





Variation in woody plant $\delta^{13}\text{C}$ along a topoedaphic gradient in a subtropical savanna parkland

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Abstract $\delta^{13}\text{C}$ values of C_3 plants are indicators of plant carbon–water relations that integrate plant responses to environmental conditions. However, few studies have quantified spatial variation in plant $\delta^{13}\text{C}$ at the landscape scale. We determined variation in leaf $\delta^{13}\text{C}$, leaf nitrogen per leaf area (N_{area}), and specific leaf area (SLA) in April and August 2005 for all individuals of three common woody species within a 308 12-m belt transect spanning an upland–lowland topoedaphic gradient in a subtropical savanna in southern Texas. Clay content, available soil moisture, and soil total N were all negatively correlated with elevation. The $\delta^{13}\text{C}$ values of *Prosopis glandulosa* (deciduous nitrogen fixing tree legume), *Condalia hookeri* (evergreen shrub), and *Zanthoxylum fagara* (evergreen shrub) leaves increased 1–4‰ with decreasing elevation, with the $\delta^{13}\text{C}$ value of *P. glandulosa* leaves being 1–3‰ higher than those of the two shrub species. Contrary to theory and results from previous studies, $\delta^{13}\text{C}$ values were highest where soil water was most available, suggesting that some other variable was overriding or interacting with water availability. Leaf N_{area} was positively correlated with leaf $\delta^{13}\text{C}$ of all species ($p < 0.01$) and appeared to exert the strongest control over $\delta^{13}\text{C}$ along this topoedaphic gradient. Since leaf N_{area} is positively related to photosynthetic capacity, plants with high leaf N_{area} are likely to have low p_i/p_a ratios and therefore higher $\delta^{13}\text{C}$ values, assuming stomatal conductance is constant. Specific leaf area was not correlated significantly with leaf $\delta^{13}\text{C}$. Following a progressive growing season drought in July/August, leaf $\delta^{13}\text{C}$ decreased. The lower $\delta^{13}\text{C}$ in August may reflect the accumulation of ^{13}C -depleted epicuticular leaf wax. We suggest control of leaf $\delta^{13}\text{C}$ along this topoedaphic gradient is mediated by leaf N_{area} rather than by stomatal conductance limitations associated with water availability.

Keywords Carbon isotope discrimination · Leaf nitrogen · Specific leaf area · Soil moisture · Photosynthetic capacity

Introduction

Quantification of spatial variability in leaf carbon isotope composition ($\delta^{13}\text{C}$) across the landscape affords an opportunity to evaluate plant–environment interactions and plant responses to environmental change (Adams and Grierson 2001; Dawson et al. 2002). Many studies have quantified leaf $\delta^{13}\text{C}$ variation along environmental gradients, particularly altitudinal gradients in mountainous regions (Körner et al. 1988; Cordell et al. 1999; Hultine and Marshall 2000). An increase in $\delta^{13}\text{C}$ with altitude has been observed in many plant species and appears to result from variations in precipitation (Van de Water et al. 2002), temperature (Panek and Waring 1995), and/or nutrient availability (Schulze et al. 1998) along these gradients.

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However, less is known about variations in leaf $\delta^{13}\text{C}$ along more subtle topographic gradients where the macroclimatic conditions are the same, but where changes in soil moisture, texture and nutrients may interact to influence plant carbon–water relations and leaf $\delta^{13}\text{C}$.

In C_3 plants, the carboxylation enzyme Rubisco (ribulose 15-bisphosphate carboxylase/oxygenase) discriminates against ^{13}C during photosynthesis. This effect is moderated by the ratio of CO_2 partial pressure at the carboxylation sites (p_i) to that in ambient air (p_a), which is strongly determined by stomatal aperture and photosynthetic capacity (Farquhar et al. 1982, 1989). Plants with lower p_i/p_a ratios usually have higher leaf $\delta^{13}\text{C}$ values (Farquhar et al. 1982). Therefore, a wide range of intrinsic plant characteristics and environmental variables play roles in determining leaf $\delta^{13}\text{C}$ through effects on p_i/p_a . For example, both leaf N_{mass} (leaf N per leaf dry mass) and leaf N_{area} (leaf N per leaf area) have been reported to be positively related to leaf $\delta^{13}\text{C}$ by elevating photosynthetic capacity and lowering p_i (Morecroft and Woodward 1996; Sparks and Ehleringer 1997; Wright et al. 2003; Hamerlynck et al. 2004). Similarly, irradiance (Zimmerman and Ehleringer 1990; Arthur et al. 2001) and air temperature have been found positively correlated to leaf $\delta^{13}\text{C}$ (Panek and Waring 1995), while precipitation is negatively correlated to leaf $\delta^{13}\text{C}$ (Stewart et al. 1995; Ferrio and Voltas 2005). Although process studies have revealed how leaf $\delta^{13}\text{C}$ responds to specific abiotic factors, we know little of how these interact to influence the $\delta^{13}\text{C}$ of the foliage in plant canopies along landscape-scale gradients during a growing season.

Since the p_i/p_a ratio is also related to plant water-use efficiency [WUE; ratio of CO_2 assimilation over transpiration (A/E)], it has often been noted that leaf $\delta^{13}\text{C}$ and WUE are positively related via p_i/p_a (Farquhar et al. 1982; Ehleringer and Cooper 1988; Mooney et al. 1989; Stewart et al. 1995; Saurer et al. 2004). In some cases, plants located on drier portions of a landscape have higher leaf $\delta^{13}\text{C}$, suggesting higher WUE (Comstock and Ehleringer 1993; Anderson et al. 1996). In other cases, the reverse is true. For example, Read and Farquhar (1991) found a positive relationship between water availability and leaf $\delta^{13}\text{C}$ of *Nothofagus* species in New Guinea. They speculated that because soil moisture was seldom limiting in their study area, other factors, such as soil nutrients, may have been dictating leaf $\delta^{13}\text{C}$. Similarly, Schulze et al. (1998) found that variation in leaf N_{mass} rather than variation in annual rainfall determined community-averaged leaf $\delta^{13}\text{C}$ in northern Australia. Zhang and Marshall (1995) reported lower leaf $\delta^{13}\text{C}$ from dry environments and speculated that there could be differential sensitivity of stomatal conductance to water availability among different species. Therefore, caution should be taken while

interpreting the link between leaf $\delta^{13}\text{C}$ and WUE for natural vegetation because numerous biotic and abiotic factors and their interactions may influence $\delta^{13}\text{C}$ (Vitousek et al. 1990; Griffiths et al. 2000).

In arid and semiarid regions, topographic variation is a strong determinant of plant carbon–water relations through its impacts on soil water availability and rates of biogeochemical processes that determine the availability of limiting nutrients (Ehleringer et al. 1998; Hamerlynck et al. 2004). The objectives of our study were to: (1) quantify landscape-scale variations of leaf $\delta^{13}\text{C}$ along a topographic gradient varying in soil texture, soil moisture, and soil fertility in a subtropical savanna parkland landscape; (2) evaluate potential controls over these isotopic variations. To examine inter- and intraspecific variability in leaf $\delta^{13}\text{C}$, we focused on three woody plant species differing in growth form that were ubiquitous along the gradient: a deciduous tree (*Prosopis glandulosa* Torr. var. *glandulosa*), and two evergreen shrubs (*Buddleia hookeri* M.C. Johnst. and *Zanthoxylum fagara* (L.) Sarg.). Leaf $\delta^{13}\text{C}$, specific leaf area (SLA), and leaf N_{area} were measured early and late in the growing season to test the following hypotheses: (1) leaf $\delta^{13}\text{C}$ is negatively correlated with the availability of soil moisture along the topographic gradient; (2) leaf N_{area} is positively correlated with leaf $\delta^{13}\text{C}$ due to its influence on photosynthetic capacity; (3) SLA is negatively correlated with leaf $\delta^{13}\text{C}$ due to its influence on leaf internal resistance to CO_2 diffusion; (4) leaf $\delta^{13}\text{C}$ of *P. glandulosa* is lower than that of the other two species due to its mixing ability; (5) seasonal drought will increase leaf $\delta^{13}\text{C}$ values of all species.

Material and methods

Study area

This research project was conducted at the Texas Agricultural Experiment Station La Copita Research Area (27°40'N; 98°12'W; elevation 80 m a.s.l.) in Jim Wells County, 15 km SW of Alice, Texas in the eastern Rio Grande Plains of Tamaulipan Biotic Province. The climate of the region is subtropical, with a mean annual temperature of 22.4°C and mean annual precipitation of 680 mm. Rainfall maxima occur in May–June and September; while July and August are typically the driest (and warmest) months of the year.

Landscapes at La Copita typically grade (1–3% slopes) from sandy loam uplands to clay loam and clay lowlands which receive runoff from the uplands and may have standing water after large rainfall events. Upland soils are primarily Typic Argiustolls with a subsurface argillic horizon; however, patches of Typic Ustochrepts lacking an

argillic horizon are also found in the uplands (Archer Plant and soil sampling and analysis 1995). Upland vegetation is savanna parkland consisting of discrete woody clusters 3–10 m in diameter (comprised of two soil cores (0–15 cm) were collected at 1-m intervals a single *P. glandulosa* trees with up to 15 understory tree/ along the transect in April 2005. One core was used to shrub species) and larger groves 10–20 m in diameter determine soil particle size distribution (pipette method; (comprised of several woody clusters that have fused together) embedded within a matrix of grassland vegetation. Clusters and groves are dominated by *P. glandulosa* EA-1108 elemental analyzer; CE Elantech, Lakewood, NJ) and *Z. fagara*. *Prosopis glandulosa* and several other following procedures outlined in Harris et al (2001). leguminous trees and shrubs present in wooded areas are another set of soil cores were collected at 3-m intervals capable of symbiotic N fixation (Zitzer et al. 1996). and weighed before and after drying at 105°C to determine

Soils in lower lying drainage woodlands are generally gravimetric (GWC) and volumetric water content (VWC). Pachic Argiustolls and are covered by closed-canopy Plant-available VWC was computed from total VWC and woodlands similar in composition to upland clusters and soil texture using the equations of Cosby et al (1984), groves. Playas are closed-basin depressions that occur assuming a matric potential at wilting point of 1.0 MPa. within the drainage woodlands and occupy the lowest Soil water content was not quantified in August 2005 portions of the landscape. Playa soils are Ustic Epiaque because significant rainfall occurred the day prior to plant erts and Vertic Argiaquolls, and vegetation there consists of a continuous grass layer dominated by *Paspalum* re ected the antecedent soil moisture conditions that would *pubiliflorum* and *Bothriochloa ischaemum*, with *P. glandulosa* and *Acacia farnesiana* trees of variable density have influenced our plant response variables in August. (Farley 2000). Three common woody species were selected for isotopic analyses: (1) *P. glandulosa*, (2) *C. hookeri*, and (3) *Z. fagara*.

Woody plant encroachment has occurred throughout Biological characteristics of these species are summarized in the study area over the past 75–100 years due to the Table 1. Leaf tissue of the three study species was sampled interaction of heavy livestock grazing and reduced re from all individuals occurring within 6 m on either side of frequency (Archer 1995). Archer (1995) and Boutton the 308-m transect in April and August 2005. For *P. glandulosa*, approximately 10 g of fully expanded, south-facing, sun-lit leaves in the upper canopy were sampled. For *C. hookeri* and *Z. fagara*, approximately 10 g of fully expanded, south-facing, leaves located in the upper canopy approximately 1–2 m above ground level were sampled. The

Transect description

A 308-m transect spanning a hillslope gradient from an spatial coordinates of each plant sampled within the upland through a drainage woodland and into a playa was 29 308-m area were recorded by measuring the distance established. All ve of the major landscape elements at the along and perpendicular to the central transect line. study site (grasslands, clusters, groves, drainage wood- Leaf area was measured on fresh tissue using a leaf area lands, and playa) occurred along the transect. The transect meter (CI-202; CID, Vancouver, WA, USA). Leaves were was marked with steel posts at 1-m intervals and was then oven dried for 48 h at 60°C and weighed. Specific leaf georeferenced by global positioning system (GPS path area was calculated as the ratio of leaf area to leaf dry nder Pro XRS; Trimble Navigation, Sunnyvale, CA) at weight. Leaves were then pulverized in a centrifugal mill 5-m intervals. Elevations were determined by surveying. (Angstrom, Belleville, MI). $\delta^{13}C$ and leaf N_{mass} (g kg⁻¹)

Table 1 Plant species characteristics in the study area

Species	Family	Growth habit (max. height, m)	Leaf texture	Initiation of spring regrowth	Timing of maximum numbers of leaves	Functional rooting depth (m)
<i>Prosopis glandulosa</i>	Fabaceae	Deciduous tree (10)	Coriaceous	March	June–July	2–3
<i>Zanthoxylum fagara</i>	Rutaceae	Evergreen shrub (4)	Coriaceous-malacophyllous	March–April	July–August	1–2
<i>Condalia hookeri</i>	Rhamnaceae	Evergreen shrub (4)	Malacophyllous	March–April	July–August	1–2

Data on growth habit, leaf texture, and phenology are from Nelson et al (2002), and functional rooting depths are based on and $\delta^{18}O$ of plant and soil water (Boutton et al. 1999)

were determined using an elemental analyzer (Carlo Erb EA-1108; CE Elantech, Lakewood, NJ) interfaced with an isotope ratio mass spectrometer (Delta Plus, Thermo Electron, Waltham, MA) operating in continuous flow mode. Carbon isotope ratios are presented in notation:

$$d = [(R_{\text{SAMPLE}} - R_{\text{STD}}) / R_{\text{STD}}] \times 10^3$$

where R_{SAMPLE} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and R_{STD} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the V-PDB standard (Coplen 1996). The precision of duplicate measurements was 1‰. Leaf N_{area} (g m^{-2}) was calculated by dividing leaf N_{mass} by SLA.

Temperature and rainfall data from December 2004 to August 2005 were obtained from the Alice, TX weather station approximately 15 km from the study site (available at <http://www.ncdc.noaa.gov>)

Statistical analyses

A univariate general linear model ANOVA was used to test for the differences in leaf ^{13}C with respect to landscape locations, sampling seasons, species, and their interactions. Fisher's least significant difference (LSD) method was used to distinguish differences following ANOVA. Regression analyses were performed to determine the significance of relationships between leaf N_{area} , SLA, plant available water, and leaf ^{13}C . Stepwise multiple linear regression was performed to determine if certain combinations of plant and/or environmental variables could be used to predict leaf ^{13}C . Analysis of covariance (ANCOVA) was used to compare the slopes of the relationship for leaf N_{area} versus leaf ^{13}C between the two sampling seasons. All statistical analyses were performed using the SPSS 10.0 program (SPSS, Chicago, IL).

Results

Temperature and rainfall during study period

Average monthly temperatures during February (17.1°C), March (19.1°C), and April (22.8°C) of 2005 were above the 110-year mean at Alice, TX (Fig. 1). Monthly rainfall in both February (64.3 mm) and March (62.2 mm) was approximately 60% above the 110-year mean, however, rainfall during April (2.54 mm) was only 6% of the 110-year mean.

Average monthly temperatures during May, June, July, and August of 2005 were 26.2, 29.4, 30.7, and 30.4°C, respectively (Fig. 1). These temperatures were all above the 110-year mean for those months. Monthly rainfall during the same period was 41.4 mm (May), 3.3 mm (June), 64.8 mm (July) and 8.6 mm (August), which was either substantially less than or comparable to the 110-year mean for those months. Hence, conditions prior to the April foliage sampling were relatively warm and mesic, while those preceding the August foliage sampling were comparatively hot and dry.

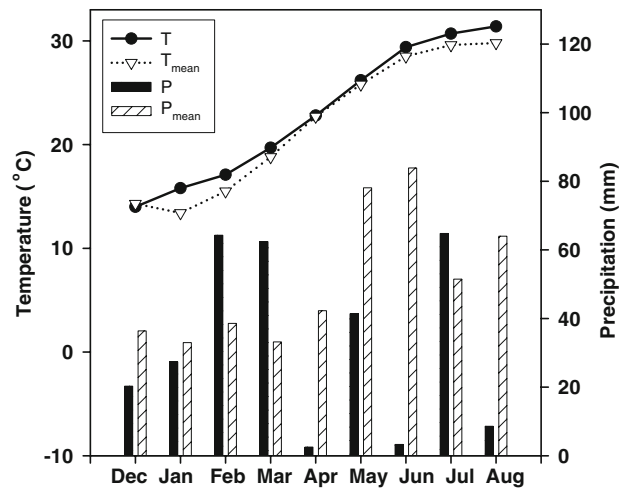
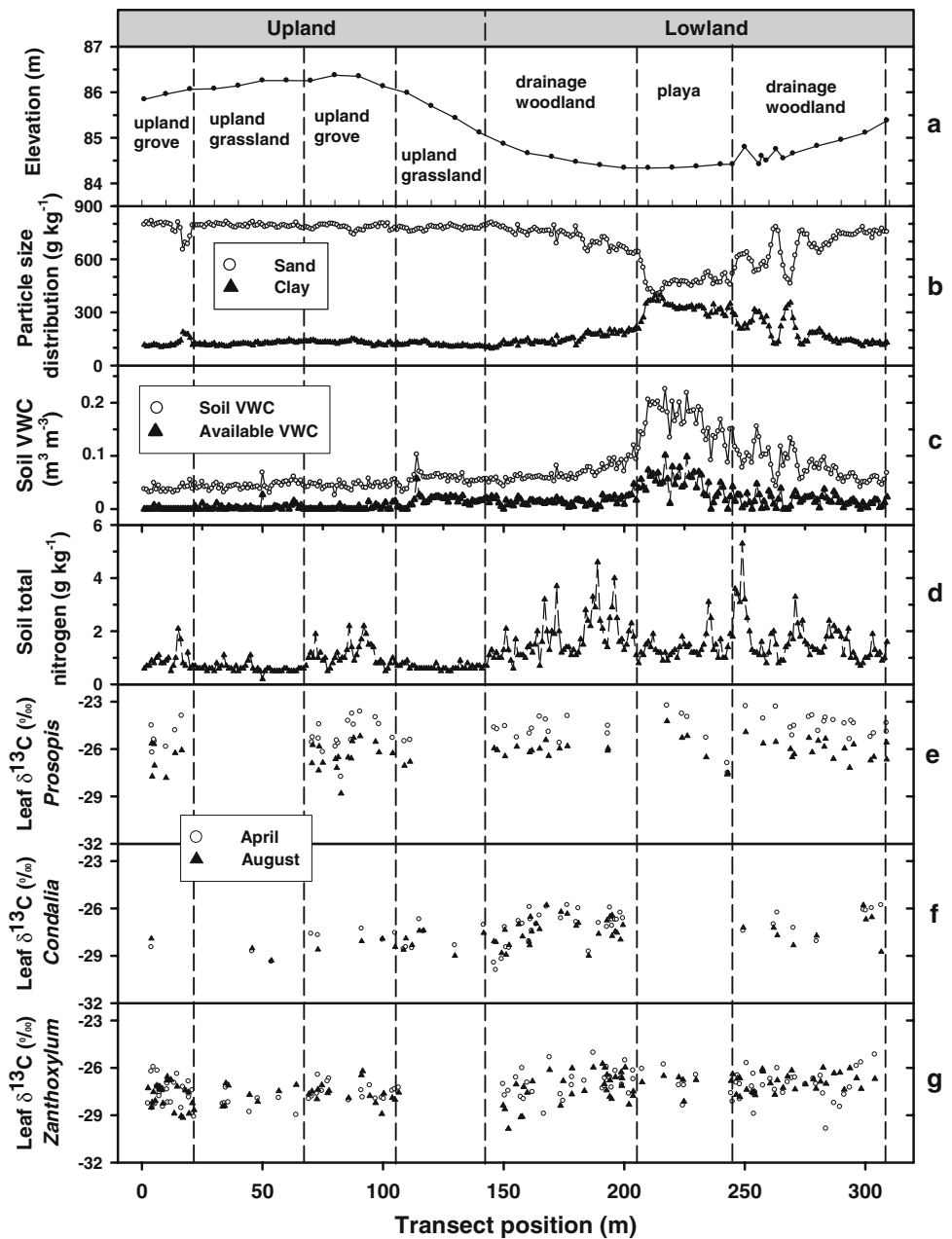


Fig. 1 Monthly mean temperature (°C) and rainfall (mm). Solid line and dark bars represent the monthly mean temperature and rainfall of the year of sampling (2005); broken line and cross-hatched bars represent 110-year means

Transect characteristics

Elevation along the transect ranged from 84.5 to 86.5 m a.s.l. (Fig. 2a). Although subtle, these variations in elevation have induced significant variation in soil particle size distribution (Fig. 2b), soil water content (Fig. 2c), and soil total N (Fig. 2d). Soil clay content, plant available water and soil total N were all negatively correlated with elevation (Table 2). Soil clay content increased as elevation decreased along the transect, with values of approximately 100 g kg^{-1} in the uplands, $100\text{--}200 \text{ g kg}^{-1}$ in the drainage woodlands, and $250\text{--}350 \text{ g kg}^{-1}$ in the playas (Fig. 2b). Both soil VWC and plant available VWC were related to elevation and texture, with the highest values in playas (plant available VWC = $0.10 \text{ m}^3 \text{ m}^{-3}$) and the lowest values in uplands (plant available VWC = $0.01 \text{ m}^3 \text{ m}^{-3}$) (Fig. 2c). Plant-available VWC was always significantly less than the total VWC, especially in the playas where clay content was the highest. Soil total N was related to both elevation and vegetation cover, with higher values in the lower lying drainage woodlands and playas ($1\text{--}5 \text{ g N kg}^{-1}$ soil) than in the uplands ($0.6\text{--}2 \text{ g N kg}^{-1}$ soil), and higher values in the woody vegetation types ($1\text{--}5 \text{ g N kg}^{-1}$ soil in grove, cluster and woodland communities) than in the grassland (Fig. 2d).

Fig. 2 Changes in elevation (m) (a), soil particle size distribution (g kg^{-1}) (b), soil volumetric water content (VWC, total and available) (mm^{-3}) (c), soil total nitrogen (g N kg^{-1}) (d) and *Prosopis glandulosa*, *Condalia hookeri* and *Zanthoxylum fagara* leaf $\delta^{13}\text{C}$ per thousand vs. V-PDB (‰) along the transect



Leaf $\delta^{13}\text{C}$ values

All three variables [species (S), landscape locations (L) and date (D)] had significant main effects on leaf $\delta^{13}\text{C}$ (Table 3). Mean leaf $\delta^{13}\text{C}$ of *P. glandulosa* (-25.7‰) was significantly lower than that of *C. hookeri* (-27.8‰) and *Z. fagara* (-27.4‰) (Table 3; Fig. 2e–g), and mean leaf $\delta^{13}\text{C}$ of woody plants in uplands (-27.2‰) was significantly lower than those in lower lying woodlands (-26.5‰) and playas (-26.3‰). Leaf $\delta^{13}\text{C}$ of all three species averaged -26.4‰ in April, which was significantly higher than that in August (-27.0‰) (Table 3). Leaf $\delta^{13}\text{C}$ of *P. glandulosa* in April ($-24.9 \pm 0.13\text{‰}$) was significantly higher than that in August ($-26.2 \pm 0.13\text{‰}$) (Table 3; Fig. 2e).

However, leaf $\delta^{13}\text{C}$ of *C. hookeri* and *Z. fagara* in April (-27.5 ± 0.13 and $-27.2 \pm 0.11\text{‰}$, respectively) and August (-27.8 ± 0.13 and $-27.2 \pm 0.11\text{‰}$, respectively) were statistically similar (*C. hookeri* was not present in the playa). Accordingly, the interaction between species and date was significant.

Correlations between plant $\delta^{13}\text{C}$ and biological and environmental variables

There was a significant negative correlation between leaf N_{area} and elevation for *P. glandulosa* in both April and August ($p < 0.05$) (Table 2). The significant negative correlations between leaf N_{area} and elevation observed for

Table 2 Pearson's correlation coefficients for elevation vs. soil properties, and elevation vs. plant properties along a hillslope gradient

		Elevation	
		April	August
Soil properties			
Clay		- 0.617**	–
Plant available VWC		- 0.575**	–
Soil total N		- 0.543**	–
Plant properties			
Leaf d ¹³ C	<i>Prosopis glandulosa</i>	- 0.260*	- 0.306*
	<i>Condalia hookeri</i>	- 0.430**	- 0.464**
	<i>Zanthoxylum fagara</i>	- 0.357**	- 0.346**
Leaf N _{area}	<i>Prosopis glandulosa</i>	- 0.379**	- 0.327*
	<i>Condalia hookeri</i>	- 0.239 ns	- 0.301*
	<i>Zanthoxylum fagara</i>	- 0.035 ns	- 0.242**
SLA	<i>Prosopis glandulosa</i>	- 0.565**	- 0.613**
	<i>Condalia hookeri</i>	- 0.552**	- 0.488**
	<i>Zanthoxylum fagara</i>	- 0.541**	- 0.308**

* p < 0.05; ** p < 0.01; ns, not significant (p > 0.05)

SLA, Specific leaf area; VWC, volumetric water content

C. hookeri and *Z. fagara* in August were not evident in April. There was a significant positive correlation between leaf N_{area} and leaf d¹³C for all species in April (*P. glandulosa*, R² = 0.15, p < 0.01; *C. hookeri*, R² = 0.20, p < 0.001; *Z. fagara*, R² = 0.19, p < 0.001) and August (*P. glandulosa*, R² = 0.11, p < 0.01; *C. hookeri*, R² = 0.23, p < 0.001; *Z. fagara*, R² = 0.23, p < 0.001) (Fig. 3c).

There was also a significant negative correlation between SLA and elevation for all species on both dates (p < 0.001) (Table 2). Leaf d¹³C tended to increase with SLA for all three species, however, this relationship was not significant (Fig. 3b). Relationships in April and August were consistent for all three species.

Plant available water was not related to leaf d¹³C for either *C. hookeri* or *Z. fagara* in April 2005. However, water availability was positively correlated with leaf d¹³C for *P. glandulosa* (R² = 0.11, p < 0.01) (Fig. 3a).

Stepwise multiple linear regression analysis was performed to test the effects of soil available water, SLA, and leaf N_{area} on leaf d¹³C of each species. Leaf N_{area} was the only variable that accounted for a significant proportion (p < 0.01) of the variation in leaf d¹³C.

Seasonal variation in plant d¹³C

Leaf d¹³C values of *P. glandulosa* were significantly higher than those of the other two species (Table 3). The d¹³C value of *P. glandulosa* leaves was more negative in August (mean = - 26.2‰) than April (mean = - 24.9‰), but it did not change with time for the other two species (Table 3).

ANCOVA comparisons of the slopes of the linear regression lines of leaf N_{area} versus leaf d¹³C in April and August revealed no significant differences for either *C. hookeri* or *Z. fagara* at α = 0.05. The regression slopes were significantly different for *P. glandulosa* between the two dates.

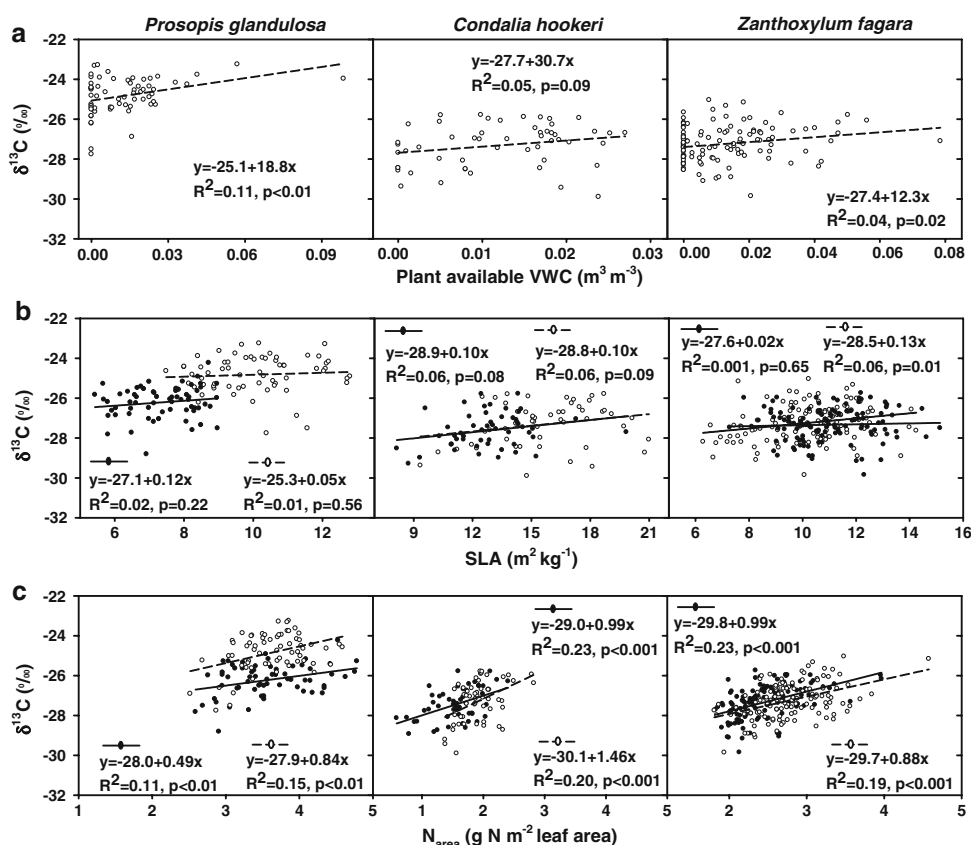
Table 3 Leaf d¹³C (‰) in different species (S), landscape locations (L) and date (D)

Source of variation	Significance (p value)	Categories	Mean	Standard error	
Species (S)	0.000	<i>Prosopis glandulosa</i>	- 25.7 a	0.13	
		<i>Condalia hookeri</i>	- 27.8 b	0.11	
		<i>Zanthoxylum fagara</i>	- 27.4 b	0.08	
Landscape location (L)	0.000	Upland	- 27.2 a	0.07	
		Drainage woodland	- 26.5 b	0.05	
		Playa	- 26.3 b	0.16	
Date (D)	0.000	April	- 26.4 a	0.07	
		August	- 27.0 b	0.07	
S × D	0.000	<i>P. glandulosa</i>	April	- 24.9 a	0.13
			August	- 26.2 b	0.13
		<i>C. hookeri</i>	April	- 27.5 a	0.13
			August	- 27.8 a	0.13
		<i>Z. fagara</i>	April	- 27.2 a	0.11
			August	- 27.2 a	0.11
S × L ^b	0.368	–	–	–	
L × D ^b	0.316	–	–	–	
S × D × L ^b	0.994	–	–	–	

^a Values followed by different letters indicate differences between means (Fisher's LSD analysis; α = 0.05), for a given source of variation, means followed by different letters were significantly different at p < 0.05

^b Since the interactions between S × L, L × D, and S × D × L were not significant, detailed comparisons were omitted

Fig. 3 Relationship between leaf $\delta^{13}\text{C}$ (‰) and plant available VWC ($\text{m}^3 \text{m}^{-3}$) (a), specific leaf area (SLA , $\text{m}^2 \text{kg}^{-1}$) (b), and leaf N_{area} (g N m^{-2} leaf area) (c) for *P. glandulosa*, *C. hookeri*, and *Z. fagara* in April (open circle) and August (filled circle) of 2005



Discussion

Spatial variability

Many studies have documented strong negative correlations between water availability and leaf $\delta^{13}\text{C}$ at ecosystem to regional scales (Comstock and Ehleringer 1993; Stewart et al. 1995; Anderson et al. 1996). Given the importance of water in this semiarid subtropical savanna parkland landscape, we hypothesized that greater soil water availability should favor greater stomatal conductance, leading to higher p_i/p_a at the sites of carboxylation and more negative leaf $\delta^{13}\text{C}$. Surprisingly, our studies showed that the overall leaf $\delta^{13}\text{C}$ values of all three woody species were actually more negative in the uplands (−27.2‰), where near-surface (0–15 cm) soil moisture was less available (Fig. 2c), than in drainage woodland (−26.5‰) and playacombos (−26.3‰), where near-surface soil moisture was relatively more available (Fig. 2c; Table 3). Regression analyses revealed that leaf $\delta^{13}\text{C}$ was negatively correlated with elevation and positively related to plant-available VWC for all three woody plant species (Fig. 3; Table 2). This result implies that these species may be relatively decoupled from near-surface soil moisture conditions or that some other variables may be overriding or interacting with water

availability to influence the observed spatial pattern of plant $\delta^{13}\text{C}$ values.

Why was the spatial pattern of plant $\delta^{13}\text{C}$ uncoupled from the spatial pattern of soil moisture availability? First of all, it should be emphasized that the assessment of soil water in this study was based on a single point in time and, as such, this assessment is clearly limited because leaf values integrate the isotopic composition of carbon acquired over a much longer period of time. Second, our assessment of soil water availability was confined to the surface soil (0–15 cm). However, *P. glandulosa*, *Z. fagara*, and *C. hookeri* all have functional roots capable of acquiring water at soil depths of 1–3 m (Table 1) (Midwood et al. 1998; Boutton et al. 1999; Zou et al. 2005). Thus, the soil moisture gradient that we have documented in the upper 15 cm of the profile (Fig. 2) may be of little or no consequence to the carbon–water relations of these deep-rooted species, which have the ability to exploit deeper—and potentially more available and more reliable—sources of soil water. Hence, we hypothesize that the variations in surface soil moisture availability across the landscape. Third, under semiarid conditions, plants have multiple adaptations for coping with water limitation, such

as high root–shoot ratios, leaf-shedding, and osmotic CO_2 diffusion; consequently, C_3 partial pressure at the adjustment. These mechanisms may dampen stomatal regulation site should be lower, and leaf $\delta^{13}\text{C}$ would be response to variations in apparent soil moisture availability negatively correlated with SLA. However, Cordell et al. (1999) found that internal resistance could not entirely indicated by vapor pressure deficit (VPD) can also be an explain the spatial variation of $\delta^{13}\text{C}$. *M. polymorpha* leaf $\delta^{13}\text{C}$ important factor in uncencing leaf water potentials and sto- and that its impact was secondary to the influence of stomatal regulation (Stewart et al. 1995; Cornwell et al. 2007). photosynthetic capacity. In our study, there was no significant relationship between leaf $\delta^{13}\text{C}$ and SLA (Fig. 3). We speculate that VPD across the landscape in our study may be similar due to the same macroclimate and that these species, which are well-adapted to water limitation and capacity (as indicated by leaf N_{area}) likely minimized any influence of SLA on leaf internal resistance to CO_2 levels, may have little stomatal response to changes in soil diffusion. moisture availability.

The robust positive correlation between leaf N_{area} and leaf $\delta^{13}\text{C}$ could account for the observed spatial pattern of leaf $\delta^{13}\text{C}$. Leaf N_{area} is highly correlated with photosynthetic capacity (Field and Mooney 1986; Hikosaka et al. 1998; Wright et al. 2003; Santiago et al. 2004). Plants with higher leaf N_{area} are likely to have lower p_i/p_a ratios due to their rapid assimilation of CO_2 during photosynthesis, resulting in higher $\delta^{13}\text{C}$ values. In our study area, leaf N_{area} increased as elevation decreased (Table 1), potentially contributing to the increase of leaf $\delta^{13}\text{C}$ along the transect. In a comparison of the linear regression lines of leaf N_{area} versus leaf $\delta^{13}\text{C}$ in April and August, ANCOVA analysis revealed no significant difference in the slopes for either *C. hookeri* or *Z. fagara*. The fact that the effects of leaf N_{area} on leaf $\delta^{13}\text{C}$ in August did not differ from that in April after a period of drought indicates that seasonal water availability had no effect on the positive relationship between leaf N_{area} and leaf $\delta^{13}\text{C}$. Therefore, we speculate that the major factor contributing to the spatial variation of leaf $\delta^{13}\text{C}$ may be photosynthetic capacity, as indicated by leaf N_{area} instead of soil moisture availability.

However, soil moisture could be influencing foliar $\delta^{13}\text{C}$ indirectly by affecting N-availability along the topographic gradient. Water and nitrogen are often co-limiting in dry-land ecosystems (Schimel et al. 1997; Hooper and Johnson 1999; Hamerlynck et al. 2004). Both soil water content and soil total N increased with decreasing elevation along our transect (Fig. 2), and both of these variables are strongly correlated with rates of N mineralization across a broad range of ecosystem types (Booth et al. 2005). Thus, water may be influencing foliar $\delta^{13}\text{C}$ by regulating soil N availability and leaf nitrogen along this gradient.

Leaf $\delta^{13}\text{C}$ may also be influenced by leaf internal resistance to CO_2 diffusion, as indicated by SLA. Vitousek et al. (1990) found that the leaf $\delta^{13}\text{C}$ of *Metrosideros polymorpha* did not reflect p_i/p_a , but it was strongly correlated with leaf mass per unit area. These researchers concluded that internal resistance to CO_2 diffusion was the main cause of the observed trend, and they speculated that lower SLA (thicker leaves) imposed greater resistance to

Interspecific variability

Interspecific variations of leaf $\delta^{13}\text{C}$ of C_3 plants are usually within the range of 2–5‰ (O'Leary 1981). We found that leaf $\delta^{13}\text{C}$ of *P. glandulosa* was up to 3‰ higher than that of *C. hookeri* and *Z. fagara* (Table 3; Fig. 2e–g). It seems unlikely that this isotopic difference is attributable to differences in environmental conditions during leaf production since these three species share relatively similar phenologies, with massive leaf production initiated in March–April resulting in peak leaf abundance between June–August (Nelson et al. 2002). However, interspecific differences in $\delta^{13}\text{C}$ could be related to the fact that *P. glandulosa* is a N_2 xer (Zitzer et al. 1996). Consistent with the results of other studies on this site (e.g. Barnes and Archer 1996; Nelson et al. 2002), leaf N_{area} of *P. glandulosa* was higher than that of *C. hookeri* and *Z. fagara*, possibly because *P. glandulosa* has access to both atmospheric and soil sources of N while the other two species are limited to the soil for their N source. The higher leaf N_{area} in *P. glandulosa* provides this plant with the potential for a higher photosynthetic capacity (Barnes and Archer 1999; Zou et al. 2005) and, therefore, higher leaf $\delta^{13}\text{C}$. This is consistent with the results of Schulze et al. (1998) who found that N_2 xers had leaf $\delta^{13}\text{C}$ values 1.2–2.4‰ higher than those of non- N_2 xing species.

Differences in plant height (Table 1) may also influence interspecific variation in leaf $\delta^{13}\text{C}$ values. Understorey plants in forest ecosystems have been reported to have lower leaf $\delta^{13}\text{C}$ values (Berry et al. 1997; Ometto et al. 2002). Two explanations for this vertical variation are possible: (1) reduction in light intensity, and/or (2) the fraction of respired CO_2 , which has much lower $\delta^{13}\text{C}$ than tropospheric CO_2 (Percy and Putsch 1991; Lai et al. 2005). In our study, the canopy of the overstorey tree *P. glandulosa* is generally fully illuminated, whereas the canopies of the relatively shorter shrub *C. hookeri* and *Z. fagara* typically receive light that has been attenuated by the overstorey *P. glandulosa* canopy (Archer 1995; Barnes and Archer 1996). Thus, variations in light intensity and the isotopic

composition of assimilated atmospheric C may both contribute to the higher leaf $\delta^{13}\text{C}$ in *P. glandulosa*.

Seasonal variability

Increases in leaf $\delta^{13}\text{C}$ during the growing season associated with progressive seasonal drought have been widely reported (Smedley et al. 1991; Ehleringer et al. 1992; Baldocchi and Bowling 2003). Because $\delta^{13}\text{C}$ can provide a relative index of instantaneous WUE and long-term transpiration efficiencies (Farquhar et al. 1989), increases in WUE after a period of drought should be accompanied by increases in leaf $\delta^{13}\text{C}$. Therefore, we hypothesized that leaf $\delta^{13}\text{C}$ values would be higher in August than in April. However, contrary to expectations, leaf $\delta^{13}\text{C}$ remained constant across time for *P. hookeri* and *Z. fagara* and were actually lower in August than in April for *P. glandulosa* (Table 3). Studies from hanging gardens in southern Utah found similar seasonal patterns (Flanagan et al. 1997), but these observations were unexplained. One potential mechanism for the decrease in leaf $\delta^{13}\text{C}$ in *P. glandulosa* during the growing season is the accumulation of epicuticular wax, which is known to occur from May through July in *P. glandulosa* (Jacoby et al. 1990). Leaf wax of *P. glandulosa* consists mostly of esters and alkanes (Mayeux and Wilkinson 1990); n-alcohol, n-acid, and n-alkane wax compounds are, on average, depleted by approximately 6.6‰ relative to total plant carbon (Conte et al. 2003). Thus, the accumulation of $\delta^{13}\text{C}$ -depleted leaf wax could account for the decreased leaf $\delta^{13}\text{C}$ in August compared to April in *P. glandulosa*.

Conclusions

In this semi-arid subtropical savanna, leaf $\delta^{13}\text{C}$ values of dominant woody species were found to be negatively correlated with elevation and near-surface soil moisture, suggesting that surface soil water has little impact on the carbon/water relations and leaf $\delta^{13}\text{C}$ values of these deeply rooted woody species. Instead, leaf $\delta^{13}\text{C}$ values along this topographic gradient appeared to be most strongly influenced by leaf N_{area} . Higher leaf $\delta^{13}\text{C}$ values at lower elevations appeared to be the result of a larger carboxylation capacity resulting from higher leaf N_{area} . Interspecific patterns of leaf $\delta^{13}\text{C}$ values also appeared to be most strongly related to leaf N_{area} . We hypothesize that surface soil water availability in this system influences landscape-scale spatial patterns of leaf $\delta^{13}\text{C}$ primarily via its effects on N-availability rather than its effects on stomatal conductance.

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