

1

2 Dear editor,

3 Many thanks. We have already improved the manuscript according to the reviewer comments  
4 and the interactive discussion suggestions. Also we have improved English by Editing Service  
5 company. Thus, we sincerely hope to publish it in BG journal.

6 Best Regards,

7 Guoan Wang

8

9 **A point-by-point response Referee #1**

10 Dear Dr. Martin J. Hodson,

11 Many thanks for your comments. We have modified the manuscript following the  
12 comments.

13

14 Response to the comments:

15 1) The reviewer felt it needs some shortening of the discussion. We think the  
16 suggestion is great, thus, some unnecessary contents have been deleted in the new  
17 discussion.

18 2) The reviewer felt that the English needs attention. Thank you. We have asked  
19 Professor Eric Posmentier in department of Earth Sciences, Dartmouth College to  
20 do the English editing again, and he had edited the English thoroughly. Thus, I  
21 believe the English must be greatly improved. Actually, Professor Eric Posmentier  
22 has done English-editing of the manuscript last year, however, we added some  
23 new contents in the manuscript after his editing, this may introduce some English  
24 mistakes.

25 3) The title was changed following the comment.

26

27

28 Best wishes

29

30 Sincerely yours,

31

32 Guoan Wang

33

34 **A point-by-point response to Referee #2**

35 Dear Dr. SL Yang,  
36 Many thanks for your comments. We have modified the manuscript following the  
37 comments.

38

39 Response to the comments:

- 40 1) Your suggestion that the discussion needs some shortening is great, thus, some  
41 unnecessary contents about C<sub>4</sub> plant distribution in China (in the first paragraph in  
42 the old version) have been deleted in the new discussion. In addition, we have  
43 deleted the discussion about the influence of precipitation on soil isotope.
- 44 2) The reviewer felt that the English needs some corrections. We had asked Professor  
45 Eric Posmentier in department of Earth Sciences, Dartmouth College to do the  
46 English editing again. We believe English was greatly improved in the new  
47 version. Thank you.

48

49 Best wishes

50

51 Sincerely yours,

52

53 Guoan Wang

54

55

### 56 A point-by-point response to Referee #3

57 Dear Referee #3.

58 Many thanks for your comments. We have modified the manuscript following the  
59 comments.

60

61 **Comment-1:** That being said, said that the discussion wanders quite a bit and discusses  
62 several topics that are irrelevant to the paper or are obvious, such as the humidity cline  
63 and the plant variation. The discussion should focus mainly on the temperature and soil  
64 main effect and that would tighten it up and strengthen it.

65 **Response:** We think the suggestion is great, thus, the influence of precipitation was  
66 deleted in new discussion (please see the lines 297-307 in the text with a mark-up);  
67 the content with respect to plant variation was greatly shortened (please see the lines  
68 244-247 and 251-256 in the text with a mark-up).

69

70 **Comment-2:** Overall, there are several grammatical errors including missed commas,  
71 etc. The writing is okay, but could be improved and it needs to be retitled.

72 **Response:** Thank you. In order to improve the English, we asked an English service  
73 company, Editage Company, to edit the manuscript thoroughly. Referee #3 suggested

74 that it needs to be retitled. Referee #1 also suggested us to change the title. Thus, we  
75 modified the title following their suggestions.

76

77

78 Best wishes

79

80 Sincerely yours,

81

82

83      **Temperature exerted no influence on the soil organic carbon**  
84      **matter  $\delta^{13}\text{C}$  isotope of surface soil along the 400 mm isopleth**  
85      **of 400-mm-mean annual precipitation in China**

带格式的: 上标

86

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

88      <sup>1</sup>College of Resources and Environmental Sciences, China Agricultural University,

89      Beijing 100193, China

90

91

92

93

94

95

96      Author for correspondence:

97      Guoan Wang

98      Tel: +086-10-62733942

99      Email:gawang@cau.edu.cn

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104

105 **Abstract**

106 Soil organic carbon is the largest pool of terrestrial ecosystem, and its carbon isotope  
107 composition is affected by many factors. However, the influence of environmental  
108 factors, especially temperature, on soil organic carbon isotope values ( $\delta^{13}\text{C}_{\text{SOM}}$ ) is  
109 poorly constrained. This impedes interpretations and application of variability of  
110 organic carbon isotopes in reconstructions of paleoclimate and paleoecology and  
111 global carbon cycling. With a Given then considerable temperature gradient along the  
112 400 mm isohyet (isopleth of mean annual precipitation – MAP) in China, this isohyet  
113 provides ideal experimental sites for studying the influence of temperature on soil  
114 organic carbon isotopes. In this study, the effect of temperature on surface soil  
115  $\delta^{13}\text{C}$  was assessed by a comprehensive investigation from at 27 sites across a  
116 temperature gradient along the isohyet. This work demonstrates that temperature did  
117 not play a role in soil  $\delta^{13}\text{C}$ . This suggests that organic carbon isotopes in sediments  
118 cannot be used for the paleotemperature reconstruction, and that the effect of  
119 temperature on organic carbon isotopes can be neglected in the reconstruction of  
120 paleoclimate and paleovegetation. Multiple regressions with MAT (mean annual  
121 temperature), MAP, altitude, latitude and longitude as independent variables, and  
122  $\delta^{13}\text{C}_{\text{SOM}}$  as the dependent variable, shows that the five environmental factors in total  
123 account for only 9% of soil  $\delta^{13}\text{C}$  variance. However, Oneone-way ANOVA analyses  
124 suggest that soil type and vegetation types are significant influential factors on soil  
125  $\delta^{13}\text{C}$ . Multiple regressions in which the above five aforementioned environmental  
126 factors were taken as quantitative variables, vegetation type, Chinese nomenclature  
127 soil type and WRB soil type were introduced as dummy variables separately, show  
128 that 36.2%, 37.4% and, 29.7% of the variability in soil  $\delta^{13}\text{C}$  are explained,  
129 respectively. Compared to the multiple regressionregressions in which only  
130 quantitative environmental variables were introduced, the multiple regressions in  
131 which soil and vegetation were also introduced explain more variance, suggesting that  
132 soil type and vegetation type really exerted significant influences on  $\delta^{13}\text{C}_{\text{SOM}}$ .

134

135 **1. Introduction**

136 Global climate change has recently received a great deal of attention, and effective  
137 predictions of future climate change depend on the relevant information from climate  
138 in the geological past. Over recent decades, stable carbon isotopes in sediments, such  
139 as loess, paleosol, lacustrine, and marine sediments, have been widely used to  
140 reconstruct paleo-vegetation and paleo-environments, and provided important insights  
141 into patterns of past climate and environment changes. For examples, many  
142 researchers have used organic carbon isotopes of loess to reconstruct paleo-vegetation  
143 and paleo-precipitation. Vidic and Montañez (2004) conducted a reconstruction of  
144 paleovegetation at the central Chinese Loess Plateau during the Last Glaciation (LG)  
145 and Holocene by using the organic carbon isotopes in loess from Jiaodao, Shanxi  
146 Province. Hatté and Guiot (2005) carried out a palaeo-precipitation reconstruction by  
147 inverse modelling using the organic carbon isotopic signal of the Nußloch  
148 loess sequence (Rhine Valley, Germany). Rao et al. (2013) reconstructed a  
149 high-resolution summer precipitation variations in the western Chinese Loess Plateau  
150 during the Last Glaciation LG using a well-dated organic carbon isotopic dataset  
151 set. Yang et al. (2015) derived a minimum 300 km northwestward migration of the  
152 monsoon rain belt from the Last Glacial Maximum to the Mid-Holocene using the  
153 organic carbon isotopes from 21 loess sections across the Loess Plateau. However, to  
154 our knowledge, almost no researchers have conducted paleo-temperature  
155 reconstructions using organic carbon isotope records of loess and paleosol, because it

156 has been argued that temperature exerts slight, or even no influence on soil organic  
157 carbon isotope values ( $\delta^{13}\text{C}_{\text{SOM}}$ ) $\delta^{13}\text{C}_{\text{SOM}}$ . While this statement may be likely, it needs  
158 to be demonstrated because only few studies have addressed the influence of  
159 temperature on organic carbon isotopes of modern surface soil; furthermore, these  
160 studies do not appear to result in a conclusive statement. Lee et al. (2005) and Feng et  
161 al. (2008) both reported no relationship between temperature and surface soil  $\delta^{13}\text{C}$  in  
162 central-east Asia. However, Lu et al. (2004) discovered a nonlinear relationship  
163 between mean annual mean temperature (MAT) and  $\delta^{13}\text{C}_{\text{SOM}}$  from the  
164 Qinghai-Tibetan Plateau; Sage et al. (1999) compiled the data from Bird and Pousai  
165 (1997) and also found a nonlinear trend for the variation in  $\delta^{13}\text{C}_{\text{SOM}}$  along a  
166 temperature gradient in Australian grasslands and savannas.

167 Plant residues are the most important source of soil organic matter.  $\delta^{13}\text{C}_{\text{SOM}}$  is  
168 generally close to plant  $\delta^{13}\text{C}$  value carbon isotope despite isotopic fractionation during  
169 decomposition of organic matter (Nadelhoffer and Fry, 1988; Balesdent et al., 1993;  
170 Ågren et al., 1996; Fernandez et al., 2003; Wynn, 2007). Thus, the influential factors  
171 of for plants  $\delta^{13}\text{C}$  might also play a role in influence  $\delta^{13}\text{C}_{\text{SOM}}$   $\delta^{13}\text{C}$  in plants,  
172 especially C<sub>3</sub> plants, is tightly associated with precipitation. (Diefendorf et al., 2010;  
173 Kohn, 2010), so precipitation should that may have influence on affect soil  $\delta^{13}\text{C}$   
174 (Diefendorf et al., 2010; Kohn, 2010). In addition to the effect of precipitation, many  
175 other factors, such as temperature, air pressure, atmospheric CO<sub>2</sub> concentration,  
176 altitude, latitude and longitude, may also influence  $\delta^{13}\text{C}$  exert influences on variancee in  
177 plants  $\delta^{13}\text{C}$  (Körner et al., 1991; Hultine and Marshall, 2000; Zhu et al., 2010; Xu et

178 al., 2015). Although patterns of variation in plants  $\delta^{13}\text{C}$  with respect to temperature  
179 are unresolved so far (e.g. Schleser et al., 1999; McCarroll and Loader, 2004; Treydte  
180 et al., 2007; Wang et al., 2013), it has been widely accepted that, ~~even if~~ temperature  
181 has a slight effect on plants  $\delta^{13}\text{C}$ , ~~this effect is slight~~. So As such, if the  $^{13}\text{C}$  enrichment  
182 during SOM-soil organic matter decomposition is a constant value, we expect a slight  
183 or no influence of temperature on soil  $\delta^{13}\text{C}$ . However, the fact is that  
184 this  $^{13}\text{C}$ -enrichment is affected by environmental and biotic factors (Wang et al.,  
185 2015). Thus, it is difficult to expect determinet whether or how temperature affects  
186 soil  $\delta^{13}\text{C}$ , and there should be it needs specific investigations ~~of~~ focusing on this issue.  
187 Although the relationship between temperature and  $\delta^{13}\text{C}_{\text{SOM}}$  has been investigated in  
188 these previous studies mentioned above, these studies were unable to effectively  
189 separate the influence of temperature from the effect of precipitation. Thus, new  
190 investigations are necessary. The present study includes an intensive investigation of  
191 the variation in  $\delta^{13}\text{C}_{\text{SOM}}$  with respect to temperature across a temperature gradient  
192 along the 400 mm isohyet (isopleth of mean annual precipitation: MAP) in China.  
193 We sampled surface soil along the specific isohyet to minimize the effect of  
194 precipitation changes on  $\delta^{13}\text{C}_{\text{SOM}}$ .

195 In addition, there are no meteorological stations near most of the sampling sites in  
196 the previous studies mentioned above; thus, they had to interpolate meteorological  
197 data, which could can be unrealistic in regions with strong topographical variability.  
198 This interpolation could have produced errors in the relationships between  
199 temperature and  $\delta^{13}\text{C}_{\text{SOM}}$  that were established in these studies. In the present

200 investigation, we collected samples only at those sites with meteorological stations;  
201 thus, the climatic data that we obtained from these stations are probably more reliable  
202 compared with to the interpolated pseudo-data derived by interpolation.

203

204 **2. Materials and methods**

205 2.1. Study site

206 In this study, we set up a transect along the 400 mm isohyet from LangKaZi (site 1,  
207 29°3.309'N, 90°23.469'E), on the Qinghai-Tibetan Plateau in southwest China, to  
208 BeiJiCun (Site27, 53°17.458'N, 122°8.752'E), in Heilongjiang Province in northeast  
209 China (Fig.1, Table 1). The straight-line distance between the above two sites is about  
210 6000 km. Twenty-seven (27) sampling sites were set along the transect. Among these  
211 sampling sites, 10 sites are-were located on the Qinghai-Tibetan Plateau, and the  
212 others are in north China. BeiJiCun and KuDuEr have-had the lowest MAT of -5.5  $^{\circ}\text{C}$   
213  $^{\circ}\text{C}$  and while ShenMu has-had the highest MAT of +8.9  $^{\circ}\text{C}$ . The average MAP of  
214 these sites is-was 402 mm. In north China, rainfall from June to September accounts  
215 for approximately 80% of the total annual precipitation, and the dominant control  
216 over the amount of precipitation is the strength of the East-Asian monsoon system. In  
217 the Qinghai-Tibetan Plateau, however, precipitation is associated with both the  
218 Southwest monsoon and the Qinghai-Tibetan Plateau monsoon, and approximately 80%  
219 — 90% rainfall occurs in the summer season (from May to October).

220

221 Fig.1

222 Table 1

223 2.2 ~~soil~~Soil sampling224 Soil samples were collected in the summer of 2013 between July 12<sup>th</sup> and August 30<sup>th</sup>.带格式的: 上标  
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225 ~~In order to~~ To avoid disturbance of human activities, sample sites ~~are~~were chosen 5-7  
 226 ~~kilometers~~km ~~far~~ from the towns where the meteorological stations are located. We  
 227 set three ~~quadrats~~squares (0.5 m×0.5 m) within 200 m<sup>2</sup> to collect surface mineral  
 228 soil (0-5 cm) using a ring knife. The O-horizon, including litters, morders and mors  
 229 ~~were~~was removed before collecting mineral soil. About 10 g ~~of~~ air dried soils ~~were~~was  
 230 sieved at 2 mm. Plant fragments and the soil fraction coarser than 2 mm were  
 231 removed. The ~~rest~~remainder of the ~~sieved~~ soil samples ~~were~~was immersed using  
 232 excessive HCl (1 mol/L) for 24 h. ~~In order to~~ To ensure that all carbonate was cleared,  
 233 we conducted ~~artificial~~ stirring ~~4~~four times during the immersion. Then, the samples  
 234 was washed to neutrality using distilled water. Finally it was oven-dried at 50°C and  
 235 ground. Carbon isotope ratios were determined on a Delta<sup>Plus</sup> XP mass spectrometer  
 236 (Thermo Scientific, Bremen, Germany) coupled with an elemental analyzer (FlashEA  
 237 1112; CE Instruments, Wigan, UK) in continuous flow mode. The elemental analyzer  
 238 combustion temperature was 1020°C<sup>°</sup>E.

239 The carbon isotopic ratios are reported in delta notation relative to the V-PDB  
 240 standard using the equation:

$$241 \delta^{13}\text{C} = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000 \quad (1)$$

242 where  $\delta^{13}\text{C}$  is the carbon isotope ratio of the sample (‰), and  $\text{R}_{\text{sample}}$  and  $\text{R}_{\text{standard}}$  are  
 243 the  $^{13}\text{C}/^{12}\text{C}$  ratios of the sample and the standard, respectively. For this measurement,

244 we obtained a standard deviation of less than 0.15‰ among replicate measurements  
245 of the same soil sample.

246

### 247 3. Results

248 Except for one  $\delta^{13}\text{C}_{\text{SOM}}$  value (-18.8‰), all other data ranged from -20.4‰ to -27.1‰  
249 with a mean value of -23.3‰ (n = 80, s.d. = 1.45). Multiple regression with MAT,  
250 MAP, altitude, latitude and longitude as independent variables, and  $\delta^{13}\text{C}_{\text{SOM}}$  as the  
251 dependent variable, shows that only 9% of the variability in soil  $\delta^{13}\text{C}$  can be explained  
252 as a linear combination of all five environmental factors ( $p = 0.205$ ;[Table 2](#)).  
253 Considering the possibility of correlations among the five explanatory variables,  
254 stepwise regression was used to eliminate the potential influence of collinearity  
255 among them. Variables were incorporated into the model with  $P$ -value < 0.05 and  
256 excluded with  $P$ -value > 0.1. [Statistical analysis showed that only latitude was](#)  
257 [included in the stepwise regression model](#)[Stepwise regression of soil  \$\delta^{13}\text{C}\$  in the](#)  
258 [model consisting only of latitude](#) ( $R^2 = 0.077$ ,  $p = 0.012$ ). In order to [better](#) constrain  
259 the relationship between soil  $\delta^{13}\text{C}$  and each environmental factor [better](#), bivariate  
260 correlation analyses of soil  $\delta^{13}\text{C}$  against some environmental factors were conducted.

261 The bivariate correlation analyses show that  $\delta^{13}\text{C}_{\text{SOM}}$  is not related to MAT ( $p = 0.114$ )  
262 or SMT ( $p = 0.697$ ) along the isohyet (Fig. 2a, b). In addition, in order to [further](#)  
263 determine [further](#) the response of  $\delta^{13}\text{C}_{\text{SOM}}$  to temperature, we considered three subsets  
264 of our soil samples defined according to the climate, topography or vegetation type:  
265 the [Qinghai-Tibetan Plateau](#) (mainly alpine meadow, including 10 sites), steppe or

266 grassland(11 sites) and coniferous forest(~~6~~six sites) (Table 1). Bivariate correlation  
267 analyses within these subsets also show no relationship between  $\delta^{13}\text{C}_{\text{SOM}}$  and MAT  
268 for all categories. The correlation analysis of  $\delta^{13}\text{C}_{\text{SOM}}$  ~~vs. with respect to~~ altitude is  
269 shown in Fig.3, which ~~displays~~ displays no relationship ( $p = 0.132$ ). Although longitude ~~is~~was  
270 not found to exert influence on  $\delta^{13}\text{C}_{\text{SOM}}$  in the above stepwise regression, bivariate  
271 correlation analyses ~~showed~~ showed that latitude and longitude were both ~~are~~ negatively  
272 related to  $\delta^{13}\text{C}_{\text{SOM}}$  ( $p = 0.012$  and  $0.034$ , respectively) (Fig. 4a, b).

273 In addition to the effects of quantifiable environmental factors, qualitative factors,  
274 such as soil type and vegetation type, ~~may~~ may have influence on  $\delta^{13}\text{C}_{\text{SOM}}$ . Varied concepts  
275 have been introduced in soil taxonomy, leaving varied soil nomenclatures in use. In  
276 this study we adopted Chinese soil nomenclature and the World Reference Base  
277 (WRB) to describe the observed soils. The soil was divided into ~~8~~eight types ~~and~~or ~~6~~  
278 six types based on the Chinese Soil Taxonomy ~~and~~or WRB, respectively (Table 1).  
279 One-way ANOVA analyses suggest that both soil ~~type~~ and vegetation type ~~both~~  
280 played a significant role in  $\delta^{13}\text{C}_{\text{SOM}}$  ( $p = 0.002$  for soil types based on the Chinese  
281 Soil Taxonomy,  $p = 0.003$  for soil type based on WRB and  $p = 0.001$  for vegetation  
282 types) (Fig. 5).

283 In order to~~To further~~ constrain further the effects of soil ~~type~~ and vegetation type on  
284  $\delta^{13}\text{C}_{\text{SOM}}$ , multiple regressions with soil ~~type~~ and vegetation type as dummy variables  
285 were conducted. Considering the ~~tight~~ relationship between soil type and vegetation  
286 type, especially in Chinese soil taxonomy, the soil variables ~~s~~ and the vegetation  
287 variables ~~s~~ were separately introduced into the statistical analyses. Multiple regression,

288 in which the above five aforementioned explanatory environmental factors were taken  
289 as quantitative variables and the 8eight soil types of the Chinese nomenclature as  
290 values of a dummy variable, shows that environmental factors and soil types in total  
291 account for 37.4% of the soil  $\delta^{13}\text{C}$  variance ( $p < 0.001$ )(Table 2). Using the 6 soil  
292 types based on WRB rather than the Chinese nomenclature, 29.7% ( $p = 0.003$ ) of the  
293 variability is explained using the 6 soil types based on WRB rather than the Chinese  
294 nomenclature(Table 2). Similarly, multiple regression with vegetation types as  
295 dummy variables shows that the five environmental factors and vegetation types in  
296 total can explain 36.2% of the variability in soil  $\delta^{13}\text{C}$  ( $p = 0.001$ )(Table 2).  
297 Compared to the multiple regressions in which only quantitative environmental  
298 variables were introduced, the multiple regressions in which soil and vegetation were  
299 also introduced explain more variance, suggesting that soil type and vegetation type  
300 really played a significant role in  $\delta^{13}\text{C}_{\text{SOM}}$  variability.

301 Table 2

302 Fig.2a,b

303 Fig.3

304 Fig.4a,b,b

305 Fig.5

306

#### 307 4. Discussion

308 Soil  $\delta^{13}\text{C}$  depends on plants  $\delta^{13}\text{C}$  of plants and on carbon isotopic fractionation during  
309 organic matter decomposition.  $\delta^{13}\text{C}$  values of  $\text{C}_3$  plants vary between -22‰ and -34‰

310 with a mean of  $-27\text{\textperthousand}$ , and C<sub>4</sub> plants range from  $-9\text{\textperthousand}$  to  $-19\text{\textperthousand}$  with a mean of  
311  $-13\text{\textperthousand}$  (Dienes, 1980). Carbon isotope fractionation occurs ~~in~~during the process of  
312 plant litter decomposition into soil organic matter in most environments, especially in  
313 non-arid environments, causing <sup>13</sup>C-enrichment in soil organic matter compared ~~with~~to the plant sources (Nadelhoffer, 1988; Balesdent et al., 1993; Ågren et al., 1996;  
314 Fernandez et al., 2003; Wynn et al., 2005; Wynn, 2007). An intensive investigation of  
315 isotope fractionation during organic matter decomposition, which was conducted in  
316 Mount Gongga, an area ~~in~~of the Qinghai-Tibetan Plateau dominated by C<sub>3</sub> vegetation  
317 with herbs, shrubs and trees, showed that the mean <sup>13</sup>C-enrichment in surface soil (0-5  
318 cm depth) relative to the vegetation was  $2.87\text{\textperthousand}$  (Chen et al., 2009). Another  
319 investigation of 13 soil profiles from the Tibetan Plateau and north China showed that  
320 the  $\delta^{13}\text{C}$  difference between surface soil (0-5 cm depth) and the original biomass  
321 varied from 0.6 to  $3.5\text{\textperthousand}$  with a mean of  $1.8\text{\textperthousand}$  (Wang et al., 2008). Thus, the  $\delta^{13}\text{C}_{\text{SOM}}$   
322 dataset data set of from this study ( $\delta^{13}\text{C}_{\text{SOM}}$  ranges from  $-20.4\text{\textperthousand}$  to  $-27.1\text{\textperthousand}$ ) indicates  
323 that the modern terrestrial ecosystem along ~~the~~ isohyet is greatly dominated by C<sub>3</sub>  
324 plants. This result is consistent with the observations of vegetation along the isohyet  
325 done completed in our previous study (Wang et al., 2013) and in this the present study.  
326 ~~Yin and Li (1997), Lu et al. (2004) and Wang et al. (2004) have reported that a small~~  
327 ~~number of C<sub>4</sub> species occurred in the Qinghai Tibetan Plateau; however, in this~~  
328 ~~present study we found no C<sub>4</sub> plants in the Qinghai Tibetan Plateau. We are also very~~  
329 ~~surprised at by such high soil <sup>13</sup>C values occurring at RiKaZe (site Site 2): Fig.3 and~~  
330 ~~Table 1) because only four C<sub>3</sub> plants grew there, and there were while, no C<sub>4</sub> species.~~  
331

332 ~~The This~~ abnormal observation suggests that a very high carbon isotope fractionation  
333 with SOM degradation ~~have taken places occurred~~ in the local ecosystem. ~~Previous~~  
334 ~~studies have also observed a similar phenomenon. Although although~~ the mechanism  
335 ~~accounted responsible~~ for the unusually high isotopic fractionation remains unclear, ~~it~~  
336 ~~is not surprising~~. For example, Wynn (2007) ~~has~~ reported that the fractionation ~~leaved~~  
337 ~~enriched~~ soil organic carbon  $^{13}\text{C}$  ~~enriched enrichment by~~ up to  $\sim 6\%$  with respect to  
338 the original biomass. ~~Rao et al. (2008) has suggested that mid-latitude area~~  
339 ~~(31°N–40°N) in east China provides relatively favorable condition for C<sub>4</sub> plant growth.~~  
340 ~~But we observed that a small number of C<sub>4</sub> species occur only in the temperate~~  
341 ~~meadow steppe and the temperate typical steppe in north China, while no C<sub>4</sub> species~~  
342 ~~are distributed in the coniferous forests in north China. In short, the contribution of C<sub>4</sub>~~  
343 ~~biomass to the local vegetation along the isohyet is very low, and can be neglected.~~

344 The MAT, MAP, altitude, latitude and longitude combined are responsible for only  
345 9% ~~of the~~ variability in soil  $\delta^{13}\text{C}$  in the multiple regression model, suggesting that the  
346 contribution of ~~these~~ five environmental factors to the soil  $\delta^{13}\text{C}$  variance is very small.  
347 Our previous study conducted along the isohyet ~~resulted~~ in a strong positive  
348 relationship ~~between the  $\delta^{13}\text{C}$  of C<sub>3</sub> plants  $\delta^{13}\text{C}$  and MAT~~ with a coefficient of 0.104‰  
349  $/^{\circ}\text{C}$  (Wang et al., 2013). The difference between maximum and minimum  
350 temperature ~~along the isohyet is 15  $^{\circ}\text{C}$~~ , so the greatest possible effect of temperature  
351 on plant  $\delta^{13}\text{C}$  along the temperature gradient is 1.56‰, which ~~is~~ not very  
352 ~~great substantial~~. ~~Since Because~~ the main source of soil organic matter along the  
353 isohyet ~~is C<sub>3</sub> plants, the induced variance in soil  $\delta^{13}\text{C}$  by plant  $\delta^{13}\text{C}$  also cannot be~~

354 very ~~greathigh~~. On the other hand, although the  $^{13}\text{C}$ -enrichment with SOM  
355 degradation follows a Rayleigh distillation process (Wynn, 2007), our recent study  
356 shows that temperature does not influence carbon isotopic fractionation during  
357 decomposition (Wang et al., 2015), which ~~is~~ also ~~a reason explains~~ for the lack of a  
358 relationship between ~~soil~~  $\delta^{13}\text{C}$  and temperature. Feng et al. (2008) and Lee et al. (2005)  
359 respectively, reported ~~no~~ relationships between ~~soil~~  $\delta^{13}\text{C}$  and MAT and SMT, which  
360 is ~~consistent~~ with our result. Their field campaigns were conducted in central Asia,  
361 which is also dominated by  $\text{C}_3$  plants, ~~similar~~ to the area along the 400 mm isohyet.  
362 This is the reason why the same pattern exists in both central Asia and in the area  
363 along the 400 mm isohyet.

364 The observations in Bird and Pousai (1997) and Sage et al. (1999) appear to be  
365 inconsistent with our findings; they found a nonlinear relationship between soil  $\delta^{13}\text{C}$   
366 and MAT in Australian grasslands. However, if ~~they~~ considered ~~ing~~ only soil with  
367 pure  $\text{C}_3$  plants (MAT is below  $16\text{ }^{\circ}\text{C}$ ), soil  $\delta^{13}\text{C}$  and temperature ~~are~~ ~~were~~ not  
368 related in Australian grasslands, which ~~is in agreement~~ ~~agrees~~ with our results.  
369 Below  $15\text{ }^{\circ}\text{C}$ , ~~the~~  $\text{C}_4$  contribution to productivity in Australian grasslands is  
370 negligible, whereas above  $23\text{ }^{\circ}\text{C}$ ,  $\text{C}_3$  contribution is negligible. Between  $14\text{ }^{\circ}\text{C}$   
371 and  $23\text{ }^{\circ}\text{C}$ , soil  $\delta^{13}\text{C}$  is positively correlated with MAT, indicating  $\text{C}_4$  representation  
372 increasing with MAT (Sage et al., 1999). Lu et al. (2004) also reported a nonlinear  
373 relationship between soil  $\delta^{13}\text{C}$  and MAT. Similarly, if the soil data with  $\text{C}_4$  plants are  
374 excluded from the nonlinear correlation, soil  $\delta^{13}\text{C}$  is also not related to MAT in Lu et  
375 al. (2004) (see Fig.5b in Lu et al., 2004). Thus, ~~this~~ ~~the~~ present study and the previous

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376 observations are consistent in showing that in a terrestrial ecosystem in which the  
377 vegetation is dominated by C<sub>3</sub> plants, temperature does not influence soil δ<sup>13</sup>C  
378 variance.

379 ~~This study shows that the contribution of precipitation to the variability in soil δ<sup>13</sup>C~~  
380 ~~is neglected. The reason for this is that the soil was sampled along the 400 mm~~  
381 ~~isohyet, and the MAP difference among sites is very small. It should be pointed out~~  
382 ~~here that the no MAP influence on the soil δ<sup>13</sup>C does not mean no moisture control of~~  
383 ~~the soil δ<sup>13</sup>C. Because the temperature varies greatly across the temperature~~  
384 ~~gradient although the MAP is almost the same for each sampling site; this would cause~~  
385 ~~a big difference in relative humidity among sites. We expect that relative humidity~~  
386 ~~would explain a great variability in soil δ<sup>13</sup>C. But we did not take relative humidity as~~  
387 ~~an explanatory variable in the statistical analyses, because we lack the complete data~~  
388 ~~of relative humidity, and we do not want to use the pseudo data derived by~~  
389 ~~interpolation.~~

390 All the soil samples were taken along the 400 mm isohyet, thus, this study shows  
391 that the contribution of precipitation to the variability in soil δ<sup>13</sup>C is negligible.

392 Although stepwise regression and correlation analysis both show a significant  
393 influence of latitude on soil δ<sup>13</sup>C, the five environmental variables including latitude  
394 were responsible for only 9% of the variability in soil δ<sup>13</sup>C in a multiple regression  
395 model (Table 2), suggesting that the contribution of latitude to soil δ<sup>13</sup>C was also  
396 slight limited. This study shows a negative correlation between latitude and δ<sup>13</sup>C<sub>SOM</sub>  
397 (p=0.012). Bird and Pausai (1997) and Tieszen et al. (1979) reported a similar pattern.

398 Latitude is a comprehensive environmental factor, and change in latitude can bring  
399 about changes in other environmental factors, such as temperature, irradiation, cloud  
400 amount, and moisture.but temperature Temperature or and irradiation, however,  
401 should be most strongly related to latitude, and obviously change with latitude. The  
402 observed significant relationship between latitude and soil  $\delta^{13}\text{C}$  (Fig.4a) suggests that  
403 environmental factors other than temperature might contribute more or less to the  
404 variance in soil  $\delta^{13}\text{C}$ .

405 Vegetation type control of the soil  $\delta^{13}\text{C}$  mainly reflected reflects the effects of  
406 life-forms on plant  $\delta^{13}\text{C}$  and the effect of substrate quality on isotope fractionation  
407 during organic matter decomposition. Communities in which life-forms of the  
408 dominant plants is are similar are generally treated as the same vegetation type. Plant  
409  $\delta^{13}\text{C}$  is tightly related to life-form (Diefendorf et al., 2010; Ehleringer and Cooper,  
410 1988) and this causes  $\delta^{13}\text{C}$  differences among varying vegetation types, consequently  
411 resulting in the observed effect of vegetation type on the soil  $\delta^{13}\text{C}$ .

412 Substrate quality partly quantifies how easily organic carbon is used by soil  
413 microbes (Poage and Feng, 2004). It can be related to plant type and is often defined  
414 using a C/N ratio, lignin content, cellulose content, and/or lignin content/N ratio  
415 (Melillo et al., 1989; Gartner et al., 2000). Our study in Mount Gongga, China, showed  
416 that litter quality played a significant role in isotope fractionation during organic  
417 matter decomposition, and the carbon isotope fractionation factor,  $\alpha$ , increases  
418 with litter quality (Wang et al., 2015). Thus, the isotope fractionation factor  
419 should be different among varying sites because litter quality is dependent on

420 vegetation and this makes soil change its  $\delta^{13}\text{C}$  with vegetation type.

421 ~~Control~~ The effect of soil type on soil  $\delta^{13}\text{C}$  ~~could~~ may be associated with the effect  
422 of soil type on isotope fractionation during organic matter decomposition, ~~and~~ which  
423 involves at least two mechanisms ([Wang et al. \[2008\] has discussed the mechanisms](#)  
424 in detail). ~~(1)First, Properties~~ properties and compositions of microbial decomposer  
425 communities are dependent ~~efon~~ soil type (Gelsomino et al., 1999). Different microbes  
426 ~~could~~ can have different metabolic pathways even when they decompose the same  
427 organic compound (Macko and Estep, 1984), and the extent of isotope fractionation  
428 during decomposition may be tightly related to the metabolic pathways of microbes  
429 (Macko and Estep, 1984). ~~For example, Morasch et al. (2001) observed a greater~~  
430 ~~hydrogen isotope fractionation for toluene degradation in growth experiments with the~~  
431 ~~aerobic bacterium *P. putida* mt 2 and less fractionation in toluene degradation by~~  
432 ~~anaerobic bacteria.~~ (2) Second, Physical ~~physical~~ and chemical properties, such as pH,  
433 particle size fraction, and water-holding capacity, display ~~striking~~ considerable  
434 differences among soil types and this causes organic compounds to ~~be~~ decayed at  
435 different rates in different soil environments. The magnitude of isotope fractionation  
436 during decomposition is linked to the degree of organic matter decomposition (Feng,  
437 2002). Thus, soil type plays a significant role in fractionation.

438

## 439 **5. Conclusions**

440 The present study measured organic carbon isotopes in surface soil along a 400 mm  
441 isohyet of mean annual precipitation in China, and observed that soil type and

442 vegetation type both had significant influence on soil organic carbon isotopes.  
443 However, temperature is was found to have no observable impact on  $\delta^{13}\text{C}_{\text{SOM}}$ ,  
444 suggesting that  $\delta^{13}\text{C}$  signals in sediments cannot be used for the reconstruction of  
445 temperature, and that the effect of temperature on  $\delta^{13}\text{C}_{\text{SOM}}$  could should be neglected  
446 in the reconstruction of paleo climate and paleo vegetation~~paleo climate and paleo~~  
447 vegetation that use using carbon isotopes of soil organic matter.

448

449

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455 College of Resources and Environment, China Agricultural University~~we~~We  
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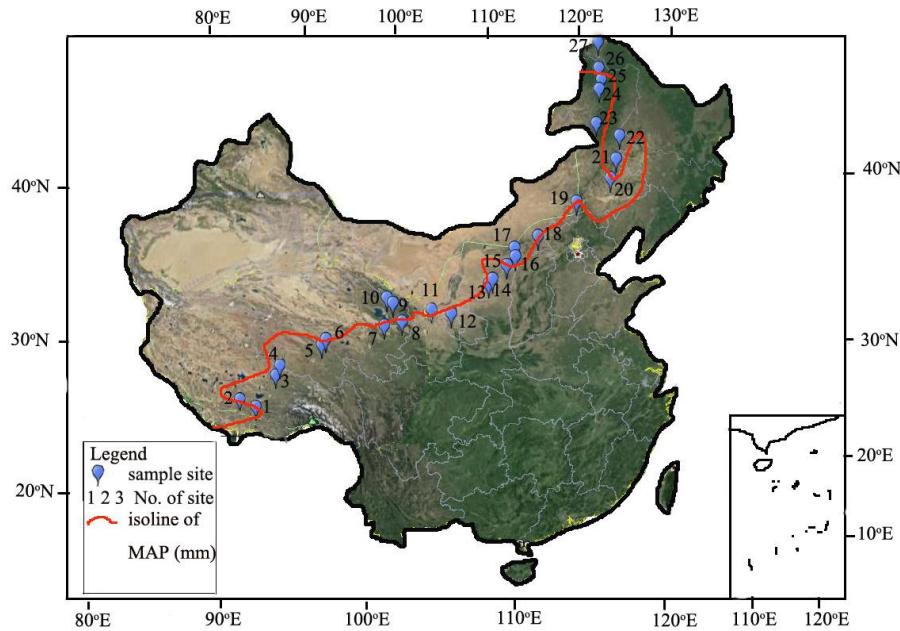
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614 **Figures**

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619 Fig.1. Sketch of sampled region. Sample sites are indicated with numbers. 1, LangKaZi; 2,RiKaZe;  
 620 3,NaQu; 4,NieRong; 5,ZhiDuo; 6,QuMaLai; 7,TongDe; 8,TongRen; 9,HuangYuan; 10,HaiYan;  
 621 11,YuZhong; 12,XiJi; 13,JingBian; 14,HengShan; 15,ShenMu; 16,HeQu; 17,ZhunGeErQi;  
 622 18,FengZhen; 19,DuoLun; 20,LinXi; 21,ZhaLuTeQi; 22,WuLanHaoTe; 23,AErShan; 24,YaKeShi;  
 623 25,KuDuEr; 26,GenHe; 27,BeiJiCun. Detailed information of sites is shown in Table 1.

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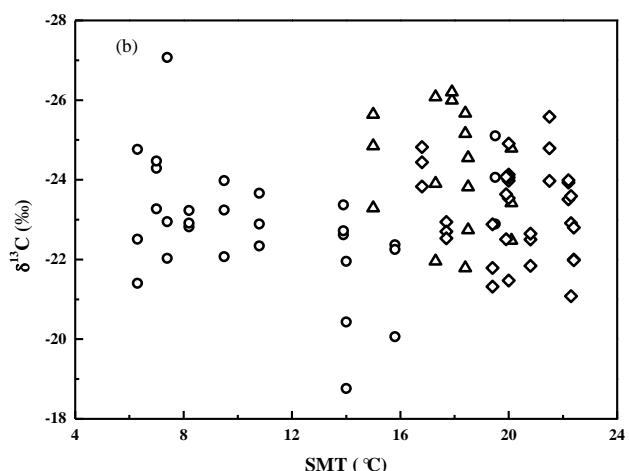
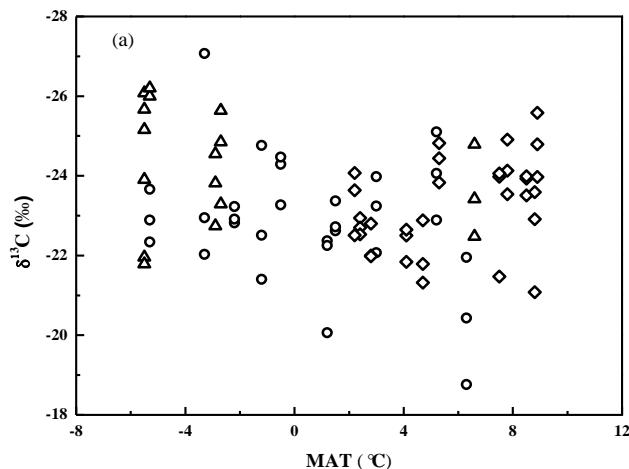
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640 Fig.2 shows the variance in surface soil  $\delta^{13}\text{C}$  with MAT(a) and SMT (b) along the 400 mm isoline  
 641 in China. Circle represents alpine and subalpine; diamond indicates temperate steppe and  
 642 grassland; triangle is coniferous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

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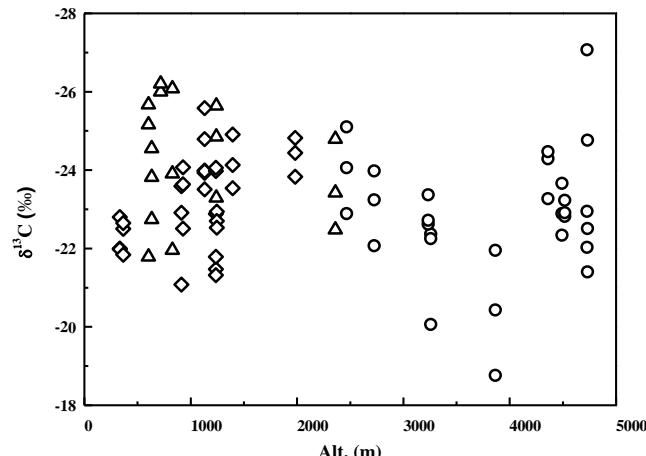
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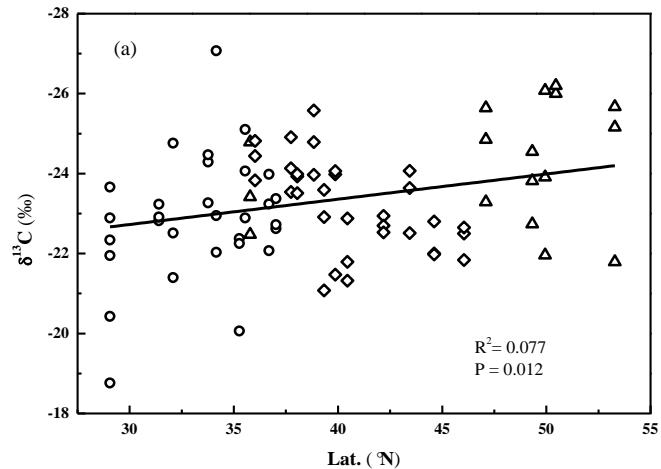
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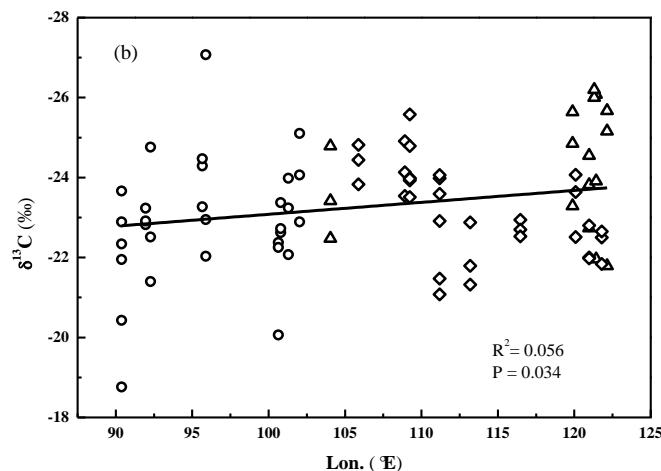
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662 Fig.3 shows the variance in surface soil  $\delta^{13}\text{C}$  with altitude.  
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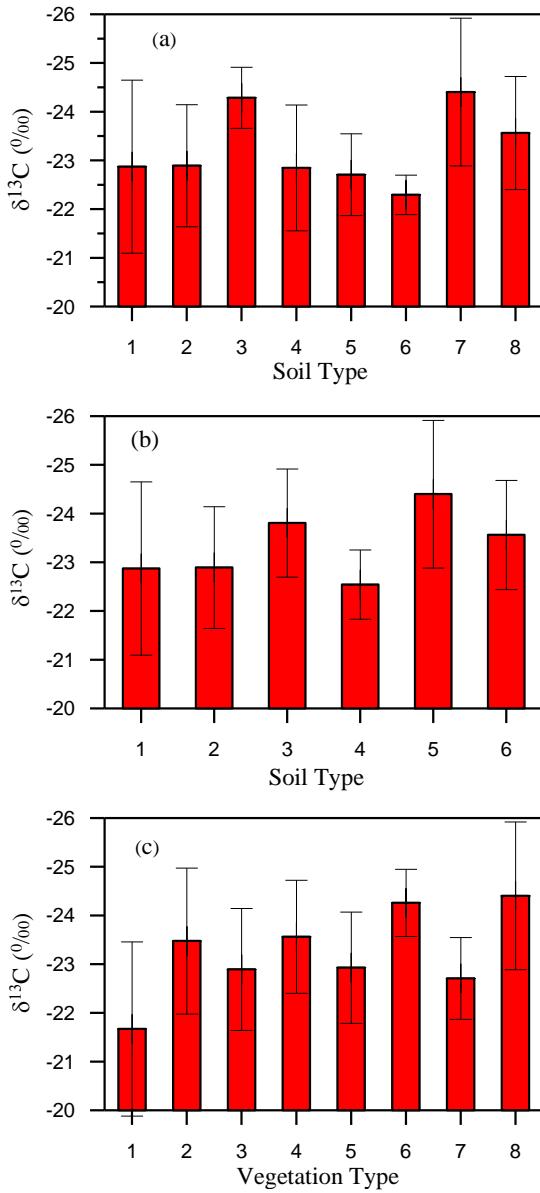
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Fig.4 shows the relationships between the soil  $\delta^{13}\text{C}$  and latitude (a) and longitude (b).

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695 Fig.5 shows the effects of soil types and vegetation types on the soil  $\delta^{13}\text{C}$ . (a.) soil types based on  
 696 Chinese soil taxonomy. 1. Matti-GelicCambosols; 2. Hapli- CryicAridosols;  
 697 3.Calci-OrthicAridosols; 4.MottlicCalci-OrthicAridosols; 5.TypicCalci-UsticIsohumosols;  
 698 6.Pachi-UsticIsohumosols; 7.Umbri-GelicCambosols; 8.Hapli-UsticArgosols.(b.)soil types based  
 699 on WRB. 1.Cambisols; 2. Leptosols; 3.Calcisols; 4.Chernozems; 5.Umbrisols; 6.Luvisols.(c.)  
 700 vegetation type. 1. Alpine grassland; 2. Alpine meadow; 3.Subalpine grassland; 4.Temperate  
 701 coniferous and broad-leaved mixed forests; 5.Temperate meadow steppe; 6.Semi-desert grasslands;  
 702 7.Temperate typical steppe; 8.Frigid temperate coniferous forest. The bar in Fig.5 indicates  $\pm 1\text{SD}$ .  
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Table 1 Information of the sampling sites

No.	Site name	Mean									
		MAT/°C	SMT/°C	MAP/mm	Alt./m	Lat./N °	Lon./E °	$\delta^{13}\text{C}$ (‰)	Vegetation type	Dominate species	Soil types
1	LangKaZi	-5.3	10.8	376	4492	29.06	90.39	-23.0	Alpine grassland	<i>Stipa</i> , <i>Festuca</i> and <i>Carex</i>	Matti-GelicCambosols (Cambisols)
2	RiKaZe	6.3	14	420	3865	29.33	88.98	-20.4	Alpine grassland	<i>Stipa</i> , <i>Festuca</i> and <i>Carex</i>	Matti-GelicCambosols (Cambisols)
3	NaQu	-2.2	8.2	406	4519	31.41	91.96	-23.0	Alpine meadow	<i>Kobresia</i>	Matti-GelicCambosols (Cambisols)
4	NieRong	-1.2	6.3	400	4731	32.09	92.27	-22.9	Alpine meadow	<i>Kobresia</i>	Matti-GelicCambosols (Cambisols)
5	ZhiDuo	-0.5	7	394	4360	33.77	95.66	-24.0	Alpine meadow	<i>Kobresia</i>	Matti-GelicCambosols (Cambisols)
6	QuMaLai	-3.3	7.4	391.7	4727	34.16	95.9	-24.0	Alpine meadow	<i>Kobresia</i>	Matti-GelicCambosols (Cambisols)
7	TongDe	1.2	15.8	371	3258	35.27	100.64	-21.6	Subalpine grassland	<i>Stipa</i> and <i>Hippolytia</i>	Hapli- CryicAridosols1 (Leptosols)
8	TongRen	5.2	19.5	425.7	2467	35.55	102.03	-24.0	Subalpine grassland	<i>Stipa</i> and <i>Hippolytia</i>	Hapli- CryicAridosols1 (Leptosols)
9	HuangYuan	3	13.9	408.9	2725	37.02	100.8	-22.9	Subalpine grassland	<i>Stipa</i> and <i>Hippolytia</i>	Hapli- CryicAridosols1 (Leptosols)
10	HaiYan	1.5	9.5	400	3233	36.69	101.3	-23.1	Subalpine grassland	<i>Stipa</i> and <i>Hippolytia</i>	Hapli- CryicAridosols1 (Leptosols)
									Temperate coniferous and broad-leaved mixed forests		
11	YuZhong	6.6	20.1	403	2361	35.78	104.05	-23.6		<i>Pinustabulaeformis</i>	Hapli-UsticArgosols (Luvisols)
12	XiJi	5.3	16.8	400	1982	36.02	105.88	-24.4	Temperate meadow steppe	<i>Stipa</i> and <i>Hippolytia</i>	Calci-OrthicAridosols(Calcisols)
13	JingBian	7.8	20	395.4	1394	37.74	108.91	-24.2	Semi-desert grasslands	<i>Stipa</i> , <i>Hippolytia</i> and <i>Ajania</i>	Calci-OrthicAridosols(Calcisols)
14	HengShan	8.5	22.2	397	1131	38.04	109.24	-23.8	Semi-desert grasslands	<i>Stipa</i> , <i>Hippolytia</i> and <i>Ajania</i>	Calci-OrthicAridosols(Calcisols)
15	ShenMu	8.9	21.5	393	1131	38.84	110.44	-24.8	Semi-desert grasslands	<i>Stipa</i> , <i>Hippolytia</i> and <i>Ajania</i>	Calci-OrthicAridosols(Calcisols)
16	HeQu	8.8	22.3	426	912	39.33	111.19	-22.5	Temperate meadow steppe	<i>Bothriochloa</i> and <i>Pennisetum</i>	MottlicCalci-OrthicAridosols(Calcisols)
17	ZhunGeErQi	7.5	20	400	1236	39.87	111.18	-23.2	Temperate meadow steppe	<i>Stipa</i> and <i>Aneuralepidium</i>	MottlicCalci-OrthicAridosols(Calcisols)
18	FengZhen	4.7	19.4	413	1236	40.45	113.19	-22.0	Temperate typical steppe	<i>Stipa</i> and <i>Aneuralepidium</i>	TypicCalci-UsticIsohumosols (Chernozems)
19	DuoLun	2.4	17.7	407	1245	42.18	116.47	-22.7	Temperate typical steppe	<i>Stipa</i> and <i>Aneuralepidium</i>	TypicCalci-UsticIsohumosols (Chernozems)
20	LinXi	2.2	19.9	370	928	43.44	110.08	-23.4	Temperate typical steppe	<i>Stipa</i> and <i>Aneuralepidium</i>	TypicCalci-UsticIsohumosols (Chernozems)
21	ZhaLuTeQi	2.8	22.4	387	332	44.61	120.97	-22.3	Temperate meadow steppe	<i>Stipa</i> , <i>Aneuralepidium</i> and	Pachi-UsticIsohumosols (Chernozems)

22	WuLanHaoTe	4.1	20.8	416	366	46.05	121.79	-22.3	Temperate meadow steppe	<i>Filifolium</i>	<i>Stipa, Aneuralepidium and</i>		
									Frigid temperate coniferous	<i>Filifolium</i>	Pachi-UsticIshumosols (Chernozems)		
23	AErShan	-2.7	15	391	1240	47.1	119.89	-24.6	forest	<i>Larixgmelinii</i>	and <i>Betulaplatyphylla Suk</i>	Umbri-GelicCambosols (Umbrisols)	
									Frigid temperate coniferous	<i>Larixgmelinii</i>			
24	YaKeShi	-2.9	18.5	379	634	49.33	120.97	-23.7	forest	<i>Larixgmelinii</i>		Umbri-GelicCambosols (Umbrisols)	
									Frigid temperate coniferous	<i>Larixgmelinii</i>			
25	KuDuEr	-5.5	17.3	402	829	49.94	121.43	-24.0	forest	and <i>Betulaplatyphylla Suk</i>		Umbri-GelicCambosols (Umbrisols)	
									Frigid temperate coniferous	<i>Betulaplatyphylla Suk</i>			
26	GenHe	-5.3	17.9	424	718	50.46	121.31	-26.1	forest	<i>Larixgmelinii and</i>		Umbri-GelicCambosols (Umbrisols)	
									Frigid temperate coniferous	<i>Pinussylvestnisvar</i>			
27	BeiJicun	-5.5	18.4	450.8	603	53.29	122.15	-24.2	forest			Umbri-GelicCambosols (Umbrisols)	

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706 Note: MAT, SMT, MAP, Alt, Lat and Lon are the abbreviations of mean annual temperature, summer mean temperature, mean annual precipitation, altitude, latitude,  
 707 longitude, respectively. Longitude, latitude and altitude of each site were from the portable GPS; MAT and MAP represent the average values of more than 30 years,  
 708 SMT presents the average value of June, July and August for more than 30 years. All climatic data were from the local meteorological stations and the China  
 709 Meteorological Data Sharing Service System (<http://cdc.cma.gov.cn/shishi/climate.jsp>); The soil types are based on Chinese soil taxonomy and WRB (in the  
 710 brackets).

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723 Table 2 shows the results from multiple regressions.

Model	R <sup>2</sup>	Adjusted R <sup>2</sup>	F	p-value
1	0.091	0.030	1.484	0.205
2	0.374	0.273	3.690	<0.001
3	0.297	0.195	2.911	0.004
4	0.362	0.247	3.164	0.001

724 Note: Model-1 is the multiple regression of soil  $\delta^{13}\text{C}$  against MAT, MAP, altitude, latitude and longitude; For Model-2, Model-3 and Model-4, in  
725 addition to taking these five environmental factors as independent variables, the soil types based on Chinese nomenclature and WRB, and the  
726 vegetation types as dummy variables were separately introduced in the multiple regressions.

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