

Costs and benefits of photosynthetic stems in desert species from southern California

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Abstract. Woody plants with green photosynthetic stems are common in dry woodlands with the possible advantages of extra carbon gain, re-assimilation of CO₂, and high water-use efficiency. However, their green stem tissue may also incur greater costs of water loss when stomata are closed. Our study focussed on evaluating the costs and benefits of having green stems in desert plants, addressing the water-use efficiency hypothesis. We measured water status, carbon and water exchange, and carbon, nitrogen and oxygen isotopic composition of 15 species in a desert wash scrub in Joshua Tree National Park, California, USA. We found that all woody species that have green stems relied on their green stems as the sole organ for carbon assimilation for most of the study period. Green stems had similar photosynthetic rate (A_{\max}), stomatal conductance (g_s) and intrinsic water-use efficiency (WUE_i) to leaves of the same species. However, A_{\max} , g_s and cuticular conductance (g_{\min}) were higher in green stems than in leaves of non-green stemmed species. Carbon isotopic composition ($\delta^{13}\text{C}$) was similar in both leaves and green stems, indicating no difference in integrated long-term WUE. Our results raise questions about the possible trade-off between carbon gain and water loss through the cuticle in green stems and how this may affect plant responses to current and future droughts.

Additional keywords: carbon isotopes, gas exchange, oxygen isotopes, water relations, water-use efficiency.

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Introduction

Woody plants with green photosynthetic stems are found in hot, dry habitats with high irradiance (Gibson 1983), which includes Mediterranean ecosystems, subtropical warm deserts and tropical arid lands worldwide. There is evidence that green stems confer advantages to plants bearing them, including (1) extra photosynthetic carbon gain, (2) re-assimilation of respired CO₂, and (3) high water-use efficiency (WUE) compared with leaves. There is support for the first and second advantages (Mooney and Strain 1964; Adams *et al.* 1967; Adams and Strain 1968; Ehleringer *et al.* 1987; Nilsen *et al.* 1989, 1990; Nilsen and Bao 1990; Nilsen 1992a, 1995; Gibson 1996; Pfanz *et al.* 2002; Aschan and Pfanz 2003; Tinoco-Ojanguren 2008), but in terms of water-use efficiency, data is more limited (Ehleringer *et al.* 1987; Osmond *et al.* 1987; Nilsen and Bao 1990; Comstock and Ehleringer 1992; Nilsen and Sharifi 1997; Santiago *et al.* 2016; Ávila-Lovera and Tezara 2018). WUE is a trait that expresses carbon gain per unit of water lost and is a critical economic trait for plants living in arid ecosystems. However, not all arid-land plants have green stems, suggesting there could be costs associated with having green stems. We

conducted a study using plant species from a desert ecosystem in southern California to evaluate the costs and benefits, in terms of water use and carbon gain, of having green stems.

There are limited data supporting the idea that green stems are more water-use efficient than leaves, and these data include both gas-exchange data (Osmond *et al.* 1987; Comstock and Ehleringer 1988; Ávila-Lovera and Tezara 2018) and carbon isotopic composition ($\delta^{13}\text{C}$) data (Ehleringer *et al.* 1987, 1992; Comstock and Ehleringer 1992; Nilsen and Sharifi 1997). Green stems have moderate transpiration rates (E) and stomatal conductance (g_s) similar to leaves on the same plant (Comstock and Ehleringer 1988). Because of the large surface area of green stems, their water requirement is high, which explains why the majority of the species inhabit desert washes and water drainage channels, or have very deep roots (e.g. phreatophytes such as *Olneya tesota* A. Gray, 1854) (Gibson 1998). Furthermore, plants can also lose water through their cuticles, although not as fast as through their open stomata (Kerstiens 1996). Like leaves, green stems are covered by an epidermis (Cannon 1908; Gibson 1983) and may lose more water through the cuticle than non-green stems, which instead are covered by a suberised

periderm. Previous work has shown that green stem cuticular conductance (g_{\min}) can have values in the same range as field measures of g_s (Ávila-Lovera *et al.* 2017), and this influences whole-plant water loss. Plants can regulate g_s in response to water stress, but not g_{\min} . Therefore, a larger water loss through the stems than the leaves is expected in periods of water deficit.

Plant species with both leaves and green stems may have greater annual carbon income than plants with only leaves. However, all plants known to have green stems inhabiting desert ecosystems are drought-deciduous, so that they only have leaves and stems photosynthesising together during a short period of time at the beginning of the growing season, corresponding to March–May in the northern hemisphere. During this time, leaf and stem areas and maximum net photosynthetic rates (A_{\max}) are similar for both organs (Adams *et al.* 1967; Adams and Strain 1968; Smith and Nobel 1986). Previous work has shown that stem net photosynthetic rates can be on average 60% of leaf photosynthetic rate (Ávila *et al.* 2014b), and that these rates are maintained throughout the dry season (Nilsen and Sharifi 1994; Ávila-Lovera *et al.* 2017). This may give an advantage to green-stemmed plants over other drought-deciduous species that do not have the extra carbon gain during the dry season. Generally, stem photo-assimilates are used for plant bud development, flowering, and recovery after herbivory (Bossard and Rejmanek 1992; Tinoco-Ojanguren 2008; Saveyn *et al.* 2010); however, woody tissue photosynthesis also contributes to maintenance of hydraulic function (Saveyn *et al.* 2010; Schmitz *et al.* 2012; Bloemen *et al.* 2016) through sugar-mediated synthesis of surfactants and proteins (De Baerdemaeker *et al.* 2017). Furthermore, within the same species, populations from habitats with higher leaf-to-air water vapour gradients produce proportionally more twigs than leaves, indicating that arid habitat populations may rely on stem photosynthesis at the expense of leaf photosynthesis (Comstock and Ehleringer 1992). These green stems have lower discrimination against ^{13}C (Δ) than leaves, suggesting a tighter stomatal control in the former than the later (Comstock and Ehleringer 1992). These results indicate that stem photosynthesis performs better than leaf photosynthesis in very dry habitats, but this ‘water-saving’ strategy may not be an advantage in wetter habitats.

The carbon isotopic composition of photosynthetic organs reflects the balance between CO_2 supply and demand, and isotope values can be used to investigate whether photosynthesis is limited by photosynthetic machinery or CO_2 availability (Scheidegger *et al.* 2000). On the one hand, there is a relationship between $\delta^{13}\text{C}$ and WUE (Farquhar *et al.* 1982) such that values of $\delta^{13}\text{C}$ in photosynthetic leaf and stem tissue can help us determine possible differences in long-term WUE between these organs. Several studies have found lower intercellular CO_2 concentration (C_i) in green stems than leaves (Osmond *et al.* 1987; Comstock and Ehleringer 1988), leading to differences in discrimination and consequently more enriched $\delta^{13}\text{C}$ values in green stems than in leaves (Ehleringer *et al.* 1987; Smith and Osmond 1987; Farquhar *et al.* 1989; Nilsen and Sharifi 1997). However, other studies have found re-assimilation in stems (Cernusak and Marshall 2000; Berveiller *et al.* 2007) and this can potentially mask the effect of WUE on $\delta^{13}\text{C}$ (Cernusak *et al.* 2001, 2009). On the other hand, oxygen isotopic composition ($\delta^{18}\text{O}$) of

organic matter depends on source water $\delta^{18}\text{O}$, evaporative enrichment and biochemical fractionation during incorporation (Scheidegger *et al.* 2000). Assuming that co-occurring plants use the same soil water source and there are no differences in biochemical fractionation among species, differences in $\delta^{18}\text{O}$ are mainly due to differences in evaporative enrichment and stomatal control (Scheidegger *et al.* 2000). Using $\delta^{18}\text{O}$ values as proxies for changes in air relative humidity, we can tease apart the effects of g_s and A_{\max} on $\delta^{13}\text{C}$ values and determine if changes in C_i , and therefore WUE, are due to stronger stomatal or metabolic limitations on photosynthesis. This has been done previously in leaves, where changes in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were used to infer carbon and water relations of herbaceous plants subjected to different land-use (Scheidegger *et al.* 2000). In the present work, we set out to test this model using green stems.

Our goal was to understand the costs and benefits of having green stems in desert species from southern California. Our research questions were: (1) Does water status differ between green-stemmed and non-green-stemmed species? (2) Do species with green stems have higher carbon gain than plants without green stems? (3) What are the water costs of stem photosynthesis? We expected that green-stemmed plants would have lower water potential than non-green-stemmed plants and higher annual carbon gain, but that this would be associated with higher water costs from both stomatal and cuticular conductance.

Materials and methods

Study site and species

This study was conducted in a desert wash scrub in Mojave Desert at Joshua Tree National Park (34°03′50.5″N, 116°03′16.3″W) during 2016 and 2017. Joshua Tree has a mean annual air temperature of 18.6°C and annual precipitation of 119.1 mm (period 1959–2012, station 044405, Joshua Tree, Western Regional ClimateCenter). During 2016, the mean annual air temperature was 19.4°C and precipitation was 82.3 mm (station 233, Joshua Tree, California Irrigation Management Information System) (Fig. 1). The site is dominated by creosote-bush scrub (*Larrea tridentata* (DC.) Coville, Zygophyllaceae). Fifteen species were studied, and they were categorised in one of two stem-type groups: (1) species with green photosynthetic stems (nine species) and (2) species without evident green stems (six species; Table 1). Most of the species studied are woody shrubs, except for two perennial herbs: *Stillingia linearifolia* S. Watson (Euphorbiaceae) and *Eriogonum inflatum* Torr. (Polygonaceae). Sampling was done in a total of nine campaigns approximately every 6 weeks from February 2016 to March 2017. Three randomly selected individuals per species were sampled each time.

Water potential measurements

Current-year terminal twigs were collected from plants between 0800 and 1200 hours, sealed in double plastic bags (Whirlpak 0.057 mm thick; Nasco) and transported to the laboratory inside a dark ice chest for measurement of water potential (Ψ) using a pressure chamber (Model 1000; PMS Instrument Co.). These twigs were collected during the time of gas-exchange measurements because we were interested in the water status of

plants when photosynthetic rate was at its maximum. Since both green- and non-green-stemmed species were randomly sampled every time, the possible effect of the variable ‘time of the day’ is randomised among all species.

Gas-exchange measurements

Photosynthetic gas exchange of CO₂ and water vapour in stems and leaves (when present) were measured between 0800 and 1200 hours using an open-system infrared gas analyser (Li-6400; LI-COR Biosciences). From these data, the following traits were calculated for both leaves and green stems: net maximum photosynthetic rate (A_{max}), g_s , E and C_i . For non-green stems, the traits measured were respiration rate

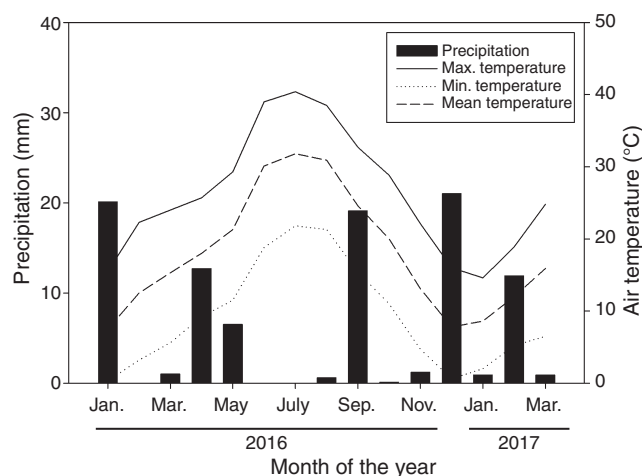


Fig. 1. Total monthly precipitation, maximum air temperature, mean air temperature, and minimum air temperature during the study period February 2016–March 2017. Weather data is from station # 233, Joshua Tree, California Irrigation Management Information System (CIMIS).

in light (R_d), non-stomatal (periderm) conductance to water vapour (g_w), periderm transpiration rate (E_{ns}), and C_i . For each individual, gas exchange was measured in one intact twig segment of <5 mm diameter using the 2×3 cm Li-6400 leaf chamber and custom-made clay-modelled gaskets that allowed a tight seal. Leak trials included (i) empty chamber with clay-modelled gaskets, which showed no changes in apparent gas-exchange measurements (constant ΔCO_2 and $\Delta\text{H}_2\text{O}$), and (ii) chamber with clay-modelled gaskets and a stem sample, which showed positive and stable measurements of A and g_s . Furthermore, blowing air at the chamber did not change A nor g_s . Leaves were measured using standard gaskets that allowed a tight seal without extra accommodation. We did not set the block temperature of the Li-6400 and let the chamber temperature vary with ambient temperature, which varied with season from 12.9°C in January 2017 to 41.0°C in June 2016. We were careful to place small leaves and stems in contact with the leaf thermocouple, so that accurate measures of temperature were recorded. The gas-exchange measurements were performed at 400 $\mu\text{mol mol}^{-1}$ of CO₂ and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically photon flux density (PPFD) provided by a red blue light source (6400–02B #SI-710, LI-COR Biosciences) (Evans and Santiago 2014). We used the same PPFD for both leaves and stems because in another similar ecosystem intercepted PPFD by leaves and stems did not differ (E Ávila-Lovera, C Bejarano-Silva, PP Garcillán, LS Santiago, unpubl. data). Leaves and especially stems did not cover the 6 cm² area of the chamber so after gas-exchange measurements were taken, leaves and stems were sectioned and transported to the laboratory, where projected area was measured using an area meter (Li-3100; LI-COR Biosciences). Gas-exchange traits were then re-calculated using the projected area and all traits for both leaves and stems were expressed on a projected area basis. We used stem projected area to relate the gas-exchange traits to expected light

Table 1. List of 15 plant species studied in a desert wash at Joshua Tree National Park, California, USA

Family, growth form, leaf phenology and stem type are indicated

Species	Family	Growth form ^A	Leaf phenology ^B	Stem type
<i>Ambrosia dumosa</i>	Asteraceae	Shrub	Deciduous	Non-green
<i>Ambrosia salsola</i>	Asteraceae	Shrub	Deciduous	Green
<i>Bebbia juncea</i>	Asteraceae	Shrub	Deciduous	Green
<i>Condea emoryi</i>	Lamiaceae	Shrub	Evergreen	Non-green
<i>Eriogonum inflatum</i>	Polygonaceae	Perennial herb	NA	Green
<i>Krameria bicolor</i>	Krameriaceae	Shrub	Evergreen	Green
<i>Larrea tridentata</i>	Zygophyllaceae	Shrub	Evergreen	Non-green
<i>Menodora spinescens</i>	Oleaceae	Shrub	Deciduous	Green
<i>Psoralea arborescens</i>	Fabaceae	Shrub	Deciduous	Non-green
<i>Scutellaria mexicana</i>	Lamiaceae	Shrub	Deciduous	Green
<i>Senegalia greggii</i>	Fabaceae	Shrub	Deciduous	Non-green
<i>Senna armata</i>	Fabaceae	Shrub	Deciduous	Green
<i>Simmondsia chinensis</i>	Simmondsiaceae	Shrub	Evergreen	Non-green
<i>Stillingia linearifolia</i>	Euphorbiaceae	Perennial herb	NA	Green
<i>Thamnosma montana</i>	Rutaceae	Shrub	Deciduous	Green

^AGrowth form information was taken from CalFlora.org.

^BLeaf phenology was determined during the study period, with deciduous plants dropping their leaves at the onset of the dry season, except for *Senegalia greggii*, which is a winter-deciduous species. NA is used for herbaceous plants, which may be perennial but died back during November 2016–January 2017.

interception (Comstock and Ehleringer 1988) and to allow for comparisons between our work and previously published work (see reviews Nilsen 1995; Gibson 1998; Ávila *et al.* 2014b; Ávila-Lovera and Ezcurra 2016). This normalisation by projected area is consistent with the bulk of the literature on photosynthetic stems (Adams and Strain 1968; Szarek and Woodhouse 1978; Ehleringer *et al.* 1987; Osmond *et al.* 1987; Comstock *et al.* 1988; Comstock and Ehleringer 1988, 1990; Nilsen *et al.* 1989; Bossard and Rejmanek 1992; Tinoco-Ojanguren 2008). However, some have argued that half (Chen and Black 1992; Valladares *et al.* 2003) or even the total (Nilsen and Bao 1990; Nilsen 1992a; Nilsen and Sharifi 1994; Nilsen *et al.* 1996) of the surface area of the stem is more appropriate because of diffuse light interception in the field. Therefore, we also provide the stem diameters (see Table S1, available as Supplementary Material to this paper) and A_{\max} and g_s values expressed on (1) projected area basis (Tables S2), (2) half surface area basis (Table S3) and (3) total surface area basis (Table S4).

Intrinsic water-use efficiency (WUE_i) was calculated for leaves and green stems as A/g_s . Originally, we wanted to compare leaves and green stems of the same species. However, in green-stemmed species, leaves were small in February 2016 and absent for the rest of the year. Hence, we compared leaf and green stem performance during the only month when both were available March 2017. Furthermore, we compared the photosynthetic organs of each stem-type group: green stems and leaves of non-green-stemmed species. Annual carbon gain of green stems and leaves of non-green-stemmed species were estimated by integrating the area under the curve of A_{\max} versus day of the year (DOY) during the study period using the trapezoidal rule (Butler 1962). Then, cumulative carbon gain curves for each organ were plotted and we tested for differences in the total cumulative carbon between them at the end of the study period. We also applied this protocol to calculate cumulative water loss of green stems and leaves of non-green-stemmed species.

Cuticular conductance and periderm permeance

Cuticular conductance (i.e. minimum epidermal conductance, g_{\min} *sensu* Kerstiens (1996)) was determined in leaves and green stems, whereas periderm permeance (P_p) was determined in non-green stems. The same individuals sampled for Ψ measurements were used for g_{\min} measurements. Cuticular conductance and P_p were measured following the methods on PrometheusWiki (Sack and Scoffoni 2011). One 20-cm long-terminal leafy twig was chosen from each individual sampled in the field and taken to the laboratory. Stem samples were cut to a length of 10 cm with a fresh razor blade, and cut ends were sealed with melted candlewax. Leaves were removed from the stems and the cuts were also covered with candlewax. Fully matured leaves were used to estimate leaf g_{\min} . Current-year green and non-green stems were used to estimate stem g_{\min} and P_p , respectively. The samples were placed near a fan, such that they swayed in the breeze, and were allowed to dry for at least 1 h at PPFD of $<10 \mu\text{mol m}^{-2} \text{s}^{-1}$ to induce stomatal closure. A sensor to measure temperature and humidity was placed next to the samples within the breeze and measurements of air temperature and air RH were recorded at the beginning of each weighing

cycle to determine the mole fraction vapour pressure deficit (VPD_{mf}). Samples were weighed for at least six intervals of 20 min during a 2-h period. A graph of water loss versus time was obtained for each sample and water loss per unit of time during the linear portion was used to calculate g_{\min} or P_p and therefore taken to represent transpiration after stomata had fully closed. Values of g_{\min} or P_p were calculated as the transpiration rate divided by the VPD_{mf} using the spreadsheet provided with the protocol, which was modified to account for the total surface area of stems (Sack and Scoffoni 2011).

Stable isotopic composition

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic composition, as well as C and N concentration (%), were measured in dry leaf and stem bark samples in three individuals per species at the end of the wet season (late spring, April 2016) and at the end of the dry season (early fall, October 2016). Stable isotopes were measured using a Delta-V Advantage isotope ratio mass spectrometer operating in continuous-flow mode and attached to either an elemental analyser for C and N (Model 4010, Costech Inc.) or a thermal conversion elemental analyser (Model TC/EA, Thermo-Fisher) for O at the Facility for Isotope Ratio Mass Spectrometry (FIRMS) of the University of California, Riverside (UCR). Stem bark included epidermis, cortex (chlorenchyma), phloem and vascular cambium in green stems, and periderm, cortex (parenchyma), phloem and vascular cambium in non-green stems. The main difference between these two types of bark is the presence of chloroplasts in the cortex of green-stemmed species (Cannon 1908). To compare leaves and green stems, only the bark was used because the wood of green stems is usually enriched in ^{13}C compared with bark (Nilsen and Sharifi 1997), and we were interested in possible differences between leaf and stem photosynthetic tissues in these species and possible implications for WUE_i . The $\delta^{13}\text{C}$ values were expressed relative to the V-PDB standard. Oxygen isotopic composition was measured in the stem bark of the nine green-stemmed species studied. The $\delta^{18}\text{O}$ values were expressed relative to the V-SMOW standard.

Using values of A_{\max} , g_s , $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ we tested a conceptual isotope model (Scheidegger *et al.* 2000) to tease apart the effects of photosynthetic capacity and stomatal conductance regulation on green stem bark $\delta^{13}\text{C}$ changes from wet to the dry season.

Statistical analyses

Because our Ψ observations in time are repeated-measures in the same sites, we could not analyse each observation as an independent replicate. Furthermore, we could not use time as a linear predictor in our ANCOVAs because seasonality recurs in a periodic and cyclic fashion. The approach to use in these cases is that of periodic regression, also known as circular-linear regression, a branch of time-series analysis (Batschelet 1981). A simple model of periodic phenomena is given by the sine curve $y = a + b \sin \omega(t - t_0)$, where a and b are the standard linear regression parameters, t is time measured as day of the year subject to a certain period T (in our case, one whole year, or 365.24 days), t_0 defines the origin of the periodic oscillation describing the point at which the sine function

becomes zero and, ω is the angular frequency that converts the period of the oscillation in days into an angular measurement in radians, so that $\omega = 2\pi/T$. The shift-of-the-origin parameter t_0 can be determined by Fourier analysis of Ψ against time for each species (Batschelet 1981; Shumway and Stoffer 2017). However, because all species have a similar seasonal behaviour decreasing their water potential during the dry summer and increasing it during the wetter winter, their Ψ values are highly correlated and cannot be considered truly independent measures. To make our hypothesis-testing more parsimonious, we grouped all species together using Principal Component Analysis of the among-species correlation matrix and used the first axis as a summary variable that condenses the periodic Ψ cycle in all species grouped together. Using Fourier analysis of the combined Ψ values for all species against time, we found a t_0 value of 30 days, which places the beginning of the wet season on 1 December. With these results, we converted the linear predictor time (t) into the periodic predictor τ , such that $\tau = \sin \omega(t - t_0)$. This predictor was used as a covariate in all analyses of covariance (ANCOVA) where species stem-type was used as a factor. Significance was tested at $P \leq 0.05$.

For gas-exchange data in March 2017, Mann–Whitney U -tests were performed to test for median differences between leaves and green stems of the same species because the Kolmogorov–Smirnov test of equal distributions failed. Also, green stem gas-exchange data was recalculated using half of the stem surface area and total surface area to test for the possibility of changes in ANCOVAs given the area used to express A_{\max} and g_s . Results from these analyses are in the supplemental materials.

For the stable isotope data, paired Student's t -tests were performed to test for mean differences between leaf and green stem bark $\delta^{13}\text{C}$ during the wet season of 2016 when leaves were present. Three-way analyses of variance (ANOVA) were performed to test the effect of season (wet and dry), stem-type group (with and without green stems), organ (leaf and stem bark) and their interactive effects on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ as well as C and N concentration. Relationships among isotopic composition and elemental concentration were assessed using linear regression with significance tested at $P \leq 0.05$.

All statistical analyses were done using R ver.3.4.0 (R Core Team 2017). All plots were made using SigmaPlot 11 (Systat Software).

Results

Leaves of green-stemmed species were small in early spring (February 2016) and fell at the onset of the dry season (May 2016), being absent during summer, fall and winter seasons (May 2016–February 2017). The high precipitation of the 2017 wet season (Fig. 1) promoted the growth of large leaf canopies in most of the green-stemmed species, and we were able to measure these leaves for gas-exchange traits only in 2017.

For both green-stemmed and non-green-stemmed plants shoot Ψ was highly seasonal ($P < 2 \times 10^{-6}$), being higher (less negative) in the wet season, and lower in the dry season (Fig. 2). However, there was a secondary peak in shoot Ψ on DOY 282 (October 2016), given the substantial rain that fell

in September 2016 (Fig. 1). The ANCOVA showed that shoot Ψ across the study period was slightly higher in green-stemmed than in non-green-stemmed species but this difference was not statistically significant ($P = 0.052$, Fig. 2).

Across the study period, green stems had higher A_{\max} ($P = 0.005$, Fig. 3a) and g_s ($P = 0.007$, Fig. 3b) values than leaves of non-green-stemmed species. Since both A_{\max} and g_s were higher in green stems than in leaves, this led to similar values of WUE_i in both organs ($P = 0.420$, Fig. 3c). Furthermore, when green stem data are expressed on a half surface area basis, there are no differences between green stems and leaves of non-green-stemmed species in values of A_{\max} and g_s (see Table S3). When we compared leaves and green stems of the same species in March 2017 we found that both have similar A_{\max} , g_s and WUE_i values (Table 2).

Cuticular conductance was highly seasonal ($P = 2.42 \times 10^{-7}$), being the highest in the wet season and the lowest in the dry season (Fig. 4). Also, leaves of green-stemmed species and green stems had higher g_{\min} values than leaves and P_p of stems of non-green-stemmed species ($P = 3.04 \times 10^{-10}$).

When we integrated A_{\max} and E over the course of the study period we found that green stems have slightly higher cumulative carbon gain per unit of projected area than leaves of non-green-stemmed species (Mann–Whitney U -test, $P = 0.061$, Fig. 5a). Green stems did have higher cumulative water loss than leaves of non-green-stemmed species (Mann–Whitney U -test, $P = 0.046$, Fig. 5b). This shows that in terms of carbon gain both organs are equally efficient, but green stems have generally higher water loss rates, evidenced by high values of both g_s and g_{\min} (Figs 3a, 4).

There were no seasonal changes in $\delta^{13}\text{C}$ and N concentration across species, stem-type groups or organs. The $\delta^{13}\text{C}$ values for green-stemmed species (leaves and stems) were enriched compared with those in non-green-stemmed species ($P = 0.002$). Green stem bark was more enriched than leaves of non-green-stemmed species ($P = 0.013$). Green stem bark $\delta^{13}\text{C}$, however, was statistically higher than same species leaf

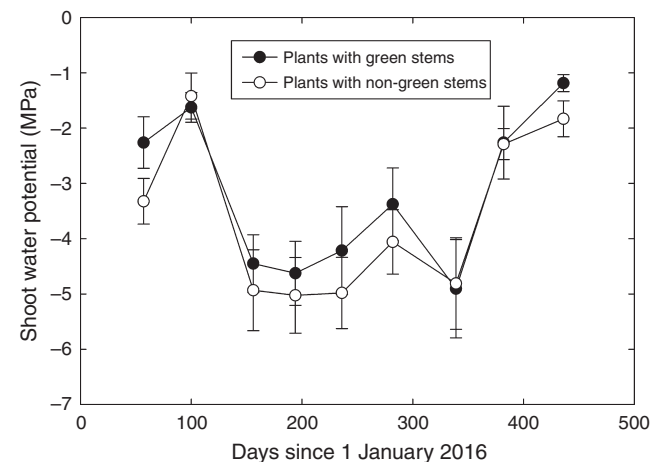


Fig. 2. Shoot water potential for green and non-green-stemmed plant species during the nine sampling campaigns from February 2016 (day 57) to March 2017 (day 436). Values shown are means of all species within each stem-type group \pm s.e. ($n = 9$ for green-stemmed species and $n = 6$ for non-green-stemmed species).

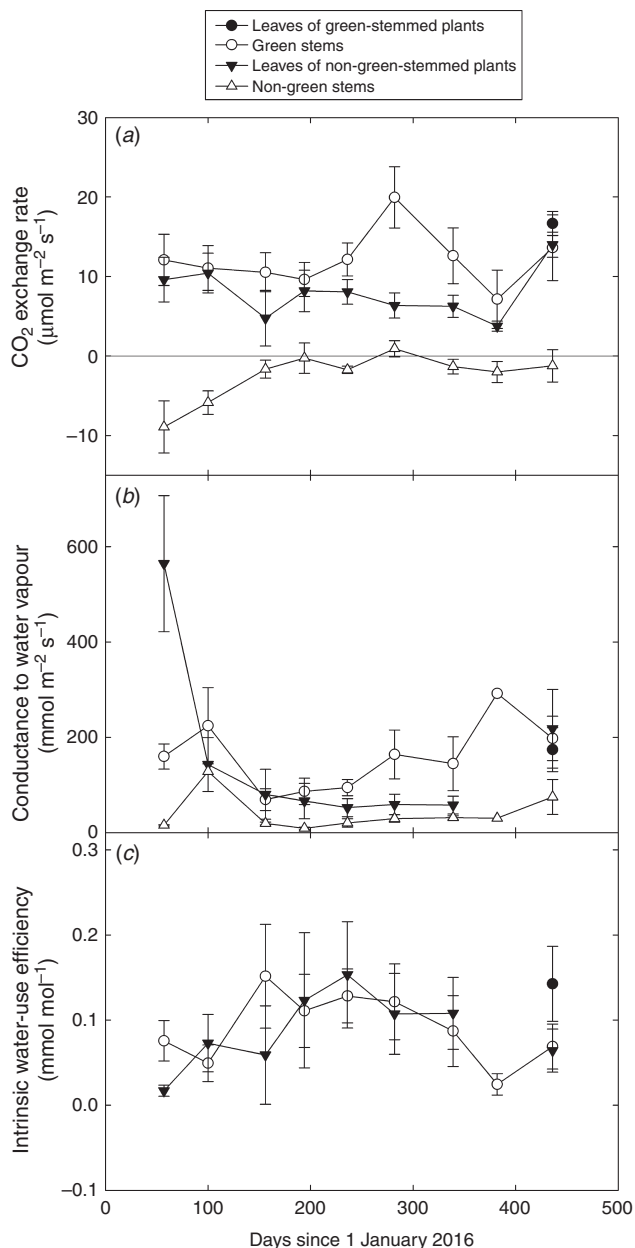


Fig. 3. Gas-exchange data for leaves (when present) and stems of green-stemmed species, and leaves and stems of non-green-stemmed species during the nine sampling campaigns from February 2016 (day 57) to March 2017 (day 436). (a) CO₂ exchange rate, where positive values indicate net photosynthetic rate (A_{\max}) and negative values indicate respiration rate (R_d). (b) Stomatal conductance to water vapour (g_s) of leaves and green stems, and non-stomatal conductance to water vapour (g_w) of non-green stems. (c) Intrinsic water-use efficiency (WUE_i) for leaves and green stems. Values shown are means of all species within each stem-type group \pm s.e. ($n=9$ for green-stemmed species and $n=6$ for non-green-stemmed species).

$\delta^{13}\text{C}$ only in one out of eight green-stemmed species that had both leaves and green stems during the wet season (Table 3). N concentration in leaves and stems of green-stemmed species was also higher than in leaves and stems of non-green-stemmed species ($P=0.003$).

Values of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in green stem bark were not significantly negatively related (Fig. 6a) ($P=0.086$). In contrast, $\delta^{13}\text{C}$ values were positively related with N concentration in both leaves and stems of green-stemmed species ($P=0.016$, Fig. 6b).

We used a conceptual isotope model to test for possible effects of A_{\max} and g_s on stem bark $\delta^{13}\text{C}$. However, seasonal differences in $\delta^{13}\text{C}$ across species were not significant, giving limited resolution for the model to work (see Fig. S1, available as Supplementary Material to this paper). The model only worked for evaluating the seasonal changes of A_{\max} and g_s of *E. inflatum*, which was the only species with significant changes in bark $\delta^{18}\text{O}$ (Table 3; Fig. S1). The decrease of bark $\delta^{18}\text{O}$ from wet to dry season with no change in bark $\delta^{13}\text{C}$ implied that relative humidity increased whereas C_i stayed the same, which could have only happened if both A_{\max} and g_s increased from wet to dry season.

Discussion

The main goal of our work was to understand the costs and benefits of having green stems, in relation to carbon gain and water loss, in desert species from southern California. We found that green-stemmed species maintained statistically similar Ψ compared with species with non-green stems. Both green stems and leaves of non-green-stemmed species had the same cumulative carbon gain per unit of area by the end of the study period. However, A_{\max} and g_s were higher in green stems than in leaves of the same species or leaves of non-green-stemmed species, leading to similar WUE_i in both photosynthetic organs. Greater A_{\max} and g_s in green-stemmed species also indicates a greater overall exchange with the environment. Furthermore, green stems had higher g_{\min} values than non-green stem P_p , suggesting a higher water cost associated with the presence of green stems when stomata are fully closed. These results suggest that leaves and green stems have a similar ratio of carbon gain to water loss during photosynthesis, and that green stems may be losing a greater amount of water than non-green stems through their cuticles. However, since green-stemmed species can maintain similar shoot Ψ values compared with non-green-stemmed species, despite greater rates of gas exchange, this higher water loss through the cuticle may not be as important for whole-plant performance during the dry season, when plants continue assimilating CO₂.

The similar shoot Ψ found in green-stemmed species compared with species with non-green stems despite greater rates of gas exchange suggests that green-stemmed plants may have access to deeper water sources in the soil (Gibson 1998; ME Allen, E Ávila-Lovera, LS Santiago, unpubl. data), that they have better stem stomatal control than leaves of non-green-stemmed plants, or simply that their vertical orientation plays a role in reducing radiation loads and minimising the water loss needed to cool high temperature tissues during the day when air VPD is high. This maintenance of shoot Ψ is important for sustaining physiological activity in green stems during the hot dry season. In this manner, plants can maintain stem stomata open, when other drought-deciduous plants without green stems would be inactive, consistent with the 'extended growth season hypothesis' previously described for

Table 2. Photosynthetic rate (A_{\max}), stomatal conductance (g_s), and intrinsic water-use efficiency (WUE_i) of leaves and green stems of seven woody species studied in a desert wash at Joshua Tree National Park, California, USA

Values correspond to the wet season (March) of 2017 when leaves were present in most species. Values shown are species mean \pm s.e. ($n=3$). P -values are shown for the comparison between organs using the Mann–Whitney U -test

Species	Organ	A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	WUE_i (mmol mol^{-1})
<i>Ambrosia salsola</i>	leaf	11.50 \pm 1.72	189.43 \pm 36.61	0.07 \pm 0.02
	stem	10.73 \pm 5.99	174.64 \pm 94.17	0.45 \pm 0.39
<i>Bebbia juncea</i>	leaf	NA	NA	NA
	stem	43.89 \pm 6.94	472.64 \pm 121.06	0.10 \pm 0.01
<i>Krameria bicolor</i>	leaf	NA	NA	NA
	stem	5.67 \pm 1.97	55.63 \pm 26.63	0.12 \pm 0.01
<i>Menodora spinences</i>	leaf	NA	NA	NA
	stem	15.51 \pm 2.47	159.37 \pm 26.58	0.10 \pm 0.00
<i>Scutellaria mexicana</i>	leaf	18.20 \pm 9.68	341.38 \pm 165.77	0.05 \pm 0.01
	stem	0.99 \pm 4.57	372.68 \pm 76.32	0.02 \pm 0.01
<i>Senna armata</i>	leaf	15.82 \pm 9.13	69.93 \pm 40.37	0.29 \pm 0.17
	stem	10.81 \pm 6.24	71.77 \pm 41.44	0.36 \pm 0.21
<i>Thamnosma montana</i>	leaf	20.58 \pm 4.93	122.26 \pm 44.00	0.19 \pm 0.03
	stem	13.73 \pm 3.94	93.24 \pm 41.49	0.33 \pm 0.20
Mean \pm s.e.	leaf	16.67 \pm 1.51	218.27 \pm 46.05	0.14 \pm 0.04
	stem	13.63 \pm 4.13	75.02 \pm 46.56	0.18 \pm 0.05
P -value		0.122	0.850	0.207

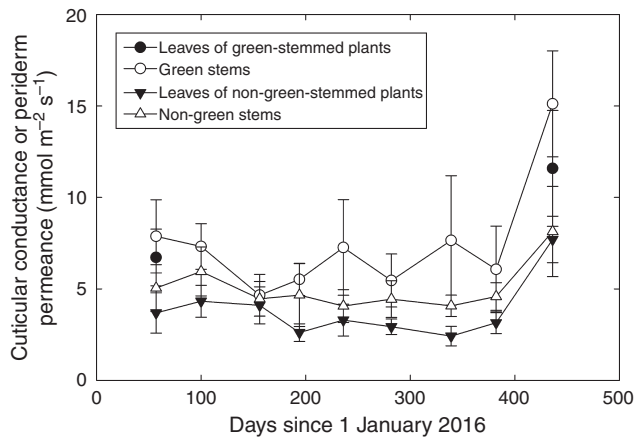


Fig. 4. Cuticular conductance (g_{\min}) of leaves and green stems and periderm permeance (P_p) of non-green stems during the nine sampling campaigns from February 2016 (day 57) to March 2017 (day 436). Values shown are means of all species within each stem-type group \pm s.e. ($n=9$ for green-stemmed species and $n=6$ for non-green-stemmed species).

species with photosynthetic stems (Nilsen and Sharifi 1997; Saveyn *et al.* 2010; Cernusak and Hutley 2011; Simbo *et al.* 2013). Furthermore, it seems that green stems may be more responsive to short-term increases in water availability than leaves, as shown by a sudden increase in A_{\max} in October 2016 (Fig. 3a) after it rained in September 2016 (Fig. 1). Photosynthetic rate of green stems has also been found to be less sensitive to water deficit than leaves of the same species (Nilsen and Bao 1990; Tinoco-Ojanguren 2008), giving green stems a performance advantage over leaves during the dry season, when soil and air water deficit are high. This may be related to the fact that many species with green stems have stomata in longitudinal grooves with an epidermis sometimes covered by trichomes (Gibson 1983), so that stomata may stay

open within a relatively humid boundary layer with low VPD compared with atmospheric VPD, and gain carbon at a relatively lower water cost compared with leaves. Furthermore, keeping leaves during the dry season would mean higher carbon losses through maintenance respiration, as well as water losses through the cuticle even when stomata are closed.

It has been noted before that as aridity increases leaf area declines, and plants rely increasingly on photosynthesis occurring in green stems (Comstock and Ehleringer 1992). Furthermore, the contribution of twigs to annual integrated carbon assimilation of green-stemmed species has been found to be 16–83% (Comstock *et al.* 1988). Our results show that stems can have photosynthetic rates as high as leaves, and that they are not structurally constrained by their shapes despite of the fact that they play additional roles, such as vascular transport and structural support, as has been previously highlighted (Comstock and Ehleringer 1988). The similar cumulative carbon gain between green and non-green-stemmed species suggests that plants can cope with the same environmental stresses through different strategies (Pivovarov *et al.* 2016), such as using leaves vs stems for assimilating CO_2 , and that this could be a mechanism leading to coexistence of the two stem-type groups at this site.

The existence of trade-offs between the benefits of having photosynthetic stems and possible constraints to plant structure and function was first proposed by Nilsen (1995). At that time, these constraints were related to the cost of having a thin bark (i.e. cortex + epidermis) to stem herbivore defence and the presence of surface stomata that could be entry points for pathogens; constraints that are structural or mechanical in nature. This thin bark can also lose water more rapidly than a bark covered by a suberised periderm, leading to a functional constraint. Across species, green stem g_{\min} values ranged from 1.42 to 33.74 $\text{mmol m}^{-2} \text{s}^{-1}$, and they overlapped with the range of 1.11–20.49 $\text{mmol m}^{-2} \text{s}^{-1}$ found for 11

green-stemmed species studied in desert sites in southern California (Ávila-Lovera *et al.* 2017). These values are significantly higher than the ones found for non-green stems

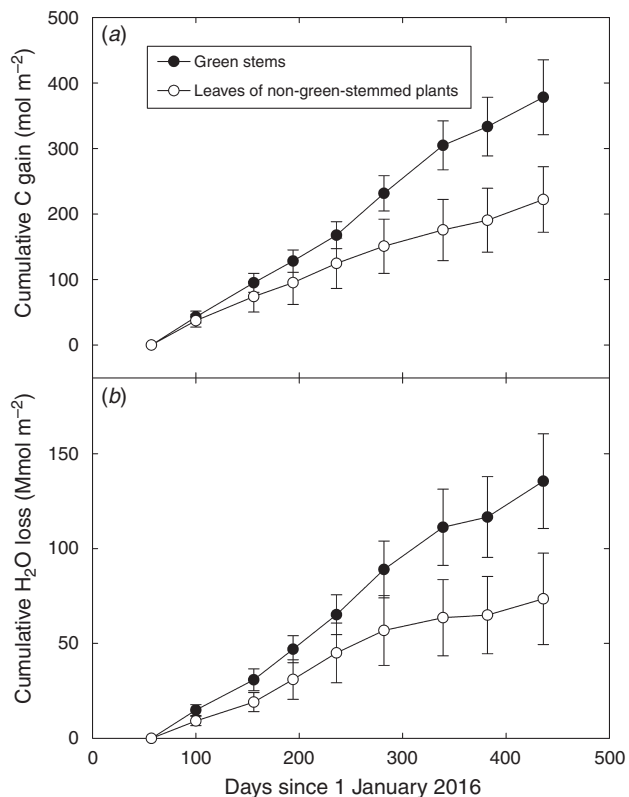


Fig. 5. (a) Cumulative carbon gain, and (b) water loss curves for green stems and leaves of non-green-stemmed species as a function of day since January 2016. Values shown are means of all species within each stem-type group \pm s.e. ($n=9$ for green-stemmed species and $n=6$ for non-green-stemmed species).

in this study, which ranged from 1.05 to 19.03 mmol m⁻² s⁻¹, and were also higher than mean periderm permeance of 2.42 mmol m⁻² s⁻¹ found in different tree and crop species (Schönherr and Ziegler 1980; Vogt *et al.* 1983; Garrec and Barrois 1992; Lulai and Orr 1994; Lulai *et al.* 1996; Groh *et al.* 2002; Schreiber *et al.* 2005; Kocurek *et al.* 2015). Our results support the idea that green stems lose more water per surface area and time through their cuticle than non-green stems do through their periderm and this may be a cost that partially offsets the benefits of having green stems.

When we compared the photosynthetic organ of each stem-type group, green stems vs leaves of non-green-stemmed species, we see that green stems have higher N concentration and $\delta^{13}\text{C}$ values than leaves. This high N concentration partially explains the high photosynthetic capacity of green stems compared with leaves (von Caemmerer and Farquhar 1981). In leaves, the proteins of the Calvin cycle and thylakoids represent the majority of leaf nitrogen (Evans 1989). Although measures of protein concentration in stems are limited (Ávila *et al.* 2014a), the positive relationship between CO₂ assimilation and N concentration has been found in stems that perform photosynthesis (Nilsen 1992b; Berveiller *et al.* 2007). In some cases, seasonal changes in stem photosynthetic rate are not related to seasonal changes in N concentration (Nilsen and Sharifi 1994), indicating that both stomatal behaviour and metabolic factors coordinate the photosynthesis occurring in stems, as it occurs in leaves (Tezara *et al.* 1999).

The fact that the relationship between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in green stem bark trended significant ($P=0.09$), provides little evidence that stems with higher long-term integrated WUE, as indicated by high $\delta^{13}\text{C}$, have lower enrichment values, as indicated by $\delta^{18}\text{O}$ values. The positive relationship between $\delta^{13}\text{C}$ and N concentration across leaves and stems of green-stemmed species on the other hand indicates that as N concentration increases, there is a higher CO₂ drawdown for a given stomatal conductance, leading to increased WUE_i and $\delta^{13}\text{C}$ values (Farquhar *et al.* 1982). These would provide a benefit for species in the high $\delta^{13}\text{C}$ -high N end because high N

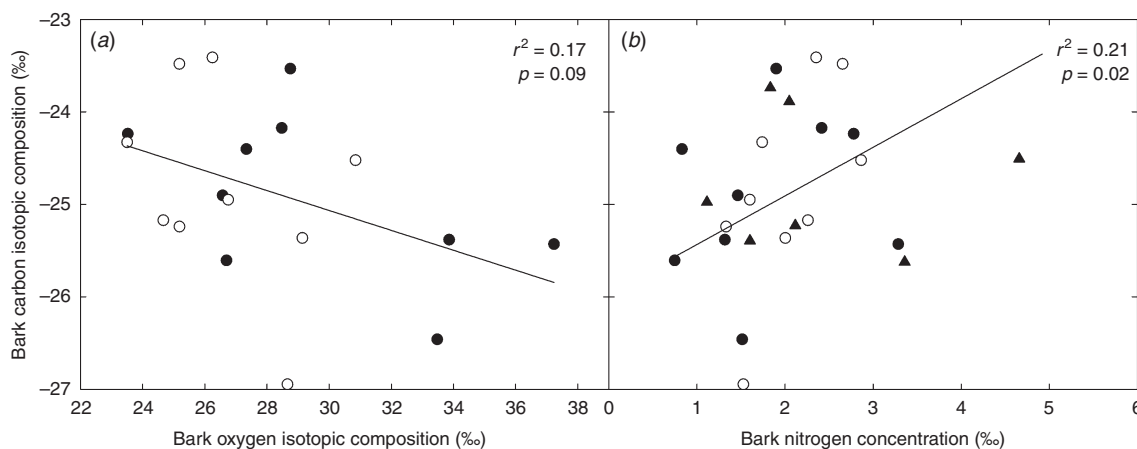


Fig. 6. (a) Carbon isotopic composition as a function of oxygen isotope composition in bark of green-stemmed species. (b) Carbon isotopic composition as a function of nitrogen concentration of both leaves and stems of green-stemmed species. Values correspond to species means. Symbols are as follows: leaves (triangles), stems (circles), wet season (closed symbols), dry season (open symbols). Lines are regressions through species means.

Table 3. Carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$) and oxygen ($\delta^{18}\text{O}$) isotopic composition, as well as carbon (C) and nitrogen (N) concentration, of leaves and stem bark of 15 species with and without green stems studied in a desert wash at Joshua Tree National Park, CA, USA
 These values correspond to both wet and dry season of 2016. Values shown are species mean \pm s.e. ($n=3$)

Season	Species	Organ	$\delta^{13}\text{C}(\text{‰})$	C(%)	$\delta^{15}\text{N}(\text{‰})$	N(‰)	$\delta^{18}\text{O}(\text{‰})$	
Wet	<i>Green-stemmed</i>							
	<i>Ambrosia salsola</i>	leaf	-25.6 \pm 0.8	44.1 \pm 0.5	3.6 \pm 0.8	3.4 \pm 0.4	32.1 \pm 1.1	
		bark	-24.2 \pm 0.7	45.6 \pm 0.6	2.6 \pm 0.8	2.8 \pm 0.2	23.5 \pm 0.6	
	<i>Bebbia juncea</i>	leaf	-27.7 \pm 0.3	37.0 \pm 1.3	4.3 \pm 0.4	1.4 \pm 0.3	38.9 \pm 0.2	
		bark	-25.4 \pm 0.2	40.5 \pm 0.3	2.6 \pm 0.4	1.3 \pm 0.4	33.9 \pm 2.9	
	<i>Eriogonum inflatum</i>	leaf	—	—	—	—	—	
		bark	-25.4 \pm 0.5	42.2 \pm 3.5	4.5 \pm 0.5	3.3 \pm 0.9	37.2 \pm 1.1	
	<i>Krameria bicolor</i>	leaf	-23.7 \pm 1.0	45.2 \pm 0.3	7.0 \pm 1.2	1.8 \pm 0.4	46.1 \pm 2.7	
		bark	-24.4 \pm 0.3	46.4 \pm 1.4	3.7 \pm 0.2	0.8 \pm 0.0	27.3 \pm 1.8	
	<i>Menodora spinescens</i>	leaf	-25.2 \pm 1.0	44.1 \pm 0.5	2.7 \pm 0.2	2.1 \pm 0.1	39.4 \pm 0.9	
		bark	-24.9 \pm 0.1	46.4 \pm 0.2	1.2 \pm 0.3	1.5 \pm 0.5	26.6 \pm 0.8	
	<i>Scutellaria mexicana</i>	leaf	-24.3 \pm 0.0	42.7 \pm 0.1	3.7 \pm 0.7	1.1 \pm 0.1	44.0 \pm 1.1	
		bark	-25.6 \pm 0.6	50.6 \pm 1.1	2.0 \pm 0.7	0.8 \pm 0.2	26.7 \pm 2.5	
	<i>Senna armata</i>	leaf	-25.4 \pm 0.4	42.8 \pm 0.4	3.1 \pm 0.3	1.6 \pm 0.2	50.4 \pm 1.4	
		bark	-26.5 \pm 0.7	48.3 \pm 1.4	2.1 \pm 0.5	1.5 \pm 0.4	33.5 \pm 1.6	
	<i>Stillingia linearifolia</i>	leaf	-24.5 \pm 0.7	41.9 \pm 0.6	1.9 \pm 0.7	4.7 \pm 0.4	43.0 \pm 3.3	
		bark	-23.5 \pm 0.3	42.7 \pm 0.9	0.5 \pm 0.8	1.9 \pm 0.2	28.8 \pm 0.5	
	<i>Thamnosma montana</i>	leaf	-23.9 \pm 0.0	44.2 \pm 0.8	2.2 \pm 0.1	2.1 \pm 0.3	50.4 \pm 6.2	
		bark	-24.2 \pm 0.9	47.5 \pm 0.3	0.8 \pm 0.5	2.4 \pm 0.6	28.5 \pm 2.3	
	<i>Non-green-stemmed</i>							
	<i>Ambrosia dumosa</i>	leaf	-25.2 \pm 0.6	39.3 \pm 0.9	4.4 \pm 0.6	2.6 \pm 0.0	33.7 \pm 1.0	
		bark	-25.5 \pm 0.4	43.5 \pm 0.4	4.3 \pm 0.6	1.0 \pm 0.1	25.8 \pm 0.6	
	<i>Condea emoryi</i>	leaf	-28.5 \pm 1.1	48.7 \pm 0.8	4.1 \pm 0.8	2.2 \pm 0.4	30.7 \pm 0.7	
		bark	-27.1 \pm 0.9	44.2 \pm 1.0	1.3 \pm 0.5	0.3 \pm 0.2	29.0 \pm 2.3	
	<i>Larrea tridentata</i>	leaf	-24.9 \pm 0.6	47.5 \pm 0.4	5.2 \pm 0.2	1.5 \pm 0.3	31.0 \pm 0.7	
		bark	-24.0 \pm 0.4	41.6 \pm 5.4	1.6 \pm 0.4	0.2 \pm 0.1	21.7 \pm 0.5	
	<i>Psoralea arborescens</i>	leaf	-23.4 \pm 0.3	42.7 \pm 0.5	2.1 \pm 0.5	2.0 \pm 0.1	35.2 \pm 1.4	
		bark	-24.4 \pm 0.1	43.7 \pm 0.7	1.1 \pm 0.8	1.6 \pm 0.2	18.2 \pm 0.6	
	<i>Senegalia greggii</i>	leaf	-29.2 \pm 0.8	45.0 \pm 1.9	5.1 \pm 1.6	3.1 \pm 1.8	50.4 \pm 1.4	
		bark	-27.4 \pm 0.8	46.5 \pm 0.6	4.3 \pm 1.6	2.2 \pm 0.6	22.3 \pm 0.6	
<i>Simmondsia chinensis</i>	leaf	-26.2 \pm 0.6	44.7 \pm 0.4	5.2 \pm 0.3	0.8 \pm 0.3	37.4 \pm 2.0		
	bark	-25.2 \pm 0.3	47.1 \pm 0.3	2.4 \pm 0.4	1.1 \pm 0.1	24.8 \pm 0.3		
Dry	<i>Green-stemmed</i>							
	<i>Ambrosia salsola</i>	leaf	—	—	—	—	—	
		bark	-25.4 \pm 0.5	46.1 \pm 0.2	3.3 \pm 0.4	1.3 \pm 0.3	25.2 \pm 1.4	
	<i>Bebbia juncea</i>	leaf	—	—	—	—	—	
		bark	-26.9 \pm 0.5	42.2 \pm 1.2	0.6 \pm 0.8	1.5 \pm 0.1	28.7 \pm 0.8	
	<i>Eriogonum inflatum</i> ^A	leaf	—	—	—	—	—	
		bark	-25.2	40.4	0.9	1.3	25.2 \pm 1.0	
	<i>Krameria bicolor</i>	leaf	—	—	—	—	—	
		bark	-24.3 \pm 0.2	47.7 \pm 1.2	2.9 \pm 0.4	1.7 \pm 0.1	23.5 \pm 0.9	
	<i>Menodora spinescens</i>	leaf	—	—	—	—	—	
		bark	-25.1 \pm 0.4	47.5 \pm 0.3	1.1 \pm 0.1	2.3 \pm 0.2	24.7 \pm 0.5	
	<i>Scutellaria mexicana</i>	leaf	—	—	—	—	—	
		bark	-25.0 \pm 0.3	50.4 \pm 0.2	2.7 \pm 0.2	1.6 \pm 0.2	26.8 \pm 0.8	
	<i>Senna armata</i>	leaf	—	—	—	—	—	
		bark	-25.4 \pm 0.2	50.5 \pm 0.2	1.2 \pm 0.1	2.0 \pm 0.2	29.1 \pm 0.6	
	<i>Stillingia linearifolia</i>	leaf	—	—	—	—	—	
		bark	-23.4 \pm 0.1	45.8 \pm 0.4	-0.8 \pm 0.6	2.3 \pm 0.3	26.2 \pm 1.8	
	<i>Thamnosma montana</i>	leaf	—	—	—	—	—	
		bark	-24.5 \pm 0.2	51.7 \pm 4.2	0.5 \pm 0.2	2.9 \pm 0.4	30.8 \pm 1.7	
	<i>Non-green-stemmed</i>							
	<i>Ambrosia dumosa</i>	leaf	—	—	—	—	—	
		bark	-25.5 \pm 0.5	46.1 \pm 0.2	3.3 \pm 0.4	1.3 \pm 0.3	25.4 \pm 0.2	
	<i>Condea emoryi</i>	leaf	-27.7 \pm 1.0	48.6 \pm 0.8	4.7 \pm 0.2	1.7 \pm 0.1	31.9 \pm 1.0	
		bark	-26.6 \pm 0.6	44.4 \pm 0.4	1.4 \pm 0.3	1.2 \pm 0.1	23.6 \pm 0.6	

(continued next page)

Table 3. (continued)

Season	Species	Organ	$\delta^{13}\text{C}(\text{‰})$	C(%)	$\delta^{15}\text{N}(\text{‰})$	N(‰)	$\delta^{18}\text{O}(\text{‰})$
	<i>Larrea tridentata</i>	leaf	-22.7 ± 0.3	51.9 ± 0.8	5.7 ± 0.7	1.5 ± 0.1	29.0 ± 0.8
		bark	-24.0 ± 0.4	50.7 ± 1.1	0.9 ± 0.7	1.6 ± 0.1	19.6 ± 1.0
	<i>Psoralea arborescens</i>	leaf	–	–	–	–	–
		bark	-25.3 ± 0.3	46.3 ± 0.2	0.4 ± 0.4	1.6 ± 0.3	17.2 ± 0.3
	<i>Senegalia greggii</i>	leaf	-26.3 ± 0.5	46.4 ± 0.3	4.4 ± 0.5	2.2 ± 0.2	33.4 ± 0.7
		bark	-26.2 ± 0.6	46.3 ± 0.6	1.5 ± 0.8	2.4 ± 0.3	20.2 ± 0.0
	<i>Simmondsia chinensis</i>	leaf	-25.5 ± 1.0	46.6 ± 1.0	5.2 ± 0.2	1.6 ± 0.1	37.2 ± 1.0
		bark	-24.4 ± 0.5	47.6 ± 0.3	0.3 ± 0.5	1.7 ± 0.1	24.7 ± 0.4

^A*Eriogonum inflatum* dry season bark isotope value is from one individual only. The other samples were lost in the isotope facility.

concentration appears to allow for a higher assimilation capacity with a relatively lower stomatal aperture.

The biochemical composition of a given plant tissue determines its $\delta^{13}\text{C}$ value (Cernusak *et al.* 2009). The $\delta^{13}\text{C}$ also varies with WUE (Nilsen and Sharifi 1997) and re-assimilation in green stems (Cernusak *et al.* 2001). In our study, $\delta^{13}\text{C}$ values were not different between leaves and green bark, indicating that both tissues had similar biochemical composition and possibly similar long-term integrated WUE.

We used the conceptual isotope model proposed by Scheidegger *et al.* (2000) and applied it to green stems to understand the effect of A_{max} and g_s on stem bark $\delta^{13}\text{C}$. Across species, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values did not change from wet to dry season, therefore, we found no match between model expectations and changes in gas exchange. The model only worked for *E. inflatum*, whose bark $\delta^{18}\text{O}$ value decreased from wet to dry season without a change in $\delta^{13}\text{C}$, corresponding to the observed gas-exchange behaviour of increased A_{max} and g_s . This model was originally proposed and used to evaluate isotope data from species in plots that differed in land-use intensity (Scheidegger *et al.* 2000) and changes in land-use are expected to have greater impact on carbon and water relations than seasonal variation in precipitation, as in our case. Furthermore, if green-stemmed species really do have access to deeper water sources in the soil (Gibson 1998; ME Allen, E Ávila-Lovera, LS Santiago, unpubl. data), seasonal variation in precipitation may not result in a strong seasonal soil water deficit, leading to changes in gas-exchange behaviour. We encourage researchers to continue applying this model in other plants and systems to understand the magnitude of physiological changes that needs to happen in order for these changes to be recorded in the stable isotope composition of photosynthetic tissues.

In summary, our results show that plants with green stems relied on their stem as the sole organ for carbon assimilation for most of the study period. These green stems can continue assimilating CO_2 at high rates throughout the dry season. We found no support for the high water-use efficiency hypothesis in green stems over leaves, but green stems did maintain higher rates of carbon assimilation than leaves of non-green stems, emphasising their overall greater transfer capacity of water and CO_2 between the plant body and the atmosphere. Green stems might be losing more water through their cuticle than leaves and non-green stems, raising further questions of how trade-offs between carbon gain and water loss through

the cuticle in green stems may affect plant responses to future droughts.

Conflict of interest

The authors declare no conflicts of interest.

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