## Title

Do Plants With Photosynthetic Stems Respond Differently to Drought? An Ecophysiological Evaluation of Desert Communities

**Author**

Avila Lovera, Eleinis

**Publication Date**

2018

UNIVERSITY OF CALIFORNIA
RIVERSIDE

# Do Plants With Photosynthetic Stems Respond Differently to Drought? An Ecophysiological Evaluation of Desert Communities

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology and Organismal Biology

by

Eleinis Alexandra Avila Lovera

June 2018

Dissertation Committee:
Dr. Louis Santiago, Chairperson
Dr. Exequiel Ezcurra
Dr. Michael Allen

The Dissertation of Eleinis Alexandra Avila Lovera is approved:

Committee Chairperson

University of California, Riverside

# ABSTRACT OF THE DISSERTATION

## Do Plants With Photosynthetic Stems Respond Differently to Drought? An Ecophysiological Evaluation of Desert Communities

by

Eleinis Alexandra Avila Lovera

Doctor of Philosophy, Graduate Program in Evolution, Ecology and Organismal Biology
University of California, Riverside, June 2018
Dr. Louis Santiago, Chairperson

In many plants from arid and semi-arid ecosystems in both temperate and tropical regions, green photosynthetic stems appear to have evolved as a response to water shortage at least during one period of the year. My work addresses the question of whether plants with photosynthetic stems respond differently to drought. In my first chapter I studied the coordination between photosynthesis and hydraulics in plants with photosynthetic stems in southern California. This topic is critical because photosynthetic stems have been proposed to be more tolerant to drought than leaves, and given the predictions of global climate change, plants in arid ecosystems may face die-back and eventual local extinction if they cannot cope with the detrimental effects of drought. Using drought survival traits, I can predict what species are more likely to survive extreme droughts. My second chapter addresses the question of how carbon and water dynamics differ in green versus non-green-stemmed plants. It is obvious that having photosynthetic stems increases the carbon gain of the whole plant, but what are the costs associated with it? They might have higher water costs than plants without green stems, raising the question of the existence of trade-offs in carbon and water resources in plants with photosynthetic stems. I found that plants with green stems have the same carbon gain as plants without green stems, and both had the same water-use efficiency. However, cuticular conductance was higher in green stems than leaves and brown stems of non-green-stemmed species, raising questions about the possible costs of having green stems in terms of water balance. In my third chapter I studied photosynthetic and hydraulic traits of plants with photosynthetic stems in a subtropical arid ecosystem in Mexico. I found that green stems had higher photosynthetic capacity than fleshy ones, but that fleshy stems had higher hydraulic capacity and water storage capacity. The results show that different photosynthetic stem syndromes can have different physiological outputs. Overall, green stem physiology resembles leaf physiology and the advantages of extra carbon gain are offset by water costs.

# Acknowledgements

The text of this dissertation, in part, is a reprint of the material as it appears in Ávila-Lovera et al. 2017. The co-author Louis Santiago listed in that publication directed and supervised the research which forms the basis for this dissertation.

I want to thank the UCR Botanic Gardens and the DeRouen family for use of their road to access the Morongo Valley study site. Special thanks to Antonio Zerpa, Mark De Guzman, Alex Pivovaroff, Aleyda Acosta-Rangel, Luis Torres, Roxana Haro, Manika Choudary and Michael Torres, for help provided in the field and in the lab. I am thankful to my Dissertation Committee, Dr. Louis Santiago, Dr. Exequiel Ezcurra and Dr. Michael Allen for their support and insightful discussions on my work. Special thanks to Marilyn Fogel for helpful discussions on stable isotopes.

Funding was provided by the Mayhew Graduate Research Award and the University of California Natural Reserve System to do field work at Boyd Deep Canyon Desert Research Center; the Robert Lee Graduate Student Research Grant to do field work in Joshua Tree National Park; and the University of California Institute for Mexico and the United States through a Grant for Dissertation Research to do field work on southern Baja California peninsula, Mexico.

To the memory of my grandparents, Leonidas de Lovera and Luis Lovera

**Table of Contents**

**Lists of Tables**

**Lists of Figures**

## Introduction

Leaves are the main photosynthetic organs in $C_3$, $C_4$ and some plants using crassulacean acid metabolism (CAM). Much is known about their physiological responses to changes in environmental variables (Cowan and Farquhar 1977; Pearcy and Ehleringer 1984), and how their carbon and water dynamics influence whole plant carbon balance and water relations (Cowan 1982; Lambers et al. 2008). However, in many deciduous plant species that remain leafless for part or most of the year in seasonally dry ecosystems, $CO_2$ fixation occurring in photosynthetic stems represents an important source for the carbon gain of the plants (Nilsen 1995).

Plant species with photosynthetic stems can be classified as having either stem net photosynthesis (SNP) or stem recycling photosynthesis (SRP) (Ávila et al. 2014a, b). Stem net photosynthesis is similar to leaf photosynthesis in which atmospheric $CO_2$ is assimilated after it has diffused through stomata on the epidermis. On the other hand, SRP serves as a re-assimilatory process where chlorenchyma under a suberized periderm assimilates $CO_2$ that comes from the respiration of root and stem tissues. Mature woody plants with chlorenchyma and a stomata-bearing epidermis in the stem have green looking stems, and they increase their carbon gain through SNP. Trees with chlorenchyma and periderm reduce their carbon loss through SRP. Both types of photosynthesis have significant positive effects in the carbon economy of plants (Nilsen 1995; Pfanz et al. 2002; Aschan and Pfanz 2003).

Woody plants with green stems (and with SNP) are very common in desert and Mediterranean climate ecosystems (Gibson 1983; Nilsen and Sharifi 1994), where water is typically the environmental factor limiting photosynthesis and growth (Schulze et al. 1987; Chaves and Pereira 1992; Chaves et al. 2002; McDowell et al. 2008). This suggests that water is the main factor regulating the distribution of these plants, and that their green stems give them some advantages over non-green-stemmed plants in these arid and semi-arid ecosystems. However, this idea is based solely on generally greater photosynthetic water-use efficiency (WUE), the ratio of carbon gain to water loss when stomata are open, in green stems compared to leaves (Ehleringer et al. 1987; Smith and Osmond 1987; Nilsen and Sharifi 1997), and this hypothesis still requires testing based on plant water relations or behavior during drought.

Having green stems (and SNP) can be advantageous in arid ecosystems because it provides extra carbon income to the plant, an extra income that other non-green-stemmed deciduous plants do not have. During favorable periods of the year, both types of plants have their leaf stomata open for photosynthetic uptake of $CO_2$, but open stomata also allow evaporative loss of water, producing a necessary trade-off between carbon gain and water loss (Cowan 1982). In stems of green-stemmed plants, the story is not different. Carbon uptake is followed by water loss, but little is known about the water costs of green-stemmed plants when comparing to non-green-stemmed plants during drought when stomata are closed. Even more important, is whether we can predict what will happen with these plants when facing more frequent, severe and longer droughts, then so-called global-change-type droughts (Breshears et al. 2005), if we do not know about their physiology and how they respond to seasonal drought.

Little is known about the differences between syndromes of photosynthetic stems and how physiology can explain their distribution. Retamoid plants have green woody stems capable of performing stem net photosynthesis because of stomata on the stem epidermis that allows for atmospheric $CO_2$ assimilation (Ávila et al. 2014b; Ávila-Lovera and Ezcurra 2016). Sarcocaulescent plants, or pachycauls, have enlarged fleshy stems with low density wood that serves as water storage, usually exfoliating bark, and green parenchyma (chlorenchyma) capable of doing stem recycling photosynthesis and in some occasions stem net photosynthesis (Ávila et al. 2014b; Ávila-Lovera and Ezcurra 2016). Because of their different morphology it is expected that they will be functionally different, and be able to respond to drought differently.

The main question I investigated is "do plants with photosynthetic stems respond differently to drought?" To address this question, I set three specific questions, which comprise the three chapters of my dissertation:

1. Do green stems behave like leaves in terms of coordinating photosynthesis and hydraulics?
2. How do the carbon and water dynamics of green-stemmed plants compare to non-green-stemmed plants?
3. What are the photosynthetic and hydraulic capacities of green and fleshy stems?

## References

Aschan G, Pfanz H (2003) Non-foliar photosynthesis – a strategy of additional carbon acquisition. *Flora - Morphology, Distribution, Functional Ecology of Plants* 198:81–97. doi: 10.1078/0367-2530-00080

Ávila E, De Almeida J, Tezara W (2014a) Comparación ecofisiológica y anatómica de los tejidos fotosintéticos de *Cercidium praecox* (Ruiz & Pav. ex Hook.) Harms (Fabaceae, Caesalpinioideae). *Acta Botánica Venezuelica* 37:59–76

Ávila E, Herrera A, Tezara W (2014b) Contribution of stem $CO_2$ fixation to whole-plant carbon balance in nonsucculent species. *Photosynthetica* 52:3–15. doi: 10.1007/s11099-014-0004-2

Ávila-Lovera E, Ezcurra E (2016) Stem-succulent trees from the Old and New World tropics. In: Goldstein G, Santiago LS (eds) Tropical tree physiology. Springer International Publishing, Switzerland, pp 45–65

Breshears DD, Cobb NS, Rich PM, et al (2005) Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* 102:15144–15148. doi: 10.1073/pnas.0505734102

Chaves MM, Pereira JS (1992) Water Stress, $CO_2$ and Climate Change. *Journal of Experimental Botany* 43:1131–1139. doi: 10.1093/jxb/43.8.1131

Chaves MM, Pereira JS, Maroco J, et al (2002) How Plants Cope with Water Stress in the Field? Photosynthesis and Growth. *Annals of Botany* 89:907–916. doi: 10.1093/aob/mcf105

Cowan IR (1982) Regulation of Water Use in Relation to Carbon Gain in Higher Plants. In: Lange PDOL, Nobel PPS, Osmond PCB, Ziegler PDH (eds) Physiological Plant Ecology II. Springer Berlin Heidelberg, pp 589–613

Cowan IR, Farquhar GD (1977) Stomatal function in relation to leaf metabolism and environment. *Symposia of the Society for Experimental Biology* 31:471–505

Ehleringer JR, Comstock JP, Cooper TA (1987) Leaf-twig carbon isotope ratio differences in photosynthetic-twig desert shrubs. *Oecologia* 71:318–320

Gibson AC (1983) Anatomy of Photosynthetic Old Stems of Nonsucculent Dicotyledons from North American Deserts. *Botanical Gazette* 144:347–362

Lambers H, Chapin FS, Pons TL (2008) Plant Physiological Ecology. Springer New York, New York, NY

McDowell N, Pockman WT, Allen CD, et al (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* 178:719–739. doi: 10.1111/j.1469-8137.2008.02436.x

Nilsen E, Sharifi M (1997) Carbon isotopic composition of legumes with photosynthetic stems from mediterranean and desert habitats. *American Journal of Botany* 84:1707–1713

Nilsen ET (1995) Stem photosynthesis: extent, patterns, and role in plant carbon economy. In: Gartner BL (ed) Plant stems: physiology and functional morphology. Academic Press, San Diego, pp 223–240

Nilsen ET, Sharifi MR (1994) Seasonal acclimation of stem photosynthesis in woody legume species from the Mojave and Sonoran deserts of California. *Plant Physiology* 105:1385–1391

Pearcy RW, Ehleringer J (1984) Comparative ecophysiology of C<sub>3<sub> and $C_4$ plants. *Plant, Cell & Environment* 7:1–13

Pfanz H, Aschan G, Langenfeld-Heyser R, et al (2002) Ecology and ecophysiology of tree stems: corticular and wood photosynthesis. *Naturwissenschaften* 89:147–162. doi: 10.1007/s00114-002-0309-z

Schulze E-D, Robichaux RH, Grace J, et al (1987) Plant Water Balance. *BioScience* 37:30–37. doi: 10.2307/1310175

Smith SD, Osmond CB (1987) Stem Photosynthesis in a Desert Ephemeral, *Eriogonum inflatum*. Morphology, Stomatal Conductance and Water-Use Efficiency in Field Populations. *Oecologia* 72:533–541

# Chapter one: Stem photosynthesis and hydraulics are coordinated in desert plant species

## Abstract

Coordination between stem photosynthesis and hydraulics in green-stemmed desert plants is important for understanding the physiology of stem photosynthesis and possible drought responses. Plants with photosynthetic stems have extra carbon gain that can help cope with the detrimental effects of drought. I studied photosynthetic, hydraulic and functional traits of 11 plant species with photosynthetic stems from three California desert locations. I compared relationships among traits between wet and dry seasons to test the effect of seasonality on these relationships. Finally, I compared stem trait relationships with analogous relationships in the leaf economics spectrum. I found that photosynthetic and hydraulic traits are coordinated in photosynthetic stems. The slope or intercept of all trait relationships were mediated by seasonality. The relationship between mass-based stem photosynthetic $CO_2$ assimilation rate ($A_{mass}$) and specific stem area (SSA, stem surface area to dry mass ratio) was statistically indistinguishable from the leaf economics spectrum. These results indicate that photosynthetic stems behave like leaves in the coordination of multiple traits related to carbon gain, water movement and water loss. Because of the similarity of the stem $A_{mass}$ – SSA relationship to the leaf $A_{mass}$ – specific leaf area relationship, I suggest the existence of a photosynthetic stem economic spectrum.

## 1.1 Introduction

The efficiency of the stem xylem to transport water to photosynthesizing tissue is of paramount importance in the life of plants. We know that water is the resource that most limits terrestrial productivity (Schulze et al. 1987; Chaves and Pereira 1992), and plants living where water is scarce need a suite of traits that allows them to persist in such harsh environments. Leaf photosynthetic traits for example are tightly related to the ability of stems to transport water (Brodribb and Feild 2000; Brodribb et al. 2002; Santiago et al. 2004). This coordination implies that investment in stem hydraulic capacity is correlated with investment in leaf carbon gain potential in plants where leaves are the main photosynthetic organs. Having high stem hydraulic conductivity is only useful if leaves can capitalize on high water transport capacity in gaining carbon through a high stomatal conductance ($g_s$). In some foliated plants, photosynthetic green stems are the organs responsible for most of the annual carbon assimilation (Ávila et al. 2014b). However, we do not know whether hydraulic capacity and carbon gain are related in the stems of these plants. In this regard, the main goal of this chapter was to evaluate the relationships between photosynthetic and hydraulic traits in green-stemmed plants from southern California desert ecosystems.

Traits of leaf photosynthetic capacity, such as relative quantum yield of photosystem II ($\Phi_{PSII}$) (Brodribb and Feild 2000), electron transport rate (ETR) (Brodribb et al. 2002), and maximum leaf photosynthetic rate ($A_{max}$) (Santiago et al. 2004) have been found to be positively related to leaf-specific stem hydraulic conductivity ($K_L$) in both tropical rainforests and dry forests. Also, soil to leaf hydraulic conductance ($K_{plant}$) is positively correlated with leaf $A_{max}$ and $g_s$ in ponderosa pine (Hubbard et al. 2001). All of these studies found positive correlations between leaf photosynthetic rate and stem hydraulic efficiency, and the mechanism coupling these traits appears to be leaf stomatal responses to changes in water potential ($\Psi$) brought about by changes in stem hydraulic conductance (Sperry 2000; Hubbard et al. 2001). High rates of stem water transport can maintain high leaf $\Psi$ and $g_s$, which in turn allow more diffusion of $CO_2$ to the sites of carboxylation. This physiological coordination may be expected to occur in photosynthetic stems as well. Yet, the functional relationship between stem photosynthesis and hydraulic conductance has not been assessed.

Woody plants with photosynthetic stems are common in dry and hot ecosystems worldwide (Gibson 1983, 1998; Nilsen 1995; Ávila et al. 2014b) and, as leaf area declines along aridity gradients, many plants increasingly rely on stem photosynthetic activity (Comstock et al. 1988; Smith et al. 2004). Plants with photosynthetic stems are represented in at least 35 dicotyledonous families and the gymnosperm family Ephedraceae (Gibson 1996), suggesting that the syndrome could have evolved more than once as an adaptation to life in arid environments (Ávila-Lovera and Ezcurra 2016). The presence of many species with photosynthetic stems in arid ecosystems suggests that photosynthetic stems confer some physiological advantages to plants bearing them, including: 1) extra carbon gain during periods of water deficit when many plants are leafless, thus extending the period for carbon gain (Osmond et al. 1987; Nilsen et al. 1989; Nilsen and Bao 1990; Nilsen and Sharifi 1997); 2) balance of respiratory costs due to re-assimilation of $CO_2$ (Pfanz et al. 2002; Aschan and Pfanz 2003), and 3) improved water use efficiency because stem photosynthesis often occurs at higher water-use efficiency (WUE) than leaf photosynthesis (Ehleringer et al. 1987; Osmond et al. 1987; Nilsen and Sharifi 1997). This higher WUE can be critical for plant survival during periods of water stress, which can last several months in most North American warm deserts.

Photosynthetic stems have positive effects on the carbon economy of plants through two main mechanisms. Photosynthetic stems can either assimilate atmospheric $CO_2$ and contribute to the net carbon gain of the plant through the process of stem net photosynthesis, or decrease respiratory losses by recycling $CO_2$ previously respired by roots and stems through the process of stem recycling photosynthesis (Ávila et al. 2014b). In this chapter, I focused on foliated plants that have stem net photosynthesis, plants that have green and photosynthetic stems year round and have positive net $CO_2$ assimilation rates ($A_{\mathrm{stem}}$). There is no doubt that stem photosynthesis contributes to the carbon economy of plants, but little is known about the water use by these organs, and how hydraulic traits scale with stem photosynthesis.

The main questions that I address in this chapter are: 1) Are photosynthetic and hydraulic traits coordinated in photosynthetic stems? 2) Are relationships between stem photosynthetic and hydraulic traits mediated by seasonality? 3) How do stem trait relationships compare with analogous relationships in the leaf economics spectrum? I hypothesized that there is a positive relationship between stem hydraulic capacity and stem photosynthesis mediated by $g_s$ and seasonality. In the dry season, when plants are leafless I expect a tighter relationship between stem hydraulics and photosynthesis. I also expect that photosynthetic stems are similar to leaves in their relationships between carbon acquisition and functional traits.

## 1.2 Materials and methods

### *Study sites*

This study was conducted in three southern California locations: 1) a Sonoran Desert scrub in Boyd Deep Canyon Desert Research Center (DC), 2) a Mojave Desert-chaparral transitional ecosystem in Morongo Valley (MV), and 3) the University of California, Riverside (UCR) Botanic Gardens Desert Collection (BG), both in wet (winter) and dry (summer) seasons during 2014 – 2016. Boyd Deep Canyon is located at 299 m a.s.l. (33°41’5” N, 116°22’8” W) and has an annual average temperature of 23.7°C (average minimum of 17.8°C and average maximum of 29.5°C) and an annual precipitation of 135.1 mm for the 1961-2015 period (http://www.wrcc.dri.edu/weather/ucde.html). Morongo Valley is located at 1000 m a.s.l. (34°2’12” N, 116°37’24” W) and has an annual average temperature of 17.6°C (average minimum of 9.3°C and average maximum of 25.1°C) and an annual precipitation of 184 mm for the 1991-2016 (information for Yucca Valley, http://www.raws.dri.edu/wraws/scaF.html). The UCR Botanic Gardens is located at 440 m a.s.l. (33°58’14” N, 117°19’20” W) and has an annual average temperature of 18.1°C (average minimum of 10.2°C, and average maximum 26.1°C) and annual precipitation of 257 mm for the 1948-2005 period (http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?carvrc). Wet season measurements were taken between December and February, whereas dry season measurements were taken between May and July in DC, and July and September for the other two sites. Soils are classified as an eutric fluvisol with loam texture at DC, a luvic yermosol with sandy loam texture at MV, and a chromic luvisol with sandy clay loam texture at BG based on (Batjes 2012).

### *Plant species*

The 11 plant species selected are known to have green photosynthetic stems year round and are frequent in the warm deserts of North America (Table 1.1). All species are drought-deciduous shrubs or small trees, except for the three species of *Ephedra*, which have scales instead of leaves. The shrubby species are usually rounded bushes of less than 3 m height with many thin green twigs. The thin twigs (0.2 – 0.5 cm thickness) are the ones used for all the measurements. All species are native to California except *Euphorbia xanti*, which is native to central and southern Baja California, Mexico (Table 1.1).

**Table 1.1**. List of species studied per location, family, symbol used in graphs, origin/growth form, and average height from The Jepson Desert Manual (Wetherwax 2002), except for *Euphorbia xanti*, which was taken from Baja California Plant Field Guide (Rebman and Roberts 2012).

| **Species** | **Family** | **Symbol** | **Origin/growth form** | **Average height (m)** |
|---|---|---|---|---|
| **Boyd Deep Canyon** | | | | |
| *Ambrosia salsola* | Asteraceae | ◆ | Native shrub | < 2 |
| *Bebbia juncea* | Asteraceae | ⬣ | Native shrub | ≤ 3 |
| *Ephedra aspera* | Ephedraceae | ♂ | Native shrub | < 1.5 |
| *Hoffmannseggia microphylla* | Fabaceae | ✖ | Native shrub | 0.5 – 2 |
| *Justicia californica* | Acanthaceae | ▲ | Native shrub | < 2 |
| *Parkinsonia florida* | Fabaceae | ▼ | Native tree | < 8 |
| **Morongo Valley** | | | | |
| *Ambrosia salsola* | Asteraceae | ◆ | Native shrub | < 2 |
| *Ephedra californica* | Ephedraceae | ★ | Native shrub | < 1.5 |
| *Scutellaria mexicana* | Lamiaceae | ✚ | Native shrub | 0.5 – 1 |
| *Thamnosma montana* | Rutaceae | ♀ | Native shrub | 0.3 – 0.6 |
| **UCR Botanic Gardens** | | | | |
| *Ephedra nevadensis* | Ephedraceae | ● | Native shrub | < 1.3 |
| *Euphorbia xanti* | Euphorbiaceae | ■ | Mexican shrub | 3 |
| *Justicia californica* | Acanthaceae | ▲ | Native shrub | < 2 |
| *Parkinsonia florida* | Fabaceae | ▼ | Native tree | < 8 |

### *Stem hydraulic conductance using the evaporative flux method*

Stem hydraulic conductance ($K_{\text{stem}}$) was measured in wet and dry seasons on twigs of four to six individuals of each species, using the evaporative flux method, which is commonly used to estimate leaf hydraulic conductance (Sack et al. 2002; Sack and Scoffoni 2012). Terminal sections of current-year photosynthetic twigs were collected from adult plants, the cut end wrapped in parafilm, and sealed in dark plastic bags inside an ice chest to be transported to the laboratory. In the laboratory, the twigs were cut under

20 mM KCl partially degassed solution. The cut end was submerged in this solution and allowed to fully rehydrate over night for *c.* 12 h. For wet season measurements, leaves were removed from the twig and the cuts covered with melted wax in order to avoid water loss through the wounds. During the dry season, twigs had no leaves.

Once rehydrated, the twigs were recut under solution to a length of 10-12 cm, and connected to tubing filled with partially degassed 20 mM KCl solution. One end of the tubing was connected to the base of the twig section, and the other was placed in a reservoir containing the solution on a balance (±0.1 mg; Denver Instrument P-214; Sartorius, Bohemia, NY, USA). It is important to note that these were terminal twigs with only one cut end, the end connected to the tubing. The twig was positioned on top of a fan and under a light source (> 1200 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density; PPFD), that stimulated stomatal opening. The amount of water evaporated from the surface of the twig was recorded every 60 s on a computer synchronized with the balance. After reaching a maximum steady-state transpiration rate ($E_{\max}$), usually after 30 min in the system, the twig continued transpiring 10 min before recording the last $E_{\max}$ measurements. The twig was then removed from the system and sealed in a hermetic plastic bag and placed in the dark for at least 20 min, to allow any Ψ gradients within the twig to equilibrate. After reaching equilibration, Ψ was rapidly measured using a pressure chamber (Model 1000; PMS Instrument Company, Alabny, OR, USA). Finally, stem projected area was measured with an area meter (Li-3100; Li-Cor Biosciences, Lincoln, NE, USA). The twig hydraulic conductance on a projected area basis ($K_{\text{stem-area}}$, mmol $H_2O$ $m^{-2}$ $s^{-1}$ $MPa^{-1}$) was calculated as:

$$K_{stem} = \frac{E_{max}}{-\Delta\Psi_{stem}} \qquad \text{Eqn 1.1}$$

where $E_{\mathrm{max}}$ is the steady-state transpiration rate per unit of stem projected area (mmol $H_2O$ $m^{-2}$ $s^{-1}$) and $-\Delta\Psi_{\mathrm{stem}}$ (MPa) is the water potential driving flow (where $\Delta\Psi_{\mathrm{stem}} = \Psi_{\mathrm{final}}$ - 0 MPa according to (Sack and Scoffoni 2012)). The dry mass of the sample was also measured, so that $K_{\mathrm{stem}}$ could be expressed on a mass basis ($K_{\mathrm{stem\text{-}mass}}$; mmol $H_2O$ $g^{-1}$ $s^{-1}$ $MPa^{-1}$).

These measures of hydraulic conductance differ from hydraulic conductivity ($K_{\mathrm{H}}$) measurements, in which only axial flow through a stem segment is measured (Sperry et al. 1988; Kolb et al. 1996), but is more appropriate for evaluating water supply capacity to support stem photosynthesis. Since $K_{\mathrm{stem}}$ was estimated through the determination of $E_{\mathrm{max}}$, $K_{\mathrm{stem}}$ refers to a total stem hydraulic conductance, which includes xylem conductance (in the axial direction) that moves water vertically, and extra-xylary conductance (in the radial direction) that moves water horizontally from the xylem to the sites of evaporation. This is important for interpreting results of $K_{\mathrm{stem}}$.

From these measurements, it is also possible to estimate maximum $g_{\mathrm{s}}$ ($g_{\mathrm{s\text{-}max}}$), $g_{\mathrm{s}}$ associated to maximum $K_{\mathrm{stem}}$, by determining the mole fraction stem-to-air vapor pressure difference ($\mathrm{VPD}_{\mathrm{stem}}$). I measured air temperature and air relative humidity near the sample using a traceable digital hygrometer/thermometer (11-661-9, Fisher Scientific), and the stem temperature using a laser infrared temperature gun (Minitemp MT6, Raytek). With these parameters, $\mathrm{VPD}_{\mathrm{stem}}$ was determined using the equation in (Pearcy et al. 2000), and this value was used in the calculation of $g_{\mathrm{s\text{-}max}}$ (mmol $H_2O$ $m^{-2}$ $s^{-1}$) as follows:

$$g_{s-max} = \frac{E_{max}}{VPD_{stem}} \qquad \text{Eqn 1.2}$$

*Stem cuticular conductance*

Cuticular conductance, also known as minimum epidermal conductance ($g_{\min}$, *sensu* (Kerstiens 1996) was determined on twigs of six individuals per species during the late wet season and late dry season following the methods of (Sack and Scoffoni 2011) on *PrometheusWiki*. One 20 cm long twig section was chosen from each individual sampled and taken to the lab. Twig samples were cut to a length of 10 cm with a fresh razor blade, and cut ends were sealed with melted candlewax. The samples were taped to a shelf above a bench and placed near a fan, such that they swayed in the breeze for at least 1 h at PPFD of <10 µmol $m^{-2}$ $s^{-1}$ to induce stomatal closure. A traceable digital hygrometer/thermometer (11-661-9, Fisher Scientific) was placed next to the samples within the breeze, and air temperature and relative humidity were recorded at the beginning of each weighing cycle to determine the mole fraction air vapor pressure deficit ($VPD_{mf}$). Samples were weighed for at least eight intervals of 20 min. A graph of sample mass versus time was obtained for each sample. These curves were linear most of the time. When they were not linear, they were concave down and the water lost per unit of time during the late linear portion, when all stomata have closed, was used to calculate $g_{\min}$ and taken to represent water loss after full stomatal closure. Values of $g_{\min}$ were calculated as transpiration rate (mmol $H_2O$ $m^{-2}$ $s^{-1}$) divided by $VPD_{mf}$ using the spreadsheet provided with the *PrometheusWiki* protocol, which was modified to estimate the total surface area of stems (Sack and Scoffoni 2011).

*Stem photosynthetic rate*

Stem photosynthetic $CO_2$ assimilation rate ($A_{\mathrm{stem}}$) was measured in the field in wet and dry seasons on intact twigs of six individuals per species using an open system infrared gas analyzer (Li-6400; Li-Cor Biosciences, Lincoln, NE, USA) between 0900 and 1200 h. For each individual, $A_{\mathrm{stem}}$ was measured in one intact twig of <0.5 cm of diameter using the 2×3 cm leaf chamber and clay-modeled gaskets that allowed hermetic seal. No leaks were found in the system using this protocol and the measurements were taken to be accurate. Measurements were performed at ambient temperature, which varied from 16.28 – 35.65 °C in the wet season and from 22.86 – 44.09 °C in the dry season, 400 $\mu$mol mol$^{-1}$ of $CO_2$ and 1500 $\mu$mol m$^{-2}$ s$^{-1}$ of PPFD provided by a red blue light source (6400-02B #SI-710, Li-Cor Biosciences) (Evans and Santiago 2014). After gas exchange measurements were taken, the stems were sectioned and transported to the lab, where projected area was measured using an area meter (Li-3100; Li-Cor Biosciences, Lincoln, NE, USA). Total stem surface area was determined by multiplying the projected area by $\pi$ (3.14). Samples were then dried in an oven at 65°C for at least 48 h to obtain dry mass. Photosynthetic rate was expressed on a total surface area basis ($A_{\mathrm{area}}$) as well as a dry mass basis ($A_{\mathrm{mass}}$).

### *Stem chlorophyll a fluorescence*

Fluorescence of chlorophyll *a* in stems was measured in wet and dry seasons on twigs of six individuals per species using a portable chlorophyll fluorometer (mini-PAM, Waltz, Germany) between 0900 and 1200 h. Minimum ($F'_0$), stable (F) and maximum ($F'_m$) fluorescence of light-adapted twigs were measured at the PPFD that induced the maximum electron transport rate ($PPFD_{\max}$), which varied between 600 and 1200 $\mu$mol m$^{-2}$ s$^{-1}$, depending on the species. Relative quantum yield of photosystem II ($\Phi_{\mathrm{PSII}}$) and electron transport rate (ETR) were calculated using fluorescence data as follows:

$$\Phi_{PSII} = \frac{(F'_m - F)}{F'_m} \qquad \text{Eqn 1.3}$$

$$ETR = \Phi_{PSII} \times PPFD \times a \times 0.5 \quad \text{(Krall and Edwards 1992)} \qquad \text{Eqn 1.4}$$

where *a* is the fraction of light absorbed by the stem, which was considered to be 0.8, a value typical found in many non-succulent leaves (Demmig and Björkman 1987).

*Functional traits: specific stem area, specific bark area, wood density, stem surface area to xylem area ratio*

I define specific stem area (SSA) as a new term to represent the ratio between stem photosynthetic surface area and whole-stem dry mass. SSA was measured for each sample stem after each gas exchange measurement. Stems were sectioned and projected area was measured using an area meter (Li-3100; Li-Cor Biosciences, Lincoln, NE, USA). Stem surface area was determined by multiplying the projected area by π (3.14). Samples were then dried in an oven at 65°C for at least 48 h to obtain whole-stem dry mass. Specific stem area (cm$^2$ g$^{-1}$; SSA) was calculated as:

$$SSA = \frac{Stem\ surface\ area}{Whole{-}stem\ dry\ mass} \qquad \text{Eqn 1.5}$$

I define specific bark area (SBA) as a new term to represent the ratio between stem photosynthetic surface area and bark dry mass. One 4-cm long twig was selected from six individuals from each species and transported to the lab. Stem surface area was estimated as in SSA. The photosynthetic bark was removed from the twig and dried separately from the wood and pith tissue in an oven at 65°C for at least 48 h to obtain dry mass. Specific bark area ($cm^2 g^{-1}$; SBA) was calculated as:

$$SBA = \frac{Stem\ surface\ area}{Bark\ dry\ mass} \qquad \text{Eqn 1.6}$$

Samples of branches longer than 25 cm were collected at the end of the dry season to estimate wood density (WD), stem surface area to xylem area ratio, and to establish functional relationships with hydraulic and photosynthetic traits. For WD, a 1 cm-long stem segment was sectioned 25 cm from the tip of the branch, and the bark and pith (if present) were removed. Since the wood segments had a regular cylinder-shape, fresh volume was determined by measuring the diameter of the wood segments using a vernier caliper. The fresh volume was calculated as the volume of a cylinder ($\pi r^2 L$, where r is diameter/2, and L is 1 cm) and subtracting the volume of the pith if present. Dry mass was obtained after drying the wood segments in an oven at 100°C for at least 48 h. Wood density was calculated as dry mass divided by fresh volume.

For stem surface area to xylem area ratio measurements, the same 25 cm-long branches were used. Xylem area was estimated by measuring the diameter of the xylem at 25 cm from the tip, and calculating the area of a circle ($\pi r^2$, where r is diameter/2). If pith was present, its area was subtracted from the total xylem area.

*Statistical analyses*

Cross-species bivariate relationships among the photosynthetic, hydraulic and functional traits were assessed using Pearson’s correlation coefficients and a significance level of $p \leq 0.05$. These analyses were done using the “Hmisc” library and the “rcorr” function in R 3.2.3. The correlation analyses were performed to assess the correlated structure of the data and to choose correlated traits for further analyses. Since the data have errors in both the predictor and response variables, I used standard major axis (SMA) regressions in significant bivariate relationships to test whether wet and dry season slopes were significantly different, and, in the case of statistically indistinguishable wet and dry season slopes, to test for shifts in elevation (changes in *y*-intercept) or shifts along the common slope (changes in *x*-range and y-range) using SMATR software (Warton et al. 2006). To evaluate the stem $A_{\mathrm{mass}}$ – SSA relationship against the analogous relationship in the leaf economic spectrum, the SMA slope and intercept of the leaf $A_{\mathrm{mass}}$ – SLA relationship was determined using the leaf "globamax" dataset (Maire et al. 2015), and compared for homogeneity against the SMA regression of stem $A_{\mathrm{mass}}$ – SSA. STATISTICA 10 (StatSoft Inc.) was used to perform ANOVAs to test the effects of season, species and season×species interactions within sites for chlorophyll fluorescence traits. All plots were made using SigmaPlot 11 (Systat Software).

## 1.3 Results

I found substantial evidence for coordination between stem photosynthesis and stem hydraulic conductance. There was a positive relationship between $K_{\mathrm{stem}}$ and $A_{\mathrm{stem}}$ when the traits were expressed on a dry mass basis ($K_{\mathrm{stem\text{-}mass}}$ and $\mathrm{A}_{\mathrm{mass}}$; Fig. 1.1a). This relationship was found in both wet ($r^2 = 0.420$, $p = 0.017$) and dry seasons ($r^2 = 0.542$, $p = 0.006$), with no difference between slopes but with significant shift in the *y*-intercept (Table 1.2). Values of $\Phi_{\mathrm{PSII}}$ were also positively related with $K_{\mathrm{stem\text{-}mass}}$, but only during the wet season ($r^2 = 0.439$, $p = 0.010$). On the other hand, ETR was positively related with $K_{\text{stem}}$, but only when this was expressed on an area basis and again only during the wet season ($r^2 = 0.339$, $p = 0.029$). Also, there was a positive relationship between $K_{\text{stem-area}}$ and $g_{\text{s-max}}$ in wet ($r^2 = 0.581$, $p = 0.002$) and dry seasons ($r^2 = 0.763$, $p < 0.0001$) (Fig. 1.1b, Table 1.2), and between $A_{\text{mass}}$ and $g_{\text{s-max}}$ in both wet ($r^2 = 0.296$, $p = 0.055$) and dry seasons ($r^2 = 0.438$, $p = 0.019$) (Fig. 1.2, Table 1.2). The coordination between $K_{\text{stem-area}}$ and $g_{\text{s-max}}$ is expected and supports the idea that stomatal opening, and therefore photosynthesis, is constrained by hydraulic conductance in these photosynthetic stems.

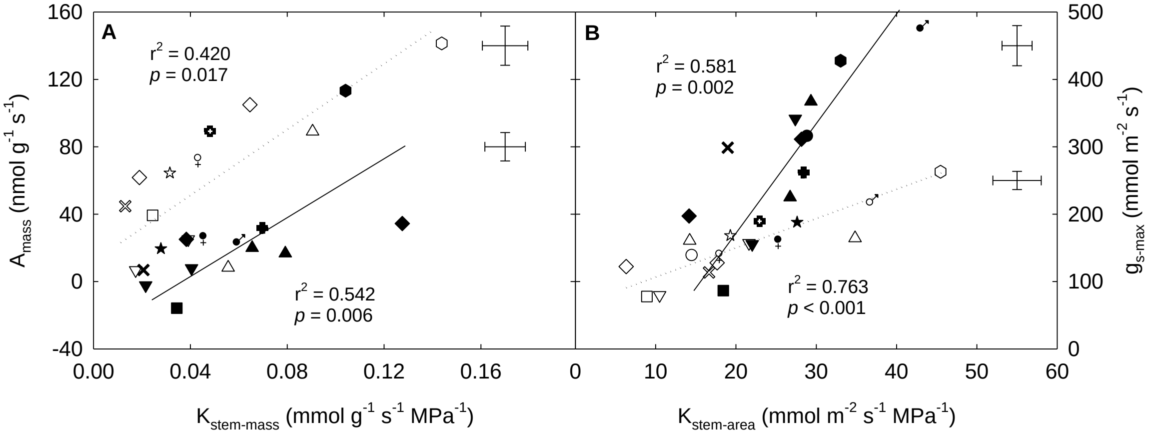

**Figure 1.1**. Relationship between (a) mass-based stem hydraulic conductance ($K_{\text{stem-mass}}$) and mass-based photosynthetic rate ($A_{\text{mass}}$), and between (b) area-based stem hydraulic conductance ($K_{\text{stem-area}}$) and maximum stomatal conductance ($g_{\text{s-max}}$) of green-stemmed species from Boyd Deep Canyon, Morongo Valley and the UCR Botanic Gardens during wet (closed symbols) and dry (open symbols) seasons. Symbols represent species means, and error bars represent standard error in both x and y variables for each season. The SMA regression lines are also shown: solid for wet season, and dotted for dry season. Results of SMA regression analyses are presented in table 1.2.

**Table 1.2**. Tests for common slope, shifts in intercept, and shifts along a standardized major axis with a common slope for main relationships among stem traits in wet and dry seasons. Traits are: maximum stomatal conductance ($g_{\text{s-max}}$; mmol m$^{-2}$ s$^{-1}$), photosynthetic photon flux density inducing maximum electron transport rate (PPFD$_{\text{max}}$; µmol m$^{-2}$ s$^{-1}$), stem photosynthetic rate in area basis ($A_{\text{area}}$; µmol m$^{-2}$ s$^{-1}$), stem photosynthetic rate in mass basis ($A_{\text{mass}}$; nmol g$^{-1}$ s$^{-1}$), stem hydraulic conductance in mass basis ($K_{\text{stem\_mass}}$; mmol g$^{-1}$ s$^{-1}$ MPa$^{-1}$), stem hydraulic conductance in area basis ($K_{\text{stem\_area}}$; mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$), specific stem area (SSA; cm$^{2}$ g$^{-1}$), specific bark area (SBA; cm$^{2}$ g$^{-1}$), dry season cuticular conductance in area basis ($g_{\text{min}}$; mmol m$^{-2}$ s$^{-1}$). Values in bold indicate a statistically significant test.

| **y** | **x** | **Slope** | | | **Intercept** | | | |
|---|---|---|---|---|---|---|---|---|
| | | Wet | Dry | Heterogeneity of slope | Wet | Dry | Shift in intercept | Shift along common slope |
| $A_{\text{mass}}$ | $K_{\text{stem-mass}}$ | 936.2 | 1082.6 | $p = 0.654$ | **-29.26** | **8.97** | **$p = 0.0001$** | $p = 0.259$ |
| $g_{\text{s-max}}$ | $K_{\text{stem-area}}$ | **15.845** | **4.472** | **$p = 0.001$** | -148.30 | 59.56 | | |
| $A_{\text{mass}}$ | $g_{\text{s-max}}$ | **0.264** | **0.802** | **$p = 0.005$** | -47.36 | -55.46 | | |
| $K_{\text{stem-mass}}$ | SSA | 9.59x10$^{-4}$ | 2.50x10$^{-3}$ | **$p = 0.011$** | 9.50x10$^{-5}$ | -0.07 | | |
| $A_{\text{mass}}$ | SSA | **0.898** | **2.702** | **$p = 0.003$** | -29.17 | -61.95 | | |
| $K_{\text{stem-mass}}$ | BSA | **8.48x10$^{-4}$** | **9.54x10$^{-4}$** | **$p < 0.001$** | -0.02 | -0.03 | | |
| $A_{\text{mass}}$ | BSA | **0.794** | **1.029** | **$p < 0.001$** | -49.54 | -12.78 | | |
| $g_{\text{s-max}}$ | PPFD$_{\text{max}}$ | **0.579** | **0.302** | **$p = 0.034$** | -108.93 | -50.82 | | |
| $A_{\text{area}}$ | PPFD$_{\text{max}}$ | **0.015** | **0.063** | **$p = 0.003$** | -6.01 | -26.75 | | |
| $A_{\text{area}}$ | Dry season $g_{\text{min}}$ | **0.488** | **1.216** | **$p = 0.006$** | -0.65 | 1.24 | | |
| $A_{\text{mass}}$ | Dry season $g_{\text{min}}$ | **4.914** | **6.659** | **$p < 0.001$** | -23.10 | -5.10 | | |

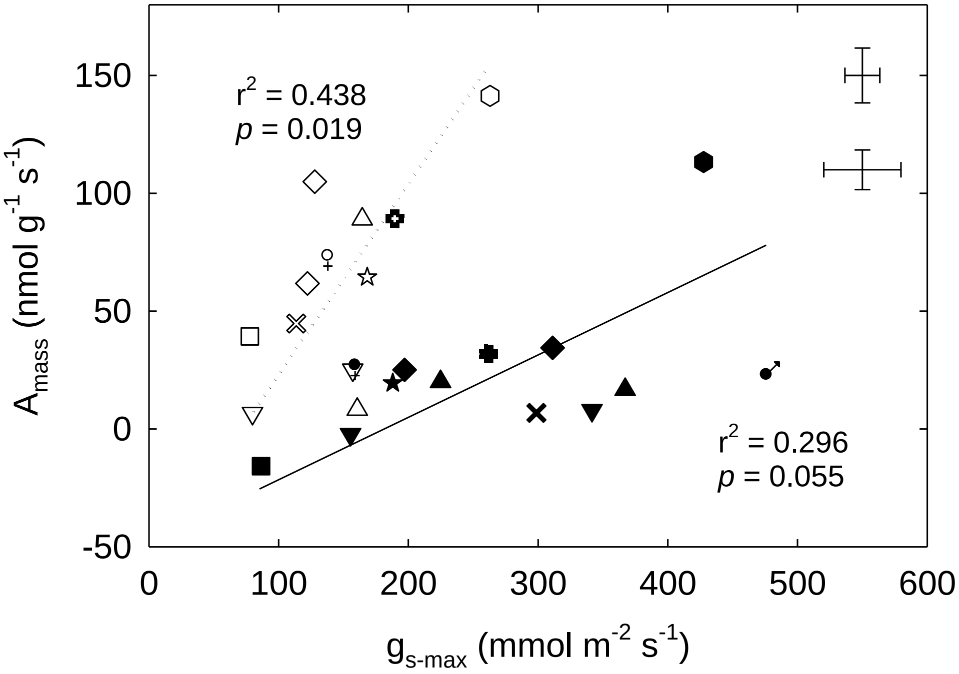

**Figure 1.2**. Relationship between maximum stomatal conductance ($g_{s\text{-max}}$) and mass-based photosynthetic rate ($A_{mass}$), of green-stemmed species from Boyd Deep Canyon, Morongo Valley and the UCR Botanic Gardens during wet (closed symbols) and dry (open symbols) seasons. Symbols represent species means, and error bars represent standard error in both x and y variables for each season. The SMA regression lines are also shown: solid for wet season, and dotted for dry season. Results of SMA regression analyses are presented in table 1.2.

Values of $K_{stem\text{-}mass}$ and $A_{mass}$ were positively related with both SSA (Fig. 1.3a-b) and SBA, with plant species with long and thin twigs (high SSA) having higher values of $K_{stem\text{-}mass}$ (Fig. 1.3a) and $A_{mass}$ (Fig. 1.3b). Responses of $K_{stem\text{-}mass}$ and $A_{mass}$ to SBA were similar in their significance, slopes and intercepts, to responses of $K_{stem\text{-}mass}$ and $A_{mass}$ to SSA (Table 1.2). These relationships were found in both wet and dry seasons with significantly steeper slopes in both cases during the dry season (Table 1.2).

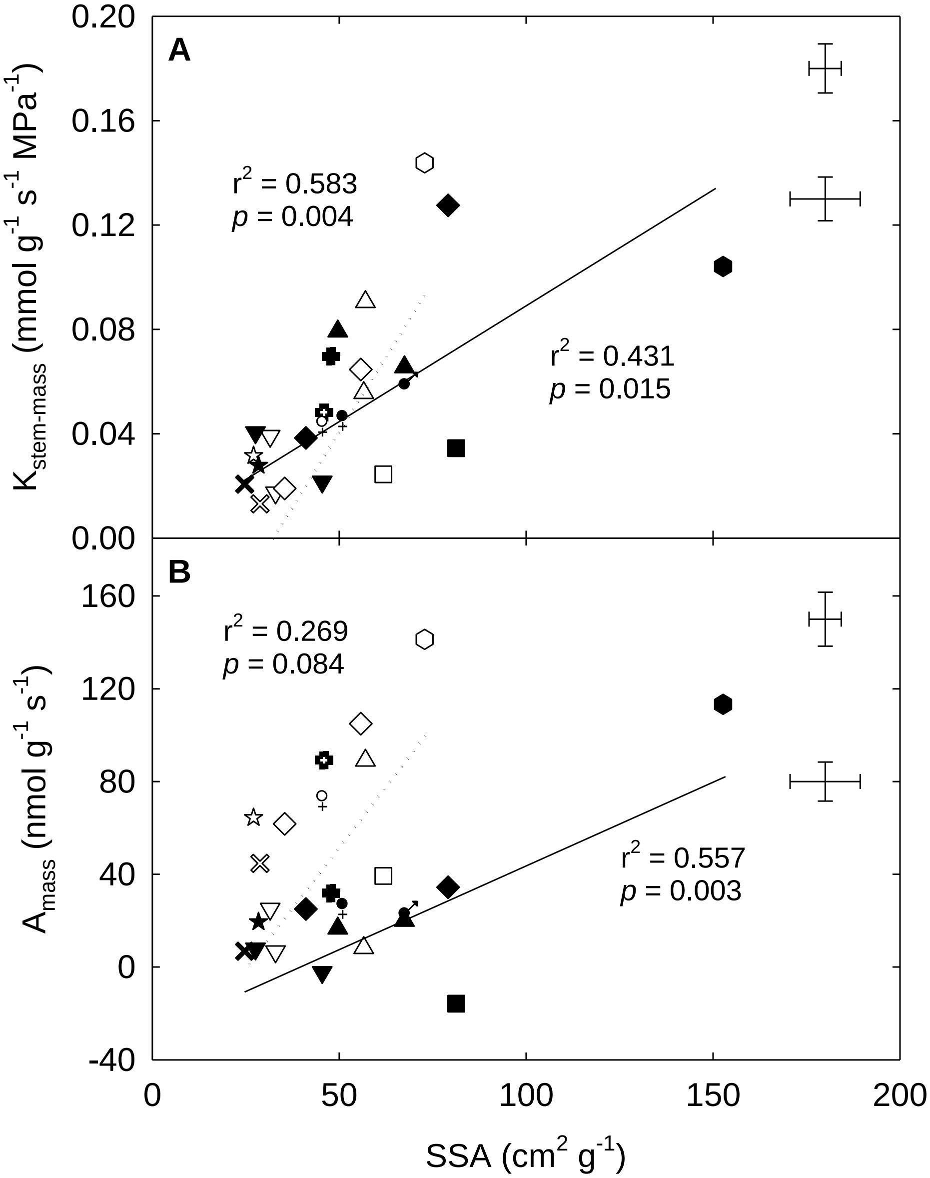

**Figure 1.3**. Relationship between (a) stem specific area (SSA) and mass-based stem hydraulic conductance ($K_{\text{stem-mass}}$), and between (b) SSA and mass- based photosynthetic rate ($A_{\text{mass}}$) of green-stemmed species from Boyd Deep Canyon, Morongo Valley and the UCR Botanic Gardens during wet (closed symbols) and dry (open symbols) seasons. Symbols represent species means, and error bars represent standard error in both x and y variables for each season. The SMA regression lines are also shown: solid for wet season, and dotted for dry season. Results of SMA regression analyses are presented in table 1.2.

Maximum stomatal conductance, measured with the evaporative flux method, and $A_{area}$ were positively correlated with the PPFD inducing maximum ETR (Fig. 1.4a-b). The relationship between $g_{s\text{-}max}$ and $PPFD_{max}$ was found in both wet ($r^2 = 0.557$, $p = 0.002$) and dry seasons ($r^2 = 0.389$, $p = 0.017$) (Fig. 1.4a), with the slope of the relationship being significantly greater during the wet season (Table 2). The relationship between $A_{area}$ and $PPFD_{max}$ was significant only during the dry season ($r^2 = 0.333$, $p = 0.049$) (Fig. 1.4b).

Traits of chlorophyll fluorescence were not compared across sites because of differences in climate and soil characteristics but also because of different species composition. Within sites, there was no effect of season, species or season×species interaction in $\Phi_{PSII}$ or ETR in DC and MV, while there was a significant effect of season on both traits in BG (for $\Phi_{PSII}$: $F_{1,39} = 45.04$, $p < 0.0001$; for ETR: $F_{1,39} = 47.96$, $p < 0.0001$), with higher values in the dry season than in the wet season (Table 1.3).

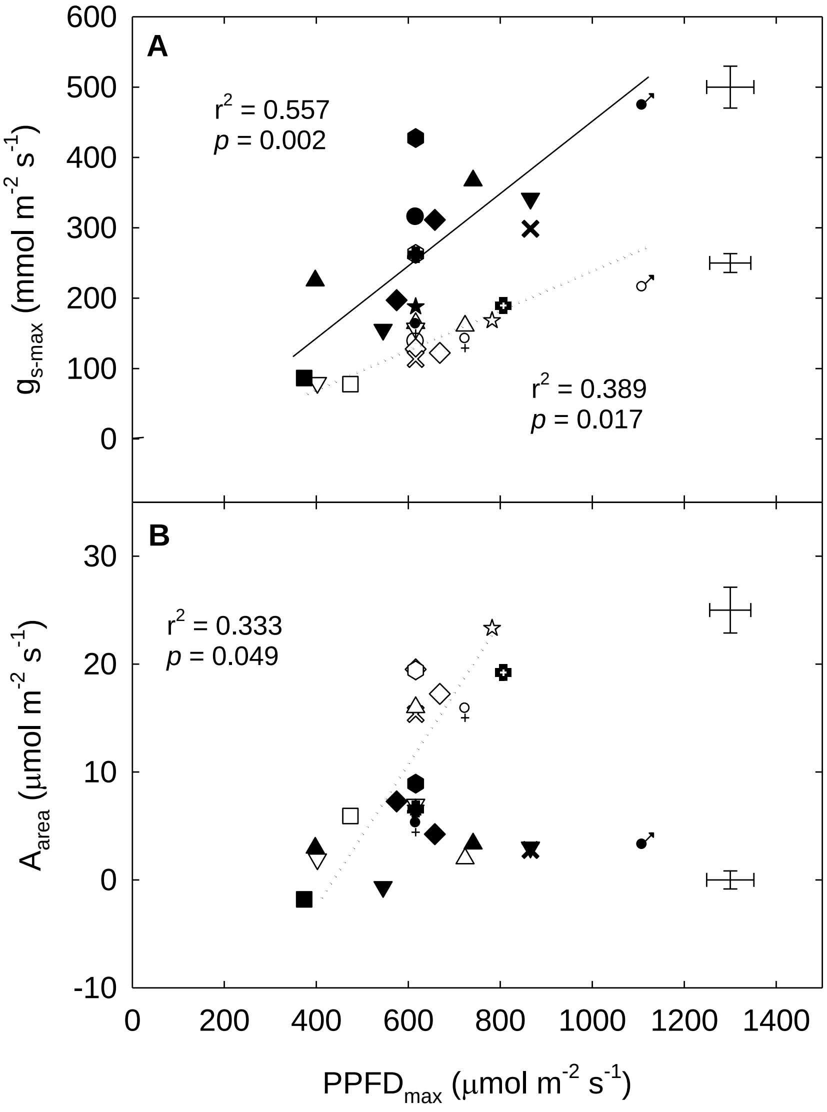

**Figure 1.4**. Relationship between (a) photosynthetic photon flux density inducing maximum electron transport rate (PPFD$_{max}$) and maximum stomatal conductance ($g_{s\text{-}max}$), and between (b) PPFD$_{max}$ and area-based photosynthetic rate ($A_{area}$) of green-stemmed species from Boyd Deep Canyon, Morongo Valley and the UCR Botanic Gardens during wet (closed symbols) and dry (open symbols) seasons. Symbols represent species means, and error bars represent standard error in both x and y variables for each season. The SMA regression lines are also shown: solid for wet season, and dotted for dry season. Results of SMA regression analyses are presented in table 1.2.

Values of $g_{min}$ ranged from 1.11 mmol $m^{-2}$ $s^{-1}$ in *Ephedra nevadensis* in BG in the wet season to 20.49 mmol $m^{-2}$ $s^{-1}$ in *Ambrosia salsola* in MV in the dry season. Mean wet season $g_{min}$ was significantly lower than dry season $g_{min}$ for all species (4.37 vs. 9.02 mmol $m^{-2}$ $s^{-1}$; $t_{13} = -4.33$, $p = 0.0008$). Wet season $g_{min}$ was found to be correlated to dry season $g_{min}$ ($r = 0.84$, $p < 0.001$), so only dry season $g_{min}$ data is shown. Dry season $g_{min}$ was found to be related with stem $A_{area}$ in both wet ($r^2 = 0.566$, $p = 0.003$) and dry ($r^2 = 0.339$, $p = 0.047$) seasons (Fig. 1.5a). Dry season $g_{min}$ was also related to $A_{mass}$ in both wet ($r^2 = 0.373$, $p = 0.027$) and dry ($r^2 = 0.409$, $p = 0.025$) seasons (Fig. 1.5b), indicating that the cost of water loss through the permeable cuticle increases with the benefit of high rates of stem photosynthetic $CO_2$ assimilation.

The SMA slope of the dry season relationship between stem $A_{mass}$ and SSA (2.102) was significantly greater than the SMA slope of the relationship between leaf $A_{mass}$ and SLA of 1.299 derived from the "globamax" dataset ($F = 7.786$, $p = 0.019$). However, the SMA slope of the wet season $A_{mass}$ – SSA relationship (0.898) was not significantly different from the $A_{mass}$ – SLA SMA "globamax" slope of 1.299 ($F = 4.294$, $p = 0.063$), indicating that during the wet season, an increase in one $cm^2$ per g of stem dry mass increases photosynthetic rate to a similar degree as one $cm^2$ per g of dry leaf mass.

Wood density was only correlated with stem cross sectional area ($r = -0.60$, $p = 0.023$) and stem diameter in the dry season ($r = -0.72$, $p = 0.009$), with plants having thick stems also having low density wood.

**Table 1.3**. Relative quantum yield of PSII ($\Phi_{PSII}$), electron transport rate (ETR), and photosynthetic photon flux density inducing maximum ETR ($PPFD_{max}$) for the species studied in both wet and dry seasons. Values are mean of six replicates ± standard error. For *Ephedra aspera*, only one individual was found and it was not included in the one-way ANOVA. Same letters within rows indicate no statistical difference in values between seasons.

| **Species** | **$\Phi_{PSII}$** | | **ETR ($\mu$mol $m^{-2}$ $s^{-1}$)** | | **$PPFD_{max}$ ($\mu$mol $m^{-2}$ $s^{-1}$)** | |
|---|---|---|---|---|---|---|
| | Wet | Dry | Wet | Dry | Wet | Dry |
| **Boyd Deep Canyon** | | | | | | |
| *Ambrosia salsola* | 0.180 ± 0.026$^a$ | 0.181 ± 0.020$^a$ | 45.38 ± 4.25$^a$ | 46.62 ± 5.05$^a$ | 657.7 ± 100.1$^a$ | 616.0 ± 0$^a$ |
| *Bebbia juncea* | 0.176 ± 0.015$^a$ | 0.150 ± 0.013$^a$ | 43.25 ± 0$^a$ | 38.62 ± 3.43$^a$ | 616.2 ± 111.5$^a$ | 616.0 ± 0$^a$ |
| *Ephedra aspera* | 0.125 | 0.187 | 58.40 | 87.50 | 1115.0 | 1115.0 |
| *Hoffmannseggia microphylla* | 0.115 ± 0.008$^a$ | 0.131 ± 0.014$^a$ | 40.43 ± 4.42$^a$ | 33.77 ± 3.59$^a$ | 865.5 ± 111.6$^a$ | 616.0 ± 0$^a$ |
| *Justicia californica* | 0.117 ± 0.015$^a$ | 0.159 ± 0.039$^a$ | 33.35 ± 4.33$^a$ | 35.10 ± 4.30$^a$ | 740.8 ± 124.7$^a$ | 616.2 ± 111.5$^a$ |
| *Parkinsonia florida* | 0.142 ± 0.011$^a$ | 0.142 ± 0.020$^a$ | 49.12 ± 3.71$^a$ | 32.80 ± 3.17$^a$ | 865.5 ± 111.6$^a$ | 616.2 ± 111.5$^a$ |
| Mean ± SE | 0.145 ± 0.008$^a$ | 0.152 ± 0.010$^a$ | 48.83 ± 2.15$^a$ | 38.66 ± 2.44$^a$ | 760.9 ± 50.1$^a$ | 632.2 ± 32.6$^a$ |
| **Morongo Valley** | | | | | | |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| *Ambrosia salsola* | 0.106 ± 0.013$^{a}$ | 0.114 ± 0.017$^{a}$ | 25.15 ± 3.51$^{a}$ | 32.90 ± 5.98$^{a}$ | 574.5 ± 41.5$^{a}$ | 668.7 ± 156.1$^{a}$ |
| *Ephedra californica* | 0.102 ± 0.015$^{a}$ | 0.141 ± 0.020$^{a}$ | 26.40 ± 3.86$^{a}$ | 34.13 ± 5.91$^{a}$ | 616.0 ± 0$^{a}$ | 782.3 ± 105.2$^{a}$ |
| *Scutellaria mexicana* | 0.121 ± 0.028$^{a}$ | 0.107 ± 0.028$^{a}$ | 31.27 ± 7.17$^{a}$ | 31.27 ± 6.88$^{a}$ | 616.0 ± 0$^{a}$ | 806.5 ± 147.7$^{a}$ |
| *Thamnosma montana* | 0.125 ± 0.016$^{a}$ | 0.115 ± 0.020$^{a}$ | 32.23 ± 4.05$^{a}$ | 34.20 ± 3.47$^{a}$ | 616.0 ± 0$^{a}$ | 723.3 ± 135.9$^{a}$ |
| Mean ± SE | 0.114 ± 0.009 | 0.119 ± 0.010 | 28.76 ± 2.36 | 30.94 ± 2.67 | 605.6 ± 10.4 | 745.2 ± 65.1 |
| **UCR Botanic Gardens** |  |  |  |  |  |  |
| *Ephedra nevadensis* | 0.097 ± 0.009$^{a}$ | 0.248 ± 0.034$^{a}$ | 24.92 ± 2.44$^{a}$ | 60.37 ± 10.44$^{a}$ | 616.0 ± 0$^{a}$ | 616.2 ± 111.5$^{a}$ |
| *Euphorbia xanti* | 0.088 ± 0.011$^{a}$ | 0.261 ± 0.065$^{a}$ | 13.27 ± 1.81$^{a}$ | 45.28 ± 7.95$^{a}$ | 373.5 ± 52.9$^{a}$ | 474.0 ± 65.4$^{a}$ |
| *Justicia californica* | 0.051 ± 0.009$^{b}$ | 0.262 ± 0.061$^{a}$ | 7.62 ± 1.63$^{b}$ | 73.23 ± 18.27$^{a}$ | 397.5 ± 71.1$^{a}$ | 723.3 ± 135.9$^{a}$ |
| *Parkinsonia florida* | 0.050 ± 0.010$^{b}$ | 0.391 ± 0.081$^{a}$ | 10.46 ± 1.63$^{b}$ | 54.20 ± 10.12$^{a}$ | 545.2 ± 70.8$^{a}$ | 402.5 ± 97.2$^{a}$ |
| Mean ± SE | 0.072 ± 0.006 | 0.290 ± 0.032 | 14.22 ± 1.65 | 58.27 ± 6.13 | 480.4 ± 33.8 | 554.0 ± 55.7 |

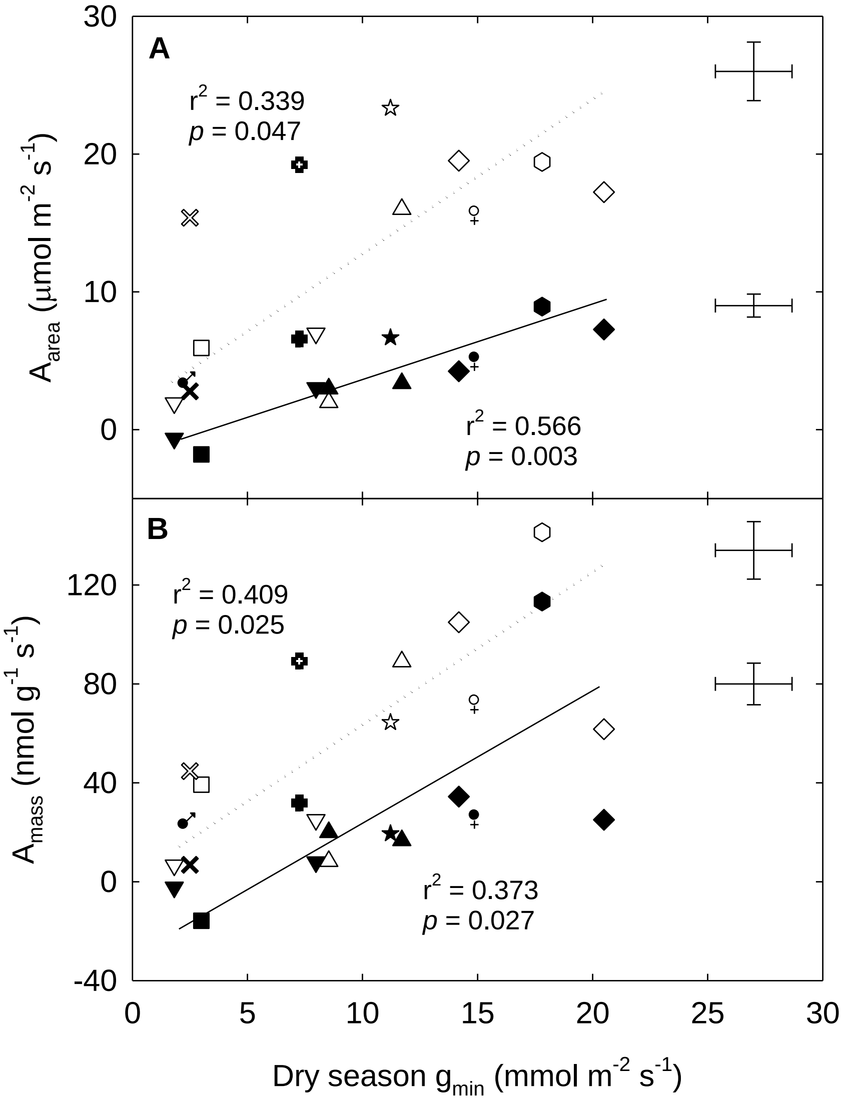

**Figure 1.5**. Relationship between (a) dry season stem cuticular conductance ($g_{\mathrm{min}}$) and area-based photosynthetic rate ($A_{\mathrm{area}}$), and between (b) $g_{min}$ and mass-based photosynthetic rate ($A_{\mathrm{mass}}$) of green-stemmed species from Boyd Deep Canyon, Morongo Valley and the UCR Botanic Gardens during wet (closed symbols) and dry (open symbols) seasons. Symbols represent species means, and error bars represent standard error in both x and y variables for each season. The SMA regression lines are also shown: solid for wet season, and dotted for dry season. Results of SMA regression analyses are presented in table 1.2.

## 1.4 Discussion

These results indicate substantial coordination between photosynthetic capacity and hydraulic efficiency. The positive relationships found between $A_{\mathrm{mass}}$ and $K_{\mathrm{stem\text{-}mass}}$ and between $g_{\mathrm{s\text{-}max}}$ and $\mathrm{PPFD}_{\mathrm{max}}$, demonstrating how photosynthetic and hydraulic traits are coordinated in the photosynthetic stems of desert plant species from southern California. I also found that the relationship between $A_{\mathrm{mass}}$ and SSA is similar to the leaf economic spectrum (Wright et al. 2004). The photosynthetic-hydraulic coordination in stems is as important as in leaves because most of these plants rely on their stems for assimilating carbon year, likely affecting whole plant carbon balance and performance. When leaves were absent during the dry season stems continued to assimilate $CO_2$ at even higher rates than during the wet season, probably because of seasonal acclimation to higher light availability and warmer temperatures (Kitajima et al. 1997).  One of the first works on stem photosynthetic responses to temperature on *Parkinsonia florida* showed that stems maintained their $A_{\mathrm{area}}$ near its maximum value over a broad range of temperatures (20-40°C) (Adams and Strain 1968). This characteristic is significant in the maintenance of metabolism during the leafless period, which is also the period of high temperature, and the decrease in shading after leaf loss increased the light interception by the stems promoting continued $CO_2$ assimilation. Therefore, these results offer strong support that the principles of investment in photosynthetic capacity and structure in leaves apply to photosynthetic stems as well.

The coordination found between $A_{\mathrm{mass}}$ and $K_{\mathrm{stem\text{-}mass}}$ and $g_{\mathrm{s}}$ and $K_{\mathrm{stem\text{-}area}}$ is in agreement with previous findings relating stem or whole-plant hydraulic conductance with leaf photosynthesis and $g_s$ in plants from different ecosystems (Meinzer et al. 1995; Brodribb and Feild 2000; Sperry 2000; Hubbard et al. 2001; Brodribb et al. 2002; Santiago et al. 2004), suggesting that stem photosynthesis is also constrained by stem water transport capacity. The coupling between stem $A_{\text{mass}}$ and $K_{\text{stem-mass}}$ is expected to be stronger, with different slopes and intercepts during the dry season compared to the wet season, because when leaves are present stems are transporting water for both downstream leaf processes and for local stem photosynthesis. The coefficient of determination ($r^2$) and the *y*-intercept of the $A_{\text{mass}} - K_{\text{stem-mass}}$ relationship is higher during the dry season, supporting the idea that relationships among stem photosynthesis and hydraulic function are mediated by seasonality and that stronger coordination exists between stem photosynthetic and hydraulic traits in the dry season than during the wet season, consistent with the need to transport water for both leaf and stem photosynthesis during the wet season, while during the dry season the water moved by the stem is only used in stem photosynthesis. This has implications for plants facing future climate change, because losses of hydraulic conductivity could limit carbon gain through the stem and possibly remove the advantage of having this extra photosynthetic organ. In this situation, higher maintenance costs of photosynthetic pigments and enzymes should be expected in plants with photosynthetic stems than in plants without photosynthetic stems. More work on the costs and benefits of having photosynthetic stems in terms of carbon gain and water loss is needed to better understand their physiology and their possible responses to future or present climate change.

A huge body of research suggest that having photosynthetic stems is advantageous for plants living in hot and dry ecosystems (see recent reviews Ávila et al. 2014; Vandegehuchte et al. 2015; Cernusak and Cheesman 2015). The stem photosynthetic contribution to whole plant carbon gain is usually high and this contribution has been measured before in some of the species studied here (Comstock et al. 1988). Two of these species did not rely highly on stem photosynthesis during most of the year; these were *A. salsola* in which the contribution of the stem was 0% in late August and reached a maximum of 44% in late May, and *Bebbia juncea*, which had a minimum of 2% during June-October and a maximum of 56 % in late March (Comstock et al. 1988). In other species, namely *Scutellaria mexicana* and *Thamnosma montana*, the contribution of the stem was larger and the contribution of leaves was significantly lower than in the other species studied; in *S. mexicana* and *T. montana* the stem contribution ranged from 17% in late August to a maximum of 67% in late May (Comstock et al. 1988). The stem contribution to whole-plant carbon gain in multiple species have been estimated to range from 23 to 100% (Ávila et al. 2014b).

In general, stems are thought to have higher WUE than leaves, but this relationship has only been evaluated on a limited amount of available data (Santiago et al. 2016). I did not measure leaves in this study, but an extensive literature review including my stem data suggests that stem intrinsic WUE is higher in stems than in leaves (Ávila-Lovera E, unpublished data). Also, carbon isotopic composition tends to be higher in stems than in leaves, suggesting that stems may have a higher long-term integrated WUE (Ehleringer et al. 1987; Osmond et al. 1987; Smith and Osmond 1987; Nilsen and Sharifi

1997), although some of this effect is likely due to within-plant carbon isotopic fractionation (Cernusak et al. 2009). Desert plants with green stems may have access to water year round considering their presence in desert washes (Adams and Strain 1968; Gibson 1996). Furthermore, if they access groundwater in spring and fall seasons (Allen ME et al., unpublished data), and the stem contribution to whole plant carbon gain is relatively high, there may be a greater selection on these stems to maximize carbon gain than to minimize water loss (Gibson 1998). Values of $g_s$ and $E$ are usually lower in stems than in leaves of the same species (Osmond et al. 1987; Smith and Osmond 1987; Comstock and Ehleringer 1988; Nilsen and Bao 1990; Franco-Vizcaíno et al. 1990; Tinoco-Ojanguren 2008), but the stems may lose more water through the cuticle. From an extensive literature survey (201 species, (Kerstiens 1996), mean leaf $g_{min}$ is 5.40 mmol m$^{-2}$ s$^{-1}$, which is lower than the dry season stem $g_{min}$ of 9.02 mmol m$^{-2}$ s$^{-1}$ found in this study, but higher than the wet season stem $g_{min}$ of 4.37 mmol m$^{-2}$ s$^{-1}$. The overall mean stem $g_{min}$ for both seasons of 6.69 mmol m$^{-2}$ s$^{-1}$ in this study was also higher than the periderm permeability found in different tree and crop species (mean of 2.42 mmol m$^{-2}$ s$^{-1}$; (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). This suggests that during periods of low water availability where stomata are partially or fully closed, water loss through the stem cuticle can be significant. Although $g_{min}$ measured here is cuticular conductance to water, $CO_2$ could possibly move through the cuticle if there is a concentration gradient between the atmosphere and the stem cortex intercellular air spaces. The positive relationships between $A_{area}$ and $A_{mass}$ with dry season $g_{min}$

partially support this idea (Table 1.2). Stomatal conductance measured in the field showed small values, usually in the order of magnitude of $g_{\mathrm{min}}$. With such small values of $g_{\mathrm{s}}$, $g_{\mathrm{min}}$ might significantly contribute to the movement of $CO_2$ from the atmosphere to the intercellular air spaces, significantly increasing the rate of photosynthesis.

Most of the relationships between hydraulic and photosynthetic traits with functional traits were significant only when using mass-based values. This has also been found in nine temperate tree species exhibiting stem recycling photosynthesis (Berveiller et al. 2007). Massive and thick stems with low SSA and SBA have a higher proportion of wood and pith tissue than bark, which can explain their lower photosynthetic capacity. Thick stems also have relatively low $K_{\mathrm{stem}}$, which may be partly due to their higher WD (although the negative relationship between $K_{\mathrm{stem}}$ and WD was not statistically significant in this study), and to the low conductance of extra-xylary tissue. The trait $K_{\mathrm{stem}}$ as measured here combines conductance of two types of tissue: 1) xylem and 2) cambium/phloem/parenchyma tissues, the latter not being specialized for conducting water. Bark thickness was estimated from measurements of twig and xylem diameter and it was found to be negatively related with $E_{\mathrm{max}}$, $g_{\mathrm{s\text{-}max}}$ and $K_{\mathrm{stem\text{-}area}}$, and recent work has shown the role of other anatomical and functional traits of leaf parenchyma on leaf hydraulic conductance (Aasamaa et al. 2005; Sack et al. 2015).

Specific stem area (SSA) and SBA are terms that I am introducing to refer to the ratio of stem photosynthetic surface area to whole-stem dry mass and to bark dry mass, respectively. As with SLA, SSA and SBA reflect investment of mass in capturing light and are expected to be associated with photosynthetic capacity and life span (Reich et al.

1997; Wright et al. 2004). I found a positive relationship between $A_{\mathrm{mass}}$ and SSA and SBA, similar to the worldwide leaf economic spectrum, which runs from a fast (high SSA and SBA) to a slow (low SSA and SBA) return on investment of dry mass, and directly influences photosynthetic capacity. Furthermore, the slope of the $A_{\mathrm{mass}}$ – SSA relationship during the wet season was similar to the global leaf $A_{\mathrm{mass}}$ – SLA relationship, indicating that stems are similar to leaves in terms of investment in structure and function. This is true not only for species with stem net photosynthesis (this study), but also for species with stem recycling photosynthesis (Berveiller et al. 2007). Assuming that life span depends on how the organs are built, it is expected that SSA and SBA could be related with stem life span, nitrogen and phosphorus concentration, and dark respiration (Berveiller et al. 2007).

Wood density has been found to be negatively correlated with $K_{\mathrm{L}}$ and $K_{\mathrm{plant}}$ in some plants (Santiago et al. 2004; Anderegg and Meinzer 2015) but not in others (Mitchell et al. 2008; Pivovaroff et al. 2014; De Guzman et al. 2017). In this study, WD was not correlated with either $K_{\mathrm{stem\text{-}area}}$ or $K_{\mathrm{stem\text{-}mass}}$. Wood density determines stem mechanical strength (Zanne et al. 2010), and has been found to be negatively related with $\Psi$ at which 50% of sapwood-specific hydraulic conductivity ($K_{\mathrm{S}}$) is lost (P50) (Jacobsen et al. 2007), with species having high WD also having low P50. I did not measure P50 but based on the global relationship between WD and P50 (Gleason et al. 2016), it is possible that stems with high WD, those with small stem diameters, are also more resistant to xylem cavitation and are able to continue transporting water at low $\Psi$.

These results indicate that photosynthetic stems behave like leaves in the coordination of multiple traits related to carbon gain and water movement and loss. Such similarity is potentially advantageous because we may be able to extend our knowledge of leaf physiology and the leaf economic spectrum to better understand photosynthetic stem physiology. Furthermore, understanding seasonal changes in photosynthetic capacity and hydraulics can help in predicting green-stemmed plant responses to global-change-type droughts. Whereas numerous models are now being parameterized for predicting drought-induced mortality of woody plants (Joetzjer et al. 2014; Christoffersen et al. 2016), most of these do not incorporate the contribution of stems and may benefit from including stem-derived carbon balances for plants during drought.

## 1.5 References

Aasamaa K, Niinemets Ü, Sõber A (2005) Leaf hydraulic conductance in relation to anatomical and functional traits during *Populus tremula* leaf ontogeny. *Tree Physiol*ogy 25:1409–1418. doi: 10.1093/treephys/25.11.1409

Adams M, Strain B (1968) Photosynthesis in Stems and Leaves of *Cercidium floridum* - Spring and Summer Diurnal Field Response and Relation to Temperature. *Oecologia Plantarum* 3:285–297.

Anderegg WRL, Meinzer FC (2015) Wood Anatomy and Plant Hydraulics in a Changing Climate. In: Hacke U (ed) Functional and Ecological Xylem Anatomy. Springer International Publishing, Switzerland, pp 235–253

Aschan G, Pfanz H (2003) Non-foliar photosynthesis – a strategy of additional carbon acquisition. *Flora - Morphology, Distribution, Functional Ecology of Plants* 198:81–97. doi: 10.1078/0367-2530-00080

Ávila E, Herrera A, Tezara W (2014) Contribution of stem $CO_2$ fixation to whole-plant carbon balance in nonsucculent species. *Photosynthetica* 52:3–15. doi: 10.1007/s11099-014-0004-2

Ávila-Lovera E, Ezcurra E (2016) Stem-Succulent Trees from the Old and New World Tropics. In: Goldstein G, Santiago LS (eds) Tropical Tree Physiology. Springer International Publishing, Switzerland, pp 45–65

Batjes NH (2012) ISRIC-WISE derived soil properties on a 5 by 5 arc-minutes global grid (ver. 1.2). ISRIC – World Soil Information, Wageningen

Berveiller D, Kierzkowski D, Damesin C (2007) Interspecific variability of stem photosynthesis among tree species. *Tree Physiology* 27:53–61.

Brodribb TJ, Feild TS (2000) Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant, Cell & Environment* 23:1381–1388.

Brodribb TJ, Holbrook NM, Gutierrez MV (2002) Hydraulic and photosynthetic co-ordination in seasonally dry tropical forest trees. *Plant, Cell & Environment* 25:1435–1444.

Cernusak LA, Cheesman AW (2015) The benefits of recycling: how photosynthetic bark can increase drought tolerance. *New Phytologist* 208:995–997. doi: 10.1111/nph.13723

Chaves MM, Pereira JS (1992) Water Stress, $CO_2$ and Climate Change. *Journal of Experimental Botany* 43:1131–1139. doi: 10.1093/jxb/43.8.1131

Christoffersen BO, Gloor M, Fauset S, Fyllas NM, Galbraith D, Bake TR, Rowland L, Fisher RA, Binks OJ, Sevanto SA et al (2016) Linking hydraulic traits to tropical forest function in a size-structured and trait-driven model (TFS v.1-Hydro). *Geoscientific Model Development* 9:4227–4255. doi: 10.5194/gmd-9-4227-2016

Comstock JP, Cooper TA, Ehleringer JR (1988) Seasonal patterns of canopy development and carbon gain in nineteen warm desert shrub species. *Oecologia* 75:327–335. doi: 10.1007/BF00376933

Comstock JP, Ehleringer JR (1988) Contrasting Photosynthetic Behavior in Leaves and Twigs of *Hymenoclea salsola*, a Green-Twigged Warm Desert Shrub. *American Journal of Botany* 75:1360–1370. doi: 10.2307/2444459

De Guzman ME, Santiago LS, Schnitzer SA, Álvarez-Cansino L (2017) Trade-offs between water transport capacity and drought resistance in neotropical canopy liana and tree species. *Tree Physiolology* 37: 1404–1414. doi: 10.1093/treephys/tpw086

Demmig B, Björkman O (1987) Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of $O_2$ evolution in leaves of higher plants. *Planta* 171:171–184. doi: 10.1007/BF00391092

Ehleringer JR, Comstock JP, Cooper TA (1987) Leaf-twig carbon isotope ratio differences in photosynthetic-twig desert shrubs. *Oecologia* 71:318–320.

Evans JR, Santiago LS (2014) PrometheusWiki Gold Leaf Protocol: gas exchange using LI-COR 6400. *Functional Plant Biology* 41:223. doi: 10.1071/FP10900

Franco-Vizcaíno E, Goldstein G, Ting IP (1990) Comparative Gas Exchange of Leaves and Bark in Three Stem Succulents of Baja California. *American Journal of Botany* 77:1272–1278. doi: 10.2307/2444588

Garrec J-P, Barrois A (1992) Caracteristiques de la fixation et de la penetration corticale. Passage du phosphite dipotassique et de l'eau au travers d'ecorces isolees. *Environmental and Experimental Botany* 32:11–23. doi: 10.1016/0098-8472(92)90025-W

Gibson AC (1983) Anatomy of Photosynthetic Old Stems of Nonsucculent Dicotyledons from North American Deserts. *Botanical Gazette* 144:347–362.

Gibson AC (1998) Photosynthetic Organs of Desert Plants. *BioScience* 48:911–920. doi: 10.2307/1313295

Gibson AC (1996) Structure-Function Relations of Warm Desert Plants. Springer Berlin Heidelberg, Berlin, Heidelberg

Gleason SM, Westoby M, Jansen S, et al (2016) Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. *New Phytologist* 209:123–136. doi: 10.1111/nph.13646

Groh B, Hübner C, Lendzian KJ (2002) Water and oxygen permeance of phellems isolated from trees: the role of waxes and lenticels. *Planta* 215:794–801.

Hubbard RM, Ryan MG, Stiller V, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell & Environment* 24:113–121. doi: 10.1046/j.1365-3040.2001.00660.x

Jacobsen AL, Pratt RB, Ewers FW, Davis SD (2007) Cavitation Resistance among 26 Chaparral Species of Southern California. *Ecological Monographs* 77:99–115.

Joetzjer E, Delire C, Douville H, Ciais P, Decharme B, Fisher R, Chrsitoffersen BO, Calvet JC, da Costa ACL, Ferreira LV, et al (2014) Predicting the response of the Amazon rainforest to persistent drought conditions under current and future climates: a major challenge for global land surface models. *Geoscientific Model Development* 7:2933–2950. doi: 10.5194/gmd-7-2933-2014

Kerstiens G (1996) Cuticular water permeability and its physiological significance. *Journal of Experimental Botany* 47:1813–1832. doi: 10.1093/jxb/47.12.1813

Kitajima K, Mulkey SS, Wright SJ (1997) Seasonal leaf phenotypes in the canopy of a tropical dry forest: photosynthetic characteristics and associated traits. *Oecologia* 109:490–498. doi: 10.1007/s004420050109

Kocurek M, Kornas A, Pilarski J, et al (2015) Photosynthetic activity of stems in two *Clusia* species. *Trees* 29:1029–1040. doi: 10.1007/s00468-015-1182-7

Kolb KJ, Sperry JS, Lamont BB (1996) A method for measuring xylem hydraulic conductance and embolism in entire root and shoot systems. *Journal of Experimental Botany* 47:1805–1810.

Krall JP, Edwards GE (1992) Relationship between photosystem II activity and $CO_2$ fixation in leaves. *Physiologia Plantarum* 86:180–187. doi: 10.1111/j.1399-3054.1992.tb01328.x

Lulai EC, Glynn MT, Orr PH (1996) Cellular changes and physiological responses to tuber pressure-bruising. *American Potato Journal* 73:197–209. doi: 10.1007/BF02854874

Lulai EC, Orr PH (1994) Techniques for detecting and measuring developmental and maturational changes in tuber native periderm. *American Potato Journal* 71:489–505. doi: 10.1007/BF02851322

Maire V, Wright IJ, Prentice IC, et al (2015) Global effects of soil and climate on leaf photosynthetic traits and rates: Effects of soil and climate on photosynthetic traits. *Global Ecology and Biogeography* 24:706–717. doi: 10.1111/geb.12296

Meinzer FC, Goldstein G, Jackson P, et al (1995) Environmental and physiological regulation of transpiration in tropical forest gap species: the influence of boundary layer and hydraulic properties. *Oecologia* 101:514–522. doi: 10.1007/BF00329432

Mitchell PJ, Veneklaas EJ, Lambers H, Burgess SSO (2008) Using multiple trait associations to define hydraulic functional types in plant communities of south-western Australia. *Oecologia* 158:385–397. doi: 10.1007/s00442-008-1152-5

Nilsen E, Sharifi M (1997) Carbon isotopic composition of legumes with photosynthetic stems from mediterranean and desert habitats. *American Journal of Botany* 84:1707–1713.

Nilsen ET (1995) Stem Photosynthesis: Extent, Patterns, and Role in Plant Carbon Economy. In: Gartner BL (ed) Plant Stems. Academic Press, San Diego, pp 223–240

Nilsen ET, Bao Y (1990) The Influence of Water Stress on Stem and Leaf Photosynthesis in *Glycine max* and *Sparteum junceum* (Leguminosae). *American Journal of Botany* 77:1007–1015. doi: 10.2307/2444572

Nilsen ET, Meinzer FC, Rundel PW (1989) Stem photosynthesis in *Psorothamnus spinosus* (smoke tree) in the Sonoran Desert of California. *Oecologia* 79:193–197.

Osmond CB, Smith SD, Gui-Ying B, Sharkey TD (1987) Stem photosynthesis in a desert ephemeral, *Eriogonum inflatum*. Characterization of leaf and stem $CO_2$ fixation and $H_2O$ vapor exchange under controlled conditions. *Oecologia* 72:542–549.

Pearcy RW, Ehleringer JR, Mooney H, Rundel PW (eds) (2000) Plant Physiological Ecology: Field methods and instrumentation, 2000 edition. Springer, London; New York

Pfanz H, Aschan G, Langenfeld-Heyser R, et al (2002) Ecology and ecophysiology of tree stems: corticular and wood photosynthesis. *Naturwissenschaften* 89:147–162. doi: 10.1007/s00114-002-0309-z

Pivovaroff AL, Sack L, Santiago LS (2014) Coordination of stem and leaf hydraulic conductance in southern California shrubs: a test of the hydraulic segmentation hypothesis. *New Phytologist* 203:842–850. doi: 10.1111/nph.12850

Rebman JP, Roberts NC (2012) Baja California Plant Field Guide, 3rd edition. Sunbelt Publications, San Diego, CA

Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America* 94:13730–13734.

Sack L, Melcher PJ, Zwieniecki MA, Holbrook M (2002) The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. *Journal of Experimental Botany* 53:2177–2184. doi: 10.1093/jxb/erf069

Sack L, Scoffoni C (2012) Measurement of Leaf Hydraulic Conductance and Stomatal Conductance and Their Responses to Irradiance and Dehydration Using the Evaporative Flux Method (EFM). *Journal of Visualized Experiments* 70:1–7. doi: 10.3791/4179

Sack L, Scoffoni C (2011) PrometheusWiki | Minimum epidermal conductance ($g_{min}$, a.k.a. cuticular conductance). http://prometheuswiki.publish.csiro.au/tiki-index.php?page=Minimum+epidermal+conductance+%28gmin%2C+a.k.a.+cuticular+conductance%29. Accessed 28 Mar 2016

Sack L, Scoffoni C, Johnson DM, et al (2015) The Anatomical Determinants of Leaf Hydraulic Function. In: Hacke U (ed) Functional and Ecological Xylem Anatomy. Springer International Publishing, pp 255–271

Santiago LS, Bonal D, De Guzman ME, Ávila-Lovera E (2016) Drought Survival Strategies of Tropical Trees. In: Goldstein G, Santiago LS (eds) Tropical Tree Physiology. Springer International Publishing, Switzerland, pp 243–258

Santiago LS, Goldstein G, Meinzer FC, et al (2004) Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* 140:543–550. doi: 10.1007/s00442-004-1624-1

Schönherr J, Ziegler H (1980) Water Permeability of *Betula* Periderm. *Planta* 147:345–354.

Schreiber L, Franke R, Hartmann K (2005) Wax and suberin development of native and wound periderm of potato (*Solanum tuberosum* L.) and its relation to peridermal transpiration. *Planta* 220:520–530.

Schulze E-D, Robichaux RH, Grace J, et al (1987) Plant Water Balance. *BioScience* 37:30–37. doi: 10.2307/1310175

Smith SD, Naumburg E, Niinemets Ül, Germino MJ (2004) Leaf to Landscape. In: Smith WK, Vogelmann TC, Critchley C (eds) Photosynthetic Adaptation. Springer New York, pp 262–294

Smith SD, Osmond CB (1987) Stem Photosynthesis in a Desert Ephemeral, *Eriogonum inflatum*. Morphology, Stomatal Conductance and Water-Use Efficiency in Field Populations. *Oecologia* 72:533–541.

Sperry JS (2000) Hydraulic constraints on plant gas exchange. *Agricultural and Forest Meteorology* 104:13–23. doi: 10.1016/S0168-1923(00)00144-1

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell & Environment* 11:35–40.

Tinoco-Ojanguren C (2008) Diurnal and seasonal patterns of gas exchange and carbon gain contribution of leaves and stems of *Justicia californica* in the Sonoran Desert. *Journal of Arid Environments* 72:127–140. doi: 10.1016/j.jaridenv.2007.06.004

Vandegehuchte MW, Bloemen J, Vergeynst LL, Steppe K (2015) Woody tissue photosynthesis in trees: salve on the wounds of drought? *New Phytologist* 208:998–1002.

Vogt E, Schönherr J, Schmidt HW (1983) Water permeability of periderm membranes isolated enzymatically from potato tubers (*Solanum tuberosum* L.). *Planta* 158:294–301.

Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. *Biological Reviews* 81:259–291. doi: 10.1017/S1464793106007007

Wetherwax M (2002) The Jepson Desert Manual: Vascular Plants of Southeastern California, First edition. University of California Press, Berkeley

Wright IJ, Reich PB, Westoby M, et al (2004) The worldwide leaf economics spectrum. *Nature* 428:821–827.

Zanne AE, Westoby M, Falster DS, et al (2010) Angiosperm wood structure: Global patterns in vessel anatomy and their relation to wood density and potential conductivity. *American Journal of Botany* 97:207–215. doi: 10.3732/ajb.0900178

# Chapter two: Costs and benefits of green photosynthetic stems in desert species from southern California

## Abstract

Plants with woody green stems are common in dry woodlands with the possible advantages of extra carbon gain, re-assimilation of $CO_2$, and high water-use efficiency. However, their green stem tissue may also incur greater costs of water loss when stomata are closed. My study focused on evaluating the costs and benefits of having green stems in desert plants, addressing the water-use efficiency hypothesis. I measured water status, carbon and water exchange, and carbon, nitrogen and oxygen isotopic composition in 15 species, in a desert wash scrub in Joshua Tree National Park, California, USA. I found that all woody species with green stems had small leaves in February-April 2016 and did not have any leaves April 2016-January 2017, indicating that these plants relied on their green stems as the sole organ for carbon assimilation for most of the study period. Green stems had higher photosynthetic rate ($A$), stomatal conductance ($g_s$) and cuticular conductance ($g_{min}$) than leaves of non-green-stemmed plants. This yielded similar values of intrinsic water-use efficiency ($WUE_i$) in green stems and leaves of non-green-stemmed species. I also found that green stems appear to lose more water through their cuticle than leaves or non-green stems. My 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.

## 2.1 Introduction

Plants with woody green 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 some evidence that green stems confer advantages to plants bearing them, including extra photosynthetic carbon gain, re-assimilation of respired $CO_2$, and high water-use efficiency compared to leaves. There is enough support for the first and second advantages (Mooney and Strain 1964; Adams et al. 1967; Ehleringer et al. 1987; Nilsen et al. 1989; Nilsen and Bao 1990; Pfanz et al. 2002; Aschan and Pfanz 2003; Tinoco-Ojanguren 2008), but in terms of water-use efficiency the 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). Water-use efficiency is an important 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 plant species have green stems, suggesting there could be costs associated with having green stems. My study focused on evaluating the carbon gain and water loss costs and benefits of having green photosynthetic stems in plants from a desert ecosystem in southern California.

There are data supporting the idea that green stems are more water-use efficient than leaves, and this has been shown with both gas exchange (Osmond et al. 1987; Comstock and Ehleringer 1988) and carbon isotope composition ($\delta^{13}C$) of photosynthetic tissues (Ehleringer et al. 1987, 1992; Comstock and Ehleringer 1992; Nilsen and Sharifi 1997). However, green stems have moderate transpiration rates and stomatal conductance, 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*) (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 suberized periderm. Previous work has shown that green stem cuticular conductance can have values in the same range as field measures of stomatal conductance (Ávila-Lovera et al. 2017a), and this influences whole-plant water loss rates. Water loss becomes even more important in periods of water deficit, when low soil water availability limits photosynthesis and stomatal conductance, and cuticular conductance can vary throughout the year (Ávila-Lovera et al. 2017a).

It is intuitive to think that 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 photosynthesizing 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 photosynthetic rates are similar for both organs (Adams et al. 1967; Adams and Strain 1968; Smith and Nobel 1986). However, further work has shown that stem photosynthetic rates can be on average 60% of leaf photosynthetic rate (Ávila et al. 2014b), and these rates are maintained throughout the dry season (Ávila-Lovera et al. 2017a). This may give an advantage to green-stemmed plants over other drought-deciduous species that do not have this extra carbon gain during the dry season. This extra carbon gain has been shown to be used for plant bud development, flowering, and recovery after herbivory (Bossard and Rejmanek 1992; Tinoco-Ojanguren 2008; Saveyn et al. 2010). More recently, it has been suggested that stem photosynthesis (woody tissue photosynthesis, (Saveyn et al. 2010)) contributes to maintenance of hydraulic function (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 vapor gradient rather than lower annual precipitation produce proportionally more twigs than leaves, indicating that arid habitat populations rely on stem photosynthesis at expense of leaf photosynthesis (Comstock and Ehleringer 1992). These green stems have lower discrimination against $^{13}C$ ($\Delta$) than leaves, suggesting a tighter stomatal control (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 use of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotope composition in organic matter of photosynthetic tissues has proven to be effective to distinguish the effects of photosynthesis ($A$) and stomatal ($g_s$) on leaf $\delta^{13}C$ (Scheidegger et al. 2000). During photosynthesis, $CO_2$ diffuses into the leaf (or stem) through open stomata, and there is discrimination against $^{13}C$ during diffusion and fixation by Rubisco. When stomata close, leaf internal $CO_2$ cannot be replenished by atmospheric $CO_2$ and discrimination against $^{13}C$ decreases, leading to enriched values of $\delta^{13}C$. This low ratio of intercellular $CO_2$ concentration (or partial pressure) ($C_i$) relative to atmospheric $CO_2$ concentration ratio ($C_a$), $C_i/C_a$, is related to high values of intrinsic water-use efficiency ($A/g_s$; $WUE_i$) and $\delta^{13}C$, hence $\delta^{13}C$ is a measure of integrated, long-term WUE (Farquhar et al. 1982, 1989; Farquhar and Richards 1984). Values of $\delta^{13}C$ in photosynthetic tissue can help determine possible differences in long-term WUE between leaves and green stems. Several studies have found lower $C_i$ values in green stems than leaves (Osmond et al. 1987; Comstock and Ehleringer 1988), leading to differences in discrimination and consequently higher $\delta^{13}C$ values in green stems (Ehleringer et al. 1987; Smith and Osmond 1987; Farquhar et al. 1989; Nilsen and Sharifi 1997). On the other hand, organic matter $\delta^{18}O$ depends on source water $\delta^{18}O$, evaporative enrichment and biochemical fractionation during incorporation (Scheidegger et al. 2000). Assuming plants use the same soil water source and there are no differences in biochemical fractionation among species, differences in $\delta^{18}O$ are mainly due to differences in evaporative enrichment (Scheidegger et al. 2000), which depends on leaf-to-air water vapor pressure difference so that dry air has a positive effect on leaf water $\delta^{18}O$, and on stomatal control so that tighter control has a negative effect on leaf water $\delta^{18}O$ (Scheidegger et al. 2000; Cernusak et al. 2008; Santiago et al. 2017) and, after assimilation, on organic matter $\delta^{18}O$. Using $\delta^{18}O$ values as proxies for changes in relative humidity, we can tease apart the effects of $g_s$ and $A$ on $\delta^{13}C$ values and determine if changes in $C_i$, and therefore WUE, are due to stronger stomatal or metabolic limitations on photosynthesis. This has been successfully done in leaves using a conceptual model (Scheidegger et al. 2000) and I tested this model in green stems.

My main goal was to understand the costs and benefits of having green photosynthetic stems. I set out to answer three different questions: (1) does water status differ between green-stemmed and non-green-stemmed species? (2) do species with green stems have higher carbon balance than plants without green stems? (3) what are the water costs of stem photosynthesis? I expected that plants with green stems would have higher annual carbon balance than plants without green stems, but that this will be associated with higher water costs.

## 2.2 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 2016–2017. Joshua Tree has a mean annual air temperature of 18.6°C and annual precipitation of 119.1 mm for the period 1959-2012 (station 044405, Joshua Tree, Western Regional Climate Center). During 2016, mean annual air temperature was 19.4°C and precipitation was 82.3 mm (station # 233, Joshua Tree, California Irrigation Management Information System) (Fig. 2.1). The site is dominated by creosote-bush scrub (*Larrea tridentata*, Zygophyllaceae). Fifteen species were studied and they were categorized 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 2.1). Most of the species studied are woody shrubs, except for two perennial herbs: *Stillingia linearifolia* (Euphorbiaceae) and *Eriogonum inflatum* (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.

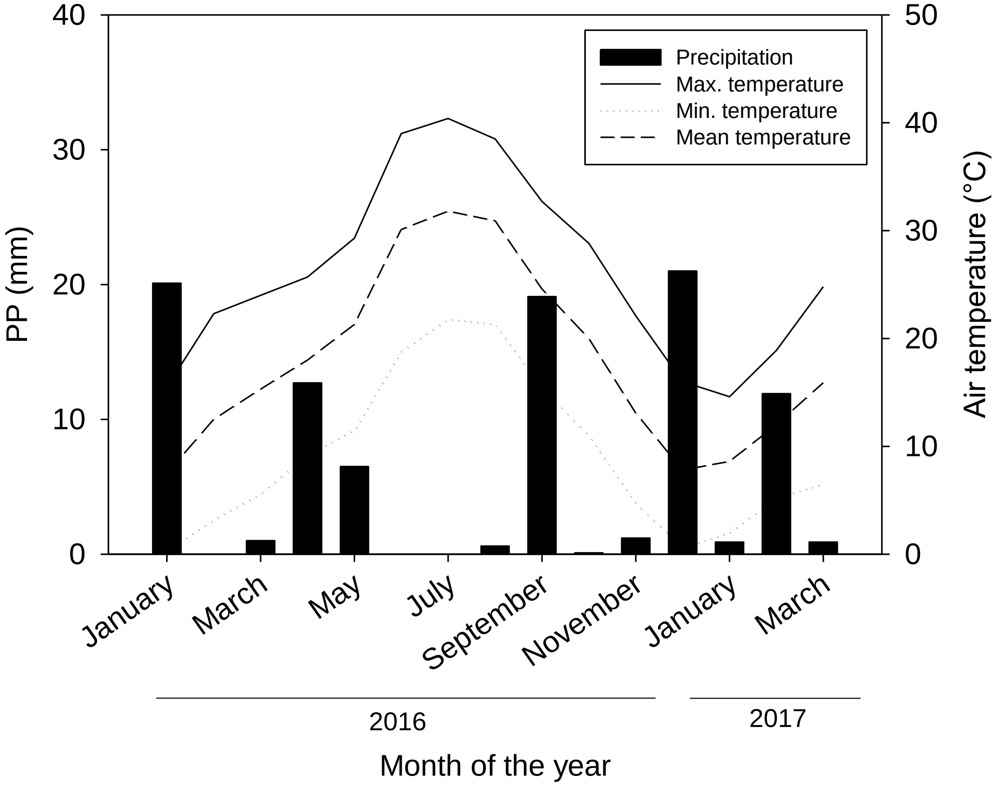

**Figure 2.1**. Total monthly precipitation, maximum air temperature, mean air temperature, and minimum air temperature during the study period February 2016 - March 2017. Weather data is from station # 233, Joshua Tree, California Irrigation Management Information System (CIMIS).

**Table 2.1**. List of fifteen plant species studied in a desert wash at Joshua Tree National Park, CA, USA. Family, growth form, leaf phenology and stem type is indicated.

| **Species** | **Family** | **Growth form**[a] | **Stem type** |
|---|---|---|---|
| *Ambrosia dumosa* | Asteraceae | Shrub | Non-green |
| *Ambrosia salsola* | Asteraceae | Shrub | Green |
| *Bebbia juncea* | Asteraceae | Shrub | Green |
| *Stillingia linearifolia* | Euphorbiaceae | Perennial herb | Green |
| *Psorothamnus arborescens* | Fabaceae | Shrub | Non-green |
| *Senna armata* | Fabaceae | Shrub | Green |
| *Senegalia greggi* | Fabaceae | Shrub | Non-green |
| *Krameria bicolor* | Krameriaceae | Shrub | Green |
| *Condea emoryi* | Lamiaceae | Shrub | Non-green |
| *Scutellaria mexicana* | Lamiaceae | Shrub | Green |
| *Menodora spinescens* | Oleaceae | Shrub | Green |
| *Eriogonum inflatum* | Polygonaceae | Perennial herb | Green |
| *Thamnosma montana* | Rutaceae | Shrub | Green |
| *Simmondsia chinensis* | Simmondsiaceae | Shrub | Non-green |
| *Larrea tridentata* | Zygophyllaceae | Shrub | Non-green |

[a] Growth form information was taken from CalFlora.org.

### *Water potential*

Morning water potential (Ψ) was measured using a pressure chamber (Model 1000; PMS Instrument Company, Albany, OR, USA) between 0800 and 1200 h, during the time of gas exchange measurements, because I was 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 randomized among all species. Current-year terminal leafy twigs were collected from the plants, sealed in double plastic bags (Whirlpak 0.057 mm thick; Nasco, Fort Atkinson, WI, USA) and transported to the lab for measurement of Ψ.

### *Gas exchange measurements*

Photosynthetic gas exchange of $CO_2$ and water vapor in stems and leaves (when present) were measured between 0800 and 1200 h using an infrared gas analyzer (Li-6400; Li-Cor Biosciences, Lincoln, NE, USA). The traits measured for leaves and green stems were photosynthetic rate ($A$), conductance to water vapor ($g_s$), transpiration rate ($E$) and internal $CO_2$ concentration ($C_i$). For non-green stems, the traits measured were respiration rate in light ($R_d$), non-stomatal (periderm) conductance ($g_w$), non-stomatal (periderm) transpiration rate ($E_{ns}$), and $C_i$. For each individual, gas exchange was measured in one intact twig segment of < 5 mm of diameter using the 2×3 cm Li-6400 leaf chamber and clay-modeled gaskets that allowed a tight seal. No leaks were found in the system using this protocol and the measurements were taken to be accurate. Leaves were measured using the standard gaskets that allowed tight seal without extra accommodation. Measurements were performed at ambient temperature, which varied with season from 12.9°C in January 2017 to 41.0 °C in June 2016, 400 $\mu$mol mol$^{-1}$ of $CO_2$ and 1500 $\mu$mol m$^{-2}$ s$^{-1}$ of photosynthetic photon flux density (PPFD) provided by a red blue light source (6400-02B #SI-710, Li-Cor Biosciences) (Evans and Santiago 2014). Leaves and especially stems did not cover the 6 cm$^2$ area of the chamber so after gas exchange measurements were taken, leaves and stems were sectioned and transported to the lab, where projected area was measured using an area meter (Li-3100; Li-Cor Biosciences, Lincoln, NE, USA). Gas exchange traits were then re-calculated using the projected area and all traits for both leaves and stems are expressed on a projected area basis. I used stem projected area instead of stem total area to be able to relate the gas exchange traits to expected light interception (Comstock and Ehleringer 1988) and to allow for comparisons between my work and previously published work. However, diameters of the stems used for gas exchange measurements are available (Table S1).

Intrinsic water-use efficiency ($\mathrm{WUE_i}$) was calculated only for green stems and leaves of non-green-stemmed species as $A/g_s$. Originally, I wanted to compare leaves and green stems of the same species to test if they were different in their photosynthetic capacity and rates of water loss. Leaves in green-stemmed species were small in February 2016 and absent for the rest of the year, hence I do not have data on leaf photosynthetic performance for 2016. I have, however, data in March 2017, so I compared leaf and green stem performance only during this sampling.

### *Cuticular conductance and periderm permeance*

Cuticular conductance (i.e. minimum epidermal conductance, $g_{\mathrm{min}}$ *sensu* (Kerstiens 1996)) was determined in leaves and green stems and periderm permeance ($P_{\mathrm{p}}$) was determined in non-green stems of three individuals per species following the methods of (Sack and Scoffoni 2011). One 20 cm long terminal leafy twig was chosen from each individual sampled in the field and taken to the lab. 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 mature leaves were used to estimate leaf $g_{\mathrm{min}}$. Current-year green and non-green stems were used to estimate stem $g_{\mathrm{min}}$ and $P_{\mathrm{p}}$, 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 $< 10$ μmol $\mathrm{m^{-2}\ s^{-1}}$ to induce stomatal closure. A temperature and humidity sensor was placed next to the samples within the breeze and measurements were recorded at the beginning of each weighing cycle to determine the mole fraction vapor 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 estimate the total surface area of stems (Sack and Scoffoni 2011).

### *Stable isotopic composition*

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratio, 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 analyzer for C and N (Model 4010, Costech Inc, Valencia, California) or a thermal conversion elemental analyzer (Model TC/EA, Thermo-Fisher, Bremen, Germany) for O at the Facility for Isotope Ratio Mass Spectrometry (FIRMS) of the University of California, Riverside (UCR). Only the stem photosynthetic tissue was used because the wood of green stems is usually enriched in $^{13}C$ compared to bark (Nilsen and Sharifi 1997), and I was interested in possible differences between leaf and stem photosynthetic tissues and implications for $WUE_i$. The $\delta^{13}C$ values were expressed relative to the V-PDB standard. Oxygen isotopic composition ($\delta^{18}O$) was measured in the stem bark of a subset of six green-stemmed species at the end of both wet and dry seasons. These species were selected because of their $g_s$ values being representatives of low, intermediate and high stomatal aperture in the wet season. The $\delta^{18}O$ values were expressed relative to the V-SMOW standard.

With values of $A$, $g_s$, $\delta^{13}C$ and $\delta^{18}O$ I used an isotope model (Scheidegger et al. 2000) to tease apart the effects of $A$ and $g_s$ on $\delta^{13}C$ changes from April 2016 to October 2016 for a subset of six green-stemmed species.

*Statistical analyses*

For the time series analyses I was interested in finding a time index that could be used as an independent variable in linear models. In order to do this, I calculated a seasonal time series predictor using the day of year (DOY) and following a sinusoidal function:

$$\text{Time series predictor} = \sin((DOY + c) \times 2\pi/365.2422) \qquad \text{Eqn. 1}$$

The parameter $c$ is the time series-shift from the origin, defined as the day of the year in which the sine function changes from negative to positive. In order to parsimoniously estimate the mean value of the shift for all species, I first did a principal component analysis on the water potential of all species pooled together, and performed a Fourier regression on the first axis. The shift value ($c$) was approximately 30 days, indicating that physiological conditions change around December 1st and was summed to the DOY value for each species.

This time series predictor ranges between -1 and 1. Values higher than zero reflect the winter-spring signal (December to May), while values lower than zero reflect the summer-fall signal (June to November). This predictor, converting the circular variable

DOY into a linear independent variable, was used as a co-variate in analyses of covariance (ANCOVA), where the stem-type groups of species with and without green stems was used as a factor. The significance was tested at $p \leq 0.05$.

For the stable isotope data, paired *t*-tests were performed to test for differences between leaf and green stem bark $\delta^{13}C$ during the wet season 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 $\delta^{13}C$ and $\delta^{15}N$, as well as C and N concentration. Relationships among isotope compositions and elemental concentrations were assessed using linear regression. The significance was tested at $p \leq 0.05$.

All statistical analyses were done using R v.3.4.0 (R Core Team 2017). All plots were made using SigmaPlot 11 (Systat Software, San Jose, CA, USA).

## 2.3 Results

Leaves of green-stemmed species were small and were present only in early spring 2016 (February), falling at the beginning of the dry season (June), and were absent for most of the study period. The high precipitation of the 2017 wet season (Fig. 2.1) promoted the growth of large leaf canopies in most of the green-stemmed species, and I was able to measure these leaves for gas exchange traits only in 2017.

For both green-stemmed and non-green-stemmed plants shoot $\Psi$ was highly seasonal ($p < 2\text{x}10^{-6}$), being higher (less negative) in the wet season, and lower in the dry season (Fig. 2.2). However, there was a secondary peak in shoot $\Psi$ in October 2016, given the substantial rain that fell in September 2016 (Fig. 2.1). The ANCOVA showed that shoot Ψ across the study period was higher in green-stemmed species than in non-green-stemmed species ($p$ = 0.052, Fig. 2.2).

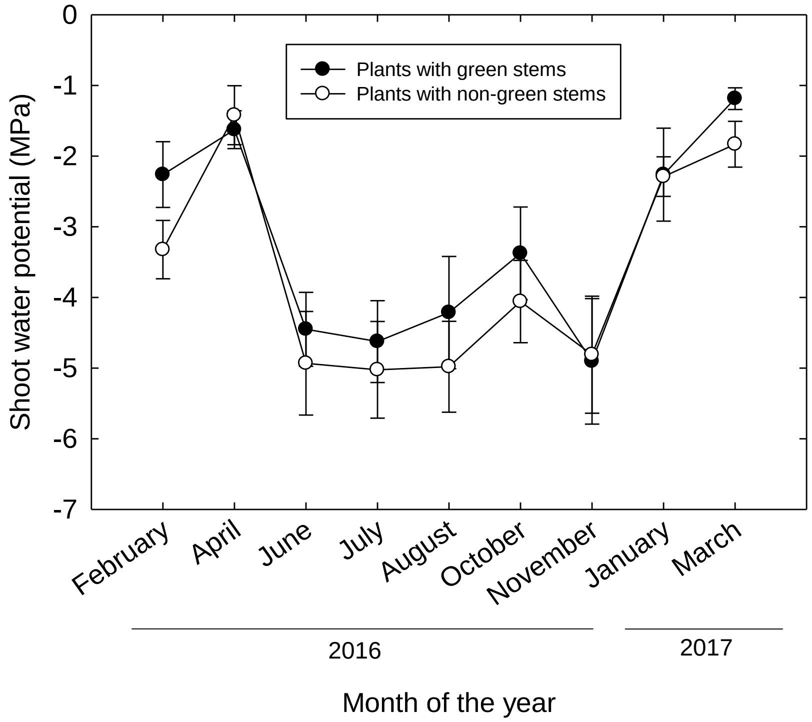

**Figure 2.2**. Shoot water potential for green and non-green-stemmed plant species during the nine sampling campaigns from February 2016 to March 2017. Values shown are means of species within each stem-type group ± standard error.

Green stems had higher $A$ ($p$ = 0.005, Fig. 2.3A) and $g_s$ ($p$ = 0.043, Fig. 2.3B) than leaves of non-green-stemmed species. Since both $A$ 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. 2.3C). Comparing leaves and stems of green-stemmed species they showed the same $A$, $g_s$ and $WUE_i$ (Table 2.2).

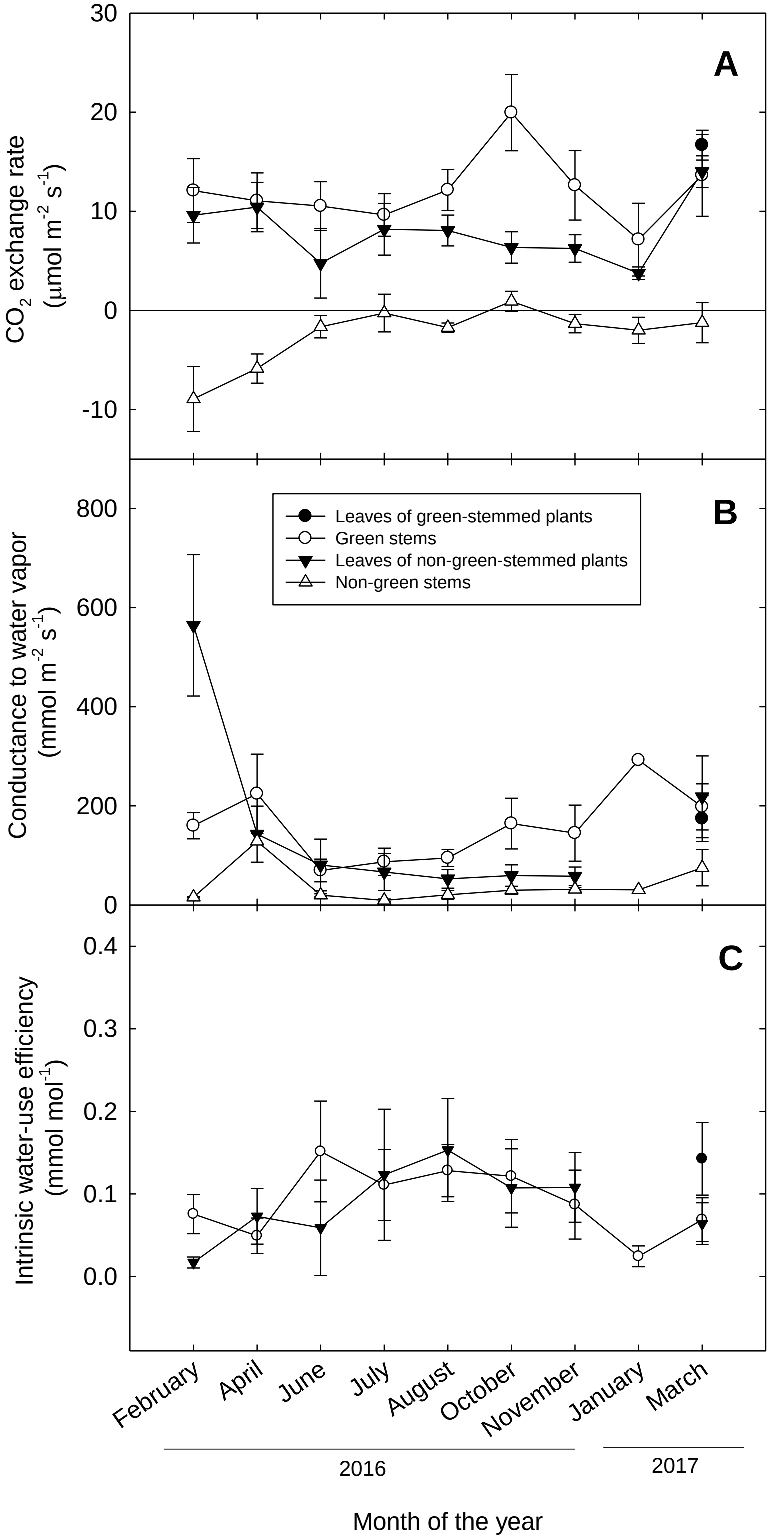
30
20
10
0
-10
A
CO$_2$ exchange rate ($\mu$mol m$^{-2}$ s$^{-1}$)
800
600
400
200
0
B
Conductance to water vapor (mmol m$^{-2}$ s$^{-1}$)
Leaves of green-stemmed plants
Green stems
Leaves of non-green-stemmed plants
Non-green stems
0.4
0.3
0.2
0.1
0.0
C
Intrinsic water-use efficiency (mmol mol$^{-1}$)
February
April
June
July
August
October
November
January
March
2016
2017
Month of the year

**Figure 2.3**. Gas exchange data for leaves (when present) and stems of green-stemmed species, and leaves and stems of non-green-stemmed species. A) $CO_2$ exchange rate, where positive values indicate net photosynthetic rate and negative values indicate respiration rate. B) Stomatal and non-stomatal conductance to water vapor. C) Intrinsic water-use efficiency for leaves and non-green stems. Values shown are means of species within each stem-type group ± standard error.

**Table 2.2**. Photosynthetic rate (*A*), stomatal conductance ($g_s$), and intrinsic water-use efficiency ($WUE_i$) of leaves and green stems of seven woody species with green stems studied in a desert wash at Joshua Tree National Park, CA, USA. These values correspond to the wet season of 2017 when leaves were present in most species. Values shown are mean ± SE. *p*-values are shown for the comparison between organs using paired Student's t-test.

| **Species** | **Organ** | ***A*** **($\mu$mol $m^{-2}$ $s^{-1}$)** | **$g_s$ (mmol $m^{-2}$ $s^{-1}$)** | **$WUE_i$ (mmol $mol^{-1}$)** |
|---|---|---|---|---|
| *A. salsola* | leaf | 11.50 ± 1.72 | 189.43 ± 36.61 | 0.07 ± 0.02 |
| | stem | 10.73 ± 5.99 | 174.64 ± 94.17 | 0.45 ± 0.39 |
| *B. juncea* | leaf | NA | NA | NA |
| | stem | 43.89 ± 6.94 | 472.64 ± 121.06 | 0.10 ± 0.01 |
| *K. bicolor* | leaf | NA | NA | NA |
| | stem | 5.67 ± 1.97 | 55.63 ± 26.63 | 0.12 ± 0.01 |
| *M. spinences* | leaf | NA | NA | NA |
| | stem | 15.51 ± 2.47 | 159.37 ± 26.58 | 0.10 ± 0.00 |
| *S. mexicana* | leaf | 18.20 ± 9.68 | 341.38 ± 165.77 | 0.05 ± 0.01 |
| | stem | 0.99 ± 4.57 | 372.68 ± 76.32 | 0.02 ± 0.01 |
| *S. armata* | leaf | 15.82 ± 9.13 | 69.93 ± 40.37 | 0.29 ± 0.17 |
| | stem | 10.81 ± 6.24 | 71.77 ± 41.44 | 0.36 ± 0.21 |
| *T. montana* | leaf | 20.58 ± 4.93 | 122.26 ± 44.00 | 0.19 ± 0.03 |
| | stem | 13.73 ± 3.94 | 93.24 ± 41.49 | 0.33 ± 0.20 |
| Mean ± SE | leaf | 16.67 ± 1.51 | 218.27 ± 46.05 | 0.14 ± 0.04 |
| | stem | 13.63 ± 4.13 | 75.02 ± 46.56 | 0.18 ± 0.05 |
| *p*-value | | 0.122 | 0.850 | 0.207 |

Cuticular conductance was highly seasonal ($p = 2.42\times10^{-7}$), being higher in the wet season and lower in the dry season (Fig. 2.4). Also, leaves of green-stemmed species (present only in February 2016 and March 2017) and green stems had higher $g_{min}$ values than leaves and stems ($P_p$) of non-green-stemmed species ($p = 3.04\times10^{-10}$).

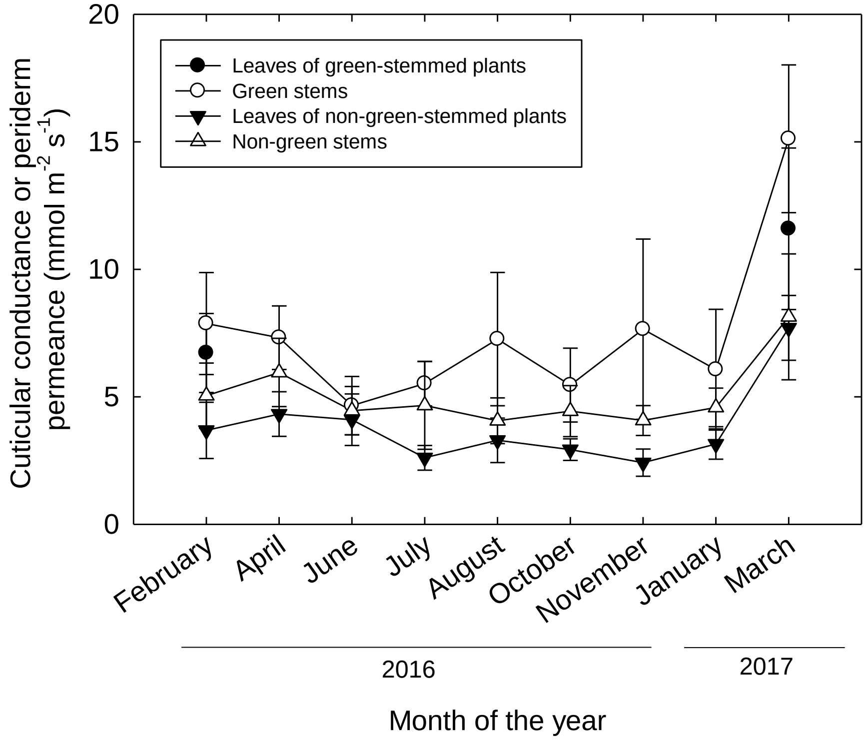

**Figure 2.4**. Cuticular conductance of leaves and green stems and periderm permeance of non-green stems. Values shown are means of species within each stem-type group ± standard error.

There were no seasonal changes in $\delta^{13}$C and N concentration across species, stem-type groups or organs. Values of $\delta^{13}$C in leaves and stems of green-stemmed species were higher than those in leaves and stems of non-green-stemmed species ($p$ = 0.002). Comparing the photosynthetic organ of each stem-type group, green stems *vs.* leaves of non-green-stemmed species, I found that green stem bark had higher $\delta^{13}$C values than leaves ($p$ = 0.013). Green stem bark $\delta^{13}$C, however, was statistically higher than same species leaf $\delta^{13}$C in only one out of eight green-stemmed species that had both leaves and green stems during the wet season (Table 2.3). 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), which may explain the higher photosynthetic performance in green stems than leaves of non-green-stemmed species.

Values of $\delta^{13}$C and $\delta^{18}$O in green stem bark were negatively related (Fig. 2.5A), but on the margin of significance (*p* = 0.09), providing little evidence that stems with higher long-term WUE, as indicated by high $\delta^{13}$C, have lower $\delta^{18}$O values. On the other hand, $\delta^{13}$C values were positively related with N concentration in both leaves and stems of green-stemmed species (Fig. 2.5B), indicating that species with high N concentration in their photosynthetic tissues are more water-use efficient.

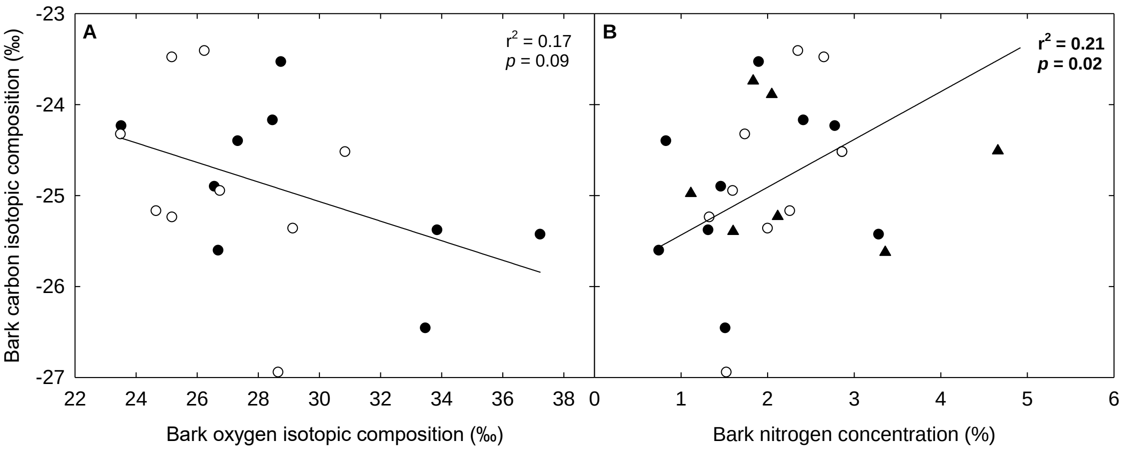

**Figure 2.5**. A) Carbon isotope composition as a function of oxygen isotope composition in bark of green-stemmed species. B) Carbon isotope composition as a function of nitrogen content of both leaves and stems of green-stemmed species. Values correspond to species means. Symbols are as follows: leaves (triangles), stems (circles), wet season (closed symbols), dry season (open symbols).

**Table 2.3**. Carbon ($\delta^{13}$C), nitrogen ($\delta^{15}$N) and oxygen ($\delta^{18}$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. Values shown are mean ± SE.

| **Season** | **Species** | **Organ** | **$\delta^{13}$C (‰)** | **C (%)** | **$\delta^{15}$N (‰)** | **N (‰)** | **$\delta^{18}$O (‰)** |
|---|---|---|---|---|---|---|---|
| **Wet** | **Green-stemmed** | | | | | | |
| | *Ambrosia salsola* | leaf | -25.6 ± 0.8 | 44.1 ± 0.5 | 3.6 ± 0.8 | 3.4 ± 0.4 | 32.1 ± 1.1 |
| | | bark | -24.2 ± 0.7 | 45.6 ± 0.6 | 2.6 ± 0.8 | 2.8 ± 0.2 | 23.5 ± 0.6 |
| | *Bebbia juncea* | leaf | -27.7 ± 0.3 | 37.0 ± 1.3 | 4.3 ± 0.4 | 1.4 ± 0.3 | 38.9 ± 0.2 |
| | | bark | -25.4 ± 0.2 | 40.5 ± 0.3 | 2.6 ± 0.4 | 1.3 ± 0.4 | 33.9 ± 2.9 |
| | *Eriogonum inflatum* | leaf | - | - | - | - | - |
| | | bark | -25.4 ± 0.5 | 42.2 ± 3.5 | 4.5 ± 0.5 | 3.3 ± 0.9 | 37.2 ± 1.1 |
| | *Krameria bicolor* | leaf | -23.7 ± 1.0 | 45.2 ± 0.3 | 7.0 ± 1.2 | 1.8 ± 0.4 | 46.1 ± 2.7 |
| | | bark | -24.4 ± 0.3 | 46.4 ± 1.4 | 3.7 ± 0.2 | 0.8 ± 0.0 | 27.3 ± 1.8 |
| | *Menodora spinescens* | leaf | -25.2 ± 1.0 | 44.1 ± 0.5 | 2.7 ± 0.2 | 2.1 ± 0.1 | 39.4 ± 0.9 |
| | | bark | -24.9 ± 0.1 | 46.4 ± 0.2 | 1.2 ± 0.3 | 1.5 ± 0.5 | 26.6 ± 0.8 |
| | *Scutellaria mexicana* | leaf | -24.3 ± 0.0 | 42.7 ± 0.1 | 3.7 ± 0.7 | 1.1 ± 0.1 | 44.0 ± 1.1 |
| | | bark | -25.6 ± 0.6 | 50.6 ± 1.1 | 2.0 ± 0.7 | 0.8 ± 0.2 | 26.7 ± 2.5 |
| | *Senna armata* | leaf | -25.4 ± 0.4 | 42.8 ± 0.4 | 3.1 ± 0.3 | 1.6 ± 0.2 | 50.4 ± 1.4 |
| | | bark | -26.5 ± 0.7 | 48.3 ± 1.4 | 2.1 ± 0.5 | 1.5 ± 0.4 | 33.5 ± 1.6 |
| | *Stillingia linearifolia* | leaf | -24.5 ± 0.7 | 41.9 ± 0.6 | 1.9 ± 0.7 | 4.7 ± 0.4 | 43.0 ± 3.3 |
| | | bark | -23.5 ± 0.3 | 42.7 ± 0.9 | 0.5 ± 0.8 | 1.9 ± 0.2 | 28.8 ± 0.5 |
| | *Thamnosma montana* | leaf | -23.9 ± 0.0 | 44.2 ± 0.8 | 2.2 ± 0.1 | 2.1 ± 0.3 | 50.4 ± 6.2 |
| | | bark | -24.2 ± 0.9 | 47.5 ± 0.3 | 0.8 ± 0.5 | 2.4 ± 0.6 | 28.5 ± 2.3 |
| | **Non-green-stemmed** | | | | | | |
| | *Ambrosia dumosa* | leaf | -25.2 ± 0.6 | 39.3 ± 0.9 | 4.4 ± 0.6 | 2.6 ± 0.0 | 33.7 ± 1.0 |
| | | bark | -25.5 ± 0.4 | 43.5 ± 0.4 | 4.3 ± 0.6 | 1.0 ± 0.1 | 25.8 ± 0.6 |

|  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
|  | *Condea emoryi* | leaf | -28.5 ± 1.1 | 48.7 ± 0.8 | 4.1 ± 0.8 | 2.2 ± 0.4 | 30.7 ± 0.7 |
|  |  | bark | -27.1 ± 0.9 | 44.2 ± 1.0 | 1.3 ± 0.5 | 0.3 ± 0.2 | 29.0 ± 2.3 |
|  | *Larrea tridendata* | leaf | -24.9 ± 0.6 | 47.5 ± 0.4 | 5.2 ± 0.2 | 1.5 ± 0.3 | 31.0 ± 0.7 |
|  |  | bark | -24.0 ± 0.4 | 41.6 ± 5.4 | 1.6 ± 0.4 | 0.2 ± 0.1 | 21.7 ± 0.5 |
|  | *Psorothamnus arborescens* | leaf | -23.4 ± 0.3 | 42.7 ± 0.5 | 2.1 ± 0.5 | 2.0 ± 0.1 | 35.2 ± 1.4 |
|  |  | bark | -24.4 ± 0.1 | 43.7 ± 0.7 | 1.1 ± 0.8 | 1.6 ± 0.2 | 18.2 ± 0.6 |
|  | *Senegalia greggii* | leaf | -29.2 ± 0.8 | 45.0 ± 1.9 | 5.1 ± 1.6 | 3.1 ± 1.8 | 50.4 ± 1.4 |
|  |  | bark | -27.4 ± 0.8 | 46.5 ± 0.6 | 4.3 ± 1.6 | 2.2 ± 0.6 | 22.3 ± 0.6 |
|  | *Simmondsia chinensis* | leaf | -26.2 ± 0.6 | 44.7 ± 0.4 | 5.2 ± 0.3 | 0.8 ± 0.3 | 37.4 ± 2.0 |
|  |  | bark | -25.2 ± 0.3 | 47.1 ± 0.3 | 2.4 ± 0.4 | 1.1 ± 0.1 | 24.8 ± 0.3 |
| **Dry** | **Green-stemmed** |  |  |  |  |  |  |
|  | *Ambrosia salsola* | leaf | - | - | - | - | - |
|  |  | bark | -25.4 ± 0.5 | 46.1 ± 0.2 | 3.3 ± 0.4 | 1.3 ± 0.3 | 25.2 ± 1.4 |
|  | *Bebbia juncea* | leaf | - | - | - | - | - |
|  |  | bark | -26.9 ± 0.5 | 42.2 ± 1.2 | 0.6 ± 0.8 | 1.5 ± 0.1 | 28.7 ± 0.8 |
|  | *Eriogonum inflatum* | leaf | - | - | - | - | - |
|  |  | bark | -25.2 | 40.4 | 0.9 | 1.3 | 25.2 ± 1.0 |
|  | *Krameria bicolor* | leaf | - | - | - | - | - |
|  |  | bark | -24.3 ± 0.2 | 47.7 ± 1.2 | 2.9 ± 0.4 | 1.7 ± 0.1 | 23.5 ± 0.9 |
|  | *Menodora spinescens* | leaf | - | - | - | - | - |
|  |  | bark | -25.1 ± 0.4 | 47.5 ± 0.3 | 1.1 ± 0.1 | 2.3 ± 0.2 | 24.7 ± 0.5 |
|  | *Scutellaria mexicana* | leaf | - | - | - | - | - |
|  |  | bark | -25.0 ± 0.3 | 50.4 ± 0.2 | 2.7 ± 0.2 | 1.6 ± 0.2 | 26.8 ± 0.8 |
|  | *Senna armata* | leaf | - | - | - | - | - |
|  |  | bark | -25.4 ± 0.2 | 50.5 ± 0.2 | 1.2 ± 0.1 | 2.0 ± 0.2 | 29.1 ± 0.6 |
|  | *Stillingia linearifolia* | leaf | - | - | - | - | - |
|  |  | bark | -23.4 ± 0.1 | 45.8 ± 0.4 | -0.8 ± 0.6 | 2.3 ± 0.3 | 26.2 ± 1.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Thamnosma montana* | leaf | - | - | - | - | - |
| | bark | -24.5 ± 0.2 | 51.7 ± 4.2 | 0.5 ± 0.2 | 2.9 ± 0.4 | 30.8 ± 1.7 |
| **Non-green-stemmed** | | | | | | |
| *Ambrosia dumosa* | leaf | - | - | - | - | - |
| | bark | -25.5 ± 0.5 | 46.1 ± 0.2 | 3.3 ± 0.4 | 1.3 ± 0.3 | 25.4 ± 0.2 |
| *Condea emoryi* | leaf | -27.7 ± 1.0 | 48.6 ± 0.8 | 4.7 ± 0.2 | 1.7 ± 0.1 | 31.9 ± 1.0 |
| | bark | -26.6 ± 0.6 | 44.4 ± 0.4 | 1.4 ± 0.3 | 1.2 ± 0.1 | 23.6 ± 0.6 |
| *Larrea tridendata* | 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 |
| *Psorothamnus arborescens* | leaf | - | - | - | - | - |
| | bark | -25.3 ± 0.3 | 46.3 ± 0.2 | 0.4 ± 0.4 | 1.6 ± 0.3 | 17.2 ± 0.3 |
| *Senegalia greggii* | leaf | -26.3 ± 0.5 | 46.4 ± 0.3 | 4.4 ± 0.5 | 2.2 ± 0.2 | 33.4 ± 0.7 |
| *Simmondsia chinensis* | 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 |

## 2.4 Discussion

The main goal of my work was to understand the costs and benefits of having green photosynthetic stems, in terms of carbon gain and water loss, in desert species from southern California. I found that green-stemmed species maintained higher $\Psi$ than 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, $A$ and $g_s$ were higher in green stems than leaves of non-green-stemmed species, leading to similar $WUE_i$ in both organs. Furthermore, green stems had higher $g_{min}$ values than non-green stems $P_p$, suggesting a higher water cost associated with the presence of green stems when stomata are closed. These results suggest that leaves and green stems have a similar ratio of carbon gain to water loss during photosynthesis, leading to similar $WUE_i$, but that stems may be losing a greater absolute amount of water than non-green stems through their cuticles. However, since green-stemmed species can maintain higher shoot $\Psi$ values than non-green-stemmed species, this higher water loss through the cuticle may not be as important for whole-plant performance during the dry season, when plants continue assimilating $CO_2$.

The higher shoot $\Psi$ found in green-stemmed species than in species with non-green stems suggests that green-stemmed plants may have access to deeper water sources in the soil (Gibson 1998), 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 minimizing the water loss needed to cool high temperature tissues during the day when vapor pressure deficit is high. This higher shoot $\Psi$ is important for green stems in maintaining their physiological activity during the hot dry season, because they can maintain stomata open, when other drought-deciduous plants without green stems would be inactive, consistent with the *extended growth season hypothesis* previously described for green-stemmed species ((Nilsen and Sharifi 1997). Furthermore, it seems that green stems may be more responsive to short-term increases in water availability than leaves, as shown by a sudden increased in $A$ 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. 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). My 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 photosynthetic rates between green and non-green-stemmed species suggests that plants can cope with the same environmental stresses through different strategies (Pivovaroff 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 Erik T. Nilsen (Nilsen 1995). At that time, these constraints were related to the cost of having a thin bark (i.e. cortex + epidermis) to stem herbivore defense 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 suberized periderm, leading to a functional constraint. Across species, green stem $g_{min}$ values ranged from 1.42 to 33.74 mmol $m^{-2}$ $s^{-1}$, and they overlap with the range of 1.11 – 20.49 mmol $m^{-2}$ $s^{-1}$ found for 11 green-stemmed species studied in desert sites in southern California (Ávila-Lovera et al. 2017a). These values are significantly higher than the ones found for non-green stems in this study, which ranged from 1.05 to 19.03 mmol $m^{-2}$ $s^{-1}$, and were also higher than mean periderm permeability 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). My results support the idea that green stems lose more water through their cuticle than non-green stem do through their periderm, this is may be a cost that partially offsets the benefits of having green stems.

When I 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 $\delta^{13}C$ values than leaves. This high N concentration partially explains the high photosynthetic capacity of green stems compared to leaves. 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 $CO_2$ assimilation and N concentration has been found in stems that perform photosynthesis (Nilsen 1992; 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), I can say that both stomatal behavior and metabolic factors coordinate the photosynthesis occurring in stems.

The positive relationship between $\delta^{13}C$ and N concentration in photosynthetic tissues of green-stemmed species indicates that as N concentration increases, there is a higher $CO_2$ drawdown for a given stomatal conductance, leading to increased $WUE_i$ and $\delta^{13}C$ values. These would provide a benefit for the species in the high $\delta^{13}C$ - high N end because the high N concentration allows for a higher assimilation capacity with a relatively lower stomatal aperture.

I used the conceptual isotope model proposed by Scheidegger et al. (2000) and applied it to green stems to understand the cause of changes in $\delta^{13}C$ from April 2016 to October 2016. Across species, $\delta^{13}C$ and $\delta^{18}O$ values did not change much in this period, giving limited resolution for the model. Therefore, I found no match between model expectations and actual gas exchange data collected on or the month before the months of sample collection for isotope analyses. The model was originally used to evaluate isotope data from species in plots that differed in land-use intensity (Scheidegger et al. 2000). Changes in land-use are expected to have greater impact on carbon and water relations than seasonal variation in precipitation, as in my case. Furthermore, if green-stemmed species really do have access to deeper water sources in the soil (Gibson 1998; M.E. Allen *et al.* unpublished data), seasonal variation in precipitation may not result in seasonal soil water deficit, and hence, large changes in gas exchange behavior.

In summary, my results showed 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 $CO_2$ throughout the dry season. I found partial support for the high water-use efficiency hypothesis, and that green stems might be losing more water through their cuticle than non-green stems. This raises questions of possible trade-offs between carbon gain and water loss through the cuticle in woody green stems and how this may affect plant responses to current and future droughts.

## 2.6 References

Adams M, Strain B (1968) Photosynthesis in stems and leaves of *Cercidium floridum* - spring and summer diurnal field response and relation to temperature. *Oecologia Plantarum* 3:285–297

Adams MS, Strain BR, Ting IP (1967) Photosynthesis in chlorophyllus stem tissue and leaves of *Cercidium floridum*: accumulation and distribution of $^{14}C$ from $^{14}CO_2$. *Plant Physiology* 42:1797-1799

Aschan G, Pfanz H (2003) Non-foliar photosynthesis – a strategy of additional carbon acquisition. *Flora - Morphology, Distribution, Functional Ecology of Plants* 198:81–97. doi: 10.1078/0367-2530-00080

Ávila E, De Almeida J, Tezara W (2014a) Comparación ecofisiológica y anatómica de los tejidos fotosintéticos de *Cercidium praecox* (Ruiz & Pav. ex Hook.) Harms (Fabaceae, Caesalpinioideae). *Acta Botánica Venezuelica* 37:59–76

Ávila E, Herrera A, Tezara W (2014b) Contribution of stem $CO_2$ fixation to whole-plant carbon balance in nonsucculent species. *Photosynthetica* 52:3–15. doi: 10.1007/s11099-014-0004-2

Ávila-Lovera E, Zerpa AJ, Santiago LS (2017) Stem photosynthesis and hydraulics are coordinated in desert plant species. New Phytologist. doi: 10.1111/nph.14737

Berveiller D, Kierzkowski D, Damesin C (2007) Interspecific variability of stem photosynthesis among tree species. *Tree Physiology* 27:53–61

Bloemen J, Vergeynst LL, Overlaet-Michiels L, Steppe K (2016) How important is woody tissue photosynthesis in poplar during drought stress? *Trees* 30:63–72. doi: 10.1007/s00468-014-1132-9

Bossard CC, Rejmanek M (1992) Why Have Green Stems? *Functional Ecology* 6:197–205. doi: 10.2307/2389755

Cannon WA (1908) The topography of the chlorophyll apparatus in desert plants. Carniege Institution of Washington

Cernusak LA, Mejia-Chang M, Winter K, Griffiths H (2008) Oxygen isotope composition of CAM and $C_3$ *Clusia* species: non-steady-state dynamics control leaf water $^{18}O$ enrichment in succulent leaves. *Plant, Cell & Environment* 31:1644–1662. doi: 10.1111/j.1365-3040.2008.01868.x

Comstock JP, Ehleringer JR (1992) Correlating genetic variation in carbon isotopic composition with complex climatic gradients. *Proceedings of the National Academy of Sciences of the United States of America* 89:7747–7751

Comstock JP, Ehleringer JR (1988) Contrasting Photosynthetic Behavior in Leaves and Twigs of *Hymenoclea salsola*, a Green-Twigged Warm Desert Shrub. *American Journal of Botany* 75:1360–1370. doi: 10.2307/2444459

De Baerdemaeker NJF, Salomón RL, De Roo L, Steppe K (2017) Sugars from woody tissue photosynthesis reduce xylem vulnerability to cavitation. *New Phytologist* 216:720–727. doi: 10.1111/nph.14787

Ehleringer JR, Comstock JP, Cooper TA (1987) Leaf-twig carbon isotope ratio differences in photosynthetic-twig desert shrubs. *Oecologia* 71:318–320

Ehleringer JR, Phillips SL, Comstock JP (1992) Seasonal Variation in the Carbon Isotopic Composition of Desert Plants. *Functional Ecology* 6:396–404. doi: 10.2307/2389277

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of $C_3$ plants. *Oecologia* 78:9–19

Evans JR, Santiago LS (2014) PrometheusWiki Gold Leaf Protocol: gas exchange using LI-COR 6400. *Functional Plant Biology* 41:223. doi: 10.1071/FP10900

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Biology* 40:503–537

Farquhar GD, O’leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Functional Plant Biology* 9:121–137

Garrec J-P, Barrois A (1992) Caracteristiques de la fixation et de la penetration corticale. Passage du phosphite dipotassique et de l’eau au travers d’ecorces isolees. *Environmental and Experimental Botany* 32:11–23. doi: 10.1016/0098-8472(92)90025-W

Gibson AC (1983) Anatomy of Photosynthetic Old Stems of Nonsucculent Dicotyledons from North American Deserts. *Botanical Gazette* 144:347–362

Gibson AC (1998) Photosynthetic Organs of Desert Plants. *Bioscience* 48:911–920. doi: 10.2307/1313295

Groh B, Hübner C, Lendzian KJ (2002) Water and oxygen permeance of phellems isolated from trees: the role of waxes and lenticels. *Planta* 215:794–801

Kerstiens G (1996) Cuticular water permeability and its physiological significance. *Journal of Experimental Botany* 47:1813–1832. doi: 10.1093/jxb/47.12.1813

Kocurek M, Kornas A, Pilarski J, et al (2015) Photosynthetic activity of stems in two *Clusia* species. *Trees* 29:1029–1040. doi: 10.1007/s00468-015-1182-7

Lulai EC, Glynn MT, Orr PH (1996) Cellular changes and physiological responses to tuber pressure-bruising. *American Potato Journal* 73:197–209. doi: 10.1007/BF02854874

Lulai EC, Orr PH (1994) Techniques for detecting and measuring developmental and maturational changes in tuber native periderm. *American Potato Journal* 71:489–505. doi: 10.1007/BF02851322

Mooney HA, Strain BR (1964) Bark photosynthesis in Ocotillo. *Madroño* 17:230–233

Nilsen E, Sharifi M (1997) Carbon isotopic composition of legumes with photosynthetic stems from mediterranean and desert habitats. *American Journal of Botany* 84:1707–1713

Nilsen ET (1995) Stem photosynthesis: extent, patterns, and role in plant carbon economy. In: Gartner BL (ed) Plant stems: physiology and functional morphology. Academic Press, San Diego, pp 223–240

Nilsen ET (1992) Partitioning Growth and Photosynthesis between Leaves and Stems During Nitrogen Limitation in *Spartium junceum*. *American Journal of Botany* 79:1217–1223. doi: 10.2307/2445047

Nilsen ET, Bao Y (1990) The Influence of Water Stress on Stem and Leaf Photosynthesis in *Glycine max* and *Sparteum junceum* (Leguminosae). *American Journal of Botany* 77:1007–1015. doi: 10.2307/2444572

Nilsen ET, Meinzer FC, Rundel PW (1989) Stem photosynthesis in *Psorothamnus spinosus* (smoke tree) in the Sonoran desert of California. *Oecologia* 79:193–197

Nilsen ET, Sharifi MR (1994) Seasonal acclimation of stem photosynthesis in woody legume species from the Mojave and Sonoran deserts of California. *Plant Physiology* 105:1385–1391

Osmond CB, Smith SD, Gui-Ying B, Sharkey TD (1987) Stem photosynthesis in a desert ephemeral, *Eriogonum inflatum*. Characterization of leaf and stem $CO_2$ fixation and $H_2O$ vapor exchange under controlled conditions. *Oecologia* 72:542–549

Pfanz H, Aschan G, Langenfeld-Heyser R, et al (2002) Ecology and ecophysiology of tree stems: corticular and wood photosynthesis. *Naturwissenschaften* 89:147–162. doi: 10.1007/s00114-002-0309-z

Pivovaroff AL, Pasquini SC, De Guzman ME, et al (2016) Multiple strategies for drought survival among woody plant species. *Functional Ecology* 517–526. doi: 10.1111/1365-2435.12518

Sack L, Scoffoni C (2011) PrometheusWiki | Minimum epidermal conductance ($g_{min}$, a.k.a. cuticular conductance). http://prometheuswiki.publish.csiro.au/tiki-index.php?page=Minimum+epidermal+conductance+%28gmin%2C+a.k.a.+cuticular+conductance%29. Accessed 28 Mar 2016

Santiago L, Silvera K, Andrade JL, Dawson TE (2017) Functional strategies of tropical dry forest plants in relation to growth form and isotopic composition. *Environmental Research Letters*. doi: 10.1088/1748-9326/aa8959

Santiago LS, Bonal D, De Guzman ME, Ávila-Lovera E (2016) Drought Survival Strategies of Tropical Trees. In: Goldstein G, Santiago LS (eds) Tropical Tree Physiology. Springer International Publishing, Cham, pp 243–258

Saveyn A, Steppe K, Ubierna N, Dawson TE (2010) Woody tissue photosynthesis and its contribution to trunk growth and bud development in young plants. *Plant, Cell & Environment* 33:1949–1958. doi: 10.1111/j.1365-3040.2010.02197.x

Scheidegger Y, Saurer M, Bahn M, Siegwolf R (2000) Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia* 125:350–357. doi: 10.1007/s004420000466

Schmitz N, Egerton JJG, Lovelock CE, Ball MC (2012) Light-dependent maintenance of hydraulic function in mangrove branches: do xylary chloroplasts play a role in embolism repair?: Rapid report. *New Phytologist* 195:40–46. doi: 10.1111/j.1469-8137.2012.04187.x

Schönherr J, Ziegler H (1980) Water Permeability of *Betula* Periderm. Planta 147:345–354

Schreiber L, Franke R, Hartmann K (2005) Wax and suberin development of native and wound periderm of potato (*Solanum tuberosum* L.) and its relation to peridermal transpiration. *Planta* 220:520–530

Smith SD, Nobel PS (1986) Deserts. In: Baker NR, Long SP (eds) Photosynthesis in Contrasting Environments. Elsevier Science Publishers B.V., Amsterdam, pp 13–62

Smith SD, Osmond CB (1987) Stem Photosynthesis in a Desert Ephemeral, *Eriogonum inflatum*. Morphology, Stomatal Conductance and Water-Use Efficiency in Field Populations. *Oecologia* 72:533–541

Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW (1999) Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* 401:914. doi: 10.1038/44842

Tinoco-Ojanguren C (2008) Diurnal and seasonal patterns of gas exchange and carbon gain contribution of leaves and stems of *Justicia californica* in the Sonoran Desert. *Journal of Arid Environments* 72:127–140. doi: 10.1016/j.jaridenv.2007.06.004

Vogt E, Schönherr J, Schmidt HW (1983) Water permeability of periderm membranes isolated enzymatically from potato tubers (*Solanum tuberosum* L.). *Planta* 158:294–301

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153:376–387. doi: 10.1007/BF00384257

# Chapter three: Photosynthetic and hydraulic traits of green *vs*. fleshy photosynthetic stems in species from the southern Baja California peninsula

## Abstract

Photosynthetic stems represent a source extra carbon in species from dry and hot vegetation types, including subtropical deserts and tropical woodlands, but little is known about the relationships between stem photosynthetic and hydraulic traits compared to the well-known relationships found in leaves. I studied physiological and functional traits in plants with two photosynthetic stem syndromes: 1) retamoid, having green photosynthetic stems, and 2) sarcocaulescent, having fleshy photosynthetic stems, in southern Baja California Sur, Mexico in both wet and dry seasons. My goals were to determine if they behave differently in terms of photosynthetic and hydraulic traits, if there are different drought responses in these traits, and if stem photosynthetic and hydraulic traits are coordinated. I measured soil water status, stem water transport efficiency, leaf-to-sapwood area ratio, leaf and stem chlorophyll *a* fluorescence, leaf and stem bark carbon and nitrogen isotopic composition, as well as wood density, specific leaf area, specific stem area, and specific bark area. I found that species with fleshy stems had higher hydraulic conductivity, lower wood density, and lower stem photosynthetic capacity than species with green stems. Plants with high stem hydraulic conductivity also had high specific leaf area and enriched leaf carbon isotopic composition in the dry season, suggesting greater long-term integrated water-use efficiency. There was no relationship between stem photosynthesis and hydraulics in either season. Overall, both

stem types use resources differently, but both are still able to respond positively to water shortage and survive the nine-month long dry season in this subtropical arid ecosystem.

## 3.1 Introduction

Photosynthetic stems are common in dry lands worldwide, including subtropical deserts and tropical woodlands. Species with photosynthetic stems belong to at least 26 plant families (Gibson 1996), indicating that the syndrome originated multiple times in unrelated families during evolution of plant lineages. The ecophysiology of plants with photosynthetic stems from subtropical deserts has been widely studied (Nilsen 1995; Nilsen and Sharifi 1997; Ávila et al. 2014b), however, little is known about the ecophysiology of these plants in more tropical areas (Tinoco-Ojanguren 2008; Ávila et al. 2014a). The main goal was to study two syndromes of photosynthetic stems in a sarcocaulescent desert scrub in subtropical Baja California and compare the syndromes in terms of their photosynthetic and hydraulic traits to identify those traits that relate to their different morphologies.

Plant physiological responses to environmental conditions depends on 1) the availability of resources such as carbon, water and nutrients, and 2) the allocation patterns of the plant when using those resources (Parolari et al. 2014). From the differences in form between green and fleshy stems, I expect differences in their hydraulic capacity (Zimmermann 1983). Furthermore, stem photosynthetic capacity could be constrained by hydraulic capacity, similar to mechanisms recently shown in desert species with photosynthetic stems (Ávila-Lovera et al. 2017b). The link between hydraulics and photosynthesis has been found not only in photosynthetic stems, but also in leaves, where parameters of leaf photosynthesis have been found to be positively related to leaf-specific stem hydraulic conductivity, including relative quantum yield of PSII ($\Phi_{PSII}$) and electron transport rate (ETR) (Brodribb and Feild 2000; Brodribb et al. 2002), and maximum leaf photosynthetic rate ($A_{max}$) (Santiago et al. 2004; Choat et al. 2011). This is expected because leaf gas exchange is constrained by its water supply from the stem (Sperry 2000), and leaf stomatal conductance and photosynthesis have been found to vary linearly with whole-plant hydraulic conductance (Hubbard et al. 2001). In addition, it is known that water stress affects molecular processes in leaves, such as negatively affecting photosynthesis by decreasing ATP-synthase and production of ATP (Tezara et al. 1999).

The sarcocaulescent desert scrub of southern Baja California Peninsula lies between arid Sonoran Desert scrub to the north and tropical dry forests of the Cape Region to the south, and shares some floristic attributes of both of these floristic regions (León de la Luz et al. 1996). The sarcocaulescent scrub is home to plant species with three syndromes of photosynthetic stems: retamoid, sarcocaulescent, and cactoid. Retamoid plants have green woody stems capable of performing stem net photosynthesis because of stomata on the stem epidermis that allows for atmospheric $CO_2$ assimilation (Ávila et al. 2014b; Ávila-Lovera and Ezcurra 2016). Sarcocaulescent plants, or pachycauls, have enlarged fleshy stems with low density wood that serves as water storage, usually exfoliating bark, and green parenchyma (chlorenchyma) capable of doing stem recycling photosynthesis and in some occasions stem net photosynthesis (Ávila et al. 2014b; Ávila-Lovera and Ezcurra 2016). Cactoid plants refer to species with green succulent stems that perform Crassulacean Acid Metabolism or $C_4$ photosynthesis, and are found in the New World Cactaceae and Old World Euphorbiaceae. In this chapter, I focused on retamoid and sarcocaulescent photosynthetic stem syndromes, hereafter green and fleshy stems, respectively.

Functional traits have been used as another approach to study plant physiological responses to environmental conditions. Functional traits are easy-to-measure plant traits that give proxy information on the physiology of the organ or individual and its performance, which in turn determine the ecological role of a species in the community (Westoby et al. 2002; McGill et al. 2006; Violle et al. 2007). Wood density is one of the most common functional traits and is related to the mechanical strength of the wood (Zanne et al. 2010). Wood density is also related to hydraulic safety and trades off with hydraulic efficiency in some species (Chave et al. 2009), and we expect hydraulic safety or efficiency to be maximized if species are to persist in their corresponding habitats (Gleason et al. 2016). Specific leaf area is one of the traits of the leaf economics spectrum, and it is positively related to $A_{\mathrm{max}}$ (Reich et al. 1997; Wright et al. 2004). Specific stem area is a relatively new term that is analogous to specific leaf area and refers to the ratio between photosynthetic surface area and dry mass in photosynthetic stems (Ávila-Lovera et al. 2017b). As with leaves, specific stem area is positively related with stem $A_{\mathrm{max}}$, in desert species (Ávila-Lovera et al. 2017b), where species with high specific stem area have more area per unit of mass that can capture light and perform photosynthesis at higher rates. The similar relationship found in leaves and stems indicate that both photosynthetic organs are constrained by the same evolutionary and biophysical forces (Berveiller et al. 2007).

Given the importance of photosynthetic stem physiology for species in arid environments that heavily rely on their stems for carbon assimilation, I evaluated the following questions: 1) how do green and fleshy stems differ in their photosynthetic and hydraulic traits in a tropical arid ecosystem? 2) Does seasonality drive distinct trait responses between green and fleshy-stemmed species? 3) Do green and fleshy stems coordinate photosynthesis and hydraulic strategies similar to leaves? I hypothesized that green and fleshy stems would differ in photosynthetic and hydraulic capacity given their different morphologies that are expected to influence their physiology. I expected green stem species to have high photosynthetic capacity, and fleshy stems to have high hydraulic efficiency. I also expected that plants from these two photosynthetic stem syndromes would present seasonal differences in photosynthetic and hydraulic traits given that water deficit in the dry season can decrease hydraulic efficiency and in turn photosynthesis. Finally, I hypothesized that plants with photosynthetic stems would coordinate photosynthetic and hydraulic traits in such a way that water and carbon resources are optimized, especially during seasonal drought. This last hypothesis has been supported in green-stemmed species from desert ecosystems (Ávila-Lovera et al. 2017b) and I sought to test whether this is also true in tropical ecosystems.

## 3.2 Materials and methods

*Study site and plant species*

This study was conducted in a sarcocaulescent desert scrub in Doctor Laura Arriaga Cabrera Biological Station (24°05´ N; 110°21´ W), managed by Centro de Investigaciones Biológicas del Noroeste (CIBNOR) in El Comitán, Baja California Sur, Mexico. The site is characterized by temperature that ranges from 18°C in January to 30°C in August, and a mean annual precipitation of 180 mm (20-460 mm) (León de la Luz et al. 1996). Precipitation falls during September-November with the advent of tropical hurricanes. I studied 12 plant species, six with green stems and six with fleshy stems. Study species were a mix of drought-deciduous and evergreen species (Table 3.1).

**Table 3.1**. List of species studied in a sarcocaulescent scrub in Doctor Laura Arriaga Cabrera Biological Station (CIBNOR) in El Comitán, Baja California Sur, Mexico.

| Species | Acronym | Family | Stem type |
|---|---|---|---|
| *Bursera epinnata* | BUEP | Burseraceae | fleshy |
| *Bursera microphylla* | BUMI | Burseraceae | fleshy |
| *Castela peninsularis* | CAPE | Simaroubaceae | green |
| *Cyrtocarpa edulis* | CYED | Anacardiaceae | fleshy |
| *Euphorbia lomelii* | EULO | Euphorbiaceae | green/fleshy* |
| *Fouquieria diguetii* | FODI | Fouquieriaceae | fleshy |
| *Jatropha cinerea* | JACI | Euphorbiaceae | fleshy |
| *Jatropha cuneata* | JACU | Euphorbiaceae | fleshy |
| *Krameria paucifolia* | KRPA | Krameriaceae | green |
| *Olneya tesota* | OLTE | Fabaceae | green |
| *Parkinsonia praecox* | PAPR | Fabaceae | green |
| *Prosopis articulata* | PRAR | Fabaceae | green |

**Euphorbia lomelii* stem was considered as green for the statistical analyses but it is important to note that it is slightly fleshy.

Sampling was performed during the dry season (March-May) and wet season of 2016 (November). Since soil water availability was extremely low during the 2016 wet season, an extra sampling campaign was performed in the wet season of 2017 (September) (Fig. 3.1). Data shown for the wet season is pooled data from the two wet season campaigns.

*Soil water availability*

Topsoil (5-10 cm) water potential was measured in the morning 0800 – 1000 h near three individuals per species in both wet and dry seasons. The soil samples were collected in plastic containers with lid and wrapped in parafilm to be transported to the lab. In the lab, soil matric potential was measured using a water potential meter in normal mode (WP4C, Decagon Devices, Inc., Pullman, WA, USA).

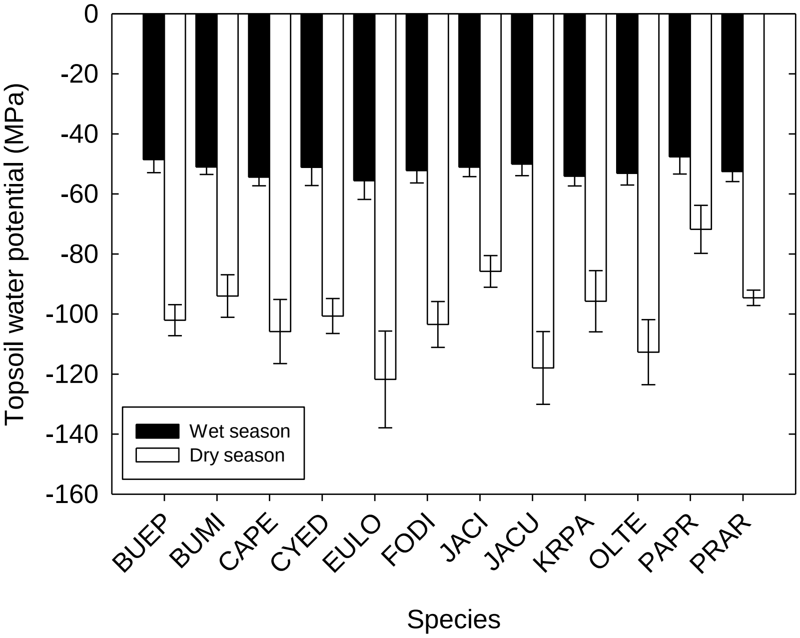

**Figure 3.1**. Topsoil water potential in fleshy- and green-stemmed species in wet (black) and dry (white) seasons. Data are means ± SE.

### *Chlorophyll* a *fluorescence traits*

Photosynthetic capacity was estimated by measuring chlorophyll $a$ fluorescence in light-adapted samples. I used a chlorophyll fluorometer (OS30p+, Opti-sciences, Hudson, NH, USA) to measure effective quantum yield of PSII ($\Phi_{PSII}$) of leaves and stems of six individuals per species in the morning between 0800 - 1100 h. Leaves and stems were light-adapted to the prevailing photosynthetic photon flux density (PPFD). Intercepted PPFD was measured using a digital light meter (Model 401025, Extech Instruments, Nashua, NH, USA). Using $\Phi_{PSII}$ and intercepted PPFD, I calculated electron transport rate (ETR) using the following equation (Krall and Edwards 1992)

$$\mathrm{ETR} = \Phi_{PSII} \times \mathrm{PPFD} \times 0.5 \times a \qquad \text{Eqn. 3.1}$$

where $a$ is the fraction of incident light estimated to be absorbed by the sample, which was assumed to be similar to the one for leaves of 0.84.

### *Native stem hydraulic conductivity*

Branch samples longer than 1 m were collected from six individuals per species in the field. The cut ends were covered with parafilm and placed inside double-dark plastic bags and transported to the lab. In the lab, samples were recut under 20 mM KCl partially degassed solution to a length of 14 cm. Stem hydraulic conductivity was measured with the pipette method (Santiago 2010). Stems were connected to tubing filled with 20 mM KCl solution, where solution flowed from an elevated source through the stem and into a 1 mL glass pipette. Stem hydraulic conductivity ($K_h$, kg m s$^{-1}$ MPa$^{-1}$) was then calculated as

$$K_h = F/(\Delta P/L) \qquad \text{Eqn. 3.2}$$

where $F$ is the flow rate (kg $s^{-1}$) measured as solution volume moved through the stem per unit of time, ΔP is the driving force (MPa), and $L$ is the length of the stem (m).

Stem hydraulic conductivity was normalized by sapwood area (SA) to estimate sapwood-specific hydraulic conductivity ($K_S$, kg $m^{-1}$ $s^{-1}$ $MPa^{-1}$) as follows

$$K_S = K_h / SA \qquad \text{Eqn. 3.3}$$

Stem hydraulic conductivity was also normalized by distal leaf area (LA) to estimate leaf-specific hydraulic conductivity ($K_L$, kg $m^{-1}$ $s^{-1}$ $MPa^{-1}$) as follows

$$K_L = K_h / LA \qquad \text{Eqn. 3.4}$$

Hydraulic architecture was estimated by calculating the LA distal to the stem section used for $K_h$ measurement and its SA, and calculating the LA to SA ratio, which is a measure of the balance between transpiration (LA) and stem water supply to those leaves (SA) (Martínez-Vilalta 2011).

*Functional traits: wood density, specific leaf area and specific stem area*

Wood density was measured at the end of the dry season by removing the bark and pith from 1 cm long stem subsamples. Since wood segments had a regular cylinder shape, fresh volume was determined by measuring the diameter of the wood segments using a vernier caliper. Fresh volume was then calculated as the volume of a cylinder ($\pi r^2 L$, where r is diameter/2, and L is 1 cm), and the wood segment was dried for 48 h at 100°C to obtain dry mass. Woody density (g $cm^{-3}$) was calculated as the ratio of wood dry mass (g) to fresh volume ($cm^3$).

Specific leaf area (SLA, $cm^2$ $g^{-1}$) was measured in a subsample of leaves from the LA determination above, by measuring leaf area using an area meter (Li-3100, Li-Cor

Biosciences, Lincoln, NE, USA) and drying leaves in an oven for 48 h at 65°C to obtain dry mass. SLA was measured as the ratio of leaf area ($cm^2$) to leaf dry mass (g).

Specific stem area (SSA, $cm^2$ $g^{-1}$) was measured following the protocol in Ávila-Lovera et al. (2017). Stems were sectioned and projected area was measured using an area meter (Li-3100C, Li-Cor Biosciences). Stem surface area was determined by multiplying the projected area by π. Samples were then dried in an oven for 48 h at 65°C to obtain whole-stem dry mass. SSA was measured as the ratio of stem surface area ($cm^2$) to whole-stem dry mass (g).

Specific bark area (SBA, $cm^2$ $g^{-1}$) was measured following the protocol in (Ávila-Lovera et al. 2017b). Stems were sectioned and projected area was measured using an area meter (Li-3100 C, Li-Cor Biosciences). Stem surface area was determined by multiplying the projected area by π. Photosynthetic bark was removed from these stem sections and weighed separately from wood. Bark samples were then dried in an oven for 48 h at 65°C to obtain bark dry mass. SBA was measured as the ratio of stem surface area ($cm^2$) to bark dry mass (g).

*Carbon and nitrogen stable isotopes*

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic composition, as well as C and N concentration (%), were measured in dry leaf and stem bark samples in three to four individuals per species in both wet and dry seasons. Stable isotopes were measured using a Delta-*V* Advantage isotope ratio mass spectrometer operating in continuous-flow mode and attached to a Costech elemental analyzer at the Facility for Isotope Ratio Mass Spectrometry (FIRMS) of the University of California, Riverside (UCR). Only the stem photosynthetic tissue was used because the wood of green stems is usually enriched in $^{13}$C compared to the bark (Nilsen and Sharifi 1997). The $\delta^{13}$C values were expressed relative to the Vienna-Pee Dee Belemnite standard, while $\delta^{15}$N values are expressed relative to atmospheric $N_2$.

*Statistical analyses*

Two- and three-ways analyses of variance (ANOVA) were used to evaluate the effect of stem syndrome, season and organ on physiological and functional traits. Pair-wise Pearson correlation analyses were also performed to test for coordination among physiological and functional traits using all of the data, as well as subsets for each season. The statistical analyses were performed using R v. 3.4.0 (R Core Team 2018).

## 3.3 Results

*How do green and fleshy stems differ in their photosynthetic and hydraulic traits?*

There was no difference in topsoil water potential adjacent to green or fleshy-stemmed species ($F_{1,106} = 0.07$; $p = 0.79$; Fig. 3.1). Green-stemmed species had higher values of $\Phi_{PSII}$ than fleshy-stemmed species across seasons ($F_{1,353} = 7.9$; $p = 5.1 \times 10^{-3}$) (Fig. 3.2b). Similarly, ETR was higher in green-stemmed species than in fleshy-stemmed ones ($F_{1,353} = 36.3$; $p = 4.3 \times 10^{-9}$) (Fig. 3.2c).

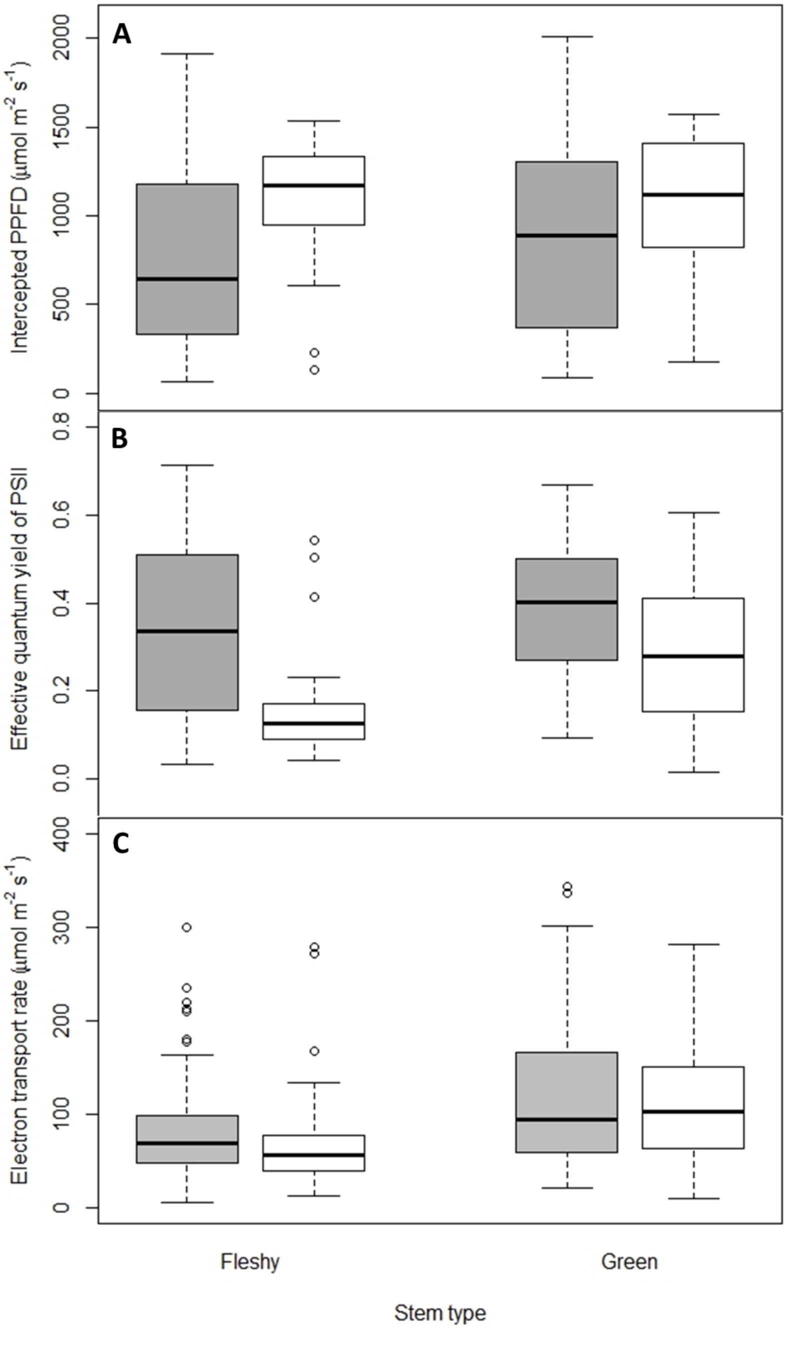

**Figure 3.2**. A) Intercepted photosynthetic photon flux density (PPFD), B) effective quantum yield of PSII, and C) electron transport rate in fleshy- and green-stemmed species in wet (grey) and dry (white) seasons.

Stem water transport efficiency traits, namely $K_h$ ($F_{1,130} = 12.0$; $p = 7.1 \times 10^{-4}$), $K_S$ ($F_{1,130} = 4.7$; $p = 0.03$) and $K_L$ ($F_{1,92} = 4.0$; $p = 0.05$), were higher in fleshy stems than in green stems (Fig. 3.3a-c). However, LA to SA ratio was higher in green stems than in fleshy ones ($F_{1,92} = 7.0$; $p = 9.6 \times 10^{-3}$) (Fig. 3.3d), indicating that green stems can support higher leaf area per unit of sapwood area.

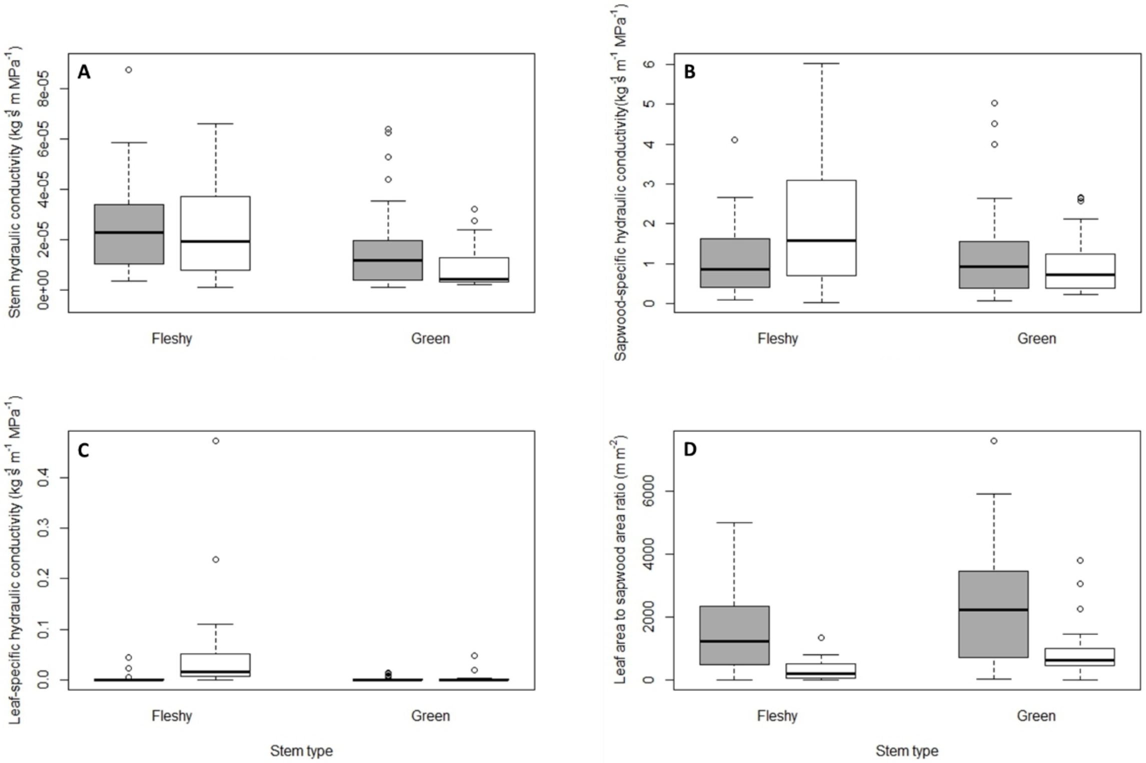

**Figure 3.3**. A) Stem hydraulic conductivity, B) Sapwood-specific hydraulic conductivity, C) leaf-specific hydraulic conductivity, and D) leaf area to sapwood area ratio in fleshy- and green-stemmed species in wet (grey) and dry (white) seasons.

Fleshy-stemmed species had the highest values of SLA (Fig. 3.4a) and the lowest values of wood density (Fig. 3.4c). Despite obvious differences in the morphology of green and fleshy stems, there was no difference in SSA between the two stem syndromes ($F_{1,100}$ = 0.3; $p = 0.56$) (Fig. 3.4b).

N concentration was higher in both leaves and stem bark of green-stemmed species than in fleshy-stemmed species ($F_{1,125}$ = 34.2; $p = 4.2 \times 10^{-8}$), and N concentration was higher in leaves than in stem bark overall ($F_{1,125}$ = 18.8; $p = 3.0 \times 10^{-5}$). Fleshy-stemmed species had higher leaf and stem bark $\delta^{15}$N values than green-stemmed species ($F_{1,125}$ = 12.4; $p = 5.9 \times 10^{-4}$), and $\delta^{15}$N was higher in leaves than in stem bark ($F_{1,125}$ = 9.7; $p = 2.3 \times 10^{-3}$). C concentration was higher in both leaves and stem bark of green-stemmed species than in fleshy-stemmed species ($F_{1,125}$ = 5.0; $p = 0.027$), and it was higher in leaves than in stem bark ($F_{1,125}$ = 4.6; $p = 0.034$). Values of stem bark $\delta^{13}$C were higher in green-stemmed species than in fleshy-stemmed species ($p = 0.02$).

*Does seasonality drive distinct trait responses between green- and fleshy-stemmed species?*

Topsoil water potential was significantly lower in the dry than in the wet season ($F_{1,106}$ = 317.6; $p = 2.2 \times 10^{-6}$) demonstrating the strong seasonality of the study site (Fig. 3.1). Intercepted PPFD by leaves and stems in both stem syndrome groups was higher in the dry season than in the wet season ($F_{1,353}$ = 27.4; $p = 2.8 \times 10^{-7}$) (Fig. 3.2a), due to the presence of clouds during the wet season.

Effective quantum yield of PSII was higher in the wet season than in the dry season ($F_{1,353}$ = 41.4; $p = 4.0 \times 10^{-10}$). Values of ETR did not differ between seasons ($F_{1,353}$ = 0.15; $p = 0.70$) (Fig. 3.2c). Values of $K_h$ and $K_S$ did not differ between seasons, but $K_L$ increased during the dry season ($F_{1,92} = 7.5$; $p = 7.4 \times 10^{-3}$). Values of LA to SA ratio were overall higher in the wet season than in the dry season ($F_{1,92} = 18.1$; $p = 5.1 \times 10^{-5}$). Values of SLA did not change with season, but SSA values were higher in the dry season than in the wet season ($F_{1,100} = 78.6$; $p = 3.3 \times 10^{-14}$) (Fig. 3.4a-b).

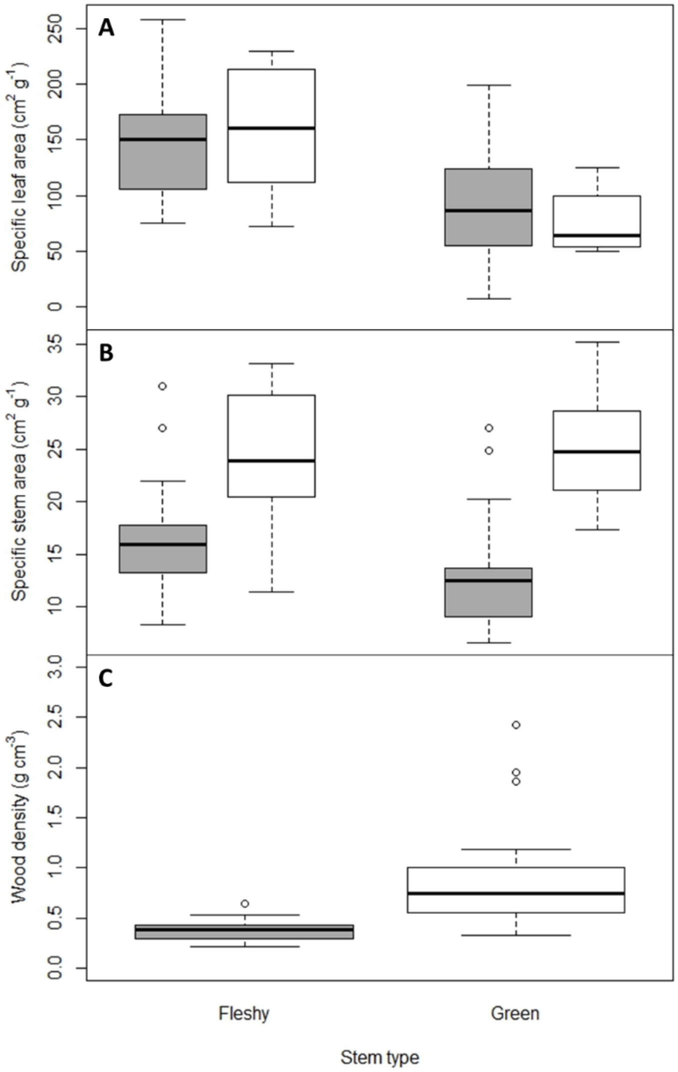

**Figure 3.4**. A) Specific leaf area and B) specific stem area in fleshy- and green-stemmed species in wet (grey) and dry (white) seasons; and C) wood density, in fleshy- (grey) and green-stemmed species (white) in the dry season.

*Do green and fleshy stems coordinate photosynthesis and hydraulic strategies in the same way it occurs in leaves?*

There was no relationship between fluorescence and hydraulic traits in either season (Fig. 3.5a). Sapwood-specific hydraulic conductivity was positively related to leaf $\delta^{13}C$ (r = 0.64; $p = 5.2 \times 10^{-3}$); species with high $K_S$ also have high leaf $\delta^{13}C$ (Fig. 3.5b). There was coordination between leaf and stem ETR in the wet season (r = 0.73; $p = 0.02$); species with high leaf ETR also had high stem ETR (Fig. 3.6).

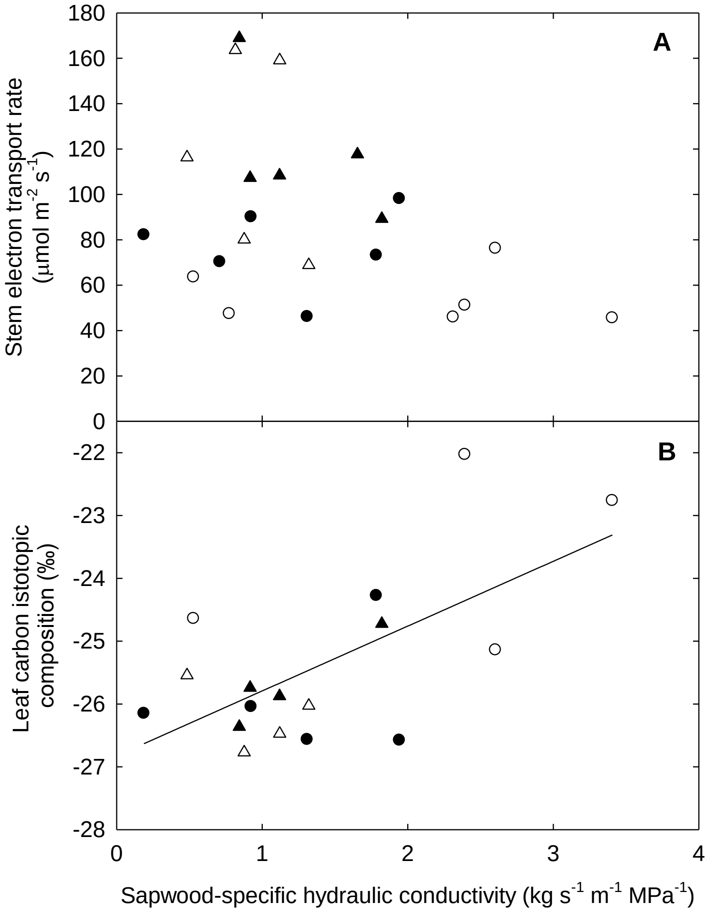

**Figure 3.5**. A) Stem electron transport rate and B) leaf carbon isotopic composition *versus* sapwood-specific hydraulic conductivity in fleshy- (circle) and green-stemmed species (triangle) in wet (black) and dry (white) seasons.

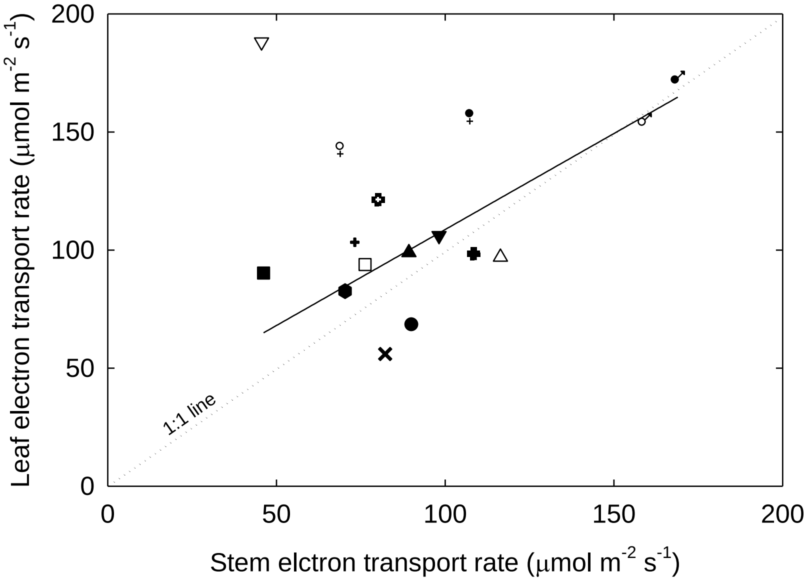

**Figure 3.6**. Leaf *versus* stem electron transport rate in fleshy- (circle) and green-stemmed species (triangle) in wet (black) and dry (white) seasons.

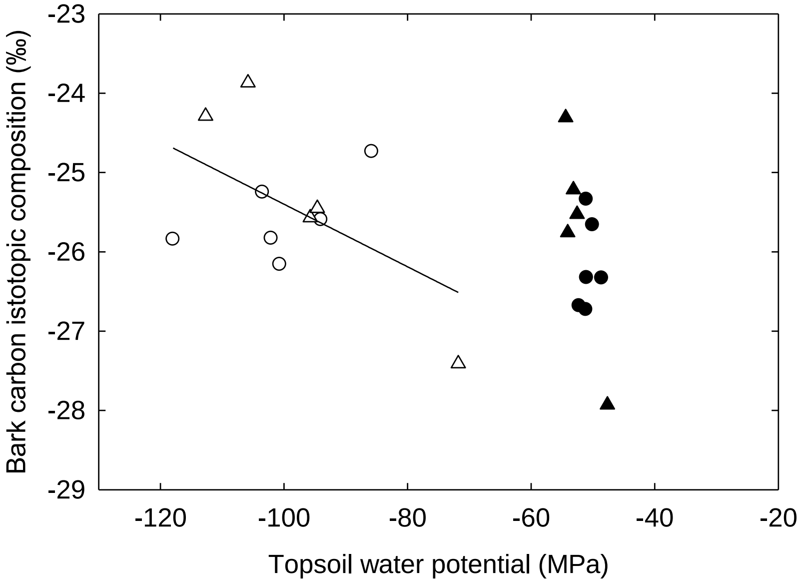

**Figure 3.7**. Stem bark carbon isotopic composition *versus* topsoil water potential in fleshy- (circle) and green-stemmed species (triangle) in wet (black) and dry (white) seasons.

## 3.4 Discussion

I studied physiological and functional traits in green and fleshy stems from a tropical arid ecosystem. The results show how the two groups of species with different photosynthetic stem syndromes use resources differently, with green-stemmed species having high leaf and stem photosynthetic capacity, whereas fleshy stems have higher hydraulic efficiency and low wood density associated with water storage. The lack of difference in topsoil water potential adjacent to green- or fleshy-stemmed species indicates that both groups of plants can occupy similar soils and that their different responses to water deficit are due to their physiological strategies rather than the soil water availability that they experience during the dry season (Parolari et al. 2014). There was a strong response to seasonality in photosynthetic traits but a weak response in hydraulic traits. There was no coordination between photosynthetic and hydraulic traits in the stems of the study species, but there was coordination between other leaf and stem traits that give information on the efficiency of resource use by the two groups of species.

Topsoil water potential was negatively correlated with stem bark $\delta^{13}C$ only in the dry season ($r = -0.59$; $p = 0.04$), indicating that those species experiencing the strongest soil water deficit were photosynthesizing at higher long-term integrated water-use efficiency (Fig. 3.7).

Green-stemmed species had higher values of $\Phi_{PSII}$ and ETR compared to fleshy-stemmed species. It has been shown recently that stem $A_{max}$ values can be up to 60% those found in leaves (Ávila et al. 2014b). This is mainly due to lower values in N and chlorophyll concentration in stems than leaves (Osmond et al. 1987; Nilsen and Bao

1990; Tinoco-Ojanguren 2008; Ávila et al. 2014a). Most species in the green-stemmed group are evergreen, so both leaves and green stems contribute to the total annual carbon balance. Fleshy-stemmed species had higher values of $K_h$, $K_S$ and $K_L$, indicating that they have larger diameter xylem vessels than green-stemmed species (Tyree and Ewers 1991). Also, green stems had high values of wood density, which implies a high cell wall to cell volume ratio (Carlquist 1977), which has a negative effect on hydraulic efficiency because of low lumen volume through which water can potentially flow.

On one hand, the low wood density of fleshy stems suggests high water storage capacity, which is typical in species with this syndrome (Borchert 1994; Borchert and Pockman 2005). On the other hand, low wood density could suggest high risk of cavitation (Li et al. 2018), especially during the dry season. However, the maintenance of high hydraulic conductivity in fleshy stems during the dry season indicates either low cavitation risk, or cavitation-refilling cycles. High hydraulic efficiency in fleshy stems is potentially beneficial for mobilizing stored water (Santiago et al. 2018), and stored water in the xylem may have been used to maintain xylem water potentials from reaching cavitation thresholds (Meinzer et al. 2008). Wood density has been found to be negatively related to capacitance across evergreen and deciduous species from tropical forests (Meinzer et al. 2003; Scholz et al. 2007), evergreen species from chaparral shrublands (Pratt and Jacobsen 2017), Amazonian canopy tree species (Santiago et al. 2018) and species with different climate-of-origin (Li et al. 2018). Furthermore, stem-succulent plants from desert ecosystems are known to use their stem stored water to buffer diurnal fluctuations in leaf turgor (Nilsen et al. 1990). Fleshy-stemmed species are usually pioneers in regenerating tropical secondary forests (Worbes et al. 2013), which implies that their efficient use of water is a key factor determining their successful establishment and survival in environments where conditions can be unfavorable.

There was downregulation in $\Phi_{PSII}$ during the dry season with no differences in ETR because intercepted PPFD increased during the dry season. Stem hydraulic efficiency traits such as $K_h$ and $K_S$ did not change between seasons, but $K_L$ increased for some species because of reduction in dry season leaf area, allowing for more water to reach remaining leaves. This was also translated into decreases of LA to SA ratio in the dry season, when xylem water supply was shared among fewer leaves than in the wet season.

The absence of coordination between photosynthetic and hydraulic traits in this study shows that the relationships found in other ecosystems are not general (Baraloto et al. 2010; Ávila-Lovera et al. 2017b). The site is particularly interesting because it has elements of the arid Sonoran Desert scrub from the north and tropical dry forests from the south, which could partially explain the different behavior of plants from these two floristic regions compared to those that are strictly found in desert ecosystems. Something else to consider is that I did not measure gas exchange; I measured chlorophyll *a* fluorescence traits as proxies for photosynthetic capacity. In leaves, parameters of photochemical activity are related to $CO_2$ assimilation (von Caemmerer 2000), but do not characterize stomatal activity and water loss. Further study is needed to determine relationships between photochemistry activity, carbon assimilation and transpiration in photosynthetic stems.

However, there was coordination among some leaf and stem traits: leaf ETR - stem ETR, and leaf $\delta^{13}C$ - stem $K_S$. The positive relationship between leaf and stem ETR, at least during the wet season when most drought-deciduous species have leaves, indicates that nutrient and water resources are allocated between leaves and stems roughly equally. During the wet season when resources are more available, the best way to optimize the use of these resources is by allocating them to all possible photosynthetic tissues (Yan et al. 2016). This way, more area is equipped to assimilate or re-assimilate $CO_2$. The positive relationship between $K_S$ and leaf $\delta^{13}C$ across species and seasons indicates that the ability of the stem to supply water to leaves is related to the ability of leaf stomata to regulate water loss, given that differences in $\delta^{13}C$ across species are driven mainly by stomatal control during photosynthesis rather than differences in source $\delta^{13}C$. Usually, stems that have high hydraulic efficiency are more vulnerable to cavitation. In this sense, these species should close stomata sooner in the dry season to avoid cavitation, leading to enriched leaf $\delta^{13}C$.

The results indicate that there is functional diversity within the photosynthetic stem syndrome with green-stemmed species having high values in traits related to photosynthesis and low hydraulic capacity, whereas fleshy-stemmed species have high hydraulic capacity and low values in traits related to photosynthesis. This finding has important implications for plants with photosynthetic stems because it suggests that contrasting photosynthetic stem syndromes do not necessarily behave similarly, indicating that they may need to be considered as different functional groups. I expect that during extreme drought, fleshy stems would be subjected to higher cavitation risk and would experience the highest decrease in $\Phi_{PSII}$. The high wood density found in green stems will likely confer higher xylem mechanical and hydraulic safety, with lower expected decrease in $\Phi_{PSII}$.

## 3.6 References

Ávila E, De Almeida J, Tezara W (2014a) Comparación ecofisiológica y anatómica de los tejidos fotosintéticos de *Cercidium praecox* (Ruiz & Pav. ex Hook.) Harms (Fabaceae, Caesalpinioideae). *Acta Botánica Venezuelica* 37:59–76

Ávila E, Herrera A, Tezara W (2014b) Contribution of stem $CO_2$ fixation to whole-plant carbon balance in nonsucculent species. *Photosynthetica* 52:3–15. doi: 10.1007/s11099-014-0004-2

Ávila-Lovera E, Ezcurra E (2016) Stem-succulent trees from the Old and New World tropics. In: Goldstein G, Santiago LS (eds) Tropical tree physiology. Springer International Publishing, Switzerland, pp 45–65

Ávila-Lovera E, Zerpa AJ, Santiago LS (2017) Stem photosynthesis and hydraulics are coordinated in desert plant species. *New Phytologist* 216:1119–1129. doi: 10.1111/nph.14737

Baraloto C, Timothy Paine CE, Poorter L, et al (2010) Decoupled leaf and stem economics in rain forest trees. *Ecology Letters* 13:1338–1347. doi: 10.1111/j.1461-0248.2010.01517.x

Berveiller D, Kierzkowski D, Damesin C (2007) Interspecific variability of stem photosynthesis among tree species. *Tree Physiology* 27:53–61

Bobich EG, North GB (2008) Structural implications of succulence: Architecture, anatomy, and mechanics of photosynthetic stem succulents, pachycauls, and leaf succulents. In: De la Barrera E, Smith W (eds) Perspectives in Biophysical Plant Ecophysiology, A Tribute to Park S. Nobel. Mildred E. Mathias Botanical Garden, University of California, Los Angeles, Los Angeles, pp 3–37

Borchert R (1994) Soil and Stem Water Storage Determine Phenology and Distribution of Tropical Dry Forest Trees. *Ecology* 75:1437. doi: 10.2307/1937467

Borchert R, Pockman WT (2005) Water storage capacitance and xylem tension in isolated branches of temperate and tropical trees. *Tree Physiology* 25:457–466

Brodribb TJ, Feild TS (2000) Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant, Cell & Environment* 23:1381–1388

Brodribb TJ, Holbrook NM, Gutierrez MV (2002) Hydraulic and photosynthetic co-ordination in seasonally dry tropical forest trees. *Plant, Cell & Environment* 25:1435–1444

Carlquist S (1977) Ecological Factors in Wood Evolution: A Floristic Approach. *American Journal of Botany* 64:887. doi: 10.2307/2442382

Chave J, Coomes D, Jansen S, et al (2009) Towards a worldwide wood economics spectrum. *Ecology Letters* 12:351–366

Choat B, Medek DE, Stuart SA, et al (2011) Xylem traits mediate a trade-off between resistance to freeze-thaw-induced embolism and photosynthetic capacity in overwintering evergreens. *New Phytologist* 191:996–1005. doi: 10.1111/j.1469-8137.2011.03772.x

De Guzman ME, Santiago LS, Schnitzer SA, Álvarez-Cansino L (2017) Trade-offs between water transport capacity and drought resistance in neotropical canopy liana and tree species. *Tree Physiology* 37:1404–1414. doi: 10.1093/treephys/tpw086

Fortunel C, Fine PVA, Baraloto C, Dalling J (2012) Leaf, stem and root tissue strategies across 758 Neotropical tree species. *Functional Ecology* 26:1153–1161. doi: 10.1111/j.1365-2435.2012.02020.x

Funk JL, Larson JE, Ames GM, et al (2017) Revisiting the Holy Grail: using plant functional traits to understand ecological processes. *Biological Reviews* 92:1156–1173. doi: 10.1111/brv.12275

Gibson AC (1996) Structure-function relations of warm desert plants. Springer Berlin Heidelberg, Berlin, Heidelberg

Gleason SM, Westoby M, Jansen S, et al (2016) Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world’s woody plant species. *New Phytologist* 209:123–136. doi: 10.1111/nph.13646

Hubbard RM, Ryan MG, Stiller V, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell & Environment* 24:113–121. doi: 10.1046/j.1365-3040.2001.00660.x

Ishida A, Nakano T, Yazaki K, et al (2008) Coordination between leaf and stem traits related to leaf carbon gain and hydraulics across 32 drought-tolerant angiosperms. *Oecologia* 156:193. doi: 10.1007/s00442-008-0965-6

Krall JP, Edwards GE (1992) Relationship between photosystem II activity and $CO_2$ fixation in leaves. *Physiologia Plantarum* 86:180–187. doi: 10.1111/j.1399-3054.1992.tb01328.x

León de la Luz JL, Benet RC, Estrada MC (1996) Fenología floral de una comunidad árido-tropical de Baja California Sur, México. *Acta Botánica Mexicana* 45–64

Li X, Blackman CJ, Choat B, et al (2018) Tree hydraulic traits are co-ordinated and strongly linked to climate-of-origin across a rainfall gradient. Plant Cell Environ n/a-n/a. doi: 10.1111/pce.13129

Maherali H, Pockman WT, Jackson RB (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85:2184–2199. doi: 10.1890/02-0538

Markesteijn L, Poorter L, Paz H, et al (2011) Ecological differentiation in xylem cavitation resistance is associated with stem and leaf structural traits. *Plant, Cell & Environment* 34:137–148

Martínez-Vilalta J (2011) PrometheusWiki | Water balance traits - leaf to sapwood area ratio. http://prometheuswiki.org/tiki-index.php?page=Water+balance+traits+-+leaf+to+sapwood+area+ratio. Accessed 22 Nov 2017

McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution* 21:178–185. doi: 10.1016/j.tree.2006.02.002

Meinzer FC, James SA, Goldstein G, Woodruff D (2003) Whole-tree water transport scales with sapwood capacitance in tropical forest canopy trees. *Plant, Cell & Environment* 26:1147–1155

Meinzer FC, Woodruff DR, Domec J-C, et al (2008) Coordination of leaf and stem water transport properties in tropical forest trees. *Oecologia* 156:31–41. doi: 10.1007/s00442-008-0974-5

Méndez-Alonzo R, Paz H, Zuluaga RC, et al (2012) Coordinated evolution of leaf and stem economics in tropical dry forest trees. *Ecology* 93:2397–2406. doi: 10.1890/11-1213.1

Nilsen E, Sharifi M (1997) Carbon isotopic composition of legumes with photosynthetic stems from mediterranean and desert habitats. *American Journal of Botany* 84:1707–1713

Nilsen ET (1995) Stem photosynthesis: extent, patterns, and role in plant carbon economy. In: Gartner BL (ed) Plant stems: physiology and functional morphology. Academic Press, San Diego, pp 223–240

Nilsen ET, Bao Y (1990) The Influence of Water Stress on Stem and Leaf Photosynthesis in *Glycine max* and *Sparteum junceum* (Leguminosae). *American Journal of Botany* 77:1007–1015. doi: 10.2307/2444572

Nilsen ET, Sharifi MR, Rundel PW, et al (1990) Water relations of stem succulent trees in north-central Baja California. *Oecologia* 82:299–303. doi: 10.1007/BF00317474

Nolf M, Creek D, Duursma R, et al (2015) Stem and leaf hydraulic properties are finely coordinated in three tropical rain forest tree species. *Plant, Cell & Environment* 38:2652–2661. doi: 10.1111/pce.12581

Osmond CB, Smith SD, Gui-Ying B, Sharkey TD (1987) Stem photosynthesis in a desert ephemeral, *Eriogonum inflatum*. Characterization of leaf and stem $CO_2$ fixation and $H_2O$ vapor exchange under controlled conditions. *Oecologia* 72:542–549

Pineda-García F, Paz H, Meinzer FC, Angeles G (2015) Exploiting water versus tolerating drought: water-use strategies of trees in a secondary successional tropical dry forest. *Tree Physiology* tpv124. doi: 10.1093/treephys/tpv124

Pineda-García F, Paz H, Tinoco-Ojanguren C (2011) Morphological and physiological differentiation of seedlings between dry and wet habitats in a tropical dry forest. *Plant, Cell & Environment* 34:1536–1547. doi: 10.1111/j.1365-3040.2011.02351.x

Pivovaroff AL, Sack L, Santiago LS (2014) Coordination of stem and leaf hydraulic conductance in southern California shrubs: a test of the hydraulic segmentation hypothesis. *New Phytologist* 203:842–850. doi: 10.1111/nph.12850

Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *American Journal of Botany* 87:1287–1299

Pratt RB, Jacobsen AL (2017) Conflicting demands on angiosperm xylem: Tradeoffs among storage, transport and biomechanics. *Plant, Cell & Environment* 40:897–913. doi: 10.1111/pce.12862

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America* 94:13730–13734

Santiago LS (2010) PrometheusWiki | Hydraulic conductivity - Pipette method. http://prometheuswiki.org/tiki-index.php?page=Hydraulic+Conductivity+-+Pipette+Method. Accessed 22 Nov 2017

Santiago LS, De Guzman ME, Baraloto C, et al (2018) Coordination and trade-offs among hydraulic safety, efficiency and drought avoidance traits in Amazonian rainforest canopy tree species. New Phytologist. doi: 10.1111/nph.15058

Santiago LS, Goldstein G, Meinzer FC, et al (2004) Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* 140:543–550. doi: 10.1007/s00442-004-1624-1

Scholz A, Klepsch M, Karimi Z, Jansen S (2013) How to quantify conduits in wood? *Frontiers in Plant Science* 4:. doi: 10.3389/fpls.2013.00056

Scholz FG, Bucci SJ, Goldstein G, et al (2007) Biophysical properties and functional significance of stem water storage tissues in Neotropical savanna trees. *Plant, Cell & Environment* 30:236–248. doi: 10.1111/j.1365-3040.2006.01623.x

Silva JLA, Souza AF, Caliman A, et al (2017) Weak whole-plant trait coordination in a seasonally dry South American stressful environment. *Ecology and Evolution*. doi: 10.1002/ece3.3547

Sperry JS (2000) Hydraulic constraints on plant gas exchange. *Agricultural and Forest Meteorology* 104:13–23. doi: 10.1016/S0168-1923(00)00144-1

Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW (1999) Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* 401:914. doi: 10.1038/44842

Tinoco-Ojanguren C (2008) Diurnal and seasonal patterns of gas exchange and carbon gain contribution of leaves and stems of *Justicia californica* in the Sonoran Desert. *Journal of Arid Environments* 72:127–140. doi: 10.1016/j.jaridenv.2007.06.004

Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. *New Phytologist* 119:345–360. doi: 10.1111/j.1469-8137.1991.tb00035.x

Violle C, Navas M-L, Vile D, et al (2007) Let the concept of trait be functional! *Oikos* 116:882–892. doi: 10.1111/j.0030-1299.2007.15559.x

von Caemmerer S (2000) Biochemical models of leaf photosynthesis. CSIRO Publishing, Collingwood, Australia

Westoby M, Falster DS, Moles AT, et al (2002) Plant Ecological Strategies: Some Leading Dimensions of Variation Between Species. *Annual Review of Ecology and Systematics* 33:125–159. doi: 10.1146/annurev.ecolsys.33.010802.150452

Wright IJ, Reich PB, Westoby M, et al (2004) The worldwide leaf economics spectrum. *Nature* 428:821–827

Yan Z, Li P, Chen Y, et al (2016) Nutrient allocation strategies of woody plants: an approach from the scaling of nitrogen and phosphorus between twig stems and leaves. *Scientific Reports* 6:. doi: 10.1038/srep20099

Zanne AE, Westoby M, Falster DS, et al (2010) Angiosperm wood structure: Global patterns in vessel anatomy and their relation to wood density and potential conductivity. *American Journal of Botany* 97:207–215

## Concluding remarks

My work shows important aspects of photosynthetic stems: 1) Photosynthetic stems behave like leaves in the coordination of multiple traits related to carbon gain and water movement and loss; 2) plants with green stems rely on their stem as the sole organ for carbon assimilation for most of the study period; 3) there is functional diversity within the photosynthetic stem syndrome with green-stemmed species having high values in traits related to photosynthesis and low hydraulic capacity, while fleshy-stemmed species have high hydraulic capacity and low values in traits related to photosynthesis.

Such similarity between photosynthetic stems are leaves is potentially advantageous because we may be able to extend our knowledge of leaf physiology and the leaf economic spectrum to better understand photosynthetic stem physiology. Furthermore, understanding seasonal changes in photosynthetic capacity and hydraulics can help in predicting green-stemmed plant responses to global-change-type droughts. I found partial support for the high water-use efficiency hypothesis, and that green stems might be losing more water through their cuticle than non-green stems. This raises questions of possible trade-offs between carbon gain and water loss through the cuticle in woody green stems and how this may affect plant responses to current and future droughts. However, most plants bearing green photosynthetic stems inhabit desert washes, and may have continuous access to water sources.