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Responses of leaf δ¹³C and leaf traits to precipitation and temperature in arid ecosystem of northwestern China

Respuestas del δ¹³C foliar y características foliares a la precipitación y temperatura en un ecosistema árido del noroeste de China

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Abstract. Leaf δ¹³C is widely used to explain plant strategies related to resource availability in different environments. However, the coupled response of leaf δ¹³C to precipitation and temperature as well as the relationship between leaf δ¹³C and leaf traits remain unclear. The leaf δ¹³C and its relationship with leaf traits [leaf size (LS), leaf length (LL), leaf width (LW), leaf length to width ratio (L:W), specific leaf area (SLA) and mass-based leaf nitrogen concentration (N_{mass})] were investigated on the dominant shrub species *Nitraria tangutorum* Bobr. in the arid region (Dengkou and Minqin) of northwestern China under the simulated increasing precipitation (PGS) and ambient temperature (TGS) in plant growing season from 2008 to 2010. Our results showed that LS, LW, LL, SLA and Nmass significantly increased with increasing PGS, but had decreasing tendencies with increasing TGS. However, the majority of the negative relationships between leaf traits and TGS were not obvious in Minqin. At the two study sites, L:W increased simultaneously with increasing PGS and TGS. There was a shift in the negative leaf δ¹³C-PGS relationship across Minqin and Dengkou, which lead to the lacking effects of precipitation on leaf δ¹³C across the two sites, and higher leaf δ¹³C at lower precipitation in Minqin. Across Minqin and Dengkou, PGS could only explain 14% of the variation in leaf δ¹³C. The combination of PGS and TGS could explain 64% of the variation in leaf δ¹³C. Leaf traits (LW and L:W) further improved the estimation of leaf δ¹³C. The combinations of PGS, TGS, LW and L:W could explain 84% of the variation in leaf δ¹³C. Our study demonstrated the importance of leaf traits in exploring the responses of leaf δ¹³C to global changes in arid ecosystems.

Keywords: Leaf traits; Leaf δ¹³C; Water use efficiency; Climate change; Arid ecosystem.

Resumen. El δ¹³C foliar es ampliamente usado para explicar estrategias relacionadas con la disponibilidad de recursos en diferentes ambientes. Sin embargo, la respuesta conjunta del δ¹³C foliar a la precipitación y temperatura así como la relación entre el δ¹³C foliar y las características foliares no están claras. El δ¹³C foliar y su relación con las características foliares [tamaño de hoja (LS), longitud foliar (LL), ancho foliar (LW), relación entre la longitud y el ancho foliar (L:W), área foliar específica (SLA) y concentración de N foliar (en una base de peso seco) (Nmass)] fueron investigadas en la especie de arbusto dominante *Nitraria tangutorum* Bobr en la región árida (Dengkou y Minqin) del noroeste de China. El estudio se efectuó bajo condiciones de varias cantidades de precipitación simuladas (PGS) y temperaturas ambientales (TGS) en las estaciones de crecimiento de 2008, 2009 y 2010. Los resultados mostraron que LS, LW, LL, SLA y N_{mass} se incrementaron significativamente cuando las cantidades de PGS se incrementaron, pero hubo tendencias de reducción en dichas características cuando las TGS aumentaron. Sin embargo, la mayoría de las relaciones negativas entre las características foliares y las TGS no fueron obvias en Minqin. En ambos sitios, L:W se incrementó cuando las PGS y TGS aumentaron. Hubo un cambio en la relación negativa entre el δ¹³C foliar-PGS a través de Minqin y Dengkou, lo cual condujo a la falta de efectos de la precipitación en el δ¹³C foliar a través de ambos sitios, y mayor δ¹³C foliar a menor precipitación en Minqin. A través de Minqin y Dengkou, PGS solo pudo explicar un 14% de la variación en el δ¹³C foliar. La combinación de PGS y TGS pudo explicar un 64% de la variación en el δ¹³C foliar. Las características foliares (LW y L:W) mejoraron aún más la estimación del δ¹³C foliar. Las combinaciones de PGS, TGS, LW y L:W pudieron explicar un 84% de la variación en el δ¹³C foliar. Nuestro estudio demostró la importancia de las características foliares en explorar las respuestas del δ¹³C foliar a cambios globales en ecosistemas áridos.

Palabras clave: Características foliares; δ¹³C foliar; Eficiencia de uso del agua; Cambio climático; Ecosistema árido.

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INTRODUCTION

Leaf δ13C (or leaf 13C discrimination), as a pivotal index in evaluating leaf water use efficiency, is widely used to explain plant strategies related to resource availability in different environments and to understand past changes in climate (Bonal et al., 2011; Mardegan et al., 2011; Sheng et al., 2011; Sarris et al., 2013). Generally, as the major factor influencing leaf δ13C, precipitation has strong negative impact on leaf δ13C at regional and global scales (Sheng et al., 2011; Pflug et al., 2015). However, the effects of precipitation on leaf δ13C are unclear in some regions, the negative relationship disappeared or even reversed in some arid ecosystems, such as those in Southwestern Australia (Schulze et al., 2006), Northern Australia (Miller et al., 2001), Eastern Asiatic Temperate Zone (Su et al., 2000; Wei et al., 2011) and tropical southern Africa (Bragg et al., 2013). Furthermore, understanding the temporal and spatial pattern of precipitation in the plant growing season can promote a more accurate evaluation of the change of leaf δ13C along precipitation gradient (Diefendorf et al., 2010).

In most cases, the effect of precipitation on leaf δ13C is often cross-correlated with other environmental factors, such as temperature (Wang & Schjoerring, 2012; Sharp et al., 2013). High temperature can increase soil evaporation and reduce precipitation availability for plants, especially in arid regions where the coverage of vegetation is universally low. Simultaneously, temperature can adjust leaf water use and photosynthetic physiology (Leon-Sanchez et al., 2016; Yun et al., 2016; Li et al., 2017). Increasing temperatures may conduce to higher leaf transpiration and respiration rates, lower water use efficiencies and uncertain photosynthetic rates because optimal photosynthesis temperature is variable for different plants or conditions (Sheng et al., 2011; Li et al., 2017). However, the coupled response of leaf δ13C to both precipitation and temperature has not been fully explored, especially in arid ecosystems with water stress and high temperature.

Furthermore, precipitation and temperature can indirectly control leaf δ13C by their directly affecting leaf traits, such as leaf size, leaf width, ratio of leaf length to width, specific leaf area, and leaf nitrogen concentration. Generally, plants under high water availability have large leaf size, leaf length, leaf width, high specific leaf area and low mass-based leaf nitrogen concentration. Instead, plants in arid environments, especially combined high temperature, generally have small leaf size, leaf width, low specific leaf area and high mass (or area)-based leaf nitrogen concentration, which usually induce high leaf water use efficiencies (Dudley, 1996; Lambers et al., 1998; Picotte et al., 2007; Leon-Sanchez et al., 2016; Liu et al., 2017). Large leaf size can synchronously increase carbon capture and transpiration area. Leaves with low specific leaf area are usually thin, which can conduce to higher transpiration rate because of increasing mesophyll conductances. Low leaf nitrogen concentration can reduce carbon dioxide assimilation. Accordingly, the plasticity of leaf traits in moist environment will induce low leaf water use efficiencies (leaf δ13C) (Parkhurst & Loucks, 1972; Lambers et al., 1998). In contrast, small leaf size and width can decrease leaf temperature because of thinner leaf boundary layer thickness (Smith & Geller, 1980; Lambers et al., 1998; Westoby et al., 2002; McDonald et al., 2003). This is important for plants under arid and high radiation conditions because it is a physical process which does not consume water (Yates et al., 2010). However, the relationship between leaf δ13C and leaf traits is still unclear up to date. Understanding the above relationship is essential to explore the water use efficiency and adaptive strategies of plants in arid regions.

In the present study, we collected the data of leaf δ13C and leaf traits of *Nitraria tangutorum* Bobr. (*N. tangutorum*) under different precipitation addition treatments as well as climatic variables (total precipitation and mean temperature in the growing seasons) at two study sites (Dengkou and Minqin) in northwestern China from 2008 to 2010. This study aimed to (1) examine the plasticity of leaf traits along precipitation and temperature gradients in arid systems to examine the effects of leaf traits on leaf δ13C, (2) test the coupled response of leaf δ13C to precipitation, temperature and leaf traits to explore a more accurate way for estimating leaf δ13C in arid ecosystems.

MATERIALS AND METHODS

Study sites. Two study sites were located nearby the (1) Experimental Center of Desert Forestry, Chinese Academy of Forestry, Dengkou County, Inner Mongolia (106° 43′ E, 40° 24′ N) and (2) Minqin Desert Control Research Station, Minqin County, Gansu Province (102° 58′ E, 38° 34′ N) in northwestern China (Fig. 1). The mean annual precipitation and mean annual temperature of Dengkou and Minqin are 145 mm and 115 mm, and 7.6 °C and 8.3 °C, respectively. During the period of 2008-2010, the annual precipitation and the precipitation during the plant growing season (from April to September) were 99.0-129.4 mm and 91.8-124.5 mm, respectively, in Dengkou, and 56.0-119.8 mm and 53.4-110.4 mm, respectively, in Minqin. Meanwhile, and from 2008 and 2010, the mean annual temperature and the mean temperature in the plant growing season were 8.0-9.3 °C and 19.5-20.6 °C, respectively, in Dengkou, and 8.0-9.1 °C and 18.6-19.3 °C, respectively, in Minqin (Table 1).

The dominant geomorphological types in the two study sites are fixed- and semi-fixed sand dunes, where vegetation coverage is about 30%. *N. tangutorum* is the dominant species in the two sites. Its leaf shape is broadly oblanceolate, 1.8-3.0 cm long, 0.6-0.8 cm wide. The margins of most leaves are entire. Some leaves have 2-3 teeth on the apex. *Nitraria. tangutorum* can fix sand and form shrub sand dunes. The diameter and height of most shrub sand dunes are 1.0-6.0 m and 0.5-2.0 m, respectively. *N. tangutorum* is generally less than 0.5 m on the dune. This species has obvious two root layers. One is

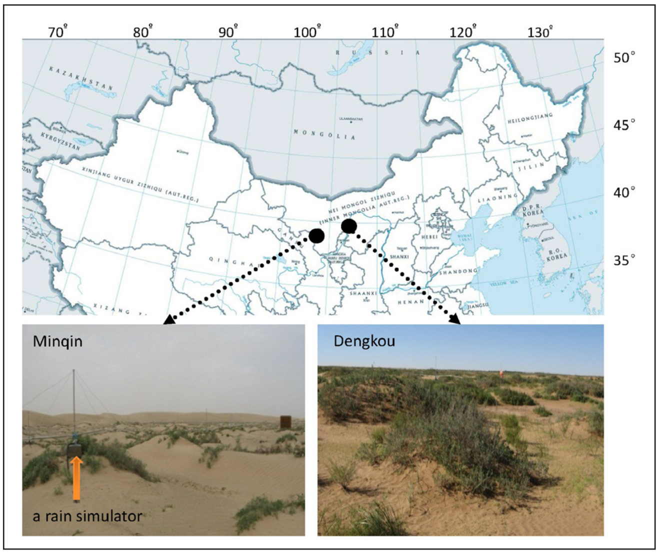

Fig. 1. Study sites at Minqin and Dengkou in northwestern China.
Fig. 1. Sitios de estudio en Minqin y Dengkou en el noroeste de China.

Table 1. Climatic data in Dengkou and Minqin from 2008 to 2010. P: annual precipitation; PGS: annual precipitation in the growing season (from April to September); T: mean annual temperature; TGS: mean annual temperature during the growing season (from April to September).
Tabla 1. Datos climáticos en Dengkou y Minqin desde 2008 a 2010. P: precipitación anual; PGS: precipitación anual en la estación de crecimiento (desde Abril a Septiembre); T: temperatura anual promedio; TGS: temperatura anual promedio durante la estación de crecimiento (desde Abril a Septiembre).

Year	Minqin		Dengkou	
	P/PGS (mm)	T/TGS (ºC)	P/PGS (mm)	T/TGS (ºC)
2008	119.8/110.4	8.0/19.1	129.4/124.5	8.0/19.5
2009	56.0/53.4	9.0/19.3	99.0/91.8	9.3/20.6
2010	85.4/74.4	9.1/18.6	103.6/96.7	9.1/20.0

under the sand dune and the other one is in the sand dune. There is a firm clay layer at 100-200 cm depth under the sand dune at the two sites, which can limit root growth to deeper soil layers. The depth of groundwater is more than 6 m at the two sites.

Experimental design. A rainfall simulator was applied for the precipitation addition treatments (Fig. S1c). The simulator was not only versatile enough to simulate various rainfall events by driving water pressure, but also more simple and lightweight. Field test data showed that the rainfall validity of the rainfall simulator was about 66% (Fig. S1a, b). The distribution of simulated rainfall was higlyh uniform as long as the hydraulic pressure exceeded 1.4 KPa, and the wind speed was below 2.5 m/s (Fig. S2), which could be achieved easily in most cases in semiarid or arid regions.

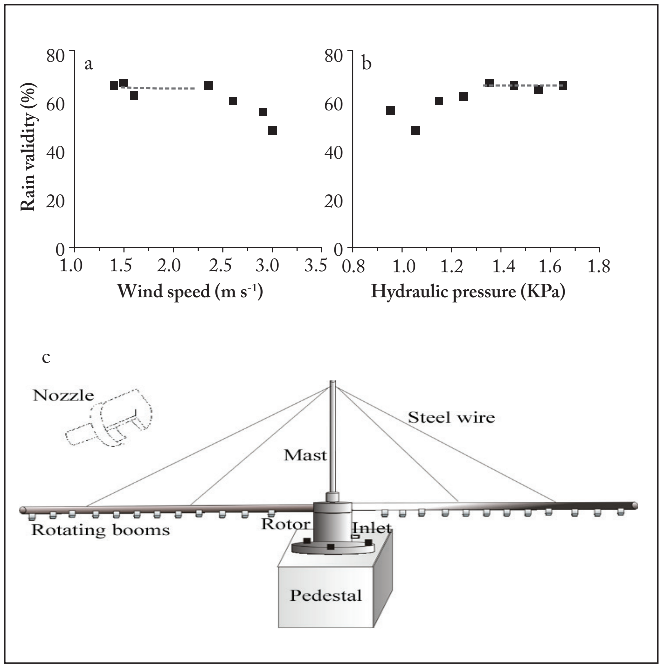

Fig. S1. A sketch map for the rain simulator (c) and its performance. Test data showed that the rain validity (ratio of observed rainfall to expected rainfall) changed with wind speed (a) and hydraulic pressure (b).
Fig. S1. Diagrama del simulador de lluvia (c) y su performance. Datos de pruebas mostraron que la validez de la lluvia (relación de la lluvia observada a la lluvia esperada) cambió con la velocidad del viento (a) y la presión hidráulica (a).

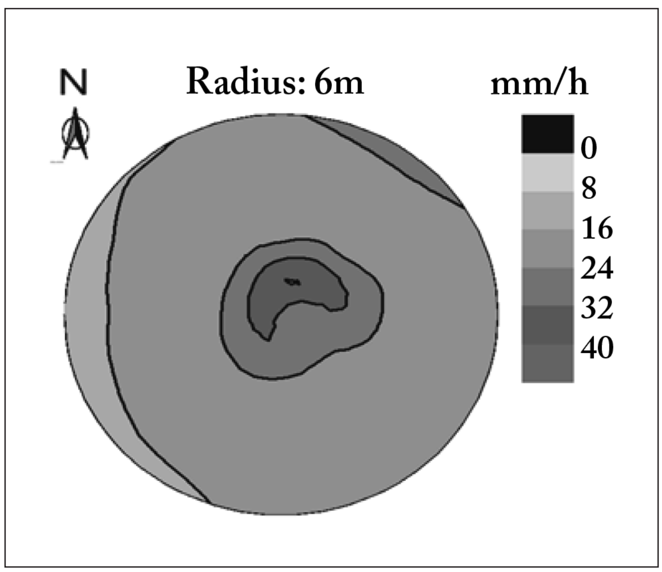

Fig. S2. Rainfall distribution in an experimental plot at hydraulic pressure 1.4-1.5 KPa and wind speed 2.1 m/s (south-west wind) within one hour.
Fig. S2. Distribución de la lluvia en una parcela experimental con una presión hidráulica de 1,4-1,5 KPa y velocidad del viento de 2,1 m/s (viento del sudoeste) durante una hora.

It is predicted that in the future the increase rate of mean annual precipitation and temperature will be about 0-10 mm and 0.25-1.0 °C, respectively, every 10 years at the northern part of northwestern China (Qian & Lin, 2005). Based on this, five precipitation addition treatments and four replicates for each treatment (113 m^2 per plot, 20 plots in total) were designed. The precipitation addition gradients were 0 mm (0%), 7.3 mm (25%), 14.5 mm (50%), 21.8 mm (75%) and 29 mm (100%) in Dengkou, and 0 mm (0%), 5.8 mm (25%), 11.5 mm (50%), 17.3 mm (75%) and 23.0 mm (100%) in Minqin. This could represent the maximal additional precipitation from about 20 to 100 years in local sites in the future. Based on the temporal distribution pattern of precipitation in the two sites, where the ratio of growing season (from April to September) to annual precipitation is more than 80%, the simulated precipitation was conducted from May (mid-spring) to September (late summer)(i.e., the major growing season) from 2008 to 2010. In each month, the set precipitations were added to each plot. For example, in Dengkou, the maximal additional precipitation (29 mm)×5 times was equal to the annual mean precipitation (145 mm).

Leaf trait measurements. In late September of each year (2008-2010), 150-300 unbroken, mature leaves were randomly collected from each plot. Leaves of each plot were placed in an envelope, labeled, scanned with 300 DPI (Uniscan A688, Unisplendour Corporation Limited, China), dried at 75 °C for 48 hours and weighed. Leaf size, leaf length and leaf width of each leaf were estimated with Image-pro Plus 6.0 (Media Cybernetics, USA). Thereafter, mean leaf size (LS), leaf width (LW), leaf length (LL), leaf length to width (L:W) ratio and specific leaf area (SLA) of each treatment were calculated. The mass-based leaf nitrogen concentration (N_{mass}) was analyzed by the micro-kjeldahl method.

Leaf δ^{13}C measurement. At least 2 g leaf sample from a subsample of each plot were chosen for the analysis of leaf δ^{13}C in the Stable Isotope Laboratory (Thermo Delta V advantage) of the Chinese Academy of Forestry, Beijing, China.

Climatic variables collection. Because precipitation heterogeneity is high in arid regions, an automatic meteorological station was installed in each study site to collect monthly natural precipitation and mean monthly temperature. Diefendorf et al. (2010) suggested that the water availability in growing season was important to evaluate leaf ^{13}C. Therefore, we calculated the mean temperature in the study growing seasons (TGS) and the total precipitation during the growing seasons (PGS) for each plot (Table 1). PGS was calculated as follows:

PGS=0.66 × (added precipitation) + natural precipitation in the growing seasons, where 0.66 was the rainfall validity of the rainfall simulator (Fig. S1 a, b).

Data analysis. One-way analysis of variance (ANOVA) was applied to detect the effect of simulated increasing PGS on leaf traits (LS, LL, LW, SLA and N_{mass}) and leaf δ^{13}C at a same time and place. Linear regression models were employed to examine the responses of leaf δ^{13}C and leaf traits (LS, LW, LL, L:W, SLA and N_{mass}) to PGS or TGS, and the relationships between leaf δ^{13}C and leaf traits. The partial correlation analysis was used to examine the effect of TGS on leaf δ^{13}C while controlling PGS, or the effects of leaf traits (LS, LW, L:W, SLA and N_{mass}) on leaf δ^{13}C while controlling PGS and TGS.

Data (30 samples) from two sites and three years were analyzed by Amos to estimate standardized regression coefficients and to create path models. Path models were employed to estimate the direct effects of climate (precipitation and temperature) or leaf traits (LS, LW, L:W, SLA and N_{mass}) on leaf δ^{13}C, and both the direct and indirect (through their effects on leaf traits) effects of precipitation and temperature on leaf δ^{13}C. Models were compared using the second-order Akaike information criterion (AIC_c) for small samples, calculated with the values from Amos analyses of each model.

All statistical analysis was performed using SPSS 18.0 (SPSS Inc. Chicago, IL, USA).

RESULTS

Leaf traits in response to precipitation and temperature during the plant growing season. Compared with the leaf traits under ambient precipitation, leaf traits significantly increased (5/24 leaf size, 7/24 leaf length, 2/24 leaf width, 10/24 specific leaf area and 0/24 mass-based leaf nitrogen concentration) most of the times under simulated precipitation addition treatments (Table 2). The effects of the 100% precipitation addition treatments on leaf traits were remarkable. In this study, all leaf traits were significantly different from the control on 19 out of 24 times at the 100% precipitation addition treatment (Table 2).

Linear regression models showed that LS, LW, LL, SLA and N_{mass} significantly increased with increasing PGS in Minqin and Dengkou (R^2=0.30-0.58, P<0.05), but had downward tendencies with increasing TGS in Minqin and Dengkou. However, the majority of the negative relationships between leaf traits and TGS were not obvious in Minqin (Fig. 2, Fig. 3). Simultaneously, L:W also had upward tendencies with increasing PGS in Minqin and Dengkou, but decreased with TGS in Dengkou. Given a PGS, LS, LW and LL were generally higher in Minqin than Dengkou, but SLA and N_{mass} were lower in Minqin than Dengkou. The shifts were just corresponding to the distribution pattern of each leaf trait along TGS (Fig. 2, Fig. 3).

Combining the data of Dengkou and Minqin, PGS and TGS could at maximum explain 68% and 83% of the variation in leaf traits, respectively. LW was the most insensitive leaf trait

Table 2. Leaf traits (LS: leaf size, LL: leaf length, LW: leaf width, SLA: specific leaf area, Nmass: mass-based leaf nitrogen concentration) and leaf δ13C (mean ± SE) under each rainfall addition treatment in Dengkou and Minqin during 2008-2010. Numbers bold highlight significant differences in leaf traits and leaf δ13C under different rainfall addition treatments compared with the control treatment. Differences were considered significant at the P<0.05 level.

Tabla 2. Características foliares (LS: tamaño de hoja, LL: longitud foliar, LW: ancho de hoja, SLA: área foliar específica, Nmass: concentración de N foliar basada en peso) y δ13C foliar (promedio ± EE) en cada tratamiento de adición de lluvia en Dengkou y Minqin durante 2008-2010. Los números en negrita destacan las diferencias significativas en las características foliares y δ13C bajo tratamientos diferentes de agregado de lluvias comparado con el tratamiento control. Las diferencias se consideraron significativas a P<0,05.

Leaf traits	Years	Minqin					Dengkou				
		ck	25%	50%	75%	100%	ck	25%	50%	75%	100%
LS	2008	0.99 ± 0.1	0.81 ± 0.1	0.98 ± 0.1	0.91 ± 0.1	1.08 ± 0.1	0.88 ± 0.1	0.91 ± 0.1	0.87 ± 0.1	1.04 ± 0.1	**1.18 ± 0.1**
	2009	0.62 ± 0.1	0.73 ± 0.1	0.80 ± 0.1	0.93 ± 0.2	**1.07 ± 0.1**	0.52 ± 0	0.47 ± 0	0.46 ± 0.1	0.57 ± 0.1	**0.77 ± 0**
	2010	0.83± 0.1	0.87 ± 0.1	0.81 ± 0.1	1.03 ± 0.1	**1.16 ± 0.1**	0.61 ± 0	0.64 ± 0	0.55 ± 0	0.66 ± 0.1	**0.90 ± 0.1**
LL	2008	2.05 ± 0	1.87 ± 0.2	2.11 ± 0.1	2.22 ± 0.1	**2.50 ± 0.1**	2.22 ± 0.1	2.35 ± 0	2.33 ± 0.1	**2.59 ± 0.1**	**2.71 ± 0.1**
	2009	1.92 ± 0.2	1.92 ± 0.2	1.73 ± 0.1	2.11 ± 0.2	**2.24 ± 0.1**	1.53 ± 0.1	1.47 ± 0	1.48 ± 0.2	1.90 ± 0.2	**2.09 ± 0.1**
	2010	1.97 ± 0	1.99 ± 0.1	1.91 ± 0.1	2.19 ± 0.1	**2.38 ± 0.1**	1.94 ± 0	2.06 ± 0.1	1.91 ± 0.1	2.17 ± 0.1	**2.47 ± 0.2**
LW	2008	0.70 ± 0	0.65 ± 0	0.69 ± 0.1	0.66 ± 0	0.71 ± 0	0.62 ± 0	0.62 ± 0	0.60 ± 0.1	0.64 ± 0	0.70 ± 0
	2009	0.62 ± 0	0.61 ± 0.1	0.59 ± 0.1	0.66 ± 0.1	0.69 ± 0.1	0.52 ± 0	0.44 ± 0	0.45 ± 0.1	0.51 ± 0	**0.55 ± 0**
	2010	0.64 ± 0	0.66 ± 0	0.64 ± 0	0.72 ± 0	**0.75 ± 0**	0.48 ± 0	0.49 ± 0	0.45 ± 0	0.47 ± 0	**0.55 ± 0**
SLA	2008	88.25 ± 2.6	90.69 ± 1.5	90.07 ± 5.1	90.66 ± 3.6	**103.89 ± 2.1**	99.73 ± 5.5	111.34 ± 3.8	105.61 ± 5.7	110.17 ± 2.2	**116.81 ± 1.5**
	2009	75.39 ± 1.2	77.01 ± 1.1	**80.44 ± 1.5**	**80.73 ± 0.6**	**89.48 ± 1.7**	85.65 ± 3.2	90.69 ± 4.1	87.32 ± 3.7	90.66 ± 4.9	**102.24 ± 5.4**
	2010	86.26 ± 1.0	89.23 ± 3.2	88.26 ± 2.1	**96.88 ± 1.2**	**110.56 ± 2.6**	90.12 ± 2.4	97.23 ± 3.4	93.65 ± 3.7	**100.38 ± 5.0**	**106.53 ± 2.5**
Nmss	2008	3.34 ± 0.1	3.37 ± 0.1	3.31 ± 0.2	3.32 ± 0.1	3.58 ± 0.1	3.67 ± 0.2	4.04 ± 0.1	3.71 ± 0.2	3.86 ± 0.1	4.11 ± 0.2
	2009	3.15 ± 0.1	3.38 ± 0.1	3.23 ± 0.2	3.20 ± 0.1	3.34 ± 0.1	3.43 ± 0.1	3.72 ± 0.1	3.43 ± 0.2	3.45 ± 0.3	3.93 ± 0.2
	2010	3.17 ± 0.1	3.18 ± 0.1	3.20 ± 0.1	3.24 ± 0.1	3.22 ± 0.1	3.49 ± 0.1	3.58 ± 0.1	3.77 ± 0.1	3.68 ± 0.1	3.87 ± 0.1
δ13C	2008	-27.06 ± 0	-27.80 ± 0.3	-27.44 ± 0.3	-27.38 ± 0.3	**-27.96 ± .03**	-26.59 ± 0.3	-26.84 ± 0.4	-27.44 ± 0.1	-27.09 ± 0.2	**-27.97 ± 0.4**
	2009	-26.91 ± 0.1	-26.97 ± 0.2	-26.79 ± 0.3	-27.03 ± 0.1	-27.39 ± 0.3	-26.40 ± 0.2	-26.48 ± 0.3	-26.91 ± 0.3	-26.59 ± 0.4	**-27.28 ± 0.3**
	2010	-27.17 ± 0.2	-27.47 ± 0.1	-27.20 ± 0.3	-27.43 ± 0.3	-27.74 ± 0.2	-26.01 ± 0.3	-26.24 ± 0.2	-26.46 ± 0.3	-26.82 ± 0.3	**-26.79 ± 0.3**

to precipitation, but was sensitive to temperature. The shift of SLA was pronounced with the variation in precipitation, but it was not related to the variation in temperature (Table 3).

Path analysis suggested that the positive and direct effects of PGS on LS, LW, L:W, SLA and N_{mass} were remarkable. Increasing TGS could conduce to lower LS and LW, but higher L:W and N_{mass}. Additionally, the direct effect of TGS on SLA was tiny. The combination of PGS and TGS could significantly improve our ability to estimate leaf traits except for LW. In the present study, PGS and TGS together could explained 79%, 79%, 48%, 70%, 64% of the variation in LS, LW, L:W, SLA and N_{mass}, respectively (Fig. 4).

Leaf δ13C in response to precipitation and temperature during the plant growing season. Compared to the leaf traits under ambient precipitation, 23/24 leaf δ13C decreased under the simulated precipitation addition treatments (Table 2). However, only 3/24 leaf δ13 decreased significantly, which all came from the plots with 100% precipitation addition treatments.

Linear regression models showed that leaf δ13C significantly decreased with the increasing PGS in Minqin and Dengkou (R^2=0.56-0.68, P<0.05), but had upward tendencies with increasing TGS. However, the positive relationships between leaf δ13C and TGS were not significant at the two sites. Given a PGS, leaf δ13C was higher in Dengkou than Minqin (Fig. 5).

Combined the data of Dengkou and Minqin, PGS and TGS could explain 14% and 37% of the variation in leaf δ13C, respectively (Table 3).

Leaf traits were closely related to leaf δ13C (R^2=0.22-0.69, P<0.05), except for L:W in Minqin and Dengkou. However, combining the data of Dengkou and Minqin, leaf δ13C was weakly influenced by SLA and N_{mass}.

Path analysis indicated that the direct effects of PGS and TGS on leaf δ13C were remarkable. Increasing precipitation could lead to lower plant water use efficiency (leaf δ13C), while the effects of temperature on leaf δ13C were opposite. The indirect effects of PGS and TGS on leaf δ13C were also notable through their direct effects on leaf traits. The combination of PGS, TGS and leaf traits explained about 84% of the variation in leaf δ13C (Fig. 4).

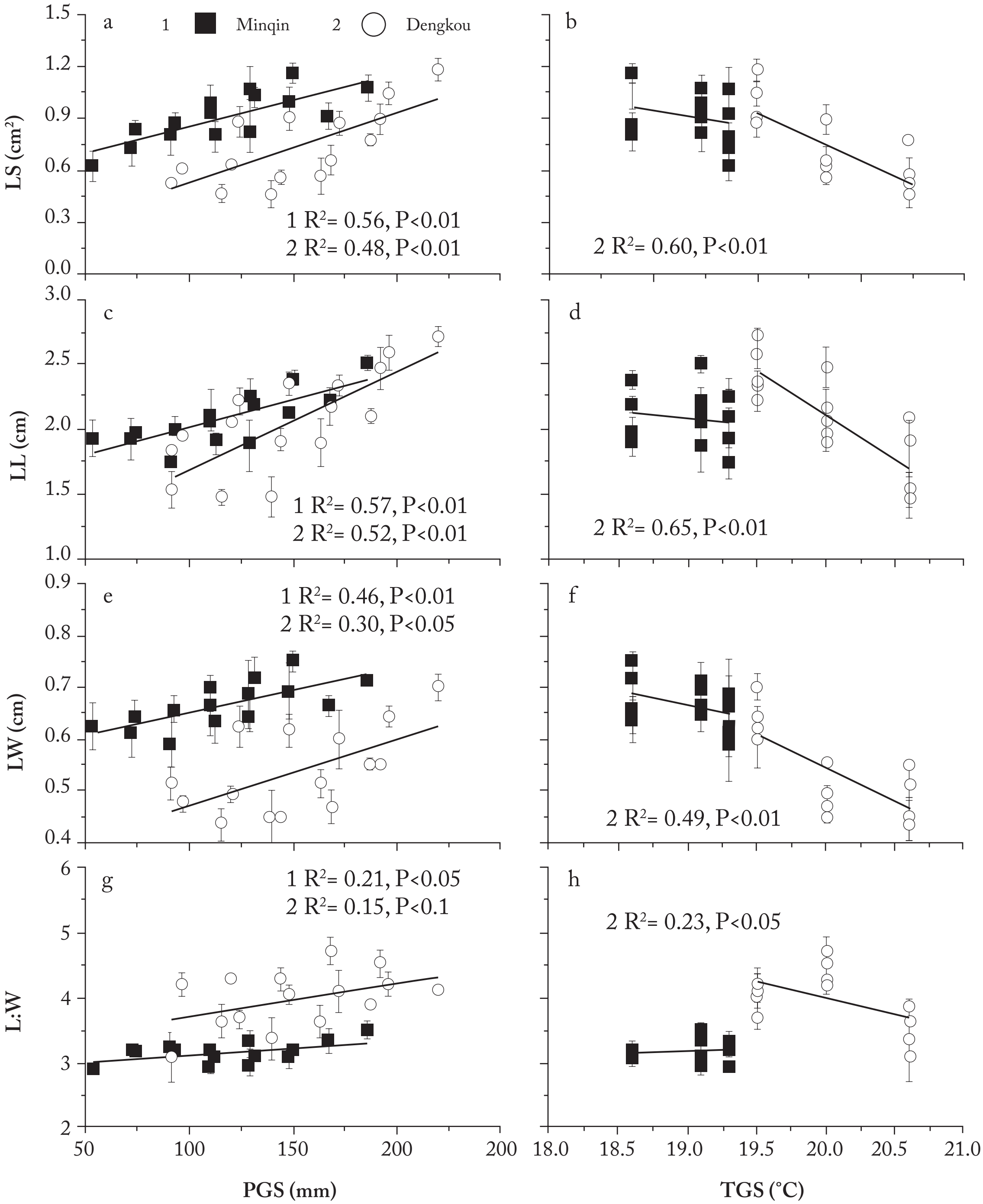

Fig. 2. Response of leaf size (LS), leaf length (LL), leaf width (LW) and leaf length to width ratio (L:W) to precipitation (PGS) and mean temperature (TGS) during the growing seasons (2008 to 2010).

Fig. 2. Respuesta del tamaño foliar (LS), longitud foliar (LL), ancho foliar (LW) y relación de longitud a ancho foliar (L:W) a la precipitación (PGS) y temperatura promedio (TGS) durante las estaciones de crecimiento (2008 a 2010).

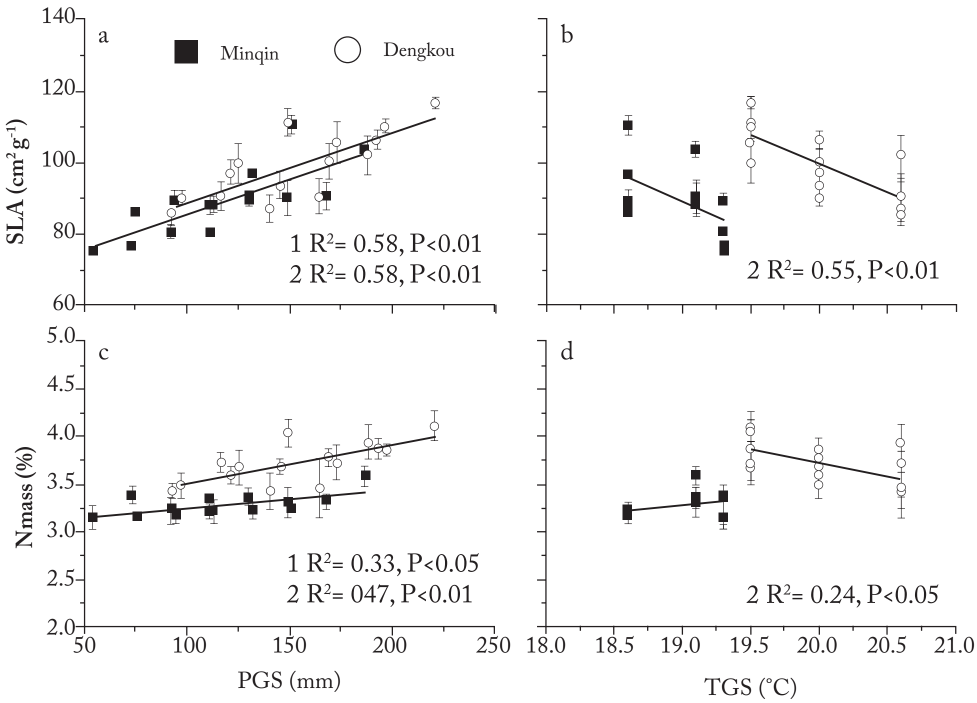

Fig. 3. Responses of specific leaf area (SLA) and mass-based leaf nitrogen concentration (Nmass) to precipitation (PGS) and mean temperature (TGS) during the growing seasons (2008-2010).

Fig. 3. Respuestas del área foliar específica (SLA) y la concentración de N foliar basado en peso (Nmass) a la precipitación (PGS) y temperatura promedio (TGS) durante las estaciones de crecimiento (2008 a 2010).

Table 3. Regression models between leaf traits and leaf $\delta^{13}C$ and PGS (or TGS) in Dengkou and Minqin. LS: leaf size, LW: leaf width, LL: leaf length, L:W: leaf length to width ratio, SLA: specific leaf area, Nmass: mass-based leaf nitrogen concentration.

Tabla 3. Modelos de regression entre las características foliares y $\delta^{13}C$ foliar y PGS (o TGS) en Dengkou y Minqin. LS: tamaño de hoja, LW: ancho foliar, LL: longitud foliar, L:W: relación de longitud a ancho foliar, SLA: área foliar específica, Nmass: concentración foliar de N basada en peso.

Leaf trait	PGS		TGS	
	Slope	R^2	Slope	R^2
LS	0.00*	0.16	-0.21**	0.47
LW	0.00	0.01	-0.12**	0.83
LL	0.01**	0.43	-0.17*	0.14
L:W	0.01**	0.34	0.40**	0.24
SLA	0.21**	0.68	0.42	0.00
N_{mass}	0.01**	0.49	0.22**	0.28
$\delta^{13}C$	-0.01*	0.14	0.47**	0.37

Note: * P<0.05, ** P<0.01.

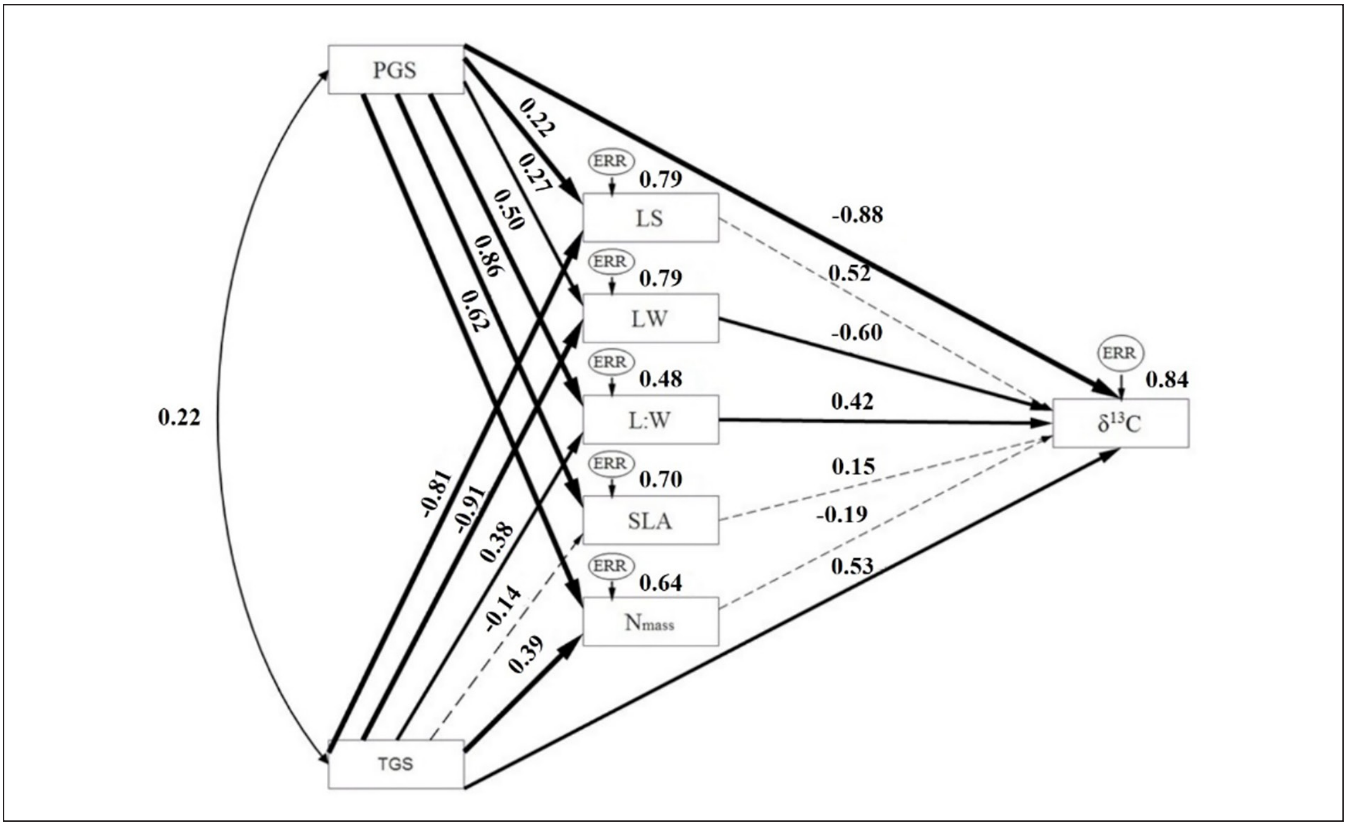

Fig. 4. Results of path models to estimate the direct effects of precipitation (PGS), temperature (TGS) and leaf traits (LS, LW, L:W, SLA and Nmass) on leaf δ13C, and both the direct and indirect (through their effects on leaf traits) effects of precipitation and temperature on leaf δ13C.

Fig. 4. Resultados de modelos de trayectoria para estimar los efectos directos de la precipitación (PGS), temperatura (TGS) y características foliares (LS, LW, L:W, SLA y Nmass) en el δ13C foliar, y los efectos directos e indirectos (a través de sus efectos sobre las características foliares) de la precipitación y la temperatura sobre el δ13C foliar.

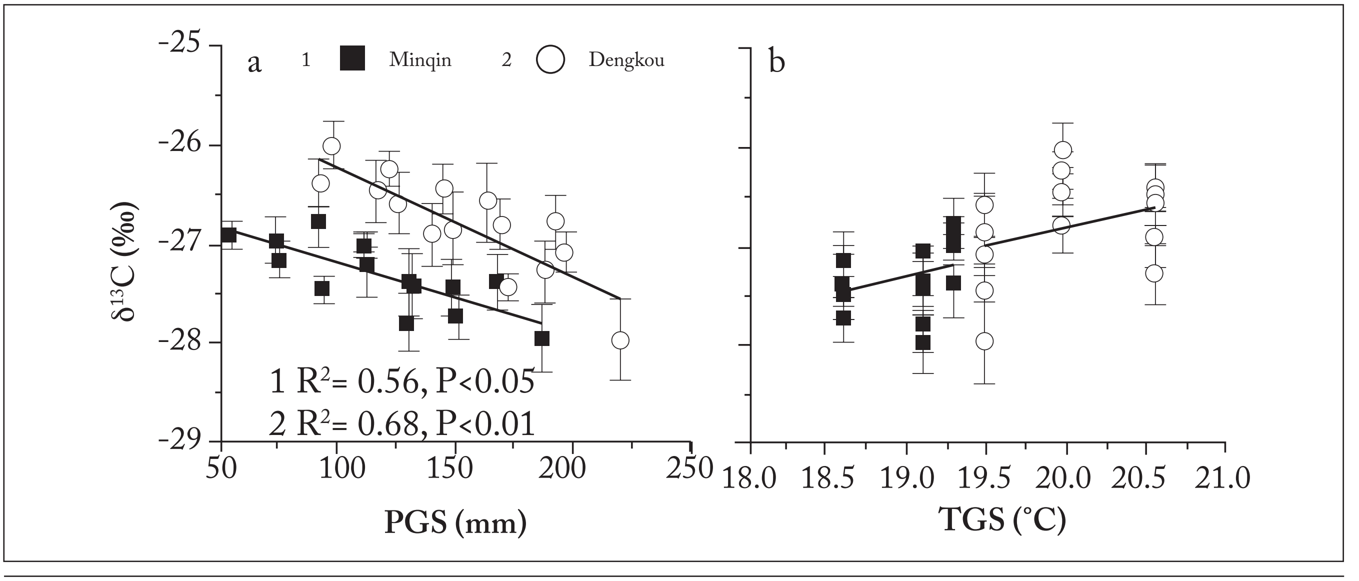

Fig. 5. Response of leaf δ13C to precipitation (PGS) and mean temperature (TGS) during the growing seasons (2008 to 2010).

Fig. 5. Respuesta de δ13C foliar a la precipitación (PGS) y temperatura promedio (TGS) durante las estaciones de crecimiento (2008 a 2010).

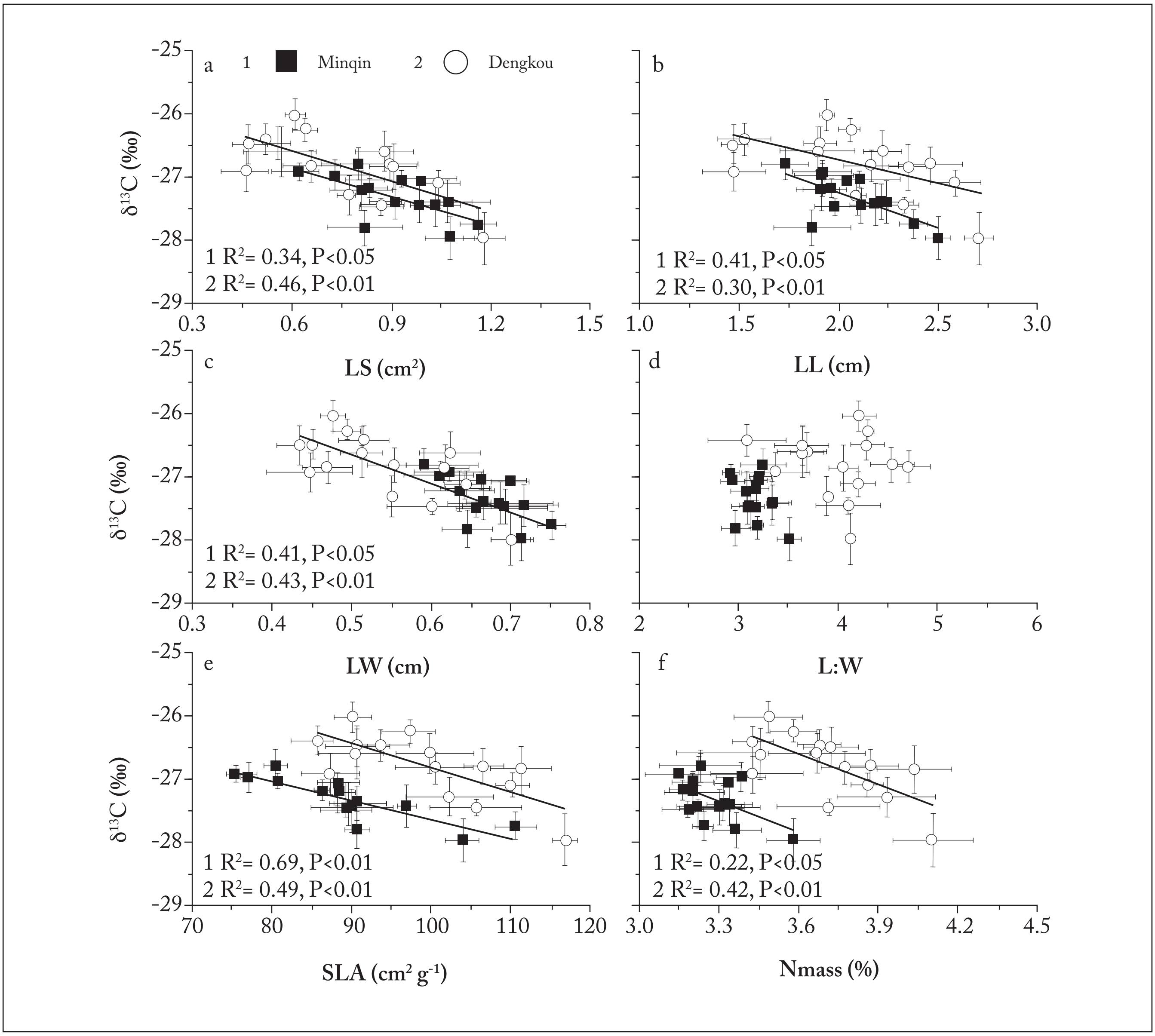

Fig. 6. The relationships between leaf size (LS), leaf length (LL), leaf width (LW), leaf length to width ratio (L:W), specific leaf area (SLA), mass-based leaf nitrogen concentration (N_{mass}) and leaf $\delta^{13}C$.
Fig. 6. Relaciones entre tamaño de hoja (LS), longitud foliar (LL), ancho foliar (LW), relación de longitud a ancho foliar (L:W), área foliar específica (SLA) y concentración de N foliar basado en peso (N_{mass}) versus $\delta^{13}C$ foliar.

DISCUSSION

Responses of leaf traits to precipitation and temperature in arid ecosystem. Leaf traits are sensitive to changes in environmental factors (Li et al., 2017; Liu et al., 2017; Meng et al., 2017). And the plasticity of leaf traits is advantageous to the survival, growth and adaptation of plants (Yates et al., 2010; Baird et al., 2017). Our results are consistent with previous studies, which suggested that high water availability could increase leaf growth and conduce to high SLA (Sheng et al., 2011; Li et al., 2015).

The plasticity in LS is directly related to the expansion of LL and LW. Previous studies found that the expansion of LL and LW is asynchronous in certain environments. For example, increasing water stress can limit leaf expansion along the short axis (LW) more severely than along the long axis (LL) (Picotte et al., 2007; Clair et al., 2013). It can incidentally promote the thermal diffusivity on the blade because of a thinner leaf boundary layer and increase leaf water use efficiency (leaf $\delta^{13}C$) (Yates et al., 2010). Picotte et al. (2007) also demonstrated that narrower and longer leaves could increase plant growth because of a higher leaf water use efficiency under drought conditions.

However, our results showed that leaf length-to-width ratio (L:W) increased with increasing precipitation by reducing the sensitivity of LW to precipitation and increasing LL rapidly under a higher water availability level. It might imply that there might be an optimized growth strategy of arid plants in carbon capture as water stress is released gradually in high temperature environments during the plant growth season. In the process, increasing LL could expand effectively photosynthetic leaf area. At the same time, a lower growth rate in LW might be good for maintaining a thin leaf boundary layer and a high leaf water use efficiency (leaf $\delta^{13}C$).

The dramatic effect of temperature on leaf traits was partly due to the relations of covariation between temperature and precipitation. Increasing temperature could not only reduce soil water availability for plants, but also accelerate transpiration (see the below discussion about TGS and soil water availability). High temperature stress not only decreased LS and LL, but also mainly restricted growth of LW. The optimized leaf shape directly reduce the water and heat stresses down to increase leaf water use efficiency.

Additionally, we observed that N_{mass} increased with increasing PGS. This could be due to the activation of roots and soil microorganisms in the plots with precipitation addition treatments. But increasing N_{mass} was insignificant to influence leaf $\delta^{13}C$. The relations of covariation between N_{mass} and leaf $\delta^{13}C$ are largely due to the co-regulation of PGS and TGS on N_{mass} and leaf $\delta^{13}C$.

Leaf $\delta^{13}C$ changes in arid region. Leaf $\delta^{13}C$ values to some extent could reflect the balance of photosynthesis (C-3 plant vs. C4- plant) and leaf conductance (including mesophyll, stomatal and leaf boundary layer conductance), and their coupled responses to the environment (Bragg et al., 2013). Thereinto, water availability for plants seems to be the first predictor of leaf $\delta^{13}C$. Besides, the relationship between leaf $\delta^{13}C$ and water availability differs among photosynthetic pathways. For example, in Australia grasses, there was a negative relationship between water availability and leaf $\delta^{13}C$ in C-3 grasses, while the relationship was opposite in C-4 grasses (Murphy & Bowman, 2009). The negative relationship between leaf $\delta^{13}C$ and precipitation was supported by theory and abundant controlled experiments and field observations at regional and global scales (Sheng et al., 2011; Pflug et al., 2015). Since the target species *N. tangutorum* is a C-3 shrub, the negative relationship was also confirmed by our data from sites located at different places in the arid region of northwestern China.

Meanwhile, our data showed that there was a shift in the negative leaf $\delta^{13}C$-PGS relationship across Minqin and Dengkou. This lead to the lacking effects of precipitation on leaf $\delta^{13}C$ across the two sites, and higher leaf $\delta^{13}C$ at lower precipitation fields in Minqin. Similarly, many studies also found the lack of effects of precipitation on leaf $\delta^{13}C$ in high water stress regions, such as southern Africa, Australia, Europe and East Asia (Su et al., 2000; Miller et al., 2001; Schulze et al., 2006; Wei et al., 2011). They implied that the changes were due to the heterogeneity of water availability or ample supply of ground water (Schulze et al., 1996) or optimal leaf traits (Wei et al., 2011) during the plant growing season.

Our results suggest to accept that the shift in the relationship between leaf $\delta^{13}C$ and precipitation is related to temperature and optimal leaf traits. Because of the effects of water availability during growing season on leaf $\delta^{13}C$ across Minqin and Dengkou, the partial correlation analysis showed that leaf $\delta^{13}C$ was positively related to TGS as PGS was controlled, and positively (or negatively) related to L:W (or LW) as PGS and TGS were controlled. The combination of PGS and TGS and the combination of PGS, TGS, LW and L:W could explain 64% and 84% of the variation in leaf $\delta^{13}C$, respectively. However, PGS could only independently explain 14% of the variation in leaf $\delta^{13}C$.

Theoretically, TGS is associated with soil evaporation and plant transpiration. The ratio of soil water availability for plants (used in transpiration) to precipitation decreases with increasing temperature, especially in fields with low vegetation coverage. Guo et al. (2015) found that ratios of evapotranspiration to precipitation on *Nitraria* dunes were generally higher than 90% in Minqin, where the ratios of soil evaporation to evapotranspiration were more than 70 % in fields with less than 25% vegetation coverage. Moreover, the ratio of evapotranspiration to precipitation is generally higher in dry (and hot) than in wet (and cool) years. As a result, the relations of covariation between temperature and soil water availability could result in the positive relationship between leaf $\delta^{13}C$ and PGS. The response of soil organic $\delta^{13}C$ to temperature had also been observed along the 400 mm isohyet of mean annual precipitation in China (Jia et al., 2016).

Leaf traits could be used to estimate the leaf $\delta^{13}C$ across different biomes and regions. However, few studies evaluated the direct effect of leaf traits on leaf $\delta^{13}C$ as the indirect effects of environmental factors (temperature and precipitation) on leaf $\delta^{13}C$ through leaf traits were neglected. In this study, we suggest that leaf traits (LW and L:W) could be used to improve the estimation of leaf $\delta^{13}C$ in arid ecosystems, since LW could regulate water, carbon dioxide and heat flow velocity on the blade.

In conclusion, our results support the optimizing theory between water cost and carbon benefit by the plasticity of leaf traits regulating water, carbon dioxide and heat flow velocity on the blade. Moreover, the combinations of PGS, TGS, LW and L:W could explain the variation of leaf $\delta^{13}C$ in arid ecosystems. Further research is required to better understand the coupled responses of soil water availability to temperature and precipitation, because soil water availability was a direct factor affecting plant water uptake and use.

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