raw $\delta^{13}\text{C}$ (‰)	Error (1stdv)	Max $\delta^{13}\text{C}$ change at 200°C (‰)	Error (1stdv)	Max $\delta^{13}\text{C}$ change at 300°C (‰)	Error (1stdv)	Max difference from raw $\delta^{13}\text{C}$ (‰)	
201	Alsophila spinulosa (Tree fern)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

205 **Table 2:** Data from this thermal maturation experiment showing the change in $\delta^{13}\text{C}$ (at 200°C and
 206 300°C) from the untreated value, highlighting the maximum $\delta^{13}\text{C}$ change recorded for each species.



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

229 **CONCLUSION**

230 The thermal maturation of four different, non-flowering plants at 200°C and 300°C
231 demonstrates a $\delta^{13}\text{C}$ value decrease of up to -0.90% , likely related to the preferential loss and
232 decomposition of isotopically heavier cellulose in relation to lighter lignin. This process
233 occurs rapidly, with the majority of $\delta^{13}\text{C}$ change occurring within the first 50 hours of
234 degradation. This carbon isotope shift occurs uniformly across all species at the higher
235 temperature but at 200°C two of the species exhibit a slight ($< +0.25\%$) but significant rise in
236 $\delta^{13}\text{C}$ values. Importantly, our experiment shows that the extent of $\delta^{13}\text{C}$ fractionation in
237 ancient non-angiosperm species is similar to fractionations which are known to occur during
238 the decomposition of modern plant material. Palaeoclimate reconstruction from plants
239 commonly found in the early geological record should therefore only need to consider a
240 relatively small isotope offset ($<1\%$) related to sedimentation and degradation of non-
241 angiosperm plant material in the early geological record.

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