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1 Holocene temperature and hydrological changes
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4

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16

17 **Abstract**

18 To achieve a sufficient understanding of the spatial dynamics of terrestrial climate
19 variability, new proxies and networks of data that cover thousands of years and run up
20 to the present day are needed. Here we show the first Gram-negative bacterial
21 3-hydroxy fatty acid (3-OH-FA) based temperature and hydrological records from any
22 palaeoclimate archive globally. The data, covering the last 9 ka before present (BP),
23 are generated from an individual stalagmite, collected from Heshang Cave, located on
24 a tributary of the Yangtze River, central China (30°27'N, 110°25'E; 294 m). Our
25 results indicate a clear early-to-middle Holocene Climatic Optimum (8.0-6.0 ka BP)
26 followed by a long-term monotonic cooling and increasing variability over the last 0.9
27 ka BP. The hydrological record shows two relatively long wet periods (8.8-5.9 ka BP
28 and 3.0-0 ka BP) and one relative dry period (5.9-3.0 ka BP) in central China. We
29 show that 3-OH-FA biomarkers hold promise as independent tools for palaeoclimate
30 reconstruction, with the potential to deconvolve temperature and hydrological signals
31 from an individual stalagmite.

32

33 **Keywords:**

34 Holocene; Paleoclimatology; Novel proxy; 3-hydroxy fatty acid; China; Monsoon;
35 Speleothems

36

37 **1. Introduction**

38 Nearly half of the Earth's population live within the influence of the modern
39 monsoon and its importance to terrestrial eco-systems, societal wellbeing and the
40 global economy can not be overstated ([Webster et al., 1998](#)). Records of past
41 Holocene rainfall and temperature, which extend the relatively short instrumental
42 record, can constrain natural monsoon variability and are particularly important for
43 the Asian monsoon region where prediction of future changes in rainfall using climate
44 models has proven challenging ([IPCC, 2014](#)). Such records can also illustrate the
45 influence of the monsoon on prehistoric cultures and settlements ([Xie et al., 2013](#)).

46 Stalagmites have become a key archive in Quaternary palaeoclimatic
47 reconstruction due to their ability to yield continuous and undisturbed records, precise
48 and absolute chronologies, and their global terrestrial distribution ([Blyth et al., 2016](#);
49 [Fairchild et al., 2006](#); [Fairchild and Baker, 2012](#); [McDermott, 2004](#); [Wong and](#)
50 [Breecker, 2015](#)). Oxygen isotopes are effectively the 'master' or standard approach
51 for speleothem analysis, but inherently encode a mix of climatic signals ([Lachniet,](#)
52 [2009](#); [McDermott, 2004](#)), including, at the regional scale: temperature changes, the
53 isotopic composition of source waters and precipitation amount. In addition, complex
54 site-specific factors must be taken into account, such as drip rate ([Dreybrodt and](#)
55 [Scholz, 2011](#)) and CaCO₃ precipitation ([Fairchild and Baker, 2012](#)). Many previous
56 studies have focused on the interpretation of oxygen isotopes in speleothems, but
57 deconvolving independent temperature and precipitation signals from speleothem
58 CaCO₃ remains highly challenging, as evidenced by the paucity of such deconvolved
59 records ([Hu et al., 2008b](#); [Yuan et al., 2004](#)).

60 Biomarker based proxies are now firmly established in the fields of
61 paleoceanography and paleolimnology (Castañeda and Schouten, 2011; Eglinton and
62 Eglinton, 2008; Schouten et al., 2013). Recently attention has turned to the potential
63 of organic matter and biomarker techniques for speleothem research (Blyth et al.,
64 2008; Blyth et al., 2016). A number of biomarkers with known paleoclimatic utility
65 have been discovered and measured in speleothems, including glycerol dialkyl
66 glycerol tetraethers (GDGTs) (Blyth et al., 2014; Blyth and Schouten, 2013; Yang et
67 al., 2011), plant derived biomarkers (Blyth et al., 2007; Blyth et al., 2011; Blyth et al.,
68 2010; Bosle et al., 2014; Xie et al., 2003), branched fatty acids and hydroxy fatty
69 acids (Blyth et al., 2006; Huang et al., 2008; Wang et al., 2012). Furthermore, Blyth
70 and Schouten (2013) recently proposed a novel GDGT calibration, based on samples
71 derived from 33 globally distributed speleothems from caves with a range of average
72 air temperatures.

73 Biomarkers in stalagmites may originate from the overlying vegetation,
74 overlying soil ecosystem, limestone aquifer and cave fauna (Blyth et al., 2008).
75 Moreover, different biomarker classes may have different sources, for example, Yang
76 et al. (2011) found that the majority of the archaeal isoprenoid and bacterial branched
77 GDGTs measured in stalagmite samples from Heshang cave were likely produced *in*
78 *situ*. Most recently, Blyth et al. (2014) found that GDGTs preserved in stalagmites in
79 the UK and Australia likely originated from the *in situ* microbial communities within
80 cave systems. An artificial irrigation experiment conducted in Cathedral Cave,
81 Australia, found different GDGT distributions among speleothem, soil and drip water

82 samples (Baker et al., 2016). In contrast, a 2 year monitoring experiment of drip
83 waters in Heshang Cave found that fatty acids in drip waters were most likely derived
84 from the overlying soil and/or groundwater system via particulate entrainment and
85 deposition (Li et al., 2011). It is noteworthy that the fatty acid ratios (ratios of
86 $nC_{16:1}/nC_{16:0}$ and $nC_{18:1}/nC_{18:0}$; the prefix n indicates normal, the number before the
87 colon specifies the number of C atoms, and the number after the colon gives the
88 number of double bonds) showed a strong negative relationship with the external
89 temperature recorded in Yichang meteoric station (located ca. 100 km east of Heshang
90 Cave), whereas the two ratios displayed no relationship with internal cave
91 temperatures recorded at the HS4 site, which suggests that *in situ* cave microbes are
92 probably not the predominant source for C_{16} and C_{18} acids in drip water collected in
93 Heshang Cave. Li et al., (2011) concluded that, based the distributional patterns of the
94 fatty acids, microbes living in the overlying soils and/or groundwater system are the
95 dominant source of fatty acids to the Heshang Cave drip waters. We note that
96 Vaughn et al. (2011) discovered microbia living on speleothem surfaces in Kartchner
97 Caverns, USA. Such consortia of microbes are an inevitable source of *in situ* fatty
98 acids. Thus fatty acids measured in stalagmites may be derived from mixed sources,
99 including overlying soils/ sediments (Li et al., 2011), the ramifying network of
100 conduits and reservoirs in the limestone and *in situ* microbes (Vaughan et al., 2011).
101 However, even though the origin and pathways of inclusion into speleothems of
102 biomarkers may be complex (Blyth et al., 2008; Blyth et al., 2016), it doesn't hinder
103 the utilization of biomarkers in paleoclimate reconstruction. Site specific

104 interpretation and ground truthing is required, but this is also true for established
105 paleoclimate techniques, as outlined above. In summary lipid biomarkers preserved in
106 speleothems show clear potential for paleoclimate reconstruction. However, very few
107 such biomarker based paleoclimatic reconstructions have been published (Blyth et al.,
108 2011; Huguet et al., 2018; Li et al., 2014; Xie et al., 2003).

109 Gram-negative bacterial 3-hydroxy fatty acids (3-OH-FAs) are abundant in
110 stalagmites (Blyth et al., 2006; Huang et al., 2008; Wang et al., 2016; Wang et al.,
111 2012) and are characteristic compounds of Lipid A, the lipid component of the
112 lipopolysaccharides (LPS) located in the outer membrane of Gram-negative bacteria
113 (Szponar et al., 2003; Szponar et al., 2002; Wollenweber and Rietschel, 1990). Based
114 on strong relationships with environmental pH and temperature from an altitudinal
115 transect of soils on Shennongjia Mountain (Mt.) central China, a number of novel
116 3-OH-FA based proxies have been proposed (Wang et al., 2016). For example, the
117 ratio of *anteiso* to *normal* C₁₅ 3-hydroxy fatty acid (RAN₁₅) was propounded to be a
118 novel temperature proxy, and the ratio of the total sum of *iso* and *anteiso* 3-OH-FAs to
119 the total amount of *normal* 3-OH-FAs (Branching Ratio) and RIAN (negative
120 logarithm of Branching Ratio) were propounded to be novel pH proxies (Wang et al.,
121 2016).

122 In this study we present inferred temperature and hydrological records, spanning
123 the last 9 ka BP, based on 3-OH-FA derived proxies from a single stalagmite
124 collected from Heshang Cave, central China (Fig. 1). This work is the first
125 demonstration of the application of 3-OH-FA based proxies for paleoclimatic

126 reconstruction and suggests that such approaches may be used to derive independent
127 quantitative temperature and qualitative hydrological signals from an individual
128 stalagmite.

129

130 **2. Materials and methods**

131 **2.1 Sampling site and sample information**

132 Heshang Cave is located at 294m above sea level (a.s.l.), on the Qing River, a
133 tributary in the middle reaches of the Yangtze River, central China (30°27'N,
134 110°25'E) (Fig. 1A). Heshang Cave is one of several caves which characterize the
135 regional karst landscape. The overlying dolomite is ca. 400 m thick and is capped
136 with a mature layer of soil (20-40 cm-thick) and reasonably dense vegetation (Fig.
137 1B). The regional climate is strongly impacted by the East Asian Monsoon, with a hot
138 and moist summer, but relatively cold and dry winter (An, 2000). Regional average
139 annual precipitation is 1161 mm, based on the recent 65 years (1951-2014) of
140 meteorological data from Yichang station. The seasonal temperature ranges, inside
141 and immediately outside the cave, were constrained by 2-hour resolution logging
142 between 2004 and 2007 using HOBO H8 Pro T loggers (Hu et al., 2008a). The
143 modern temperature immediately outside the cave varies seasonally from 3°C to 30°C,
144 with an annual average of 18°C and is statistically identical to that of the nearest
145 government meteorological station in Changyang county (Hu et al., 2008a). The
146 annual mean temperature inside the cave is identical to the outside measurements.
147 However, the amplitude of the internal temperature range is about one fifth of the

148 external cycle and lags the external temperatures by about 10 days (Hu et al., 2008a).
149 Heshang cave extends a distance of ≈ 250 m, roughly horizontally from its opening
150 (see Fig. 1C) and is well decorated with stalagmites, rimstone pools, and less frequent
151 stalactites (including an exquisite ‘Lotus Flower’ stalactite).

152 The HS4 stalagmite is 2.5 m long, and was actively growing when collected
153 from ca. 150 m within Heshang Cave in 2001 (Fig. 1C). It shows clear annual banding
154 throughout its growth axis, generated by the strong seasonal cycle at this site (Johnson
155 et al., 2006). Highlights of previous work on this stalagmite include a quantitative
156 Holocene Asian monsoon rainfall record (Hu et al., 2008b) and high resolution 8.2 ka
157 event record (Liu et al., 2013; Owen et al., 2016). The HS4 stalagmite was divided
158 longitudinally into 4 sections. Each section was dedicated to a different branch of
159 analyses (e.g. $\delta^{18}\text{O}$, trace elements, organic geochemistry etc.). 206 subsamples were
160 taken from the organic geochemistry section along the stalagmite growth axis and
161 73 subsamples were selected at intervals for biomarker analysis. All the outer layers
162 of the subsample were removed during sampling to avoid any potential contamination.
163 Based on annual layering each sample has a resolution of several decades to >100
164 years.

165 In 2013 seventeen cave sediment samples were collected within Heshang cave
166 from the entrance to the deepest accessible part of the cave (Fig. 1C) and nine
167 overlying soil samples were collected from the land-surface immediately above the
168 cave (which slopes upwards from an altitude of 457 m to 489 m) (Fig. 1B). The
169 sediment inside the cave is oligotrophic with < 6 g/kg total organic carbon (Gong et

170 [al., 2015](#)).

171 **2.2 Chronology**

172 The chronology of HS-4 was established independently by U-Th dating and layer
173 counting. Twenty-one subsamples were sampled and prepared in a class-1000 clean
174 lab before being analyzed by multi-collector inductively coupled plasma mass
175 spectrometry (MC-ICP-MS) at Oxford University (Nu Instruments), following the
176 techniques of Robinson et al. (2002). Layer counting was used for the uppermost 150
177 years and U-Th dating for the period ca. 378 ± 57 to 9446 ± 146 years BP. The
178 chronology of each sample is based on linear interpolation between the ^{230}Th dates,
179 the average age uncertainty is 67 yrs. Further details on the chronological techniques
180 and model are reported by Hu et al. (2008b).

181 **2.3 pH measurement**

182 The pH of cave surface sediments and overlying soils was measured following
183 the methods of Yang et al. (2014). Samples were mixed with ultrapure water in a ratio
184 of 1:2.5 (g/mL). After standing for 30 min, the pH of the supernatant was measured
185 using a meter with a precision of ± 0.01 .

186 **2.4 Lipid extraction and work-up**

187 The stalagmite samples were treated with an optimized acid digestion method
188 following Wang et al. (2012). In brief, 10 grams of stalagmite sample were digested
189 with 3M HCl, then re-fluxed at 130°C for 3 hours with a condenser/ heating mantle
190 assembly. An internal standard (pregn-5-en-3.beta.-ol) was quantitatively added to
191 each sample to quantify the amount of lipids in the stalagmite. After cooling, the

192 residue was extracted by dichloromethane (15mL×4) and the extracts combined.
193 Solvents were removed by rotary evaporation (Buchi R210) under reduced pressure.
194 Soil samples and cave surface sediments underwent the same work-up protocol as the
195 stalagmite samples. The condensed lipids were further derivatized by BF₃-methanol
196 (14% BF₃/methanol, Sigma) and BSTFA (N, O-bis(trimethylsilyl) trifluoroacetamide,
197 Supelco) before undergoing gas chromatography-mass spectrometry (GC-MS). In
198 order to minimize contamination, all glassware was soaked in a decontamination
199 solution, rinsed with ultra purified water, and heated for 6 h at 500 °C. The HCl was
200 pre-extracted with dichloromethane (DCM, ×4), and all other reagents were tested for
201 background contaminants.

202 **2.5 Instrumental analysis**

203 All the samples were analyzed using GC-MS with a Hewlett Packard 6890 gas
204 chromatograph coupled to a Hewlett Packard 5973 mass selective detector. Separation
205 was performed on a ZB-5MS fused silica capillary column (60 m×0.25 mm id.; 0.25
206 µm film thickness). The GC oven temperature was programmed from 70°C to 200°C
207 at 10°C per min, then from 200°C to 300°C at 2°C per min, and finally held at 300°C
208 for 27 min. The carrier gas was He (1 mL/min). The spectrometers were operated in
209 electron-impact (EI) mode, the ionization energy was set at 70 eV and the scan range
210 was from 50 to 550 aum.

211 **2.6 Proxy calculation**

212 The RAN₁₅ and RIAN in the HS4 stalagmite samples were calculated using the
213 relative abundances of the 3-OH-FAs with carbon numbers from C₁₀ to C₁₈, which are

214 derived from Gram-negative bacteria. Standard deviations for the RAN₁₅-MAAT and
215 RIAN were calculated by a duplicate extraction and analyses on 9 randomly selected
216 samples.

217

218 **3. Results and Discussion**

219 **3.1 Distribution and source of 3-OH-FAs**

220 Below we discuss the distributional characteristics of 3-OH-FAs in Heshang
221 cave sediments and overlying soils and, with consideration of recent bacterial
222 monitoring of the cave environment and drip waters (Liu et al., 2010; Yun et al.,
223 2016b), constrain their possible sources and pathways.

224 The average distributions of 3-OH-FAs in the overlying soils, cave surface
225 sediments and the HS4 stalagmite samples are illustrated in [Figure 2](#). There is an
226 overall similarity in the distribution patterns of the three sample sets (with some
227 differences discussed below), with the C₁₀, C₁₂, C₁₄, C₁₆ and C₁₈ homologues being
228 typically most abundant. The carbon number of the detectable 3-OH-FA homologues
229 varies from C₈ to C₃₀ ([Fig. 2](#)), however only the overlying soil samples contain the
230 lowest carbon numbers of the 3-OH-FAs (C₈, *i*-C₉, *a*-C₉, C₉) ([Fig. 2A](#)). The
231 distribution of 3-OH-FAs in the HS-4 stalagmite reported here agrees with previous
232 studies of the HS4 stalagmite from Heshang cave ([Huang et al., 2008](#); [Wang et al.,](#)
233 [2012](#)). The only other reports of 3-OH-FAs in speleothem samples comes from an
234 Ethiopian stalagmite ([Blyth et al., 2006](#)) and a British stalagmite ([Blyth et al., 2011](#)).
235 The molecular distributions reported by Blyth et al. ([2006](#)) and ([2011](#)) are similar

236 (maxima at C₁₂, C₁₄, C₁₆ etc.) to the HS4 distributions, but the higher molecular
237 weight 3-OH-FAs (>C₂₀) we detected were not previously reported.

238 Normal and branched 3-OH-FAs homologues of C₁₀ to C₁₈ chain length are
239 abundant constituents of Lipid A, a constituent of Lipopolysaccharide (LPS), the main
240 component of the outer membrane of Gram-negative bacteria (Lee et al., 2004;
241 Szponar et al., 2003). 3-OH-FAs have been used to quantify and characterize the
242 Gram-negative bacterial community in atmospheric aerosols (Lee et al., 2004), marine
243 dissolved organic matter (DOM) (Wakeham et al., 2003) and snow samples (Tyagi et
244 al., 2016; Tyagi et al., 2015), and have recently been utilized to define a number of
245 novel terrestrial paleoclimate proxies (Wang et al., 2016). The fractional abundance of
246 the individual 3-OH-FA homologues varies from 0 to 30% in the studied samples. The
247 3-OH-FAs generally show a strong even/odd predominance (Fig. 2). In the HS4
248 stalagmite, this general even/ odd predominance is accentuated for the homologues in
249 the range from C₁₁ to C₁₅, but is reversed for the *i*-C₁₇ 3-OH-FA which is notably
250 higher than the *n*-C₁₆ 3-OH-FAs (Fig. 2C). Furthermore, the *n*-C₁₂ 3-OH-FAs account
251 for ca.18% in the stalagmite samples, but only ca.11% in both the overlying soils and
252 cave surface sediments. The higher proportion of *n*-C₁₂ and *i*-C₁₇ homologues in the
253 stalagmite may derive from *in situ* bacterial production or possibly better preservation
254 of these compounds in stalagmites.

255 Culturable bacteria in drip waters from Heshang Cave are dominated by
256 Gram-negative heterotrophs, derived from Proteobacteria with the dominance of
257 Gamma proteobacteria (Liu et al., 2010). A two-year drip water monitoring

258 experiment in Heshang Cave likely demonstrates a pathway for transporting
259 3-OH-FAs from the overlying soil microbial community to cave sediments and
260 speleothem surfaces (Li et al., 2011). Meanwhile, a recent molecular survey of
261 bacterial communities in Heshang cave drip waters, over the period of 2008 to 2013,
262 confirms a diverse Gram-negative bacterial community and reveals a seasonal control
263 on Proteobacteria, whereby Beta-proteobacteria are supplied in the summer and
264 Gamma-proteobacteria in the winter (Yun et al., 2016b). A latest investigation shows
265 that Proteobacteria are both abundant in Heshang cave overlying soils and drip waters
266 (Yun et al., 2016a). This demonstrates that the seasonal signal of changes in the
267 Gram-negative bacterial community is transmitted readily through the Heshang cave
268 system to drip waters and to the cave and speleothems. This seasonal cycle is positive
269 from a paleoclimate perspective, suggesting minimal attenuation of bacterial based
270 climate signals transmitted from the overlying soils to the HS4 stalagmite, at least
271 sufficient for centennial to millennial scale paleoclimate studies.

272 As noted above, a distinctive feature of the 3-OH-FA distribution in the HS4
273 samples, is the greater relative abundance of the *n*-C₁₂ and *i*-C₁₇ 3-OH-FAs compared
274 to both overlying soils and cave sediments (Fig. 2). This may suggest an additional
275 contribution of 3-OH-FAs, derived from microbes from the cave drip water, which are
276 sequestered and trapped in the calcite matrix (Supplementary Information). We note
277 that Paction et al. (2013) report that microbial activity can initiate calcite deposition in
278 the aphotic zone of caves before inorganic precipitation of carbonates. We also note
279 the low abundances of long chain 3-OH-FAs (C₂₀-C₂₆), which might originate from

280 fungi, and/or Gram-positive actinomycetes (Keinänen et al., 2003). However, we
281 suggest that the broad similarity of 3-OH-FA distributions in the overlying soils and
282 stalagmites, supported by the site-specific analyses of bacterial diversity and transport
283 pathways (Liu et al., 2010; Yun et al., 2016b), supports a major contribution of
284 3-OH-FAs from Gram-negative bacteria dwelling in the overlying soils to the HS4
285 stalagmite samples. This is consistent with previous findings that lipids preserved in
286 speleothems are principally derived from the overlying soil ecosystem and vegetation,
287 having been transported from the surface by percolating groundwater, although a
288 proportion may be derived from the cave ecosystem (Blyth et al., 2014; Xie et al.,
289 2005; Xie et al., 2003; Yang et al., 2011).

290

291 **3.2 Holocene hydrological reconstruction**

292 Recent work has demonstrated that pH is a key environmental parameter in
293 controlling soil bacterial community structure and diversity (Bååth and Anderson,
294 2003; Griffiths et al., 2011; Lauber et al., 2009; Shen et al., 2013; Zhang et al., 2015).
295 Notably, Giotis et al. (2007) found that a strain of Gram-negative bacterium
296 increased/decreased the proportion of branched-chain fatty acids in higher pH/lower
297 pH conditions. Recently, a novel pH proxy RIAN which is based on Gram-negative
298 bacterial derived 3-OH-FAs in soils from Shennongjia Mountain was proposed by
299 Wang et al. (2016), with a low RIAN value when pH is high. Based on our finding
300 that 3-OH-FAs in the HS4 stalagmite are mainly derived from the overlying soils,
301 here we interpret the HS4 RIAN record as reflecting local changes of pH (Fig. 3A).

302 We note that the HS4 RIAN record is, for the period of mutual overlap, consistent
303 with hydrological records based on Hopanoids from the Dajiuhu peatland (Xie et al.,
304 2013) (Fig. 3B; Supplementary Information), situated 130 km to the NW of Heshang
305 Cave and the proportion of soil-derived magnetic minerals ($IRM_{\text{soft-flux}}$) incorporated
306 into the HS4 stalagmite, which Zhu et al. (2017) interpret as a proxy for rainfall
307 amount and intensity, with sensitivity to extreme precipitation events (Fig. 3C). These
308 consistent lines of evidence demonstrate RIAN could be used as qualitative
309 hydrological proxy in stalagmites. We argue below that, for the HS4 record, when
310 effective precipitation was higher in the past, the RIAN value is lower and likely
311 indicates leaching of soils and the influence of higher groundwater pH on the
312 3-OH-FA producing Gram-negative bacteria.

313 In soil environments pH reflects the balance between precipitation and
314 evaporation (e.g. hydrologically effective precipitation) and water movement through
315 the soil. Rainwater is naturally acidic due to the reaction with CO_2 in the atmosphere
316 to form carbonic acid. Excess rainfall leaches base cations increasing the relative
317 percentage of H^+ and Al^{3+} ions (and thus acidity) in water. Soil temperatures, pH and
318 aeration conditions also affect soil microbial activity and diversity which determines
319 bacterial respiration of CO_2 (and the formation of carbonic acid), further influencing
320 the pH of soil water and the degree of leaching (Fairchild and Baker, 2012). Thus we
321 argue that in the soils above Heshang cave, during the Holocene, pH was lower with
322 higher effective precipitation rates (and vice versa). This is consistent with the
323 generally observed relationship between effective precipitation and pH in global soils

324 (Slessarev et al., 2016; Yang et al., 2014) and wider evidence of the substantial
325 influence of pH on bacterial communities at both local and continental scales (Lauber
326 et al., 2009; Rousk et al., 2010). However, on the contrary, changes of pH in
327 groundwater systems may display in an opposite trend to that of the overlying soils in
328 response to increased effective precipitation. In a well-drained karst landscape
329 increases in precipitation will also be associated with the increased movement of
330 material (including organic matter and biomarkers), in dissolved or colloidal form to
331 the groundwater system, which may ultimately percolate or flush into cave systems
332 (Blyth et al., 2008). The pH of the groundwater is greatly affected by soil-derived
333 colloids and fine sands flushed into the groundwater system (Fairchild and Baker,
334 2012). The soil processes outlined above influence the latter source of material and
335 thus contribute to the pH of the groundwater system. More importantly, increased
336 rainfall can result in a fall in the total cation content which will lead to an increase of
337 pH in the groundwater system (Fairchild and Baker, 2012). Thus increased effective
338 precipitation may lead to antiphased pH variations between the overlying soils and
339 underlying groundwater systems. Here we interpret Holocene changes in RIAN in the
340 HS4 stalagmite record as primarily reflecting local changes in precipitation regime
341 which control the pH of the soil and groundwater systems. Specifically, we suggest
342 that RIAN records increases in groundwater pH in response to increased rainfall,
343 whereby microbes either initially derived from the overlying soils or already present
344 in the groundwater system modified their membrane lipids to adapt to higher pH in
345 the groundwater system resulting from increased rainfall and soil leaching.

346 From the above, hydrological changes during the last 9 ka BP were qualitatively
347 reconstructed from the HS4 stalagmite using the RIAN proxy (Fig. 3), which we
348 interpret as reflecting local changes in pH of the groundwater, originally driven by
349 changes in precipitation. The RIAN record from HS4 reveals two (relatively long)
350 wetter periods in central China, between 8.8-5.9 ka BP and 3.0-0 ka BP and one
351 relative dryer period from 5.9-3.0 ka BP (Fig. 3A). Both the HS4 and the Dajiuhu
352 peatland records reconstruct a dry middle Holocene with decreased precipitation
353 centered on ca. 5.5, 4.8 and 3.5 ka BP in central China (Fig. 3B, C) and overlap for
354 two long wet periods between > 9 ka to 6 ka BP and 3 to 0.6 ka BP. These drying
355 events occur simultaneously with colder RAN₁₅-MAATs and heavier $\delta^{18}\text{O}$ values in
356 HS4 (Fig. 4) and coeval cold/dry events recognized in a number of global NH and
357 regional paleoclimate records (Liu et al., 2013; Ljungqvist, 2010; Mayewski et al.,
358 2004; Owen et al., 2016; Rohling and Palike, 2005). These proxy-inferred
359 hydrological reconstructions re-enforce the conclusion of Xie et al. (2013) that the
360 overturn of distinctive cultures in the Neolithic Period to Iron Age in central China (as
361 observed in the distributions of >1600 prehistoric settlement sites) correlates with wet
362 or flood episodes.

363

364 **3.3 Holocene temperature reconstruction**

365 Temperature changes during the last 9 ka BP were reconstructed from the HS4
366 stalagmite using the RAN₁₅ index (Wang et al., 2016). RAN₁₅ is a novel temperature
367 proxy based on 3-OH-FA distributions measured in the soils from an altitudinal

368 transect on Shennongjia Mountain located 120 km to the NW of Heshang Cave (Wang
369 et al., 2016). Higher/lower RAN₁₅ values (higher/lower ratio of the *anteiso* to *normal*
370 C₁₅ 3-OH-FAs) are obtained in soils with cooler/warmer MAATs. The quantitative
371 correlation between MAAT and RAN₁₅ is expressed in the following equation (Wang
372 et al., 2016):

$$373 \text{ MAAT} = 23.03 - 3.03 \times \text{RAN}_{15} \quad (R^2 = 0.51, p < 0.001, \text{RMSE} = 2.6^\circ\text{C}) \quad (3)$$

374 RAN₁₅ in the HS4 stalagmite varies from 0.79 to 2.14 during the last 9 ka BP,
375 with the lowest value at ca. 7.4 ka BP and highest value at ca. 0.5 ka BP (Fig. 4A). By
376 applying equation (3) to the HS4 samples, we obtain RAN₁₅-MAAT reconstructions
377 over the last 9 ka BP (Fig. 4A). The average RAN₁₅-MAAT of 18.4°C over the most
378 recent part of the record (<0.8 ka BP) overlaps with the range of MAATs, ca. 16.2 to
379 18.7 (av. 17.5°C) measured since 1952 at the nearest meteorological station (Yichang,
380 located ca. 100 km away) and is very close to the av. MAAT of 18°C measured
381 directly outside the cave by a temperature logger between 2004 and 2007 (Hu et al.,
382 2008a). This agreement between reconstructed temperatures and instrumental
383 measurements increases our confidence in the potential of the RAN₁₅ proxy.
384 RAN₁₅-MAATs in HS4 vary from 16.5 to 20.6°C (av. 19°C), during the last 9 ka BP,
385 and broadly follow a long-term trend of declining temperatures in line with declining
386 solar insolation at 30°N in July (Laskar et al., 2004) (Fig. 4B). The temperature
387 variation (4.1°C) in our record is larger than the calibration error of the RAN₁₅ proxy
388 (RMSE = 2.6°C; Wang et al., 2016). The Holocene Climate Optimum (HCO) shown
389 in the RAN₁₅-MAAT record is from 8 to 6 ka BP, with the highest temperature at ca.

390 7.0 ka BP (Fig. 4A). Superimposed on the orbital-scale Holocene trend are centennial
391 to millennial scale climate fluctuations of ca. 1 to 2°C (Fig. 4A). Interestingly, the
392 most recent 0.9 ka BP is distinguished by greater variability with the highest (20.5°C)
393 and lowest (16.5°C) RAN₁₅-MAATs occurring consecutively at 0.6 ka BP and 0.5 ka
394 BP.

395 Our reconstructed RAN₁₅-MAAT follows a similar trend to the $\delta^{18}\text{O}$ record (Hu
396 et al., 2008b) from the HS4 stalagmite (Fig. 4C). The high resolution HS4 $\delta^{18}\text{O}$
397 record encodes a mixture of temperature and hydrological signals and clearly defines
398 a series of centennial scale episodes of heavier $\delta^{18}\text{O}$ (dry/cool events) superimposed
399 on the longer term Holocene trend (Hu et al., 2008b). Although the novel biomarker
400 based proxy has a relatively low resolution it's worth noting that a number of cooler
401 episodes observed in the HS4 RAN₁₅-MAAT record, centered on ca. 8.2 ka, 3.4 ka
402 and 0.5 ka BP (little ice age, LIA) occur simultaneously with heavier values in the
403 high resolution $\delta^{18}\text{O}$ record in HS4. Coeval cooling events are broadly recognized in a
404 number of global NH and regional (monsoonal) paleoclimate records (Ljungqvist,
405 2010; Mayewski et al., 2004; Rohling and Palike, 2005). Notably our RAN₁₅-MAAT
406 record is consistent with globally distal $\delta^{18}\text{O}$ ice-core record from Greenland (Johnsen
407 et al., 2001) (Fig. 4D) and the Northern Hemisphere Holocene stacked temperature
408 anomalies record (30° to 90°N) (Marcott et al., 2013) (Fig. 4E). Although our
409 sampling resolution is necessarily low due to biomarker sampling requirements (at
410 this stage of analytical development), the HS4 age model is well constrained and we
411 note that these RAN₁₅-MAAT maxima and minima at 0.6 ka BP and 0.5 ka BP

412 coincide with Northern Hemisphere (NH) scale warm and cold episodes during the
413 late medieval warm periods (MWP) and LIA respectively (Ljungqvist, 2010; Mann et
414 al., 2008; Moberg et al., 2005).

415

416 **4. Conclusion**

417 Hydrological and temperature changes in the middle reaches of the Yangtze
418 River during the last 9 ka BP were reconstructed using Gram-negative membrane
419 lipids extracted from the HS4 stalagmite from Heshang Cave, central China. RAN₁₅ is
420 a temperature proxy while RIAN is interpreted as qualitative hydrological proxy.
421 Temperatures varied from 16.5 to 20.6°C during the last 9 ka BP, with a relatively
422 warm period in the early to middle Holocene (8.0-6.0 ka BP), and then a relative cool
423 period in the late Holocene. The hydrological record shows two relatively long wet
424 periods and one relative dry period in central China, 8.8-5.9 ka BP, 3.0-0 ka BP and
425 5.9-3.0 ka BP respectively. The HS4 Holocene Climatic Optimum (HCO) between
426 8.0-6.0 ka BP is warmer and wetter than any other period in the Holocene and
427 supports a conclusion of the seminal review of Monsoon palaeoclimate by An et al.
428 (2000) that an early Holocene Optimum in Monsoon strength occurred in the middle
429 and lower reaches of the Yangtze River, China, centered on ca. 6 ka BP. Moreover,
430 this agrees with an ensemble of 18 different model simulations (Joussaume et al.,
431 1999) for 6 ka, which all indicate enhanced low level convergence into the monsoon
432 low over Eurasia, with the summer monsoon flow extending further inland. The
433 present study demonstrates both the first paleoclimate application of 3-OH-FA based

434 proxies and how such biomarker tools can record independent hydrological and
435 temperature signals in speleothems.

436

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449

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648

649 **FIGURE CAPTIONS**

650 **Figure 1** The location of Heshang Cave and sample sites. (A) Schematic map
651 showing the main regional surface drainage, revised after Hu et al. (2008a). The red
652 star shows the location of Heshang Cave. (B) The view of Heshang Cave entrance
653 from the opposite site of the Qing River. (C) Sampling locations of HS4 stalagmite
654 and cave surface sediments. Black solid triangle denotes the location of HS4 which
655 was collected in 2001 (Hu et al., 2008a). Black solid circles denote the sampling sites
656 of stream surface sediments; hollow squares denote the sampling sites of cave surface
657 sediment.

658

659 **Figure 2** Distribution and fractional abundance of hydroxy fatty acid homologues in
660 (A) cave overlying soils, (B) cave sediments, and (C) HS4 stalagmites.

661

662 **Figure 3** Comparison of the HS4 RIAN record with other local and regional
663 palaeo-hydrological records. A) Heshang Cave hydrological record inferred from the
664 RIAN record during the last 9 ka BP. U–Th dating errors (Hu et al., 2008b) are shown

665 on the top of the RIAN curve as red line segments. B) Hydrological record based on
666 hopanoids biomarkers from the Dajiuhu peatland (Xie et al., 2013), raw data are
667 shown as triangle symbols, the black line is a locally weighted scatter plot smoothing
668 with a quadratic polynomial (lowess) using a span of 5%. C) $IRM_{\text{soft-flux}}$ in stalagmite
669 HS4. Peaks in $IRM_{\text{soft-flux}}$ indicate intervals with increased precipitation events (Zhu et
670 al., 2017).

671

672 **Figure 4** Comparison of the HS4 stalagmite RAN_{15} -MAAT record with other
673 time-series and proxy records. A) RAN_{15} and RAN_{15} -MAAT record reconstructed
674 from the HS4 stalagmite. The standard deviation of the RAN_{15} -MAAT record is \pm
675 0.1°C . The green star represents the mean annual air temperature (MAAT)
676 immediately outside the cave, as by a temperature logger between 2004 and 2007 (Hu
677 et al., 2008a). U–Th dating errors (Hu et al., 2008b) are shown on the bottom of the
678 RAN_{15} -MAAT curve as red line segments. B) Solar insolation changes at 30°N in July
679 during the last 9 ka BP (Laskar et al., 2004). C) The CaCO_3 oxygen isotope record
680 from the HS4 stalagmite (Hu et al., 2008b). D) Ice core $\delta^{18}\text{O}$ record from North GRIP
681 (Johnsen et al., 2001). E) The Northern Hemisphere stacked temperature anomalies
682 (30° to 90°N) for the $5^{\circ} \times 5^{\circ}$ area-weighted mean calculation with its 1σ
683 uncertainty (Marcott et al., 2013).

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