

# Speleothem biomarker evidence for a negative terrestrial feedback on climate during Holocene warm periods

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## Highlights

- The first compound specific  $\delta^{13}\text{C}$  analysis of fatty acids from a stalagmite
- Proportional increases in  $\text{C}_3$  plants during warmer/wetter Holocene intervals
- Soil respiration is more substrate selective during warmer/wetter Holocene intervals
- Primary production outpaces soil respiration during warmer/wetter Holocene intervals
- Subtropical mineral soils act as a negative feedback in a warmer/wetter climate

1 **Speleothem Biomarker Evidence for a Negative Terrestrial Feedback on Climate During**  
2 **Holocene Warm Periods**

3

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15 **Abstract**

16 Understanding how terrestrial carbon storage feeds back on warm climate states is critical for  
17 improving global warming projections. Soils may act as a positive feedback on climate if  
18 warming increases soil carbon decomposition rates. Conversely, if increases in net primary  
19 production (NPP) exceed increases in decomposition, the climate feedback will be negative. Here  
20 we utilize the first palaeoclimatic application of compound-specific  $\delta^{13}\text{C}$  measurements on *n*-fatty  
21 acid biomarkers (extracted from a stalagmite from central China) to constrain the response of  
22 catchment terrestrial carbon cycle feedbacks during warmer phases of the Holocene. We resolve  
23 proportional increases in  $\text{C}_3$  plants in the catchment area during these warmer/wetter intervals.  
24 Moreover, we infer that heterotrophic soil respiration was highly substrate selective, indicating  
25 that NPP outpaced decomposition and the catchment behaved as a carbon sink (mediated and  
26 enhanced by changes in the relative proportion of  $\text{C}_3$  vs  $\text{C}_4$  plants). Thus, we provide  
27 palaeoclimate evidence that subtropical soils in a warmer/wetter climate acted as a sink for  
28 organic carbon, and thus as a negative climate feedback, during warmer climatic phases.

29

30 **Keywords**

31 Speleothem; Fatty acids; Carbon isotope; Vegetation; Soil respiration; Negative feedback

32 **1. Introduction**

33         Constraining the effect of climate change on terrestrial respiration and associated feedbacks  
34 is critical to furthering our understanding of the global carbon cycle ([Mahecha et al., 2010](#)).  
35 Globally, soil respiration contributes ca. 100 PgC/yr from the soil to the atmosphere and is  
36 sensitive to changes in temperature and precipitation ([Bond-Lamberty and Thomson, 2010](#)). If  
37 warming increases decomposition rates and transfers carbon stored belowground to the  
38 atmosphere, a positive feedback to climate change will occur. Conversely, if increases of  
39 plant-derived carbon inputs to soils exceed increases in decomposition, the feedback will be  
40 negative. Laboratory and mesocosm experiments to interrogate the response of soil carbon to  
41 climate change show highly variable results (see review by [Davidson and Janssens \(2006\)](#) and  
42 refs therein). Moreover, laboratory and mesocosm experiments have limited ecosystem  
43 complexity and operate on limited timescales, from years to decades ([Melillo et al., 2017](#)). Thus,  
44 the longer-term climate sensitivity of soil organic matter and global soil carbon stocks, at the  
45 whole ecosystem level, is still subject to debate ([Davidson and Janssens, 2006](#)). An alternative to  
46 laboratory and mesocosm studies is to use palaeoclimate data, which inherently incorporates the  
47 response of the whole system. A recent ice-core based study used centennial scale data to derive  
48 an estimate for the response of gross primary production and ecosystem respiration to cold  
49 climate state during the Little Ice Age cooling (LIA) ([Rubino et al., 2016](#)). However, the  
50 sensitivity of ecosystem respiration to past warm climate states has not yet been investigated as  
51 we lack geological proxies to quantify net primary production and terrestrial respiration.  
52 Constraining the intensity of feedback mechanisms between terrestrial ecosystems and warmer  
53 climates, on longer timescales, and in natural settings, is central to understanding the global  
54 carbon cycle, and thus a prerequisite for reliable future climate projections.

55

56         Speleothems are versatile terrestrial archives of palaeoclimate because they have the ability  
57 to record changes in ambient environmental conditions at the time of deposition, and can preserve

58 material transported from overlying ecosystems via karst drip waters ([Fairchild and Baker, 2012](#)).  
59 *Normal (n-)* alkyl lipid biomarkers are produced by higher plants, algae, and bacteria, and are  
60 preserved in various palaeoclimate archives. In speleothems, however, *n*-alkyls have the potential  
61 to constrain these ambiguities and isolate catchment vegetation and bacterial changes. The  $\delta^{13}\text{C}$  of  
62 high molecular weight *n*-alkyls (leaf waxes predominantly produced by higher plants) preserved  
63 in lake ([Huang et al., 2001](#)) and marine ([Hughen et al., 2004](#)) sediments, along with palaeosols  
64 ([Zhang et al., 2006](#)), has been used extensively to reconstruct changes in the relative abundance  
65 of  $\text{C}_3$  vs  $\text{C}_4$  plants. Low molecular weight *n*-alkyl  $\delta^{13}\text{C}$  records are typically overlooked in  
66 palaeoclimate archives because they are produced by three end-members (plants, algae, and  
67 bacteria), rendering interpretation challenging. In speleothems though, algal contributions are  
68 likely minimal ([Fairchild and Baker, 2012](#)), meaning that low molecular weight *n*-alkyls derive  
69 from a simpler two end-member system, produced by higher plants and bacteria. Therefore, the  
70 compound-specific records of  $\delta^{13}\text{C}$  entrapped in the calcite can be more directly linked to sources  
71 and processes (e.g. bacterial respiration, catchment vegetation, etc.) ([Blyth et al., 2016](#)),  
72 something which cannot, for example, be achieved from the carbon isotope compositions of bulk  
73 calcite ([Fairchild and Baker, 2012](#); [Genty et al., 2003](#)), operationally defined organic matter (e.g.  
74 acid-soluble organic matter (ASOM) ([Li et al., 2014](#)), or non-purgeable organic carbon (NPOC)  
75 ([Blyth et al., 2013](#))). Hence, the  $\delta^{13}\text{C}$  of lipid biomarkers in speleothems represents a uniquely  
76 direct line of evidence for vegetation and bacterial changes in terrestrial ecosystems.

77

78 Here we present the first record of soil bacterial respiration (a critical component of  
79 terrestrial ecosystem respiration) and vegetation changes from central East Asia using  
80 compound-specific carbon isotopes extracted from a cave speleothem covering the past 9,000  
81 years. Our novel record is based on the  $\delta^{13}\text{C}$  of low molecular weight (LMW;  $\leq\text{C}_{20}$ ) and high  
82 molecular weight (HMW;  $>\text{C}_{20}$ ) *n*-fatty acids (a subset of *n*-alkyls) from a previously-reported  
83 Holocene speleothem (HS4) recovered from Heshang Cave ([Hu et al., 2008b](#)) ([Fig. 1](#)), located in

84 the East Asian monsoon region of central China ([Wang et al., 2018](#)). The temperature and  
85 hydrological conditions in this region have been reconstructed by multiple proxies from the HS4  
86 stalagmite ([Wang et al., 2016](#); [Wang et al., 2018](#); [Xie et al., 2013](#); [Zhu et al., 2017](#)). The  
87 3-hydroxy fatty acid (3-OH-FA) biomarker based RAN<sub>15</sub> proxy reconstructs mean annual  
88 temperature variations of ca.16 to 21°C during the last 9 ka BP, with a relatively warm period in  
89 the early to middle Holocene (8.0–6.0 ka BP), and then a relatively cool period in the late  
90 Holocene ([Wang et al., 2018](#)) (see [Fig. 5d](#)). The hydrological conditions for the region have also  
91 been reconstructed from multiple archives and proxies, including the 3-OH-FA biomarker-based  
92 RIAN proxy from the HS4 stalagmite ([Fig. 5g](#)), which, for the Holocene, indicate two relatively  
93 long wet periods and one relatively dry period, 8.8–5.9 ka BP, 3.0–0 ka BP and 5.9–3.0 ka BP  
94 respectively ([Wang et al., 2018](#); [Xie et al., 2013](#); [Zhu et al., 2017](#)). In this current study we  
95 demonstrate a marked increase in C<sub>3</sub> vegetation during warm periods of the Holocene epoch,  
96 namely the Holocene Climate Optimum (HCO; ca. 4.4 to 8.8 ka BP) and Medieval Warm Period  
97 (MWP). Moreover, a deconvolved record of soil bacterial respiration and substrate selectivity  
98 implies that the catchment behaved as a net carbon sink during these warm intervals, thus acting  
99 as a negative climate feedback.

100

## 101 **2. Materials and Methods**

### 102 *2.1 Study site and sample information*

103 Heshang Cave is located at 294m above sea level (a.s.l.), in the Qing River, a tributary in the  
104 middle reaches of the Yangtze River, central China (30°27'N, 110°25'E). Heshang Cave is a  
105 dissolutional cave system formed in Cambrian dolomite, the overlying dolomite is ca. 400 m  
106 thick and is capped with a mature layer of soil (20-40 cm-thick) and reasonably dense vegetation  
107 ([Hu et al., 2008a](#)). The regional climate is strongly impacted by the East Asian Monsoon, with a  
108 hot and moist summer, but relatively cold and dry winter ([An, 2000](#)). The average annual  
109 precipitation in this region is 1161 mm, based on the recent 64 years (1951-2014) of

110 meteorological data from Yichang station (located ca.100 km east of Heshang Cave). The  
111 seasonal temperature ranges, inside and immediately outside the cave, were constrained by 2-h  
112 resolution logging between 2003 - 2007 using HOBO H8 Pro T loggers ([Hu et al., 2008a](#)). The  
113 modern temperature immediately outside the cave varies seasonally from 3°C to 30°C, with an  
114 annual average of 18°C and is statistically identical to that of the nearest government  
115 meteorological station at Changyang county ([Hu et al., 2008a](#)).

116 The HS4 stalagmite is 2.5 m long, and was actively growing when collected from ca. 150 m  
117 within Heshang Cave in 2001. Soil samples were collected in 2013 from the thin soil layers  
118 overlying Heshang Cave, more details were reported in [Wang et al. \(2018\)](#). A detailed description  
119 of the age model of HS4 stalagmite can be found in [Hu et al. \(2008b\)](#).

120

## 121 *2.2 Lipid Extraction and Work-Up*

122 In order to prevent external contamination during the experiments, all the glassware was first  
123 soaked overnight in a decontamination solution, then rinsed in deionised water, dried and  
124 combusted for 6h at 500°C. All solvents were purchased at the highest purity available (Absolv,  
125 TEDIA) and were checked for purity using gas chromatography–mass spectrometry prior to use.  
126 The HCl was pre-extracted with dichloromethane (DCM, ×4), and all other reagents were tested  
127 for background contaminants.

128 The stalagmite samples were treated with an optimized acid digestion method following  
129 [Wang et al. \(2012\)](#). In brief, 10 grams of stalagmite sample were digested with 3M HCl, then  
130 re-fluxed at 130°C for 3 hours with a condenser/ electrothermal heating mantle assembly. An  
131 internal standard (pregn-5-en-3,β-ol) was quantitatively added to each sample to quantify the  
132 amount of lipids in the stalagmite. After cooling, the residue was extracted by dichloromethane  
133 (15mL×4) and the extracts combined. Solvents were removed by rotary evaporation (Buchi R210)  
134 under reduced pressure.



135 The condensed lipids were further derivatized by BF<sub>3</sub>-methanol (14% BF<sub>3</sub>/methanol, Sigma)  
136 before undergoing column separation. The elute solvent are successively in Hexane,  
137 Hexane:DCM (2:1, v/v), DCM and Methanol. The fatty acid methyl esters are in the DCM eluted  
138 fraction.

139

### 140 2.3 Instrumental Analysis

141 Identification of *n*-alkanoic acids was performed on an Agilent 7890B gas chromatograph  
142 (GC) coupled to an Agilent 5977A mass spectrometer (MS) using a BPI fused silica capillary  
143 column (60 m×0.25 mm id.; 0.25 μm film thickness). The GC oven temperature was programmed  
144 from 70°C to 130°C at 10°C per min, then from 130°C to 340°C at 3°C per min, and finally held  
145 at 340°C for 10 min. The carrier gas was Helium (2.7 mL/min). The MS was operated in  
146 electron-impact (EI) mode, the ionization energy was set at 70 eV and the scan range was from 50  
147 to 550 aum.

148 All the stalagmite samples were quantified on an Agilent 7890B gas chromatograph-FID  
149 detector for quantification, separation was performed on a BPI fused silica capillary column (60  
150 m×0.25 mm id.; 0.25 μm film thickness). The GC oven temperature was programmed from 70°C  
151 (1min) to 150°C ramped at 30°C per min, then from 150°C to 340°C at 3°C per min, and finally  
152 held at 340°C for 10 min.

153 Compound-specific δ<sup>13</sup>C analyses of the C<sub>16</sub>, C<sub>18</sub>, C<sub>22</sub>, C<sub>24</sub> *n*-fatty acids were performed on  
154 an Agilent 7890A GC coupled to an Isoprime GC5 furnace and an IsoPrime100 isotope ratio  
155 mass spectrometer. The Isoprime GC5 contains a CuO furnace tube and is kept at 850°C. The GC  
156 was equipped with a 60 m BPI column (SGE) (i.d. = 0.25 mm, film thickness = 0.25 μm), with  
157 helium as the carrier gas, set at a constant flow of 1.7 ml/min, the oven was programmed from  
158 70°C (1 min) to 150°C ramped at 30°C /min, then from 150°C to 340°C at 3°C per min, and  
159 finally held at 340°C for 5 min.

160 The carbon isotope values are reported as per mil (‰) deviations from Vienna Pee Dee  
161 Belemnite (VPDB) in standard delta notation. A homemade mixture of *n*-alkanes standard and an  
162 authenticated standard *n*-fatty acid methyl and ethyl esters mixture with known  $\delta^{13}\text{C}$  (F8; Arndt  
163 Schimmelmann, Indiana University) were measured regularly between a maximum of 5 sample  
164 injections to test the conditions of the instrument and determine the  $\delta^{13}\text{C}$  values of the *n*-alkanoic  
165 acids. Each sample was run at least in duplicate.

166 Correcting the derivatisation effect on  $\delta^{13}\text{C}$  isotopic signature of the fatty acids was done  
167 following [Polissar and D'Andrea \(2014\)](#). The determination of the  $\delta^{13}\text{C}$  of the methanol is  
168 calibrated by a phthalic acid standard with a known  $\delta^{13}\text{C}$  value bought from A. Schimmelmann,  
169 Indiana University.

170

#### 171 *2.4 Calculation of carbon isotopic values of fatty acids derived from bacteria*

172 We isolate the bacterial contribution to the *n*-C<sub>18</sub> chain length fatty acid, because this chain  
173 length is known to be produced by higher plants in low relative abundances ([Chikaraishi and](#)  
174 [Naraoka, 2007](#); [Chikaraishi et al., 2004a](#); [Liu and Liu, 2017](#); [Wang and Liu, 2012](#)). Equation (1)  
175 describes the measured  $\delta^{13}\text{C}_{18\text{FA}}$  as a function of the higher plant and bacterial *n*-C<sub>18</sub> chain length  
176 fatty acid carbon isotopic composition ( $\delta^{13}\text{C}_{18\text{FA(P)}}$  and  $\delta^{13}\text{C}_{18\text{FA(B)}}$ , respectively) and F, the  
177 fractional contribution of higher plants to  $\delta^{13}\text{C}_{18\text{FA}}$ .

$$178 \quad \delta^{13}\text{C}_{18\text{FA}} = F * \delta^{13}\text{C}_{18\text{FA(P)}} + (1 - F) * \delta^{13}\text{C}_{18\text{FA(B)}} \quad (1)$$

179 Higher plants do not fractionate differentially during the production of fatty acids within  
180 even or odd chain lengths ([Chikaraishi and Naraoka, 2007](#)). Therefore, if we presume that  
181 exclusively plant-derived  $\delta^{13}\text{C}_{24\text{FA}}$  and the higher plant-derived contribution to  $\delta^{13}\text{C}_{18\text{FA}}$  ( $\delta^{13}\text{C}_{18\text{FA(P)}}$ )  
182 derived from predominantly the same higher-plant sources in the catchment, then  $\delta^{13}\text{C}_{24\text{FA}}$  and  
183  $\delta^{13}\text{C}_{18\text{FA(P)}}$  should have the same carbon isotopic signature ([Chikaraishi and Naraoka, 2007](#)).  
184 Rearranging and substituting  $\delta^{13}\text{C}_{24\text{FA}}$  into equation (1) gives us equation (2), which expresses the  
185 carbon isotopic signature of the bacterial fraction of C<sub>18FA</sub> ( $\delta^{13}\text{C}_{18\text{FA(B)}}$ ) as a function of measured

186  $\delta^{13}\text{C}_{18\text{FA}}$ , measured  $\delta^{13}\text{C}_{24\text{FA}}$ , and F.

187 
$$\delta^{13}\text{C}_{18\text{FA(B)}} = (\delta^{13}\text{C}_{18\text{FA}} - F * \delta^{13}\text{C}_{24\text{FA}}) / (1 - F) \quad (2)$$

188 F, the fractional contribution of higher plants  $\delta^{13}\text{C}_{18\text{FA}}$ , can be expressed as the quotient of the  
189 relative abundance of higher plant-derived *n*-C<sub>18</sub> chain length fatty acids ( $\text{C}_{18\text{FA(P)}}$ ) divided by the  
190 relative abundance of C<sub>18</sub> chain length fatty acids ( $\text{C}_{18\text{FA}}$ ) in each sample:

191 
$$F = \text{C}_{18\text{FA(P)}} / \text{C}_{18\text{FA}} \quad (3)$$

192 We do not know the relative abundance of higher plant-derived C<sub>18FA</sub> ( $\text{C}_{18\text{(P)}}$ ) *a priori*, but we  
193 can estimate this using the mean ratio of C<sub>18FA</sub>:C<sub>24FA</sub> (R) produced by higher plants. The mean R  
194 value is 1.14 for the compiled global dataset (see [Supplementary Datasheet 1](#)) ([Chikaraishi et al.,](#)  
195 [2004a](#); [Liu and Liu, 2017](#); [Wang and Liu, 2012](#)). Thus, in equation (4) we express  $\text{C}_{18\text{FA(P)}}$  as the  
196 product of R and the measured relative abundance of C<sub>24FA</sub>.

197 
$$\text{C}_{18\text{FA(P)}} = \text{R} * \text{C}_{24\text{FA}} \quad (4)$$

198 Combining equations (2), (3), & (4) gives equation (5), which expresses the bacterial carbon  
199 isotope signature of each sample ( $\delta^{13}\text{C}_{18\text{FA(B)}}$ ) as a function of the sampled relative abundances of  
200 C<sub>18FA</sub> and C<sub>24FA</sub>, the mean C<sub>18FA</sub>:C<sub>24FA</sub> ratio of higher plants (R), and the measured carbon isotopic  
201 signatures of the C<sub>18</sub> and C<sub>24</sub> chain length fatty acids in the sample.

202 
$$\delta^{13}\text{C}_{18\text{FA(B)}} = (\delta^{13}\text{C}_{18\text{FA}} - (\text{R} * \text{C}_{24\text{FA}} / \text{C}_{18\text{FA}}) \delta^{13}\text{C}_{24\text{FA}}) / (1 - \text{R} * \text{C}_{24\text{FA}} / \text{C}_{18\text{FA}}) \quad (5)$$

203 The uncertainty in the calculated  $\delta^{13}\text{C}_{18\text{FA(B)}}$  is propagated from the uncertainties of  $\delta^{13}\text{C}_{18\text{FA}}$   
204 and  $\delta^{13}\text{C}_{24\text{FA}}$  according to equation (2). Since there is considerable variability in reported global R  
205 values in the literature ([Supplementary Datasheet 1](#)), putting an error estimate on this would be  
206 highly speculative (and perhaps much too conservative, given that we are operating in a single  
207 catchment where the spatial and temporal variability is unlikely to approach the global spread in  
208 reported R values. Future research efforts for better constraints on the value of R and soil bacterial  
209 biomarkers are needed with the ultimate goal of producing more quantitative estimates of  
210 palaeo-respiration). Thus the propagated error is calculated according equation (6) shown below:

211 
$$\delta(\delta^{13}\text{C}_{18\text{FA(B)}}) = \sqrt{\left(\frac{1}{1-F} \delta a\right)^2 + \left(\frac{F}{1-F} \delta b\right)^2} \quad (6)$$

212 where  $\delta a$  represents the uncertainty of  $\delta^{13}\text{C}_{18\text{FA}}$ ,  $\delta b$  represents the uncertainty of  $\delta^{13}\text{C}_{24\text{FA}}$ .

213 Our  $\delta^{13}\text{C}_{18\text{FA(B)}}$  curve is derived by subtracting the  $\text{C}_{18\text{FA(P)}}$  contribution from the  $\text{C}_{18\text{FA}}$  record  
 214 (e.g. correcting for the direct contribution of higher plants to the  $\text{C}_{18\text{FA}}$ ). As a data exploration  
 215 exercise, we further subtracted the  $\delta^{13}\text{C}_{24\text{FA(P)}}$  record from the  $\delta^{13}\text{C}_{18\text{FA(B)}}$  (see Figure S1). An  
 216 argument for this approach is that the resultant  $\Delta\delta^{13}\text{C}_{18\text{FA(B)} - 24\text{FA}}$  is a more constrained record of  
 217 soil bacterial respiration—by doing this, one is attempting to remove the influence of changes in  
 218  $\text{C}_3/\text{C}_4$  vegetation on the bacterial substrate pool. However, calculating  $\Delta\delta^{13}\text{C}_{18\text{FA(B)} - 24\text{FA}}$  entails a  
 219 two-step subtraction in which a (weighted)  $\delta^{13}\text{C}_{24\text{FA(P)}}$  is added to itself (less  $\delta^{13}\text{C}_{18\text{FA}}$ ). Given the  
 220 much larger isotopic range for the  $\delta^{13}\text{C}_{24\text{FA(P)}}$  record compared with the raw  $\delta^{13}\text{C}_{18\text{FA}}$  record, the  
 221 end result is simply to produce a curve that largely resembles the original  $\delta^{13}\text{C}_{24\text{FA(P)}}$  record with  
 222 amplified variability. In light of this, we restrict our discussion and interpretation to our  $\delta^{13}\text{C}_{18\text{FA(B)}}$   
 223 record.

224

### 225 **3. Results and Discussion**

#### 226 *3.1 Composition and distribution of fatty acids*

227 The chain length of fatty acids ranges from  $\text{C}_{12}$  to  $\text{C}_{32}$  in the overlying soils and from  $\text{C}_{12}$  to  
 228  $\text{C}_{28}$  in the HS4 stalagmite. In all the sample sets, fatty acids exhibit a bimodal distribution, with a  
 229 strong predominance of even-carbon-numbered homologues maximizing at  $n\text{-C}_{16}$  and  $n\text{-C}_{24}$ . Trace  
 230 amounts of branched and monounsaturated fatty acids were found along with the abundant  $n$ -fatty  
 231 acids in both the overlying soils and HS4 stalagmite. However, in this study, HMW branched  
 232 fatty acids were only detected in the HS4 stalagmite (Fig. 2 and Supplementary Datasheet 1), the  
 233 absence of HMW branched fatty acids in the overlying soils may indicate a contribution of HMW  
 234 branched fatty acids to the HS4 stalagmite from microorganisms living in the karst or cave  
 235 environment (Matsumoto et al., 1992). The bimodal distribution pattern of fatty acids in the HS4

236 stalagmite is the same as the distribution pattern from the same stalagmite reported by [Huang et al.](#)  
237 [\(2008\)](#) and a stalagmite from a British cave ([Blyth et al., 2011](#)), all of which show a dominant  
238 carbon maximizing at  $n\text{-C}_{16}$  in the LWM homologues and at  $n\text{-C}_{24}$  in the HMW homologues. The  
239 bimodal distribution pattern of fatty acids is similar to that of  $n$ -alkanes and fatty alcohols which  
240 indicates a mixed origin from higher plants and microbes ([Xie et al., 2003](#)). We did not detect  
241 long chain  $n$ -fatty acids from  $\text{C}_{29}$  to  $\text{C}_{32}$  in the stalagmite samples, suggesting either low  
242 concentrations below the detection limit or that they were totally absent. The bimodal distribution  
243 of  $n$ -fatty acids in the HS4 stalagmite is akin to that in the overlying soils, suggesting both share a  
244 similar source of fatty acids. The implication is that the  $n$ -fatty acids in HS4 are dominantly  
245 sourced from the overlying soil, with a minor contribution of microbes from inside the karst/cave  
246 system, as suggested by the minor component of branched homologues (see discussion in Section  
247 3.3 below).

248 The percentage of low molecular weight (LMW,  $\leq\text{C}_{20}$ ) fatty acids we measured is  
249 significantly higher than high molecular weight (HMW,  $>\text{C}_{20}$ ) fatty acids. The LMW fatty acids  
250 show a similar pattern in the overlying soils and the HS4 stalagmite. The dominant compound  
251  $n\text{-C}_{16}$  fatty acid occupied ca. 35% of the total fatty acids in both the soils and stalagmite samples.  
252 The  $n\text{-C}_{18}$  fatty acid is slightly higher in the HS4 stalagmite (ca. 15%) than that of in the  
253 overlying soils (ca. 6%) ([Fig. 2a, c](#)). HMW fatty acids from cave overlying soils are mainly  
254 dominated by  $n$ -fatty acids with non-detected branched fatty acids, however, trace amount of *iso*-  
255 (*i*-) and *anteiso*- (*a*-) fatty acids were found in the HS4 stalagmite accounting for an average  
256 around 1% of all fatty acids. The most abundant HMW fatty acid  $n\text{-C}_{24}$  accounted for an average  
257 of ca. 7.5% in cave overlying soils and ca. 5.5% in the HS4 stalagmite ([Fig. 2b, d](#)).

258

### 259 3.2 Carbon isotopic compositions of fatty acids

260 We analysed the  $\delta^{13}\text{C}$  values of the major  $n$ -fatty acids with 16, 18, 22 and 24 carbon atoms

261 from the HS4 stalagmite subsamples. The  $\delta^{13}\text{C}$  of the  $n\text{-C}_{16}$  fatty acid ( $\delta^{13}\text{C}_{16\text{FA}}$ ) varies from -27.5‰  
262 to -25.2‰. The  $n\text{-C}_{18}$  fatty acid ( $\delta^{13}\text{C}_{18\text{FA}}$ ) varies from -27.0‰ to -23.4‰, and is slightly enriched  
263 in  $^{13}\text{C}$  across the whole time series (Fig. 6a). The  $n\text{-C}_{22}$  and  $n\text{-C}_{24}$  fatty acid are relatively more  
264 depleted in  $^{13}\text{C}$  than the short chain fatty acids  $n\text{-C}_{16}$  and  $n\text{-C}_{18}$ , with  $\delta^{13}\text{C}_{22\text{FA}}$  varying from -32.9‰  
265 to -25.7‰ and  $\delta^{13}\text{C}_{24\text{FA}}$  varying from -33.5‰ to -25.2‰ over the past 8.8 ka BP (Figs. 4, 6a). The  
266 variation of  $\delta^{13}\text{C}_{16\text{FA}}$  and  $\delta^{13}\text{C}_{18\text{FA}}$  show a generally parallel trend over the past 8.8 ka BP ( $R^2 =$   
267 0.79,  $n = 70$ ; Fig. 3a), while the correlation between the HS4  $\delta^{13}\text{C}_{16\text{FA}}$  and  $\delta^{13}\text{C}_{24\text{FA}}$  is much  
268 weaker ( $R^2 = 0.55$ ,  $n = 61$ ; Fig. 3b). In addition, the  $\delta^{13}\text{C}_{22\text{FA}}$  and  $\delta^{13}\text{C}_{24\text{FA}}$  are very strongly  
269 correlated within the available data set ( $R^2 = 0.94$ ,  $n = 49$ ; Fig. 3c), while the correlation between  
270 the HS4  $\delta^{13}\text{C}_{18\text{FA}}$  and  $\delta^{13}\text{C}_{24\text{FA}}$  is much weaker ( $R^2 = 0.61$ ,  $n = 59$ ; Fig. 3d). The  $^{13}\text{C}$  values of all  
271 the analysed  $n$ -fatty acids from 8.8 to 4.4 ka BP and 0.8 to 0.6 ka BP are more negative than those  
272 from 4.4 to 0.8 ka BP and 0.6 to 0 ka BP (Figs. 4, 6a.). Overall, the  $\delta^{13}\text{C}_{16\text{FA}}$  and  $\delta^{13}\text{C}_{18\text{FA}}$  values  
273 are relatively positive and vary within a smaller range than that of  $\delta^{13}\text{C}_{22\text{FA}}$  and  $\delta^{13}\text{C}_{24\text{FA}}$  (Fig. 6a).

274

### 275 3.3 Sources of fatty acids in the HS4 stalagmite

276 We have compared the average distributions of fatty acids within the soils above Heshang  
277 Cave ( $n = 9$ ) to those from HS4 ( $n = 73$ ) (Fig. 2 and Supplementary Datasheet 1). Strong  
278 similarities in distributions between the soil and cave interior suggest that the  $n$ -fatty acids are  
279 primarily sourced from microbes and higher plants living above the cave and/or within the  
280 groundwater system, with some minor *in-situ* contribution stalagmite microbial community  
281 possible (Wang et al., 2018). This inference is supported by previous work which concluded that  
282 the broad similarity of 3-OH-FA lipid distributions in the overlying soils and stalagmites and the  
283 site-specific bacterial 16S rRNA analyses of bacterial diversity and transport pathways (Liu et al.,  
284 2010; Yun et al., 2016), demonstrated a major contribution of lipid biomarkers from  
285 Gram-negative bacteria dwelling in the overlying soils to the HS4 stalagmite samples.

286 Furthermore, the 16S rRNA analyses demonstrated that changes in the Gram-negative bacterial  
287 community were rapidly transmitted through the Heshang Cave system to drip waters and to the  
288 cave and speleothems on seasonal timescales ([Yun et al., 2016](#)). Such seasonal responsiveness  
289 suggests minimal attenuation of climate signals transmitted from the overlying soils to the HS4  
290 stalagmite (at least sufficient for centennial to millennial scale paleoclimate studies) ([Wang et al.,  
291 2018](#)). Furthermore, quantitative PCR from Heshang Cave weathered rock yields bacterial 16S  
292 rRNA gene abundances of about  $10^8$  to  $10^9$  copies  $g^{-1}$  dry sample ([Zhao et al., 2018](#)), while values  
293 in soils are about  $10^{10}$  copies  $g^{-1}$  dry sample ([Wessén et al., 2010](#)), indicating that bacterial  
294 biomass in soils are an order of magnitude higher than in the Heshang cave environment. This  
295 site specific work on Heshang cave is consistent with the general observation that fatty acids  
296 preserved in speleothems are principally derived from the overlying soil ecosystem and  
297 vegetation, having been transported from the surface by percolating groundwater, and with only a  
298 minor proportion derived from cave ecosystems ([Blyth et al., 2007](#); [Huang et al., 2008](#); [Li et al.,  
299 2011](#); [Xie et al., 2005](#)).

300 Numerous studies have established that, in environmental settings, HMW *n*-fatty acids  
301 ( $C_{20}$ - $C_{32}$ ) with an even number predominance are mainly derived from higher plants ([e.g.  
302 Eglinton and Hamilton, 1967](#)), whereas LMW *n*-fatty acids (such as  $C_{16}$ ,  $C_{18}$ ) are sourced from  
303 microbes ([Lichtfouse et al., 1995](#)) and higher plants ([Chikaraishi and Naraoka, 2006](#)). Hence, this  
304 suggests that the HMW *n*-fatty acids in HS4 were primarily derived from higher plants in the  
305 overlying catchment, whilst the LMW *n*-fatty acids originated from mixed sources:  
306 predominantly microbes in soil-karst system, with some contribution from higher plants.  
307 Therefore, changes in the isotopic ratios of stalagmite *n*-fatty acids primarily reflect processes  
308 occurring at the subaerial catchment ecosystem above the cave.

309 Compound-specific isotope analysis is an additional tool to identify the sources of biomarker  
310 lipids. For example, significant differences in  $\delta^{13}C$  values between the long chain and short chain  
311 lipids would indicate they likely originated from different sources ([Rieley et al., 1991](#)), because

312 no significant kinetic isotope effect is associated with chain elongation during bacterial fatty acid  
313 biosynthesis ([Monson and Hayes, 1982](#)), and research shows that the carbon isotope composition  
314 of LMW and HMW fatty acids from the same plants are essentially the same ([Chikaraishi et al.,  
315 2004b](#)). Overall, the strong correlation between  $\delta^{13}\text{C}_{16\text{FA}}$  and  $\delta^{13}\text{C}_{18\text{FA}}$  and between  $\delta^{13}\text{C}_{22\text{FA}}$  and  
316  $\delta^{13}\text{C}_{24\text{FA}}$  ( $R^2 = 0.79, 0.94$ , respectively, [Fig. 3a, b](#)), and the considerable isotopic differences  
317 between the LMW and HMW *n*-fatty acids indicate different biological sources for the LMW and  
318 HMW *n*-fatty acids ([Figs. 4, 6a](#))

319

#### 320 *3.4 Holocene vegetation changes derived from $\delta^{13}\text{C}_{24\text{FA}}$*

321  $\text{C}_3$  plants use the Rubisco enzyme to fix carbon during photosynthesis, which discriminates  
322 against the  $^{13}\text{CO}_2$  isotopologue and therefore results in  $^{13}\text{C}$  depletion (relative to  $\text{C}_4$  plants). By  
323 contrast,  $\text{C}_4$  plants (most grasses, sedges and dicots) developed a carbon concentration  
324 mechanism during the Miocene ([Sage, 2004](#)) that permits them to discriminate less between  
325  $^{12}\text{CO}_2$  and  $^{13}\text{CO}_2$ ; as a result,  $\text{C}_4$  plants are more enriched in  $^{13}\text{C}$  compared with  $\text{C}_3$  plants  
326 ([Farquhar et al., 1989](#); [Sage, 2004](#)). The resulting isotopic difference between  $\text{C}_3$  and  $\text{C}_4$  plants is  
327 propagated to the *n*-fatty acids they synthesize: typical  $\delta^{13}\text{C}$  values for *n*-fatty acids from  $\text{C}_3$   
328 plants range from -30.8‰ to -47.7‰, whilst *n*-fatty acids from  $\text{C}_4$  plants range from -16.3‰ to  
329 -28.2‰ ([Ballentine et al., 1998](#); [Chikaraishi et al., 2004b](#)). Given that  $\text{CO}_2$  changes are relatively  
330 subtle during the Holocene (changes of ca. 20ppm) ([Indermühle et al., 1999](#)), we argue that the  
331 general increase in HS4  $\delta^{13}\text{C}_{24\text{FA}}$  values through the Holocene ([Fig. 5a](#)) reflects broad changes in  
332  $\text{C}_3$  vs  $\text{C}_4$  vegetation in the HS4 catchment.

333 Temperature, precipitation and  $\text{CO}_2$  concentrations are the three main controls on  $\text{C}_3/\text{C}_4$   
334 vegetation changes ([Ehleringer et al., 1997](#); [Huang et al., 2001](#)). Due to their greater water use  
335 efficiency and carbon concentrating mechanism,  $\text{C}_4$  plants can theoretically outcompete  $\text{C}_3$  plants  
336 under conditions of lower  $p\text{CO}_2$  and/or higher daytime growing season temperatures ([Ehleringer](#)



337 [et al., 1997](#)). However, the dominant control factor may vary spatially and temporally ([Huang et](#)  
338 [al., 2001](#)). Here we expect changes in the  $\delta^{13}\text{C}$  of the plant wax-derived  $n\text{-C}_{24}$  fatty acid to  
339 primarily reflect relative changes in  $\text{C}_3$  vs  $\text{C}_4$  vegetation driven by changes of temperature and  
340 precipitation ([Fig. 5a](#)). Our  $\delta^{13}\text{C}_{24\text{FA}}$  data displays a trend towards heavier values that is broadly  
341 consistent with the monotonic decline in NH summer insolation ([Fig. 5b](#)), along with a general  
342 increase in HS4 stalagmite  $\delta^{18}\text{O}$  ([Fig. 5c](#)) which, based on prior work ([Cheng et al., 2016](#)), is  
343 interpreted to reflect a weakening of the East Asian monsoon during the middle Holocene. The  
344  $\delta^{13}\text{C}_{24\text{FA}}$  trend is also coeval with declining temperatures inferred from the 3-OH-FA based  $\text{RAN}_{15}$   
345 temperature record from the same stalagmite ([Fig. 5d](#)) ([Wang et al., 2018](#)), along with changes in  
346 hydrology represented by the 3-OH-FA based RIAN proxy (RIAN, the negative logarithm of  
347 Branching Ratio; [Fig. 5g](#)) ([Wang et al., 2018](#)) and dead carbon percentage (DCP, [Fig. 5h](#))  
348 ([Noronha et al., 2014](#)) from the HS4 speleothem. [Noronha et al. \(2014\)](#) interpret higher DCP to  
349 reflect higher precipitation and increased soil moisture, limiting  $\text{CO}_2$  diffusion and open-system  
350 dissolution, and thus leading to a higher proportion of carbon derived from the  $^{14}\text{C}$ -free bedrock.

351 Closer inspection of our  $\delta^{13}\text{C}_{24\text{FA}}$  record reveals that values are more negative during the  
352 HCO (ca. 4.4 to 8.8 ka BP), indicating a greater abundance of  $\text{C}_3$  vs.  $\text{C}_4$  plants during this  
353 relatively wet ([Fig. 5c](#)) and warm ([Fig. 5d](#)) phase. Following the HCO around ca. 4.4 ka,  $\delta^{13}\text{C}_{24\text{FA}}$   
354 values become more positive, indicating a proportional increase in  $\text{C}_4$  vegetation in the Heshang  
355 Cave catchment. Critically, the inferred maximum in  $\text{C}_3$  abundance at our site during the early  
356 Holocene is consistent with maximum expansion of evergreen forest in the middle reaches of the  
357 Yangtze River ([Sun and Chen, 1991](#)), and is regionally consistent with pollen records from  
358 northern China ([Ji et al., 2005](#); [Zhou et al., 2010](#)), which show significantly higher percentages of  
359 tree pollen during the HCO and a relative decrease in tree pollen during the late Holocene ([Fig.](#)  
360 [5e-f](#)); the latter shift infers a concomitant increase in grasses and other vegetation types (sedges  
361 and dicots), many of which utilise the  $\text{C}_4$  pathway. An additional feature of our record worth  
362 noting is a rapid excursion to lighter  $\delta^{13}\text{C}_{24\text{FA}}$  values towards the end of the MWP (between ca. 0.6

363 and 0.8 ka BP), which is again consistent with an increase in tree pollen in Tianchi Lake to the  
364 north (Fig. 1, 5f). In light of these phase relationships between regional proxy records in central  
365 and northern China, we are confident that temperature and monsoon rainfall were the dominant  
366 factors controlling vegetation in the middle reaches of Yangtze River during the Holocene.

367

### 368 *3.5 Changes in soil-karst bacterial respiration and substrate selectivity due to Holocene climate* 369 *change*

370 Holocene variations in  $\delta^{13}\text{C}$  for the HS4 HMW and LMW *n*-fatty acids are shown in Figure  
371 3a. In contrast to the higher plant derived HMW *n*-fatty acids, the LMW fatty acids are relatively  
372 heavier and show more subtle changes in carbon isotope values. Higher plants produce fatty acids  
373 with a broad range of chain lengths, characterised by a predominance of HMW even-numbered  
374 fatty acids (e.g.  $\text{C}_{24}$ ,  $\text{C}_{26}$ ,  $\text{C}_{28}$ ). However, what is sometimes overlooked is that they also produce  
375 shorter chain length compounds (e.g.  $\text{C}_{16}$ ,  $\text{C}_{18}$ ) generally in lower abundances (Chikaraishi et al.,  
376 2004b). This means that typically there are no *n*-fatty acids exclusively produced by bacteria  
377 preserved in palaeoclimate archives in abundances sufficient for compound-specific isotope  
378 analyses. Therefore, in order to constrain bacterial  $\delta^{13}\text{C}$  signatures we must deconvolve the  
379 bacterial  $\delta^{13}\text{C}$  component from the net  $\delta^{13}\text{C}$  value (typically some combination of inputs from  
380 bacteria, higher plants, and algae) for any given fatty acid.

381 Unlike in marine or lacustrine systems, algal contributions to the *n*-fatty acid pool in a  
382 soil-karst cave system are negligible; thus, the only remaining sources to account for are higher  
383 plants and microbes. Archaeal tetra-ether bonded membrane lipids are fundamentally different  
384 from bacteria and a negligible source of fatty acids (Koga, 2011). We note that fungi also produce  
385  $\text{C}_{18:0}$  fatty acid (Zelles, 1997), but the diagnostic biomarker for fungi ( $\text{C}_{18:2\omega 6,9}$ ) (Frostegård and  
386 Bååth, 1996) was found only in trace amounts or not detected at all in our samples, suggesting the  
387 contribution of  $\text{C}_{18}$  fatty acids from fungi is negligible. Thus, we argue that we isolate the first

388 order bacterial contribution to the C<sub>18</sub> fatty acid, which we term  $\delta^{13}\text{C}_{18\text{FA(B)}}$  (see [Section 2.4](#)), and  
389 subsequently use this to reconstruct a unique record of Holocene changes in terrestrial bacterial  
390 respiration and substrate selectivity.  $\delta^{13}\text{C}_{18\text{FA(B)}}$  varies between -19‰ to -27‰ during the  
391 Holocene, becoming generally lighter from 8.5 ka BP (-21‰) to 8 ka BP (-25‰), then heavier to  
392 6.1 ka BP (ca. -19‰), lighter to 2.7 ka BP (-27‰), and finally maintaining values of ca. -22‰ to  
393 -25‰ with a notable heavy isotope excursion at ca. 0.6 ka BP (-22‰). Superimposed on these  
394 broad trends are several single point outliers. Overall, the  $\delta^{13}\text{C}_{18\text{FA(B)}}$  is relatively heavier during  
395 the warm/wet phases of the HCO and MWP ([Fig. 6b](#)).

396 Soil bacterial respiration is primarily driven by temperature, with a secondary influence of  
397 soil moisture ([Raich and Tufekciogul, 2000](#)). Warmer climate episodes, such as the HCO and the  
398 MWP, are expected to increase rates of bacterial decomposition of soil organic matter (SOM)  
399 ([Crowther et al., 2016](#)). The stock of SOM in the soil-karst system results from the balance  
400 between inputs and outputs of carbon within the belowground environment. Inputs are primarily  
401 from leaf and root detritus (including root exudates). Outputs are controlled by the temperature  
402 sensitivity of decomposition and leaching. The intrinsic temperature sensitivity of decomposition  
403 for a particular soil environment depends on the inventory of the thousands of different organic  
404 compounds residing in the soil, each with its own kinetic properties and potential rates of  
405 decomposition ([Sollins et al., 1996](#)). In most environments, the stocks of labile and recalcitrant  
406 compounds are not equal, with recalcitrant compounds being much more abundant than labile  
407 compounds ([Tjoelker et al., 2001](#)).

408 Modern biogeochemical studies on  $\delta^{13}\text{C}$  fractionation between SOM substrates and bacterial  
409 CO<sub>2</sub> (as a product) help us to interpret our HS4  $\delta^{13}\text{C}_{18\text{FA(B)}}$  record. A recent synthesis of studies  
410 highlights considerable variability, but suggests a <sup>13</sup>C enrichment of bacterial CO<sub>2</sub> of up to +5‰  
411 compared to the bulk substrate in most cases for C<sub>3</sub> plant dominated soils. Such positive offsets  
412 indicate that bacteria preferentially utilise <sup>13</sup>C-enriched compounds in the SOM fraction, e.g.  
413 sugars, starch, cellulose etc, which are relatively labile ([Werth and Kuzyakov, 2010](#)). Šantrůčková

414 et al. ([Šantrůčková et al., 2000](#)) found that the enriching effect associated with the preferential use  
415 of organic compounds in C<sub>3</sub> soils is more pronounced than the <sup>13</sup>C-depletion effect of metabolism  
416 itself. Hence, by preferential substrate utilization in C<sub>3</sub> soils bacterial biomass gets enriched in <sup>13</sup>C,  
417 but respire CO<sub>2</sub> that is isotopically depleted in <sup>13</sup>C relative to bacterial biomass (but still enriched  
418 compared to SOM). This effect therefore additionally enriches soil bacteria in <sup>13</sup>C ([Werth and](#)  
419 [Kuzyakov, 2010](#)).

420 Our reconstructed  $\delta^{13}\text{C}_{18\text{FA(B)}}$  in the HCO and MWP portions of our HS4 record are relatively  
421 isotopically heavy ([Fig. 6](#)).  $\delta^{13}\text{C}_{18\text{FA(B)}}$  also shows a remarkably coherent, anti-phased trend with  
422 the carbon isotope values of the acid-soluble organic matter ( $\delta^{13}\text{C}_{\text{ASOM}}$ ) from the same stalagmite  
423 ([Fig. 6c](#)) ([Li et al., 2014](#)). We interpret this anti-phased trend between  $\delta^{13}\text{C}_{18\text{FA(B)}}$  and  $\delta^{13}\text{C}_{\text{ASOM}}$   
424 record in the HS4 stalagmite as reflecting an increase in substrate selectivity by bacteria of  
425 <sup>13</sup>C-enriched labile substrates (sugars, starches etc) within the soil organic matter (SOM) pool.  
426 This evidence of increased substrate selectivity is intriguing given that the warmer conditions in  
427 the HCO and MWP would be conducive to higher rates of soil bacterial activity ([Lloyd and](#)  
428 [Taylor, 1994](#); [Luo, 2007](#)). If inputs from leaf and root detritus and the size of substrate pool  
429 remained equal (during the HCO and MWP), we would expect an increase in soil bacterial  
430 activity to lead to greater competition for substrates, less selectivity and increased utilization of  
431 recalcitrant <sup>13</sup>C-depleted substrates (e.g. lipids, wax esters, macro-molecular material etc).  
432 However, our results are consistent with increases in gross primary production (GPP) outpacing  
433 bacterial respiration (overall increase in net primary production), leading to greater inputs of plant  
434 and root detritus, greater selectivity of <sup>13</sup>C-enriched labile substrates, and an enriched  $\delta^{13}\text{C}_{18\text{FA(B)}}$   
435 signal. The corollary of this is a greater proportion of recalcitrant <sup>13</sup>C-depleted substrates being  
436 sequestered in the remaining SOM ([Benner et al., 1987](#); [Werth and Kuzyakov, 2010](#)) as  
437 represented by the bulk  $\delta^{13}\text{C}_{\text{ASOM}}$  record from HS4 ([Li et al., 2014](#)). Our interpretation is  
438 consistent with our higher plant  $\delta^{13}\text{C}$  record ([Fig. 5a](#)). We infer the highest proportions of C<sub>3</sub> plant  
439 biomass in the HCO and HWP, coeval with our heaviest  $\delta^{13}\text{C}_{18\text{FA(B)}}$  and greatest selectivity of

440 <sup>13</sup>C-enriched labile substrates. Preferential substrate utilization is more important in C<sub>3</sub>  
441 plant-dominated soils because C<sub>4</sub> soils, typical of arid and semiarid climates, contain generally  
442 less labile SOM and thus soil bacteria consume the SOM more completely than in C<sub>3</sub> soils  
443 ([Šantrůčková et al., 2000](#)). This has been demonstrated in modern field studies (see review by  
444 [Werth and Kuzyakov \(2010\)](#)) but our reconstruction from HS4 is the first evidence of this  
445 relationship on Holocene timescales.

446 To summarize, we argue that because we have isolated the contribution of C<sub>18</sub> fatty acids  
447 from higher plants, the residual  $\delta^{13}\text{C}$  signal ( $\delta^{13}\text{C}_{18\text{FA(B)}}$ ) is most parsimoniously explained by  
448 greater soil bacterial respiration and decomposition rates, in response to a warmer and wetter  
449 local climate, driven by a stronger regional Asian summer monsoon. If net bacterial respiration is  
450 substrate-selective, this implies that the more recalcitrant phases are likely escaping heterotrophic  
451 degradation. Thus the system acts as a net carbon sink in the early Holocene and would act as a  
452 negative feedback on the warmer/wetter local early Holocene climate. This process is likely  
453 mediated and enhanced by changes in the relative proportion of C<sub>3</sub> vs C<sub>4</sub> plants. This  
454 interpretation of LMW fatty acids is uniquely applicable to speleothems because compared to  
455 lake and marine sediments there is minimal contribution from algae, resulting in a simpler two  
456 end-member system. This work represents the first such application in a speleothem or indeed  
457 any paleoclimate archive. As such, it provides key insights into links between East Asian  
458 palaeoclimate, vegetation, terrestrial ecosystem respiration and the carbon cycle during the  
459 Holocene.

460

#### 461 **4. Conclusion**

462 We have produced the first reconstruction of vegetation and bacterial activity from the  
463 important East Asian monsoon region using compound specific carbon isotopes of fatty acids  
464 from a stalagmite. Critically, our study finds that soil bacteria selectively degrade more labile  
465 (<sup>13</sup>C-enriched) substrates during warm periods of the Holocene, suggesting that gross primary

466 production outpaced soil respiration under higher temperatures. Our findings therefore show that  
467 mineral soils in subtropical karst settings may represent a net carbon sink during warmer climate  
468 states, thus acting as a negative feedback on Earth's climate.

469

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655

656

657 **Figure Captions**

658

659 **Fig. 1.** Location of Heshang Cave, Qinghai Lake and Tianchi Lake. The distribution of  $C_4$   
660 vegetation in Asia is after [Still et al. \(2009\)](#). The inset is map shows the main regional surface  
661 drainage and location of Heshang cave on the Qing River tributary of the Yangtze (revised after  
662 [Hu et al. \(2008a\)](#)).

663

664 **Fig. 2.** Distribution and relative abundance of fatty acids in (a, b) cave overlying soil, and (c, d)  
665 HS4 stalagmite. “*i-*” refers to “*iso*”, “*a-*” refers to “*anteiso*” and “*n-*” refers to “*normal*”.  $n-C_{16}$   
666 fatty acid is dominant in low molecular weight fatty acids ( $\leq C_{20}$ ),  $n-C_{24}$  fatty acid is dominant in  
667 high molecular weight fatty acids ( $>C_{20}$ ).

668

669 **Fig. 3.** Plots showing the relationship between  $\delta^{13}C$  values of  $n$ -fatty acids from HS4 stalagmite. **a**,  
670 Linear correlation between  $\delta^{13}C_{16FA}$  and  $\delta^{13}C_{18FA}$  based on a data set of 70 samples ( $R^2 = 0.79$ ,  $p <$   
671  $0.001$ ). **b**, Linear correlation between  $\delta^{13}C_{16FA}$  and  $\delta^{13}C_{24FA}$  based on a data set of 61 samples ( $R^2$   
672  $= 0.55$ ,  $p < 0.001$ ). **c**, Linear correlation between  $\delta^{13}C_{22FA}$  and  $\delta^{13}C_{24FA}$ , based on a data set of 49  
673 samples ( $R^2 = 0.94$ ,  $p < 0.001$ ). **d**, Linear correlation between  $\delta^{13}C_{18FA}$  and  $\delta^{13}C_{24FA}$  based on a  
674 data set of 59 samples ( $R^2 = 0.61$ ,  $p < 0.001$ ).

675

676 **Fig. 4.** Box chart showing the carbon isotope values of  $C_{16}$ ,  $C_{18}$ ,  $C_{22}$  and  $C_{24}$  *normal* ( $n$ -) fatty  
677 acids (FAs) from HS4 stalagmite.

678

679 **Fig. 5.** Vegetation changes and climatic drivers in central China during the last 9 ka BP. **a**,  
680 Variation of  $\delta^{13}C_{24FA}$  showing vegetation changes during the last 9 ka BP. **b**, Insolation changes at  
681  $30^\circ N$  in July during the last 9 ka BP ([Laskar et al., 2004](#)). **c**, Calcite  $\delta^{18}O$  of HS4 stalagmite over  
682 the past 9 ka BP ([Hu et al., 2008b](#)). **d**, Temperature variation during the last 9 ka BP

683 reconstructed by ratio of *anteiso* to *normal* C<sub>15</sub> 3-hydroxy fatty acid (RAN<sub>15</sub>; temperature proxy)  
684 from HS4 stalagmite from Heshang Cave, central China (Wang et al., 2018). **e**, Percentage of tree  
685 pollen from Qinghai Lake sediment (Ji et al., 2005). **f**, Percentage of deciduous tree pollen from  
686 Tianchi Lake (Zhou et al., 2010). **g**, Heshang Cave hydrological conditions inferred from the  
687 RIAN proxy (RIAN, the negative logarithm of Branching Ratio) from HS4 stalagmite during the  
688 last 9 ka BP (Wang et al., 2018). **h**, Dead carbon proportion (DCP) from HS4 stalagmite  
689 (Noronha et al., 2014). Blue shading highlights two periods with relative low percentage of C<sub>3</sub>  
690 plants during the HCO. Orange shading highlights relatively high percentages of C<sub>3</sub> plants during  
691 the HCO and MWP. Black line segments showing the U-Th dating errors.

692

693 **Fig. 6.** Vegetation changes and soil bacterial heterotrophic selectivity and respiration rates in  
694 response to climate changes. **a**, Compound specific  $\delta^{13}\text{C}$  values measured on the *n*-C<sub>16</sub>, *n*-C<sub>18</sub>,  
695 *n*-C<sub>22</sub>, *n*-C<sub>24</sub> fatty acids extracted from the HS4 stalagmite. **b**, Variation in  $\delta^{13}\text{C}_{18\text{FA(B)}}$  over the last  
696 9 ka BP with locally weighted scatterplot smoothing of 25% (LOWESS). **c**, Acid-soluble organic  
697 matter (ASOM) carbon isotope ( $\delta^{13}\text{C}_{\text{ASOM}}$ ) sequence derived from HS4 stalagmite (grey line) and  
698 three-point running average (red line) (Li et al., 2014). **d**, Temperature variation during the last 9  
699 ka BP reconstructed by RAN<sub>15</sub> from HS4 stalagmite from Heshang Cave, central China (Wang et  
700 al., 2018). **e**, Calcite  $\delta^{18}\text{O}$  of HS4 stalagmite over the past 9 ka BP (Hu et al., 2008b). U-Th dating  
701 errors (Hu et al., 2008b) are shown as black line segments.

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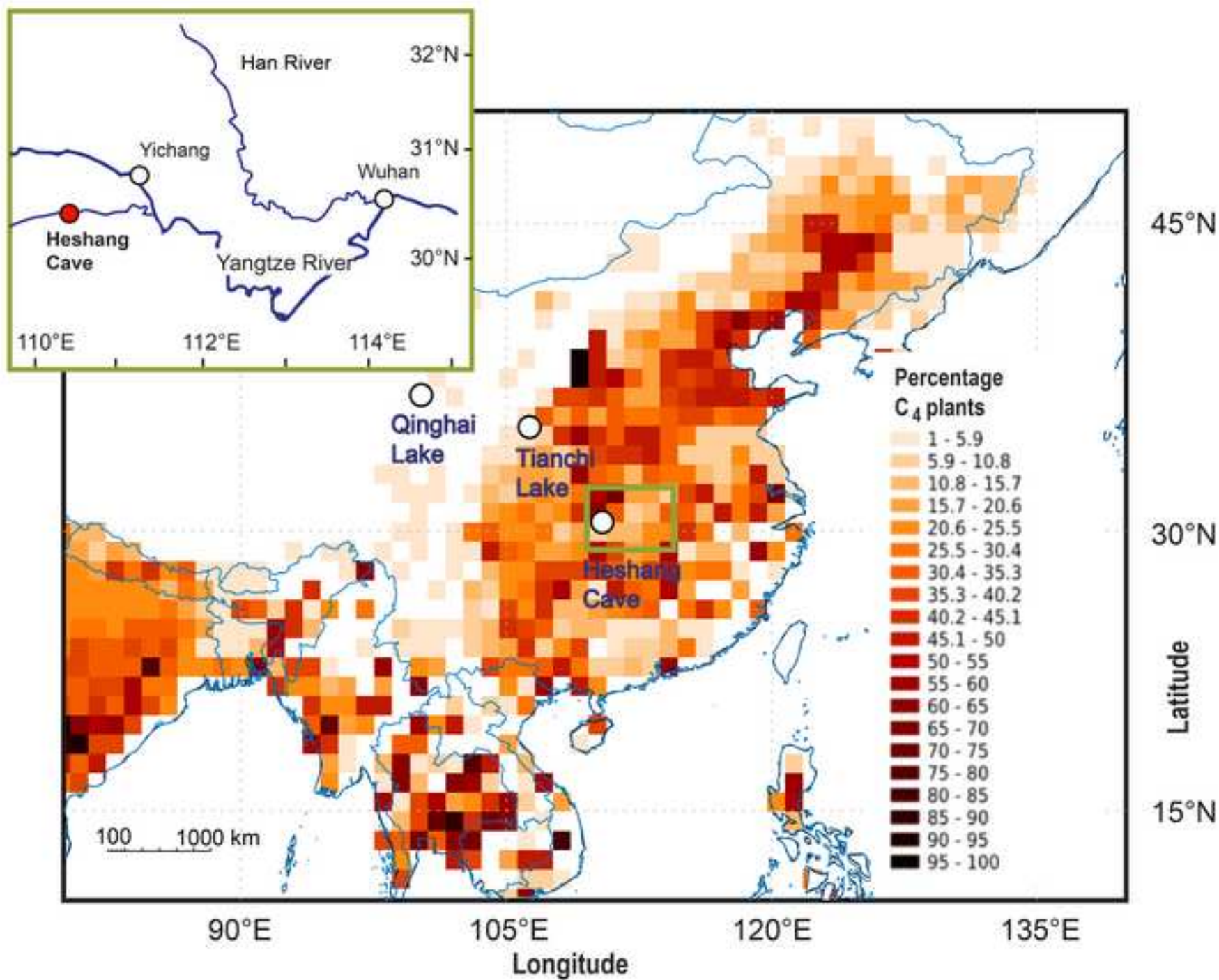




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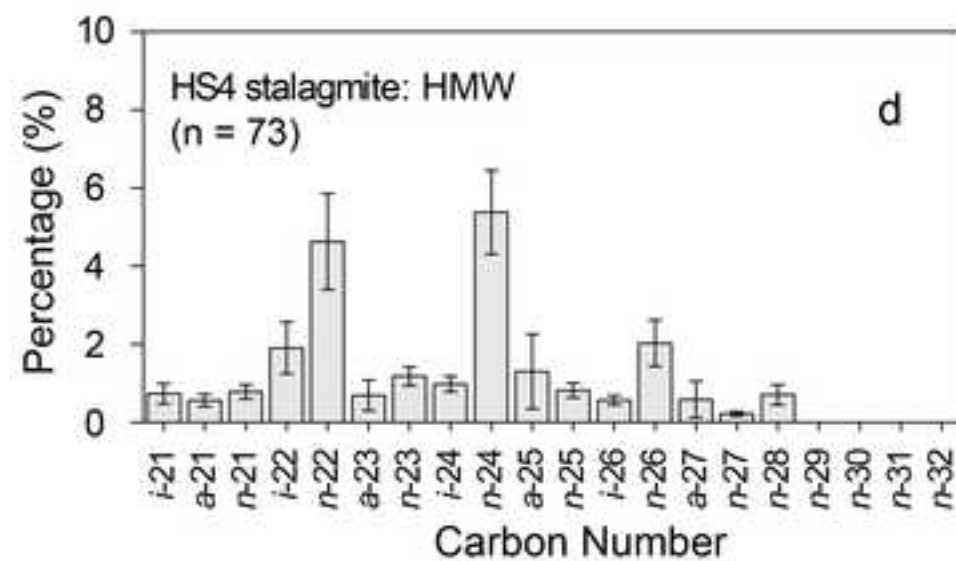
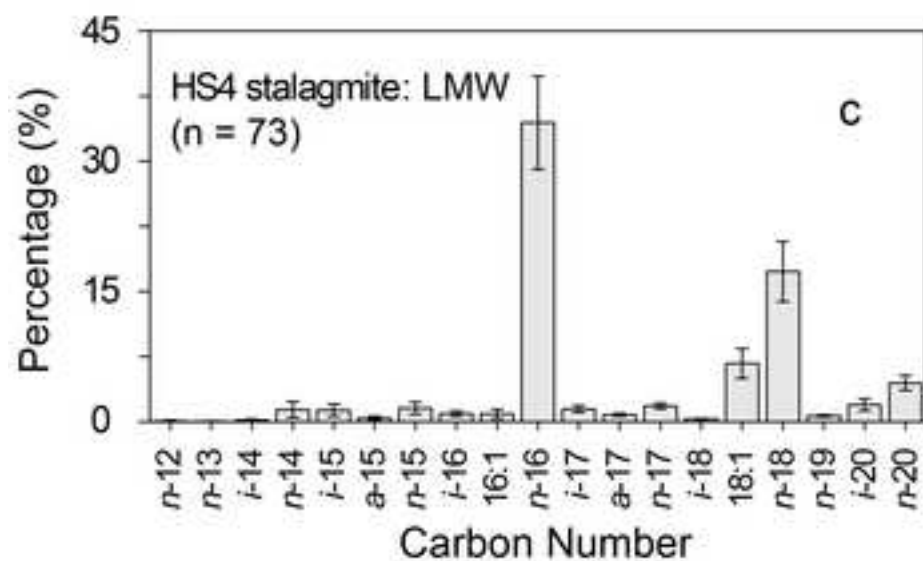
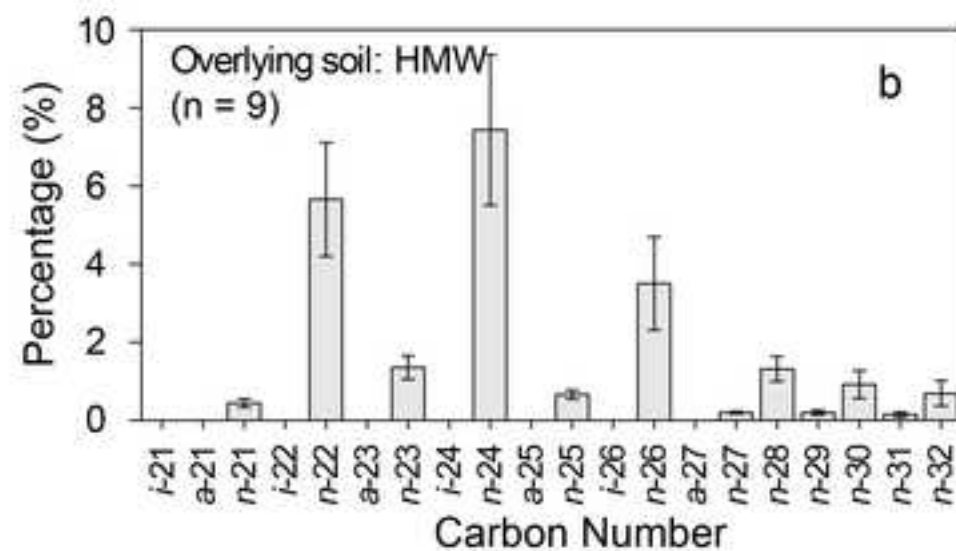
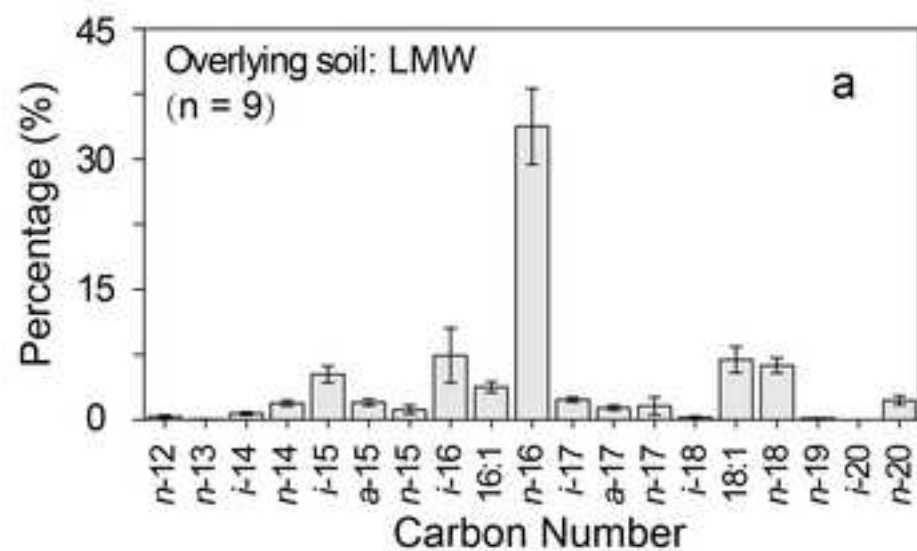


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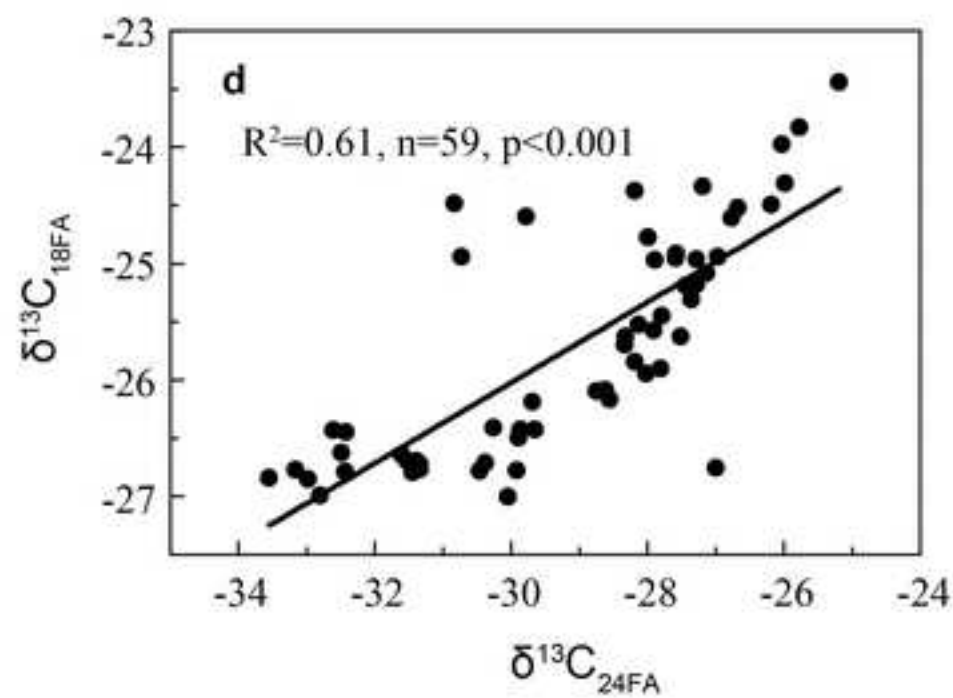
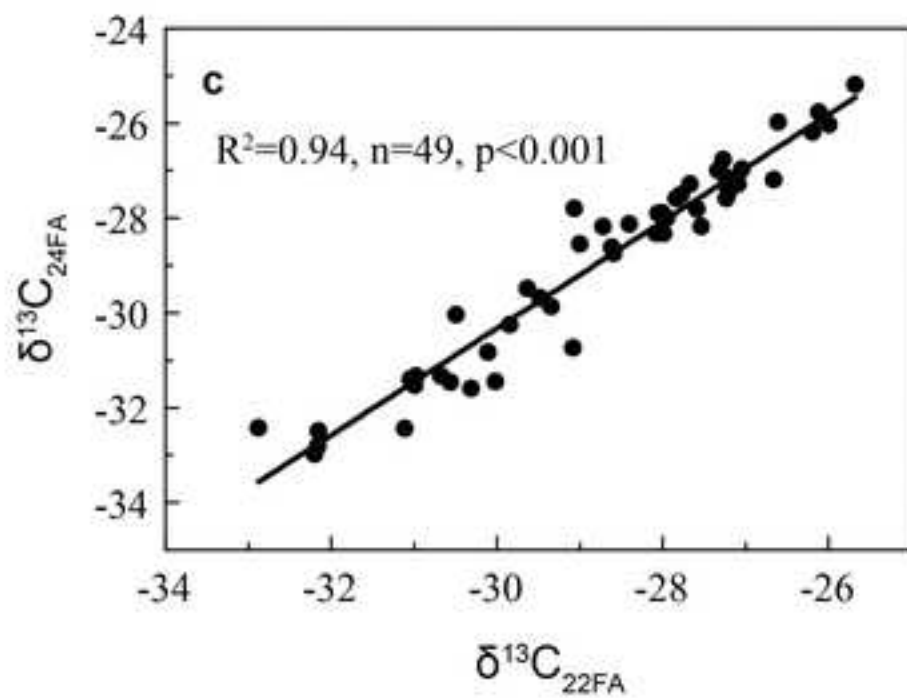
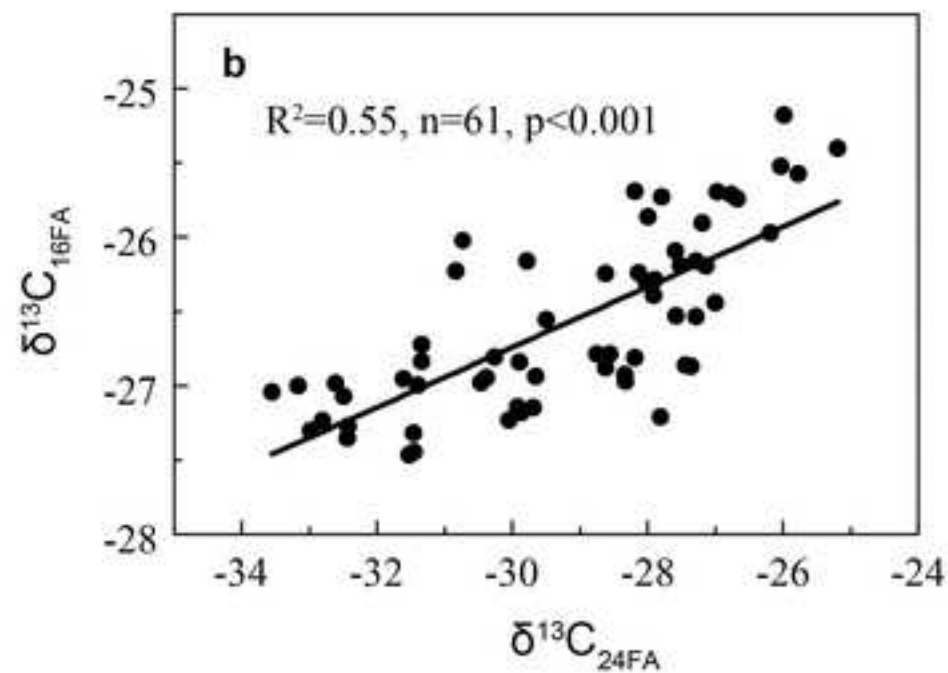
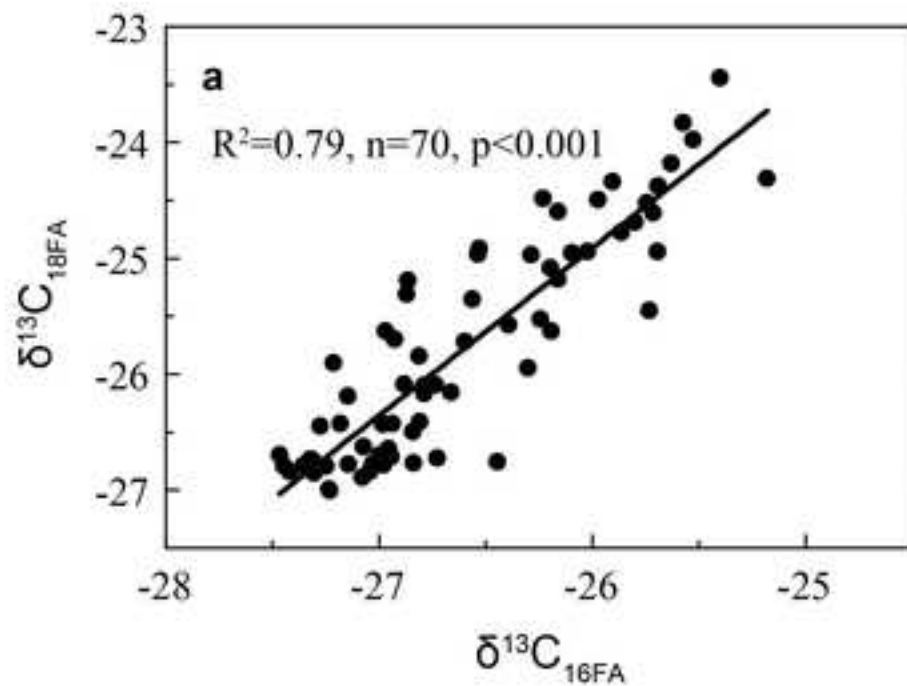


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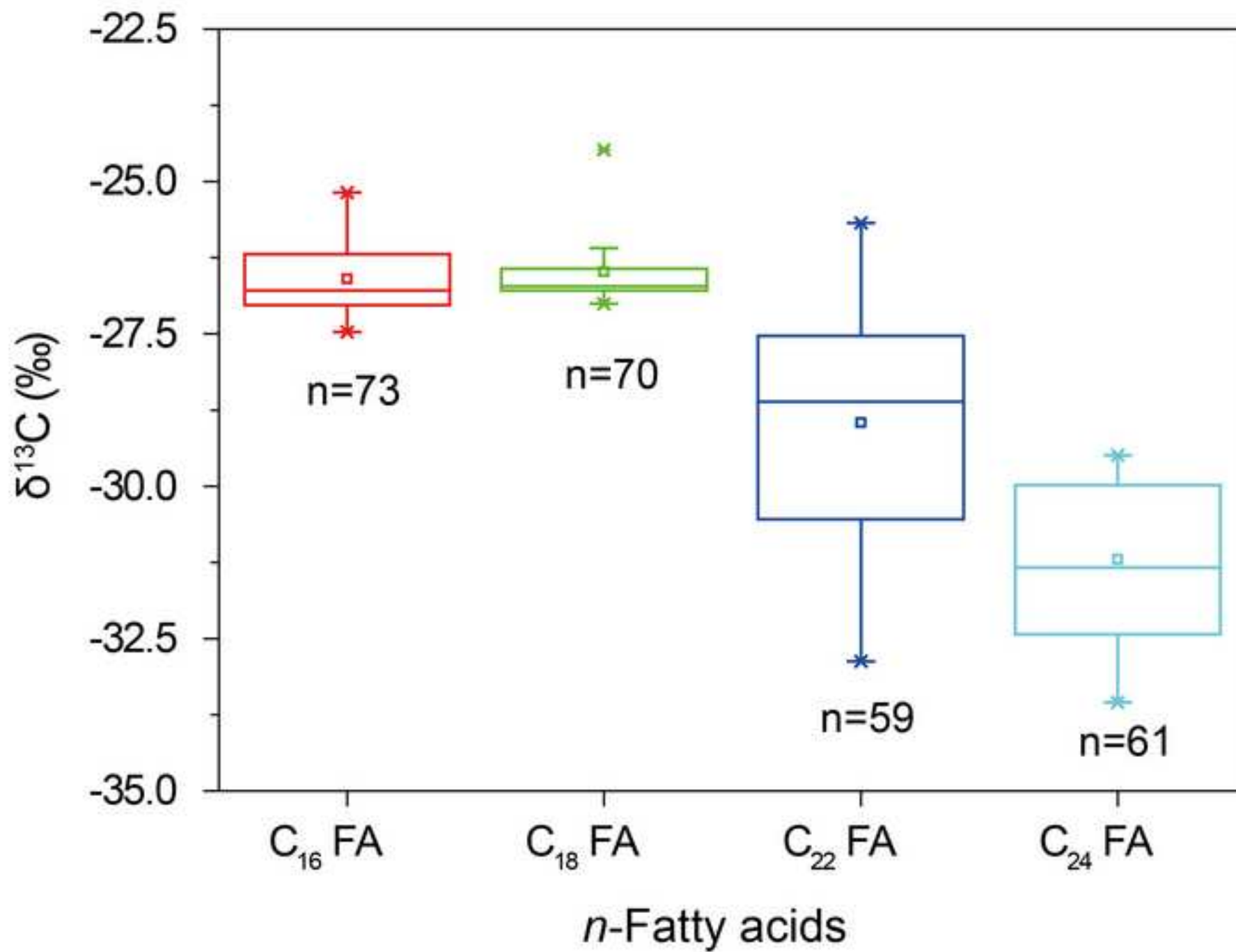


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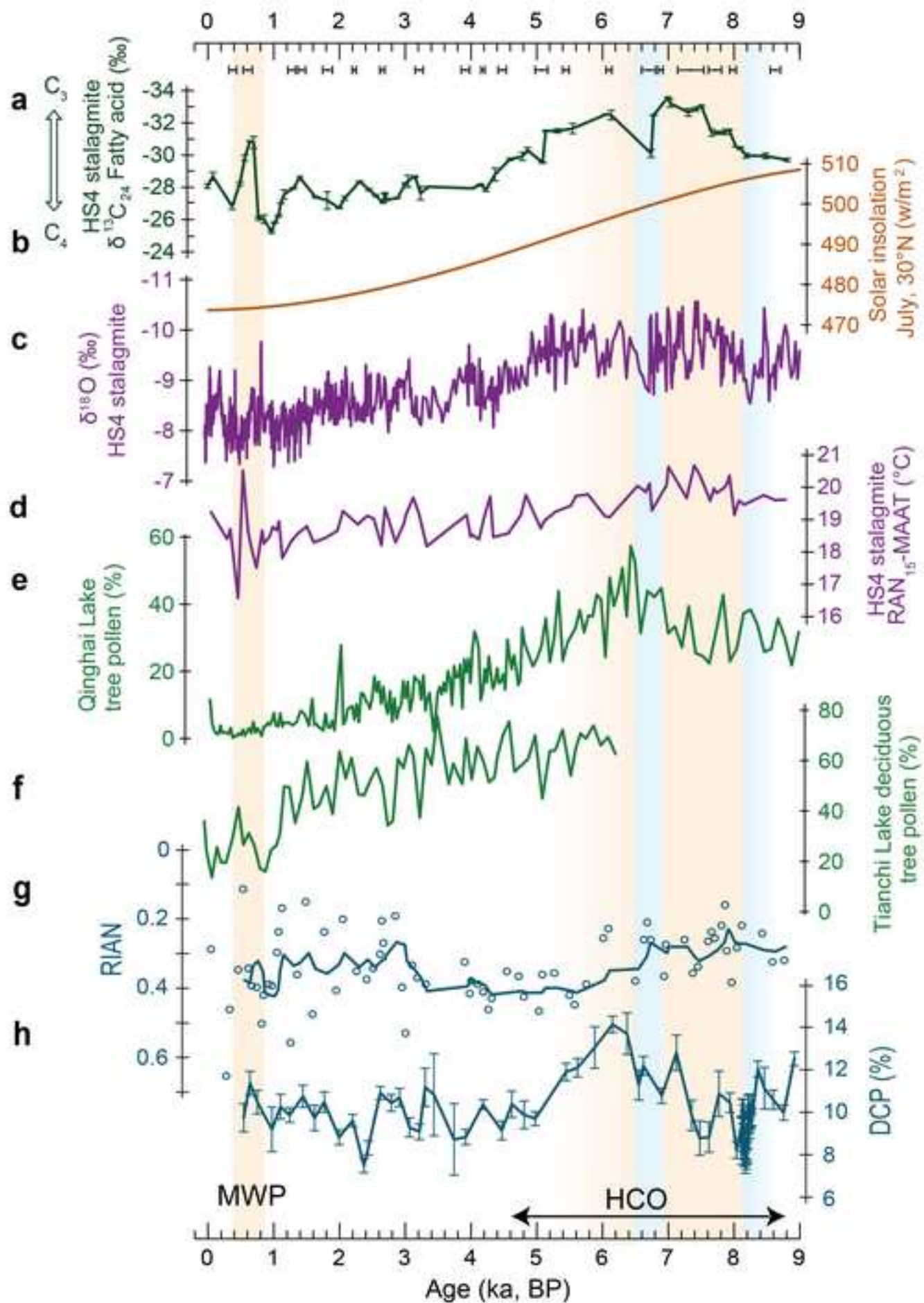
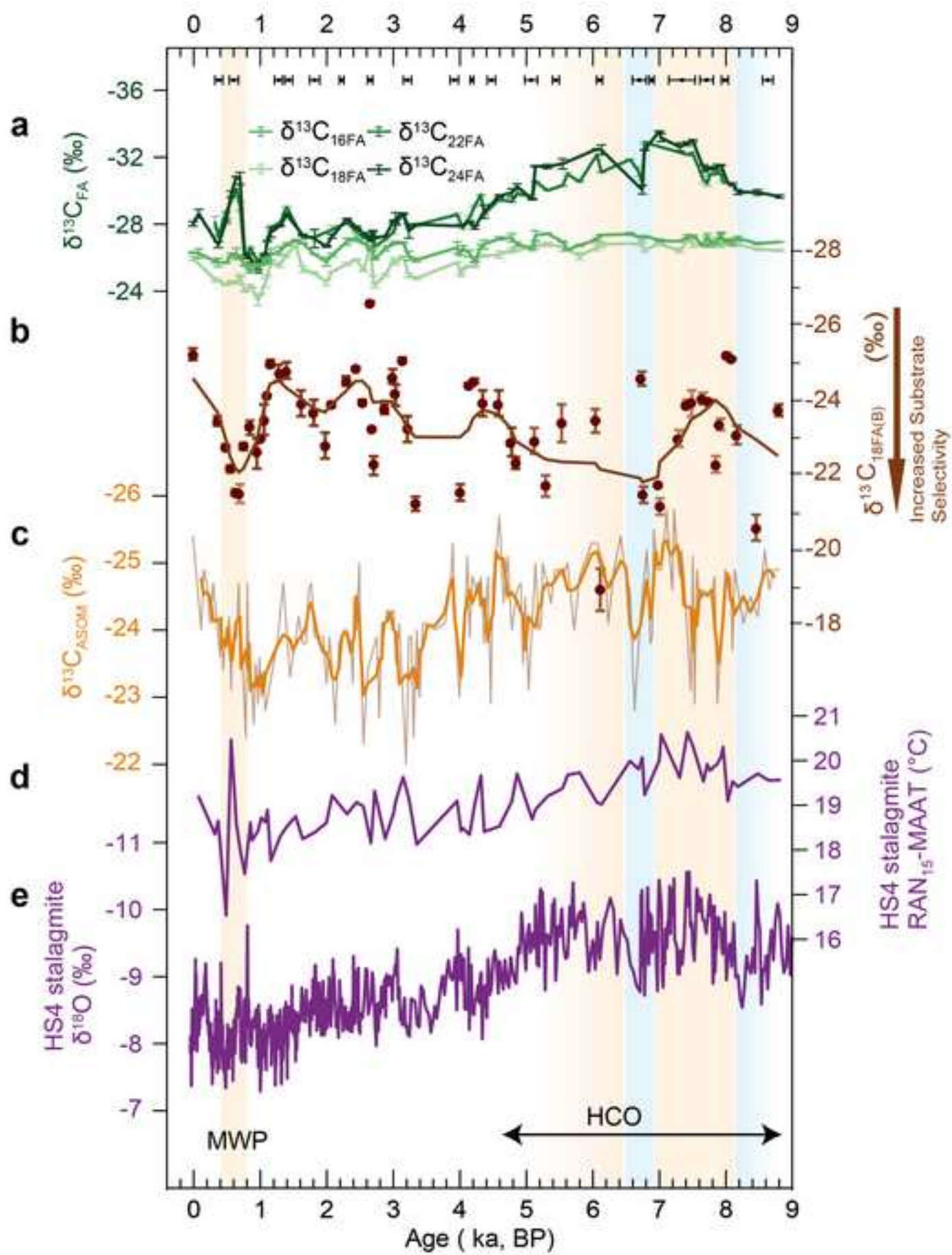


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