

Compound and Simple Leaf Woody Species of The Chilean Matorral Are Equally Affected by Extreme Drought

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Abstract

Two main leaf types are recognized among vascular plant species: compound and simple. Compound leaves are believed to be photosynthetically more productive than simple ones, by diluting mass tissue in more projected area. Conversely, simple leaves are believed to be more stress tolerant by packing mass tissue in less projected area during stress like drought. Nevertheless, convective cooling is more efficient in compound than simple leaves, a process that could alleviate water loss in drought periods. In Central Chile, woody species with simple and compound leaves coexist. This zone is facing a mega-drought event, causing browning and tree mortality. However, photosynthetic limitations on both types of leaves in drought conditions have not been addressed so far. We measured photosynthetic limitations in well-watered and drought conditions in three compound and three simple leaf species, and drought response ratio were obtained. We hypothesized that with no water limitation compound leaf species will show higher net photosynthesis (A_N) than simple leaf species associated with a higher mesophyll conductance (g_m). Nevertheless, opposite results are expected for simple leaves because their stress-tolerant physiology, showing fewer changes in their photosynthetic traits. We found that (g_m) and (A_N) were larger in compound leaves in well-watered conditions. Under drought conditions, both types of leaves were negatively affected despite foliar temperature in compound leaf species was 4°C lower. Our result suggests that regardless leaf shape matorral species in Central Chile will be seriously affected in their A_N due to the megadrought currently affecting this zone.

Introduction

There are two basic leaf types among the vascular plants realm: simple and compound leaves (Givnish, 1979). Whilst in simple leaves a single blade is inserted directly on the petiole, in compound leaves a blade has two or more subunits called leaflets that vary in number, form and connection to the petiole (e.g. palmately compound leaves vs. pinnately compound leaves). Compound leaves has been regarded as more productive than simple leaves due to their lower production cost (Givnish, 1979; Niinemets et al. 1999; Whitfield, 2006; Malhado et al. 2010). With the dissection of the photosynthetic area, compound leaves can maximize foliar area (diluting mass tissue in more projected area) for light capture and hence increase the growth rates (Givnish et al. 1979; Sack et al. 2003; Malhado et al. 2010). Further, the highly dissected venation usually found on compound leaves contributes with the mechanic support through the hydraulic force inside veins avoiding alterations in the leaf mass area (LMA) as occurs in simple leaves (Givnish et al. 1979; Li et al. 2008; Niinemets et al. 2010). For a similar area, compound leaves are more efficient in convective heat exchange, and thus less transpiration is required for cooling (Gates, 1968; Moya & Flexas, 2012; Michaletz et al. 2015). In fact, under identical environmental conditions, the temperature of dissected leaves can be 4°C lower than that of simple leaves (Stokes et al. 2004). Convective heat exchange allows compound leaves to decrease water loss (Gurevitch et al. 1990; Xu et al. 2009), and to tolerate a wider range of temperatures for biochemical reactions to occur such as carbon assimilation (Michaletz et al. 2015). Thus, based on leaf thermoregulation trade-off related to leaf area

and transpirational cooling, different responses to drought can be expected between compound and simple leaves (Givnish, 1979; Michaletz et al. 2015).

Compound leaves are on average thinner than simple leaves (Li et al. 2008; de la Riva et al. 2016). Thinner leaves tend to have higher mesophyll conductances (g_m), but lower tolerance to drought (Niinemets et al. 2011; Flexas et al. 2014). It has been shown that, when exposed to drought simple leaf species increase LMA by packing mesophyll cells to avoid cellular lysis (Wright et al. 2004; Galmés et al. 2007; Xu et al. 2009), whilst compound leaf species do not change LMA (Xu et al. 2009). Thus, whilst in well-watered conditions higher carbon assimilation can be achieved in compound leaves compared to simple leaves, the latter are more drought tolerant (Galmés et al. 2007; Alonso-Forn et al. 2020b).

It is well known that photosynthesis decreases with drought, but whether drought equally affects the two diffusive components of photosynthesis (i.e. stomatal and mesophyll conductance), remains somehow controversial (Grassi et al. 2005; Galle et al. 2009; Ferrio et al. 2012; Nadal et al. 2018a; Alonso-Forn et al. 2020a). While it seems almost universal that plants exposed to drought close stomata to avoid water losses in detriment of carbon assimilation (A_N) (Cornic et al. 2000; Nadal et al. 2018b; Alonso-Forn et al. 2020a), for the mesophyll conductance (g_m) some studies have shown that g_m decreases with drought (e.g. Galle et al. 2009; Cano et al. 2013; Ouyang et al. 2017) whilst others have found no changes (Galmés et al. 2007; Hommel et al. 2014; Ouyang et al. 2017). Further, to what extent leaf-shape related differences in diffusion photosynthetic traits affect carbon assimilation during drought remain elusive.

Mediterranean-type ecosystem are characterized by severe droughts during summer, and they occur only in five regions of the world: California, South Africa, southeast of Australia, the Mediterranean basin, and central Chile (Lawrence, 1987; Arroyo et al. 1995; Mooney et al. 2001; Armesto et al. 2007). Mediterranean plant species exhibit several morpho-physiological traits to deal with drought such as sclerophyllous leaves, low leaf areas, increased efficiency in photosystem II, increases in the water use efficiency, and high RuBisCO specificity (Delfine et al. 2001; Galmés et al. 2005, 2007; Medrano et al. 2009; Galle et al. 2011; Flexas et al. 2014; Alonso-Forn et al. 2020ab). In the Mediterranean-type climate zone of central Chile species with simple and compound leaves coexist (Mooney & Dunn, 1970; Arroyo et al. 1995). Unlike other Mediterranean-type climate zones where some rainfall events usually occur during the growth season, in central Chile plants must cope with long droughts with no rain during months (Parson, 1976; Schultz, 2005). Moreover, central Chile has experienced an uninterrupted sequence of dry years since 2010 with mean rainfall deficits of 20–40% (Garreaud et al. 2020). The so-called Mega Drought (MD) is the longest event on record and with few analogues in the last millennia, with detrimental effects on water availability (Bozkurt et al. 2018), vegetation and forest fires that have scaled into social and economic impacts (CR2 2017). Recently, Miranda et al. (2020) used temporal trends in the Normalized Difference Vegetation Index (NDVI) to show that the extreme drought of 2019 significantly reduced NDVI (browning) in near one-third of the region's forests and that the highest browning was observed in sclerophyllous forest dominated by species that have been catalogued as tolerant to drought. Further, global climate models project that observed climate trends are likely to be preserved and the number of extreme drought events will be increasing during the rest of the 21st century, which may have a

detrimental impact on these ecosystems (Matskovsky et al. 2021). Therefore, it is important to understand how different representative species of this ecosystem would be affected in their photosynthesis to future increased episodes of extreme drought as well as to assess the underlying mechanisms.

In the present study, we evaluated the photosynthetic response to an extreme experimental drought in compound and simple leaf species of the Central Chile matorral. We hypothesized that with no water limitation compound leaf species will show a higher A_N than simple leaf species associated with a higher CO_2 diffusion inside the leaves (g_m). Nevertheless, with an extreme drought simple leaf species will be less affected than compound leaf species because of their stress-tolerant physiology, showing fewer changes in their photosynthetic traits.

Materials And Methods

Study species and growth conditions

We selected six tree species abundant in the central Chile Mediterranean-type zone; three of them have simple leaves whilst the other three compound leaves. The species with simple leaves were: *Peumus boldus* Mol. (Monimiaceae), *Lithraea caustica* Mol. (Hook et. Arn) (Anacardiaceae) and *Cryptocaria alba* (Mol.) Looser (Laureaceae), all of them characterized by ovoid to oval sclerophyllous coriaceous leaves (Fig. 1). Compound leaves species were: *Prosopis chilensis* (Mol.) Stuntz (Fabaceae), *Acacia caven* Mol. (Fabaceae) and *Sophora cassioides* (Phil) Sparre (Fabaceae). *P. chilensis* and *A. caven* have bipinnate leaves, while *S. cassioides* have compound paripinnate leaves (Fig. 1). All the study species are distributed between 30° and 41° south latitude (SI Fig. 1; Rodriguez et al. 1983).

For each species, twenty individuals of similar age (1.5 or 2 years) and size were selected. Plants were acquired from a commercial garden ("Encanto Salvaje" <http://www.encantosalvaje.cl>), and transplanted into a 30 height x 15 cm diameter pots with soil taken from a natural matorral community near Farellones village (33°S), located at 50 km east of Santiago, Chile. Plants were kept for 21 days in a greenhouse at 33/13 °C (day/night mean temperature) and a PPFD of 1100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with a 12/12 h light/dark cycle, and 40–60% relative humidity. Pots were periodically irrigated (three times a week) at field capacity. After this period, plants of each species were randomly divided in two groups of 10 individuals, one group was maintained at field capacity (control treatment), whilst the other was exposed to a severe drought. For this, individuals under this treatment received no irrigation for 45 days. Then, to maintain this drought intensity comparable while we were conducting the gas exchange measures, pots were weighed every day and the water lost by evapotranspiration was refilled (usually < 50 ml). Plants were maintained 30 days under this water condition before measurements.

Leaf gas exchange and chlorophyll a fluorescence measurement

Leaf gas exchange and chlorophyll a fluorescence measurements were performed with a portable gas exchange system Li 6400XT (LI-COR Inc., Lincoln, NE, USA) equipped with a leaf chamber fluorometer (Li-6400-40; LI-COR Inc.).

The response of the net photosynthesis CO₂ uptake (A_N) to varying substomatal CO₂ concentration (C_i) was studied with A_N - C_i curves. For each species and growth condition, 10 replicates were performed. For this, a fully expanded leaf was introduced in the IRGA's chamber and after stabilization (15 minutes) of A_N and g_s a measurement was recorded. Curves were performed by increasing CO₂ concentrations from 0 to 50, 100, 200, 300, 400, 600, 900, 1400 and 2000 $\mu\text{mol CO}_2 \text{ mol}^{-1}$, at 1500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and between 40 to 60% humidity. A_N - C_i curves were performed at both 21% and 2% of O₂, the latter to suppress photorespiration.

The actual photochemical efficiency of photosystem II (Φ_{PSII}) was determined, simultaneously to the A_N - C_i curves, by measuring steady-state fluorescence (F_s) and maximum fluorescence (F_m') during a light-saturating pulse of c.a. 8,000 photons $\text{m}^{-2} \text{ s}^{-1}$ following the procedure of Genty et al. (1989):

$$\Phi_{\text{PSII}} = (F_m' - F_s) / F_m'$$

Φ_{PSII} was used for the calculation of the linear rate of electron transport (ETR) according to Krall and Edwards (1992):

$$\text{ETR} = \Phi_{\text{PSII}} \cdot \text{PPFD} \cdot \alpha \cdot \beta$$

where α is the leaf absorbance and β is partitioning of absorbed quanta between photosystems I and II. The product $\alpha \cdot \beta$ was determined from PAR/ A_N curves performed with 2% O₂ and at increasing PAR values of 30, 50, 75, 100, 150, 200, 500, 100, 1500, 2000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Yin et al. 2009; Bellasio et al. 2016). Corrections for the leakage of CO₂ into and out of the leaf chamber were applied to all gas-exchange data (Flexas et al., 2007).

Dark respiration (R_d) was measured by darkening the measuring leaf for 30 minutes with CO₂ concentration of 400 $\mu\text{mol mol m}^{-1}$ at 25 °C. Measured R_d value was used to correct the A_N - C_i curves.

Estimation of mesophyll conductance

From the combined A_N / C_i curves and chlorophyll a fluorescence the *in vivo* value of g_m was obtained following Harley et al. (1992):

$$g_m = A_N / (C_i - (\Gamma^*(\text{ETR} + 8 (A_N + R_L)) / (\text{ETR} - 4 (A_N + R_L)))$$

where A_N and C_i were obtained from gas exchange measurements at saturating light. The rate of non-photorespiratory CO₂ evolution in the light (R_L) was determined as half of dark respiration. Chloroplast compensation point Γ^* was obtained following Bernacchi et al. (2002), which used kinetic properties of

RuBisCO and specificity factor ($S_{c/o}$) (von Caemmerer, 2000). ETR is the electrons that are managed by PSII, calculated with the $q\beta$ values obtained from the PAR/ A_N curves (Yin et al. 2009).

Leaf temperature

To assess differences in the heat exchange between compound and simple leaves, infra-red thermal images were taken in six replicates per species at each growth condition. Leaf temperature was calculated by choosing 3 points in each leaf. Thermal images were obtained with a thermographic camera Testo 875 (Testo 875-2i, Germany) equipped with a display 3.5" LCD of with a resolution of 320 x 250 pixels and a field of view of 32°x 23°. All images were taken at midday when leaves reached their maximum temperature.

Statistical analyses

Linear mixed models were used to analyze the effect of drought on each parameter (photosynthetic rate (A_N), mesophyll conductance (g_m), stomatal conductance (g_s)) where leaf-type was the fixed factor and species a random factor. Log response ratios (LnRR) were calculated to assess the magnitude of the effect of drought on the different photosynthetic parameters between compound and simple leaves as:

$$LRR = \log(X_d/X_c)$$

where X_d corresponded to the parameter X measured on plants exposed to drought and X_c to that parameter measured in control plants. Response ratios were calculated for photosynthetic rate (A_N), mesophyll conductance (g_m), stomatal conductance (g_s). Linear mixed models were used to analyze the response ratio of each parameter where leaf-type was the fixed factor and species a random factor. The analyses were done in R 3.0 using Ranova library.

Results

Under well-watered conditions (control), on average, compound leaf species had greater photosynthetic capacity than simple leaf species, where the compound leaf tree species *Prosopis chilensis* and *Acacia caven* were those showing the highest A_N values, corresponding with the highest g_s value for *A. caven* and the highest g_m value for *P. chilensis* (Fig. 2, SI Tables 1 and 2). Notwithstanding, the simple leaf species *Lithrea caustica* also showed a high A_N value corresponding with high g_s and g_m values (Fig. 2, SI Tables 1 and 2).

Table 1.

A. Mixed linear model for photosynthesis, A_N , mesophyll conductance, g_m , and stomatal conductance, g_s . Asterisks indicates statistical effects Ranova Test ($p < 0.05$).

Source:				
		Df	t value	Pr(> t)
A_N				
	Type of Leaf	5	-0.061	0.954
	Treatment	64	14.08	0.000 *
	Type of leaf x Treatment	64	-4.937	0.000 *
g_m				
	Type of Leaf	5	-0.242	0.816
	Treatment	64	7.538	0.000
	Type of leaf x Treatment	64	-4.151	0.000 *
g_s				
	Type of Leaf	5	-0.033	0.975
	Treatment	64	12.350	0.000 *
	Type of leaf x Treatment	64	-2.200	0.031 *

B. Mixed Lineal model for LnRR of carbon assimilation, A_N. Mesophyll conductance, g_m. Stomatal conductance, g_s.

Source		Df	t value	Pr(> t)
A_N	Type of Leaf	4	-0.396	0.712
g_m	Type of Leaf	4	0.165	0.876
	Type of Leaf	4	0.483	0.654
g_s				

Table 2. Leaf temperature of compound and simple leaf species from the central Chile matorral growing under control and drought conditions. Values are mean \pm S.D. (n = 6).

Leaf type	Species	Condition	Leaf Temperature (C°)
Compound	<i>Acacia caven</i>	Control	27.4 \pm 0.21
		Drought	27.2 \pm 0.31
	<i>Prosopis chilensis</i>	Control	32 \pm 0.44
		Drought	29.8 \pm 0.07
Simple	<i>Sophora cassioides</i>	Control	31.8 \pm 0.54
		Drought	28.5 \pm 0.18
	<i>Peumus boldus</i>	Control	28.4 \pm 0.62
		Drought	32.7 \pm 0.55
<i>Cryptocaria alba</i>	Control	28.8 \pm 0.6	
	Drought	33.3 \pm 0.56	
<i>Lithrea caustica</i>	Control	32.1 \pm 0.55	
	Drought	33.9 \pm 0.47	

Drought negatively affected A_N , g_s and g_m in both leaf type species (Fig. 2, Table 1A), but leaf-type had no statistical effect on any of parameter evaluated (Table 1A). Size effect estimations with the LRR showed that drought affected the photosynthetic parameters in similar magnitudes (Fig. 3, SI Table 3), with no statistical effects of leaf-type (Table 1B).

Regarding species-specific responses, the compound leaf species *Sophora cassioides* and the simple leaf species *Peumus boldus* were those relatively less affected in terms of A_N , g_s and g_m (Fig. 3, SI Tables 1 and 2). In contrast, the compound leaf species *Prosopis chilensis* and the simple leaf *Cryptocaria alba* were the more negatively affected on these parameters (Fig. 3, SI Tables 1 and 2).

Under well-watered conditions, foliar temperature of compound-leaf species did not differ significantly from that of simple leaf species (30.4 vs 29.8°C in compound and simple leaf species, respectively). Nonetheless, the foliar temperature of control plants in compound leaf species was significantly lower (4°C) than that of plants under drought, whilst no differences between treatments were observed in simple leaf species (Table 2, SI Figures).

Discussion

Plants possess a great diversity of leaf shapes and sizes (Nicoitra et al. 2011; Shi et al. 2020), and several studies remark that leaf shape plays crucial roles in process such as photosynthesis, thermoregulation, hydraulic conductivity, nitrogen content and growth (Wright et al. 2004; Michaletz et al. 2015; Oguchi et al. 2018; Alonso-Forn et al. 2020b). Nevertheless, few studies have characterized the photosynthetic responses to drought between compound and simple leaf species, which is particularly important for Mediterranean species that are increasingly exposed to severe droughts due to climate change (Miranda et al. 2020). We found that a severe drought negatively affect in a similar magnitude compound and simple leaf species from the Chilean matorral.

We observed that with no soil moisture limitations (control conditions), on average, compound leaf species had greater photosynthetic capacity than simple leaf species, where the compound leaf tree species *Prosopis chilensis* and *Acacia caven* were the species showing the highest A_N values. In *P. chilensis*, this could be due to the high values of g_m observed ($0.29 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, SI Table 1) that are on the range of values typically found on herbs (Tomás et al. 2013; Nadal et al. 2018b), being greater than the g_m values reported for other Mediterranean species that typically ranged between 0.18 and $0.08 \text{ CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (SI Table 1) (Niinemets et al. 2005; Galmes et al. 2007; Niinemets et al. 2009b; Peguero-Pina et al. 2012; Tomás et al. 2013; Flexas et al. 2014; Peguero-Pina et al. 2017; Alonso-Fern et al. 2020b). In *A. caven*, the high A_N value seems to be related to a greater stomatal conductance that would allow higher CO_2 diffusion inside leaves consequently increasing the amount of carbon in carboxylation sites (Cc). Therefore, it seems that compound leaf species tend to show higher A_N and g_m than simple leaf species, as expected due to their lower LMA values (SI Table 4). As an exception, the compound leaf species *Sophora cassioides* showed the lowest A_N , analogous to the values observed in simple leaf species, probably due to their low g_m .

The lower A_N values were observed on the simple leaf species *Peumus boldus* and *Cryptocaria alba*, and these species have high LMA values (SI Tables 1 and 4), suggesting a strong constraints for CO_2 diffusion inside their leaves, and thus for A_N (Niinemets et al. 2009b; Tosens et al. 2012; Niinemets et al. 2015; Veroman-Jüergenson et al. 2019, 2020). Indeed, the g_m values obtained for simple leaf species in well-watered conditions are near to the limit of the foliar spectrum for g_m , and similar to values found in other Mediterranean sclerophyllous species ($< 0.1 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (SI Table 1) (Niinemets et al. 2009a; Peguero-Pina et al. 2012; Flexas et al. 2014; Peguero-Pina et al. 2018; Alonso-Forn et al. 2020a). On the other hand, the simple leaf species *L. caustica* showed the highest values of A_N (SI Table 1), with values similar to those reported in previous studies (e.g. Dunn, 1975; Lawrence, 1987, Brito et al. 2014). The g_m value of $0.1 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ *L. caustica* (SI Table 1) was similar to that expected for a LMA of 160 g m^{-2} as proposed by Flexas et al. (2009), and to the value modelled for sclerophyllous species for a similar LMA value (Hassioutou et al. 2010). However, despite the high value of LMA in *Lithrea caustica*, higher A_N value found on this species are due to low diffusional limitation of photosynthesis according to the high g_s and g_m value respect the other simple leaves species.

In contrast to our expectations, leaf-type did not affect photosynthetic plant responses to drought. Although compound leaves species were 4°C cooler under drought compared to simple leaves species, supporting that a dissected leaf anatomy offer a great advantage in drought conditions by convective heat exchange (Givnish, 1979), all compound leaf species were strongly affected by drought in A_n , g_s and g_m . In addition, *A. caven* is a winter-deciduous species (Specht, 1988; Aronson, 1992), thus a high control of evaporative demands is critical for maintaining the carbon assimilation during short leaf life span (Mooney & Dunn, 1970).

In this study we focused on the gas exchange response of Chilean matorral plants to severe drought conditions where no differences were observed between compound and simple-leaf species. This suggests that the “browning” of vegetation (Miranda et al. 2020) caused by the intense and extended mega-drought (Gerraud et al. 2020) is a general response of vegetation where all species, regardless their leaf-type, are seriously affected. However, to forecast how these species will respond to further increases in drought require further analyses. For example, anatomy and mesophyll arrangement data are required if there exists some type of adaptation in the drought, or enzymatic RuBisCO properties/concentration of this species related to leaf (Onoda et al. 2017; Galmes et al. 2017, 2019; Alonso-Forn et al. 2020). Experiments that account for the recovery phase are needed to obtain a rate of recovery, and if compound and simple leaves show differences in the recovery time.

Declarations

Conflict of interest statement

Authors declare no conflict of interest

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Authors Contribution

JO and LC designed the idea of this experiment. JO and LC wrote the main manuscript text. NF, PS, and CHF collaborated with the inputs to the manuscript. All authors reviewed the manuscript.

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Figures

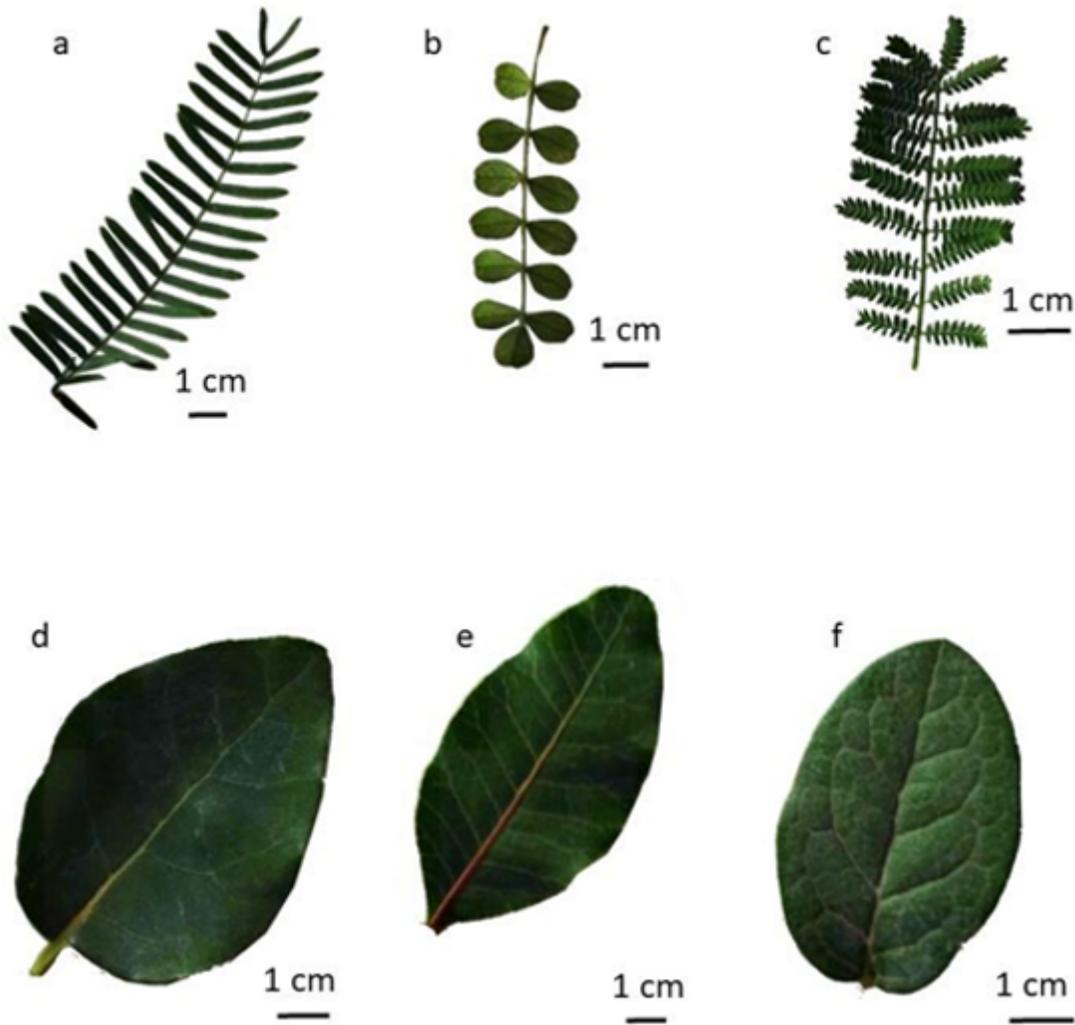


Figure 1

Compound (A-C) and simple leaves (D-E) used in this study. **A**, *Prosopis chilensis*. **B**, *Sophora cassioides*. **C**, *Acacia caven*. **D**, *Cryptocarya alba*. **E**, *Lithraea caustica*. **F**, *Peumus boldus*. Straight line indicates scale in cm.

Figure 2

Photosynthesis (A_N), stomatal conductance (g_s) and mesophyll conductance (g_m) of simple and compound leaf species of the central Chile matorral. Values are mean \pm two standard errors.

Figure 3

Log response ratio (LnRR) to drought on photosynthesis (A_N), stomatal conductance (g_s), mesophyll conductance (g_m) of two leaf-type species. Values are mean \pm two standard errors. Zero line indicates no change.

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