AVOIDING DESICCATION IN TROPICAL FORESTS: MECHANISMS THAT MAINTAIN TREE WATER STATUS DURING SEASONAL DROUGHTS

by

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ABSTRACT

Droughts pose a problem for plants because as plants dry (i.e., decrease in water potential, Ψ_{plant}), they lose hydraulic conductivity (K_s) due to cavitation within xylem conduits. Critically low levels of Ψ_{plant}, such as the Ψ_{plant} at 80% loss of K_s (P80), are associated with mortality. In seasonally dry tropical forests (SDTF), diverse tree communities endure several months of annual drought. This dissertation examines the mechanisms by which SDTF trees regulate Ψ_{plant} during seasonal droughts and their efficacy in preventing mortality during extreme drought conditions.

Patterns of dry-season leaf shedding vary widely among tree species. Leaf shedding is predicted to be a mechanism to slow water loss and maintain Ψ_{plant} above P80. Supporting this, among six species that I tracked, the Ψ_{plant} at which saplings shed leaves was correlated with P80. Furthermore, a hydraulic model showed that species converge in shedding leaves as they approached the point that hydraulic limits stopped transpiration. These results suggest that a universal mechanism drives leaf shedding among SDTF saplings. However, after shedding leaves, species varied in their maintenance of Ψ_{plant} and K_s, suggesting that the role of leaf shedding varies among species.

Water stored within stems is predicted to buffer stem water potential (Ψ_{stem}) against dry-season water loss. Indeed, among saplings of six species in two SDTFs, most lost stem water during the dry season; however, the amount of water varied among species and forests. In addition, species with low wood density used stored water to produce leaves at the onset of the wet season. Bark tissues showed seasonal fluctuations in relative water content while xylem tissues did not, suggesting an important role for bark in buffering Ψ_{stem}.

In a potted-plant experiment, three tree species with traits that support water storage and retention maintained high Ψ_{stem} and survived in extremely dry soil while three tree species without these traits reached low Ψ_{stem} and had high mortality. These results suggest that species differ in their ability to regulate Ψ_{stem} and that performance during normal dry-season droughts does not reflect mortality during extreme droughts. Combinations of multiple traits in addition to P80 predict extreme-drought performance.

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CHAPTER 1

INTRODUCTION

Background

Plants depend on water in many ways, including the diffusion of solutes, temperature regulation, structural support, and as a reactant in photosynthesis (Nobel 2009). Those processes consume relatively minor quantities of water whereas, for all actively growing land plants, relatively large volumes of water are consumed by evaporation. Specifically, plants must allow water to evaporate freely from their leaves, termed transpiration, in order to efficiently absorb carbon dioxide from the air for photosynthesis.

Most plants absorb this water from soil and transport it to their leaves through xylem conduits. The water within the conduits is under tension (i.e., negative pressure), in part, because it is pulled downward by gravity and it is pulled upward by the pressure gradient produced by transpiration. When soil dries, the remaining soil water is held more tightly within pore spaces, so if the transpiration rate is constant, the pull on the water column is harder, that is, the tension in the xylem conduits increases. Since water under negative pressure is in a metastable state, it is prone to cavitation (i.e., liquid water is converted to water vapor) if xylem tension becomes too high. This breaks the water column, blocking the transport of water through the conduit. As more xylem conduits cavitate, the plant's ability to transport water to downstream stems and leaves decreases, putting them at risk hydraulic failure, or total loss of the ability to transport water, which causes desiccation and dieback. Plants counteract this process by reducing xylem tension through stomatal closure, which decreases the transpiration rate and therefore the upward pull of water. Stomatal control, combined with various morphological and physiological adaptations, allows plants to inhabit regions where there may be several months or more without rainfall during annual dry seasons.

Avoiding excessive cavitation is particularly critical for plants with a permanent stem, such as trees, because hydraulic failure may lead to death (McDowell et al. 2008). Trees that are able to survive prolonged droughts are predicted to follow, to varying degrees, three divergent strategies: drought escape, desiccation tolerance, and desiccation avoidance. Various names have been given to these strategies, but the paradigm has a long history (Levitt 1972). Drought escape involves maintaining access to soil water during droughts. This is accomplished mainly by growing deep roots and is limited to sites with accessible soil water during droughts. Desiccation tolerance involves surviving at low plant water potential (Ψ_{plant}). This requires xylem conduits that resist cavitation. In trees, a commonly measured proxy for desiccation tolerance is the Ψ_{plant} that induces a 50% loss of conductivity (water flow rate per pressure gradient) in the stems (P50). Among tree species, P50 ranges from -0.04 to -14 MPa (Choat et al. 2012). A more direct measurement of desiccation tolerance is the Ψ_{plant} that induces mortality, though this has rarely been measured (Kursar et al. 2009). Even the most desiccation-tolerant tree species can only survive to about Ψ_{plant} of -12 MPa, while non-desiccation-tolerant tree species survive to $\Psi_{plant} > -2$ MPa (Kursar et al. 2009). However, non-desiccation-tolerant trees may survive droughts if they exhibit desiccation avoidance, or the ability to maintain high Ψ_{plant} despite a dry environment. This involves greatly slowing water loss along with buffering against incidental water loss with stored water.

Wood density is a key trait that may correlate with strategies for drought survival. High resistance to cavitation is related to higher wood density (Hacke et al. 2001). Conversely, water storage capacity is negatively related to wood density (Simpson 1993). This indicates a tradeoff that prevents trees from being both resistant to cavitation (or tolerant of desiccation) and able to store water in the stem (or avoiding desiccation).

Seasonally dry tropical forests are intriguing since they contain tree species that exhibit each of these strategies (Borchert 1994). These forests typically receive <1800 mm of rainfall annually and experience annual dry seasons that last 3–6 months with very little or no rainfall (Murphy and Lugo 1986). All seasonally dry tropical forests have a closed canopy; that is, they lack the extensive zones with high light and grasses that typify tropical savannahs. Nevertheless, seasonally dry tropical forests range widely in physiognomy; for example, one structural trait, canopy height, ranges 10–40 m among forests (Murphy and Lugo 1986).

Seasonally dry tropical forests are critical for biodiversity and conservation. Regarding their biodiversity, wet-forest species are excluded from seasonally dry forests because of their poor survival during droughts (Brenes-Arguedas et al. 2009), and tree-species richness is generally lower in seasonally dry forests than in wetter forests, typically 35–90 vs. 50–200 species ha^{-1} (Murphy and Lugo 1986). As a result, seasonally dry and wetter forests of the tropics share few tree species, even when they are in close proximity (Condit et al. 2002).

Seasonally dry forests once accounted for > 42% of all forested land in the tropics, but their extent has been disproportionately reduced compared to wetter forests (Murphy and Lugo 1986). In many regions, remaining dry forests cover < 5% of their original extent, prompting a conservation imperative (Miles et al. 2006). Various factors threaten the remaining patches of seasonally dry tropical forests, such as continued conversion to non-forest uses, anthropogenic fires, and climate change (Miles et al. 2006).

Climate change will likely have major effects on these ecosystems. Aridity in seasonally dry tropical regions is expected to increase this century in association with climate change (Sherwood and Fu 2014). Recent long-term droughts have been linked to directional shifts in tree species composition in seasonally dry tropical forests (Enquist and Enquist 2011, Fauset et al. 2012). Predictions for how tropical forests, and seasonally dry tropical forests in particular, will respond to shifts in rainfall patterns are limited by our knowledge in how tropical trees respond to drought.

Within this context, I asked how trees that inhabit seasonally dry tropical forests survive droughts. More specifically, I asked how do they regulate Ψ_{plant} such that it does not drop to levels that would cause hydraulic failure and mortality. I focused on the role of leaf shedding in slowing water loss, the role of stored water in buffering Ψ_{plant} against water loss, and finally how these two factors influence survival during extreme drought conditions.

Chapter summaries

In Chapter 2, as annual dry seasons progressed and leaves were shed, I determined the Ψ_{plant} at which saplings shed leaves. I found that species that shed leaves at high Ψ_{plant}, usually early in the dry season, tend to have a high P50, or xylem that is sensitive to cavitation. To further study leaf shedding, I repeatedly censused leaf area, Ψ_{plant}, and stomatal conductance on saplings of six species distributed across two seasonally dry forests, combined with measurements of hydraulic conductance and stem vulnerability to cavitation. From these data, I parameterized a hydraulic model and predicted the maximum rate of transpiration that does not cause hydraulic failure. I show that saplings shed leaves as their maximum transpiration rate approaches zero. The convergent patterns of leaf shedding among species support the hypothesis that leaf shedding is a response to Ψ_{plant} that is coordinated with maintaining hydraulic conductance in the stem. However, after shedding leaves, some species maintained constant Ψ_{plant} while in other species, Ψ_{plant} continued to decline, putting stems at risk of hydraulic failure. Differences among species in the response of Ψ_{plant} after shedding leaves suggest that the ultimate physiological function of leaf shedding as the soil dries may vary among species, with some stopping water loss and others maximizing carbon dioxide assimilation.

In Chapter 3, I show that most saplings depend on water stored within their stems to buffer stem water potential (Ψ_{stem}) against incidental water loss during seasonal droughts. I measured the water content of stems of saplings growing in two seasonally dry forests during the wet season, the dry season, and for species that flushed leaves at the onset of the wet season, during leaf flush. I found that the dependence on stored water varies among species and with environmental conditions. Two deciduous species with low wood density appeared to rely on stem-stored water to flush leaves during the onset of the wet season. One of these species did not lose stem water during the dry season.

The other species, in a drier forest, lost a moderate amount of stem-stored water but maintained high Ψ_{stem} through high hydraulic capacitance. Two deciduous species with moderate stem density reached low Ψ_{stem} and lost relatively high amounts of stem water during the dry season. Two evergreen species with moderate stem density, which were both measured in a transitional and a dry forest, reached moderate Ψ_{stem}, but lower in the dry forest than the transitional forest, and lost moderate amounts of stem water during the dry season. In the transitional forest, one of the evergreen species appeared to rely on soil water during the dry season because it had diurnal patterns of stem water content and Ψ_{stem}. These results show that low wood density (and associated high hydraulic capacitance) may be an adaptation that allows leaf flushing early during the onset of the wet season. For other species, although I found that the amount of stored water that saplings use during seasonal droughts is related to their wood density and leaf phenology, they differ in the extent to which stored water actually buffers Ψ_{stem}.

In Chapter 4, I further investigated the physiology of desiccation-avoiding species in a potted-plant experiment in which saplings were either watered or droughted for 3–4 months during a natural dry season and compared to pre-dry-season, reference plants. I show that when saplings of putative desiccation-avoiding species are exposed to extreme drought conditions (Ψ_{soil} < -10 MPa), they survive by decoupling Ψ_{plant} from Ψ_{soil}, maintaining Ψ_{plant} near -1 MPa, having low lateral root surface area per stem basal area during the dry season, storing large amounts of water in their roots and stems, and retaining stored water. In contrast, species that show desiccation tolerance under moderate droughts are susceptible to desiccation under extreme drought. They reach low Ψ_{plant} (< -6 MPa), experience high mortality, grow lateral roots in response to drought, store less water in their tissues, and retain less stored water compared to desiccation-avoiding species. These findings suggest that species vary widely in their ability to regulate Ψ_{plant} during extreme drought. Therefore, predicting species performance under extreme drought will require knowledge of this ability rather than commonly measured traits such as safety margins of Ψ_{plant} during moderate droughts, P50, or even drought performance under moderate droughts. Among saplings of tropical dry forest species, the ability to avoid desiccation during extreme drought was associated with low tissue density, low leaf mass fraction, high stem mass fraction, and low lateral root surface area per stem basal area.

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CHAPTER 2

DOES LEAF SHEDDING PROTECT STEMS FROM CAVITATION DURING SEASONAL DROUGHTS? A TEST OF THE HYDRAULIC FUSE HYPOTHESIS

Abstract

Drought-induced tree dieback and death are associated with plant water potentials (Ψ_{plant}) at which xylem cavitation causes critically low stem hydraulic conductivity (K_s). The hydraulic segmentation hypothesis predicts that leaves act as ‘hydraulic fuses’ during droughts by shedding when stems approach critical levels of cavitation, thus stopping water loss and stabilizing Ψ_{plant}. We asked whether the hydraulic fuse hypothesis explains the wide range in timing and extent of leaf shedding among trees in seasonally dry tropical forests. We tracked leaf phenology, Ψ_{plant}, and K_s in saplings of six tree species distributed across two forests to test whether leaf shedding during seasonal droughts coincides with key points of hydraulic stress, stabilizes Ψ_{plant}, and maintains K_s. Species converged in shedding leaves as they approached the Ψ_{plant} associated with a 50% loss of K_s and at which their modeled maximum steady-state transpiration rate approached zero. However, after shedding all leaves, one species, *Genipa americana*, continued to decline in Ψ_{plant}. K_s was highly variable among saplings within species and seasons, suggesting a

minimal influence of seasonal drought on K_s. Together, these results show that hydraulic limitations underlie leaf-shedding phenology among tropical trees but that leaf shedding is not universally effective in stabilizing Ψ_{plant}.

Introduction

Trees must allow water to evaporate from their leaves (i.e., transpire) in order to maintain the CO_2 uptake that supplies photosynthesis. Water is held under tension in a "tug of war" within the soil-plant-atmosphere continuum (Sperry et al. 2002). Since water moves down gradients of water potential, plant water potential (Ψ_{plant}) must remain below soil water potential (Ψ_{soil}) for water uptake to feed the transpiration stream. But, as Ψ_{plant} decreases, hydraulic conductance within the soil-leaf continuum decreases due to cavitation within soil pore spaces and the xylem conduits. These two processes result in a constraint, such that trees must regulate their transpiration rate (E) below the critical level at which soil-to-leaf conductance reaches zero (E_{crit}), when 'hydraulic failure' occurs. On short timescales, stomata act as valves to control E and Ψ_{plant} (Sperry et al. 2002). But as Ψ_{soil} decreases, E_{crit} decreases, and at some point E_{crit} reaches zero, when any amount of transpiration would cause hydraulic failure. Since closed stomata may leak and since water evaporates from leaf cuticles, additional water-conserving responses enhance survival during droughts (Levitt 1972).

The hydraulic segmentation hypothesis states that, during droughts, the most distal organs of plants (e.g., leaves and fine roots) experience the lowest water potential and therefore, if their vulnerability to cavitation is the same as or greater than more basal organs (e.g., the main stem or bole), then the distal organs will cavitate before the basal organs (Zimmermann 1983, Tyree et al. 1993, Tyree and Zimmermann 2002). Since cavitation lowers hydraulic conductivity, this response would serve to retain water within the basal organs and protect them from cavitation. Moreover, since cavitation renders the distal organs non-functional, the hydraulic segmentation hypothesis also predicts, at some point during drought, these organs may be shed or abscised (Tyree et al. 1993). In theory, this hydraulic architecture is adaptive because basal organs such as the bole are more costly for plants to replace than distal organs such as leaves. Thus, leaves are predicted to act as “hydraulic fuses” that break the soil-plant-atmosphere continuum before damage occurs to more costly stems. While the role of stomatal control has received much attention (Klein 2014), the role of leaf shedding in the context of the hydraulic fuse hypothesis has rarely been tested (Tyree et al. 1993).

Seasonally dry tropical forests contain many tree species that shed some or all of their leaves during annual dry seasons (Axelrod 1966, Frankie et al. 1974, Condit et al. 2000). In these ecosystems, leaf shedding during drought (i.e., drought deciduousness) may represent a critical response that protects stems as predicted by the hydraulic fuse hypothesis. But, despite its potential importance, the hydraulic function of leaf shedding in tropical plants is poorly understood (Brodribb et al. 2002). Also quite intriguing is the fact that the timing, rate, and extent of leaf shedding vary considerably among species (Bullock and Solis-Magallanes 1990, Williams et al. 2008). Such diversity suggests multiple physiological mechanisms for avoiding drought-induced mortality. If the hydraulic fuse hypothesis is correct (i.e., if leaf shedding is a drought response that protects stems from hydraulic failure), then diverse leaf phenologies correspond to divergence among species in hydraulic limits within the soil-plant continuum. For example, the stems of deciduous species may be more vulnerable to cavitation than those of co-occurring evergreen species. But, this simple prediction has received equivocal support. Some studies have found no difference in vulnerability to cavitation between deciduous and evergreen species (Machado and Tyree 1994, Sobrado 1997, Brodribb et al. 2002, Markesteijn et al. 2011), while others have found higher vulnerability in deciduous species than in evergreen species (Brodribb et al. 2003, Choat et al. 2005, Lopez et al. 2005, Fu et al. 2012). This suggests that the role of leaf shedding in maintaining hydraulic systems of tropical trees deserves a closer mechanistic analysis.

In order to address how leaf shedding relates to the regulation of Ψ_{plant} and to the avoidance of stem hydraulic failure, we tracked saplings in two seasonally dry tropical forests for leaf phenology, stomatal conductance, Ψ_{plant}, and stem hydraulic conductivity (K_s). These data were combined with additional measurements of stem vulnerability to cavitation to parameterize a hydraulic model that predicted saplings' E_{crit} given their environmental conditions. We then tested the following predictions of the hydraulic fuse hypothesis: (1) the timing of leaf shedding coincides with key points of hydraulic stress (e.g., E_{crit} near zero), (2) leaf shedding stabilizes Ψ_{plant} during seasonal droughts, and (3) saplings maintain stable K_s during seasonal droughts.

Materials and methods

Study sites and species

This study was conducted in two seasonally dry forests in Panama. One forest, the Parque Natural Metropolitano located in Panama City, is transitional between moist forest and dry forest ('transitional forest'), with annual rainfall of 1800 mm. The other forest, the Eugene Eisenmann Reserve located in Coronado, is a dry forest, with annual rainfall of 1590 mm. Both are mature secondary forests and experience an annual dry season from mid-December to May. During the study, we measured rainfall in the dry forest with an automated rain gauge (Onset Corporation, Bourne, MA, USA). We obtained rainfall data collected within the transitional forest from the Smithsonian Tropical Research Institute Physical Monitoring Program. In each forest, we placed a temperature and relative humidity sensor (models HMP50 and CS500; Campbell Scientific, Logan, UT, USA) in the understory (50 cm height). Air temperature and relative humidity measurements were made every 10 minutes and hourly mean temperature and minimum and maximum relative humidity were recorded on CR200 data loggers (Campbell Scientific). During the full year encompassed by the study (i.e., 2012), rainfall was 1938 mm in the transitional forest and 1259 mm in the dry forest. In general, the dry season in the dry forest is slightly longer, hotter, and higher in vapor pressure deficit than in the transitional forest. The two dry seasons encompassed by the study (2012 and 2013) fit this pattern, except that the 2013 dry season in the transitional forest extended for ~30 days longer than normal (Fig. 2.1, Table 2.S1). During the dry seasons, Ψ_{soil} was lower in the dry forest than in transitional forest (e.g., in 2013, soil at 50 cm depth was, mean ± SD, -4.6 ± 0.7 and -2.1 ± 0.9 MPa in the dry and transitional forest, respectively; Fig. 2.S1). In each forest, we chose four species that were common as saplings and that were reported to vary in deciduousness and wood density as adults (Table 2.1). Two species were shared between the forests, so six species were studied in total. Throughout the text, the study species are referred to by genus name.

Censuses of leaf area, stomatal conductance, and plant water potential

Ten healthy-looking saplings (15–225 cm height, 3.5–57 mm basal diameter) of each species were selected for censuses of leaf area and stomatal conductance. Censuses were conducted every 3–6 weeks from November 2011 to July 2013, more often during the two dry seasons. In the transitional forest, three *Cojoba*, one *Cavanillesia*, and one *Annona* died, all after November 2012; so sample size was reduced for these species towards the end of the project. On each census plant, the position of the leaves along the stems was diagramed and the length of each leaf was measured to the nearest 1 mm with a ruler. For compound-leaved species (Table 2.1), the length of the most distal leaflet was measured and the number of leaflets on each leaf was counted. In each census, the presence or absence of previously measured leaves and the number of leaflets in compound leaves was recorded and any newly produced leaves were measured for length and number of leaflets. If leaves were not fully expanded during a census, then they were re-measured for length in the subsequent census.

For each species, 23–94 leaves were collected from nearby saplings and measured for leaf area with an LI-3100 area meter (LI-COR Biosciences, Lincoln, NE, USA). The relationship between leaf length and area was modeled with simple linear regression on log-transformed values, including leaflet number as a cofactor for compound-leaved species (Fig. 2.S2). The models were used to estimate the area of each census leaf, which was summed for each sapling to obtain its total leaf area in each census.

During each census, saplings with leaves (i.e., not deciduous species that were leafless) were measured for stomatal conductance. On each sapling, three leaves were randomly selected and measured for stomatal conductance with a porometer (LI-1600, LI-COR Biosciences) between 1000 and 1400 h. If saplings had fewer than three leaves during a census, then all leaves were measured. During measurements, the porometer's cuvette was maintained at < 70% relative humidity to reduce measurement error (McDermitt 1990). Stomatal conductance was measured within 60 s of placing the cuvette over the leaf, before it was affected by the difference in relative humidity between the air and the cuvette. The porometer was regularly calibrated with a dew point generator (LI-610, LI-COR Biosciences).

At each census, four saplings of each species were measured for leaf water potential (Ψ_{leaf}) at predawn (0400–0630 h) and midday (1100–1400 h). Saplings used for Ψ_{leaf} measurements were located within ~50 m of the saplings that were censused for leaf area and stomatal conductance. Most saplings were measured for Ψ_{leaf} in only one census; those that were measured more than once were left > 5 months between measurements to avoid possible measurement effects on Ψ_{leaf}. At both predawn and midday, two leaves were collected from each sapling, sealed in humidified plastic bags, and placed in a cooler with ice. After all the leaves were collected, they were measured for Ψ_{leaf} with a Scholander pressure chamber < 2 h after collection. Ψ_{leaf} for each sapling was taken as the average of the two leaves. Within each census, the same saplings were measured for Ψ_{leaf} at predawn and midday. When species were leafless, terminal twigs were collected from saplings to measure twig water potential (Ψ_{twig}) following the same protocol as for Ψ_{leaf}, except that Ψ_{twig} was measured only at predawn. Comparisons with psychrometric measurements of Ψ_{twig} confirmed that the pressure chamber measurements accurately assessed Ψ_{twig} (Chapter 3). For each species within each census, the mean of Ψ_{leaf} at predawn and midday and Ψ_{twig} was calculated to represent Ψ_{plant}.

Hydraulic conductivity and vulnerability to cavitation

In each forest, we searched the area near the census plants (~2 ha) for healthy looking saplings of each study species that were 110–400 cm tall for measurements of stem hydraulic conductivity. During the 2012 wet season (July–December) and during the subsequent dry season (March–April 2013), 8 saplings of each species at each site were collected (4 at predawn and 4 at midday, n = 128). For the three species that flushed leaves near the onset of the wet season (*Cavanillesia*, *Bursera*, and *Genipa*; Fig. 2.1) we also collected 8 stems (4 at predawn and 4 at midday, n = 24) that were flushing leaves at the onset of the 2013 wet season (May). To collect the stems, the saplings were cut near the base with pruning shears, sealed in opaque plastic bags that were humidified with wet paper towels, and brought to the lab for measurements of native hydraulic conductivity. An additional set of stems that were collected during the 2012 and 2014 wet seasons were bench dried for assessment of vulnerability to cavitation. They were allowed to air dry in the laboratory for 2–300 h, then re-resealed in opaque plastic bags for 2 h before subsequent measurements (n = 101, 9–16 per species in each forest).

In the laboratory, Ψ_{leaf} was measured with a Scholander pressure chamber on three leaves and averaged. Stem water potential (Ψ_{stem}) was assumed to equal Ψ_{leaf} because sealing the stems in opaque bags stopped transpiration. For the stems of deciduous species collected during the dry season, which were leafless, we measured Ψ_{stem} as described above for the Ψ_{twig} of leafless census plants.

The stem was submerged in tap water and a segment 30 cm in length, located > 70 cm from the end that was cut in the field, was removed with pruning shears. The ends of the segment were re-cut with a fresh razor and the bark was removed to 2 cm from the ends. The segment was attached to a hydraulic conductivity apparatus (Sperry et al. 1988) and perfused with filtered (0.1 μm) and degassed 10 mM KCl solution. To correct for passive water uptake, flow rates were measured under four pressure heads, ranging 0.98–8.6 kPa, and hydraulic conductivity was calculated as the slope of the regression of the flow rate on the pressure gradient across the stem segment (Torres-Ruiz et al. 2012). Stem-area specific hydraulic conductivity (K_s) was calculated by dividing conductivity by the segment's cross-sectional area. Stem cross-sectional area was calculated using the mean of two diameter measurements made with a dial caliper (to 0.1 mm) on each end of the segment.

Vulnerability to cavitation was assessed as the decrease in K_s with decreasing Ψ_{stem}. A three-parameter Weibull function was fit as:

$$K_s = a \times \exp(-(-\Psi_{stem}/b)^c) \qquad \text{Equation 2.1}$$

through all K_s measurements for each species (including wet-season, dry-season, leaf-flushing, and bench-dried samples). Since multiple factors limit K_s independently of the Ψ_{stem} at which K_s is measured (e.g., previous drought stress, pathogen attack, stem bending), we fit regression curves through the 90th quantile of the measurements, which represents the envelope of K_s values of as a function of Ψ_{stem}, or the upper limit of K_s at a given Ψ_{stem} (Cade and Noon 2003). We fit the regressions with the R package quantreg (Koenker 2013). For comparisons among species, we used the Weibull functions to calculate the Ψ_{stem} at 50% and 80% loss of K_s (P50 and P80, respectively).

Using ‘native’ conductivity measurements (i.e., without refilling embolized vessels in the laboratory) on field-collected and bench-dried samples to assess vulnerability to cavitation had the advantage of avoiding techniques that may introduce bias in vulnerability curves (Christman et al. 2012). However, it was recently reported that excising stem segments for K_s measurements while the xylem is under tension produces artifactually low K_s values for some species (Wheeler et al. 2013). Since our initial K_s measurements were liable to this excision artifact, for the measurements made in 2014 (which were all on bench-dried stems), we relaxed the xylem tension by sequentially cutting back the stems towards the K_s segment while the stem was under water. The values of K_s did not differ between the 2014 measurements and the earlier measurements (visual inspection, Fig. 2.2), so we included the full dataset in our analyses.

To test whether K_s varied among seasons and time of day within season (i.e., predawn vs. midday), we set up a two-way ANOVA for each species with season, time of day, and their interaction as fixed effects. Linear contrasts were used to compare K_s between seasons and between times of day within season. The false discovery rate method of Benjamini and Hochberg (1995) was used to correct for multiple comparisons to $\alpha = 0.05$.

Relationships among leaf shedding, stomatal conductance, and vulnerability to cavitation

In order to test for relationships between leaf shedding and Ψ_{plant}, we first calculated the percent leaf area of each sapling relative to the November censuses preceding the dry seasons (PNLA). The November censuses were used as reference points because that is when most species had maximum leaf area and because some *Cavanillesia* and *Bursera* saplings lost leaf area in December, prior to the onset of the dry season (Fig. 2.1). We then calculated the mean PNLA for each species in each census and used standardized-major-axis regression to assess its relationship with Ψ_{plant} within each census year (1^{st} year = Nov. 2011–Aug 2012; 2^{nd} year = Nov. 2012–Aug 2013). To compare among species for sensitivity of leaf shedding in response to Ψ_{plant}, we calculated the Ψ_{plant} at which PNLA was 50% (LA50; analogous to the P50 value for sensitivity of K_s to Ψ_{stem}).

Similarly, we tested for relationships between stomatal conductance and Ψ_{plant}. We took the mean stomatal conductance for each species at each census and plotted it against Ψ_{plant}. Then for each census year, we fit a Weibull function through the points with nonlinear regression. The Ψ_{plant} at which stomatal conductance was 50% of the maximum (maximum taken as the intercept of the regression) was calculated as the SC50. We then tested whether leaf responses (SC50 and LA50) were correlated with points of hydraulic stress in the stem (P50 and P80) among species using Pearson's correlation analysis.

Soil-plant-atmosphere hydraulic model

We used the hydraulic model developed by Sperry et al. (1998) and expanded by Sperry and Love (2015) to estimate the E_{crit} of the census plants throughout the two dry seasons. The model incorporates hydraulic conductance as a function of water potential in the soil, rhizosphere, roots, stem, and leaves. Since details of the model are described elsewhere, here we describe only our methods for parameterizing it.

To estimate soil hydraulic conductance, the model uses van Genuchten's (1980) equations for unsaturated soil fit with the soil texture parameters of Leij et al. (1996). Although the model can incorporate multiple soil layers, we used a simplified version with a single soil layer whose Ψ_{soil} was equal to the census values of predawn Ψ_{leaf}. It was parameterized with the soil texture of the top horizon measured in pits near the census plants in each forest (< 200 m). The transitional-forest soil texture was clay while the dry-forest soil texture was sandy clay loam (B. L. Turner, personal communication).

The saturated-whole-plant hydraulic conductance (k_{sat}) was initially set to an arbitrarily high value (50 kg h^{-1} m^{-2} basal area) with roots, stems, and leaves set to 50%, 25%, and 25% of k_{sat}, respectively. Each component was parameterized with the stem vulnerability curves (Table 2.2). To predict E_{crit}, the model was tuned to fit measured midday Ψ_{leaf} and whole-plant diffusive conductance (G, kg h^{-1} m^{-2} basal area). Measured G was calculated for each census plant at each census by multiplying stomatal conductance (measured on a leaf-area basis with a porometer, see above) by leaf area and dividing by stem basal area. Midday Ψ_{leaf} values were fit by adjusting the rhizosphere hydraulic limitation, which was unknown. We ran the model while iteratively adjusting the Ψ_{soil} at which the rhizosphere hydraulic conductance equals zero (P_{rhizo}) in order to minimize the mean square error (MSE) between the modeled and measured midday Ψ_{leaf}. Increasing P_{rhizo} (setting at less negative Ψ_{soil}) had the effect of making total soil-plant conductance more sensitive to low Ψ_{soil}. This accounted for the unknown rhizosphere limitation as well as any deviations in vulnerability of roots and leaves from the known vulnerability of the stems. After fitting midday Ψ_{leaf} via P_{rhizo} adjustment, we iteratively ran the model while adjusting k_{sat} to minimize the MSE between modeled and measured G. The best-fit parameterizations of P_{rhizo} and k_{sat} are listed in Table 2.S2.

We used the model's output of E_{crit} as a measure of the census plants' capacity for hydraulically safe gas exchange during each census. If leaf shedding occurs as a result of leaves being cut off from the soil-plant water continuum due to hydraulic failure, then model-predicted E would exceed E_{crit} prior to leaf shedding. Conversely, if leaf shedding is a response that maintains stem K_s as predicted by the hydraulic fuse hypothesis, then leaf shedding would occur as E_{crit} approaches zero. To test this, we plotted PNLA as a function of E_{crit} and used nonlinear regression to fit Weibull functions through the points for each census year. To test the prediction that leaf shedding slows water-loss rates through reduced hydraulic conductance, we plotted the model's output of the percent loss of total soil-plant hydraulic conductance (PLC_{total}) as a function of measured PNLA and used nonlinear regression to fit a function of the form:

$$PLC_{total} = b - \exp(PNLA/a) \qquad \text{Equation 2.2}$$

Finally, to test whether the saplings regulated Ψ_{plant} such that loss of hydraulic conductance in the stem was low compared to that of more distal components in the plant, we plotted the modeled-predicted percent loss of stem conductance (PLC_{stem}) against PLC_{total}.

Results

Seasonal changes in leaf area, stomatal conductance, and water potential

In both the transitional forest and the dry forest, species exhibited a wide range of dry-season deciduousness (Fig. 2.1). *Cavanillesia* and *Bursera* saplings shed their leaves early during the dry seasons (Fig. 2.1a, b), while at the other extreme, *Cojoba* saplings did not reduce their leaf area during the dry seasons in either forest (Fig. 2.1g, h). To varying degrees, the other species were intermediate in deciduousness: *Genipa* shed its leaves later than *Bursera* and remained leafless for ~2 months (Fig, 2.1d); *Annona* shed its leaves gradually through the dry season but flushed new leaves within days of shedding the old ones (Fig. 2.1c); *Astronium* maintained its leaf area during the dry season in the transitional forest, but in the dry forest, it shed nearly 50% of its leaf area during the dry season (Fig. 2.1e, f). All of these patterns were similar between the 2012 and 2013 dry seasons.

All species in both forests had lower stomatal conductance during the dry season than during the wet season (Fig. 2.S3). The species that shed their entire canopies had reduced stomatal conductance before shedding their leaves. *Cojoba* and *Astronium* had lower dry-season stomatal conductance in the dry forest than in the transitional forest (Fig. 2.S3).

Among species, there was a wide range in seasonal patterns of Ψ_{plant} (Fig. 2.S4). *Cavanillesia* and *Bursera* maintained Ψ_{plant} above -1 MPa throughout the dry seasons. At the other extreme, *Genipa* reached -6.5 MPa during the 2012 dry season and -4.3 MPa during the 2013 dry season. Meanwhile, *Annona*, *Astronium,* and *Cojoba* had lower Ψ_{plant}

during the dry season than during the wet season. Both *Astronium* and *Cojoba* reached lower Ψ_{plant} in the dry forest than in the transitional forest (Fig. 2.S4).

Stem hydraulic conductivity and vulnerability to cavitation

Among species, maximum stem K_s ranged from 1.4 to 4.4 kg s^{-1} MPa^{-1} m^{-1} (estimated as the intercept with 90th quantile regression, Table 2.2). Samples within species had a wide range in K_s during all seasons, creating envelopes of K_s values as a function of Ψ_{stem} (Fig. 2.2). Using quantile regression to quantify vulnerability to cavitation as the margin of these envelopes, we found a wide range of P50 and P80 values among species (Fig. 2.2). For *Astronium* in the dry forest, K_s was low across the range of Ψ_{stem} and we could not fit a Weibull function to the data, so this species was excluded from the analyses that included vulnerability to cavitation. K_s did not differ significantly between predawn and midday for any species in any season (Fig. 2.3). K_s differed significantly between seasons in only two cases: *Cojoba* in the transitional forest had lower K_s in the dry season than in the wet season and *Bursera* had lower K_s in the dry season than during leaf flush at the onset of the wet season (linear contrasts, $P < 0.05$; Fig. 2.3).

Coordination between drought responses and vulnerability to cavitation among species

Within species, stomatal conductance generally decreased as Ψ_{plant} decreased during the dry seasons (Fig. 2.S5). However, during 2012, the nonlinear regressions for *Cavanillesia* and *Bursera* did not converge on a Weibull function, apparently because the saplings had a wide range in stomatal conductance throughout the relatively small range in Ψ_{plant} (Fig. 2.S5). Since these species likely had a threshold response that could not be fit via regression, we used the minimum Ψ_{plant} as a conservative estimate of SC50 for *Cavanillesia* and *Bursera* during 2012. As for stomatal conductance, the percent leaf area (relative to the November census before the dry season; PNLA) generally decreased as Ψ_{plant} decreased (Fig. 2.4). However, in the dry forest, *Cojoba* had higher PNLA at lower Ψ_{plant} (Fig. 2.4h), because this species added leaf area in the late wet season and early dry season, then lost leaf area near the onset of the wet season (Fig. 2.1h). Since *Cojoba* in the dry forest never reached LA50, we excluded this datum from the LA50 analysis. In general, LA50, SC50, and minimum Ψ_{plant} values were similar between the 2012 and 2013 dry seasons (Figs. 2.3, 2.S4, 2.S5). Among species, a lower SC50 was significantly correlated with lower P50 and P80 in both census years (Fig. 2.5a, b). Similarly lower LA50 was correlated with lower P50 and P80 during both census years (Fig. 2.5c, d). Minimum Ψ_{plant} was not correlated with P50 or P80 during either census year (Fig. 2.5e, f).

Hydraulic limits to gas exchange

The hydraulic model successfully predicted seasonal patterns in species' mean G (Fig. 2.S6). However, its accuracy varied among species; Pearson's correlation coefficients between measured and modeled G ranged among species from 0.50 to 0.98 (mean = 0.81) and reduced-major-axis regression slopes ranged from 0.38 to 0.99 (mean = 0.80) (Fig. 2.S7). The reasonable model fit implied equally reasonable estimates of E and E_{crit}. All species in both forests were predicted to be hydraulically limited during seasonal droughts; minimum E_{crit} values ranged 0.1–5.3 kg h^{-1} m^{-2} among species while maximum E_{crit} values ranged 6.7–42 kg h^{-1} m^{-2} (Fig. 2.6a). None of the species were predicted to experience $E > E_{crit}$, indicating strong reduction of E during seasonal drought (Fig. 2.6a). During both dry seasons, PNLA approached zero, full deciduousness, near the lowest values of E_{crit}, 0.5–2 kg h^{-1} m^{-2} (Fig. 2.6b), supporting the prediction that leaf shedding is associated with E_{crit} approaching zero. The relationship between PNLA and E_{crit}, assessed with Weibull functions, was similar during the two dry seasons (F test, P = 0.10; Table 2.S3). PLC_{total} predicted by the model was related to measured PNLA such that, at PNLA > 80%, only slight leaf loss, PLC_{total} was not yet severe, 0–60%. With greater leaf loss, at PNLA < 60%, PLC_{total} increased only slightly (Fig. 2.6c, Table 2.S4). As a result, on average, measured leaf area reached zero before complete loss of modeled water transport capacity. All species reached relatively high values of PLC_{total} during the dry seasons, while *Genipa* reached the highest (range = 40–96% excluding *Genipa*; *Genipa* = 99.4%), yet PLC_{stem} remained relatively low in all species except *Genipa* (range = 7–56% excluding *Genipa*; *Genipa* = 97%; Fig. 2.6d).

Discussion

We found that each species and population had distinct patterns of leaf phenology (Fig. 2.1). Although species fit within ‘evergreen’ and ‘deciduous’ functional groups (evergreen: *Astronium* and *Cojoba*; deciduous: *Cavanillesia*, *Bursera*, *Annona*, and *Genipa*), this grouping misses important differences among species. For example, in the transitional forest, *Cavanillesia* reached zero leaf area about 5 weeks earlier than *Annona* (Fig. 2.1). Also, while *Astronium* and *Cojoba* both maintained leaves throughout the dry seasons, *Astronium* saplings in the dry forest shed nearly 50% of their leaf area (relative to the November census before the dry season; PNLA) whereas *Cojoba* saplings maintained 100% PNLA (Fig. 2.1). These phenological differences are likely linked to physiological and morphological differences among species and they likely influence species performance, distinctions that would be obfuscated by functional grouping. Quantifying deciduousness among populations, such as the time lag between the onset of the dry season leaf loss, may be more informative than grouping (Kushwaha and Singh 2005, Méndez-Alonzo et al. 2012). Furthermore, we found that comparisons among species for their rate of leaf shedding as a function of Ψ_{plant} and E_{crit} (Figs. 2.4, 2.6) helped to elucidate mechanisms that underlie the diverse patterns of deciduousness among tropical trees.

Leaf shedding occurs at key points of hydraulic stress

Various models predict that the optimal time for leaf abscission is when net CO_2 assimilation reaches zero (reviewed by Givnish 2002). Other models predict that leaf abscission occurs in response to water stress, such as the loss of hydraulic conductance in petioles (Tyree and Sperry 1988, Tyree et al. 1993, Brodribb and Holbrook 2003). Distinguishing between these models under natural drought conditions is difficult because water stress, stomatal closure, and resultant reduced net CO_2 assimilation co-occur. Additionally, during progressive drought, the process of nutrient translocation from leaves may be impeded if leaves experience hydraulic failure and phloem turgor loss. In this case, the most adaptive response may be to abscise leaves before water stress prevents translocation. Therefore, trees adapted to seasonally dry conditions may be "programmed" to shed leaves before hydraulic failure cuts the leaves from their water source. Indeed, none of the saplings that we studied were predicted to experience $E > E_{crit}$ (Fig. 2.6a), indicating that they shed leaves without experiencing hydraulic failure.

We found that LA50 was correlated with P50 and P80 among species (Fig. 2.5c, d). LA50 was above the 1:1 line with P50, indicating that saplings shed half of their leaf area before they experienced levels of cavitation that can lead to hydraulic failure in their stems. Likewise, PNLA decreased precipitously when E_{crit} was reduced to < 5 kg h^{-1} m^{-2} (Fig. 2.6b). These results are similar to those from potted seedlings of Mexican dry forest tree species, among which, the Ψ_{plant} at 80% leaf loss was correlated with P80 (Pineda-García et al. 2013). This suggests that hydraulic limits drive a universal pattern of leaf shedding among trees in seasonally dry tropical forests, at least among juvenile trees.

Most of the decrease in measured leaf area (from 100 to 0% PNLA) was associated with the decrease in model-estimated PLC_{total} from 50 to 75% (Fig. 2.6c). Since we did not directly parameterize vulnerability to cavitation in the rhizosphere, roots, or leaves, we cannot predict where within the soil-plant continuum the bottleneck forms that leads to higher PLC_{total} as the dry season progresses. However, many studies have shown that leaves and roots are more vulnerable to cavitation than stems (see references within Sperry and Love 2015), suggesting that as the dry season progresses, loss of hydraulic conductance occurs first in leaves and roots. Thus, this result is consistent with the hydraulic fuse hypothesis—the loss of hydraulic conductance associated with leaf shedding occurred only after major declines hydraulic conductance elsewhere within the continuum, such as in petioles. Indeed, most leaf shedding occurred (i.e., decline in

PNLA) when PLC_{total} approached levels that are associated with stem dieback and tree mortality (i.e., 60–90%; Sperry and Love 2015).

Hydraulic stress appears to induce senescence and abscission before hydraulic failure occurs in the soil-leaf continuum. In contrast, trees that are not adapted to seasonally dry condition may lack this response. For example, during extreme droughts in temperate forests, leaves attached to stems that experience high losses in K_s are often described as turning brown on the stem or abscising while green rather than going through senescence (Hoffmann et al. 2011, Nardini et al. 2013), suggesting that a normal senescence process and nutrient translocation is blocked by the loss of conductance in the stem or leaf.

Leaf shedding may not stabilize Ψ_{plant} during seasonal droughts

During both dry seasons, the Ψ_{plant} of *Genipa* declined after saplings shed all of their leaves, putting them at risk of hydraulic failure (i.e., $\Psi_{plant} < P80$; Figs. 2.5f, 2.S4). In contrast to the case of dynamic failure caused by $E > E_{crit}$, this would be static failure (sensu Tyree and Sperry 1988), where Ψ_{stem} and K_s decline without a xylem pressure gradient produced by transpiration. This result does not support the central prediction of the hydraulic fuse hypothesis—that leaf shedding protects stems from hydraulic stress. Similarly, after adult trees of several species in Costa Rican dry forests shed all of their leaves, they experienced stem-diameter contraction that is associated with water loss (Daubenmire 1972, Reich and Borchert 1984). The Costa Rican observations also suggests that Ψ_{plant} was not stabilized by leaf shedding; however, data on adult trees may be conflated with dry-season flower production, which uses stored water, causing a decline in stem diameter.

Although *Cavanillesia* and *Bursera* had stable Ψ_{plant} after shedding leaves, they also had relatively stable Ψ_{plant} during the onset of the dry season, before shedding leaves (Fig. 2.S4). These species have high stem hydraulic capacitance that buffers Ψ_{plant} against water loss, making Ψ_{plant} a poor indicator of water loss (Chapter 3), so it is unclear whether leaf shedding retained stem water. In contrast, *Annona* had Ψ_{plant} that decreased at the onset of the dry season, before the saplings shed leaves, and then stabilized after leaf shedding (Fig. 2.S4). This pattern suggests that leaf shedding was effective at stopping water loss in *Annona*.

The effectiveness of leaf abscission in stopping water loss depends on several factors that we did not measure. For example, the permeability of the stem surface to water vapor would affect the rate at which leafless stems lose water. This trait likely varies widely among tropical tree species, considering the great diversity of bark traits (Rosell et al. 2014). Radial hydraulic conductance in the roots is also likely to be important, yet this is also largely unknown for tropical trees (Holbrook et al. 1995). To maintain $\Psi_{plant} > \Psi_{soil}$, plants must prevent water from diffusing from their roots into the soil. Root radial hydraulic conductance is highly dynamic on timescales of minutes to hours through aquaporin regulation (Javot and Maurel 2002) and on longer time scales through various mechanisms such as root shrinkage (Nobel and Cui 1992, Carminati et al. 2009), cavitation (Sperry and Ikeda 1997), and suberin deposition in the root cortex (Lo Gullo et al. 1998). In order for leaf shedding to stop water loss from trees rooted in dry soil, leaf shedding must be coordinated with root responses. The lack of correlation between minimum Ψ_{plant} and P50 among species (Fig. 2.5e) could reflect differences among species in this coordination.

Do saplings maintain stable K_s during normal dry seasons?

Throughout the dry season, for all species except *Genipa*, the modeled PLC_{stem} remained < 60% while PLC_{total} reached 40–96% (Fig. 2.6d). This result is consistent with the hydraulic segmentation hypothesis, in that stem hydraulic conductance was protected from cavitation relative to the more-distal components. For most species, the model predictions of low PLC_{stem} concurred with direct measurements of K_s, which did not vary between the wet and dry season (Fig. 2.3). Contrary to the model predictions, *Genipa* did not experience catastrophic loss of K_s; measured K_s was not significantly different between the wet and dry season (Fig. 2.3b). The difference between modeled and measured loss of K_s likely resulted because the census plants that were used to parameterize Ψ_{soil} in the model reached lower water potential than did the stems used to measure K_s (2013 census, mean ± SE: -4.3 ± 1.1 vs. -3.2 ± 0.4 MPa). The difference in water potential could have resulted because we used smaller plants in the census than in the K_s measurements (range of heights: 15–225 vs. 120–400 cm). We measured K_s on taller saplings to prevent measuring K_s on segments with vessels that were cavitated when the stems were cut in the field. Still, two of eight *Genipa* measured for K_s during the dry season had Ψ_{stem} < P80, whereas the those with relatively high K_s had near-wet-season Ψ_{stem} (Fig. 2.2d), further supporting the model output that the census saplings that reached lower Ψ_{plant} indeed experienced high PLC_{stem}. Considering that none of the

Genipa census saplings died during the study, this species likely has a mechanism to recover K_s, either through embolism refilling or xylem production.

Another difference between modeled PLC_{stem} and measured K_s is that *Cojoba* in the transitional forest was predicted to have PLC_{stem} of just 13% (Fig. 2.6c), yet its mean K_s was 50% lower during dry season than during the wet season (Fig. 2.3). Unlike the case with *Genipa*, the census plants that were used to parameterize Ψ_{soil} in the model and the saplings measured for K_s reached similar water potential (-2.6 ± 0.9 vs. -2.2 ± 0.4 MPa), making the cause of the loss of K_s unclear. It may reflect processes other than water-stress induced cavitation, for example, resin deposition in aging vessels.

Previous studies in seasonally dry tropical forests have found that, in adult trees, the distal branches of deciduous species experience large losses of K_s during the dry season while those of evergreen species maintain stable K_s seasonally. One such study included petioles in the segments that were measured for K_s (Brodribb et al. 2002), precluding a test of whether leaf shedding protected stems from hydraulic stress. In contrast, in a study of adult trees in a Venezuelan dry forest, Sobrado (1993) measured segments that only included stems (similar to our K_s measurements). Within four deciduous species, there was a significant loss of K_s in the dry season compared to the wet season (65–92% loss in mean K_s), while two evergreen species did not have a significant loss in K_s. At the time of leaf shedding, the deciduous species had Ψ_{leaf} of -5.0 to -4.7 MPa while the evergreen species had Ψ_{leaf} of -2.5 and -2.2 MPa (Sobrado 1993). Thus, comparing deciduous species, those in Sobrado (1993) shed their leaves at much lower water potential and lost more K_s than did the saplings in our study (Fig. 2.3). The results of Sobrado (1993) do not support the prediction that leaf shedding protects stems from hydraulic stress, yet it is unclear whether K_s in more basally located stems is more stable than in distal branches, which would support the more general hydraulic segmentation hypothesis. In the only other study that we are aware of in which seasonal patterns of K_s were measured in tropical trees, Ishida et al. (2010) found that the twigs in an adult tree of one deciduous species had a 55% loss in K_s during the late dry season compared to the mid-wet season while adult trees of another deciduous species and two evergreen species did not have reduced dry-season K_s. Together with our results, these studies show that reduced-dry season K_s occurs in the stems of some species and not others, more so in deciduous species than evergreen species; yet the conditions, traits, and behaviors that are associated with seasonal loss of K_s will require more research.

Conclusions

The diverse patterns of leaf shedding in species of tropical dry forests allow for critical tests of the hydraulic fuse hypothesis. We found that dry-season leaf shedding occurs as saplings reach hydraulic limits that reduce transpiration rates (and by extension net CO_2 assimilation) to near zero. This is consistent with the prediction that leaf shedding is cued to occur before leaves are cut off from their water source through hydraulic failure. The cues for dry-season leaf senescence and abscission remain unclear (e.g., water stress, day length, endogenous rhythms). However, when saplings of our study species were grown in well-watered pots, leaf abscission was impeded such that saplings maintained nearly 100% leaf area throughout the dry season (Chapter 4). This suggests that, among dry forest tree species, hydraulic limitation to gas exchange is a universal mechanism that underlies complex patterns of leaf-shedding phenology, at least among saplings. Extending this relationship to adult trees could improve leaf phenology parameters in terrestrial biosphere models, which are currently a source of uncertainty in projections of forest carbon fluxes (Powell et al. 2013). However, our results and those of other studies suggest that leaf shedding does not stop water loss or the loss of K_s in stems, contrary to the predictions of the hydraulic fuse hypothesis. Rather than drought-deciduousness acting universally as a water-conservation strategy, its main function (or 'ultimate cause') may vary among species, acting as a means for respiration reduction, nutrient conservation, and leaf-to-root-area balancing.

Table 2.1. Study species traits, descriptions of adult leaf phenology (Pérez 2008), and adult wood density (S. J. Wright, unpublished).

Family	Species	Leaf shape	Adult leaf phenology	Adult wood density (g cm^{-3})
Anacardiaceae	*Astronium graveolens*	Compound	Deciduous	0.82
Annonaceae	*Annona hayesii*	Simple	Not reported	0.50
Burseraceae	*Bursera simaruba*	Compound	Deciduous	0.43
Fabaceae	*Cojoba rufescens*	Compound	Semi-deciduous	0.69
Malvaceae	*Cavanillesia platanifolia*	Simple	Deciduous	0.19
Rubiaceae	*Genipa americana*	Simple	Deciduous	0.69

Table 2.2. Parameters (mean ± SE) from Weibull functions (Equation 2.1) used to quantify vulnerability to cavitation in the stem of the study species and the number of samples included in the regression. A Weibull function could not be fit to *Astronium* in the dry forest because the nonlinear regression did not converge (see Fig. 2.2f).

Species	Forest	a	b	c	n
Annona	Transitional	1.5 ± 0.2	5.0 ± 0.8	4.3 ± 4.1	32
Astronium	Transitional	1.4 ± 0.3	4.9 ± 3.2	3.3 ± 6.0	28
Astronium	Dry	-	-	-	29
Bursera	Dry	2.6 ± 0.7	1.4 ± 0.3	5.8 ± 9.3	38
Cavanillesia	Transitional	2.5 ± 0.8	1.3 ± 3.1	2.3 ± 1.4	34
Cojoba	Transitional	2.0 ± 0.3	5.3 ± 1.6	2.7 ± 2.8	30
Cojoba	Dry	4.5 ± 5.2	3.8 ± 4.2	1.2 ± 2.5	28
Genipa	Dry	1.7 ± 0.6	2.7 ± 0.9	1.3 ± 0.8	33

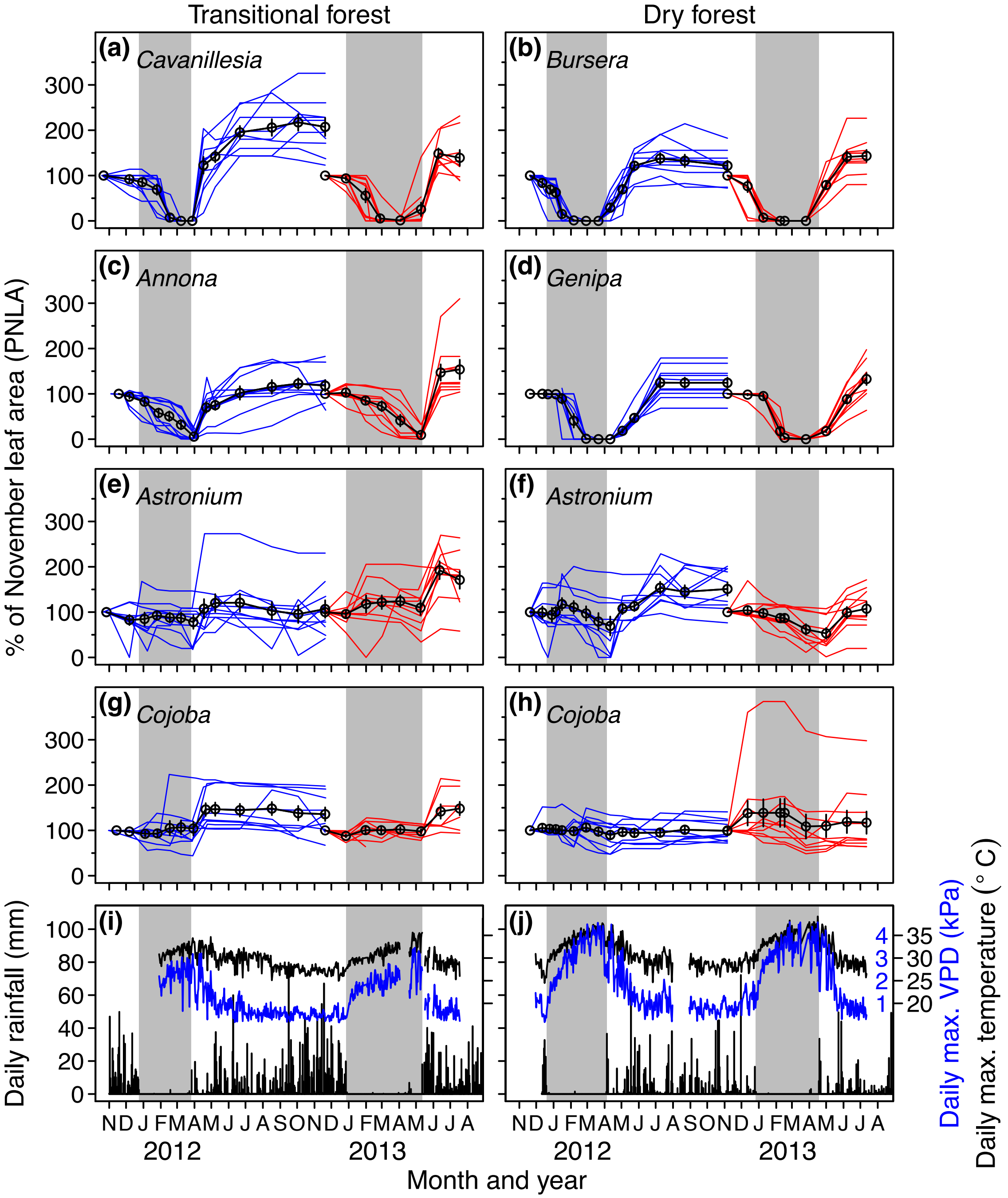

Fig. 2.1. Seasonal trends in percent leaf area (relative to November leaf area, PNLA) among saplings of six tree species (a–h) and weather (i, j) in two seasonally dry tropical forests in Panama. Dry seasons are shaded in grey. In panels a–h, PNLA for individual saplings is represented by blue and red lines for the 1st and 2nd census years, respectively. Black circles represent means (± SE). November censuses were used as a pre-dry-season baseline for the calculation of PNLA. Note that 1st year lines extend to November 2012 for reference only, 2nd year values (i.e., 100% leaf area) were used for analyses for November 2012. In panels i and j, bars represent daily rainfall, black lines represent daily maximum temperature, and blue lines represent daily maximum vapor pressure deficit (VPD).

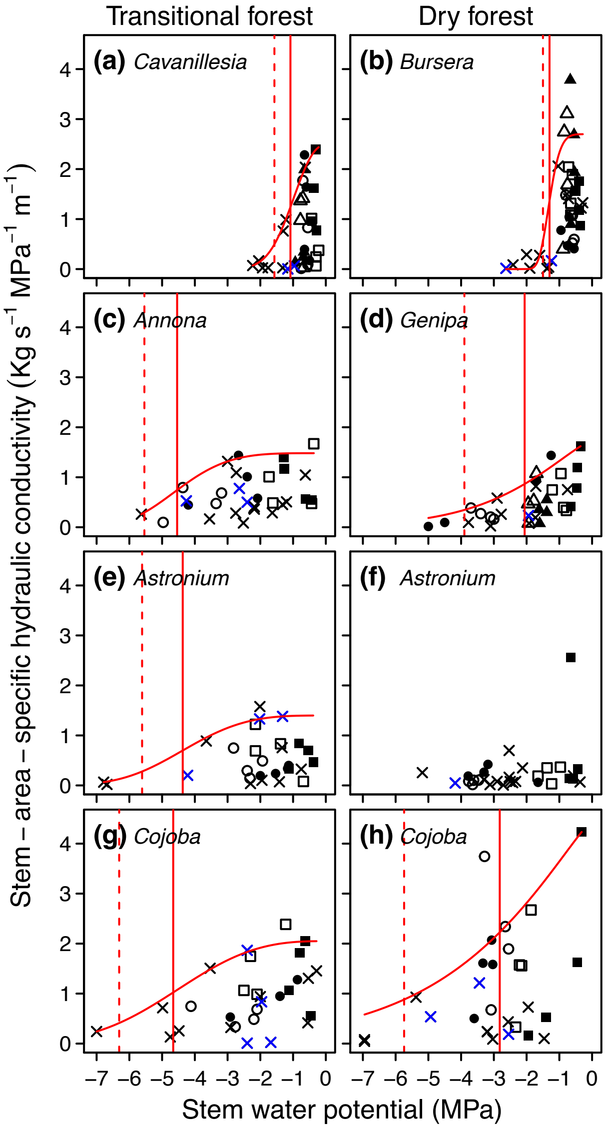

Fig. 2.2. Stem-area-specific hydraulic conductivity (K_s) as a function of stem water potential (Ψ_{stem}) for saplings of six tree species distributed across two seasonally dry forests. Vulnerability to cavitation was assessed as the 90th quantile regression with a Weibull function (Equation 2.1), drawn as red lines. At the Ψ_{stem} where the regression predicts 50% loss of K_s (P50) and an 80% loss of K_s (P80), vertical solid and dashed lines are drawn, respectively. A regression line is not drawn in panel f because a quantile regression could not be fit to the data. Squares, circles, and triangles represent samples collected during the wet and dry seasons, and during early-wet-season leaf flushing, respectively. Closed and open symbols represent samples collected during predawn and midday, respectively. Crosses represent samples collected during the wet season and bench-dried before measuring Ψ_{stem} and K_s. Blue crosses represent samples in which xylem tension was deliberately relaxed prior to excising the K_s segment, which were used to test for an excision artifact (see Materials and methods).

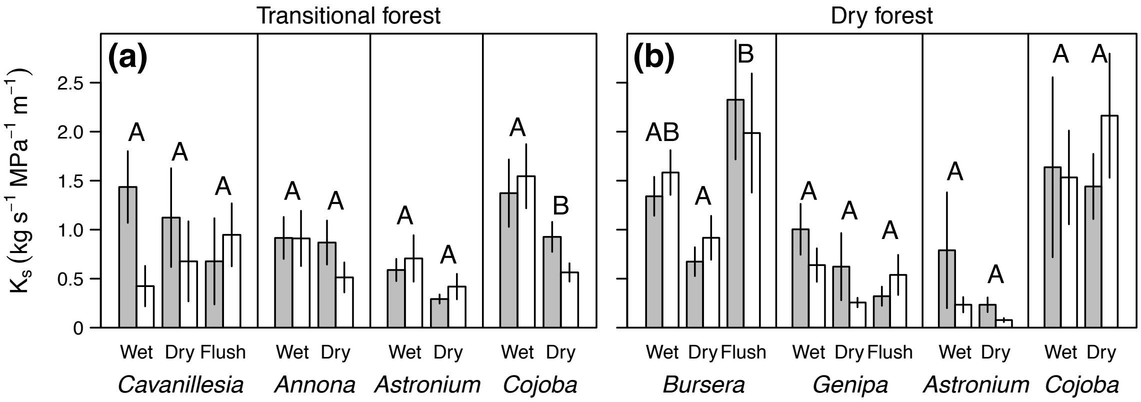

Fig. 2.3 Stem-area-specific hydraulic conductivity (K_S) among seasons and time of day within season for four species in a transitional forest (a) and four species in a dry forest (b). Each bar represents mean ± SE (n = 4). Shaded and open bars represent predawn and midday, respectively. Wet, dry, and flush are the wet season, dry season, and during early-wet-season leaf flushing, respectively. Seasons that share letters are not significantly different. Within season, no differences were detected between predawn and midday.

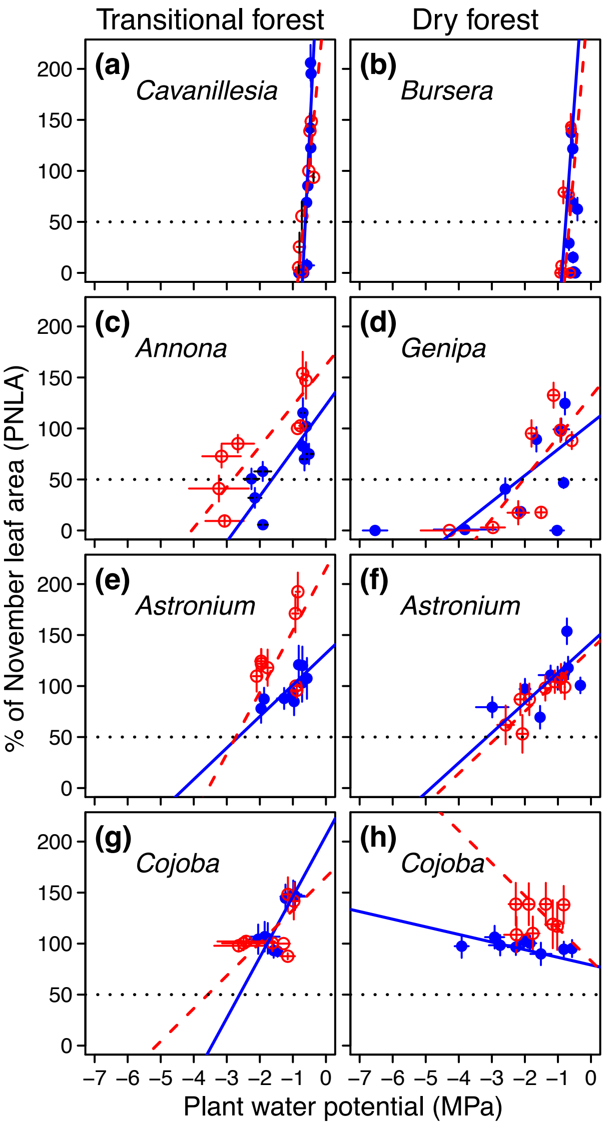

Fig. 2.4. Percent leaf area (relative to November leaf area, PNLA) as a function of plant water potential (Ψ_{plant}; mean of predawn and midday leaf- or leafless-twig water potential) for six species distributed across two seasonally dry tropical forests measured over 21 months (see Figs. 2.1, 2.S4). Filled blue and open red circles represent means (± SE) for censuses within the 1st and 2nd census years (2012 and 2013), respectively. Standardized-major-axis regressions are shown with solid blue and dashed red lines for the 1st and 2nd year, respectively. The Ψ_{plant} at which leaf area reached 50% (LA50) was calculated as the point where the regression line intersects 50% PNLA (dotted lines).

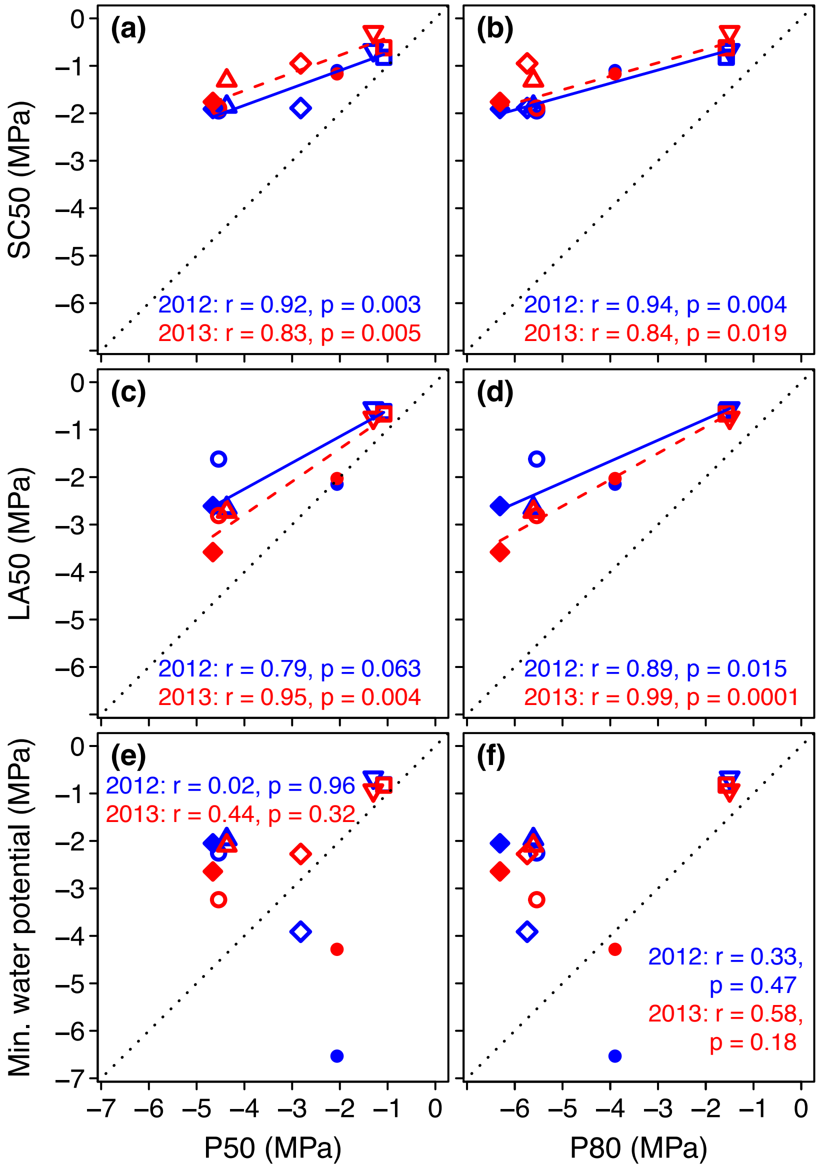

Fig. 2.5. Correlations among species between the stem water potential associated with 50% and 80% loss of hydraulic conductivity (P50 and P80, respectively) and the plant water potential (Ψ_{plant}) at which stomatal conductance is 50% of maximum (SC50; panels a, b), the Ψ_{plant} at which leaf area is 50% of the November census before the dry season (LA50; panels c, d), and the minimum Ψ_{plant} reached during the dry season (panels e, f). Species are represented as *Annona*, open circle; transitional-forest *Astronium*, up-pointed triangle; *Bursera*, down-pointed triangle; *Cavanillesia*, open square, transitional-forest *Cojoba*, closed diamond; dry-forest *Cojoba*, open diamond; *Genipa*, closed circle. Blue and red symbols represent values obtained in the 1st and 2nd years of the census (2012 and 2013), respectively. When Pearson's product-moment correlation was $P < 0.10$ (statistics shown in each panel), the standardized-major-axis regression line is drawn as a solid blue or dashed red line for 2012 and 2013, respectively. Dotted 1:1 lines are shown for reference.

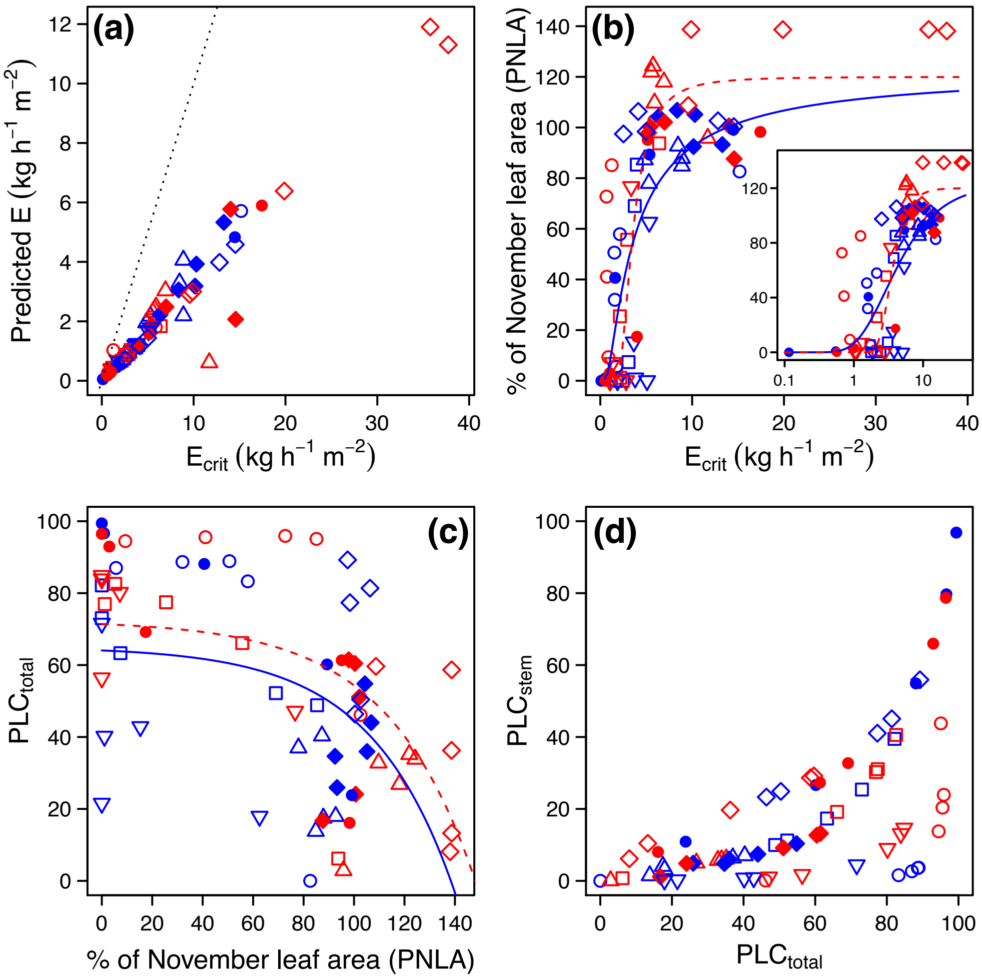

Fig. 2.6. Relationships among modeled hydraulic parameters and measured percent leaf area (relative to November leaf area, PNLA). (a) Model-predicted transpiration rate (E) as a function of the transpiration rate that would cause hydraulic failure in the soil-plant continuum (E_{crit}). (b) PNLA as a function of E_{crit}. (c) Modeled percent loss of hydraulic conductance in the whole soil-plant continuum (PLC_{total}) as a function of PNLA. (d) Modeled percent loss of hydraulic conductance in the stem (PLC_{stem}) as a function of PLC_{total}. Symbols represent values from censuses within the dry seasons (i.e., within the shaded regions in Fig. 2.1). Symbols represent species as drawn in Fig. 2.5. In panel a, the dotted 1:1 line is shown for reference. In panels b and c, functions fit with nonlinear regression are drawn as solid blue and dashed red lines for the 2012 and 2013 dry seasons, respectively. The inset in panel b shows PNLA as a function of E_{crit} with the x-axis on a log scale.

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Supplemental materials

Table 2.S1. Dry season characteristics during the study period in two seasonally dry tropical forests in Panama. Dry season length is defined here as the number of consecutive days with < 10 mm of rainfall. Values are mean ± SD.

		Dry season characteristics		
Site	Year	Length (days)	Daily max. temp. (°C)	Daily max. VPD (kPa)
Eisenmann Reserve	2012	107	33.9 ± 2.3	3.1 ± 0.9
Eisenmann Reserve	2013	113	34.7 ± 2.0	3.2 ± 0.8
Parque Metropolitano	2012	93	31.2 ± 1.5	2.2 ± 0.5
Parque Metropolitano	2013	136	31.2 ± 2.0	1.9 ± 0.6

Table 2.S2. Hydraulic model parameters that were fit with iteration. k_{sat} was fit assuming atmospheric pressure of 100 kPa, predawn Ψ_{leaf} of 0 MPa, and midday Ψ_{leaf} of -1 MPa for all species except *Bursera* and *Cavanillesia*, for which a midday Ψ_{leaf} of -0.5 MPa was used. The Ψ_{leaf} parameters used to fit k_{sat} were chosen arbitrarily to roughly match measured values of Ψ_{leaf} during the wet-season (see Fig. 2.S3).

Species	Forest	P_{rhizo} (MPa)	k_{sat} (kg s^{-1} m^{-2})
Annona	Transitional	11	12
Astronium	Transitional	32	3.5
Bursera	Dry	2.6	48
Cavanillesia	Transitional	3.5	75
Cojoba	Transitional	29	6.5
Cojoba	Dry	13	12
Genipa	Dry	9.5	10.5

Table 2.S3. Parameters of Weibull functions fit with nonlinear regression for leaf area (percent of the November census preceding the dry season, PNLA) as a function of the transpiration rate that would cause hydraulic failure in the soil-plant continuum (E_{crit}). PNLA = a × $\exp(-(E_{crit}/b)^c)$).

Year	a	b	c	R^2
1st	124 ± 46	2.89 ± 1.28	-1.01 ± 0.67	0.55
2nd	120 ± 11	3.17 ± 0.35	-2.94 ± 1.16	0.74
Combined	137 ± 23	3.00 ± 0.65	-1.04 ± 0.34	0.61

Table 2.S4. Parameters of functions fit with nonlinear regression for the percent loss of hydraulic conductance in the soil-plant continuum (PLC_{total}) as a function of leaf area (percent of the November census preceding the dry season, PNLA). $PLC_{total} = b - \exp(PNLA/a)$.

Year	a	b	R^2
1st	65 ± 6.6	33 ± 5.4	0.12
2nd	72 ± 5.1	35 ± 2.0	0.38
Combined	67 ± 3.8	35 ± 2.1	0.24

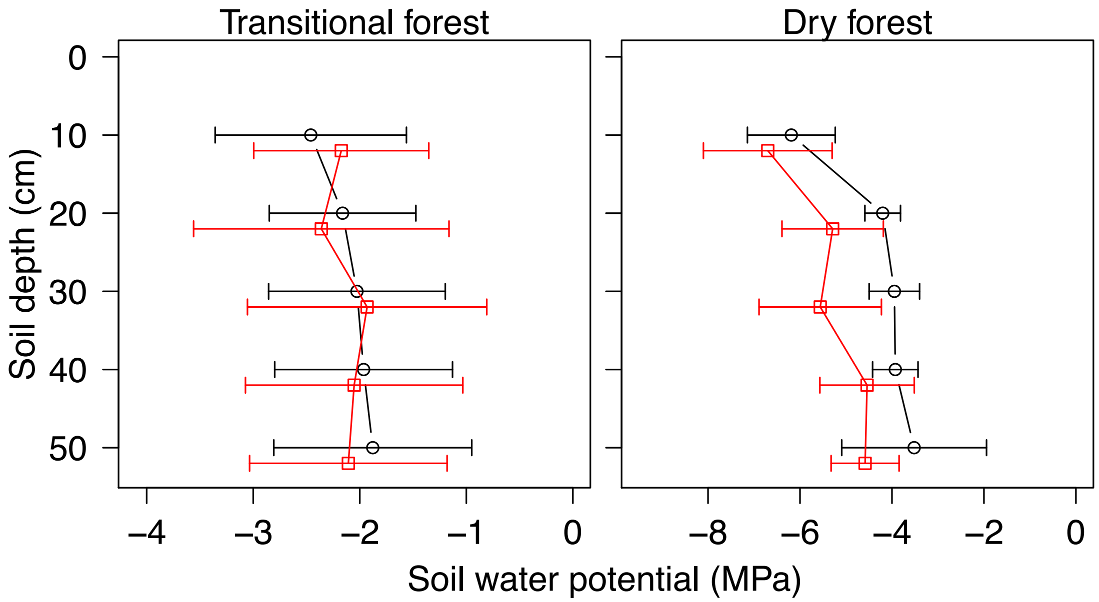

Fig. 2.S1. Soil water potential measured in late March 2012 and 2013, near the end of the dry seasons. Each year, four pits were dug with an auger within the area encompassed by the census plants. In each pit, soil from each depth (10, 20, 30, 40, 50 cm) was immediately sealed in within two aluminum chambers (25 mm diameter × 45 mm in depth) that were later (24-48 hours) attached to leaf-cutter psychrometers (Merrill Engineering, Logan, UT) and measured for Ψ_{soil} with the protocol of Kursar et al. (2005). The two Ψ_{soil} measurements at each depth in each pit were averaged. In 2013, one of the pits had Ψ_{soil} less than the measurement limit of the psychrometers (-10 MPa) and another pit could only be dug to 30 cm because the deeper soil was too hard. Errors bars represent 1 SD. Black circles and red squares represent 2012 and 2013 values, respectively. Values from 2013 are drawn 2 cm below the actual depth in order to prevent the error bars from overlapping. Reference: Kursar T.A., B.M.J. Engelbrecht, and M.T. Tyree. 2005. A comparison of methods for determining soil water availability in two sites in Panama with similar rainfall but distinct tree communities. Journal of Tropical Ecology 21:297–305.

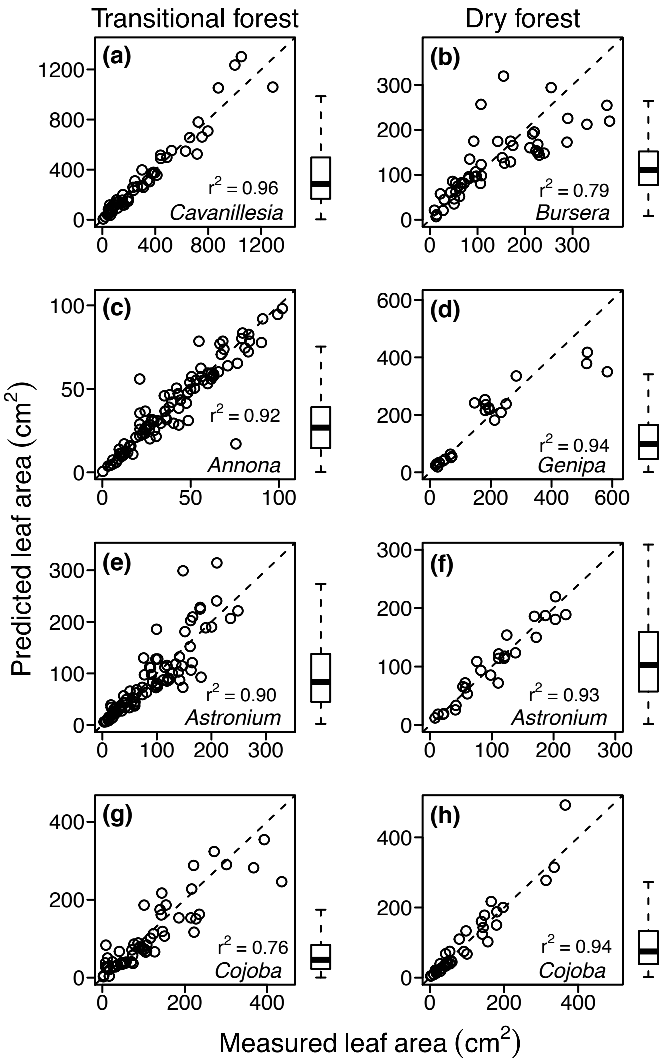

Fig. 2.S2. Relationship between measured leaf area and predicted leaf area for saplings of six tree species distributed across two seasonally dry forests. Each point represents an individual leaf. Leaf area was measured with an LI-3100 area meter (LI-COR Biosciences, Lincoln, NE, USA) after removing the petiole and rachis. Leaf area was predicted using simple linear regression with leaf length as the independent variable and leaf area as the dependent variable. For compound-leaved species, the number of leaflets per leaf was included as a cofactor. Values of leaf length and area were log transformed prior to analysis. The r^2 values listed refer to the linear regression models. Boxplots show the predicted leaf areas of leaves on the census plants. Boxes extend to the 1st and 3rd quartiles and are bisected by the median. Bars extend to the most extreme value within 1.5 times the interquartile length.

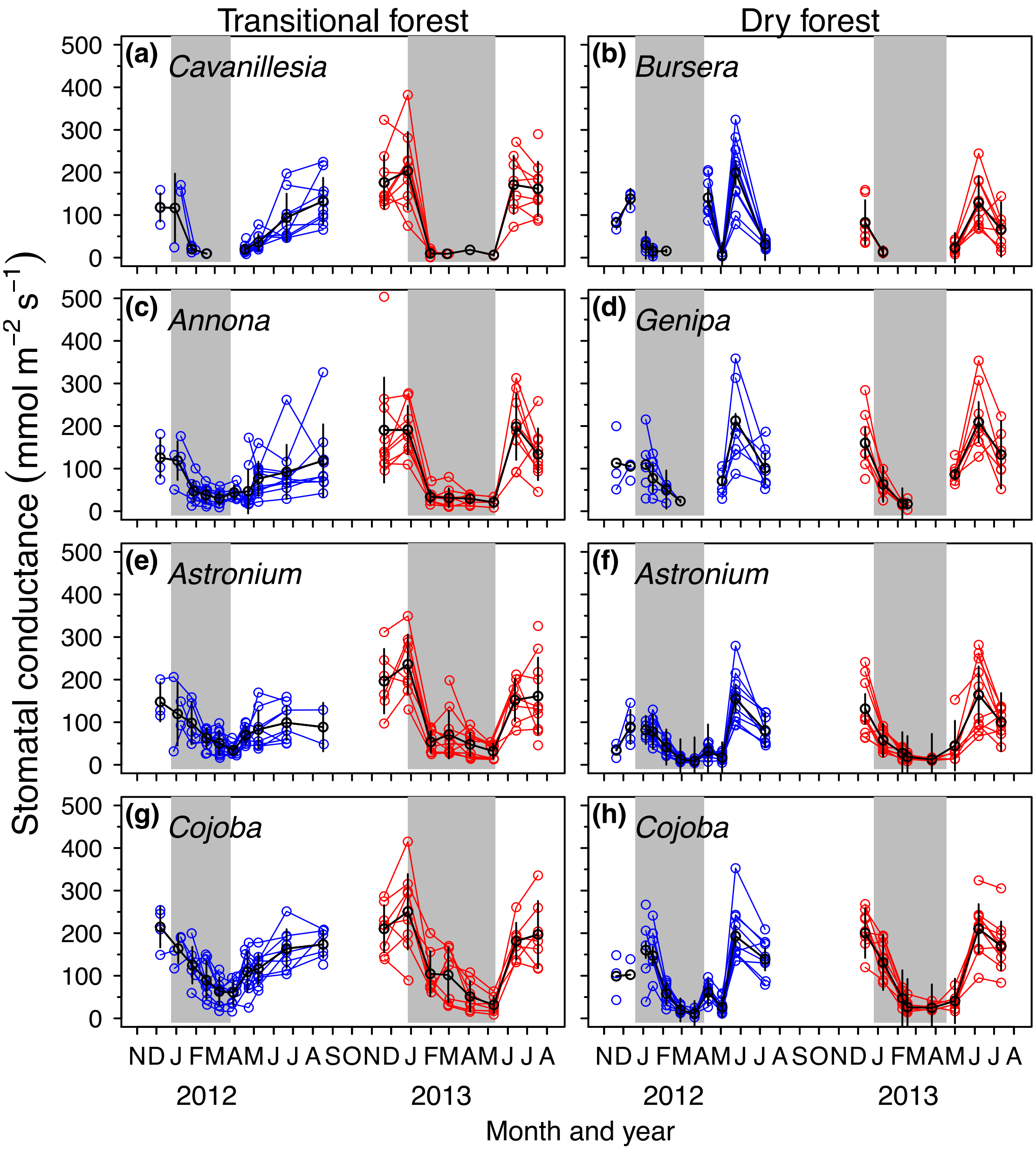

Fig. 2.S3. Stomatal conductance among saplings of six tree species distributed across two seasonally dry forests over 21 months. Each series of connected blue and red circles represents a sapling that was repeatedly measured in 2012 and 2013, respectively. Black circles represent means. Bars extend to 1 SE.

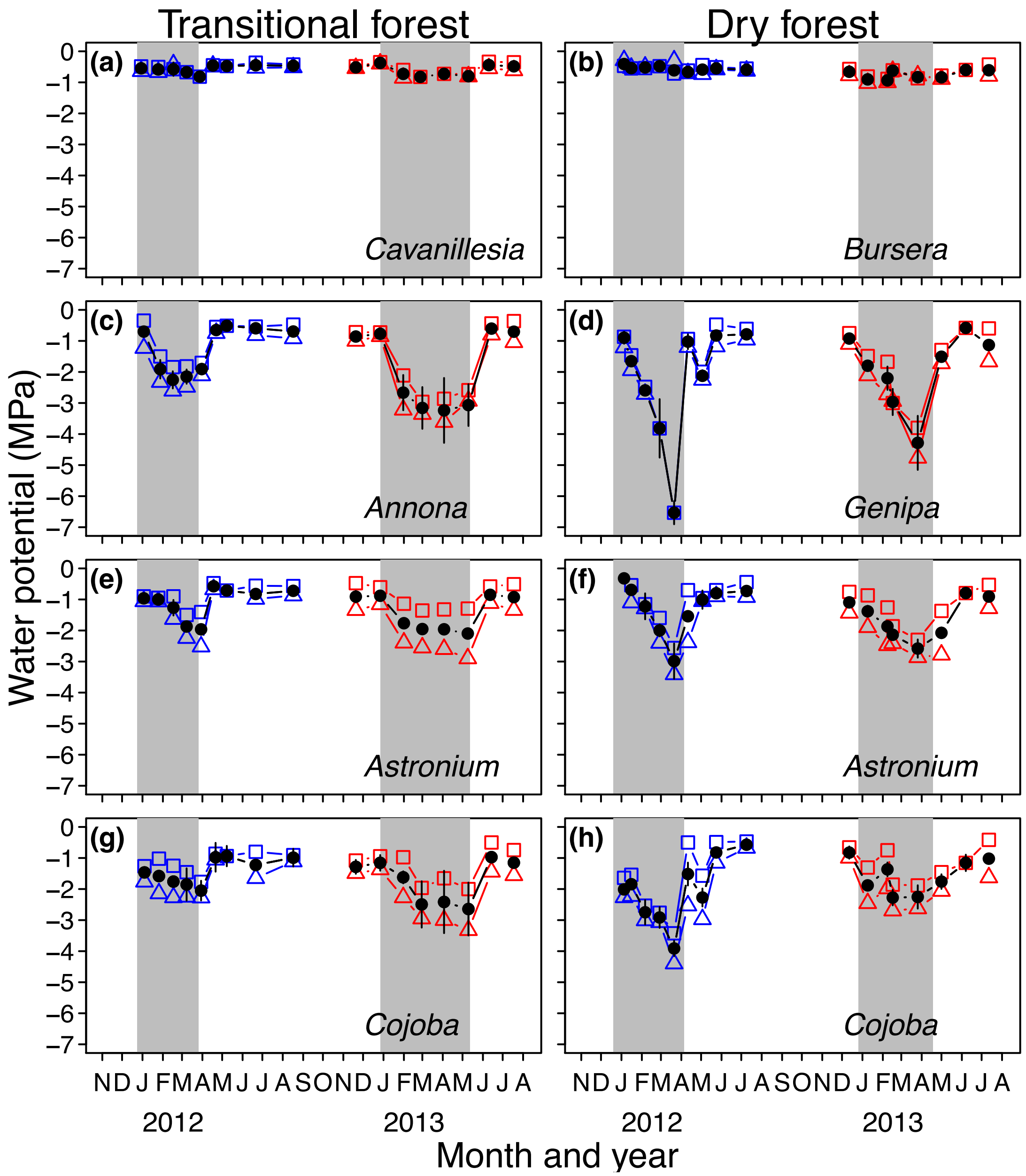

Fig. 2.S4. Leaf- or leafless-twig water potential among saplings of six species distributed across two seasonally dry forests over 21 months. Squares represent the mean predawn water potential and triangles represent the mean midday water potential. Blue and red symbols represent measurements within the 1st and 2nd census years (2012 and 2013), respectively Filled circles represent the mean of predawn and midday values, termed plant water potential. Bars are drawn to 1 SE and are drawn only for plant water potential to improve legibility.

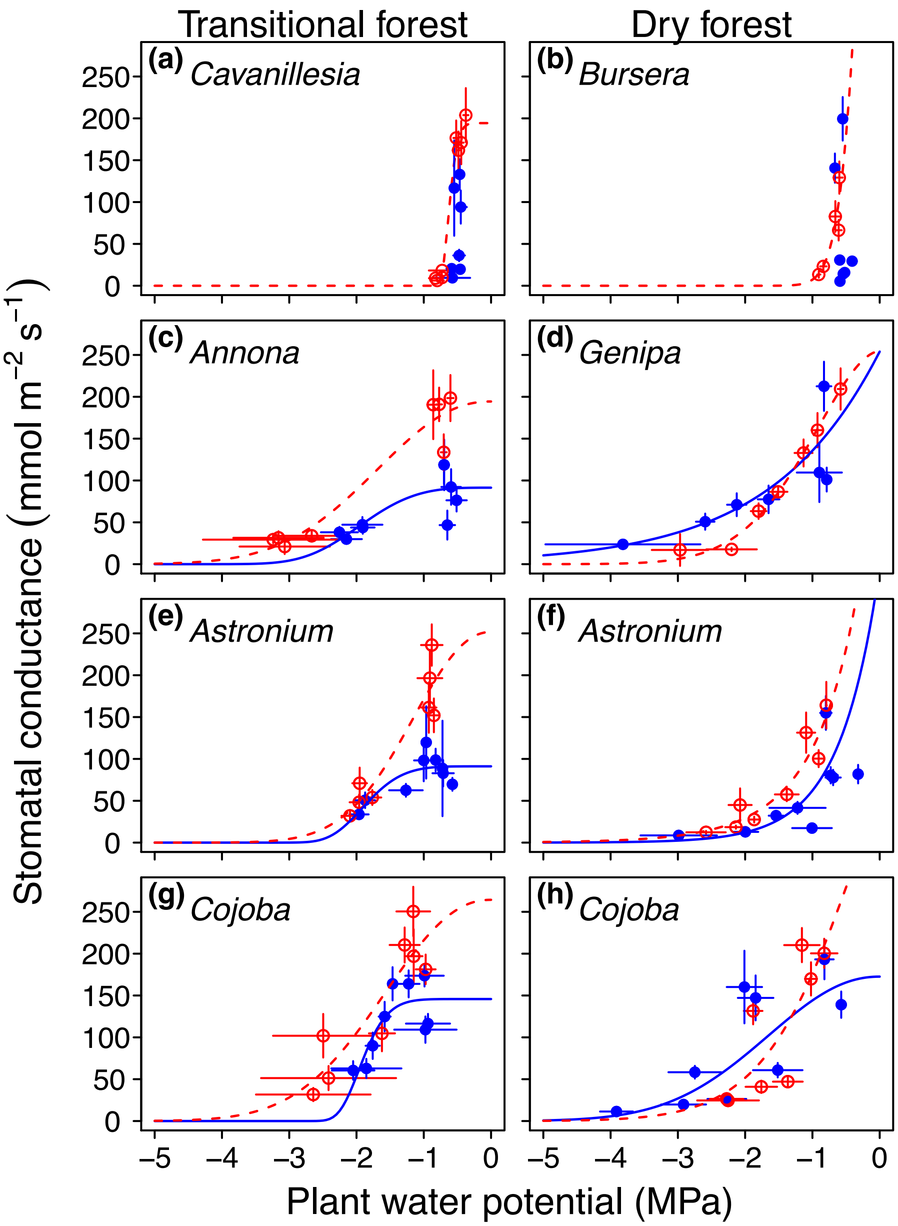

Fig. 2.S5. Stomatal conductance as a function of plant water potential for six species distributed across two seasonally dry forests measured over 21 months. Circles represent means within censuses. Bars extend to 1 SE. Closed blue and open red circles represent measurements within the 1st and 2nd census years (2012 and 2013), respectively. Solid and dashed lines represent Weibull functions fit through the 2012 and 2013 points, respectively.

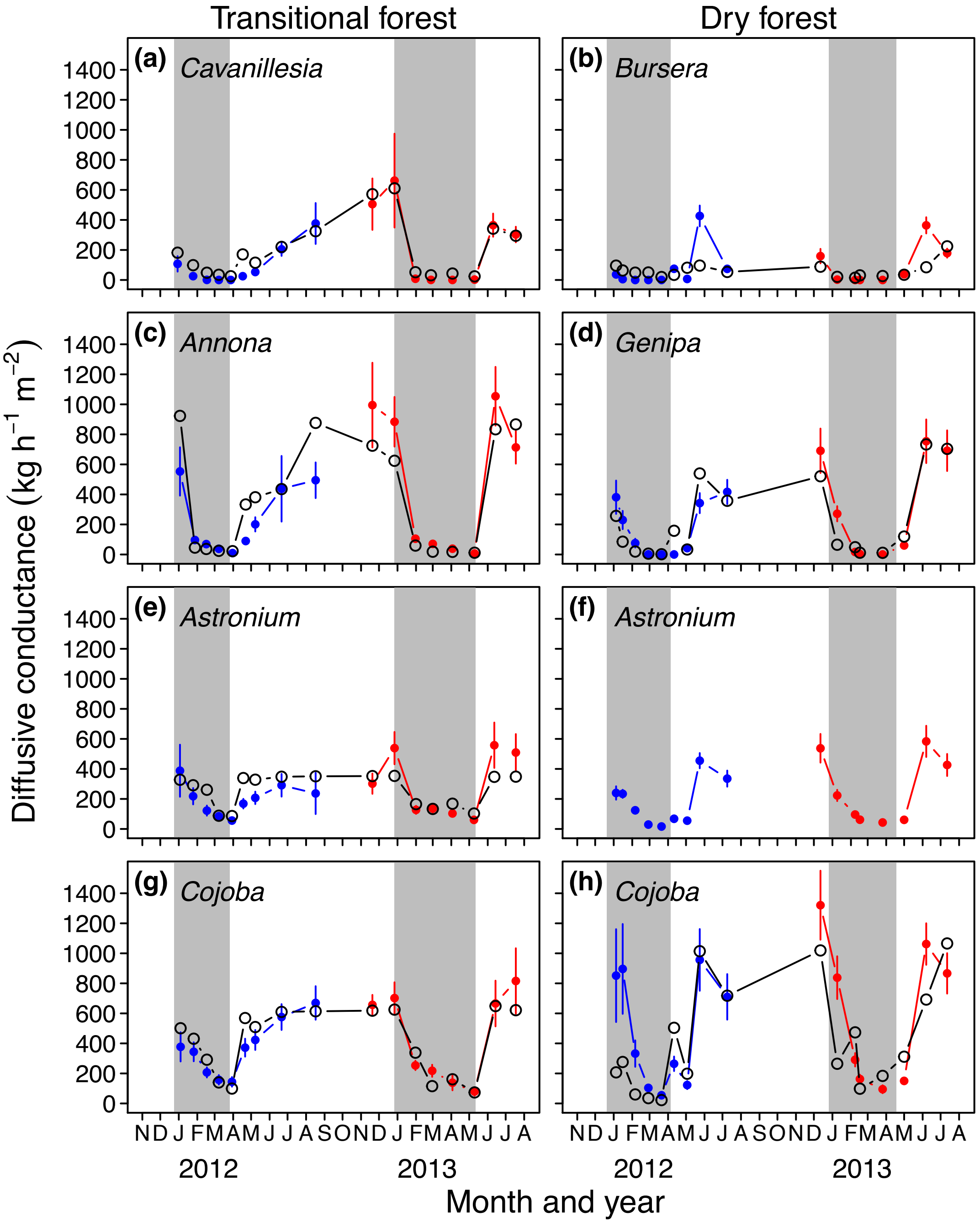

Fig. 2.S6. Measured vs. modeled whole-plant canopy diffusive conductance (G) over 21 months. Filled circles represent the mean of measured G (estimated from leaf-level measurements, see Material and methods). Bars extend to 1 SE. Blue and red symbols represent measurements within the 1st and 2nd census years (2012 and 2013), respectively. Open black circles represent modeled G. Shaded regions represent the dry seasons (see Fig. 2.1). In panel f, modeled G is not drawn because the hydraulic model could not be fit to this species (see Results).

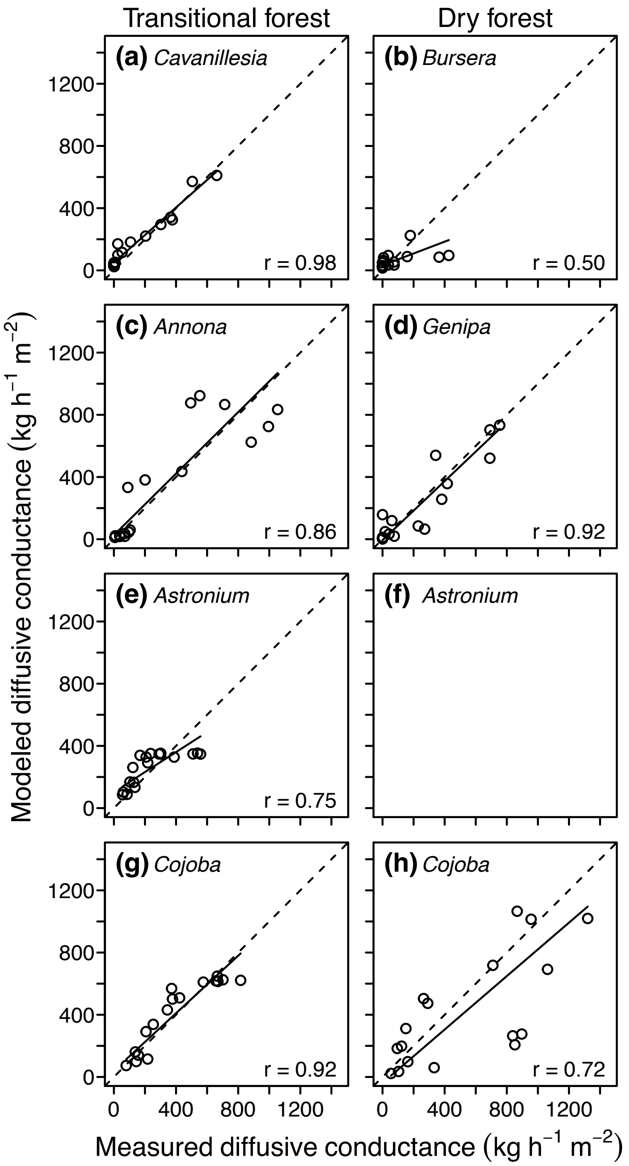

Fig. 2.S7. Measured vs. modeled whole-plant canopy diffusive conductance. Solid lines represent fits with standardized-major-axis regressions. Dashed lines are 1:1. Pearson's correlation coefficients (r) are shown.

CHAPTER 3

DIVERSE PATTERNS OF STORED WATER USE AMONG SAPLINGS IN SEASONALLY DRY TROPICAL FORESTS

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HIGHLIGHTED STUDENT RESEARCH

Diverse patterns of stored water use among saplings in seasonally dry tropical forests

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Received: 15 September 2014 / Accepted: 20 April 2015

Abstract Tree species in seasonally dry tropical forests likely vary in their drought-survival mechanisms. Drought-deciduousness, which reduces water loss, and low wood density, which may permit dependence on stored water, are considered key traits. For saplings of six species at two distinct sites, we studied these and two associated traits: the seasonal amount of water released per stem volume ("water released") and the hydraulic capacitance of the stem (*C*). Two deciduous species with low stem density, *Cavanillesia platanifolia* and *Bursera simaruba*, had high *C* and high dry-season stem water potential (Ψ_{stem}), but differed in dry-season water released. *C. platanifolia* did not use stored water during the dry season whereas *B. simaruba*, in a drier forest, released stored water. In both, water released was highest while flushing leaves, suggesting that stored water supports leaf flushing. In contrast, two deciduous species with intermediate stem density, *Annona hayesii* and *Genipa americana*, had intermediate *C*, low dry-season Ψ_{stem}, and high seasonal change in water released. Meanwhile, two evergreen species with intermediate stem density, *Cojoba rufescens* and *Astronium graveolens*, had relatively low *C*, low dry-season Ψ_{stem}, and intermediate seasonal change in water released. Thus, at least three, distinct stored-water-use strategies were observed. Additionally, bark relative water content (RWC) decreased along with Ψ_{stem} during the dry season while xylem RWC did not change, suggesting that bark-stored water buffers Ψ_{stem} seasonally. Together these results suggest that seasonal use of stored water and change in Ψ_{stem} are associated with functional groups that are characterized by combinations of deciduousness and stem density.

Keywords Capacitance · Drought · Functional groups · Leaf phenology · Tree physiology

Introduction

Seasonally dry tropical forests (SDTF) occupy areas at the climate transition between moist forest and savanna, making them vulnerable to conversion to savanna if areas become drier (Murphy and Bowman 2012). In fact, aridity is increasing in many regions due to temperature increases associated with climate change (Sherwood and Fu 2014). Furthermore, the extent of SDTF, which may have historically approximated the area of wetter forests, has been inordinately reduced through conversion to agricultural and urban uses, creating a conservation imperative (Murphy and Lugo 1986; Miles et al. 2006). Predicting

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In this work we offer new insights into the drought responses of trees by focusing on the critical sapling stage, developing a novel technique to measure hydraulic capacitance, and comparing multiple stem tissues for seasonal water use. The results expand our knowledge of the functional traits that influence drought performance in trees and suggest new dimensions for understanding how climate change will potentially drive forest community dynamics.

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how the remaining patches of SDTF will respond to drying, including possible expansion of drier habitats (Feng and Fu 2013), requires knowledge of the mechanisms by which tropical trees survive drought.

To avoid dieback and death during droughts, trees must maintain their stem water potential (Ψ_{stem}) above levels that would cause hydraulic failure (Kursar et al. 2009; Urli et al. 2013). Species with low wood density tend to incur hydraulic failure at higher Ψ_{stem} than species with high wood density (Hacke et al. 2001; Markesteijn et al. 2011; Pineda-García et al. 2013). Yet, species with low wood density also maintain higher Ψ_{stem} during droughts than species with high wood density. Consequently, all species tend to maintain a safety margin between the Ψ_{stem} reached during seasonal droughts and the Ψ_{stem} causing hydraulic failure (Choat et al. 2012). Likewise, wood density, which ranges widely among SDTF species (Markesteijn et al. 2011; Pineda-García et al. 2013), is predicted to form an important axis of variation in drought-survival strategies. Specifically, species with low wood density, which are vulnerable to hydraulic failure yet maintain high Ψ_{stem}, survive drought through desiccation avoidance, whereas species with high wood density, which are resistant to hydraulic failure yet reach low Ψ_{stem}, survive drought through desiccation tolerance (Ludlow 1989; Poorter and Markesteijn 2008; Markesteijn and Poorter 2009; Pineda-García et al. 2013).

One mechanism by which desiccation-avoiding species can maintain high Ψ_{stem} during droughts is by accessing moist soil. But, in dry soil, the mechanisms by which desiccation-avoiding species maintain high Ψ_{stem} are unclear. Since water storage capacity (i.e., water stored per volume of wood) and stem capacitance [C (water released per $\Delta\Psi_{\text{stem}}$); see "Materials and methods"] increase as wood density decreases (Simpson 1993; Meinzer et al. 2008a), desiccation-avoiding species may rely on stored water to maintain high Ψ_{stem} during droughts (Poorter and Markesteijn 2008; Pineda-García et al. 2013). Many SDTF tree species with low-density wood also close their stomata or shed their leaves, traits that help to retain water within stems during droughts (Stratton et al. 2000; Brodribb et al. 2003; Poorter and Markesteijn 2008). Hence, desiccation-avoiding species may minimize the release of stem water and use C to buffer the effects of incidental water loss on Ψ_{stem} (Borchert 1994a). In these species, without access to soil water, stem water release could occur very slowly over the dry season followed by recharge in the wet season, as opposed to the more commonly studied case of daily loss and recharge of stem water (Meinzer et al. 2003; Scholz et al. 2011).

The sapling life stage is highly responsive to reductions in rainfall (Enquist and Enquist 2011) and strongly influences the species composition of forests through habitat filtering (Baldeck et al. 2013). We addressed the issue of divergent mechanisms of drought survival among saplings of SDTF species by comparing two sites that differ in rainfall and by choosing species that likely differ in physiology. Hence, we studied responses of saplings in two SDTF: a dry forest and in a forest that is intermediate between moist and dry forest, hereafter termed "transitional forest."

Because we are particularly interested in the role of deciduousness, C, and the release of stored water in drought survival, we chose six common species that have a wide range of deciduousness and stem density (analogous

Table 1 List of study species, the sites where they were sampled in Panama, and their stem traits

Species	Forest	Leaf phenology[a]	Height (cm)	Basal diam. (mm)	Stem density[b] (g cm^{-3})	Stem composition (% of cross-sectional area)		
						Bark	Xylem	Pith
Annona hayesii	PNM	Brevi-deciduous	279 ± 19a	21.5 ± 1.5b	0.44 ± 0.08	41.4 ± 10.6	54.1 ± 9.7	4.6 ± 6.3
Astronium graveolens	Coronado	Semi-deciduous	179 ± 9cd	13.1 ± 0.7c	0.55 ± 0.07	27.3 ± 5.6	61.9 ± 8.7	10.8 ± 7.2
	PNM	Evergreen	203 ± 19bcd	15.2 ± 1.1bc	0.45 ± 0.05	30.4 ± 6.2	58.7 ± 7.7	11.0 ± 6.1
Bursera simaruba	Coronado	Deciduous	187 ± 7cd	15.5 ± 0.5bc	0.26 ± 0.04	30.3 ± 9.0	61.5 ± 6.4	8.0 ± 3.9
Cavanillesia platanifolia	PNM	Deciduous	231 ± 16abc	42.3 ± 2.9a	0.23 ± 0.03	48.6 ± 7.7	40.7 ± 6.6	10.7 ± 5.0
Cojoba rufescens	Coronado	Evergreen	203 ± 10bcd	15.8 ± 0.9bc	0.64 ± 0.05	33.3 ± 5.5	61.2 ± 6.4	5.5 ± 6.0
	PNM	Evergreen	240 ± 15ab	22.0 ± 1.6b	0.58 ± 0.04	30.8 ± 8.9	60.4 ± 10.5	8.8 ± 6.6
Genipa americana	Coronado	Deciduous	152 ± 8d	12.6 ± 0.5c	0.45 ± 0.07	46.1 ± 8.1	32.6 ± 7.5	21.4 ± 8.1

For height and basal diameter (diam.), values are mean ± SE. Differences among species were tested with one-way ANOVAs followed by Tukey's honestly significant difference test; species that *share letters* do not differ

For stem density and stem composition, values are mean ± SD

PNM Parque Natural Metropolitano

[a] Leaf phenology descriptions refer to saplings (see Fig. S1)

[b] Stem density is dry weight per fresh volume of stem, including bark, xylem, and pith

to wood density; see Table 1). We measured *C* and tracked leaf area, Ψ_{stem}, and stem water released during seasonal or monthly intervals. Specifically, our objectives were to determine:

1. The extent to which the seasonal release of stored water and seasonal change in Ψ_{stem} are related to *C*.
2. How the proportion of bark, xylem and pith tissue in the stem influences *C* among species.
3. Which stem tissues release water during seasonal droughts.

Materials and methods

Study sites, species, and censuses

Samples were collected in two forests in the Republic of Panama—a dry forest and a transitional forest. The dry forest was the Eugene Eisenmann Reserve in Coronado (8°31′N, 79°53′W), a 43-ha private reserve with mean rainfall of 1592 mm year^{-1} (measured from 2001 to 2010 by the Empresa de Transmision Electrica in Chame, 8 km west of the study site). The transitional forest was the Parque Natural Metropolitano in Panama City (8°59′N, 79°32′W), a 232-ha protected area with mean rainfall of 1800 mm year^{-1}. Both forests are mature secondary forests and experience an annual dry season from mid-December to May. In each forest, four species were selected for study (Table 1). Two species were shared between the forests, so six species were sampled in total: *Astronium graveolens* Jacq. (Anacardiaceae); *Annona hayesii* Saff. in Standl. (Annonaceae); *Bursera simaruba* (L.) Sarg. (Burseraceae); *Cojoba rufescens* (Benth.) Britton and Rose (Fabaceae); *Cavanillesia platanifolia* (Bonpl.) Kunth (Malvaceae); and *Genipa americana* L. (Rubiaceae). Henceforth, the species will be referred to by genus name only. Since there are few data on leaf phenology, particularly of saplings, we characterized the phenology of saplings in both forests by comparing leaf area during the wet season with four measurements during the 2013 dry season (Electronic supplementary material, Fig. S1).

Sample collection

At each site, we searched an area of ~2 ha and selected healthy looking saplings that were 120–400 cm in height. During the mid-late wet season (August–December 2012) and near the end of the subsequent dry season (March–April 2013), we collected stems from eight saplings (four at predawn, four at midday) of each species in each forest ($n = 128$ stems). For the three deciduous species (Table 1), which flushed leaves near the onset of the wet season, we also collected stems from eight saplings (four at predawn, four at midday, $n = 24$ stems) during leaf flush (May 2013; Fig. S1). Species differed in basal diameter and height due to differences in allometry (Table 1). Each stem, at least 110 cm long, was collected by cutting near the base with pruning shears, quickly sealed in opaque plastic bags that were humidified with wet paper towels, and transported to the laboratory to measure water content. An additional set of stems ($n = 89$) was collected during the 2012 wet season and bench-dried for assessing *C*; they were removed from their bags in the laboratory to allow them to air dry for 2–120 h and then re-sealed in the bags for 2 h before subsequent measurements.

Stem water potential

In the laboratory, leaf water potential was measured with a Scholander pressure chamber on three leaves per stem and averaged. Leaf water potential was assumed to equal Ψ_{stem} because sealing the stems in opaque bags stopped transpiration. For the stems of deciduous species collected during the dry season, which were leafless, we measured Ψ_{stem} by cutting the stem segment 10 cm from the apical meristem, placing the section with the apical meristem in the pressure chamber, and then pressurizing until equilibrium pressure. We verified the accuracy of these leafless Ψ_{stem} measurements on a subset of samples that was also measured with psychrometers (Fig. S2).

Stem water released

After measuring Ψ_{stem}, each stem was submerged in tap water and a 15-cm-long segment at >50 cm from the cut base was removed for measurement of stem water released. On the distal end, the stem, cambium, and pith diameters were measured with calipers to calculate areas of bark, xylem and pith (Table 1). The segment was blotted dry and measured for fresh mass, then for volume using water displacement on a digital balance. The segments were submerged in distilled water for 26.4 ± 0.7 h (mean ± SE), then removed, blotted dry, and measured for saturated mass. After the first saturated mass measurement, 197 of the 241 samples were submerged again in distilled water and measured again for saturated mass after an additional 35.4 ± 1.8 h. Interpolation was used to calculate the saturated mass of samples at a standardized time of 48 h of submersion. For samples with one measurement, we used the species-specific mean slope of the initial to final saturated mass to calculate the saturated mass at 48 h (Fig. S3).

While it is common to measure saturated mass at 24 h, the ideal submersion time is equivocal because over-saturation of capillary spaces may produce artifactual results (Tyree and Yang 1990). We used 48 h because our stem segments were wider and longer than in other studies. Moreover, we verified that the stems were near the theoretical maximum saturation at 48 h and that the values of saturated water content were not affected by the season in which stems were collected (Fig. S4). The segments were oven dried at 60 °C to constant mass to obtain the dry mass. Stem water released was normalized on a stem volume basis by calculating (saturated mass − fresh mass)/stem segment volume.

Hence, all values for water released are relative to saturated stems. Since saturated stems are unlikely to occur in the field, the use of stored water is indicated by differences between sets of water-released measurements. These include dry season minus wet season to infer seasonal use of stored water, midday minus predawn to infer daily use of stored water, or flush minus dry season to infer stored water use during leaf flush at the onset of the wet season.

Relative water content in bark, xylem, and pith

Tissue-level relative water content (RWC) was measured on the stems collected from the dry forest during the wet and dry seasons, and during leaf flush for the deciduous species ($n = 80$ stems), but not on stems that were bench-dried to calculate C or on stems collected from the transitional forest. A 2-cm segment basal to that used for stem water released was cut under water, then dissected into bark (all tissue radially distal to the xylem, including the cambium), xylem, and pith. To minimize water loss during dissection, we enveloped the stem segments in moist paper towels and completed each dissection in <10 min. We measured the mass of the segment before dissection and then compared it to the sum of the dissected masses of bark, xylem and pith. The sum of the dissected tissue masses was 96.2 ± 0.2 % of the whole-segment mass. Considering that the loss also represents pieces of tissue that were lost, very little or no water was lost during dissection. After each tissue was measured for fresh mass, it was submerged in distilled water for 31.4 ± 1.3 h, and weighed for saturated mass. Samples from 36 of the 80 stems were then submerged in distilled water for an additional 25.6 ± 2.1 h and measured again for saturated mass in order to standardize saturated mass to 48 h of submersion as described above for intact stems (Fig. S3). The tissue samples were then oven dried at 60 °C to constant mass. RWC was calculated as (fresh mass − dry mass)/(saturated mass − dry mass). Two *Astronium* and six *Cojoba* had piths that were too small to assess with our dissection method, reducing the sample size for the RWC of pith in these species.

Calculation of C and statistical analyses

All analyses were completed in R version 3.0.1 (R Core Team 2013). Since parameters such as C may vary with plant size, we tested, for each species, whether basal diameter or height differed among seasonal or diurnal samples (two-way ANOVAs, $P > 0.1$). Since none differed, we did not include diameter or height as explanatory factors for water-relation parameters.

We calculated C for each species in each forest as the slope of the plot of water released as a function of Ψ_{stem} using standardized-major-axis regression in the smatr package (Warton et al. 2006). In order to test whether C values generated from water-release curves of bench-dried stems accurately predict water-release curves of saplings in the field, we used only stems collected during the wet season and either measured these directly or bench-dried them in order to calculate C. Since C decreases as Ψ_{stem} decreases, we fit the regression only on the samples within the Ψ_{stem} range that each species reached in the field (Richards et al. 2014). To our knowledge, assessing C by bench drying long stem segments, each to a single Ψ_{stem}, is a novel technique, so we compared our C values to others that we produced with the common method of sequentially drying short stem segments to obtain repeated measures of water released and psychrometrically determined Ψ_{stem}. For the three species on which we compared the two methods, the C values were either indistinguishable or were higher by 27–79 % using the independent sample/long-stem method (Fig. S5). The difference may be due to artifacts introduced by sequentially drying short stem segments (see "Discussion"; Tyree and Yang 1990).

For each water-relation parameter, a two-way ANOVA was fit for each species, with season, time of day (predawn vs. midday), and their interaction as fixed effects. Response variables were Ψ_{stem}, stem water released, and tissue-level RWC. Linear contrasts were used to test for the a priori hypotheses that the parameters differed between seasons and between times of day within season, correcting for multiple comparisons to $\alpha = 0.05$ with the false discovery rate method of Benjamini and Hochberg (1995). Finally, to test whether C predicts seasonal changes in Ψ_{stem} and water released among species, we used simple linear regression with C as the independent variable and species' means of the difference between wet and dry-season Ψ_{stem} and water released as dependent variables, respectively.

Results

Stem capacitance

C ranged among species from 36.7 to 140.5 kg m^{-3} MPa^{-1} in the transitional forest and 32.3–195.2 kg m^{-3} MPa^{-1} in

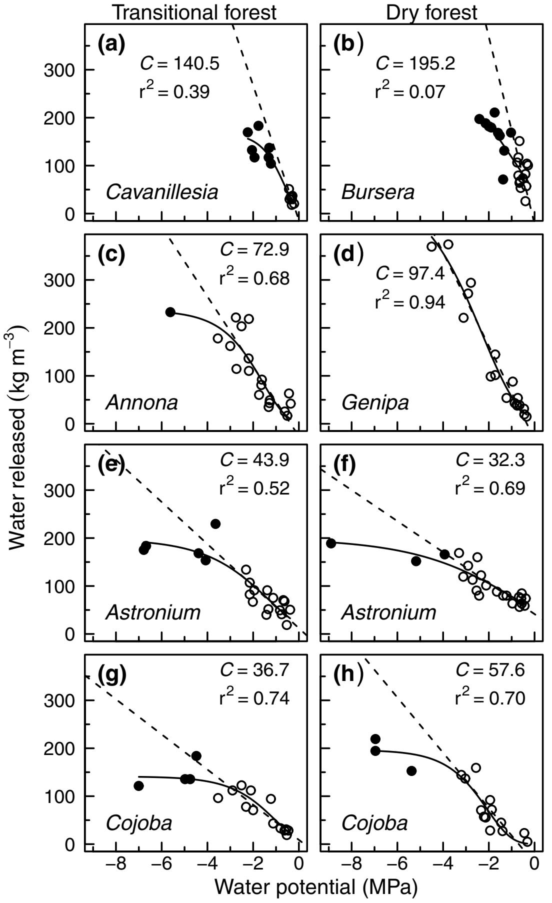

Fig. 1 The relationship between stem water potential (Ψ_{stem}) and stem water released for four species (*Cavanillesia platanifolia*, *Annona hayesii*, *Astronium graveolens*, *Cojoba rufescens*) in a transitional forest (**a**, **c**, **e**, **g**) and four species (*Bursera simaruba*, *Genipa americana*, *A. graveolens*, *C. rufescens*) in a dry forest (**b**, **d**, **f**, **h**). Henceforth, the species will be referred to by genus name only. *Each symbol* represents a separate plant. *Open circles* are samples within the range of Ψ_{stem} that species reached in the field (see Fig. 2) and were included in the standardized-major-axis regression analysis to compute capacitance (*C*) as the absolute value of the slope of the dashed line. **a–h** *C* (kg m^{-3} MPa^{-1}) and the r^2 are indicated. *Filled circles* Stems were bench-dried to lower the Ψ_{stem} relative to that reached in the field and were not included in the calculation of *C*, s*olid lines* indicate Gompertz functions fit through both open and closed circles

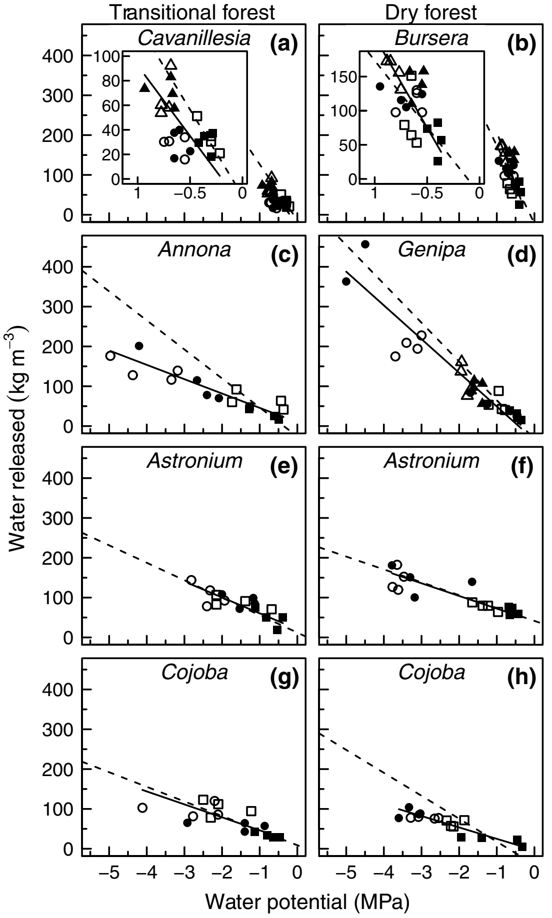

Fig. 2 Daily and seasonal relationship between Ψ_{stem} and stem water released for four species in a transitional forest (**a**, **c**, **e**, **g**) and four species in a dry forest (**b**, **d**, **f**, **h**). *Each symbol* represents a separate plant. Samples collected during the wet season (*squares*), dry season (*circles*), leaf flush (*triangles*), predawn (*filled symbols*), and midday (*open symbols*). *Dashed lines* represent the water-release curves of bench-dried samples used to calculate capacitance (see Fig. 1), *solid lines* represent standardized-major-axis regressions fit through all *points* in the scatter plots. **a**, **b** *Insets* have smaller axis ranges to show detail

the dry forest (Fig. 1). Among species, *C* decreased with increasing stem density at a slope and intercept that did not differ between forests (analysis of covariance—stem density, $F = 28.4$, $P = 0.006$; forest, $F = 4.9$, $P = 0.09$; stem density × forest, $F = 0.48$, $P = 0.53$; simple linear regression with all species combined, $C = -339 \times \text{stem density} + 273.4$, $r^2 = 0.68$). For the two species measured in both the transitional forest and the dry forest, *C* differed between the two forests (Fig. 1e–h). For *Astronium*, *C* was 36 % higher in the transitional forest than the dry forest (smatr analysis, $P = 0.006$). In contrast, for *Cojoba*, *C* was 57 % higher in the dry forest than in the transitional forest (smatr analysis, $P = 0.053$).

Stem water released

For most species, the amount of stem water released per decrease in Ψ_{stem} during the dry season closely tracked that

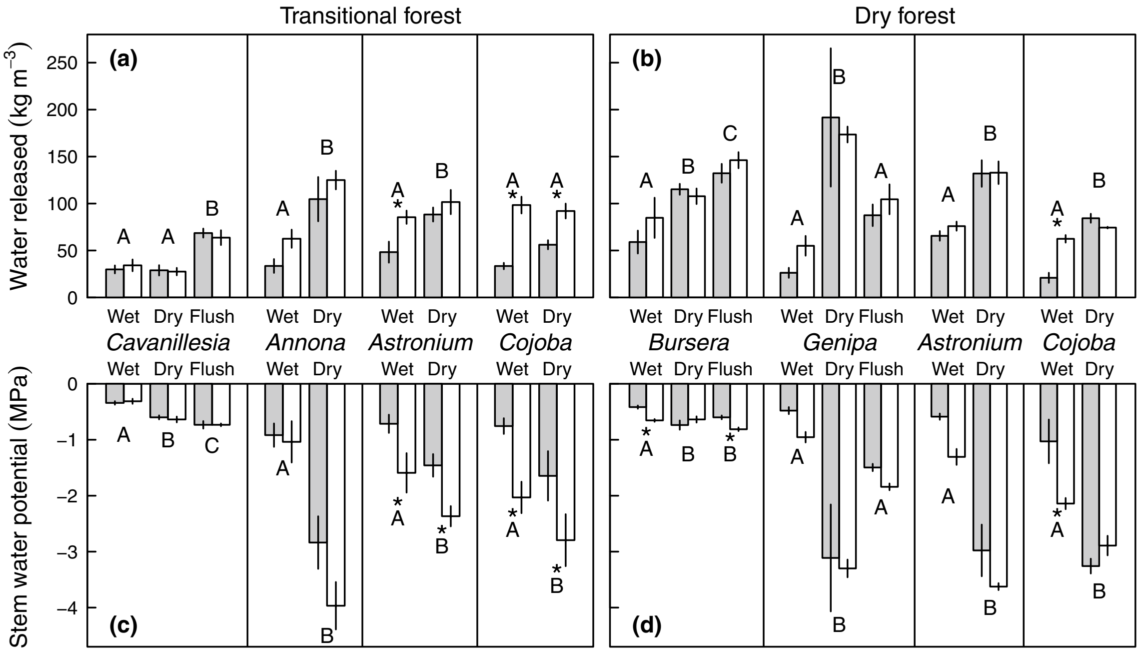

Fig. 3 Daily and seasonal stem water released (**a**, **b**) and Ψ_{stem} (**c**, **d**). *Filled bars* Predawn measurements, *open bars* midday measurements ($n = 4$ per bar). *Error bars* extend to ±1 SE. *Asterisks* indicate significant differences between predawn and midday values within seasons ($P < 0.05$). Seasons that *share letters* did not differ significantly. Because water released is relative to saturated stems, our measure of stored water use is the difference between two conditions. The key comparisons are predawn vs. midday, wet vs. dry, and dry vs. flush (see "Materials and methods"). *Wet* Wet season, *Dry* dry season, *Flush* leaf flushing at the end of the dry season

of the bench-dried stems used to compute C (cf. solid and dashed lines in Fig. 2). However, *Annona* in the transitional forest and *Cojoba* in the dry forest had significantly lower water released per decrease in Ψ_{stem} in the field than predicted by C (smatr analysis, $P < 0.001$).

All species had significantly higher stem water released during the dry season than during the wet season, except for *Cavanillesia* and *Cojoba* in the transitional forest (Fig. 3a, b; P-values for all ANOVAs are in Table S1). Since our measure of seasonal use of stored water is the difference in water released between wet and dry seasons, aside from the exceptions noted, all other species used stored water during the dry season. Water released at midday was significantly higher than at predawn during the wet season for *Astronium* in the transitional forest and for *Cojoba* in both forests and also during the dry season for *Cojoba* in the transitional forest (Fig. 3a, b). *Annona*, *Bursera*, and *Genipa* had similar, yet non-significant, trends for higher water released at midday than at predawn during the wet season; however, during the dry season this trend did not occur within most species.

While flushing leaves in early May 2013, *Cavanillesia* and *Bursera* had significantly higher water released than during the preceding dry season (compare flush vs. dry in Fig. 3a, b; linear contrasts $P < 0.001$ and $P = 0.039$, respectively). Hence, stem water was used during leaf flush. In contrast, *Genipa* had less water released during leaf flush than during the dry season (Fig. 3b; linear contrast $P = 0.004$). This indicates that during leaf flush, *Genipa* stems were actually more hydrated than during the dry season (late March–early April). *Genipa* saplings were likely partially rehydrated by 85 mm of rain that fell in several events in April and May before we conducted our analyses of leaf-flushing stems (Fig. S1b).

Stem water potential

At both sites, all species had significantly lower Ψ_{stem} during the dry season than during the wet season (Fig. 3c, d). Dry-season Ψ_{stem} ranged widely among species, for example, at the transitional forest, from −0.62 ± 0.03 to −3.40 ± 0.036 MPa (mean ± SE, predawn and midday combined). Within seasons, Ψ_{stem} was significantly lower at midday than at predawn for *Astronium* and *Cojoba* in the transitional forest during the wet and dry seasons, *Bursera* during the wet season and during leaf flush, and *Cojoba* in the dry forest during the wet season (Fig. 3c, d). Trends for Ψ_{stem} to be lower at midday than at predawn were common

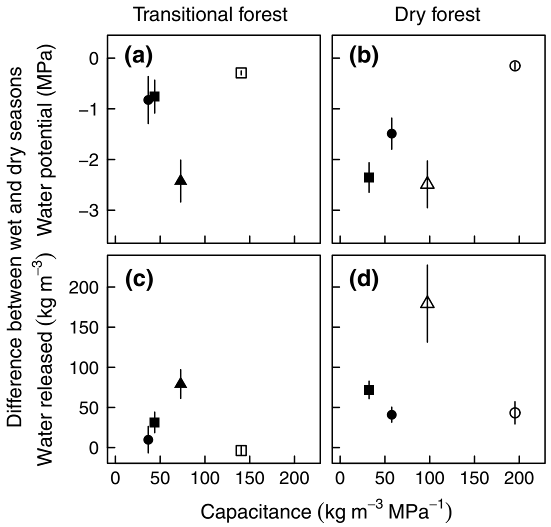

Fig. 4 The difference between wet and dry seasons for Ψ_{stem} (**a**, **b**) and stem water released (**c**, **d**) in relation to capacitance. *Points* represent means for species. *Filled circle Cojoba*, *filled square Astronium*, *filled triangle Annona*, *open square Cavanillesia*, *open triangle Genipa*, *open circle Bursera*. *Bars* extend to ±1 SE

among the other species, yet they were not significant (Fig. 3c, d).

When *Cavanillesia* flushed leaves near the onset of the wet season, its Ψ_{stem} was lower than during the dry season (linear contrast $P = 0.039$), while *Bursera* Ψ_{stem} did not differ from that of the dry season (linear contrast $P = 0.7$; Fig. 3c, d). In contrast, while *Genipa* flushed leaves near the onset of the wet season, it had significantly higher Ψ_{stem} than during the dry season (Fig. 3d). Again, for *Genipa*, this may reflect stem rehydration from rainfall early in the wet season (Fig. S1b).

Seasonal change in water released and Ψ_{stem} in relation to C

Among all four species in each forest, C was not related to the seasonal change in Ψ_{stem} or the amount of stored water used during the dry season (difference in water released between wet and dry seasons, $P > 0.14$; Fig. 4). *Cojoba* and *Astronium* in the transitional forest were distinct in that they had low C but showed a small change in Ψ_{stem} (Fig. 4a). In both forests, the deciduous species with high C, *Cavanillesia* and *Bursera*, also had distinctive responses. These had the lowest seasonal changes in Ψ_{stem} (Fig. 4a, b), yet these species differed in seasonal change in stem water released. In the transitional forest, *Cavanillesia* had essentially zero change in water released between the wet and dry seasons (Fig. 4c), meaning that its stems did not lose water during the dry season. In the dry forest, *Bursera* showed an increase in water released between the wet and dry seasons (Fig. 4d), meaning that, in contrast to *Cavanillesia*, significant water was lost from its stems. *Astronium* and *Cojoba* lost similar amounts of water as *Bursera*, whereas *Genipa* registered the highest loss of stem water during the dry season for any species (Fig. 4c, d).

Water storage in bark, xylem, and pith

Species ranged widely in the proportion of stem composed of bark, xylem, and pith (Table 1), yet C was not correlated with stem-tissue composition (bark proportion vs. C, Pearson's $r = 0.35$, $P = 0.39$; xylem proportion vs. C, $r = -0.29$, $P = 0.49$; pith proportion vs. C, $r = 0.06$, $P = 0.88$). We measured tissue level RWC in the dry-forest species. Bark RWC was correlated with Ψ_{stem} in all species (Fig. 5a, d, g, j). Bark RWC was significantly lower during the dry season than during the wet season in all species except *Bursera*, in which it was lower during leaf flush than during the wet and dry seasons. In contrast, xylem RWC was not correlated with Ψ_{stem} and did not differ between wet and dry seasons for any species (Fig. 5b, e, h, k). Pith RWC was correlated with Ψ_{stem} only in *Genipa* (Fig. 5f). The only significant decrease in RWC from predawn to midday occurred in *Bursera* bark during leaf flush (Fig. 5a; linear contrast $P = 0.016$).

Discussion

Variation in C within and among species and ontogenetic stages

In adult trees, the C of sapwood generally decreases among species as sapwood density increases, but the slope of the relationship varies greatly among sites (Meinzer et al. 2008a; Richards et al. 2014). We measured saplings and found that stem C decreased with increasing stem density at a slope that was similar between the transitional and dry forests. Our C values for any given stem density were about 25–230 % lower than values previously reported for the sapwood of adult trees of other species in other forests (Meinzer et al. 2008a) and in adult trees in one of our study sites, Parque Metropolitano [four trees measured had ranges of sapwood density and C of 0.28–0.52 g cm^{-3} and 80–415 kg m^{-3} MPa^{-1}, respectively (Meinzer et al. 2003)]. This difference might reflect an ontogenetic shift, as C tends to increase with tree height (Scholz et al. 2011), possibly due to changes in xylem structure (Gartner 1995). Tall trees may benefit more than saplings from high C because they use stored water to overcome the time lag in transporting soil water to the canopy, which increases with tree height (Scholz et al. 2011).

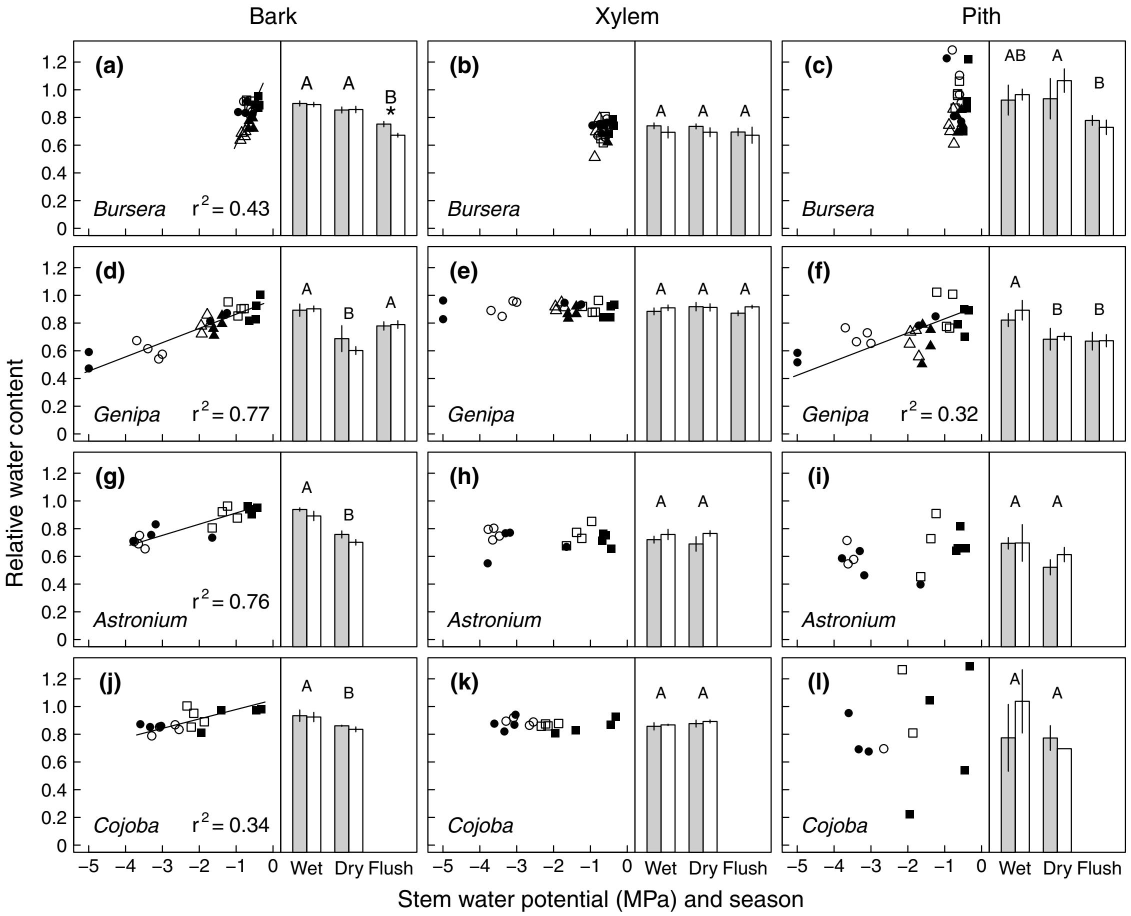

Fig. 5 Relative water content of bark (**a**, **d**, **g**, **j**), xylem (**b**, **e**, **h**, **k**), and pith tissues (**c**, **f**, **i**, **l**) in relation to Ψ_{stem} and season in the dry forest. *Symbols* in scatter plots and bar plots are drawn as in Figs. 2 and 3, respectively. For tissues with correlation coefficients with $P < 0.05$, the standardized-major-axis regression lines and r^2-values are given

Other factors may have led to lower *C* in our study than in previous studies. For example, we measured the *C* of whole stem segments as opposed to the common method of using sapwood cores. Using sapwood cores excludes the bark and pith. Possibly, these tissues have lower *C* than sapwood (Scholz et al. 2007). Using sapwood cores may also introduce bias because the water column within xylem vessels is broken during core extraction, converting water held tightly within vessels into easily extractable capillary water (Tyree and Yang 1990). Finally, studies vary in how *C* is calculated. Many have included values of Ψ_{stem} above those experienced in the field, including 0 MPa. Because *C* commonly declines with declining Ψ_{stem}, this may inflate *C* values (Richards et al. 2014). While we require more research on how methodology influences the value for *C*, we posit that bench-drying long, intact stem segments rather than sapwood cores more closely simulates drying in vivo, and therefore likely produces more reliable *C* measurements (Tyree and Yang 1990).

None of the saplings that we measured in the field reached the Ψ_{stem} at which *C* decreased (i.e., they did not reach the portion of the water release curves with shallow slopes in Fig. 1). This result coincides with results from adult trees measured by Meinzer et al. (2008b), who predicted that regulation of Ψ_{stem} above the shift to low *C* is a convergent trait among species that acts to optimize stored water use while preventing hydraulic failure (but see Richards et al. 2014). Although desiccation tolerance is little studied, *Cojoba* can tolerate desiccation to −8.1 MPa with a 50 % survival rate (Kursar et al. 2009). Hence, both phases of our water release curves that are fit to a Gompertz function may have physiological significance (Fig. 1; solid lines). For *Cojoba*, the steeper phase down to −3 MPa may indicate the Ψ_{stem} for which water storage is important. In the range from −3 to −8 MPa, with a very low value for *C* (a shallow slope), desiccation tolerance may be more critical.

The two species measured in both forests had *C* values that varied between forests; *Astronium* had higher *C* in the transitional forest while *Cojoba* had higher *C* in the dry forest (Fig. 1e–h). Similarly, Barnard et al. (2011) found plasticity in *C* among conifers in forests that varied in rainfall, yet both species they studied had higher *C* at low-rainfall sites. Our result that *C* shifted to be higher or lower in the dry forest is consistent with either greater desiccation avoidance (higher *C*) or greater desiccation tolerance (lower *C*) in drier environments (discussed below).

Comparisons among species and forests of stored water use during the dry season

First, we tested the assumption that most species did not have access to soil water during the dry season. For species with access to soil water, we predicted release of water and decrease in Ψ_{stem} during the daytime (i.e., significant differences between midday and predawn for water released and Ψ_{stem}). Only *Cojoba* in the transitional forest showed this pattern (Fig. 3), suggesting that *Cojoba* saplings likely maintained their stems hydrated during the dry season by accessing soil water. In fact, its stems are hydraulically functional at much lower Ψ_{stem} than that experienced in the transitional forest during the dry season; severe wilting associated with loss of hydraulic conductance occurs at −6.9 MPa in this species (Kursar et al. 2009).

By contrast, it appears that the other species and *Cojoba* in the dry forest did not have access to soil water during the dry season. For these, we predicted that stem water would be released very slowly in the dry season (and recharged during the wet season). Consistent with that, we found that, for most species in both forests, water released in the dry season exceeded water released in the wet season (Fig. 3a, b). This pattern indicates gradual (seasonal) water loss occurred despite shedding leaves or closing stomata. For many species, it seems that it may be impossible for saplings to avoid significant water loss during seasonal droughts. Instead, we suggest that many species rely on the capacitive effect of stored water to buffer Ψ_{stem} against water loss during the dry season (Figs. 2, 3). In other words, although stem water is lost, the rate is low and the stored water buffers Ψ_{stem} in order to provide a margin of safety against hydraulic failure during drought.

In the transitional forest, the species with the highest *C*, *Cavanillesia*, did not have higher water released during the dry season than the wet season (Fig. 3a), suggesting that it does not rely on stored water to buffer Ψ_{stem} against seasonal water loss. *Cavanillesia* may have accessed soil water during the dry season as *Cojoba* appears to have done, but the water would have to have been from much deeper soil because *Cavanillesia* maintained much higher Ψ_{stem} than *Cojoba* did (predawn Ψ_{stem}, -0.60 ± 0.04 vs. -1.65 ± 0.44 MPa). Since tropical deciduous species tend to have shallow roots (Eamus and Prior 2001), it is more likely that dry-season water released was low in *Cavanillesia* due to deciduousness and high resistance to water loss from the stem. Thus, despite its relatively high *C*, *Cavanillesia* appears to rely on water retention to maintain Ψ_{stem} during the dry season. In contrast, the other species with high *C*, *Bursera*, appears to rely on capacitive effects to maintain Ψ_{stem} during the dry season; its stems lost a similar amount of water as those of the co-occurring evergreen species, yet its Ψ_{stem} remained near wet-season levels (Fig. 4b, d). This contrast between the two deciduous, high-*C* species could have resulted because they differ in their water-use strategies; for example, *Bursera* bark may be more permeable to water than *Cavanillesia* bark. Alternatively, the drier conditions that *Bursera* experienced in the dry forest may have caused it to lose more water than *Cavanillesia* in the transitional forest.

Timing and use of stored water in leaf flush

Cavanillesia and *Bursera* are similar to three *Adansonia* species (baobab trees) in Madagascar in that they have higher water released during leaf flush than during the dry season, meaning that they used stored water to flush leaves (Chapotin et al. 2006). This suggests that the use of stored water to flush leaves is common among species with high *C*. Indeed, among 19 tree species in a Brazilian SDTF, only the six species with the lowest wood density (<0.55 g cm^{-3}) initiated leaf and flower production before the onset of the wet season (Lima and Rodal 2010). Since water from early wet-season rain is inaccessible to plants until soil moisture is recharged to $\Psi_{\mathrm{soil}} > \Psi_{\mathrm{stem}}$, and since saplings with high *C* maintain very high Ψ_{stem}, leaf flushing in these saplings would be greatly delayed if it relied on soil water. Thus high *C* in these species is likely an adaptation that permits leaf flushing early in the transition to the wet season. Although their stomata likely remain closed until soil water is available, flushing leaves early would extend their productive season (Chapotin et al. 2006), and could facilitate photosynthesis during the early wet season, when understory light levels are higher due to reduced leaf area among canopy trees (Brenes-Arguedas et al. 2011). Also, early leaf flushing could help trees to escape herbivore pressure on susceptible, expanding leaves, which is higher during the wet season (Aide 1988).

In contrast to *Cavanillesia* and *Bursera*, *Genipa* saplings had lower water released during leaf flush than during the preceding dry season, suggesting that they did not rely on stem-stored water to flush leaves. Since *Genipa* saplings reached relatively low Ψ_{stem} during the dry season (Fig. 3b), early wet-season rains would rehydrate them sooner than *Cavanillesia* and *Bursera* Still, *Genipa* saplings initiated leaf flush before their stems were fully hydrated, evidenced by Ψ_{stem} that was lower during leaf flush than during the wet season (predawn Ψ_{stem}, -1.50 ± 0.07 vs. -0.48 ± 0.06 MPa; Fig. 2d). In contrast to *Genipa*, adult trees of various deciduous species in a Costa Rican dry forest initiated leaf flush only after their tissues were fully hydrated (Borchert 1994b). Our results suggest that full stem hydration is not a universal prerequisite to flushing leaves. However, *Annona*, *Astronium*, and *Cojoba* saplings flushed leaves only after several wet-season rainfall events (Fig. S1), which is a common observation among trees in SDTF (Daubenmire 1972; Lieberman 1982; Borchert et al. 2002).

The role of pith and bark in buffering xylem tension

Most studies of stored water use in tropical trees have focused on the role of sapwood, which, in adult trees, composes a larger proportion of stem volume than bark and pith (Schulze et al. 1988; Meinzer et al. 2003). However, bark and pith composed 38–67 % of the stem volume of the saplings we studied (Table 1). In other plants that have relatively large pith, such as giant rosettes (*Espeletia* spp.), daily xylem tension is buffered by water stored within the pith (Goldstein et al. 1984). In the desert shrubs *Pittocaulon* spp., which also have relatively large bark and pith, a similar role has been hypothesized, whereby water stored within the bark and pith buffers xylem tension seasonally (Olson 2005).

Our results support the hypothesis that water stored in bark helps to buffer xylem tension in SDTF saplings: bark RWC decreased with Ψ_{stem} seasonally while xylem RWC remained constant for all species (Fig. 4). Yet we cannot confirm whether the water released from the bark during the dry season entered the xylem. Alternatively, xylem RWC could be uncorrelated with Ψ_{stem} due to differences among plants in their drought histories and extent of cavitated xylem vessels. If embolisms form and do not refill when Ψ_{stem} rises, then subsequent xylem RWC will be anomalously low. In our study, such a process is unlikely since xylem RWC showed little variability in relation to Ψ_{stem} (Fig. 5b, e, h, k). Similar to our results, sapwood RWC in adult trees of *Adansonia* species did not vary between wet and dry seasons (Chapotin et al. 2006). However, since the water-use strategies of *Adansonia* may differ from species with higher wood density (Chapotin et al. 2006), it is not clear that the absence of seasonal change in xylem RWC is applicable more generally to SDTF trees.

Scholz et al. (2007) found that daily changes in bark RWC were less than or the same as in sapwood RWC during the dry season in Brazilian savanna tree species, suggesting that water stored within bark does not buffer xylem tension. However, these results do not directly contrast with ours since Scholz et al. (2007) did not measure seasonal changes in RWC. Dry-forest trees experience relatively large reductions in stem diameter during seasonal droughts (Lieberman 1982; Reich and Borchert 1984), which are caused mostly by changes in water content of the inner bark (De Schepper et al. 2012). It is possible that xylem RWC fluctuates in response to changes in Ψ_{stem} on hourly and daily timescales but remains nearly constant on seasonal timescales through water exchange with pith and bark. Studies that have measured xylem RWC in SDTF trees have generally only made measurements during the dry season, and so do not provide information on seasonal flux. The relatively large seasonal flux in bark RWC (Fig. 5) suggests that bark-stored water is important in the drought response of tropical trees and should be addressed with future studies.

Fitting capacitance and stored water use with plant functional types

Choat et al. (2012) showed with a meta-analysis that a key trait, resistance to cavitation, varies considerably among species from dry biomes. Consistent with this observation, we found considerable variation in traits related to water storage. For example, a simple prediction is that reliance on stored water to buffer Ψ_{stem} during seasonal droughts increases from desiccation-tolerant, evergreen species to desiccation-avoiding, deciduous species. Our results do not support this prediction. Instead, we found two distinct patterns of stored water use among deciduous species, both of which varied from those of evergreen species. *Cavanillesia* and *Bursera* had high C (Fig. 1), shed their leaves early in the dry season (Fig. S1), maintained high dry-season Ψ_{stem}, used a moderate amount (*Bursera*) or no (*Cavanillesia*) stored water during the dry season (i.e., moderate or no difference in water released between the wet and dry seasons; Fig. 4), and used stored water to flush leaves (i.e., water released during leaf flush > dry-season water released; Fig. 3). In contrast *Annona* and *Genipa* had moderate C, shed their leaves later in the dry season, reached low dry-season Ψ_{stem}, had relatively large differences in water released between the wet and dry seasons, and, for *Genipa*, had lower water released during the dry season than when flushing leaves.

This dichotomy among deciduous species is similar to that described by Borchert (1994a) for adult trees in a Costa Rican dry forest. He found that deciduous species with low-density wood—which he termed “deciduous lightwood” species—maintained high Ψ_{stem} during the dry season and that stem water was depleted during flowering and leaf flush before the onset of the wet season. Others have termed such species “stem-succulent trees” or “caudiciform succulents” (Ogburn and Edwards 2010). Borchert (1994a) also identified “deciduous hardwood” species that reached low Ψ_{stem}, shed leaves gradually, and lost a large fraction of stem water during the dry season. Such species, represented in our study by *Annona* and *Genipa*, appear to be highly reliant on stem water during the dry season as they had the highest seasonal change in water released among species in each forest (Fig. 4c, d). Despite their deciduous behavior, these species do not stop water loss during the dry season. Instead their moderate *C* lessens the impact of water loss on Ψ_{stem} and, presumably, their xylem is resistant to cavitation. Similarly, in a SDTF, even after shedding their leaves during the dry season, the stems of several deciduous species contracted in association with water loss (Daubenmire 1972). These observations contrast with the prediction that deciduous species avoid desiccation and maintain high Ψ_{stem} during droughts through deciduousness (Markesteijn and Poorter 2009). Rather, in terms of Ψ_{stem}, non-stem-succulent deciduous species appear to experience the most desiccation and fit better into a strategy of desiccation tolerance.

Borchert (1994a) proposed that “evergreen softwood” trees that maintain green leaves through the dry season and have moderate wood density, such as the *Cojoba* and *Astronium* in our study, depend on soil water throughout the dry season. Our results are partially consistent with this prediction. *Cojoba* and *Astronium* had lower dry-season Ψ_{stem} in the dry forest than in the transitional forest, suggesting that Ψ_{stem} tracked Ψ_{soil}. Furthermore, in the transitional forest, *Cojoba* had a pattern of daily flux in Ψ_{stem} and water released during the dry season (Fig. 3a, c), suggesting that it relied on soil water. However, this was not the case for *Astronium* in either forest or for *Cojoba* in the dry forest, where they may not have had access to soil water during the dry season (Fig. 3b, d). Yet, these species also had lower seasonal change in water released than *Annona* and *Genipa* despite maintaining leaves through the dry season (Fig. 4). These species may have better access to soil water through deeper roots, a common trait among evergreen, dry-forest species (Markesteijn and Poorter 2009). However, between the two evergreen species that we studied, there was a divergence in water-use patterns between the transitional and dry forests. *Cojoba* had higher *C* in the dry forest than in the transitional forest while *Astronium* had lower *C* in the dry forest than in the transitional forest (Fig. 1). Also, *Cojoba* and *Astronium* had similar seasonal change in Ψ_{stem} and water released in the transitional forest, but in the dry forest *Astronium* had greater seasonal change in Ψ_{stem} and water released than *Cojoba* (Fig. 4). Hence, in the drier forest, it appears that *Cojoba* shifts toward desiccation avoidance while *Astronium* is more desiccation tolerant. This divergence in strategies is consistent with comparisons of congeneric species in which one species inhabits wetter valleys and the other drier plateaus of Mexican dry forest. Surprisingly, suites of functional traits, including wood density, varied in opposite directions between habitats depending on the species pair (Pineda-García et al. 2011). Taken together, our results suggest that stored water use and seasonal changes in Ψ_{stem} are not directly related to *C*, but rather to the combination of *C* and other key traits such as resistance to cavitation, leaf phenology, and rooting depth. Hence, for habitats that experience drought, a better understanding of water storage will be important for understanding species distributions in relation to soil water availability and predicting responses to future drying.

Author contribution satement B. T. W. and T. A. K. conceived and designed the experiments. B. T. W. performed the experiments and analyzed the data. B. T. W. and T. A. K. wrote the manuscript.

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Table S1 *P*-values from two-way ANOVA models for the effects of season (wet, dry, and leaf flush) and time (predawn and midday) on water-relation parameters in saplings of six species distributed across two seasonally dry forests in Panama. PNM = Parque Natural Metropolitano. RWC = relative water content.

Species	Forest	Factor	Stem water released	Stem water potential	Bark RWC	Xylem RWC	Pith RWC
Annona	PNM	Season	0.001	0.000			
		Time	0.145	0.127			
		Season × time	0.850	0.210			
Astronium	Coronado	Season	0.000	0.000	0.000	0.745	0.089
		Time	0.561	0.017	0.055	0.142	0.528
		Season × time	0.708	0.885	0.838	0.605	0.551
Astronium	PNM	Season	0.013	0.008			
		Time	0.022	0.003			
		Season × time	0.327	0.948			
Bursera	Coronado	Season	0.000	0.004	0.000	0.596	0.015
		Time	0.251	0.007	0.088	0.218	0.595
		Season × time	0.408	0.004	0.081	0.942	0.557
Cavanillesia	PNM	Season	0.000	0.000			
		Time	0.898	0.941			
		Season × time	0.740	0.758			
Cojoba	Coronado	Season	0.199	0.000	0.014	0.272	0.671
		Time	0.000	0.130	0.546	0.514	0.608
		Season × time	0.078	0.007	0.782	0.898	0.554
Cojoba	PNM	Season	0.000	0.039			
		Time	0.004	0.005			
		Season × time	0.000	0.892			

Table S1 (continued)

Species	Forest	Factor	Stem water released	Stem water potential	Bark RWC	Xylem RWC	Pith RWC
Genipa	Coronado	Season	0.002	0.000	0.000	0.547	0.005
		Time	0.895	0.317	0.569	0.308	0.477
		Season × time	0.686	0.937	0.51	0.53	0.814

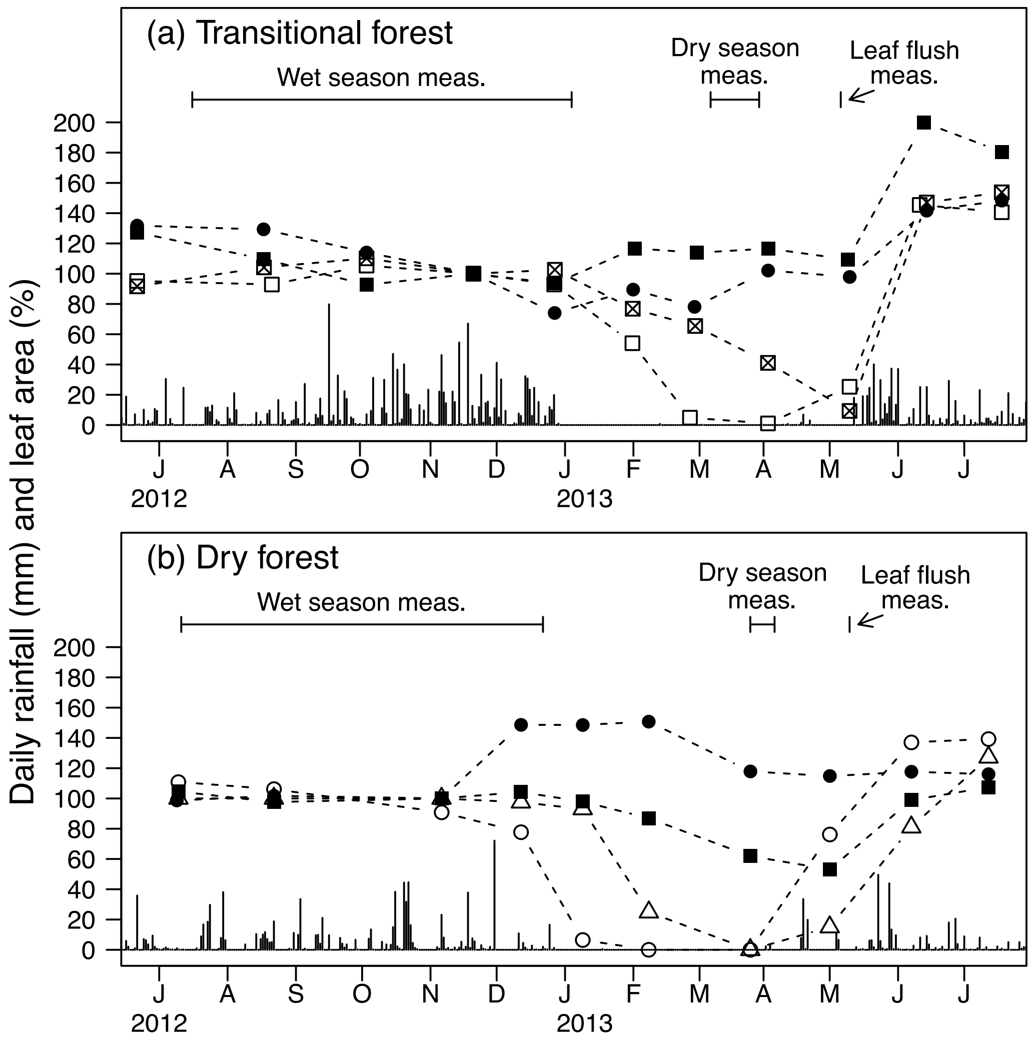

Fig. S1 Sampling dates, daily rainfall, and leaf phenology of study species in (a) the transitional forest, and (b) the dry forest. Each *symbol* represents the mean leaf area of 10 saplings as a percentage of their leaf area in the November 2012 census. *Horizontal lines* extend to the start and end of each sampling season. *Symbols* represent species as: *filled circle*, *Cojoba rufescens*; *filled square*, *Astronium graveolens*; *crossed square*, *Annona hayesii*; *open square*, *Cavanillesia platanifolia*; *open triangle*, *Genipa americana*; *open circle*, *Bursera simaruba*

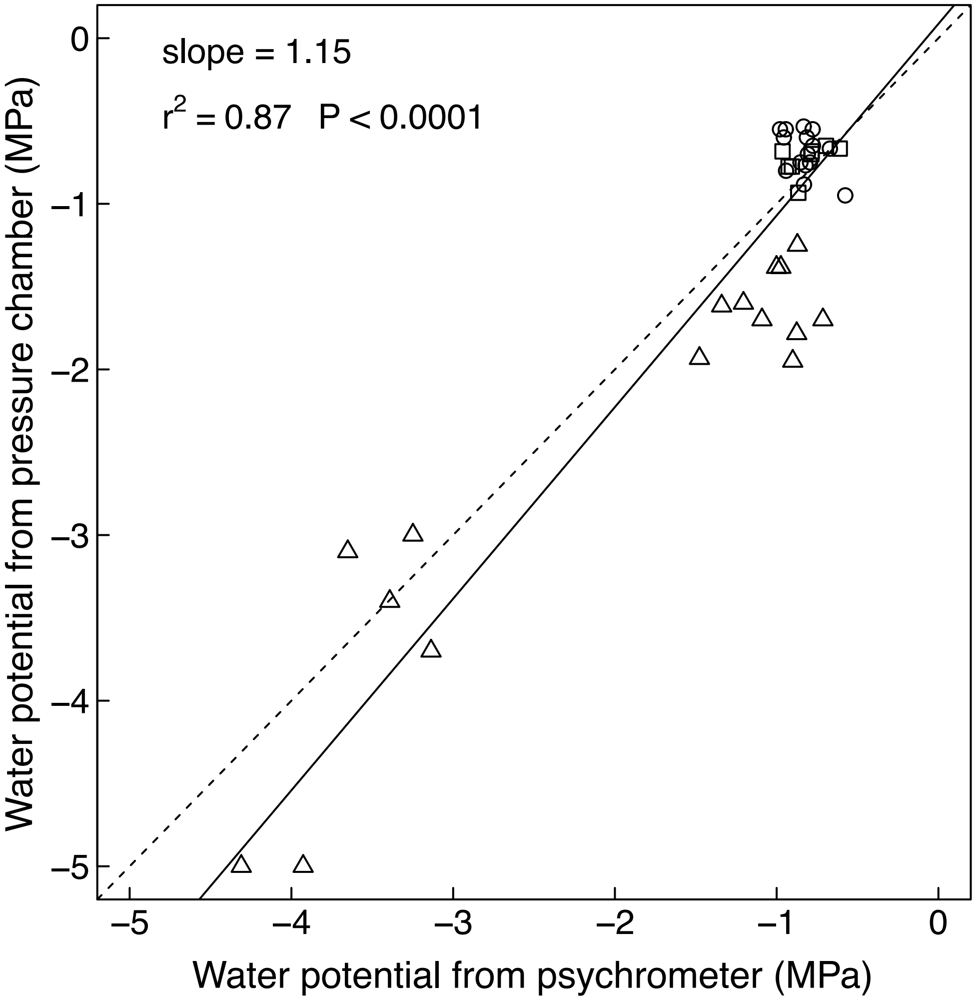

Fig. S2 Comparisons of water potential measurements of leafless stems of deciduous saplings made with psychrometers and a pressure chamber. Each *symbol* represents a separate stem; species are represented as: *triangles*, *Genipa americana*; *circles*, *Bursera simaruba;* and *squares*, *Cavanillesia platanifolia.* Pressure chamber measurements were performed on the most distal 10 cm long section of the stem. For each stem, the mean of three psychrometric measurements is represented. For each measurement, stem segments were placed in a chamber (25 mm diameter by 45 mm length) and measured for water potential following Kursar *et al.* (2005). Regression statistics are presented for a reduced major axis regression

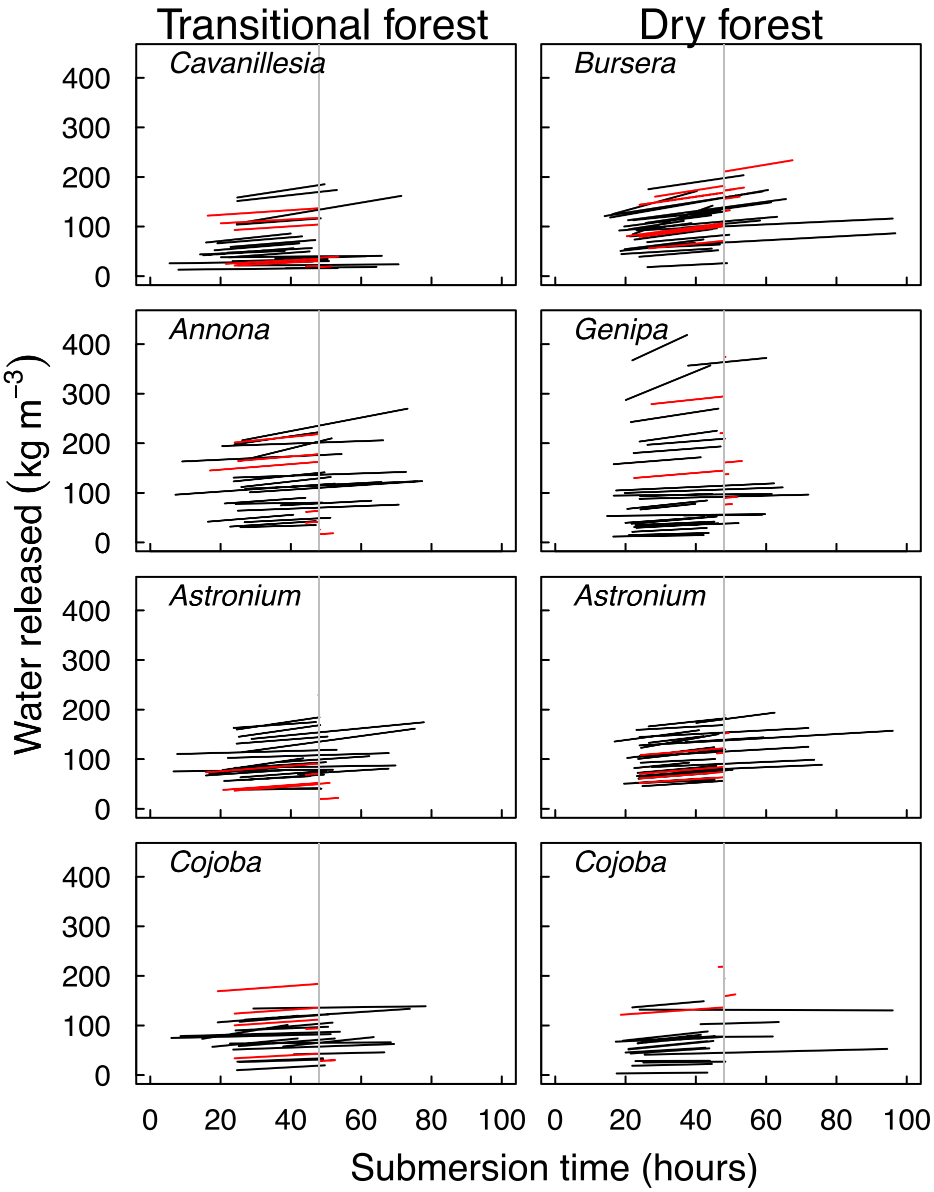

Fig. S3 The relationship between the duration that stem segments were submerged in water and values of stem water released. Each *line* represents an independent sample, that is, a separate plant. Those in *black* were measured twice for saturated water content while those in *red* were measured once. Water released was standardized among samples by calculating the saturated water content at 48 hours of submersion. For samples on which saturated water content was measured twice, we used the slope of submersion time versus water released to interpolate the water released at 48 hours. For samples on which saturated water content was measured once we used the species' mean slope of submersion time versus saturated water content to adjust water released to that of 48 hours

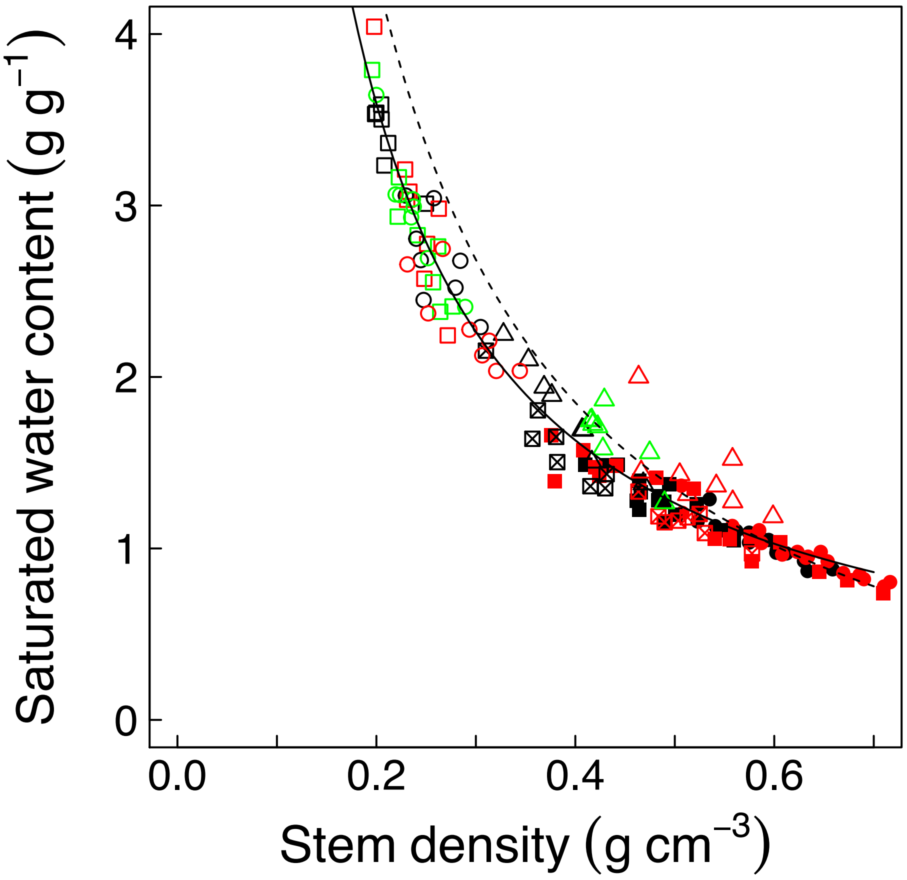

Fig. S4 The relationship between saturated water content (SWC) and stem density among study species. Each *point* represents a separate stem. *Symbols* represent species as in Fig. S1. *Black*, *red*, and *green* symbols represent samples collected in the wet, dry, and leaf flushing seasons, respectively. The *solid line* represents the fit of a least-squares regression through log-transformed values. The *dashed line* represents the theoretical maximum SWC for wood (Simpson 1993). Our samples included bark and pith, which are more elastic than wood, so SWC occasionally exceeded the theoretical maximum for wood. No difference in SWC was found among seasons with a mixed model ANOVA on log-transformed values that included stem density as a co-factor and species and site as random effects ($P = 0.6$)

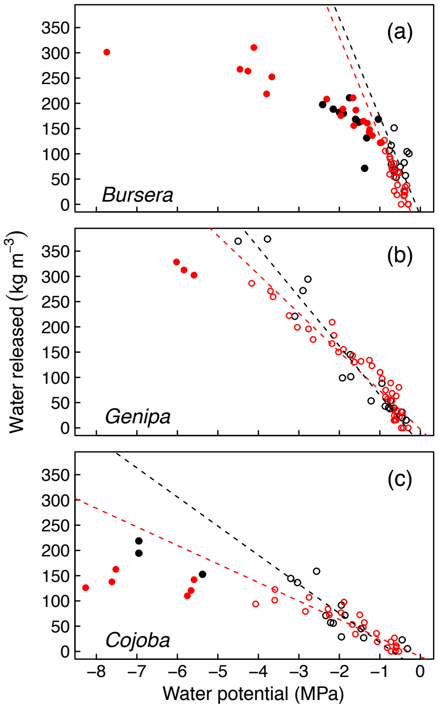

Fig. S5 Comparison of two methods for producing the water release curves that were used to compute stem capacitance. Three species from the dry forest were studied: (a) *Bursera simaruba*, (b) *Genipa americana*, and (c) *Cojoba rufescens*. *Red circles* represent short stem segments (~4 cm long) that were soaked in distilled water for 24 hours then repeatedly dried and measured for water potential and water released following the drying methods and calculation of water released of Meinzer *et al.* (2003) and the psychrometric methods of Kursar *et al.* (2005). *Black circles* represent long stem segments (> 110 cm), each from a separate plant, that were dried to a particular water potential before a 15 cm-long section was removed to measure water released (see Methods). *Open circles* represent stem segments that were within the range of water potential that the species reached in the field and were used to calculate capacitance as the slope of the curves using standardized-major-axis regression. *Filled circles* represent stem segments that were dried to water potentials lower than stems reached in the field and were excluded from the regressions. The *red and black dashed lines* represent regressions produced with short and long stem segments, respectively. Differences between slopes within each species were tested with likelihood methods in smatr. For *Bursera*, the slopes did not differ between the long- and short-segment methods (195.2 [95% CI = 106.8-357.0] vs. 196.7 [156.0-247.9], *P* = 0.98). For *Genipa*, the slope was significantly higher with the long-segment method than with the short-segment method (97.4 [85.4-111.1] vs. 76.7 [69.6-84.6], *P* = 0.005). For *Cojoba*, the slope was also significantly higher with the long-segment method than with the short-segment method (57.6 [40.4-82.1] vs. 36.7 [30.3-44.5], *P* = 0.028). Kursar TA, Engelbrecht BMJ, Tyree MT (2005) A comparison of methods for determining soil water availability in two sites in Panama with similar rainfall but distinct tree communities. J Trop Ecol 21:297–305

CHAPTER 4

WATER STORAGE AND RETENTION PROMOTE DESICCATION AVOIDANCE IN TROPICAL TREE SAPLINGS DURING EXTREME DROUGHT

Abstract

Tree species converge in maintaining similar, small safety margins between the stem water potential (Ψ_{stem}) that they reach during seasonal droughts and the Ψ_{stem} associated with their mortality. This pattern has led to the prediction that forests are at similar risk of dieback during extreme droughts. However, if tree species vary in their ability to regulate Ψ_{stem}, then safety margins would be poor predictors of survival during extreme droughts. To explore variation among species in Ψ_{stem} regulation, I subjected potted saplings of six tropical tree species to extreme drought and compared their responses to well-watered plants and pre-treatment reference plants. I assessed the disequilibrium between Ψ_{stem} and soil water potential (Ψ_{soil}), the amount of water released from tissues, and the functional traits associated with Ψ_{stem} regulation. In the drought treatment, Ψ_{soil} reached < -10 MPa, yet three species, *Bursera simaruba*, *Cavanillesia platanifolia*, and *Cedrella odorata* had 100% survival and maintained Ψ_{stem} near -1 MPa (i.e., desiccation-avoiding species). Three other species, *Cojoba rufescens*, *Genipa americana*, and *Hymenaea courbaril* had 50, zero, and 25% survival, respectively, and survivors had Ψ_{stem} < -6 MPa (i.e., desiccation-susceptible species). In the drought

treatment, the desiccation-avoiding species had more water released from all organs and tissues (root, stem, bark, xylem) compared to the reference plants (100–200 vs. 0–50 kg m^{-3}), but the survivors of the desiccation-susceptible *Cojoba* had much higher water released in the drought treatment (300–400 kg m^{-3}). Compared to the desiccation-susceptible species, the desiccation-avoiding species had lower-density tissues and lower lateral root surface area (scaled to stem basal area; LRA), especially in the drought treatment. Together these results suggest that the ability to regulate Ψ_{stem} during extreme drought is associated with functional traits that favor water storage and retention and that safety margins under seasonal droughts are poor predictors of survival under extreme drought.

Introduction

Given that water is critical for plants and that their responses to water are complex, predicting the effects that future shifts in water availability will have on ecosystems is a pressing challenge in ecology. Recent droughts have been linked to extensive tree die-offs and altered species composition in forests worldwide (Allen et al. 2010); however, predicting drought impacts is limited by knowledge in how trees endure drought. The mechanisms by which droughts kill trees remain unresolved (McDowell et al. 2008, Anderegg et al. 2012), yet mounting evidence indicates that trees must maintain the water potential of their stems (Ψ_{stem}) above levels that cause an appreciable loss of hydraulic conductivity (e.g., the Ψ_{stem} at 50% and 80% loss of conductivity; P50 and P80, respectively) in order to prevent stem dieback and death (Sperry and Love 2015). There is a global convergence among forest ecosystems in that trees tend to maintain a similar safety margin between Ψ_{stem} and P50 of about 2 MPa, leading to the prediction that forests worldwide are at similar risk of dieback during extreme droughts (Choat et al. 2012). However, if species are able to maintain a high Ψ_{stem} during extreme droughts, then survival would be higher than that estimated by safety margins under normal or non-stressed conditions.

Regulation of Ψ_{stem} during drought varies widely among species and has been described as strategies of “drought avoidance” and “drought tolerance” (Levitt 1972). Throughout this paper, these are referred to as “desiccation avoidance” and “desiccation tolerance” to avoid confusion with related terms like “drought resistance” (c.f., Pineda-García et al. 2013). Desiccation avoidance involves maintaining homeostasis in Ψ_{stem} by increasing water supply through root extension, storing water within tissues, and decreasing water loss through stomatal control and deciduousness. Desiccation tolerance involves sustaining transpiration despite low Ψ_{stem} by fortifying tissues to maintain hydraulic conductance. Tradeoffs in tissue structure and resource allocation limit drought responses such that species exhibit a range between desiccation avoidance and desiccation tolerance (Borchert 1994, Poorter and Markesteijn 2008, Pineda-García et al. 2013). During extreme droughts, when soil water potential (Ψ_{soil}) reaches below that which trees can extract water (i.e., < ~P80), strategies for survival are limited to desiccation avoidance. Therefore, predicting how forests will respond to extreme droughts will require understanding the mechanisms by which trees avoid desiccation. Yet, the mechanisms of desiccation avoidance are currently understood much more poorly than those of desiccation tolerance (e.g., Kursar et al. 2009).

To explore mechanisms of desiccation avoidance among tropical tree saplings, I subjected six dry-forest tree species with a wide range of putative drought-response strategies to conditions equivalent to an extreme drought. The experiment was conducted within pots in order to control the saplings' access to soil water. Drought responses were compared to those of well-watered saplings. The ability to avoid desiccation and the physiological and functional traits associated with this ability were the major focus of the experiment. Specifically, the following questions were addressed with regard to extreme drought: (1) Can saplings achieve disequilibrium between Ψ_{stem} and Ψ_{soil}? (2) To what degree does desiccation avoidance rely on preventing water loss versus relying on stored water to maintain Ψ_{stem} despite water loss? (3) What organs and tissues are the most important sources of stored water? (4) Does leaf shedding and lateral-root shedding prevent water loss? And (5) what functional traits are associated with desiccation avoidance?

Materials and methods

Plant material and growing conditions

Tree species were selected to represent a variety of putative drought-response strategies based on their wood density and leaf phenology. The species are widespread in moist and dry forests of Panama. Seeds were collected from March to May 2012. *Cavanillesia platanifolia*, *Cedrela odorata*, and *Hymenaea courbaril* seeds were collected from Parque Natural Metropolitano, a forest that is transitional between moist and dry located in Panama City, Panama, with mean annual rainfall of 1800 mm. *Bursera simaruba*, *Genipa americana*, and *Cojoba rufescens* seeds were collected from the

Eugene Eisenmann Reserve, a dry forest in Coronado, Panama, with mean rainfall of 1592 mm. Hereafter the focal species will be referred to by genus name. For each species, seeds were collected from > 5 trees, with the exception of *Hymenaea*, for which seeds were collected beneath a single individual. Upon collection, the seeds were sown in flats with a 1:1 mixture of river sand and topsoil collected from Barro Colorado Island, a seasonally dry moist forest in central Panama. Germinates were transferred to 1.7 L pots with a 1:9 mixture of sand and topsoil. They were grown in approximately 20% full sunlight and allowed to receive rainwater. During the 2013 dry season (January to May 2013), the pots were watered to saturation with lake water every 2–3 days. In July 2013, the saplings were transferred to 6.5 L (30 cm depth) pots to prevent root binding. The pots were then arranged so that 6 saplings of each species were located on each of four tables in a shadehouse. The tables received approximately 20% of full sunlight.

Near the onset of the 2014 dry season (3–18 December 2013), 8 saplings per species were harvested for pre-treatment baseline reference measurements (“reference plants”). Subsequently (18 December 2013), a transparent plastic sheet was placed over the shadehouse roof to exclude rainwater from the pots. Eight saplings per species were randomly selected to be watered to soil saturation every 2–3 days (“watered treatment”) while the other 8 saplings of each species were subjected to drought by excluding water (“drought treatment”). The watered- and drought-treatment saplings were harvested near the end of the dry season (17 March 2014 to 16 April 2014), so that water was excluded from the drought-treatment saplings for 89–119 days. The harvest dates were spread over several weeks because the extensive post-harvest measurements (described below) could only be achieved on four saplings per day.

The saplings were harvested in the morning (0800–0900 hours). Their pots were cut open lengthwise and soil was collected from 10 cm and 20 cm depth to measure soil water potential (Ψ_{soil}). Soil from each depth was used to fill two aluminum chambers (25 mm diameter × 45 mm in depth) that were attached to leaf-cutter psychrometers (Merrill Engineering, Logan, UT) and measured for Ψ_{soil} with the protocol of Kursar et al. (2005).

When the saplings were harvested, soil in the drought treatment was extremely dry; Ψ_{soil} was commonly below the range that the psychrometers could measure (-10 MPa). In order to assess soil moisture in the drought treatment, gravimetric soil water content (WC_{soil}) was also measured. The soil that was placed in the psychrometer chambers was weighed for fresh mass then dried at 100°C for > 72 hours and weighed for dry mass. WC_{soil} was calculated as 100 × (fresh mass – dry mass) / dry mass. I tested whether soil moisture in the drought treatment varied among species with ANOVA, with WC_{soil} as the response variable, species as a fixed effect, and table as a random block effect, followed by Tukey HSD tests. At 10 cm depth, *Cojoba* had significantly higher WC_{soil} than the other species, otherwise there were no differences among species (Fig. 4.S1). At 20 cm depth, *Cojoba* had significantly higher WC_{soil} than all other species except *Cavanillesia*, while *Cavanillesia* had significantly higher WC_{soil} than *Genipa* (Fig. 4.S1). These results indicate that saplings in the drought treatment were generally exposed to similar drought conditions, except for *Cojoba*, which was exposed to slightly milder drought conditions than the other species.

To test whether Ψ_{soil} varied among species in the reference plants and the watered treatment, ANOVA was used as described above for WC_{soil}. There were no differences among species in Ψ_{soil} at either 10 cm or 20 cm depth in either the reference plants or the watered treatment (Fig. 4.S2). In the reference plants, mean ± SE of Ψ_{soil} was -0.66 ± 0.02 and -0.70 ± 0.02 MPa at 10 cm and 20 cm depths, respectively. In the watered treatment, Ψ_{soil} was -0.52 ± 0.03 and -0.59 ± 0.02 MPa at 10 cm and 20 cm depths, respectively. These results indicate that all species had similar growing conditions and were well watered in these treatments.

Growth, phenology, and CO_2 assimilation

The number of leaves was counted on all saplings and their basal diameter was measured in December 2013, before the reference plants were harvested. Stems were marked with a permanent marker at 5 cm above the soil line, where diameter was measured twice perpendicularly with calipers to the nearest 0.05 mm and averaged. Diameter and leaf-number measurements were repeated on saplings in the watered and drought treatments every 15–30 days and again when they were harvested.

Maximum CO_2 assimilation rate (A_{max}) was measured on 4 randomly selected saplings per species in December 2013, before the reference plants were harvested. A_{max} was measured on the most recent fully expanded leaf with an LI-6400 portable photosynthesis device (Li-Cor Biosciences, Lincoln, NE, USA) set to 1200 µmol m^{-2} s^{-1} photosynthetic photon flux density provided by a red blue light source (6400-02B, Li-Cor Biosciences) with air temperature and relative humidity at ambient levels and CO_2 at 400 ppm.

Plant water potential, water released, and morphology

Upon harvesting the saplings, they were rinsed of soil and the rinse water was passed through a 2 mm mesh screen to collect disconnected roots. Each sapling was divided into leaves, petioles, stem, taproot (i.e., axis), and lateral roots. The fresh mass of each was measured. Live and dead roots were distinguished by their color and friability (Powers and Peréz-Aviles 2013) and dead roots were not included in analyses. Stem water potential (Ψ_{stem}) was measured at 4–5 cm height by excising sections of the stem, placing them in the leaf-cutter psychrometer chambers, and following the psychromatic protocol described above for Ψ_{soil}. Three replicates per sapling were measured and Ψ_{stem} was taken as the mean. Likewise, root water potential (Ψ_{root}) was measured on the axis at 2–3 cm depth.

On each sapling, the volume-normalized mass of water released (relative to saturation) was measured on the stem and taproot and also on bark and xylem taken from the stem (WR_{stem}, WR_{root}, $WR_{bark,}$ WR_{xylem}, respectively). For WR_{stem}, a stem section 2 cm in length was collected at 6 cm height. For WR_{root}, a section 2 cm in length was collected at 4 cm depth. For WR_{bark} and WR_{xylem}, a stem section 2 cm in length at 8 cm height was cut and the bark and pith were removed from the sapwood with razor blades. The fresh mass of each segment was taken and its volume was measured with water displacement on a digital balance. Then the segment was submerged in distilled water for 24 hours and measured for saturated mass. Dry mass was taken after drying at 60°C for > 72 hours. WR was calculated as (saturated mass – fresh mass) / fresh volume.

Leaf area was measured with an LI-3100 area meter (Li-Cor Biosciences). The lateral roots were arranged flat on a sheet of white paper that was back lit with fluorescent lights and photographed from above with a digital camera. The photographs were processed with GiA Roots software to measure the total length and surface area of the lateral roots (Galkovskyi et al. 2012). For comparisons among saplings, lateral root surface area was scaled by stem basal area (lateral root surface area per stem basal area, LRA) as a proxy for the ability to take up water compared to transport it in the stem (Sperry and Love 2015). All plant parts were oven dried at 60°C for > 72 hours and weighed for dry mass. The dry masses of the segments extracted to measure WR and Ψ were summed with the remaining sections of stem and axis to calculate the total dry mass of these organs.

Analysis

Data analysis was guided, in part, by the outcome of the experiment: of the eight saplings of each species in the drought treatment, mortality occurred in eight, six, and four saplings of *Genipa*, *Hymenaea*, and *Cojoba*, respectively, while *Bursera*, *Cavanillesia*, and *Cedrela* had no mortality. Saplings were determined as dead when their stems were not green after scraping the bark. This determination was verified by measuring Ψ_{stem} and Ψ_{root} of 2–3 apparently dead saplings of each species, which were < -10 MPa (below the range of our psychrometers and likely below the saplings' survival threshold). So, due to a lack of survivors in the drought treatment, *Genipa* and *Hymenaea* saplings from the experimental treatments were not harvested to measure water-relation parameters (i.e, Ψ, WR, LRA). For *Cojoba*, water-relation parameters were measured on the four saplings that survived the drought treatment and the eight saplings from the watered treatment.

For each water-relation parameter, we used ANOVA with a randomized complete block design for each species, with treatment (i.e., reference, watered, and drought) as a fixed effect and shadehouse table as a randomized block. Differences between treatments were tested with Tukey HSD tests. Differences between the reference plants and watered treatment were interpreted as seasonal or ontogenetic effects independent of drought, whereas differences between the drought treatment and the reference plants and the watered treatment were interpreted as drought effects. Response variables were Ψ_{stem}, Ψ_{root}, WR_{stem}, WR_{root}, $WR_{bark,}$ WR_{xylem}, and LRA.

If taproots are an important reservoir of stored water that buffers Ψ_{stem} and Ψ_{root} against water loss, then during drought, more water would be released from the taproot vs. the stem. In order to test this, the total mass of water released from the taproot was estimated by multiplying WR_{root} by the volume of the taproot (calculated by dividing total taproot dry mass by taproot density measured on the WR segment). The total mass of water released from the stem was estimated the same way. Then, for each species, total water released from the taproot was plotted against total water released from the stem and a regression was fit with standardized-major-axis regression. The smatr package in R was used to test whether the slope varied from unity (Warton et al. 2012). A slope > 1 would indicate that saplings released more water from taproots than stems when exposed to drought.

Similarly, in order to test whether saplings released more water from bark vs. xylem, the mass of water released from the bark tissue segment (bark saturated mass – bark fresh mass) was plotted against the mass of water released from the xylem tissues segment (xylem saturated mass – xylem fresh mass). Since, for each sapling, the bark and xylem tissue segments were extracted from the same section of stem, this controlled for the proportion of stem composed of each tissue. Standardized-major-axis regression was used to test whether the slope of water released from the bark vs. xylem tissue segments differed from unity as described above for the total mass of water released from the taproot vs. stem.

The strong pattern of divergent drought survival among species (high: *Bursera*, *Cavanillesia*, and *Cedrela* vs. low: *Cojoba*, *Genipa*, and *Hymenaea*) enabled an exploration of the functional traits associated with desiccation avoidance. From the references plants, the following functional traits were derived: wood density, bark density, ratio of bark volume to xylem volume (measured on the WR segments), leaf mass per area, $A_{\max}$, leaf mass fraction, stem mass fraction, root mass fraction (each organ mass fraction is the dry weight of the organ divided by the dry weight of the entire plant), specific root length (total lateral root length divided by total lateral root dry mass), and LRA. An additional functional trait, leaf retention time (LRT) of droughted saplings, was calculated as the number of drought days at which saplings shed 50% of their leaves, following Méndez-Alonzo et al. (2012). Number of leaves (as percent of pre-drought; PNL) was plotted as a function of Julian date (JD) and a sigmoidal function was fit with the form $\text{PNL} = 100/(1 + \exp(a \times (\text{JD} - b)))$. Also, an index of dry-season dormancy was calculated assuming that saplings that exhibit dry-season dormancy would not grow during the dry season even in environmental conditions that are conducive to growth, such as in the watered treatment. For each sapling in the water treatment, relative growth was calculated as (final basal diameter – initial basal diameter) / initial basal diameter × 100. Initial basal diameter was measured before the dry season and the final basal diameter was taken before the saplings were harvested, 15 March 2013, near the end of the dry season. Each functional trait was compared between drought-response types (desiccation-avoiding vs. desiccation susceptible) with a mixed model ANOVA, with drought response type as a fixed effect and species and shade house table as random effects.

Results

When the saplings were harvested 10–15 weeks after the onset of the dry season, soil in the droughted pots was extremely dry. All pots of the surviving plants, which were assessed for water relation parameters (see Materials and methods), had Ψ_{soil} that was lower than the psychrometers' detection limit (i.e., < -10 MPa) at 10 cm depth, except for three *Cojoba* saplings for which Ψ_{soil} ranged from -6.90 to -9.26. All had Ψ_{soil} < -10 MPa at 20 cm depth except one *Cojoba* sapling for which Ψ_{soil} was -6.63 MPa. Despite the dry conditions in the drought treatment, the saplings of *Bursera*, *Cavanillesia*, and *Cedrela* maintained Ψ_{stem} and Ψ_{root} near -1 MPa, which did not differ from the Ψ_{stem} and Ψ_{root} of the pre-treatment reference plants (Table 4.1). In contrast, the four *Cojoba* saplings that survived the drought treatment (out of eight) had Ψ_{stem} and Ψ_{root} near -6 MPa, significantly lower than the reference plants and the saplings in the watered treatment (Table 4.1). All of the *Genipa* saplings and 6 of 8 *Hymenaea* saplings in the drought treatment died, so their Ψ_{stem} and Ψ_{root} were not measured. The two surviving *Hymenaea* saplings had Ψ_{stem} of -4.98 and -8.28 MPa, Ψ_{root} of -4.84 and -6.20 MPa, 10 cm-depth Ψ_{soil} of -5.71 and < -10 MPa, and 20 cm-depth Ψ_{soil} of -7.15 and < -10 MPa.

For all of the species with sufficient survival rates in the drought treatment to assess water-relation parameters, all tissues and organs showed similar patterns of water release among treatments (Fig. 4.1): WR_{stem}, WR_{root}, $WR_{bark,}$ and WR_{xylem} were higher in the drought treatment than in the reference plants and the watered treatment (Tukey HSD, $P < 0.018$), while the watered-treatment did not differ from the reference plants (Tukey HSD, $P > 0.11$). Among species, the drought-treatment WR values were generally similar for *Bursera, Cavanillesia*, and *Cedrela*; WR ranged from 74.4 ± 4.6 kg m^{-3} (mean ± SE) in *Cavanillesia* stem to 226.8 ± 31.5 kg m^{-3} in *Bursera* bark (Fig. 4.1). However, for *Cojoba*, WR values were generally higher than those of the other species; WR values ranged from 207.8 ± 71.4 kg m^{-3} in xylem to 678.7 ± 50.4 kg m^{-3} in bark (Fig. 4.1).

The relationship between the total mass of water released from the taproot vs. the stem varied among species. *Bursera* and *Cedrela* released more water from the stem (Fig. 4.2a,c; slopes < 1) while *Cavanillesia* and *Cojoba* released similar amounts of water from the taproot and stem (Fig. 4.2b,d; slopes do not differ from 1). Likewise the relationship between the mass of water released from bark vs. xylem tissues within stem segments varied among species. *Bursera* and *Cedrela* released more water from bark than xylem (Fig. 4.2e, g; slopes > 1) while *Cavanillesia* and *Cojoba* released more water from the xylem than the bark (Fig. 4.2f, h; slopes < 1).

The response of lateral root surface area to the watered and drought treatments was different for each species (Fig. 4.3). *Bursera* had lateral root surface area per stem basal area (LRA) that was low compared to the other species and that did not differ from the reference plants in either the watered or drought treatments (Fig. 4.3). For *Cavanillesia* and *Cedrela*, LRA was lower in both the watered and drought treatment compared to the reference plants (Fig. 4.3). For *Cedrela*, LRA was also lower in the drought treatment than the watered treatment while *Cavanillesia* had a similar, yet non-significant, trend. In contrast, for *Cojoba,* LRA was nearly twice as high in the drought treatment than in the reference plants and the watered treatment (Fig. 4.3).

The three species that had 100% survival and maintained high Ψ_{stem} and Ψ_{root} in the drought treatment (*Bursera*, *Cavanillesia*, and *Cedrela*; i.e., desiccation-avoiding species) differed from the three species with low survival and low Ψ_{stem} and Ψ_{root} (*Cojoba*, *Genipa*, and *Hymenaea*; i.e., desiccation-susceptible species) in many of the functional traits that were compared among species (Table 4.2). The desiccation-avoiding species had lower wood density, bark density, leaf mass per area, leaf mass fraction, LRA, and, marginally, watered-treatment growth; and they had higher stem mass fraction. However, the two groups did not differ in A_{max}, specific root length, root mass fraction, or, surprisingly, leaf retention time (Table 4.2; Fig. 4.S3).

Discussion

Desiccation avoidance during extreme drought

The length of the drought period imposed on saplings in this experiment (89–119 days) was typical for seasonally dry tropical forests, where dry seasons last 3–6 months (Murphy and Lugo 1986). The intensity of the drought ($\Psi_{\mathrm{soil}} < -10$ MPa) was extreme for seasonally dry tropical forests, but not unrealistic. In a companion study, Ψ_{soil} was measured in the transitional- and dry-forest sites where seeds were collected for this experiment (Chapter 2). Near the end of the 2013 dry season in the transitional forest, at 10 and 20 cm depths, Ψ_{soil} was -2.2 ± 0.4 and -2.4 ± 0.6 MPa (mean ± SE), respectively, while in the dry forest Ψ_{soil} was -6.1 ± 0.8 and -5.7 ± 0.6 MPa, respectively. In the dry forest, one of the four sampling pits had Ψ_{soil} < -10 MPa at 10 cm depth. Similarly, during the dry season in a Bolivian dry forest, soil at 0–20 cm depth on hill tops had Ψ_{soil} of -5.5 ± 1.5 MPa (mean ± SE) with a range to < -10 MPa (Markesteijn et al. 2010). During the late dry season in in a Mexican dry forest, soil at 0–10 cm depth had mean ± SD Ψ_{soil} of -49.9 ± 12.5 MPa (Lebrija-Trejos et al. 2010); although this probably does not represent Ψ_{soil} within the soil profile since Ψ_{soil} generally becomes less negative with depth (Engelbrecht and Kursar 2003, Markesteijn et al. 2010). In a drier ecosystem, the Sonoran Desert, Ψ_{soil} at 0–10 cm depth reached < -10 MPa after 30 days without rain (Nobel and Cui 1992). Together, these results suggest that the drought intensity in this experiment was higher than normal for shallow soil layers in tropical dry forests, but may be increasingly common as climate change continues to influence droughts.

In a companion study, four species from this study were tracked for their responses to drought in the forests from which their seeds were collected for this experiment (Chapters 2 and 3). In the dry season, at predawn, saplings of *Bursera, Cavanillesia, Cojoba,* and *Genipa* had Ψ_{stem} of -0.78 ± 0.05, -0.60 ± 0.04, -2.89 ± 0.35, and -3.11 ± 1.00 MPa, respectively. Their P80 values were -1.50, -1.57, -5.75, and -3.90 MPa, respectively. Therefore, during a normal seasonal drought, *Bursera, Cavanillesia, Cojoba,* and *Genipa* maintained hydraulic safety margins of 0.72, 0.97, 2.86, and 0.79 MPa, respectively, which is similar to the global average (Choat et al. 2012). If hydraulic safety margins were used to predict how these species would respond to an increase in drought intensity, *Bursera* and *Cavanillesia* would be incorrectly predicted to have higher mortality than *Cojoba*, since they maintain lower safety margins. However, *Cojoba* would be correctly predicted have lower mortality than *Genipa* (although this result is confounded by the fact soil in the *Cojoba* pots dried less that soil in the *Genipa* pots (Fig. 4.S1)). These results suggest that hydraulic safety margins during normal droughts do not accurately predict survival under extreme drought and, in contrast to recent predictions (Choat et al. 2012, Delzon and Cochard 2014), safety margins may not be particularly useful for predicting how forests will respond to climate change.

Likewise, species' drought performance, in terms of survival, under normal drought conditions may not predict their performance under extreme drought. For example, Engelbrecht and Kursar (2003) compared drought performance among species by excluding water from seedlings planted in a forest understory. After 22 weeks, topsoil in the droughted plots reached -2.5 MPa. *Hymenaea* seedlings had high drought performance; their survival rate in droughted plots was not significantly different than in watered control plots (60% vs. 77% survival; Engelbrecht and Kursar 2003). These results contrast strongly with mine from the drought vs. watered treatments (25% vs. 100% survival; Chi-square test, $P = 0.01$). It appears that *Hymenaea* survives relatively mild drought well, but its performance under extreme drought is much worse. This is likely the case for all species that follow a desiccation-tolerance strategy, since their performance depends on extracting water from soil. However, for desiccation-avoiding species such as *Bursera*, *Cavanillesia* and *Cedrela*, drought intensity does not appear to affect drought performance, at least within relatively extreme conditions imposed in the current experiment. In order to predict how forests will respond to climate change, understanding variability among species in their ability to regulate Ψ_{stem} under extreme drought may be more important than quantifying safety margins or drought performance under normal dry-season drought conditions.

Root segmentation as a mechanism to slow water loss

Cavanillesia and *Cedrela* had lower lateral root surface area per stem basal area (LRA) in both the watered- and drought treatment compared to the reference plants (Fig. 4.3). Although the other desiccation-avoiding species, *Bursera*, did not have lower LRA in the experimental treatments compared to the reference plants, all three groups had very low LRA (Fig. 4.3). It is unclear why *Bursera* saplings had such low LRA. They also had the lowest total biomass and A_{max} (Table 4.1). *Bursera* is a strict pioneer species that may have been light limited during the experiment. Although these plants appeared healthy, one hypothesis is that, at the experiment's initiation, the *Bursera* saplings were not healthy. Nonetheless, *Cojoba* showed a strikingly different pattern than the desiccation-avoiding species by having substantially higher LRA in the reference and watered plants, and then nearly doubling LRA in the drought treatment (Fig. 4.3).

Root growth in response to drought is commonly observed in crop plants and has been interpreted as a desiccation-avoidance strategy because it facilitates water uptake, which, when combined with reduced transpiration through stomatal closure, helps to maintain plant water status (Verslues et al. 2006). However, when Ψ_{soil} is below that from which plants can extract water, high root surface area is disadvantageous because roots may leak water into the soil, speeding plant desiccation (Holbrook 1995). Thus, by growing lateral roots in drying soil, *Cojoba* relies on Ψ_{soil} within its rooting zone remaining within the range at which it can extract water. *Cojoba* is able to extract water from relatively dry soil; it experiences severe wilting associated with loss of hydraulic conductance at Ψ_{leaf} of -6.9 MPa (Kursar et al. 2009), so under normal dry-season conditions, this strategy appears adaptive. However, under the extreme drought in the drought treatment, the increased lateral root surface area likely leads to higher water-loss rates for *Cojoba*, and ultimately to its high mortality rate compared to the desiccation-avoiding species.

For *Cavanillesia* and *Cedrela*, reduced LRA in the experimental treatments compared to the reference plants likely reflects root dieback and abscission. Alternatively, since LRA was scaled by stem basal area, higher stem growth relative root growth in the experimental treatments could reduce LRA. To test for this, I performed an additional analysis in which lateral root surface area measured at the time of harvest was scaled by stem basal area measured in November, before the experimental treatments were imposed. *Cavanillesia* and *Cedrela* still had reduced LRA in the drought treatment compared to the reference plants, supporting the hypothesis that reduced LRA in the experimental treatments reflects root dieback. Root dieback would slow water loss during seasonal droughts by decreasing the surface area from which water can leak. This represents a form of plant segmentation, whereby organs located distally on the plant (lateral roots) are lost, slowing water loss from the remaining, more basally located organs of the plant (taproot and trunk), which are more costly for the plant to replace if they are damaged by desiccation (Alder et al. 1996, Tyree and Zimmermann 2002).

Root dynamics in response to drought have rarely been reported for tropical trees, yet Rojas-Jimenez (2007) found that the density of live fine roots decreased and the density of dead fine roots increased under *Enterolobium cyclocarpum* trees during the dry season in a Costa Rican dry forest. *E. cyclocarpum* is more resistant to xylem cavitation than *Bursera* and *Cavanillesia*; its P80 is -3.2 MPa (Brodribb et al. 2003), but like *Bursera* and *Cavanillesia,* it generally sheds its leaves and replaces them during the dry season (Rojas-Jiménez et al. 2007), so it is unclear how common seasonal reduction in lateral root surface area is among species or which traits are associated with this behavior. In a Costa Rican dry forest, Powers and Peréz-Aviles (2013) found no pattern for increased or decreased fine-root density during the dry season compared to the wet season among soil cores randomly placed within samplings plots. This may indicate that species that grow lateral roots in response to drought and those that shed them during the dry season balance out the community-wide density of fine roots.

Stored water buffers plant water status

Saplings in the drought treatment released more water from their stems than saplings did during a normal dry season in seasonally dry forests. In a companion study, WR_{stem} was measured near the end of the dry season, at predawn, in the forests from which the seeds were collected for this study (Chapter 3). *Bursera*, *Cavanillesia*, and *Cojoba* had WR_{stem} of 115.2 ± 5.8, 28.9 ± 5.5, and 84.3 ± 4.8 kg m^{-3}, respectively. In the drought treatment they had WR_{stem} of 165.5 ± 13.7, 74.8 ± 12.9, and 333.2 ± 41.0 kg m^{-3}, respectively (Fig. 4.1); so, compared to a normal dry season, WR_{stem} in the drought treatment was 50.3, 45.9, and 248.9 kg m^{-3} higher for *Bursera*, *Cavanillesia*, and *Cojoba*, respectively. Apparently, *Bursera* and *Cavanillesia* lost less water than *Cojoba* in extreme drought due to the desiccation-avoiding responses described above. However, *Bursera* and *Cavanillesia* still had higher WR_{stem} in the drought treatment than in a normal dry season, suggesting that their water-loss rate is still dependent on soil aridity.

Thus, desiccation-avoiding species are at risk of desiccation in droughts that are more intense or longer than that of the drought treatment in this experiment. Quantifying the drought conditions under which desiccation-avoiding species can survive will require a better understanding of the factors that determine their water-loss rate and the maximum amount of water loss that they can sustain without dying.

There was no convergence among species in the organ (stem vs. taproot) or the tissue (bark vs. xylem) from which more water was released in response to drought (Fig. 4.2). However, none of the species released more water from the taproot than from the stem (Fig. 4.2a–d), suggesting that the taproot is not a particularly important storage organ for saplings of these species. Although taproots are commonly implicated as important storage organs for seedlings (e.g., Poorter and Markesteijn 2008), as trees grow, their root mass becomes smaller relative to stem mass (Tomlinson et al. 2013). The saplings in this experiment may have outgrown the stage at which taproots are more important than stems as sources of stored water during drought.

Wolfe and Kursar (Chapter 3) found that the relative water content of xylem did not change between the wet and dry season for saplings in the dry forest where seeds were collected for the saplings in this study, including *Bursera* and *Cojoba*. In contrast, the *Bursera* and *Cojoba* in the drought treatment had higher WR_{xylem} in the drought treatment than in the reference plants and watered treatment (Fig. 4.1). It is possible that water is not lost from xylem during normal dry season droughts, but only during extreme droughts. Consistent with this, Zweifel et al. (2000) found that as stems of *Picea abies* (Norway spruce) dried, water was released only from the bark until a transition point of Ψ_{stem} at which water was released from both bark and xylem. For *P. abies*, the transition point was near to the Ψ_{stem} at which cavitation began to occur. Since *Bursera* and *Cojoba* had lower Ψ_{stem} in the drought treatment than during the seasonal drought in the dry forest (see above), it is possible that they passed the transition point and began to release water from the xylem as the drought progressed. Thus, during extreme drought, water stored in both the bark and the xylem appears to be important for buffering Ψ_{stem}. Indeed, for *Cavanillesia*, more water was released from the xylem than from the bark (Fig. 4.2f).

Functional traits associated with desiccation avoidance

There is much interest in using functional traits to predict community responses to novel disturbances such as extreme droughts associated with climate change (Lavorel and Garnier 2002). I found that desiccation-avoiding species (*Bursera*, *Cavanillesia*, and *Cedrela*) shared several functional traits that were not found in desiccation-susceptible species (*Cojoba*, *Genipa*, and *Hymenaea*), suggesting that combinations of certain traits can predict mortality during extreme drought. The desiccation-avoiding species had low wood density (Table 4.1), similar to the "deciduous light-wood species" from a Costa Rican dry forest described by Borchert (1994). During a dry season, deciduous light-wood species, including *Bursera,* maintained high Ψ_{stem} and stem water content (Borchert 1994). Although low wood density is associated with vulnerability to cavitation (Hacke et al. 2001), among tropical dry-forest tree species, it also appears to be associated with better regulation of Ψ_{stem} during extreme droughts. Similarly, during recent extreme droughts in a semi-arid shrubland and a temperate deciduous forest, high mortality rates were associated with high wood density (Hoffmann et al. 2011, Kukowski et al. 2012). Since species with low wood density must avoid equilibrium with Ψ_{soil} even during relatively mild droughts, when droughts progress to be extremely dry, the saplings likely have already gone into a dormant state with low gas exchange and water loss, which protects them from desiccation.

When Poorter and Markesteijn (2008) subjected tropical tree seedlings to extreme drought, they found that the best predictors of survival time were whether species had thickened taproots and whether they shed leaves. This contrasts with the results of my study, which found that root mass fraction and leaf retention time did not differ between desiccation-avoiding and desiccation-susceptible species (Table 4.2). This difference may be due to ontogenetic differences between seedlings and saplings, whereby root-to-shoot morphology and leaf-shedding behavior change with plant size. As noted above, root mass per stem mass generally becomes smaller with plant size (Tomlinson et al. 2013). In response to drought, seedlings of evergreen or drought-deciduous species are probably less prone to shedding leaves than saplings because they likely have fewer carbon reserves to replace leaves and thus would be less likely to recover from leaf abscission.

It is striking that the desiccation-susceptible species shed their leaves at similar rates to the desiccation-avoiding species, revealing that leaf shedding did not protect them against desiccation. For deciduousness to protect trees from desiccation during extreme drought, it appears to require coordination with root shedding or low LRA during drought (Fig. 4.3). The desiccation-avoiding species also had lower LRA than the desiccation-susceptible species in the reference plants (Table 4.2). Since desiccation-avoiding species maintain photosynthesis only when soils are well hydrated, they can likely invest less in lateral root surface area without risk of incurring a hydraulic bottleneck (Sperry and Love 2015).

Table 4.1. Stem and root water potential (Ψ_{stem} and Ψ_{root}, respectively) for each of the study species in the reference plants and experimental treatments. Values are mean ± SE, n = 8 except for *Cojoba* in the drought treatment, where n = 4. Since no *Genipa* and only two *Hymenaea* survived the drought treatment, most saplings from the experimental treatments were not harvested to measure Ψ_{stem} and Ψ_{root}. Within species, values that share letters are not significantly different (ANOVA followed by Tukey HSD, $P > 0.05$).

	Ψ_{stem} (MPa)			Ψ_{root} (MPa)		
Species	Reference	Watered	Drought	Reference	Watered	Drought
Bursera	-0.58 ± 0.04 a	-0.57 ± 0.06 a	-0.64 ± 0.10 a	-0.79 ± 0.04 a	-0.78± 0.08 a	-0.92 ± 0.06 a
Cavanillesia	-0.79 ± 0.06 a	-0.57 ± 0.08 b	-0.78 ± 0.05 ab	-0.85 ± 0.02 a	-0.69 ± 0.06 b	-0.78 ± 0.05 ab
Cedrela	-1.00 ± 0.03 a	-0.84 ± 0.11 a	-0.98 ± 0.09 a	-0.96 ± 0.03 ab	-0.77 ± 0.09 a	-1.06 ± 0.05 b
Cojoba	-0.97 ± 0.05 a	-0.75 ± 0.07 a	-5.57 ± 0.34 b	-1.38 ± 0.06 a	-1.13 ± 0.08 a	-6.73 ± 0.50 b
Genipa	-0.90 ± 0.06	NA	NA	-1.18 ± 0.04	NA	NA
Hymenaea	-0.99 ± 0.07	NA	NA	-0.99 ± 0.06	NA	NA

Table 4.2. Functional traits among the study species. Values are mean ± SE, n = 8, except for maximum CO_2 assimilation rate (A_{max}), where n = 4. *P* value refers to the test for a difference between desiccation-avoiding species (*Bursera*, *Cavanillesia*, and *Cedrela*) and desiccation-susceptible species (*Cojoba*, *Genipa*, and *Hymenaea*), using ANOVA with drought-response strategy as a fixed effect and species as a random effect. Leaf retention time was compared between drought-response strategies with a t-test. LRA is lateral root surface area per stem basal area.

Trait	*Bursera*	*Cavanillesia*	*Cedrela*	*Cojoba*	*Genipa*	*Hymenaea*	*P* value
Total dry mass	0.32 ± 0.05	13.9 ± 1.5	6.88 ± 0.63	1.82 ± 0.32	14.1 ± 1.1	5.96 ± 1.28	0.97
Wood density (g cm^{-3})	0.34 ± 0.04	0.29 ± 0.02	0.40 ± 0.01	0.49 ± 0.03	0.66 ± 0.01	0.55 ± 0.02	0.021
Bark density (g cm^{-3})	0.12 ± 0.00	0.22 ± 0.01	0.15 ± 0.01	0.36 ± 0.01	0.31 ± 0.02	0.45 ± 0.04	0.015
Bark:xylem volume ratio	1.46 ± 0.21	2.08 ± .014	2.13 ± 0.08	1.11 ± 0.14	2.18 ± 0.28	0.68 ± 0.09	0.31
Leaf mass per area (g cm^{-2})	2.70 ± 0.50	3.12 ± 0.11	1.95 ± 0.09	4.81 ± 0.36	3.68 ± 0.11	4.77 ± 0.25	0.025
A_{max} (µmol m^{-2} s^{-1})	4.41 ± 0.98	8.27 ± 1.28	7.52 ± 0.87	5.88 ± 0.87	9.64 ± 1.03	7.32 ± 1.17	0.61
Specific root length (cm g^{-1})	438 ± 43	55 ± 3	177 ± 11	443 ± 36	142 ± 33	133 ± 10	0.92
Leaf mass fraction	0.07 ± 0.04	0.19 ± 0.02	0.25 ± 0.01	0.44 ± 0.01	0.34 ± 0.03	0.36 ± 0.03	0.022
Stem mass fraction	0.75 ± 0.04	0.43 ± 0.01	0.50 ± 0.01	0.27 ± 0.01	0.23 ± 0.02	0.30 ± 0.02	0.042
Root mass fraction	0.18 ± 0.01	0.38 ± 0.02	0.25 ± 0.01	0.30 ± 0.01	0.43 ± 0.04	0.43 ± 0.04	0.31
LRA (cm^{2} mm^{-2})	0.5 ± 0.1	2.3 ± 0.2	3.4 ± 0.4	5.8 ± 0.3	13.3 ± 1.1	8.8 ± 1.3	0.036
Watered treatment growth (%)	4.9 ± 2.3	10.6 ± 3.11	11.5 ± 2.60	32.9 ± 7.16	22.9 ± 3.56	14.5 ± 4.68	0.065
Leaf retention time (days)	51 ± 4	42 ± 1	19 ± 1	65 ± 3	30 ± 1	68 ± 2	0.34

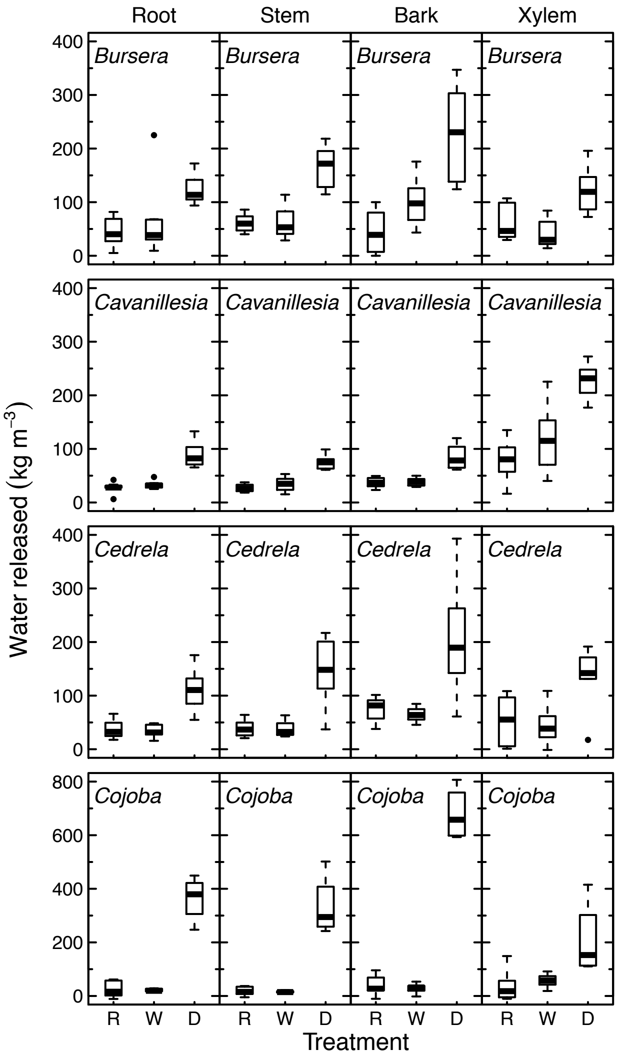

Fig. 4.1. Water released from roots, stems bark, and xylem in the reference plants (R), watered (W), and drought (D) treatments. Boxes delineate the first to third quartiles and are bisected by the median; bars extend to minimum and maximum observations within 1.5 times interquartile length; and filled circles represent outliers. Within each panel, the drought treatment had significantly higher water released than the reference plants and the watered treatment (ANOVA followed by Tukey HSD, $P < 0.05$), while reference plants and the watered treatment were not significantly different ($P > 0.05$). Note that the range of the y-axis in the *Cojoba* panels is twice that of the other species.

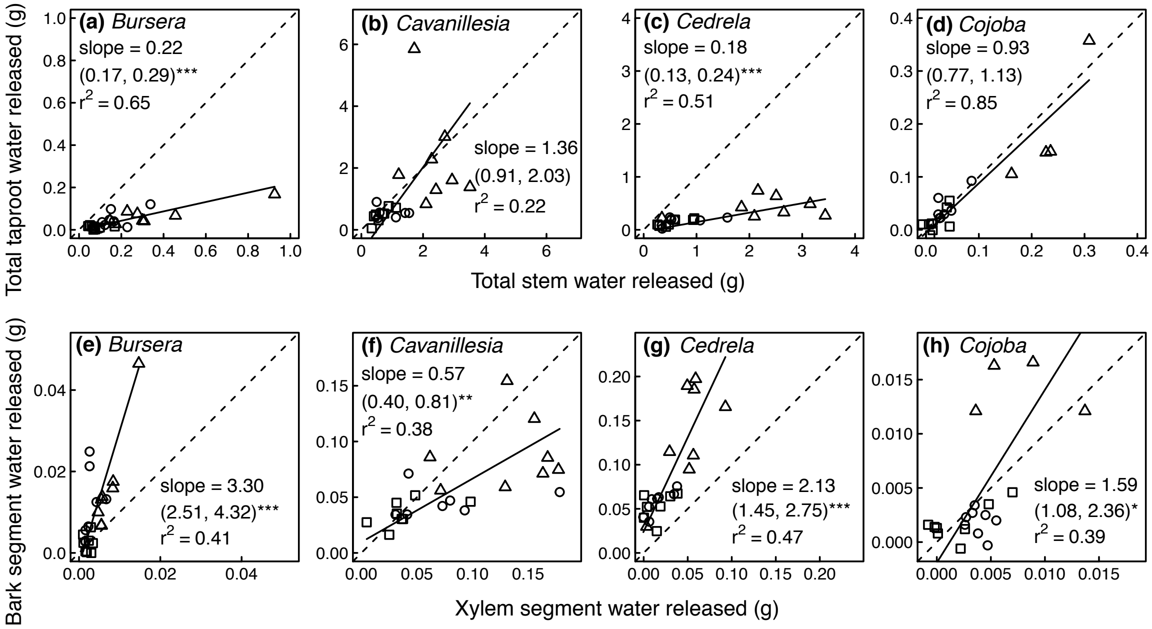

Fig. 4.2. Total mass of water released (i.e., saturated mass – fresh mass; see Materials and methods) in the stem vs. the taproot (a–d) and in segments of xylem vs. bark (e–h). Each point represents an independent sample taken from a different plant. Squares represent reference plants; circles, watered treatment; and triangles, drought treatment. The standardized-major-axis regression slope of each relationship is shown with the 95% confidence interval in parentheses. Asterisks indicate that the slope is significantly different from unity; *, $P < 0.05$; ** $P < 0.01$; ***, $P < 0.0001$. In panels a–d, slopes < 1 indicate that taproots release less water than stems during droughts. In panels e–h, slopes > 1 indicate that bark releases more water than xylem during drought. Note that axes ranges vary among panels, reflecting differences among species in plant size.

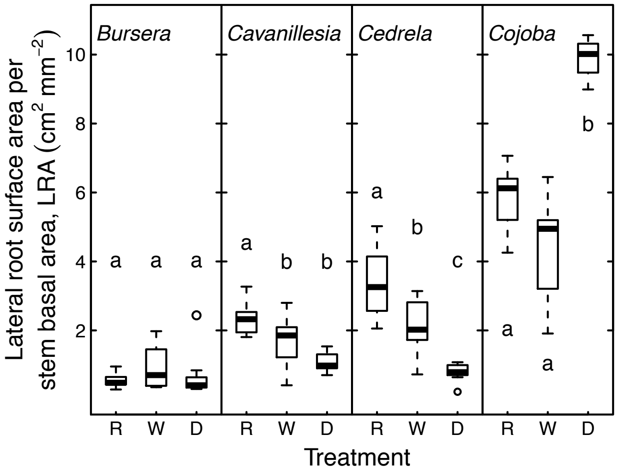

Fig. 4.3. Lateral root surface area per stem basal area in reference plants (R), watered (W), and drought (D) treatments. Boxes are drawn as in Fig. 4.1. Boxes that share letters do not differ significantly when tested with ANOVA followed by Tukey HSD test (*P* > 0.05). N = 8 within each box except for within the *Cojoba* drought treatment, where n = 4.

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Supplemental materials

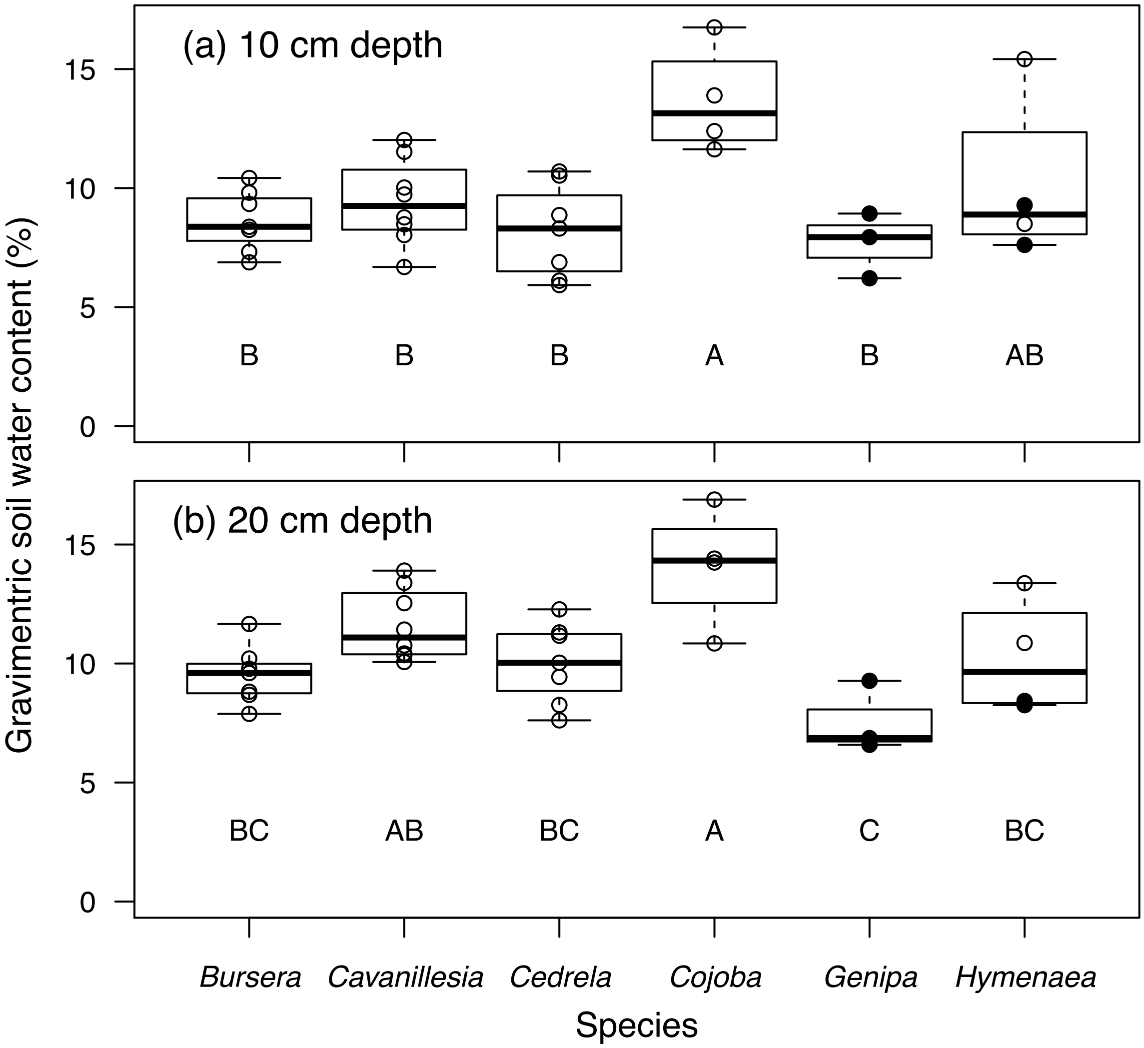

Fig. 4.S1. Drought treatment gravimetric soil water content at (a) 10 cm and (b) 20 cm depth. Open and filled circles represent pots with saplings that survived and died, respectively. Species that share letters beneath boxes do not differ significantly (Tukey HSD, $P > 0.05$). Sample sizes for *Coboja*, *Genipa*, and *Hymenea* are < 8 because most pots with saplings that were dead were not measured (see Material and methods).

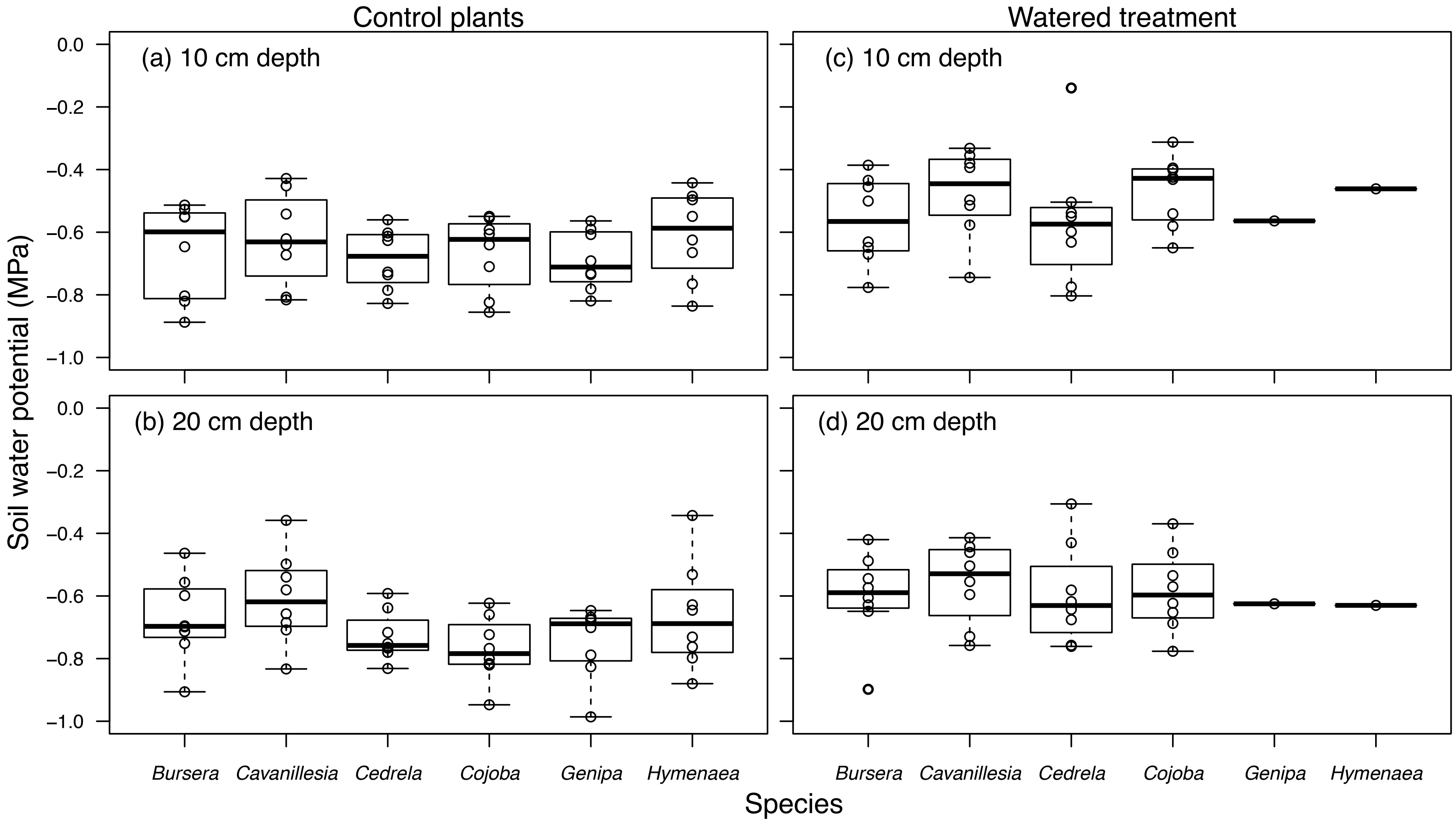

Fig. 4.S2. Soil water potential at 10 cm and 20 cm depth in the pots of the control plants and the watered treatment. Within depth andtreatment, there were no significant differences among species (Tukey HSD, $P > 0.05$). Sample sizes for *Genipa* and *Hymenaea* in the watered treatment are < 8 because most of the saplings were not harvested due to mortality in the drought treatment (see Materials and methods).

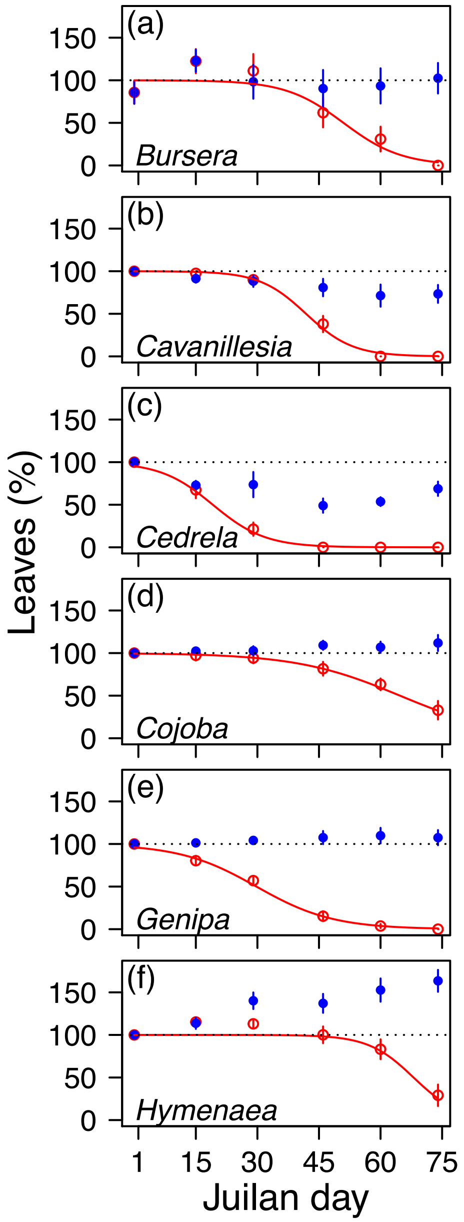

Fig. 4.S3. The number of leaves (percent relative to the start of the experimental treatments) over time. Closed blue circles represent the watered treatment. Red open circles represent the dry treatment. Values are the means and bars extend to 1 SE (n = 8). The red lines represent sigmoidal functions fit through the drought treatment data.