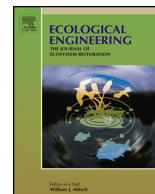




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# The effects of short-term rainfall variability on leaf isotopic traits of desert plants in sand-binding ecosystems



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## ABSTRACT

Sand-binding vegetation is effective in stabilizing sand dunes and reducing soil erosion, thus helps minimize the detrimental effects of desertification. The aim of this study is to better understand the relationships between water and nutrient usage of sand-binding species, and the effects of succession and rainfall variability on plants' water–nutrient interactions. We examined the effects of long-term succession (50 years), inter-annual rainfall variability (from 65% of the mean annual precipitation in 2004 to 42% in 2005) and seasonality on water–nutrient interactions of three major sand-binding species (*Artemisia ordosica*, *Hedysarum scoparium* and *Caragana korshinskii*) by measuring foliar  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and [N]. Long-term succession in general did not significantly alter  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and [N] of the three species. Short-term rainfall variability, however, significantly increased foliar  $\delta^{13}\text{C}$  levels of all three species by 1.0–1.8‰ during the severely dry year. No significant seasonal patterns were found in foliar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the three species, whereas foliar [N] varied by season. For the two leguminous shrubs, the correlations between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were positive in both sampling years, and the positive correlation between [N] and  $\delta^{13}\text{C}$  was only found in the severely dry year. The results indicate that these sand-binding plants have developed into a relatively stable stage and they are able to regulate their nitrogen and water use in responding to environmental conditions, which reinforces the effectiveness of plantation of native shrubs without irrigation in degraded areas. However, the results also indicate that short-term climate variability could have severe impact on the vegetation functions.

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## 1. Introduction

Desertification is one of the major environmental problems worldwide (Reynolds et al., 2007) and it occurs in almost every habitable region such as North America (e.g., Schlesinger et al., 1990; Li et al., 2008; Ravi et al., 2009), Africa (e.g., Thomas et al., 2008; Wang et al., 2010a) and northern China (e.g., Chen et al., 1996; Mitchell et al., 1998; Liu et al., 2012). The deserts in northern China

are expanding at an estimated rate of 2100 km<sup>2</sup> year<sup>−1</sup> (Chen et al., 1996; Mitchell et al., 1998).

Revegetation of native shrubs, a massive ecological engineering project (Mitsch et al., 1993), has been one of the most effective methods to reduce desertification. Large areas of China's desert regions have been reclaimed through planting of native plants since the 1950s (Shapotou Desert Research and Experiment Station, 1986). For example, the 1000-km long Baotou-Lanzhou railway that passes through Shapotou region has been protected by sand-binding shrubs that were planted in 1956, 1964, 1981 and 1987, respectively (Shapotou Desert Research and Experiment Station, 1986). The success of these efforts suggests that it is an effective approach to control desertification and to restore the ecological environment along transportation corridors in the desert regions of China (Xiao et al., 2003a; Li et al., 2006). Many revegetated

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species, such as *Caragana intermedia*, *Calligonum arborescens* and *Tamarix ramosissima*, disappeared over the years, whereas some native species such as *Artemisia ordosica*, *Hedysarum scoparium* and *Caragana korshinskii* survived during succession and have become the dominant shrubs (Ma et al., 2002). As a result of long-term succession, the planted vegetation ecosystem is expected to develop into a stable system (Xiao et al., 2003b). The effects of long-term succession and short-term climate variability (e.g., rainfall) on the function of these successful sand-binding species (e.g., *A. ordosica*, *H. scoparium*, and *C. korshinskii*) are not yet fully understood, which hinders our ability to predict future successional pathways of such planted ecosystems under the circumstances of potential warming and drought in this region (Solomon et al., 2007).

In this study, we measured dual isotope ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) compositions and nitrogen concentrations ([N]) of three dominant species, i.e., *A. ordosica*, *H. scoparium*, and *C. korshinskii*, and analyzed their interactions to investigate the effects of long-term succession (50 years) and short-term drought (2 years) on plant uptake of water and nitrogen at a desert stabilized by sand-binding plants in northern China. Besides providing valuable isotopic information of these sand-binding species, this study will have both theoretical and applied importance in the restoration of degraded ecosystems and in improving our ability to predict the response of planted sand-binding vegetation to climate change.

Stable isotopes are used in the investigation because they provide integrated information on plant water and nutrient use and serve as powerful tracers in ecosystem studies. The stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) of plant tissues reflects the relations between plant carbon and soil water. The effect of water availability on carbon isotope discrimination during photosynthesis of  $\text{C}_3$  plant is relatively well understood (e.g., Farquhar et al., 1982). For example, photosynthesis depends on water availability, and low water availability can decrease the assimilation of carbon, thereby reducing plant productivity and the corresponding nitrogen requirements or assimilation rates, leading to different patterns of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variation (Yoneyama et al., 2001). Foliar  $\delta^{13}\text{C}$  typically increases when water availability is low, as a result of stomatal closure and reduced transpiration (Peuke et al., 2006). And foliar  $\delta^{13}\text{C}$  is higher in the water-limited treatment relative to the well-watered treatment (Grant et al., 2012). Analyses of  $\delta^{13}\text{C}$  have greatly increased our understanding of the relationships between water and carbon use by desert plants (Ehleringer, 1993). Nitrogen isotope ratio ( $\delta^{15}\text{N}$ ) provides information on ecosystem nitrogen cycling (Högberg et al., 1995; Roggy et al., 1999; Ometto et al., 2006; Bai et al., 2009) and plant nitrogen isotope discrimination is related to the availability of nutrients and water (Högberg, 1997; Swap et al., 2004; Aranibar et al., 2008; Wang et al., 2010b). Previous studies showed that succession affected N cycling and it was reflected in foliar  $\delta^{15}\text{N}$  (Davidson et al., 2007; Wang et al., 2007), and foliar  $\delta^{15}\text{N}$  signatures decreased as successional age increased at both the plant community and species levels (Wang et al., 2007).

Plant  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures are often regulated by two or more factors (BassiriRad et al., 2003; Murphy and Bowman, 2009). For example, Schulze et al. (1991) point out that the significant correlation between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of nitrogen-fixing African trees is caused by decreased water use efficiency, compared with that of the non-nitrogen-fixers. The decreased water use efficiency in nitrogen fixer usually is a result of the extra cost of carbohydrate supply to the nitrogen-fixing diazotroph (Farquhar and Richards, 1984).

Previous research also shows that photosynthetic capacity is strongly correlated with  $\text{C}_3$  plant foliar [N] because photosynthetic enzymes, such as RuBP carboxylase, contain large amounts of nitrogen (Reich et al., 1998; Tognetti and Penuelas, 2003). It is also found that higher photosynthetic activity leads to enriched foliar  $\delta^{13}\text{C}$

values (O'Leary, 1981). Because of these two reasons, positive correlations between foliar  $\delta^{13}\text{C}$  and [N] have been found in many cases (e.g., Sparks and Ehleringer, 1997; Wang et al., 2008). Such relationships together with the positive correlation between foliar  $\delta^{15}\text{N}$  and [N] in non-nitrogen-fixing plants (Hobbie et al., 2000; Wang et al., 2007) suggest that foliar  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and [N] levels are closely linked.

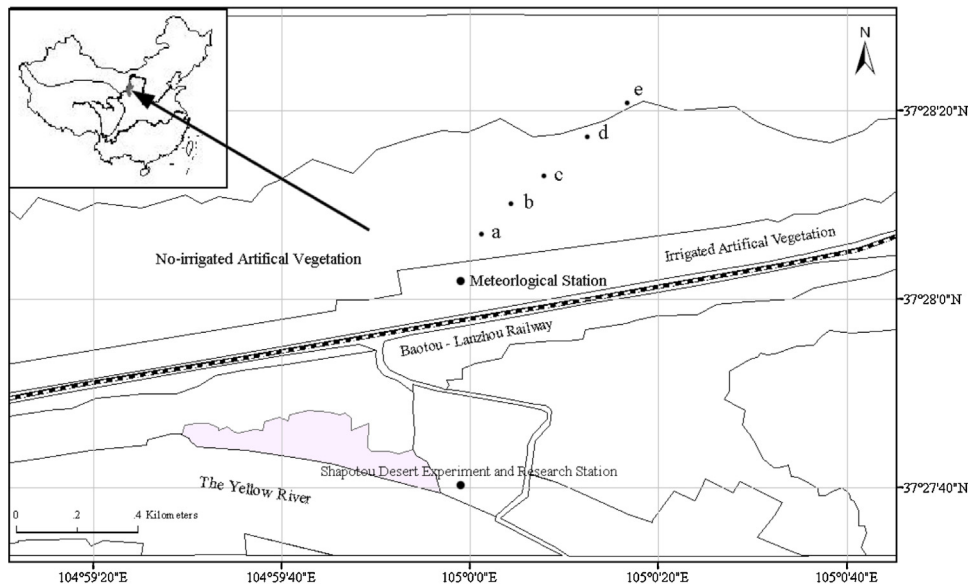
This study is to investigate the interactions of plant nitrogen and water use in planted sand-binding vegetation after long-term succession and to test whether there are functional group (potential nitrogen-fixer and non nitrogen-fixer) dependent adaptation strategies to water and nitrogen variations in the harsh environments. Specifically, the objectives of this study are to investigate (1) the long-term effect (up to 50 years) of successional age on the isotopic signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and [N] of three dominant shrubs in planted sand-binding vegetation communities; (2) the effects of seasonality and short-term drought on variations in isotopic signatures and [N] of each species; and (3) the relationships between  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and [N] of the three shrub species. The results will provide important implications on how plant species adapt to harsh environments in terms of water use efficiency and nutrient use during succession and how plant species respond to environmental changes.

## 2. Materials and methods

### 2.1. Study area

Shapotou region is located at the southeastern border of the Tengger Desert in China (Fig. 1). The region receives high solar radiation and is low in relative humidity. Average annual precipitation is 180.2 mm, with 80% of the rainfall occurring from May to September. The mean annual temperature is 10.0 °C, with a mean January temperature of -6.9 °C and a mean July temperature of 24.3 °C. The water table is more than 80 m below ground, thus rainfall is usually the only source of water for plants.

The planting of sand-fixing vegetation in the mobile dunes of the Shapotou region began in 1956 and subsequent revegetation occurred in 1964, 1981 and 1987, respectively. There was no irrigation or fertilization for these plantings. We calculated the successional age as of the year 2005 (the final year of the sampling), and the four sites selected were 50 year-old, 42 year-old, 25 year-old and 19 year-old for 1956, 1964, 1981 and 1987 planting, respectively. As a result of long-term succession, the mobile dunes were already stabilized at the time of the experiment. Local ecosystems have changed into a grass dominated semi-desert landscape with increasing rates of biological processes and an accumulation of soil organic matter despite the extreme environment (Table 1) (Xiao et al., 2003b). In all planted zones, the three dominant species survived long-term succession were *A. ordosica*, *H. scoparium* and *C. korshinskii*. *A. ordosica* is a succulent, non-nitrogen-fixing semi-shrub xerophyte, and is found in desert and semi-desert biomes. *H. scoparium* and *C. korshinskii* are leguminous xerophytic shrubs and have been reported to have nitrogen fixing activities (Tan et al., 1996; Ma, 2003). The soil physical and chemical properties also improved after revegetation (Table 1) though levels of variation remained high. The vegetated soil profile in the area were composed of a surface biological crust (up to 13.0 mm thick), a transitional layer (up to 50 mm thick) and an original layer of shifting sand (Duan et al., 2003). The biological crust may supply most of the fixed nitrogen to desert plant and soil communities. It may also reduce the loss of nutrient-rich fine soil particles to erosion by binding soil particles together, promoting the formation and increasing the size of soil aggregates.



**Fig. 1.** Study area and sampling sites in the south-eastern margin of the Tengger Desert (a, b, c and d indicate sand-fixing zone planted in 1956, 1964, 1981 and 1987 without irrigation and fertilization).

**Table 1**

Variations of vegetation, soil water and nutrient in the planted sand-binding vegetation at Shapotou. The data were from Ma (2003) and Li et al. (2004).

Year of planting (age)	Vegetation cover (%)	Biomass density (kg 100 m <sup>-2</sup> )	Clay content (%)	Crust thickness (cm)	Soil moisture (%)	Nutrient Content (g kg <sup>-1</sup> )		
						N	P <sub>2</sub> O <sub>5</sub>	Organic matter
1956 (50)	21.6	17.05	11.0	2.5	1.16	1.02	1.59	13.4
1964 (42)	27.7	13.18	6.9	2.2	1.17	0.74	1.50	13.2
1981 (25)	36.4	36.41	4.9	1.4	1.52	0.54	1.51	7.7
1987 (19)	30.0	39.74	–	–	2.03	0.38	1.04	–

## 2.2. Plant sampling

The field sampling took place at the Shapotou Desert Experimental Research Station (37°27.55'N, 105°00.64'E), which is located on the southeastern border of the Tengger Desert. The field sampling was conducted at successional sites of 50 year-old, 42 year-old, 24 year-old and 19 year-old (Fig. 1). At each successional site, we established eight (four for the 19 year-old site due to its low vegetation coverage) 5 m × 5 m plots including different microhabitats such as windward slope, ridge and hollow. Each plot had five to six individuals of *A. ordosica* shrubs and two to three individuals of *C. korshinskii* and *H. scoparium* shrubs, with similar heights and canopy dimensions for each species. At the selected sites, we sampled the mature leaves of *A. ordosica*, *C. korshinskii*, and *H. scoparium* at a monthly interval from the beginning of the growing season in April (except mid-May was used in 2004) to the end of the growing season in September according to the growth seasonality. Specifically, in 2004, the mature leaves were sampled on May 13, May 28, June 30, July 28, August 28 and September 23; and in 2005, the

mature leaves were sampled on April 23, May 28, June 27, July 27, August 27, and September 29, respectively. Each time, 20 leaves or assimilating shoots of each species from each plot were collected between 8 and 10 a.m. All the leaf samples were fully developed and current-year leaves. For each successional age, samples from the same plot were pooled for each species.

## 2.3. Climatic conditions

The De Martonne's (1926) aridity index (AI),  $AI = P/(T + 10)$ , was used to indicate the long-term and short-term climate conditions, where  $P$  was precipitation in mm and  $T$  was temperature in degree C. The mean AI from 1950 to 1988 was 9.2, which indicated that the climate was dry (i.e.,  $AI < 10.0$ ). The climate was dry during the 2004 growing season ( $AI = 3.9$ , 65% of the long-term mean precipitation) and was extremely dry during the 2005 growing season ( $AI = 2.2$ , 42% of the long-term mean precipitation). Climate conditions of our sites during 2004 and 2005 growing seasons were summarized in Table 2.

**Table 2**

Growing season meteorological conditions of the study site during the two sampling years.

	May	June	July	August	September	Growing season	Annual total
2004							
Mean temperature (°C)	19.3	22.0	24.7	22.0	19.0	20.5	–
Rainfall (mm)	2.9	25.5	26.4	48.7	2.1	107.1	116.2
Relative humidity (%)	28.9	41.0	44.3	64.0	46.7	41.5	–
2005							
Mean temperature (°C)	19.3	25.3	25.5	22.5	18.6	20.9	–
Rainfall (mm)	15.6	3.5	14.3	10.7	15.9	61.3	74.9
Relative humidity (%)	34.0	28.0	44.0	53.0	52.0	37.8	–

#### 2.4. Leaf trait analyses

The sampled leaves were wiped carefully with deionized water, and then oven-dried (70 °C, 48 h) and homogenized into a fine powder in a ball mill (Pulverisette 23, Fritsch, Idar-Oberstein, Germany). A 2000–3000 µg sub-sample of the plant tissues was used for the  $\delta^{15}\text{N}$  analysis and a 100–200 µg sub-sample was used for the  $\delta^{13}\text{C}$  analysis. Foliar  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were determined using an elemental analyzer (EA 3000, Euro Vector, Milan, Italy) coupled to a continuous-flow isotope spectrometer (Isoprime, Micromass Ltd., Manchester, UK) at Heihe Key Laboratory of Ecohydrology and River Basin Science, Cold and Arid Regions Environmental and Engineering Research Institute. The standard deviations of repeated measurements using working standards (USGS 24 for  $\delta^{13}\text{C}$  and IAEA-No-3 for  $\delta^{15}\text{N}$ ) were  $\pm 0.2\%$  for  $\delta^{15}\text{N}$  and  $\pm 0.1\%$  for  $\delta^{13}\text{C}$ . All  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were reported based on the international standards (*V-PDB* for  $\delta^{13}\text{C}$  and atmospheric nitrogen for  $\delta^{15}\text{N}$ ).  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1]$ , where  $R$  was the molar ratio ( $^{15}\text{N}:^{14}\text{N}$  or  $^{13}\text{C}:^{12}\text{C}$ , respectively). The dried samples were ground in a stainless steel mill, and wet-digested in concentrated  $\text{H}_2\text{SO}_4$  to determine total N (Li et al., 2010). The [N] was measured using semi-micro Kjeldahl analysis (Bremner and Mulvaney, 1982).

#### 2.5. Statistical analysis

The effects of successional age, sampling year and seasonality on leaf  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and [N] were tested using three-way nested analysis of variance (ANOVA) for each species, with seasonality nested within sampling year. If there were significant interactions, differences in foliar  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and [N] would be compared using two-way ANOVA with successional age and sampling year, or sampling year and seasonality as main factors. If the results of two-way ANOVA were significant, the differences in foliar  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and [N] among species would be compared using one-way ANOVA. The Tukey's honestly significant difference (HSD) post hoc test was used to separate the means. For each species, correlations were conducted between foliar  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ , and [N]. All analyses were performed using Statistica (StatSoft, Inc., Tulsa, OK).

### 3. Results

#### 3.1. Foliar $\delta^{15}\text{N}$ , $\delta^{13}\text{C}$ , and [N] of each species

Foliar  $\delta^{15}\text{N}$  values of *A. ordosica*, *H. scoparium*, and *C. korshinskii* differed significantly ( $p < 0.05$ ), with the most negative value in *A. ordosica* ( $-3.1 \pm 0.1\%$  (mean  $\pm$  SE)) and the most positive value in *C. korshinskii* ( $-1.5 \pm 0.1\%$ ) (Table 3). Foliar  $\delta^{13}\text{C}$  values of *A. ordosica* ( $-24.4 \pm 0.1\%$ ) and *H. scoparium* ( $-24.5 \pm 0.1\%$ ) did not show significant difference, but both values were significantly more negative than that of *C. korshinskii* ( $-23.2 \pm 0.1\%$ ,  $p < 0.05$ ) (Table 3). Foliar [N] of *C. korshinskii* ( $16.1 \pm 0.98 \text{ g kg}^{-1}$ ) was significantly lower than those of *A. ordosica* ( $24.2 \pm 0.36 \text{ g kg}^{-1}$ ) and *H. scoparium* ( $22.7 \pm 0.43 \text{ g kg}^{-1}$ ), which did not differ significantly from each other (Table 3).

**Table 3**

Values of foliar  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ , and nitrogen concentration ([N]) in *Artemisia ordosica*, *Hedysarum scoparium*, and *Caragana korshinskii* in China's Tengger Desert. Values of a parameter followed by different letters differ significantly ( $P < 0.05$ ). Capital letters, boldfaced lowercase letters, and italic lowercase letters represent the differences in  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and [N] among species. SE is the standard error, and  $n$  is the sample size.

	<i>Artemisia ordosica</i>			<i>Hedysarum scoparium</i>			<i>Caragana korshinskii</i>		
	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	[N] ( $\text{g kg}^{-1}$ )	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	[N] ( $\text{g kg}^{-1}$ )	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	[N] ( $\text{g kg}^{-1}$ )
Mean	-3.1A	<b>-24.4a</b>	24.2a	-2.7B	<b>-24.5a</b>	22.7a	-1.5C	<b>-23.2b</b>	16.1b
SE (n)	0.10 (131)	0.08 (131)	0.36 (131)	0.09 (135)	0.09 (135)	0.43 (135)	0.08 (54)	0.14 (54)	0.98 (54)

#### 3.2. Effects of successional age

There were no significant changes in foliar  $\delta^{13}\text{C}$  values of all three species along succession except for a higher (less negative) foliar  $\delta^{13}\text{C}$  value of *H. scoparium* at the 25 year-old site compared to the other successional sites. There were no significant changes in foliar  $\delta^{15}\text{N}$  values and [N] of both leguminous shrubs (*H. scoparium* and *C. korshinskii*) along succession ( $p > 0.05$ , Fig. 2). The  $\delta^{15}\text{N}$  value of *A. ordosica* was the highest at the 50 year-old site in 2004, which was in accordance with the higher foliar [N] at the same period (Fig. 2). The  $\delta^{15}\text{N}$  value of *A. ordosica* was also higher at the 19 year-old site in 2005.

#### 3.3. Effects of short-term rainfall variations

In 2004, foliar  $\delta^{13}\text{C}$  values of all three species were remarkably lower than those in 2005, which was an extremely dry year ( $p < 0.05$ , Table 4). Mean foliar  $\delta^{15}\text{N}$  values did not differ significantly between the two years for all three species (Table 4). The foliar [N] of *C. korshinskii* was significantly lower ( $9.8 \pm 0.6 \text{ g kg}^{-1}$ ) in the extremely dry year than in the dry year ( $23.6 \pm 0.5 \text{ g kg}^{-1}$ ,  $p < 0.05$ ), but foliar [N] of the other species did not differ significantly between the two years (Table 4).

#### 3.4. Effects of seasonality

Foliar  $\delta^{15}\text{N}$  of the three species did not differ seasonally in either year (Fig. 3a, d and g). With the exception of early May, the seasonal differences in foliar  $\delta^{13}\text{C}$  of the three species were also not significant (Fig. 3b, e and h). The effects of seasonality on foliar [N] varied for different species and year combination. In the arid year of 2004, the highest foliar [N] levels of *A. ordosica* were found in mid May, August and September, and the lowest levels were found at the end of July. In the extremely arid year of 2005, foliar [N] of *A. ordosica* was relatively stable before July, with the highest [N] in August and September (Fig. 3c). For *H. scoparium* and *C. korshinskii*, the foliar [N] levels differed significantly by season in both years, and the seasonal patterns were similar. The [N] was the highest at the beginning of the growing season, decreased gradually until the end of the growing season, and then remained relatively constant until late August (Fig. 3f and i).

#### 3.5. Relationships between foliar $\delta^{15}\text{N}$ , $\delta^{13}\text{C}$ , and [N]

Table 5 presents the correlations between foliar  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and [N] of all three species in both sampling years. In both years, the correlations between foliar  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of *A. ordosica* were not significant. Foliar  $\delta^{15}\text{N}$  was significantly correlated with  $\delta^{13}\text{C}$  of both leguminous shrubs (*H. scoparium* and *C. korshinskii*) in both years ( $p < 0.01$ ). The correlations in *C. korshinskii* ( $r = 0.74$  in 2004 and  $0.77$  in 2005) were stronger than those in *H. scoparium* ( $r = 0.31$  in 2004 and  $0.38$  in 2005). Foliar [N] of *A. ordosica* was significantly positively correlated with  $\delta^{15}\text{N}$  in both years ( $r = 0.41$  in 2004 and  $0.28$  in 2005,  $p < 0.01$ ). Foliar  $\delta^{13}\text{C}$  and [N] of *H. scoparium* and *C.*



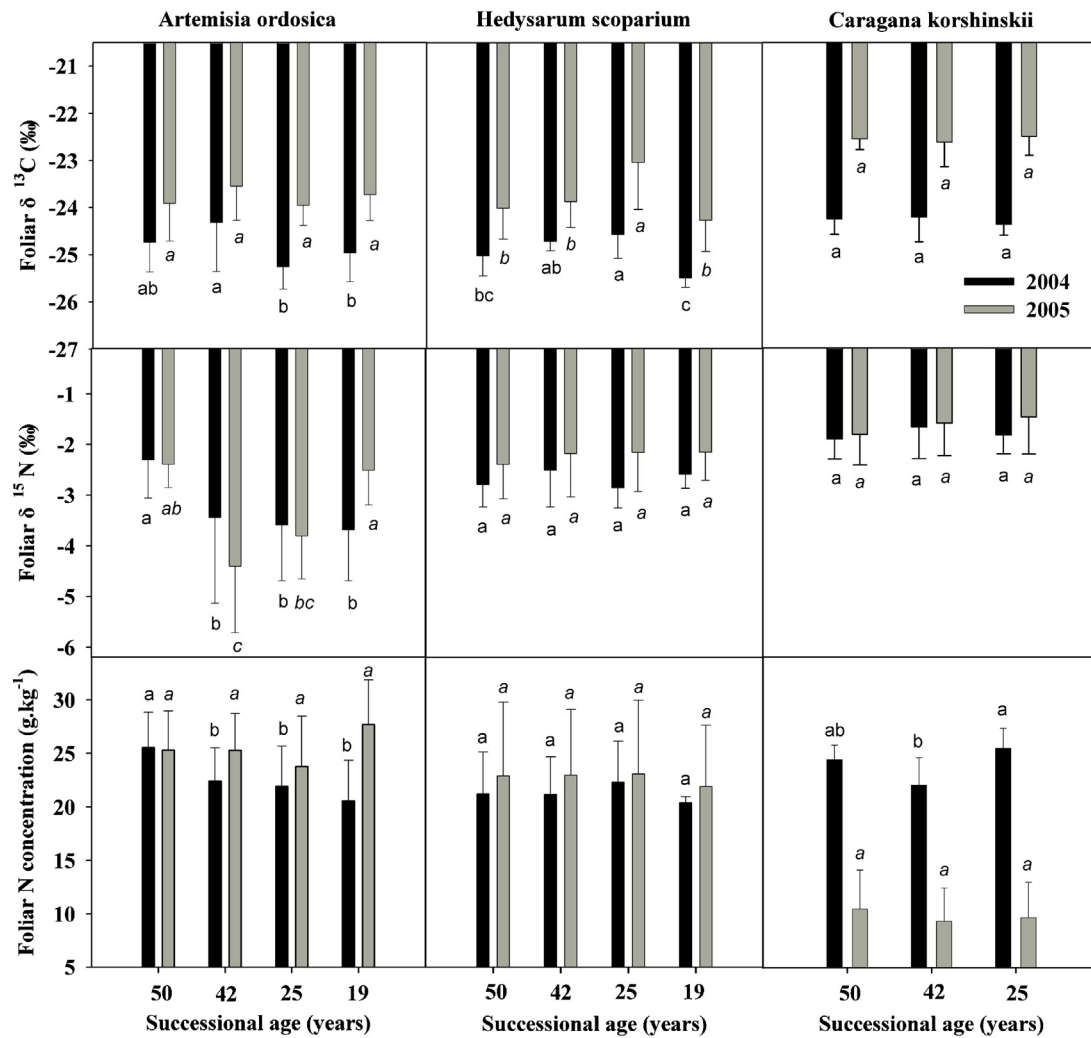


Fig. 2. Effects of successional age on foliar  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and [N] in *Artemisia ordosica*, *Hedysarum scoparium* and *Caragana korshinskii* in the Tengger Desert. Different lowercase and italic lowercase letters indicate significant differences among the successional age in 2004 and 2005, respectively. The error bars represent standard deviations.

Table 4

Values of foliar  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ , and nitrogen concentration ([N]) in *Artemisia ordosica*, *Hedysarum scoparium*, and *Caragana korshinskii* in 2004 and 2005. SE is the standard error. Values of a parameter followed by different letters differ significantly ( $P < 0.05$ ) between the two sampling years. Difference represents the values in 2005 minus those in 2004.

Species	<i>Artemisia ordosica</i>			<i>Hedysarum scoparium</i>			<i>Caragana korshinskii</i>			
		$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	[N] ( $\text{g.kg}^{-1}$ )	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	[N] ( $\text{g.kg}^{-1}$ )	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	[N] ( $\text{g.kg}^{-1}$ )
2004 (dry)	Mean	-2.8 A	-25.0 A	23.4 A	-3.0 A	-25.2 A	22.1 A	-1.7A	-24.3 A	23.6 A
	SE	0.2	0.1	0.5	0.1	0.1	0.4	0.1	0.1	0.5
2005 (extremely dry)	Mean	-3.3 A	-24.0 B	24.9 A	-2.4 A	-24.1 B	23.2 A	-1.4 A	-22.5 B	9.8 B
	SE	0.1	0.1	0.5	0.1	0.1	0.7	0.1	0.1	0.6
Difference		-0.5	1.0	1.5	0.6	1.1	1.1	0.3	1.8	-13.7

*korshinskii* were only positively correlated in the extremely arid year ( $r = 0.39$  for *H. scoparium* and  $0.49$  for *C. korshinskii*,  $p < 0.01$ ).

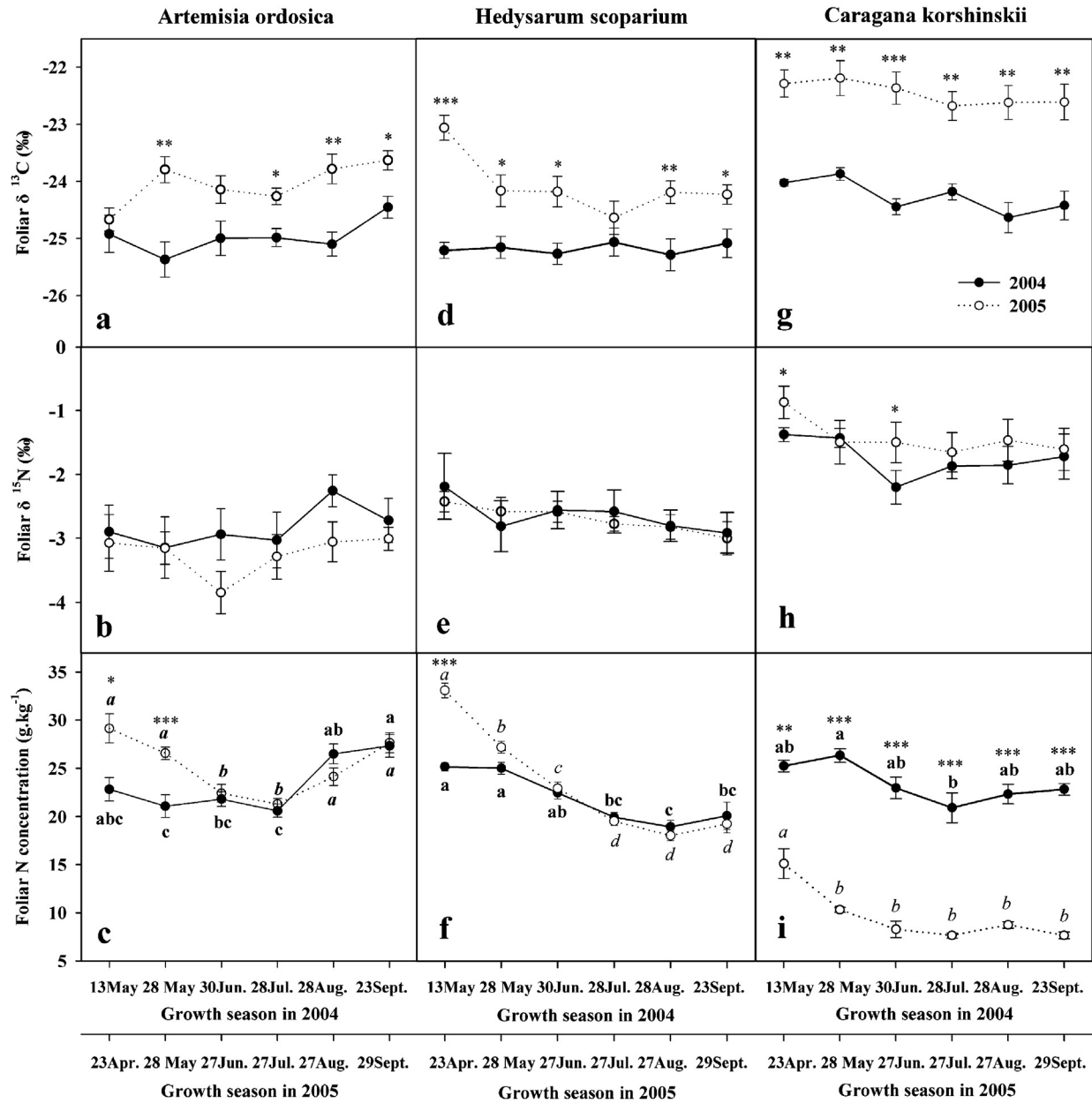
#### 4. Discussion

##### 4.1. Variations in three plant species

Foliar  $\delta^{13}\text{C}$  is an indicator of long-term plant water use efficiency (Farquhar et al., 1989). Our results showed that the  $\delta^{13}\text{C}$  values (ranging from  $-21.1$  to  $-26.6\%$ ) of the dominant shrubs in the Tengger Desert (a cold desert) were more positive than the values reported for other desert  $\text{C}_3$  plants, which ranged

from  $-21.0$  to  $-28.0\%$  in the northern Namaqualand Desert of South Africa (Rundel et al., 1999), from  $-22.0$  to  $-29.0\%$  in the Arizona deserts of the United States (Ehleringer and Cooper, 1988) and from  $-24.8$  to  $-30.2\%$  in the Kalahari, southern Africa (Wang et al., 2010b).

The three shrubs differed significantly in  $\delta^{15}\text{N}$  values (Table 3), with *C. korshinskii* being significantly less negative ( $-1.5\%$ ), and *A. ordosica* being more negative ( $-3.1\%$ ). The  $\delta^{15}\text{N}$  values of nitrogen-fixing plants have been shown to approximate atmospheric  $\delta^{15}\text{N}$  value ( $0\%$ ) (Shearer and Kohl, 1986; Dawson et al., 2002). Less negative  $\delta^{15}\text{N}$  values (close to  $0\%$ ) of *C. korshinskii* suggest that its main nitrogen source is the fixation of atmospheric nitrogen.



**Fig. 3.** The effects of seasonality on the foliar  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and [N] in *Artemisia ordosica* (a, b and c), *Hedysarum scoparium* (d, e and f) and *Caragana korshinskii* (g, h and i) in 2004 and 2005. Different boldfaced lowercase letters indicate there are significant differences among the seasons in 2004, and different italic lowercase letters indicate there are significant differences among the seasons in 2005. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  indicate that there are significant differences between two years.

*C. korshinskii* and *H. scoparium* are both leguminous shrubs, but the  $\delta^{15}\text{N}$  values of *H. scoparium* were significantly lower ( $-2.7\text{‰}$ ) (Table 3), which indicates that its nitrogen-fixing activity is low or it uses alternative nitrogen sources. Golluscio et al. (2006) reported that some leguminous shrubs with the presence of nodules did not appear to be actively fixing nitrogen in arid ecosystems, but nitrogen fixation can occur in some favorable years. Similar observations are reported for southern African ecosystems where foliar  $\delta^{15}\text{N}$  values of some leguminous *Acacia* trees reach 8‰ (Wang et al., 2010b), indicating minimum nitrogen fixing activities in normal years (vs. wet years). Because atmospheric nitrogen tends to have more negative  $\delta^{15}\text{N}$  values, more negative  $\delta^{15}\text{N}$  values in *A. ordosica* suggest that this shrub may obtain a large amount of its nitrogen from precipitation and deposition (Skinner et al., 2004).

#### 4.2. Effect of successional age on leaf isotopic characteristics

The relatively constant foliar [N] and  $\delta^{13}\text{C}$  values of the three species along succession indicate that these plant species are able to regulate their nitrogen and water use in response to environmental conditions from as early as 19 years after succession (the youngest successional stage in this study). The stable nitrogen uptake and assimilation along succession are further supported by the constant foliar  $\delta^{15}\text{N}$  values of both leguminous shrubs (*H. scoparium* and *C. korshinskii*). In addition to the isotope data, soil water capacity data also provide support for a stable stage after 19 years of succession (Fig. 4). It clearly showed that there was a significant decrease in soil water capacity until 19 years and after that the soil water capacity became stable. This provides a strong and alternative evidence for the conclusion of stable succession after 19 years.

**Table 5**

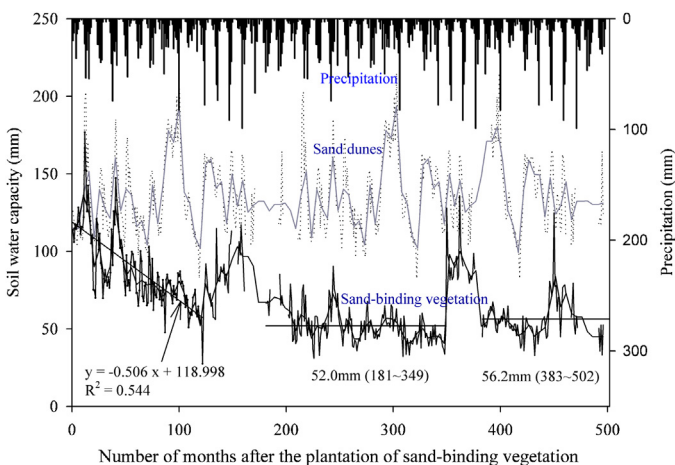
Correlation coefficients of the foliar [N] ( $\text{g kg}^{-1}$ ),  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰) in *Artemisia ordosica*, *Hedysarum scoparium* and *Caragana korshinskii* in 2004 and 2005.

		2004			2005		
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	[N]	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	[N]
<i>Artemisia ordosica</i>							
2004	$\delta^{13}\text{C}$	1.0					
	$\delta^{15}\text{N}$	0.21	1.0				
	[N]	0.25	0.41***	1.0			
2005	$\delta^{13}\text{C}$	0.70***	-0.003	0.02	1.0		
	$\delta^{15}\text{N}$	0.11	0.54**	0.24	0.03	1.0	
	[N]	0.15	0.08	0.25	0.16	0.28**	1.0
<i>Hedysarum scoparium</i>							
2004	$\delta^{13}\text{C}$	1.0					
	$\delta^{15}\text{N}$	0.31*	1.0				
	[N]	-0.19	0.10	1.0			
2005	$\delta^{13}\text{C}$	0.47**	0.08	0.13	1.0		
	$\delta^{15}\text{N}$	0.10	0.20	-0.03	0.38**	1.0	
	[N]	0.006	0.13	0.53**	0.39***	0.22	1.0
<i>Caragana korshinskii</i>							
2004	$\delta^{13}\text{C}$	1.0					
	$\delta^{15}\text{N}$	0.74***	1.0				
	[N]	0.17	0.20	1.0			
2005	$\delta^{13}\text{C}$	0.58**	0.57**	0.21	1.0		
	$\delta^{15}\text{N}$	0.64**	0.60**	0.32	0.77***	1.0	
	[N]	0.36	0.12	0.54**	0.49**	0.29	1.0

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

\*\*\*  $p < 0.001$ .



**Fig. 4.** The monthly precipitation (the bars on the top) and soil water capacity of top three meters in the planted site (the solid line) and sand dunes (the dashed line). The x-axis represents the number of months after the plantation of sand-binding vegetation. The thicker solid lines are the six-month running averages of the vegetated and sand dune data. The linear regression equations and average soil water capacity values of three distinct periods were also reported for the vegetated site. This figure is modified based on the data from Xiao et al. (2004).

There was no clear decreasing trend in foliar  $\delta^{15}\text{N}$  during succession as found in secondary successional ecosystems under a temperate climate (Wang et al., 2007), indicating that the openness (e.g., the ratio between external loss and internal cycling) of the ecosystem nitrogen cycle does not change along the succession. For *A. ordosica*, there was higher foliar  $\delta^{15}\text{N}$  value at the 50 year-old site in 2004, which is linked to higher soil [N] (Table 2) and foliar [N] (Fig. 2) at this successional age. The higher foliar  $\delta^{15}\text{N}$  value at the

19 year-old site in 2005 is unexpected and the cause is inconclusive, but probably related to local conditions (e.g., microhabitat).

#### 4.3. Effect of interannual rainfall and seasonality on leaf isotopic characteristics

A large body of literature has reported negative correlations between plants  $\delta^{13}\text{C}$  values and the availability of water (e.g., precipitation) (Miller et al., 2001; Van de Water et al., 2002; Swap et al., 2004; Murphy and Bowman, 2009; Wang et al., 2010b). In our study, foliar  $\delta^{13}\text{C}$  values of the three shrubs in the extremely arid year of 2005 (61.3 mm precipitation during the growing season) were significantly more positive than those in the arid year of 2004 (107.1 mm precipitation during the growing season). This may be resulted from decreased stomatal and mesophyll conductance under extremely arid conditions, which in turn increased water use efficiency (e.g., Choi et al., 2005). The difference in the  $\delta^{13}\text{C}$  values of *C. korshinskii* between years was 1.8‰, which was greater than those of *A. ordosica* (1.0‰) and *H. scoparium* (1.1‰) (Table 4). Higher foliar  $\delta^{13}\text{C}$  value of *C. korshinskii* and its higher sensitivity to rainfall variation indicate that *C. korshinskii* is more sensitive to water availability than the other two species. Overall, with the exception of *H. scoparium* in 2005, there was no significant variation in  $\delta^{13}\text{C}$  of the three shrubs during the growing season (Fig. 3b, e and h). This suggests that seasonal differences in this dry and hot environment have a relatively minor effect on the photosynthetic capacity and stomatal conductance of these species.

Foliar  $\delta^{15}\text{N}$  values did not vary much between the dry and the extremely dry year (Fig. 3). These two years were different in rainfall (116.2 mm in 2004 and 74.9 mm in 2005). The constant annual foliar  $\delta^{15}\text{N}$  values suggest that water availability does not play an important role in nitrogen cycles and/or plant uptake in these two years. This conclusion is different from many earlier studies which demonstrate strong rainfall/soil moisture controls on soil nitrogen cycle in water-limited systems (e.g., Wang et al., 2009). The likely reason for this discrepancy is that both years were drought years (65% of the mean annual precipitation in 2004 and 42% in 2005), and the effect of soil moisture on nitrogen cycle (e.g., plant uptake) diminished significantly. The relatively constant seasonal patterns in  $\delta^{15}\text{N}$  in both years support the basic assumptions that seasonality does not affect plant foliar  $\delta^{15}\text{N}$  (Garten, 1993; Swap et al., 2004; Ometto et al., 2006).

Many studies have shown that a large proportion of the foliar nitrogen is incorporated into photosynthesis-related enzymes (Evans, 1989), and the leaves with higher leaf nitrogen concentrations have higher photosynthetic rates (Field and Mooney, 1986). The foliar [N] of *A. ordosica* and *H. scoparium* did not change between the dry and extreme dry years, indicating either nitrogen is not limiting or water limitation effect suppresses nitrogen effect for these two species. The foliar [N] of *C. korshinskii* was significantly lower than in 2004 and was significantly lower than foliar [N] of the other two species (Fig. 3c, f and i, Table 4). The lower foliar [N] of *C. korshinskii* during the extremely dry year may be related to less active nitrogen fixation and assimilation, indicating the *C. korshinskii* nitrogen uptake is less sensitive to water availability than other two species and only the extreme arid year affects its nitrogen uptake. Under such conditions, high energy cost of nitrogen fixation limits the potential of nitrogen fixation in natural ecosystems when photosynthesis decreases under water stress (Vitousek and Howarth, 1991). The seasonal patterns of foliar [N] of all three species varied remarkably during the growing season (Fig. 3c, f, and i). Foliar [N] of *A. ordosica* decreased from May to July reaching approximately the initial level. The decreasing trend is probably due to the dilution effects which resulted from low nitrogen assimilation capacity under drought and high temperature as

growing season develops. Foliar [N] of *H. scoparium* and *C. korshinskii* decreased throughout the growing season, probably resulting from the decreased nitrogen-fixing activity.

#### 4.4. Relationships of leaf characteristics

Foliar  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values are often correlated through their links with the growth and nutrition of plants, and with carbon and nitrogen mineralization processes (e.g., Perroni-Venture et al., 2009). The correlations between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the two leguminous shrubs (*H. scoparium* and *C. korshinskii*) were significant and positive in both years (Table 5). These results suggest that carbon and nitrogen isotope discrimination of these plants are regulated by similar factors and that carbon and nitrogen cycling are connected.

Foliar  $\delta^{13}\text{C}$  is affected by both internal factors (e.g., foliar [N]) and external factors (e.g., water availability). Sparks and Ehleringer (1997) suggest that the increase in  $\delta^{13}\text{C}$  value with increasing foliar [N] reflects a positive effect of high nitrogen availability on photosynthetic rate. Many other studies have reported that nitrogen fertilization tends to increase  $\delta^{13}\text{C}$  by lowering the internal to ambient  $\text{CO}_2$  concentration ratio ( $C_i/C_a$ ) mainly through enhanced carboxylation efficiency (Hogberg and Alexander, 1995; Livingston et al., 1999; Choi et al., 2005). This phenomenon is particularly obvious for seedlings or natural stands where nitrogen is the primary limiting factor (Hogberg and Alexander, 1995; Livingston et al., 1999; Choi et al., 2005). In our study, positive correlation between [N] and  $\delta^{13}\text{C}$  was only found in *C. korshinskii* and *H. scoparium* in the extremely arid year ( $r=0.49$  and  $0.39$ , respectively, Table 5). It is possible that the two leguminous shrubs reduce nitrogen-fixing activity during the extremely arid year and nitrogen starts to limit the photosynthetic rates. This is particularly evident for *C. korshinskii* as its foliar [N] was much lower in the extremely arid year (Table 4). Lower photosynthetic capacity causes more discrimination against  $^{13}\text{C}$  and results in lower  $\delta^{13}\text{C}$ . At the same time, foliar  $\delta^{13}\text{C}$  was higher in the extremely dry year for all three species (Table 4), contradicting the foliar [N] effect since lower foliar [N] supposedly results in lower  $\delta^{13}\text{C}$  values. The higher foliar  $\delta^{13}\text{C}$  in the extremely dry year corroborates with the water effect on foliar  $\delta^{13}\text{C}$ , indicating that water availability is the predominant factor in this system and the water effect suppresses the nitrogen effect.

There were significant positive relationships between  $\delta^{15}\text{N}$  and [N] of *A. ordosica*. This pattern has been widely reported for non-nitrogen-fixing plants (Vitousek et al., 1989; Garten and Van Miegruet, 1994; Johannisson and Högberg, 1994; Högberg et al., 1995; Hobbie et al., 2000; Wang et al., 2007). Hobbie et al. (2000) suggest that the positive correlations reflect the changes in the proportion of nitrogen obtained through mycorrhizae. The mycorrhizae association status of the studied shrubs was not certain, but presumably the association was weak. We think that the variation in soil nitrogen availability (e.g., Table 2) is the major cause of the observed correlations between  $\delta^{15}\text{N}$  and [N], which is similar to what happens during secondary succession in northern Virginia (Wang et al., 2007).

## 5. Summary

To better understand the long-term succession and short-term climate variability effects on the functions of three major sand-binding species (*A. ordosica*, *H. scoparium* and *C. korshinskii*), we examined the effects of succession, rainfall variability, and seasonality on foliar  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and [N]. The study has both theoretical and applied importance in the restoration of degraded lands and

in improving our ability to predict the response of planted sand-binding vegetation to climate change. The results showed that,

- (1) Long-term succession did not significantly alter  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and [N] levels of the three species in general. The study indicates that these sand-binding ecosystems have developed into a stable stage. The relatively constant foliar  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and [N] levels of the three species along succession indicate that these species are able to regulate their nitrogen and water use in response to environmental conditions.
- (2) Short-term rainfall variability, however, significantly increased foliar  $\delta^{13}\text{C}$  of all three species by 1.0–1.8%, indicating higher water use efficiency during a more severe drought. The results indicate that short-term drought significantly affects the plant functions.
- (3) No significant seasonal patterns were found in foliar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the three species. However, foliar [N] levels varied by season and differed between leguminous and non-leguminous species.
- (4) The correlations between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were significant and positive for two leguminous shrubs (*H. scoparium* and *C. korshinskii*) in both years. A positive correlation between [N] and  $\delta^{13}\text{C}$  was found in both species only in the extremely arid year. These results suggest that carbon and nitrogen isotope discrimination in these leguminous plants are controlled by similar factors and leguminous shrubs might be limited by soil nitrogen during the extremely dry year.
- (5) There are functional type (leguminous shrubs vs. non-leguminous shrubs) dependent adaptation strategies to the extreme environments. Interestingly, the functions of *H. scoparium*, a leguminous shrub share similarities with both leguminous *C. korshinskii* and non-leguminous *A. ordosica*. For example, in terms of its water and nitrogen use responses to inter-annual rainfall variability, *H. scoparium* is similar to *A. ordosica* indicated by their similar responses in foliar  $\delta^{13}\text{C}$  and foliar [N]. In terms of its nitrogen and water relationship within a growing season, *H. scoparium* is similar to *C. korshinskii* indicated by similar foliar  $\delta^{13}\text{C}$ -[N] and foliar  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  relationships of these two species.

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