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Trade-off between leaf turnover and biochemical responses related to drought tolerance in desert woody plants

Zijuan Zhou [a], Peixi Su [a,*], Luciana González-Paleo [b], Tingting Xie [a], Shanjia Li [a], Haina Zhang [a]

[a] Key Laboratory of Land Surface Process and Climate Change in Cold and Arid Regions, Linze Inland River Basin Research Station, Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences, Donggang West Road, No. 320, Lanzhou 730000, China
[b] CONICET-Museo Paleontológico Egidio Feruglio, Av. Fontana 140, 9100, Argentina

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ABSTRACT

We describe differences in leaf photo-protection mechanisms in a group of arid adapted C_3 and C_4 shrubs that differ in their leaf life-span and compared these mechanisms to known differences in drought tolerance. The experiments were carried out in the field with fourteen woody species native to the Hexi Corridor region, northwestern China. We assessed water status, chlorophyll content, antioxidant enzymes activity, and solute content. We found that differences in photo-protection mechanism among species were not a consequence of differences in photosynthetic pathway, but they were related to leaf life-span. Further, we found evidence that supports the concept of a trade-off between leaf turnover and photo-protective mechanism: species with a longer leaf life-span (leaves with low turnover rate) had higher values of enzymatic (POD and CAT) and no-enzymatic (Chl a, Chl b, Car, and soluble sugars-SS) compounds, than species with a shorter life-span (high turnover rate). These different photoprotective strategies are in accordance with known differences in morphological and physiological leaf attributes that allow for rapid acquisition resources (i.e. acquisitive type) or permit conservation of resources within well protected tissues (i.e. conservative type).

1. Introduction

Plants native to arid environments have developed an array of adaptations to drought, resulting in a high diversity of growth forms, from deep-rooted evergreen sclerophyllous, to deciduous shrubs and geophytes and winter annual herbs, which escape drought by finishing their annual cycle before the onset of drought (Ehleringer and Mooney, 1983). In desert shrubs, differences in the physiology of water, nutrients and carbon acquisition provide different strategies of resource use and partition to cope with water stress (Sala et al., 2012).

Shrubs with C_4 photosynthetic pathway tend to have high radiation, nitrogen, and water use efficiencies, and are thus, able to tolerate higher levels of stresses than shrubs with C_3 photosynthetic pathway (Ghannoum, 2009). It is well-established that the physiological advantages, conferred by the higher photosynthetic efficiency under high light and temperature of C_4 relative to C_3, are crucial for the ecological dominance of C_4 plants in open, hot and arid environments (Long, 1999; Osmond et al., 1982). Still, terrestrial vegetation is composed of about 95% C_3 plants and 5% C_4 and CAM plants, but primary productivity of C_4 plants is high and accounts for 20% of the total primary productivity (Ward et al., 1999). Predictions of global warming and changes in precipitation patterns are likely to expose plants to increase water stress (IPCC, 2007). The consequences of this change are likely to be on the form of lower water availability and higher water demand from the atmosphere, leading to an increase in the proportion of land area covered by C_4 plants (Henderson et al., 1994). In particular, in the desert of the central Hexi Corridor, C_4 woody plants tend to be dominant (Moore, 1994) and environmental change has the potential to increase this dominance. The understanding of how physiological and biochemical traits differ between C_3 and C_4 plants and how variable within these two groups these traits are, could help us predict potential changes in species composition and ecosystem carbon cycling under global warming scenarios.

Low water availability and/or high salt concentration typical in the soils of arid environments are manifested primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cells of plants growing in dry environments (Serrano et al., 1999). Oxidative stress, which frequently accompanies

* Corresponding author. Tel.: +86 931 4967211; fax: +86 931 8273894.
E-mail address: supx@lzb.ac.cn (P. Su).

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Abbreviations

Car	carotenoid
CAT	catalase
Chl a	chlorophyll a
Chl b	chlorophyll b
Chl a/Chl b	chlorophyll a/chlorophyll b
LWP	leaf water potential
MDA	monodehydroascorbate
NR	nitrate reductase
POD	peroxidase
Pro	proline
RWC	relative water content
SOD	superoxide dismutase
SP	soluble protein
SS	soluble sugar
TFA	total free amino acid
$\delta^{13}C$	stable carbon isotope ratio (‰).

drought and temperature stress, may cause denaturation of functional and structural proteins. As a consequence, these diverse environmental stresses often activate similar cell signaling pathways or cellular responses, such as the production of stress proteins, up-regulation of anti-oxidants and accumulation of compatible solutes (Knight and Knight, 2001). Plant tissue dehydration induces reactive oxygen species (ROS) synthesis, which could disrupt normal metabolism of plants through oxidative damage to lipids, proteins, nucleic acids and photosynthetic pigments (Ozkur et al., 2009). In order to overcome oxidative stress, plants have developed enzymatic (such as superoxide dismutase-SOD, catalase-CAT, and peroxidase-POD; Smirnoff, 1993; Reddy et al., 2004) and non-enzymatic (such as carotenoids; Adams et al., 1999; Munné-Bosch and Peñuelas, 2003) antioxidant defense mechanisms to quench ROS and stabilize photosynthetic complexes. Reduction in the content of pigments (such as chlorophyll a and b, and carotenoid) as a result of either slow synthesis or fast breakdown has been considered as a protective mechanism to avoid oxidative stress (Smirnoff, 1993). Another energy dissipative mechanism, distinguished by its faster kinetics, is related to the energy-dependent chlorophyll fluorescence quenching (Krause and Weis, 1991). All these photo-protective mechanisms help to maintain the high oxidative state of the primary electron acceptors of PSII, reducing the probability of photo-damage and photo-oxidative stress in chloroplasts.

In C_3 plants exposed to water stress, photorespiration may act as an alternative electron sink, protecting them from over reduction of the photosynthetic electron transport chain (Cornic and Fresneau, 2002; Osmond and Grace, 1995). In C_4 plants, the scope for photorespiration acting as a protective electron sink is minimal, and it is expected that the activity of antioxidant enzymes should be greater than in C_3 plants, contributing to their greater drought tolerance (Osmond and Grace, 1995). The multiple roles of metabolic mechanisms to tolerate drought in plants with different photosynthetic pathways is not completely understood and raises the question if C_4 plants are better adapted to severe drought than C_3 plants (Ripley et al., 2007).

While C_3 and C_4 shrubs are expected to adjust differently to low water availability, it is also expected that shrubs with leaves that differ in life span have different photo-protective mechanisms (Ishida et al., 2006). Leaf longevity modifies photosynthetic capacity, foliage nitrogen concentration and specific leaf area (SLA), and thus, structural and functional adaptation to cope with the stress factors should also differ for leaves of different life span. Long-lived leaves tend to have low SLA and a prolonged nutrient retention (Wright et al., 2002), that allow the plant to buffer the cost of construction of low-productivity leaves, over a longer period (Kikuzawa, 1991). However, structural and chemical protection is necessary for leaves with a long life span to enhance their tolerance to physical hazards (Coley, 1988). In contrast, short-lived leaves have high SLA and traits that allow for a greater carbon acquisition capacity (high nitrogen and chlorophyll content; Poorter et al., 2009; González-Paleo and Ravetta, 2011).

These functional differences related to leaf-longevity (i.e. inverse of leaf turnover) should be also reflected in differences in their photo-protective mechanisms (Ain-Lhout et al., 2004; Hamerlynck and Huxman, 2009). The dissipation of excess absorbed radiation by photorespiration and/or antioxidant enzymes when CO_2 uptake is reduced by stomata closure is essential for avoiding chronic photo-inhibition. It is expected that this response should be better developed in leaves with a longer life span. Ishida et al. (2006) proposed that in deciduous trees, due to the low leaf construction cost, the dissipation of excess-absorbed light energy is mainly achieved by photorespiration. By contrast, long-lived leaves in trees exhibit down-regulation of photochemical capacity through the synthesis of non-enzymatic (i.e. carotenoids) and enzymatic compounds.

Our objective was to evaluate, in a group of C_4 and C_3 shrubs differing in leaf-span, whether the biochemical photoprotective strategy fits the world-wide leaf economics spectrum described by Wright et al. (2002). A general empirical quantification of the differences in energy dissipation and the links to photosynthetic pathway and leaf-type is lacking.

Thus, in this study, we hypothesized that: 1) C_4 shrubs have a higher capacity for antioxidant production (enzymatic and non-enzymatic) than C_3 shrubs and this capacity should be positively associated with drought tolerance (relative water content and leaf water potential) and water use efficiency (higher $\delta^{13}C$) under drought stress; 2) the tolerance to photo-inhibition during drought-stress is more conspicuous in leaves with long leaf-span than in leaves with a short leaf-span (i.e. there is a trade-off between leaf turnover and photo-protective mechanism).

2. Materials and methods

2.1. Study site and plant material

This study was conducted in the Hexi Corridor region, Gansu province, northwestern China (38°42′–39°25′ N, 100°03′–100°24′ E). The area is characterized by a temperate arid desert climate with an average temperature of 7.6 °C, while the absolute maximum and minimum may reach 39.1 °C and −27 °C, respectively with a mean annual precipitation of 116.8 mm (Su et al., 2004). We studied fourteen native shrubs that were chosen because they are the most abundant in this ecosystem (Table 1). Species were classified according to two criteria: 1) photosynthetic pathway, C_3 or C_4; and 2) leaf type. Although leaves have traditionally been categorized as corresponding to evergreen or deciduous plants, we defined three categories based on longevity: short-lived leaves, long-lived leaves and intermediate-lived leaves (Table 1). For each species, we collected and pooled three samples of leaves for 10 to 20 individuals (mixed samples), in mid-July 2011. In July, the midday photosynthetic photon flux density can exceed 2000 μmol m^{-2} s^{-1}, and the air temperature can exceed 40 °C. Leaves were immediately frozen in liquid nitrogen and stored at −70 °C until analyzed.

Table 1
Classification of fourteen woody desert species found in the study site, according to their leaf type and photosynthetic pathway.

Photosynthetic pathway	Leaf type	Species	Family
C_3	Long-lived leaves	*Caragana roborovskyi* Kom.	Fabaceae
		Caragana korshinskii Kom.	Fabaceae
		Tamarix ramosissima Ledeb.	Tamaricaceae
		Asterothamnus alyssoides (Turcz.) Nov	Asteraceae
		Artemisia sphaerocephala Krasch.	Asteraceae
		Alhagi sparsifolia Shap.	Fabaceae
		Hedysarum scoparium Fisch. et Mey.	Papilionaceae
C_3	Intermediate-lived leaves	*Sympegma regelii* Bunge	Chenopodiaceae
		Kalidium cuspidatum (Ung.-Sternb.) Grub.	Chenopodiaceae
		Reaumuria soongorica (Pall.) Maxim.	Tamaricaceae
		Nitraria sphaerocarpa Maxim.	Zygophyllaceae
C_4	Short-lived leaves	*Haloxylon ammodendron* (C. A. Mey.) Bge.	Chenopodiaceae
		Calligonum mongolicum Turcz.	Polygonaceae
C_4	Intermediate-lived leaves	*Salsola passerina* Bunge	Chenopodiaceae

2.2. Water status and $\delta^{13}C$

2.2.1. Determination of the relative water content

The relative water contents (RWC, %) of leaves and assimilating shoots were estimated based on the fresh weight (FW), saturated weight (SW) and dry weight (DW). Fresh leaf samples was weighed immediately and then kept in distilled water to rehydrate for 12 h. The leaves were subsequently dried at 80 °C for 24 h, and the DW was determined. The RWC was determined according to the method of Barrs and Weatherley (1962) and was based on the following calculation:

$$\mathrm{RWC}\,(\%) = [(\mathrm{FW}-\mathrm{DW})/(\mathrm{SW}-\mathrm{DW})] \times 100 \quad (1)$$

2.2.2. Determination of leaf water potential

The leaf water potential (LWP, MPa) was measured using a WP4-T dew point meter (Decagon, USA). Plant leaves or assimilating shoots were collected at pre-dawn and then placed in a disposable sample cup of the equipment, completely covering the bottom of the cup to measurement the water potential. Before determination, 0.5 mol L^{-1} potassium chloride solution (water potential of −2.22 MPa) was used to calibrate. We waited for 30 min before measurement until stability, and each measuring time was no more than 5 min.

2.2.3. Measurement of $\delta^{13}C$ values

Plant leaves and assimilating shoots were transported back to the laboratory, dried at 80 °C for 24 h, ground, sieved using an 80-μm mesh screen, and sealed in plastic bags for analysis. The stable carbon isotope ratio was analyzed with an MAT-252 mass spectrometer, and the sensitivity of this instrument is 5×10^{-3} A/Pa and its measurement error is ±0.05‰ (Ghosh and Brand, 2003; Kloeppel et al., 1998; Su et al., 2004). The measured results were determined using the following formula:

$$\delta^{13}\mathrm{C}\,(‰) = \left[\left(R_{\mathrm{sample}} - R_{\mathrm{standard}}\right)/R_{\mathrm{standard}}\right] \times 10^3 \quad (2)$$

where R_{sample} and R_{standard} are the abundance ratios, $^{13}C/^{12}C$, of the sample and the standard, Pee Dee belemnite (PDB), respectively.

2.3. Measurement of chlorophyll contents

Chlorophyll (Chl a and Chl b) and total carotenoids (Car) were extracted from 0.2 g samples of leaf tissue that were homogenized in 95% ethanol. The absorbance of the supernatant was measured at 470, 649, and 665 nm. The contents of Chl a, Chl b, and total Car were determined according to the method described by Lichtenthaler (1987).

2.4. Measurement of antioxidant enzymes activities

Plant samples (0.5 g) were homogenized in 50 mmol L^{-1} phosphate buffer (pH 7.8) for measurement of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity and then centrifuged at 5000× g for 20 min at 4 °C. The supernatant was immediately assayed for activity.

SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to Giannopolitis and Ries (1977). The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm. CAT activity was assayed by measuring the initial rate of disappearance of hydrogen peroxide (H_2O_2) by measuring the absorbance at 240 nm according to the method of Aebi (1984). POD activity was assayed by measuring the initial rate of the increase in H_2O_2 based on the absorbance at 470 nm to detect the formation of tetra-guaiacol (Rao et al., 1996). Lipid peroxidation was estimated by measuring the malondialdehyde (MDA) content (Aravind and Prasad, 2003). The absorbance of the supernatant was recorded at 450, 532 and 600 nm.

2.5. Measurement of osmotic adjustment solute contents

Soluble sugars were extracted and detected using phenol according to Dubois et al. (1956), and the absorbance was then measured at 485 nm. The soluble protein content was assayed using the Coomassie Brilliant Blue G-250 chromogenic method reported by Bradford (1976) and measured at a wavelength of 595 nm. Total free amino acids were extracted and detected following the ninhydrin coloration method of Lee et al. (2003), and the absorbance was measured at 570 nm. Proline was determined following the method of Bates et al. (1973), and the absorbance of the extract was measured at 520 nm.

2.6. Measurement of nitrate reductase activity

Nitrate reductase activity was measured using the in vitro determination method. A 0.5 g sample was homogenized in 4 ml of 3% extraction buffer and then centrifuged. 0.4 ml of the supernatant was added to 1 ml of 0.1 mol L^{-1} potassium nitrate and 0.4 ml of NADH. The mixture was kept warm for 30 min with 25 °C. Then 1 ml of sulfonamides and 1 ml of naphthalene base ethylene amines were added to the mixture and the absorbance was measured at 540 nm.

All absorbance measurements for the determination of enzyme activities and the contents of various osmotic adjustment solutes were conducted using a GeneQuant 1300 spectrophotometer (Britain), and all measurements were repeated in triplicate.

2.7. Statistical analysis

We performed a one way ANOVA to compare differences in all measured traits between: 1) photosynthetic pathway (C_3 and C_4) and 2) leaf type (short-, intermediate-, and long-lived leaves). Assumptions of ANOVA were calculated using Shapiro–Wilks test for normality and Levene's test for homogeneity of variance.

The presented values are the means ± one standard error (SE) of three replicates. We estimated the principal components (PCs) of the two-way standardized matrix of 14 individual woody shrubs species and 14 traits. For this analysis, a biplot of the first two PCs of species and traits was constructed.

3. Results

3.1. Leaf water status and $\delta^{13}C$ values

We did not find significant differences in the relative water content (RWC, $F = 0.23$, $p = 0.63$) and the leaf water potential (LWP, $F = 0.38$, $p = 0.84$) between shrubs with different photosynthetic pathway (C_3 and C_4; Table 2), although both attributes differed with leaf type ($F = 5.11$, $p = 0.03$ for RWC and $F = 4.60$, $p = 0.04$). Independently of photosynthetic pathway, shrubs with intermediate-lived leaves had the highest RWC than long-lived leaves. Intermediate-lived leaves also had lowest LWP while short-lived leaves had the highest values (Table 2).

There was a positive correlation between $\delta^{13}C$ and water use efficiency (WUE). Shrubs with C_4 photosynthetic pathway exhibited higher $\delta^{13}C$ and thus presented a higher WUE than C_3. In relation with leaf-type, long-lived leaves and intermediate-lived leaves had a lower $\delta^{13}C$ than that of short-lived leaves ($F = 157.18$; $p = 0.0001$, Table 2).

3.2. Chlorophyll content

C_4 shrubs had the lowest chlorophyll and carotenoid contents (Car) than C_3 shrubs. C_3 shrubs had 45% more chlorophyll a (Chl a; $F = 4.4$, $p = 0.046$), chlorophyll b (Chl b; $F = 4.8$, $p = 0.048$) and Car ($F = 5.1$, $p = 0.04$). Chlorophyll a/Chlorophyll b (Chl a/Chl b) ratio did not differ between C_3 and C_4 shrubs (Fig. 1; $F = 0.69$, $p = 0.42$).

Long-lived leaves had a higher chlorophyll and Car content than other two groups, and the differences was significant ($F = 8.5$, $p = 0.006$ for Chl a; $F = 8.1$, $p = 0.007$ for Chl b, and $F = 8.5$, $p = 0.006$ for Car). No differences were associated with leaf type in Chl a/Chl b ($F = 2.2$, $p = 0.16$, Fig. 1).

3.3. Antioxidant enzymes and lipid peroxidation

There was no significant difference between C_3 and C_4 shrubs (Table 3) in antioxidant enzymes activity, which differed in relation to leaf type. Long-lived leaves had higher values of CAT ($F = 7.2$, $p = 0.02$) and POD ($F = 8.5$, $p = 0.02$, Table 3) than that of intermediate- and short-lived leaves, and also had more efficient antioxidant system functioning to overcome the damage of tissue metabolism by reducing the toxic level of H_2O_2. There was no difference between leaf types in SOD activity. MDA content is usually used to measure the extent of lipid peroxidation resulting from oxidative stress (Smirnoff, 1993). MDA content differed between C_3 and C_4 shrubs ($F = 8.2$, $p = 0.02$), and C_3 shrubs had a higher MDA content than C_4 shrubs. MDA did not differ significantly with leaf type ($F = 1.63$, $p = 0.25$; Table 3).

3.4. Osmotic adjustment solute and nitrate reductase activity

There were no significant differences between C_3 and C_4 plants, or between leaf type in osmotic adjustment or nitrate reductase activity (Table 4). We found that C_3 plants had a higher proline accumulation than C_4 plants ($F = 4.87$, $p = 0.04$). In relation to the leaf type, the only difference in osmotic adjustment was that long-lived leaves had more soluble sugar content that the other leaf types ($F = 8.39$, $p = 0.03$, Table 4). We found that C_3 plants had a higher proline accumulation than C_4 plants ($F = 4.87$, $p = 0.04$). Higher soluble proteins content was also found in long-lived leaves, but the differences was not significant ($F = 0.29$, $p = 0.75$). There were no significant differences between C_3 and C_4 plants, or related with the leaf type in nitrate reductase activity (Table 4)

3.5. Multivariate analysis

The first and the second principal component (PC) accounted for 44% and 20% of the total variation, respectively. Each point represents a woody species labeled according to leaf type and photosynthetic pathway. As we expected, the PC1 axis segregated species mainly according to photosynthetic pathway or leaf longevity. At the left end of the PC1 we found species with short-lived leaves, characterized by higher RWC, LWP, and $\delta^{13}C$. On the right side of PC1, woody species with long-lived leaves had higher soluble sugars and proteins, higher CAT and POD activities, and higher pigment content (Chl a, Chl b and Car). Between these two extremes we found species with intermediate-lived leaves (Fig. 2).

The determinant traits of variation in relation to leaf longevity were confirmed by the strong correlation found between the first PC and RWC ($r^2 = -0.74$), LWP ($r^2 = -0.71$); $\delta^{13}C$ ($r^2 = -0.65$); Chl a ($r^2 = 0.88$), Car ($r^2 = 0.88$); POD ($r^2 = 0.78$), CAT ($r^2 = 0.94$), soluble sugars ($r^2 = 0.66$) and soluble proteins ($r^2 = 0.64$).

The second PC was responsible for 20% of the variation and was determined by: LWP ($r^2 = -0.63$), soluble proteins ($r^2 = 0.63$) and nitrate reductase ($r^2 = 0.80$). The grouping of species according to this axis was not related to the hypotheses, and was species-specific.

4. Discussion

Although in arid environments, C_4 species and species with long leaf life-span can be more efficient in the use of resources such as

Table 2
Leaf water status and $\delta^{13}C$ values for shrubs with C_3 and C_4 photosynthetic pathway, and according to their leaf type (short-, intermediate- and long-lived leaves). RWC – Relative water content; LWP – Leaf water potential. Values are means ± SE of three replications per species and all species belonging to a category. Different lowercase in the same line indicate significant differences among groups ($p < 0.05$). ns, non-significant difference.

	Photosynthetic pathway		Leaf type		
	C_3	C_4	Long-lived	Intermediate-lived	Short-lived
RWC (%)	74.6 ± 2.7^{ns}	77.4 ± 5.3^{ns}	70.2 ± 2.6^{b}	83.0 ± 3.1^{a}	76.1 ± 4.9^{a}
LWP (MPa)	-5.5 ± 0.9^{ns}	-3.7 ± 1.7^{ns}	-5.2 ± 0.9^{b}	-7.6 ± 1.1^{a}	-2.3 ± 1.7^{a}
$\delta^{13}C$ (‰)	-26.3 ± 0.03^{a}	-16.8 ± 0.01^{b}	-23.6 ± 0.02^{b}	-23.9 ± 0.02^{b}	-16.5 ± 0.01^{a}

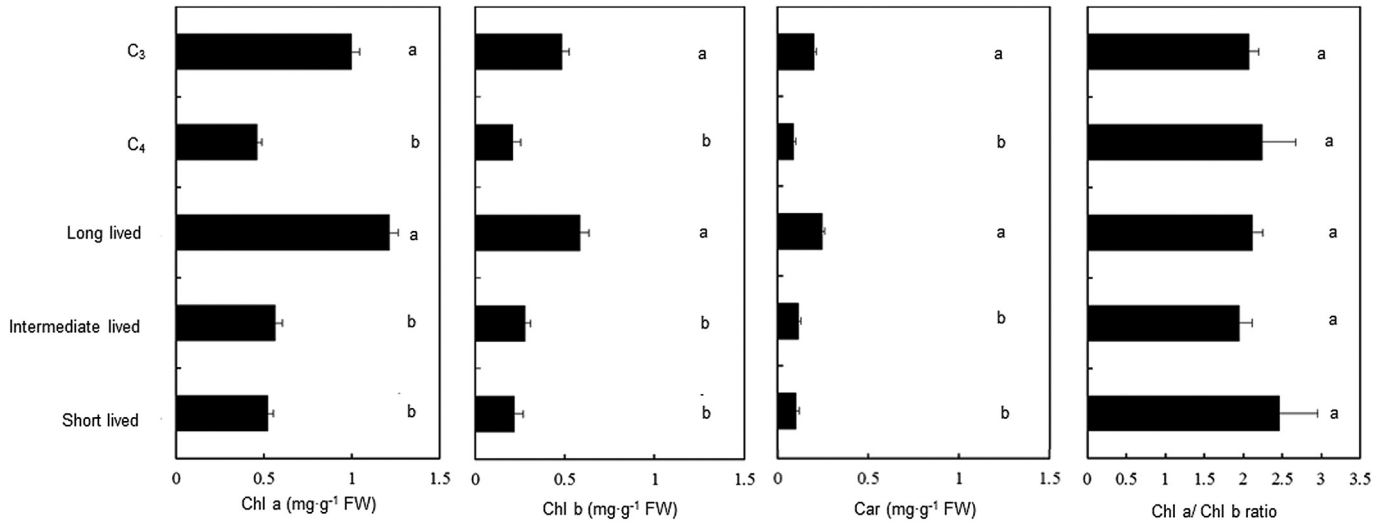

Fig. 1. Chlorophyll contents for the shrubs with C_3 and C_4 photosynthetic pathway, and for shrubs with different leaf type. Chl a – chlorophyll a, Chl b – chlorophyll b, Car – carotenoid. Values represent means of three individual plants of each species in each category ± SE. Different lowercase in the same column indicate significant differences among groups ($p < 0.05$). ns, non-significant difference.

water, nutrients and radiation (Cerling et al., 1993), the biochemical mechanisms behind their successful adjustment to these environments is not fully understood. Our aim was to analyze the possible differences in photo-protective mechanisms among woody species as influenced by photosynthetic pathway (i.e. C_3 and C_4) and leaftype. The biochemical data from fourteen desert woody plants support the hypothesis that a trade-off exists between leaf turnover and biochemical responses, and suggest that the biochemical mechanism of protection can be related to the world wide leaf economic spectrum modulated by specific leaf area and leaf longevity (Wright et al., 2002). Plants that are not successful in escaping excessive stress by architectural or morphological protection, are likely to develop a diversity of biochemical photo-protective mechanisms (Anderson et al., 1997). We did not find differences between C_3 and C_4 woody shrub species in the main traits that describe the biochemical mechanism responsible for photo-protection. As expected, we found that C_3 and C_4 woody species showed differences in $\delta^{13}C$, indicating differences in WUE. However, this result was not related with a more efficient photo-protective mechanism (enzymatic and no-enzymatic oxidant compounds) or a better plant water status (RWC or LWP) of C_4 species.

Species that differ in leaf life-span cope with low water availability through strategies that include structural, anatomical, physiological, and biochemical adaptations (Tattini et al., 2000). Morphological and structural differences, such as these, frequently coincide with differences in physiological tolerance and acclimation to drought and light (Abrams et al., 1994; Adams et al., 1999; Wright et al., 2001). An inverse relationship between photosynthetic and photo-protective capacity in tree species has been shown (Faria et al., 1998), but a few studies have considered the trade-off between leaf turnover and photo-protective mechanisms (Ishida et al., 2006). Our results show evidence that supports the concept of this trade-off. We found that woody species with a longer leaf life-span (or leaves with low turnover rate) had higher values of enzymatic (POD and CAT) and no-enzymatic (Chl a, Chl b, Car, and soluble sugars-SS) compounds, in relation to the species with a shorter life-span (or high turnover rate).

Multivariate analysis showed the existence of a continuum of strategies (i.e. traits profile) that reflected this trade-off. The first two axis of the principal components analysis were easily interpreted: the first axis was related to differences in leaf type, while the second was species-dependent. The presence on the left side of the ordination, of woody species with short or medium life-span leaves with a low level of photo-protective compounds is to be noted. This result agrees with previous studies (Ain-Lhout et al., 2004; Hamerlynck and Huxman, 2009; Ishida et al., 2006) that suggest that long-lived leaves or with a low turnover rate are more protected than leaves with shorter life-span.

The degree of activity of the antioxidant system under drought stress is extremely variable, and is modulated, among other factors, by the growth form of the plant (evergreen and deciduous shrubs or trees, herbs, etc.), and the level of stress that is experienced (Chaves et al., 2003). Long-lived leaves have prolonged nutrient

Table 3
Antioxidant enzymes activity and MDA content of the desert woody plants. Values represent means ± SE of three replications per species and all species belonging to each category. Different lowercase in the same line indicate significant differences among groups ($p < 0.05$). ns, non-significant difference.

	Photosynthetic pathway		Leaf type		
	C_3	C_4	Long-lived	Intermediate-lived	Short-lived
SOD (U g^{-1} FW)	129.9 ± 7.5^{ns}	159.4 ± 14.2^{ns}	126.4 ± 8.5^{ns}	134.3 ± 10.1^{ns}	153.3 ± 15.9^{ns}
CAT (U g^{-1} min^{-1})	3.8 ± 1.3^{ns}	3.2 ± 2.4^{ns}	4.85 ± 1.6^{a}	2.7 ± 1.9^{b}	1.9 ± 2.9^{b}
POD (U g^{-1} min^{-1})	76.5 ± 37.2^{ns}	52.9 ± 21.2^{ns}	104.2 ± 60.9^{a}	50.4 ± 23.2^{ab}	9.6 ± 3.6^{b}
MDA (nmol g^{-1} FW)	17.1 ± 2.6^{a}	9.6 ± 4.9^{b}	19.4 ± 3.2^{ns}	12.8 ± 3.8^{ns}	8.8 ± 5.9^{ns}

Table 4
Osmotic adjustment and nitrate reductase content. SS – soluble sugar; SP – soluble protein; TFA – Free amino acid; Pro – proline; NR – nitrate reductase. Values represent means of three plants of each species belonging to each group ± SE. Different lowercase in the same line indicate significant differences among groups ($p < 0.05$). ns, non-significant difference.

	Photosynthetic pathway		Leaf type		
	C_3	C_4	Long-lived	Intermediate-lived	Short-lived
SS (mg g^{-1} FW)	58.7 ± 7.1^{ns}	43.9 ± 14.2^{ns}	66.0 ± 8.9^{a}	43.4 ± 10.6^{b}	49.2 ± 16.8^{b}
SP (mg g^{-1} FW)	1.8 ± 0.4^{ns}	1.5 ± 0.7^{ns}	2.0 ± 0.5^{ns}	1.8 ± 0.6^{ns}	1.2 ± 0.9^{ns}
TFA (mg g^{-1} FW)	93.0 ± 17.9^{ns}	118.4 ± 33.5^{ns}	82.2 ± 22.3^{ns}	113.7 ± 26.3^{ns}	117.0 ± 42^{ns}
Pro (μg g^{-1} FW)	140.1 ± 20.6^{a}	60.6 ± 16.8^{b}	68.2 ± 16.5^{ns}	218.5 ± 71.2^{ns}	76.1 ± 11.3^{ns}
NR (μg g^{-1} FW)	5.2 ± 1.0^{ns}	5.8 ± 1.9^{ns}	4.1 ± 1.23^{ns}	6.9 ± 1.5^{ns}	5.7 ± 2.3^{ns}

retention allowing the plant to amortize the construction of these less productive leaves (Escudero et al., 1992). However, structural and chemical reinforcements are necessary for these leaves to enhance their tolerance to physical hazards (Coley, 1988). For these reason, we expected that minimal levels of water stress should trigger the biochemical mechanisms of photo-protection. In contrast, in species with shorter leaf life-span the strategy to cope with drought stress would be to maximize carbon acquisition and to maintain a higher water status (reflecting by the higher values of LWP and RWC), without investments in chemical protection. In these species the cost of constructing enzymatic and/or no-enzymatic photo-protective compounds should be higher than the cost of constructing new leaves. Differences in enzymatic mechanisms of photo-protection were mainly due to CAT and POD activity, both highest in long life-span leaves. These two enzymes play an important role in the fine-regulation of ROS concentration in the cell through activation and deactivation of H_2O_2, and the increase in CAT and POD activity helps to overcome the damage of tissue metabolism by reducing the toxic level of H_2O_2 (Cornic et al., 1989).

As described above leaf traits related with interception (e.g. SLA), uptake (e.g. leaf N, photosynthetic rate), use (chemical compounds involved in protection or conservation of resources) and turnover of resources (e.g. leaf life-span), form a fundamental spectrum of variation among plant species (i.e. world-wide economic leaf spectrum; Reich et al., 1999; Niinemets, 1999). Spectra of variation underpinned by this causal trade-offs represent conflicting costs and benefits (Kikuzawa, 1991; Reich et al., 1991). Any given strategy along the strategy-spectrum, therefore, represents a compromise. In this context shrubs species with long leaf life-span would have a higher cost of construction and a higher investment in biochemical compounds to protect the photosynthetic apparatus, than species with less longeve leaves. This leaf trait syndrome is also associated with important whole-plant characteristics (Craine et al., 2002; Lambers and Poorter, 1992), some of which provide drought tolerance.

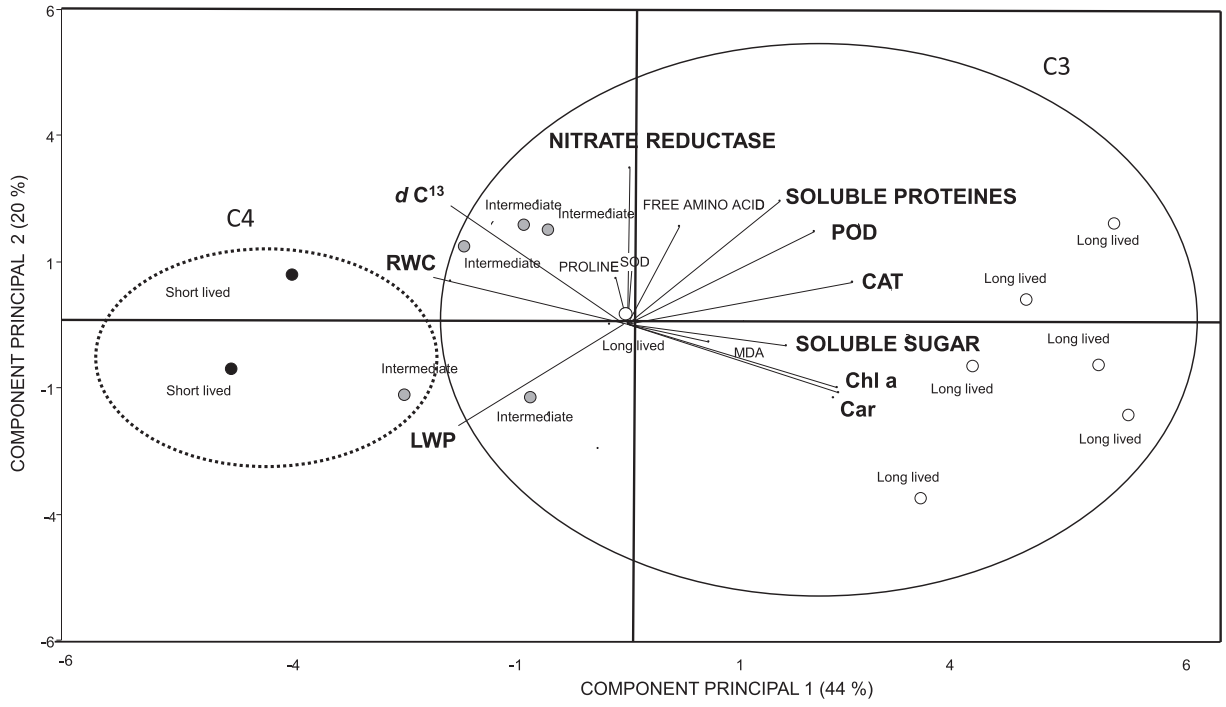

Fig. 2. A species by trait bi-plot for 14 woody species that differ in leaf longevity (i.e. black circles: short-lived, gray circles: intermediate-lived and white circles: long-lived leaf type) and in photosynthetic pathway (C_3 or C_4, enclosed by solid and dotted lines, respectively). The measure bio-chemical traits are related with osmoregulation and photo-protective mechanisms. The first two principal components are plotted, each accounting for a proportion of variance in the original data set, shown in parenthesis. Species labeled according their leaf type and photosynthetic pathway are presented by points and traits by vectors. RWC, relative water content; LWP, leaf water potential; Ca, Chlorophyll a content; Cb, chlorophyll b content; Car, Carotenoid content; Ca/Cb, Chlorophyll a/Chlorophyll b ratio; CAT, catalase; MDA, malondialehyde content; POD, peroxidase; SOD, superoxide dismutase; $\delta^{13}C$, stable carbon isotope ratio.

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