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RELATIONSHIP BETWEEN THE CARBON ISOTOPE COMPOSITION OF GRASSLAND PLANT COMMUNITIES AND ALTITUDE IN BARKOL, XINJIANG PROVINCE, CHINA

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Abstract

Several environmental factors potentially influence the stable isotope composition of plants. Identifying the relationship between the stable carbon isotope composition ($\delta^{13}C$) of plants and soils along altitudinal gradients is important in the context of reconstructing past climates. In this study, we investigated the patterns of $\delta^{13}C$ variation among plant leaves and along an altitudinal gradient (1597～2137 m a.s.l.) in the Barkol area of Xinjiang Province, China. At the community level, leaf $\delta^{13}C$ values decreased with increasing altitude while root $\delta^{13}C$ values had no significant relationship with altitude. We infer that the lower leaf $\delta^{13}C$ values at high altitudes were related to variations in humidity conditions and atmospheric pressure.

Key words: Carbon isotopes; $\delta^{13}C$; Altitude.

Introduction

Ongoing global climate and environmental change has resulted in increasing interest in the storage, distribution and transformation of carbon in terrestrial ecosystems. Carbon exists in two principal forms, ^{12}C and ^{13}C, with ^{13}C only accounting for 1.11% of the total. The two stable isotopes have the same chemical properties but differences in atomic mass cause differences in physical and some chemical properties, such as gas phase transmission rate, molecular bond energy and biochemical synthesis. During photosynthesis, plants preferentially assimilate atmospheric $^{12}CO_2$ compared to $^{13}CO_2$, and this isotopic discrimination depends on the CO_2 partial pressure inside the leaf and the ratio compared to that in the ambient air (Farquhar *et al.*, 1989; O'Leary *et al.*, 1992). In other words, the factors that can affect plant photosynthesis efficiency or stomatal conductance can influence plant $\delta^{13}C$ values. Therefore, during plant growth, the carbon isotopic composition can record information about the ambient climate and environment, as well as providing a comprehensive measure of plant physiological and ecological characteristics. Thus, $\delta^{13}C$ analyses are increasingly being used in studies of plant physiological ecology and global climate change.

Leaf $\delta^{13}C$ has been found to correlate with environmental factors such as precipitation (Diefendorf *et al.*, 2010), temperature (Wang *et al.*, 2013), irradiance (Geßler *et al.*, 2001) and soil properties such as water content (Wei *et al.*, 2014). Altitude, which reflects the combined effect of these factors, has been found to positively influence leaf $\delta^{13}C$ at regional and global scales (Chen *et al.*, 2015; Chen *et al.*, 2005; Hultine & Marshall, 2000).

Although the response of the $\delta^{13}C$ of plant leaves to increasing altitude varies with species, life-form and distribution area, research shows that in the absence of drought stress, the $\delta^{13}C$ values of plant leaves generally increase with increasing altitude (Wang *et al.*, 2008; Guo & Xie *et al.*, 2006; Körner *et al.*, 1991; Kogami *et al.*, 2001; Zhu *et al.*, 2010; Li *et al.*, 2009; Shi *et al.*, 2006). However, it is unclear which is the dominant factor responsible. For example, (Li *et al.*, 2009) found that in humid regions at an altitude of 2000 m or more, such as on the eastern slopes of the Gonggashan Mountains in China, temperature was the main environmental factor causing $\delta^{13}C$ values to increase with altitude, while rainfall and atmospheric pressure had only a small influence. However, Zhu *et al.* (2010) concluded that the increase in plant $\delta^{13}C$ values with altitude primarily resulted from the reduction of atmospheric pressure, and not from decreasing temperature. Van de Water *et al.* (2002) attributed the observed altitudinal pattern of leaf $\delta^{13}C$ values mainly to variations in soil water availability. Under drought conditions, the influence of water content on plant $\delta^{13}C$ values is greater than the influence of the physiological adaptations of plants to changing altitude; in contrast, in wetter environments, the situation was the reverse (Zhou *et al.*, 2013). However, other workers have concluded that the reduction in temperature with increasing altitude had a greater influence on plant $\delta^{13}C$ values, and would reduce the diffusion conductance of the CO_2 of plants growing at high altitude, thus increasing the $\delta^{13}C$ values (Shi *et al.*, 2006). Thus, it is clear that the changes in the $\delta^{13}C$ values of plants along an altitudinal gradient are not the result of a single factor, but rather that they result from the interaction of several factors (Warren *et al.*, 2001). In the case of the Barkol mountain grassland studied here, if the change of altitude gradient of the researched sample land is not very significant, it is worth in-depth thinking whether or not the similar research conclusion can be got — that is, carbon isotopes effectively indicate water use efficiency.

The response of the $\delta^{13}C$ of plant leaves to altitudinal changes varies with plant species and study location. Several studies have shown that the $\delta^{13}C$ of C_3 plants increases with altitude (Chen *et al.*, 2003; Wang *et al.*, 2003; He *et al.*, 2010). The work of Morecroft *et al.* (1992), on *Alchemilla alpina* growing at different altitudes in eight areas of the Scottish Highlands, indicated the following relationship between the $\delta^{13}C$ values of the plant leaves and altitude: $Y=-27.24‰ + 1.906X$, $R^2=0.31$, $Y=\delta^{13}C$ values of leaves (‰), X= altitude (km); thus, the $\delta^{13}C$ values of plant leaves would increase by about 1.9‰ per 1000 m increase in altitude. The research of Kogami *et al.* (2001) on *Polygonum cuspidatum* in the Fuji Mountain area of Japan, and of Shi *et al.* (2010) on big leaf buddleia (*Buddleja da vidii*) in Wolong Nature Reserve, produced similar conclusions. However, other studies have revealed no significant relationship between the δ^{13}C values of C$_3$ plant leaves and altitude. For example, the work of Su & Yan (2008) on plants in the inland Heihe River Basin showed that although δ^{13}C values of most of the plant leaves increased with altitude, this was not the case for *Potentilla fruticosa* and *Salix gilashanica.* In addition, the study of Wang *et al*. (2008) on Dong LingShan Mountain reached similar conclusions.

The arid and semi-arid grasslands in northern China comprise more than 70% of the national grassland area and they play an important role in providing ecosystem services and in maintaining terrestrial carbon balances (Kang *et al*., 2007; Lal, 2004). Water is the main limiting factor for plant growth in the region and is highly variable over both space and time. Research has shown that if water content is not the main limiting factor for plant growth, then the water content does not affect the δ^{13}C of plant leaves (Li, 2009; Feng, 2003). In addition to being related to changes in water content, changes in altitude also result in changes in temperature; moreover, existing studies have shown that that the δ^{13}C values of plant leaves have a very complex relationship with temperature (Schleser, 1999) because different species have different optimum photosynthetic temperatures. Several studies have found that plant δ^{13}C values are positively related to temperature (e.g., Li, 2007), while other studies observed either a negative relationship (Zheng, 2007), or no relationship (Sheu & Chiu, 1995).

The primary objectives of the present study of the Barkol grassland ecosystem in North China are (i) to use the biomass weighting method and the direct measuring method to obtain the δ^{13}C values of plant leaves and root systems at the community level, (ii) to assess any altitudinal trend in δ^{13}C values of horizontal plant leaves and root systems at the community level, and (iii) to determine the response of leaf δ^{13}C values to temperature, moisture availability and atmospheric pressure, and (iv) thereby to determine the main factors responsible for changes in the δ^{13}C values of plant leaves at the community level with altitude.

Study area: Barkol County is located at the north foot of the eastern section of Tianshan Mountains, on the plain between the east section of Tianshan Mountains and the fault block mountains of the east Junggar. The coordinates of the sampling area are 91° 19'30″ ~ 91°48'30"E, 43°21' ~ 45°5'19″N. The topographic characteristics can be summarized as “three mountains and two basins”; the three mountains are the Tianshan Mountains (Barkol Mountains), Moyin Wulashan Mountains (Beishan Mountains), and the Altai Mountains and its extension; and the two basins are Barkol Basin and Three Pond Lake Basin. The elevation of the terrain is high in the southeast and low in the northwest. The area belongs to the temperate continental cold and arid climate zone, with the climate characterized by cool summers and cool winters, with annual precipitation of 202-250 mm and annual evaporation of 1622 mm. There is a high degree of temperature variability on both annual and diurnal scales. Barkol County has an average annual temperature of 0.8°C, maximum temperature of 42°C (Santang Lake, Hanshui Spring), and a minimum temperature of -43.6°C; the frost-free interval is short, about 98-104 days, and the four seasons are not clearly demarcated.

The grassland type varies significantly with altitude. The following sequence occurs with decreasing altitude: “*Kobresia capillifolia* (*Kobresia capimfolia*) + *Carex oxyleuca* (*Carex atrofusca* Schkuh) + *Polygonum viviparum* L. (*Polygonum viviparum*)” and other types of alpine meadow steppe; “Spikes cold fescue (*Festuca ovim* L. subsp. *Sphagnicola* (B. Kdler)Tzve) + grassland carex (*C. lipawcarpos* Gaudin), short leaf fescue (*F. brachyphylla* Schult) + miscellaneous grasses” and other types of alpine grassland; “*Ceratoides latens* (*Cemtoidedatem revealet* Holmgren) + pipa firewood (*Reaumuria soongonica* (*PalL*) Maxim) - *Stipa glareosa* (*Stipa glareosa P. smirn*), *Ceratoides latens* + *Anabasis brevifolia* (*Anabasis brevifolia* C.A. Mey) - *Stipa glareosa*, *Artemisia frigida* (*Artemisia frigida* milld.Sp. Pl) + *Stipa glareosa*” and other types of desert grassland; Pipa firewood (*Reaumuria soongonica* (PalL)Maxim) + *Anabasis brevifolia* + *Stipa glareosa*, *Kalidium schrenkianum* (*Kalidiwn schrenkianum* Bge. ex Ung.-Sternb) + *Stipa glareosa*, *Seriphidium kaschgaricum* (*Seriphidium kaschgariam* (Krasch.) Poljak) + *Anabasis brevifolia*, *Stipa glareosa* + *Ceratoides latens* +*Anabasis brevifolia* + *Stipa glareosa*” and other types of desert steppe. In addition, “*Blysmus sinocompressus* (*Blysmus sinocompressus* Tang et Wang) + grasses (Poaceae), *Leymus secalinus* (*Leymus secalinus* (Georgi)Tzvel) + grasses + miscellaneous grasses” and other types of intrazonal low meadow steppe, which are not adapted to a temperate zone continental climate, occur in the interior.

Sample collection: In the Barkol area, the grassland is cut in mid-August for use as winter feed, and therefore, our sampling was carried out in early August of 2013 when the grass biomass was at a maximum. Sampling was conducted from north to the south with increasing altitude from the piedmont plain of the Barkol area along the eastern section of the Tianshan Mountains. The 1:3,000,000 grassland map of Xinjiang Uyghur Autonomous Region was used as a basis for determining sampling locations together with a field survey of the dominant grassland types. Sample plots were located in warm desert grassland, warm steppe desert grassland, warm steppe grassland, and low plain meadow grassland, spanning an altitudinal range of 1597~2137 m. All altitudes were measured using a UniStrong G190 GPS. At each location, three major quadrats of size 10 m × 10 m were used, with an interval of at least 100 m. In the four corners and the center of each major quadrat, an additional five small observation quadrats, of dimensions 1 m × 1 m, were used. The harvesting method was used to sample the aboveground biomass, with the dominant species of each sample placed in a separate envelope. In addition to the dominant species, other aboveground living parts and dry parts were also separately stored in an envelope. A soil drilling method was used to obtain the underground biomass; the drill had an internal diameter of 33 mm. Each quadrat was divided into three sampling layers (0~10 cm, 10~20 cm, 20~30 cm); each layer uses three drills, and the same quadrat/sample and the same layer of underground biomass was placed in a sealed bag.

Plant samples from a total of 54 sample plots were collected from five types of grassland: low plain meadow, steppe desert, desert steppe, alpine steppe and alpine meadow grassland (the classification of grassland types is based on the Chinese Grassland Resources (Liao & Jia, 1996).

Sample preparation: In the laboratory, the aboveground biomass was divided into leaves, stems and flowers/fruits. Then, all the plant samples (including the aboveground and underground biomass) were washed with de-ionized water, air-dried, and then heated in an oven at 80°C for 48 h. The samples were then weighed with an electronic balance (to the nearest 0.0001g) to obtain the underground (root) biomass in the sample plot for each dominant species, and the aboveground biomass (leaves, stems and flowers/fruits) of the other living parts. After weighing, the leaves and the underground root system samples were ground to a powder using a plant crusher. The δ^{13}C values of the plant leaves and root system were measured using an isotope mass spectrometer (Isoprime 100) in the State Key Laboratory of Desert and Oasis Ecology. The δ^{13}C values are expressed as:

$$\delta^{13}\mathrm{C}\ (‰) = [(\mathrm{R}_{\mathrm{sample}} - \mathrm{R}_{\mathrm{standard}}) / \mathrm{R}_{\mathrm{standard}}] \times 1000 \qquad (1)$$

where $\mathrm{R}_{\mathrm{sample}}$ and $\mathrm{R}_{\mathrm{standard}}$ are the carbon isotope ratios of test samples and standard samples respectively, relative to a PDB standard.

Climate data: The annual average temperature, average temperature, annual average rainfall, average monthly rainfall and the cumulative temperature greater than 0°C at each sampling location were extracted from the National Land Ecological Information and Meteorological Grid Database (1970~2000, resolution 1 km × 1 km) constructed by the Ecosystem Research Network Comprehensive Center of the Chinese Academy of Sciences (Han, 2000). Growing season temperature (GST) and the growing season rainfall (GSP) were calculated using equations (2) and (3), respectively:

$$GST = \sum_{i=5}^{9} MMT_i/5 \qquad (2)$$

$$GSP = \sum_{i=5}^{9} MMP_i \qquad (3)$$

where $MMTi$ and $MMP_i$are the average monthly temperature and the rainfall in month i, respectively.

Sample point dryness index (*DI*) was calculated following Ren *et al*. (1965):

$$DI = \frac{0.1\sum \theta}{MAP} \qquad (4)$$

where $\sum\theta$ is the cumulative temperature greater than 0°C and *MAP* is the average annual rainfall. Atmospheric pressure was calculated following Jones (1983):

$$p = 101.325/\mathrm{e}^{[(z/29.3)/T_k]} \qquad (5)$$

where p is atmospheric pressure (kPa), z is altitude and $_k$ isair temperature (in this study, the growing season temperature was taken as the air temperature. The relationship between climatic parameters and altitude in the study area is shown in Table 1 and Fig. 1

Data analysis: SPSS 18.0, Microsoft Excel, and DPS data processing software were used to perform single factor analysis of variance, correlation analysis and stepwise regression analysis to determine differences between grassland types and the relationships between variables. ArcGIS was used for layer superposition and interpolation analysis of the relationship at the community level between the δ^{13}C values of plant leaves and roots and altitude, and the relationships between leaf δ^{13}C values and temperature, precipitation and atmospheric pressure.

Results

δ^{13}C values of community level plant leaves and roots: The δ^{13}C values of community level plant leaves and root systems are listed in Table 2. The δ^{13}C of the plant leaves in alpine grassland is significantly greater than in alpine meadow and low plain meadow, and similar to the values of desert steppe grassland and steppe desert grassland. There is no significant difference between the δ^{13}C values of steppe desert grassland, desert steppe and alpine steppe grassland. There are significant differences between the values for desert steppe and alpine steppe and the other grassland types. In addition, the leaf δ^{13}C values are lower than those of the root system.

Table 1. Altitude of precipitation and the relationship between climatic parameters in the Barkol area.

	Altitude (m)	MAP (mm)	MMT (℃)	DI	GSP (mm)
Altitude (m)					
MAP (mm)	0.457				
MMT (℃)	-0.431	-0.997**			
DI	0.450	0.997**	-0.999**		
GSP (mm)	0.461	1.000**	-0.997**	0.998**	
GST (℃)	-0.521	-0.989**	0.977**	-0.981**	-0.990**

Note: ** 0.01 significance level (2-tailed)

Table 2. Community level δ^{13}C characteristics of plant leaves and roots.

Grassland types	Leaves δ^{13}C(‰)	Root δ^{13}C(‰)
Lower-flat meadows	(-27.62 ± 0.34) BCb	(-25.91 ± 0.90) Aa
Steppe desert	(-25.25 ± 0.45) AEa	(-25.54 ± 1.09) Aa
Desert steppe	(-25.29 ± 0.77) AEa	(-22.37 ± 0.96) Aa
Alpine steppe	(-25.58 ± 0.63) ABDa	(-24.07 ± 0.57) Aa
Alpine meadow	(-27.45 ± 0.53) BCDb	(-25.80 ± 0.60) Aa

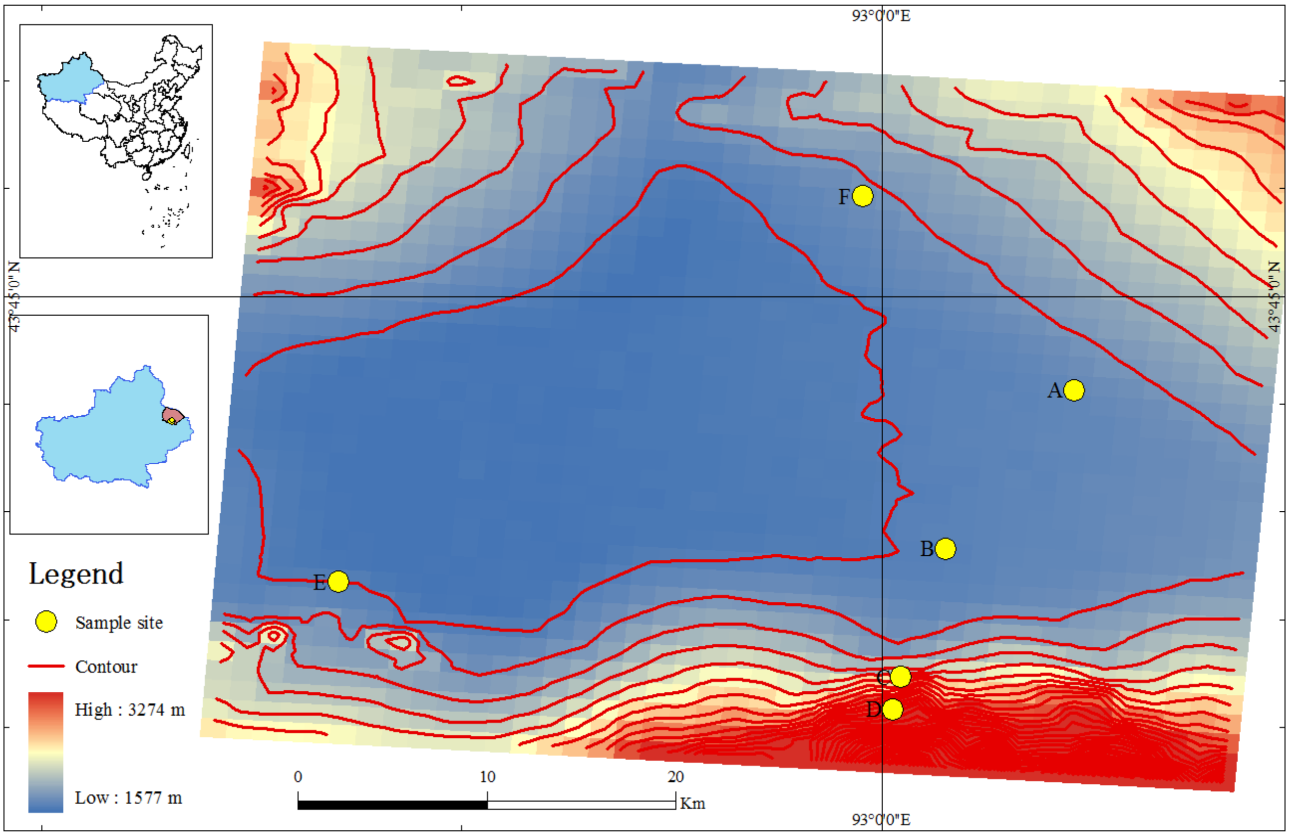

Fig. 1. Topography of the study area and location of sampling sites. Lower inset map shows the location of the study area in Xinjiang Province and the upper map the location of Xinjiang in China.

Relationship between the δ^{13}C of community level plant leaves and root systems and altitude: At the community level, the δ^{13}C values of the leaves of the plants of alpine meadow grassland are negatively correlated with altitude (Fig. 2). However, although the δ^{13}C values of the equivalent root systems also decrease with altitude the relationship is not statistically significant.

Relationship between theδ^{13}C values of community level plant leaves and moisture availability: The δ^{13}Cvalues of the grassland ecosystem plant leaves are positively correlated with average annual rainfall (Fig. 3a) and negatively correlated with growing season rainfall (Fig 3b); in addition, there is a negative (but not statistically significant) relationship with dryness index (Fig. 3c).

Relationship between the δ^{13}C of community level plant leaves and temperature: Overall, the leaf δ^{13}C values are not significantly related to temperature (Fig. 4); however, in the case of alpine meadow and alpine grassland, temperature has a larger effect. In addition, the influence of growing season temperature on leaf δ^{13}C is greater than that of average annual temperature.

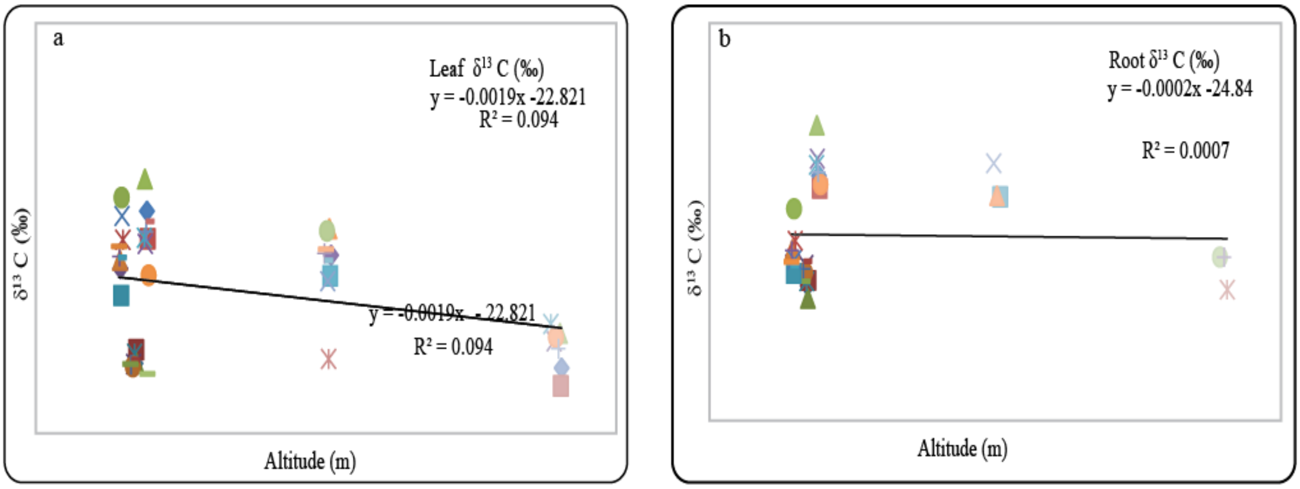

Fig. 2. Relationship between altitude and the δ^{13}C values of community level plant leaves (top) and roots (bottom).

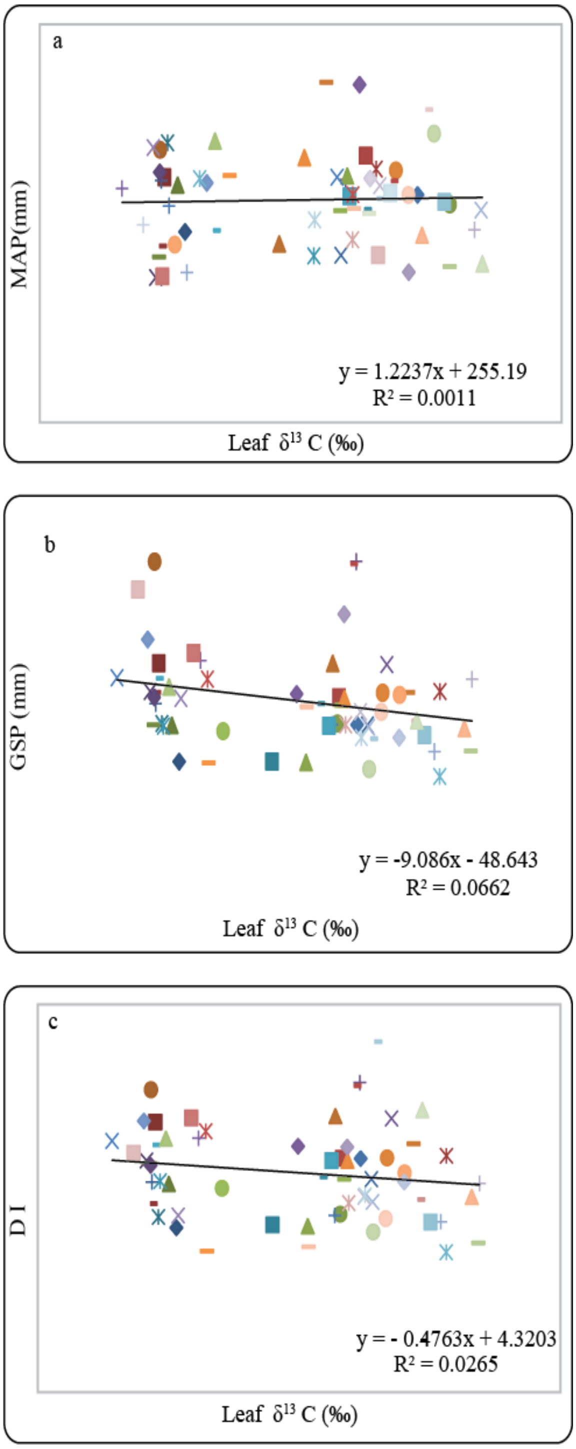

Fig. 3. Community level plant leaves δ^{13}C relations with *MAP*、*GSP* and *DI* a *MAP*; b *GSP*; c *DI*

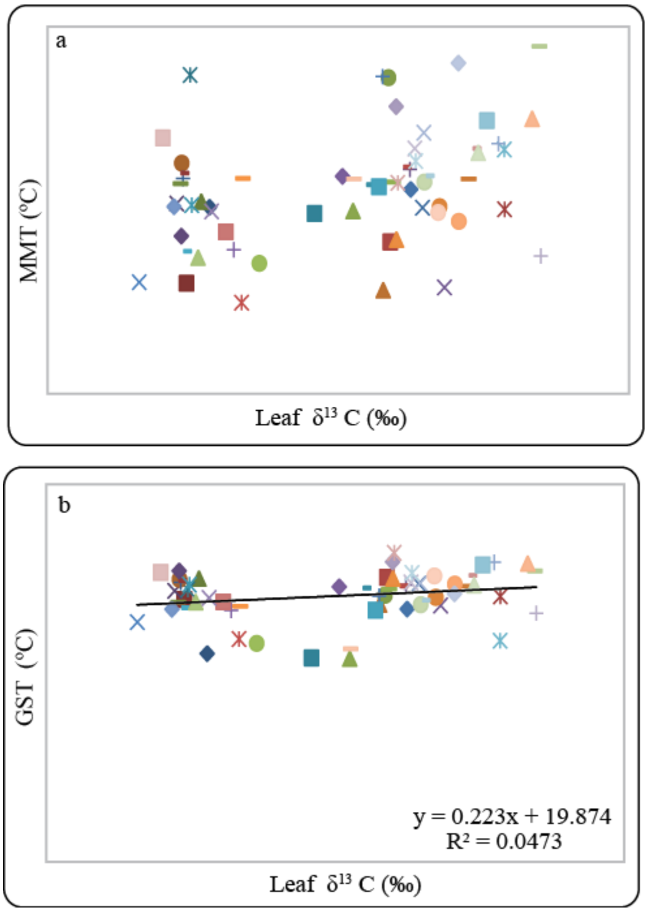

Fig. 4. Relationship between the δ^{13}C values of community level plant leaves and (a) *MMT* and (b) *GST*.

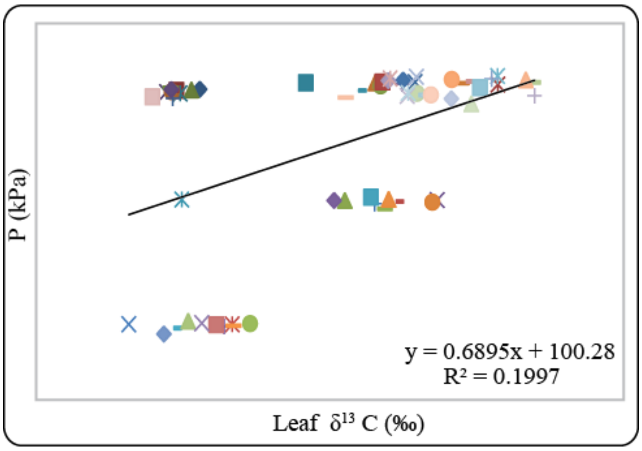

Fig. 5. Relationship between the δ^{13}C values of community level plant leaves and atmospheric pressure.

Relationship between the δ^{13}C of community level plant leaves with atmospheric pressure: The δ^{13}C values of the grassland ecosystem plant leaves are positively correlated with atmospheric pressure (Fig. 5), in accordance with a relationship between plant leaf δ^{13}C and altitude.

Discussion

δ^{13}C characteristics of plant leaves and root systems at the community level: The leaf δ^{13}C values of alpine grassland plants are higher than those of alpine meadow plants, at the community level. This may be the result of differences in plant species composition between the two types of grassland. In this study, sedges are the dominant species in alpine meadow, while *Stipa* L. is the dominant species in alpine grassland. A previous study on the species level found that the δ^{13}C values of Gramineae leaves were significantly higher than in sedges (Zhou *et al.*, 2011). Although the alpine grasslands and temperate grasslands are both dominated by *Stipa* species, there are differences between the δ^{13}C values of the plant leaves of different species; this may be because they exhibit different altitudinal distributions. In the present study, the altitudinal range of temperate steppe is 1597-1636 m, while that of alpine steppe is 1846-1861 m. Alpine grassland is thus distributed at higher altitudes than temperate grasslands and the decrease in leaf δ^{13}C values with increasing altitude may be the cause of the observed differences. However, there is a only a small altitudinal difference between alpine grassland and warm steppe and thus the difference in the plant leaf δ^{13}C values between the two is insignificant.

In the present study, for alpine meadow plants at the community level, the leaf δ^{13}C values are lower than those of the roots; this is consistent with the results of previous research carried out at the species level (Farquhar *et al.*, 1984; Feng *et al.*, 2003; Fang *et al.*, 2009; Zhao *et al.*, 2004). Differences in the δ^{13}C values of plant leaves and roots may arise for two reasons. First, there is a difference in the biochemical composition of plant leaves and roots, which will lead to different δ^{13}C values (Dungait *et al.*, 2008). The main chemical composition of the photosynthetic apparatus (leaves) is lignin and lipids, and the main chemical composition of the other non-photosynthetic apparatus, such as roots, is cellulose and hemicellulose. The δ^{13}C value of lignin and lipids is lower than that of cellulose and hemicellulose (Winkler *et al.*, 1978). This may be because carbon with δ^{13}C values lower than that of the initial carbon products of photosynthesis are used for the synthesis of lignin and lipids, while the remaining carbon, with significantly higher δ^{13}C, is used to produce synthetic celluloses and is transferred to the outside of the leaf (Hobbie, 2004). Second, the respiratory characteristics of the different organs are significantly different. In theory, during the respiration of plant organs, the substances containing ^{12}C are used preferentially, leaving ^{13}C enriched in the plant tissue (Leavitt & Long, 1986). According to the determination of various respiration rates of non-photosynthetic plant organs in the mountainous area of Beijing, different species (or different organs of the same species) had a different respiration rate, which was the reason for the corresponding differences in δ^{13}C values (Han *et al.*, 2000). However, several studies found that differences in δ^{13}C values between the leaves and roots of some species were not significant (Han *et al.*, 2000), indicating that the δ^{13}C characteristics of plant organs are highly related to the species and that not all species exhibit differences in δ^{13}C values between different organs. In the present study, the dominant species of alpine grassland, temperate grassland or even in alpine meadow and alpine meadow may be different. This may be an important reason for the significant difference observed in the δ^{13}C values between leaves and root systems at the community level between alpine meadow and lowland meadow grassland, while in the other three grassland types the difference was not significantly different.

Relationship between community level plant leaf δ^{13}C and elevation: At the community level, the leaf δ^{13}C values of grassland ecosystems in the Barkol area are negatively related with altitude; however, the relationship is not statistically significant. The degree of respond to elevation are different for species diversity and plant leaves on community level and may be the reason that the leaf δ^{13}C changing rate with altitude are lower than that of species. Many studies have confirmed that the δ^{13}C response of plant leaves to altitude was species specific. For example, Hultine & Marshall (2000) found that rate of change for Los Angeles fir (*Abies lasiocarpa*) was 0.9‰ km^{-1}, while that of lodgepole pine (*Pinus contorta*) was 2.68‰ km^{-1}. Zhu *et al.* (2010) reported that the rate of change of *Potentilla* and *Carex* species was about 1.2‰ km^{-1}, while that for *Saxifraga* spp. was 1.8‰ km^{-1}. Liu *et al.* (2004) observed a rate of change for edelweiss (*Leontopodium nanum*) of 0.7‰ km^{-1} in the Qinghai-Tibetan Plateau, while the rate for *Astragalus membranaceus* (*Astragalus tolycladus*) was 3.2‰ km^{-1}. Clearly, plant communities are composed of a variety of species and different species exhibit different rates of change of δ^{13}C with altitude. This results in the leaf δ^{13}C values at the community level being relatively stable with changing environmental conditions. It also confirms the importance of community structure, in terms of species diversity, in ecosystem stability.

Relationship between community level plant leaf δ^{13}C and water availability: The δ^{13}C values of the leaves of alpine grassland plants are positively correlated with dryness which may be due to the development of alpine grassland in a relatively arid climate and environment (Liao & Jia, 1996). Moisture is a major limiting factor for plant growth and with increasing aridity and water stress, plants will reduce their rate of water evaporation and improve their water use efficiency (WUE) by closing their stomata, thus decreasing stomatal conductance (Seibt *et al*., 2008; Diefendorf *et al*., 2010; Yang *et al*., 2015). At the same time, because the leaf stomata are the channel of atmospheric CO_2 transport into the leaves, partial stomatal closure will cause a decrease in the internal CO_2 concentration of plant leaves (C_i), resulting in increased leaf δ^{13}C (Morecroft *et al*., 1990). In the present study, there is no correlation between the leaf δ^{13}C values of alpine meadow plants, which may be due to the growth of the alpine meadow in relatively cold and humid climatic conditions (Liao & Jia, 1996).

The δ^{13}C of the leaves of the entire grassland ecosystem is negatively correlated with growing season precipitation, which is consistent with the results of previous studies (Wang *et al*., 2010; Zhou *et al*., 2011); however, the relationship is not significant (Fig. 3b). We infer that this may be a statistical aberration because in the present study precipitation and elevation are positive correlated (Table 1), whereas elevation is negatively correlated with plant leaf δ^{13}C (Fig. 2). The reasons for this can be summarized as follows: (1) Moisture is not the main limiting factor for plant growth in the entire grassland ecosystem in the Barkol area and thus, water will not significantly affect the plant leaf δ^{13}C values. In addition, in the present study, dryness (which more accurately reflects the moisture status of an area than precipitation or evaporation amount) and leaf δ^{13}C exhibit a significant negative correlation (Fig. 3c). (2) In a study of the Qinghai-Tibetan Plateau there was no correlation between plant leaf δ^{13}C and rainfall (Wang *et al*., 2003; Li *et al*., 2007).

Relationship between community level plant leaf δ^{13}C and temperature: In the studied grassland ecosystem, annual average temperature and plant leaf δ^{13}C are not significantly correlated, but there is a non-significant positive correlation between leaf δ^{13}C and growing season temperature (Fig. 4a and 4b). This shows that temperature is not the main factor responsible for the decrease in leaf δ^{13}C with increasing altitude, which may instead be attributed to differences in the species composition of grassland ecosystems. In this context, it has been clearly demonstrated that different species have different photosynthetic optimum temperatures, resulting in a complex relationship between plant leaf $\delta^{13}C$ and temperature (Schleser *et al*., 1999). Several studies found that plant $\delta^{13}C$ values and temperature were positively correlated (Li *et al*., 2007; Loader & Hemming, 2001; Saurer *et al*., 1995), while others found either a negative or no clear relationship between leaf $\delta^{13}C$ and temperature (Zheng & Shangguan, 2007; Sheu & Chiu, 1995; Wang *et al*., 2013).

The relationship between plant leaf $\delta^{13}C$ and growing season temperature is stronger than that with annual mean temperature in the studied grassland ecosystem, which may be due to the low winter temperatures in the Barkol area. Plants are dormant or die resulting in the cessation of gas exchange caused by photosynthesis, so that winter temperature does not affect plant $\delta^{13}C$. However, winter temperature makes a large contribution to the mean annual temperature, resulting in a weak, or even no correlation, between plant leaf $\delta^{13}C$ and MAT.

Relationship between community level plant leaf $\delta^{13}C$ and atmospheric pressure: CO_2, as the raw material for plant photosynthesis, is an important limiting factor for plant photosynthesis when its concentration is low (O'Leary, 1981). The altitudinal range of the present study (1597-2137 m) corresponds to a range of 83.89-78.40% of standard atmospheric pressure. Therefore, atmospheric pressure (and thus the atmospheric CO_2 concentration) cannot be a major limiting factor for plant growth. (Notably, a limiting factor for plant growth is often a key factor that controls changes in plant $\delta^{13}C$.) In contrast, leaf $\delta^{13}C$ was positively related to altitude in the Tibetan Plateau, where the reduction of atmospheric pressure with increasing altitude is likely the main cause of the relationship (Zhou *et al*., 2011; Zhu *et al*., 2010). Therefore, there does not always have to be a general pattern of changing leaf $\delta^{13}C$ along an environment gradient such as MAP, MAT or altitude (Wang C *et al*., 2016). Detailed analysis of the underlying controlling factors is necessary for a better understanding the altitudinal pattern of leaf carbon isotopes.

Conclusions

In the Barkol region of Xinjiang, the main driving factors for the decrease in leaf $\delta^{13}C$ with increasing altitude are related to moisture levels and atmospheric pressure discrimination, or most cases, the most limiting factor is generally the most important factor for variations of leaf $\delta^{13}C$ such as rainfall along our studied transect and atmospheric CO_2 concentration with high altitude. Overall, our results indicate that the environmental factors controlling leaf $\delta^{13}C$ vary among different ecosystems, depending on the most important limiting factors.

References

Chen, L.T., D.F.B. Flynn, X.W. Zhang, X.L. Gao, L. Lin, J. Luo and C.M. Zhao. 2015. Divergent patterns of foliar $\delta^{13}C$ and $\delta^{15}N$ in Quercus aquifolioides with an altitudinal transect on the Tibetan Plateau: an integrated study based on multiple key leaf functional traits. *J. Plant Ecol.*, 8: 303-12.

Chen, S.P., Y.F. Bai, G.H. Lin and X.G. Han. 2005. Variations in life-form composition and foliar carbon isotope discrimination among eight plant communities under different soil moisture conditions in the Xilin River Basin, Inner Mongolia, China. *Ecol. Res.*, 20: 167-76.

Chen, T., M.X. Yang, H.Y. Feng, S.J. Xu, W.Y. Qiang, Y.Q. He and L.Z. An. 2003. Spatial distribution of stable carbon isotope compositions of plant leaves in the north of the Tibetan Plateau. *J. Glaciology & Geocryology*, 25: 83-87.

Diefendorf, A.F., K.E. Mueller, S.L. Wing, P.L. Koch and K.H. Freeman. 2010. Global patterns in leaf ^{13}C discrimination and implications for studies of past and future climate. *Proc. Natl. Acad. Sci. USA.*, 107: 5738-43.

Dungait, J.A.J., G. Docherty, V. Straker and R.P. Evershed. 2008. Interspecific variation in bulk tissue, fatty acid and monosaccharide $\delta^{13}C$ values of leaves from a mesotrophic grassland plant community. *Phytochem.*, 69: 2041-2051.

Fang, X.J., J.Y. Li, L.S. Nie, Y.B. Shen and Z.Y. Zhang. 2009. The characteristics of stable carbon isotope and water use efficiency for *Populus tomentosa* hybrid clones. *Ecol. & Environ. Sci.*, 18: 2267-2271.

Farquhar, G.D. and R.A. Richards. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Funct. Plant Biol*., 11: 539-552

Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Ann. Rev. Plant Physiol. & Plant Mol. Biol.*, 40: 503-537.

Feng, H.Y., L.Z. An, T. Chen, S.J. Xu, W.Y. Qiang, G.X. Liu and X.L. Wang. 2003. The relationship between foliar stable carbon isotope composition in *Pedicularis* L. and environmental factors. *J. Glaciology & Geocryology*, 25: 88-93.

Geßler, A., S. Schrempp, A. Matzarakis, H. Mayer, H. Rennenberg and M.A. Adams. 2001. Radiation modifies the effect of water availability on the carbon isotope composition of beech (Fagus sylvatica). *New Phytol.*, 150: 653-64.

Guo, G. and G. Xie. 2006. The relationship between plant stable carbon isotope composition, precipitation and satellite data, Tibet Plateau, China. *Quat. Int.*, 144: 68-71.

Han, X. G., C.R. Yan, L.Q. Chen and X. Mei. 2000. Stable carbon isotope characteristics of some woody plants in warm temperate zone. *Chinese J. App. Ecol.*, 11: 497-500.

He, C.X., J.Y. Li, P. Meng and Y. Zhang. 2010. Changes in leaf stable carbon isotope fractionation of trees across climatic gradients. *Acta Ecologica Sinic*, 30: 3828-3838.

Hobbie, E.A. 2004. Intramolecular, compound-specific, and bulk carbon isotope patterns in C_3 and C_4 plants: A review and synthesis. *New Phytol.*, 161: 371-385.

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

Jones, H.G. 1983. Plants and Microclimate. *Cambridge: Cambridge University Press.*

Kang, L., X.G. Han, Z.B. Zhang and O.J. Sun. 2007. Grassland ecosystems in China: review of current knowledge and research advancement. *Philos. Trans. R Soc. B.*, 362: 997-1008.

Kogami, H., Y.T. Hanba, T. Kibe, I. Terashima and T. Masuzawa. 2001. CO_2 transfer conductance, leaf structure and carbon isotope composition of *Polygonum cuspidatum* leaves from low and high altitudes. *Plant Cell Enviro.*, 24: 529-538.

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

Lal, R. 2004. Carbon sequestration in dryland ecosystems. *Environ. Manag.*, 33: 528-44.

Leavitt, S. and A. Long. 1986. Stable carbon isotope variability in tree foliage and wood. *Ecology*, 67: 1002-1010.

Li, J.Z., G.A. Wang, X.Z. Liu, J.M. Han, M. Liu and X.J. Liu. 2009. Variations in carbon isotope ratios of C_3 plants and distribution of C_4 plants along an altitudinal transect on the eastern slope of Mount Gongga. *Sci China Ser D-Earth Sci.*, doi:10.1007/s11430-009-0170-4.

Li, M.C., H.Y. Liu, L.X. Li, X.F. Yi and X.J. Zhu. 2007. Carbon isotope composition of plants along altitudinal gradient and its relationship to environmental factors on the Qinghai-Tibet Plateau. *Pol. J. Ecol.*, 55: 67-78.

Li, Y. B., T. Chen, Y.F. Zhang and L.Z. An. 2007. The relation of seasonal pattern in stable carbon compositions to meteorological variables in the leaves of *Sabina przewalskii* Kom. And *Sabina chinensis* (Lin.) Ant. *Environ. Geol.*, 51: 1279-1284.

Liao, G. F. and Y.L. Jia. 1996. The grassland resources of China. *Beijing: Science and technology of China press*.

Liu, G. X., T. Chen and L.Z. An. 2004. The environmental significance of stable carbon isotope composition of modern plant leaves in the northern part of the Tibetan Plateau. *Adv. in Earth Sci.*, 19: 749-753.

Loader, N. J. and D.L. Hemming. 2001. Spatial variation in pollen $\delta^{13}C$ correlates with temperature and seasonal development timing. *Holocene*, 11: 587-592.

Morecroft, M.D. and F.I. Woodward. 1990. Experimental investigations on the environmental determination of $\delta^{13}C$ at different altitudes. *J. Exp. Bot.*, 41: 1303-1308.

Morecroft. M.D., F.I. Woodward and R.H. Marrs. 1992. Altitudinal trends in leaf nutrient contents, leaf size and ^{13}C of Alchemilla alpine. *Funct. Ecol.*, 6: 730-740.

O'Leary, M. 1981. Carbon isotope fractionation in plants. *Phytochem.*, 20: 553-567.

O'Leary, M., S. Madhavan and P. Paneth. 1992. Physical and chemical basis of carbon isotope fractionation in plants. *Plant Cell Environ.*, 15: 1099-104.

Ren, J.Z., Z.Z. Hu and X.D. Mu. 1965. Grassland types in our country the first level classification bioclimatic indicator. *Acta Inst. Agri. Gansu.*, 2: 48-64.

Saurer, M., U. Siegenthaler and F. Schweingruber. 1995. The climate carbon isotope relationship in tree rings and the significance of site conditions. *Tell. Ser. B-Chem. Phys. Meteorol.*, 47: 320-330.

Schleser, G., G. Helle, A. Luk and H. Vos. 1999. Isotope signals as climate proxies: The role of transfer functions in the study of terrestrial archives. *Quat. Sci. Rev.*, 18: 927-943.

Seibt, U., A. Rajabi, H. Griffths and J.A. Berry. 2008. Carbon isotopes and water use effciency: sense and sensitivity. *Oecologia*, 155: 441-54.

Sheu, D. and C. Chiu. 1995. Evaluation of cellulose extraction procedures for stable carbon isotope measurement in tree ring research. *Int. J. Environ. Anal. Chem*., 59: 59-67.

Shi, Z.M., Q.H. Feng, R.M. Cheng and S.R. Liu. 2010. The research progress of mesophyll conductance. *Acta Ecologica Sinic*., 3: 4792-4803.

Shi, Z.M., S.R. Liu, X.L. Liu and M. Centritto. 2006. Altitudinal variation in photosynthetic capacity, diffusional conductance, and $\delta^{13}C$ of butterfly bush (*Buddleja davidii*) plants growing at high elevations. *Physiologia Plantarum*, 128: 722-731.

Su, P.X. and Q.D. Yan. 2008. Stable carbon isotope variation in plants and their indicating significances along the inland Heihe River basin of northwestern China. *Ecologica Sinic*., 28: 1616-1624.

Van de Water, P. K., S.W. Leavitt and J.L. Betancourt. 2002. Leaf $\delta^{13}C$ variability with elevation, slope aspect, and precipitation in the southwest United States. *Oecologia*, 132: 332-343.

Wang, C., D.W. Liu, W.T. Luo, Y.T. Fang, X.B. Wang, X.T. Lü, Y. Jiang, X.G. Han and E. Bai. 2016. Variations in leaf carbon isotope composition along an arid and semi-arid grassland transect in northern china. *J. Plant Ecol.*, 9: 576-585.

Wang, G.A., J.M. Han, A. Faiia, W.B. Tan, W.Q. Shi and X.Z. Liu. 2008. Experimental Measurements of Leaf Carbon Isotope Discrimination and Gas Exchange in the Progenies of Plantago depressa and Setaria viridis Collected from a Wide Altitudinal Range. *Physiologia Plantarum*, 134: 64-73.

Wang, G.A., J.Z. Li, X.Z. Liu and X.Y. Li. 2013. Variations in carbon isotope ratios of plants across a temperature gradient along the 400 mm isoline of mean annual precipitation in north China and their relevance to paleovegetation reconstruction. *Quat. Sci. Rev*., 63:83-90.

Wang, L., H.Y. Lü, N.Q. Wu, H.B. Wu and T.S. Liu. 2003. Altitudinal trends of stable carbon isotope composition for poeceae in Qinghai-Xizang plateau. *Quat. Sci.*, 23: 573-580.

Wang, L.X., P. D'Odorico, L. Ries and S.A. Macko. 2010. Patterns and implications of plant-soil $\delta^{13}C$ and $\delta^{15}N$ values in African savanna ecosystems. *Quat. Res*., 73: 77-83.

Wang, N., S.S. Xu, X. Jia, J. Gao, W.P. Zhang, Y.P. Qiu and G.X. Wang. 2013. Variations in foliar stable carbon isotopes among functional groups and along environmental gradients in China a meta analysis. *Plant Biol*., 15: 144-51.

Warren, C.R., J.F. McGrath and M.A. Adams. 2001. Water availability and carbon isotope discrimination in conifers. *Oecologia*, 127: 476-486.

Wei, J., W.G. Liu, J.M. Cheng and W.J. Li. 2014. $\delta^{13}C$ values of plants as indicators of soil water content in modern ecosystems of the Chinese Loess Plateau. *Ecol. Eng.*, 77: 51-9.

Winkler, F.J., E. Wirth, E. Latzko, H.-L. Schmidt, W. Hoppe and P. Wimmer. 1978. Influence of growth conditions and development on $\delta^{13}C$ values in different organs and constituents of wheat, oat and maize. *J. Plant Physiol*., 87: 255-263.

Yang, Y.H., C.J. Ji, L.Y. Chen, J.Z. Ding, X.L. Cheng and D. Robinson. 2015. Edaphic rather than climatic controls over ^{13}C enrichment between soil and vegetation in alpine grasslands on the Tibetan Plateau. *Funct. Ecol*., 29: 839-48.

Zhao, B., M. Kondo, M. Maeda, Y. Ozaki and J.B. Zhang. 2004. Water-use efficiency and carbon isotope discrimination in two cultivars of upland rice during different developmental stages under three water regimes. *Plant & Soil*, 261: 61-75.

Zheng, S. and Z. Shangguan. 2007. Spatial patterns of foliar stable carbon isotope compositions of C_3 plant species in the Loess Plateau of China. *Ecol. Res*., 22: 342-353.

Zhou, Y.C., J.W. Fan, H.P. Zhong and W.Y. Zhang. 2013. Relationships between altitudinal gradient and plant carbon isotope composition of grassland communities on the Qinghai-Tibet Plateau, China. *Science China: Earth Sciences*, 56: 311-320.

Zhou, Y.C., J.W. Fan, W.Y. Zhang, W. Harris, H.P. Zhong, Z.M. Hu and L.L. Song. 2011. Factors influencing altitudinal patterns of C_3 plant foliar carbon isotope composition of grasslands on the Qinghai-Tibet Plateau, China. *Alp. Bot.*, 121: 79-90.

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

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