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Diversity of phenolic compounds and plant traits in coexisting Patagonian desert shrub species of Argentina

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Abstract Desert shrubs often accumulate different types of phenolic compounds but what determines the amount and diversity of these compounds is an issue scarcely explored. The aim of this study was to assess differences in the amount and diversity of phenolic compounds in leaves among coexisting shrub species differing in rooting depth and leaf turnover. We hypothesized that the diversity and amount of phenolic compounds in leaves of desert shrubs are related to access to soil water through rooting depth, and to leaf turnover. The study was carried out in the Patagonian Monte of Argentina. We collected green leaves of six species representing the dominant shrub morphotypes (tall evergreen, tall deciduous, and medium evergreen shrubs) and assessed lignin concentration and groups of soluble phenols obtained by sequential extraction with ethyl ether, ethyl acetate, and amyl alcohol. We

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H. Saraví Cisneros · M. B. Bertiller · A. L. Carrera Universidad Nacional de la Patagonia-UNPSJB, U9120ACF Puerto Madryn, Chubut, Argentina also assessed nitrogen concentration in leaves and leaf mass per unit area (LMA) as traits related to leaf lifespan. The diversity of phenolic compounds was higher in green leaves of tall shrubs with deep rooting depth than in those of medium evergreen shrubs with shallow rooting depth. Diversity of phenolic compounds in green leaves was negatively related to lignin concentration. Evergreen shrubs had higher amount of phenolic compounds in green leaves than deciduous ones and the total amount of phenolic compounds in green leaves was positively related to LMA. We concluded that access to soil water sources and leaf turnover were related to the amount and diversity of phenolic compounds in green leaves of desert shrub species and these results are consistent with those predicted by the resource availability theory for plants from resource-rich and resource-poor habitats.

 $\label{eq:keywords} \begin{array}{l} \text{Deciduous shrubs} \cdot \text{Evergreen} \\ \text{shrubs} \cdot \text{Lignin} \cdot \text{Rooting depth} \cdot \text{Shrub tallness} \cdot \\ \text{Soluble phenolics} \end{array}$

Introduction

Aridity is related to increasing diversity of shrub morphotypes differing in traits such as plant height, rooting depth, leaf mass per unit area (LMA), leaf longevity, and physiological adjustment, among others (Wright et al. 2002; Barboni et al. 2004; Campanella and Bertiller 2009; Moreno et al. 2010; Scholz et al. 2012). Desert shrubs are slow growing species and may have either long lived leaves, increasing nutrient residence time in plants, or drought deciduous leaves with high nutrient resorption (Aerts and Chapin 2000; Bertiller et al. 2006; Campanella and Bertiller 2011). Particularly, evergreen shrubs may accumulate large amounts of secondary compounds in green leaves (Coley et al. 1985; Hättenschwiler and Vitousek 2000; Campanella and Bertiller 2011; Carrera and Bertiller 2010; Estell 2010). Among secondary metabolites, phenolics are the most widely distributed in plants (Hättenschwiler and Vitousek 2000). Phenolics may be involved in UV absorption, tolerance to water shortage, thermal protection, allelopathy, defenses against herbivores or pathogens, soil microbial activity, soil organic matter decomposition and mineralization, among others (Sugai and Schimel 1993; Hartley and Jones 1998; Aerts and Chapin 2000; Souto et al. 2000; González-Rodríguez 2004).

Phenolics range from monomeric compounds with low molecular weight and high solubility, such as simple phenols, to complex compounds (oligomers and polymers) with high molecular weight and low solubility such as polymeric condensed tannins or lignin (Harborne 1998). Soluble phenolics are important anti-herbivore defenses and could also act as defenses against drought, could increase freeze tolerance (Coley et al. 1985; Hartley and Jones 1998; Hättenschwiler and Vitousek 2000), and could protect membranes against desiccation and free radicalinduced oxidation in resurrection plants (Moore et al. 2005). In contrast, lignin has low solubility and it is an important cell wall component mostly related to cell wall rigidity and tissue elasticity helping to enhance drought and freezing tolerance (Hättenschwiler and Vitousek 2000; Scholz et al. 2012). Although lignin may be also effective in deterring animal feeding (Harborne 1998; Distel et al. 2005), high lignin contents in plants are primarily associated with selection by drought (Hartley and Jones 1998).

Investment in chemical defenses is costly for plants since it reduces resources available for growth and reproduction being the magnitude of the reduction dependent on the nature of defense compounds (i.e. C based or N based compounds). Accordingly, these compounds could be the result from natural selection in those habitats where the cost of production is lower than the benefit resulting from drought and/or herbivore protection (Coley et al. 1985; Crawley 1998; Hartley and Jones 1998). The resource availability hypothesis suggested that a major determinant of the amount and type of plant defense is the resource availability in the environment, expecting higher levels of defense compounds in resource limited than in resource rich environments (Coley et al. 1985; Hartley and Jones 1998). However, what determines the amount and diversity of defense compounds in plants is an issue that still remains unclear (Hartley and Jones 1998).

Tall evergreen shrubs (TES), tall deciduous shrubs (TDS), and medium evergreen shrubs (MES) are the dominant coexisting shrub morphotypes in the arid Patagonian Monte of Argentina (Moreno et al. 2010). These shrub morphotypes, defined by stature and leaf lifespan, also differ in other traits such as LMA, leaf N, and rooting depth (Bertiller et al. 1991; Bucci et al. 2009; Campanella and Bertiller 2011; Scholz et al. 2012). Since plant attributes and plant defenses are highly interrelated (Coley et al. 1985; Aerts and Chapin 2000; Campanella and Bertiller 2011), we asked whether diversity in shrub morphotypes differing in leaf turnover, rooting depth, leaf N, and LMA was associated with diversity in the patterning of chemical defenses in green leaves. The aim of this study was to assess differences in the amount and diversity of phenolic compounds, N concentration in green leaves, and LMA among dominant coexisting Patagonian shrub species representing the three dominant morphotypes (TES, TDS, MES). We speculated that there is a trade-off between lignin (insoluble phenol) and soluble phenols in green leaves of shrub species resulting from species differences in the local access to soil resources and water conservation strategies, and that this trade-off is consistent with the predictions of the resource availability hypothesis (Coley et al. 1985). Accordingly, we hypothesized that the diversity and amount of phenolic compounds in green leaves are related to access to soil water, through rooting depth, and to leaf turnover. We predicted that (1) shrub species with the deepest rooting depth would have the highest diversity of phenolic compounds (i.e. different types of soluble phenolics and lignin) in green leaves (TES = TDS > MES), and (2) every every shrub species would have higher amount of phenolic compounds than deciduous ones (TES = MES > TDS). To our knowledge this is an issue scarcely explored in coexisting woody plants characteristic of cold arid ecosystems.

Study area and species

The study area is located in the Patagonian Monte, Argentina (ca. 48,000 km²). Within this area, we selected a representative site of about 30,000 m² ($42^{\circ}46.076'S$, $65^{\circ}53.211'W$, 133 m a.s.l.). Mean annual temperature is 13.2 °C, and mean annual precipitation is 125 mm (Barros and Rivero 1982; Moreno et al. 2010). Vegetation is representative of the shrubland of *Larrea divaricata* Cav. and *Stipa* spp., characteristic of the southern portion of the Monte Phytogeographic Province (Cabrera 1976). Plant canopy covers ca. 30–40 % of the soil and presents a random patchy structure (Carrera and Bertiller 2010).

The study was carried out on six dominant shrub species corresponding to three different dominant morphotypes in the Patagonian Monte: *L. divaricata* and *Schinus johnstonii* (TES more than 1 m tall with evergreen leaves, rooting depth >2 m), *Bougainvillea spinosa*, and *Lycium chilense* (TDS more than 1 m tall with deciduous leaves, rooting depth between 1 and 2 m) and *Chuquiraga avellanedae* and *Atriplex lampa*, (MES, height between 0.5 and 0.8 m with evergreen leaves, rooting depth <1 m, eventually up to 1.5 m) (Bertiller et al. 1991; Peláez et al. 1994; Rodríguez et al. 2007; Bucci et al. 2009; Scholz et al. 2012).

Sampling and leaf traits

We randomly selected three individuals of modal size (most frequent crown diameter and height) of each study species and collected fully expanded, young to medium aged green leaves from four branches of the external canopy crown (sunny and partially sunny leaves) of each individual (Bertiller et al. 2006). All leaves were collected at the same plant phenological stage (late vegetative growth-mid reproductive growth; Bertiller et al. 1991) in November 2007. For TDS species all expanded green leaves corresponded to the current year and for MES and TES species sampling also included green leaves from the previous year. One set of 10 fully expanded green leaves, without signs of deterioration, (two-three leaves per branch) of each individual were re-hydrated up to full expansion and placed on the flat bed of a HP ScanJet ADF scanner and scanned. The leaf area was calculated from the images obtained by means of the AxioVision 4 program. After that, leaves were oven dried at 45 °C for 48 h, and weighed to assess leaf dry mass and to calculate the mean LMA of each individual and species (Moreno et al. 2010).

The other set of collected leaves (without signs of deterioration) per individual and species was oven dried at 45 °C for 48 h and used for chemical analyzes. We analyzed lignin by the van Soest (1963) procedure and total soluble phenolic concentration by the Folin-Ciocalteu method using 50 % methanol as extract solution and tannic acid as standard (Waterman and Mole 1994). We further assessed groups of soluble phenols by sequential extraction with ethyl ether, ethyl acetate, and amyl alcohol (Harborne 1984) in aliquots of the 50 % methanol extract used for total soluble phenolic concentration, previously hydrolyzed with 2 M HCl during 0.5 h. After each extraction, solvents were evaporated (except for amyl alcohol requiring extreme high heating), and all measurements were performed by the Folin-Ciocalteu method using 50 % methanol as extract solution and tannic acid as standard (Waterman and Mole 1994). According to Harborne (1984), phenolic group 1 (ethyl ether extraction) is preponderantly composed by phenolics of low molecular weight such as simple phenols, phenolic acids, hydroxycinnamic acids, and hydroxycoumarins; phenolic group 2 (ethyl acetate extraction) is mainly represented by flavonols and flavones; and phenolic group 3 (amyl alcohol) by anthocyanidins and glycosylflavones. We carried out three replicates of chemical analyses per individual. Further, we calculated phenolic group 4 (soluble phenolics remaining after the sequential extraction) as the difference between total soluble phenolics and the sum of phenolic groups 1, 2, and 3. We assumed that this fraction is mainly constituted by complex soluble phenolics. It should be noted that the use of Folin-Ciocalteu reactant for soluble phenol assessment could induce some bias when comparing plant species with different chemistry since this reagent could react with non-phenolic reducing compounds (Box 1983; Ikawa et al. 2003). We calculated the total phenolic compound concentration (all soluble phenolic groups and lignin) and the proportion of each soluble phenolic group and lignin in the total, and computed the Shannon diversity index (Begon et al. 1996) of phenolic compounds in each

Table 1 Mean values ± one standard error of total concentration of phenolic compounds (lignin plus soluble phenolics), and concentration of lignin, and groups of soluble phenolics (Ph1 ethyl ether extraction, Ph2 ethyl acetate extraction, Ph3 anyl alcohol, Ph4 soluble phenolics remaining after the sequential extraction) in green leaves of the study

individual of each species. We analyzed N concentration in green leaves by semi-micro Kjeldahl (Coombs et al. 1985). We assumed that LMA and leaf N are traits closely related to leaf lifespan and growth rates (Aerts and Chapin 2000; Campanella and Bertiller 2011).

Statistical analyses

The significance of the differences in leaf traits, phenolic compounds, and Shannon diversity index of phenolic compounds among shrub morphotypes and species was evaluated by ANOVA. LSD test was used for multiple comparisons. In those cases in which assumptions of ANOVA were not met, variables were square-root transformed (Sokal and Rohlf 1995). The relationships among species, phenolic compounds, N and LMA were analyzed by principal component analysis (PCA) and correlation analysis. All statistical analyses were performed with the SPSS 7.5 package for windows (Norusis 1997).

Results

Phenolic compounds

The total concentration of phenolic compounds in green leaves (lignin and total soluble phenols) differed significantly among the three shrub morphotypes $(TES > MES > TDS; F_{2.18} = 83.6, p < 0.01)$ and species within them $(F_{1,18} = 65.6,$ between p < 0.01). TDS species and Atriplex lampa (MES) had the lowest values of total phenolics (Table 1). Morphotypes differed also in the concentration of lignin in green leaves (MES > TES > TDS) but there was some overlapping among species between morphotypes (L. divaricata = TDS species, and S. johnstonii = A. lampa). TES species had the largest concentration of soluble phenolics in green leaves while MES species and TDS species did not differ in concentration of soluble phenolics between them. Also, there was some overlapping in the concentration of soluble phenolics among species of different morphotypes (Table 1). Lignin dominated among phenolic compounds in MES species ranging between 85 and 86 % of the total. Lignin contributed with 49-50 % of the total phenolic compounds in green leaves of TDS species and with 20-31 % in green leaves of TES species. In this latter, soluble phenolics

Species	Morphotype	Total phenolics (mg g^{-1})	Lignin (mg g ⁻¹)	Soluble phenolic	s (mg g ⁻¹)		
			1	Ph1	Ph2	Ph3	Ph4
Chuquiraga avellanedae	MES	206.0 ± 17.9 b	175.3 ± 17.5 a	$11.0 \pm 1.5 c$	$11.7 \pm 0.3 \text{ cd}$	$4.0\pm0.6~{\rm c}$	$4.0\pm3.0~{ m c}$
Atriplex lampa	MES	$117.0 \pm 0.9 c$	$104.7 \pm 1.4 \text{ b}$	$6.7\pm0.9~{ m d}$	3.7 ± 0.3 d	$2.0\pm0.0~{ m c}$	0.0 ± 0.0
Mean MES		$161.5\pm21.5~\mathrm{B}$	$140.0\pm17.7~\mathrm{A}$	$8.8\pm1.2~\mathrm{B}$	$7.7 \pm 1.8 \text{ B}$	$3.0\pm0.5~\mathrm{B}$	$2.0 \pm 1.6 \text{ B}$
Lycium chilense	TDS	$115.4 \pm 15.8 c$	$56.1 \pm 1.0 \text{ c}$	$20.0\pm3.0~{ m c}$	$22.3 \pm 3.8 \text{ c}$	7.3 ± 3.4 bc	9.7 ± 6.3 bc
Bougainvillea spinosa	TDS	$70.1 \pm 5.6 d$	$35.1 \pm 5.2 \text{ c}$	$12.7 \pm 2.6 \text{ cd}$	$10.3 \pm 2.0 \text{ cd}$	$3.3\pm0.3~{ m c}$	8.7 ± 3.3 bc
Mean TDS		92.8 ± 12.6 C	$45.6\pm5.2~\mathrm{C}$	$16.3 \pm 2.4 \text{ B}$	16.3 ± 3.3 B	$5.3 \pm 1.7 \text{ B}$	9.2 ± 3.2 B
Larrea divaricata	TES	$192.5 \pm 10.8 \text{ b}$	$38.5 \pm 4.3 \text{ c}$	59.7 ± 7.9 a	58.7 ± 2.0 b	14.7 ± 0.3 b	$21.0 \pm 14.1 \text{ b}$
Schinus johnstonii	TES	301.0 ± 20.8 a	$93.3 \pm 5.2 \text{ b}$	$32.3 \pm 2.8 \text{ b}$	76.7 ± 10.2 a	34.0 ± 4.9 a	$64.7 \pm 1.9 a$
Mean TES		$246.8 \pm 25.3 \text{ A}$	$65.9 \pm 12.6 \text{ B}$	$46.0 \pm 7.2 \text{ A}$	$67.7 \pm 6.1 \text{ A}$	$24.3\pm4.9~\mathrm{A}$	$42.8\pm10.4~\mathrm{A}$

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Fig. 1 Mean concentration of lignin and groups of soluble phenolics (*bars*), and phenolic compound diversity index H' (*line*) in green leaves of shrub species and morphotypes. Medium evergreen shrubs (MES): Ca, *Chuquiraga avellanedae*; Al, *Atriplex lampa*; tall deciduous shrubs (TDS): Bs, *Bougain-villea spinosa*; Lc, *Lycium chilense*; tall evergreen shrubs (TES):

Ld, *Larrea divaricata*; Sj, *Schinus johnstonii*. Different capital and lowercase letters indicate significant differences in diversity index (H') of phenolic compounds among shrub morphotypes and plant species, respectively. Acronyms of soluble phenolic groups as in Table 1

were the dominant phenolic compounds and soluble phenolic groups and lignin were almost evenly represented in green leaves (Table 1; Fig. 1). The diversity of phenolic compounds (H') differed significantly among morphotypes (TES > TDS > MES) and did not vary between species within morphotypes. However, the diversity of phenolic compounds in green leaves of *L. divaricata* did not significantly differ from that of TDS species (Fig. 1).

Leaf mass per unit area (LMA) and N concentration in green leaves

The LMA differed significantly among the three shrub morphotypes (MES > TES > TDS; $F_{2,18} = 58.1$, p < 0.01) and between species within them ($F_{1,18} =$ 18.7, p = 0.01) (Table 2). The N concentration in green leaves significantly varied between TDS and evergreen shrub morphotypes (TDS > TES = MES). Both leaf traits were significantly negatively related (r = -0.68, p = 0.02, n = 18). Relationships among phenolic compounds, nitrogen and LMA

The PCA showed a negative relationship between lignin (negative values of PCA axis 1, 48.0 % of the total variance) and soluble phenolic groups or diversity of phenolic compounds (positive values of PCA axis 1). Nitrogen concentration (negative values of PCA axis 2, 35.5 % of the total variance) was negatively related to both lignin and soluble phenolic groups (Fig. 2). According to loading coefficients with respect to the two PCA axes, species of each morphotype were separately grouped. MES species (C. avellanedae and A. lampa) were clustered with high values of lignin, and high LMA (negative values of PCA axis 1, and positive or near zero values of PCA 2). TES species (L. divaricata and S. johnstonii) were clustered with high concentration of soluble phenolics, and high diversity of secondary compounds (positive values of PCA axis 1, and positive or near zero values of PCA 2). TDS (B. spinosa and L. chilense) were

Species	Morphotype	LMA (g m^{-2})	Nitrogen (mg g^{-1})
Chuquiraga avellanedae	MES	380.0 ± 11.6 a	$7.8\pm0.3~{ m c}$
Atriplex lampa	MES	$201.9 \pm 14.7 \; \mathrm{bc}$	$14.1 \pm 2.0 \text{ ab}$
Mean MES		$290.9 \pm 40.7 \text{ A}$	$13.4\pm0.9~\mathrm{B}$
Lycium chilense	TDS	$78.0 \pm 17.7 \text{ d}$	$16.0 \pm 1.3 \text{ ab}$
Bougainvillea spinosa	TDS	$161.3 \pm 7.4 \text{ c}$	17.9 ± 2.8 a
Mean TDS		$119.7 \pm 20.5 \text{ C}$	$16.9\pm1.4~\mathrm{A}$
Larrea divaricata	TES	$173.7 \pm 5.9 \text{ c}$	13.3 ± 1.5 ab
Schinus johnstonii	TES	$247.3 \pm 27.7 \text{ b}$	12.0 ± 0.6 bc
Mean TES		$210.5\pm20.7~\mathrm{B}$	$12.6\pm0.8~\mathrm{B}$

Table 2 Mean values \pm one standard error of LMA and N concentration in green leaves in the study species

Lowercase letters indicate significant differences among species and capital letters indicate significant differences among plant morphotypes. *TES* tall evergreen shrubs, *TDS* tall deciduous shrubs, *MES* medium evergreen shrubs. (n = 3 for species and n = 6 for morphotype)



Fig. 2 Ordination of species according to loading coefficients calculated with respect to the two first principal components of the correlation matrix of leaf traits (concentration of lignin, phenol groups: Ph1, Ph2, Ph3 and Ph4, and N, LMA, and H: diversity index of phenolic compounds). Acronyms of species as in Fig. 1 and acronyms of soluble phenolic groups as in Table 1

clustered with high N concentration in green leaves (near zero values of PCA 1 and negative values of PCA 2) (Fig. 2). Further loading coefficients of evergreen species (MES and TES) showed lower overlapping than those of TDS morphotype, due to differences in lignin and total concentration of phenolic compounds in green leaves in MES and in the phenolic groups in TES. In this latter, loading coefficients of *L. divaricata* were closer to those of phenolic group 1 (low molecular weight) than in *S. johnstonii* with high positive loading coefficients more related to values of phenolic groups with complex structure or high molecular weight (Figs. 1, 2).

Discussion

Our study addressed the variation of phenolic compounds among coexisting shrub morphotypes with different ecological strategies (Moreno et al. 2010). These compounds along with terpenoids are important plant defenses against drought and herbivores (Harborne 1998). Our findings provided evidence that the diversity and amount of phenolic compounds in green leaves of coexisting shrub morphotypes was related to plant traits such as rooting depth, leaf turnover, LMA, and leaf N. The values of soluble phenolics, lignin and N found in the study species were within the range of values of soluble phenolics and lignin reported for these species in Patagonian deserts (Campanella and Bertiller 2008, 2009, 2011; Carrera et al. 2009). We found a negative relationship between lignin and soluble phenolic groups or diversity of phenolic compounds and also between N concentration and both lignin and soluble phenolics in green leaves. Similar phytochemical patterns with prevalence of lignin or soluble phenolics depending on the species, and a negative relationship between lignin and N concentration in plant tissues were found in grasslands and forests (Horner et al. 1987; Lebreton et al. 2001).

As predicted, the diversity of phenolic compounds was higher in tall shrubs (both TES and TDS) than in MES, with deep and shallow rooting depths, respectively. Moreover, evergreen shrub species with higher LMA and lower N concentration in leaves had higher amount of phenolic compounds than deciduous ones.

MES species showed the lowest diversity of phenolic compounds and the highest lignin concentration. Lignin is an important structural component in dense leaves of MES serving as structural support and probably increasing cell wall rigidity helping these species with limited access to deep soil water to cope with drought (Coley et al. 1985; Coronato and Bertiller 1997; Lambers et al. 2008). This finding could indicate that lignin could be primarily selected by drought (Hartley and Jones 1998). However, in contrast to this idea, Scholz et al. (2012) found that the bulk leaf tissue elastic modulus was lower in shallow-than in deeprooted shrub species of Patagonia. Probably, the difference in this result could be explained by the fact that shallow-rooted species considered in Scholz et al. (2012) had other structures such as dense leaf pubescence contributing to reduce water or green biomass losses during unfavorable climatic periods. Moreover, the combination of high lignin, and low N concentration along with high LMA in MES species could also be effective in deterring animal feeding (Harborne 1998; Hartley and Jones 1998; Distel et al. 2005). Previous results indicated that MES species are more resistant to herbivore damage than some TES species and may replace these latter under high grazing disturbance (Bisigato and Bertiller 1997). These results reinforce the evidence that many plant phenolic compounds may have different roles in plant-environment interactions (Bennet and Wallsgrove 1994; Fischer et al. 2006).

Tall shrubs had the highest diversity of phenolic compounds in green leaves. Plant height reflects the biomass cost for supporting a unit of leaf area at a given height (Westoby 1998; Westoby et al. 2002) and is mostly positively related to rooting depth in deserts and Mediterranean ecosystems (Bertiller et al. 1991; Silva and Rego 2004; Bucci et al. 2009; Scholz et al. 2012). Deep rooting depth in tall desert shrubs allows an adequate water supply to maintain long waterconducting pathways to cope with high evaporative demand in arid environments (Grace 1998; Scholz et al. 2012). Therefore, tall shrubs with access to stable water sources in deep soil could be less affected by drought and could invest more C in diverse and probably more mobile defenses such as soluble phenolics, thus constructing multiple defensive mechanisms. Soluble phenolics could protect leaves not only from herbivores but also from climatic adversity (low-high temperatures and drought) (Hättenschwiler and Vitousek 2000). Moreover, differences in LMA, leaf N, and total amount of phenolic compounds between TES and TDS could be related to construction

costs of leaves varying in turnover and growth rates (Aerts and Chapin 2000; Carrera et al. 2000; Westoby et al. 2002; Wright et al. 2002; Lambers et al. 2008; Campanella and Bertiller 2011).

High costs of construction of dense leaves of TES species with large amount of defensive compounds may be compensated with long leaf lifespan (Campanella and Bertiller 2011). On the other hand, C gain in TDS is primarily invested in the production of shortlived leaves with low LMA and high N concentration. These leaves are protected from herbivores by a small amount of highly diverse secondary metabolites (trade-off between growth and defense, Coley et al. 1985; Bennet and Wallsgrove 1994; Hättenschwiler and Vitousek 2000). Moreover, TDS leaves are also protected by structural defenses as spiny stems (Moreno et al. 2010) thus, expressing multiple defense mechanisms involving both chemical and physical components. Also, TES species differed in the total amount of secondary compounds in green leaves. This contrast could be associated with differences in plant and leaf longevity. S. johnstonii had larger LMA, stronger woody architecture, and probably higher plant longevity than L. divaricata. Also, soluble phenolics of high molecular weight (phenolic groups 2, 3, and 4) prevailed in leaves of S. johnstonii while soluble phenolics of low molecular weight (phenolic group 1) were more represented in leaves of L. divaricata. These findings provided evidence on the existence of multiple defensive systems in some desert shrubs (Korikeva et al. 2004) which are strongly related to the ability of shrubs to capture soil water and to leaf turnover. The identification of the diversity of defensive compounds is important since single phenolic compounds could play important roles in plant functioning. In this sense, Moore et al. (2005) found a polyphenol in the leaves of the resurrection plant Myrothamnus flabellifolius, protecting membranes against desiccation and free radical-induced oxidation.

In summary, the patterns of phenolic compounds in green leaves found in coexisting shrub morphotypes with different access to soil resources, leaf turnover and growth rates are consistent with those predicted by the resource availability theory (Coley et al. 1985) for plants from resource-rich and resource-poor habitats. In this context, the total amount of phenolic compounds was mainly related to LMA (positively) and to leaf turnover and N concentration in green leaves (negatively). Moreover, the diversity of phenolic compounds was primarily associated with rooting depth varying from low diversity with dominance of lignin in shallow rooted MES species to high diversity of phenolic compounds with prevalence of soluble phenolics in deep rooted TES and TDS species. Accordingly, these findings provided evidence about the relationship between the amount and diversity of phenolic compounds in shrub species and the access to soil water sources, patterns of leaf turnover, N concentration, and probably shrub growth rates. An analysis in a wider context such as the systemsbiology approach proposed by Moore et al. (2008) integrating datasets from genomic, transcriptomic, proteomic, and metabolomics studies would allow the identification of the processes associated with the allocation of phenolic compounds among coexisting shrub species with different ecological strategies and access to water resources.

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