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Temperature exerted no influence on the organic carbon isotope of surface soil along the isopleth of 400 mm mean annual precipitation in China

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Abstract

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

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1. Introduction

Global climate change has recently received a great deal of attention, and effective predictions of future climate change depend on the relevant information from climate in the geological past. Over recent decades, stable carbon isotopes in sediments, such as loess, paleosol, lacustrine and marine sediments, have been widely used to reconstruct paleovegetation and paleoenvironments, and provided important insights into patterns of past climate and environment changes. For examples, many researchers have used organic carbon isotopes of loess to reconstruct paleovegetation and paleoprecipitation. Vidic and Montañez (2004) conducted a reconstruction of paleovegetation at the central Chinese Loess Plateau during the Last Glaciation (LG) and Holocene by using the organic carbon isotopes in loess from Jiaodao, Shanxi Province. Hatté and Guiot (2005) carried out a palaeoprecipitation reconstruction by inverse modelling using the organic carbon isotopic signal of the Nuβloch loess sequence (Rhine Valley, Germany). Rao et al. (2013) reconstructed a high-resolution summer precipitation variations in the western Chinese Loess Plateau during the Last Glaciation using a well-dated organic carbon isotopic dataset. Yang et al. (2015) derived a minimum 300 km northwestward migration of the monsoon rain belt from the Last Glacial Maximum to the Mid-Holocene using the organic carbon isotopes from 21 loess sections across the Loess Plateau. However, to our knowledge, almost no researchers have conducted paleotemperature reconstructions using organic carbon isotope records of loess and paleosol, because it has been argued that temperature exerts slight, or even no influence on $\delta^{13}C_{SOM}$. While this statement may be likely, it needs to be demonstrated because only few studies have addressed the influence of temperature on organic carbon isotopes of modern surface soil; furthermore, these studies do not appear to result in a conclusive statement. Lee et al. (2005) and Feng et al. (2008) both reported no relationship between temperature and surface soil $\delta^{13}C$ in central-east Asia. However, Lu et al. (2004) discovered a nonlinear relationship between annual mean temperature (MAT) and $\delta^{13}C_{SOM}$ from the Qinghai-Tibetan Plateau; Sage et al. (1999) compiled the data from Bird and Pousai (1997) and also found a nonlinear trend for the variation in $\delta^{13}C_{SOM}$ along a temperature gradient in Australian grasslands and savannas.

Plant residues are the most important source of soil organic matter. $\delta^{13}C_{SOM}$ is generally close to plant carbon isotope despite isotopic fractionation during decomposition of organic matter (Nadelhoffer and Fry, 1988; Balesdent et al., 1993; Ågren et al., 1996; Fernandez et al., 2003; Wynn, 2007). Thus, the influential factors of plants $\delta^{13}C$ might also play a role in $\delta^{13}C_{SOM}$, $\delta^{13}C$ in plants, especially C_3 plants, is tightly associated with precipitation (Diefendorf et al., 2010; Kohn, 2010), so, precipitation should have influence on soil $\delta^{13}C$. In addition to effect of precipitation, many factors, such as temperature, air pressure, atmospheric CO_2 concentration, altitude, latitude and longitude, may also exert influences on variance in plants $\delta^{13}C$ (Körner et al., 1991; Hultine and Marshall, 2000; Zhu et al., 2010; Xu et al., 2015). Although patterns of variation in plants $\delta^{13}C$ with temperature are unresolved so far (e.g. Schleser et al., 1999; McCarroll and Loader, 2004; Treydte et al., 2007; Wang et al., 2013), it has been widely accepted that, even if temperature has effect on plants

δ^{13}C, this effect is slight. So, if the ^{13}C enrichment during SOM decomposition is a constant value, we expect a slight or no influence of temperature on soil δ^{13}C. However, the fact is that this ^{13}C-enrichment is affected by environmental and biotic factors (Wang et al., 2015). Thus, it is difficult to expect whether or how temperature affects soil δ^{13}C, and it needs specific investigations of focusing on this issue. Although the relationship between temperature and $\delta^{13}C_{SOM}$ has been investigated in these previous studies mentioned above, these studies were unable to effectively separate the influence of temperature from the effect of precipitation. Thus, new investigations are necessary. The present study includes an intensive investigation of the variation in $\delta^{13}C_{SOM}$ with temperature across a temperature gradient along the 400 mm isohyet (isopleth of mean annual precipitation - MAP) in China. We sampled surface soil along the specific isohyet to minimize the effect of precipitation changes on $\delta^{13}C_{SOM}$.

In addition, there are no meteorological stations near most of the sampling sites in the previous studies mentioned above; thus, they had to interpolate meteorological data, which could be unrealistic in regions with strong topographical variability. This interpolation could produce errors in the relationships between temperature and $\delta^{13}C_{SOM}$ established in these studies. In the present investigation, we collected samples only at those sites with meteorological stations; thus, the climatic data that we obtained from these stations are probably more reliable compared with the pseudo-data derived by interpolation.

2. Materials and methods

2.1. Study site

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

Fig.1

Table 1

2.2 soil sampling

Soil samples were collected in the summer of 2013 between July 12th and August 30th. In order to avoid disturbance of human activities, sample sites are 5-7 kilometers far from the towns where the meteorological stations are located. We set three quadrats (0.5 m×0.5 m) within 200 m^2 to collect surface mineral soil (0-5 cm) using a ring knife. The O-horizon, including litters, moders and mors were removed before collecting mineral soil. About 10 g air dried soils were sieved at 2 mm. Plant fragments and the soil fraction coarser than 2 mm were removed. The rest of the soil samples were immersed using excessive HCl (1 mol/L) for 24 h. In order to ensure that all carbonate was cleared, we conducted artificial stirring 4 times during the immersion. Then, the sample was washed to neutrality using distilled water. Finally it was oven-dried at 50℃ and ground. Carbon isotope ratios were determined on a Delta^{Plus}XP mass spectrometer (Thermo Scientific, Bremen, Germany) coupled with an elemental analyzer (FlashEA 1112; CE Instruments,Wigan, UK) in continuous flow mode. The elemental analyzer combustion temperature was 1020 °C.

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

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000 \qquad (1)$$

where $\delta^{13}C$ is the carbon isotope ratio of the sample (‰), and R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively. For this measurement, we obtained a standard deviation of less than 0.15‰ among replicate measurements of the same soil sample.

3. Results

Except for one $\delta^{13}C_{SOM}$ value (-18.8‰), all other data range from -20.4‰ to -27.1‰

with a mean value of -23.3‰ (n =80, s.d. =1.45). Multiple regression with MAT, MAP, altitude, latitude and longitude as independent variables, and $\delta^{13}C_{SOM}$ as the dependent variable, shows that only 9% of the variability in soil $\delta^{13}C$ can be explained as a linear combination of all five environmental factors ($p = 0.205$) (Table 2). Considering the possibility of correlations among the five explanatory variables, stepwise regression was used to eliminate the potential influence of collinearity among them. Variables were incorporated into the model with *P*-value < 0.05 and exclude with *P*-value > 0.1. Stepwise regression of soil $\delta^{13}C$ in the model consisting only of latitude ($R^2 = 0.077$, $p = 0.012$). In order to constrain the relationship between soil $\delta^{13}C$ and each environmental factor better, bivariate correlation analyses of soil $\delta^{13}C$ against some environmental factors were conducted. The bivariate correlation analyses show that $\delta^{13}C_{SOM}$ is not related to MAT ($p = 0.114$) or SMT ($p = 0.697$) along the isohyet (Fig. 2a, b). In addition, in order to determine further the response of $\delta^{13}C_{SOM}$ to temperature, we considered three subsets of our soil samples defined according to the climate, topography or vegetation type: the Qinghai–Tibetan Plateau (mainly alpine meadow, including 10 sites), steppe or grassland (11 sites) and coniferous forest (6 sites) (Table 1). Bivariate correlation analyses within these subsets also show no relationship between $\delta^{13}C_{SOM}$ and MAT for all categories. The correlation analysis of $\delta^{13}C_{SOM}$ vs. altitude is shown in Fig.3, which displays no relationship ($p = 0.132$). Although longitude is not found to exert influence on $\delta^{13}C_{SOM}$ in the above stepwise regression, bivariate correlation analyses show that latitude and longitude both are negatively related to $\delta^{13}C_{SOM}$ (p =0.012 and 0.034,

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respectively) (Fig. 4a,b).

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

In order to constrain further the effects of soil type and vegetation type on $\delta^{13}C_{SOM}$, multiple regressions with soil type and vegetation type as dummy variables were conducted. Considering the tight relationship between soil type and vegetation type, especially in Chinese soil taxonomy, the soil variable and the vegetation variable were separately introduced into the statistical analyses. Multiple regression, in which the above five explanatory environmental factors were taken as quantitative variables and the 8 soil types of the Chinese nomenclature as values of a dummy variable, shows that environmental factors and soil types in total account for 37.4% soil $\delta^{13}C$ variance ($p < 0.001$) (Table 2). 29.7% ($p = 0.003$) of the variability is explained using the 6 soil types based on WRB rather than the Chinese nomenclature (Table 2). Similarly, multiple regression with vegetation types as dummy variables shows that the five environmental factors and vegetation types in total can explain 36.2% of the

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variability in soil $\delta^{13}C$ (p = 0.001) (Table 2). Compared to the multiple regressions in which only quantitative environmental variables were introduced, the multiple regressions in which soil and vegetation were also introduced explain more variance, suggesting that soil type and vegetation type really played a significant role in $\delta^{13}C_{SOM}$ variability.

Table 2

Fig.2a, b

Fig.3

Fig.4a, b

Fig.5

4. Discussion

Soil $\delta^{13}C$ depends on plants $\delta^{13}C$ and carbon isotopic fractionation during organic matter decomposition. $\delta^{13}C$ values of C_3 plants vary between −22‰ and −34‰ with a mean of −27‰, and C_4 plants range from −9‰ to −19‰ with a mean of −13‰ (Dienes,1980). Carbon isotope fractionation occurs in the process of plant litter decomposition into soil organic matter in most environments, especially in non-arid environments, causing ^{13}C-enrichment in soil organic matter compared with the plant sources (Nadelhoffer, 1988; Balesdent et al., 1993; Ågren et al., 1996; Fernandez et al., 2003; Wynn et al., 2005; Wynn, 2007). An intensive investigation of isotope fractionation during organic matter decomposition, which was conducted in Mount Gongga, an area in the Qinghai-Tibetan Plateau dominated by C_3 vegetation with herbs, shrubs and trees, showed that the mean ^{13}C-enrichment in surface soil (0-5 cm depth) relative to the vegetation was 2.87‰ (Chen et al., 2009). Another investigation of 13 soil profiles from the Tibetan Plateau and north China showed the δ^{13}C difference between surface soil (0-5 cm depth) and the original biomass varied from 0.6 to 3.5‰ with a mean of 1.8‰ (Wang et al., 2008). Thus, the $\delta^{13}C_{SOM}$ data set of this study ($\delta^{13}C_{SOM}$ ranges from -20.4‰ to -27.1‰) indicates that the modern terrestrial ecosystem along the isohyet is greatly dominated by C_3 plants. This result is consistent with the observations of vegetation along the isohyet done in our previous study (Wang et al., 2013) and in this present study. Yin and Li (1997), Lu et al. (2004) and Wang et al. (2004) have reported that a small number of C_4 species occurred in the Qinghai-Tibetan Plateau; however, in this present study we found no C_4 plants in the Qinghai-Tibetan Plateau. We are also very surprised at such high soil δ^{13}C values at RiKaZe (site 2) (Fig.3 and Table 1) because only four C_3 plants grow there, no C_4 species. The abnormal observation suggests that a very high carbon isotope fractionation with SOM degradation have taken place in the local ecosystem. Although the mechanism accounted for the unusually high isotopic fractionation remains unclear, it is not surprising. For example, Wynn (2007) has reported that the fractionation leaved soil organic carbon ^{13}C-enriched by up to ~6‰ with respect to the original biomass. Rao et al. (2008) has suggested that mid-latitude area (31 °N-40 °N) in east China provides relatively favorable condition for C_4 plant growth. But we observed that a small number of C_4 species occur only in the temperate meadow steppe and the temperate typical steppe in north China, while no C_4 species are distributed in the coniferous forests in north China. In short, the contribution of C_4 biomass to the local vegetation along the isohyet is very low, and can be neglected.

The MAT, MAP, altitude, latitude and longitude, combined, are responsible for only 9% variability in soil $\delta^{13}C$ in the multiple regression model, suggesting that the contribution of the five environmental factors to the soil $\delta^{13}C$ variance is very small. Our previous study conducted along the isohyet resulted in a strong positive relationship between C_3 plant $\delta^{13}C$ and MAT with a coefficient of 0.104‰/^{o}C (Wang et al., 2013). The difference between maximum and minimum temperature along the isohyet is 15^{o}C, so the greatest possible effect of temperature on plant $\delta^{13}C$ along the temperature gradient is 1.56‰, which is not very great. Since the main source of soil organic matter along the isohyet is C_3 plants, the induced variance in soil $\delta^{13}C$ by plant $\delta^{13}C$ also cannot be very great. On the other hand, although the ^{13}C-enrichment with SOM degradation follows a Rayleigh distillation process (Wynn, 2007), our recent study shows that temperature does not influence carbon isotopic fractionation during decomposition (Wang et al., 2015), which is also a reason for the lack of a relationship between soil $\delta^{13}C$ and temperature. Feng et al. (2008) and Lee et al. (2005) respectively, reported no relationships between soil $\delta^{13}C$ and MAT and SMT, which is consistent with our result. Their field campaigns were conducted in central Asia, which is also dominated by C_3 plants, similar to the area along the 400 mm isohyet. This is the reason why the same pattern exists in central Asia and the area along the 400 mm isohyet.

The observations in Bird and Pousai (1997) and Sage et al. (1999) appear to be inconsistent with our findings; they found a nonlinear relationship between soil $\delta^{13}C$ and MAT in Australian grasslands. However, if considering only soil with pure C_3 plants (MAT is below 16℃), soil $\delta^{13}C$ and temperature are not related in Australian grasslands, which is in agreement with our result. Below15℃, C_4 contribution to productivity in Australian grasslands is negligible, whereas above 23 ℃, C_3 contribution is negligible; Between 14℃ and 23℃, soil $\delta^{13}C$ is positively correlated with MAT, indicating C_4 representation increasing with MAT (Sage et al., 1999). Lu et al. (2004) also reported a nonlinear relationship between soil $\delta^{13}C$ and MAT. Similarly, if the soil data with C_4 plants are excluded from the nonlinear correlation, soil $\delta^{13}C$ is also not related to MAT in Lu et al. (2004) (see Fig.5b in Lu et al., 2004). Thus, this present study and the previous observations are consistent in showing that in a terrestrial ecosystem in which the vegetation is dominated by C_3 plants, temperature does not influence soil $\delta^{13}C$ variance.

This study shows that the contribution of precipitation to the variability in soil $\delta^{13}C$ is neglected. The reason for this is that the soil was sampled along the 400 mm isohyet, and the MAP difference among sites is very small. It should be pointed out here that the no MAP influence on the soil $\delta^{13}C$ does not mean no moisture control of the soil $\delta^{13}C$. Because the temperature varies greatly across the temperature gradient although the MAP is almost the same for each sampling site ; this would cause a big difference in relative humidity among sites. We expect that relative humidity would explain a great variability in soil $\delta^{13}C$. But we did not take relative humidity as an explanatory variable in the statistical analyses, because we lack the complete data of

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relative humidity, and we do not want to use the pseudo-data derived by interpolation.

Although stepwise regression and correlation analysis both show a significant influence of latitude on soil δ^{13}C, the five environmental variables including latitude were responsible for only 9% variability in soil δ^{13}C in a multiple regression model (Table 2), suggesting that the contribution of latitude to soil δ^{13}C was also slight. This study shows a negative correlation between latitude and $\delta^{13}C_{SOM}$ ($p=0.012$). Bird and Pausai (1997) and Tieszen et al. (1979) reported a similar pattern. Latitude is a comprehensive environmental factor, and change in latitude can bring about changes in other environmental factors, such as temperature, irradiation, cloud amount, and moisture, but temperature or irradiation should be most strongly related to latitude, and obviously change with latitude. The observed significant relationship between latitude and soil δ^{13}C (Fig.4a) suggests that environmental factors other than temperature might contribute more or less to the variance in soil δ^{13}C.

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

Substrate quality partly quantifies how easily organic carbon is used by soil microbes (Poage and Feng, 2004). It can be related to plant type and is often defined using a C/N ratio, lignin content, cellulose content, and/or lignin content/N ratio (Melillo et al., 1989; Gartern et al., 2000). Our study in Mount Gongga, China, showed that litter quality play a significant role in isotope fractionation during organic matter decomposition, and the carbon isotope fractionation factor, α, increases with litter quality (Wang et al., 2015). Thus, the isotope fractionation factor should be different among varying sites because litter quality is dependent on vegetation and this makes soil change its δ^{13}C with vegetation type.

Control of soil type on soil δ^{13}C could be associated with the effect of soil type on isotope fractionation during organic matter decomposition, and involve at least two mechanisms. (1) Properties and compositions of microbial decomposer communities are dependent on soil type (Gelsomino et al., 1999). Different microbes could have different metabolic pathways even when they decompose the same organic compound (Macko and Estep, 1984), and the extent of isotope fractionation during decomposition may be tightly related to the metabolic pathways of microbes (Macko and Estep, 1984). For example, Morasch et al. (2001) observed a greater hydrogen isotope fractionation for toluene degradation in growth experiments with the aerobic bacterium *P. putida* mt-2 and less fractionation in toluene degradation by anaerobic bacteria. (2) Physical and chemical properties, such as pH, particle size fraction, water-holding capacity, display striking differences among soil types and this causes organic compounds to be decayed at different rate in different soil environments. The magnitude of isotope fractionation during decomposition is linked to degree of organic matter decomposition (Feng, 2002), thus, soil type plays a significant role in fractionation.

5. Conclusions

The present study measured organic carbon isotopes in surface soil along a 400 mm isohyet of mean annual precipitation in China, and observed that soil type and vegetation type both had significant influence on soil organic carbon isotopes. However, temperature is found to have no observable impact on $\delta^{13}C_{SOM}$, suggesting that $\delta^{13}C$ signals in sediments cannot be used for the reconstruction of temperature, and that the effect of temperature on $\delta^{13}C_{SOM}$ could be neglected in the reconstruction of paleoclimate and paleovegetation using carbon isotopes of soil organic matter.

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Figures

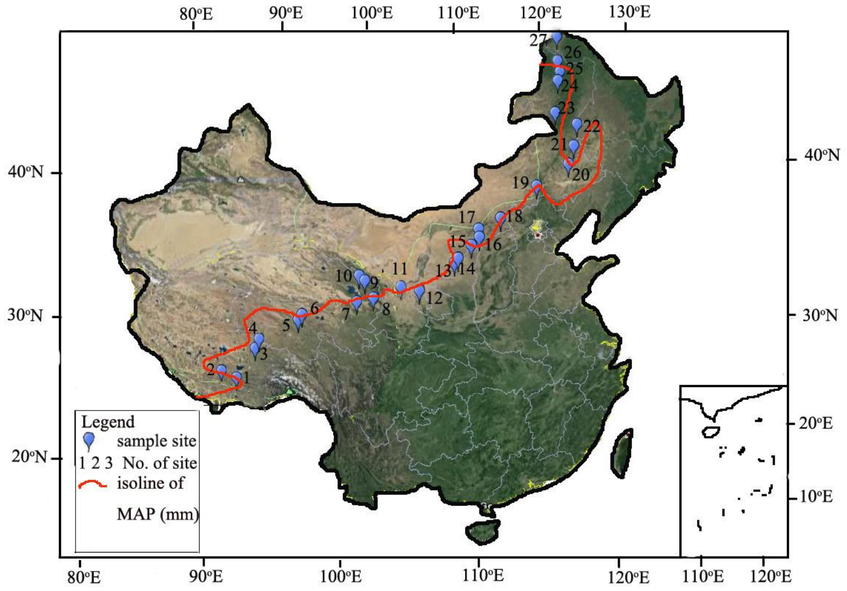

Fig.1. Sketch of sampled region. Sample sites are indicated with numbers. 1, LangKaZi; 2, RiKaZe; 3, NaQu; 4, NieRong; 5, ZhiDuo; 6, QuMaLai; 7, TongDe; 8, TongRen; 9, HuangYuan; 10, HaiYan; 11, YuZhong; 12, XiJi; 13, JingBian; 14, HengShan; 15, ShenMu; 16, HeQu; 17, ZhunGeErQi; 18, FengZhen; 19, DuoLun;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

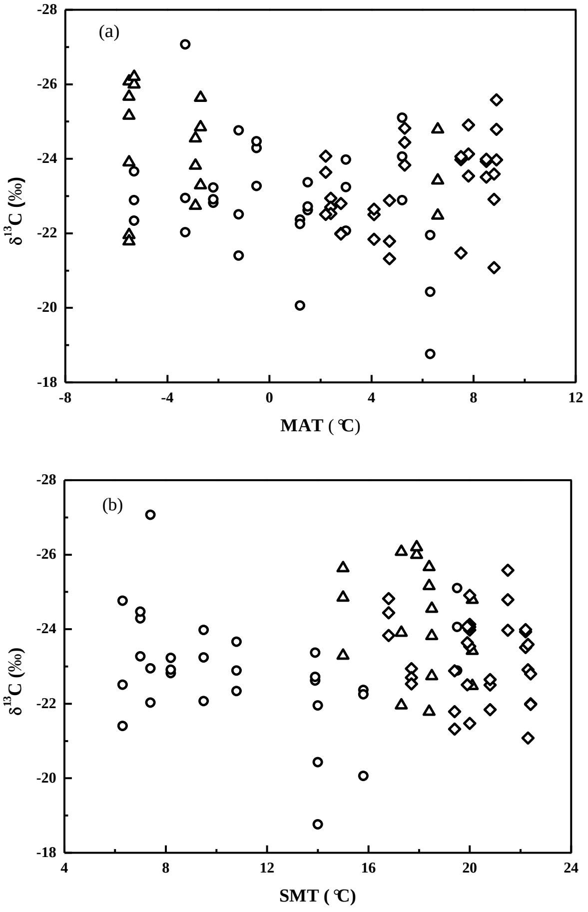

Fig.2 shows the variance in surface soil $\delta^{13}C$ with MAT (a) and SMT (b) along the 400 mm isoline in China. Circle represents alpine and subalpine; diamond indicates temperate steppe and grassland;, triangle is coniferous forest.

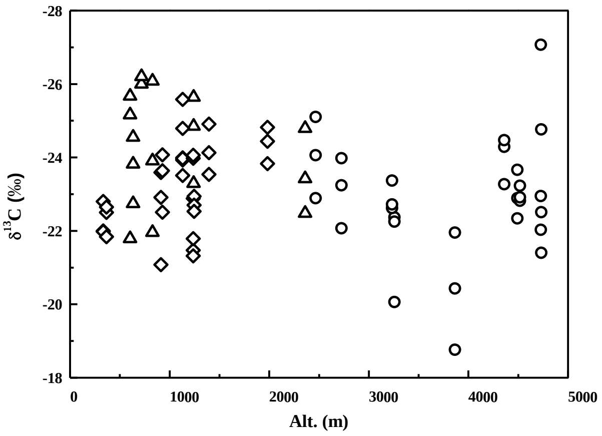

Fig.3 shows the variance in surface soil $\delta^{13}C$ with altitude.

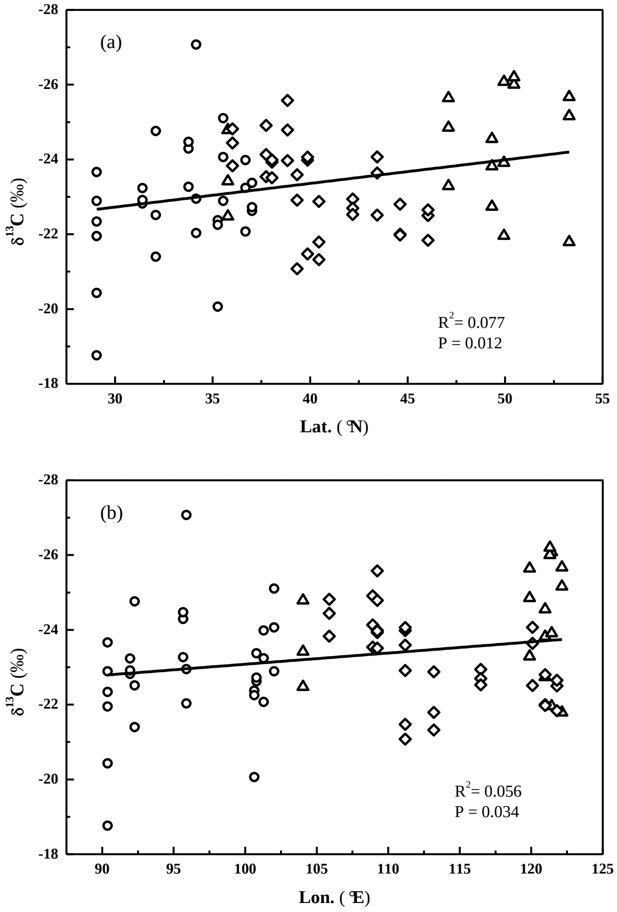

Fig.4 shows the relationships between the soil $\delta^{13}C$ and latitude (a) and longitude (b).

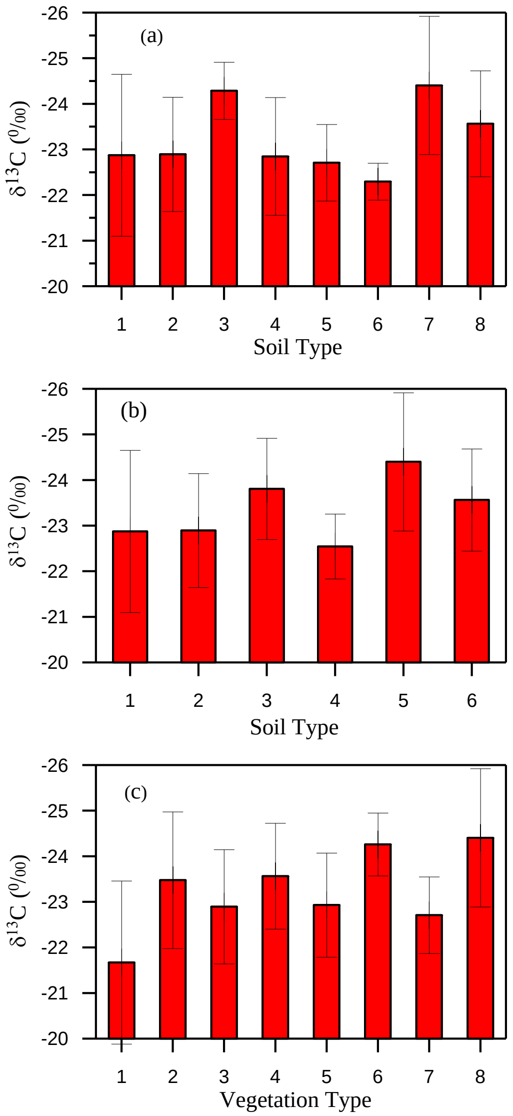

Fig.5 shows the effects of soil types and vegetation types on the soil $\delta^{13}C$. (a.) soil types based on Chinese soil taxonomy. 1. Matti-Gelic Cambosols; 2. Hapli- Cryic Aridosolsl; 3. Calci-Orthic Aridosols; 4. Mottlic Calci-Orthic Aridosols; 5. Typic Calci-Ustic Isohumosols; 6. Pachi-Ustic Isohumosols; 7. Umbri-Gelic Cambosols; 8. Hapli-Ustic Argosols. (b.) soil types based on WRB. 1. Cambisols; 2. Leptosols; 3. Calcisols; 4. Chernozems; 5. Umbrisols; 6. Luvisols. (c.) vegetation type. 1. Alpine grassland; 2. Alpine meadow; 3. Subalpine grassland; 4. Temperate coniferous and broad-leaved mixed forests; 5. Temperate meadow steppe; 6. Semi-desert grasslands; 7. Temperate typical steppe; 8. Frigid temperate coniferous forest. The bar in Fig.5 indicates ±1SD.

Table 1 Information of the sampling sites

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

										Filifolium	
22	WuLanHaoTe	4.1	20.8	416	366	46.05	121.79	-22.3	Temperate meadow steppe	*Stipa*、*Aneuralepidium and Filifolium*	Pachi-Ustic Isohumosols (Chernozems)
23	AErShan	-2.7	15	391	1240	47.1	119.89	-24.6	Frigid temperate coniferous forest	*Larix gmelinii* and *Betula platyphylla Suk*	Umbri-Gelic Cambosols (Umbrisols)
24	YaKeShi	-2.9	18.5	379	634	49.33	120.97	-23.7	Frigid temperate coniferous forest	*Larix gmelinii*	Umbri-Gelic Cambosols (Umbrisols)
25	KuDuEr	-5.5	17.3	402	829	49.94	121.43	-24.0	Frigid temperate coniferous forest	*Larix gmelinii* and *Betula platyphylla Suk*	Umbri-Gelic Cambosols (Umbrisols)
26	GenHe	-5.3	17.9	424	718	50.46	121.31	-26.1	Frigid temperate coniferous forest	*Betula platyphylla Suk*	Umbri-Gelic Cambosols (Umbrisols)
27	BeiJicun	-5.5	18.4	450.8	603	53.29	122.15	-24.2	Frigid temperate coniferous forest	*Larix gmelinii and Pinus sylvestnis var*	Umbri-Gelic Cambosols (Umbrisols)

Note: MAT, SMT, MAP, Alt, Lat and Lon are the abbreviations of mean annual temperature, summer mean temperature, mean annual precipitation, altitude, latitude, 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, 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 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 brackets).

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 $\delta^{13}C$ 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.