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DOI:

[10.1016/j.epsl.2019.115754](https://doi.org/10.1016/j.epsl.2019.115754)

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Document Version

Peer reviewed version

Citation for published version (Harvard):

Wang, C, Bendle, J, Greene, S, Griffiths, ML, Huang, J, Moossen, H, Zhang, H, Ashley, K & Xie, S 2019, 'Speleothem biomarker evidence for a negative terrestrial feedback on climate during Holocene warm periods', *Earth and Planetary Science Letters*, vol. 525, 115754. <https://doi.org/10.1016/j.epsl.2019.115754>

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Highlights

- The first compound specific $\delta^{13}\text{C}$ analysis of fatty acids from a stalagmite
- Proportional increases in 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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6

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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 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 C_3 vs 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 C_3 vs 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 RAN₁₅ 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 C₃ 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 BF₃-methanol (14% BF₃/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 δ¹³C analyses of the C₁₆, C₁₈, C₂₂, C₂₄ *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*-C₁₈ 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*-C₁₈ 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_{18FA} ($\delta^{13}\text{C}_{18\text{FA(B)}}$) 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*-C₁₈ chain length fatty acids ($\text{C}_{18\text{FA(P)}}$) divided by the
190 relative abundance of C₁₈ 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_{18FA} ($\text{C}_{18\text{(P)}}$) *a priori*, but we
193 can estimate this using the mean ratio of C_{18FA}:C_{24FA} (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_{24FA}.

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

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_{18FA} and C_{24FA}, the mean C_{18FA}:C_{24FA} ratio of higher plants (R), and the measured carbon isotopic
201 signatures of the C₁₈ and C₂₄ chain length fatty acids in the sample.

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

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 δa represents the uncertainty of $\delta^{13}\text{C}_{18\text{FA}}$, δb 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 C_3/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 C_{12} to C_{32} in the overlying soils and from C_{12} to
 228 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 C_{29} to 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}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}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}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 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}C depletion (relative to C_4 plants). By
323 contrast, 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, C_4 plants are more enriched in ^{13}C compared with C_3 plants
326 ([Farquhar et al., 1989](#); [Sage, 2004](#)). The resulting isotopic difference between C_3 and C_4 plants is
327 propagated to the *n*-fatty acids they synthesize: typical $\delta^{13}\text{C}$ values for *n*-fatty acids from C_3
328 plants range from -30.8‰ to -47.7‰, whilst *n*-fatty acids from C_4 plants range from -16.3‰ to
329 -28.2‰ ([Ballentine et al., 1998](#); [Chikaraishi et al., 2004b](#)). Given that 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 C_3 vs C_4 vegetation in the HS4 catchment.

333 Temperature, precipitation and CO_2 concentrations are the three main controls on C_3/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, C_4 plants can theoretically outcompete 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 C_3 vs 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 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 CO_2 diffusion and open-system
350 dissolution, and thus leading to a higher proportion of carbon derived from the ^{14}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 C_3 vs. 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 C_4 vegetation in the Heshang
355 Cave catchment. Critically, the inferred maximum in 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 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. C_{24} , C_{26} , C_{28}). However, what is sometimes overlooked is that they also produce
375 shorter chain length compounds (e.g. C_{16} , 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 C_{18} fatty acids from fungi is negligible. Thus, we argue that we isolate the first

388 order bacterial contribution to the C₁₈ 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 CO₂ (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 ¹³C enrichment of bacterial CO₂ of up to +5‰
411 compared to the bulk substrate in most cases for C₃ plant dominated soils. Such positive offsets
412 indicate that bacteria preferentially utilise ¹³C-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 C₃ soils is more pronounced than the ¹³C-depletion effect of metabolism
416 itself. Hence, by preferential substrate utilization in C₃ soils bacterial biomass gets enriched in ¹³C,
417 but respire CO₂ that is isotopically depleted in ¹³C relative to bacterial biomass (but still enriched
418 compared to SOM). This effect therefore additionally enriches soil bacteria in ¹³C ([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 ¹³C-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 ¹³C-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 ¹³C-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 ¹³C-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 C₃ 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 ^{13}C -enriched labile substrates. Preferential substrate utilization is more important in C_3
441 plant-dominated soils because C_4 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_3 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_{18} 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_3 vs C_4 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 (^{13}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

470 **Acknowledgements**

471 This work was supported by the National Natural Science Foundation of China (Grant Nos.
472 41807419, 41821001, 41830319, 41773135), the Key R&D Project of Ministry of Science and
473 Technology (grant no. 2016YFA0601100), the 111 project (National Bureau of Foreign Experts
474 and the Ministry of Education of China; Grant No. B08030), and the Fundamental Research
475 Funds for National University, China University of Geosciences, Wuhan (Grant Nos.
476 CUGL170815, CUGCJ1807). We thank the China Scholarship Council (CSC) (Grant No.
477 201306410031) for supporting Canfa Wang's studies at the University of Birmingham. S.G. was
478 supported by NERC Independent Research Fellowship NE/L011050/1 and NERC large grant
479 NE/P01903X/1 while working on this manuscript. M.L.G. acknowledges support from the
480 National Science Foundation award 1805544. H.Z. acknowledges support from the Hubei
481 Provincial Natural Science Foundation of China (Grant No. 2018CFB398).

482 **References**

- 483 An, Z., 2000. The history and variability of the East Asian paleomonsoon climate. *Quaternary*
484 *Science Reviews* 19, 171-187.
- 485 Ballentine, D.C., Macko, S.A., Turekian, V.C., 1998. Variability of stable carbon isotopic
486 compositions in individual fatty acids from combustion of C₄ and C₃ plants: implications for
487 biomass burning. *Chemical Geology* 152, 151-161.
- 488 Benner, R., Fogel, M.L., Sprague, E.K., Hodson, R.E., 1987. Depletion of ¹³C in lignin and its
489 implications for stable carbon isotope studies. *Nature* 329, 708.
- 490 Blyth, A.J., Asrat, A., Baker, A., Gulliver, P., Leng, M.J., Genty, D., 2007. A new approach to
491 detecting vegetation and land-use change using high-resolution lipid biomarker records in
492 stalagmites. *Quaternary Research* 68, 314-324.
- 493 Blyth, A.J., Baker, A., Thomas, L.E., Van Calsteren, P., 2011. A 2000-year lipid biomarker record
494 preserved in a stalagmite from north-west Scotland. *Journal of Quaternary Science* 26, 326-334.
- 495 Blyth, A.J., Hartland, A., Baker, A., 2016. Organic proxies in speleothems – New developments,
496 advantages and limitations. *Quaternary Science Reviews* 149, 1-17.
- 497 Blyth, A.J., Smith, C.I., Drysdale, R.N., 2013. A new perspective on the δ¹³C signal preserved in
498 speleothems using LC–IRMS analysis of bulk organic matter and compound specific stable
499 isotope analysis. *Quaternary Science Reviews* 75, 143-149.
- 500 Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil
501 respiration record. *Nature* 464, 579.
- 502 Cheng, H., Edwards, R.L., Sinha, A., Spotl, C., Yi, L., Chen, S., Kelly, M., Kathayat, G., Wang,
503 X., Li, X., Kong, X., Wang, Y., Ning, Y., Zhang, H., 2016. The Asian monsoon over the past
504 640,000 years and ice age terminations. *Nature* 534, 640-646.
- 505 Chikaraishi, Y., Naraoka, H., 2006. Carbon and hydrogen isotope variation of plant biomarkers in
506 a plant-soil system. *Chemical Geology* 231, 190-202.
- 507 Chikaraishi, Y., Naraoka, H., 2007. δ¹³C and δD relationships among three *n*-alkyl compound

508 classes (*n*-alkanoic acid, *n*-alkane and *n*-alkanol) of terrestrial higher plants. *Organic*
509 *Geochemistry* 38, 198-215.

510 Chikaraishi, Y., Naraoka, H., Poulson, S.R., 2004a. Carbon and hydrogen isotopic fractionation
511 during lipid biosynthesis in a higher plant (*Cryptomeria japonica*). *Phytochemistry* 65, 323-330.

512 Chikaraishi, Y., Naraoka, H., Poulson, S.R., 2004b. Hydrogen and carbon isotopic fractionations
513 of lipid biosynthesis among terrestrial (C_3 , C_4 and CAM) and aquatic plants. *Phytochemistry* 65,
514 1369-1381.

515 Crowther, T.W., Todd-Brown, K.E., Rowe, C.W., Wieder, W.R., Carey, J.C., Machmuller, M.B.,
516 Snoek, B.L., Fang, S., Zhou, G., Allison, S.D., Blair, J.M., Bridgham, S.D., Burton, A.J., Carrillo,
517 Y., Reich, P.B., Clark, J.S., Classen, A.T., Dijkstra, F.A., Elberling, B., Emmett, B.A., Estiarte, M.,
518 Frey, S.D., Guo, J., Harte, J., Jiang, L., Johnson, B.R., Kroel-Dulay, G., Larsen, K.S., Laudon, H.,
519 Lavallee, J.M., Luo, Y., Lupascu, M., Ma, L.N., Marhan, S., Michelsen, A., Mohan, J., Niu, S.,
520 Pendall, E., Penuelas, J., Pfeifer-Meister, L., Poll, C., Reinsch, S., Reynolds, L.L., Schmidt, I.K.,
521 Sistla, S., Sokol, N.W., Templer, P.H., Treseder, K.K., Welker, J.M., Bradford, M.A., 2016.
522 Quantifying global soil carbon losses in response to warming. *Nature* 540, 104-108.

523 Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and
524 feedbacks to climate change. *Nature* 440, 165-173.

525 Eglinton, G., Hamilton, R., 1967. Leaf epicuticular waxes. *Science* 156, 1322.

526 Ehleringer, R.J., Cerling, E.T., Helliker, R.B., 1997. C_4 photosynthesis, atmospheric CO_2 , and
527 climate. *Oecologia* 112, 285-299.

528 Fairchild, I.J., Baker, A., 2012. *Speleothem science: from process to past environments*. Wiley,
529 Chichester.

530 Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and
531 photosynthesis. *Annual review of plant biology* 40, 503-537.

532 Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial
533 and fungal biomass in soil. *Biology Fertility of Soils* 22, 59-65.

534 Genty, D., Blamart, D., Ouahdi, R., Gilmour, M., Baker, A., Jouzel, J., Van-Exter, S., 2003.
535 Precise dating of Dansgaard-Oeschger climate oscillations in western Europe from stalagmite
536 data. *Nature* 421, 833-837.

537 Hu, C., Henderson, G., Huang, J., Chen, Z., Johnson, K., 2008a. Report of a three-year
538 monitoring programme at Heshang Cave, Central China. *International Journal of Speleology* 37,
539 143-151.

540 Hu, C., Henderson, G.M., Huang, J., Xie, S., Sun, Y., Johnson, K.R., 2008b. Quantification of
541 Holocene Asian monsoon rainfall from spatially separated cave records. *Earth and Planetary
542 Science Letters* 266, 221-232.

543 Huang, X., Cui, J., Pu, Y., Huang, J., Blyth, A.J., 2008. Identifying "free" and "bound" lipid
544 fractions in stalagmite samples: An example from Heshang Cave, Southern China. *Applied
545 Geochemistry* 23, 2589-2595.

546 Huang, Y., Street-Perrott, F.A., Metcalfe, S.E., Brenner, M., Moreland, M., Freeman, K.H., 2001.
547 Climate change as the dominant control on glacial-interglacial variations in C₃ and C₄ plant
548 abundance. *Science* 293, 1647-1651.

549 Huguen, K.A., Eglinton, T.I., Xu, L., Makou, M., 2004. Abrupt tropical vegetation response to
550 rapid climate changes. *Science* 304, 1955-1959.

551 Indermühle, A., Stocker, T., Joos, F., Fischer, H., Smith, H., Wahlen, M., Deck, B., Mastroianni,
552 D., Tschumi, J., Blunier, T., 1999. Holocene carbon-cycle dynamics based on CO₂ trapped in ice
553 at Taylor Dome, Antarctica. *Nature* 398, 121-126.

554 Ji, S., Xingqi, L., Sumin, W., Matsumoto, R., 2005. Palaeoclimatic changes in the Qinghai Lake
555 area during the last 18,000 years. *Quaternary International* 136, 131-140.

556 Koga, Y., 2011. Early evolution of membrane lipids: how did the lipid divide occur? *Journal of
557 Molecular Evolution* 72, 274-282.

558 Laskar, J., Robutel, P., Joutel, F., Gastineau, M., Correia, A., Levrard, B., 2004. A long-term
559 numerical solution for the insolation quantities of the Earth. *Astronomy and Astrophysics* 428,

560 261-285.

561 Li, X., Hu, C., Huang, J., Xie, S., Baker, A., 2014. A 9000-year carbon isotopic record of
562 acid-soluble organic matter in a stalagmite from Heshang Cave, central China: Paleoclimate
563 implications. *Chemical Geology* 388, 71-77.

564 Li, X., Wang, C., Huang, J., Hu, C., Xie, S., 2011. Seasonal variation of fatty acids from drip
565 water in Heshang Cave, central China. *Applied Geochemistry* 26, 341-347.

566 Lichtfouse, É., Berthier, G., Houot, S., Barriuso, E., Bergheaud, V., Vallaeys, T., 1995. Stable
567 carbon isotope evidence for the microbial origin of C₁₄-C₁₈ *n*-alkanoic acids in soils. *Organic*
568 *Geochemistry* 23, 849-852.

569 Liu, H., Liu, W., 2017. Concentration and distributions of fatty acids in algae, submerged plants
570 and terrestrial plants from the northeastern Tibetan Plateau. *Organic Geochemistry* 113, 17-26.

571 Liu, Q., Wang, H., Zhao, R., Qiu, X., Gong, L., 2010. Bacteria isolated from dripping water in the
572 oligotrophic Heshang cave in central China. *Journal of Earth Science* 21, 325-328.

573 Lloyd, J., Taylor, J., 1994. On the temperature dependence of soil respiration. *Functional ecology*,
574 315-323.

575 Luo, Y., 2007. Terrestrial Carbon–Cycle Feedback to Climate Warming. *Annual Review of*
576 *Ecology, Evolution, and Systematics* 38, 683-712.

577 Mahecha, M.D., Reichstein, M., Carvalhais, N., Lasslop, G., Lange, H., Seneviratne, S.I., Vargas,
578 R., Ammann, C., Arain, M.A., Cescatti, A., 2010. Global convergence in the temperature
579 sensitivity of respiration at ecosystem level. *Science* 329, 838-840.

580 Matsumoto, G.I., Friedmann, E.I., Watanuki, K., Ocampo-Friedmann, R., 1992. Novel long-chain
581 anteiso-alkanes and anteiso-alkanoic acids in Antarctic rocks colonized by living and fossil
582 cryptoendolithic microorganisms. *Journal of Chromatography A* 598, 267-276.

583 Melillo, J.M., Frey, S.D., Deangelis, K.M., Werner, W.J., Bernard, M.J., Bowles, F.P., Pold, G.,
584 Knorr, M.A., Grandy, A.S., 2017. Long-term pattern and magnitude of soil carbon feedback to the
585 climate system in a warming world. *Science* 358, 101-105.

586 Monson, K.D., Hayes, J., 1982. Carbon isotopic fractionation in the biosynthesis of bacterial fatty
587 acids. Ozonolysis of unsaturated fatty acids as a means of determining the intramolecular
588 distribution of carbon isotopes. *Geochimica et Cosmochimica Acta* 46, 139-149.

589 Noronha, A.L., Johnson, K.R., Hu, C., Ruan, J., Southon, J.R., Ferguson, J.E., 2014. Assessing
590 influences on speleothem dead carbon variability over the Holocene: Implications for
591 speleothem-based radiocarbon calibration. *Earth and Planetary Science Letters* 394, 20-29.

592 Polissar, P.J., D'Andrea, W.J., 2014. Uncertainty in paleohydrologic reconstructions from
593 molecular δD values. *Geochimica et Cosmochimica Acta* 129, 146-156.

594 Raich, J.W., Tufekciogul, A., 2000. Vegetation and soil respiration: correlations and controls.
595 *Biogeochemistry* 48, 71-90.

596 Rieley, G., Collier, R.J., Jones, D.M., Eglinton, G., Eakin, P.A., Fallick, A.E., 1991. Sources of
597 sedimentary lipids deduced from stable carbon-isotope analyses of individual compounds. *Nature*
598 352, 425.

599 Rubino, M., Etheridge, D., Trudinger, C., Allison, C., Rayner, P., Enting, I., Mulvaney, R., Steele,
600 L., Langenfelds, R., Sturges, W., 2016. Low atmospheric CO₂ levels during the Little Ice Age due
601 to cooling-induced terrestrial uptake. *Nature Geoscience* 9, 691-694.

602 Sage, R.F., 2004. The evolution of C₄ photosynthesis. *New phytologist* 161, 341-370.

603 Šantrůčková, H., Bird, M., Lloyd, J.J.F.E., 2000. Microbial processes and carbon- isotope
604 fractionation in tropical and temperate grassland soils. *Functional Ecology* 14, 108-114.

605 Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic
606 matter: mechanisms and controls. *Geoderma* 74, 65-105.

607 Still, C.J., Berry, J.A., Collatz, G.J., Defries, R.S., 2009. ISLSCP II C₄ Vegetation Percentage.
608 ORNL Distributed Active Archive Center.

609 Sun, X., Chen, Y., 1991. Palynological records of the last 11,000 years in China. *Quaternary*
610 *Science Reviews* 10, 537-544.

611 Tjoelker, M.G., Oleksyn, J., Reich, P.B., 2001. Modelling respiration of vegetation: evidence for a

612 general temperature- dependent Q_{10} . *Global Change Biology* 7, 223-230.

613 Wang, C., Bendle, J., Yang, Y., Yang, H., Sun, H., Huang, J., Xie, S., 2016. Impacts of pH and
614 temperature on soil bacterial 3-hydroxy fatty acids: Development of novel terrestrial proxies.
615 *Organic Geochemistry* 94, 21-31.

616 Wang, C., Bendle, J.A., Zhang, H., Yang, Y., Liu, D., Huang, J., Cui, J., Xie, S., 2018. Holocene
617 temperature and hydrological changes reconstructed by bacterial 3-hydroxy fatty acids in a
618 stalagmite from central China. *Quaternary Science Reviews* 192, 97-105.

619 Wang, C., Zhang, H., Huang, X., Huang, J., Xie, S., 2012. Optimization of acid digestion
620 conditions on the extraction of fatty acids from stalagmites. *Frontiers of Earth Science* 6,
621 109-114.

622 Wang, Z., Liu, W.G., 2012. Carbon chain length distribution in n-alkyl lipids: A process for
623 evaluating source inputs to Lake Qinghai. *Organic Geochemistry* 50, 36-43.

624 Werth, M., Kuzyakov, Y., 2010. ^{13}C fractionation at the root–microorganisms–soil interface: a
625 review and outlook for partitioning studies. *Soil Biology and Biochemistry* 42, 1372-1384.

626 Wessén, E., Hallin, S., Philippot, L., 2010. Differential responses of bacterial and archaeal groups
627 at high taxonomical ranks to soil management. *Soil Biology and Biochemistry* 42, 1759-1765.

628 Xie, S., Evershed, R.P., Huang, X., Zhu, Z., Pancost, R.D., Meyers, P.A., Gong, L., Hu, C., Huang,
629 J., Zhang, S., 2013. Concordant monsoon-driven postglacial hydrological changes in peat and
630 stalagmite records and their impacts on prehistoric cultures in central China. *Geology* 41,
631 827-830.

632 Xie, S., Huang, J., Wang, H., Yi, Y., Hu, C., Cai, Y., Cheng, H., 2005. Distributions of fatty acids
633 in a stalagmite related to paleoclimate change at Qingjiang in Hubei, southern China. *Science in*
634 *China Series D: Earth Sciences* 48, 1463-1469.

635 Xie, S., Yi, Y., Huang, J., Hu, C., Cai, Y., Collins, M., Baker, A., 2003. Lipid distribution in a
636 subtropical southern China stalagmite as a record of soil ecosystem response to paleoclimate
637 change. *Quaternary Research* 60, 340-347.

638 Yun, Y., Xiang, X., Wang, H., Man, B., Gong, L., Liu, Q., Dong, Q., Wang, R., 2016. Five-year
639 monitoring of bacterial communities in dripping water from the Heshang Cave in central China:
640 Implication for paleoclimate reconstruction and ecological functions. *Geomicrobiology Journal*
641 33, 553-563.

642 Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial
643 communities. *Chemosphere* 35, 275-294.

644 Zhang, Z., Zhao, M., Eglinton, G., Lu, H., Huang, C.-Y., 2006. Leaf wax lipids as
645 paleovegetational and paleoenvironmental proxies for the Chinese Loess Plateau over the last 170
646 kyr. *Quaternary Science Reviews* 25, 575-594.

647 Zhao, R., Wang, H., Cheng, X., Yun, Y., Qiu, X., 2018. Upland soil cluster γ dominates the
648 methanotroph communities in the karst Heshang Cave. *FEMS Microbiology Ecology* 94, fiy192.

649 Zhou, A., Sun, H., Chen, F., Zhao, Y., An, C., Dong, G., Wang, Z., Chen, J., 2010. High-resolution
650 climate change in mid-late Holocene on Tianchi Lake, Liupan Mountain in the Loess Plateau in
651 central China and its significance. *Chinese Science Bulletin* 55, 2118-2121.

652 Zhu, Z., Feinberg, J.M., Xie, S., Bourne, M.D., Huang, C., Hu, C., Cheng, H., 2017. Holocene
653 ENSO-related cyclic storms recorded by magnetic minerals in speleothems of central China.
654 *Proceedings of the National Academy of Sciences of the United States of America* 114, 852-857.

655

656

657 **Figure Captions**

658

659 **Fig. 1.** Location of Heshang Cave, Qinghai Lake and Tianchi Lake. The distribution of C₄
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-C₁₆
666 fatty acid is dominant in low molecular weight fatty acids (≤C₂₀), n-C₂₄ fatty acid is dominant in
667 high molecular weight fatty acids (>C₂₀).

668

669 **Fig. 3.** Plots showing the relationship between δ¹³C values of n-fatty acids from HS4 stalagmite. **a**,
670 Linear correlation between δ¹³C_{16FA} and δ¹³C_{18FA} based on a data set of 70 samples (R² = 0.79, p <
671 0.001). **b**, Linear correlation between δ¹³C_{16FA} and δ¹³C_{24FA} based on a data set of 61 samples (R²
672 = 0.55, p < 0.001). **c**, Linear correlation between δ¹³C_{22FA} and δ¹³C_{24FA}, based on a data set of 49
673 samples (R² = 0.94, p < 0.001). **d**, Linear correlation between δ¹³C_{18FA} and δ¹³C_{24FA} based on a
674 data set of 59 samples (R² = 0.61, p < 0.001).

675

676 **Fig. 4.** Box chart showing the carbon isotope values of C₁₆, C₁₈, C₂₂ and C₂₄ 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 δ¹³C_{24FA} showing vegetation changes during the last 9 ka BP. **b**, Insolation changes at
681 30°N in July during the last 9 ka BP ([Laskar et al., 2004](#)). **c**, Calcite δ¹⁸O 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* C₁₅ 3-hydroxy fatty acid (RAN₁₅; 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 C₃
690 plants during the HCO. Orange shading highlights relatively high percentages of C₃ 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*-C₁₆, *n*-C₁₈,
695 *n*-C₂₂, *n*-C₂₄ 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 RAN₁₅ 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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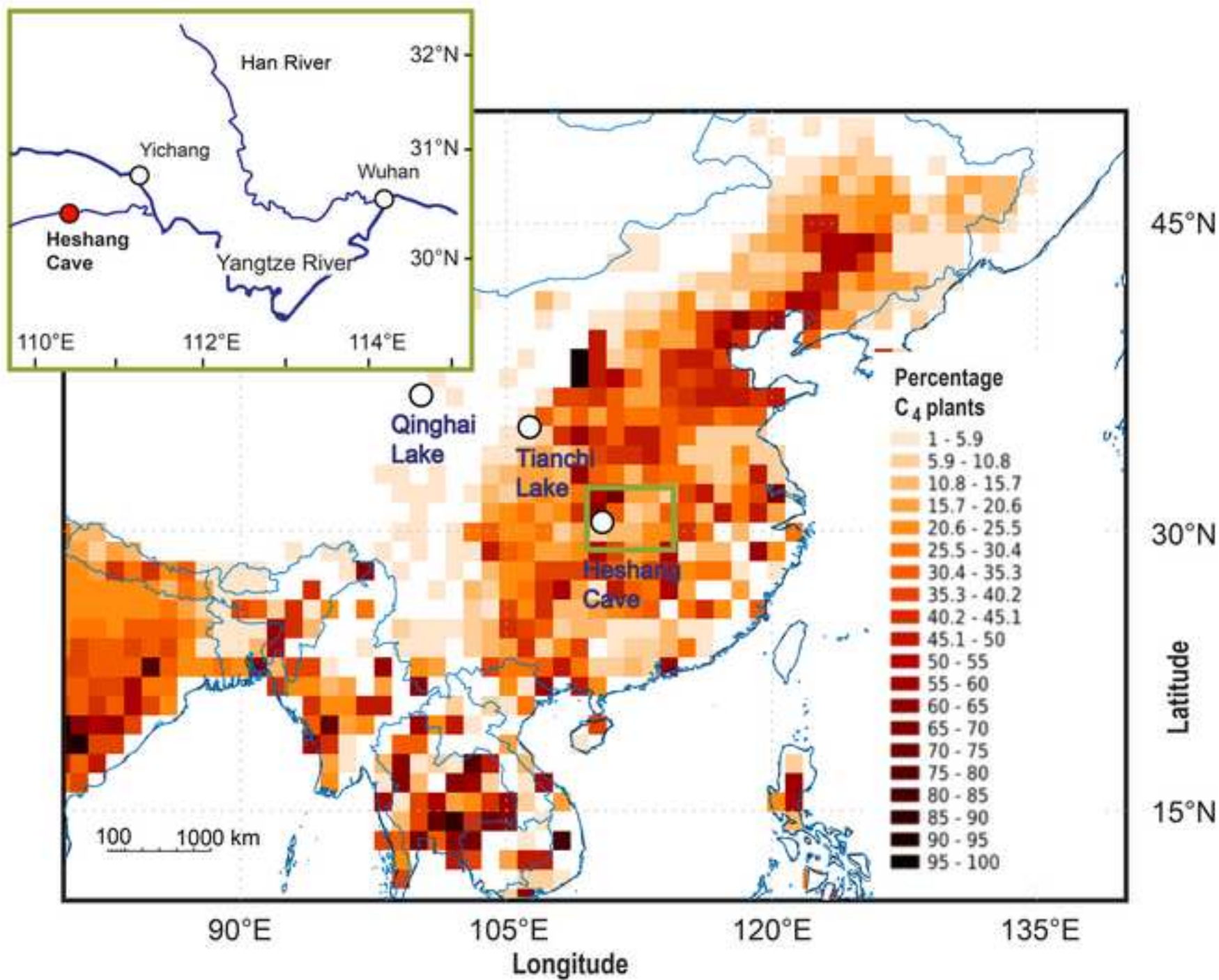


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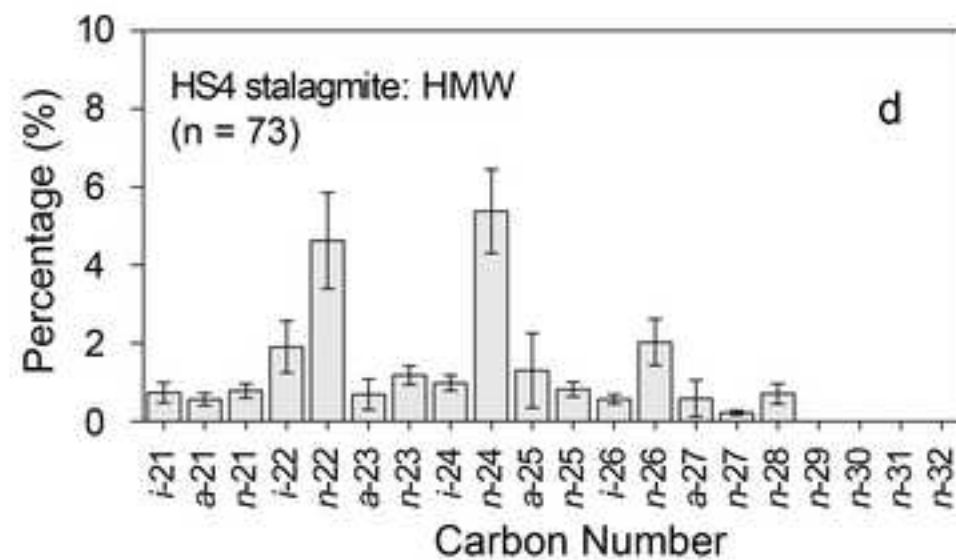
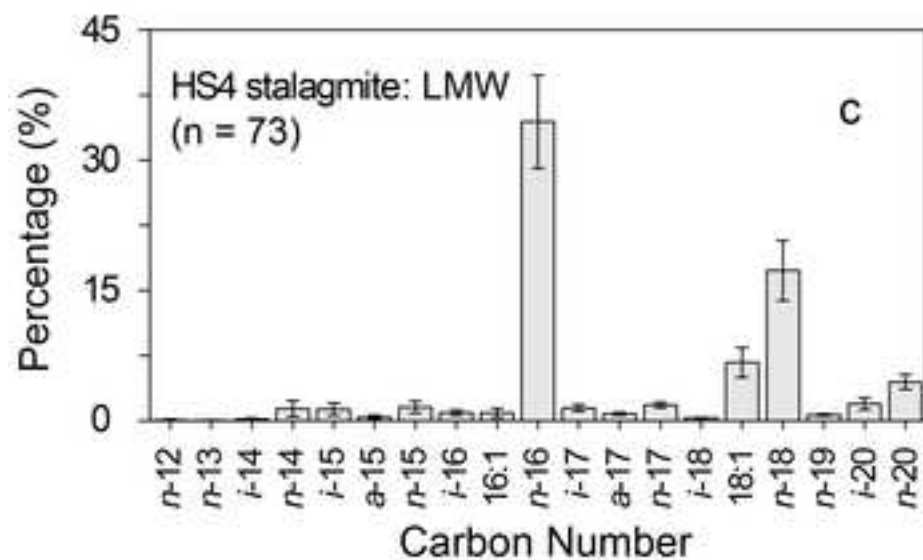
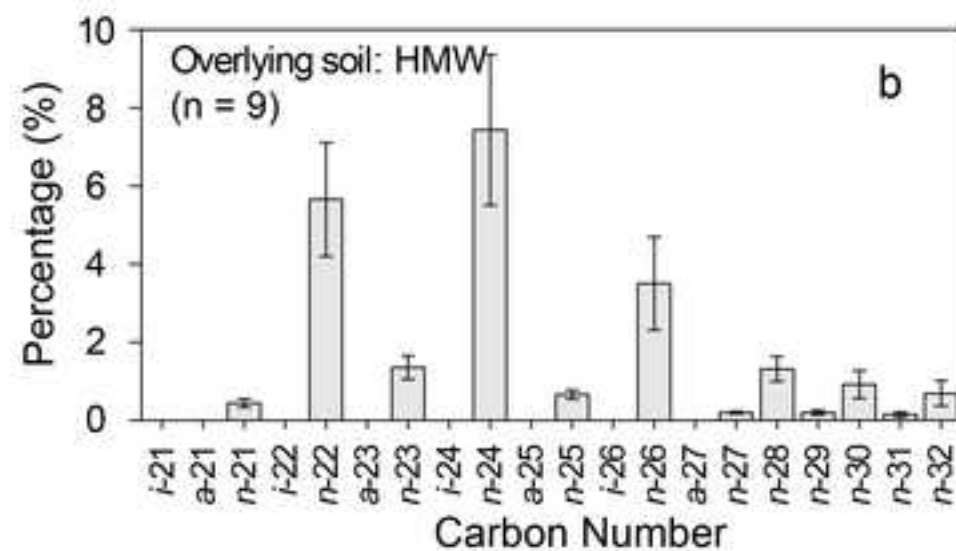
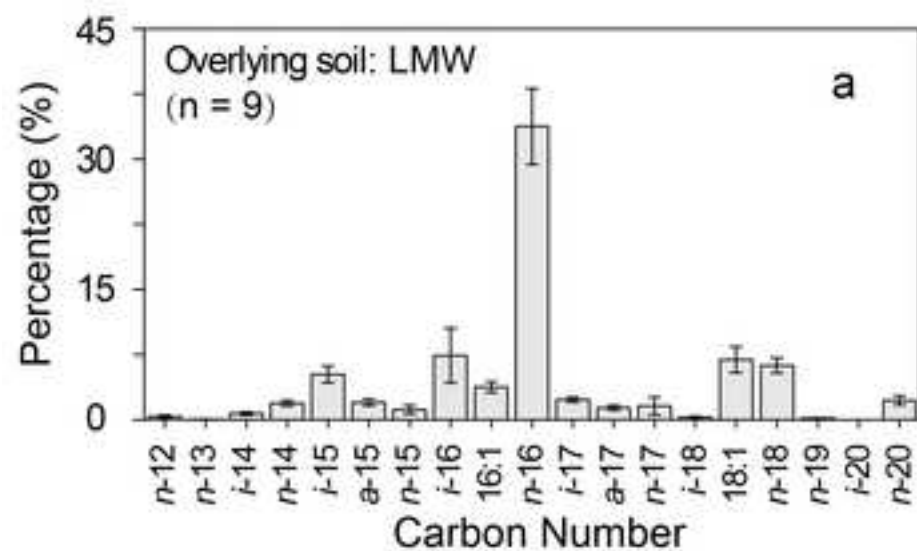


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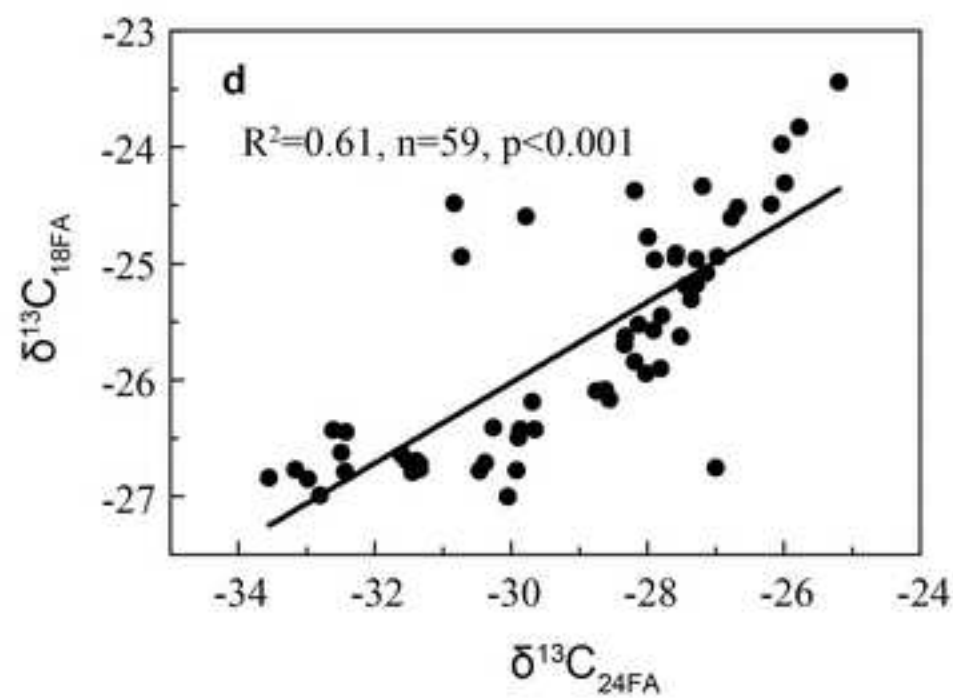
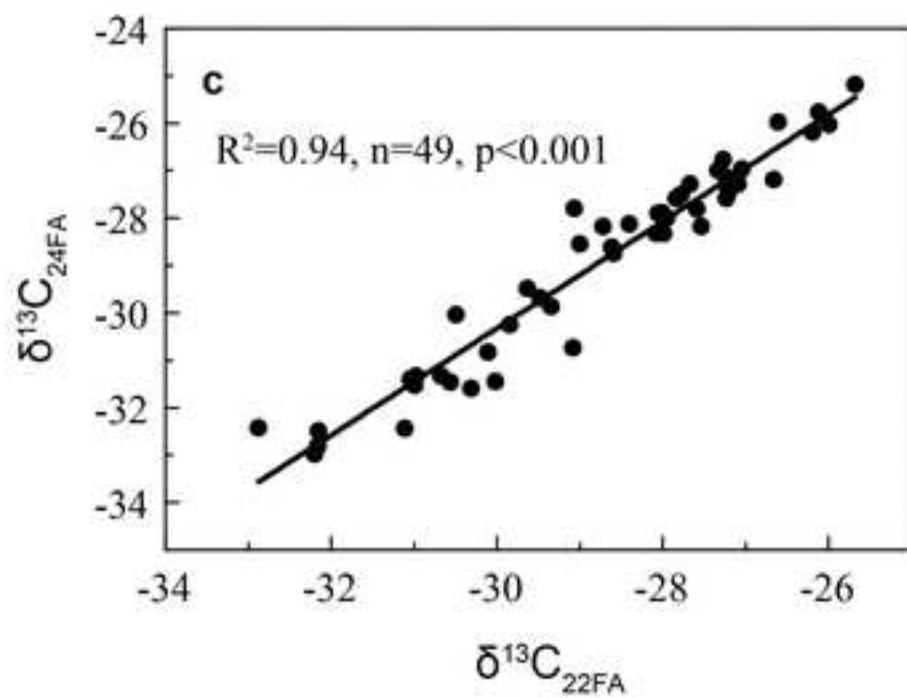
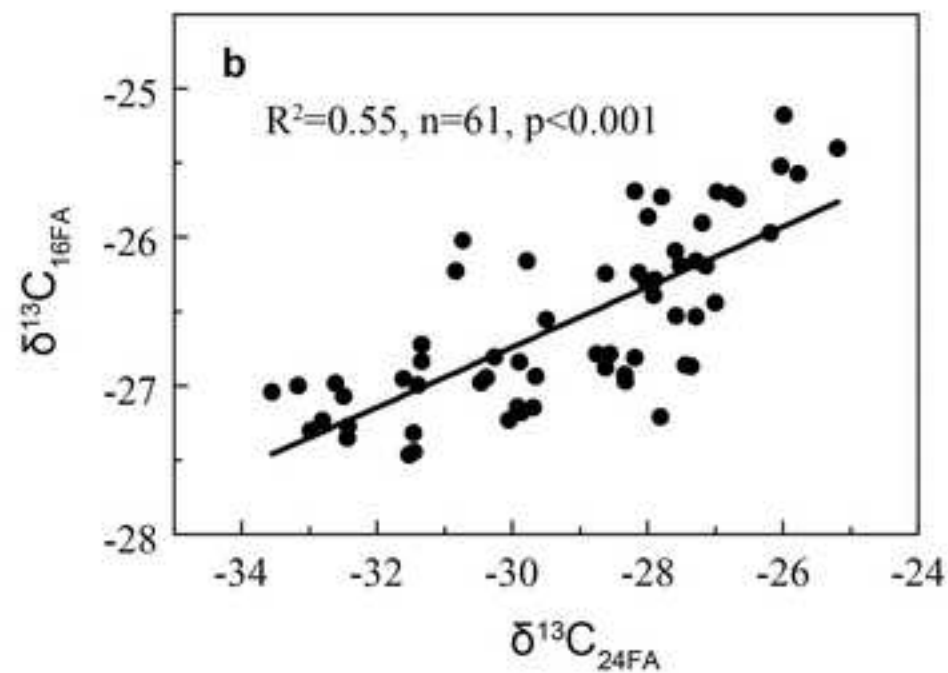
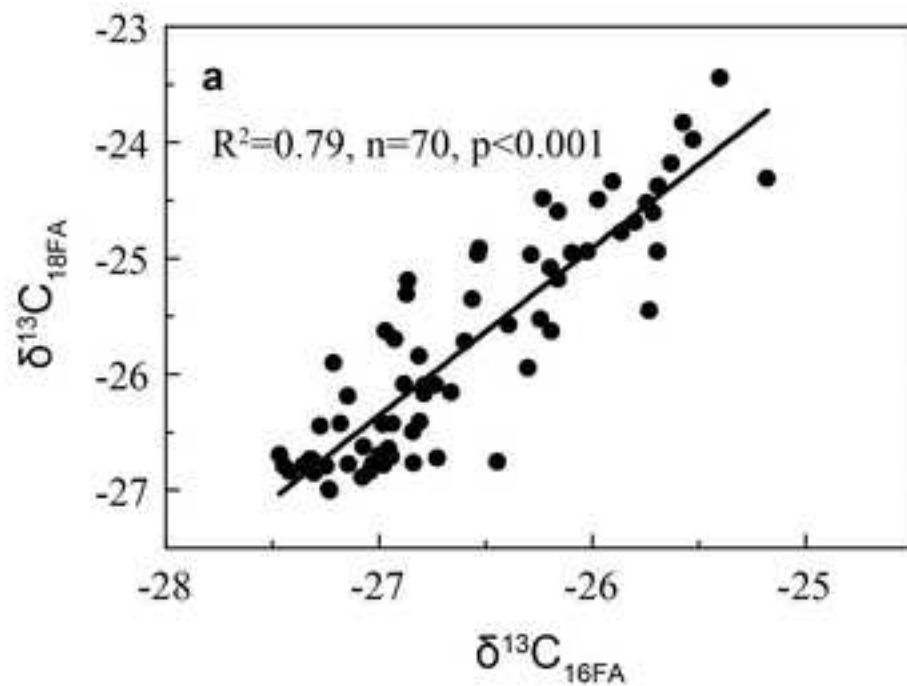


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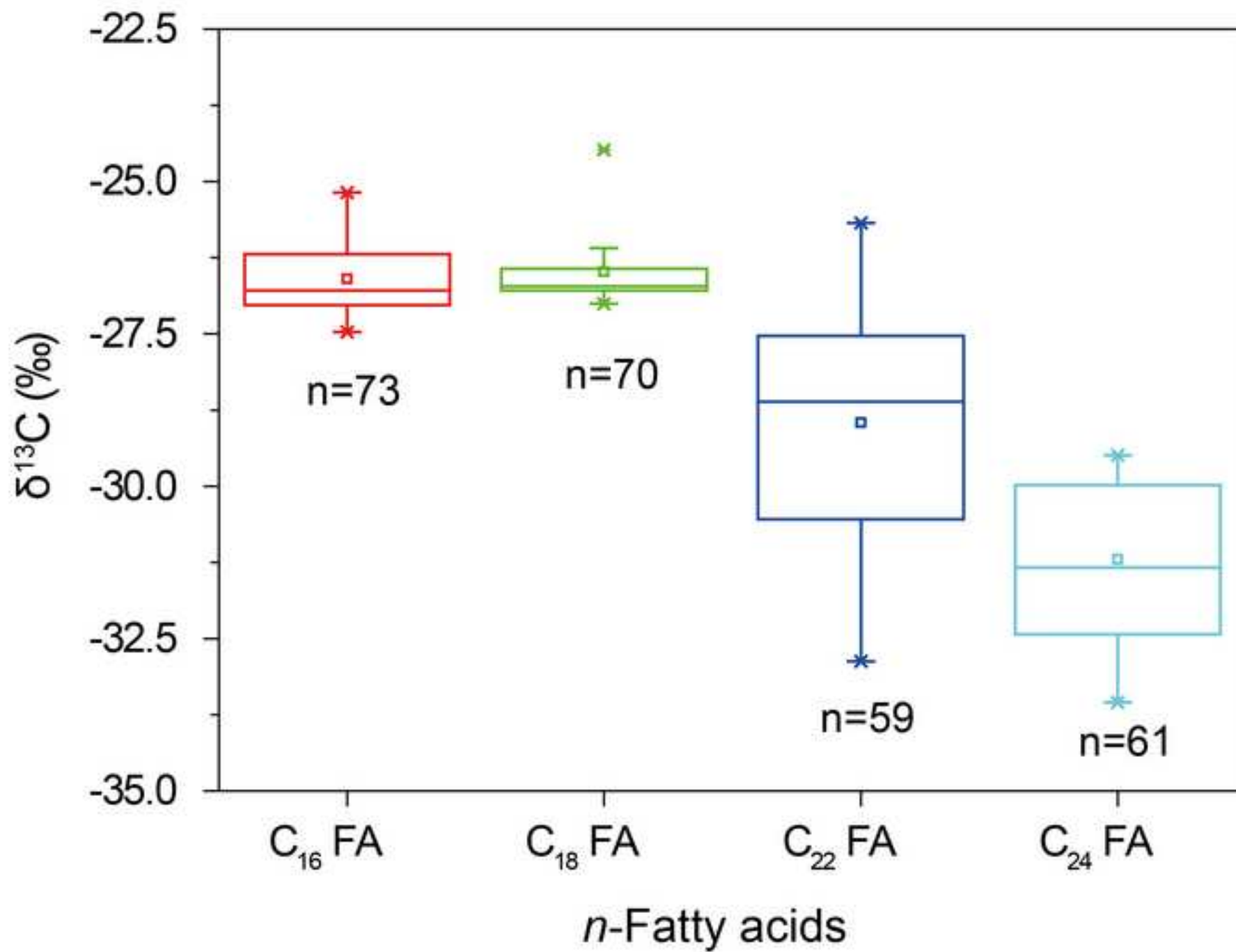


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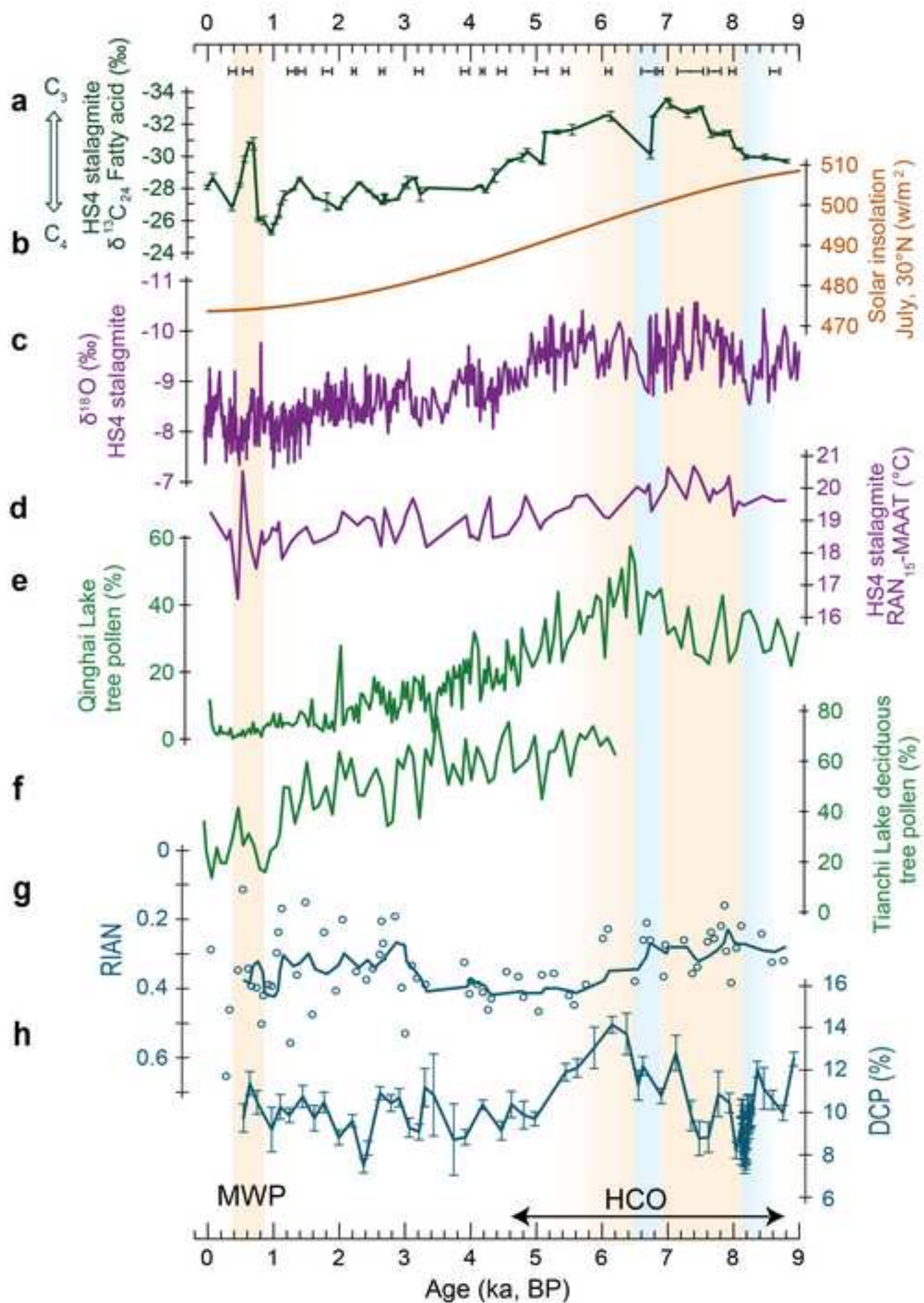


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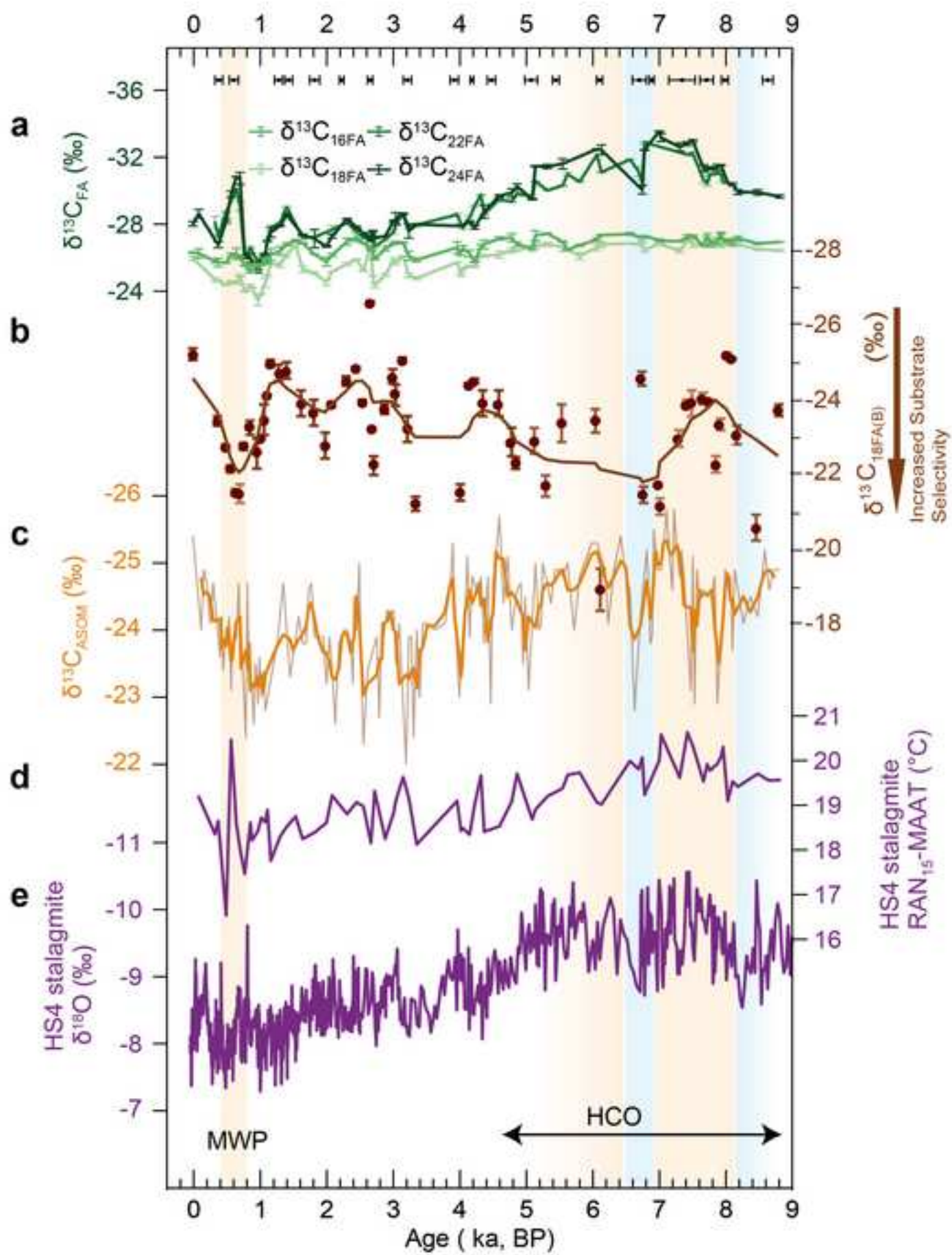


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