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Title: Differences in xylem and leaf hydraulic traits explain differences in drought tolerance among mature Amazon rainforest trees

Running head: Hydraulic traits of Amazon forest trees

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Abstract

Considerable uncertainty surrounds the impacts of anthropogenic climate change on the composition and structure Amazon forests. Two large-scale ecosystem drought experiments in the eastern Brazilian Amazon observed increases in mortality rates among some tree species but not others; and therefore, the physiological traits underpinning these differential demographic responses were investigated. Xylem pressure at 50% conductivity (xylem-P$_{50}$), leaf turgor loss point (TLP), cellular osmotic potential ($\pi_o$) and cellular bulk modulus of elasticity ($\varepsilon$), all traits mechanistically linked to drought tolerance, were measured on upper canopy branches and leaves of mature trees from selected species growing at the two drought experiment sites. Each species was placed a priori into one of four plant functional type (PFT) categories: drought-tolerant versus -intolerant based on observed mortality rates, subdivided into early- versus late-successional based on wood density. We tested the hypotheses that the measured traits would be significantly different between the four PFTs, and that they would be spatially-conserved across the two experimental sites. Xylem-P$_{50}$, TLP, and $\pi_o$, but not $\varepsilon$, occurred at significantly higher water potentials for the drought-intolerant PFT compared to the tolerant PFT; however, there were no significant differences between the early- and late-successional PFTs. These results suggest that these three traits are important for determining drought tolerance, and are largely independent of wood density—a trait commonly associated with successional status. Differences in these physiological traits that occurred between the drought-tolerant and -intolerant PFTs were conserved between the two research sites, even though they had different soil types and dry season lengths. This more detailed understanding of how xylem and leaf hydraulic traits vary between drought-tolerant and -intolerant tropical tree species that are in direct competition with each other promises to facilitate a much-needed improvement in the representation of plant
hydraulics within terrestrial biosphere models, which will enhance our ability to make robust
predictions of how future changes in climate will affect tropical forests.

Key Words: Amazon rainforest, plant traits, plant hydraulics, drought, turgor loss point

Introduction

Climate model predictions indicate that dry seasons are likely to lengthen across much of
the Amazon basin, with considerable overall drying in the eastern region by the end of this
century (Malhi et al., 2008; Joetzjer et al., 2013; Boisier et al., 2015). Widespread deforestation
is also predicted to cause lower precipitation through an extensive central corridor of the basin
(Coe et al., 2013). How these predicted changes in precipitation will affect the Amazon
rainforest is, however, highly uncertain. Dynamic vegetation models are the major tool for
assessing the consequences of chronic droughts; but presently, they are poorly formulated to
represent contrasting physiological responses of different tree species to water stress (Gailbraith
et al., 2010; Sakaguchi et al., 2011; Powell et al., 2013). Resolving this problem requires more
detailed understanding of the mechanisms that cause mature trees to succumb to drought, and the
time scale over which these mechanisms induce mortality in different species (McDowell et al.,
2008; Hartman, 2011; McDowell et al., 2013, Meir et al., 2015a).

Two in-situ ecosystem scale drought experiments were established in the eastern
Brazilian Amazon, at the Tapajós (TNF) and Caxiuanã (CAX) National Forests, to directly
assess the ecological impact of a chronic 50% reduction in precipitation (Nepstad et al., 2007; da
Costa et al., 2010). Two key and consistent findings emerged from both experiments: first,
mortality rates of the largest trees increased by 3.0 to 4.5-fold in the drought plots, resulting in a
20% reduction in aboveground biomass after three years of drought (Nepstad et al., 2007; da
Costa et al., 2010); second, mortality rates only increased in certain species (da Costa et al., 2010; Rowland et al., 2015a). These patterns also appear to be more general across Amazonia during severe natural droughts (Phillips et al., 2010; Meir et al., 2015b). The mechanistic cause (or causes) for the differential mortality rates between species, however, has not been isolated, but plant hydraulic traits are thought to have played a key role, likely signaling a cascade of processes leading to death (Rowland et al., 2015b, Binks et al., 2016a). The similarity in mortality results amongst taxa from the two drought experiments (Nepstad et al., 2007, da Costa et al., 2010) have since made it possible to test for differences in key plant traits linked to drought sensitivity in different species.

Maintaining connectivity of water through the soil-plant-atmosphere continuum is essential for vascular plants to maintain photosynthesis. Plants manage the risk of cavitation both anatomically and through stomatal regulation of transpiration. Anatomically, wider xylem elements, larger pit membrane pores, and a higher number density of pit membranes between vessel elements can increase the efficiency of water transport and hence carbon gain, but also increase the vulnerability for embolisms to form in the xylem as tension builds (Wheeler et al., 2005, Choat et al., 2008; Poorter et al., 2010). The xylem pressure when 50% of conductivity is lost (xylem -P50) is a useful quantitative metric for directly comparing the relative vulnerabilities of different species to cavitation during episodes of drought. Higher xylem-P50 values have been associated with trees growing in wetter climates in the tropics (Choat et al., 2007) and in larger-sized drought-intolerant tree species compared to smaller-sized trees and drought tolerant species growing at CAX (Rowland et al., 2015b). However, it is unclear if this pattern is conserved across ecosystems with contrasting soils types or if it is coordinated with other plant traits.
Plants also minimize the risk of cavitation through stomatal closure. Turgor loss point (TLP), the point when the leaf becomes so dehydrated its cells lose turgor, is an easily measured second order trait that correlates with the leaf water potential ($\psi_{lf}$) when the stomata are 50% closed (Brodribb et al., 2003). TLP has been used as an indicator of drought tolerance to low soil moisture (e.g. Lenz et al., 2006; Blackman et al., 2006). Cellular osmotic potential at full hydration ($\pi_o$) and the bulk modulus of elasticity of leaf cell walls ($\varepsilon$) are two first order traits that contribute to TLP. $\pi_o$ is the amount $\psi_{lf}$ is lowered by solutes in the cells. $\varepsilon$ is inversely related to the volumetric change in the leaf cell as turgor declines and thus is a metric of the amount of water available from cellular storage between full hydration and TLP. Species with lower TLP and $\pi_o$ are found to be more prominent in the drier climates across the tropics (Choat et al., 2007; Baltzer et al., 2008) and between dry versus wet biomes (Bartlett et al., 2012), as well as ecosystems with soils having comparatively lower water retention capacity (Maréchaux et al., 2015). Conclusive patterns for $\varepsilon$ are more variable between these same studies (e.g Baltzer et al., 2008 versus Bartlett et al., 2012), which likely arises from other ecological reasons to have stiff leaves that correlate with drought tolerance, such as herbivory. The role of TLP during acute or prolonged droughts and the mechanism through which it is achieved—i.e. investment in solutes ($\pi_o$) versus cellular structure ($\varepsilon$) (Bartlett et al., 2012)—is unclear in determining mortality risk of mature tropical trees growing in competition in the same ecosystem. Furthermore, understanding the physiological determinants of TLP may have important implications for modeling leaf carbon-dynamics during drought, where observations indicate water-stress elevates leaf respiration; although the mechanism explaining the respiration increase has yet to be identified (Metcalfe et al., 2010; Rowland et al., 2015a).
Phenotypic plasticity of hydraulic traits of tropical species has been poorly studied in general; however, a few studies have indicated that it may be ecologically important for some species to quickly adjust to acute droughts or survive dry seasons (e.g. Fonti et al., 2010; Maréchaux et al., 2017), while others have indicated a minor role for canopy trees (Bartlett et al., 2014; Binks et al., 2016a,b, Maréchaux et al., 2016, 2017). Interestingly, Campanello et al., (2008) showed xylem conductivity of saplings increased quickly in early-successional tropical species, but remained the same in late-successional species in response to higher light levels in the understory after a simulated disturbance (Campanello et al., 2008). This result suggests a need to test for potential interactions between traits that select for drought tolerance and successional status. Also, another study at CAX (Binks et al., 2016a) found evidence for adjustment in foliar osmotic parameters to long-term (>10 yrs) experimental drought, with drought-intolerant species showing greater long-term adjustment than drought-tolerant ones.

It has been proposed that wood density may be a useful proxy for drought tolerance in tropical tree species (Fearnside, 1997), and that it is negatively correlated with the precipitation gradient that exists across the Amazon basin (Baker et al., 2004; but see also ter Steege et al., 2006). Moreover, in a pan-tropical synthesis of moist tropical forest species, a weak but significant negative relationship between \( \pi_o \) and wood density was found (Christoffersen et al., 2016). Other observational studies have found that variation in stem economic traits, such as wood density, is largely independent of hydraulic traits (Baraloto et al., 2010; Maréchaux et al., 2015). Wood density also correlates with successional type, where species with lower wood density tend to be early-successional, and species with higher wood density tend to be late-successional (Poorter et al., 2010). However, it has not been determined for mature trees...
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responses to severe and chronic drought (Nepstad et al., 2002, 2007; Brando et al., 2008; da Costa et al., 2010). The Caxiuanã TFE experiment commenced in January 2002 and was still running at the time of this study. The Tapajós TFE experiment ran from 2000 to 2004 (Nepstad et al., 2007); therefore post hoc measurements were made on the four selected species in the forest adjacent to the former TFE plots, and within-species phenotypic plasticity was not evaluated. Physical and biological characteristics of each site are summarized in Table 1.

A brief description of the TFE experimental design is given here; more detailed descriptions are provided in Nepstad et al. (2002) and Fisher et al. (2007). Two 1-ha plots, one control and one treatment plot, were established in each of the CAX and TNF forests. Both plots in each forest were selected to be structurally and floristically similar. A 1-2 m high leaky system of plastic panels and gutters was constructed over the entire understory of the treatment plot. The panels prevented approximately 50% of the rainfall from reaching the soil (Nepstad et al., 2002). The remaining rainfall was diverted off site through the gutters and a 1 x 1 m drainage ditch lined with plastic. The drainage ditch encircled each plot to also prevent lateral roots from accessing soil moisture from outside the plots (Nepstad et al., 2002). Soil moisture measurements verified the efficacy of this design to create a soil moisture drought throughout the rooting zone in the treatment plots (Fisher et al., 2007; Brando et al., 2008).

**Species selection and sampling**

The species measured in this study were strategically selected as representatives of four contrasting plant functional type (PFT) categories that reflect two aspects of functional diversity: drought-tolerant versus drought–intolerant, and early-successional versus late-successional (Table 2). Drought-tolerant and drought-intolerant species were identified based on mortality
rates observed in the Caxiuanã and Tapajós drought experiments (see Table 2 of da Costa et al., 2010). These species were then classified as early-successional or late-successional, based on whether their wood density was relatively low (<0.6 g cm\(^{-3}\)), or high (>0.8 g cm\(^{-3}\)), respectively. The early- versus late-successional classification used here is with respect to secondary successional dynamics that occurs after gap formation in tropical forests—studies have shown that light-demanding species (i.e. early successional PFTs) tend to have lower wood densities than shade tolerant species (i.e. late successional PFTs) (Muller-Landau, 2004; King et al., 2006; Poorter et al., 2010; Wright et al., 2010). The species selected at each site and assigned to each functional group are summarized in Table 2.

*Eschweilera coriacea* was found at both research sites with a sufficient number of canopy-sized individuals to construct robust pressure-volume (p-v) and xylem vulnerability curves. There were no common species for *Protium* and *Licania* between the two research sites, thus two different species were selected for each. *Inga alba* was present at both sites, but only two canopy sized individuals were present in the drought plots at CAX in 2011. Therefore, *I. gracilifolia* was measured to supplement the *Inga alba* TLP data at CAX. No significant differences were detected between the *I. alba* and *I. gracilifolia* p-v curves (data not shown).

One or two branches approximately 3 cm in diameter, 2.5 m in length, containing several branchlets and more than 100 leaves, were harvested from each individual at a height of 14 – 25 m. All branches were located in the upper crown and in full sun during the afternoon. The branches were harvested between 6:30 and 7:30 am local time, and the canopy was generally wet from rain or condensation from the night before, thus indicating minimal transpiration had yet occurred. After harvest, the branches were wrapped in plastic and the distal end was placed in water and transported for 30 to 60 minutes to the lab. The whole branches were stored in the lab.
in large plastic bags with the distal ends submerged in water until the leaves or branches could be prepared for measurements on the morning of the harvest. The exact number of trees, leaves and branches sampled from each species at each site is provided in Table S1.

A total of three measurement campaigns were carried out at each site. Each campaign was over a 2-4 week period, with at least one campaign in each of the wet and dry seasons. Seasonality in the measurements was not detected and therefore the data were pooled across seasons. Measurements for CAX were made in May 2011, November 2011, and October 2012; measurements for TNF were made in January 2011, July 2011, and October 2012. Dawn and midday leaf water potential measurements were carried out on November 18, 2011, a mostly sunny day and five days since the last rain event.

Leaf hydraulic traits

Dawn and midday leaf water potentials ($\psi_l$) were measured in the forest immediately following the branch harvest using a PMS model 600 pressure chamber (PMS Instrument Co., Albany, OR). Pressure-volume curves were constructed for each species to estimate TLP, $\pi_o$ and $\epsilon$. The p-v curves were composites of all of the leaves sampled for each species. Only fully expanded healthy leaves, either second or third from the tip, were used. Initial rehydration of the leaves was not necessary since the leaves were often wet from rain or morning dew at the time of harvest (this was also confirmed by the initial chamber balance pressure being consistently < 0.2 MPa). Each leaf was patted dry, excised from its branch, weighed to 0.0001g and then pressurized in the pressure chamber to measure the leaf water potential. The leaves were allowed to desiccate on the bench between each set of mass and pressure measurements.
TLP, $\pi_o$ and $\varepsilon$ were estimated using a change-point detection algorithm on the composite p-v curves of each species at each site (Barr et al., 2013). The change-point in the p-v curves occurred at the $\psi_l$ values where the F-scores were maximized for two linear models recursively fit to the upper and lower portions of the data series in each composite p-v curve. During the recursive fitting, one data point at a time was switched from the upper curve to the lower curve, where the initial upper curve and the final lower curve each included 10 data points. Then, using only the data below the $\psi_l$ value where the change-point occurred, a linear model was fit to the relationship between $1/\psi_l$ (MPa) and percent water loss of the leaf (i.e. $[100 - \text{RWC}]$ (%), where RWC was the water content of the leaf relative to saturation and equaled 100% when $\psi_l$ was 0). TLP was taken as the predicted value of $\psi_l$ when the lowest measured $[100 - \text{RWC}]$ value in the relationship between $1/\psi_l$ and $[100 - \text{RWC}]$ was applied to the linear model of the relationship. $\pi_o$ was equal to the inverse of the y-intercept of the linear model of relationship between $1/\psi_l$ and $[100 - \text{RWC}]$. The slope of the line between $[1, -\pi_o]$ and $[(\text{RWC at TLP})/100, 0]$ was equal to $\varepsilon$. Confidence intervals (CI) of 95% were established for TLP, $\pi_o$ and $\varepsilon$ for each species from each site by bootstrap sampling the composite p-v curves and then running the change-point detection routine for 10000 iterations. Since the bootstrapped estimates of were not normally distributed around the mean, all CIs mark the boundaries containing 95% of the bootstrapped estimates (Fig. S1). TLP, $\pi_o$ and $\varepsilon$ were also estimated using the methods described on PrometheusWiki (Sack et al., 2011). The principle difference between the change-point detection and bootstrap method and the PrometheusWiki method is in how the error of the p-v curve parameters are determined. The PrometheusWiki approach accounts for error arising from variation between leaves. In contrast, in addition to accounting for variation between leaves, the change-point detection and bootstrap method also accounts for error arising from the
uncertainty of where TLP actually occurs within each p-v curve of each individual leaf. The results are qualitatively the same between the two methods (see Results and Discussion); but the change-point detection results are emphasized in this report because the error estimates are more conservative (see Supporting Information, Appendix 1 for additional details about how the error estimates differ).

Xylem vulnerability

Xylem vulnerability was measured using the air-injection method (Sperry & Saliendra, 1994). This method measures the resistance of xylem vessels to air seeding as a pressure gradient builds across pit membranes separating sap-filled and air-filled vessels. This method assumes that the vulnerability of the membrane is equivalent regardless of whether the pressure gradient is pulling or pushing air through the pores. Upper canopy branch segments, approximately 11 to 13 cm in length and 0.6 cm in diameter, were inserted in a 2.7 cm double-ended pressure chamber constructed from a stainless steel, union tee, pipe adapter for 0.5 in OD inline tubes. Rubber plugs held in place with washers were used to make a seal between the branch segments and the chamber. A small section (0.5 cm) of bark was removed from each end and the middle of the branch segment to prevent flow along the bark and enable air entry into the xylem. The distal tip was shaved with a razor and then attached to the plumbing system using a 4 cm silicon coupling. The plumbing system consisted of a 1 l lactated ringer and Tygon® tubing filled with filtered (0.2 μm) well water. The ion content of the well water was not measured and may have been different between sites. We assumed that any effects of ions on pit membrane properties were equal across all four species. Many studies have used a weak solution of KCl to control for effects of ionized water on pit membranes (e.g. Choat et al., 2010), which includes shrinkage of
the gelatinous film covering the microfibrils (Lee et al., 2012). But, it is unclear how such shrinkage impacts membrane resistance to air seeding (Rockwell et al., 2014).

Flow rates were measured for 10 minutes from the proximal end of the branch segment for each measurement period. On each branch segment, the native flow rate was measured first followed by a 10 minute flushing with water under ~0.1 MPa to refill highly vulnerable vessels that may have filled with an air embolism during the harvesting and preparation process. At the beginning of the campaign, we flushed 1 to 2 branch segments of each species for 30 minutes and found that flow rates equilibrated after only a few minutes in all cases. The flow rate measured after flushing was taken as the maximum flow rate. Subsequent flow rates were measured after each incremental increase in air pressure in the pressure chamber. The stem segments were disconnected from the plumbing system, pressurized for 10 minutes and then depressurized before being reconnected to the plumbing system and resuming flow. It was found on a subset of stem segments for each species that flow rates equilibrated after approximately 15 minutes after the plumbing system was reconnected. Therefore, water was allowed to flow through the stem segments for 30 minutes prior to commencing each measurement. The pressure head was between 15 and 20 cm when water was flowing through the branch.

The relationship between xylem pressure and percent loss of conductance (PLC) relative to maximum conductance of the branch segment was characterized using a Weibull function with the point where PLC reached 50% being defined as the xylem-P_{50} (Meinzer et al., 2009). The error estimate for xylem-P_{50} was calculated using the maximum and minimum P_{50} values that could be established from the error on the parameter estimates of the Weibull functions. An indicator variable (Ott, 1993) was included with each parameter of the Weibull function to test for significant categorical differences between the xylem vulnerability curves of the PFTs.
Raw data are archived in the U.S. Department of Energy NGEE-Tropics Data Archive (Powell and Moorcroft a; Powell and Moorcroft b).

**Results**

For all four species measured at CAX, no significant differences were detected between the control versus drought plots in either the composite pressure-volume curves (using change-point detection) or the measured xylem vulnerability curves (Figs. S2 and S3 respectively). Accordingly, data collected from the control and drought plots at CAX were combined for each set of measurements. Because the TNF drought experiment had concluded by the time of this study, within-species phenotypic plasticity during drought was not evaluated for the hydraulic traits of the species measured at TNF.

*Leaf traits*

Median values are the sample statistic reported for leaf traits because the bootstrapped estimates are not normally distributed; however, the significance of our results are the same if mean values are used. While the whiskers in the boxplots of Figures 1 to 3 help to identify outliers generated during the bootstrapping simulations, they do not approximate the 95% CIs because the estimates are not normally distributed around Q1 and Q3 of the box plots. Therefore, Table 3 reports the CIs marking the boundaries containing 95% of the bootstrapped estimates of the \( TLP \) and \( \pi_0 \).

A clear pattern was apparent in how \( TLP \) varied across the different plant functional types (Fig. 1). Median \( TLPs \) of the drought-intolerant species, *Inga* and *Eschweilera*, were higher than the drought-tolerant species, *Protium* and *Licania*, (ranges: -1.64 to -1.82 MPa versus -2.52 to -2.66 MPa respectively) at both TNF and CAX (Table 3, Fig. 1). In contrast, \( TLP \) was not
different between the early- versus late-successional PFTs. The pattern in Figure 1 showing the differences between the drought-tolerant and -intolerant PFTs is significant at the 95% CI level for all species (Table 3).

At both sites, the median cellular osmotic potentials at full hydration ($\pi_o$) of the drought-intolerant species were significantly higher (range: -1.51 to -1.76 MPa) than the drought-tolerant species (range: -2.31 to -2.40 MPa); but no significant difference was found between the early-versus late-successional species (Table 3, Fig. 2). The pattern between the drought-tolerant and -intolerant PFTs shown in Figure 2 is significant at the 95% CI level for all species (Table 3). In contrast to TLP and $\pi_o$, median $\varepsilon$ was not organized by either drought tolerance or successional status and was not significantly different between any of the species at either site (Fig. 3).

$TLP$ and $\pi_o$ values were also estimated for the four species from CAX following the protocol described on PrometheusWiki (Sack et al., 2011) as a point of reference for the change-point detection protocol used in this study. The magnitudes of the $TLP$ and $\pi_o$ estimated here are comparatively lower using the change-point detection routine, and the error estimates are comparatively larger (Table 3 versus S2); however, the results are qualitatively similar with the drought-intolerant species having significantly higher values compared to the drought-tolerant species and no consistent differentiation between successional types (Tables 3 and S2).

Dawn and midday leaf water potential ($\psi_{lf}$) estimates for the four CAX species are reported for both the control and drought treatment plots in Table 4. Dawn $\psi_{lf}$ for all four species were near zero in the control plots and only Protium was considerably (and significantly) more negative than zero (-1.15 MPa) in the drought plot (Table 4). These near-zero $\psi_{lf}$ values at dawn were also reflected in the p-v curves for each species (Fig. S2). The control plot Inga and Licania midday $\psi_{lf}$ were considerably higher (-0.38 and -0.14 MPa, respectively) than
*Eschweilera* and *Protium* $\psi_f$ (-0.74 and -1.42 MPa, respectively). There was also a significant reduction in midday $\psi_f$ of *Eschweilera*, *Protium* and *Licania* (Table 4) growing in the drought plots.

**Xylem vulnerability**

With the exception of the two late-successional species (*Eschweilera* and *Licania*) at TNF, the drought-intolerant species (*Inga* and *Eschweilera*) were significantly more vulnerable to xylem cavitation under decreasing xylem pressure compared to the drought-tolerant species (*Protium* and *Licania*) (IS vs. PS, CAX: F(3, 103)=225, p<0.01 and TNF: F(3, 83)=221, p<0.01; EC vs LS, CAX: F(3, 119)=166, p<0.01) (Fig. 4). Xylem-$P_{50}$ of the drought-intolerant species was higher (range: -1.1 ± 0.1 to -1.4 ± 0.1 MPa) than that of the drought-tolerant species (range: -1.8 ± 0.1 to -2.3 ± 0.1 MPa (Table 3). The two late-successional species at TNF were a significant exception to this pattern, where the xylem-$P_{50}$ of the drought-tolerant species (*Licania*) was -1.6 ± 0.2 MPa compared to -2.0 ± 0.1 MPa for the drought-intolerant species (*Eschweilera*) (F(3,99)=150, p<0.01). The steeper slopes of the drought-intolerant PFTs compared to the -tolerant PFTs (except at TNF) that are visible in Figure 4 are significant (see statistics above), thus demonstrating that, compared to the drought tolerant PFTs, the drought-intolerant PFTs maximizes water use for as long as possible before abruptly exceeding a catastrophic embolism threshold.
Geographic and taxonomic conservation of traits

Although the two sites were separated by 400 km and soil properties markedly differed (CAX: 78% sand versus TNF: 60% clay, Table 1), the TLP, $\pi_0$, and xylem-$P_{50}$ values were generally conserved at the genus level across the two sites for all plant functional types (Table 3), with the exception of xylem-$P_{50}$ of Eschweilera and Licania (Figs. 1, 2 and 4). The pattern and magnitude of how the traits differed between drought-tolerant versus intolerant plant functional types were also conserved for all three traits, again with the exception of Eschweilera and Licania xylem-$P_{50}$ at TNF: at both sites the TLP, $\pi_0$ and xylem-$P_{50}$ of the species belonging to the drought-intolerant plant functional type were always 0.7 to 0.9 MPa higher than the values of the species belonging to the drought-tolerant plant functional type (Table 3).

Median values of $\pi_0$ from each PFT in this study were compared to a recently published (Christoffersen et al., 2016) relationship between wood density and $\pi_0$ that includes an extensive pan-tropical collection of moist tropical forest species (Fig. 5). The species measured in this study are clearly segregated within the data cloud, where the drought-tolerant PFTs are in the lower region of the relationship and the drought-intolerant PFTs are in the upper region (Fig. 5).

Discussion

In this study, we investigated how variations in leaf hydraulic traits ($TLP, \pi_0, \varepsilon$) and xylem vulnerability (xylem-$P_{50}$) correlate with drought tolerance and successional type of mature tropical trees growing in two evergreen wet tropical forests in the eastern Brazilian Amazon that were experimentally exposed to chronic drought. The tree species selected for this study represent one of four different plant functional types (Table 2). Variation in these traits has been quantified in prior studies, including tropical forests (Choat et al., 2007, Baltzer et al., 2008;
Meinzer et al., 2008; Bartlett et al., 2012; Anderegg, 2014, Maréchaux et al., 2015; Christoffersen et al., 2016). However, this study, like Rowland et al. (2015b) and Binks et al. (2016a), explicitly links measured differences in these four hydraulic traits to variation in mortality rates under experimental drought of mature tropical tree species growing in direct competition. This study also demonstrates (a) that the link between hydraulic traits and mortality is not strongly coordinated with wood density, and (b) that these observed patterns are consistent across evergreen Amazonian ecosystems with different soil characteristics.

Our results support hypothesis one (H1) that TLP (Fig. 1), πo (Fig. 2) and xylem-P50 (Fig. 4) are generally more negative in drought-tolerant species (Table 3). Drought-tolerant PFTs appear to be gaining lower TLPs through differences in πo (Fig. 2) rather than ε (Fig. 3). Our within-ecosystem results support a more general hypothesis that πo is the dominant control over TLP in tree species across biomes (Lenz et al., 2006; Bartlett et al., 2012). Gaining lower TLP through πo requires active transport of solutes into the cell. The observed differences in leaf cellular solute concentrations between drought-tolerant and -intolerant tree species (Fig. 2) appears to be set during leaf development and expansion, and then largely maintained until abscission, as overall respiration rates of mature fully-expanded leaves are not significantly different between the two groups under normal precipitation (Rowland et al., 2015a).

Interestingly, drought-intolerant species measured at CAX do, however, have increased leaf respiration rates during the dry season compared to the wet season during extended drought, noting that the reported respiration rates were normalized to 25 °C (Rowland et al., 2015a). Therefore, when drought leads to a reduction in stomatal conductance and evaporative cooling, and thus, higher leaf temperatures in drought-intolerant trees, any concomitant increase in leaf respiration is likely to be exacerbated by other factors (Rowland et al., 2015a). The apparent
lack of plasticity in $\pi_o$ observed in this study (Fig. S2), except for possibly in *Inga*, excludes active solute regulation as an explanation for such additional increases in leaf respiration.

However, the relative increase in leaf respiration is notable in drought-intolerant species and may be attributable to energy expenditure required to repair embolized leaf xylem vessels as a consequence of having higher xylem-$P_{50}$ and more severe dry-season water-stress.

The second hypothesis (H2), that leaf hydraulic traits ($TLP$, $\pi_o$ and $\epsilon$) and xylem-$P_{50}$ differ in a consistent manner between early and late-successional species, is not supported by this study (Table 3). Our results, therefore, do not support the suggestion of Fearnside (1997) that variation in wood density is a useful proxy for establishing a combined growth/drought-tolerance axis of variation for evergreen wet tropical forests (Figs. 1, 2 and 4). Our results also contrast a strong correlation between both $\pi_o$ and xylem-$P_{50}$ and wood density that is found in deciduous dry tropical forests, which experience much more acute soil water deficits and have half as much precipitation as CAX and TNF (Zhu and Cao, 2009; Markesteijn *et al*., 2011). More generally, our results are consistent with the view that the functional trait space for evergreen tropical trees is multi-dimensional (Laughlin, 2014). For example, an earlier study of 668 neotropical tree species found that a suite of 16 leaf and stem traits operate along two distinct axes: leaf economics and wood economics (Baraloto *et al*., 2010). Or, a study of 9 leaf and hydraulic traits sampled from 85 old world tropical tree species concluded that the species were organized along a leaf economics axis and leaf hydraulics axis (Li *et al*., 2015; see also Maréchaux *et al*., 2015).

Our results taken together with these earlier studies imply that there are at least three axes of functional variation in tropical forest: leaf economics, stem economics, and plant hydraulics.

It appears that the three axes of functional variation may not be entirely independent: a recent pan-tropical synthesis of tree wood density and hydraulic traits revealed a significant, but
weak, negative relationship between wood density and $\pi_o$ ($R^2 = 0.08$), and a much stronger relationship ($R^2 = 0.68$) between wood density and stem capacitance (Christoffersen et al., 2016). By way of comparison, when the median values from Figure 2 are included with the pantropical wood density and $\pi_o$ data for wet tropical forests, the species classified as drought-tolerant in this study occupy the lower region of the data cloud and the species classified as drought-intolerant occupy the upper region (Fig. 5), thus suggesting that ecological differences in drought vulnerability may account for some of the unexplained variation in the wood density–$\pi_o$ relationship identified by Christoffersen et al. (2016). Furthermore, this study illustrates how a targeted species-selection approach can complement meta-analyses, as shown by Figure 5, by controlling for the methodological and site-level differences that confound aggregated data.

Leaf economic traits reflect the plants’ investment of resources into the leaf for assimilating carbon (Wright et al., 2004; Reich, 2014; Li et al., 2015, Maréchaux et al., 2015), and stem economic traits reflect the plants investment into structure and pathogen resistance—indeed, variation in rainforest wood density appears to be better explained by the density of fiber cells and fiber cell-wall thickness compared to vessel cross-sectional area (Poorter et al., 2010).

Plant hydraulic traits reflect how plants balance the supply of water against atmospheric demand, while not necessarily trading photosynthetic performance for water conservation (Li et al., 2015). Ecologically, this multidimensional functional trait space helps to explain how tropical forests can attain such high levels of taxonomic diversity since it allows for a much wider range of resource-acquisition and stress-avoidance or tolerance strategies compared to a single fast-slow axis (Reich, 2014).
We first designated the species used in this study as drought-tolerant or -intolerant based on the combined mortality-response data from the CAX and TNF drought experiments (da Costa et al., 2010) prior to collecting any trait information. The CAX mortality results were subsequently verified by Rowland et al., (2015a) and consistent with a review (Meir et al., 2015b) that includes the Sulawesi drought experiment. The selected species were then designated as early- or late-successional based on their wood density, which has been correlated with successional type (Poorter et al., 2010). Consequently, in the strictest sense, the hypothesis of successional status-related differences in hydraulic traits (H2) was only tested if the species evaluated in this study adhere to the general relationship between wood density and successional type (Ewel, 1980; Muller-Landau, 2004; King et al., 2006; Poorter et al., 2010, Wright et al. 2010). Nonetheless, as discussed above, our finding that wood density is not correlated with the stem and leaf hydraulic traits measured in this study implies the existence of an additional, largely independent, trait axis within evergreen tropical forest tree species.

CAX and TNF are separated by approximately 400 km, with differing meteorological conditions, and contrasting soil types and soil and water table depths (Table 1). Surprisingly, both drought experiments had similarly large increases in mortality rates and losses in aboveground biomass after 3 years despite site differences in physical properties (Nepstad et al., 2007; da Costa et al., 2010). However, because of the similarity in ecosystem responses, we tested the hypothesis that the relative differences in the plant hydraulic traits occurring between the PFTs would be spatially conserved across the two study sites (H3). The results from this study support H3 with one exception: the difference in xylem-P_{50} between Licania and Eschweilera at CAX versus TNF (Table 3, Figs. 1, 2, 4). It should be noted that our two study sites were both terra firme forests in the eastern portion of the Amazon basin. At present it is
unclear if these traits are similarly conserved in seasonally inundated forests or forests along the eastern flank of the Andes where on average precipitation is higher and wood density is lower (Baker et al., 2004; ter Steege et al., 2006).

Our results did not support the fourth research hypothesis (H4) that the hydraulic traits of the early-successional and drought-tolerant PFTs have plasticity in response to chronic soil water-stress: both the p-v curves and xylem vulnerability curves of the control and drought plots at CAX almost completely overlapped for each species measured (Figs. S2, S3). Limited plasticity was also reported for leaf anatomical traits (Binks et al., 2016b) and leaf photosynthetic capacity (Rowland et al., 2015a) measured on the same species at CAX, although leaf dark respiration was found to be plastic in response to long-term drought in drought-intolerant species at CAX (Rowland et al., 2015a). For leaf hydraulic traits, Binks et al. (2016a) found a relatively small (~15%), but statistically significant, degree of acclimation to long-term drought in TLP and πo in a selection of drought-intolerant species that partially overlapped with those in this study. Our results of limited acclimation to drought contrast with observations of tree species endemic to seasonally dry tropical forests, which have been observed to adjust their osmotic potential by ~50% between seasons (Zhu and Cao, 2009). The reasons for the differences regarding the presence or absence of acclimation to long-term drought in TLP and πo at CAX is, at present, unclear; however it may in part be due to the different species studied: specifically, Binks et al. (2016a) measured multiple species from Eschweilera, Protium and Licania genera, as well as Manilkara bidentata, Pouteria anomala, and Swartzia racemosa, but did not measure any Inga species. The differences may also reflect differences in how TLP and πo were estimated from p-v curves—this issue is discussed in more detail in Appendix S1.

Nonetheless, these studies, taken together with a study in French Guiana (Maréchaux et al.,...
2017) and a pan-tropical sampling of 59 tree species (Bartlett et al., 2014)—both studies of evergreen tropical forests that report limited plasticity—suggest that the hydraulic traits of evergreen tropical trees have a relatively low capacity to acclimate to both acute droughts and a drier climate. In each of the evergreen tropical forest studies presented here, the mean seasonal adjustment in TLP was <16% (this study, Bartlett et al., 2014; Binks et al., 2016a; Maréchaux et al., 2017).

The $\psi_{lf}$ measurements were taken near the end of the 2011 dry season at CAX (Table 4); a period when the ecosystem approaches its lowest available soil moisture (da Costa et al., 2010). Soil moisture measurements during this period indicated significantly less available soil water in the drought plots through at least 2 m rooting depth (Rowland et al., 2015b). Protiüm tenuifolium (early-successional drought-tolerant) was the only species measured in the treatment plot that began the day of measurement with its leaves under water-stress (Table 4). A return of $\psi_{lf}$ to near zero is also reflected in the first points of the p-v curves for all species (Fig. S2) (noting that leaf rehydration in the lab was not part of the measurement protocol). Rehydration from morning dew may explain the near-zero $\psi_{lf}$ values at dawn, but this should be experimentally evaluated to determine its role in alleviating water-stress. Fisher et al. (2006) also measured $\psi_{lf}$ on seven tree species growing in the CAX experimental plots (Hirtela bicornis, Lecythis confertiflora, Licania heteromorpha, Licaria ameniaca, Manilkara bidentate, Mezilaurus mahuba, Swartzia racemose) and concluded that all of the species measured used an isohydric strategy to protect themselves from drought—i.e. where the stomata close at a $\psi_{lf}$ well above the xylem-$P_{50}$ thereby maintaining a relatively large safety margin. In this study, Eschweilera and Protiüm in the drought plots had significant decreases in their midday $\psi_{lf}$ (Table

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thus creating relatively narrow safety margins between $\psi_{lf}$ and xylem-$P_{50}$; implying that anisohydric strategies also occur within the ecosystem.

There is a critical need for improving the representation of plant hydraulics in dynamic vegetation models that are used to predict how tropical forests will respond to severe and chronic drought (Sakaguchi et al., 2011; Powell et al., 2013; Anderegg, 2014). Significant progress has been made recently in incorporating hydrodynamics into individual-based ecosystem (e.g. Duursma and Medlyn, 2012; Christoffersen et al., 2016) and terrestrial biosphere (e.g. Xu et al., 2016) models. However, the water-stress parameterizations in these models still lack diversity in xylem cavitation curves (e.g. Fig. 4) and stomatal sensitivity to TLP (e.g. Fig. 1) as show by Brodribb et al. (2003). Prior to this study, Rowland et al. (2015b), and Binks et al., (2016a), it was not clear if the observed variation in hydraulic traits correlated with differential mortality rates during extended severe droughts, or if it was related to a separate ecological process such as successional dynamics (Campanello et al., 2008; Markesteijn et al., 2011). Therefore, the evidence from these studies argue for dynamic vegetation models to include multiple tropical tree PFTs with contrasting water-stress parameterizations when they are used to evaluate the effects of severe drought in the tropics. Moreover, the PFT specific traits measured in this study map directly onto the parameters used in hydrodynamic model formulations (e.g. SPA-Williams et al., 1996; FETCH-Bohrer et al., 2005; and ED2 with hydrodynamics-Xu et al., 2016), thus enabling a mechanistically-based evaluation of the role of functional diversity in hydrodynamic traits in tropical forest ecosystems. For example, preliminary analyses using xylem-$P_{50}$ and TLP values from this study to parameterize drought-tolerant and -intolerant PFTs in ED2 with hydrodynamics (Xu et al., 2016, a terrestrial biosphere model with size-structured dynamic vegetation,) indicate that the observed differences of 0.75 MPa for these traits are ecologically
significant for facilitating functional diversity along a drought tolerance axis of competition, but when the differences are <0.75 MPa, coexistence of both drought-tolerant and -intolerant PFTs fails to occur (Powell, 2016). This study also provides information about the uncertainty of TLP (Table 3, Figs. 1, S1) and xylem-P$_{50}$ (Table 3) estimates for drought-tolerant and drought-intolerant tropical species, which is critical for constraining hydrodynamic formulations of different PFTs. While our study supports including a greater number of hydraulically defined PFTs in dynamic vegetation models, it does not provide strong support for having the hydraulic traits of each modeled PFT vary spatially (Figs. 1-4) or temporally (Figs. S2, S3).

This study has found a characteristic pattern in the measured leaf and xylem traits of several tropical tree species that is consistent with their demographic responses to experimentally-imposed drought conditions. Therefore, this study provides valuable insight into the traits controlling drought tolerance of tropical rainforest trees and provides much needed information for parameterizing more realistic water-stress functions in terrestrial biosphere models. Evidence from this study supports H1 thereby suggesting that the leaf traits, TLP and $\pi_o$, and the stem trait, xylem-P$_{50}$, are associated with drought tolerance in tropical trees. This study, however, does not support H2 in that these three traits do not vary with wood density, an important life history trait associated with succession; thus supporting the hypothesis that variation in plant hydraulic traits is largely independent from both the stem and leaf economic traits axes for tropical rainforest trees. The evidence from this study also supports H3 and rejects H4, thus suggesting that hydraulic traits of terra firme Amazonian trees are largely conserved spatially and temporally. However, although there appears to be some species variation in the plasticity of hydraulic traits, the overall limited degree of plasticity indicates that mature trees belonging to the drought-intolerant functional type are vulnerable to reductions in precipitation.

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because of their limited ability to adjust. Finally, we find that variability in plant hydraulic traits does exist among tropical tree species, and this variability may have been—and will be—critical in determining the fate of the Amazon rainforest if precipitation patterns change substantially.

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**Tables and Figures Captions**

**Table 1.** Physical and biological characteristics of the Caxiuanã and Tapajós National Forest throughfall exclusion sites.

**Table 2.** Species selected to represent each plant functional group. Sites are noted by CAX for Caxiuanã and TNF for Tapajós National Forests.

**Table 3.** Leaf and xylem trait estimates ±95% confidence intervals for each species representing one of the four plant functional types at the Caxiuanã (CAX) and Tapajós (TNF) National Forests. IS: *Inga* species. EC: *Eschweilera coriacea*. PS: *Protium* species. LS: *Licania* species. TLP: leaf turgor loss point. \( \pi_o \): leaf osmotic potential. Xylem-\( P_{50} \): water potential when 50% of conductance is lost. The leaf traits estimates are the median bootstrapped value using the change-point detection routine. Xylem-\( P_{50} \)s are based on a Weibull-curve best-fit for each species.

**Table 4.** Mean ± SE dawn and midday leaf water potential (\( \psi_l \), MPa) measured at Caxiuanã in the control and treatment plots on 11/18/2011. Each species represents one of the four plant functional types evaluated in this study: drought-tolerant versus -intolerant and early- versus late-successional (Table 2).

**Figure 1.** Distribution of turgor loss point (TLP, MPa) estimates for each plant functional type measured at the Tapajós (TNF) and Caxiuanã (CAX) National Forests. IS: *Inga* species, early-successional drought-intolerant. EC: *Eschweilera coriacea*, late-successional drought-intolerant. PS: *Protium* species, early-successional drought-tolerant. LS: *Licania* species, late-successional drought-tolerant. The boxes show the interquartile range (IQR) between 25% (Q1) and 75% (Q3) of the values, horizontal black bars are the median value, whiskers define the “fence” = [Q1, Q3] + 1.57 × IQR, the symbols are outliers beyond the fence.

**Figure 2.** Distribution of bulk leaf osmotic potentials (\( \pi_o \), MPa) for each plant functional type measured at the Tapajós (TNF) and Caxiuanã (CAX) National Forests. IS: *Inga* species, early-
successional drought-intolerant. EC: *Eschweilerora coriacea*, late-successional drought-intolerant. PS: *Protium* species, early-successional drought-tolerant. LS: *Licania* species, late-successional drought-tolerant. The boxes show the interquartile range (IQR) between 25% (Q1) and 75% (Q3) of the values, horizontal black bars are the median value, whiskers define the “fence” = [Q1, Q3] + 1.57 × IQR, the symbols are outliers beyond the fence.

**Figure 3.** Distribution of the bulk leaf modulus of elasticity (ε, MPa) for each plant functional type measured at the Tapajós (TNF) and Caxianã (CAX) National Forests. IS: *Inga* species, early-successional drought-intolerant. EC: *Eschweilerora coriacea*, late-successional drought-intolerant. PS: *Protium* species, early-successional drought-tolerant. LS: *Licania* species, late-successional drought-tolerant. Note: outliers for LS at CAX extend to 186 MPa. The boxes show the interquartile range (IQR) between 25% (Q1) and 75% (Q3) of the values, horizontal black bars are the median value, whiskers define the “fence” = [Q1, Q3] + 1.57 × IQR, the symbols are outliers beyond the fence.

**Figure 4.** Xylem vulnerability curves showing the percent loss of conductivity (PLC) with decreasing xylem pressure (MPa) of the selected species representing the four plant functional types measured at the Tapajós (TNF, open symbols) and Caxianã (CAX, closed symbols) National Forests. (a) *Inga*: early-successional drought-intolerant. (b) *Eschweilerora*: late-successional drought-intolerant. (c) *Protium*: early-successional drought-tolerant. (d) *Licania*: late-successional drought-tolerant. Vertical lines indicate point when 50% loss of conductance (P50) was reached for the TNF (dashed) and CAX (solid) species (see Table 3 for mean±CI values).

**Figure 5.** Relationship between bulk leaf osmotic potential (π0, MPa) and wood density. Open black symbols are pan-tropical values for moist tropical forests from Figure 2b of Christoffersen et al. (2016). The closed colored symbols are the species measured in this study representing each plant functional type (PFT) from the Caxianã (CAX, circles) and Tapajós (TNF, triangles) National Forests. IS: *Inga* species, early-successional drought-intolerant (green). EC: *Eschweilerora coriacea*, late-successional drought-intolerant (gold). PS: *Protium* species, early-successional drought-tolerant (blue). LS: *Licania* species, late-successional drought-tolerant (red).
Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Caxiuanã</th>
<th>Tapajós</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Mean annual precipitation</td>
<td>2272 mm</td>
<td>2000 mm</td>
<td>Fisher et al., 2007,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nepstad et al., 2002</td>
</tr>
<tr>
<td>Wet season (&gt;100 mm mo⁻¹)</td>
<td>Dec. to mid-Jul.</td>
<td>Dec. to mid-Jun.</td>
<td>Rosolem et al., 2008</td>
</tr>
<tr>
<td>Soil type</td>
<td>clay: 15% sand: 78%</td>
<td>clay: 60% sand: 38%</td>
<td>Fisher et al., 2007, Nepstad et al., 2002</td>
</tr>
<tr>
<td>Water table depth</td>
<td>10 m</td>
<td>&gt;80 m</td>
<td>Fisher et al., 2007, Nepstad et al., 2002</td>
</tr>
<tr>
<td>Aboveground biomass of trees &gt;10 cm dbh</td>
<td>214 t C ha⁻¹</td>
<td>150 t C ha⁻¹</td>
<td>da Costa et al., 2010, Nepstad et al., 2002</td>
</tr>
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Table 2.

<table>
<thead>
<tr>
<th>Drought Sensitivity</th>
<th>Succession</th>
<th>Early</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intolerant</strong></td>
<td><strong>Genus: Inga</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CAX: <em>I. alba</em>, <em>I. gracilifolia</em> Ducke</td>
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<td></td>
</tr>
<tr>
<td><strong>Tolerant</strong></td>
<td><strong>Genus: Protium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CAX: <em>P.tenuifolium</em> Engl.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TNF: <em>P. robustum</em> (Swart.) Porter</td>
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<td></td>
</tr>
<tr>
<td><strong>Genus: Eschweilera</strong></td>
<td>CAX: <em>E. coriacea</em> (DC.) S.A.Mori</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TNF: <em>E. coriacea</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Genus: Licania</strong></td>
<td>CAX: <em>L. octandra</em> (Kuntze)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TNF: <em>L. canescens</em> R. Benoist</td>
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Table 3.

<table>
<thead>
<tr>
<th>Drought sensitivity (Species ID)</th>
<th>Trait</th>
<th>TLP</th>
<th>$\pi_0$</th>
<th>Xylem-P$_{50}$</th>
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<td></td>
<td>Site:</td>
<td>CAX</td>
<td>TNF</td>
<td>CAX</td>
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<tr>
<td>Intolerant Early (IS)</td>
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<tr>
<td></td>
<td>-1.82</td>
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<td>-1.76</td>
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<tr>
<td></td>
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<td>+0.13,−0.23</td>
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<tr>
<td>Intolerant Late (EC)</td>
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<td></td>
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<td>+0.13,−0.13</td>
<td>+0.40,−0.08</td>
<td>+0.07,−0.08</td>
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<tr>
<td>Tolerant Early (PS)</td>
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</tr>
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<td>-2.31</td>
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<td>+0.45,−0.22</td>
<td>+0.18,−0.30</td>
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<tr>
<td>Tolerant Late (LS)</td>
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<td>-2.55</td>
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<tr>
<td></td>
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<td>+0.36,−0.74</td>
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Table 4

<table>
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<tr>
<th>Drought Tolerance Succession Species</th>
<th>Midday $\psi_{lf}$ (MPa) Control</th>
<th>Treatment</th>
<th>Dawn $\psi_{lf}$ (MPa) Control</th>
<th>Treatment</th>
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<tr>
<td>Intolerant Early Inga alba</td>
<td>-0.38±0.06</td>
<td>-0.44±0.17</td>
<td>-0.04±0.01</td>
<td>-0.03±0.01</td>
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<tr>
<td>Intolerant Late Eschweilera coriacea</td>
<td>-0.74±0.08*</td>
<td>-1.95±0.13*</td>
<td>-0.08±0.01*</td>
<td>-0.04±0.01*</td>
</tr>
<tr>
<td>Tolerant Early Protium tenuifolium</td>
<td>-1.42±0.03*</td>
<td>-1.91±0.04*</td>
<td>-0.18±0.04*</td>
<td>-1.15±0.10*</td>
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<tr>
<td>Tolerant Late Licania octandra</td>
<td>-0.14±0.02*</td>
<td>-0.35±0.04*</td>
<td>-0.07±0.01</td>
<td>-0.06±0.01*</td>
</tr>
</tbody>
</table>

*Indicates significant treatment effect where $|t| > t_{a/2}$, $\alpha = 0.05$. 

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Supporting Information

The following Supporting Information may be found in the online version of this article:

Appendix S1. Ancillary discussion of methods.

Table S1. Leaf and branch sample sizes for each species at each site. Leaves were used to construct the pressure-volume curves shown in Figure S2 and branches were used to construct the xylem vulnerability curves shown in Figure S3. Sites are indicated by CAX for Caxiuanã and TNF for Tapajós National Forests.

Table S2. Turgor loss point (TLP) and bulk leaf osmotic potential ($\pi_o$) estimates (mean ± SE) using the methods described by Sack et al. (2011) on PrometheusWiki. Trait values are given for the four species representing one of the four plant functional types characterized in this study for Caxiuanã National Forest (CAX). IS: *Inga* species. EC: *Eschweilera coriacea*. PS: *Protium* species. LS: *Licania* species.

Table S3. Turgor loss point (TLP) and bulk leaf osmotic potential ($\pi_o$) estimates (mean ± SE) using the methods described by Sack et al. (2011) on PrometheusWiki. Trait values are given for the four species representing one of the four plant functional types characterized in this study for Caxiuanã National Forest (CAX). Data for each species are separated by drought treatment. IS: *Inga* species. EC: *Eschweilera coriacea*. PS: *Protium* species. LS: *Licania* species.

Figure S1. Frequency distribution of turgor loss point (TLP) estimates from a 10000 iteration bootstrap of the change point detection routine. Tapajós (TNF): grey bars. Caxiuanã (CAX): textured bars. (a) *Inga* represents early-successional drought-intolerant. (b) *Eschweilera* represents late-successional drought-intolerant. (c) *Protium* represents early-successional drought-tolerant. (d) *Licania* represents late-successional drought-tolerant.

Figure S2. Pressure-volume curves showing the relationship between leaf water potential (MPa) and leaf water loss (mmol H$_2$O cm$^{-2}$) for species from each plant functional type measured in the control and drought plots at Caxiuanã. (a) *Inga*: early-successional drought-intolerant. (b) *Eschweilera*: late-successional drought-intolerant. (c) *Protium*: early-successional drought-tolerant. (d) *Licania*: late-successional drought-tolerant. No significant differences were detected between treatments for any species using the change-point detection routine, but *Inga* was significantly different between treatments using the PrometheusWiki protocol.

Figure S3. Xylem vulnerability curves showing the percent loss in conductance (PLC, %) with decreasing xylem pressure (MPa) for species from each plant functional type measured in the control and drought treatment plots at Caxiuanã. (a) *Inga*: early-successional drought-intolerant.
(b) *Eschweilera*: late-successional drought-intolerant. (c) *Protium*: early-successional drought-tolerant. (d) *Licania*: late-successional drought-tolerant.

**Figure S4.** Leaf pressure-volume (p-v) curve with the leaf water potential inverted (1/Ψ, MPa⁻¹) on the y-axis versus the reduction in relative leaf water content (100-RWC, %). The “linear” portion of each curve is shown by closed black dots, which is determined by the *acceptance criteria* of 5 data points and an $R^2 > 0.90$. (a) Full p-v curve using data from the PrometheusWiki template (Sack et al. 2011). (b) The same p-v curve as panel (a), except the y-axis is expanded to reveal the curvature in the “linear” portion. The turgor loss point (*TLP*) and osmotic potential (*$\pi_o$*) estimates from these data are given in the inset. (c) The same p-v curve as panel (b), except the data selected for the “linear” portion are shifted by two to the left. In this scenario, we assume the data points on the right were not measured because the acceptance criteria had been met. *TLP* and *$\pi_o$* increase by 0.49 and 0.38 MPa, respectively. (d) The same p-v curve as panel (b), except one additional, and hypothetical, measurement is added to the curve (denoted by red star) to give a “linear” portion that is shifted to the right by one data point. The new data point was linearly extrapolated from the two adjacent data points. *TLP* and *$\pi_o$* decrease by 0.17 and 0.15 MPa, respectively.

![Figure 1](image-url)
Figure 2.

Figure 3.
Figure 4.
Figure 5.