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Leaf $\delta^{13}C$ variability with elevation, slope aspect, and precipitation in the southwest United States

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Abstract Leaves from several desert and woodland species, including gymnosperms and angiosperms with both C_3 and C_4 physiology, were analyzed to detect trends in $\delta^{13}C_{leaf}$ with elevation and slope aspect along two transects in southeastern Utah and south-central New Mexico, USA. The main difference between the two transects is the steeper elevational gradient for mean annual and summer precipitation in the southern transect. For any given species, we found that isotopic differences between individual plants growing at the same site commonly equal differences measured for plants along the entire altitudinal gradient. In C_3 plants, $\delta^{13}C_{leaf}$ values become slightly enriched at the lowest elevations, the opposite of trends identified in more humid regions. Apparently, increasing water-use efficiency with drought stress offsets the influence of other biotic and abiotic factors that operate to decrease isotopic discrimination with elevation. For some species shared by the two transects (e.g., *Pinus edulis* and *Cercocarpus montanus*), $\delta^{13}C_{leaf}$ values are dramatically depleted at sites that receive more than 550 mm mean annual precipitation, roughly the boundary (pedalfer-pedocal) at which soils commonly fill to field capacity in summer and carbonates are leached. We hypothesize that, in summer-wet areas, this may represent the boundary at which drought stress overtakes other factors in determining the sign of $\delta^{13}C_{leaf}$ with elevation. The opposition of isotopic trends with elevation in arid versus humid regions cautions against standard correction for elevation in comparative studies of $\delta^{13}C_{leaf}$.

Keywords Carbon isotope composition · Elevation · Slope aspect · Precipitation · C3 and C4 plants

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Introduction

Plant performance along environmental gradients offers one way to evaluate potential plant responses to climate change. Because natural plant populations and field conditions are inherently variable, the number of physiological measurements needed to characterize population variability limits inferences from gradient analyses. Isotopic measurements linked to plant performance (e.g., water-use efficiency and $\delta^{13}C$ of leaves or wood) allow multiple observations and replications along distinct environmental gradients, such as those produced by elevation. Most isotopic studies along altitudinal gradients are concentrated in humid regions where water availability may play a role secondary to other biotic and abiotic factors. These studies have consistently shown a shift towards increasing $\delta^{13}C_{leaf}$ at upper elevations (Körner et al. 1988, 1991; Vitousek et al. 1990; Marshall and Zhang 1994; Sparks and Ehleringer 1997; Hultine and Marshall 2000). This pattern is correlated with physiological and morphological changes such as leaf thickness (Vitousek et al. 1990; Cordell et al. 1998), leaf nitrogen content (Morecroft and Woodward 1996), stomatal conductance (Meinzer et al. 1992), and stomatal density (Körner et al. 1989). In addition, correlations are reported for abiotic factors such as soil moisture (Beerling et al. 1996; Sun et al. 1996), air temperature (Panek and Waring 1995), and gradients of atmospheric pCO_2 and pO_2 (partial pressures) (Morecroft and Woodward 1990; Marshall and Zhang 1994; Ehleringer and Cerling 1995; Kelly and Woodward 1995; Marshall and Monserud 1996).

In semi-arid regions, such as the southwestern United States, moisture availability and heat load typically determine plant performance, abundance and distribution, which vary predictably with elevation and slope aspect. Higher heat loads and potential evapotranspiration make soil moisture less available at lower elevations and on south-facing slopes. In semi-arid environments, drought stress should systematically increase the $\delta^{13}C_{leaf}$ at progressively lower elevations, counteracting the influence of non-drought factors. Intra-plant and site variability may also change systematically if environmental conditions select against less water-use efficient (WUE) plants at more xeric sites (Ehleringer 1993a). Extending the argument, areas with decreased warm-season precipitation should show the greatest differentiation with elevation and slope aspect.

This study reports stable carbon isotopic composition of 668 individual plants, representing 7 genera and 11 species growing along two elevational transects in Utah and New Mexico, which differ primarily in the amount of summer precipitation. Included in the samples are gymnosperms and angiosperms with both C_3 and C_4 physiology. We tested the following hypotheses: (1) drought stress produces systematic shifts in $\delta^{13}C_{leaf}$ values with elevation, slope aspect, and latitude; (2) $\delta^{13}C_{leaf}$ differences with elevation and slope aspects among taxonomic and functional groups are consistent between the two transects; (3) any differences between the two transects for the same species can be attributed to differences in precipitation amounts; and (4) C_4 plants are insensitive to changing environmental conditions and result in more or less constant values and limited variability. At stake is the ability to compare and interpret $\delta^{13}C_{leaf}$ values in modern (Leavitt and Long 1982, 1983; Jaindl et al. 1993; Lajtha and Getz 1993), historical (Pedicino et al. 2002) and paleobotanical surveys (Van de Water et al. 1994) that rely on data from disparate localities.

Materials and methods

Background

Plants balance their need for carbon against the conductance of plant-water from their tissue by regulating gas exchange through the stomata. The efficiency at which they maintain this balance can be compared among plants by analyzing the ratio of ^{13}C to ^{12}C fixed during photosynthesis. In plants with the C_3 photosynthetic pathway, the $\delta^{13}C_{leaf}$ signature results from enzymatic fractionation mediated by stomatal and mesophyll resistance to CO_2 diffusion (Farquhar 1980; O'Leary and Osmond 1980; Farquhar et al. 1982a; Vogel 1993). When stomatal resistance is high because of closure or increased photosynthetic demand, the influence of enzymatic discrimination is diminished and $\delta^{13}C_{leaf}$ values are enriched. On the other hand, if resistance is reduced the relative influence of enzymatic discrimination increases, favoring ^{13}C depletion during fixation. Isotopic fractionation during photosynthesis can be expressed by the relationship

$$\delta^{13}C_{\text{plant}} = \delta^{13}C_{\text{air}} - a\,(b-a)\,C_{\text{i}}/C_{\text{a}} \tag{1}$$

where a is 4.4‰, b which represents a combination of both RuBP and PEP carboxylase is 27‰, and C_i/C_a is the ratio of internal to atmospheric CO_2 and is a balance between diffusion as well as stomatal and mesophyll resistance to CO_2 conductance (Farquhar 1980; Ehleringer 1991; Ehleringer and Cerling 1995).

Some plant species have evolved additional physiological mechanisms to maximize carbon acquisition while reducing water loss. In C_4 plants, CO_2 enters the mesophyll where it is carboxylated by PEP carboxylase, transported to the site of fixation, decarboxylated and then fixed using the C_3 photosynthesis cycle. These added processes bring CO_2 to the site of fixation, dampen enzymatic fractionation and therefore $\delta^{13}C_{leaf}$ values predominantly reflect fractionation from diffusion. However, during fixation decarboxylated CO_2 can leak from the bundle sheath cells back into the mesophyll tissue where it is fixed directly by C_3 photosynthesis adding a fraction of carbon that shows greater discrimination. C_4 carbon discrimination can be described as

$$\delta^{13}C_{\text{plant}} = \delta^{13}C_{\text{air}} - a - [b_4\,(b_3 - s)\,\phi - a]\,C_{\text{i}}/C_{\text{a}} \tag{2}$$

where a and the C_i/C_a ratio are the same as Eq.1, b_4 is fractionation by the enzyme PEP carboxylase (–5.7‰ at 30°C), b_3 is the fixation of carbon using the C_3 Rubisco pathway (Farquhar 1983), s is the fractionation occurring during leakage out of the bundle sheath cells (1.8‰) (Henderson et al. 1998), and ϕ is the fraction of carbon fixed in the mesophyll after leaking from the bundle sheath cells.

Carbon isotopic values distinctly differ between the two photosynthesis systems. In C_3 plants, $\delta^{13}C_{leaf}$ values of whole tissue are reported from –20‰ to –35‰ whereas C_4 plants range from –7‰ to –15‰ (Ehleringer 1993a). However, within these groupings intra- and inter-plant and species variability remains high. Differences within each photosynthetic group result from biochemical variations such as leaf nitrogen content, photosynthetic capacity, stomatal conductance, and hydraulic conductivity as well as biochemical variants in C_4 plants (Woodward 1986; Körner et al. 1988; Farquhar et al. 1989; Ehleringer 1993a; Buchmann et al.1996). An additional source of variability results from differences in life-history strategies and life-form (perennial versus annual; tree versus shrub versus herb, etc.) (Ehleringer 1993b). However, because CO_2 conductance into the plant is accompanied by release of plant water, environmental conditions such as solar radiation, leaf temperature, vapor pressure deficit, as well as soil moisture availability and salinity may exert an even greater influence over $\delta^{13}C_{leaf}$ values (Farquhar et al. 1982b; Winter et al. 1982; Madhaven et al. 1991; Ehleringer 1993a; Sandquist and Ehleringer 1995; Williams and Ehleringer 1996). Plants growing along steep environmental gradients common in semi-arid regions should show isotopic evidence of the constraints that aridity exerts on carbon acquisition.

Environmental setting

Plants were sampled along elevational gradients (~150 m altitudinal site difference) on north- and south-facing slopes in Utah and New Mexico. Each gradient spans the transition from desert scrub to mixed-conifer forest. The Utah gradient spans 1,525 m, from the uppermost site (2,745 m) at Bear's Ears (37°37′31″ N, 109°52′29″ W) to Blue Notch Canyon (1,220 m) near the Colorado River, (37°44′52″ N, 110°23′24″ W). The area is underlain by sandstone, siltstone and shale eroded to form a landscape of plateaus dissected by deep and intricate canyons. The New Mexico gradient spans 1,675 m along the western limestone escarpment of the Sacramento Mountains, with the highest site at 2,895 m near Cloudcroft (32°56′38″ N, 105°58′21″ W) and the lowest site in the Tularosa Basin at 1,220 m near Alamogordo (33°48′56″ N, 105°47′28″ W). Climatic conditions for each gradient are cooler and drier in Utah versus warmer and wetter in New Mexico (Table 1). Winter climatic conditions at both sites exhibit cold-season temperature and precipitation influenced by anticyclonic storms generated in the Gulf of Alaska, which become imbedded in the upper-air westerlies crossing the area. Warm season climatic conditions result from humid tropical air-masses invading from the Gulf of Mexico and California along the western edge of the Bermuda High. New Mexico's proximity to the moisture source results in greater warm season precipitation than Utah.

Methods

Modern vegetation was sampled in 1995 during the early summer in Utah and late summer along the New Mexico transect. At each site, five plants of each species of interest were identified and the

Table 1 Average climate conditions for selected sites near each elevational transect along with calculated temperature and precipitation lapse rates derived from climatic data available for each region

	Average mean monthly					Record length
	Temperature		Precipitation			
	Maximum (°C)	Minimum (°C)	Mean (mm)	Percent cold season	Percent warm season	
Bluff Utah (1,315 m)	21.3	3.7	199	56	44	1928–1998
Natural Bridges National Monument Utah (1,980 m)	17.5	3.1	320	51	49	1965–1998
Alamogordo, NM (1,325 m)	24.7	8.2	290	36	64	1914–1998
Cloudcroft, N.M. (2,690 m)	14.3	0.4	673	36	64	1914–1987

Calculated lapse rates

	Temperature (°C)			Precipitation Mean (mm)
	Maximum (°C)	Minimum (°C)	Mean (mm)	
Utah[a]	–0.72/100 m	–0.28/100 m	–0.50/100 m	19 mm/100 m
New Mexico[a]	–0.78/100 m	–0.55/100 m	–0.66/100 m	33 mm/100 m

[a] Calculated using 18 weather stations (1,220–2,150 m) in Utah and 14 weather station (1,220–2690 m) from New Mexico

photosynthetic tissue sampled from each. The samples consist of an equal number of plant parts collected from positions of full-irradiance at each of the four cardinal directions to ensure complete representation of intra-plant variability (Leavitt and Long 1983). The collective age of available leaves varied among species. For example, *Pinus edulis* could contain more than eight annual cohorts of needles but deciduous angiosperms have only a year's complement of leaves. To ensure full extension and needle maturity at both sites, this study analyzed *P. edulis* needles from the 1994 cohort. The leaves from the deciduous angiosperm *Amelanchier utahensis* and *Cercocarpus montanus* were formed during the year of collection, 1995. The twigs of *Juniperus* spp. and *Ephedra viridis*, as well as the leaves of *Cowania mexicana* and *Atriplex* spp. are evergreen, therefore the collected samples included tissue formed during an indeterminate period. To minimize this effect, only the outermost, recent leaves were incorporated into the samples used for analysis.

The isolation of leaf tissue components employed standard chemical extraction techniques (Pettersen 1984; Leavitt and Danzer 1993). Samples were ground in a Wiley mill until the material passed a 20-mesh screen. The ground material was then pouched using commercial filter material, heat-sealed to resemble a tea-bag. Waxes and resins were removed in a soxhlet extraction apparatus using ethanol/toluene then ethanol. The samples were then "bleached" to holocellulose with acidified Na-chlorite to remove lignin (Green 1963; Leavitt and Danzer 1993; Sheu and Chiu 1995). Significant contributions of carbon from Ca-oxalate crystals, with consequent influence on bulk $\delta^{13}C_{leaf}$, have been reported in select C_4 and CAM species (Rivera and Smith 1979; Van de Water 2002). Ca-oxalate from the *Atriplex canescens* tissue was removed following a procedure modified after Baker (1952). Holocellulose was soaked in 10% HCl for more than 24 h with two to three changes of the solution. After each treatment, samples were rinsed until neutral with deionized distilled water.

The samples were dried and removed from their pouches. The holocellulose (up to 3 mg) was placed into 6-mm Vycor glass tubes with copper oxide to provide excess oxygen. Sample tubes were evacuated, sealed, then combusted to CO_2 at 900°C and 650°C in a muffle furnace for two successive 2-h periods. All tubes were cracked in vacuo, and the CO_2 gas cryogenically isolated then measured on a Finnigan MAT Delta-S mass spectrometer. The $\delta^{13}C_{leaf}$ results are reported with respect to the internationally accepted PDB standard (Craig 1957). All values are reported as "whole-tissue" by correcting back from the analyzed "holocellulose" and "treated holocellulose (Ca-oxalate removed)" tissue fraction. The correction factors among standard preparation techniques were previously determined for the different taxa (Van de Water 2002). The C_3 gymnosperms were corrected by –0.7‰, –0.5‰ for C_3 angiosperms and –2.5‰ for C_4 angiosperms.

Laboratory standards were run with every ten samples as an internal precision check. No significant difference in the C_3 and C_4 standard values were detected compared to long-term standard values. The standard deviations associated with C_3 and C_4 standards fell below 0.2‰, the estimated overall error for preparation and analysis. Additionally, a 10% replication of samples verified reproducibility with the correlation of all species replicates exceeding r^2=0.80. In eight of the ten species r^2 values were greater than 0.90.

Results

Gymnosperms

The gymnosperms show mean isotopic values for the different genera (*Pinus* spp., *Juniperus* spp. and *Ephedra* spp.) ranging from –19.0‰ to –28.3‰ and with considerable intra- and inter-site variability (Fig. 1, Table 2). Among individual species *P. edulis* has the highest mean $\delta^{13}C_{leaf}$ and is significantly different from other gymnosperm genera (*t*-test; $P<0.01$) as well as the C_3 angiosperms (*t*-test; $P<0.01$). However, analysis of the $\delta^{13}C_{leaf}$ data for the *Pinus* spp. samples shows that trees growing at the 2,440 m site in New Mexico are particularly negative (Fig. 1). Needle morphology revealed characteristics intermediate between *P. edulis* and *P. cembroides*, the latter common in southern Arizona and northern Mexico (Martin and Hutchins 1980). The population appears isolated because needle characteristics from nearby sites are consistent with *P. edulis* morphological traits. Exclusion

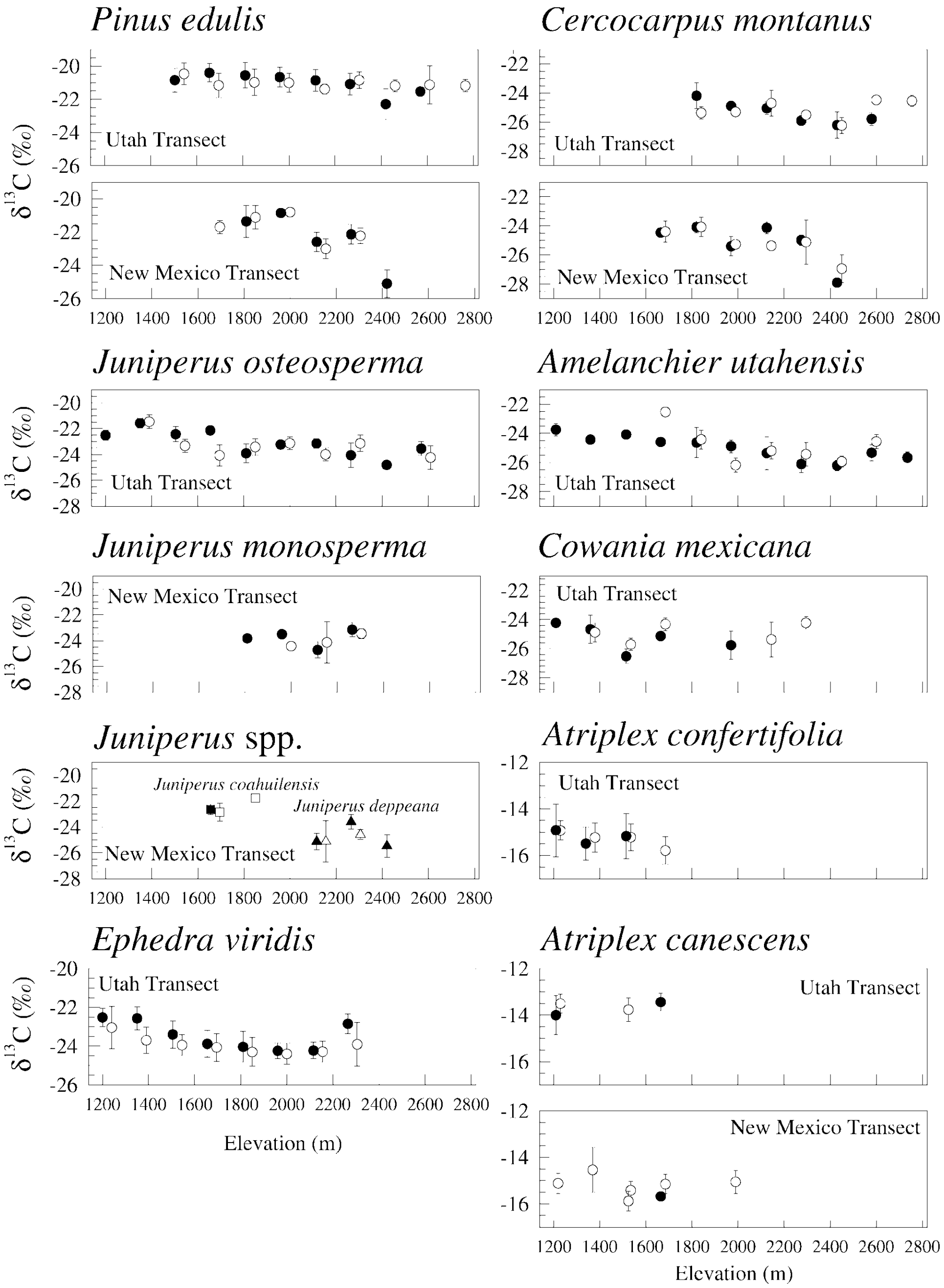


Fig. 1 Carbon isotope values from species collected along the Utah and New Mexico transects. Each set of points was collected at the same elevation, but they are plotted on each side of the elevation to emphasize similarities and differences. *Solid black symbols* represent samples from the north-facing aspect whereas *white symbols* are from the south-facing aspect. Note that the *Atriplex canescens* and *A. confertifolia* samples were collected on north and south, as well as in flat areas (*gray symbols*) along their limited distribution

of the 2,440 m site results in a less negative mean $\delta^{13}C_{leaf}$ value for the New Mexico population and a statistically stronger difference between *P. edulis* and the other gymnosperm and angiosperm species. The *P. edulis* populations along each transect are also statistically different (*t*-test; $P<0.01$), with more negative mean $\delta^{13}C_{leaf}$ values in New Mexico versus the Utah population (Fig. 1).

Within the gymnosperms all species trend toward less negative $\delta^{13}C_{leaf}$ values at lower elevations (Fig. 1, Table 3). *E. viridis* (New Mexico only), *J. coahuilensis,* and *J. deppeana* were excluded from statistical analysis because of limited sample numbers. However, in three of the five remaining species significant relationships were found between $\delta^{13}C_{leaf}$ and elevation (Table 3). Although non-significant, the steepest gradient occurs in *P. edulis* from New Mexico (–0.41‰/100 m). Exclusion of the anomalous site at 2,440 m reduces the gradient change (–0.21‰/100 m), yet the results remain three times the shift (–0.07‰/100 m) recorded in the Utah population. The Utah populations of *J. osteosperma* and *E. viridis* also show significant isotopic changes (Table 3). Of these species *J. osteosperma* shows the greatest absolute change (1.92‰) across an expanded distribution (1,370 m). Regression analysis of each site split by aspect is confined to the Utah species *J. osteosperma* (south-facing slope) and *E. viridis* (north-facing slope) (Table 3).

Table 2 Mean (±1σ) $\delta^{13}C_{leaf}$ values for each of the taxonomic groups and individual species collected along the elevational gradients in Utah and New Mexico. Maximum and minimum $\delta^{13}C_{leaf}$ values as well as the number of plants sampled for each of the groups and sites are listed as well

	Mean (‰)	Maximum (‰)	Minimum (‰)	*n*
C_3 plants				
Gymnosperms	–23.0±1.5	–19.0	–28.3	413
Angiosperms	–25.1±1.0	–22.2	–27.9	255
C_4 plants				
Angiosperms	–16.1±1.0	–14.0	–18.2	111
Utah gymnosperms				
Pinus edulis	–21.0±0.8	–19.0	–23.2	83
Juniperus osteosperma	–23.3±1.1	–20.9	–25.8	121
Ephedra viridis	–23.7±0.9	–21.3	–25.6	82
New Mexico gymnosperms				
Pinus edulis[a]	–21.8±0.9	–20.1	–24.0	44
Juniperus coahuilensis	–22.8±0.6	–22.0	–24.0	9
J. deppeana	–25.0±1.3	–23.0	–28.3	24
J. monosperma	–23.9±0.8	–21.5	–25.1	35
Ephedra viridis	–23.3±1.1	–22.1	–25.6	15
Utah C_3 angiosperms				
Amelanchier utahensis	–25.0±1.1	–22.2	–27.1	88
Cercocarpus montanus	–25.3±0.8	–22.8	–27.5	64
Cowania mexicana	–25.1±1.0	–23.3	–27.2	48
New Mexico C_3 angiosperm				
Cercocarpus montanus	–24.9±1.0	–23.3	–27.9	55
Utah C_4 angiosperms				
Atriplex canescens	–14.7±0.6	–14.0	–16.6	20
A. confertifolia	–16.4±0.8	–14.4	–18.2	57
New Mexico C_4 angiosperms				
A. canescens	–16.3±0.7	–14.4	–17.6	34

[a] Values calculated with the site at 2,440 m removed. See text for explanation

Inter-site variability was expected to show a smaller range of values at lower elevations because of increasing environmental extremes. However, the overall $\delta^{13}C_{leaf}$ variability of gymnosperms, judged by the standard deviation of the calculated mean site value (Fig. 1), lacks any systematic reduction with elevation. In addition, comparison of values from site pairs also fails to show consistent enrichment of $\delta^{13}C_{leaf}$ on the warmer, drier south-facing slopes, except for *E. viridis* from Utah (Fig. 1).

C_3 angiosperms

Three species of C_3 angiosperms were collected from Utah and a single species from New Mexico (Fig. 1). The overall individual isotope values range from –22.2‰ to –27.9‰ with the least negative values overlapping the more negative tail of the gymnosperm distribution. Overall, mean $\delta^{13}C_{leaf}$ of the C_3 angiosperms is 2.1‰ lower than the C_3 gymnosperms. No statistically significant differences were found among the sampled C_3 populations (Table 2). Overall, each of the C_3 angiosperms trends toward less negative $\delta^{13}C_{leaf}$ values at lower elevations (Fig. 1, Table 3). Two sampled populations, *A. utahensis* from Utah and *C. montanus* from New Mexico, show statistically significant increases in $\delta^{13}C_{leaf}$ at lower elevations (Table 3). The *C. montanus* collected in Utah also becomes less negative at lower elevation but shows a smaller, non-significant shift. Analysis of the data grouped by slope aspect shows significant relationships with elevation on north-facing Utah slopes for *A. utahensis* and *C. montanus* (Table 3). *A. utahensis* grows along a significant portion of the gradient (1,525 m) compared to a more restricted *C. montanus* distribution of only 765 m.

Analysis of site pairs, north- versus south-facing aspects, shows greater $\delta^{13}C_{leaf}$ enrichment in south-facing sites compared to the C_3 gymnosperm species tested. This is especially true in Utah where 75% of south-facing sites show less negative mean $\delta^{13}C_{leaf}$ compared to the north-facing values. In New Mexico this differentiation is unsupported with nearly indistinguishable mean $\delta^{13}C_{leaf}$ values in *C. montanus* except for sites at 2,285 and 2,440 m (Fig. 1). Whereas a greater number of sites have less negative isotopic values, no statistically significant difference in mean values was detected. The single, most consistent characteristic of the gradients is that the mean isotopic difference between north- and south-facing sites at the same elevation often falls within one standard deviation of the means. A hypothesized reduction of variability with decreasing elevation is only evident in *A. utahensis* sites below 1,830 m, and occurs in both the single south-facing site as well as the north-facing sample localities (Fig. 1).

Table 3 The table contains statistically significant results of linear regression analysis on $\delta^{13}C_{leaf}$ values versus elevation for each species. Results labeled “Both” combine all the data at each elevation. The data were then split by slope aspect and only those gradients that showed statistically significant correlation are reported. The “gradient change” is the isotopic difference between lowest and highest site based on calculated values. A mixed model ANOVA was used to determine the relationship between $\delta^{13}C_{leaf}$ values, elevation and slope-aspect. For each species all data (*C*) were analyzed where more than three data pairs were collected along each gradient. Statistical significance indicates that a combined effect of elevation and aspect occurs within each data set. The data was then split by slope-aspect to determine significant trends among each component (*N* north, *S* south)

Species	Aspect	*Y* intercept	Slope (‰) 100 m	r^2	*P*	Gradient change (‰)	Elevational range (m)	Mixed model ANOVA (*P*)		
								C	N	S
Utah										
C_3 gymnosperms										
Pinus edulis	Both	–18.87	–0.07	0.37	0.01	–0.85	1,220	0.04	0.01	0.71
Juniperus osteosperma	Both	–19.96	–0.14	0.46	<0.01	–1.92	1,370	<0.01	<0.01	<0.01
	South	–19.26	–0.17	0.52	0.04	–2.07	1,220			
Ephedra viridis	Both	–21.39	–0.09	0.28	0.04	–0.96	1,065	<0.01	<0.01	0.13
	North	–21.83	–0.08	0.50	0.05	–0.85	1,065			
C_3 angiosperms										
Amelanchier utahensis	Both	–21.49	–0.15	0.46	<0.01	–2.29	1,525	<0.01	<0.01	<0.01
	North	–21.36	–0.17	0.84	<0.01	–2.59	1,525			
Cercocarpus montanus	Both	–23.43	–0.05	0.05	0.47	–0.46	915	<0.01	<0.01	<0.01
	North	–19.35	–0.24	0.82	0.01	–1.82	760			
Cowania mexicana	Both	–25.93	–0.08	0.12	0.36	–0.73	915			
C_4 angiosperms										
Atriplex canescens	Both	–12.34	0.01	0.04	0.81	0.05	455			
A. confertifolia	Both	–11.39	–0.17	0.57	0.03	–0.77	455			
New Mexico										
C_3 gymnosperms										
Pinus edulis[a]	Both	–16.80	–0.21	0.33	0.11	–1.28	610	<0.01	<0.01	<0.01
Juniperus monosperma	Both	–25.38	0.01	0.10	0.50	0.05	455			
C_3 angiosperms										
Cercocarpus montanus	Both	–20.10	–0.20	0.36	0.05	–1.53	765	<0.01	<0.01	<0.01
C_4 angiosperms										
Atriplex canescens	Both	–13.27	–0.03	0.06	0.61	–0.23	760			

[a] Values calculated with the 2,440 m site removed

C_4 angiosperms

Plants with C_4 physiology are represented by two species of *Atriplex*, *A. canescens* from Utah and New Mexico and *A. confertifolia* from Utah. The overall range of $\delta^{13}C_{leaf}$ in the C_4 samples is 4.2‰, but $\delta^{13}C_{leaf}$ ranges over 3.8‰ in *A. confertifolia* and 2.6‰ to 3.2‰ in *A. canescens* from Utah and New Mexico, respectively. Mean $\delta^{13}C_{leaf}$ of *A. canescens* in New Mexico is similar to that of *A. confertifolia* from Utah. However, the $\delta^{13}C_{leaf}$ values for the two *A. canescens* populations differ significantly (*t*-test, $P<0.01$) with the Utah population more enriched in ^{13}C (Table 2). The *Atriplex* species were collected at a limited number of low elevation sites that included both north- and south-facing slopes and flat alluvial plains. Of the three populations only *A. confertifolia* shows significant isotopic changes in mean site values with elevation, but the shift and elevational range are limited (Fig. 1, Table 3). A decrease in site isotope variability at lower elevations was not detected in any of the sampled populations. The limited data set did not allow comparisons of north- and south-facing pairs.

Discussion

Taxonomic variability

Analysis included gymnosperm and angiosperm species with both C_3 and C_4 photosynthetic pathways. Results show that C_3 angiosperms are significantly depleted in $\delta^{13}C_{leaf}$ compared to co-occurring gymnosperms as seen in other studies using leaf tissue (DeLucia et al. 1988; DeLucia and Schlesinger 1991; Lajtha and Barnes 1991; Marshall and Zhang 1994), as well as wood (Stuiver and Brazianus 1987; Leavitt and Newberry 1992). The mechanisms responsible for this differentiation remain unclear. However, Leavitt and Newberry (1992) suggested that dissimilar plant traits (leaf morphology, deciduous versus evergreen leaf habits) as well as physiological mechanisms of carbon acquisition, mediated by climatic conditions, play an important role. DeLucia and Schlesinger (1991) showed arid-land angiosperm shrubs differentiating themselves isotopically from co-occurring gymnosperms with increased $\delta^{13}C_{leaf}$ values.

Within each of the taxonomic groupings significant differences between species only occurred within the C_3 gymnosperms. Isotopic values are significantly different between *Pinus edulis* versus *Juniperus* spp. and *Ephedra* spp. Differentiation of the pinyon pines (*P. monophylla*, *P. edulis*) and *Juniperus* spp. is apparent in studies of modern plant communities (DeLucia and Schlesinger 1991) as well as analysis of herbarium material from the Four-Corners region near the Utah transect site (Pedicino et al. 2002). Physiologically, pinyon pines show a greater potential for carbon gain than *Juniperus* spp., but the latter can tolerate greater levels of water stress (Nowak et al. 1999). Increased water stress on *P. edulis* may result in reduced stomatal conductance and could account for less negative $\delta^{13}C_{leaf}$ values. However, the similarity in $\delta^{13}C_{leaf}$ values between *Juniperus* and *Ephedra* is surprising considering their disparate growth forms and differences in their dominant photosynthetic tissue, scale-leaf versus stem. Previous studies have shown divergent $\delta^{13}C_{leaf}$ values between these plant traits in C_3 angiosperm species (Ehleringer et al. 1987; Comstock and Ehleringer 1988; Delucia et al. 1988; Delucia and Schlesinger 1991) but not in gymnosperms. Analysis of the C_3 angiosperms species from this study show no significant differences although they differ by leaf type, habit (winter deciduous versus evergreen) and elevational distribution across each transect (Fig. 1).

Inter-site variability

In the semi-arid southwestern United States, high heat loads and arid conditions with decreasing elevation are hypothesized to influence $\delta^{13}C_{leaf}$ values. In addition, selective pressure to maximize carbon acquisition, water-use efficiency, and drought tolerance at lower elevations (Ehleringer 1993a) was also hypothesized to reduce plant to plant $\delta^{13}C$ variability. Results from this study found that $\delta^{13}C_{leaf}$ values at lower elevations increased in C_3 plants as expected. However, mean isotopic changes over the elevational range of each C_3 species barely exceeded the isotopic variability between plants at each site. Enrichment in $\delta^{13}C_{leaf}$ occurred on south-facing C_3 samples in over half of the sites. Yet, heavier isotopic values showed no statistically significant difference from the paired north-facing value in most cases. In C_4 plants, $\delta^{13}C_{leaf}$ changed little in *A. canescens* as predicted for this photosynthesis physiology. However, a limited *A. confertifolia* data set does show less negative $\delta^{13}C_{leaf}$ values at lower elevations.

Previous studies from humid regions report isotopic trends opposite of this study with increased $\delta^{13}C_{leaf}$ values at higher elevations (Körner et al. 1988, 1991; Vitousek et al. 1988, 1990; Marshall and Zhang 1994; Kelly and Woodward 1995; Sparks and Ehleringer 1997; Hultine and Marshall 2000). Increased $\delta^{13}C_{leaf}$ content with increasing elevation is argued to result from changes in biochemical processes resulting from reduced CO_2 and O_2 partial pressures (Körner et al. 1988, 1991; Kelly and Woodward 1995), or increased photosynthetic capacity as plants strive to maintain constant interior to exterior CO_2 gradients (Körner and Diemer 1987; Friend et al. 1989; Marshall and Zhang 1994; Hultine and Marshall 2000). As a measure of increased photosynthetic capacity, increased leaf-nitrogen levels have been found in upper elevation populations (Körner and Deimer 1987) and in plants from wetter, more productive riparian areas in arid environments (Sparks and Ehleringer 1997). However, Hultine and Marshall (2000) failed to find a similar relationship in three of four conifers sampled from the northern Rocky Mountains, but suggest that redistribution of leaf nitrogen occurred post-leaf expansion.

Beyond carbon acquisition physiology, morphological changes may influence carbon acquisition physiology at upper elevations (Körner et al. 1989, 1991) and may be reflected in $\delta^{13}C_{leaf}$ values (Hultine and Marshall 2000). Changes in leaf morphology and increased mesophyll resistance with lowered pCO_2 conditions correlate to less negative $\delta^{13}C$ values in upper elevation *Metrosideros polymorpha* populations from Hawaii (Vitousek et al. 1988, 1990). Further analysis, however, failed to find a similar relationship in additional sites studied by Meinzer et al. (1992). Changes in $\delta^{13}C_{leaf}$ were ascribed to reduced moisture availability at lower elevations and hypoxic bog conditions at upper sampling sites. The effect of reduced moisture availability is the dominant environmental effect at low elevation sites tested in this study. Increased $\delta^{13}C_{leaf}$ values are linked to decreased hydraulic conductance where moisture availability is reduced (Farquhar et al. 1980; Waring and Silvester 1994; Warren and Adams 2000). In addition, low moisture conditions can lead to sapwood cavitation that also reduces stomatal conductance contributing to greater $\delta^{13}C_{leaf}$ values at lower elevations (Waring and Silvester 1994; Warren and Adams 2000).

Isotopic values from this study show less negative $\delta^{13}C_{leaf}$ values at lower elevations, suggesting that physiological and morphological adjustments with increasing elevation are secondary to the physiological effect of restricted water availability at lower elevations. These findings are consistent with previous studies that sampled gymnosperms along arid elevational gradients. Lajtha and Getz (1993) found $\delta^{13}C_{leaf}$ enrichment in *P. edulis* (–0.04‰/100 m) and *J. monosperma* (–0.03‰/100 m) growing in northern New Mexico, USA. In addition, Leavitt and Long (1982, 1983) reported similar results for *P. edulis* and a mix of *Juniperus* spp. (*J. monosperma*, *J. deppeana*, *J. osteosperma*) growing in Arizona, USA. A similar increase in $\delta^{13}C$ values in *Ephedra* spp. with drier conditions is apparent in plants collected from a wet wash habitat, a middle transition zone and upper dry-slope area (Ehleringer and Cooper 1988). Isotopic change with elevation in these other studies is consistently less than isotopic trends from our more comprehensive data set (Table 2, Fig. 1), and probably results from limited sampling.

The C_4 species of *Atriplex* spp. were restricted to lower elevations and collected on both north- and south-facing slopes as well as in flat areas. As expected, *A. canescens* shows an invariant $\delta^{13}C_{leaf}$ response to elevation and slope aspect along both transects. However, a statistically significant increase in $\delta^{13}C_{leaf}$ values was found in limited number of *A. confertifolia* samples. In C_4 plants, the C_i/C_a ratio depends on physiological and structural controls (O'Leary 1981; Evans et al. 1986; Buchmann et al. 1996), although it is relatively invariant to changes in light, humidity, and nutrients (Wong et al. 1985a, b, c). Changes in the isotopic composition of C_4 plants and the C_i/C_a ratio depend upon the leakage factor (ϕ) (Sandquist and Ehleringer 1995). In this study *A. confertifolia*, shows a shift toward increased $\delta^{13}C_{leaf}$ at lower elevations, a trend opposite to that found with increasing salinity along gradients near the Great Salt Lake, Utah (Sandquist and Ehleringer 1995). This increase can be explained if leakage is low, and drier conditions at low elevations affect the C_i/C_a ratio (Henderson et al. 1992). Modeled C_i/C_a ratio and isotopic discrimination suggest that leakage values must be below 0.32 to explain increasing $\delta^{13}C_{leaf}$ values at lower elevations (Sandquist and Ehleringer 1995). Samples of *A. canescens* show little response with elevation. At lower elevations, we sampled *A. canescens* in flat or convex terrain, where runoff accumulates, and thus we did not find comparable enrichment to *A. confertifolia*.

Along with species differences, the two populations of *A. canescens* are statistically distinct, with the population in Utah showing increased $\delta^{13}C_{leaf}$ values compared to the New Mexico population. These differences are another measure of the complex taxonomy of *A. canescens* where populations show distinct ploidy levels between these two areas. For example, Stutz and Sanderson (1979) report tetraploids ($4n$=36) growing in Utah and diploid ($2n$=18) populations in New Mexico. Dunford (1984, 1985) further differentiated the populations in south-central New Mexico and west Texas by soil type, diploid populations growing on sandy soils, hexaploids on heavier clay-rich alluvial soils and tetraploids in intermediate areas. Neither the ploidy level of the two populations sampled in this study nor the effect of ploidy differences and/or environmental conditions on $\delta^{13}C_{leaf}$ values are known. Differentiation of the two populations warrants further study.

Intra-site variability

Ehleringer (1993a) suggested that the interplay between carbon acquisition and water loss puts arid-land plants under strong selective pressure to maximize stomatal physiology, water-use efficiency, and drought tolerance. Environmental changes occurring along gradients are mimicked at each elevation as increased solar radiation results in warmer, drier conditions on south than north-facing aspects. Although microclimatic differences with slope aspect produce conspicuous differences in plant community composition and density, they do not yield significant isotopic differences. In fact, most mean site values fell within 1σ of each other (Fig. 1). Nevertheless, the majority of south-facing sites are less negative isotopically than their north-facing counterparts at the same elevation. But these sites occur randomly across each gradient and are not found consistently at the lowest elevations. The single exception is the significant difference between members of the lowest *A. utahensis* aspect pair. Also the single north-facing sites below 1,675 m show reduced variability (Fig. 1). Standard deviations at each site below 1,675 m (0.3‰ per site) are half of what they are above 1,675 m (0.6‰).

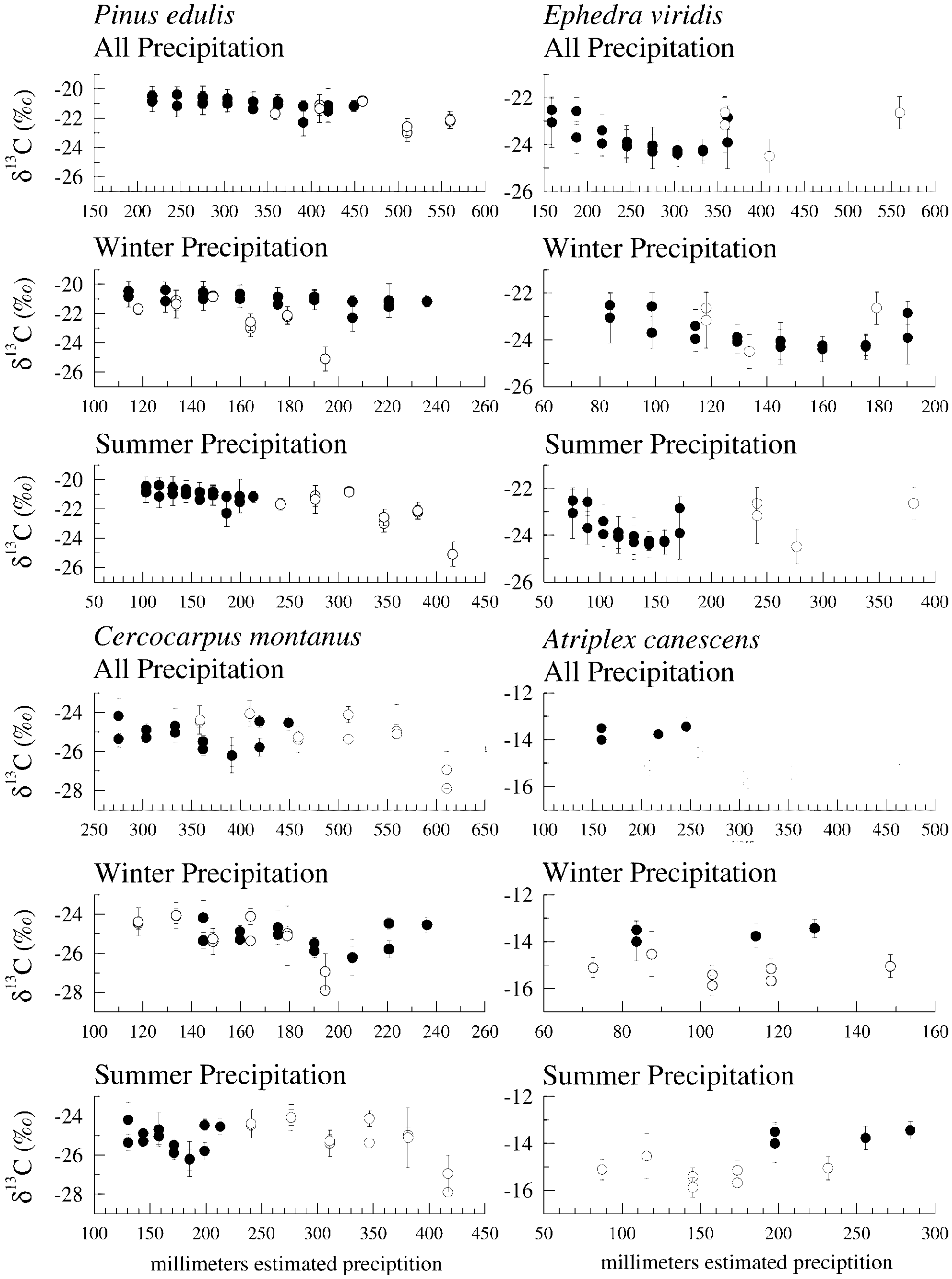
Intra-site variability in the C_4 genera was expected to be low following the results of Marino et al. (1992). However, analysis of leaf material showed increased variability with mean site values having a standard deviation (1σ) as high as 1.13‰. The differences may result from sampling strategies employed. Marino et al. (1992) pooled leaves from five plants at each site into a single $\delta^{13}C_{leaf}$ sample compared to individual plant $\delta^{13}C_{leaf}$ determinations that were then averaged into each site value in this study. The limited number of samples from this study showed no apparent trend of intra-site isotopic variability with slope aspect.

Isotopic variability with precipitation

Precipitation gradients compared to $\delta^{13}C_{leaf}$ values elsewhere in the world have produced either negative (Stewart et al. 1995; Anderson et al. 1996; Korol et al. 1999; Miller et al. 2001) or no correlation (Schulze et al. 1998). Moore et al. (1999) measured gas exchange and $\delta^{13}C_{leaf}$ of *J. osteosperma* and *J. occidentalis* along altitudinal and regional environmental gradients in the Great Basin of North America. Our results most closely match those of Moore et al. (1999) who found that $\delta^{13}C_{leaf}$ was negatively correlated with annual precipitation, although they did not find a significant correlation with elevation. Carbon isotopic values for species collected along gradients in both Utah and New Mexico (*P. edulis*, *E. viridis*, *C. montanus*, and *A. canescens*) are compared to the mean monthly annual, winter and summer precipitation values (Table 1, Fig. 2). *P. edulis* and *C. montanus* both show clear trends of decreasing $\delta^{13}C_{leaf}$ with increasing precipitation, particularly in summer. There is considerable overlap between the Utah and New Mexico transects for similar precipitation values. This probably reflects the fact that summer aridity characterizes all elevations in southeastern Utah and the lower elevations in southern New Mexico.

For some species, we measured a dramatic decrease in $\delta^{13}C_{leaf}$ above mean annual precipitation levels of about 550 mm. Similar results have been found in *Eucalyptus* spp. (Miller et al. 2001) and *Pinus radiata* (Korol

Fig. 2 $\delta^{13}C_{leaf}$ of species shared by the Utah and New Mexico transects versus estimated summer, winter and annual precipitation at each of the sampling sites. Winter precipitation consists of values from October to March whereas summer precipitation includes April to September. *Solid circles* are sites from the Utah gradient whereas *open circles* are from New Mexico



et al. 1999). Interestingly, the 550 mm boundary coincides with the mid-continent zone at which $CaCO_3$ begins to accumulate in soils of summer-wet regions, the so-called pedalfer-pedocal boundary (Birkeland 1974). This boundary, which indicates the amount of precipitation leaching downward into the soil and into the vadose zone, generally separates the humid eastern from the semi-arid western United States. In interfluve areas of semi-arid southwestern USA, both physical monitoring (Gee et al. 1994) and tracer studies (Phillips 1994) show that desert vegetation efficiently extracts all water that infiltrates through the top soil layers, and that no water reaches the vadose zone. Desert soils seldom reach field capacity (20% H_2O), demanding high water-use efficiency in desert plants. We suggest that isotopic depletion above 550 mm indicates the point at which soils commonly fill to field capacity during the growing season, reducing xylem cavitation, and allowing plants to increase discrimination against ^{13}C. At the very least, we suggest that isotopic responses may vary nonlinearly along precipitation gradients that span local or regional boundaries between pedocal and pedalfer soils. In summer-wet areas, the pedalfer-pedocal boundary may mark the point at which drought stress overtakes other biotic and abiotic factors in determining the sign of $\delta^{13}C_{leaf}$ trends with elevation.

Conclusions

In the southwestern United States, $\delta^{13}C_{leaf}$ values in selected C_3 species decrease with elevation. The isotopic shift is consistent with reduced hydraulic conductance from increased drought stress at lower elevations in this climatically extreme environment. However, the absolute change occurring across gradients often barely exceeds the variability of $\delta^{13}C_{leaf}$ values measured in individual plants growing at any given elevation. Comparison of seasonal precipitation against the $\delta^{13}C_{leaf}$ content for species growing along both gradients shows an isotopic shift towards increasing discrimination where soils fill to field capacity during the growing season. This elevation coincides with the boundary between pedalfer and pedocal soils, suggesting that moisture availability at low elevation sites is increasingly important in driving $\delta^{13}C_{leaf}$ values. Finally, the unsystematic variability found in this and other studies strongly suggest that there is little justification in correcting for $\delta^{13}C_{leaf}$ variations with elevation in comparative studies that encompass semi-arid environments.

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