Plant water use responses along secondary forest succession during the 2015-2016 El Niño drought in Panama

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March 2018

New Phytologist

DOI10.1111/nph.15071

The definitive version of this article is available at:

Summary

- Tropical forests are increasingly subjected to hotter, dryer conditions due to global climate change. The effects of drought on forests along successional gradients remain poorly understood.
- We took advantage of the 2015/16 El Niño event to test for differences in drought response along a successional gradient by measuring sap flow in 76 trees, representing 42 different species, in 8, 25, and 80-year old secondary forests in the 15 km² “Agua Salud Project” study area, located in central Panama.
- Average sap velocities and sapwood-specific hydraulic conductivities were highest in the youngest forest. During the dry-season drought, sap velocities increased significantly in the 80-year old forest due to higher evaporative demand but not in younger forests. Main drivers of transpiration shifted from radiation to vapor pressure deficit with progressing forest succession. Soil volumetric water content was a limiting factor only in the youngest forest during the dry season, likely due to less root exploration in the soil.
- Trees in early-successional forests displayed stronger signs of regulatory responses to the 2015/16 El Niño drought, and the limiting physiological processes for transpiration shift from operating at the plant-soil interface to the plant-atmosphere interface with progressing forest succession.

Keywords
Agua Salud, drought, El Niño, plant hydraulics, sap flow, seasonal tropics, secondary forest, succession
Introduction

Ecosystems worldwide are subjected to increasing pressure from climate change and plant water use traits are a crucial component towards developing a predictive understanding of plant responses to these changes. Globally, 2015 marked the warmest year since the beginning of instrumental data collection with temperature anomalies exceeding two standard deviations in the tropics (Hansen & Sato, 2016). Tropical forests are particularly sensitive to drought and respond with considerable changes in species distribution and composition (Engelbrecht et al., 2007; Nepstad et al., 2007; Phillips et al., 2009; Wright, 2010). The 2015/16 El Niño-Southern Oscillation (ENSO) event provided an excellent research opportunity to study the responses of tropical forests to severe drought conditions. In Panama, the 2015/16 ENSO event resulted in the third longest dry season on record (173 days) with over 90% of the country experiencing severe drought conditions. A mechanistic understanding of responses to drought of tropical forests is critical to land management and conservation efforts, and to improve the predictive power of global models of carbon and water fluxes that generally perform poorly under drought conditions (Powell et al., 2013).

Hydraulic failure is a main driver of drought-induced tree mortality in tropical forests (Rowland et al., 2015). However, studies that assess drought responses of tropical trees across a successional gradient are often based on seedlings/saplings or monospecific stands (e.g. Huc et al., 1994; Tyree et al., 2003; Engelbrecht et al., 2006; Markesteijn et al., 2011; Pineda-Garcia et al., 2012; Pineda-García et al., 2015), and field data are sparse. Due to the high taxonomic diversity and lack of species dominance in most tropical forests, a trait-based approach to data analysis (McGill et al., 2006; Escudero & Valladares, 2016) is often the only cost-effective way to study these forests. For example, leaf and stem hydraulic traits explained drought tolerance across species in Amazon rainforest trees (Powell et al., 2017), and similar morphological characteristics (e.g. sapwood depth, tree size, phenology) appear to outweigh taxonomic affiliation, as indicated by considerable convergence in sap flow among phylogenetically diverse but morphologically similar species in tropical systems (Meinzer et al., 2001; O'Brien et al., 2004; McJannet et al., 2007; Kunert et al., 2010; Moore et al., 2017). Recent work on sap flow in subtropical and tropical biomes includes assessments of responses to drought (Luo et al., 2016), environmental factors (Eller et al., 2015; Aparecido et al., 2016), and seasonal variability (Kunert et al., 2010; Schwendenmann et al., 2015).

Early-successional, light-demanding species are generally characterized by lower wood density, larger vessel diameter and specific leaf area, coinciding with and higher hydraulic conductivity and sap velocities compared to late-successional species (Bazzaz & Pickett, 1980; Poorter et al., 2004; Poorter et al., 2010; Markesteijn et al., 2011; Apgaua et al., 2015; Schönbeck et al., 2015). Although these traits suggest that early-successional species are more vulnerable to xylem cavitation (Tyree & Sperry, 1989),
some studies report no difference (Pineda-Garcia et al., 2012; Pineda-Garcia et al., 2015; Powell et al., 2017) or lower drought tolerance in late-successional species (Apagaua et al., 2015; Schönbeck et al., 2015). Independent of their successional classification, larger trees have the potential to mitigate drought effects through stem capacitance and access to deep soil water (Phillips et al., 2003; Čermák et al., 2007; Schwendenmann et al., 2015).

Recent studies suggest that drought-induced mortality is most common at the end of the growth spectrum, with highest mortalities reported at the seedling stage and in large trees (Engelbrecht et al., 2006; Nepstad et al., 2007; Rowland et al., 2015; Meakem et al., 2017). In the moist lowland forests of Panama, transpiration in canopy trees is generally energy limited as a result of frequent cloud cover and abundant soil water content, especially during the rainy season (Graham et al., 2003), and regulated by structural (leaf area) rather than physiological (stomatal control) means (Phillips et al., 2001; Wolfe et al., 2016). Understanding both plant hydraulic conductivity and response to environmental drivers is crucial to improve predictive understanding of drought responses. When soil-water and plant-water transport are non-limiting, transpiration is a function of available energy (radiation) and atmospheric dryness (vapor pressure deficit), i.e. atmospheric demand (Penman, 1948; Monteith, 1965). Diel transpiration is approximately linearly related to radiation or vapor pressure deficit – whichever is more limiting – until maximum hydraulic conductivity occurs and saturation of transpiration is reached (Oren et al., 1999). Thus, deviations from these linear relationships can be indicative of regulatory responses, such as stomatal closure or hydraulic limitations, and statistical modeling of these deviations can be used to detect parameters that limit transpiration, such as soil volumetric water content (Oren et al., 1998; Eller et al., 2015). Moreover, time lags and hysteresis patterns in the diurnal relationship between transpiration, vapor pressure deficit, and photosynthetic photon flux density can be used to determine the biotic and abiotic factors that limit transpiration (O’Grady et al., 1999; Phillips et al., 1999; Matheny et al., 2014; Zhang et al., 2014; Mallick et al., 2016). In addition, nocturnal sap flow can be an indicator of stem refilling of capacitance storage in drought-stressed trees (Pfautsch & Adams, 2013) and has been shown to be significantly higher in dry season compared to wet season periods in tropical biomes (Forster, 2014).

The main objective of this study is to elucidate the interactions between plant hydraulics, successional stage, and environmental drivers in species-diverse secondary tropical forests during the severe drought of the 2015/16 ENSO event in central Panama. Utilizing a chronosequence approach, we measured sap flow in 8-, 25-, and 80-year old secondary forests and tested the following hypotheses: (1) Early-successional forests exhibit highest overall sap velocities and sapwood-specific hydraulic conductivities due to typically higher leaf area allocation and leaf-level water demand of fast-growing, shade-intolerant pioneer species. (2) Early-successional forests experience reduced sap velocities during the dry-season drought, due to presumed shallower rooting depth in 8-year old trees compared to 80-year
old trees, and exhibit strong regulatory responses and opportunistic water use strategies. (3) Late-successional forests exhibit no decrease in sap velocities during drought periods due to presumed access to deep soil water reservoirs and stem water storage in larger trees. (4) Sap velocities in early-successional forests are mainly driven by radiation due to high exposure in a single-layered forest and prevalence of opportunistic pioneer species, whereas vapor pressure deficit is the main driver of sap velocities in late-successional forests due to the presence of a multi-layered, partially-shaded canopy with a higher proportion of shade-adapted species.

Materials and Methods

Study Area

All sites were located in the “Agua Salud Project” study area (AS), located centrally in the Panama Canal Watershed (PCW; 9°13’ N, 79°47’W, 330 m a.s.l.). The study area borders Soberania National Park to the west and comprises a mosaic of land uses types that are typical for the PCW, including cattle pastures, fallows, timber plantations, and secondary forests patches. Local topography is characterized by rolling hills with steep slopes and a dense network of small streams. Soils are deep Oxisols with relatively low fertility (Turner & Engelbrecht, 2011). The climate is sub-humid tropical, with an annual mean precipitation of 2700 mm and a pronounced dry season from late-December to mid-May (Ogden et al., 2013). In 2015, strong ENSO conditions resulted in only 1800 mm precipitation, and 2014-2016 marked the driest contiguous 3-year period since the beginning of instrumental data collection in 1925 on Barro Colorado Island, located ~12 km southwest from the study area (data provided by the Physical Monitoring Program of the Smithsonian Tropical Research Institute).

We used a chronosequence approach to monitor transpiration across a successional gradient in secondary forests throughout the 2015/16 ENSO event. Despite known limitations of chronosequence studies (Johnson & Miyanishi, 2008) and the reported uncertainties of successional trajectories in neotropical forest succession (Norden et al., 2015), there is sufficient support to this approach in the literature. In the AS project area, early-successional forests are more similar to one another than late-successional forests, both in regards to composition (van Breugel et al., 2013) and functional traits (Craven et al., 2015). In addition, general trends in biophysical traits are largely independent of taxonomic affiliation and a tight link exists between these traits and water use strategies (Ewers et al., 2011). We studied three stands of secondary forest including young (hereafter SF8 for secondary forest of 8 years age at the beginning of data collection), 25-year-old, and 80-year-old forest (hereafter SF25 and SF80). All three stands were utilized as a cattle pasture prior to stand initiation; basal area was 9.4, 16.0, and 31.5 m² h⁻¹ in SF8, SF25, and SF80, respectively (Table S1; van Breugel et al., 2013). Sap flow was measured on one hillslope each in SF8 and SF80, and two hillslopes in SF25 at the halfway point between
ridgetop and valley bottom, with total slope distances of 139, 103/104, and 60 m in SF8, SF25, and SF80, respectively. Slopes were 29, 29/33, and 24 degrees and aspects were 219, 242/70 and 202 degrees in SF8, SF25, and SF80, respectively. Elevations at the center of each sap flow site were 264, 276/246, and 190 m a.s.l. in SF8, SF25, and SF80, respectively.

*Sap Flow Measurements*

We used heat-ratio sap flow sensors (Marshall, 1958) to measure sap flow in 76 trees, representing 46 species, across all sites (Table S2). Initial tree selection was based on diameter at breast height (DBH) to represent local size distribution rather than species. In a landscape-scale study of forest succession in the AS project area, van Breugel et al. (2013) found that only six of the 526 plant species made up more than 5% of all plants ≥ 1 cm diameter in more than 10% of their 108 plots. Thus, most locally dominant species were not dominant across the metacommunity and selecting species based on local dominants can result in biased samples. We included small and understory trees to ensure adequate representation of establishing late-successional trees to capture a wider range of canopy positions, especially in older forests. Based on relative canopy position, 13 of the 27 instrumented trees in SF80 were classified as sub-canopy trees, compared to three and zero sub-canopy trees in SF25 and SF8, respectively. Lastly, tree size has been shown to be more important than species affiliation when determining sap velocities in a multi-species forest (Hernandez-Santana et al., 2015; Moore et al., 2017).

All selected trees were identified to species level and compared to their local abundance based on basal area data from nearby secondary succession plots (van Breugel et al., 2013). Based on these data, we identified two species in SF8 (*Conostegia xalapensis*, *Vismia macrophylla*) and two species in SF25 (*Xylopia frutescens*, *Vismia macrophylla*) that each account for at least 5% of basal area in all four and all three nearby secondary succession plots in SF8 and SF25, respectively. As a result, two additional individuals of *Conostegia xalapensis* and one individual of *Vismia macrophylla* were instrumented in SF8 to better represent these locally (pre-) dominant species, whereas *Xylopia frutescens* and *Vismia macrophylla* were already adequately represented in SF25 with 5 and 4 instrumented trees, respectively (Table S2). No dominant species were identified in SF80.

Sensors were installed at breast height (~1.37 m) on the upslope-facing side of the tree and were placed equidistantly from the heater using a drill guide. Despite potential radial variation in sap flow, we used one sensor per tree rather than multiple sensors per tree. Given a limited number of sensors, this approach has been shown to reduce uncertainties in estimates of stand-scale sap flow measurements (Komatsu et al., 2017). Sensors were constructed using stainless steel hypodermic needles (1.3 mm diameter) cut to either 26 or 13 mm length. A copper-constantan thermocouple junction was inserted into the needle with an effective measurement depth of 20 mm (for use in trees with DBH > 5 cm) and 10 mm (for use in trees with DBH < 5 cm) for 26 and 13 mm needles, respectively. Heaters were constructed
using slightly larger needles (1.6 mm diameter) to accommodate constantan coils. All needles were sealed
with epoxy at the tip and with hot glue at the fitting. To account for expectedly high flow rates, we chose
a sensor spacing of 0.5 cm (Burgess et al., 2001).

Data were logged in 30-minute intervals following a 3-second heat pulse and downloaded weekly
beginning in 2015 on March 12 (SF25), May 21 (SF8), and July 29 (SF80) until August 31, 2016 (Table
S3). To minimize noise and to allow for equilibration of temperature ratios (Burgess et al., 2001), 20
measurements were logged and averaged after a 60-second delay following the pulse of heat. At each site,
sensors were connected via differential channels to two AM16/32 multiplexers (Campbell Scientific,
Logan, UT), controlled by a single CR1000 data logger (Campbell Scientific, Logan, UT). Measured
temperature ratios were converted to heat pulse velocities \( V_h \) (cm h\(^{-1}\)) according Burgess et al. (2001):

\[
V_h = \frac{k}{x} \ln \left( \frac{v_2}{v_1} \right) 3600 ,
\]

Eqn 1

where \( k \) is thermal diffusivity of wood (cm\(^2\) s\(^{-1}\)), \( x \) is the distance between heater and either sensor (cm),
and \( v_1/v_2 \) is the temperature ratio between downstream and upstream sensor. \( V_h \) was corrected for
wounding according to the numerical solution by Burgess et al. (2001) and converted to sap velocity \( V_s \)
(cm h\(^{-1}\); Marshall, 1958; Barrett et al., 1995):

\[
V_s = \frac{V_c \rho_b (c_w + m_c c_s)}{\rho_s c_s} ,
\]

Eqn 2

where \( \rho_b \) is wood density (g cm\(^{-3}\)), \( c_w \) and \( c_s \) are specific heat capacities of wood and water (J kg\(^{-1}\) K\(^{-1}\)),
respectively, \( m_c \) is water content of sapwood, and \( \rho_s \) in the density of water (g cm\(^{-3}\)). Wood properties of
instrumented tree species were taken from a local dataset (Wright et al., 2010). To account for potential
probe misalignment, we induced zero-flow conditions by severing xylem vessels using a battery-powered
oscillating saw (DeWALT, model DCS355D1). Incisions were 4 cm wide, 6 cm deep, and located 3 cm
above and below sensors on a subset of trees with large enough diameters to reduce risk of wound-
induced mortality. After validation of zero-flow, a new set of sensors was installed on the same tree.

Zero-flow conditions were also assumed to occur pre-dawn (03:00-4:30) during periods with low
atmospheric demand for water (VPD < 0.2 kPa). Data from induced zero-flow measurements were used
to validate data from assumed zero-flow conditions. Of the 76 instrumented individuals, data from six
individuals were excluded from the analysis due to mortality (four trees) and high noise levels in the data
(two trees; Table S2). No gap filling was performed.

**Micrometeorological Measurements**

A meteorological station within the AS project area provided local climate data, including net
radiation (W·m\(^{-2}\)) using a CN4 net radiometer (Kipp & Zonen, Delft, Netherlands), air temperature (°C)
and relative humidity (RH, %) using an HMP60 (Vaisala, Vantaa, Finland), photosynthetic photon flux
density (PPFD, µmol m\(^{-2}\) s\(^{-1}\)) using a PSQ1 quantum sensor (Kipp & Zonen, Delft, Netherlands),
precipitation (mm) using a 260-250-A tipping bucket (NovaLynx, CA, USA), and wind speed (WS, m s\(^{-1}\)) using a 05103 wind anemometer (R. M. Young, Michigan, USA). Vapor pressure deficit (VPD, kPA) was calculated from these data following Allen et al. (1998). In each forest, soil volumetric water content (VWC) was measured in three locations along the hillslope using GS1 sensors (Decagon Devices, Pullman, WA) at three measurement depths (10, 30, and 50 cm) each. A single term for VWC was calculated, based on the weighted-by-depth average of data from 10, 30, and 50 cm depth.

Hydraulic Conductivity

Leaf water potentials \(\Psi_L\) were measured in the dry (March 6-16, 2016) and the wet season (July 26-28, 2016) using a pressure chamber (PMS Instrument Company, Albany, OR). In all forests, data were collected pre-dawn (3:00-5:00), midday (11:00-14:00), and pre-dusk (17:00-19:00). Data collection was limited to instrumented trees of which the canopy could be clearly distinguished from neighboring trees and that could be reached using 5 m long pole pruners (\(N_{SF80} = 7\), \(N_{SF25} = 8\), \(N_{SF30} = 11\)). To test for within-tree heterogeneity of \(\Psi_L\), we sampled 36 leaves from different positions within the canopy (lower-canopy sun, lower-canopy shade, upper-canopy sun, upper-canopy shade) from nine trees representing eight species in SF80 using a sling shot at midday (11:00-13:00) during the dry season on February 22, 2017, when \(\Psi_L\) gradients in the canopy are likely most pronounced. Lower-canopy leaves were sampled between 2 to 5 m height, upper-canopy leaves were sampled between 15 and 25 m height. ANOVA results show no significant difference in \(\Psi_L\) at different canopy positions (\(p = 0.57\), Figure S1), suggesting that lower canopy samples can be used as a proxy for average \(\Psi_L\) throughout the canopy. Similarly small gradients in \(\Psi_L\) have been found by Oberbauer et al. (1987) in Costa Rica.

Darcy’s law approximated sapwood-specific conductivity \(K_s\) was calculated for each tree (Tyree & Ewers, 1991) from the slope parameter of simple linear-regression of sap flux density and leaf water potentials (Martínez-Vilalta et al., 2014). Several assumptions are associated with this approach: First, we assumed homogeneous sap flow throughout the sapwood and accordingly calculated the volume of sap flowing across an area of sapwood based on conversion of \(V_s\) (cm h\(^{-1}\)) to sap flux density \(J\) (cm\(^3\) m\(^{-1}\) s\(^{-1}\)). Second, we assume that \(\Psi_L\) measurements are representative of average canopy \(\Psi_L\) (see above). Third, the approach accounts for the assumptions of obtaining \(\Psi_L\) at true peak sap flow and uncertainties of equilibration of soil water potential \(\Psi_S\) and \(\Psi_L\) at predawn (Martínez-Vilalta et al., 2014; Hochberg et al., 2017). Lastly, to account for vertical heterogeneity in \(V_s\) (i.e. stem hydraulic capacitance), we used sap flow data at the timestamp with the highest correlation coefficient between evaporative demand and up to 90-minutes time-lagged sap flow rates on the day of water potential measurements for each tree.

Evaporative demand (m s\(^{-1}\)) was calculated according Van Bavel (1966) and Bladon et al. (2006).

\[
ETp = \frac{\Delta Q^* + \rho_a L_a u D}{\rho_a L_a (\Delta + \gamma)}, \quad \text{Eqn 3}
\]
where $\Delta$ is the slope of the saturation vapor pressure curve (Pa K$^{-1}$), $Q^*$ is net radiation (J m$^{-2}$ s$^{-1}$), $\rho_a$ is density of air (kg m$^{-3}$), $c_a$ is heat capacity of air (J kg$^{-1}$ K$^{-1}$), $u$ is wind speed (m s$^{-1}$), $D$ is vapor pressure deficit (Pa), $\rho_w$ is density of water (kg m$^{-3}$), $\lambda_v$ is the latent heat of vaporization (J kg$^{-1}$), and $\gamma$ is the psychrometric constant (Pa K$^{-1}$). A total of 203 measurements were included in the analysis and resulting $K_s$ values were averaged by forest age.

**Statistical Analysis**

Due to the robustness of linear mixed models in dealing with longitudinal data with missing data as well as temporal autocorrelation (von Ende, 2001), a linear mixed model (M-1) was used to assess differences in average seasonal $V_s$ or $K_s$ (response) between seasons, forest age, and the interaction between season and forest age (fixed effects), while accounting for individual trees (random effect). Seasons were classified according to official dates provided by the Meteorological and Hydrological Branch of the Panama Canal Authority, with the wet seasons starting on May 17 (2015) and April 27 (2016), and the dry seasons starting on November 27 (2015) and December 19 (2016). Analysis was limited to a subset of trees for which data were available in all seasons ($N_{SF8} = 15$, $N_{SF25} = 25$, $N_{SF80} = 26$). Differences of $V_s$ and $K_s$ between seasons for a given forest age were assessed via least square means pairwise comparisons. One-sample t-tests were used to test whether nocturnal flow was significantly different from zero for each forest and season. Where multiple tests were performed, the Bonferroni correction was applied to account for multiple comparisons. A second linear measure mixed model (M-2) was used to assess differences in the effects of VPD, PPFD, WS, Precip., and VWC on average daily $V_s$ between forest ages, accounting for random effects of seasons and individual trees. Despite the known interaction between VPD and PPFD (i.e. evaporative demand; Eqn 3) and their separate mechanistic impacts on transpiration (Bladon et al., 2006), we chose to separate these parameters to be able to test for potential differences due to successional shifts in shade tolerance and a canopy structure. Model parameters were statistically evaluated using the Markov Chain Monte Carlo method with 1000 iterations (R-package "MCMCglmm"; Hadfield, 2010). For comparative analyses between forest ages using models M-1 and M-2, data were limited to July 29, 2015 to August 31, 2016 when data were collected in all forests. Wood densities were assessed for differences between forests via ANOVA. Differences in $\Psi_L$ and soil VWC between forests were assessed via linear mixed models and least square means pairwise comparisons. Mixed effect model analyses were performed using the R-package “lme4” (Bates et al., 2014) and pairwise comparisons using the R-package “multcomp” (Hothorn et al., 2008).

A linear regression model was developed with VPD, PPFD, WS, Prec., and VWC as predictors and average daily $V_s$ across trees per forest age as response. To minimize within-forest variance, the relationship between standard deviation of daily mean $V_s$ and sample size was assessed (Figure S2).

Subsequently, only days with data from at least 10 (SF8 & SF25) or 15 (SF80) trees were included in the
analysis. Linear regression models were based on Box-Cox transformed data beginning from the earliest time of measurement in each forest (Box & Cox, 1964). Relative importance metrics of environmental predictors were calculated (R-package "relaimpo"; Grömping, 2006), including each predictor’s usefulness (model contribution given all other predictor already included in the model) and overall model contribution averaged over orderings of predictors (Chevan & Sutherland, 1991). To account for a potential size-effect, we performed a separate analysis of relative importance metrics on a subset of trees smaller than 15 cm DBH in SF25 and SF80. Confidence intervals for relative importance metrics were calculated via bootstrapping (1000 runs). All data processing and analyses were performed in R 3.3.2 (R Core Team, 2015).

**Results**

Diel patterns of $V_s$ closely follow VPD and PPFD in all forests, with $V_s$ lagging slightly behind PPFD in SF8 and SF80, but not in SF25 (Fig. 1). In all forests, a clockwise hysteresis is evident between normalized half-hourly $V_s$ and VPD, especially during the dry season, and a counter-clockwise hysteresis is evident in the relationship between $V_s$ and PPFD, especially in SF8 and SF80 (Fig. 2). Average $\Psi_L$ during the dry season was similar among forests, ranging from -0.4 to -0.6 and from -1.6 to -1.7 MPa at pre-dawn and midday, respectively (Fig. 3a). Pre-dusk $\Psi_L$ was significantly less negative in SF8 than SF80 ($p = 0.04$), with -0.9 and -1.4 MPa, respectively. In the wet season, average $\Psi_L$ was similar among all forests, ranging from -0.3 to -0.4 and from -0.4 to -0.5 MPa at pre-dawn and pre-dusk, respectively (Fig. 3b). Midday $\Psi_L$ was significantly less negative in SF80 compared to SF8 ($p < 0.001$) and SF25 ($p = 0.018$), with -0.4, -0.7, and -0.9 MPa, respectively. Significant night-time sap flow occurred during the dry season in SF25 (2.01 cm h$^{-1}$; $p = 0.002$) and SF80 (1.90 cm h$^{-1}$; $p < 0.001$), reaching 11% and 8.7% of average daytime $V_s$ in SF25 and SF80, respectively (Table 1). Night-time flow was not significant in SF8 or during wet season periods in any forest.

Seasonality had a significant effect on $V_s$, as reflected in clear seasonal patterns across all forests (M-1, $p < 0.001$; Table 2, Fig. 4). Although average $V_s$ tended to be highest in SF8 and decreased with increasing forest age, the differences among forests are non-significant (Table 2, Fig. 5). In SF80, average $V_s$ was significantly higher in the dry season compared to both wet seasons ($p < 0.01$), whereas no significant differences between seasons were detected in SF8 and SF25 (Table 2). Seasonality also had a significant effect on $K_s$ ($p = 0.013$). In July 2016 (wet season), $K_s$ values were 5.81 ± 2.14, 5.22 ± 2.2, and 1.75 ± 1.3 mol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ in SF8, SF25, and SF80, respectively (Fig. 6). Compared to wet season values, $K_s$ in the dry season (March 2016) decreased significantly by 69% ($p = 0.013$) and 75% ($p < 0.001$) to 1.8 ± 1.17 and 1.3 ± 0.42 mol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ in SF8 and SF25, respectively. In SF80, the wet-to-dry-season increase of $K_s$ by 5% to 1.84 ± 0.44 mol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ was not significant. Wood densities
were not significantly different between forests \( (p = 0.06) \), with mean and standard error values of 0.59 ± 0.02, 0.53 ± 0.02, and 0.59 ± 0.02 g cm\(^{-3}\) in SF8, SF25, and SF80, respectively.

When accounting for seasonal and daily variance (M-2), significant differences in age-specific behavior and responses to environmental variables were detected. All environmental main effects (VPD, PPFD, WS, Precip., VWC) and the interactions between forest age and VPD, PPFD, WS, and VWC are significant (Table S4). Model parameter estimates suggest that VPD was a strong driver of \( V_s \) in all forests, with slopes of 3.81, 4.74, and 5.58 in SF8, SF25, and SF80, respectively. Precipitation is the only main effect with a negative slope parameter (-0.05). The effect of VPD on \( V_s \) was significantly greater in SF80 than in SF8 and the effect of PPFD was significantly greater in both SF8 and SF25 compared to SF80. WS had a significantly greater effect on \( V_s \) in SF25 compared to both SF8 and SF80. Soil VWC parameter estimates are significantly different between all forests and are the only model parameters with changing signs, with positive slopes for SF8 (0.2) and SF25 (0.08) and a negative slope for SF80 (-0.11).

Single-variable linear regression of VPD and \( V_s \) explains 48%, 54%, and 71% in SF8, SF25, and SF80, respectively (Fig. 7a,c,e). In SF8 and SF25, residuals show a distinct pattern when plotted against VWC below 40% (Fig. 7b,d,f). Below this threshold, VWC explains 42% \( (p < 0.001) \) and 7% \( (p < 0.001) \) of the residuals in SF8 and SF25, respectively, whereas no significant relationship between residuals and VWC was detected in SF80. Single-variable regression of PPFD and \( V_s \) explained 60%, 59%, and 66% in SF8, SF25, and SF80, respectively, with similar relationships between residuals and VWC as above (Figure S3). The proportion of variance explained by linear regression including all predictors is 69%, 66%, and 86% in SF8, SF25, and SF80, respectively. Overall model contributions averaged over orderings of predictors show PPFD and VPD as the main drivers of \( V_s \), together accounting for 77%, 71%, and 63% of model strength in SF8, SF25, and SF80, respectively (Table 3). With progressing forest age, VPD replaces PPFD as primary driver of \( V_s \). Parameter usefulness of WS increases with increasing forest age from 3% in SF8, to 12% and 13% in SF25 and SF80, respectively, whereas parameter usefulness of VWC decreases from 31% in SF8 to 11% and 4% in SF25 and SF80, respectively (Table 3).

Of the species that were classified as deciduous or semi-deciduous (Table S2), most exhibited only brief periods of dormancy at varying times throughout the year. However, the simultaneous, prolonged dormancy of four Annona spraguei and one Casearia arborea caused a sharp decline in average \( V_s \) in SF25 at the dry-wet transition in April 2016 (Fig. 4). Excluding aforementioned trees from the analysis resulted in a less dramatic drop and overall higher \( V_s \) at the end of the dry season in SF25 (Figure S4), improved linear regression model performance in SF25 to 72%, and reduced parameter usefulness of VWC to 2% (Table S6).


Discussion

Differences along the Chronosequence

Our results show that tropical secondary forests in central Panama exhibited different seasonal behavior and response to drought-induced water limitations during the 2015/2016 ENSO event, with clear differences along the successional gradient. Amidst species-specific differences in hydraulic architecture, early-successional and light-demanding species are generally characterized by low wood densities and wide vessel diameters, resulting in higher hydraulic conductivity and transpiration rates that facilitate their typical fast growth behavior (Finegan, 1984; Granier et al., 1996; Tyree et al., 1998; Sack et al., 2005; Poorte et al., 2010). Forests in the AS project area reflect these patterns as they exhibit a shift of functional strategies from resource acquisition to resource conservation with progressing succession (Craven et al., 2015). Although the observed differences in \( V_s \) and \( K_s \) between forest ages are non-significant and thus fail to support the first hypothesis – higher overall \( V_s \) and \( K_s \) due to higher water demands early-successional forests – our data agree with aforementioned general trends as average \( V_s \) as well as \( K_s \) during the wet season decrease with progressing forest age (Fig. 5). The lack of significant differences in \( V_s \) between forests can in part be attributed to similar wood densities of instrumented trees in all forests, suggesting similar hydraulic architecture (Santiago et al., 2004), as well as the strong seasonal, inversely-phased fluctuations in \( V_s \) between forest ages (Fig. 4). Including additional trees from mature or primary forest to the chronosequence would further clarify if the hypothesized trend is found along a broader successional gradient.

Our results partially lend support to the second hypothesis, that water use is reduced in early-successional forest during the 2015/16 ENSO dry-season drought. Pronounced hysteresis loops, differences in \( \Psi_t \), and significant reductions in \( K_s \) during the dry season (Fig. 2, Fig. 3a, Fig. 5) suggest stomatal regulation to prevent hydraulic failure in SF8 and SF25. Although \( K_s \) measurements are associated with uncertainties due to potential species-specific structural differences, \( K_s \) was not significantly related to wood density, thus further supporting environmental factors as drivers of observed differences. Similar to our study, Huc et al. (1994) found that early-successional tropical rainforest species in French Guiana exhibit significantly decreased stomatal and plant-intrinsic hydraulic conductances and less negative midday \( \Psi_t \) whereas late-successional species exhibit no change in the dry season. Although increasingly becoming the subject of debate (Hochberg et al., 2017), isohydric behavior, i.e. maintenance of constant \( \Psi_t \) through regulation of stomatal conductance, is a typical drought avoidance strategy (Bucci et al., 2005) and has been shown to be predominantly a trait in pioneer and early-successional species in a tropical dry forest in Bolivia (Markesteijn et al., 2011). In central Panama, drought-intolerant species are associated with little tolerance to low leaf water status and relatively higher hydraulic stem conductances (Kursar et al., 2009). Thus, although we did not directly assess drought
tolerance by means of mortality or percent loss of hydraulic conductivity, the greater response to soil VWC in SF8 (Fig. 7, Table 3) suggests that trees in early-successional forests experienced more drought stress than trees in late-successional forests, and consequently regulated water use during the 2015/16 ENSO dry-season drought.

The third hypothesis, that late-successional forests do not limit water use during the 2015/16 ENSO dry-season drought, was supported by our data (Fig. 5A). Studies on seasonal differences of transpiration in tropical forests report inconsistent results, including higher whole-tree transpiration in the dry season (Meinzer et al., 1999; O’Grady et al., 1999; Schwendenmann et al., 2015), similar canopy transpiration between seasons (Kumagai et al., 2004), or higher stand transpiration in the wet season (McJannet et al., 2007), suggesting complex interactions between taxonomic, physiognomic, microclimatic, edaphic, and topographic factors. Canopy trees in Panama have been shown to exhibit little stomatal control and CO₂ uptake is limited by light rather than water during the wet season (Phillips et al., 2001; Graham et al., 2003). In our study, Vₛ increased considerably in all forests at the beginning of the dry season when soils are still water-saturated and cloud cover is low, lending support to light as the limiting factor of transpiration (Fig. 4). Several studies have found direct or indirect evidence that trees with access to deep soil water maintain a favorable plant water status and higher transpiration throughout periods of reduced moisture availability (Jackson et al., 1995; Jackson et al., 1999; Meinzer et al., 1999; Stahl et al., 2013; Schwendenmann et al., 2015). In Panama, trees and lianas in old-growth forest utilize a higher proportion of deeper soil water at the end of the dry season (Andrade et al., 2005). Although root architecture varies considerably by species in Panama, with some species allocating significantly more resources to tap roots compared to lateral roots (Sinacore et al., 2017), it can safely be assumed that long-established trees in SF80 have deeper, larger root systems compared to younger trees in SF8, providing one explanation for sustained higher Vₛ during the dry season in SF80. Despite clear evidence for differences in behavior with forest age during the 2015/16 ENSO drought, long-term data are required to elucidate whether observed differences between forests are a direct result of the drought or fall within the typical seasonal behavior.

Trees in Panama exhibit considerable stem water storage capacitance that is linearly related to sapwood area, with 10 kg of stored water per 0.1 m² sapwood area (Goldstein et al., 1998). The significant nocturnal sap flow observed in SF25 and SF80 during the dry season, when VPD was comparatively low, could be indicative of stem refilling in large trees with greater stem water capacitance (Forster, 2014). However, the benefits of stem water storage could be size-independent, as stored stem water improved the tolerance to soil drought of only 1-year old late-successional species of a tropical dry forest in Mexico (Pineda-Garcia et al., 2012), and had similar potential to alleviate hydraulic constraints in small and large trees in Panama (Phillips et al., 2001). More research on whether stem capacitance has
a disproportionally greater effect on drought avoidance in large trees compared to small trees is required, such as simultaneous measurements of canopy conductance as well as sap flow at different heights in the stem and branches (Meinzer et al., 2004) and in roots across the chronosequence.

Environmental Drivers

Our data lend support to the fourth hypothesis, that VPD replaces PPFD as the main driver of $V_s$ in late-successional forests (Table 3). Light availability in the understory of secondary forest in Panama is reduced to less than 10% of above-canopy values after 20 years of growth (van Breugel et al., 2013). Consequently, the proportion of species with low light saturation points increases with progressing forest succession. As these species are adapted to low light conditions, VPD rather than PPFD becomes the primary limiting factor of transpiration in late-successional forests, making it one of the biological mechanisms that cause a feedback between microclimate and succession (Lebrija-Trejos et al., 2011).

Taller canopies are generally more exposed to wind and thus better coupled to the atmosphere (Jarvis, 1984). In addition, transpiration in well-coupled canopies is mainly driven VPD rather than radiation (Jarvis, 1984; Zhang et al., 2014). The increasing relative importance of WS with progressing forest age paired with the higher sensitivity of $V_s$ to VPD suggest a higher degree of canopy coupling in older forests due to increasing height. This is further supported by the smaller magnitude of the hysteresis between $V_s$ and VPD in SF80 compared to SF8 and SF25, indicating that $V_s$ is largely in phase with VPD in SF80. Estimates of omega decoupling coefficients (Jarvis, 1984) agree with this trend, with highest decoupling coefficients in SF8 and lowest in SF80 (Figure S5).

The negative effect of precipitation on $V_s$ is likely linked to leaf wetness, which had a strong inhibitory effect on sap flow in a tropical could forest tree in Brazil (Eller et al., 2015) and reduced transpiration by up to 28% in a tropical moist forest in central Costa Rica (Aparecido et al., 2016). In the AS project area, canopy interception values approach levels of mature lowland forests after approximately 10 years of growth (Zimmermann et al., 2013), explaining the observed significant difference in the effect of precipitation between SF8 and SF80. The counterclockwise hysteresis between PPFD and $V_s$ in all forests, specifically the morning lag, could be indicative of either stem capacitance or the inhibition of diffusion due to leaf wetness from dew that accumulated on the leaves at night (O'Brien et al., 2004).

The different effect of soil VWC on $V_s$ between forests indicates that trees in SF8 and SF25 experience some degree of water limitation, whereas higher soil VWC had a negative effect on $V_s$ in SF80. Soil VWC in SF80 remained comparatively wet throughout the dry season, never dropping below 35% (Fig. 7f, Fig. 8). If soil VWC remains well above the threshold for trees to maintain a favorable plant water status, increases in soil VWC beyond that point will have no positive effect on $V_s$ but instead have the potential to reduce $V_s$ as increases in VWC coincide with leaf-wetting precipitation events. The difference in soil VWC during the dry season between forests (Fig. 8) could be indicative of a higher
proportion of deep water usage of trees in SF80. Although not directly tested in this study, hydraulic
redistribution by larger trees can relocate water from deep to more shallow soil layers, potentially
facilitating water access of understory trees (Dawson, 1996; Caldwell et al., 1998; Oliveira et al., 2005).
The observed difference in soil VWC data during the dry season drought, paired with our sap flow data,
indicate a feedback effect between soil properties and succession that has the potential to alleviate
drought severity in older regrowing secondary forests in central Panama. Furthermore, soil water
availability is a direct determinant of local and regional species distribution in tropical forest of Panama,
and even short dry-spells can cause significant mortality in establishing seedlings (Engelbrecht et al.,
2006; Engelbrecht et al., 2007). Water stress is a major factor in shaping geographic distributions of large
trees in Panama (Meakem et al., 2017). Contrary to SF8, both SF25 and SF80 have previously
experienced droughts, including the severe 1997/1998 (SF25 & SF80) and 1982/1983 ENSO (SF80)
events, potentially shifting species composition towards more drought-tolerant species in the older
forests.

Conclusion

Our study shows that trees in early-successional forests displayed stronger signs of regulatory
responses to the 2015/16 ENSO drought, and that the limiting physiological processes for V, shift from
operating at the plant-soil interface to the plant-atmosphere interface with progressing forest succession,
likely as a result of favorable soil characteristics and access to deeper soil water in late-successional
forests. Knowledge of the resilience of establishing secondary forests to drought is not only important for
optimizing reforestation efforts but also for development and optimization of models to predict water and
carbon fluxes in a dynamic landscape that comprises a mosaic of pastures and forest fragments at
different successional stages.

Acknowledgements

This work is a contribution of the Agua Salud Project of the Smithsonian Tropical Research
Institute (STRI). Agua Salud is part of ForestGEO and is a collaboration with the Panama Canal
Authority (ACP), the Ministry of the Environment (MiAmbiente) of Panama, and other partners. The
authors thank Fred Ogden, Edward Kempema, Daniel Beverly, Jazlyn Hall (University of Wyoming),
Robert Stallard, Holly Barnard (University of Colorado), Steven Paton, Alicia Entem, Estrella Yanguas,
Anabel Rivas, and Adriana Tapia (STRI) for their significant contribution to this study. For their help in
the field and laboratory, we thank Sergio Dos Santos, Federico Davies, Mario Bailon, Andres Hernandez,
Joana Balbuena, Eric Diaz, Guillermo Fernandez, Arnulfo Hernandez, Jorge Batista, Adam Bouché,
Laura Lyon, Joan Herrmann, Catalina Guerra, Emily Purvis, Katherine Sinacore, Ethan Miller, Heather
Speckman, and John Frank. Funding for this research was provided by the U.S. National Science
Foundation (NSF) EAR-1360384, Stanley Motta, the Silicon Valley Foundation, and the Heising-Simons Foundation.

**Author Contribution**

M.B., B.E.E., and J.S.H. designed the study. M.B. collected data; M.B. carried out data analysis with input from B.E.E.; M.B. wrote the manuscript with revisions by all coauthors.


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forest: uncertainty sources, stand structure effect, and future scenarios. *Canadian Journal of


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Table 1 Summary statistics and t-test results (Bonferroni-adjusted alpha for 9 tests: 0.0056) for average nocturnal sap velocities by seasons and forest age. All analyses based on trees for which data was collected in all seasons ($N_{SF8}$ = 15, $N_{SF25}$ = 25, $N_{SF80}$ = 26). Data from July 29, 2015 to August 31, 2016.

<table>
<thead>
<tr>
<th>Forest</th>
<th>Season</th>
<th>N</th>
<th>Nocturnal $V_s$ [cm h$^{-1}$]</th>
<th>Standard Error</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF8</td>
<td>Wet 2015</td>
<td>15</td>
<td>0.86</td>
<td>0.52</td>
<td>1.12</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Dry 2016</td>
<td>15</td>
<td>0.51</td>
<td>0.56</td>
<td>1.20</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Wet 2016</td>
<td>15</td>
<td>-0.26</td>
<td>0.67</td>
<td>1.44</td>
<td>0.71</td>
</tr>
<tr>
<td>SF25</td>
<td>Wet 2015</td>
<td>25</td>
<td>-0.61</td>
<td>0.64</td>
<td>1.32</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Dry 2016</td>
<td>25</td>
<td>2.01</td>
<td>0.57</td>
<td>1.78</td>
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</tr>
<tr>
<td></td>
<td>Wet 2016</td>
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<td>0.53</td>
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</tr>
<tr>
<td>SF80</td>
<td>Wet 2015</td>
<td>26</td>
<td>0.18</td>
<td>0.44</td>
<td>0.91</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>Dry 2016</td>
<td>26</td>
<td>1.90</td>
<td>0.39</td>
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<tr>
<td></td>
<td>Wet 2016</td>
<td>26</td>
<td>0.16</td>
<td>0.35</td>
<td>0.72</td>
<td>0.65</td>
</tr>
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</table>

* significant (p < 0.05)
Table 2 ANOVA table of generalized linear mixed model results of average sap velocities per season (M-1) and least square means pairwise comparison for average diel sap velocities between seasons by forest age (Tukey’s single step). Analyses based on trees for which data was collected in all seasons (N_{SF8} = 15, N_{SF25} = 25, N_{SF80} = 26). Data from July 29, 2015 to August 31, 2016.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>Degrees of Freedom</th>
<th>F-value</th>
<th>p-value</th>
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<td>Forest Age</td>
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<td>13.13</td>
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<td>1.28</td>
<td>0.28</td>
</tr>
<tr>
<td>Season</td>
<td>156.78</td>
<td>78.39</td>
<td>2</td>
<td>7.65</td>
<td>&lt;0.001*</td>
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<tr>
<td>Forest Age : Season</td>
<td>45.36</td>
<td>11.34</td>
<td>4</td>
<td>1.11</td>
<td>0.36</td>
</tr>
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<table>
<thead>
<tr>
<th>Forest</th>
<th>Contrast</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF8</td>
<td>Wet 2015 – Dry 2016</td>
<td>-0.54</td>
<td>1.17</td>
<td>-0.46</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Wet 2015 – Wet 2016</td>
<td>1.40</td>
<td>1.17</td>
<td>1.19</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Dry 2016 – Wet 2016</td>
<td>1.94</td>
<td>1.17</td>
<td>1.66</td>
<td>0.22</td>
</tr>
<tr>
<td>SF25</td>
<td>Wet 2015 – Dry 2016</td>
<td>-1.55</td>
<td>0.91</td>
<td>-1.72</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Wet 2015 – Wet 2016</td>
<td>-0.28</td>
<td>0.91</td>
<td>-0.31</td>
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<tr>
<td></td>
<td>Dry 2016 – Wet 2016</td>
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<tr>
<td>SF80</td>
<td>Wet 2015 – Dry 2016</td>
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<td>0.89</td>
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<td>Dry 2016 – Wet 2016</td>
<td>3.15</td>
<td>0.89</td>
<td>3.55</td>
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* significant (p < 0.05)
Table 3 Relative importance metrics and confidence intervals. Methods are AVRG (overall model contribution averaged over orderings of predictors) and LAST (model contribution given all other predictor already included in the model). Data include all trees (N_{SF8} = 19, N_{SF25} = 28, N_{SF80} = 27). Predictors are PPFD (photosynthetic photon flux density), VPD (vapor pressure deficit), Precip. (precipitation), VWC (soil volumetric water content), and WS (wind speed). Note that predictors are sorted by relative importance for each metric and forest combination. Overall model performance was 69, 66, and 86% for SF8, SF25, and SF80, respectively. A separate analysis of relative importance metrics on a subset of trees smaller than 15 cm DBH in SF25 and SF80 largely agrees with results from respective full models (Table S5).

<table>
<thead>
<tr>
<th>Forest</th>
<th>Method</th>
<th>Predictor</th>
<th>Rel. imp. [%]</th>
<th>95% CI upper</th>
<th>95% CI lower</th>
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<td>SF8</td>
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<td>VWC</td>
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<tr>
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<tr>
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<tr>
<td></td>
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<td>VWC</td>
<td>11.35</td>
<td>5.11</td>
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<td>SF80</td>
<td>AVRG</td>
<td>VPD</td>
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<td>LAST</td>
<td>VWC</td>
<td>3.72</td>
<td>0.37</td>
<td>10.57</td>
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</table>
Fig. 1 Diel patterns of sap velocities (cm h⁻¹) based on half-hourly averages from all trees in SF8 (a, b), SF25 (c, d), and SF80 (e, f). Shaded areas represent 95% confidence intervals. The dashed lines indicate half-hourly averages of vapor pressure deficit (VPD in kPa; a, c, e) and photosynthetic photon flux density (PPFD in µmol m⁻² s⁻¹; b, d, f).
**Fig. 2** Hysteresis loops between normalized sap velocities and normalized vapor pressure deficit (VPD; a, c, e) and normalized sap velocities and normalized photosynthetic photon flux density (PPFD; b, d, f) in SF8 (a, b), SF25 (c, d), and SF80 (e, f). Arrows indicate direction of hysteresis.
Fig. 3 Leaf water potentials $\Psi_L$ in MPa in the dry season (a; March 6-16, 2016) and wet season (b; July 26-28, 2016) for SF8 (orange), SF25 (green), and SF80 (blue). Horizontal lines inside boxes correspond to the median, the lower and upper box boundaries correspond to first and third quartiles (25th and 75th percentile, respectively), lower and upper whiskers extend no further than 1.5×IQR (inner quartile range) from the first and third quartiles, and dots represent data points beyond this range. Letters indicate significant differences in average leaf water potential between forests at a given sampling time as assessed via linear mixed model and least square means pairwise comparison. Data from 9:00 and 20:00 were only collected in SF25 in the dry season.
Fig. 4 Time series of 3-week running mean sap velocities (cm h\(^{-1}\); a) and precipitation (mm day\(^{-1}\); b). Shaded areas denote seasons (tan: dry, blue: wet). The dashed line indicates earliest date from which data across all forest ages are available (July 29, 2015). Data from all trees are shown, with a minimum of 10 trees providing data per day out of 19, 28, and 27 trees in SF8, SF25, and SF80, respectively.
Fig. 5 Linear mixed model results of diel (a) and nocturnal (b) sap velocities in cm h\(^{-1}\) based on trees for which data was collected in all seasons \((N_{SF8} = 15, N_{SF25} = 25, N_{SF80} = 26)\). Letters in A denote grouping, with different letters indicating significant differences in sap velocities between seasons within a given forest types \((\alpha < 0.05; \text{Tukey method})\). *** in B denotes nocturnal sap velocities that were significantly different from 0. Data from July 29, 2015 to August 31, 2016. Horizontal lines inside boxes correspond to the median, the lower and upper box boundaries correspond to first and third quartiles \((25^{\text{th}} \text{ and } 75^{\text{th}} \text{ percentile, respectively})\), lower and upper whiskers extend no further than \(1.5 \times \text{IQR} \) (inner quartile range) from the first and third quartiles, and dots represent data points beyond this range.
Fig. 6 Leaf water potential $\Psi_L$ (MPa) and corresponding sap flow $J$ (cm$^3$ m$^{-2}$ s$^{-1}$) in SF8 (orange), SF25 (green), and SF80 (blue) in the dry season (a) and wet season (b). $J$ values were lagged up to 90 minutes, based on highest correlation coefficient between (lagged) $J$ and evaporative demand for a given tree on the day of measurement. Darcy’s law approximations and respective standard errors of sapwood-specific conductivity $K_s$ (mol m$^{-2}$ s$^{-1}$ MPa$^{-1}$) are given in the graphs.
**Fig. 7** Linear regression of daily average VPD (kPa) and sap velocity $V_s$ (cm h$^{-1}$; a, c, e), and residuals plotted against volumetric water content (%) in the top 50 cm of the soil (b, d, f) in SF8 (a), SF25 (b), and SF80 (c). VPD explained 48% (SF8; a), 54% (SF25; c), and 71% (SF80; e) of variance in $V_s$. Linear regression between residuals and soil VWC was significant in SF8 (b; $R^2 = 0.42, p < 0.001$) and SF25 (d; $R^2 = 0.7, p < 0.001$), and was non-significant in SF80 (f). Note how soil VWC does not go below 40% in the wet season in either SF8 (b) or SF80 (f). Single-variable regression of PPFD and $V_s$ explained 60%, 59%, and 66% in SF8, SF25, and SF80, respectively, with similar relationships between residuals and VWC as above (Figure S2).
Fig. 8 Soil volumetric water content (%) in the top 50 cm of the soil in SF8 (orange), SF25 (green), and SF80 (blue). Data from July 29, 2015 to August 31, 2016. Horizontal lines inside boxes correspond to the median, the lower and upper box boundaries correspond to first and third quartiles (25th and 75th percentile, respectively), lower and upper whiskers extend no further than 1.5×IQR (inner quartile range) from the first and third quartiles, and dots represent data points beyond this range. Letters indicate significant differences in VWC between forests per season as assessed via linear mixed model and least square means pairwise comparison.
Supporting Information

**Fig. S1** Boxplots of leaf water potentials at different canopy positions from eight species (N=36) in SF80.

**Fig. S2** Minimum number of working sap flow sensors and resulting standard deviation of average sap velocity data for all forests.

**Fig. S3** Linear regression of daily average PPFD (µmol m⁻² s⁻¹) and sap velocity V_s (cm h⁻¹), and model residuals plotted against volumetric water content (%) in the top 50 cm of the soil.

**Fig. S4** Time series of 3-week running mean sap velocities and sum of daily precipitation, excluding four Annona spraguei and one Casearea arborea in SF25 due to their long-term dormancy at the end of the dry season 2016.

**Fig. S5** Boxplot of average daily Omega decoupling coefficients for all forests and seasons.

**Table S1** Overview of sap flow site characteristics, including GPS coordinates, slope, aspect, slope length, and elevation.

**Table S2** Overview of instrumented species and their phenology, relative canopy position, diameter at breast height (cm), and wood density (g cm⁻³).

**Table S3** Overview of instrumented species, respective sampling periods, and number of days where no data was collected (e.g. due to equipment failure).

**Table S4** ANOVA table of generalized linear mixed model results (M-2) and parameter estimates and p-values based on Markov Chain Monte Carlo method with 1000 iterations.

**Table S5** Relative importance metrics and confidence intervals of trees < 15 cm DBH trees (NSF8 = 19, NSF25 = 11, NSF80 = 13).

**Table S6** Relative importance metrics and confidence intervals for SF25, excluding four Annona spraguei and one Casearia arborea due to their long-term dormancy at the end of the dry season.