

1 **Temperature exerts no influence on organic matter $\delta^{13}\text{C}$ of**
2 **surface soil along the 400-mm isopleth of mean annual**
3 **precipitation in China**

4

5 Yufu Jia, Guoan Wang, Qiqi Tan, and Zixun Chen

6 ¹College of Resources and Environmental Sciences, China Agricultural University,
7 Beijing 100193, China

8

9

10

11

12

13

14 Author for correspondence:

15 Guoan Wang

16 Tel: +086-10-62733942

17 Email: gawang@cau.edu.cn

18

19

20

21

22

23 **Abstract**

24 Soil organic carbon is the largest pool of carbon in the terrestrial ecosystem, and its
25 isotopic composition is affected by a number of factors. However, the influence of
26 environmental factors, especially temperature, on soil organic carbon isotope values
27 ($\delta^{13}\text{C}_{\text{SOM}}$) is poorly constrained. This impedes the application of the variability of
28 organic carbon isotopes to reconstructions of paleoclimate, paleoecology, and global
29 carbon cycling. Given the considerable temperature gradient along the 400-mm
30 isohyet (isopleth of mean annual precipitation – MAP) in China, this isohyet provides
31 ideal experimental sites for studying the influence of temperature on soil organic
32 carbon isotopes. In this study, the effect of temperature on surface soil $\delta^{13}\text{C}$ was
33 assessed by a comprehensive investigation of 27 sites across a temperature gradient
34 along the isohyet. Results demonstrate that temperature does not play a role in soil
35 $\delta^{13}\text{C}$. This suggests that organic carbon isotopes in sediments cannot be used for
36 paleotemperature reconstruction and that the effect of temperature on organic carbon
37 isotopes can be neglected in the reconstruction of paleoclimate and paleovegetation.
38 Multiple regressions with MAT (mean annual temperature), MAP, altitude, latitude,
39 and longitude as independent variables and $\delta^{13}\text{C}_{\text{SOM}}$ as the dependent variable show
40 that these five environmental factors together account for only 9% of soil $\delta^{13}\text{C}$
41 variance. However, one-way ANOVA analyses suggest that soil type and vegetation
42 type are significant factors influencing soil $\delta^{13}\text{C}$. Multiple regressions, in which the
43 five aforementioned environmental factors were taken as quantitative variables and
44 vegetation type, soil type based on the Chinese Soil Taxonomy, and World Reference
45 Base (WRB) soil type were separately used as dummy variables, show that 36.2%,
46 37.4%, and 29.7%, respectively, of the variability in soil $\delta^{13}\text{C}$ are explained.
47 Compared to the multiple regressions in which only quantitative environmental
48 variables were introduced, the multiple regressions in which soil and vegetation were
49 also introduced explain more of the isotopic variance, suggesting that soil type and
50 vegetation type exert significant influence on $\delta^{13}\text{C}_{\text{SOM}}$.

52

53 **1. Introduction**

54 While global climate change has received a great deal of attention in recent years,
55 effective predictions of future climate change depend on relevant information about
56 climate in the geological past. Over recent decades, stable carbon isotopes in
57 sediments such as loess, paleosol, as well as in lacustrine and marine sediments have
58 been widely used to reconstruct paleovegetation and paleoenvironments, and have
59 provided important insights into patterns of past climate and environmental changes.
60 For examples, numerous researchers have used organic carbon isotopes of loess to
61 reconstruct paleovegetation and paleoprecipitation. Vidic and Montañez (2004)
62 conducted a reconstruction of paleovegetation of the central Chinese Loess Plateau
63 during the Last Glaciation (LG) and Holocene using organic carbon isotopes in loess
64 from Jaodao, Shanxi Province. Hatté and Guiot (2005) carried out a
65 paleoprecipitation reconstruction by inverse modeling using the organic carbon
66 isotopic signal of the Nußloch loess sequence (Rhine Valley, Germany). Rao et al.
67 (2013) reconstructed high-resolution summer precipitation variations on the western
68 Chinese Loess Plateau during the LG using a well-dated organic carbon isotopic
69 dataset. Yang et al. (2015) reconstructed a minimum 300-km northwestward migration
70 of the monsoon rain belt from the Last Glacial Maximum to the Mid-Holocene using
71 organic carbon isotope data from 21 loess sections across the Loess Plateau. However,
72 to our knowledge, there are no paleotemperature reconstructions using organic carbon
73 isotope records of loess and paleosol because it has been argued that temperature

74 exerts only a slight, or even no influence on soil organic carbon isotope values
75 ($\delta^{13}\text{C}_{\text{SOM}}$). While this may be likely, it needs to be investigated because few studies
76 have addressed the influence of temperature on organic carbon isotopes of modern
77 surface soil. Lee et al. (2005) and Feng et al. (2008) both reported no relationship
78 between temperature and surface soil $\delta^{13}\text{C}$ in central-east Asia. However, Lu et al.
79 (2004) discovered a nonlinear relationship between mean annual temperature (MAT)
80 and $\delta^{13}\text{C}_{\text{SOM}}$ for the Qinghai–Tibetan Plateau. Sage et al. (1999) compiled the data
81 from Bird and Pousai (1997) and also found a nonlinear trend for the variation in
82 $\delta^{13}\text{C}_{\text{SOM}}$ along a temperature gradient in Australian grasslands and savannas.

83 Plant residues are the most important source of soil organic matter. Values for
84 $\delta^{13}\text{C}_{\text{SOM}}$ are generally close to plant $\delta^{13}\text{C}$ values, despite isotopic fractionation during
85 decomposition of organic matter (Nadelhoffer and Fry, 1988; Balesdent et al., 1993;
86 Ågren et al., 1996; Fernandez et al., 2003; Wynn, 2007). Thus, the factors influencing
87 plant $\delta^{13}\text{C}$ might also influence $\delta^{13}\text{C}_{\text{SOM}}$. Plant $\delta^{13}\text{C}$ values, especially those of C_3
88 plants, are tightly associated with precipitation, suggesting that precipitation may also
89 affect soil $\delta^{13}\text{C}$ (Diefendorf et al., 2010; Kohn, 2010). In addition to the effect of
90 precipitation, numerous other factors such as temperature, air pressure, atmospheric
91 CO_2 concentration, altitude, latitude, and longitude may also influence $\delta^{13}\text{C}$ in plants
92 (Körner et al., 1991; Hultine and Marshall, 2000; Zhu et al., 2010; Xu et al., 2015).
93 Although variation patterns of plant $\delta^{13}\text{C}$ with respect to temperature are so far
94 unresolved (e.g., Schleser et al., 1999; McCarroll and Loader, 2004; Treydte et al.,
95 2007; Wang et al., 2013), it is widely accepted that temperature has a slight effect on

96 plant $\delta^{13}\text{C}$. Therefore, if the ^{13}C enrichment during soil organic matter decomposition
97 is a constant value, we expect only a slight or no influence of temperature on soil $\delta^{13}\text{C}$.
98 However, ^{13}C -enrichment is affected by environmental and biotic factors (Wang et al.,
99 2015). Thus, it is difficult to determine whether or how temperature affects soil $\delta^{13}\text{C}$,
100 and there should be specific investigations focusing on this issue. Although the
101 relationship between temperature and $\delta^{13}\text{C}_{\text{SOM}}$ has been investigated in the studies
102 mentioned above, these studies were unable to effectively separate the influence of
103 temperature from the effect of precipitation. In addition, there are no meteorological
104 stations near most of the sampling sites in the aforementioned studies, suggesting that
105 meteorological data had to be interpolated, which can lead to unrealistic precipitation
106 data in regions with strong topographical variability. This interpolation could have
107 introduced errors in the relationships between temperature and $\delta^{13}\text{C}_{\text{SOM}}$ that were
108 established in these studies.

109 The present study includes a detailed investigation of the variation in $\delta^{13}\text{C}_{\text{SOM}}$ with
110 respect to temperature across a temperature gradient along the 400-mm isohyet
111 (isopleth of mean annual precipitation; MAP) in China. We sampled surface soil
112 along a specific isohyet to minimize the effect of precipitation changes on $\delta^{13}\text{C}_{\text{SOM}}$.

113 In addition, we collected samples only at sites with meteorological stations. Thus,
114 the climatic data we obtained from these stations are likely more reliable than
115 interpolated values.

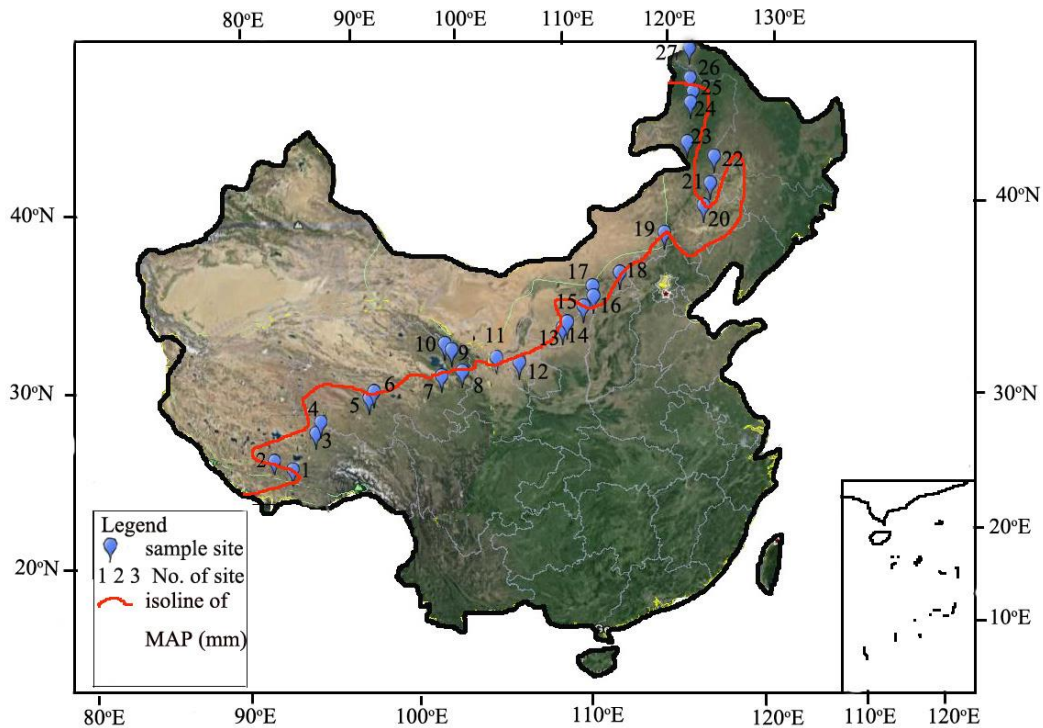
116

117 **2. Materials and methods**

118 2.1. Study site

119 In this study, we set up a transect along the 400 mm isohyet from Langkazi (site 1,
120 29°3.309'N, 90°23.469'E) on the Qinghai–Tibetan Plateau in southwest China to
121 Bejjicun (Site27, 53°17.458'N, 122°8.752'E) in Heilongjiang Province, northeast
122 China (Fig. 1, Table 1). The straight-line distance between the two sites is about 6000
123 km. Twenty-seven (27) sampling sites were set along the transect. Among these
124 sampling sites, 10 sites are located on the Qinghai–Tibetan Plateau and the remaining
125 sites are in north China. Bejjicun and Kuduer have the lowest MAT of -5.5 °C, while
126 Shenmu has the highest MAT of +8.9 °C. The average MAP of these sites is 402 mm.
127 In north China, rainfall from June to September accounts for approximately 80% of
128 the total annual precipitation, and the dominant control over the amount of
129 precipitation is the strength of the East Asian monsoon system. On the Qinghai–
130 Tibetan Plateau, however, precipitation is associated with both the Southwest
131 monsoon and the Qinghai–Tibetan Plateau monsoon; approximately 80–90% of
132 rainfall occurs in the summer season (from May to October).

133



134

135 Fig.1. Sketch of sampled region. Sample sites are indicated with numbers. 1, LangKaZi; 2,RiKaZe;
 136 3,NaQu; 4,NieRong; 5,ZhiDuo; 6,QuMaLai; 7,TongDe; 8,TongRen; 9,HuangYuan; 10,HaiYan;
 137 11,YuZhong; 12,XiJi; 13,JingBian; 14,HengShan; 15,ShenMu; 16,HeQu; 17,ZhunGeErQi;
 138 18,FengZhen; 19,DuoLun; 20,LinXi; 21,ZhaLuTeQi; 22,WuLanHaoTe; 23,AErShan; 24,YaKeShi;
 139 25,KuDuoEr; 26,GenHe; 27,BeiJiCun. Detailed information of sites is shown in Table 1.

140

141 2.2 Soil sampling

142 Soil samples were collected in the summer of 2013 between 12 July and 30 August.

143 To avoid disturbance by human activities, sample sites were chosen 5–7 km from the

144 towns where the meteorological stations are located. We set three squares (0.5×0.5

145 m) within a 200-m^2 area to collect surface mineral soil (0–5 cm) using a ring knife.

146 The O-horizon, including litters, moders, and mors, was removed before collecting

147 mineral soils. About 10 g of air-dried soil was sieved using a 2-mm mesh. Plant

148 fragments and the soil fraction coarser than 2 mm were removed. The remainder of

149 the sieved sample was immersed in HCl (1 mol L^{-1}) for 24 hours. To ensure that all

150 carbonate was removed, the samples were stirred four times during the immersion.

151 Then, the samples were washed to neutrality using distilled water, oven-dried at 50 °C,
152 and ground. Carbon isotope ratios were determined using a Delta^{Plus} XP mass
153 spectrometer (Thermo Scientific, Bremen, Germany) coupled with an elemental
154 analyzer (FlashEA 1112; CE Instruments, Wigan, UK) in continuous flow mode. The
155 elemental analyzer combustion temperature was 1020 °C.

156 Carbon isotopic ratios are reported in delta notation relative to the V-PDB standard
157 using the following equation:

$$158 \quad \delta^{13}\text{C} = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000 \quad (1)$$

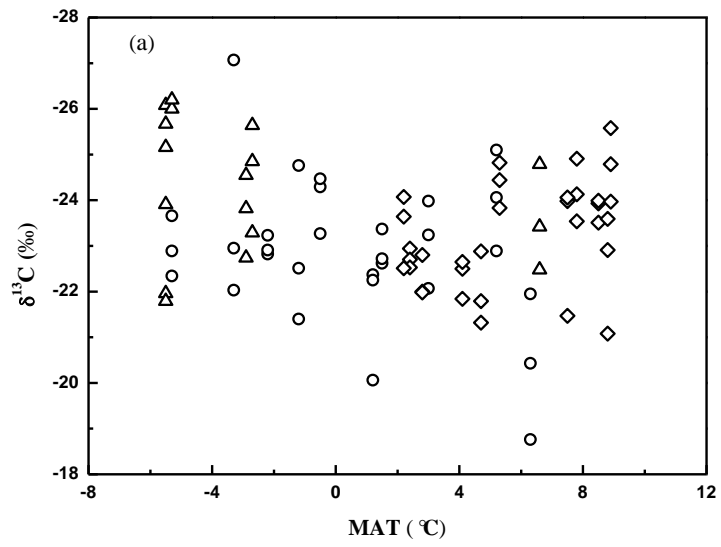
159 where $\delta^{13}\text{C}$ is the carbon isotope ratio of the sample (‰) and R_{sample} and $\text{R}_{\text{standard}}$ are
160 the $^{13}\text{C}/^{12}\text{C}$ ratios of the sample and the standard, respectively. We obtained a standard
161 deviation of less than 0.15‰ among replicate measurements of the same soil sample.

162

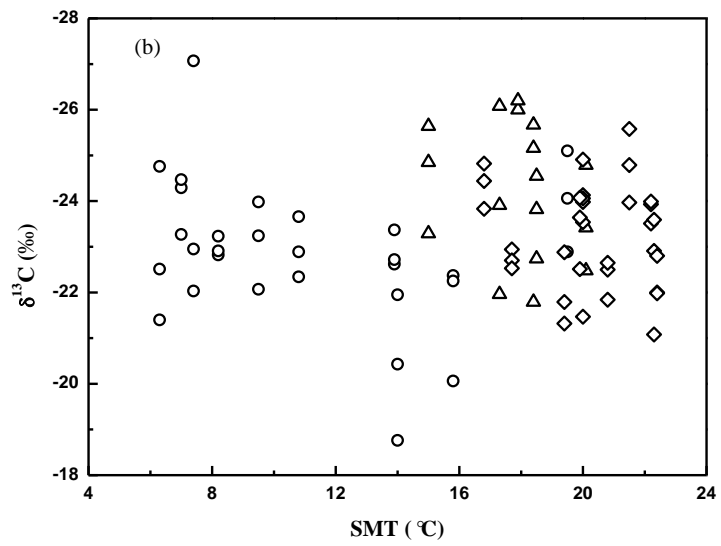
163 **3. Results**

164 Except for one $\delta^{13}\text{C}_{\text{SOM}}$ value (-18.8‰), all other data vary between -20.4‰ and -27.1‰
165 with a mean value of -23.3‰ (n = 80, s.d. = 1.45). Multiple regressions with MAT,
166 MAP, altitude, latitude, and longitude as independent variables and $\delta^{13}\text{C}_{\text{SOM}}$ as the
167 dependent variable show that only 9% of the variability in soil $\delta^{13}\text{C}$ can be explained
168 by a linear combination of all five environmental factors (p = 0.205; Table 2).
169 Considering the possibility of correlations among the five explanatory variables,
170 stepwise regression was used to eliminate the potential influence of collinearity
171 among them. Variables with *P*-values < 0.05 were incorporated into the model and
172 variables with *P*-values > 0.1 were excluded. Statistical analysis shows that only

173 latitude is included in the stepwise regression model ($R^2 = 0.077$, $p = 0.012$). In order
174 to better constrain the relationship between soil $\delta^{13}\text{C}$ and each environmental factor,
175 bivariate correlation analyses of soil $\delta^{13}\text{C}$ against some of the environmental factors
176 were conducted. The bivariate correlation analyses show that $\delta^{13}\text{C}_{\text{SOM}}$ is not related to
177 MAT ($p = 0.114$) or SMT ($p = 0.697$) along the isohyet (Fig. 2a, b). In addition, in
178 order to further determine the response of $\delta^{13}\text{C}_{\text{SOM}}$ to temperature, we considered
179 three subsets of our soil samples defined according to the climate, topography, or
180 vegetation type of the Qinghai–Tibetan Plateau (mainly alpine meadow, 10 sites),
181 steppe or grassland (11 sites), and coniferous forest (six sites; Table 1). Bivariate
182 correlation analyses within these subsets also show no relationship between $\delta^{13}\text{C}_{\text{SOM}}$
183 and MAT for all categories. The correlation analysis of $\delta^{13}\text{C}_{\text{SOM}}$ with respect to
184 altitude is shown in Fig. 3, which displays no relationship ($p = 0.132$). Although
185 longitude is not found to influence $\delta^{13}\text{C}_{\text{SOM}}$ in the above stepwise regression, bivariate
186 correlation analyses show that both latitude and longitude are negatively correlated
187 with $\delta^{13}\text{C}_{\text{SOM}}$ ($p = 0.012$ and 0.034 , respectively; Fig. 4a, b).



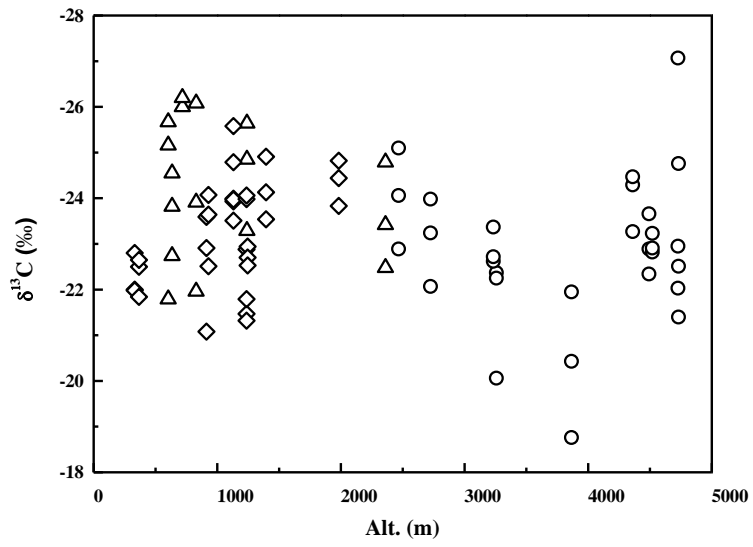
188



189

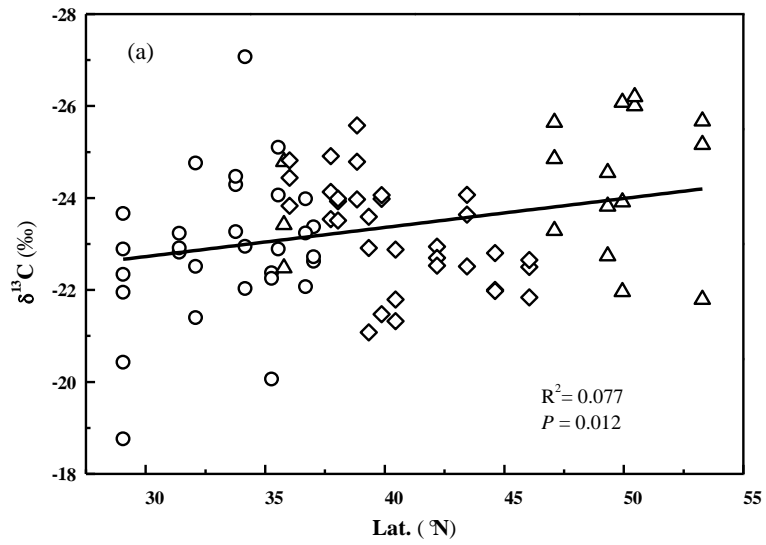
190 Fig.2 shows the variance in surface soil $\delta^{13}\text{C}$ with MAT (a) and SMT (b) along the 400 mm isoline
 191 in China. Circle represents alpine and subalpine; diamond indicates temperate steppe and
 192 grassland; triangle is coniferous forest.

193

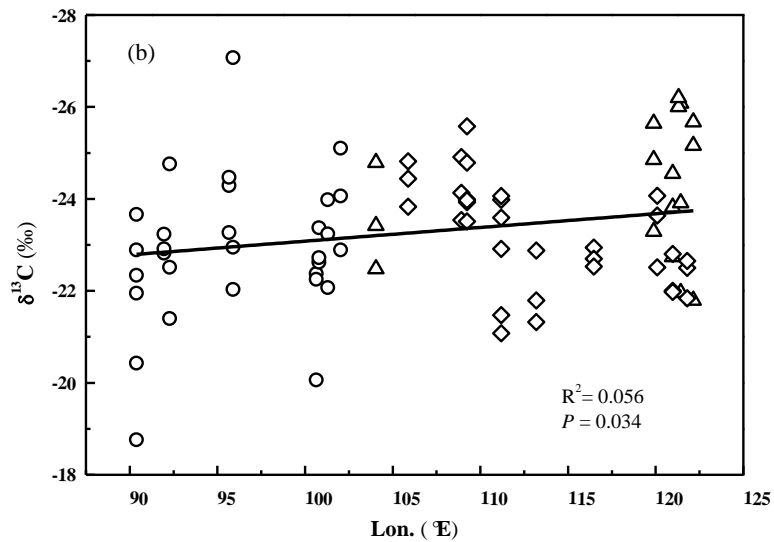


194
 195
 196

Fig.3 shows the variance in surface soil $\delta^{13}\text{C}$ with altitude.



197

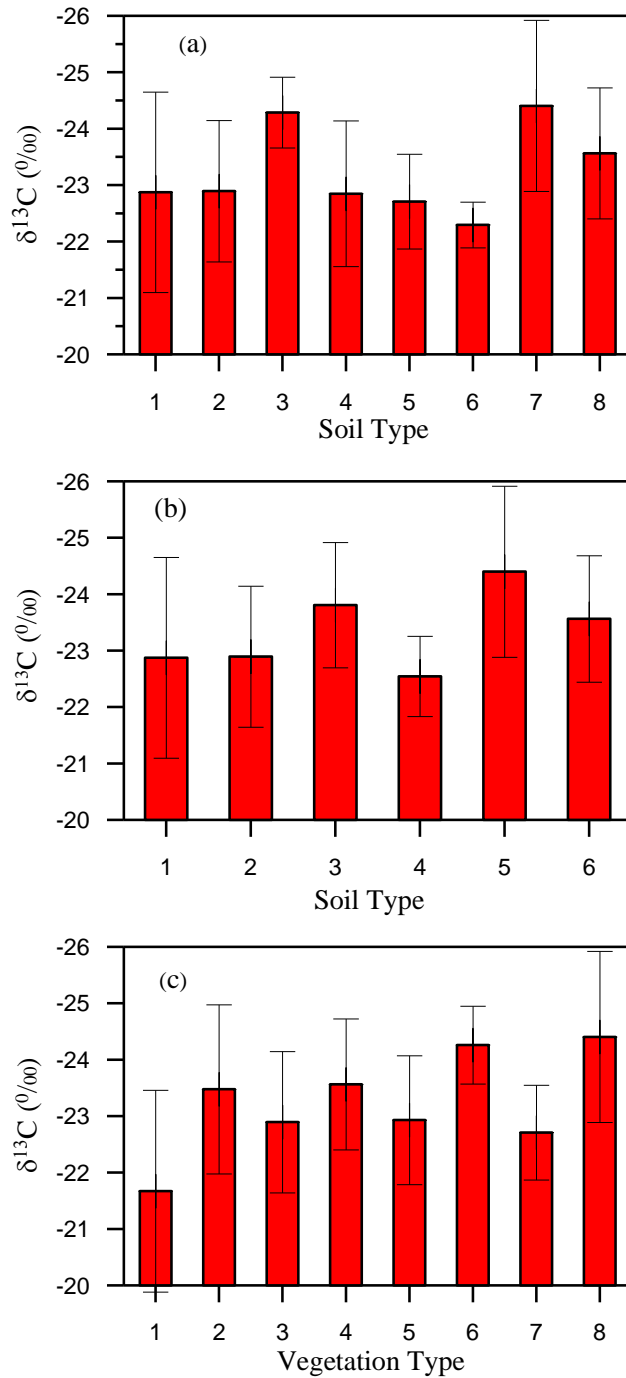


198

199 Fig.4 shows the relationships between the soil $\delta^{13}\text{C}$ and latitude (a) and longitude (b).

200

201 In addition to the effects of quantifiable environmental factors, qualitative factors
 202 such as soil type and vegetation type may influence $\delta^{13}\text{C}_{\text{SOM}}$. Various concepts have
 203 been introduced in soil taxonomy, leaving varied soil nomenclatures in use. In this
 204 study, we adopted the Chinese Soil Taxonomy and the World Reference Base (WRB)
 205 to describe the observed soils. The soil samples can be divided into eight or six types
 206 based on the Chinese Soil Taxonomy or WRB, respectively (Table 1). One-way
 207 ANOVA analyses suggest that both soil and vegetation type play a significant role for
 208 $\delta^{13}\text{C}_{\text{SOM}}$ ($p = 0.002$ for soil type based on the Chinese Soil Taxonomy, $p = 0.003$ for
 209 soil type based on WRB, and $p = 0.001$ for vegetation type; Fig. 5).



210

211 Fig.5 shows the effects of soil types and vegetation types on the soil $\delta^{13}\text{C}$. (a.) soil types based on
 212 Chinese soil taxonomy. 1. Matti-GelicCambosols; 2. Hapli- CryicAridosolsl;
 213 3.Calci-OrthicAridosols; 4.MottlicCalci-OrthicAridosols; 5.TypicCalci-UsticIsohumosols;
 214 6.Pachi-UsticIsohumosols; 7.Umbri-GelicCambosols; 8.Hapli-UsticArgosols. (b.) soil types based
 215 on WRB. 1.Cambisols; 2. Leptosols; 3.Calcisols; 4.Chernozems; 5.Umbrisols; 6.Luvisols. (c.)
 216 vegetation type. 1. Alpine grassland; 2. Alpine meadow; 3.Subalpine grassland; 4.Temperate
 217 coniferous and broad-leaved mixed forests; 5.Temperate meadow steppe; 6.Semi-desert grasslands;
 218 7.Temperate typical steppe; 8.Frigid temperate coniferous forest. The bar in Fig.5 indicates $\pm 1\text{SD}$.

219

220 To further constrain the effects of soil and vegetation type on $\delta^{13}\text{C}_{\text{SOM}}$, multiple
221 regressions with soil and vegetation type as dummy variables were conducted.
222 Considering the tight relationship between soil type and vegetation type, especially in
223 the Chinese Soil Taxonomy, soil variables and vegetation variables were separately
224 introduced into the statistical analyses. Multiple regression, in which the five
225 aforementioned explanatory environmental factors were taken as quantitative
226 variables and the eight soil types of the Chinese nomenclature as values of a dummy
227 variable, shows that environmental factors and soil types account for 37.4% of the soil
228 $\delta^{13}\text{C}$ variance ($p < 0.001$; Table 2). Using the six soil types based on WRB rather than
229 the Chinese nomenclature, 29.7% ($p = 0.003$) of the variability is explained (Table 2).
230 Similarly, multiple regression with vegetation types as dummy variables shows that
231 the five environmental factors and vegetation types together can explain 36.2% of the
232 variability in soil $\delta^{13}\text{C}$ ($p = 0.001$; Table 2). Compared to the multiple regressions in
233 which only quantitative environmental variables were introduced, the multiple
234 regressions in which soil and vegetation were also introduced explain more of the
235 variance, suggesting that soil type and vegetation type play a significant role in
236 $\delta^{13}\text{C}_{\text{SOM}}$ variability.

237

238 **4. Discussion**

239 Soil $\delta^{13}\text{C}$ depends on the $\delta^{13}\text{C}$ of plants and on carbon isotopic fractionation during
240 organic matter decomposition. $\delta^{13}\text{C}$ values of C_3 plants vary between -22‰ and -34‰
241 with a mean of -27‰, and C_4 plants range from -9‰ to -19‰ with a mean of -13‰

242 (Dienes, 1980). Carbon isotope fractionation occurs during the process of plant litter
243 decomposition to soil organic matter in most environments, especially in non-arid
244 environments, causing ^{13}C -enrichment in soil organic matter compared to the plant
245 sources (Nadelhoffer, 1988; Balesdent et al., 1993; Ågren et al., 1996; Fernandez et al.,
246 2003; Wynn et al., 2005; Wynn, 2007). A detailed investigation of isotope
247 fractionation during organic matter decomposition, which was conducted on Mount
248 Gongga, an area of the Qinghai–Tibetan Plateau dominated by C_3 vegetation with
249 herbs, shrubs, and trees, showed that the mean ^{13}C -enrichment in surface soil (0–5 cm
250 depth) relative to the vegetation was 2.87‰ (Chen et al., 2009). Another investigation
251 of 13 soil profiles from the Tibetan Plateau and north China showed that the $\delta^{13}\text{C}$
252 difference between surface soil and the original biomass varied from 0.6 to 3.5‰ with
253 a mean of 1.8‰ (Wang et al., 2008). Thus, the $\delta^{13}\text{C}_{\text{SOM}}$ dataset from this study
254 ($\delta^{13}\text{C}_{\text{SOM}}$ ranges from -20.4‰ to -27.1‰) indicates that the modern terrestrial
255 ecosystem along the isohyet is dominated by C_3 plants. This result is consistent with
256 the observations of vegetation along the isohyet completed in our previous study
257 (Wang et al., 2013). The relatively heavy soil $\delta^{13}\text{C}$ values (mean: -20.4‰) at Rikaze
258 (Site 2; Fig. 3 and Table 1) are surprising because only four species of C_3 plants grow
259 there, and C_4 species are absent. This observation suggests that very large carbon
260 isotope fractionation during SOM degradation has occurred in the local ecosystem.
261 Previous studies have observed a similar phenomenon, although the mechanism
262 responsible for the unusually large isotopic fractionation remains unclear. For
263 example, Wynn (2007) reported that isotopic fractionation enriched soil organic

264 carbon by up to $\sim 6\%$ with respect to the original biomass.

265 The MAT, MAP, altitude, latitude, and longitude combined are responsible for only
266 9% of the variability in soil $\delta^{13}\text{C}$ in the multiple regression model, suggesting that the
267 contribution of these five environmental factors to soil $\delta^{13}\text{C}$ variance is very small.
268 Our previous study conducted along the same isohyet indicated a strong positive
269 relationship between the $\delta^{13}\text{C}$ of plants and MAT, with a coefficient of $0.104\% \text{ } ^\circ\text{C}^{-1}$
270 (Wang et al., 2013). The difference between the maximum and minimum temperature
271 along the isohyet is $15 \text{ } ^\circ\text{C}$, so the greatest possible effect of temperature on plant $\delta^{13}\text{C}$
272 along the temperature gradient is 1.56% , which is not very substantial. Because the
273 main source of soil organic matter along the isohyet is C_3 plants, the induced variance
274 in soil $\delta^{13}\text{C}$ by plant $\delta^{13}\text{C}$ can also not be very high. On the other hand, although the
275 ^{13}C -enrichment during SOM degradation follows a Rayleigh distillation process
276 (Wynn, 2007), our recent study shows that temperature does not influence carbon
277 isotopic fractionation during decomposition of organic matter (Wang et al., 2015),
278 which also explains the lack of a relationship between soil $\delta^{13}\text{C}$ and temperature. Feng
279 et al. (2008) and Lee et al. (2005) reported no relationship between soil $\delta^{13}\text{C}$ and
280 MAT and SMT, respectively, which is consistent with our results. Their field
281 campaigns were conducted in central Asia, which is also dominated by C_3 plants,
282 similar to the area along the 400-mm isohyet. This is the reason why the same pattern
283 exists in both central Asia and in the area along the 400-mm isohyet.

284 Observations by Bird and Pousai (1997) and Sage et al. (1999) appear to be
285 inconsistent with our findings; the authors found a nonlinear relationship between soil

286 $\delta^{13}\text{C}$ and MAT in Australian grasslands. However, if they considered only soil with
287 pure C_3 plants (MAT is below 16°C), soil $\delta^{13}\text{C}$ and temperature were not related in
288 Australian grasslands, which agrees with our results. Below 15°C , the C_4 contribution
289 to productivity in Australian grasslands is negligible, whereas above 23°C , C_3
290 contribution is negligible. Between 14°C and 23°C , soil $\delta^{13}\text{C}$ is positively correlated
291 with MAT, indicating an increase in C_4 representation with increasing MAT (Sage et
292 al., 1999). Lu et al. (2004) also reported a nonlinear relationship between soil $\delta^{13}\text{C}$
293 and MAT. Similarly, if their soil data with C_4 plants are excluded from the nonlinear
294 correlation, soil $\delta^{13}\text{C}$ is also not related to MAT (see Fig. 5 b in Lu et al., 2004). Thus,
295 the present study and the previous observations are consistent in showing that in a
296 terrestrial ecosystem in which the vegetation is dominated by C_3 plants, temperature
297 does not influence soil $\delta^{13}\text{C}$ variance.

298 Because all soil samples were taken along the 400-mm isohyet, this study shows
299 that the contribution of precipitation to the variability in soil $\delta^{13}\text{C}$ is negligible.
300 Although stepwise regression and correlation analysis both show a significant
301 influence of latitude on soil $\delta^{13}\text{C}$ ($p = 0.012$; Fig. 4a), which was also described by
302 Bird and Pausai (1997) and Tieszen et al. (1979), the five environmental variables,
303 including latitude, are responsible for only 9% of the variability in soil $\delta^{13}\text{C}$ in a
304 multiple regression model (Table 2), suggesting that the contribution of latitude to soil
305 $\delta^{13}\text{C}$ is also limited. Latitude is a comprehensive environmental factor, and change in
306 latitude can bring about changes in other environmental factors, such as temperature,
307 irradiation, cloud amount, and moisture. Among those, temperature and irradiation

308 should be most strongly related to latitude. The observed relationship between latitude
309 and soil $\delta^{13}\text{C}$ suggests that environmental factors other than temperature might also
310 contribute to the variance in soil $\delta^{13}\text{C}$.

311 Control of soil $\delta^{13}\text{C}$ by vegetation type mainly reflects the effect of plant life forms
312 on plant $\delta^{13}\text{C}$, which in turn influences isotope fractionation during organic matter
313 decomposition. Communities in which life forms of dominant plants are similar are
314 generally treated as the same vegetation type. Plant $\delta^{13}\text{C}$ is closely related to plant
315 form (Diefendorf et al., 2010; Ehleringer and Cooper, 1988), which causes $\delta^{13}\text{C}$
316 differences among varying vegetation types, resulting in the observed effect of
317 vegetation type on soil $\delta^{13}\text{C}$.

318 Substrate quality partly quantifies how easily organic carbon is used by soil
319 microbes (Poage and Feng, 2004). It can be related to plant type and is often defined
320 using the C/N ratio, lignin content, cellulose content, and/or lignin content/N ratio
321 (Melillo et al., 1989; Gartern et al., 2000). Our study of Mount Gongga, China,
322 showed that litter quality plays a significant role in isotope fractionation during
323 organic matter decomposition, and that the carbon isotope fractionation factor α
324 increases with litter quality (Wang et al., 2015). Thus, the isotope fractionation factor
325 should differ among sites because litter quality is dependent on vegetation, which
326 causes changes in soil $\delta^{13}\text{C}$ with vegetation type.

327 The effect of soil type on soil $\delta^{13}\text{C}$ may be associated with the effect of soil type on
328 isotope fractionation during organic matter decomposition, which involves at least
329 two mechanisms (see Wang et al. [2008] for a detailed discussion). First, properties

330 and compositions of microbial decomposer communities are dependent on soil type
331 (Gelsomino et al., 1999). Different microbes can have different metabolic pathways,
332 even when they decompose the same organic compound (Macko and Estep, 1984),
333 and the extent of isotope fractionation during decomposition may be closely related to
334 the metabolic pathways of microbes (Macko and Estep, 1984). Second, physical and
335 chemical properties such as pH, particle size fraction, and water-holding capacity are
336 considerably different among soil types, which causes organic compounds to decay at
337 different rates in different soil environments. The magnitude of isotope fractionation
338 during decomposition is linked to the degree of organic matter decomposition (Feng,
339 2002). Thus, soil type plays a significant role in soil carbon isotopic fractionation.

340

341 **5. Conclusions**

342 The present study analyzed organic carbon isotopes in surface soil along a 400-mm
343 isohyet of mean annual precipitation in China. Our results indicate that both soil type
344 and vegetation type significantly influence soil organic carbon isotopes. However,
345 temperature is found to have no observable impact on $\delta^{13}\text{C}_{\text{SOM}}$, suggesting that $\delta^{13}\text{C}$
346 signals in sediments cannot be used for temperature reconstructions and that the effect
347 of temperature on $\delta^{13}\text{C}_{\text{SOM}}$ should be neglected in reconstructions of paleoclimate and
348 paleovegetation that use carbon isotopes of soil organic matter.

349

350

351 **Acknowledgments**

352 This research was supported by grants from the National Basic Research Program
353 (2014CB954202), the National Natural Science Foundation of China (No. 41272193),
354 and the China Scholarship Council (File No.201506355021). We would like to thank
355 Ma Yan for analyzing the stable carbon isotope ratios in the Isotope Lab at the
356 College of Resources and Environment, China Agricultural University. We would
357 also like to thank Professor Eric Posmentier in the department of Earth Sciences,
358 Dartmouth College, for his constructive suggestions.

359

360

361 **References**

362 Ågren, G.I., Bosatta, E., and Balesdent, J.: Isotope discrimination during
363 decomposition of organic matter: A theoretical analysis. *Soil Sci. Soc. Am. J.*, 60,
364 1121-1126, 1996.

365 Balesdent, J., Girardin, C., and Mariotti, A.: Site-related $\delta^{13}\text{C}$ of tree leaves and soil
366 organic matter in a temperate forest. *Ecology*, 74, 1713-1721, 1993.

367 Bird, M.I., and Pousai, P.: Variations of delta C-13 in the surface soil organic carbon
368 pool. *Global Biogeochemical Cycles*, 11, 313-322, 1997.

369 Chen, P.N., Wang, G.A., Han, J.M., Liu, X.J., and Liu, M.: $\delta^{13}\text{C}$ difference between
370 plants and soil organic matter along the eastern slope of Mount Gongga. *Chinese*
371 *Science Bulletin*, doi:10.1007/s11434-009-0405-y, 2009.

372 Deines, P. The isotopic composition of reduced organic carbon. *Handbook of*
373 *environmental isotope geochemistry I, The terrestrial environment* (eds. by P. Fritz &

374 J.C. Fontes), pp. 329–406. Elsevier, Amsterdam, 1980.

375 Diefendorf, A.F., Mueller, K.E., Wing, S.L., Koch, P.L., Freeman, K.H.: Global
376 patterns in leaf ^{13}C discrimination and implications for studies of past and future
377 climate. *Proceedings of the National Academy of the Sciences of the United States of*
378 *America*, 107, 5738–5743, 2010.

379 Ehleringer, J. R., and Cooper, T. A.: Correlations between carbon isotope ratio and
380 microhabitat in desert plants. *Oecologia*, 76, 62–66, 1988.

381 Feng, X.H.: A theoretical analysis of carbon isotope evolution of decomposing plant
382 litters and soil organic matter. *Global Biogeochemical Cycles*, 16, 4–1119. doi: 10.
383 1029/2002GB001867, 2002.

384 Feng, Z.D., Wang, L.X., Ji, Y.H., Guo, L.L., Lee, X.Q., and Dworkin, S. I.: Climatic
385 dependency of soil organic carbon isotopic composition along the S-N Transect from
386 34°N to 52°N in central-east Asia. *Palaeogeography Palaeoclimatology Palaeoecology*,
387 257, 335–343, 2008.

388 Fernandez, I., Mahieu, N., and Cadisch, G.: Carbon isotopic fractionation during
389 decomposition of plant materials of different quality. *Glo. Biogeochem. Cyc.*, 17, 1075,
390 doi: 10. 1029/2001GB001834, 2003.

391 Gartern, C.T.Jr., Cooper, L.W., Post, W.M., and Hanson, P.J.: Climate controls on
392 forest soil C isotope ratios in the Southern Appalachian Mountains. *Ecology*,
393 81, 1108–1119, 2000.

394 Gelsomino, A., Keijzer-Wolters, A.C., Cacco, G., and van Elsas, J.D.: Assessment of
395 bacterial community structure in soil by polymerase chain reaction and denaturing

396 gradient gel electrophoresis. *Journal of Microbiological Methods*, 38,1–15, 1999.

397 Hatt é C., and Guiot, J.: Palaeoprecipitation reconstruction by inverse modelling using
398 the isotopic signal of loess organic matter: application to the Nußloch loess sequence
399 (Rhine Valley, Germany). *Climate Dynamics*, 25, 315-327,
400 doi:10.1007/s00382-005-0034-3, 2005.

401 Hultine, K.R., and Marshall, J.D.: Altitude trends in conifer leaf morphology and
402 stable carbon isotope composition. *Oecologia*, 123, 32–40, 2000.

403 Kohn, M.J.: Carbon isotope compositions of terrestrial C₃plants as indicators of
404 (paleo)ecology and (paleo)climate. *Proceedings of the National Academy of Sciences*
405 of the United States of America, 107: 19691–19695, 2010.

406 Körner, C., Farquhar, G.D., and Wong, S.C.: Carbon isotope discrimination by plants
407 follows latitudinal and altitude trends. *Oecologia*, 88,30-40,1991.

408 Lee, X.Q., Feng, Z.D., Guo, L.L., Wang, L.X., Jin, L.Y., Huang, Y.S., Chopping, M.,
409 Huang, D.K., Jiang, W., and Jiang, Q.: Carbon isotope of bulk organic matter: A
410 proxy for precipitation in the arid and semiarid central East Asia. *Global*
411 *Biogeochemical cycles*: 19, GB4010, doi: 10.1029/2004GB002303, 2005.

412 Lu, H.Y., Wu, N.Q., Gu, Z.Y., Guo, Z.T., Wang, L., Wu, H.B., Wang, G., Zhou, L.P.,
413 Han, J.M. and Liu, T.S.: Distribution of carbon isotope composition of modern soils on
414 the Qinghai-Tibetan Plateau. *Biogeochemistry*, 70, 273–297.2004.

415 Macko, S.A., and Estep, M.L.F.: Microbial alteration of stable nitrogen and carbon
416 isotopic composition of organic matter. *Organic Geochemistry*, 6, 787–790, 1984.

417 McCarroll, D., Loader, N. J.: Stable isotopes in tree rings. *Quaternary Science Reviews*

418 23,771–801, 2004.

419 Melillo, J.M., Aber, J.D., Kinkins, A.E., Ricca, A., Fry, B., and Nadelhoffer, K.J.
420 Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic
421 matter. *Plant and Soil*, 115, 189–198, 1989.

422 Nadelhoffer, K.J., and Fry, B.: Controls on natural nitrogen-15 and carbon-13
423 abundances in forest soil organic matter. *Soil Science Society of American Journal*,
424 52, 1633–1640, 1988.

425 Poage, M.A., and Feng, X.H.: A theoretical analysis of steady state $\delta^{13}\text{C}$ profiles of
426 soil organic matter. *Global Biogeochemical Cycles*, 18, GB2016, doi: 10.
427 1029/2003GB002195, 2004.

428 Rao, Z.G., Chen, F.H., Cheng, H., Liu, W.G., Wang, G.A., Lai, Z.P., and Bloemendal,
429 J.: High-resolution summer precipitation variations in the western Chinese Loess
430 Plateau during the last glacial. *Scientific Reports*, 3, 2785, doi: 10.1038/srep02785,
431 2013.

432 Saga, R.F., Wedin, D.A., and Li, M.: The biogeography of C_4 photosynthesis: patterns
433 and controlling factors (pp. 313-373.). In: Saga, R.F., Monson, R.K. (Eds.), *C_4 Plants*
434 *Biology*. Academic Press, San Diego, California, 1999.

435 Schleser, G. H., Helle, G., Lucke, A., and Vos, H.: Isotope signals as climate proxies:
436 the role of transfer functions in the study of terrestrial archives. *Quaternary Science*
437 *Reviews*, 18, 927–943, 1999.

438 Tieszen, L.L., Senyimba, M.M., Imbamba, S.K., Troughton, J.H.: Distribution of
439 C_3 and C_4 gases and carbon isotope discrimination along an altitudinal and Moisture

440 Gradient in Kenya. *Oecologia*, 37, 337–350, 1979.

441 Treydte, K., Frank, D.; Esper, J., Andreu, L., Bednarz, Z., Berninger, F., Boettger, T.,
442 D'Alessandro, C. M., Etien, N., Filot, M., Grabner, M., Guillemin, M. T., Gutierrez,
443 E., Haupt, M., Helle, G., Hiltunen, E., Jungner, H., Kalela-Brundin, M., Krapiec, M.,
444 Leuenberger, M., Loader, N. J., Masson-Delmotte, V., Pazdur, A., Pawelczyk, S.,
445 Pierre, M., Planells, O., Pukiene, R., Reynolds-Henne, C. E., Rinne, K. T., Saracino,
446 A., Saurer, M., Sonninen, E., Stievenard, M., Switsur, V. R., Szczepanek, M.,
447 Szychowska-Krapiec, E., Todaro, L., Waterhouse, J. S., Weigl, M., and Schleser, G.
448 H.: Signal strength and climate calibration of a European tree-ring isotope
449 network. *Geophysical Research Letters* 34: L24302, doi:10.1029/2007GL031106,
450 2007.

451 Vidic, N.J., and Montanez, I.P.: Climatically driven glacial-interglacial variations in
452 C-3 and C-4 plant proportions on the Chinese Loess Plateau. *Geology*, 32, 337–340,
453 2004.

454 Wang, G.A., Feng, X.H., Han, J.M., Zhou, L.P., Tan, W.B., and Su,
455 F.: Paleovegetation reconstruction using $\delta^{13}\text{C}$ of Soil organic matter. *Biogeosciences*,
456 5, 1325–1337, 2008.

457 Wang, G.A., Li, J.Z., Liu, X.Z., and Li, X.Y.: Variations in carbon isotope ratios of
458 plants across a temperature gradient along the 400 mm isohet of mean annual
459 precipitation in north China and their relevance to paleovegetation reconstruction.
460 *Quaternary Science Reviews*, 63, 83–90, 2013.

461 Wang, G.A., Jia, Y.F., and Li, W.: Effects of environmental and biotic factors on

462 carbon isotopic fractionation during decomposition of soil organic matter. *Scientific*
463 *Reports*,5, 11043,doi: 10.1038/srep11043, 2015.

464 Wynn, J.G., Bird, M.I., Wong, V.N.L.: Rayleigh distillation and the depth profile of
465 $^{13}\text{C}/^{12}\text{C}$ ratios of soil organic carbon from soils of disparate texture in Iron Range
466 National Park, Far North Queensland, Australia. *GeochimicaetCosmochimicaActa*, 69,
467 1961-1973, 2005.

468 Wynn, J.G.: Carbon isotope fractionation during decomposition of organic matter in
469 soils and paleosols: Implications for paleoecological interpretations of paleosols.
470 *Palaeogeography, Palaeoclimatology, Palaeoecology*, 251, 437-448, 2007.

471 Xu, M., Wang, G.A., Li, X.L., Cai, X.B., Li, X.L., Christie, P., Zhang, J.L.: The key
472 factor limiting plant growth in cold and humid alpine areas also plays a dominant role
473 in plant carbon isotope discrimination. *Frontiers in Plant Science*, 6,
474 doi:10.3389/fpls.2015.00961,2015.

475 Yang, S.L., Ding, Z.L., Li, Y.Y., Wang, X., Jiang, W.Y., Huang,
476 X.F.:Warming-induced northwestward migrationof the East Asian monsoon rain belt
477 from theLast Glacial Maximum to the mid-Holocene.*Proceedings of the National*
478 *Academy of Sciences of the United States of America*,
479 www.pnas.org/cgi/doi/10.1073/pnas.1504688112

480 Zhu, Y., Siegwolf, R. T. W., Durka, W., and Körner, C.:Phylogenetically balanced
481 evidence for structural and carbon isotope responses in plants along elevational
482 gradients. *Oecologia*, 162, 853-863, 2010.