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Variations in leaf carbon isotope composition along an arid and semi-arid grassland transect in northern China

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Abstract

Aims

We aimed to quantify the variation of leaf $\delta^{13}\text{C}$ along an arid and semi-arid grassland transect in northern China. We also evaluated the effects of environmental factors (i.e. precipitation, temperature and altitude) on the spatial variation of leaf $\delta^{13}\text{C}$ in northern grasslands and Tibetan Plateau, China.

Method

We sampled leaves of plant species belonging to three herb genera (*Stipa spp.*, *Leymus spp.* and *Cleistogenes spp.*) and three shrub genera (*Caragana spp.*, *Reaumuria spp.* and *Nitraria spp.*) for carbon isotope analysis from 50 locations along a 3200-km arid and semi-arid grassland transect in northern China. Leaf $\delta^{13}\text{C}$ data in Tibetan Plateau and northern grasslands in China were also compiled from studies in literature.

Important Findings

Along the transect, leaf $\delta^{13}\text{C}$ for C_3 plants ranged from -28.0‰ to -23.3‰ , and from -16.3‰ to -13.8‰ for C_4 plant *Cleistogenes*

spp.. The change in leaf $\delta^{13}\text{C}$ ranged from -0.26‰ to -3.51‰ with every 100 mm increase of annual precipitation, and leaf $\delta^{13}\text{C}$ of shrubs (*Nitraria spp.*, *Reaumuria spp.* and *Caragana spp.*) responded more markedly to climatic factors (precipitation and temperature) than that of herbs (*Stipa spp.*, *Leymus spp.* and *Cleistogenes spp.*), indicating higher sensitivity of shrub $\delta^{13}\text{C}$ to climatic changes. The most important factor regulating spatial variations of leaf $\delta^{13}\text{C}$ in Tibetan Plateau was altitude, while it was precipitation in northern grasslands. Our results suggested that shrubs are more adapted to increasing drought in arid and semi-arid grassland. Controls of environmental factors on leaf $\delta^{13}\text{C}$ depended on the most limiting factors in arid grassland (precipitation) and Tibetan grasslands (atmospheric CO_2 concentration).

Keywords: carbon isotope, water use efficiency, plant life-form, rainfall gradient, climate change

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INTRODUCTION

Arid and semi-arid biomes currently occupy approximately 41% of the terrestrial land surface (Reynolds *et al.* 2007), and recent observations and models predicted that the global drylands would expand in the future (Feng and Fu 2013). Modeling studies suggested drylands are experiencing more variable rainfall and more drought events with increasing

atmospheric CO_2 level and temperature (Easterling *et al.* 2000). Drier climate not only limits plant growth, but may also affect plant community composition because the water use efficiency (WUE) and the ability to tolerate low soil moisture or to compete for this limiting resource of different plants are different (Austin 2011; Yahdjian and Sala 2006). Therefore, understanding the WUE of different plants and their responses to climatic factors are of great significance to

predict the primary productivity and carbon cycling in the arid and semi-arid ecosystems under changing global climate.

Leaf carbon isotope ratio ($\delta^{13}\text{C}$) has been considered as an informative indicator to evaluate plant–environment interactions and plant WUE (Diefendorf et al. 2010; Donovan and Ehleringer 1994a; Farquhar et al. 1989; Yang et al. 2015). Plants assimilate atmospheric $^{12}\text{CO}_2$ more than $^{13}\text{CO}_2$ during photosynthesis, and this isotopic discrimination depends on the CO_2 partial pressure inside the leaf and its ratio to that in the ambient air (p_i/p_a) (Farquhar et al. 1989; O'Leary et al. 1992). It has been proven that leaf $\delta^{13}\text{C}$ is positively related to plant WUE via p_i/p_a (Farquhar et al. 1989; Seibt et al. 2008). For example, due to their low ratio of p_i/p_a , plants in drier environment have been found to have higher leaf $\delta^{13}\text{C}$, suggesting higher WUE (Donovan and Ehleringer 1994a; Ehleringer and Cooper 1988).

Leaf $\delta^{13}\text{C}$ 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 nitrogen (N) (Sparks and Ehleringer 1997) and water content (Wei et al. 2014). Altitude, which reflects the combined effect of these factors, has been found to positively influence leaf $\delta^{13}\text{C}$ at regional and global scales (Chen et al. 2015; Chen et al. 2005; Hultine and Marshall 2000; Körner et al. 1988). Körner et al. (1991) suggested that both temperature and atmospheric pressure were responsible for the positive correlation between leaf $\delta^{13}\text{C}$ and altitude, while Van de Water et al. (2002) attributed the altitudinal pattern of leaf $\delta^{13}\text{C}$ they observed mainly to the variations on soil water availability. Hultine and Marshall (2000) concluded that leaf morphological and physiological traits might be the key to understand variations of plant $\delta^{13}\text{C}$ along the altitude gradient. Therefore, controls of environmental factors on leaf $\delta^{13}\text{C}$ may be different for different plant species or plant functional groups.

In fact, plant intrinsic characteristics play an important role in determining leaf $\delta^{13}\text{C}$ (Comstock and Ehleringer 1992; Marshall and Zhang 1994). For example, leaf N content has been found to correlate with leaf $\delta^{13}\text{C}$, which is because photosynthetic enzymes, such as RuBP carboxylase, contain large quantities of N (Sparks and Ehleringer 1997) and the increase of photosynthesis could induce less discrimination against ^{13}C and therefore higher leaf $\delta^{13}\text{C}$. Likewise, leaf mass per area was also found to be coupled with leaf $\delta^{13}\text{C}$ (Vitousek et al. 1990). Besides, plant genetic types (Marshall and Zhang 1994) and life-forms (Ehleringer and Cooper 1988; Smedley et al. 1991) could influence leaf $\delta^{13}\text{C}$ by their controls on photosynthetic capacity and p_i (Farquhar et al. 1989).

The arid and semi-arid grasslands in northern China cover more than 70% of the national grassland areas and they play an important role in ecological services and terrestrial carbon balance (Kang et al. 2007; Lal 2004). Water is the most limiting factor for plant production in this region and is highly variable both in space and over time. Mean annual precipitation (MAP) increases from 30 mm per year in western part of Gansu province to nearly 400 mm per year in eastern part of Inner Mongolia (Wang et al. 2014). This precipitation gradient

provides an ideal 'natural experiment' to explore spatial pattern and environmental drivers of leaf $\delta^{13}\text{C}$, especially for the plants living in areas with MAP <100 mm. Thus, the primary objectives of this study were: (i) to quantify the variation of leaf $\delta^{13}\text{C}$ along the arid and semi-arid grassland transect in northern China; (ii) to evaluate effects of MAP, mean annual temperature (MAT) and leaf N content on this variation. Besides, Tibetan Plateau is another important grassland region in China, where the average altitude is over 4000 m (Tang et al. 2009). We also explored the effects of climatic factors and altitude on leaf $\delta^{13}\text{C}$ in Tibetan Plateau grasslands, which are helpful to fully understand variations of grassland plant $\delta^{13}\text{C}$ in China.

MATERIALS AND METHODS

Study sites

This study was conducted along an arid and semi-arid grassland transect in northern China, which has been previously described (Luo et al. 2013; Wang et al. 2014). This grassland transect was 3200 km long from the west to the east, which covered approximately 24° ranging from $96^\circ 40'$ E to $120^\circ 28'$ E and latitude ranged from $39^\circ 51'$ N to $50^\circ 30'$ N (Fig. 1). The climate is predominantly arid and semi-arid continental; MAP ranged from 34 to 436 mm and MAT ranged from -3 to $+10^\circ\text{C}$. The main vegetation types are desert steppe, typical steppe and meadow steppe distributed from the west to east. Soil types are predominantly arid, sandy, brown loessials rich in calcium and belong to Kastanozem soil group in the FAO classification system.

Plant sampling

We conducted the field sampling campaign during July and August in 2012, and a total of 50 locations with a mean interval of 65 km were selected along the transect (Fig. 1). The sampling locations were far away from human habitations and they were representative of the natural grassland communities of the regions. At each location, a large plot (50×50 m) was setup first and then five 1×1 m subplots were selected (or 5×5 m subplots in areas with shrub) at each corner and the center of the large plot. Leaves of three herb genera (*Stipa*, *Leymus* and *Cleistogenes*) and three shrub genera (*Caragana*, *Reaumuria* and *Nitraria*) were sampled for carbon isotope analysis. Since only some species in each genus were sampled along the transect, *spp.* were added after the genus name to represent the plants in a specific genus. The plants included five dominating C_3 (*Nitraria spp.*, *Reaumuria spp.*, *Caragana spp.*, *Leymus spp.* and *Stipa spp.*) and one dominating C_4 (*Cleistogenes spp.*) genera, and their biomass accounted for more than 60% of total biomass at each sampling site. Ten individual fresh leaves of each sampled genus were randomly collected from the five subplots and then pooled together as one sample.

Laboratory analyses

Leaf samples were washed with deionized water to remove dust particles, then oven-dried at 65°C for 48 h, and finally

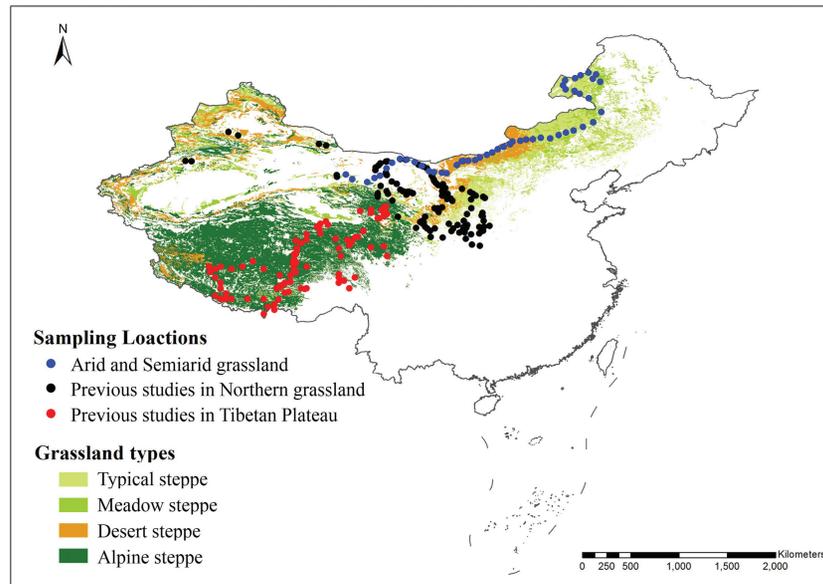


Figure 1: locations of data used in this study. Blue points represent arid and semi-arid grassland transect we sampled in Inner Mongolia and Gansu province. Black and red points represent literature data on leaf $\delta^{13}\text{C}$ in northern grassland and in Tibetan Plateau, China, respectively.

milled to powder for analysis. Leaf carbon isotope composition and N content were measured using an Elementar Vario EL Cube (Elementar Analysis system GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Faculty of University of California, Davis. Isotope value was expressed in parts per thousand (‰) relative to Pee Dee Belemnite (PDB) for $^{13}\text{C}/^{12}\text{C}$, using standard delta (δ) notation.

Meteorological data

The spatial geographical coordinates and elevation of each sampling location were recorded by GPS (eTrex Venture, Garmin, USA). MAP and MAT of each sampling location were calculated from the WorldClim database (Hijmans *et al.* 2005) using ArcGIS 10.0 Spatial Analysis tool (SERI, Redlands, CA) based on the geographical coordinates (latitude and longitude).

Literature data

We compiled leaf $\delta^{13}\text{C}$ of 17 species/genera in northern grasslands and Tibetan Plateau in China from studies in literature (supplementary Table S1). Leaf $\delta^{13}\text{C}$ data must be derived from field investigations, not including studies under manipulation or any controls; and data must be reported in numerical or graphical terms. The information of MAP, MAT and altitude for each compiled study either derived from original studies or reconstructed from WorldClim database (Hijmans *et al.* 2005) using the Spatial Analysis method described above.

Data analyses

Statistical analyses were carried out with the SPSS 16.0 package (SPSS, Chicago, IL). One-way ANOVA analysis was performed to compare the difference in leaf $\delta^{13}\text{C}$ among six

sampled genera. Ordinary least squares (OLS) regressions were conducted to examine the relationships of leaf $\delta^{13}\text{C}$ and MAP, MAT, altitude as well as leaf N content. We then used stepwise regression to explore the combined effects of climatic (MAT and MAP), altitude and leaf N content variables on leaf $\delta^{13}\text{C}$.

RESULTS

Variations in leaf $\delta^{13}\text{C}$ along the transect

Leaf $\delta^{13}\text{C}$ of C_3 plants (*Nitraria spp.*, *Reaumuria spp.*, *Caragana spp.*, *Leymus spp.* and *Stipa spp.*) ranged from -28.0‰ to -23.3‰ , while that of C_4 plant *Cleistogenes spp.* varied from -16.3‰ to -13.8‰ (Table 1 and supplementary Table S2). Among the five C_3 plants, mean leaf $\delta^{13}\text{C}$ of *Nitraria spp.* and *Reaumuria spp.* were significantly higher than other C_3 plants (*Caragana spp.*, *Leymus spp.* and *Stipa spp.*) (Table 1). Mean leaf N content was highest in *Caragana spp.* (3.41%) and lowest in *Cleistogenes spp.* (1.54%), respectively (Table 1).

Relationship between leaf $\delta^{13}\text{C}$ and environmental factors

Leaf $\delta^{13}\text{C}$ along the transect varied in a systematic way with climatic variables, i.e. MAP and MAT (Figs 2 and 3). For all leaf samples, MAP was negatively correlated with leaf $\delta^{13}\text{C}$ and accounted for 21–66% of its variations. The effect of MAP on leaf $\delta^{13}\text{C}$ was stronger on shrubs of *Nitraria spp.* (slope = -0.0351 , $P = 0.020$), *Reaumuria spp.* (slope = -0.0276 , $P = 0.017$) and *Caragana spp.* (slope = -0.0097 , $P = 0.017$) than on herbs of *Leymus spp.* (slope = -0.0071 , $P = 0.003$), *Stipa spp.* (slope = -0.0026 , $P = 0.005$) and *Cleistogenes spp.* (slope = -0.0056 , $P = 0.001$) (Fig. 2). Leaf $\delta^{13}\text{C}$ also was positively related to MAT

except for *Leymus spp.* (Fig. 3). Similarly, the responses of leaf $\delta^{13}\text{C}$ to MAT were also greater for shrubs than that for herbs.

Unlike climatic factors, altitude and leaf N content did not show apparent correlations with leaf $\delta^{13}\text{C}$ along the transect (Figs 4 and 5). Among the six genera, only $\delta^{13}\text{C}$ of *Cleistogenes spp.* was positively correlated with altitude and leaf N content (Figs 4 and 5). Stepwise regression analyses revealed that MAP was the primary factor regulating leaf $\delta^{13}\text{C}$ among six genera (data not shown).

Comparison of leaf $\delta^{13}\text{C}$ between northern grassland and Tibetan Plateau

In northern grasslands, leaf $\delta^{13}\text{C}$ of all examined genera decreased with increasing MAP with an average rate of -1.10‰ per 100 mm (Fig. 6), and the mean slope increasing with MAP for shrubs (-1.60‰ per 100 mm) was higher than that for herbs (-0.60‰ per 100 mm). In Tibetan grasslands, only leaf $\delta^{13}\text{C}$ of *Stipa spp.* and *Kobresia spp.* were significantly negatively correlated with MAP, showing an average rate of

Table 1. Mean leaf carbon isotope ratio ($\delta^{13}\text{C}$) and nitrogen (N) content for each individual genus collected along the grassland transect in northern China

Genus	C ₃ /C ₄	Life form	Max $\delta^{13}\text{C}$ (‰)	Min $\delta^{13}\text{C}$ (‰)	Mean $\delta^{13}\text{C}$ (‰)	Leaf N (%)	Number of samples
<i>Nitraria spp.</i>	C ₃	Shrub	-23.9	-27.4	-25.2 ± 1.1 b	2.94 ± 0.51 b	9
<i>Reaumuria spp.</i>	C ₃	Shrub	-23.3	-27.8	-25.6 ± 1.2 b	2.51 ± 0.38 c	11
<i>Caragana spp.</i>	C ₃	Shrub	-25.5	-27.3	-26.3 ± 0.7 c	3.41 ± 0.18 a	11
<i>Leymus spp.</i>	C ₃	Grass	-25.3	-28.0	-26.6 ± 0.6 c	2.23 ± 0.37 cd	19
<i>Stipa spp.</i>	C ₃	Grass	-25.5	-27.8	-26.6 ± 0.5 c	1.88 ± 0.45 de	35
<i>Cleistogenes spp.</i>	C ₄	Grass	-13.8	-16.3	-15.1 ± 0.7 a	1.54 ± 0.36 e	26

Values followed by different letters indicate differences between means in each column ($P < 0.05$) (mean \pm SD).

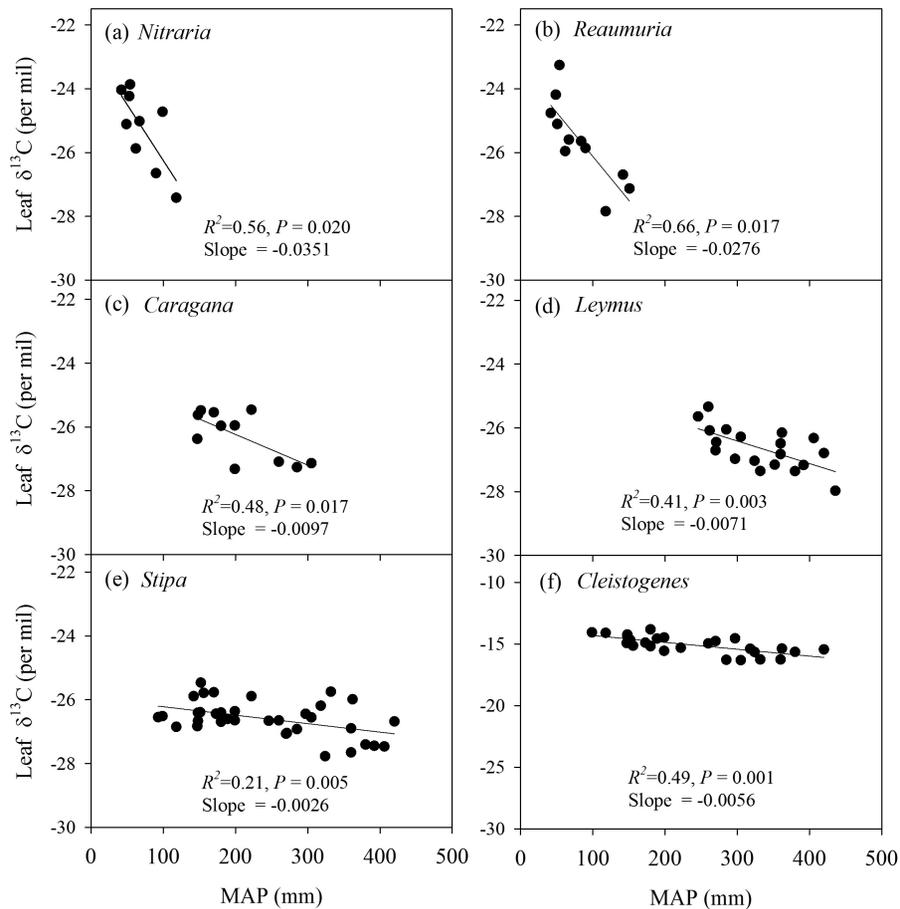


Figure 2: relationships between leaf $\delta^{13}\text{C}$ of each genus and MAP for C₃ plant (a–e) and C₄ (f) along the arid and semi-arid grassland transect in northern China.

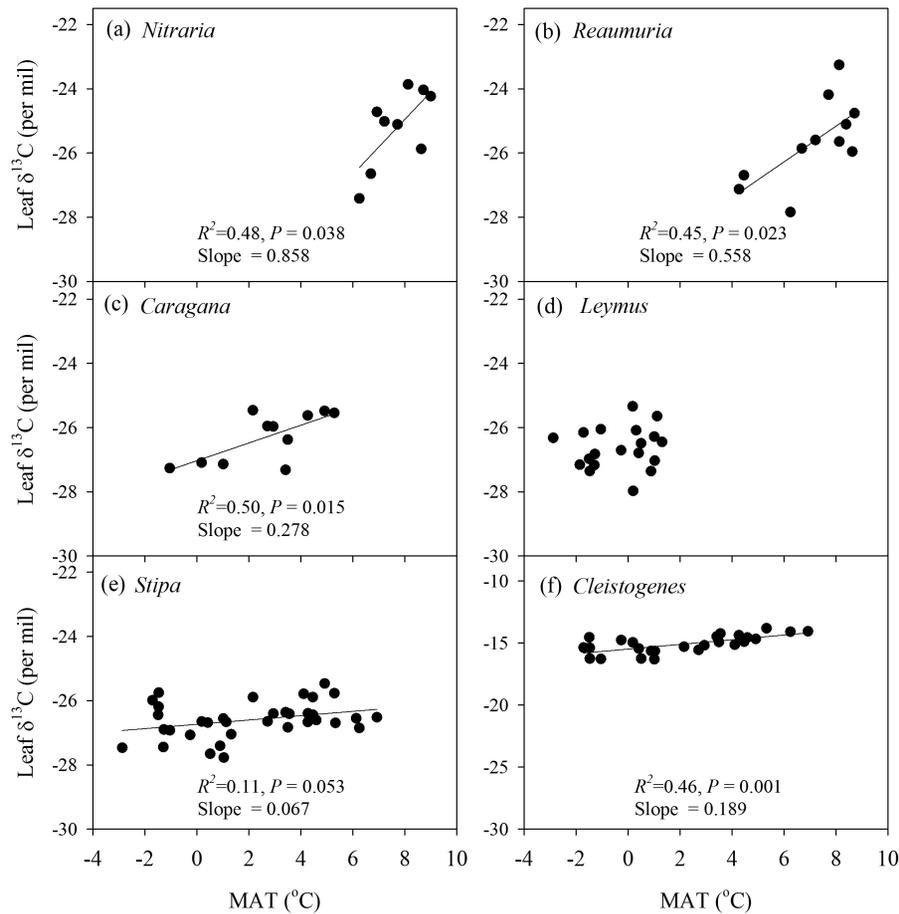


Figure 3: relationships between leaf $\delta^{13}\text{C}$ of each genus and MAT for C_3 plant (a–e) and C_4 (f) along the arid and semi-arid grassland transect in northern China.

–0.42‰ per 100 mm increase of MAP (Fig. 6). Both positive and negative relationships were observed between leaf $\delta^{13}\text{C}$ and MAT in northern grasslands, while only negative linear correlations were observed in Tibetan Plateau (Fig. 6). On average, leaf $\delta^{13}\text{C}$ changed with MAT at the rate of 1.14‰ and –2.20‰ per 10°C, respectively, in northern regions and Tibetan Plateau. Leaf $\delta^{13}\text{C}$ of all plants studied increased with altitude in Tibetan Plateau, showing an average rate of 1.42‰ per 1000 m increase of altitude; while it only correlated with leaf $\delta^{13}\text{C}$ for two cases in northern grasslands, with an average rate of –1.00‰ per 1000 m increase of altitude (Fig. 6).

DISCUSSION

Change of leaf $\delta^{13}\text{C}$ along the transect

Leaf $\delta^{13}\text{C}$ of C_3 plants along the arid and semi-arid grassland transect showed relatively wide variations, ranging from –28.0‰ to –23.3‰ (mean –26.1‰). Among five examined C_3 plants, we found that leaf $\delta^{13}\text{C}$ of shrubs (*Nitraria* spp. and *Reaumuria* spp.) was higher than that of herbs (*Stipa* spp. and *Leymus* spp.), which is consistent with previous studies (Smedley *et al.* 1991). Shorter lived herbs generally tend to be

active during the initial months of growing season and discriminate ^{13}C significantly more than the longer lived shrubs with longer growing season, resulting in lower $\delta^{13}\text{C}$ (Ehleringer and Cooper 1988; Smedley *et al.* 1991). *Caragana* spp., which is an N_2 -fixing shrub, had the highest leaf N content among all plants we studied (Table 1), and was hypothesized to have the highest leaf $\delta^{13}\text{C}$ because leaf N availability could affect leaf $\delta^{13}\text{C}$ (Bai *et al.* 2008; Sparks and Ehleringer 1997). For example, both leaf N_{mass} (leaf N per leaf dry mass) and leaf N_{area} (leaf N per leaf area) have been reported to be positively related to leaf $\delta^{13}\text{C}$ by elevating photosynthetic capacity and lowering p_i (Bai *et al.* 2008; Morecroft and Woodward 1996). However, we did not find *Caragana* spp. had the highest leaf $\delta^{13}\text{C}$ among C_3 plants (Table 1). In fact, we did not find any significant relationship between leaf $\delta^{13}\text{C}$ and leaf N for all sampled C_3 genera (Fig. 5). Along our transect, water is the most limiting factor, and plant intrinsic characteristics related to water utilization (i.e. leaf thickness) were probably the dominating factor influencing plant photosynthesis and then leaf $\delta^{13}\text{C}$.

Leaf $\delta^{13}\text{C}$ of plants in six genera along the transect was all negatively correlated with MAP (Fig. 2), which is consistent

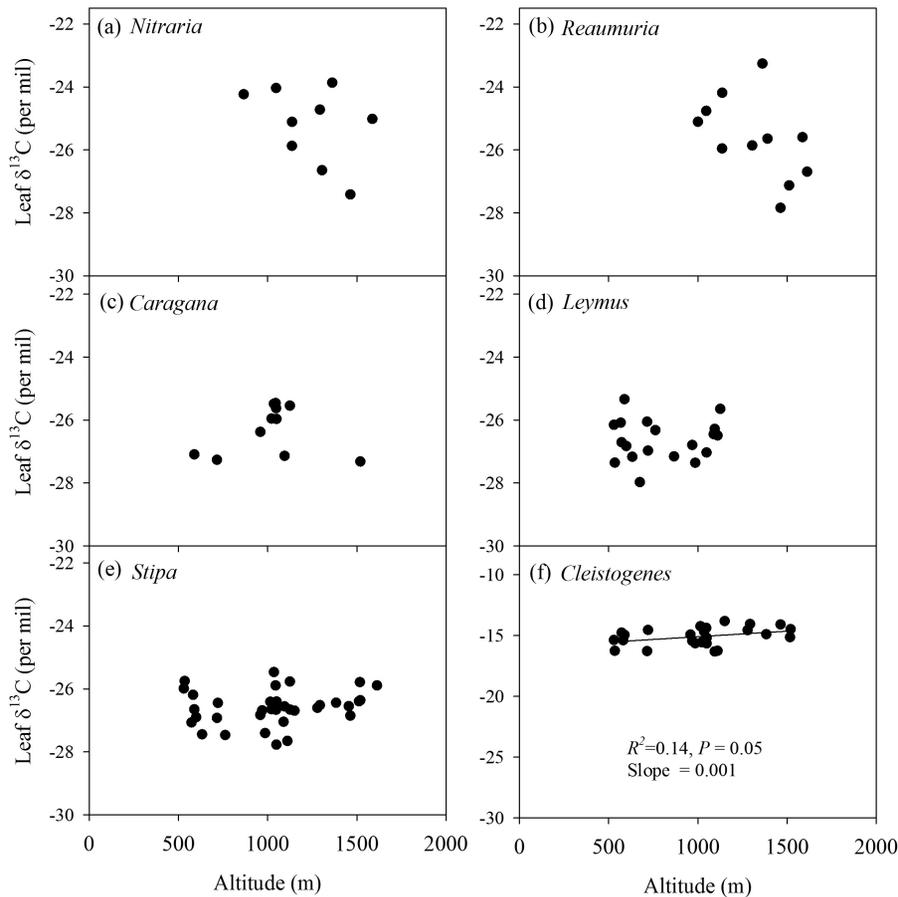


Figure 4: relationships between leaf $\delta^{13}\text{C}$ of each genus and altitude for C_3 plant (a-e) and C_4 (f) along the arid and semi-arid grassland transect in northern China.

with previous studies (Liu *et al.* 2005; Marshall and Zhang 1994; Wang *et al.* 2010; Zhou *et al.* 2011). However, few prior studies were conducted in drylands, especially in arid grassland areas with MAP <100mm in northern China, where water extremely limited plant growth. Nonetheless, our study supplemented the existing knowledge by extending study sites and plants to drylands with severe drought. The negative relationship was mainly because low precipitation and/or high evaporation may induce drought stress and lead to stomatal closure, decreasing p_i/p_a and increasing leaf $\delta^{13}\text{C}$ (Farquhar *et al.* 1982).

Temperature could be another important factor influencing leaf $\delta^{13}\text{C}$ along the arid and semi-arid grassland transect. Some previous studies found there were negative or no clear relationship between leaf $\delta^{13}\text{C}$ and temperature (Troughton and Card 1975; Wang *et al.* 2013; Welker *et al.* 1993). While along our transect, except for *Leymus spp.*, leaf $\delta^{13}\text{C}$ increased with increasing MAT for all other plants investigated. Our results are consistent with the observations of Wang *et al.* (2013) and the positive relationship between tree ring $\delta^{13}\text{C}$ and temperature summarized by Heaton (1999). The positive

relationship between leaf $\delta^{13}\text{C}$ and MAT are most likely due to the balance between stomatal conductance and carbon assimilation (carboxylation demand): higher temperature could cause (i) smaller stomatal conductance without changing assimilation, (ii) a greater relative increase in assimilation compared to conductance or (iii) simply an increase in assimilation without changing stomatal conductance (Welker *et al.* 1993). Moreover, higher MAT usually means that plants get closer to their optimal photosynthetic temperature and higher photosynthetic rate, which would lead to higher leaf $\delta^{13}\text{C}$ (Schleser *et al.* 1999). For *Leymus spp.*, they mainly occurred in the regions with a short range (-2 to 2°C) of MAT, the low MAT probably did not affect photosynthetic rates.

We found a positive relationship between leaf $\delta^{13}\text{C}$ of C_4 plants and temperature (Fig. 3). Previous studies on the effects of environmental factors on the $\delta^{13}\text{C}$ of C_4 plants were few and reported inconsistent results. Wang *et al.* (2013) found no clear relationship between $\delta^{13}\text{C}$ of C_4 plants and temperature across a temperature gradient in northern China. Another study showed a non-linear relationship between $\delta^{13}\text{C}$ of C_4 plants and temperature along a Kalahari savanna Transect

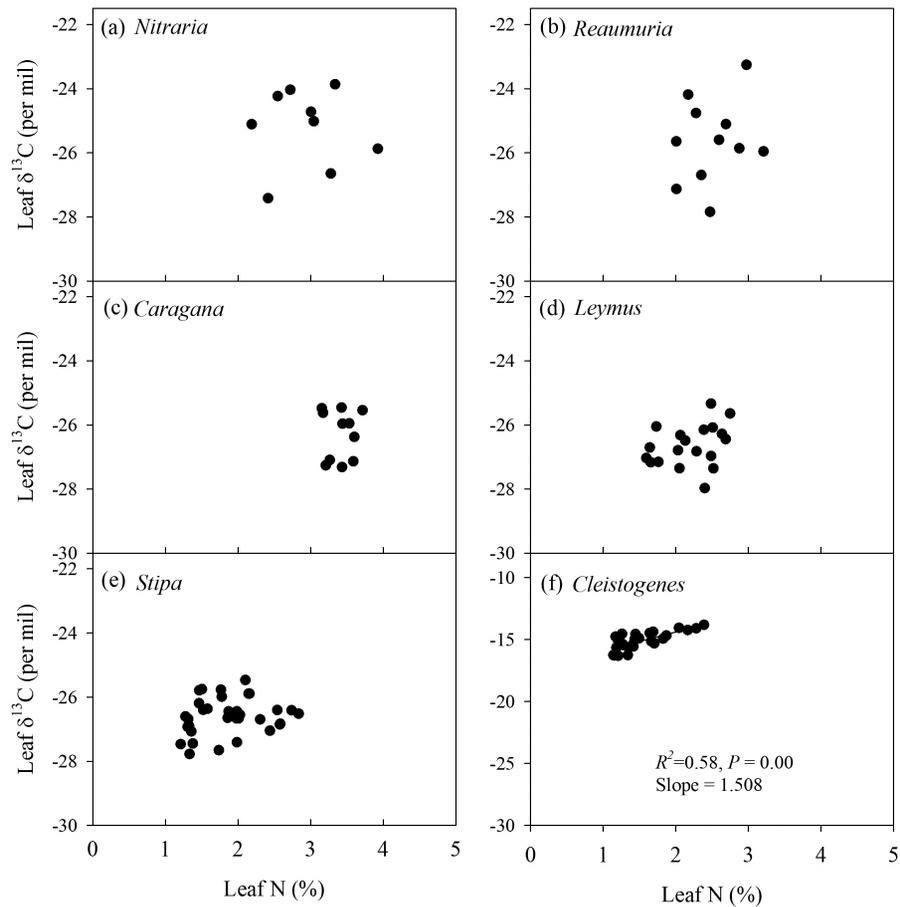


Figure 5: relationships between leaf $\delta^{13}\text{C}$ of each genus and leaf N content for C_3 plant (a–e) and C_4 (f) along the arid and semi-arid grassland transect in northern China.

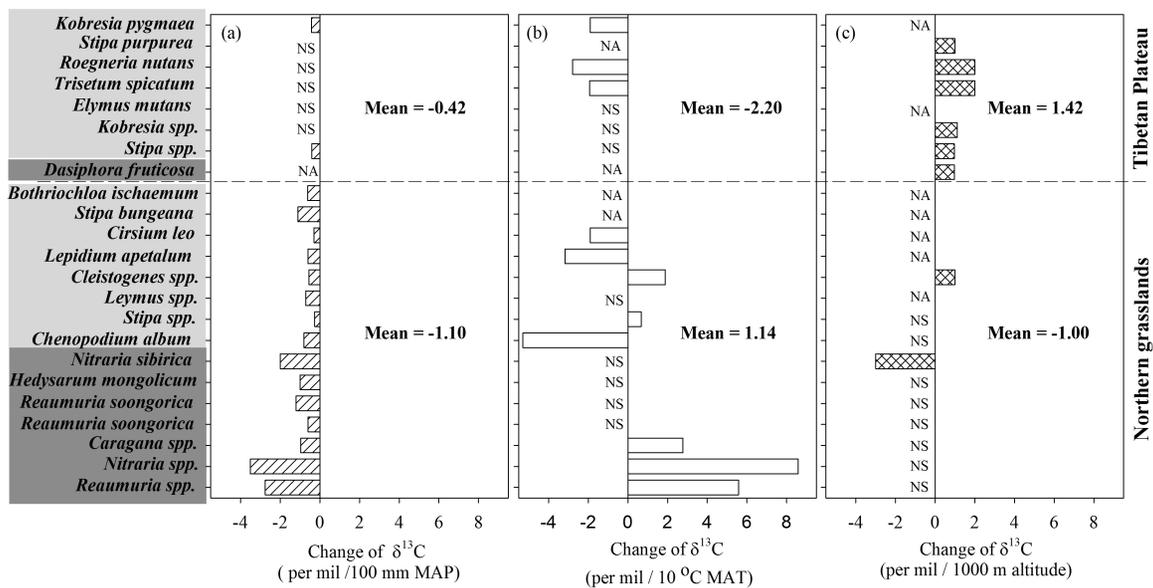


Figure 6: the responses of leaf $\delta^{13}\text{C}$ of each genus or species to changes of MAP (a, per 100 mm), MAT (b, per 10 °C) and altitude (c, per 1000 m) in northern grassland and in Tibetan Plateau, China. References from previous studies are listed in [supplementary Table S1](#). NA, data is not available; NS, statistical analysis is not significant ($P > 0.05$). French grey and dark grey backgrounds represent herbs and shrubs, respectively.

(Wang et al. 2010). Since we only sampled one genus of C_4 plant along the transect, it is hard to make a general conclusion for the temperature sensitivity of $\delta^{13}C$ values of C_4 plants. We believe that although C_4 plants are generally insensitive to environmental changes due to their photosynthetic pathway, the environmental gradient along our transect was so strong that temperature and precipitation could affect $\delta^{13}C$ of C_4 plants to some degree in this study. More research should be carried out to investigate on the underlying mechanisms of the effects of environmental factors on C_4 plants.

Leaf carbon isotope of grasslands in China

In northern grassland of China, our results showed that on average, leaf $\delta^{13}C$ decreased by -1.10‰ for every 100 mm increase in MAP and increased by 1.14‰ for every increase of 10°C MAT based on our data and literature reviews (Fig. 6). However, in Tibetan Plateau, for most cases, the effect of MAP or MAT on leaf $\delta^{13}C$ was not significant. We also found no clear relationship between leaf $\delta^{13}C$ and altitude in northern China, while most observations in Tibetan Plateau showed positive relationship between leaf $\delta^{13}C$ and altitude (Fig. 6). Of course, we could not rule out other factors we did not examine. Among MAT, MAP and altitude, it appeared that altitude was the most important factor on leaf $\delta^{13}C$ in Tibetan Plateau and MAP was the most important factor on leaf $\delta^{13}C$ along the studied transect in northern grassland.

The rate of increases in leaf $\delta^{13}C$ with altitude in Tibetan Plateau ranged from 0.97‰ per 1000 m to 2.00‰ per 1000 m with a mean of 1.42‰ per 1000 m, which was similar to results of plants of three genera (*Saxifraga*, *Potentilla* and *Carex*) obtained from south-central Europe (1.40‰ per 1000 m) (Zhu et al. 2010), but was significantly higher than rate obtained from a global relationship between leaf $\delta^{13}C$ and altitude (0.70‰ per 1000 m) (Körner et al. 1988). Altitude is a comprehensive index integrating many factors that could affect leaf $\delta^{13}C$. For example, carboxylation efficiency (Körner et al. 1988), water availability (Van de Water et al. 2002), temperature and oxygen partial pressure (Körner et al. 1991), leaf thickness (Vitousek et al. 1990), N content per leaf dry mass (Morecroft and Woodward 1996) and stomatal conductance (Hultine and Marshall 2000) may all change with altitude and cause the positive relationship between leaf $\delta^{13}C$ and altitude. The reasons that we did not find a clear relationship between leaf $\delta^{13}C$ and altitude in northern grassland in China were probably because: (i) The effects of these factors mentioned above on leaf $\delta^{13}C$ were not at the same direction and they cancelled each other out; or 2) Although the altitude along the transect varies about 1000 m, the changes of partial pressure of CO_2 in atmosphere is less important on leaf carbon isotope than changes in precipitation, which ranged from 40 to 400 mm. On the contrary, leaf $\delta^{13}C$ was positively related to altitude in Tibetan Plateau (Fig. 6), where the reduction of atmospheric pressure with increasing altitude seemed to be the main reason of the pattern (Zhou et al. 2011; Zhu et al. 2010). For 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 arid and semi-arid transect and atmospheric CO_2 concentration in Tibetan Plateau with high altitude. Therefore, there does not have to be a general pattern for the change of leaf $\delta^{13}C$ along an environment gradient such as MAP, MAT or altitude. Detailed analysis of the underlying controlling factors is necessary for better understanding the spatial pattern of leaf carbon isotopes.

Implication for the WUE of plants

Leaf $\delta^{13}C$ is related with plant WUE via π/pa ratio, with higher (less negative) values of $\delta^{13}C$ corresponding to higher WUE (Farquhar et al. 1989; Farquhar et al. 1982; Toft et al. 1989). Our results indicated that leaf $\delta^{13}C$ and therefore WUE of the six studied plants along the arid and semi-arid transect decreased with increasing MAP, which is consistent with previous studies conducted in Inner Mongolia grasslands and Mohave desert (Chen et al. 2005; Ehleringer and Cooper 1988). It is worth noticing that leaf $\delta^{13}C$ of shrubs (*Nitraria spp.*, *Reaumuria spp.* and *Caragana spp.*) (mean decrease of 2.4‰ per 100 mm increase of MAP) was more sensitive to MAP than leaf $\delta^{13}C$ of herbs (*Stipa spp.*, *Leymus spp.* and *Cleistogenes spp.*) (mean decrease of 0.5‰ per 100 mm increase of MAP) (Fig. 2). This result suggested that shrubs may have more ecological strategies to increase their WUE and adapt to drought conditions in drylands (Dodd et al. 1998). For example, shrubs generally have deeper roots and more extensive lateral roots than herbs, which can utilize water from deeper soil profile and from different seasons (Dodd et al. 1998; Donovan and Ehleringer 1994b). Besides, phenotypic selection analysis showed plants with smaller leaves (most shrubs have smaller leaves than herbs) can adapt to dry environments by reducing water losses and maximizing WUE (Dudley 1996). Therefore, under global climate change, shrubs may be more adapted to increasing drought in arid and semi-arid grasslands.

CONCLUSIONS

Along the arid and semi-arid grassland transect in northern China, leaf $\delta^{13}C$ values of six dominating genera decreased with increasing MAP, showing a down trend of plant WUE along the MAP gradient. MAT was also an important factor determining leaf $\delta^{13}C$ along the transect, but leaf N content and altitude were not. The spatial pattern of leaf $\delta^{13}C$ in Tibetan Plateau was different, which was mainly controlled by altitude. These results indicate environmental factors controlling leaf $\delta^{13}C$ varied among different ecosystems, depending on the most limiting factors. Shrubs had higher change in leaf $\delta^{13}C$ with decreasing MAP along the transect than herbs, indicating their better adaptation to more severe drought and water-stressed conditions. Shrub encroachment in the area is likely with increasing drought under global climate change. Our study has important implications on plant WUE and plant community structure change in the future in the arid and semi-arid northern grassland, China.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *Journal of Plant Ecology* Online.

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