Coordination of stem and leaf hydraulic conductance in southern California shrubs: a test of the hydraulic segmentation hypothesis

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Summary

- Coordination of water movement among plant organs is important for understanding plant water use strategies. The hydraulic segmentation hypothesis (HSH) proposes that hydraulic conductance in shorter lived, ‘expendable’ organs such as leaves and longer lived, more ‘expensive’ organs such as stems may be decoupled, with resistance in leaves acting as a bottleneck or ‘safety valve’.
- We tested the HSH in woody species from a Mediterranean-type ecosystem by measuring leaf hydraulic conductance (K_leaf) and stem hydraulic conductivity (K_s). We also investigated whether leaves function as safety valves by relating K_leaf and the hydraulic safety margin (stem water potential minus the water potential at which 50% of conductivity is lost (Ψ_stem − Ψ_50)). We also examined related plant traits including the operating range of water potentials, wood density, leaf mass per area, and leaf area to sapwood area ratio to provide insight into whole-plant water use strategies.
- For hydrated shoots, K_leaf was negatively correlated with K_s, supporting the HSH. Additionally, K_leaf was positively correlated with the hydraulic safety margin and negatively correlated with the leaf area to sapwood area ratio.
- Consistent with the HSH, our data indicate that leaves may act as control valves for species with high K_s, or a low safety margin. This critical role of leaves appears to contribute importantly to plant ecological specialization in a drought-prone environment.

Introduction

Hydraulic architecture refers to the arrangement of hydraulic resistances in the xylem of plants, and is a critical determinant of plant function (Zimmermann, 1978; McCulloh & Sperry, 2005). Hydraulic traits regulate the use and loss of water and influence other physiological processes, including gas exchange and growth (Zimmermann, 1978; Sperry, 2000; Santiago et al., 2004a; McCulloh & Sperry, 2005; Brodribb et al., 2010). The key parameter in describing hydraulic relationships is hydraulic conductance, a measure of the efficiency of water movement through a material such as plant stems or leaves (Sperry et al., 1988; Choa & PrometheusWiki contributors, 2011). The definition of hydraulic conductance is based on an analogy with Ohm’s law:

\[ R = \frac{\Delta V}{I} \]  
Eqn 1

where R is resistance, ΔV is the difference in voltage (potential difference), and I is current (flow of electric charge). For plant water transport, we take plant hydraulic conductance as the inverse of hydraulic resistance, pressure analogous to voltage, and flow rate analogous to current. Hence we can model plant hydraulic conductance as the flow of water for a given pressure or water potential difference:

\[ k_h = \frac{F}{\Delta P} \]  
Eqn 2

where \( k_h \) is hydraulic conductance, \( F \) is flow rate, and \( \Delta P \) is pressure difference.

Water moves through plants within the soil–plant–atmosphere continuum (SPAC) as a continuous column of liquid from areas of less negative water potential to more negative water potential, as described by the cohesion-tension theory (Dixon & Joly, 1895). However, studies have most often focused on hydraulic conductance in single plant organs, that is, within only leaves, stems, or roots. This typical focus on individual plant organs has led to a lack of understanding of how hydraulic conductance is coordinated between organs such as stems and leaves. One might assume that, because stems supply leaves with water, stem and leaf hydraulic conductances would increase in tandem. Conversely, discrete organs could vary in their hydraulic conductance.
For example, if distal organs such as leaves had lower hydraulic conductance than large, main stems, this decoupling would maintain the water status of longer lived and more ‘expensive’ organs that represent a significant carbon investment at the expense of shorter lived and ‘cheaper’ organs that can be more easily replaced (Zimmermann, 1983). This ‘hydraulic segmentation’ hypothesis (HSH), as first proposed by Zimmermann, is in fact one explanation for the distribution of relative hydraulic resistance in woody plants (Tyree & Zimmermann, 2002), because greater hydraulic resistance in distal organs may function as a ‘bottleneck’ to water flow through the whole plant (Zimmermann, 1978; Sack & Holbrook, 2006). Early research on some diffuse-porous trees indicated that leaves and small, terminal branches represented a ‘distinct hydraulic constriction’ in woody plants, with lower hydraulic conductance than main stems (Zimmermann, 1978). This segmentation principle was later separated into two distinct mechanisms: hydraulic segmentation and vulnerability segmentation (Tyree & Ewers, 1991; Tyree & Zimmermann, 2002). Vulnerability segmentation refers to distal organs being more vulnerable to cavitation than basal portions, and several studies have shown that leaves tend to be more vulnerable to hydraulic decline than stems (Tyree et al., 1993; Tsuda & Tyree, 1997; Choo et al., 2005; Hao et al., 2007; Chen et al., 2009; Johnson et al., 2011; Bucci et al., 2012). The putative purpose of segmentation, whether hydraulic or vulnerability, relates to the concept that ‘leaves function as a safety valve’ (Chen et al., 2009, 2010), and previous studies have demonstrated support for this ‘safety valve’ hypothesis (Chen et al., 2009, 2010; Johnson et al., 2009, 2011, 2012; McCulloh et al., 2014), although these data generally come from relatively humid environments. The aim of this study was to test the relationship between water movement in stems and leaves in the context of the HSH in a water-limited and highly seasonal environment.

Understanding coordination of water movement between the stem and leaf may be of particular importance, especially in ecosystems with seasonal water availability. In Mediterranean-type ecosystems, plants face the dual challenge of providing water to support rapid growth during the brief wet winter and conducting water across drastically different water potential gradients during the extended dry summer (Cowling et al., 2005). We first examined whether the relative resistance to water transport varied between stems and leaves, and investigated the ecological implications. We hypothesized that leaf hydraulic conductance would be negatively correlated with stem hydraulic conductivity, in support of the HSH. In addition, we investigated whether leaves function in a manner similar to a ‘safety valve’, hypothesizing that species with a lower hydraulic safety margin would also have a lower leaf hydraulic conductance (Meinzer et al., 2009; Delzon & Cochard, 2014). Finally, we quantified additional functional traits, including leaf mass per area (LMA), leaf area to sapwood area ratio (LA : SA), and wood density, along with operating stem and leaf water potentials, to evaluate the relationships among these traits, to characterize overall plant water use strategies in the context of hydraulic segmentation, and to explore new implications for the cross-species coordination of hydraulic conductance between stems and leaves.

Materials and Methods

Study site and species

The study was conducted from 2011 to 2013 at the Santa Margarita Ecological Reserve (SMER; 33°29′N, 117°09′W), located at the Riverside-San Diego county line, California, USA. The reserve size is 1790 ha, with elevation ranging from 150 to 700 m. SMER has a Mediterranean-type climate and receives 36 cm of mean annual precipitation, mostly during the winter months (November–March), resulting in an annual seasonal drought between April and October. SMER has a mean annual temperature of 16.4°C (Vourlitis & Pasquini, 2009) and last burned >35 yr ago. This study focused on the 17 most commonly occurring woody species at SMER (Table 1), with reported maximum vessel lengths varying from 0.29 m in *Adenostoma fasciculatum* to 1.96 m in *Quercus agrifolia* (Jacobsen et al., 2012). All samples were collected from the field during morning hours (08:00–12:00 h).

Water potentials

Predawn and midday water potentials (Ψ; MPa) were measured to establish seasonal variation in the water potential gradient driving flow. Measurements were taken on bagged and unbagged branches during the 2012 wet season (February; 15.1 cm of precipitation from November 2011 to February 2012; National Oceanic and Atmospheric Administration (NOAA) Fallbrook 5 and 6.5 NE CA US stations) and dry season (September; 0.5 cm of precipitation from June to September 2012; NOAA Fallbrook 5 and 6.5 NE CA US stations) with a pressure chamber (Model 1000; Plant Moisture Stress Instruments, Albany, OR, USA) to determine ranges of plant water status. The day before measurements, two branches on each of five individuals per species were

<table>
<thead>
<tr>
<th>Species Family Symbol Native or NN</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Adenostoma fasciculatum</em> Rosaceae ○ Native</td>
</tr>
<tr>
<td><em>Ceanothus tomentosus</em> Rhamnaceae ○ Native</td>
</tr>
<tr>
<td><em>Heteromeles arbutifolia</em> Rosaceae ○ Native</td>
</tr>
<tr>
<td><em>Keckiella antirrhinoides</em> Plantaginaceae ● Native</td>
</tr>
<tr>
<td><em>Malosma laurina</em> Anacardiaceae ● Native</td>
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<tr>
<td><em>Mimusulus aurantiacus</em> Phrymaceae ● Native</td>
</tr>
<tr>
<td><em>Nicotiana glauca</em> Solanaceae ● Native</td>
</tr>
<tr>
<td><em>Olea europaea</em> Oleaceae ● Native</td>
</tr>
<tr>
<td><em>Quercus agrifolia</em> Fagaceae ● Native</td>
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<tr>
<td><em>Quercus berberidifolia</em> Fagaceae ● Native</td>
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<tr>
<td><em>Rhamnus ilicifolia</em> Rhamnaceae ● Native</td>
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<tr>
<td><em>Rhus ovata</em> Anacardiaceae ● Native</td>
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<tr>
<td><em>Sambucus nigra</em> Adoxaceae ● Native</td>
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<tr>
<td><em>Salvia melifera</em> Lamiaceae ● Native</td>
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<tr>
<td><em>Schinus molle</em> Anacardiaceae ● Native</td>
</tr>
<tr>
<td><em>Senna artemisioides</em> Fabaceae ● Native</td>
</tr>
<tr>
<td><em>Xylecoccus bicolor</em> Ericaceae ● Native</td>
</tr>
</tbody>
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covered with plastic bags and aluminum foil to stop those selected samples from transpiring and allow leaf and stem water potentials to equilibrate. Immediately following collection, branches were placed in plastic bags before being sealed and placed in a dark cooler. For predawn \( \Psi \), one transpiring and one nontranspiring sample pair were measured from each individual. This was repeated for midday \( \Psi \) measurements. The nontranspiring \( \Psi \) measurements were taken to represent stem water potential (\( \Psi_{stem} \)) and the transpiring \( \Psi \) measurements were taken to represent leaf water potential (\( \Psi_{leaf} \)). We did not measure dry season water potentials for species that did not have leaves at the time of sampling, that is, *Koeckliella antirrhinoides*, *Mimulus aurantiacus*, *Sambucus nigra*, and *Salvia mellifera*. For each species within each season, we calculated the diurnal range in \( \Psi_{stem} \) and \( \Psi_{leaf} \) as the difference between midday and predawn \( \Psi_{stem} \) and that for \( \Psi_{leaf} \) (\( \Delta \Psi_{leaf} \)) as the difference between midday and predawn \( \Psi_{leaf} \). We also calculated \( \Delta \Psi_{stem-leaf} \) as the difference between midday \( \Psi_{stem} \) and \( \Psi_{leaf} \). Finally, we calculated seasonal differences in both \( \Psi_{stem} \) and \( \Psi_{leaf} \).

### Leaf hydraulic conductance

Leaf hydraulic conductance (\( K_{leaf} \)) was measured during the spring (March–June) on newly mature leaves from at least four individuals of each species, using the evaporative flux method (Sack *et al.*, 2002; Sack & Scoffoni, 2013). In the laboratory, leaves from the same branches that were used for stem hydraulic conductivity (see below; ‘Stem hydraulic conductivity and xylem vulnerability’) were cut from the stem under water with a fresh razor blade. Leaves were immediately placed in beakers with filtered (0.2 \( \mu \)m), degassed water that covered the petiole, but did not touch the lamina. Samples were covered with plastic and allowed to hydrate overnight for 8 h.

Following rehydration, leaves were connected to tubing containing filtered (0.2 \( \mu \)m), degassed water that ran from a reservoir on a balance (\( \pm 0.1 \text{ mg}; \) Denver Instrument P-214; Sartorius, Bohemia, NY, USA) to the leaf. Leaves were held in place with a wood frame strung with fishing line, and placed over a fan to reduce the boundary layer around the leaf. Light sources arranged above the sample, who light was diffused through a plexiglass container filled with water that acted as a heat trap, produced a photon flux density of \( > 1200 \text{ \mu mol m}^{-2} \text{s}^{-1} \) at the leaf level to induce stomatal opening.

The transpiration-driven flow of water into the leaf was measured from the balance interfaced to a computer, logging data every 60 s. Transpirational flow increased for 30 min at the beginning of the measurements before steady-state transpiration was achieved (coefficient of variance <5%) for 10 min. Leaves were then placed in a Whirlpak bag (Nasco, Fort Atkinson, WI, USA) and rapidly removed from the tubing to measure leaf water potential with a pressure chamber (Model 1000; Plant Moisture Stress Instruments). Finally, leaf area was measured with a leaf area meter (Li-3100; Li-Cor Biosciences, Lincoln, NE, USA). The leaf hydraulic conductance (\( K_{leaf}; \text{ mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1} \)) was calculated as:

\[
K_{leaf} = \frac{E}{\Delta \Psi_{leaf}} \quad \text{Eqn 3}
\]

normalized by leaf area (\( m^2 \)), where \( E \) is the steady-state transpiration flow rate (mmol s\(^{-1}\)) and \( \Delta \Psi_{leaf} \) is the water potential driving force (\( -\Psi_{leaf}; \text{ MPa} \)).

### Stem hydraulic conductivity and xylem vulnerability

Large branch samples \( c. 1 \text{ m} \) in length were collected from at least four individuals of each species in the field. Cut ends were covered with parafilm and samples were placed in opaque plastic bags with wet paper towels until transported to the laboratory. Once in the laboratory, stem samples were cut under water to a length of \( c. 16 \text{ cm} \). Emboli were removed from stems by vacuum infiltration under filtered (0.2 \( \mu \)m) water for 8 h. Stem ends were then re-cut under water and ends cleanly shaved with a razor blade for a final sample length of 14.2 cm.

Maximum stem hydraulic conductivity (\( K_{max} \)) was determined by connecting stems to tubing filled with filtered (0.2 \( \mu \)m), de-gassed water flowing from an elevated source, through the stem, and into a reservoir on a balance (\( \pm 0.1 \text{ mg}; \) Denver Instrument P-214; Sartorius) that was interfaced with a computer to record the flow rate, allowing the calculation of conductivity (Sperry *et al.*, 1988). Following Torres-Ruiz *et al.* (2012), we corrected for stem passive water uptake by beginning and ending each conductivity measurement with a ‘background’ measurement. Stem hydraulic conductivity (\( K_s; \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1} \)) was calculated as:

\[
K_s = \frac{F \times L}{\Delta P} \quad \text{Eqn 4}
\]

where \( F \) is the flow rate (kg s\(^{-1}\)), \( L \) is the stem length (m), and \( \Delta P \) is the driving force (MPa). Stem hydraulic conductivity was also normalized by sapwood area (\( A_{sw}; \text{ m}^2 \)) to determine stem sapwood-specific hydraulic conductivity (\( K_s; \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1} \)), and distal leaf area (\( A_l; \text{ m}^2 \)) to determine leaf specific hydraulic conductivity (\( K_l; \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1} \)).

Stem xylem vulnerability to cavitation, as determined by vulnerability curves, was measured using the ‘static’ centrifugation method (Alder *et al.*, 1997; Jacobsen & Pratt, 2012; Sperry *et al.*, 2012) to determine the water potential at which 50% of conductivity is lost (\( \Psi_{50} \)) and calculate the stem hydraulic safety margin (Meinzer *et al.*, 2009; Delzon & Cochard, 2014). The static centrifugation method differs from the cavitron, or flow centrifugation, method in that hydraulic conductivity is measured between spinning, whereas conductivity is measured during spinning with the cavitron (Cochard *et al.*, 2005).

Following the determination of \( K_{max} \) stems were spun using a custom-built, 14.2-cm-diameter rotor in a refrigerated centrifuge (Sorvall RC-5Cplus; Thermo Scientific, Waltham, MA, USA) at progressively more negative stem water potentials to cause embolisms. Foam cosmetic sponges soaked with water were added to the vertical end of the plexiglass reservoirs to keep the ends of the stems wet, even when the rotor was not spinning (Jacobsen & Pratt, 2012; Tobin *et al.*, 2012). \( \Delta P \) was always kept below 2 kPa to prevent possible flushing of
embolisms induced by centrifugation in wide and/or open vessels. Hydraulic conductivity was repeatedly measured on each stem after spinning at increasing speeds, and \( K_b \) declined with more negative water potentials and increasing levels of xylem cavitation. This was repeated until the stem lost > 90% conductivity. Per cent loss of conductivity (PLC) was calculated at each water potential step as:

\[
\text{PLC} = 100 \times \left( 1 - \left( \frac{K_b}{K_{\text{max}}} \right) \right)
\]

Vulnerability curves were constructed by plotting water potential versus PLC and fitting a Weibull model (Pammenter & Willigen, 1998):

\[
\text{PLC} = \frac{100}{\left( 1 + \exp \left( a(\Psi - b) \right) \right)}
\]

from which stem \( \Psi_{50} \) was determined for each species. Stem hydraulic safety margin as calculated as midday \( \Psi_{\text{stem}} - \Psi_{50} \) (Meinzer et al., 2009; Delzon & Cochard, 2014).

Functional traits: leaf area to sapwood area ratio, leaf mass per area, and wood density

Branch-level measurements of LA : SA were made during the spring (March–June) to determine the relationship between total transpiring leaf area and the area of sapwood supplying water to those leaves (Shinozaki et al., 1964; Martínez-Vilalta & PrometheusWiki contributors, 2011). The cross-sectional sapwood area of excised branch samples was determined by removing bark and measuring the diameter of the sapwood with digital calipers to calculate the cross-sectional area. If pith was present, its cross-sectional area was subtracted from the total sapwood area. All distal leaves were removed from the stem segment, and their total area was measured using a leaf area meter (Li-3100; Li-Cor Biosciences).

LMA was determined for individual leaves by measuring leaf area using a leaf area meter (Li-3100), drying leaves for 48 h at 65°C before measuring dry mass, and dividing dry mass (g) by fresh leaf area (m²).

Wood density was measured by removing the bark and pith from stem subsamples and determining the fresh volume with the water displacement method (Osazuwa-Peters et al.; Williamson & Wiemann, 2010), then drying the xylem for 48 h at 65°C before measuring dry mass. Woody density (g cm⁻³) was calculated as the ratio of xylem dry mass (g) to fresh volume (cm³).

Statistics

We used Pearson’s product-moment correlation coefficients to evaluate interspecific bivariate relationships among physiological variables using SAS ver. 9.3 (SAS Institute, Cary, NC, USA). Before testing correlations, when trait values were not normally distributed, they were \( \log_{10} \) transformed, which resulted in normal distributions. Negative values were converted to positive values to enable \( \log_{10} \) transformation when necessary (e.g. \( \Psi \)). The overall difference between \( \Delta \Psi_{\text{stem}} \) and \( \Delta \Psi_{\text{leaf}} \) was analyzed using a paired t-test. Rhamnus ilicifolia \( \Psi_{50} \) was removed from hydraulic safety margin analyses because its calculated \( \Psi_{50} \) was beyond the range of collected data. We also evaluated the impact of phylogenetic relationships for our analyzed trade-offs using phylogenetically independent contrasts (PICs) with the R `ape` package (ver. 3.0-11; Paradis et al., 2004). PHYLOMATIC (ver. 3) was used to build the tree and PHYLOCOM (ver. 4.2) to add branch lengths (Webb et al., 2008).

Results

Measurement of \( \Psi_{\text{leaf}} \) and \( \Psi_{\text{stem}} \) indicated that the study species experienced a wide range of tissue water status on an annual basis (Fig. 1). Midday \( \Psi_{\text{leaf}} \) (Fig. 1c) ranged from −1.19 MPa in Schinus molle during the wet season to −9.39 MPa in Olea europaea during the dry season. Midday \( \Psi_{\text{stem}} \) (Fig. 1d) ranged from a maximum of −0.95 MPa in Schinus molle during the wet season to a minimum of −8.66 MPa in Xyllococcus bicolor during the dry season. Seasonal differences (Fig. 1) of midday \( \Psi \) between the wet and dry seasons also varied, with Malosma laurina exhibiting the smallest difference at 0.05 MPa for leaves and 0.15 MPa for stems and X. bicolor exhibiting the largest difference at 6.64 MPa for leaves and 7.08 MPa for stems. The diurnal range in \( \Psi_{\text{leaf}} \) (\( \Delta \Psi_{\text{leaf}} \)) was always greater in magnitude than that of the stem (\( \Delta \Psi_{\text{stem}} \)) for both the wet (\( P < 0.0001 \)) and dry seasons (\( P = 0.0025 \); Fig. 2). \( \Delta \Psi_{\text{stem-leaf}} \) was not correlated with any measured traits.

Values for \( K_{\text{leaf}} \) were negatively correlated with \( K_s \) (\( r = -0.54; P = 0.02; \text{PIC} \ r = -0.51; P = 0.04; \) Fig. 3), with species having greater \( K_s \) exhibiting lower \( K_{\text{leaf}} \), supporting the HSH. The operating hydraulic safety margin (February midday \( \Psi_{\text{stem}} - \Psi_{50} \)) and \( K_{\text{leaf}} \) were positively correlated (Fig. 4), with species having stems that operate within a smaller safety margin also having lower \( K_{\text{leaf}} \), consistent with leaves acting as a safety valve. We also found a negative relationship between \( K_{\text{leaf}} \) and LA : SA (\( r = -0.56; P = 0.02; \text{PIC} \ r = -0.66; P = 0.004 ; \) Fig. 5a) and a positive relationship between \( K_s \) and LA : SA (\( r = 0.62; P = 0.008; \) Fig. 5b). Additional significant relationships included \( K_s \) and stem \( \Psi_{50} \) (−MPa) which were negatively related (\( r = -0.48; P = 0.06; \text{PIC} \ r = -0.61; P = 0.009 \)) and \( K_{\text{leaf}} \) and stem \( \Psi_{50} \) (−MPa) which were positively related (\( r = 0.52; P = 0.04; \text{PIC} \ r = 0.55; P = 0.02 \)). \( K_s \) was not correlated with any other measured traits.

There was a positive correlation between LMA and wood density (\( r = 0.66; P = 0.004; \text{PIC} \ r = 0.66; P = 0.003; \) Fig. 6). LMA was negatively correlated with wet season predawn \( \Psi_{\text{stem}} \) and \( \Psi_{\text{leaf}} \) (\( r = -0.65; P = 0.005, \) and \( r = -0.58; P = 0.01, \) respectively) and midday \( \Psi_{\text{stem}} \) (\( r = -0.56; P = 0.02; \) Table 2), but not with any dry season \( \Psi \) measurements. Wood density was negatively correlated with wet season predawn and midday \( \Psi_{\text{stem}} \) (\( r = -0.70; P = 0.002, \) and \( r = -0.61; P = 0.009, \) respectively) and \( \Psi_{\text{leaf}} \) (\( r = -0.71; P = 0.001, \) and \( r = -0.58; P = 0.01, \) respectively), and dry season midday \( \Psi_{\text{stem}} \) (\( r = -0.60; P = 0.03; \) Table 2), but not with dry season predawn \( \Psi_{\text{stem}} \) or \( \Psi_{\text{leaf}} \), or midday \( \Psi_{\text{leaf}} \).
Discussion

We found a negative relationship between $K_S$ and $K_{leaf}$ (Fig. 3), indicating that, for woody shrubs in the California Mediterranean-type ecosystem, species with higher stem conductivity rely on low leaf hydraulic conductance to place limits on water loss during maximum gas exchange and thus act to maintain the water status of basal organs at the expense of distal organs.
For a given sapwood area exhibited lower maximum $K_{\text{leaf}}$ on an individual leaf area basis, while conversely species with lower LA : SA had higher $K_{\text{leaf}}$ as there was less leaf area on a shoot competing for water from the transpirational stream. This finding is consistent with our sampling across species within a community that are subject to approximately the same precipitation and water availability. The negative relationship points to a constraint on total leaf area by the capacity of stems to support leaf water loss among these co-existing species. By contrast, a previous study of six _Nothofagus_ species along five sites spanning a precipitation and elevation gradient reported a positive correlation between $K_{\text{leaf}}$ and LA : SA (Bucci et al., 2012). Yet, the _Nothofagus_ system can receive more than four times the precipitation of the current study and is arranged along an altitudinal gradient, suggesting that altitudinal constraints limit both LA : SA and $K_{\text{leaf}}$. We also found a positive relationship between $K_{\text{leaf}}$ and LA : SA (Fig. 5b), as previously reported for different species sets (Choat et al., 2011; Gleason et al., 2012). LA : SA is a major determinant of plant hydraulic architecture (Martinez-Vilalta & PrometheusWiki contributors, 2011), consistent with the pipe model theory (Shinozaki et al., 1964), and previous studies have shown similar cases of balancing transpiring leaf area with stem conductive supply through the plant (Meinzer & Grantz, 1990).

Our results indicate large variation in plant water use strategies among co-occurring species. This pattern indicates that withstanding drought in this semi-arid Mediterranean-type climate can be accomplished by adjustment in multiple traits. For example, _S. mellifera_ and _K. antirrhinoides_ are both deciduous species that drop their leaves during the dry season and physiologically ‘shut down’. While they both ‘avoid’ drought through deciduous leaf phenology, they operate within different safety margins, with _K. antirrhinoides_ having a much larger safety margin than _S. mellifera_ (Fig. 4). Similarly, _L. laurina_ and _X. bicolor_ are both evergreen species that ‘tolerate’ drought by holding onto their leaves and continuing to function during the dry season, but _X. bicolor_ operates in a much larger safety margin than _L. laurina_ (Fig. 4). As such, our data are consistent with coordination of multiple traits, including water acquisition, stomatal control of water loss, and resistance to cavitation, to maintain hydraulic function (Meinzer & Grantz, 1991; Santiago et al., 2004b; Meinzer et al., 2009; Jones et al., 2010). Thus, species with similarity in one trait can have large variation in other traits, allowing for complex combinations of hydraulic strategy variation that may promote species coexistence at this site (Wright, 2002; Sack et al., 2005; Marks & Lechowicz, 2006; Angert et al., 2009). Additionally, new attention has been focused on the hydraulic safety margin of woody plants (Delzon & Cochard, 2014), with differences in species’ susceptibility to mortality found to be related to differences in their hydraulic safety margins (Plaut et al., 2012), although drought-induced tree mortality is a complex process with multiple interacting factors (McDowell et al., 2008; Anderegg et al., 2012). These differences in hydraulic safety margin for co-occurring species at our site may have implications for shifts in community structure under climate change.

As water potential is the driving force behind hydraulic conductance as explained by Ohm’s law, we also examined how organisms. The role of leaves as safety valves was further clarified by the positive relationship between the hydraulic safety margin and $K_{\text{leaf}}$ (Fig. 4), as species with stems that operate in a more narrow safety range may rely on hydraulic resistance in the leaves to act as a bottleneck to water flow, whereas species that operate within a wider safety range have higher $K_{\text{leaf}}$ consistent with less need of protection. Thus, the resistance to water transport at the leaf level has the potential to protect longer lived and more costly woody stems farther down the soil–plant–atmosphere continuum from reaching critical xylem water potentials that could produce catastrophic hydraulic failure, as hypothesized by Zimmermann (1983) in the original formulation of the HSH.

The inverse arrangement of hydraulic conductances of stems and leaves was related to shoot allometry. We found a negative relationship between $K_{\text{leaf}}$ and LA : SA (Fig. 5a), demonstrating a balance between water movement in individual leaves and overall shoot transpirational demand. Plants with overall more leaf area

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**Fig. 3** Relationship between stem sapwood-specific hydraulic conductivity and leaf hydraulic conductance ($n = 17$) in a California Mediterranean-type climate region. Each symbol represents a different species mean. Corresponding species and symbols can be found in Table 1.

**Fig. 4** Relationship between hydraulic safety margin and leaf hydraulic conductance ($n = 16$) in a California Mediterranean-type climate region. Each symbol represents a different species mean. Corresponding species and symbols can be found in Table 1.
water status and water transport capacity were related to LMA and wood density. LMA and wood density are relatively easy-to-measure functional traits that offer a potentially rapid way to characterize physiological tendencies for many species. Yet, how well these traits perform for predicting physiological processes for a particular species varies greatly across sites and species (Wright et al., 2006). Species with high LMA tend to exhibit longer leaf lifespans, relatively low leaf nitrogen concentration, and low maximum photosynthetic rates (Wright et al., 2004), whereas species with high wood density tend to exhibit low maximum hydraulic conductivity (Santiago et al., 2004b) and greater stem xylem resistance to cavitation (Hacke et al., 2001; Delzon et al., 2010). Therefore, our data indicating that LMA and wood density were correlated with each other (Fig. 6) and with various measures of water stress (Table 2) are consistent with previous findings in the literature (Westoby & Wright, 2006; Wright et al., 2006). Species with higher wood density had higher LMA, showing greater investment in structure of stems and leaves, while species with lower wood density had lower LMA, possibly to maximize resource acquisition at the cost of safe structure. Overall, wood density and LMA were more correlated with wet season than dry season measures of water potential (Table 2), suggesting that wood density and LMA are optimized for resource capture during seasonally brief environmentally favorable conditions. Furthermore, species with more negative wet season water potentials also had greater wood density (Table 2), which is consistent.
with previous findings implicating wood density in drought tolerance (Hacke et al., 2001; Delzon et al., 2010; Bucci et al., 2012).

Our result of a negative correlation between water transport in stems and leaves, which supports the HSH, has important implications for plant hydraulic strategies. Suites of traits, including resistance to cavitation, leaf phenology, and rooting depth (Hellmers et al., 1955; Ackerly, 2004), promote tolerance of periods of low water availability, whether on a seasonal basis or during prolonged events. Our findings highlight the role of leaf hydraulics in overall plant drought adaptation. This is especially important in the wake of environmental change, with warmer temperatures leading to global change-type drought and vegetation dieback (Allen, 2009). It should be mentioned that, while we measured maximum stem and leaf hydraulic conductance, which may not represent performance achieved in the field, especially as plant water supply declines, such maximum capacity measurements generally scale with integrated exposure to stress and resource availability (Field, 1991). Studying how stem and leaf hydraulic conductivities vary in vivo diurnally and seasonally would provide additional understanding of coordination of hydraulic and functional traits, as well as plant ecological strategy variation with respect to balancing water use to maximize growth and maintenance of water potential in the face of seasonal water deficit. Future studies may focus on additional plant growth forms and biomes to address the ecosystem dependence of established site-based patterns (Reich, 1993).

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