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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 C₄ 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

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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~~ 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 $\delta^{13}\text{C}$. ~~T~~ 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, ~~One~~ one-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 ~~mentioned~~ 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 ~~regression~~ regressions 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 paleovegetation and paleoenvironments, 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 paleovegetation
143 and paleoprecipitation. 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 palaeoprecipitation 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 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 paleotemperature
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_3 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_2 concentration,
176 | altitude, latitude and longitude; may also ~~influence $\delta^{13}\text{C}$~~ ~~exert influences on~~ ~~variance~~ 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}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}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 °C
213 ~~°C and while~~ ShenMu ~~has had~~ the highest MAT of +8.9 °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 ~~is~~ 90% rainfall occurs in the summer season (from May to October).

220

221 Fig.1

222 Table 1

223 2.2 ~~soil~~-Soil sampling

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

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~~quadrates (0.5 m×0.5 m) within 200 m² to collect surface mineral

228 soil (0-5 cm) using a ring knife. The O-horizon, including litters, moders and mors

229 ~~were~~-was removed before collecting mineral soil. About 10 g ~~of~~ air dried soils ~~were~~

230 ~~was~~ 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^{Plus}-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°C.

239 The carbon isotopic ratios are reported in delta notation relative to the V-PDB

240 standard using the equation:

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

242 where $\delta^{13}\text{C}$ is the carbon isotope ratio of the sample (‰), ~~and~~ R_{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,

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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 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 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 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 and the vegetation
287 variables 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 ~~8-eight~~ 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 C_3 plants vary between -22‰ and -34‰

310 with a mean of -27‰ , and C_4 plants range from -9‰ to -19‰ with a mean of
311 -13‰ (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 ^{13}C -enrichment in soil organic matter compared ~~with~~
314 ~~to~~ the plant sources (Nadelhoffer, 1988; Balesdent et al., 1993; Ågren et al., 1996;
315 Fernandez et al., 2003; Wynn et al., 2005; Wynn, 2007). An intensive investigation of
316 isotope fractionation during organic matter decomposition, which was conducted in
317 Mount Gongga, an area ~~in~~ of the Qinghai-Tibetan Plateau dominated by C_3 vegetation
318 with herbs, shrubs and trees, showed that the mean ^{13}C -enrichment in surface soil (0-5
319 cm depth) relative to the vegetation was 2.87‰ (Chen et al., 2009). Another
320 investigation of 13 soil profiles from the Tibetan Plateau and north China showed ~~that~~
321 the $\delta^{13}\text{C}$ difference between surface soil (0-5 cm depth) and the original biomass
322 varied from 0.6 to 3.5‰ with a mean of 1.8‰ (Wang et al., 2008). Thus, the $\delta^{13}\text{C}_{\text{SOM}}$
323 ~~dataset data set of from~~ this study ($\delta^{13}\text{C}_{\text{SOM}}$ ranges from -20.4‰ to -27.1‰) indicates
324 that the modern terrestrial ecosystem along the isohyet is greatly dominated by C_3
325 plants. This result is consistent with the observations of vegetation along the isohyet
326 ~~done completed~~ in our previous study (Wang et al., 2013) ~~and in this the present study.~~
327 ~~Yin and Li (1997), Lu et al. (2004) and Wang et al. (2004) have reported that a small~~
328 ~~number of C_4 species occurred in the Qinghai Tibetan Plateau; however, in this~~
329 ~~present study we found no C_4 plants in the Qinghai Tibetan Plateau.~~ We are ~~also very~~
330 surprised ~~at~~ by such high soil $\delta^{13}\text{C}$ values ~~occurring~~ at RiKaZe (site Site 2) (Fig. 3 and
331 Table 1) because only four C_3 plants ~~grow~~ there, ~~and there were while,~~ no C_4 species.

332 ~~The~~ This abnormal observation suggests that a very high carbon isotope fractionation
333 with SOM degradation ~~have taken place~~ 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}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_4 plant growth.~~
340 ~~But we observed that a small number of C_4 species occur only in the temperate~~
341 ~~meadow steppe and the temperate typical steppe in north China, while no C_4 species~~
342 ~~are distributed in the coniferous forests in north China. In short, the contribution of C_4~~
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_3 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°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_3 plants, the induced variance in soil $\delta^{13}\text{C}$ by plant $\delta^{13}\text{C}$ also cannot be

354 very ~~great~~high. On the other hand, although the ^{13}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 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~~only soil with
367 pure C_3 plants (MAT is below 16 ~~$^{\circ}\text{C}$~~), 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 ~~$^{\circ}\text{C}$~~ , the C_4 contribution to productivity in Australian grasslands is
370 negligible, whereas above 23 ~~$^{\circ}\text{C}$~~ , C_3 contribution is negligible. Between 14 ~~$^{\circ}\text{C}$~~
371 and 23 ~~$^{\circ}\text{C}$~~ , soil $\delta^{13}\text{C}$ is positively correlated with MAT, indicating 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 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 C₃ plants, temperature does not influence soil δ¹³C
378 variance.

379 ~~This study shows that the contribution of precipitation to the variability in soil δ¹³C~~
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 δ¹³C does not mean no moisture control of~~
383 ~~the soil δ¹³C. 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 δ¹³C. 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 δ¹³C is negligible.

392 Although stepwise regression and correlation analysis both show a significant
393 influence of latitude on soil δ¹³C, the five environmental variables including latitude
394 were responsible for only 9% of the variability in soil δ¹³C in a multiple regression
395 model (Table 2), suggesting that the contribution of latitude to soil δ¹³C was also
396 slight limited. This study shows a negative correlation between latitude and δ¹³C_{SOM}
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, α , increases
418 increased with litter quality (Wang et al., 2015). Thus, the isotope fractionation factor
419 should ~~be different~~ differ 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 ~~of~~ on 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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458

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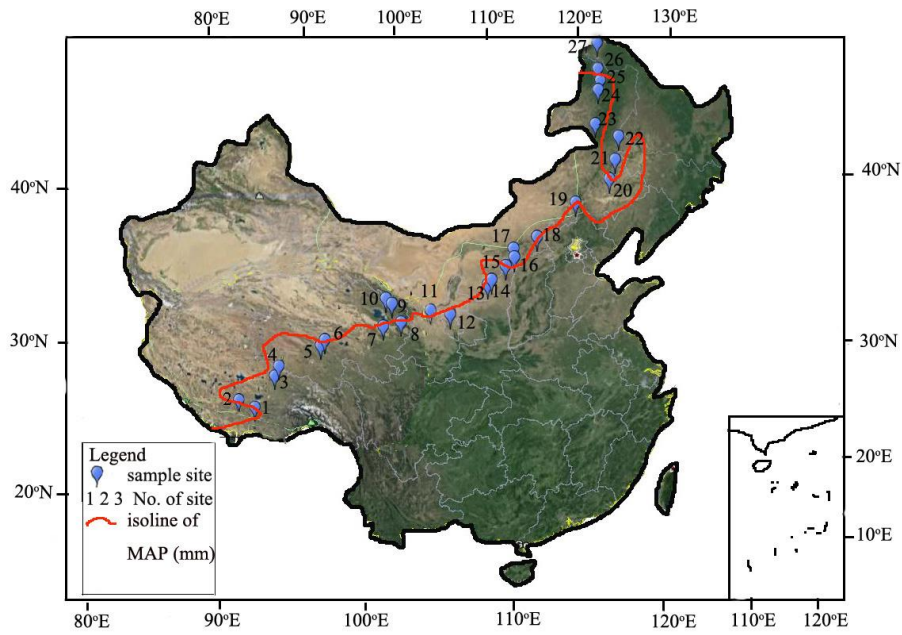
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614 **Figures**

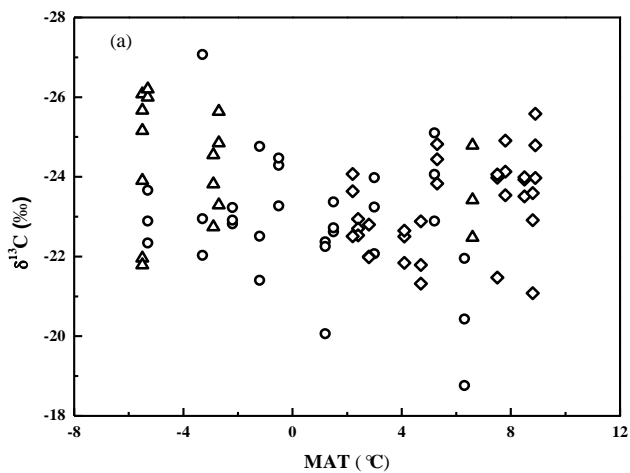
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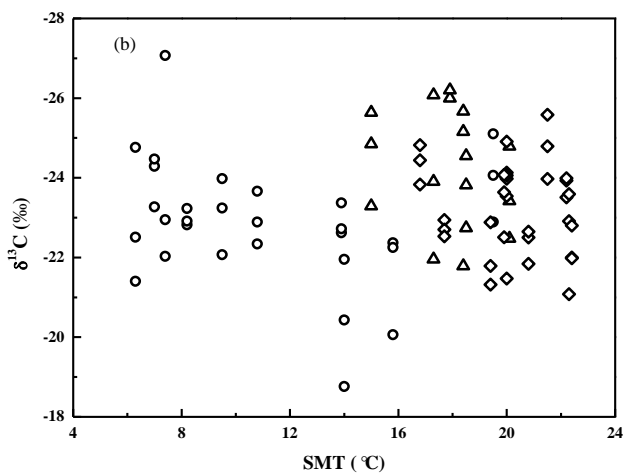


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Fig.1. Sketch of sampled region. Sample sites are indicated with numbers. 1, LangKaZi; 2,RiKaZe; 3,NaQu; 4,NieRong; 5,ZhiDuo; 6,QuMaLai; 7,TongDe; 8,TongRen; 9,HuangYuan; 10,HaiYan; 11,YuZhong; 12,XiJi; 13,JingBian; 14,HengShan; 15,ShenMu; 16,HeQu;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



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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 isohaline
 641 in China. Circle represents alpine and subalpine; diamond indicates temperate steppe and
 642 grassland; triangle is coniferous forest.

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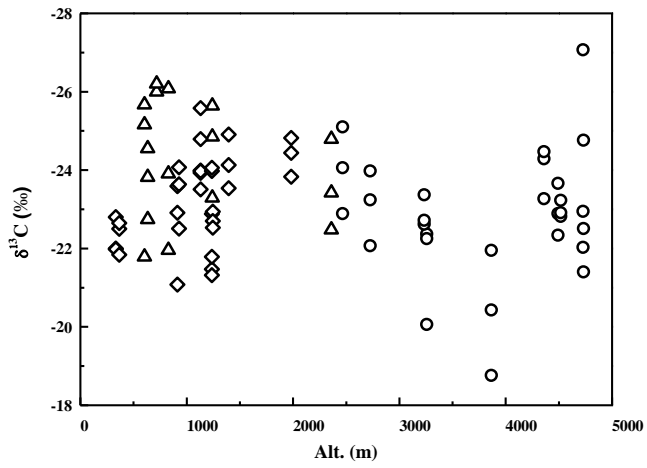
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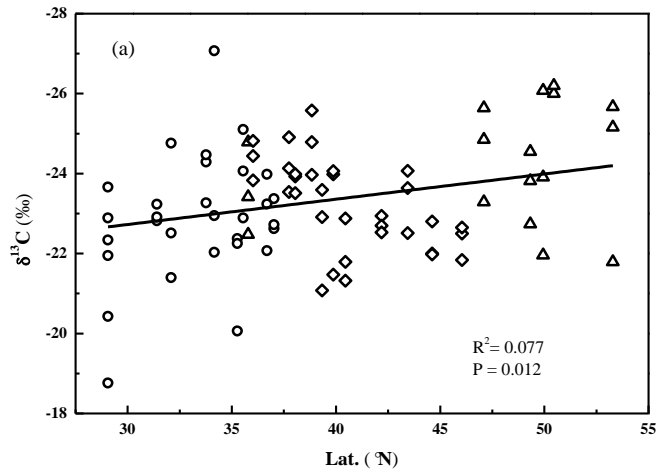
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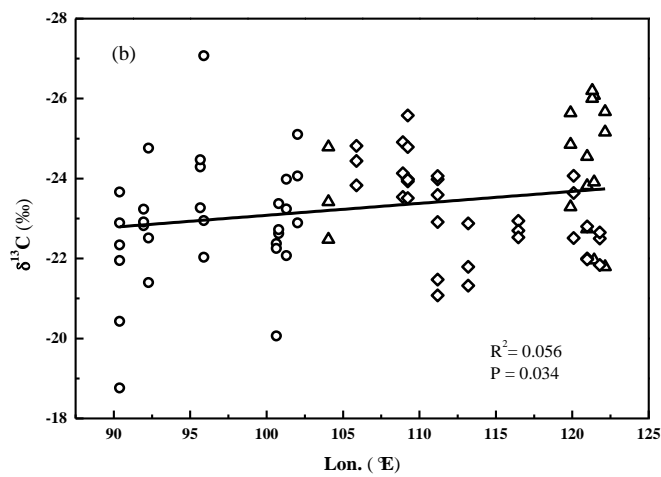
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Fig.3 shows the variance in surface soil $\delta^{13}\text{C}$ with altitude.

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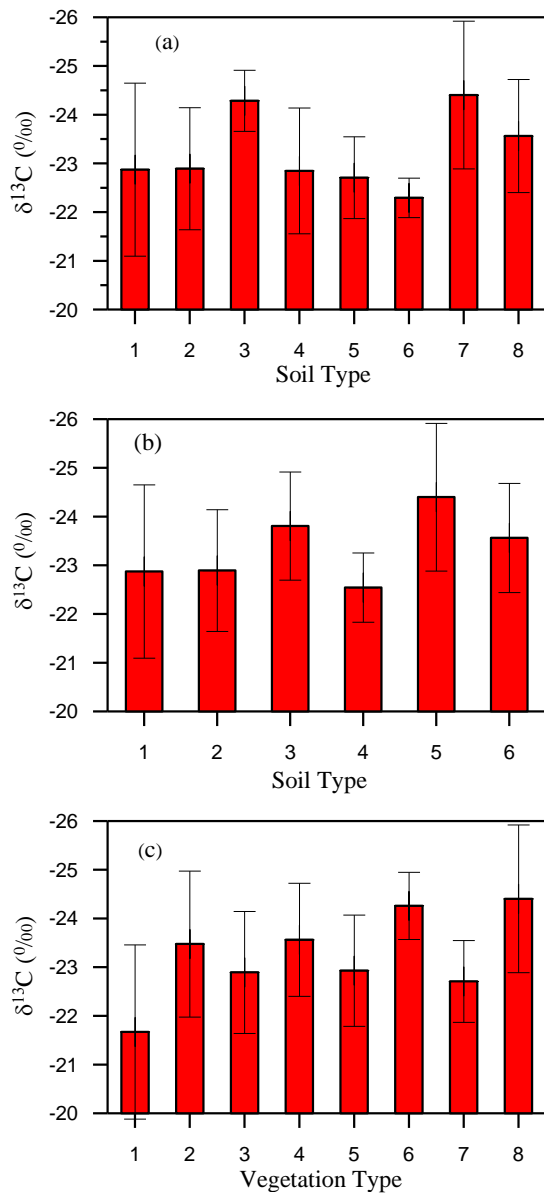
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Fig.4 shows the relationships between the soil $\delta^{13}\text{C}$ and latitude (a) and longitude (b).



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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- CryicAridosolsl;

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	MAT/°C	SMT/°C	MAP/mm	Alt./m	Lat./N°	Lon./E°	Mean	Vegetation type	Dominate species	Soil types
								δ ¹³ C (‰)			
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- CryicAridosolsI (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- CryicAridosolsI (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- CryicAridosolsI (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- CryicAridosolsI (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>	Pachi-UsticIsohumosols (Chernozems)
23	AErShan	-2.7	15	391	1240	47.1	119.89	-24.6	Frigid temperate coniferous forest	<i>Larixgmelinii</i> and <i>Betulaplatyphylla Suk</i>	Umbri-GelicCambosols (Umbrisols)
24	YaKeShi	-2.9	18.5	379	634	49.33	120.97	-23.7	Frigid temperate coniferous forest	<i>Larixgmelinii</i>	Umbri-GelicCambosols (Umbrisols)
25	KuDUEr	-5.5	17.3	402	829	49.94	121.43	-24.0	Frigid temperate coniferous forest	<i>Larixgmelinii</i> and <i>Betulaplatyphylla Suk</i>	Umbri-GelicCambosols (Umbrisols)
26	GenHe	-5.3	17.9	424	718	50.46	121.31	-26.1	Frigid temperate coniferous forest	<i>Betulaplatyphylla Suk</i>	Umbri-GelicCambosols (Umbrisols)
27	BeiJicun	-5.5	18.4	450.8	603	53.29	122.15	-24.2	Frigid temperate coniferous forest	<i>Larixgmelinii</i> and <i>Pinussylvestrisvar</i>	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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Table 2 shows the results from multiple regressions.

Model	R ²	Adjusted R ²	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

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 addition to taking these five environmental factors as independent variables, the soil types based on Chinese nomenclature and WRB, and the vegetation types as dummy variables were separately introduced in the multiple regressions.