

Environmental and species-specific controls on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in dominant woody plants from central-western Argentinian drylands

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Abstract Spatial variation in mean annual precipitation is the principal driver of plant water and nitrogen status in drylands. The natural abundance of carbon stable isotopes ($\delta^{13}\text{C}$) in photosynthetic tissues of C3 plants is an indicator of time-integrated behaviour of stomatal conductance; while that of nitrogen stable isotopes ($\delta^{15}\text{N}$) is an indicator of the main source of plant N (soil N *vs.* atmospheric N_2). Previous studies in drylands have documented that plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values increase with decreasing mean annual precipitation due to reductions in stomatal conductance, and soil enriched in ^{15}N , respectively. However, evidence for this comes from studies focused on stable isotopes measurements integrated at the plant community level or on dominant plants at the site level, but little effort has been made to study C and N isotope variations within a species growing along rainfall gradients. We analysed plant $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C/N values of three woody species having different phenological leaf traits (deciduous, perennial and aphyllous) along a regional mean annual precipitation gradient from the central-western Argentinian drylands. Noticeably, plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the three woody species did not increase towards sites with low precipitation or at the start of the growing season (drier period), as we expected. These results suggest that environmental factors other than mean annual precipitation may be affecting plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The short-term environmental conditions may interact with species-specific plant traits related to water and nitrogen use strategies and override the predictive influence of the mean annual precipitation on plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ widely reported in drylands.

Key words: desert woody plants, photosynthetic capacity, plant nitrogen use strategies, plant water use strategies, stomatal conductance *proxy*.

INTRODUCTION

Spatial variation in precipitation patterns at a regional scale are expected to alter plant water and nitrogen (N) status, and consequently, the physiological performance in plants located in drylands (Reynolds *et al.* 2004; Newman *et al.* 2006). Studies of variations in plant C ($\delta^{13}\text{C}$) and leaf N ($\delta^{15}\text{N}$) stable isotopes along rainfall gradients have been widely used to infer the role of precipitation on plant water–C and water–N relationships, and thus, plant responses to precipitation changes (Canadell *et al.* 2002; Dawson *et al.* 2002). Several studies conducted in drylands had shown increases in plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with decreasing mean annual precipitation (Stewart *et al.* 1995; Austin & Sala 1999; Aranibar *et al.* 2004; Swap *et al.* 2004; Weiguo *et al.* 2005; Hartman &

Danin 2010). The pattern observed in plant $\delta^{13}\text{C}$ is associated with reductions in plant stomatal conductance (Farquhar *et al.* 1989) and changes in $\delta^{15}\text{N}$ have been related to plant N uptake in soils enriched in ^{15}N (Robinson 2001), both as a consequence of increasing water deficits. These studies have focused on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variation at community level or on locally abundant plant species. However, the importance of C and N isotope variations in the same plant species growing along rainfall gradients has been neglected (but see Lázaro-Nogal *et al.* 2013), and studies of this variation could enhance our understanding of species-specific plant responses to precipitation changes in water-limited ecosystems.

Plant $\delta^{13}\text{C}$ reflects time-integrated effects of stomatal conductance and photosynthetic capacity on the ratio between leaf intercellular and ambient partial pressure of CO_2 (c_i/c_a) in C3 plants (Farquhar *et al.* 1989; Seibt *et al.* 2008; Cernusak *et al.* 2013). When soil moisture is low and atmospheric demand is high,

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Accepted for publication October 2016.

stomatal closure is produced to prevent water loss; which in turn reduces CO₂ diffusion from air to leaf intercellular space (Cernusak *et al.* 2013). These decreases in c_i/c_a minimize the discrimination against ¹³C exerted by Rubisco during photosynthesis and consequently increase $\delta^{13}\text{C}$ in photosynthetic tissues (Farquhar *et al.* 1989; Cernusak *et al.* 2013). In addition, leaf nitrogen content (leaf N per mass, leaf N per area or leaf N percentage) can positively influence plant $\delta^{13}\text{C}$ by enhancing photosynthetic capacity (Wright *et al.* 2003; Cernusak *et al.* 2013). Thus, when leaf N is relatively high, its photosynthetic capacity is also high; which in turn reduces the c_i/c_a and increases plant $\delta^{13}\text{C}$ (Bai *et al.* 2008). Therefore, plant $\delta^{13}\text{C}$ variations along precipitation gradients would be driven by changes in soil and atmosphere moisture conditions and species-specific leaf N content variation.

Previous studies have shown species-specific variations in plant $\delta^{13}\text{C}$ associated with spatial and temporal (or seasonal) precipitation changes in drylands (Golluscio & Oesterheld 2007; Lázaro-Nogal *et al.* 2013). These species-specific responses are related to inter-specific differences in water use strategies (Golluscio & Oesterheld 2007; Moreno-Gutiérrez *et al.* 2012) and intra-specific variation due to phenotypic plasticity (Lázaro-Nogal *et al.* 2013). In general, stress-tolerant woody species tolerate different water stress levels due to very low stomatal conductance and insensitivity to water availability changes (Orians & Solbrig 1977; Wright *et al.* 2003; Lázaro-Nogal *et al.* 2013). On the other hand, drought avoiding woody species generally have plant structures that allow them to escape from temporal and spatial water shortage; for example, leaf deciduousness, stomatal closure sensitive to soil water changes and dimorphic root systems to access more stable water sources (Orians & Solbrig 1977; Lambers *et al.* 2008; Giordano *et al.* 2011). Thus, studying patterns of $\delta^{13}\text{C}$ in species with different water use strategies and widespread distribution along precipitation gradients can be useful to understand species-specific stomatal responses to precipitation changes.

In drylands, increases in leaf $\delta^{15}\text{N}$ are mainly associated with declines in annual precipitation and concomitant increases in soil $\delta^{15}\text{N}$ (Handley *et al.* 1999; Aranibar *et al.* 2004). These results indicate that the effect of precipitation on leaf $\delta^{15}\text{N}$ is mediated by soil $\delta^{15}\text{N}$ because the soil is the main source of plant N (Evans 2001; Robinson 2001). Increases in aridity are accompanied by enrichment in soil ¹⁵N as a consequence of increases in N losses relative to N turnover, given by low and infrequent rainfall events; that is more open N cycling (Austin & Sala 1999; Handley *et al.* 1999). In addition, plant physiological factors that determine N uptake, assimilation and allocation may explain variation in leaf $\delta^{15}\text{N}$ (Evans

2001) and mask trends observed along precipitation gradients (Schulze *et al.* 1991). In general, plant N₂-fixing species show lower $\delta^{15}\text{N}$ values than non-N₂-fixing species (Schulze *et al.* 1991; Aranibar *et al.* 2004; Bai *et al.* 2009) in arid and mesic environments, with soils with positive $\delta^{15}\text{N}$ values due to the use of recently fixed atmospheric N₂ (Robinson 2001). Therefore, species-specific responses of N uptake may determine different responses of $\delta^{15}\text{N}$ to spatial variation in annual precipitation.

In this study, we explored the spatial and seasonal variation in plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of three dominant woody plant species along a precipitation gradient in central arid and semiarid ecosystems from Argentina. Because of the importance of rainfall on plant stomatal conductance and N losses/turnover, we hypothesized that plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of woody plants will be mainly driven by mean annual precipitation. Otherwise, despite differences in functional traits among species, we predicted increases in plant $\delta^{13}\text{C}$ values with increasing aridity, and in the dry season, due to declines in stomatal conductance. We also predicted higher plant $\delta^{15}\text{N}$ values with increasing aridity, and in the dry season, due to soil ¹⁵N enrichment. In addition, we expected that plant $\delta^{15}\text{N}$ in leguminous species would not vary along the aridity gradient due to their use of atmospheric N₂ and relative independence of soil N. Our objective was to evaluate inter- and intra-specific differences in plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and nutrient status (C/N ratio) of three dominant woody plant species having different phenological leaf traits (deciduous, perennial and aphyllous) in sites with different levels of mean annual precipitation. We used plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of dominant woody plants widely distributed in Argentinian drylands (Monte and Arid Chaco eco-regions).

MATERIALS AND METHODS

Study sites

Our study was conducted at four sites located along a wide precipitation gradient in central-western Argentina. These sites were selected to represent arid conditions typical of the central Monte desert in the western sector of the precipitation gradient (Roig *et al.* 2009), and semiarid conditions in the transition from Monte to Arid Chaco in the eastern sector of the precipitation gradient (Cabido *et al.* 1993). The gradient covers a significant range in annual mean rainfall levels, with long-term (1950–2000) mean annual precipitation ranging from 130 to 357 mm and mean annual temperature from 17.7 to 19.2°C (Table 1). More than 70% of the total annual rainfall occurs during the summer (from October to March) and this coincides with start and end of the growing season in the study area (Labraga & Villalba 2009). Soils are sandy Entisols (Abraham *et al.* 2009).

Table 1. Description of the four study sites located along precipitation gradient from central-western Argentinian drylands

	Sites			
	Very low precipitation (Médanos Grandes)	Low precipitation (Bermejo)	Middle precipitation (Marayes)	High precipitation (Chepes)
Latitude (South)	31°42'55.44"	31°39'30.74"	31°29'19.97"	31°21'1.12"
Longitude (West)	68°8'47.44"	67°46'12.94"	67°19'0.59"	66°49'24.17"
Mean annual precipitation (coefficient of variation) in mm	130 (79%)	188 (86%)	262 (84%)	357 (85%)
Mean annual temperature in °C (standard deviation)	17.7 (±6.2)	18.1 (±6.1)	18.8 (±5.8)	19.2 (±5.6)
Aridity Index (mean annual precipitation/evapotranspiration potential)	0.08	0.12	0.17	0.23
Winter vapour pressure deficit in kPa	1.32 (±0.97)	1.05 (±0.71)	0.99 (±0.86)	1.23 (±0.98)
Summer vapour pressure deficit in kPa	2.39 (±1.45)	2.16 (±1.16)	2.28 (±1.24)	2.75 (±1.63)
Species richness	4 (± 0.82)	4.2 (± 0.96)	6.5 (± 1.73)	12.7 (± 1.53)
Cover Vegetation (%)	31.17 (±3.44)	42.35 (±11.4)	61.82 (±11.3)	96.53 (±3.46)
<i>Bulnesia retama</i> cover (%)	14.34 (±11.17)	11.6 (±4.93)	1.7	7.92 (±5.48)
<i>Larrea divaricata</i> cover (%)	19.77 (±10.62)			32.29 (±19.5)
<i>Larrea cuneifolia</i> cover (%)		25.1 (±6.60)	26.59 (±10.33)	20 (±24.1)
<i>Prosopis flexuosa</i> cover (%)	7.23 (±6.13)	10.46 (±4.31)	9.7 (± 9.81)	11.2 (±8.35)
Soil type	Haplic yermosols	Haplic yermosols	Haplic yermosols	Haplic xerosols
Soil sand content (%)	91.17	81.75	84.69	71.52
Soil organic carbon (%)	0.25	0.26	0.34	0.43
Soil total nitrogen (%)	0.017	0.014	0.038	0.051

Climatic characteristics (mean annual precipitation and their coefficient of variation, mean annual temperature and their standard deviation, and aridity index) for the period 1950–2000 were obtained from WorldClim global database (Hijmans *et al.* 2005). Vapour pressure deficit was averaged ($n = 100$ days) for winter season (from July to October) and summer season (from October to March). Vapour pressure deficit was calculated using temperature and relative humidity from iButton sensors. Plant species richness, total vegetation and species-specific covers were calculated from Gatica (2015). Soil type, soil sand content, soil organic content and soil total nitrogen were obtained from BIOCUM database published in Maestre *et al.* (2012).

The sites were located more than 2 km away from houses and paddocks to reduce land use effects on the response variables (Goirán *et al.* 2012). The sites were classified according to mean annual precipitation values, as follows: Médanos Grandes is a very low precipitation site, Bermejo is a low precipitation site, Marayes is a middle precipitation site and Chepes is a high precipitation site; see Table 1 for complementary information.

Variation in vegetation physiognomy and floristic composition is observed along the precipitation gradient. In the western sites, Médanos Grandes and Bermejo, the vegetation is conformed as open shrublands dominated by shrubs of the genus *Larrea*, accompanied by sparse shrubs such as *Bulnesia retama*, *Capparis atamisquea*, and the subshrubs *Lycium* and *Atriplex* species (Cabido *et al.* 1993; Gatica 2015). In these sites, the herbaceous layer is dominated by ephemeral annuals, and grasses are scarce (Cabido *et al.* 1993). In the more humid sites (Marayes and Chepes), the vegetation is presented as open woodlands dominated by scattered *Prosopis flexuosa* trees, with a low abundance of *Geoffroea decorticans*, *Cercidium praecox* and *Aspidosperma quebracho-blanco* trees (Cabido *et al.* 1993; Gatica 2015). In these sites, the shrub layer is co-dominated by *Larrea* species and *Mimozyanthus carinatus* tall shrubs, and the herbaceous layer is composed of perennial and annual grasses (Cabido *et al.* 1993; Gatica 2015). Differences among sites in vegetation structure and plant community attributes are shown in Table 1.

Species selection

Although an evident change in vegetation structure occurs along the precipitation gradient, several woody plant species are conspicuous in all the study sites (Cabido *et al.* 1993). We used data from a previous study to detect dominant woody plant species and identify ubiquitous species along the precipitation gradient (Gatica 2015). In this previous study, the vegetation survey was conducted in several 50 m long transects per study site using the intercept method, recording the cover of all perennial plants (woody, grasses and forbs) intercepting the line transect. The species cover, estimated as per cent cover of each species in the 50-m line transect, showed that *Larrea divaricata*, *Larrea cuneifolia*, *B. retama* and *P. flexuosa* were the most abundant woody plant species in the four study sites. These species differ in plant functional traits related to water and nitrogen use strategies (Table 3). *Larrea divaricata* is more abundant in very low precipitation and high precipitation sites, while *L. cuneifolia* presents an opposite pattern, being more abundant in low and middle precipitation sites (Table 1). However, both *Larrea* species have similar physiological characteristics and functional traits (Table 3), and therefore in this study they are treated together (hereafter, '*Larrea* sp.'). *Larrea* sp. showed higher cover values in very low, low and middle precipitation sites than in the high precipitation site (Table 1). In contrast, *P. flexuosa* cover increased from 10% to more than 20% with

increasing precipitation, and *B. retama* showed lower cover values in very low, low and middle precipitation than in high precipitation sites (Table 1).

Data collection

At each site, we collected more than 10 fully expanded and healthy leaves from each of five individuals of *P. flexuosa* and *Larrea* sp. plants, and terminal photosynthetic twigs of the aphyllous *B. retama* in October 2011 ($n = 60$); and from 10 different individuals of the same species in March 2012 ($n = 120$), representing the start and the end of the growing season, respectively. At each site, individuals were spaced more than 10 m from each other, therefore, representing independent sampling units. Leaves from each individual were collected from different height and canopy positions, and were composited into one sample per individual. The leaves were stored in paper bags and oven dried at 60°C for 72 h in the laboratory. Later, dry samples were ground to powder and sent to the University of Arkansas Stable Isotope Laboratory for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C and %N determinations. The samples were analysed on a Delta Plus Isotope Ratio Mass Spectrometer coupled with an elemental analyser, and the natural abundance of stable isotopes was expressed with the delta notation, considering Pee Dee Belemnite (PDB) and atmospheric N_2 as standard material for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (Farquhar *et al.* 1989). The overall precision of the determinations was better than 0.2 per mil for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Higher values of these parameters indicate an enrichment and low biological discrimination against the heavy C or N isotopes.

Data analysis

Plant $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C/N values were compared among woody species, between sampling dates (start *vs.* end of the growing season) and among sites (four mean annual precipitation levels) using generalized linear models (Pinheiro & Bates 2000). Woody species, sampling dates and precipitation levels were included as categorical fixed factors and we considered all possible interactions among the fixed factors. Generalized linear model is a statistical approach appropriate for unbalanced data set structures (Pinheiro & Bates 2000), which was our case. To account for variance heterogeneity among groups (categories within each fixed factor; Appendix S3), we fitted models differing in their variance structure component (varIdent) for each response variable and, thus, we selected the models that minimized the Akaike information criterion (AIC; Pinheiro & Bates 2000). The response variables ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C/N) were modelled using Gaussian error distribution with identity link and we evaluated the significance of fixed factors with the F test, following Type III analysis of deviance (Pinheiro & Bates 2000). In Type III analysis of deviance the significance of factors does not depend on the order of inclusion of fixed factors (Pinheiro & Bates 2000). Statistically significant mean differences (contrast with $P < 0.05$) were tested with the Wald test (Bolker *et al.* 2008).

Linear regressions were used to evaluate the relationships between plant $\delta^{13}\text{C}$ and plant N (percentage) within

precipitation levels (or sites), within species and within species in each precipitation level (Crawley 2013). All statistical analyses were performed with R 3.0.3 statistical software (R Core Team 2014). Generalized linear models and Wald tests were fitted with 'nlme' version 3.1-128 (Pinheiro *et al.* 2015) and 'contrast' version 0.19 (Kuhn *et al.* 2013) packages in R, respectively.

RESULTS

Spatial and seasonal variations in plant $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C/N

We found significant differences in plant $\delta^{13}\text{C}$ among species, between sampling dates and among precipitation levels (or sites) (Table 2). At the start of the growing season, *P. flexuosa* showed highest $\delta^{13}\text{C}$ values in the high precipitation site, whereas at the end of the growing season, it showed higher $\delta^{13}\text{C}$ values in the very low and high precipitation sites than in the low and middle precipitation sites (Fig. 1a). In addition, this species showed significantly higher $\delta^{13}\text{C}$ at the start than at the end of the growing season in all sites except at the low precipitation site, where $\delta^{13}\text{C}$ did not differ significantly between sampling dates (Fig. 1a). In *Larrea* sp., $\delta^{13}\text{C}$ values were significantly higher in the low and middle precipitation sites than in the very low and high precipitation sites, whereas they did not differ significantly among sites at the end of the growing season (Fig. 1b). In the case of *B. retama*, values of $\delta^{13}\text{C}$ did not differ significantly among sites; either at the start or at the end of the growing season, and no differences were found between sampling dates in all sites (Fig. 1c). Values of $\delta^{13}\text{C}$ for *B. retama* were significantly higher than those of *P. flexuosa* and *Larrea* sp. in all sites and in both sampling dates (see comparisons across panels Fig. 1a–c).

Plant $\delta^{15}\text{N}$ values differed significantly among species and among sites, but they did not differ between sampling dates (Table 2). *Prosopis flexuosa* showed higher values in low and high precipitation sites at the start as well as at the end of the growing season (Fig. 1d). In *Larrea* sp., values of $\delta^{15}\text{N}$ did not differ among sites at the start of the growing season, whereas they were lower at very low and middle precipitation sites of the gradient at the end of the growing season (Fig. 1e). In contrast, no differences in plant $\delta^{15}\text{N}$ values were found in *B. retama* (Fig. 1f). In general, plant $\delta^{15}\text{N}$ values were lower in *P. flexuosa* than *Larrea* sp. and *B. retama* at very low and low precipitation sites on both sampling dates (see comparisons across panels Fig. 1d–f).

Similarly to plant $\delta^{13}\text{C}$, significant differences in plant C/N were found among species, between

Table 2. Results of the Type III analysis of deviance of the models fitted to evaluate the variation in natural abundance of carbon stable isotopes ($\delta^{13}\text{C}$ ‰), nitrogen stable isotopes ($\delta^{15}\text{N}$ ‰) and carbon/nitrogen (C/N) associated with woody plant species with different water and nitrogen use strategies (see Table 3), sampling dates (start of the growing season *vs.* end of the growing season) and mean annual precipitation

Fixed factors	Degree of freedom	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		C/N	
		<i>F</i>	<i>P</i> value	<i>F</i>	<i>P</i> value	<i>F</i>	<i>P</i> value
Intercept	1	1685.6	<0.0001	318.8	<0.0001	1390.6	<0.0001
Species	2	83.7	<0.0001	23.7	<0.0001	68.5	<0.0001
Sampling dates	1	18.0	<0.0001	0.5	0.47	26.2	<0.0001
Mean annual precipitation	3	3.1	0.03	13.2	<0.0001	47.3	<0.0001
Species \times sampling dates	2	25.5	<0.0001	2.9	0.054	2.42	0.092
Species \times mean annual precipitation	6	7.6	<0.0001	4.6	0.0003	6.6	<0.0001
Sampling dates \times mean annual precipitation	3	7.5	0.0001	7.2	0.0002	8.44	<0.0001
Species \times sampling dates \times mean annual precipitation	6	6.6	<0.0001	4.5	0.0003	10.2	<0.0001

The woody plant species are distributed along precipitation gradient from central-western Argentinian drylands. *F* is the value of the *F* statistics for each model term.

sampling dates and among precipitation levels (Table 2). In *P. flexuosa*, plant C/N values were higher in the middle precipitation site at the start and at the

end of the growing season (Fig. 1g). At the start of the growing season, plant C/N in *Larrea* sp. was higher in the very low and the low precipitation sites, whereas

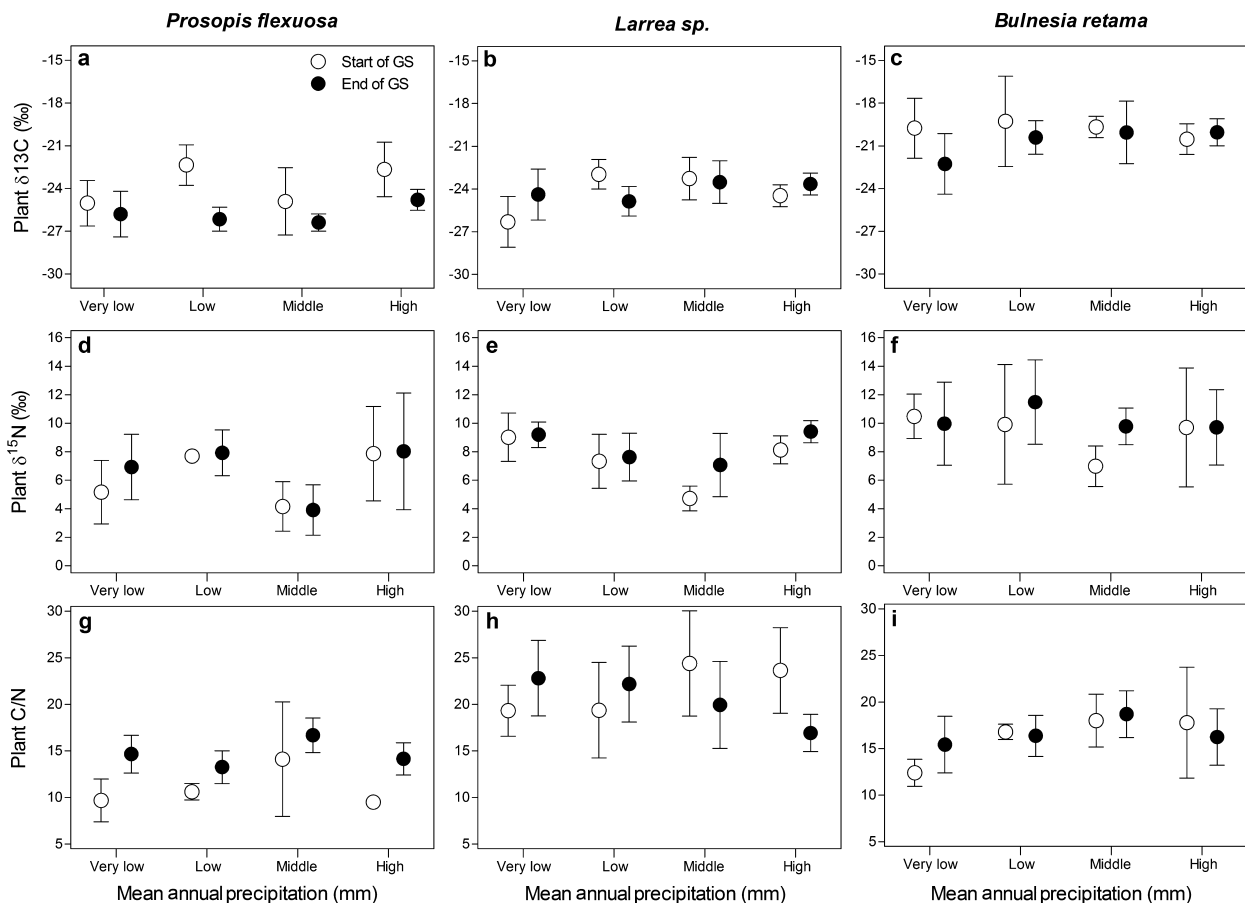


Fig. 1. Values of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C/N of woody plant species at the start (white circles) and end (black circles) of the growing season (GS) and distributed in four study sites located along a mean annual precipitation (MAP, mm) gradient from central-western Argentinian drylands. Values are mean and bars indicate 95% confidence intervals.

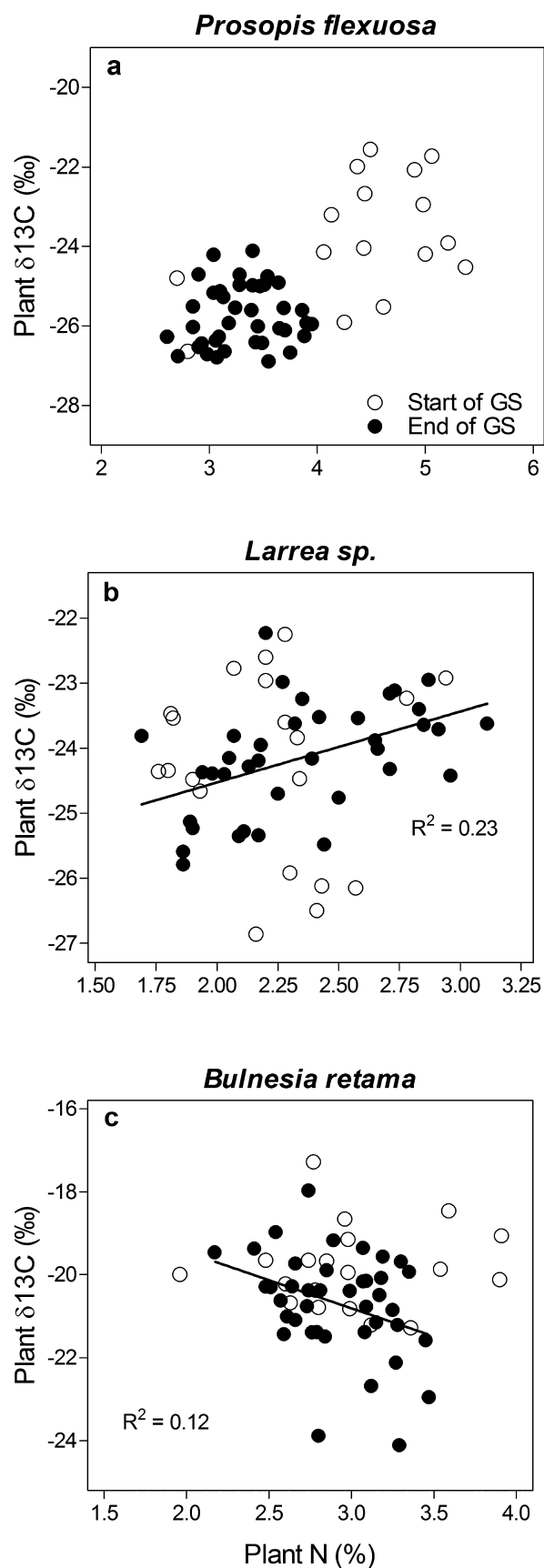


Fig. 2. Relationships between $\delta^{13}\text{C}$ and leaf N content (%) for individuals of (a) *Prosopis flexuosa* (deciduous woody plant species), (b) *Larrea* sp. (perennial woody plant species) and (c) *Bulnesia retama* (aphyllous woody plant species) at the start (white circles) and end (black circles) of the growing season (GS). Values correspond to individuals present in four study sites located along mean annual precipitation (MAP, mm) gradient from central-western Argentinian drylands. Line and coefficient of determination (R^2) are shown when significant ($P < 0.05$) lineal regression parameters were found.

the opposite trend was found at the end of the growing season (Fig. 1h). This species showed significant differences between sampling dates in all sites. In the very low and low precipitation sites, higher plant C/N values were found at the end of the growing season, whereas at the start of the growing season, higher values were found in the middle and high precipitation sites (Fig. 1h). In contrast, plant C/N in *B. retama* did not differ significantly between sampling dates in all sites, but spatial significant differences were found at the start of the growing season, where the lowest values were recorded in the very low precipitation site (Fig. 1i). Overall inter-specific differences showed lower plant C/N values in *P. flexuosa* than *Larrea* sp. and *B. retama* in all sites and in both sampling dates (see comparisons across panels Fig. 1g–i).

Plant $\delta^{13}\text{C}$ and leaf nitrogen content relationships

For the three woody species analysed, plant $\delta^{13}\text{C}$ values did not change as a function of plant N when analyses were performed grouping individuals within each precipitation level, both at the start and at the end of the growing season (data not shown). However, significant relationships between plant $\delta^{13}\text{C}$ and plant N were found when analyses were performed grouping individuals of all precipitation levels for *Larrea* sp. and *B. retama* and only at the end of the growing season (Fig. 2b,c). In *Larrea* sp., plant $\delta^{13}\text{C}$ values increased significantly as plant N increased (Fig. 2b). In contrast, in *B. retama* a slight but significant decrease in plant $\delta^{13}\text{C}$ occurred when plant N increased (Fig. 2c).

DISCUSSION

Based on our hypothesis, we expected an increase in plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with decreasing mean annual precipitation and at the start of the growing season (drier conditions). In addition, we did not expect leaf $\delta^{15}\text{N}$ variation associated with precipitation levels in *P. flexuosa* (N_2 -fixing tree species). Although we found subtle inter-specific mean differences in plant

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among the different precipitation levels, the more relevant results were that intra-specific isotope values did not increase towards sites with low precipitation or at the start of the growing season. Moreover, similar results were found when plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the three species were averaged at the site (or precipitation) level (data not shown). These results contrast with our hypothesis and previous studies conducted in drylands with similar precipitation levels (Austin & Sala 1999; Aranibar *et al.* 2004; Swap *et al.* 2004; Weiguo *et al.* 2005; Hartman & Danin 2010), and suggest that environmental factors other than mean annual precipitation may be affecting plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ under high aridity levels (mean annual precipitation <400 mm).

Spatial and seasonal variations in plant $\delta^{13}\text{C}$

The direction and magnitude of spatial and seasonal variations in plant $\delta^{13}\text{C}$ varied among the woody species studied. Particularly, leaf $\delta^{13}\text{C}$ values in *P. flexuosa* (a drought avoiding species) were higher at very low precipitation and at high precipitation sites, where vapour pressure deficit values were relatively higher than in low and middle precipitation sites during the study period (Table 1). Noticeably, leaf $\delta^{13}\text{C}$ values at very low and at high precipitation sites were similar at the end of the growing season, indicating that individuals located in sites with a threefold precipitation difference incurred similar stomatal regulation and water stress. Low accumulated rainfalls during the growing season (compared to historical data, Fig. S1 in Appendix S1) recorded in the high precipitation site could have produced an atypically low soil water moisture and, possibly, lower stomatal conductance towards the long-term wetter sites. Populations of drought avoiding woody species located in humid sites have been shown to have lower tolerance to drought than populations located in xeric environments (Lázaro-Nogal *et al.* 2013). In these species, the phenotypic plasticity may lead to differential drought-adapted populations along aridity gradients, where individuals present in wetter sites have plant traits less adapted to cope with drought events (Guevara *et al.* 2010; Matesanz *et al.* 2010; Giantomasi *et al.* 2013; Lázaro-Nogal *et al.* 2013). Furthermore, lower leaf $\delta^{13}\text{C}$ values at the start than at the end of the growing season in almost all sites (except at low precipitation site) suggest an association of stomatal regulation with seasonal rainfall patterns, as expected. Although the roots of *P. flexuosa* may access groundwater due to a dimorphic root system, previous studies on this species showed a reduced stomatal conductance and higher leaf $\delta^{13}\text{C}$ in years and seasons with markedly low rainfall and soil moisture (Giordano *et al.* 2011; Aranibar *et al.* 2014; Meglioli

et al. 2015). This sensitivity to temporal rainfall patterns is related to the fact that a greater proportion of the water uptake in *P. flexuosa* comes from lateral roots, which are distributed in the surface soil layers (generally ranged from 30 to 60 cm of depth, see Guevara *et al.* 2010). These results and the positive relationship found between vapour pressure deficits and $\delta^{13}\text{C}$ at the end of the growing season (Fig. S4a in Appendix S4) suggest that the leaf $\delta^{13}\text{C}$ in *P. flexuosa* responds to short-term environmental factors such as atmospheric demand, which are not always reflected in long-term environmental average characteristics such as mean annual precipitation.

We found that leaf $\delta^{13}\text{C}$ in *Larrea* sp. and *B. retama* (drought-tolerant species) did not vary among precipitation levels at the end of the growing season, suggesting that these species reached similar stomatal regulation in all precipitation levels during the wet and warm season. Although we found subtle differences in leaf $\delta^{13}\text{C}$ in these species among precipitation levels at the start of the growing season, this pattern was not associated with the atmospheric demand of approx. 3 months before the sampling date (see vapour pressure deficit values in Table 1 and Fig. S4b,c in Appendix S4). The results observed in *Larrea* sp. coincide with those reported by Ogle and Reynolds (2002), who showed that stomatal conductance in *Larrea tridentata* did not vary with changes in vapour pressure deficit and were independent of plant water status (pre-dawn water potential). However, the estimated time period affecting $\delta^{13}\text{C}$ used to calculate vapour pressure deficit (100 days) may not reflect the time period of carbon fixation in *Larrea* sp. perennial leaves. Nevertheless, we found that increasing leaf $\delta^{13}\text{C}$ in *Larrea* sp. was associated with an increase in leaf N content at the end of the growing season, at least partially ($R^2 = 0.23$; Fig. 2b). Leaf N may increase $\delta^{13}\text{C}$ by increasing photosynthetic capacity, which lowers c_i/c_a and isotope discrimination, due to high CO_2 demand in the leaf mesophyll (internal conductance) (Wright *et al.* 2003; Flexas *et al.* 2008; Cernusak *et al.* 2013). In drought-tolerant species, such as *Larrea* sp. (Table 3), increments in photosynthetic capacity allow CO_2 uptake with lower stomatal conductance under high water stress (Prentice *et al.* 2014). In *B. retama*, photosynthetic twigs are specialized to take up CO_2 at reduced water loss rates (Gibson 1983; Palacios & Hunziker 1984; Pfanz *et al.* 2002). Thus, the lack of spatial and seasonal variations in *B. retama* $\delta^{13}\text{C}$ could be associated with a lower sensitivity of stomatal conductance to changes in water availability (Ehleringer *et al.* 1987). In general, species with highly drought-tolerant strategies are less sensitive to changes in water availability and atmospheric demand (Orians & Solbrig 1977; Reynolds *et al.* 2004). Our results suggest that, unlike

Table 3. Qualitative and quantitative plant functional traits of woody plant species studied and located in central-western Argentinian drylands

Species	Family	Growth form	Drought tolerance	Palatability	Phenology	N fixing	Specific leaf area (mm ² mg ⁻¹)	Maximum height (cm)
<i>Bulnesia retama</i>	Zygophyllaceae	Tall shrub	Tolerant	Null	Aphyllous	No	1.94	600
<i>Larrea cuneifolia</i>	Zygophyllaceae	Shrub	Tolerant	Low	Perennial	No	5.22	150
<i>Larrea divaricata</i>	Zygophyllaceae	Shrub	Tolerant	Low	Perennial	No	5.97	167
<i>Prosopis flexuosa</i>	Fabaceae	Tree	Avoider	Middle	Deciduous	Yes	14.47	414

Qualitative traits (growth form, drought tolerance, palatability, phenology and N-fixation) were obtained from Díaz *et al.* (1998) and quantitative traits (specific leaf area and maximum height) were extracted from Conti and Díaz (2013).

P. flexuosa, biological rather than environmental controls, may affect stomatal conductance and $\delta^{13}\text{C}$ in *Larrea* sp. and *B. retama* along the precipitation gradient.

Spatial and seasonal variations in plant $\delta^{15}\text{N}$

Contrary to our expectations, plant $\delta^{15}\text{N}$ in *P. flexuosa*, *Larrea* sp. and *B. retama* did not increase with decreasing precipitation, either at the start or at the end of the growing season. Moreover, we found slightly higher plant $\delta^{15}\text{N}$ values at wetter than drier sites in the three studied woody species, patterns rarely documented (but see Wang *et al.* 2014). These results suggest that, at least for the temporal period that we analysed, precipitation (long-term averaged) had a low role in regulating plant $\delta^{15}\text{N}$. Our results are similar to those of Wang *et al.* (2014), who observed that leaf $\delta^{15}\text{N}$ of grasses and shrubs did not vary with variation in an aridity index (AI = mean annual precipitation/potential evapotranspiration). These authors suggested that in sites with rainfall below 400 mm a year, gaseous N losses are more important than N leaching losses. Thus, we hypothesize that a drought in the rainfall season recorded at the wettest sites of the gradient (Fig. S1 in Appendix S1) would lead to soils enriched in ^{15}N due to increases in process of N losses such as nitrification, denitrification and abiotic ammonia volatilization (Austin *et al.* 2004; Wang *et al.* 2014). This would consequently lead to increased plant $\delta^{15}\text{N}$ towards wetter sites. Changes in plant $\delta^{15}\text{N}$ precipitation relationships between wet and dry years have been observed in drylands of the Kalahari (Aranibar *et al.* 2004). Hence, processes leading to gaseous N losses driven by short-term drought events, and soil wet-dry pulses, may be more important drivers of plant $\delta^{15}\text{N}$, and could be independent of long-term precipitation (environmental) conditions.

It is important to highlight that significant differences in $\delta^{15}\text{N}$ were found among the woody species studied, and these differences were more important at the drier sites. When species co-exist under the same source of N availability, significant inter-specific

differences in $\delta^{15}\text{N}$ suggest that those species use different N sources (Robinson 2001). Low $\delta^{15}\text{N}$ values in *P. flexuosa* suggest that part of the N was fixed from atmospheric N_2 fixation; possibly due to their capacity to form nodules with N_2 -fixing symbiotic bacteria (Villagra *et al.* 2011). While high $\delta^{15}\text{N}$ values in *Larrea* sp. and *B. retama* suggest a predominantly mineral, ^{15}N -enriched soil N source (Robinson 2001). Partitioning of limiting resources is a widely recognized mechanism that allows plant co-existence in drylands (Carrera *et al.* 2000; James & Richards 2006). Therefore, variation in the degree of N partitioning among species influenced by changes in N availability and mean annual precipitation may partly explain inter-specific differences in plant $\delta^{15}\text{N}$ values observed.

Spatial and seasonal variations in plant carbon/nitrogen (C/N)

Like $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, we found that intra-specific C/N variation did not show a clear trend along the precipitation gradient, indicating that mean annual precipitation did not influence nitrogen status of the woody species studied. However, seasonal C/N variation within each species deserves special attention. Higher C/N values at the end than at the start of the growing season in *P. flexuosa* in all sites suggest N resorption before leaf abscission. This mechanism is associated in deciduous species with N conservation, and reflects high N requirements for short leaf life spans and to maintain higher growth rates (Aerts 1990; Carrera *et al.* 2000; Wright & Westoby 2003). Leaves in *Larrea* sp. showed higher C/N at the end than at the start of the growing season towards the most arid sites, suggesting a tendency of N re-distribution to new leaves during the rainfall of the growing season with increasing aridity, which can be influenced by low soil N availability (see N total concentration values in Table 1; Carrera *et al.* 2000). In contrast, we did not find seasonal and spatial differences in C/N within *B. retama*; perhaps due to lower N requirements given by their low growth rates and a lack of green leaves (Lambers *et al.* 2008). Given that

plant C/N has been shown to be independent of precipitation in arid and semi-arid ecosystems (Schulze *et al.* 1998; Aranibar *et al.* 2004), our results suggest that N conservation strategies were not driven by precipitation, and that intra-specific plant variation may be a more important driver of plant carbon and nitrogen use strategies.

Our observational approach showed that $\delta^{13}\text{C}$ did not increase with decreasing precipitation or with the dry season, as has been widely reported for drylands (Swap *et al.* 2004; Weiguo *et al.* 2005; Hartman & Danin 2010, among others). Two possible explanations could arise behind the observed plant $\delta^{13}\text{C}$. First, the low annual rainfall recorded at the wettest sites, markedly below the historical averages (Fig. S1 in Appendix S1) could have reduced the linear predictive effect of mean annual precipitation on plant $\delta^{13}\text{C}$, particularly in *P. flexuosa*. Drought-avoiding species such as *P. flexuosa* commonly show a stomatal regulation strongly linked to spatial and seasonal rainfall patterns (Giordano *et al.* 2011; Lázaro-Nogal *et al.* 2013; Meglioli *et al.* 2015). Second, biotic controls on plant $\delta^{13}\text{C}$ seem to be more important than spatial and seasonal variations in precipitation, both for *Larrea* sp. and *B. retama*. Drought-tolerant species are generally characterized by foliar and root functional traits that allow photosynthesis at low and constant stomatal conductance, being less sensitive to changes in water availability than other species. On the other hand, the $\delta^{15}\text{N}$ values of the three woody species did not vary linearly with precipitation variation, and concur with novel evidence that other processes independent (at least partially) of annual rainfall patterns may influence plant $\delta^{15}\text{N}$ in drylands (Wang *et al.* 2014). These processes are related to N losses, which do not always vary linearly with precipitation (Aranibar *et al.* 2004; Wang *et al.* 2014). Future studies relating intra-specific plant trait variation with soil isotope measurements along precipitation gradients could enhance our understanding of the role of phenotypic plasticity and N sources as drivers of carbon, water and nitrogen uptake, use and losses. However, the results we have reported here highlight the idea that responses of dryland plants to precipitation changes are not as simple to understand and cannot be generalized to all plant species present along these ecosystems.

ACKNOWLEDGEMENTS

We thank Edgardo Melián, Andrés Rolhauser and Agostina Lucero for invaluable collaboration in the field. We greatly acknowledge Margarita Nievas and Sr. Paredes for their permission to work on their properties. We thank anonymous associate editor and two anonymous reviewers for helpful comments on

the manuscript. Servicio Meteorológico Nacional (Secretaría de Planeamiento del Ministerio de Defensa de la Nación, Argentina) provided unpublished meteorological data of San Juan and Chepes, La Rioja. This work was supported by grants from FONCyT to EP (PICT No. 2013-2449) and to JA (PICT No. 2011-2703) and from CICITCA-UNSJ (21E/891) to EP. MGG was supported by a doctoral fellowship from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Climate characterization.

Appendix S2. Atmospheric characterisation of study sites.

Appendix S3. Selection of models fitted to plant $\delta^{13}\text{C}$, plant $\delta^{15}\text{N}$ and plant C:N of dominant woody plants located along a precipitation gradient.

Appendix S4. Atmospheric control on plant $\delta^{13}\text{C}$.