

Investigation of the controlled factors influencing carbon isotope composition of foxtail and common millet on the Chinese Loess Plateau

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To apply carbon isotope composition ($\delta^{13}\text{C}$) analyses of C_4 plants to the quantitative reconstruction of paleoclimate, the functional mechanism linking plant $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_\text{p}$) to the environment, which is based on the plants' physiological characteristics and morphological adaptability, must be thoroughly understood. Foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*), as C_4 plants, are representative crops of the rain-fed agriculture present in northern China. Fossil millets are ideal for paleoclimatic studies because of the ease of acquisition and identification to the species level. Modern seeds of foxtail and common millet collected from different habitats of the Chinese Loess Plateau, and their carbon isotope compositions, were analyzed and correlated with environmental factors, such as latitude, altitude, temperature, precipitation, water availability, and relative humidity. The results showed that the $\delta^{13}\text{C}$ of foxtail millet had a significantly negative correlation with latitude ($R=-0.46$), which may indicate the influence of light. The effect of light on the $\delta^{13}\text{C}$ of foxtail millet accounted for only 21% of variability, while other climatic factors did not exert significant influences. Thus, the $\delta^{13}\text{C}$ of foxtail millet was not suitable for extracting climatic information. The $\delta^{13}\text{C}$ of common millet was significantly and positively correlated with precipitation during the growing period ($R=0.75$), explaining 56% of variability. The functional mechanisms analyzed, using the plants' physiological characteristics and morphological adaptability, indicated that common millet can adapt to environmental changes because of stomatal sensitivity and some non-stomatal factors. Therefore, the $\delta^{13}\text{C}$ of common millet can record precipitation during growth and is a promising factor for paleoclimatic reconstruction.

foxtail millet, common millet, stable carbon isotope, water availability, C_4 plant, physiological characteristics

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C_4 plants, as a significant component of the modern global ecosystem, expanded rapidly some 7 to 8 million years ago (Cerling et al., 1997) because of a decrease in the atmospheric CO_2 concentration combined with high temperature and/or moisture stress (Cerling et al., 1993). The evolution of the C_4 photosynthetic pathway reduces photorespiration

compared with the ancestral C_3 pathway (Farquhar, 1983). C_4 plants have the ability to maintain high photosynthetic efficiencies in high light intensity, high temperature and low CO_2 concentration conditions (Dengler and Nelson, 1999; Hatch, 1999), hence C_4 plants play a significant role in the global carbon budget (Still et al., 2003). The carbon isotope composition of organic materials of terrestrial plants is affected by physiological characteristics and environmental factors, thus $\delta^{13}\text{C}$ can be used to differentiate C_3 and C_4

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types of plant as well as environment conditions in which one species of plant grew (Farquhar et al., 1989; Cerling et al., 1997; Cernusak et al., 2013). Therefore, a $\delta^{13}\text{C}$ analysis of plants provides an effective approach in studies of climate change, carbon emissions and sinks, and physiological ecology (Ciais et al., 1995; Drake et al., 1997; Anderson et al., 1998; Valentini et al., 2000; Liu et al., 2005; West et al., 2006).

The ^{13}C fractionation that occurs during photosynthesis is a useful technique for investigating the relationship between plant $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_p$) and environmental factors, such as atmospheric CO_2 concentration, temperature, precipitation, humidity, and soil composition, during the plants' growth period (McCarroll and Loader, 2004). The relationship between the $\delta^{13}\text{C}$ of C_3 plants and the environment has been widely surveyed and thoroughly researched (Körner et al., 1988; Miller et al., 2001; Hatté et al., 2001; Leffler and Enquist, 2002; Wang et al., 2003; Macfarlane et al., 2004; Liu et al., 2005; Roden et al., 2005; Guo and Xie, 2006; Ning et al., 2008; Song et al., 2008; Li et al., 2009; Gouveia and Freitas, 2009; Diefendorf et al., 2010). Based on a comprehensive compilation of the modern $\delta^{13}\text{C}$ of the investigated C_3 plant types, a model showing the relationship between $\delta^{13}\text{C}_p$ and both climatic factors and descriptive parameters (e.g. latitude, altitude) has been established (Kohn, 2010). The establishment of this model has had a profound effect on paleodietary and paleoecological studies, stimulating intensive studies of tectonic, climatic and biotic changes (Wang et al., 2013a).

However, the mathematical equation between the $\delta^{13}\text{C}$ of C_4 plants and climatic factors cannot be elucidated directly because of the decreased sensitivity of C_4 plants compared with C_3 plants. However, based on qualitative descriptions of the natural occurrence and geographical distribution of C_4 plants and their climatic significances (Tieszen et al., 1979; Schulze et al., 1996; Akhani et al., 1997; Kalapos et al., 1996; Pyankov et al., 2000; Liu et al., 2002; Wang et al., 2006; Li et al., 2009), great efforts have been made to understand the relationship between the abundance of C_3/C_4 plants and climate change (Hattersley, 1983; Goodfriend, 1990; Gu et al., 2003; Swap et al., 2004; Liu et al., 2005; Ning et al., 2008), as well as the quantitative relationship between the $\delta^{13}\text{C}$ composition of C_4 plants and climatic parameters (Schulze et al., 1998; Wang et al., 2006; Ma et al., 2012; Wang et al., 2013b). With the purpose of applying $\delta^{13}\text{C}$ of C_4 plants to reconstruct paleoclimate, the particular $\delta^{13}\text{C}$ fractionation imposed by the climatic factors should be analyzed entirely based on the C_4 plants' physiological characteristics and morphological adaptability. It is crucial, therefore, that the functional mechanisms of chemical and biochemical processes linking $\delta^{13}\text{C}_p$ to their ambient environments should be thoroughly understood. Taking into consideration the existing variations in ^{13}C discrimination among interspecies and even intraspecies (Comstock and Ehleringer, 1992), any genetically determined discrimina-

tion of interspecies will be eliminated once a single species is adopted for the $\delta^{13}\text{C}$ analysis and, hence, any environmental information derived from the carbon isotope composition associated with this plant species will be more authentic and reliable. Marino et al. (1992) used the $\delta^{13}\text{C}$ of the C_4 shrub *Atriplex confertifolia* to reconstruct the glacial-to-interglacial variations of the $\delta^{13}\text{C}$ of atmospheric CO_2 , thus providing essential and reliable data to validate results derived from ice cores. However, owing to the limited distribution of *Atriplex confertifolia*, it is necessary to evaluate new species of plants to obtain the high resolution of quantitative climate reconstruction over a more extensive area.

Foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*), belonging to the Panicoideae family of C_4 plant, are representative crops of the early rain-fed agriculture in northern China. Even today, they remain important cereal crops in arid and semi-arid areas, including China, Japan, Russia, and India (You, 1993; Lu, 1998; Crawford, 2006). They originated around 10000 a BP in northern China (Lu et al., 2009) and appeared in Eurasia before rice and wheat. They usually have similar ecological habitats and are adapted for most soil types, except for alkaline-saline soils (Liang and Kang, 1996; Gu et al., 2001). They were widely and continuously cultivated during the Neolithic period in northern China (An, 1988). Charred millet kernels buried in sediment layers can preserve their chemical properties and maintain their stable $\delta^{13}\text{C}$ (DeNiro and Hastorf, 1985; Yang et al., 2011). They also have readily obtained and identified characteristics at the species level (Liu and Kong, 2004). Thus, they are ideal for studying paleoclimate, paleoenvironment and early agricultural activities.

The climate during the Holocene epoch in northern China, especially in the Loess Plateau, which is highly sensitive to climatic changes, could be quantitatively reconstructed if the $\delta^{13}\text{C}$ of foxtail or common millet can be correlated with climatic factors. In the work described in this paper, modern seeds of foxtail and common millet cultivated on the Loess Plateau and its surrounding area were systematically sampled and their $\delta^{13}\text{C}$ determined. Statistical analyses were carried out to explore whether the $\delta^{13}\text{C}$ of organic materials was correlated with climatic factors. Then, the explicit functional mechanisms linking $\delta^{13}\text{C}$ and environmental parameters were defined based on plant physiological characteristics and morphological adaptability. The dominant factors were determined and the relationship between the $\delta^{13}\text{C}$ of millet and climatic factors was established. These will provide a foundation for the quantitative reconstruction of paleoclimate, and early agricultural activities and adaptations (Li, 2013).

1 Materials and methods

1.1 Sampling

All modern foxtail and common millet seed samples were

collected from the Loess Plateau and its surrounding areas in the maturation period of September to October 2008. Sampling spots were divided into two cross-sections, aligned in north-south and east-west profiles. The north-south cross-section traversed the hinterland of the Loess Plateau and spanned four latitudes; the east-west cross-section covered the arid area from the south-western Loess Plateau to the Hexi corridor, spanning seven longitudes (Figure 1). All of the sampling spots were located between 990 and 2200 m above sea level (Table 1) and were selected in flat, open and non-irrigated land far from villages to avert the influence of local geomorphology or microhabitats. The possibilities of fertilization and irrigation, which are anthropic factors that may affect the $\delta^{13}\text{C}_p$, were excluded by inquiring the local villagers. Three spikes with plump seeds, from different living millet plants that were spaced about 5 m from each other, were sampled randomly from each site. All three spikes were mixed into one sample for each spot. In total, 22 samples of foxtail millet and 15 samples of common millet were collected.

1.2 Stable carbon isotope composition analysis

Five grains from the 22 samples of foxtail millet and 15 samples of common millet were selected randomly and their

lemma and palea were shucked to avoid the $\delta^{13}\text{C}$ bias caused by the mixture of lemma, palea and caryopsis (seeds) (Yang et al., 2011). The $\delta^{13}\text{C}$ of seeds can provide the integrated estimation information during the period when organic materials are synthesized, which is from the beginning of the growing period to the sampling time in this study.

The organic carbon isotope analysis was used to measure the $\delta^{13}\text{C}$ of the seeds. Each sample portion was placed in a beaker and covered with 1% hydrochloric acid to remove any carbonates. The samples were then washed with distilled water to a pH>5 and oven dried at 40°C for 24 h. The dried samples were ground in an agate mortar and homogenized, then vacuum sealed in a quartz tube with copper oxide and silver foil, and combusted for at least 4 h at 850°C. The CO_2 gas from the combustion tube was extracted and cryogenically purified. The isotope ratio of the extracted CO_2 gas was determined at the Institute of Earth Environment, Chinese Academy of Sciences using a MAT-251 gas source mass spectrometer with a dual inlet system.

All isotope ratios are expressed using the following δ notation:

$$\delta^{13}\text{C}(\text{‰}) = \left[\frac{R_{\text{sample}} - R_{\text{std}}}{R_{\text{std}}} \right] \times 1000. \quad (1)$$

The isotope standard used is Vienna Pee Dee Belemnite

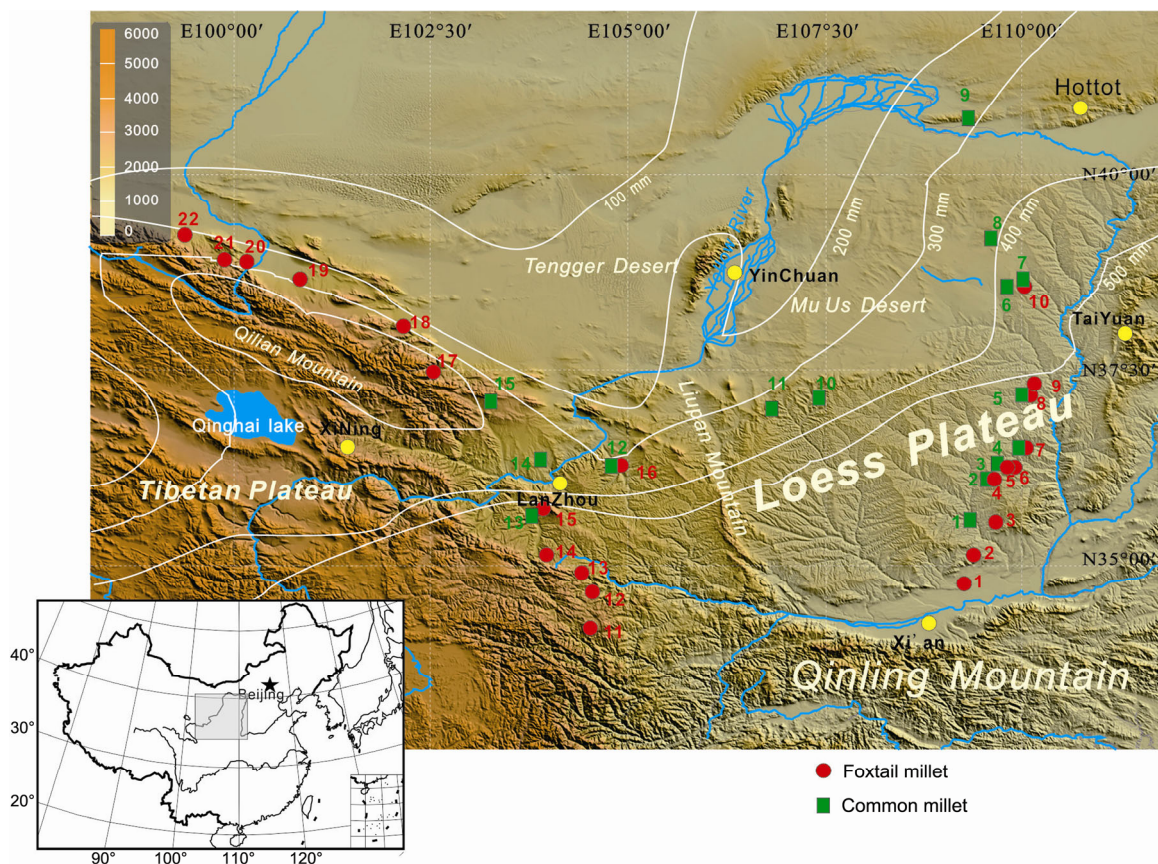


Figure 1 Map of the sampling sites.

(V-PDB), and the analytical precision at the 1 σ level is reported as 0.2‰.

1.3 Meteorological stations and data

The sampling area, located in an arid to semi-arid region of northern China, is characterized by high precipitation in the summer and low precipitation in the winter because of the Asian monsoon. The mean annual precipitation is ~50–600 mm, which decreases gradually from the southeast to the northwest. All meteorological data for 2008 were collected from meteorological stations in the vicinity of the sampling sites, which had the similar geomorphological features according to the principle of proximity. Temperatures at the sampling spots were corrected from those of meteorological stations since the temperature decreases as altitude increases. The data included mean temperature (T), precipitation (P) and relative humidity (RH) for the whole year (January to December) and for the growing season (July to September or August to October) (Table 1). The growing season of foxtail and common millet was from July to September in the north-south cross-section, but from August to October in the Hexi Corridor (Chai, 1999; Lin et al., 2002; Yao et al., 2004).

Water availability is an important indicator of plant growth conditions. The monthly ratio of precipitation to potential evapotranspiration (P/E_p) was calculated as a weighting factor of the water availability to plants. E_p was calculated based on temperature and latitude according to the algorithm of Thornthwaite (1948), which has proved to be robust and can be calculated from easily available meteorological data. $P/E_p < 1$ indicates that the available monthly water does not limit growth; $P/E_p = 1$ means that plants probably achieve a complete growth cycle because soil water levels are adequate; and $P/E_p > 1$ indicates that the monthly excess precipitation over potential evapotranspiration is assumed to be lost as runoff, or stored in the soil profile until lost as E_p (Comstock and Ehleringer, 1992). The latter assumption would yield an increase in moisture availability for evapotranspiration in the warmer months. Therefore, the evapotranspiration gradient is the only factor related to moisture availability when soil humidity is able to meet the demand of plant respiration. In this study, the P/E_p for all of the sampling sites was > 1 , indicating the whole-plant samples are subjected to water stress. The greater the P/E_p value, the greater the water availability for growth.

1.4 Statistical analysis

The $\delta^{13}C_p$ can be affected by many factors working together. To clarify the extent of the $\delta^{13}C_p$ impacted from each climatic factor, all of the data were analyzed using statistical analysis methods. First, a cluster analysis was applied to classify all of the sampling sites based on the similarity of each climate factor. Strongly similar sites were then placed in the same group, ensuring that each group was signifi-

cantly different. According to the clustering results, box plots of the $\delta^{13}C$ values of each group of both millets were drawn, followed by the application of the non-parameter test to pair-wise groups of two independent samples.

Correlation analyses using the $\delta^{13}C$ of millets and latitudes, altitude, temperature, precipitation, P/E_p , and RH were performed. Linear regression using the linear fit method was adopted to reveal the correlation between the $\delta^{13}C$ of millet and each climatic factor. Partial correlation analyses and path analyses of $\delta^{13}C$ of millets and their marked influence factors were then performed. Finally, multiple linear regression (MLR) analyses of $\delta^{13}C$ of millet with all of the environmental factors were carried out.

SPSS 19.0 for Windows and OriginPro 8.0 were used for the statistical analyses. Unless otherwise stated, differences were considered statistically significant at $p < 0.05$.

2 Results

2.1 $\delta^{13}C$ of foxtail and common millet in different habitats

Various habitats were grouped using a cluster analysis based on environmental factors, including latitude, altitude, temperature, precipitation, water availability and relative humidity of the sampling sites. The habitats of foxtail and common millet sampling sites were grouped into five and three types, respectively (Table 2). For foxtail millet, Group A consisted of 10 sites (Nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) and Group B contained only the No. 18 site. Group C comprised seven sites (Nos. 15, 16, 16, 17, 19, 20, 21, and 22), Group D included three sites (Nos. 12, 13, and 14), and Group E contained only No. 11, which had a special habitat. For common millet, Group I consisted of eight sites (Nos. 1, 2, 3, 4, 5, 6, 7, and 9), Group II included three sites (Nos. 8, 10, and 11), and Group III contained four sites (Nos. 12, 13, 14, and 15). Based on the principle of cluster analysis, highly similar sites were gathered into the same group, ensuring that significant differences existed between each group.

To show the average $\delta^{13}C$ of the organic materials collected from different habitats, a boxplot analysis was applied to the $\delta^{13}C$ of each group. For foxtail millet, the average $\delta^{13}C$ values of Group A, B, C, and D were $-12.60‰ \pm 0.26‰$, $-12.56‰$, $-13.02‰ \pm 0.62‰$, $-12.62‰ \pm 0.43‰$, and $-11.57‰$, respectively. For common millet, the average $\delta^{13}C$ values of Group I, II, and III were $-12.91‰ \pm 0.38‰$, $-12.95‰ \pm 0.38‰$ and $-13.76‰ \pm 0.50‰$, respectively (Table 2, Figure 2).

The non-parameter test between independent samples was applied to verify whether each pair-wise group, from different habitats, had significantly different $\delta^{13}C_p$ values. For foxtail millet, the distributions of $\delta^{13}C$ between each pair had no significant differences at $P < 0.05$, indicating that they all came from the same ensemble. For common millet,

Table 1 The $\delta^{13}\text{C}$ values of foxtail and common millet, and the geographic and climatic parameters of the sampling sites^{a)}

Sample	Latitude	Longitude	Altitude (m)	T ($^{\circ}\text{C}$)		P (mm)		P/E_p		RH (%)		$\delta^{13}\text{C}_p$ (‰)
				Growing season	Annual	Growing season	Annual	Growing season	Annual	Growing season	Annual	
Foxtail millet												
1	35.48°N	109.29°E	1088	19.8	10.7	272.2	552.4	9.7	7.8	70.9	-12.91	
2	35.55°N	109.26°E	1129	18.9	9.1	272.2	522.4	9.9	8.2	70.9	-12.57	
3	35.68°N	109.39°E	1064	20.1	10.5	265.4	501.3	9.2	7.3	71.3	-12.58	
4	36.20°N	109.71°E	1009	18.3	7.6	207.1	396.9	7.6	7.0	68.0	-12.92	
5	36.69°N	109.71°E	911	21.1	10.7	225.2	416.8	7.4	6.3	68.0	-12.21	
6	36.80°N	109.90°E	1030	18.1	6.6	198.8	415	7.6	8.1	68.0	-12.84	
7	37.01°N	110.12°E	987	19.6	7.8	202.5	363.1	7.5	7.2	68.0	-12.44	
8	37.58°N	110.24°E	910	21.5	9.8	202.9	310.9	7.3	5.3	63.4	-12.37	
9	37.58°N	110.24°E	919	21.4	9.8	202.9	310.9	7.3	5.3	63.4	-12.34	
10	38.75°N	110.20°E	1215	18.6	6.5	323.9	463.7	11.7	7.9	60.9	-12.84	
11	35.09°N	104.58°E	2216	14.0	4.5	184.7	457.8	8.2	8.0	80.8	-11.57	
12	35.50°N	104.63°E	1935	16.5	6.5	202.2	425.5	9.9	7.8	80.8	-12.83	
13	35.71°N	104.49°E	1873	16.9	6.9	176.1	314.1	8.7	6.0	74.6	-12.12	
14	35.92°N	104.05°E	1895	16.7	6.9	215.7	372.7	10.7	7.3	74.6	-12.9	
15	36.39°N	103.96°E	1706	20.3	10.4	157.1	293.5	6.8	4.8	65.6	-13.01	
16	36.93°N	104.95°E	1771	17.9	7.2	97.7	187.1	5.0	3.9	66.9	-12.13	
17	37.94°N	102.55°E	1583	20.2	8.9	46.3	112.5	2.2	2.2	52.2	-13.01	
18	38.43°N	102.16°E	1242	18.1	7.3	74.9	184.9	3.4	3.4	55.6	-12.56	
19	38.86°N	100.78°E	1565	19.8	8.1	91.5	205.4	4.1	3.7	50.6	-13.67	
20	38.96°N	100.19°E	1579	19.6	7.7	79.5	137.1	4.0	2.5	56.8	-13.53	
21	38.97°N	99.90°E	1884	16.8	5.0	19.6	40.9	1.4	1.0	43.9	-12.24	
22	39.20°N	99.47°E	1826	16.8	5.0	37.9	90.3	2.6	2.2	57.3	-13.53	
Common millet												
1	36.20°N	109.37°E	1009	18.3	7.6	207.1	396.9	7.8	7.7	68.0	-12.74	
2	36.70°N	109.71°E	915	21.1	10.6	225.2	416.8	7.7	6.9	68.0	-13.00	
3	36.79°N	109.89°E	1029	18.1	6.6	198.8	415.0	9.2	9.1	68.0	-13.46	
4	37.01°N	110.12°E	982	19.6	7.8	202.5	363.1	7.9	7.7	68.0	-12.58	
5	37.58°N	110.24°E	903	21.5	9.9	202.9	310.9	8.4	6.0	63.4	-13.18	
6	38.70°N	110.07°E	1169	18.9	6.8	323.9	463.7	12.5	8.5	60.9	-12.63	
7	38.76°N	110.19°E	1223	18.5	6.4	323.9	463.7	12.7	8.7	60.9	-12.46	
8	39.29°N	109.82°E	1364	18.8	6.7	267.2	366.0	7.7	6.5	61.4	-13.36	
9	40.63°N	109.65°E	1025	20.2	7.9	220.6	317.0	8.3	6.9	59.8	-13.36	
10	37.64°N	107.55°E	1346	19.9	8.9	207.5	235.6	8.1	4.2	60.9	-12.87	
11	37.60°N	106.94°E	1485	18.5	7.0	228.2	251.8	8.5	4.6	64.6	-12.61	
12	36.94°N	104.96°E	1768	17.9	7.2	157.1	293.5	5.0	3.9	66.9	-13.39	
13	36.32°N	103.88°E	1701	20.3	10.4	97.7	187.1	6.7	4.8	65.6	-13.87	
14	36.95°N	103.99°E	1953	18.0	7.2	157.1	293.5	5.6	3.9	57.0	-13.93	
15	37.57°N	103.30°E	1901	17.4	6.3	107.2	176.0	8.1	6.2	70.7	-13.85	

a) All temperature and relative humidity data shown above are mean values of diurnal temperatures and diurnal moisture readings in the year or growing season. All original meteorological data were provided by the National Climate Center.

Table 2 Cluster analysis of the sampling sites

	Latitude (N)	Altitude (m)	T (°C)	P (mm)	P/E _p	RH (%)	δ ¹³ C (‰)	Sample No.
Foxtail millet								
A	36.7°±1.1°	1026±100	19.7±1.3	237.3±43.3	8.5±1.5	67.3±3.6	-12.60±0.26	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
B	38.4°	1242	18.1	74.9	3.4	55.6	-12.56	18
C	38.2°±1.1°	1702±130	18.8±1.6	75.7±46.2	3.7±1.8	56.2±8.2	-13.02±0.62	15, 16, 17, 19, 20, 21, 22
D	35.7°±0.2°	1901±31	16.7±0.2	198.0±20.1	9.8±1.0	76.7±3.6	-12.62±0.43	12, 13, 14
E	35.09°	2216	14.0	187.7	8.2	80.8	-11.57	11
Common millet								
I	37.8°±1.5°	1032±113	19.5±1.3	238.1±53.7	9.3±2.1	64.6±3.7	-12.91±0.38	1, 2, 3, 4, 5, 6, 7, 9
II	38.2°±1.0°	1831±117	19.1±0.7	234.3±30.3	8.1±0.4	62.3±2.0	-12.95±0.38	8, 10, 11
III	36.9°±1.2°	1318±365	18.4±1.3	129.8±31.8	6.4±1.4	65.1±5.8	-13.76±0.50	12, 13, 14, 15

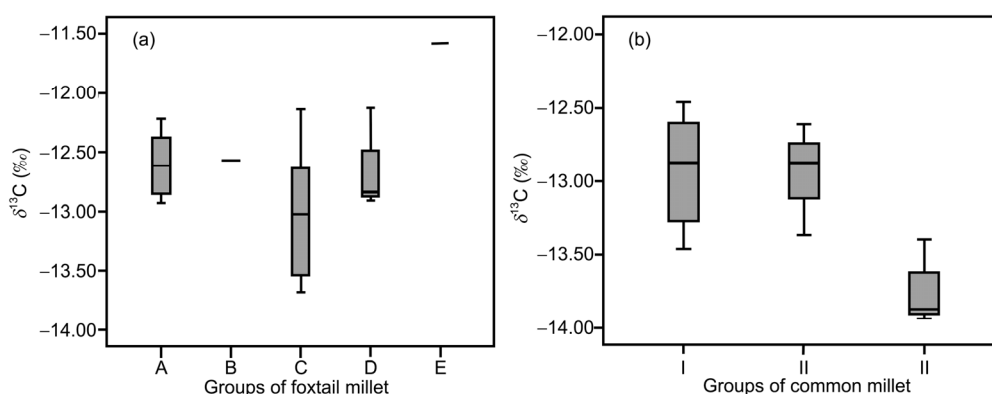


Figure 2 Boxplots of the δ¹³C of each group of foxtail millet (a) and common millet (b).

the distributions of δ¹³C in Group I and III were significantly different, indicating that they came from observably different distributions; Group I and II had no significant differences, indicating they came from the same distribution with high probability; the distribution of δ¹³C between Group II and III had no significant differences at *P*<0.05 but was significantly different at *P*<0.1. Thus, there was a 90% probability they came from two different ensembles.

2.2 Correlations between δ¹³C_p and climatic factors

The δ¹³C_p may be affected by latitude, altitude, temperature, precipitation, water availability, and/or relative humidity. Correlation analyses were performed by examining the relationships of millet δ¹³C and each of these factors, including geographical factors, such as latitude and altitude, and climatic factors, such as mean temperature, precipitation, *P*/*E*_p and RH, throughout the whole year as well as during the growing season (Table 3, Figures 3 and 4). The results demonstrated that there were no significant correlations between the δ¹³C of foxtail millet and any factor, except altitude. The δ¹³C of foxtail millet was negatively related to latitude, and the Pearson R correlation coefficient was -0.46 (*P*<0.05), indicating that the altitude influenced the δ¹³C of

Table 3 Correlations between the δ¹³C of foxtail and common millet, and parameters^{a)}

Parameter	δ ¹³ C of foxtail millet (‰)	δ ¹³ C of common millet (‰)
Latitude	-0.46 [*]	0.14 ^{ns}
Altitude	0.02 ^{ns}	-0.61 [*]
<i>T</i>	Annual	-0.08 ^{ns}
	Growing season	0.14 ^{ns}
<i>P</i>	Annual	0.27 ^{ns}
	Growing season	0.75 ^{**}
<i>P</i> / <i>E</i> _p	Annual	0.29 ^{ns}
	Growing season	0.61 [*]

a) * *P*<0.05, ** *P*<0.01; ns, correlation coefficient not significantly different from zero.

foxtail millet, and the δ¹³C value would decrease at higher latitudes. For common millet, the δ¹³C was negatively related to altitude (*R*=-0.61, *P*<0.05) and positively related to precipitation over the whole year (*R*=0.57, *P*<0.05), precipitation over the growing period (*R*=0.75, *P*<0.01), as well as water availability during the growing period (*P*/*E*_p) (*R*=0.61, *P*<0.05). The δ¹³C of common millet had no significant relationships with the other factors, such as latitude, temperature,

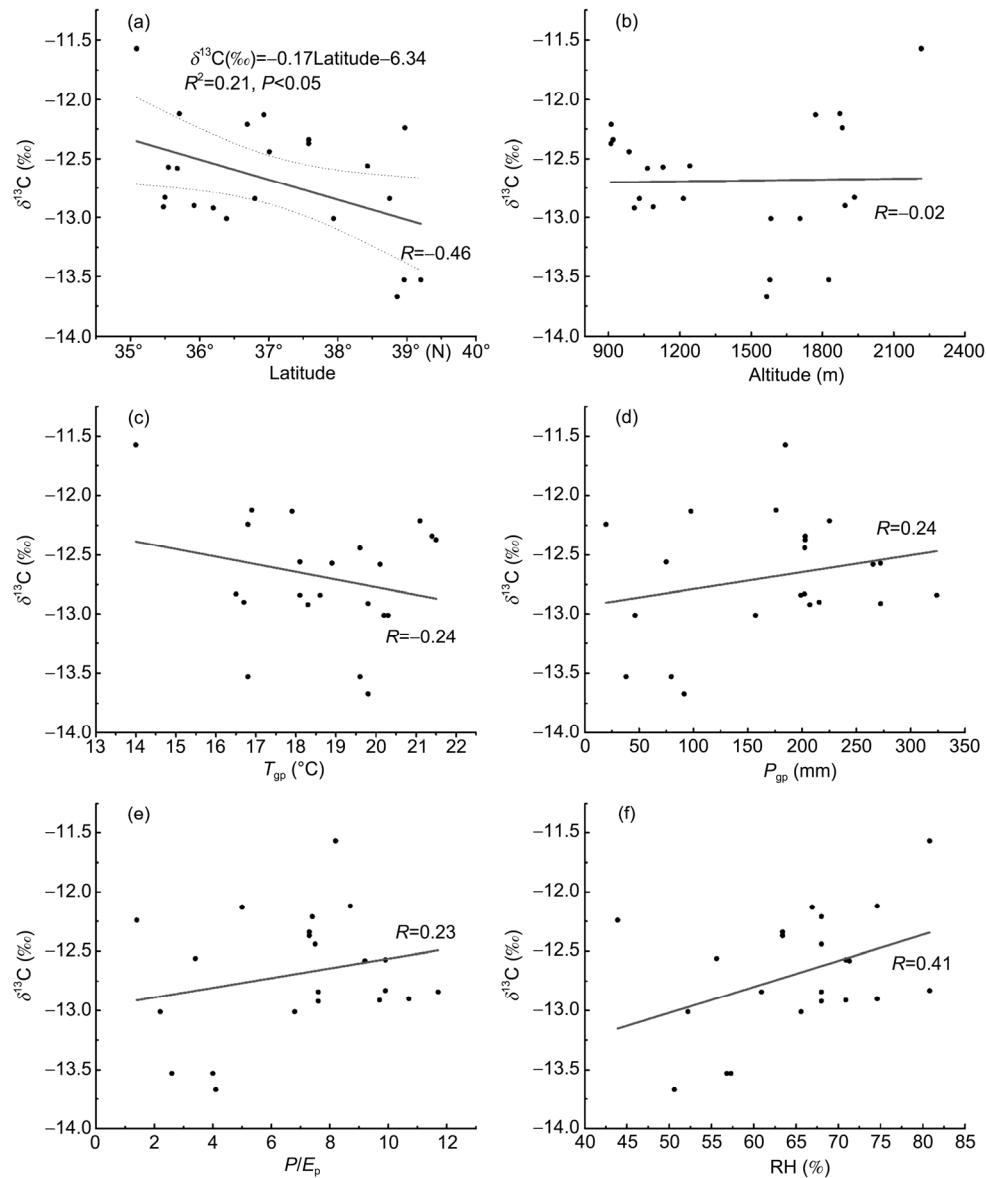


Figure 3 Linear diagrams between the $\delta^{13}\text{C}$ of foxtail millet and latitude, altitude, temperature, precipitation, P/E_p , and relative humidity. The $\delta^{13}\text{C}$ of foxtail millet shows a good linear relationship with latitude (a).

relative humidity and water availability during the whole year.

Accordingly, linear regression analyses were applied to the $\delta^{13}\text{C}_p$ and their strongly correlated climatic factors. The linear fit clearly showed that the $\delta^{13}\text{C}$ of foxtail millet relates linearly to latitude (Figure 3(a)), as follows:

Negative relationship between $\delta^{13}\text{C}$ and latitude: $\delta^{13}\text{C}(\text{‰}) = -0.17 \text{ Latitude} - 6.34$, $R^2 = 0.21$, $P < 0.05$.

In the same way, the $\delta^{13}\text{C}$ of common millet relates linearly to altitude, precipitation and P/E_p (Figure 4(b)–(e)), as follows:

Negative relationship between $\delta^{13}\text{C}$ and altitude: $\delta^{13}\text{C}(\text{‰}) = -8.34 \times 10^{-4} \text{ Altitude} - 12.05$, $R^2 = 0.37$, $P < 0.05$;

Positive relationship between $\delta^{13}\text{C}$ and precipitation

during the growing period (P_{gp}): $\delta^{13}\text{C}(\text{‰}) = 0.006 P_{gp} - 14.36$, $R^2 = 0.56$, $P < 0.01$;

Positive relationship between $\delta^{13}\text{C}$ and water availability during the growing period (P/E_p): $\delta^{13}\text{C}(\text{‰}) = 0.15 P/E_p - 14.37$, $R^2 = 0.37$, $P < 0.05$.

More than one environment factor exerts its effect on the $\delta^{13}\text{C}$ of common millet based on the above analysis. A partial correlation analysis was applied based on these factors being correlated with each other. The results demonstrated that the relationship between $\delta^{13}\text{C}$ and precipitation during the growing period did not have an effect when altitude was controlled, and the positive relationship between $\delta^{13}\text{C}$ and water availability during the growing period was absent. Likewise, the significant correlations of the $\delta^{13}\text{C}$ of

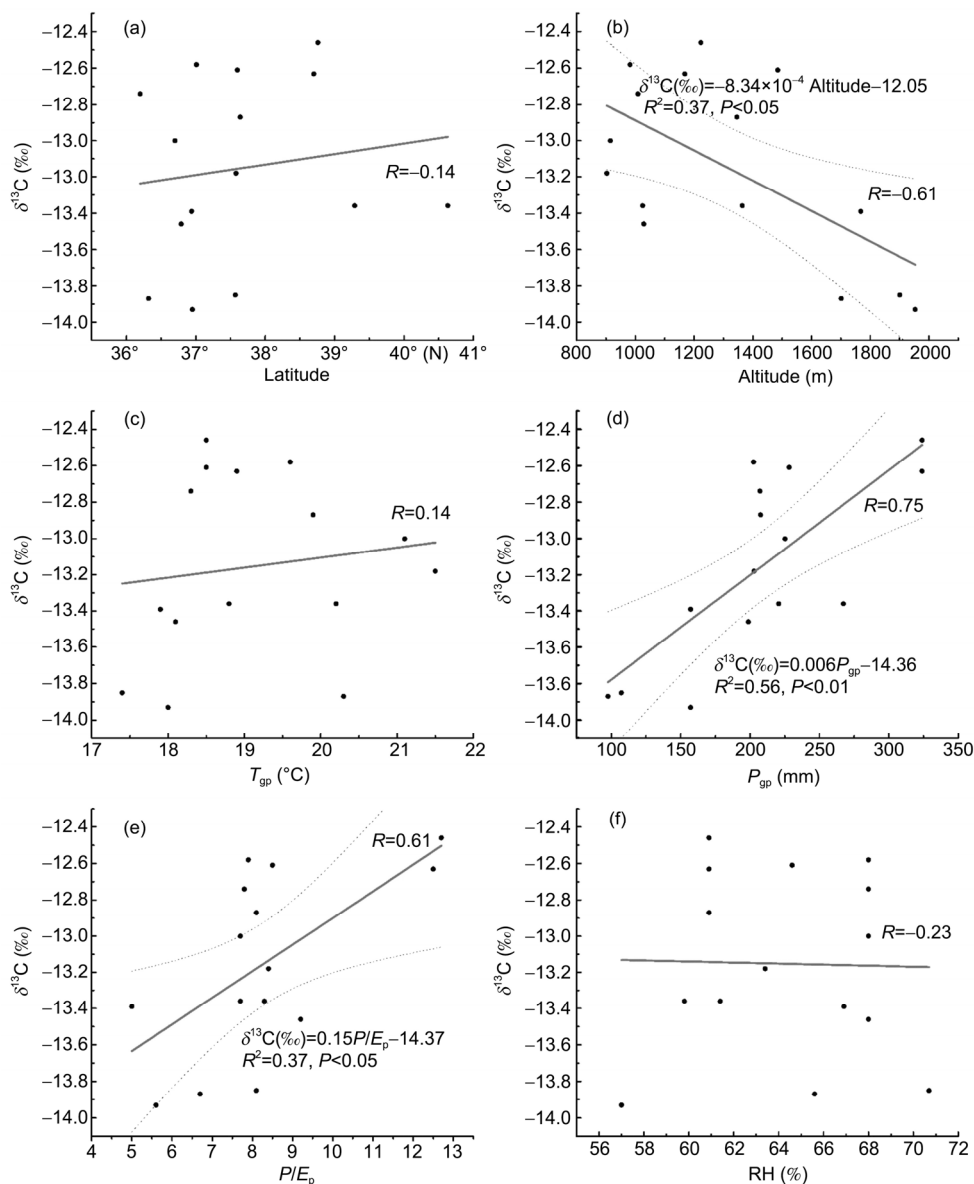


Figure 4 Linear diagrams between the $\delta^{13}\text{C}$ of common millet and latitude, altitude, temperature, precipitation, P/E_p , and relative humidity. The $\delta^{13}\text{C}$ of common millet had good linear relationships with altitude (b), precipitation (d), and P/E_p (e).

common millet with altitude and water availability were absent when precipitation was controlled. Thus, altitude and water availability did not act upon the $\delta^{13}\text{C}$ of common millet directly.

Since the results from simple correlations are one-sided to some extent, a further analysis was necessary for testing the stability of correlations linking these factors with $\delta^{13}\text{C}$. Accordingly, a stepwise multiple regression analysis was conducted between $\delta^{13}\text{C}_p$ and latitude, altitude, temperature, precipitation, P/E_p and RH for foxtail and common millet separately. For foxtail millet, the results demonstrated that only latitude entered the regression equation, as follows: $\delta^{13}\text{C}(\text{‰})=-0.17\text{Latitude}-6.34$, $R=-0.46$ ($P<0.05$); coefficient of determination (R^2)=0.21 and the remaining variable $e=\sqrt{1-r^2}=0.88$, which is great, indicating that latitude is

not the major factor controlling the $\delta^{13}\text{C}$ value of foxtail millet and that the main factor has not yet been discerned. For common millet, only P_{gp} entered the regression equation, as follows: $\delta^{13}\text{C}(\text{‰})=0.006P_{gp}-14.36$, $R=0.75$ ($P<0.01$); $R^2=0.56$ and $e=\sqrt{1-r^2}=0.66$, which is relatively large, indicating that the $\delta^{13}\text{C}$ of common millet was mainly controlled by the P_{gp} . However, some other factors also affected $\delta^{13}\text{C}_p$ to some extent. The indirect path coefficient of $\delta^{13}\text{C}$ imposed by P_{gp} through changes in altitude was -0.41 .

3 Discussion

The $\delta^{13}\text{C}_p$ can be affected not only by the inherent physiological properties but also by many environmental factors,

such as atmospheric CO₂ pressure, O₂ partial pressure, temperature, light and precipitation (Farquhar et al., 1989; Dawson et al., 2002). Additionally, soil nutrition and chemical properties, such as salinity, can affect $\delta^{13}\text{C}_p$ as well. Both physiological characteristics and the environmental factors influence the variation of $\delta^{13}\text{C}_p$, with a degree of play assigned to their contributions (Cernusak et al., 2013). C₃ and C₄ plants, with different photosynthetic pathways, have significantly different $\delta^{13}\text{C}$ ranges, with the average of -28‰ in C₃ plant and -12.5‰ in C₄ plants (Cerling et al., 1998; Kohn, 2010). Based on the differences in bundle sheath permeability and the decarboxylation reaction during photosynthesis in leaves (Farquhar, 1983; Hattersley and Watson, 1992), carbon assimilation in C₄ plants is grouped into three biochemical subtypes, NAD-ME, NADP-ME and PCK (Hatch, 1987). Foxtail millet belongs to the NADP-ME subtype while common millet belongs to the NAD-ME subtype (Hattersley, 1982). The NADP-ME subtype C₄ species usually have significantly higher $\delta^{13}\text{C}$ values than those in their NAD-ME counterparts (Hattersley, 1982; Schulze et al., 1996).

The $\delta^{13}\text{C}_p$ is determined by the combined action of carbon isotope fractionation and source isotope composition (Araus and Buxó, 1993). The $\delta^{13}\text{C}_p$ composition depends largely on the isotope composition of atmospheric CO₂ during its growing period. The $\delta^{13}\text{C}$ of atmospheric CO₂ changes only a little with increasing altitude and latitude (Francey and Farquhar, 1982; Körner et al., 1988), and their influence on $\delta^{13}\text{C}_p$ is synchronous. Therefore, the effects can be ignored in this work.

Latitude and altitude, as descriptive parameters, did not exert a direct influence on the plants' metabolism, but they did affect $\delta^{13}\text{C}$ indirectly by exerting influences on temperature, moisture, precipitation, and other related factors. For foxtail millet, the $\delta^{13}\text{C}$ of organic material is significantly related to latitude, which might be the consequence of light duration and intensity. Likewise, the influence of altitude upon $\delta^{13}\text{C}$ varies with precipitation. Decreasing precipitation contributes 41% to the decreasing $\delta^{13}\text{C}$ of common millet, with increasing altitude, based on the indirect path coefficient -0.41 between P_{gp} and $\delta^{13}\text{C}$.

In C₃ plants, $\delta^{13}\text{C}_p$ responds to environmental factors by dominating the ratio of the intercellular and ambient partial pressure of CO₂ (c_i/c_a) with the opening or closing of leaf stomata (Körner and Diemer, 1987; Körner and Larcher, 1988; Körner et al., 1989). c_i/c_a represents the equilibrium of photosynthetic carbon fixation and biochemical constraints, and usually decreases with stomatal closure during an increase in drought stress (Farquhar et al., 1989). If $\delta^{13}\text{C}_a$ is relatively constant, then the $\delta^{13}\text{C}$ of C₃ plants has consistent negative responses to c_i/c_a (Orchard et al., 2010). In C₄ species, the $\delta^{13}\text{C}$ depends not only on c_i/c_a but also on how much CO₂ and HCO₃⁻ in bundle sheath cells leaks into the mesophyll cells (called leakiness ϕ), which is deter-

mined by its physiological characteristics. c_i/c_a is related to the light-use efficiency of carbon fixation and leakiness is related to transpiration efficiency (Hubick et al., 1990). There is either a positive or negative correlation between the $\delta^{13}\text{C}$ of C₄ plants and c_i/c_a , depending on whether ϕ is larger or smaller than c. 0.37, respectively (Farquhar et al., 1989). This contributes to the complicated physiological characteristics of C₄ plants. The key feature of C₄ photosynthesis is the operation of a CO₂-concentrating mechanism in the leaves of C₄ plants, which consists of a series of biochemical and structural modifications around the ancestral C₃ photosynthetic pathway (Hatch, 1987). The simplified equation characterizing the ¹³C discrimination of C₄ plants based on Farquhar (1983) is as follows:

$$\delta_p = \delta_a - a - [b_4 + (b_3 - s)\phi - a]p_i/p_a, \quad (2)$$

where δ_a denotes the isotope value of atmospheric CO₂ used by the plant during photosynthesis (‰), δ_p denotes the isotope value of the photosynthate produced in the leaf, from which plant issue is made, a is the fractionation during the diffusion of CO₂ in the air ($\sim 4.4\text{‰}$), b_4 is the isotope shift associated with the combined influences of carbonic anhydrase and PEP ($\sim 7.9\text{‰}$ at 25°C), b_3 is the fractionation introduced by Rubisco (27‰), s is the fractionation during CO₂ leakage from the bundle sheath (2‰), and ϕ is the fractionation of CO₂ released in the bundle sheath cells returned to mesophyll (the amount recycled in mesophyll). The value of ϕ has been shown to vary among subtypes, and its variation relates to morphological features, such as the presence of suberized lamellae around the bundle sheath cells.

Limited precipitation and soil humidity are the most important environmental elements affecting the growth of plants in arid and semi-arid areas (Hadley and Szarek, 1981; Ehleringer and Mooney, 1983; Smith and Nowak, 1990; IPCC, 2007; Murphy and Bowman, 2009). For C₃ plants, the increase of water stress usually induces stomata to close and conductance (g) to decrease, so that, consequently, the intercellular partial pressure of CO₂ (c_i) decreases and the $\delta^{13}\text{C}$ value increases. Therefore, c_i/c_a directly affects $\delta^{13}\text{C}$, and $\delta^{13}\text{C}$ has a negative correlation with water availability (Farquhar et al., 1989). However, for C₄ plants, the response of $\delta^{13}\text{C}$ to drought depends not only on c_i/c_a but also on ϕ . The relationship can be positive, negative, or insignificant, depending on ϕ . Under most environmental conditions, ϕ is less than 0.37, and $\delta^{13}\text{C}_4$ is expected to increase with decreasing water availability as a result of decreasing c_i/c_a (Ubierna et al., 2011), which causes a negative correlation between $\delta^{13}\text{C}$ and water availability. However, the $\delta^{13}\text{C}$ value increases with rising water availability, leading to a positive relationship between them when ϕ is greater than 0.37 (Ubierna et al., 2011). For C₄ species in the arid regions of northwest China, $\delta^{13}\text{C}$ tends to decrease with decreasing soil water availability (Wang et al., 2005), and the positive correlation between $\delta^{13}\text{C}$ of common millet and

water availability clearly indicates its φ is greater than 0.37.

In the arid regions of northwestern China, the water-use efficiency (WUE) of C_4 plants increased with decreasing soil moisture (Wang et al., 2005). WUE, as a component of the drought resistance of a crop, involves the increased production of dry matter per unit of water consumed by rain-fed plants (Blum, 2009) and is measured by instantaneous rates of assimilation per unit of transpiration (Farquhar and Richards, 1984). The WUE of plants are usually affected by soil fertility, which in turn influences their growth (Livingston et al., 1999; Chen et al., 2005; Brueck, 2008). No definite conclusion has been made yet on the effects of soil fertility on $\delta^{13}C_p$ (Sheriff and Nambiar, 1991; Mitchell and Hinckley, 1993; Guehl et al., 1995; Korol et al., 1999; Yao et al., 2011), but it may not be the decisive factor affecting $\delta^{13}C_p$ or WUE (Morecroft and Woodward, 1996; Yao et al., 2011).

Similarly to C_3 plants under water stress, the stomatal conductance of C_4 plants decreases with declining leaf water status, and this invariably coincides with reduced photosynthetic rates. Thus, the $\delta^{13}C$ value increases with decreasing c_i (Kalapos et al., 1996; Maroco et al., 2000; Ghannoum et al., 2003; Carmo-Silva et al., 2008). The physiological adaptations resulting from the variability of soil water availability and atmospheric moisture are similar, thus their effects on $\delta^{13}C$ are similar (Farquhar and Sharkey, 1982; Farquhar and Richards, 1984; Hubick and Fauquhar, 1987; Smith and Osmond, 1987; DeLucia et al., 1988). For common millet, the correlation analysis between $\delta^{13}C$ and environmental factors demonstrated that precipitation was the controlling factor for $\delta^{13}C$. The parameter values, except elevation, of Group I and II were close to each other, especially precipitation, which produced the closest $\delta^{13}C$ values in the two groups. Meanwhile, in contrast with Group I and II, Group III had the lowest precipitation and the greatest drought stress, producing the most negative $\delta^{13}C$ value, which was significantly different from the values of the other two groups. For foxtail millet, the precipitation and water availability of Group A and B were markedly different, but their $\delta^{13}C$ values had similar distributions, suggesting that precipitation and water availability were not the dominating factors affecting the $\delta^{13}C$. The habitats of Group B and C had the same characteristics, such as the lowest precipitation, P/E_p and relative humidity, and the greatest drought stress. They also had the closest temperatures, but the $\delta^{13}C$ differed greatly, with Group B (-12.56‰) being significantly more positive than Group C ($-13.02\text{‰} \pm 0.62\text{‰}$), indicating that precipitation, water availability, relative humidity and temperature were not the controlling factors affecting the $\delta^{13}C$ of foxtail millet. Group A and Group D had small differences in precipitation, water availability and relative humidity, but a large difference in altitude and temperature. However, the $\delta^{13}C$ values were not significantly different, suggesting that altitude and temperature

were not the dominating factors controlling the $\delta^{13}C$ of foxtail millet. The only factor significantly correlated with the $\delta^{13}C$ of foxtail millet was latitude, which may represent the influence of light duration and intensity.

In addition to stomatal factors, non-stomatal factors also contribute to the inhibition of C_4 photosynthesis under water stress. Water stress may lead to a decrease in the activity ratio of C_3/C_4 cycle enzymes in C_4 plants and induce increased leakiness (Bowman et al., 1989; Saliendra et al., 1996; Ghannoum, 2009). In particular, if the carboxylation activity decreases more than the decarboxylation activity, CO_2 consumption will fall in the bundle sheath, leading to an increase in the CO_2 concentration in the bundle sheath ($[CO_2]_{BS}$) (von Caemmerer and Furbank, 1999). A greater $[CO_2]_{BS}$ gradient across the bundle sheath cell walls causes a greater leakage of CO_2 . Consequently, the $\delta^{13}C$ of both millets tended to become more negative as water stress increased. In addition, the effects of drought on bundle sheath leakiness and $\delta^{13}C$ can be ameliorated by reducing transpiration, prolonging soil water availability, and enhancing plant water status by morphological adaptability, such as increasing the root weight and/or extending the root, reducing the specific leaf area by thickening leaves, and/or developing intensive porosities (Tang, 1983; Williams et al., 2001).

C_4 grasses with different biochemical pathways have different WUE or drought tolerances (Ghannoum, 2009). The NADP-ME subtype occurs in mesic environments, while the NAD-ME subtype dominates in drier areas (Hattersley, 1982; Schulze et al., 1996). The latter has a greater WUE under water stress than the former (Ghannoum et al., 2002). The $\delta^{13}C$ values of NADP-ME grasses are less influenced by changes in environmental variables than those of the NAD-ME subtype (Buchmann et al., 2006). The instantaneous $\delta^{13}C$ of online measurements did not differ between subtypes, which might indicate post-photosynthetic fractionations due to the 'leakiness' of the bundle sheath cells to CO_2 (Bowman et al., 1989; Henderson et al., 1992).

The different responses of the $\delta^{13}C$ of foxtail and common millet to environmental factors can be attributed to their physiological characteristics. The NADP-ME subtype has suberized lamellae around the bundle sheath cells, which reduce leakiness, and reduced grana in the bundle sheath chloroplasts, which results in less photosystem II activity and O_2 evolution than in the other subtypes. As a general rule, plants adapt to altered environments by opening or closing leaf stomata, which results in a change in c_i/c_a , which in turn changes $\delta^{13}C$. The inverse is also true, with the $\delta^{13}C$ of leaves not being significantly affected by environmental factors, suggesting that leaf stomata are insensitive to climate change. The $\delta^{13}C$ in biomass accumulation of seed-producing foxtail millet is influenced by latitude, accounting for only 21% of variability, which may be affected mainly by light duration and intensity, while the other

environmental factors, such as temperature, precipitation, water availability and relative humidity, did not seem to significantly affect the $\delta^{13}\text{C}$ of foxtail millet. All of this indicated that the stomatal factor in foxtail millet was insensitive to climate change and was not suitable for extracting climatic factors. Meanwhile, common millet, such as the NAD-ME subtype, had a higher WUE under water stress, and the sensitivity of stomatal and some non-stomatal factors, such as enzyme activity, had the ability to resist water stress. Therefore, the $\delta^{13}\text{C}$ of organic materials is significantly affected by precipitation with a 56% influence coefficient. Therefore, the $\delta^{13}\text{C}$ of common millet can be used to record precipitation during the growth process and serve as an effective indicator of paleoclimatic reconstruction.

4 Conclusion

This study employed correlation analyses to examine the relationships of the $\delta^{13}\text{C}$ of foxtail and common millet cultivated in the Loess semi-arid and arid regions with environmental factors, such as latitude, altitude, temperature, precipitation, water availability, and relative humidity of the whole year and the growing period. The $\delta^{13}\text{C}$ of foxtail millet was negatively correlated with latitude ($R=-0.46$), but there were no significant correlations between the $\delta^{13}\text{C}$ of foxtail millet and the altitude, temperature, precipitation, water availability or relative humidity. The $\delta^{13}\text{C}$ of common millet was significantly and positively correlated with precipitation during the growing period ($R=0.75$), which also caused the negative correlation with altitude ($R=-0.61$) and the positive correlation with water availability ($R=0.61$). There were no significant correlations between the $\delta^{13}\text{C}$ of common millet and the latitude, temperature and relative humidity.

Under water stress in the semi-arid and arid region, the negative correlation between the $\delta^{13}\text{C}$ of foxtail millet and latitude indicated that the light factor exerted an influence on $\delta^{13}\text{C}$, accounting for 21% of variability, while other climatic factors did not affect the $\delta^{13}\text{C}$ of foxtail millet significantly. Therefore, the $\delta^{13}\text{C}$ of foxtail millet was not suitable for extracting climatic information.

Under water stress, the $\delta^{13}\text{C}$ of common millet significantly correlated with P_{gp} and 56% of variation can be explained by the change in P_{gp} . Common millet had a higher WUE and can resist water stress because of the sensitivity of stomata and some non-stomatal factors. Therefore, the $\delta^{13}\text{C}$ of common millet can record precipitation during growth and serves as a promising indicator of paleoclimatic reconstruction.

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