Edaphic rather than climatic controls over ^{13}C enrichment between soil and vegetation in alpine grasslands on the Tibetan Plateau

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Summary

1. Soil organic carbon (SOC) dynamics is crucial for evaluating ecosystem carbon balance and its feedback to climate warming. However, it is difficult to detect statistically significant changes in SOC stock over short-time period due to its large pool size, slow turnover time and huge spatial heterogeneity. Stable isotopic measurements, such as $\Delta \delta^{13}$C (i.e. the difference of natural abundance of ^{13}C and ^{12}C (δ^{13}C) between surface soils and source plants) and its variations along environmental gradients provide an alternative approach inferring soil carbon dynamics over broad geographical scale. However, current isotopic evidence is primarily derived from temperate and tropical regions, with very limited measurements available in alpine regions.

2. Here, we examined spatial variations of $\Delta \delta^{13}$C in alpine grasslands on the Tibetan Plateau, using large-scale isotopic measurements obtained from consecutive field samplings. We aimed to test whether previously observed isotopic patterns in temperate and tropical regions still hold true in alpine regions and whether climatic or edaphic variables regulated large-scale patterns of ^{13}C enrichment between soil and vegetation in alpine ecosystems.

3. Our results showed that topsoil stable carbon isotope composition in alpine steppe and meadow ranged from −26·1‰ to −19·7‰ and from −25·7‰ to −22·2‰, with an average of −23·7‰ and −24·1‰, respectively. As previously observed in temperate forests, soil δ^{13}C exhibited linear increases with plant δ^{13}C in alpine grasslands.

4. In contrast to earlier findings, our results revealed that edaphic rather than climatic factors regulated spatial variability of the $\Delta \delta^{13}$C in high-altitude regions. Moreover, edaphic controls over $\Delta \delta^{13}$C exhibited meaningful differences between alpine steppe and meadow. The $\Delta \delta^{13}$C exhibited an initial increase and a subsequent decrease with soil carbon content in alpine steppe, but was negatively associated with silt content and carbon: nitrogen ratio in alpine meadow.

5. Our results confirmed the association between the δ^{13}C of surface soils and vegetation across contrasting ecosystems, but revealed that edaphic rather than climatic variables were better explanations of ^{13}C enrichment between soil and vegetation at high altitudes. Changes in soil texture and substrate quality could therefore induce soil carbon dynamics in alpine ecosystems.

Key-words: alpine ecosystems, carbon cycle, carbon-climate feedback, soil organic carbon, stable carbon isotope

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Introduction

Alpine ecosystems belong to an important component of terrestrial ecosystems around the world and play a vital role in the terrestrial carbon balance. This is because high-altitude ecosystems are experiencing faster rates of warming and their soils store large amounts of organic carbon (Yang *et al.* 2009). For instance, soil organic carbon (SOC) stock in the top 1 m across alpine grasslands on the Tibetan Plateau was estimated at a range of 7·4–23·2 Pg C (1 Pg = 10^{15} g; Wang *et al.* 2002; Yang *et al.* 2008). It has been suggested that continuous climate warming is likely to accelerate the decomposition of SOC which would increase carbon release from alpine soils to the atmosphere (Lin *et al.* 2011; Lu *et al.* 2013). Warming-induced carbon losses from alpine soils could even offset enhanced carbon sequestration by vegetation, triggering a positive feedback to climate warming (Tan *et al.* 2010; Chen *et al.* 2013). Therefore, our understanding of the mechanisms governing SOC dynamics is crucial for evaluating ecosystem carbon balance in high-altitude regions and its feedbacks to climate warming (Yang *et al.* 2009; Averill, Turner & Finzi 2014).

During the past several years, soil carbon dynamics in alpine ecosystems has received particular interest from the global change research community. Inventory-based approach, that is compare soil carbon stocks among different periods based on regional inventory data sets, is the most common method used to quantify SOC dynamics over broad geographical scale (e.g. Yang *et al.* 2009). However, it is difficult to make direct comparisons of SOC stocks among various sampling periods due to the lack of detailed historical or concurrent measurements at the same sites (Yang *et al.* 2014). It is also difficult to detect statistically significant changes in SOC stock over short-time periods because of the large pool size, slow turnover time and huge spatial heterogeneity (van Groenigen *et al.* 2014). Moreover, inventory-based studies usually do not analyse the potential drivers for different magnitude of SOC changes over broad geographical scale (e.g. Bellamy *et al.* 2005; Yang *et al.* 2009). Therefore, it is still a great challenge to depict an explicit picture of spatial patterns and environmental drivers of SOC dynamics in alpine ecosystems.

Stable isotopic measurements, that is the natural abundance of ^{13}C and ^{12}C ($\delta^{13}C$) along environmental gradients, could indirectly reflect soil carbon dynamics over the large geographic areas and over relatively long time periods (e.g. Bird, Chivas & Head 1996; Bird & Pousai 1997; Ehleringer, Buchmann & Flanagan 2000; Staddon 2004; Powers & Veldkamp 2005; Bai *et al.* 2012; Wang *et al.* 2013). For instance, the slope of a linear regression relating isotopic composition to the logarithm of SOC concentration was used to characterize the changes associated with the transformation of fresh organic carbon into soil organic matter: a more negative slope indicates faster soil carbon turnover through microbial decomposition

(e.g. Garten *et al.* 2000; Powers & Schlesinger 2002; Campbell *et al.* 2009; Acton *et al.* 2013). Likewise, spatial variations in the difference of $\delta^{13}C$ values between surface soils and source plants along environmental gradients were also adopted to infer soil carbon dynamics over broad geographical scale (e.g. Garten *et al.* 2000; Peri *et al.* 2012). These two types of isotopic studies have demonstrated that climatic (Garten *et al.* 2000; Peri *et al.* 2012), edaphic variables (Powers & Schlesinger 2002) and their combination (Acton *et al.* 2013) could shape large-scale patterns of soil carbon dynamics in terrestrial ecosystems. However, current isotopic evidence is mainly derived from temperate (Garten *et al.* 2000; Peri *et al.* 2012) and tropical regions (Powers & Schlesinger 2002), with very limited coverage of alpine regions. It is therefore a matter of priority to test whether isotopic patterns observed in temperate and tropical regions still hold true in alpine regions.

The Tibetan Plateau provides an ideal region to explore spatial patterns and environmental drivers of $\Delta \delta^{13}C$, that is the difference between topsoil and plant $\delta^{13}C$ in alpine ecosystems. However, a comprehensive understanding of this issue remains elusive since previous studies focused on large-scale patterns of soil $\delta^{13}C$ (Lu *et al.* 2004; Wang *et al.* 2013) but not of $\Delta \delta^{13}C$ which have more direct and profound implications for soil carbon dynamics along environmental gradients. Consequently, the present study was designed to examine spatial variability of $\Delta \delta^{13}C$ in alpine grasslands on the Tibetan Plateau using large-scale isotopic measurements derived from consecutive field sampling campaigns. We measured isotope composition of both plant tissues and soil samples in the top 10 cm, and then quantified the individual and combined effects of climatic and edaphic variables on spatial variation of $\Delta \delta^{13}C$. Overall, these isotopic patterns are expected to clarify whether large-scale patterns of ^{13}C enrichment between soil and vegetation in alpine ecosystems are mainly determined by climatic or edaphic variables and whether previously observed isotopic patterns in temperate and tropical regions still hold true in alpine regions. These isotopic patterns could then provide clues for soil carbon dynamics and its potential feedback to climate warming.

Materials and methods

STUDY AREA

The Tibetan Plateau is the highest and largest plateau on the Earth. It has an average elevation of 4000 m above sea level, and covers an area of ~2·0 × 10^6 km², about 1·4 times the size of Alaska (Li & Zhou 1998; Yang *et al.* 2008). The study area is located at 29·25°~37·64°N in latitude and 80·75°~101·31°E in longitude. The mean annual temperature (MAT) ranges between −3·09 and 4·43 °C, with the lowest mean monthly temperature in January and the highest in July. The mean annual precipitation (MAP) is between 103 and 694 mm, about 90% of which falls in the growing season from May to September. Along a south-east/north-west precipitation gradient, the study area covers two

vegetation types: alpine steppe and alpine meadow. The alpine steppe, located at the dry end of the gradient, is dominated by *Stipa purpurea* and *Carex moorcroftii* (Zhang *et al.* 1988), with low species richness, productivity and SOC content. Despite the high altitude and low temperature, C_4 species such as *Orinus kokonorica, Orinus thoroldii, Pennisetum centrasiaticum, Salsola abrotanoides* (Wang *et al.* 2004; Li *et al.* 2005) occur across the steppe sites surveyed in this study. The alpine meadow at the wet part of the precipitation gradient is dominated by *Kobresia pygmaea, K. humilis* and *K. tibetica*, with relatively high species richness, aboveground productivity and SOC content (Zhang *et al.* 1988). Accordingly, two soil types are found in this region, including cold calcic and felty soils according to the Chinese soil classification system (Xiong & Li 1987), equivalent to cambisols in the soil taxonomy system of the Food and Agriculture Organization (FAO) of the United Nations (Yang *et al.* 2010).

REGIONAL VEGETATION AND SOIL SURVEY

Field sampling was carried out at 135 sites during the summers (July and August) of 2001–2004 (see Fig. S1, Supporting information), covering all major grassland types across the study area. Many of the sampling sites suffered from the pastoral grazing by cattle and sheep. To minimize the grazing effect, field sampling was restricted to sites with minimal recent disturbance (Prentice *et al.* 2010). At each site, we set up five 1 m × 1 m quadrats located at each corner and the centre of a 10 m × 10 m area. Aboveground standing biomass of herbaceous plants was sampled from these quadrats. For each quadrat, aboveground biomass was clipped at the ground level and pooled for isotope analysis. Three replicate soil samples in the top 10 cm were collected from three of five quadrats which locate along the diagonal line of the sampling site. A detailed description of vegetation and soil survey was documented in Yang *et al.* (2008).

CARBON ISOTOPE AND SOIL PROPERTIES ANALYSIS

We measured $\delta^{13}C$ values in surface soils for 124 sites and $\delta^{13}C$ values in plant tissues for 113 sites due to the exhaust of samples in the remaining sites. Soil samples were sieved to 2 mm, with plant residuals being removed. Then, they were finely ground using a ball mill (NM200; Retsch, Hann, Germany), treated with 0·1 N HCL solution for 24 h at room temperature to remove any carbonates, washed with distilled water, and oven dried at 65 °C for 24 h prior to isotopic measurements (Bird, Haberle & Chivas 1994; Lu *et al.* 2004; Cheng *et al.* 2006). Similarly, plant samples were ground and oven dried before analysis. The carbon and nitrogen concentrations and $\delta^{13}C$ values were measured using an elemental analyzer (Flash EA1112; Thermo Finnigan, Milan, Italy) coupled to an isotope ratio mass spectrometer (Finnigan MAT-253; Thermo Electron, Bremen, Germany). $\delta^{13}C$ expresses the ^{13}C content relative to the standard, and is calculated as follows:

$$\delta^{13}C(‰) = (R_{sample}/R_{std} - 1) \times 10^3, \qquad \text{eqn 1}$$

where R_{sample} is the ratio of stable carbon ($^{13}C/^{12}C$) of the sample and R_{std} is $^{13}C/^{12}C$ of a Vienna Pee Dee Belemnite standard (Coplen 2011). The overall precision for C and $\delta^{13}C$ measurements is ±0·2‰ and ±0·1‰, respectively.

We also determined other soil physical and chemical properties. Of them, the percentage of clay, silt and sand contents was measured using a particle size analyzer (Malvern Mastersizer 2000; Malvern Instruments Ltd, Worcestershire, UK) after removal of organic matter and calcium carbonates. Soil pH was analysed using a pH electrode in a mixture of soil and water, with a soil:

water ratio of 1:2.5 (Bao 2005). Soil carbon: nitrogen (C: N) ratio was calculated as the quotient of carbon and nitrogen concentrations per unit mass of dry soil.

CLIMATE VARIABLES

Based on kriging interpolation, mean monthly air temperature (MMT) and mean monthly precipitation (MMP) of our sampling sites were estimated from the records at 43 climatic stations located above an elevation of 3000 m across the plateau (Yang *et al.* 2008). Using MMT and MMP, we then calculated the MAT and MAP of our study sites during the sampling period.

DATA ANALYSES

We conducted ordinary least squares (OLS) regression analyses to examine the relationship between soil and plant $\delta^{13}C$ values, and also the relationships of plant $\delta^{13}C$, soil $\delta^{13}C$, and $\Delta \delta^{13}C$ with climatic and edaphic variables. We then used stepwise regression to explore the combined effects of climatic and edaphic variables on soil $\delta^{13}C$ and $\Delta \delta^{13}C$. The MAT, MAP, pH, silt content, SOC content and C:N ratio were included in the stepwise regression model as independent variables. We further used regression tree analysis, as implemented in the 'rpart' packages in R, to explore primary driving factors of spatial variations in soil $\delta^{13}C$ and $\Delta \delta^{13}C$. An iterative cross-validation approach was used to prune trees to a size that best represented relationships with the smallest cross-validated relative error (CVRE). All statistical analyses were performed using the software package R (R Development Core Team 2012).

Results

VARIATIONS OF PLANT AND SOIL $\delta^{13}C$ AMONG SAMPLING SITES

Plant stable carbon isotope composition in alpine steppe ranged from −27·2‰ to −21·8‰ (Fig. 1a), while that in alpine meadow varied from −26·8‰ to −24·0‰ (Fig. 1b). Likewise, topsoil $\delta^{13}C$ exhibited large variations among different sampling sites, ranging from −26·1‰ to −19·7‰, and −25·7‰ to −22·2‰ in alpine steppe and meadow, respectively (Fig. 1c,d). Soil $\delta^{13}C$ exhibited linear increases with plant $\delta^{13}C$ in both alpine steppe ($r^2 = 0·22$, $P < 0·05$; Fig. 2a) and alpine meadow ($r^2 = 0·49$, $P < 0·05$; Fig. 2b). The mean plant and soil $\delta^{13}C$ signatures in alpine steppe were estimated at −25·4‰ and −23·7‰, while those in alpine meadow equalled to −25·6‰ and −24·1‰, respectively.

On average, topsoil was 1·6‰ and 1·5‰ enriched in ^{13}C relative to source plants in alpine steppe and alpine meadow, respectively. The difference between topsoil and plant $\delta^{13}C$ values was larger than 1·3‰ (the Suess effect, a shift to isotopically lighter carbon in the atmosphere due to biomass burning; Garten *et al.* 2000) across 65% of our sampling sites. Moreover, one-sample t-test revealed that the average $\Delta \delta^{13}C$ was significantly higher than 1·3‰ in both alpine steppe and alpine meadow ($P < 0·05$). These analyses demonstrate that the Suess effect cannot be the only mechanism responsible for the isotopic discrimination observed in this study and other mechanisms such as isotopic fractionation
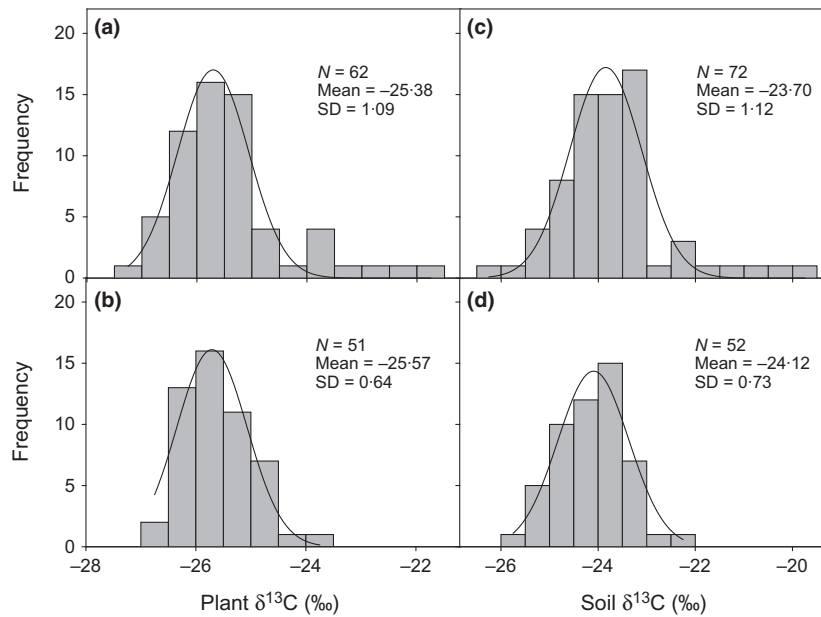
Fig. 1. Frequency distributions of plant $\delta^{13}C$ (a, b) and topsoil $\delta^{13}C$ (c, d) in alpine grasslands on the Tibetan Plateau. Panel (a, c) is for steppe sites, while panel (b, d) is for meadow sites.
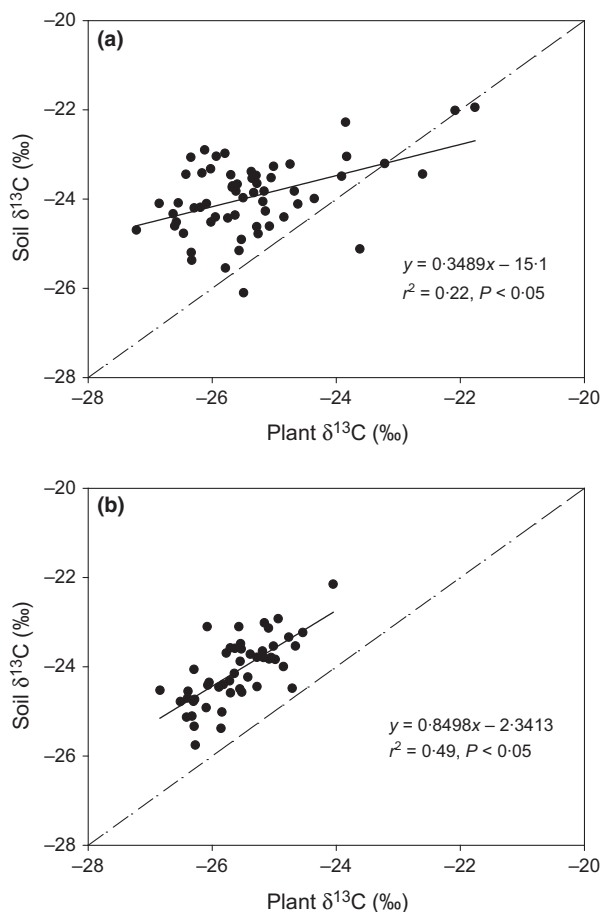

Fig. 2. Relationships between topsoil $\delta^{13}C$ and plant $\delta^{13}C$ in alpine steppe (a) and alpine meadow (b) on the Tibetan Plateau. The thick line is the regression line, and the dashed line is 1:1 line.

during microbial decomposition should play an important role (Garten *et al.* 2000; Powers & Schlesinger 2002).

VARIATIONS OF PLANT $\delta^{13}C$, SOIL $\delta^{13}C$ AND $\Delta\,\delta^{13}C$ ALONG CLIMATIC AND EDAPHIC GRADIENTS

Plant $\delta^{13}C$ in alpine steppe did not vary in a systematic way either with climatic (Fig. 3a,b) or edaphic variables (Fig. 3c–f). Similarly, the plant isotopic composition in alpine meadow showed weak relationships with most environmental parameters (Fig. 3h–l). The only exception was that plant $\delta^{13}C$ in alpine meadow decreased linearly by 0·14‰ per 1 °C increase in MAT ($r^2 = 0.16$, $P < 0.05$; Fig. 3g).

Soil $\delta^{13}C$ in alpine steppe was only related to one of all environmental parameters (Fig. 4a–f), exhibiting negative association with topsoil C:N ratio ($r^2 = 0.13$, $P < 0.05$; Fig. 4f). In contrast, the soil isotopic composition in alpine meadow was negatively correlated with several climatic and edaphic variables, including MAT ($r^2 = 0.31$, $P < 0.05$; Fig. 4g), soil pH ($r^2 = 0.14$, $P < 0.05$; Fig. 4i), silt content ($r^2 = 0.24$, $P < 0.05$; Fig. 4j), and SOC content ($r^2 = 0.16$, $P < 0.05$; Fig. 4k). A model containing MAT and silt content explained 46% of the variation in surface soil $\delta^{13}C$ in alpine meadow (Table 1). The regression tree analysis confirmed that MAT and silt content were the most important contributors for spatial variation of soil $\delta^{13}C$ across the meadow sites (Fig. 6a).

The $\Delta\,\delta^{13}C$ in alpine steppe did not exhibit any associations with most climatic (Fig. 5a,b) and edaphic variables except SOC content (Fig. 5c–f). The $\Delta\,\delta^{13}C$ in alpine steppe had an initial increase and a subsequent decrease with SOC content ($r^2 = 0.20$, $P < 0.05$; Fig. 5e), with a

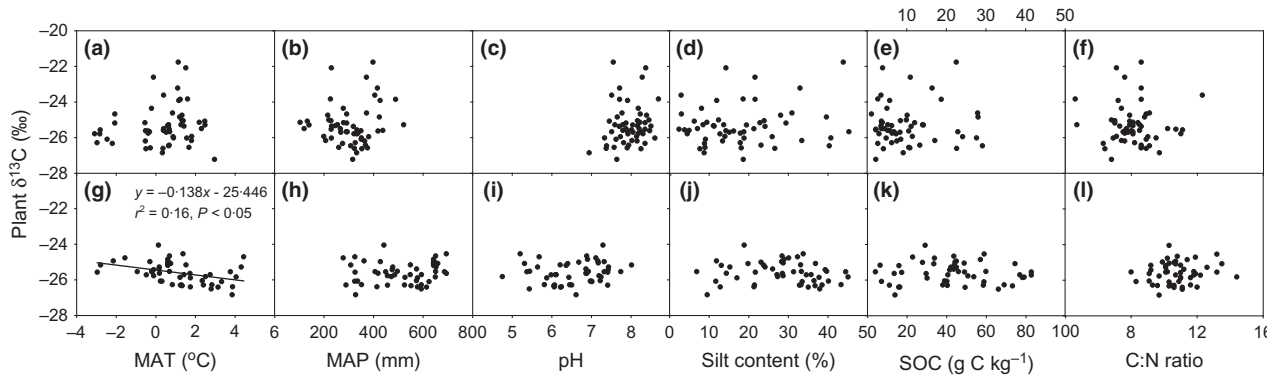**Fig. 3.** Relationships of plant $\delta^{13}C$ with climatic and edaphic factors in alpine grasslands on the Tibetan Plateau. Panel (a–f) is for steppe sites, while panel (g–l) is for meadow sites. MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; C:N ratio, topsoil carbon: nitrogen ratio.
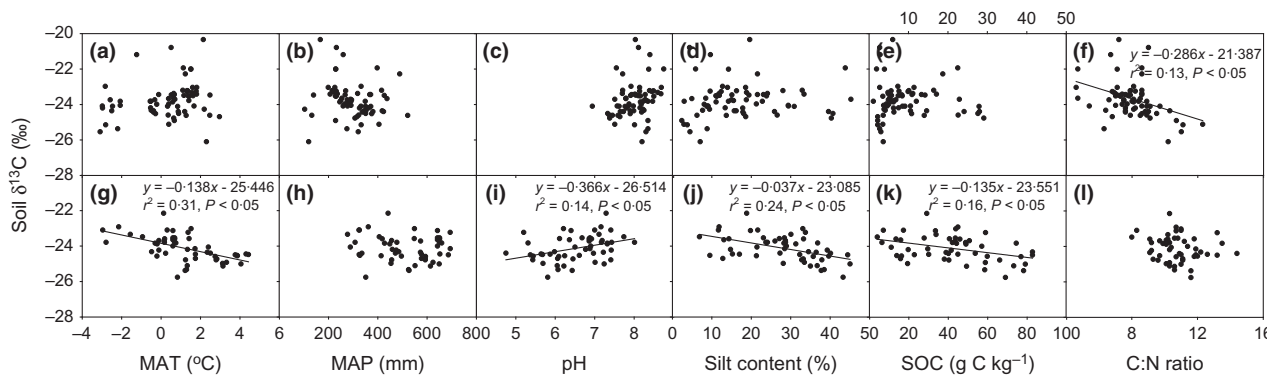

Fig. 4. Relationships of topsoil $\delta^{13}C$ with climatic and edaphic factors in alpine grasslands on the Tibetan Plateau. Panel (a–f) is for steppe sites, while panel (g–l) is for meadow sites. MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; C:N ratio, topsoil carbon: nitrogen ratio.

Table 1. Stepwise regression analyses for topsoil $\delta^{13}C$ and $\Delta\,\delta^{13}C$ (i.e. the difference between topsoil and plant $\delta^{13}C$ values) in alpine meadow on the Tibetan Plateau. MAT, mean annual temperature; C:N ratio, topsoil carbon: nitrogen ratio

Predicted variables	Model parameters	Coefficients	Standard errors	r^2	Significance
Soil $\delta^{13}C$	Intercept	−23·052	0·232	0·46	< 0·001
	MAT	−0·201	0·045		
	Silt content	−0·030	0·008		
$\Delta\,\delta^{13}C$	Intercept	3·399	0·513	0·39	< 0·001
	Silt content	−0·027	0·007		
	C:N ratio	−0·104	0·050		

maximum $\Delta\,\delta^{13}C$ occurring at the SOC concentration of 21 g C kg^{-1}. Notably, a linear model failed to fit the relationship between $\Delta\,\delta^{13}C$ and SOC content in alpine steppe (see Fig. S2a, Supporting information), while the 95% confidence interval of the nonlinear model prediction captured the variation across the steppe sites (see Fig. S2b), and thus, we finally used the nonlinear model to simulate the trend.

The $\Delta\,\delta^{13}C$ in alpine meadow exhibited systematic shifts with both climatic and edaphic variables (Fig. 5g–l). Stepwise regression analyses revealed that silt content and topsoil C:N ratio were the primary factors regulating the $\Delta\,\delta^{13}C$; however, climatic variables did not enter into the final model (Table 1). The $\Delta\,\delta^{13}C$ in alpine meadow was negatively associated with both silt content ($r^2 = 0.34$, $P < 0.05$; Fig. 5j) and topsoil C:N ratio ($r^2 = 0.17$, $P < 0.05$; Fig. 5l). The combination of silt content and C:N ratio explained about 40% of spatial variability in $\Delta\,\delta^{13}C$ (Table 1).

Regression tree analysis also captured the effects of edaphic factors including silt content and C:N ratio on $\Delta\,\delta^{13}C$ (Fig. 6b). The pruned tree with four terminal nodes

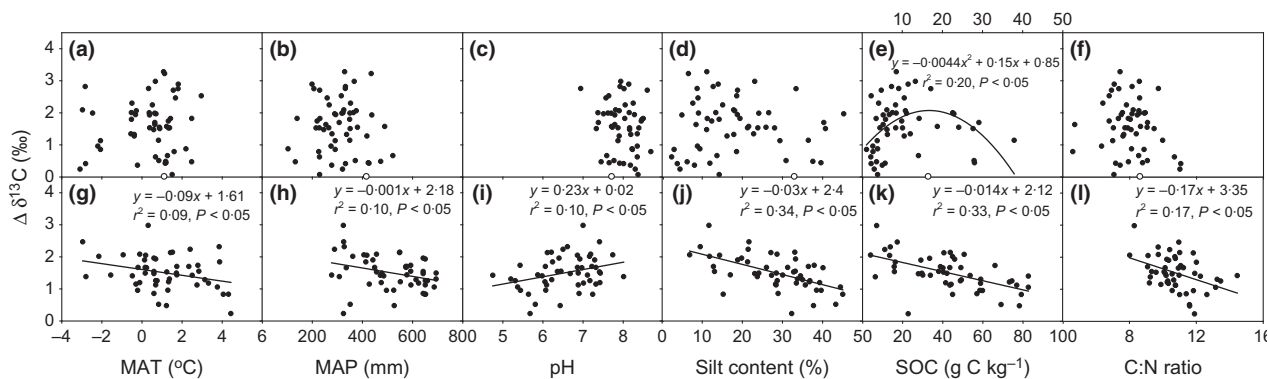**Fig. 5.** Relationships of Δ δ¹³C, that is the difference between topsoil and plant δ¹³C with climatic and edaphic factors in alpine grasslands on the Tibetan Plateau. Panel (a–f) is for steppe sites, while panel (g–l) is for meadow sites. MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; C:N ratio, topsoil carbon: nitrogen ratio. The open circle in panel (a-f) shows an outlier.

explained 42·2% of the variance of Δ δ¹³C in alpine meadow. Of this, 29·2% was accounted for by the first split; the second split explained an additional 9·8% of the variance in Δ δ¹³C. Silt content >= 27·2% was identified as the criterion for the first split, reflecting that the sites with higher silt content for the most part ($n = 31$) also tended to have smaller Δ δ¹³C value. The C:N ratio entered the final model as the second explanatory factor, indicating that lower C:N ratio (<11·3) resulted in higher mean Δ δ¹³C under certain conditions (silt content >=27·2%). Overall, these results demonstrate that edaphic controls over Δ δ¹³C were different between alpine steppe and meadow: the Δ δ¹³C was regulated by SOC content in alpine steppe whereas it was related more strongly to silt content and C:N ratio in alpine meadow.

Discussion

A GENERAL POSITIVE RELATIONSHIP BETWEEN SOIL AND PLANT δ¹³C

Soil δ¹³C values exhibited linear increases with the corresponding plant δ¹³C values in both alpine steppe and meadow. The positive relationship between soil δ¹³C and plant δ¹³C, to a large degree, is a reflection that soil organic matter is derived mainly from plant litter inputs (Balesdent, Girardin & Mariotti 1993; Ehleringer, Buchmann & Flanagan 2000; Peri *et al.* 2012). Our large-scale measurements also support that SOC content exhibits an increasing trend with the forage production in alpine grasslands on the Tibetan Plateau (Yang *et al.* 2008). Greater primary productivity would then produce more litter, and thus drive greater carbon accumulation in mineral soils.

The isotopic pattern between surface soils and source plants observed in alpine grasslands accords well with those reported in temperate forests around the world. For instance, using isotopic data obtained from 14 sites in a temperate forest of French, Balesdent, Girardin & Mariotti (1993) showed that δ¹³C values of tree leaves was positively and linearly associated with soil δ¹³C values. Likewise, based on stable isotope natural abun-

dance measurements along a climosequence of 33 forest stands in Argentina, Peri *et al.* (2012) observed strong positive correlations between soil δ¹³C and foliar δ¹³C. The consistence among three individual studies suggests that the positive soil δ¹³C vs. plant δ¹³C trend was independent of vegetation type and climatic characteristics. The general isotopic pattern, coupled with carbon content, has been proposed to estimate soil δ¹³C from plant δ¹³C and vice versa (Balesdent, Girardin & Mariotti 1993).

WEAK CLIMATIC EFFECTS ON Δ δ¹³C IN ALPINE ECOSYSTEMS

To our surprise, climatic variables, particularly temperature, did not explain the spatial variation of Δ δ¹³C values in alpine grasslands; although temperature has been widely considered to limit microbial decomposition in cold regions (Hobbie *et al.* 2000). The weak climate effects on Δ δ¹³C may be partly induced by the potential uncertainties involved in climate interpolation, since the weather data for most sampling sites had to be extrapolated across a very topographically complex region. Then, an interesting question arises that how good the extrapolation with respect to geographic location was. To evaluate the reliability of climate interpolation, we explored the associations between kriging-based dataset and other independent parameters. Specifically, we analysed the relationship between MAT and the land surface temperature (LST) derived from Moderate Resolution Imaging Spectro-radiometer (MODIS), and the association between MAP and measured soil moisture across our sampling sties. Our analyses indicated that LST exhibited linear increases with MAT ($r^2 = 0.30$, $P < 0.05$; see Fig. S3a, Supporting information). Moreover, soil moisture was closely associated with MAP ($r^2 = 0.59$, $P < 0.05$; see Fig. S3b). These results demonstrated that kriging interpolation captured the regional climate trend across the study area. Therefore, kriging error should not be the main reason for the weak climatic effects on isotopic characteristics in alpine ecosystems.
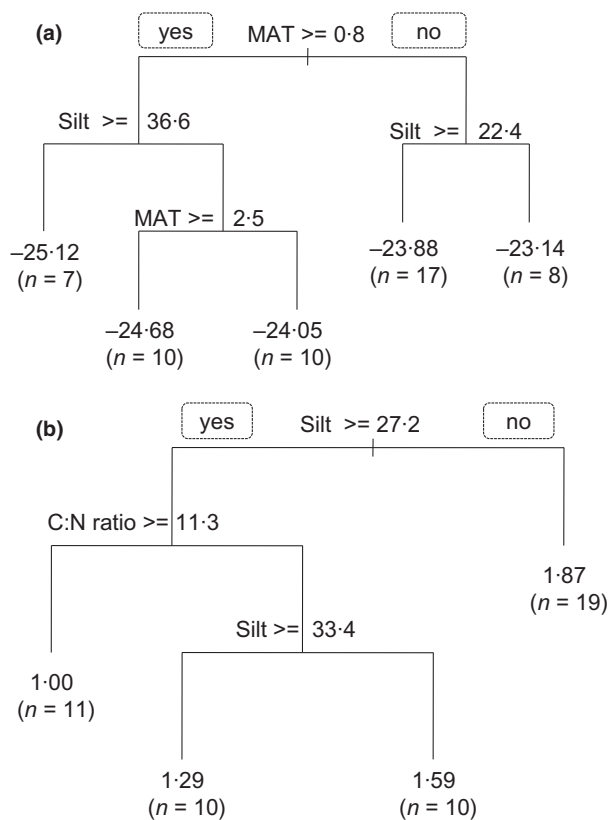
Fig. 6. Summary of regression tree results for relationships between soil $\delta^{13}C$ and environmental variables (a) and between Δ $\delta^{13}C$ and environmental variables (b) in alpine meadow on the Tibetan Plateau. Branches are labelled with criteria used for data classification. Values in terminal leaves indicate mean Δ $\delta^{13}C$ values of sites grouped within each leaf. n = number of sites within each leaf. MAT, mean annual temperature; C:N ratio, topsoil carbon: nitrogen ratio.

The weak climatic effects on Δ $\delta^{13}C$ observed in this study may also be ascribed to the inter-annual climate variability during the sampling period. To test this point, we used the related sampling year's climate data set rather than the average climate conditions during 2001-2004, to re-evaluate the relationships between Δ $\delta^{13}C$ and climate variables (see Fig. S4, Supporting information). Our analyses confirmed previously observed isotopic patterns that used the average climate during 2001–2004 (Fig. 5), demonstrating that the inter-annual climate variability did not significantly alter the relationships between isotopic characteristics and climate parameters. In addition, climate seasonality could also modify the variations of Δ $\delta^{13}C$ along climatic gradients, since $\delta^{13}C$ values of plants can vary by several per mil during the course of a single growing season (Walcroft *et al.* 1997). However, similar to many other studies in grassland ecosystems (e.g. Prentice *et al.* 2010), vegetation and soil samplings were conducted at the height of the growing season. Consequently, the potential effects of climate seasonality on isotope signatures should be minimized in this study.

The isotopic evidence for relatively weak temperature effects on Δ $\delta^{13}C$ observed in this study was supported by

two recent observations which concluded that both soil carbon stock (Yang *et al.* 2008) and soil respiration (Geng *et al.* 2012) in Tibetan alpine grasslands were slightly modified by temperature. However, the isotopic pattern observed in study conflicts with several lines of isotopic evidence that climatic variables played vital roles in regulating ^{13}C enrichment between soil and vegetation over broad geographical scale. For instance, Garten *et al.* (2000) demonstrated that temperature exerted dominant control over the extent of isotopic fractionation during SOC decomposition in temperate forests in the Southern Appalachian Mountains. Similarly, Peri *et al.* (2012) revealed that climatic variables particularly mean annual precipitation, but not soil properties explained the largest proportion of spatial variability of the isotopic difference between the $\delta^{13}C$ values in leaves and soils in Southern Patagonia's native forests. The discrepancy between current study and previous observations illustrates that environmental controls over ^{13}C enrichment between soil and vegetation are region-specific, probably depending on the width of the regional climate gradient. Higher possibility of statistically significant isotopic patterns tends to occur along the wider climate gradient, and vice versa. Consistent with this deduction, a recent synthesis of 11 individual studies across a range of cool temperate to tropical forest soils, demonstrated that the slope of the linear regression relating isotopic composition to the logarithm of SOC concentration exhibited strong negative associations with MAT, with temperature alone explained 73% variations of the slope of the regression between $\delta^{13}C$ and the logarithm of SOC (Acton *et al.* 2013).

EDAPHIC CONTROLS OVER Δ $\delta^{13}C$ IN ALPINE ECOSYSTEMS

Edaphic controls over Δ $\delta^{13}C$ exhibited meaningful differences between alpine steppe and meadow, with Δ $\delta^{13}C$ regulated by SOC content in alpine steppe while related more closely to silt content and C:N ratio in alpine meadow. It has been suggested that microbial decomposition of soil organic matter can be limited by poor availability of labile substrates in soils with low SOC contents but it tends to be constrained by both substrate availability and chemical quality in soils with rich SOC (Chapin, Matson & Mooney 2011). Based on this principle, the different isotopic patterns observed between the two grassland types in relation to edaphic factors could be explained by the larger amounts of organic carbon stored in alpine meadow compared with alpine steppe soils (Yang *et al.* 2008).

The Δ $\delta^{13}C$ in alpine steppe exhibited an initial increase and a subsequent decrease with SOC content. The quadric pattern observed in alpine steppe could be explained by the following three aspects. First, the initial increase of Δ $\delta^{13}C$ with SOC content may reflect that substrate quantity limits microbial decomposition under lower SOC conditions (Chapin, Matson & Mooney 2011). Under low soil fertility conditions, increased substrate quantity could be

favourable for microbial decomposition, and thus lead to the greater isotopic difference between surface soils and source plants (Ehleringer, Buchmann & Flanagan 2000). Second, the subsequent decline of $\Delta \delta^{13}C$ with SOC content may be driven by the higher proportion of fresh carbon which has less isotopic discrimination against source plants in those soils with higher SOC content (Bai *et al.* 2012). Third, the unimodal pattern between $\Delta \delta^{13}C$ and SOC content may be due to the changing contribution of microbe *vs.* plant components along the fertility gradient. One possibility is that the proportion of microbe-derived component may increase with SOC content under barren conditions (SOC ≤ 21 g C kg^{-1}), while the proportion of plant-derived organic matter may increase with SOC content under fertile environments (SOC ≥ 21 g C kg^{-1}). The 'mixing effect' hypothesis predicts that microbe-derived organic matter is usually more enriched in $\delta^{13}C$ than plant-derived component (Ehleringer, Buchmann & Flanagan 2000). Higher microbial portions in soil organic matter may thus lead to the initial increase of $\Delta \delta^{13}C$ with SOC content whereas the increasing proportion of plant-derived organic matter may result in the subsequent decline of $\Delta \delta^{13}C$ with SOC content. Nevertheless, these hypotheses need to be tested by more experimental results.

The $\Delta \delta^{13}C$ in alpine meadow was negatively correlated with silt content, which was supported by previous observations in tropical rainforests of north-eastern Costa Rica. Specifically, Powers & Schlesinger (2002) demonstrated that silt content played an important role in regulating the slope of the regression between $\delta^{13}C$ and the logarithm of SOC in tropical rain forest soils of Costa Rica, with slower increases in ^{13}C enrichment with soil depth occurring in those sites with higher silt content. The decrease of $\Delta \delta^{13}C$ with silt content could be caused by the direct stabilizing effects of silt on soil organic matter (Schimel *et al.* 1994). Increasing silt content could, therefore, reduce gaseous carbon losses to the atmosphere and dissolved organic carbon leaching, both of which are products of microbial decomposition (Yang *et al.* 2014). This would lead to a smaller carbon isotopic discrimination between mineral soils and source plants. In addition, the decrease of $\Delta \delta^{13}C$ with silt content observed in alpine meadow could also be induced by the reduced contribution of microbe *vs.* plant components along the texture gradient. As mentioned above, microbe-derived organic matter has higher $\delta^{13}C$ values than plant-derived component (Ehleringer, Buchmann & Flanagan 2000). A negative pattern between the $\Delta \delta^{13}C$ and silt content would occur once plant-derived and microbe-derived components become dominant in fine-textured and coarse-textured soils, respectively. Again, these deductions need to be tested in future studies.

The decline of $\Delta \delta^{13}C$ with topsoil C:N ratio observed in alpine meadow could be largely driven by the generally faster microbial decomposition in soils with lower C:N ratios. As an index of substrate quality, soil C:N ratio is often associated inversely with the rate of organic carbon decomposition (Chapin, Matson & Mooney 2011). In sup-

port of this deduction, a recent study demonstrated that the rate of organic matter decomposition was negatively associated with the C:N ratio in alpine grasslands on the Tibetan Plateau, with higher rates of soil-surface CO_2 fluxes occurring from soils with lower C:N ratios (Jiang *et al.* 2013). According to the 'fractionation' hypothesis, higher rates of organic matter decomposition could then lead to isotope fractionation between residual organic matter and any labile products (e.g. CO_2 flux and dissolved organic carbon), since chemical bonds involving ^{12}C are usually more easily broken during enzyme-catalysed reactions than are those involving ^{13}C (Garten *et al.* 2000; Powers & Schlesinger 2002). Consequently, greater carbon isotopic discrimination between mineral soils and source plants was observed in soils with low C:N ratios. In addition, the negative association between the $\Delta \delta^{13}C$ and soil C:N ratio observed in alpine meadow may also reflect the greater degree of humification, because lower soil C:N ratio usually indicates more decomposed soil organic matter (Powers & Schlesinger 2002).

In summary, using large-scale isotopic measurements derived from alpine grasslands on the Tibetan Plateau, this study tested whether previously observed isotopic patterns in temperate and tropical regions still hold true in alpine regions and whether climatic or edaphic variables regulated ^{13}C enrichment between soil and vegetation in alpine ecosystems. Similar to previous studies in temperate forests, we found that soil $\delta^{13}C$ values exhibited positive associations with plant $\delta^{13}C$ values in alpine grasslands. The consistence suggests that the isotopic pattern between surface soils and source plants was independent of vegetation type and climatic characteristics. However, in contrast to earlier observations, our results revealed that edaphic rather than climatic variables are better explanations of ^{13}C enrichment between soil and vegetation in high-altitude regions. These differences highlight that environmental controls over ^{13}C enrichment between soil and vegetation are region-specific. The isotopic patterns observed in this study, that is both silt content and C:N ratio were associated most strongly with carbon isotope discrimination in surface soils against source plants, provide clue that systematic shifts in soil texture and substrate quality may lead to potential fluctuations of soil carbon stock in alpine ecosystems.

Data accessibility

Isotope data set used in this study is available in Table S1 (Supporting information).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1 Geographic location of 135 sampling sites in alpine grasslands on the Tibetan Plateau.

Fig. S2 Relationships of Δ δ^{13}C, i.e. the difference between topsoil and plant δ^{13}C with soil organic carbon (SOC) content in alpine steppe on the Tibetan Plateau. Panel (a) and (b) shows linear and quadratic regressions for the same dataset, respectively. The thick

line is the regression line, and the thin lines are the 95% confidence interval. The open circle shows an outlier.

Fig. S3 Relationships between land surface temperature (LST) extracted from Moderate Resolution Imaging Spectro-radiometer (MODIS) and mean annual temperature (MAT) derived from kriging interpolation (a), and between measured soil moisture and mean annual precipitation (MAP) derived from kriging interpolation (b) across our sampling sites. Soil moisture was determined gravimetrically after 24 h desiccation at 105 °C (Yang *et al.* 2008).

Fig. S4 Relationships between $\Delta \delta^{13}$C (i.e. the difference between topsoil and plant δ^{13}C) and climatic factors in alpine grasslands on the Tibetan Plateau. The sampling year's climate datasets rather than the average over the sampling period were used to explore their associations with $\Delta \delta^{13}$C. Panel (a-b) is for steppe sites, while panel (c-d) is for meadow sites. MAT, mean annual temperature; MAP, mean annual precipitation. The open circle shows an outlier.

Table S1 Large-scale isotopic dataset in alpine grasslands on the Tibetan Plateau. MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; C:N ratio, topsoil carbon: nitrogen ratio; $\Delta \delta^{13}$C, the difference of δ^{13}C between surface soils and source plants; AS, alpine steppe; AM, alpine meadow.