Relationships between foliar carbon isotope composition and elements of C₃ species in grasslands of Inner Mongolia, China

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Abstract Foliar elements and their ratios can be related to foliar carbon isotope composition ($\delta^{13}\text{C}$) through their involvement in plant transpiration, photosynthesis, and osmotic adjustment. In order to investigate these relationships in grasslands of Inner Mongolia, China, $\delta^{13}\text{C}$ and element contents of dominant C₃ species at 47 grassland sites were

determined. For C₃ species, $\delta^{13}\text{C}$ showed no correlation with carbon (C), positive correlations with nitrogen (N), nitrogen:phosphorus ratio (N/P) and carbon:phosphorus ratio (C/P), and negative correlations with phosphorus (P), potassium (K), and carbon:nitrogen ratio (C/N), while correlation with P was subsidiary to positive correlation between P and K. These correlations indicate that plants in Inner Mongolia that survive in dry conditions may profit from their higher water use efficiency via stomatal regulation and N-related photosynthetic capacity rather than K-related osmotic adjustment and P-related photosynthetic capacity. Further, plants adapt to severe environments by having higher water and phosphorus use efficiency at the expense of nitrogen use efficiency. However, these correlations differed among plant functional groups (PFGs), e.g., $\delta^{13}\text{C}$ was negatively correlated with N in shrubs in contrast to other life forms. A possible explanation is that shrubs adapt to low N availability by having lower photosynthetic nitrogen use efficiency (PNUE), so that N is not positively related to photosynthetic rate in shrubs. Obviously, data on stomatal conductance, PNUE, and cell osmotic pressure are needed to fully understand these correlations and the strategies of plants adapted to arid environments.

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Introduction

Foliar carbon isotope composition ($\delta^{13}\text{C}$) can provide integrated information on how plants interact with and respond to their biotic and abiotic environment, and can also enhance our understanding of ecosystem carbon cycles (Dawson et al. 2002). Thus, foliar $\delta^{13}\text{C}$ has been increasingly employed in plant ecological and global change studies, and its patterns and controlling factors have been often studied (Körner et al. 1991; Li et al. 2015; Liu et al. 2014; Ma et al. 2012; Peri et al. 2012; Zhou et al. 2011).

However, compared with environmental factors, fewer studies have been conducted for leaf elements (Zhou et al. 2013), even though these may have important roles in controlling foliar $\delta^{13}\text{C}$ through their effect on stomatal conductance (g_s) and photosynthetic capacity (A). For example, foliar nitrogen (N) is closely related to plant growth, and positive correlations between N and photosynthetic rates have been reported (Domingues et al. 2010; Springer et al. 2005; Walker et al. 2014). Phosphorus (P) can affect photosynthesis indirectly through its effects on Calvin cycle enzymes and enzymes related to the synthesis of protein, RNA, and DNA (Walker et al. 2014, 1986; Warren and Adams 2002). Potassium (K) plays an important role related to stomatal behavior, enzyme activity, photosynthesis, and osmotic adjustment (Amrutha et al. 2007; Si et al. 2015). Further, foliar element ratios, e.g., C/N (foliar carbon:nitrogen ratio), C/P (foliar carbon:phosphorus ratio), and N/P (foliar nitrogen:phosphorus ratio), are also key traits of ecological relevance and link with $\delta^{13}\text{C}$ through their correlation with water use efficiency (WUE). For example, Cernusak et al. (2010) observed a positive correlation between leaf N/P ratio with WUE and that with $\delta^{13}\text{C}$.

However, previous studies have suggested that relationships between foliar $\delta^{13}\text{C}$ and elements differ for study areas and plant species (Cao et al. 2009; Zhao et al. 2006, 2007; Zhou et al. 2013). For example, although most studies have reported that $\delta^{13}\text{C}$ values positively correlate with N (Hamerlynck et al. 2004; Ma et al. 2012; Prasolova et al. 2005; Sparks and Ehleringer 1997), Zhou et al. (2013) found that the low atmospheric pressure and temperatures of Qinghai-Tibet Plateau may preclude expression of positive relationship between $\delta^{13}\text{C}$ and N. Li et al. (2015) also found no correlation between $\delta^{13}\text{C}$ and N for *Ranunculus natans*, while Li et al. (2011) found that $\delta^{13}\text{C}$ of

Pinus tabulaeformis is negatively related to N. Negative relationships between $\delta^{13}\text{C}$ and K were reported for *Quercus aquifolioides* (Li et al. 2009), *Pinus tabulaeformis* (Li et al. 2011), sugar beet (Tsialtas and Maslaris 2006), and grassland species on Qinghai-Tibet Plateau (Zhou et al. 2013). However, while Cao et al. (2009) and Zhao et al. (2007) found negative relationships between $\delta^{13}\text{C}$ and K for *Tamarix ramosissima*, *Artemisia ordosica*, and *Hedysarum scoparium*, as well, they also found positive relationships for *Populus euphratica* and *Caragana korshinskii*.

The variable relationships between $\delta^{13}\text{C}$ and elements indicate that further research on them is needed for better understanding of interactions between plant nutrition and ecophysiological processes indicated by $\delta^{13}\text{C}$. Moreover, high $\delta^{13}\text{C}$, due to its positive relationship with WUE (Farquhar et al. 1989), is considered as a trait contributing to the successful growth and production of species in arid and semiarid environments. Thus, investigating the relationships between $\delta^{13}\text{C}$ and nutrient elements is important for ascertaining potential mechanisms of plant adaptation to arid and semiarid environments and the main factors limiting plant growth. Although some previous studies have undertaken related research in arid regions, they have mostly involved a limited number of tree or shrub species and have not involved grassland herb species (Cao et al. 2009; Zhao et al. 2006, 2007).

Inner Mongolia is the major dry-semiarid region in China, it contains the main area of temperate grassland of the country, and its low precipitation is the main factor affecting plant growth and $\delta^{13}\text{C}$ (Hu et al. 2010; Zhou et al. 2016).

Thus, this study was conducted to analyze the relationships of foliar $\delta^{13}\text{C}$ with C, N, P, and K concentrations, as well as foliar C/N, C/P, and N/P ratios of C_3 species in grasslands of Inner Mongolia. Further, these relationships were analyzed when species were classified by life form (graminoids, forbs, and shrubs) and for the grass genus *Stipa* and species *Leymus chinensis* (hereafter collectively grouped as plant functional groups, PFGs). The goals of this study were (1) to examine interactions between plant nutrition and ecophysiological processes, and then to discuss potential mechanisms of plant adaption to arid and semiarid environments; and (2) to explore whether these strategies differ among PFGs.

Methods

Study area

The study was conducted in Inner Mongolia, northern China (Fig. 1). This region is characterized by an arid and semiarid continental climate, with water availability being the major abiotic factor limiting plant growth. Mean annual precipitation (MAP) ranges from 50 to 450 mm, and mean annual temperature (MAT) ranges from -3 to 9 °C. Grazing for livestock production is the typical land use of the region. The main temperate grassland types are meadow steppe, typical steppe, desert steppe, steppe–desert, and desert. Dominant species are *Stipa baicalensis*, *S. grandis*, *S. krylovii*, *S. tianschanica*, *Leymus chinensis*, *Filifolium sibiricum*, *Cleistogenes squarrosa*, *C. songorica*, *Agropyron cristatum*, *A. desertorum*, *Artemisia frigida*, *Caragana microphylla*, *Hippolytia yunnanensis*, *Sal-sola passerine*, *S. arbuscula*, *Reaumuria songarica*, *Ceratoides latens*, *Allium victorialis*, *Haloxylon persicum*, *Calligonum squarrosum*, *Zygophyllum xanthoxylum*, *Nitraria sphaerocarpa*, *Anabasis truncata*, *Kalidium gracile*, and *Nanophyton erinaceum*. Chernozem, chestnut, calcic brown, and desert soils predominate (Hu et al. 2010).

Sampling

Leaf samples were obtained from actively growing plants during August 2003 and 2004 at 28 and 19 sample sites, respectively (Fig. 1). The sites, varying in altitude from 500 to 1700 m, were located at intervals of about 50 km in a northeast to southwest direction, and the geographic position of each was recorded using a Magellan GPS (Garmin, Kansas, USA). Mostly, three $1\text{ m} \times 1\text{ m}$ quadrats were randomly located within each sample site, but for sites with shrubs, $5\text{ m} \times 5\text{ m}$ or larger quadrats were used. The dominant species in each quadrat were defined, and were separated and placed in different envelopes; every common dominant species in three quadrats of each site was considered as one sample for chemical analysis. A total of 126 samples were obtained from the 47 sites (for more information see Supplement 1).

Leaf element concentrations and stable carbon isotope analysis

Leaf element concentrations and stable carbon isotope determinations of the 126 samples were conducted at the central physicochemical laboratory of the Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing. Depending on leaf size, 3–20 leaves (mostly five) from at least three different adult individual plants were selected for each sample from each site. Leaves were rinsed with deionized water to remove dust particles and then air-dried before oven drying at 80 °C for 48 h and grinding to fine powder for analysis. The $^{13}\text{C}/^{12}\text{C}$ ratio and total concentrations of N and carbon (C) were determined by an isotope mass spectrometer (Finnigan MAT-253, Thermo Electron). Carbon isotope composition ($\delta^{13}\text{C}$, ‰) of leaf samples was calculated as

$$\delta^{13}\text{C}(\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000, \quad (1)$$

where R_{sample} and R_{standard} , respectively, are the $^{13}\text{C}/^{12}\text{C}$ ratios in the leaf and the standard. The universally accepted standard of Vienna Pee Dee Belemnite (VPDB) was used.

Leaf powder was digested by concentrated nitric acid, and then extracted solutions were determined for concentrations of P and K on an Inductively Coupled Plasma Optical Emission Spectrometer (PerkinElmer, Optima 5300DV) (Matejovic and Durackova 1994).

Data analyses

Data analyses were conducted using SPSS (ver.18.0; SPSS Inc., USA). Simple correlation and regression analysis was applied to investigate relationships between $\delta^{13}\text{C}$ and nutrient concentrations and their ratios for the 126 leaf samples. To explore whether these relationships differed among PFGs, life-form groups of graminoids (grasses and sedges), forbs (herbaceous species other than graminoids), and shrubs, and the grass genus *Stipa* and the grass *Leymus chinensis* were analyzed separately. Multiple regressions were applied to determine whether relationships of foliar $\delta^{13}\text{C}$ with nutrient elements and ratios remained significant after the MAP effect was taken into account.

Fig. 1 Locations of the sampling sites for leaf elements and $\delta^{13}\text{C}$ of C_3 species in grasslands of Inner Mongolia, China

Results

Correlations between foliar elements and ratios

The correlations between foliar elements and ratios are presented in Table 1. Potassium was negatively correlated with C, and the C/N, C/P and N/P ratios, and positively correlated with N and P. A positive correlation between N and P was found, while there was no correlation of C with N, P, and the N/P ratio. The C/N ratio was negatively correlated with P and the C/P ratio with N.

Correlations between MAP and foliar elements and ratios

Carbon was positively correlated with MAP for all C_3 species pooled, graminoids, and *Stipa*. While shrubs showed positive correlation between N and MAP, this correlation was not shown for the other comparisons.

Correlations between P and MAP were significantly positive for graminoids and shrubs. Potassium significantly increased with the increasing MAP, but this trend was not significant for forbs. The C/N ratio was positively correlated with MAP when C_3 species were pooled, but this correlation was negative for shrubs. No significant correlations between C/P and MAP were found except for the positive correlation for shrubs. The N/P ratio decreased with MAP, but this trend was not significant for shrubs and *Leymus chinensis* (Table 2).

Relationships of foliar $\delta^{13}\text{C}$ with C, N, P, and K

Apart from the negative correlation shown by *Stipa*, there were no significant correlations between $\delta^{13}\text{C}$ and C (Fig. 2). However, there were two obvious outlier points for *Stipa*, and when these were excluded, the correlation was not significant (Fig. 2e).

Table 1 Correlations (r) and significance of the correlations between leaf elements and ratios of C_3 species sampled from 47 grassland sites in Inner Mongolia, China

	N	P	K	C/N	C/P	N/P
C	−0.08 ^{ns}	−0.15 ^{ns}	−0.29**	–	–	0.04 ^{ns}
N		0.38***	0.37***	–	−0.40***	–
P			0.63***	−0.36***	–	–
K				−0.41***	−0.60***	−0.36***
C/N					–	–
C/P						–

C/N carbon:nitrogen ratio, C/P carbon:phosphorus ratio, N/P nitrogen:phosphorus ratio, *ns* not significant. “–” correlation rejected because of functional connection; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Nitrogen was positively correlated with $\delta^{13}\text{C}$ for all C_3 species, *Stipa*, and less strongly for *Leymus chinensis* (Fig. 3a, e, f). Correlations between $\delta^{13}\text{C}$ and N differed among life forms with positive correlations for graminoids and forbs and a negative correlation for shrubs (Fig. 3b–d). Phosphorus was negatively correlated with $\delta^{13}\text{C}$ for all comparisons although not significantly for *Leymus chinensis* (Fig. 4). However, when outlier points for *Leymus chinensis* were excluded, the negative correlation was significant (Fig. 4f). Potassium was negatively correlated with $\delta^{13}\text{C}$ for all the comparisons (Fig. 5).

After the effect of MAP on $\delta^{13}\text{C}$ and interaction between elements were taken into account, the relationships of $\delta^{13}\text{C}$ with N and K were in accord with the results of simple regressions for all C_3 samples pooled. These showed that N and K were, respectively, positively and negatively correlated with $\delta^{13}\text{C}$. This was also the case for graminoids, forbs, and *Stipa*. However, N was not significantly

related to $\delta^{13}\text{C}$ for shrubs and *Leymus chinensis*. Differing from the negative correlation in simple regression, P was not related to $\delta^{13}\text{C}$ (Table 3). The results indicate that among the foliar elements, regardless of PFGs or MAP, K had the strongest relationship with $\delta^{13}\text{C}$.

Relationships between $\delta^{13}\text{C}$ and C/N, N/P, and C/P ratios

Relationships between $\delta^{13}\text{C}$ and nutrient ratios differed among PFGs (Table 4). For C_3 species pooled, $\delta^{13}\text{C}$ was negatively correlated with the C/N ratio, and positively correlated with the C/P and N/P ratios. This was also the case for graminoids, forbs, and *Stipa*. However, in shrubs, $\delta^{13}\text{C}$ was positively correlated with C/N but correlations with C/P and N/P ratios were not significant. In *Leymus chinensis*, $\delta^{13}\text{C}$ was not significantly related to the C/N ratio but positively related to the N/P and C/P ratios.

Table 2 Correlations (r) and significance of the correlations between MAP and leaf elements and ratios of C_3 species sampled from 47 grassland sites in Inner Mongolia, China

Plant functional groups	C	N	P	K	C/N	C/P	N/P
C_3 species (126)	0.21*	−0.13 ^{ns}	0.17 ^{ns}	0.22*	0.22*	−0.03 ^{ns}	−0.25**
Graminoids (63)	0.29*	−0.13 ^{ns}	0.33**	0.44***	0.18 ^{ns}	−0.22 ^{ns}	−0.42***
Forbs (31)	0.23 ^{ns}	−0.27 ^{ns}	0.34 ^{ns}	0.15 ^{ns}	0.34 ^{ns}	−0.29 ^{ns}	−0.42*
Shrubs (32)	0.03 ^{ns}	0.79***	0.56***	0.73***	−0.61***	−0.36*	0.02 ^{ns}
<i>Stipa</i> (31)	0.44*	−0.20 ^{ns}	0.33 ^{ns}	0.53**	0.24 ^{ns}	−0.30 ^{ns}	−0.47**
<i>Leymus chinensis</i> (13)	0.04 ^{ns}	−0.29 ^{ns}	0.24 ^{ns}	0.66*	0.12 ^{ns}	−0.38 ^{ns}	−0.54 ^{ns}

Numbers in brackets are the sample sizes of C_3 species, graminoids, forbs, shrubs, *Stipa*, and *Leymus chinensis*. C/N carbon:nitrogen ratio, C/P carbon:phosphorus ratio, N/P nitrogen:phosphorus ratio, *ns* not significant. *** $P < 0.001$; ** $P < 0.01$, * $P < 0.05$

Fig. 2 Relationships between $\delta^{13}\text{C}$ and C of C_3 species sampled from grasslands in Inner Mongolia, China: **a** C_3 species, **b** graminoids, **c** forbs, **d** shrubs, **e** *Stipa*, and **f** *Leymus chinensis*

Fig. 3 Relationships between $\delta^{13}\text{C}$ and N of C_3 species sampled from grasslands in Inner Mongolia, China: **a** C_3 species, **b** graminoids, **c** forbs, **d** shrubs, **e** *Stipa*, and **f** *Leymus chinensis*

Fig. 4 Relationships between $\delta^{13}\text{C}$ and P of C_3 species sampled from grasslands in Inner Mongolia, China: **a** C_3 species, **b** graminoids, **c** forbs, **d** shrubs, **e** *Stipa*, and **f** *Leymus chinensis*

Fig. 5 Relationships between $\delta^{13}\text{C}$ and K of C_3 species sampled from grasslands in Inner Mongolia, China: **a** C_3 species, **b** graminoids, **c** forbs, **d** shrubs, **e** *Stipa*, and **f** *Leymus chinensis*

Table 3 Multiple regressions between $\delta^{13}\text{C}$, N, P, K, and MAP for the dominant C_3 species, graminoids, forbs, shrubs, *Stipa*, and *Leymus chinensis* sampled from 47 grassland sites in Inner Mongolia, China

Plant functional groups	a (constant)	bx_1 (MAP)	Standardized coefficients	cx_2 (N)	Standardized coefficients	dx_3 (K)	Standardized coefficients	F	p regression	R^2
C_3 species (126)	−23.16 $P < 0.001$	−0.01	−0.61 $P < 0.001$	0.05	0.32 $P < 0.001$	−0.08	−0.47 $P < 0.001$	126.39	$P < 0.001$	0.75
Graminoids (63)	−24.37 $P < 0.001$	−0.01	−0.34 $P < 0.001$	0.06	0.37 $P < 0.001$	−0.12	−0.61 $P < 0.001$	63.93	$P < 0.001$	0.75
Forbs (31)	−23.70 $P < 0.001$	−0.01	−0.60 $P < 0.001$	0.07	0.27 $P = 0.01$	−0.06	−0.35 $P = 0.001$	31.62	$P < 0.001$	0.75
Shrubs (32)	−22.33 $P < 0.001$	−0.01	−0.57 $P < 0.001$			−0.07	−0.36 $P = 0.01$	43.83	$P < 0.001$	0.73
<i>Stipa</i> (31)	−24.11 $P < 0.001$	−0.01	−0.53 $P < 0.001$	0.05	0.33 $P = 0.004$	−0.09	−0.34 $P = 0.007$	25.93	$P < 0.001$	0.71
<i>Leymus chinensis</i> (13)	−22.37 $P < 0.001$	−0.01	−0.55 $P < 0.001$			−0.12	−0.53 $P = 0.002$	94.63	$P < 0.001$	0.94

Numbers in brackets are the sample sizes of C_3 species, graminoids, forbs, shrubs, *Stipa*, and *Leymus chinensis*. The regression models are $y = a + bx_1 + cx_2 + dx_3$. x_1 , x_2 , and x_3 are MAP, N, and K, respectively. With stepwise regression analysis, P did not enter the regression equations and N did not enter the regressions for shrubs and *Leymus chinensis*

Table 4 Relationships between $\delta^{13}\text{C}$ and element ratios of C_3 species sampled from 47 grassland sites in Inner Mongolia, China

Plant functional groups	Element ratios	Intercept	Slope	R^2
C_3 species (126)	C/N	−25.62	−0.016	0.06**
	N/P	−26.87	0.040	0.15***
	C/P	−26.43	0.001	0.03*
Graminoids (63)	C/N	−25.66	−0.015	0.10*
	N/P	−27.22	0.046	0.39***
	C/P	−26.92	0.001	0.15**
Forbs (31)	C/N	−23.91	−0.115	0.22**
	N/P	−27.73	0.072	0.32***
	C/P	−27.67	0.003	0.19*
Shrubs (32)	C/N	−26.69	0.057	0.14*
	N/P	−25.41	−0.001	0.00 ^{ns}
	C/P	−26.05	0.002	0.10 ^{ns}
<i>Stipa</i> (31)	C/N	−25.13	−0.015	0.17*
	N/P	−26.62	0.037	0.41***
	C/P	−26.34	0.001	0.11*
<i>Leymus chinensis</i> (13)	C/N	−26.12	−0.023	0.11 ^{ns}
	N/P	−27.79	0.049	0.54**
	C/P	−27.96	0.002	0.32*

Numbers in brackets are the sample sizes of C_3 species, graminoids, forbs, shrubs, *Stipa*, and *Leymus chinensis*. C/N carbon:nitrogen ratio, C/P carbon:phosphorus ratio, N/P nitrogen:phosphorus ratio, ^{ns} not significant. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Discussion

Relationships between $\delta^{13}\text{C}$ and C

Previous studies have reported negative correlations between $\delta^{13}\text{C}$ and ash or mineral content (Cao et al.

2009; Merah et al. 2001; Zhao et al. 2007; Zhu et al. 2010). Generally, C was negatively correlated with ash or mineral content (Zhou et al. 2013). Thus, positive correlation between $\delta^{13}\text{C}$ and C was expected. A previous study on Qinghai-Tibet Plateau found that $\delta^{13}\text{C}$ was positively related to C (Zhou et al. 2013).

However, in the present work, there were no correlations between $\delta^{13}\text{C}$ and C (Fig. 2), although $\delta^{13}\text{C}$ and C were both negatively related to K (Table 1; Fig. 5). This was because C increased with MAP, and this trend was significant for all C_3 species, graminoids, and *Stipa* (Table 2). Further, previously it was found that MAP was negatively related to $\delta^{13}\text{C}$ in Inner Mongolia (Zhou et al. 2016), indicating that MAP can counteract the positive association between $\delta^{13}\text{C}$ and C.

To verify this finding, a partial correlation analysis between $\delta^{13}\text{C}$ and C with MAP as a controlling factor was conducted. This revealed that over all C_3 species $\delta^{13}\text{C}$ was positively related with C ($r = 0.18$, $p < 0.05$). This confirmed the finding that MAP can influence the association of $\delta^{13}\text{C}$ with C in dry areas such as Inner Mongolia.

Relationships between $\delta^{13}\text{C}$ and N

The positive relationship between $\delta^{13}\text{C}$ and N over all C_3 species (Fig. 3a) is in accordance with most previous studies (Hamerlynck et al. 2004; Li et al. 2009; Walia et al. 2010). However, it is inconsistent with findings of studies in high altitude areas where low atmospheric pressure and temperature alter the expression of the relationship between N and photosynthetic capacity and thus the $\delta^{13}\text{C}$ –N relation (Ares and Fownes 1999; Zhou et al. 2013). The main cause of the positive relationships is that photosynthetic capacity increases with leaf N (Grassi and Minotta 2000; Mae 1997), and $\delta^{13}\text{C}$ is positively related with photosynthetic capacity (Farquhar et al. 1989).

Unlike the other PFGs, foliar $\delta^{13}\text{C}$ of shrubs was negatively related to foliar N (Fig. 3). To explore whether this negative correlation was induced by the presence of nitrogen-fixing species in the samples (*Lespedeza bicolor* and *Caragana microphylla*), we reanalyzed the relationship between N and $\delta^{13}\text{C}$ excluding these species, and the negative association persisted ($r = -0.59$, $p = 0.02$). However, this relationship was subsidiary and coincidental to the positive relationship between MAP and N in shrubs (Table 2), as it was not significant when the MAP effect was taken into account (Table 3). This finding is supported by Tsialtas et al. (2001), and they also interpreted the negative correlation between foliar $\delta^{13}\text{C}$ and N as an autocorrelation with water availability in a semiarid environment.

Consistent with our results, Qiu et al. (2013) found that the relationship between $\delta^{13}\text{C}$ and N was not significant for the shrub *Nitraria tangutorum*. A possible explanation is that $\delta^{13}\text{C}$ of shrubs is mainly controlled by stomatal conductance rather than photosynthetic capacity (Zhao et al. 2006). Moreover, the average N/P ratio of shrubs of 17 (data not shown) indicates N and P co-limitation, given that critical values of less than 10 and greater than 20 correspond to N- and P-limitation, respectively (Güsewell 2004). Further, Wang and Moore (2014) suggested that plants adapt to extremely low nutrient availability by maintaining low nutrient concentrations in photosynthetically active tissues, and this consequently reduces photosynthetic nitrogen use efficiency (PNUE), especially for shrubs. Thus, another possible explanation is that N content is not positively related to photosynthetic rate in shrubs because of their lower PNUE (Hikosaka et al. 2002). However, determinations of stomatal conductance, photosynthetic rate, and PNUE were beyond the scope of our study but are indicated to be subjects for further study to interpret the relationships we observed.

Relationships between $\delta^{13}\text{C}$ and P

Research relating foliar P to $\delta^{13}\text{C}$ is limited, and the findings have been inconsistent. Some studies have found $\delta^{13}\text{C}$ to be positively related to P (Ma et al. 2008), and this was attributed to the effect of leaf P on photosynthetic capacity via Rubisco (Warren and Adams 2002). Conversely, other studies have found $\delta^{13}\text{C}$ to be negatively related to P (Li et al. 2011; Zhou et al. 2013), and this was linked to the movement of soil P to root surfaces being partly dependent on the mass flow of the soil solution caused by plant transpiration (Cernusak et al. 2007; Cramer et al. 2009). Thirdly, Merah (2001) found no correlation between $\delta^{13}\text{C}$ and P. In our study, $\delta^{13}\text{C}$ was negatively related to P in simple regression (Fig. 4), but in multiple regression, the correlation between $\delta^{13}\text{C}$ and P was not significant (Table 3). This indicates that variation in $\delta^{13}\text{C}$ values was more likely caused by stomatal limitation rather than P-related changes in photosynthetic efficiency, although plants exhibited N and P co-limitation (the average N/P ratio being 18).

Relationship between foliar $\delta^{13}\text{C}$ and K

Potassium is an osmoticum that plays an important role in osmotic adjustment by its active accumulation in cells to reduce osmotic potential, thence attracting water into cells (Si et al. 2015). Thus, plants subjected to drought stress could actively accumulate large quantities of K for the purpose of osmotic adjustment and in concomitance show higher WUE, and correspondingly $\delta^{13}\text{C}$ (Guy et al. 1989). Consequently, $\delta^{13}\text{C}$ may be expected to be positively correlated with K in dry regions. However, contrary to expectation, K was negatively related to $\delta^{13}\text{C}$ in the present study, and this correlation was robust and did not differ among PFGs (Fig. 5; Table 3). This negative correlation is in accordance with findings of previous studies (Li et al. 2009; Tsialtas et al. 2002; Tsialtas and Maslaris 2006; Zhou et al. 2013). The negative correlation could be attributed to the accumulation of K, at least in part passively through the transpiration stream, into vegetative tissues (Masle et al. 1992).

The negative relationships between $\delta^{13}\text{C}$ and K suggest that the capacity to uptake K increases with improved water availability. This suggestion is supported by the positive correlation between K and MAP (Table 2). This result also indicates the possibility that species in Inner Mongolia surviving in dry conditions may profit from their higher WUE through partial stomatal closure rather than osmotic adjustment through more K absorption. Alternatively, K may not be the primary osmoticum in species of Inner Mongolia, but instead organic osmotica such as sugars and amino acids may constitute the solute (Guy et al. 1989; Si et al. 2015).

Relationship between $\delta^{13}\text{C}$ and element ratios

The finding of a negative relationship between $\delta^{13}\text{C}$ and C/N ratio is in agreement with Li et al. (2009) and indicates that plants in Inner Mongolia achieve a higher WUE at the expense of decreased nitrogen use efficiency (NUE). However, Zhou et al. (2013) reported a positive correlation between $\delta^{13}\text{C}$ and C/N ratio on Qinghai-Tibet Plateau, indicating that a high WUE is not in discordance with a high NUE. This difference between C_3 species in Inner Mongolia and those on Qinghai-Tibet Plateau indicates different strategies to adapt to the different severe environmental limitations of these regions. A possible explanation

for this discrepancy is that plants on Qinghai-Tibet Plateau not only need to adapt to drought, as is the case for plants in Inner Mongolia, but also need to adapt to low temperature stress. Thus, they have both high WUE and NUE. However, based on the Temperature-Plant Physiological Hypotheses (TPPH), which proposes that plants compensate for decreases in metabolic rate at low temperatures by increasing N content (Reich and Oleksyn 2004), NUE should be low in low-temperature environments. These considerations indicate that the mechanisms determining correlation between $\delta^{13}\text{C}$ and C/N ratio require further investigation.

For all C_3 species, $\delta^{13}\text{C}$ was positively related with C/P ratio (Table 4), as was the relationship for Qinghai-Tibet Plateau (Zhou et al. 2013). This indicates that plants with high WUE may also have high phosphorus use efficiency (PUE) and that this pattern is common to different regions. In accordance with Cernusak et al. (2007), $\delta^{13}\text{C}$ and N/P ratio were positively correlated, a relationship not found by Zhou et al. (2013) for Qinghai-Tibet Plateau. Cernusak et al. (2010) suggested that a positive correlation was due to that the N/P ratio being positively related to WUE as plant C gains is positively related to N, and the uptake of P partly depends on plant transpiration. This assumption is supported by our findings of the positive correlation between $\delta^{13}\text{C}$ and N (Fig. 3) and the negative correlation between $\delta^{13}\text{C}$ and P (Fig. 4).

The lack of significant correlation between $\delta^{13}\text{C}$ and N/P ratio for Qinghai-Tibet Plateau (Zhou et al. 2013) could be ascribed to that $\delta^{13}\text{C}$ being not related to N as photosynthetic capacity remains constant with variations of N (Cordell et al. 1998). The difference between Inner Mongolia and Qinghai-Tibet Plateau further indicates that N relates to $\delta^{13}\text{C}$ via photosynthetic capacity and P is linked to $\delta^{13}\text{C}$ via plant transpiration, and that these relationships are the basis of the correlations between $\delta^{13}\text{C}$ and N/P ratio.

Conclusion

We hypothesize that correlations between $\delta^{13}\text{C}$ and nutrient elements indicate that C_3 species in Inner Mongolia surviving in dry conditions profit from their higher WUE via stomatal regulation and N-related photosynthetic capacity rather than K-related osmotic adjustment and P-related photosynthetic capacity.

This is indicated by the positive correlation between $\delta^{13}\text{C}$ and N, negative correlation between $\delta^{13}\text{C}$ and K, and non-significant correlation between $\delta^{13}\text{C}$ and P, when the effects of MAP on $\delta^{13}\text{C}$ and interactions between elements are taken into account. Moreover, correlations between $\delta^{13}\text{C}$ and element ratios suggest that plants adapt to dry condition by achieving higher water and phosphorus use efficiency at the expense of nitrogen use efficiency. Differences of correlations between $\delta^{13}\text{C}$ and nutrient elements among PFGs suggest that different PFGs have different strategies to adapt to drought conditions in Inner Mongolia.

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