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Running title: Tree size strongly controls plant hydraulic responses in a droughted tropical forest

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Abstract

Future climate change predictions for tropical forests highlight increased frequency and intensity of extreme drought events. However, it remains unclear whether large and small trees have differential strategies to tolerate drought due to the different niches they occupy. The future of tropical forests is ultimately dependent on the capacity of small trees (<10 cm in diameter) to adjust their hydraulic system to tolerate drought. To address this question, we evaluated whether the drought tolerance of neotropical small trees can adjust to experimental water stress and was different from tall trees. We measured multiple drought resistance-related hydraulic traits across nine common neotropical genera at the world's longest-running tropical forest throughfall-exclusion experiment and compared their responses with surviving large canopy trees. Small understorey trees in both the Control and the throughfall exclusion treatment (TFE) had lower minimum stomatal conductance and maximum hydraulic leaf-specific conductivity relative to large trees of the same genera, as well as greater hydraulic safety margin (HSM), percentage loss of conductivity (PLC) and embolism resistance, demonstrating they occupy a distinct hydraulic niche. Surprisingly, in response to the drought treatment, small trees increased specific hydraulic conductivity by 56.3% and leaf:sapwood area ratio by 45.6%. The greater HSM of small understorey trees relative to large canopy trees likely enabled them to adjust other aspects of their hydraulic systems to increase hydraulic conductivity and take advantage of increases in light availability in the understorey resulting from the drought-induced mortality of canopy trees. Our results demonstrate that differences in hydraulic strategies between small understorey and large canopy trees drive hydraulic niche segregation. Small understorey trees can adjust their hydraulic systems in response to changes in water and light availability indicating natural regeneration of tropical forests following long-term drought may be possible.

Key-words: Long-term drought; Understorey trees; Hydraulic Safety margin; P50; Maximum conductivity; Acclimation; Amazon forest.

Introduction

Climate change predictions for tropical forests comprise increased frequency and intensity of extreme drought events (Aragão et al., 2018; Brodribb, Powers, Cochard, & Choat, 2020) and long-term reductions in soil moisture availability (Corlett 2016, Christensen et al. 2017). Most studies relating to drought focus on the impacts on large trees that comprise the highest proportion of forest biomass (Meir et al. 2015, Rowland, da Costa, et al. 2015), often finding the effect of drought stress on a plant's hydraulic system is a key driver of tree mortality (Bittencourt et al., 2020; Brodribb et al., 2020; Rowland et al., 2015). However, small understorey trees not only are responsible for up to 20% of the forest carbon sink (Hubau et al. 2019) but have a fundamental role in recruitment and the maintenance of tree populations, as they will effectively compose the future pool of large tree in the forest. Thus, small trees may be critical in determining long-term drought responses if there is extensive loss of large canopy trees (Rowland, da Costa, et al. 2015, Esquivel-Muelbert et al. 2017).

Large trees occupy canopy positions (hereafter, large trees) with high light levels and high vapor pressure deficit. In contrast, small trees from the same genus occupy understory positions (hereafter small trees), grow slowly, generally in shaded conditions and experience a lower atmospheric vapor pressure deficit (Sterck et al. 2011). The distinct resource partitioning between small and large trees, (Brum et al., 2019; Poorter, Bongers, Sterck, & Wöll, 2005) could cause strong differences in their water supply and demand relative to large trees. Reduced water supply from the roots, alongside lower capacitance, is likely to cause more negative water potentials in small trees relative to larger ones, during periods of low soil moisture (Salomón et al. 2017). Large trees are more likely to

buffer periods of water deficit with greater water access by deep roots (Brum et al. 2019), higher capacitance (Mcculloh et al. 2014), and elevated carbohydrate storage that allow to maintain either prolonged stomatal opening (deep roots) or prolonged stomatal closure (greater storage) (McDowell et al., 2008). These potential size-dependent variations in structural and physiological traits suggest tree size potentially influences a tree's capacity to acclimate in response to severe drought stress.

Several key traits of the hydraulic system of a plant are essential in determining the capacity of a tree to survive prolonged drought stress. These traits are often related to preventing hydraulic failure, via emboli formation, in the xylem vessels (Sperry and Tyree 1988), which can lead to severe decreases in leaf water supply, photosynthesis and other physiological functions (Sperry et al. 2002, McDowell et al. 2008, Martinez-Vilalta et al. 2019). These key traits include the water potentials at which the xylem lose 50% or 88% of their conductance (P50 or P88, respectively) and the hydraulic safety margin (HSM) (Meinzer et al. 2009),i.e., the difference between the minimum leaf water potential that is naturally experienced and P50, effectively a metric of the risk of a plant crossing a critical hydraulic threshold. Following sustained periods of drought stress, a tree's capacity to survive is likely to be related to its capacity to acclimate certain key drought tolerance traits or to limit its demand for water, via traits such as minimum stomatal conductance, thus reducing stress on its hydraulic system (Sala et al. 2010, Meir et al. 2018). Existing studies on large trees show limited capacity for tropical trees to adjust plant hydraulic traits in response to drought stress (Binks et al., 2016; Bittencourt et al., 2020; Powell et al., 2017; Schuldt et al., 2011). Some studies have shown that the risk of embolism can be reduced by increasing HSMs under drought conditions (Awad et al. 2010, Tomasella et al. 2018, Prendin et al. 2018). However, in a tropical forest drought experiment, large trees

were found to have limited plasticity in leaf level anatomy (Binks et al., 2016) and no capacity to acclimate their hydraulic systems, especially in traits relating to embolism resistance (Bittencourt et al., 2020; Powell et al., 2017; Rowland et al., 2015). Yet, to our knowledge, no studies have evaluated whether small trees (<10 cm diameter at breast height, DBH), contrary to adult trees, have the capacity to adjust their hydraulic system to prolonged drought stress. Following high mortality losses in large, more drought-intolerant, trees, small trees can increase photosynthetic capacity (Bartholomew et al., 2020;) and lower canopy trees can increase growth rates, even following drought (Brando et al., 2008; Rowland et al., 2015). This suggests that small trees can increase performance in response to elevated light, despite drier conditions. Increased light availability would also require these small trees to the increased atmospheric water demand, implying the need to increase water supply from their hydraulic system and/or to sustain a lower xylem water potential. However, these adjustments to conditions of severe drought only seem to be possible if small trees have a greater drought tolerance, functioning with higher levels of embolism resistance and hydraulic safety margin (HSM). Consequently, consideration of ecosystem changes, such as canopy loss and shifting light availability, is likely to be as important as the consideration of the direct impact of soil moisture stress following long-term drought, as both factors may influence hydraulic acclimation within small trees.

Here we take advantage of a unique drought experiment located in northeast Amazonia (Meir et al. 2015, 2018) to evaluate the response of small trees to combined changes in water and light availability. Previous research at this site has shown that large trees (>40 cm DBH) had significantly higher mortality rates, when compared to small trees and to trees in adjacent control forest, leading to a 40% reduction in biomass following 14 years

of experimentally-imposed soil drought (da Costa et al., 2010; Rowland et al., 2015, Meir et al. 2018). This biomass loss was almost entirely from trees reaching the upper canopy, which led to increased levels of light in the understory and increased growth rates of small understory trees in the wet season (da Costa et al., 2014; Metcalfe, et al., 2010; Rowland et al., 2015, Meir et al. 2018). Furthermore elevated radiation loads are likely to have increased leaf vapour pressure deficit and temperature, increasing the atmospheric drought effect these small trees experience (Mulkey & Pearcy 1992a; Kamaluddin & Grace 1992; Krause, Virgo & Winter 1995). Using new data from this soil drought experiment (henceforth *throughfall-exclusion experiment* – TFE), we explore how small trees adjust hydraulic traits in response to increases in light availability coupled with increased drought stress, specifically, if small trees are able to adjust traits to novel light conditions whilst under drought stress. Thus, we test whether small trees (1-10 cm DBH) alter their plant hydraulic system in response to prolonged soil moisture stress and increased canopy openness, and determine how these responses vary relative to those of large trees (>20 cm DBH). We address the following hypotheses:

1) Considering the same genus we hope that the hydraulic systems of small trees adjust to the combined soil-drought and radiation-load conditions imposed in the TFE relative to the Control. We expect small trees in TFE treatment to take advantage of the increased canopy openness by increasing their water transport efficiency (greater hydraulic specific conductivity and leaf-sapwood ratios). At the same time, we predict that small trees will have more negative water potentials resulting from drought conditions and the capacity to compensate this by adjusting hydraulic traits to maintain higher hydraulic safety margins to meet the elevated canopy water demands in support of photosynthesis.

2) Small trees have different hydraulic strategies from large trees. Specifically, we predict that, independent of the drought and radiation responses in the TFE, small trees have greater drought tolerance, higher xylem embolism resistance and larger hydraulic safety margins, relative to large trees. We therefore predict that, as a consequence of those trait differences, small trees occupy a different hydraulic trait space from large trees.

Methods

Site and plant material

Our study site is a lowland tropical rainforest located in the Caxiuanã National Forest, state of Pará, north-east Brazil (1°43'S, 51°27 W). It has an annual rainfall of 2000-2500mm, with a dry season (< 120 mm monthly rainfall) from July to December. A throughfall exclusion (TFE) experiment was established in 2002, where 50% of canopy throughfall is excluded by a plastic panel structure installed at 1-2m height over a 1 ha area (Meir et al. 2018). The TFE plot was studied alongside a 1 ha Control plot, where no throughfall exclusion took place. The plots have been monitored continuously since 2001 and further information on the experimental set-up can be found in earlier papers (da Costa *et al.*, 2010; Fisher *et al.*, 2007; Meir *et al.*, 2015 and Rowland *et al.*, 2015b).

From August-September 2017, during the peak of the dry season, we sampled 74 small trees with diameters ranging from 1 to 10 cm at breast height (1.3 m). We measured 41 small trees on the Control plot and 33 on the TFE, all taken from nine genera (20 species), replicated in each plot (two to five individuals per genera per plot). While we tried to maintain the same range of tree heights within each genus between plots, small trees had more variable height in the TFE, with light-exposed individuals reaching over 15 meters height, whilst no individuals in the Control reached 15 metres height (See Fig. S1). It was not possible to know the age of each sampled individual, because (destructive) sampling for age determination (tree-ring analyses; e.g., Brienen et al., 2016) was not possible. Consequently, we must assume that our sampled trees may have strongly varying ages (Groenendijk et al. 2014). We thus test the influence of tree stature and

position within the forest strata (van der Sleen et al. 2015), while assuming that most of our sampled trees are likely to be young.

For each individual, we collected two branches from the top of the crown, representing the point maximally exposed to light. The branches were third to fourth order (30-55 mm of diameter), counting from the tip. We collected one set of branches before sunrise (0400 to 0600 hours) and used these to measure embolism resistance and predawn leaf water potential. We collected a second set of branches at midday (1130 to 1330 hours) and used these to measure midday leaf water potential, native embolism, leaf-to-sapwood area, xylem and leaf specific conductivity, minimum leaf conductance and wood density measurements. Immediately after collection, branches were bagged in thick black plastic sacks with moist paper to humidify internal air and minimise leaf transpiration. Branches were transported 100m from the plots to measure leaf water potential, and for the remaining measurements the branches were transported to a laboratory ~1km walk away.

We measured predawn leaf water potential (Ψpd), taken to represent the time-point when transpiration is at its minimum and the water potential of the plant is closest to equilibrium with that of the soil. Ψpd can be considered an integrated metric of soil water availability across the rooting depth (Bartlett et al. 2016). We also determined midday water potential (Ψmd), to capture the minimum Ψ of the plant in the dry season. This measure is affected by any cuticular or stomatal transpiration and, thus, broadly captures the integrated effects of plant traits and the environment water demand on the minimum water potential a plant reaches in natural conditions. We also measured the native dry-season percentage loss of conductivity (PLC). We used the difference between

the minimum leaf water potential (Ψ_{md}) and P_{50}, to calculate the branch hydraulic safety margin (HSM). These two values (native PLC and HSM) were used as indicators of the cumulative damage from embolism.

Predawn and midday water potential

Predawn and midday leaf water potentials were measured in the field immediately after collection, using a pressure chamber (Model 1505, PMS). Branches collected for predawn water potential measures were sampled before sunrise, and for midday water potential, the sampling took place between 1130 to 1330 hours. For each tree we measured water potential of two leaves, or three leaves if the first two measures differed substantially (>0.5 MPa difference) from one another. Measurements from multiple leaves were averaged to create a single value per tree. All water potential measurements were taken on the same day for small trees and across three days for large trees.

Wood density, leaf to sapwood area ratio and minimum stomatal conductance

We measured wood density (W_D) on woody sections 40 to 80 mm long with a diameter of 4 to 7 mm. We debarked samples, immersed them in water for 24 hours to rehydrate and measured the saturated volume using the water displacement method (Pérez-Harguindeguy *et al.*, 2013). We then oven dried the samples at 60ºC until they were a constant mass and measured their dry weight with a precision balance to 3 decimal places.

We determined the leaf to sapwood area ratio (A_L:A_{SW}), on all branches by measuring leaf area and calculating sapwood area from two diameter measurements of the debarked basal part of the branch using precision callipers at a standardised distance from the tip. To avoid overestimation we checked the absence of pith area in all branches

per species before the measurement. We measured leaf area by scanning all leaves on the branch and quantifying their area using Image J software (version 1.6.0_20; Schneider et al., 2012). We calculated the leaf area to sapwood area ratio as total branch leaf area divided by its basal sapwood area. All branches had a similar size and were standardised by distance to the tip (~40-70 cm). The A_L:A_{SW} is a key indicator of the balance between transpirative demand and water supply capacity (Mencuccini et al. 2019).

For minimum leaf conductance (G_{min}), we used the leaf conductance to water vapour measured on the abaxial surface of leaves kept 30 minutes in the dark, using an infrared gas analyser (Li-COR 6400, US). All measured leaves were fully formed and undamaged leaves. G_{min} is a measure key indicator of residual leaf water loss and likely a due to a combination of leakage stomatal conductance from partially from leakage of partially closed stomata and cuticular conductance (Duursma et al. 2019, Binks et al. 2020, Márquez et al. 2021)., see Rowland *et al.* (2020)and Bartholomew et al. (2020), provide further details on gas exchange measurement.

Hydraulic efficiency and native embolism

We used maximum hydraulic specific conductivity (K_s) as a measure of xylem hydraulic efficiency and maximum leaf specific conductivity (K_{sl}) as a measure of leaf water supply capacity. We used the native percentage loss of conductivity of the collected branches (PLC) as a measure of native embolism. To PLC, we measured branch xylem hydraulic conductivity before (K_{snat} – native conductivity) and after flushing to remove emboli (Ks). We quantified the leaf area distal to each sample to obtain K_{sl} from K_l (leaf conductance). Using samples from the branches collected at midday, we put the entire branch underwater and discarded a 10 cm long segment from the base. After this, we cut

another 10-15 cm long segment from the base of each branch underwater, standard distance from the tip of the branch and let them rehydrate for 15 min to release tension and avoid artefacts (Venturas et al. 2015). Subsequently, to relax the tension in the branch we cut 1-1.5 cm of branch from base to leaves underwater, in steps of ~15 cm, and used the distal end of the branch for hydraulic measurements to ensure no artificially embolised vessels were present in the measured sample. All samples used for hydraulic measurements were second or third order branches, between 30-55 mm in length and 3-5 mm diameter and were recut underwater with a sharp razor blade before connecting to the apparatus, to ensure all vessels were open at both ends. We then measured flow in the sample using the Ventury tube method (Tyree et al. 2002, Pereira and Mazzafera 2013), where known resistance (PEEK capillary) is connected in series with the sample and the pressure drop in the capillary is proportional to flow in the sample. K_{snat} is then calculated from the pressure head applied and water flow. The samples are then flushed to remove emboli and estimate Ks (Martin-StPaul et al., 2014). We used pressure transducers (26PCCFA6G, Honeywell; read with a OM-CP-VOLT101A data logger, Omega Engineering) to measure pressure drop in the capillary and measured the capillary resistance prior to measurements using precision scales. The samples remained under-water throughout the entire procedure. We calculated PLC as the ratio of K_{snat} to K_s multiplied by 100. We calculated K_{sl} as the sample hydraulic conductivity (i.e., sample conductance times sample length) after flushing divided by the leaf area distal to the measured sample.

Embolism resistance and hydraulic safety

As an index of xylem embolism resistance, we used P_{50} and P_{88}, the xylem water potentials where, respectively, 50% and 88% of hydraulic conductivity is lost. We also used P_{50} to calculate the hydraulic safety margin - the difference between P_{50} and Ψ_{md}, an index of tree hydraulic safety. Branches collected before sunrise were rehydrated for 24 hours and from each branch we cut two or three smaller branches of approximately 40-70 cm. We measured the xylem embolism resistance of each branch using the pneumatic method (Pereira et al. 2016, Zhang et al. 2018). With this method, the loss of hydraulic conductance is estimated from the increase in air volume inside the wood caused by embolism formation as the branch dehydrates. Air volume is estimated from the air discharge from the cut end of the branch into a vacuum reservoir (~50 kPa absolute pressure) of known volume during a given amount of time (2.5 minutes). We measured initial and final pressure inside the vacuum reservoir with a pressure transducer (163PC01D75, Honeywell) and calculated the volume of air discharged using the ideal gas law. A detailed protocol is presented in (Pereira et al. 2016, Bittencourt et al. 2018) and revised by Pereira et al. (2021). Percentage loss of conductance for each branch is estimated from percentage air discharged (PAD) during its dehydration. PAD is calculated by standardising air discharge for each branch by its minimum (fully hydrated) and maximum (most dehydrated) air discharge state. We dehydrated branches using the bench dehydration method (Sperry et al. 1988). Before each air discharge measurement, branches were sealed in thick black plastic bags for one hour for leaf and wood xylem water potential to equilibrate. Directly after the air discharge was measured, we estimated wood xylem water potential by measuring the leaf water potential of one to two leaves. Drought embolism resistance is then given by the increase in PAD with decreasing xylem water potential for each tree. To calculate P_{50}, we pooled data from the

two-to-three branch replicates from the same tree and fitted a sigmoid curve to the data (Pammenter and Van der Willigen 1998) where P_{50} and slope (a) are the fitted parameters and P_{88} is predicted from the fit (Eqn 1):

$$PAD = 100/\left(1 + \exp\left(a\left(\Psi - P50\right)\right)\right) \quad (1)$$

Eqn1. Percentage air discharge equation (PAD). Ψ Water potential. P_{50} (xylem embolism resistance (MPa)

Data analysis

By comparing trees found on the Control and TFE experimental plots, we measure the effect of the experimental drought on our drought stress indicators ($\Psi_{pd,}$ Ψ_{md} - midday water potential; HSM – branch hydraulic safety margin to P_{50}; PLC – native dry season percentage loss of conductivity) and plant traits (W_D – wood density; A_L:A_{SW} - leaf to sapwood area; P_{50} - xylem embolism resistance; P_{88} - xylem embolism resistance; G_{min} – minimum stomatal conductance; K_s – maximum hydraulic specific conductivity; K_{sl} - maximum hydraulic leaf -specific conductivity) in small trees. We used linear mixed effects models to test for plot (TFE vs Control) and taxonomic effects (genus and species) on hydraulics traits in small trees (n = 66) using the R package lme4 (Bates et al. 2015). We tested the significance of the random effect by removing it and evaluating if the model significantly worsened using log likelihood tests using the *ranova* function for *lmerTest* objects (Zuur et al. 2009). We tested sequentially for the random effect of genus on: (a) the model intercept; (b) the fixed Plot effect (drought effect, difference between plots) on slope without intercept; and (c) both intercept and plot. When either the genus effect on plot, slope or both did not show the significance, we kept the multilevel approach using

genus as a random effect on the intercept (1|genus), as it controls for experimental design (Burnham and Anderson 2004). After testing the random effects, we tested the fixed TFE effect on variables. When taxonomy was included as a random effect in our models, we tested for both genus-only and species-nested-within-genus effects. We tested the complete model (genus and species as a random effect) against a General Linear Model (GLM) containing only the fixed effects. In all variables genus was significant as random effect. Therefore, linear models with genus as a random effect were used to test the significance of the fixed effects. To quantify model goodness of fit, we considered the marginal and conditional R^2 (Mulkey and Pearcy 1992b). The marginal R^2 indicates how much of the model variance is explained by the fixed effects only, whereas the conditional R^2 indicates how much of the model variance is explained by the complete model with fixed and random effects. All the analyses were done in R (version 3.3.0; R Core Team, 2016)

Small and large tree comparisons

We tested for differences in individual tree-level responses to the TFE treatment for large (n = 72) and small trees (n = 39). We use the large trees data from Bittencourt et al. (2020) conducted in the same experimental plots and collected during 2017 with the same methodological procedures. For this comparison we restrict the samples to those trees whose genera are replicated on both plots and replicated between the large and small trees, with a minimum sample size of 2 individuals per size group per plot and genus. Consequently, the number of genera and individuals employed in this comparison is lower than the available number of individual small trees and the full dataset published in Bittencourt et al., (2020). In total we use five genera (*Eschweilera, Inga, Licania, Protium,*

Swartzia), with 15 small trees on the Control and 24 small trees on the TFE, and 35 large trees on the Control and 37 large trees on the TFE. We used linear mixed-effect models to test the effects of the tree size with two classes (large and small), and tree size on drought stress indicators and hydraulic traits. Taxonomic effects were included by using genus as random effects, following the same protocol used for the small tree analyses, presented above. Within this paper, all data presented represent the mean and standard errors of the mean. A summary of available trait data by genus is presented in Table 1.

To test for an overall difference in the hydraulic strategy between small and large trees, we used the multivariate approach conducting non-metric multidimensional scaling (NMDS) using an individual-traits matrix (McCune & Grace 2002). We construct a matrix of data consisting of rows of individuals of each species and columns of traits values. We standardized the individual trait values for each genus and built the similarity matrix using Gower distance. NMDS searches for the best position of individuals variables on k dimensions (axes) to minimize the “stress” of the resulting k-dimensional configuration. We use k axes = 2 from that ordination as the initial configuration. The "stress" is obtained by comparison among the pair-wise distances (differences) of each individual's variables in reduced ordination space (expressed in terms of axes) and the original distance matrix (Gower distance). The regression is fitted using least-squares regressions and the goodness of fit is measured as the sum of squared differences between ordination-based distances and the distances predicted by the regression. A goodness of fit, or stress value, between 0.1 to 0.2 represent a good fit within the specified number of dimensions analysed to enable points to be interpreted relative to the NMDS axes. Therefore, the axis represents the data in a way that best represents their dissimilarity, points on the graph that are closer together are more similar. In addition, we use MANOVA to test the

difference in multidimensional space filled by tree size (small and large groups) and by plot effect (TFE and Control groups) separately (Anderson 2001). We use a MANOVA to compare Gower distance among observations in the same group versus those in different groups. We conducted a MANOVA first using small and large tree groups and then using TFE and Control groups using both tree sizes together. The size and plot effects were tested separately. Finally, we use permutations of the observations to obtain a probability associated with the null hypothesis of no differences between groups.

Results

The reduced soil moisture availability and increased canopy openness caused by 15 years of the TFE (Fig. S2) caused significant changes in the hydraulic traits of the small trees (Fig. 1). Maximum specific conductivity (K_s) increased significantly, by 56.3±41.5%, in the TFE small trees relative to the Control (Fig. 1f, $p<0.01$), similarly there was a significant (45.6±38.2%) increase in the leaf: sapwood area ratio (A_L:A_{SW}, Fig 1b.; $p<0.001$). The TFE also had significant effects on key physiological indicators of drought stress in small trees (Fig. 1) with Ψ_{pd} 0.24 MPa lower on the TFE relative to the Control ($p <0.001$) and Ψ_{md} 0.67 MPa lower ($p < 0.001$, Table S2). In contrast, other key hydraulic traits including xylem embolism resistance (P_{50} and P_{88}), leaf specific conductivity (K_{sl}), minimum stomatal conductance (G_{min}) and wood density (WD) showed no significant difference between the TFE and the Control plots for small trees (Fig. 1; Table 2; Table S3).

Taxonomic effects on hydraulic traits and their interactions with drought

Using mixed-effect modelling analysis we found that the variance explained by taxonomy had only a limited role in affecting the overall drought responses. When genus by genus responses to the drought effect were examined separately, it was clear that there were highly variable responses to the treatment among genera and sometimes these were inconsistent in terms of direction, as well as magnitude. We cannot separate the taxonomic effect from the residual variance because genus-specific influences on the plot effect were highly variable (Fig. 2 and 3). Given the low replication (between 2 and 5 for each genus on each plot treatment) and high variation within each genus, it was not always statistically viable to test the plot effect within each genus (Fig. 2 and 3), however where this was possible, clear statistical differences were seen for some genera but not for others (Table 2 Fig. 2). For example, *Licania* showed consistent responses in P_{50} and HSM while *Ocotea* did not show differences between plots (Fig. 2 and 3). The patterns described here were also maintained when we analysed the data at a species level (data not shown).

Large versus small trees

We compared the responses of hydraulic traits between large (>20 cm DBH) and small trees (1-10 cm DBH). Except for Ψ_{pd}, the results we obtain considering only the five genera that overlap between the small and large size classes, were similar to when considering all nine genera of trees present in Control plot and TFE experiment (see Fig. S3 supplementary material and Table S3 for n values for the small to large tree comparisons). Using all of the trait data for five overlapping genera, we applied NMDS ordination which demonstrated that the niche space occupied by the small trees was significantly different from the trait space of large trees. The traits space separated on to a clear 2-dimensional axis with a stress score of 0.18, indicating a good fit between the data and an analysis consisting of two axes (Fig. 4). Different associations amongst the nine hydraulic traits separated the individuals in the small and large tree groups. This result was driven predominantly by the first axis, which was positively related to PLC, P_{50} and P_{88} that influenced small tree grouping (Fig. 4). While the first axis was negatively related to K_s, K_{sl}, G_{min} influencing large trees grouping (Fig 4, Table S4). Using the complete set of hydraulic traits, we show that the hydraulic niche of small trees was significantly different from that of large trees ($MANOVA_{(1,66)}$; F=7.96; p<0.001; Table 1). However, there was no difference in hydraulic niche space occupied by the Control and TFE groups ($MANOVA_{(1,64)}$; F=1.22; p=0.30), except for K_s that showed plot and tree size effects ($MANOVA_{(1,64)}$; F=3.5; p=0.05).

In contrast to the large increase in K_s observed between small trees in Control and TFE trees (Figs 1 & S2), the plot level average values of K_s were similar among large trees in both Control and droughted conditions (4.82±3.93 TFE and 4.86±2.79 Control plot). Similar to Ψ_{md}, notable plot level differences were present in small trees, but these were absent

in the large trees (-1.72 ± 0.48 MPa TFE and -1.70±0.48 MPa Control treatment). However, small trees had values of Ψ_{md} which were 17.12±0.03% higher (values closer to 0) than the large trees. Furthermore, for the variables which had no treatment effect amongst the small trees, we find on average, across both the TFE and Control plots, the small trees had a 38.20±32.10% ($p<0.01$) more negative P_{50} and a 68.40±58.80% and 20.70±30.40% lower G_{min} and K_{sl}, respectively, than the large trees (Fig. 5b, 5d, 5f; $p<0.001$). Also across the plot we found that HSM increased by 72.97±36.34% and PLC increased by 44.41±14.62% in the small trees relative to large trees (Fig. 5g, 5i, 5j; $p<0.01$).

We analysed the influence of genus on the combined effect of treatment and tree size effect (i.e., large and small trees on the Control and TFE plot) for the five genera we could replicate across plots and tree size classes. We found that the effects of tree size varied substantially among genera and between traits and stress indicators (Fig. 6 and 7, table S5 and S6). For example, the difference in P_{50} between large trees and the small trees was 61.48±52.51% for *Licania* and 38.96±3.7% for *Inga* (Fig. 6). In contrast, G_{min} was significantly lower in the small trees relative to large trees across almost all genera (Fig. 6b). The drought-response pattern also changes when doing within-genus comparisons between large and small trees, for example the mean P_{50} response for *Inga* was different between small and large trees (Fig. 6). A difference in trait values between the Control and TFE plots that was present either for small tree or large trees, but not for both size classes simultaneously, occurred multiple times (Fig. 6 and 7), especially for the genus *Inga*. Mixed effect modelling results identify a strong influence of genus on trait variation between our two size classes (Table 3), yet there are limited cases where we find significant models demonstrating trait differences between the Control and the TFE plot with a significant tree size and genus effect (Table 3).

To test for size (small vs. large) and genus effects in each treatment (Control and TFE), we created a model with both size and genus as fixed effects. In the Control plot the full model (trait ~ genus*size) was a better predictor of variation across almost all traits, except for K_s, where there was a genus only effect and G_{min}, P_{50} and P_{88} where there was a size only effect. An interaction between size and genus was only significant for PLC (Table S7). The full model was also the best predictor of trait variation in the TFE plot, although only HSM, W_D and G_{min} showed a significant size effect. Significant interactions between genus and size were found for P_{50} and P_{88} (Table S7).

Discussion

Our results provide the evidence that small trees can adjust their functioning in response to drought, allowing them to maximize carbon gain in the higher-light levels following mortality of large trees in the TFE. We find that small trees (1-10 cm DBH) have the capacity to increase maximum specific hydraulic conductivity and leaf-sapwood area ratio in response to prolonged (15 year) soil moisture deficit. Despite having significantly lower pre-dawn and midday leaf water potentials, small trees had the capacity to adjust key hydraulic traits to allow a positive response to a higher light environment. This suggests that despite soil drought stress, small trees can still increase water transport efficiency and canopy water use in response to increases in light availability, following drought-induced mortality of large trees, potentially allowing them to maximise productivity in periods of the year when water is available. We also show the different components of a hydraulic strategy that provides niche segregation between small and large trees, with small trees being more drought tolerant than large canopy trees.

Studying the effects of multiple factors (here, imposed drought and size) on the physiology of hyper-diverse tropical forests is challenging. Here, we successfully addressed this problem by using genus and species nested in genus as random factors in linear mixed models and show that variability of species within genera is generally small. We nonetheless acknowledge our sample size limitations and the possibility that greater sampling depth may discover significant species-by-species variability in these traits.

The impact of drought on the hydraulic system of small trees

The substantial drought-related mortality of large trees (da Costa et al., 2010; Rowland et al., 2015) in the 15 years preceding this study led to an increase in the light availability in the lower canopy of the TFE, driving increases in the maximum photosynthetic capacity (71.1% and 29.2% increase in J_{max} and Vc_{max} respectively) and a 15.1% increase in the LMA of the same small trees we study here (Bartholomew et al. 2020). These differences in response to the prevailing light environment have also been observed elsewhere in tropical tree canopies (Ruggiero et al. 2002, Domingues et al. 2010, Cavaleri et al. 2010) and are indicative of plants changing their allocation strategy in response to increased light availability (Poorter et al., 2009; Wright et al., 2004). Critically, these allocation shifts are likely to result in a net increase in photosynthesis and growth (Metcalfe, Meir, Aragão, et al. 2010, Rowland, da Costa, et al. 2015, Meir et al. 2018), which require higher water supply to the canopy of each individual. The elevated soil moisture stress in the TFE relative to the Control trees manifested itself as significantly more negative pre-dawn and midday leaf water potential values (Figs 1h-1i), key indicators of plant water stress (Bhaskar & Ackerly, 2006; Kramer, 1988; Martínez-vilalta & Garcia-Forner, 2017). Interestingly however, these more negative water potentials did not translate into a significant change in HSM between plots, which would imply that the small trees converge to have the same vulnerability to drought (Choat et al. 2012). This could occur because of a trend, albeit not statistically detectable, towards more negative P50 values in the TFE plot for small trees, relative to the Control trees (Fig. 1), making a significant difference in HSM less likely. When examined at the genus level, five of the nine genera have consistently more negative P_{50} values on the TFE relative to the Control, with two remaining roughly equal and two less negative on the TFE (Fig. 2). These data

suggest that, despite operating at more negative water potentials, it is still possible for small trees to adjust their hydraulic system to support the increased growth in response to greater light availability.

Consistent with increases in photosynthetic capacity (Bartholomew et al., 2020), we observe an increase in leaf area to sapwood area ratio (A_L:A_{SW}) in the small trees on the TFE, relative to the Control. Combined with greater hydraulic specific conductivity, small trees in the TFE are therefore able to supply water to more photosynthetic tissue without increasing the volume of sapwood. A global study, including multiple sites from the tropics showed plant hydraulic systems are highly sensitive to changes in this ratio (A_L:A_{SW}) and may be one of the main factors controlling trade-offs in other plant hydraulic traits (Mencuccini et al. 2019). Increasing leaf area increases the total water demand of the tree; however, the observed increases in photosynthetic capacity (high values of Vc_{max} and J_{max}, Bartholomew et al. 2020), may allow slightly lower stomatal conductance for any given CO_2 concentration (Bartholomew et al., 2020; Sperry et al., 2017). This may, in part, compensate for the increase in demand for water that increased leaf areas could cause. However, even with the observed increases in photosynthetic capacity, these small trees probably still experience increased total water demand due to increased exposure to higher temperatures and VPD, suggesting that small trees must increase maximum hydraulic conductivity and/or tolerate reductions in water potential and therefore greater embolism risk (Sperry et al., 2017). In our study, small trees sampled in the TFE were slightly taller than the small trees in the Control plot (Fig. S1). This difference may in part-contribute to the slightly elevated conductance in the branches, as taller trees can have larger vessels at the base and greater vessel tapering from the trunk to branch tip (Olson

and Rosell 2013, Olson et al. 2020). It is, however, unlikely that these differences had a large influence on our K_s results, overall, the difference in height were small and the genera with the greatest height differences between the TFE and Control (*Protium, Octea, Voucoupoa*, Fig. S1) showed no changes in Ks (Fig. 2).

Differential hydraulic strategy between small and large trees

The comparison between small trees and large trees multidimensional hydraulic trait space, using NMDS and MANOVA, indicate they occupy different hydraulic niche spaces, despite some overlap. This revealed that smaller trees do indeed have a different water use strategy to larger canopy trees (Fig 3). The differences in the traits we observed were far greater, and in most cases significantly so, between the large and the small trees than for trees of the same size class between treatments (Fig 4). In addition, we show that smaller trees across both the Control and the TFE plot have significantly more negative P_{50} values and lower G_{min} values and significantly greater hydraulic safety margins (HSM), midday leaf water potentials and PLC (Fig. 4). This may imply that the small trees converge to the same vulnerability to drought, consistent with the results from large scale studies (e.g. Choat et al., 2012). However, the HSM is 1.94 MPa more positive in the small trees relative to large trees, indicative of a lack of convergence of the vulnerability of large and small trees (i.e. Fig 4i), potentially suggesting vulnerability to drought is driven by the ontogenetic stage of a tree. In addition, our results are consistent with the hypothesis that the smaller trees are shallow rooted and compensate for the lack of access to deep water through developing greater xylem embolism resistance and greater stomatal control (Brum et al., 2019; Tardieu, 1996, Sperry et al. 2017). It is possible that the greater hydraulic safety margin in small trees enables them to adjust more effectively to increased

light availability, despite the lower water availability in the TFE, as it enables these trees to tolerate greater drought stress without passing critical thresholds.

The carbon gain associated with greater photosynthesis under higher light environments may be translated into new xylem growth in smaller trees. This growth could rapidly replace damaged tissues and is likely to be a more viable strategy for smaller trees, relative to large trees (Damián et al. 2018, Trugman et al. 2018), which would reduce the risk associated with higher PLC levels. Furthermore, small trees maintained significantly lower G_{min} and higher midday leaf water potentials (Fig. 5d, 5g), relative to the large trees, despite having similar pre-dawn leaf water potentials, suggesting that small trees are able to more tightly regulate water loss, during both the day and night. Probably, the high regulate water loss in small tree is associate a lower water storage capacity to buffer short-term variation of water availability (Goldstein et al. 1998, Meinzer et al. 2003). The greater degree of control further reduces the risk of runaway embolism when photosynthesising during periods with low water potential, particularly if these trees can repair cavitated vessels (Nardini et al., 2011; Salleo et al., 2004; Salleo et al., 1995) or grow new vessels between consecutive dry seasons (Eller *et al.* 2018). Also, small trees also have fewer structural constraints than large trees, so small changes in hydraulic traits in a small tree could have bigger effects on overall performance during drought, because the marginal effect of each unit change is larger relative to the size of the tree (Mencuccini 2002). Combined, these factors are likely to allow small trees to have greater flexibility in terms of the strategy they use to adjust to combined changes in water and light availability. However, as we highlight in our results, there is considerable variability both within and between taxonomic groups with respect to how small trees may alter their traits and their resulting drought tolerance strategy.

This study highlights the importance of forest structural changes in controlling the traits of what are likely to be the next generation of trees growing up during prolonged drought stress. We show that, relative to large trees, small trees have a larger capacity to acclimate their hydraulic systems to increases in light availability following drought-induced mortality of large canopy-dominant trees. Our results suggest that small trees are able to acclimate the hydraulic conductance and leaf area to sapwood area ratio despite experiencing prolonged soil moisture stress, which resulted here in lower leaf water potentials and greater PLC. Also, our results demonstrate that there is a consistent and larger shift in the plant hydraulic strategy of saplings relative to large trees across most of Amazonia's hyper-abundant taxonomic groups. Whilst we find adjustment of traits in response to the drought treatment, it remains unknown whether all small trees community can respond in the same way or only the long-term drought surviving trees. In this way, a key uncertainty that remains to be answered are relates to the long-term development of these trees. Assuming these small trees continue to develop under the experimental drought stressed conditions, it would be of interest to know if the trajectory of change in hydraulic traits we observe can be sufficient to increase the hydraulic resistance to drought of these trees as they approach full size.

Ultimately, continued acclimation of hydraulic systems throughout a the lifespan of a tree may allow a more drought-resilient ecosystem to develop following the negative impacts of drought on pre-existing larger trees. Therefore, even the current generation of trees showing huge mortality rates, the next generation might be followed by a new stable community composed of those small trees that can adapt to drought. This implies that for prediction of the future of tropical ecosystems function we needs to consider trait adjustment in the future forest instead currently forest.

Data and Materials Availability

We are in the process of making this data publically available through the main funding bodies data centre, NERC EIDC (https://eidc.ac.uk/), if accepted the data will be fully publically available on a link we will provide.

Supplementary Data

Supplementary tables

Table S1. Linear mixed effect model analysis of stress indicator variables from small tress.

Table S2. Linear mixed effect model analysis of hydraulic traits from small tress.

Table S3 Numbers of individuals for small and large tree in each treatment (TFE an Control) and mean and standard deviation of all variables measured.

Table S4. Statistics from the NMDS modelling shown in Figure 3.

Table S5 Results of linear effect models of size (Size vs TFE) on the stress indicator variables on Control plot.

Table S6 Results of linear effect models of size (Size vs TFE) on the hydraulic traits variables on TFE plot.

Table S7 Results of linear effect models of size (Size vs TFE) and genus on the stress indicator and hydraulic variables on TFE plot.

Supplementary figures

Figure S1. Height and diameter in each treatment (TFE vs. Control) and by genus for the most common small tree genera in this study (9 genera)

Figure S2. Soil water content during 2016 in the Throughfall Exclusion Experiment plot and in the Control plot at 10 cm and at 100 cm.

Figure S3. Comparison between the small trees and large trees from the throughfall exclusion (TFE) and Control plots from grouping all 9 genera available within the large and small tree groupings.

Conflict of interest

The authors have no conflict of interest to declare.

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Authors' Contributions

ALG collected and compiled the data alongside LR, PRLB, IC, PBC, PG, LVF, DDV, JASJ, DCB and ACLdC. LR designed the study with MM, ACLdC, PM, ALG and RO. ALG, MM, PRLB and LR performed the statistical analysis and ALG, LR and RO wrote the paper, all authors substantially contributed to revisions.

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Table 1 – Mean and standard deviation from small trees: P_{50} - xylem embolism resistance (MPa); P_{88} - xylem embolism resistance (MPa); G_{min} – minimum stomatal conductance (mol m^{-2} s^{-1}); K_s – maximum hydraulic specific conductivity (kg m^{-2} s^{-1} MPa^{-1}); K_{sl} - maximum hydraulic leaf-specific conductivity (kg m^{-2} s^{-1} MPa^{-1}); A_L:A_{SW} – leaf to sapwood area ratio (m^2 m^{-2}); W_D- Woody density; Ψ_{pd} - predawn water potential (MPa); Ψ_{md} - midday water potential (MPa); HSM – branch hydraulic safety margin to P_{50} (MPa); PLC – native dry season percentage loss of conductivity (%), separated by genus and treatment.

		Hydraulic traits										
Genus (n individuals)	Treatment	P_{50}	P_{88}	G_{min}	K_s	K_{sl}	$A_L:A_{SW}$	W_D	Ψ_{pd}	Ψ_{md}	HSM	PLC
***Eschweilera* (3)**	**Control**	-2.91±0.07	-5.08±0.31	0.028±0.023	1.12±0.19	0.57±0.30	112.07±32.41	0.73±0.12	-0.32±0.23	-1.68±0.24	1.13±0.32	49.14±4.9
***Eschweilera* (3)**	**TFE**	-3.66±2.01	-6.32±3.80	0.026±0.019	2.80±2.62	4.71±6.12	92.12±65.44	0.59±0.09	-0.52±0.24	-1.87±0.26	1.89±2.28	9.22±4.36
***Inga* (4)**	**Control**	-4.60±1.63	-7.84±3.10	0.02±0.014	2.3±1.43	1.56±0.85	84.59±47.51	0.64±0.18	-0.37±0.26	-1.51±0.57	3.09±2.07	11.33±10.19
***Inga* (3)**	**TFE**	-3.48±0.58	-6.22±1.62	0.02±0.006	4.56±1.68	1.93±0.73	160.81±58.68	0.63±0.08	-0.39±0.22	-1.35±0.78	2.13±0.4	19.28±13.21
***Licania* (4)**	**Control**	-5.28±1.98	-9.62±4.40	0.025±0.014	0.15±0.04	0.12±0.07	66.15±24.41	0.76±0.062	-0.25±0.07	-1.65±0.85	3.62±2.75	38.29±28.52
***Licania*(4)**	**TFE**	-6.18±1.59	-9.07±1.77	0.024±0.02	2.17±2.19	0.37±0.40	104.90±45.99	0.761±0.014	-0.85±0.81	-1.388±0.78	5.183±1.70	68.667±28.13
***Mouriri* (3)**	**Control**	-4.77±0.54	-7.69±1.31	0.025±0.017	0.62±0.05	0.22±0.20	154.33±59.51	0.867±0.003	-0.24±0.09	-0.943±0.08	3.829±0.48	58.031±27.65
***Mouriri* (3)**	**TFE**	-5.55±0.74	-7.35±2.18	0.077±0.022	3.63±3.03	1.32±0.88	143.30±92.13	0.751±0.17	-1.07±1.32	-2.583±0.95	2.972±0.77	60.769±15.83
***Ocotea* (3)**	**Control**	-3.59±1.49	-8.72±2.63	0.007±0.003	1.63±0.81	0.84±0.17	125.38±54.22	0.638±0.05	-0.36±0.4	-0.6±0.364	2.994±1.26	36.718±18.42
***Ocotea* (3)**	**TFE**	-5.04±2.08	-8.61±4.88	0.03±0.024	1.58±0.66	0.60±0.46	84.83±32.64	0.68±0.13	-1.44±1.17	-2.41±0.81	2.62±2.45	65.27±24.19
***Protium* (5)**	**Control**	-2.30±0.71	-4.16±2.40	0.017±0.01	1.68±0.94	0.75±0.41	78.60±6.37	0.74±0.07	-0.31±0.3	-1.23±0.31	1.07±0.78	54.73±17.02
***Protium* (3)**	**TFE**	-3.64±1.47	-5.65±0.73	0.013±0.01	1.10±0.07	0.44±0.07	90.57±17.71	0.72±0.049	-0.48±0.16	-1.00±0.24	2.55±1.73	49.74±11.94
***Swartzia* (3)**	**Control**	-3.17±1.28	-5.98±1.89	0.06±0.04	1.67±0.26	0.78±0.55	72.45±18.20	0.73±0.02	-0.23±0.12	-1.57±0.16	1.60±1.36	59.73±9.94
***Swartzia* (3)**	**TFE**	-4.34±0.57	-6.94±0.06	0.06±0.02	2.78±0.51	0.89±0.54	210.45±67.51	0.72±0.005	-0.79±0.48	-2.36±0.09	1.98±0.66	49.13±8.57
***Tetragastris* (5)**	**Control**	-2.31±1.48	-4.34±1.81	0.03±0.01	2.22±1.66	2.29±3.12	83.86±59.38	0.64±0.05	-0.28±0.13	-1.06±0.58	1.25±1.31	22.12±15.60
***Tetragastris* (3)**	**TFE**	-4.36±1.19	-6.52±2.90	0.016±0.01	1.33±0.62	1.04±0.45	88.10±34.28	0.58±0.04	-1.43±0.40	-2.44±0.13	1.92±1.06	43.24±6.33
***Vouacapoa* (3)**	**Control**	-3.57±0.13	-5.37±1.45	0.015±0.003	1.00±0.16	0.95±0.64	56.71±22.69	0.69±0.13	-0.39±0.18	-1.59±0.19	1.97±0.31	43.78±11.37
***Vouacapoa* (3)**	**TFE**	-2.22±0.79	-3.54±1.63	0.012±0.004	0.83±0.51	0.67±0.78	229.76±101.26	0.70±0.01	-0.77±0.35	-2.07±0.24	0.15±0.72	33.24±19.67

Table 2 Results of linear mixed effect models of plot (Control versus TFE) on the stress indicators and hydraulic traits for small trees (1-10 cm DBH) measured in dry season (Oct/2017) on the Control plot and through-fall exclusion TFE. WD - wood density; AL:ASW - leaf to sapwood area ratio; P50 - xylem embolism resistance; P88 - xylem embolism resistance; Gmin - minimum stomatal conductance; Ks - maximum hydraulic specific conductivity; Ksl - maximum hydraulic leaf -specific conductivity; Ψpd - predawn water potential; Ψmd - midday water potential. HSM - branch hydraulic safety margin to P50; PLC - native dry season percentage loss of conductivity. The table shows the least square means for the Control and TFE, and the random genus effects (see analysis section in Methods for details). The first row of each trait gives the mean and second row gives one standard error for the fixed effects and the 95% confidence interval for genus-level random effects. Traits for which plot had a significant effect, and species for which the random effects were different from zero, are marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) and ns (non-significant).

	Plot-level Coefficients		Genus-level Coefficients								
Variable	**Control**	**TFE**	**Eschweilera**	**Inga**	**Licania**	**Mouriri**	**Ocotea**	**Protium**	**Swartzia**	**Tetragastris**	**Vouacapoa**
P_{50}	3.56 (-4.12/-2.99)	-4.26 (-5.761/-2.79) ns	-3.29 (-4.67/-1.91) ***	0.83 (-2.56/0.90)	2.38 (-4.11/-0.65) **	1.87 (-3.65/-0.09) *	-1.03 (-2.81/0.75)	0.60 (-1.13/2.33)	-0.35 (-2.20/1.50)	0.21 (-1.48/1.90)	0.39 (-1.39/2.17)
P_{88}	6.49 (-7.45/-5.42)	-6.58 (-9.34/-4.00) ns	-5.71 (-8.18/-3.24) ***	1.44 (-4.54/1.65)	3.69 (-6.78/-0.59) *	-1.82 (-5.01/1.37)	-2.96 (-6.15/0.22)	1.11 (-1.98/4.21)	-0.66 (-3.97/2.65)	0.54 (-2.48/3.57)	1.25 (-1.94/4.43)
G_{min}	0.027 (0.02/0.03)	0.030 (0.008/0.053) ns	0.03 (0.01/0.04) ***	0.01 (-0.03/0.01)	0.00 (-0.02/0.02)	0.03 (0.01/0.05) **	-0.01 (-0.03/0.01)	-0.01 (-0.03/0.01)	0.04 (0.02/0.06) ***	0.00 (-0.02/0.02)	-0.01 (-0.03/0.01)

K_s	1.46 (0.95/2.00)	2.32 (0.98/3.64) *	1.79 (0.46/3.13) **	1.48 (-0.28/3.23)	-0.49 (-2.24/1.26)	0.34 (-1.47/2.15)	-0.19 (-2.00/1.63)	-0.33 (-2.03/1.38)	0.33 (-1.56/2.22)	0.17 (-1.58/1.92)	-0.88 (-2.69/0.93)
K_{sl}	1.00 (0.45/1.55)	1.22 (0.21/2.58) ns	2.23 (0.86/3.61) **	0.51 (-2.31/1.29)	-1.96 (-3.76/-0.17)	-1.45 (-3.31/0.40)	-1.51 (-3.37/0.35)	-1.59 (-3.34/0.16)	-1.41 (-3.35/0.54)	-0.29 (-2.09/1.50)	-1.42 (-3.28/0.44)
$A_L:A_{SW}$	90.77 (69.76/110.74)	131.70 (81.14/183.50) **	104.09 (48.95/159.23) ***	13.17 (-59.03/85.37)	-18.57 (-88.86/51.73)	44.73 (-29.93/119.39)	1.02 (-73.64/75.68)	-21.00 (-91.29/49.29)	23.56 (-54.42/101.54)	-18.64 (-88.93/51.65)	39.15 (-35.52/113.81)
W_D	0.71 (0.68/0.75)	0.68 (0.59/0.78) ns	0.68 (0.60/0.75) ***	0.03 (-0.14/0.07)	0.09 (-0.01/0.19)	0.13 (0.03/0.24) **	-0.01 (-0.12/0.10)	0.06 (-0.04/0.16)	0.05 (-0.06/0.16)	-0.05 (-0.15/0.06)	0.03 (-0.08/0.13)
Ψ_{pd}	0.31 (0.14/0.48)	0.86 (0.63/1.52) ***	-0.42 (-0.05/0.90)	0.04 (-0.69/0.61)	0.13 (-0.50/0.76)	0.24 (-0.44/0.91)	0.48 (-0.19/1.15)	-0.03 (-0.66/0.60)	0.03 (-0.67/0.74)	0.29 (-0.34/0.92)	0.17 (-0.51/0.84)
Ψ_{md}	1.29 (1.09/1.54)	1.93 (1.50/2.19) **	-1.78 (1.19/2.37) ***	0.34 (-1.14/0.46)	-0.26 (-1.03/0.52)	-0.02 (-0.85/0.81)	-0.27 (-1.10/0.56)	-0.63 (-1.41/0.14)	0.11 (-0.76/0.98)	-0.20 (-0.98/0.57)	0.05 (-0.78/0.88)
HSM	2.25 (1.64/2.86)	2.35 (0.74/3.96) ns	1.52 (0.05/2.99) *	1.16 (-0.69/3.01)	2.77 (0.93/4.62) **	1.88 (-0.02/3.78)	1.29 (-0.61/3.19)	-0.02 (-1.87/1.83)	0.24 (-1.74/2.21)	-0.02 (-1.82/1.79)	-0.46 (-2.36/1.45)
PLC	41.315 (32.38/49.21)	45.82 (24.14/67.78) ns	33.18 (15.62/50.74) ***	18.44 (-41.43/4.56)	22.47 (-0.52/45.47)	26.22 (2.44/50.00) *	17.82 (-5.96/41.60)	19.68 (-2.70/42.07)	22.31 (-2.52/47.15)	-4.01 (-27.79/19.77)	5.33 (-18.45/29.11)

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Table 3. Results of linear mixed effect models of size (Large versus Small) on the stress indicators and hydraulic traits for small trees (1-10 cm DBH) and large trees (>20 cm DBH) measured in dry season (Oct/2017) on the Control plot and through-fall exclusion TFE. WD - wood density; AL:ASW - leaf to sapwood area ratio; P50 - xylem embolism resistance; P88 - xylem embolism resistance; Gmin - minimum stomatal conductance; Ks - maximum hydraulic specific conductivity; Ksl - maximum hydraulic leaf -specific conductivity; Ψpd - predawn water potential; Ψmd - midday water potential. HSM - branch hydraulic safety margin to P50; PLC - native dry season percentage loss of conductivity. The table shows the least square means for the Control and TFE, and the random genus effects (see analysis section in Methods for details). The first row of each trait gives the mean and second row gives one standard error for the fixed effects and the 95% confidence interval for genus-level random effects. Traits for which plot had a significant effect, and species for which the random effects were different from zero, are marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) and ns (non-significant).

Plot-level Coefficients			**Genus-level Coefficients**				
Variable	**Large**	**Small**	**Eschweilera**	**Inga**	**Licania**	**Protium**	**Swartzia**
P_{50}	-2.66 (-3.24/-2.08)	-4.06 (-5.20/-2.93) ***	-2.654 (-3.382/-1.926)***	-0.824 (-1.798/0.150) ns	-1.526 (-2.540/-0.513) **	0.014 (-0.985/1.013) ns	-0.593 (-1.592/0.406) ns
P_{88}	-4.83 (-5.87/-3.80)	-7.08 (-9.18/-5.02))***	-4.83 (-6.16/ -3.49) ***	-1.35 (-3.13/0.44) ns	-2.53 (-4.39/ -0.67) **	0.21 (-1.62 /2.04) ns	-1.02 (-2.85 /0.81) ns
G_{min}	0.08 (0.06/0.09)	0.02 (0.007/0.06) ***	0.07482 (0.053/0.09) ***	-0.01930 (-0.049/0.010)ns	-0.02935 (-0.05941/0.00071)*	-0.03583 (-0.066/-0.005) *	0.01851 (-0.011/0.048) ns
K_s	4.60 (2.20/5.58)	2.10 (1.09/4.33))***	3.88 (2.59/5.16) ***	1.98 (0.28/3.68) *	-2.81 (-4.69/ -0.94) **	-0.90 (-2.64/0.83) ns	-0.30 (-2.11/1.52) ns
K_{sl}	6.13 (5.06/7.19)	5.00 (3.09/6.89) *	4.35 (3.42/5.28) ***	0.90 (-0.41/2.21)	1.55 (0.24/2.86) **	1.56 (0.29/2.83) **	2.98 (1.69/4.28)***
W_D	0.70 (0.61/0.70)	0.60 (0.52/0.67) ***	0.63 (0.59/0.67) ***	0.01 (-0.04/0.06) ns	0.05 (0.00/0.11)*	-0.05 (-0.11/0.00)*	0.05 (-0.01/0.10) ns
Ψ_{pd}	-0.44 (-0.50/-0.38)	-0.44 (-0.61/-0.28)ns	-0.48 (-0.59/-0.36) ***	0.04 (-0.12/0.20) ns	0.09 (-0.07/0.25) ns	-0.05 (-0.21/0.11) ns	0.08 (-0.08/0.24) ns
Ψ_{md}	-1.75 (-2.04/-1.46)	-1.50 (-1.98/-1.02)**	-1.85 (-2.07/-1.63) ***	0.17 (-0.12 to 0.47) ns	0.54 (0.24/0.84) ***	0.41 (0.11/0.71) ***	-0.23 (-0.54/0.08) ns
HSM	0.90	2.70	0.92	0.97	1.96	0.21	0.27

	(1.09/2.32)	(1.32/3.91)***	(0.10/1.74) **	(-0.13/2.08) ns	(0.82/3.11) ***	(-0.92/1.34) ns	(-0.85/1.40) ns
PLC	19.50 (8.91/30.75)	42.03 (23.13/60.94)***	19.19 (8.87/29.50) ***	-4.71 (-18.36/8.93) ns	12.90(-2.14/27.94) ns	20.93 (7.02/34.84) **	16.15 (1.56/ 30.74) *

Figures

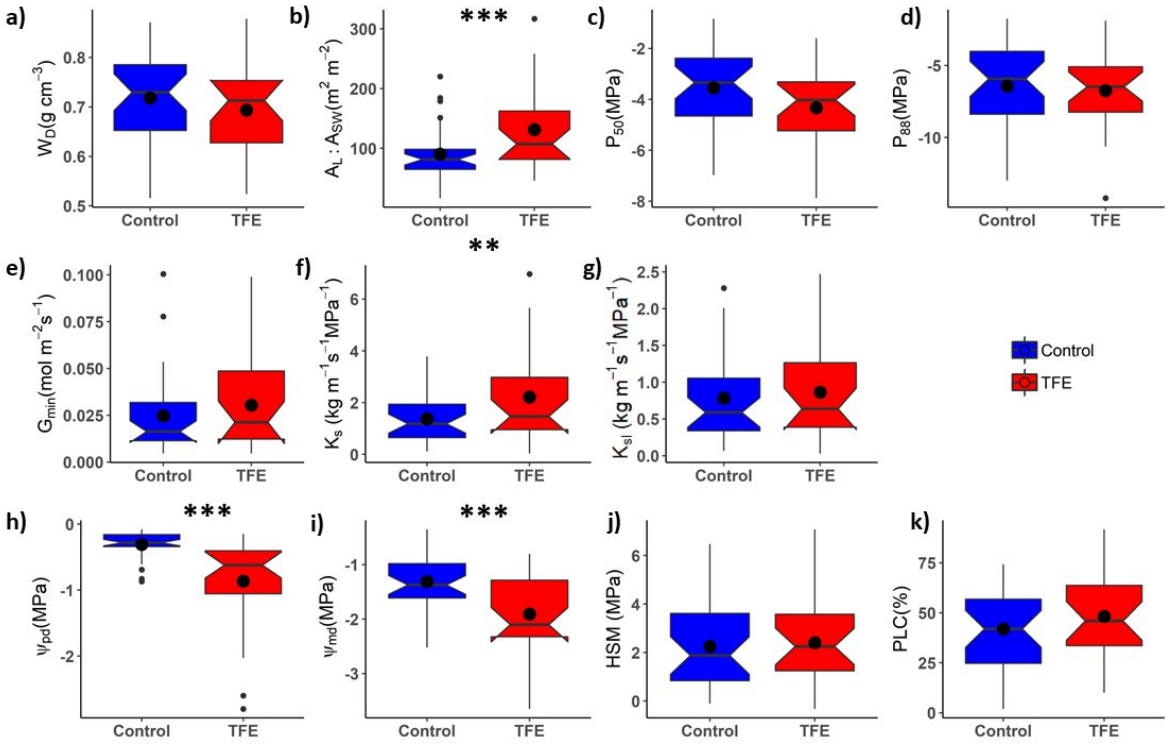

Figure 1 Stress indicators and hydraulic traits for small trees (1-10 cm DBH) measured in dry season oct/2017 on the Control plot (blue) and through-fall exclusion (TFE, red). a) W_D – wood density b) A_L:A_{SW} - leaf to sapwood area ratio c) P_{50} - xylem embolism resistance; d) P_{88} - xylem embolism resistance; e) G_{min} – minimum stomatal conductance; f) K_s – maximum hydraulic specific conductivity; g) K_{sl} - maximum hydraulic leaf -specific conductivity; h) Ψ_{pd} - predawn water potential; i) Ψ_{md} - midday water potential. j) HSM – branch hydraulic safety margin to P_{50}; l) PLC – native dry season percentage loss of conductivity. The boxes represent quartiles 1 and 3, the central line indicates the median and the black points the mean of each treatment. Whiskers are either maximum value or 1.5 interquartile range above quartile 3, if outliers are present and notches represents a confidence interval around the median represented by central line. Traits for which plot had a significant effect are marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). P-values are from mixed effects analysis (see Table 2 for models and analysis section in Methods

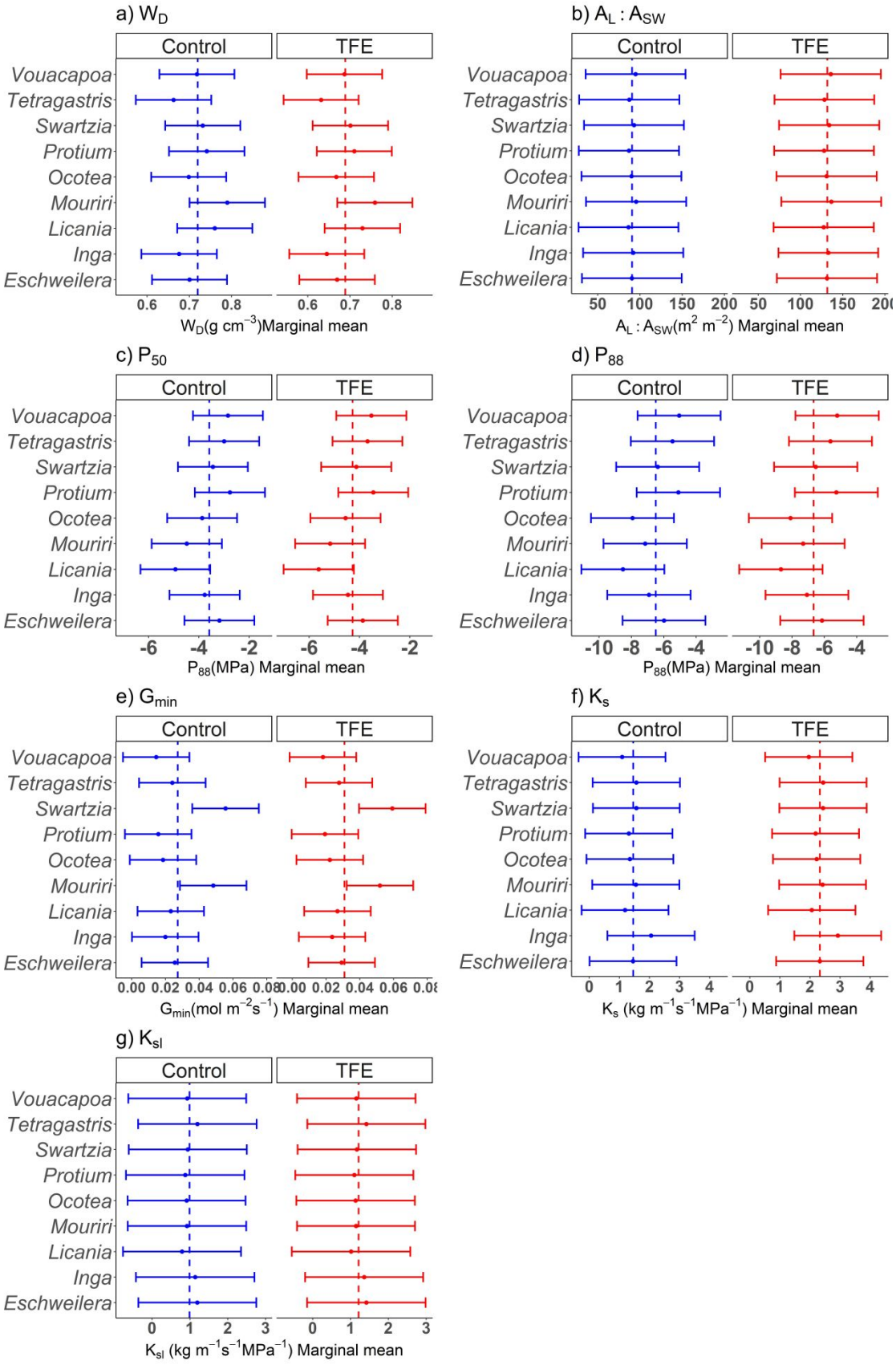

Figure 2 Hydraulic traits by genus for small trees (1-10 cm DBH) surviving after 15 years of throughfall exclusion (TFE – red) and the Control plot (blue). a) W_D - wood density b) A_L:A_{SW} - leaf to sapwood area ratio c) P_{50} - xylem embolism resistance; d) P_{88} - xylem embolism resistance; e)

G_{min} - minimum stomatal conductance; f) K_s - maximum hydraulic specific conductivity; g) K_{sl} - maximum hydraulic leaf -specific conductivity. The vertical dashed coloured lines represent the marginal fixed effects for plot. The points represent random effects plus fixed effect mean by genus and the horizontal lines represents standard error for each genus (see Table 2 for models and analysis section in Methods).

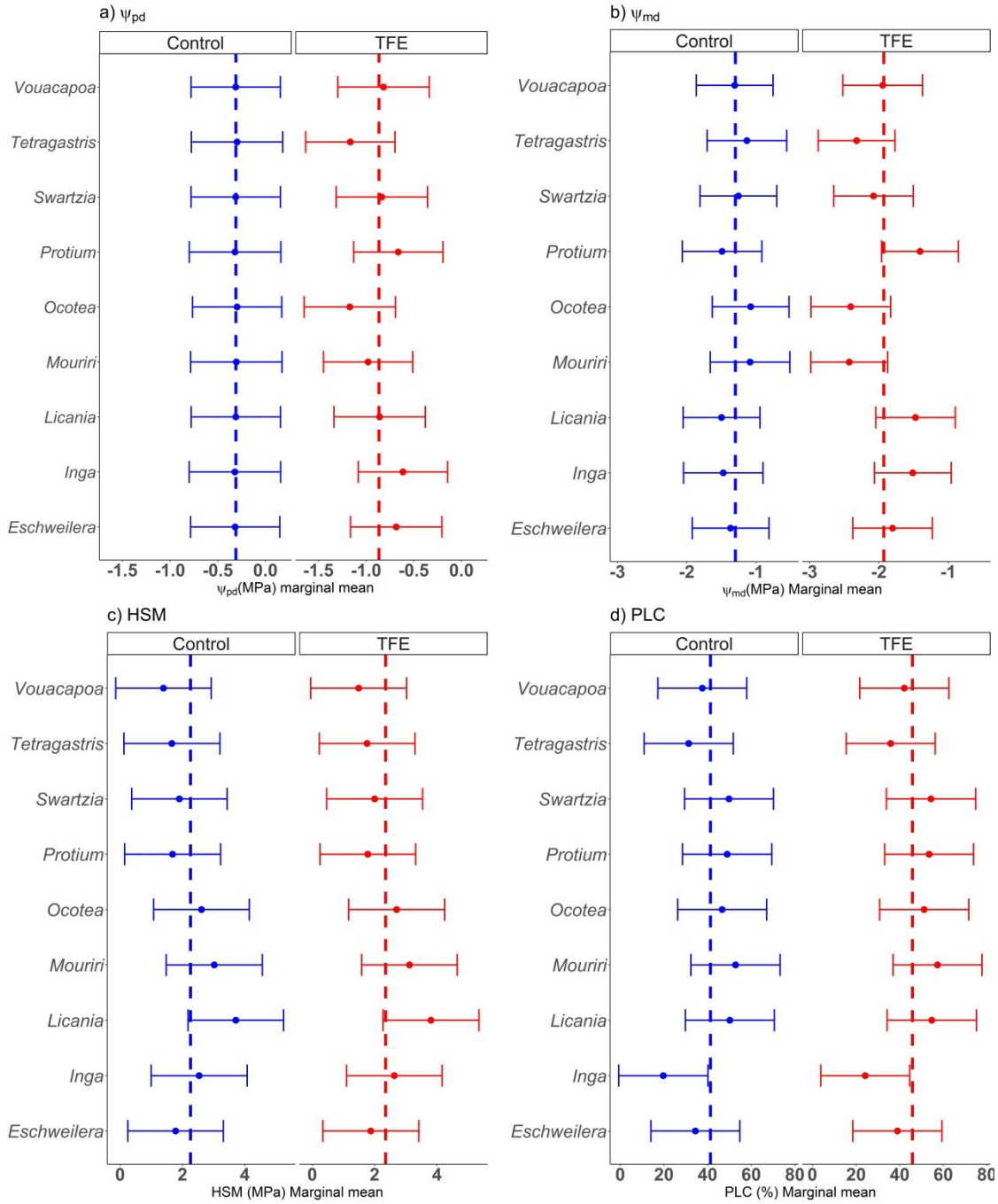

Figure 3 Drought stress indicators considered by genus for small trees (1-10 cm DBH) surviving after 15 years of throughfall exclusion (TFE – red) and the Control plot (blue). a) Ψ_{pd} - predawn water potential; b) Ψ_{md} - midday water potential. c) HSM – branch hydraulic safety margin to P_{50}; d) PLC – native dry season percentage loss of conductivity. The vertical dashed lines represents marginal fixed effects for plot, the points represents random effects plus fixed effect mean by genus and the horizontal lines represents standard error by genus (see Table 2 for models and analysis section in Methods).

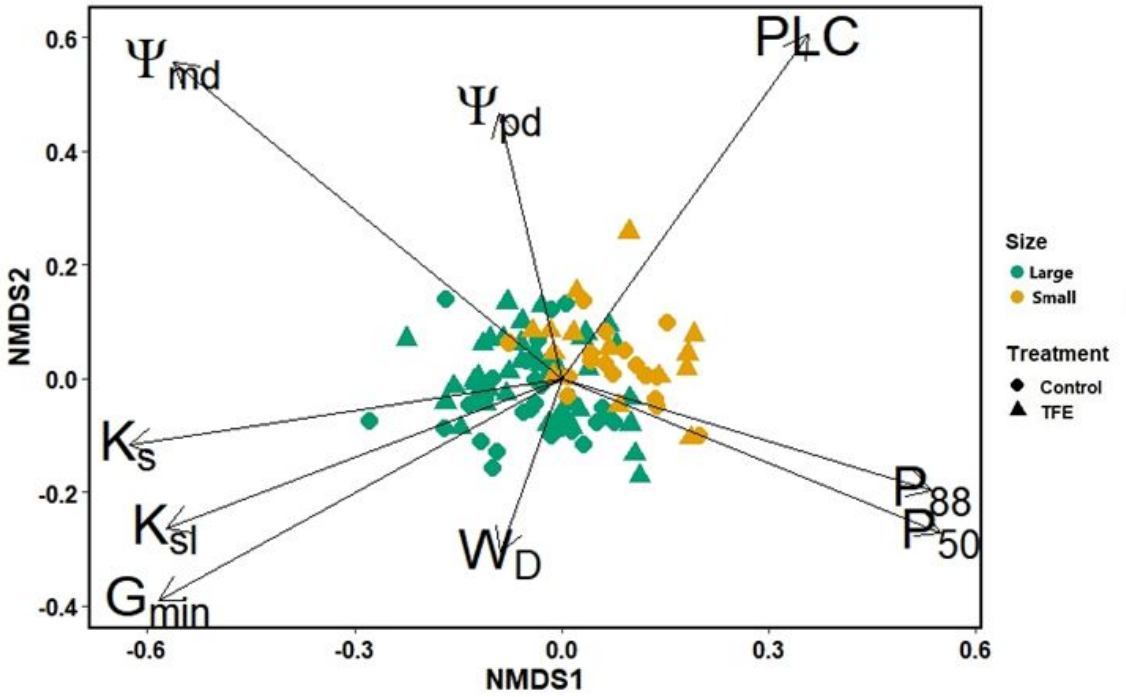

Figure 4 Non-metric multidimensional scaling (NMDS) of drought stress indicators and hydraulic traits. Ordination showing multidimensional space filled by small (yellow) and large (green) trees indicating distinct hydraulic ecological strategies (MANOVA; $P < 0.05$) between trees from the TFE and Control. Hydraulic traits represented by arrows (Arrow length represent predictor "strength"). Dots represent individuals in Control and triangles individuals in TFE treatment. The green colour represents large trees and yellow represents small trees.

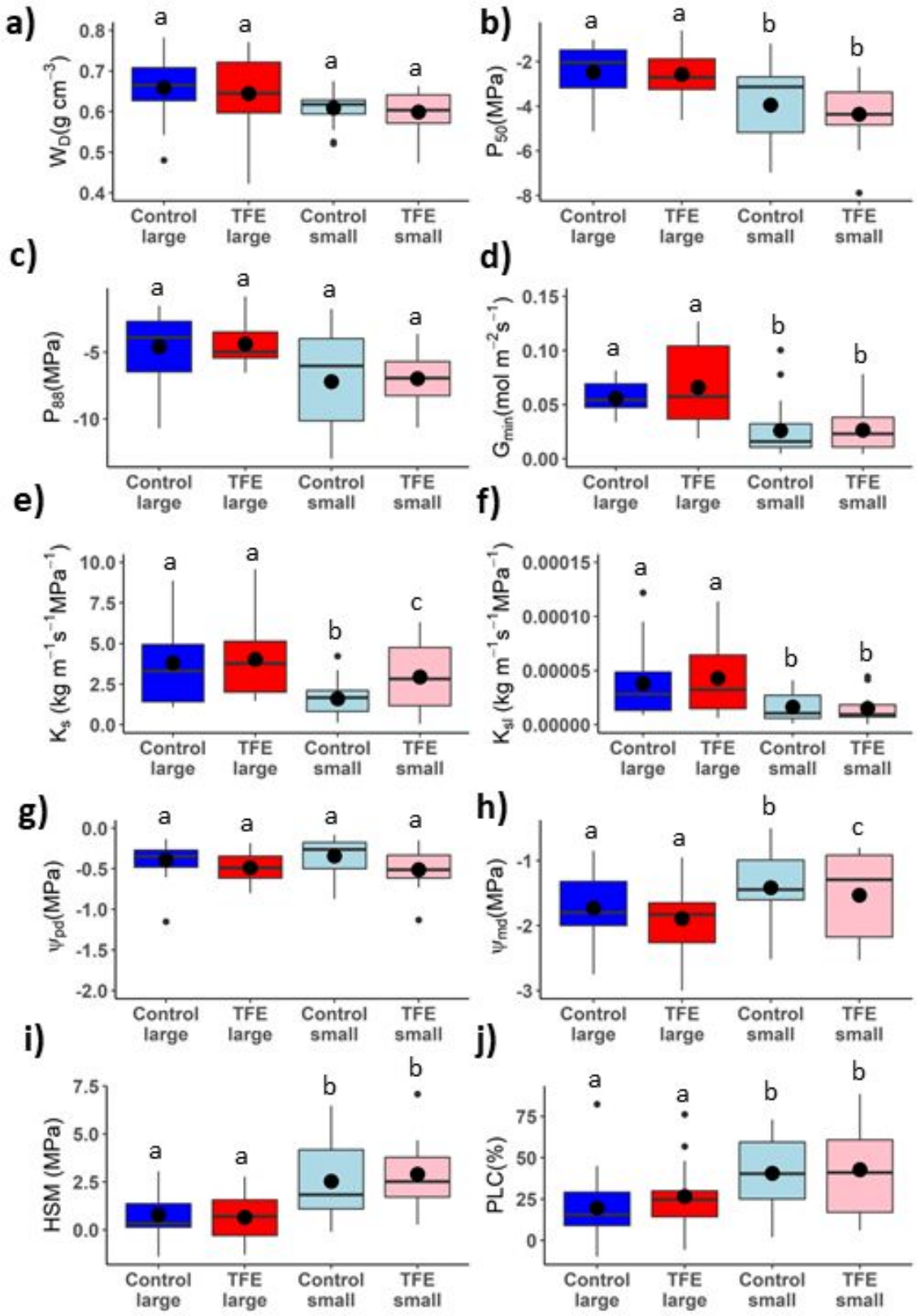

Figure 5 Comparison between small trees and large trees from the throughfall exclusion (TFE) and Control plots. a) W_D – wood density; b) P_{50} - xylem embolism resistance; c) P_{88} - xylem embolism resistance; d) G_{min} – minimum stomatal conductance; e) K_s – maximum hydraulic specific conductivity; f) K_{sl} - maximum hydraulic leaf -specific conductivity; g) Ψ_{pd} - predawn water potential; h) Ψ_{md} midday water potential; i) HSM – branch hydraulic safety margin to P_{50}; j) PLC –

native dry season percentage loss of conductivity. The boxes represent quartiles 1 and 3, the central line indicates the median and the black points the mean of each treatment. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present. Different letters indicate significant differences within each graph, $p<0.05$.

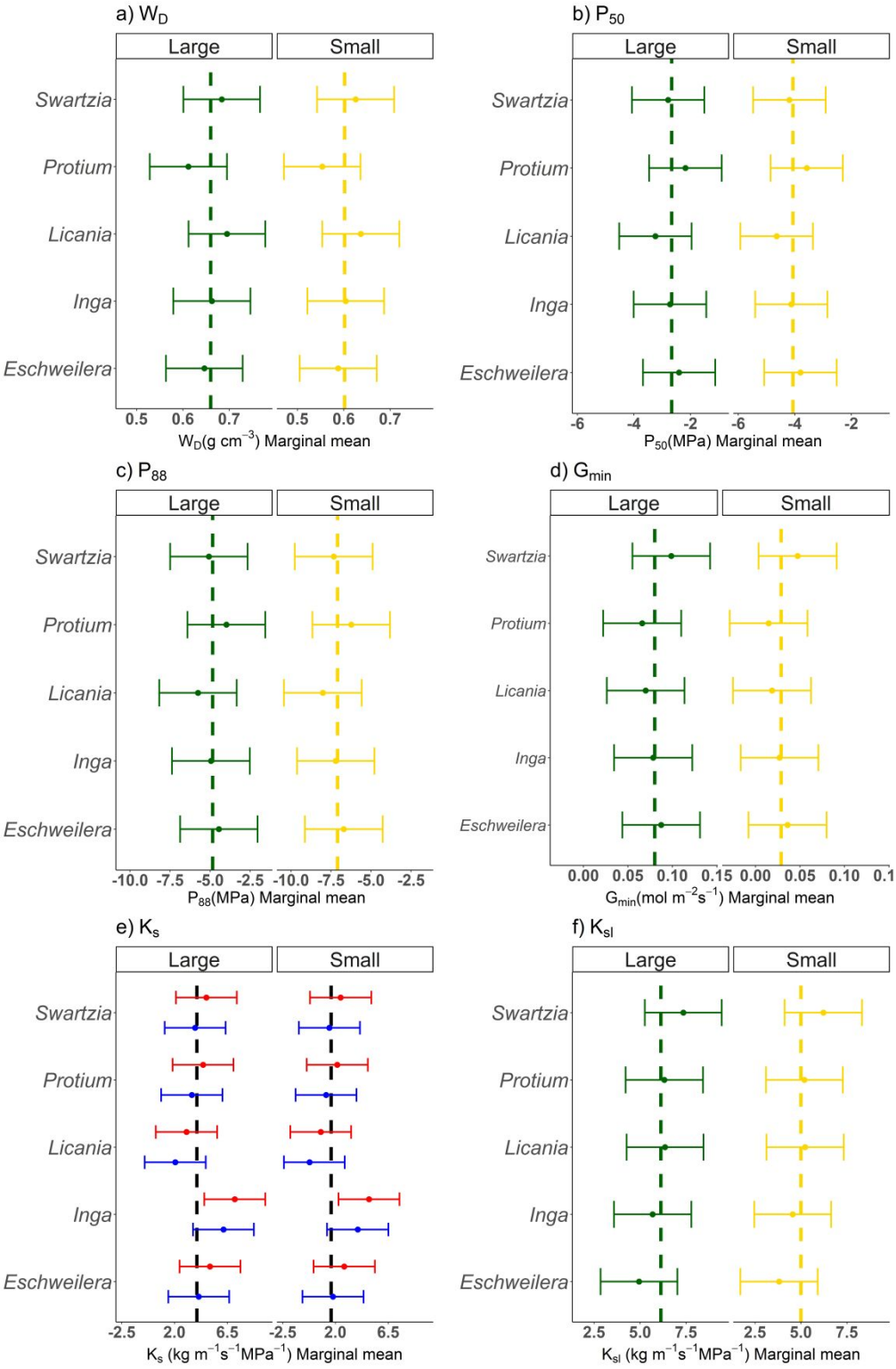

Figure 6 Hydraulic traits comparison between small trees and large trees from throughfall exclusion (TFE) and Control plot. a) W_D – wood density; b) P_{50} - xylem embolism resistance; c) P_{88} - xylem embolism resistance; d) G_{min} – minimum stomatal conductance; e) K_s – maximum hydraulic

specific conductivity; f) K_{sl} - maximum hydraulic leaf-specific conductivity. The vertical dashed lines represents marginal fixed effect mean, green vertical lines represents large trees and yellow vertical lines, the points represents random plus fixed effect mean by each level (by genus) and the horizontal lines represents standard error by each random effects level. The blue and red in horizontal lines represents Control and TFE plot, respectively and are show when a significant plot effect was found. All points and lines represent genus in each treatment (see Table 3 for models and analysis section in Methods).

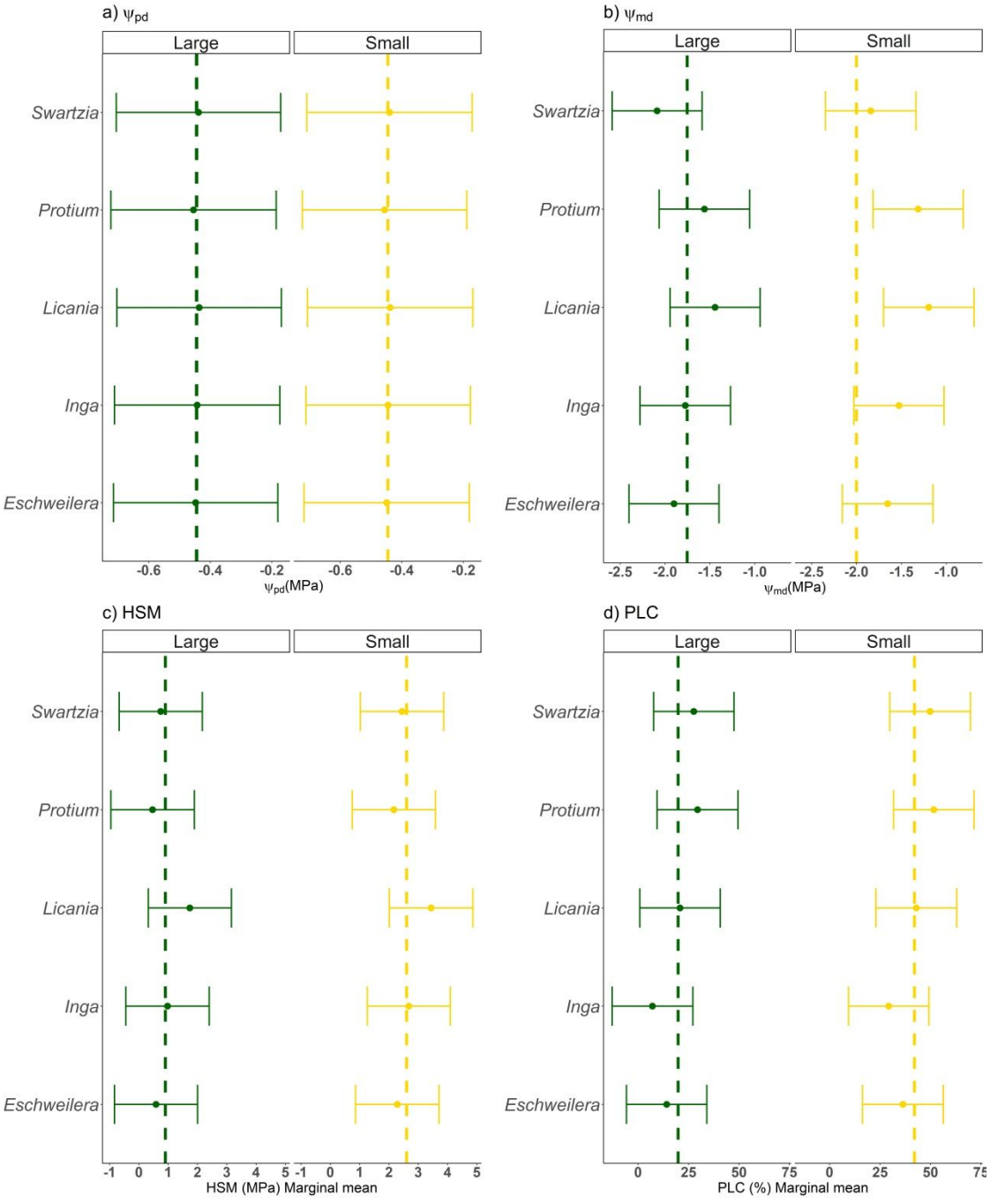

Figure 7 Drought stress indicators comparison between small trees and large trees from throughfall exclusion (TFE) and Control plot. a) Ψpd - predawn water potential; b) Ψmd - midday water potential. c) HSM – branch hydraulic safety margin to P50; d) PLC – native dry season percentage loss of conductivity. The vertical dashed lines represents marginal fixed effect mean, the points represents random effects plus fixed effect mean by each level (by genus) and the

horizontal lines represents standard error by each random effects level. All points and lines represent genus in each treatment. P-values are from mixed effects analysis (see Table 3 for models and analysis section in Methods).

Small understorey trees have greater capacity than canopy trees to adjust hydraulic traits following prolonged experimental drought in a tropical forest

Running title: Tree size strongly controls plant hydraulic responses in a droughted tropical forest

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Abstract

Future climate change predictions for tropical forests highlight increased frequency and intensity of extreme drought events. However, it remains unclear whether large and small trees have differential strategies to tolerate drought due to the different niches they occupy. The future of tropical forests is ultimately dependent on the capacity of small trees (<10 cm in diameter) to adjust their hydraulic system to tolerate drought. To address this question, we evaluated whether the drought tolerance of neotropical small trees can adjust to experimental water stress and was different from tall trees. We measured multiple drought resistance-related hydraulic traits across nine common neotropical genera at the world's longest-running tropical forest throughfall-exclusion experiment and compared their responses with surviving large canopy trees. Small understorey trees in both the Control and the throughfall exclusion treatment (TFE) had lower minimum stomatal conductance and maximum hydraulic leaf-specific conductivity relative to large trees of the same genera, as well as greater hydraulic safety margin (HSM), percentage loss of conductivity (PLC) and embolism resistance, demonstrating they occupy a distinct hydraulic niche. Surprisingly, in response to the drought treatment, small trees increased specific hydraulic conductivity by 56.3% and leaf:sapwood area ratio by 45.6%. The greater HSM of small understorey trees relative to large canopy trees likely enabled them to adjust other aspects of their hydraulic systems to increase hydraulic conductivity and take advantage of increases in light availability in the understorey resulting from the drought-induced mortality of canopy trees. Our results demonstrate that differences in hydraulic strategies between small understorey and large canopy trees drive hydraulic niche segregation. Small understorey trees can adjust their hydraulic systems in response to changes in water and light availability indicating natural regeneration of tropical forests following long-term drought may be possible.

Key-words: Long-term drought; Understorey trees; Hydraulic Safety margin; P50; Maximum conductivity; Acclimation; Amazon forest.

Introduction

Climate change predictions for tropical forests comprise increased frequency and intensity of extreme drought events (Aragão et al., 2018; Brodribb, Powers, Cochard, & Choat, 2020) and long-term reductions in soil moisture availability (Corlett 2016, Christensen et al. 2017). Most studies relating to drought focus on the impacts on large trees that comprise the highest proportion of forest biomass (Meir et al. 2015, Rowland, da Costa, et al. 2015), often finding the effect of drought stress on a plant's hydraulic system is a key driver of tree mortality (Bittencourt et al., 2020; Brodribb et al., 2020; Rowland et al., 2015). However, small understorey trees not only are responsible for up to 20% of the forest carbon sink (Hubau et al. 2019) but have a fundamental role in recruitment and the maintenance of tree populations, as they will effectively compose the future pool of large tree in the forest. Thus, small trees may be critical in determining long-term drought responses if there is extensive loss of large canopy trees (Rowland, da Costa, et al. 2015, Esquivel-Muelbert et al. 2017).

Large trees occupy canopy positions (hereafter, large trees) with high light levels and high vapor pressure deficit. In contrast, small trees from the same genus occupy understory positions (hereafter small trees), grow slowly, generally in shaded conditions and experience a lower atmospheric vapor pressure deficit (Sterck et al. 2011). The distinct resource partitioning between small and large trees, (Brum et al., 2019; Poorter, Bongers, Sterck, & Wöll, 2005) could cause strong differences in their water supply and demand relative to large trees. Reduced water supply from the roots, alongside lower capacitance, is likely to cause more negative water potentials in small trees relative to larger ones, during periods of low soil moisture (Salomón et al. 2017). Large trees are more likely to

buffer periods of water deficit with greater water access by deep roots (Brum et al. 2019), higher capacitance (Mcculloh et al. 2014), and elevated carbohydrate storage that allow to maintain either prolonged stomatal opening (deep roots) or prolonged stomatal closure (greater storage) (McDowell et al., 2008). These potential size-dependent variations in structural and physiological traits suggest tree size potentially influences a tree's capacity to acclimate in response to severe drought stress.

Several key traits of the hydraulic system of a plant are essential in determining the capacity of a tree to survive prolonged drought stress. These traits are often related to preventing hydraulic failure, via emboli formation, in the xylem vessels (Sperry and Tyree 1988), which can lead to severe decreases in leaf water supply, photosynthesis and other physiological functions (Sperry et al. 2002, McDowell et al. 2008, Martinez-Vilalta et al. 2019). These key traits include the water potentials at which the xylem lose 50% or 88% of their conductance (P50 or P88, respectively) and the hydraulic safety margin (HSM) (Meinzer et al. 2009),i.e., the difference between the minimum leaf water potential that is naturally experienced and P50, effectively a metric of the risk of a plant crossing a critical hydraulic threshold. Following sustained periods of drought stress, a tree's capacity to survive is likely to be related to its capacity to acclimate certain key drought tolerance traits or to limit its demand for water, via traits such as minimum stomatal conductance, thus reducing stress on its hydraulic system (Sala et al. 2010, Meir et al. 2018). Existing studies on large trees show limited capacity for tropical trees to adjust plant hydraulic traits in response to drought stress (Binks et al., 2016; Bittencourt et al., 2020; Powell et al., 2017; Schuldt et al., 2011). Some studies have shown that the risk of embolism can be reduced by increasing HSMs under drought conditions (Awad et al. 2010, Tomasella et al. 2018, Prendin et al. 2018). However, in a tropical forest drought experiment, large trees

were found to have limited plasticity in leaf level anatomy (Binks et al., 2016) and no capacity to acclimate their hydraulic systems, especially in traits relating to embolism resistance (Bittencourt et al., 2020; Powell et al., 2017; Rowland et al., 2015). Yet, to our knowledge, no studies have evaluated whether small trees (<10 cm diameter at breast height, DBH), contrary to adult trees, have the capacity to adjust their hydraulic system to prolonged drought stress. Following high mortality losses in large, more drought-intolerant, trees, small trees can increase photosynthetic capacity (Bartholomew et al., 2020;) and lower canopy trees can increase growth rates, even following drought (Brando et al., 2008; Rowland et al., 2015). This suggests that small trees can increase performance in response to elevated light, despite drier conditions. Increased light availability would also require these small trees to the increased atmospheric water demand, implying the need to increase water supply from their hydraulic system and/or to sustain a lower xylem water potential. However, these adjustments to conditions of severe drought only seem to be possible if small trees have a greater drought tolerance, functioning with higher levels of embolism resistance and hydraulic safety margin (HSM). Consequently, consideration of ecosystem changes, such as canopy loss and shifting light availability, is likely to be as important as the consideration of the direct impact of soil moisture stress following long-term drought, as both factors may influence hydraulic acclimation within small trees.

Here we take advantage of a unique drought experiment located in northeast Amazonia (Meir et al. 2015, 2018) to evaluate the response of small trees to combined changes in water and light availability. Previous research at this site has shown that large trees (>40 cm DBH) had significantly higher mortality rates, when compared to small trees and to trees in adjacent control forest, leading to a 40% reduction in biomass following 14 years

of experimentally-imposed soil drought (da Costa et al., 2010; Rowland et al., 2015, Meir et al. 2018). This biomass loss was almost entirely from trees reaching the upper canopy, which led to increased levels of light in the understory and increased growth rates of small understory trees in the wet season (da Costa et al., 2014; Metcalfe, et al., 2010; Rowland et al., 2015, Meir et al. 2018). Furthermore elevated radiation loads are likely to have increased leaf vapour pressure deficit and temperature, increasing the atmospheric drought effect these small trees experience (Mulkey & Pearcy 1992a; Kamaluddin & Grace 1992; Krause, Virgo & Winter 1995). Using new data from this soil drought experiment (henceforth *throughfall-exclusion experiment* – TFE), we explore how small trees adjust hydraulic traits in response to increases in light availability coupled with increased drought stress, specifically, if small trees are able to adjust traits to novel light conditions whilst under drought stress. Thus, we test whether small trees (1-10 cm DBH) alter their plant hydraulic system in response to prolonged soil moisture stress and increased canopy openness, and determine how these responses vary relative to those of large trees (>20 cm DBH). We address the following hypotheses:

1) Considering the same genus we hope that the hydraulic systems of small trees adjust to the combined soil-drought and radiation-load conditions imposed in the TFE relative to the Control. We expect small trees in TFE treatment to take advantage of the increased canopy openness by increasing their water transport efficiency (greater hydraulic specific conductivity and leaf-sapwood ratios). At the same time, we predict that small trees will have more negative water potentials resulting from drought conditions and the capacity to compensate this by adjusting hydraulic traits to maintain higher hydraulic safety margins to meet the elevated canopy water demands in support of photosynthesis.

2) Small trees have different hydraulic strategies from large trees. Specifically, we predict that, independent of the drought and radiation responses in the TFE, small trees have greater drought tolerance, higher xylem embolism resistance and larger hydraulic safety margins, relative to large trees. We therefore predict that, as a consequence of those trait differences, small trees occupy a different hydraulic trait space from large trees.

Methods

Site and plant material

Our study site is a lowland tropical rainforest located in the Caxiuanã National Forest, state of Pará, north-east Brazil (1°43'S, 51°27 W). It has an annual rainfall of 2000-2500mm, with a dry season (< 120 mm monthly rainfall) from July to December. A throughfall exclusion (TFE) experiment was established in 2002, where 50% of canopy throughfall is excluded by a plastic panel structure installed at 1-2m height over a 1 ha area (Meir et al. 2018). The TFE plot was studied alongside a 1 ha Control plot, where no throughfall exclusion took place. The plots have been monitored continuously since 2001 and further information on the experimental set-up can be found in earlier papers (da Costa *et al.*, 2010; Fisher *et al.*, 2007; Meir *et al.*, 2015 and Rowland *et al.*, 2015b).

From August-September 2017, during the peak of the dry season, we sampled 74 small trees with diameters ranging from 1 to 10 cm at breast height (1.3 m). We measured 41 small trees on the Control plot and 33 on the TFE, all taken from nine genera (20 species), replicated in each plot (two to five individuals per genera per plot). While we tried to maintain the same range of tree heights within each genus between plots, small trees had more variable height in the TFE, with light-exposed individuals reaching over 15 meters height, whilst no individuals in the Control reached 15 metres height (See Fig. S1). It was not possible to know the age of each sampled individual, because (destructive) sampling for age determination (tree-ring analyses; e.g., Brienen et al., 2016) was not possible. Consequently, we must assume that our sampled trees may have strongly varying ages (Groenendijk et al. 2014). We thus test the influence of tree stature and

position within the forest strata (van der Sleen et al. 2015), while assuming that most of our sampled trees are likely to be young.

For each individual, we collected two branches from the top of the crown, representing the point maximally exposed to light. The branches were third to fourth order (30-55 mm of diameter), counting from the tip. We collected one set of branches before sunrise (0400 to 0600 hours) and used these to measure embolism resistance and predawn leaf water potential. We collected a second set of branches at midday (1130 to 1330 hours) and used these to measure midday leaf water potential, native embolism, leaf-to-sapwood area, xylem and leaf specific conductivity, minimum leaf conductance and wood density measurements. Immediately after collection, branches were bagged in thick black plastic sacks with moist paper to humidify internal air and minimise leaf transpiration. Branches were transported 100m from the plots to measure leaf water potential, and for the remaining measurements the branches were transported to a laboratory ~1km walk away.

We measured predawn leaf water potential (Ψpd), taken to represent the time-point when transpiration is at its minimum and the water potential of the plant is closest to equilibrium with that of the soil. Ψpd can be considered an integrated metric of soil water availability across the rooting depth (Bartlett et al. 2016). We also determined midday water potential (Ψmd), to capture the minimum Ψ of the plant in the dry season. This measure is affected by any cuticular or stomatal transpiration and, thus, broadly captures the integrated effects of plant traits and the environment water demand on the minimum water potential a plant reaches in natural conditions. We also measured the native dry-season percentage loss of conductivity (PLC). We used the difference between

the minimum leaf water potential (Ψ_{md}) and P_{50}, to calculate the branch hydraulic safety margin (HSM). These two values (native PLC and HSM) were used as indicators of the cumulative damage from embolism.

Predawn and midday water potential

Predawn and midday leaf water potentials were measured in the field immediately after collection, using a pressure chamber (Model 1505, PMS). Branches collected for predawn water potential measures were sampled before sunrise, and for midday water potential, the sampling took place between 1130 to 1330 hours. For each tree we measured water potential of two leaves, or three leaves if the first two measures differed substantially (>0.5 MPa difference) from one another. Measurements from multiple leaves were averaged to create a single value per tree. All water potential measurements were taken on the same day for small trees and across three days for large trees.

Wood density, leaf to sapwood area ratio and minimum stomatal conductance

We measured wood density (W_D) on woody sections 40 to 80 mm long with a diameter of 4 to 7 mm. We debarked samples, immersed them in water for 24 hours to rehydrate and measured the saturated volume using the water displacement method (Pérez-Harguindeguy *et al.*, 2013). We then oven dried the samples at 60°C until they were a constant mass and measured their dry weight with a precision balance to 3 decimal places.

We determined the leaf to sapwood area ratio (A_L:A_{SW}), on all branches by measuring leaf area and calculating sapwood area from two diameter measurements of the debarked basal part of the branch using precision callipers at a standardised distance from the tip. To avoid overestimation we checked the absence of pith area in all branches

per species before the measurement. We measured leaf area by scanning all leaves on the branch and quantifying their area using Image J software (version 1.6.0_20; Schneider et al., 2012). We calculated the leaf area to sapwood area ratio as total branch leaf area divided by its basal sapwood area. All branches had a similar size and were standardised by distance to the tip (~40-70 cm). The A_L:A_{SW} is a key indicator of the balance between transpirative demand and water supply capacity (Mencuccini et al. 2019).

For minimum leaf conductance (G_{min}), we used the leaf conductance to water vapour measured on the abaxial surface of leaves kept 30 minutes in the dark, using an infrared gas analyser (Li-COR 6400, US). All measured leaves were fully formed and undamaged leaves. G_{min} is a measure key indicator of residual leaf water loss and likely a due to a combination of leakage stomatal conductance from partially from leakage of partially closed stomata and cuticular conductance (Duursma et al. 2019, Binks et al. 2020, Márquez et al. 2021)., see Rowland *et al.* (2020)and Bartholomew et al. (2020), provide further details on gas exchange measurement.

Hydraulic efficiency and native embolism

We used maximum hydraulic specific conductivity (K_s) as a measure of xylem hydraulic efficiency and maximum leaf specific conductivity (K_{sl}) as a measure of leaf water supply capacity. We used the native percentage loss of conductivity of the collected branches (PLC) as a measure of native embolism. To PLC, we measured branch xylem hydraulic conductivity before (K_{snat} – native conductivity) and after flushing to remove emboli (Ks). We quantified the leaf area distal to each sample to obtain K_{sl} from K_l (leaf conductance). Using samples from the branches collected at midday, we put the entire branch underwater and discarded a 10 cm long segment from the base. After this, we cut

another 10-15 cm long segment from the base of each branch underwater, standard distance from the tip of the branch and let them rehydrate for 15 min to release tension and avoid artefacts (Venturas et al. 2015). Subsequently, to relax the tension in the branch we cut 1-1.5 cm of branch from base to leaves underwater, in steps of ~15 cm, and used the distal end of the branch for hydraulic measurements to ensure no artificially embolised vessels were present in the measured sample. All samples used for hydraulic measurements were second or third order branches, between 30-55 mm in length and 3-5 mm diameter and were recut underwater with a sharp razor blade before connecting to the apparatus, to ensure all vessels were open at both ends. We then measured flow in the sample using the Ventury tube method (Tyree et al. 2002, Pereira and Mazzafera 2013), where known resistance (PEEK capillary) is connected in series with the sample and the pressure drop in the capillary is proportional to flow in the sample. K_{snat} is then calculated from the pressure head applied and water flow. The samples are then flushed to remove emboli and estimate Ks (Martin-StPaul et al., 2014). We used pressure transducers (26PCCFA6G, Honeywell; read with a OM-CP-VOLT101A data logger, Omega Engineering) to measure pressure drop in the capillary and measured the capillary resistance prior to measurements using precision scales. The samples remained under-water throughout the entire procedure. We calculated PLC as the ratio of K_{snat} to K_s multiplied by 100. We calculated K_{sl} as the sample hydraulic conductivity (i.e., sample conductance times sample length) after flushing divided by the leaf area distal to the measured sample.

Embolism resistance and hydraulic safety

As an index of xylem embolism resistance, we used P_{50} and P_{88}, the xylem water potentials where, respectively, 50% and 88% of hydraulic conductivity is lost. We also used P_{50} to calculate the hydraulic safety margin - the difference between P_{50} and Ψ_{md}, an index of tree hydraulic safety. Branches collected before sunrise were rehydrated for 24 hours and from each branch we cut two or three smaller branches of approximately 40-70 cm. We measured the xylem embolism resistance of each branch using the pneumatic method (Pereira et al. 2016, Zhang et al. 2018). With this method, the loss of hydraulic conductance is estimated from the increase in air volume inside the wood caused by embolism formation as the branch dehydrates. Air volume is estimated from the air discharge from the cut end of the branch into a vacuum reservoir (~50 kPa absolute pressure) of known volume during a given amount of time (2.5 minutes). We measured initial and final pressure inside the vacuum reservoir with a pressure transducer (163PC01D75, Honeywell) and calculated the volume of air discharged using the ideal gas law. A detailed protocol is presented in (Pereira et al. 2016, Bittencourt et al. 2018) and revised by Pereira et al. (2021). Percentage loss of conductance for each branch is estimated from percentage air discharged (PAD) during its dehydration. PAD is calculated by standardising air discharge for each branch by its minimum (fully hydrated) and maximum (most dehydrated) air discharge state. We dehydrated branches using the bench dehydration method (Sperry et al. 1988). Before each air discharge measurement, branches were sealed in thick black plastic bags for one hour for leaf and wood xylem water potential to equilibrate. Directly after the air discharge was measured, we estimated wood xylem water potential by measuring the leaf water potential of one to two leaves. Drought embolism resistance is then given by the increase in PAD with decreasing xylem water potential for each tree. To calculate P_{50}, we pooled data from the

two-to-three branch replicates from the same tree and fitted a sigmoid curve to the data (Pammenter and Van der Willigen 1998) where P_{50} and slope (a) are the fitted parameters and P_{88} is predicted from the fit (Eqn 1):

$$\mathrm{PAD} = 100/\left(1 + \exp\left(a\left(\Psi - \mathrm{P50}\right)\right)\right) \quad (1)$$

Eqn1. Percentage air discharge equation (PAD). Ψ Water potential. P_{50} (xylem embolism resistance (MPa)

Data analysis

By comparing trees found on the Control and TFE experimental plots, we measure the effect of the experimental drought on our drought stress indicators ($\Psi_{pd,}$ Ψ_{md} - midday water potential; HSM – branch hydraulic safety margin to P_{50}; PLC – native dry season percentage loss of conductivity) and plant traits (W_D – wood density; A_L:A_{SW} - leaf to sapwood area; P_{50} - xylem embolism resistance; P_{88} - xylem embolism resistance; G_{min} – minimum stomatal conductance; K_s – maximum hydraulic specific conductivity; K_{sl} - maximum hydraulic leaf -specific conductivity) in small trees. We used linear mixed effects models to test for plot (TFE vs Control) and taxonomic effects (genus and species) on hydraulics traits in small trees (n = 66) using the R package lme4 (Bates et al. 2015). We tested the significance of the random effect by removing it and evaluating if the model significantly worsened using log likelihood tests using the *ranova* function for *lmerTest* objects (Zuur et al. 2009). We tested sequentially for the random effect of genus on: (a) the model intercept; (b) the fixed Plot effect (drought effect, difference between plots) on slope without intercept; and (c) both intercept and plot. When either the genus effect on plot, slope or both did not show the significance, we kept the multilevel approach using

genus as a random effect on the intercept (1|genus), as it controls for experimental design (Burnham and Anderson 2004). After testing the random effects, we tested the fixed TFE effect on variables. When taxonomy was included as a random effect in our models, we tested for both genus-only and species-nested-within-genus effects. We tested the complete model (genus and species as a random effect) against a General Linear Model (GLM) containing only the fixed effects. In all variables genus was significant as random effect. Therefore, linear models with genus as a random effect were used to test the significance of the fixed effects. To quantify model goodness of fit, we considered the marginal and conditional R^2 (Mulkey and Pearcy 1992b). The marginal R^2 indicates how much of the model variance is explained by the fixed effects only, whereas the conditional R^2 indicates how much of the model variance is explained by the complete model with fixed and random effects. All the analyses were done in R (version 3.3.0; R Core Team, 2016)

Small and large tree comparisons

We tested for differences in individual tree-level responses to the TFE treatment for large (n = 72) and small trees (n = 39). We use the large trees data from Bittencourt et al. (2020) conducted in the same experimental plots and collected during 2017 with the same methodological procedures. For this comparison we restrict the samples to those trees whose genera are replicated on both plots and replicated between the large and small trees, with a minimum sample size of 2 individuals per size group per plot and genus. Consequently, the number of genera and individuals employed in this comparison is lower than the available number of individual small trees and the full dataset published in Bittencourt et al., (2020). In total we use five genera (*Eschweilera, Inga, Licania, Protium,*

Swartzia), with 15 small trees on the Control and 24 small trees on the TFE, and 35 large trees on the Control and 37 large trees on the TFE. We used linear mixed-effect models to test the effects of the tree size with two classes (large and small), and tree size on drought stress indicators and hydraulic traits. Taxonomic effects were included by using genus as random effects, following the same protocol used for the small tree analyses, presented above. Within this paper, all data presented represent the mean and standard errors of the mean. A summary of available trait data by genus is presented in Table 1.

To test for an overall difference in the hydraulic strategy between small and large trees, we used the multivariate approach conducting non-metric multidimensional scaling (NMDS) using an individual-traits matrix (McCune & Grace 2002). We construct a matrix of data consisting of rows of individuals of each species and columns of traits values. We standardized the individual trait values for each genus and built the similarity matrix using Gower distance. NMDS searches for the best position of individuals variables on k dimensions (axes) to minimize the “stress” of the resulting k-dimensional configuration. We use k axes = 2 from that ordination as the initial configuration. The "stress" is obtained by comparison among the pair-wise distances (differences) of each individual's variables in reduced ordination space (expressed in terms of axes) and the original distance matrix (Gower distance). The regression is fitted using least-squares regressions and the goodness of fit is measured as the sum of squared differences between ordination-based distances and the distances predicted by the regression. A goodness of fit, or stress value, between 0.1 to 0.2 represent a good fit within the specified number of dimensions analysed to enable points to be interpreted relative to the NMDS axes. Therefore, the axis represents the data in a way that best represents their dissimilarity, points on the graph that are closer together are more similar. In addition, we use MANOVA to test the

difference in multidimensional space filled by tree size (small and large groups) and by plot effect (TFE and Control groups) separately (Anderson 2001). We use a MANOVA to compare Gower distance among observations in the same group versus those in different groups. We conducted a MANOVA first using small and large tree groups and then using TFE and Control groups using both tree sizes together. The size and plot effects were tested separately. Finally, we use permutations of the observations to obtain a probability associated with the null hypothesis of no differences between groups.

Results

The reduced soil moisture availability and increased canopy openness caused by 15 years of the TFE (Fig. S2) caused significant changes in the hydraulic traits of the small trees (Fig. 1). Maximum specific conductivity (K_s) increased significantly, by 56.3±41.5%, in the TFE small trees relative to the Control (Fig. 1f, $p<0.01$), similarly there was a significant (45.6±38.2%) increase in the leaf: sapwood area ratio (A_L:A_{SW}, Fig 1b.; $p<0.001$). The TFE also had significant effects on key physiological indicators of drought stress in small trees (Fig. 1) with Ψ_{pd} 0.24 MPa lower on the TFE relative to the Control ($p <0.001$) and Ψ_{md} 0.67 MPa lower ($p < 0.001$, Table S2). In contrast, other key hydraulic traits including xylem embolism resistance (P_{50} and P_{88}), leaf specific conductivity (K_{sl}), minimum stomatal conductance (G_{min}) and wood density (WD) showed no significant difference between the TFE and the Control plots for small trees (Fig. 1; Table 2; Table S3).

Taxonomic effects on hydraulic traits and their interactions with drought

Using mixed-effect modelling analysis we found that the variance explained by taxonomy had only a limited role in affecting the overall drought responses. When genus by genus responses to the drought effect were examined separately, it was clear that there were highly variable responses to the treatment among genera and sometimes these were inconsistent in terms of direction, as well as magnitude. We cannot separate the taxonomic effect from the residual variance because genus-specific influences on the plot effect were highly variable (Fig. 2 and 3). Given the low replication (between 2 and 5 for each genus on each plot treatment) and high variation within each genus, it was not always statistically viable to test the plot effect within each genus (Fig. 2 and 3), however where this was possible, clear statistical differences were seen for some genera but not for others (Table 2 Fig. 2). For example, *Licania* showed consistent responses in P_{50} and HSM while *Ocotea* did not show differences between plots (Fig. 2 and 3). The patterns described here were also maintained when we analysed the data at a species level (data not shown).

Large versus small trees

We compared the responses of hydraulic traits between large (>20 cm DBH) and small trees (1-10 cm DBH). Except for Ψ_{pd}, the results we obtain considering only the five genera that overlap between the small and large size classes, were similar to when considering all nine genera of trees present in Control plot and TFE experiment (see Fig. S3 supplementary material and Table S3 for n values for the small to large tree comparisons). Using all of the trait data for five overlapping genera, we applied NMDS ordination which demonstrated that the niche space occupied by the small trees was significantly different from the trait space of large trees. The traits space separated on to a clear 2-dimensional axis with a stress score of 0.18, indicating a good fit between the data and an analysis consisting of two axes (Fig. 4). Different associations amongst the nine hydraulic traits separated the individuals in the small and large tree groups. This result was driven predominantly by the first axis, which was positively related to PLC, P_{50} and P_{88} that influenced small tree grouping (Fig. 4). While the first axis was negatively related to K_s, K_{sl}, G_{min} influencing large trees grouping (Fig 4, Table S4). Using the complete set of hydraulic traits, we show that the hydraulic niche of small trees was significantly different from that of large trees ($MANOVA_{(1,66)}$; $F=7.96$; $p<0.001$; Table 1). However, there was no difference in hydraulic niche space occupied by the Control and TFE groups ($MANOVA_{(1,64)}$; $F=1.22$; $p=0.30$), except for K_s that showed plot and tree size effects ($MANOVA_{(1,64)}$; $F=3.5$; $p=0.05$).

In contrast to the large increase in K_s observed between small trees in Control and TFE trees (Figs 1 & S2), the plot level average values of K_s were similar among large trees in both Control and droughted conditions (4.82±3.93 TFE and 4.86±2.79 Control plot). Similar to Ψ_{md}, notable plot level differences were present in small trees, but these were absent

in the large trees (-1.72 ± 0.48 MPa TFE and -1.70±0.48 MPa Control treatment). However, small trees had values of Ψ_{md} which were 17.12±0.03% higher (values closer to 0) than the large trees. Furthermore, for the variables which had no treatment effect amongst the small trees, we find on average, across both the TFE and Control plots, the small trees had a 38.20±32.10% ($p<0.01$) more negative P_{50} and a 68.40±58.80% and 20.70±30.40% lower G_{min} and K_{sl}, respectively, than the large trees (Fig. 5b, 5d, 5f; $p<0.001$). Also across the plot we found that HSM increased by 72.97±36.34% and PLC increased by 44.41±14.62% in the small trees relative to large trees (Fig. 5g, 5i, 5j; $p<0.01$).

We analysed the influence of genus on the combined effect of treatment and tree size effect (i.e., large and small trees on the Control and TFE plot) for the five genera we could replicate across plots and tree size classes. We found that the effects of tree size varied substantially among genera and between traits and stress indicators (Fig. 6 and 7, table S5 and S6). For example, the difference in P_{50} between large trees and the small trees was 61.48±52.51% for *Licania* and 38.96±3.7% for *Inga* (Fig. 6). In contrast, G_{min} was significantly lower in the small trees relative to large trees across almost all genera (Fig. 6b). The drought-response pattern also changes when doing within-genus comparisons between large and small trees, for example the mean P_{50} response for *Inga* was different between small and large trees (Fig. 6). A difference in trait values between the Control and TFE plots that was present either for small tree or large trees, but not for both size classes simultaneously, occurred multiple times (Fig. 6 and 7), especially for the genus *Inga*. Mixed effect modelling results identify a strong influence of genus on trait variation between our two size classes (Table 3), yet there are limited cases where we find significant models demonstrating trait differences between the Control and the TFE plot with a significant tree size and genus effect (Table 3).

To test for size (small vs. large) and genus effects in each treatment (Control and TFE), we created a model with both size and genus as fixed effects. In the Control plot the full model (trait ~ genus*size) was a better predictor of variation across almost all traits, except for K_s, where there was a genus only effect and G_{min}, P_{50} and P_{88} where there was a size only effect. An interaction between size and genus was only significant for PLC (Table S7). The full model was also the best predictor of trait variation in the TFE plot, although only HSM, W_D and G_{min} showed a significant size effect. Significant interactions between genus and size were found for P_{50} and P_{88} (Table S7).

Discussion

Our results provide the evidence that small trees can adjust their functioning in response to drought, allowing them to maximize carbon gain in the higher-light levels following mortality of large trees in the TFE. We find that small trees (1-10 cm DBH) have the capacity to increase maximum specific hydraulic conductivity and leaf-sapwood area ratio in response to prolonged (15 year) soil moisture deficit. Despite having significantly lower pre-dawn and midday leaf water potentials, small trees had the capacity to adjust key hydraulic traits to allow a positive response to a higher light environment. This suggests that despite soil drought stress, small trees can still increase water transport efficiency and canopy water use in response to increases in light availability, following drought-induced mortality of large trees, potentially allowing them to maximise productivity in periods of the year when water is available. We also show the different components of a hydraulic strategy that provides niche segregation between small and large trees, with small trees being more drought tolerant than large canopy trees.

Studying the effects of multiple factors (here, imposed drought and size) on the physiology of hyper-diverse tropical forests is challenging. Here, we successfully addressed this problem by using genus and species nested in genus as random factors in linear mixed models and show that variability of species within genera is generally small. We nonetheless acknowledge our sample size limitations and the possibility that greater sampling depth may discover significant species-by-species variability in these traits.

The impact of drought on the hydraulic system of small trees

The substantial drought-related mortality of large trees (da Costa et al., 2010; Rowland et al., 2015) in the 15 years preceding this study led to an increase in the light availability in the lower canopy of the TFE, driving increases in the maximum photosynthetic capacity (71.1% and 29.2% increase in J_{max} and Vc_{max} respectively) and a 15.1% increase in the LMA of the same small trees we study here (Bartholomew et al. 2020). These differences in response to the prevailing light environment have also been observed elsewhere in tropical tree canopies (Ruggiero et al. 2002, Domingues et al. 2010, Cavaleri et al. 2010) and are indicative of plants changing their allocation strategy in response to increased light availability (Poorter et al., 2009; Wright et al., 2004). Critically, these allocation shifts are likely to result in a net increase in photosynthesis and growth (Metcalfe, Meir, Aragão, et al. 2010, Rowland, da Costa, et al. 2015, Meir et al. 2018), which require higher water supply to the canopy of each individual. The elevated soil moisture stress in the TFE relative to the Control trees manifested itself as significantly more negative pre-dawn and midday leaf water potential values (Figs 1h-1i), key indicators of plant water stress (Bhaskar & Ackerly, 2006; Kramer, 1988; Martínez-vilalta & Garcia-Forner, 2017). Interestingly however, these more negative water potentials did not translate into a significant change in HSM between plots, which would imply that the small trees converge to have the same vulnerability to drought (Choat et al. 2012). This could occur because of a trend, albeit not statistically detectable, towards more negative P50 values in the TFE plot for small trees, relative to the Control trees (Fig. 1), making a significant difference in HSM less likely. When examined at the genus level, five of the nine genera have consistently more negative P_{50} values on the TFE relative to the Control, with two remaining roughly equal and two less negative on the TFE (Fig. 2). These data

suggest that, despite operating at more negative water potentials, it is still possible for small trees to adjust their hydraulic system to support the increased growth in response to greater light availability.

Consistent with increases in photosynthetic capacity (Bartholomew et al., 2020), we observe an increase in leaf area to sapwood area ratio (A_L:A_{SW}) in the small trees on the TFE, relative to the Control. Combined with greater hydraulic specific conductivity, small trees in the TFE are therefore able to supply water to more photosynthetic tissue without increasing the volume of sapwood. A global study, including multiple sites from the tropics showed plant hydraulic systems are highly sensitive to changes in this ratio (A_L:A_{SW}) and may be one of the main factors controlling trade-offs in other plant hydraulic traits (Mencuccini et al. 2019). Increasing leaf area increases the total water demand of the tree; however, the observed increases in photosynthetic capacity (high values of Vc_{max} and J_{max}, Bartholomew et al. 2020), may allow slightly lower stomatal conductance for any given CO_2 concentration (Bartholomew et al., 2020; Sperry et al., 2017). This may, in part, compensate for the increase in demand for water that increased leaf areas could cause. However, even with the observed increases in photosynthetic capacity, these small trees probably still experience increased total water demand due to increased exposure to higher temperatures and VPD, suggesting that small trees must increase maximum hydraulic conductivity and/or tolerate reductions in water potential and therefore greater embolism risk (Sperry et al., 2017). In our study, small trees sampled in the TFE were slightly taller than the small trees in the Control plot (Fig. S1). This difference may in part-contribute to the slightly elevated conductance in the branches, as taller trees can have larger vessels at the base and greater vessel tapering from the trunk to branch tip (Olson

and Rosell 2013, Olson et al. 2020). It is, however, unlikely that these differences had a large influence on our K_s results, overall, the difference in height were small and the genera with the greatest height differences between the TFE and Control (*Protium, Octea, Voucoupoa*, Fig. S1) showed no changes in Ks (Fig. 2).

Differential hydraulic strategy between small and large trees

The comparison between small trees and large trees multidimensional hydraulic trait space, using NMDS and MANOVA, indicate they occupy different hydraulic niche spaces, despite some overlap. This revealed that smaller trees do indeed have a different water use strategy to larger canopy trees (Fig 3). The differences in the traits we observed were far greater, and in most cases significantly so, between the large and the small trees than for trees of the same size class between treatments (Fig 4). In addition, we show that smaller trees across both the Control and the TFE plot have significantly more negative P_{50} values and lower G_{min} values and significantly greater hydraulic safety margins (HSM), midday leaf water potentials and PLC (Fig. 4). This may imply that the small trees converge to the same vulnerability to drought, consistent with the results from large scale studies (e.g. Choat et al., 2012). However, the HSM is 1.94 MPa more positive in the small trees relative to large trees, indicative of a lack of convergence of the vulnerability of large and small trees (i.e. Fig 4i), potentially suggesting vulnerability to drought is driven by the ontogenetic stage of a tree. In addition, our results are consistent with the hypothesis that the smaller trees are shallow rooted and compensate for the lack of access to deep water through developing greater xylem embolism resistance and greater stomatal control (Brum et al., 2019; Tardieu, 1996, Sperry et al. 2017). It is possible that the greater hydraulic safety margin in small trees enables them to adjust more effectively to increased

light availability, despite the lower water availability in the TFE, as it enables these trees to tolerate greater drought stress without passing critical thresholds.

The carbon gain associated with greater photosynthesis under higher light environments may be translated into new xylem growth in smaller trees. This growth could rapidly replace damaged tissues and is likely to be a more viable strategy for smaller trees, relative to large trees (Damián et al. 2018, Trugman et al. 2018), which would reduce the risk associated with higher PLC levels. Furthermore, small trees maintained significantly lower G_{min} and higher midday leaf water potentials (Fig. 5d, 5g), relative to the large trees, despite having similar pre-dawn leaf water potentials, suggesting that small trees are able to more tightly regulate water loss, during both the day and night. Probably, the high regulate water loss in small tree is associate a lower water storage capacity to buffer short-term variation of water availability (Goldstein et al. 1998, Meinzer et al. 2003). The greater degree of control further reduces the risk of runaway embolism when photosynthesising during periods with low water potential, particularly if these trees can repair cavitated vessels (Nardini et al., 2011; Salleo et al., 2004; Salleo et al., 1995) or grow new vessels between consecutive dry seasons (Eller *et al.* 2018). Also, small trees also have fewer structural constraints than large trees, so small changes in hydraulic traits in a small tree could have bigger effects on overall performance during drought, because the marginal effect of each unit change is larger relative to the size of the tree (Mencuccini 2002). Combined, these factors are likely to allow small trees to have greater flexibility in terms of the strategy they use to adjust to combined changes in water and light availability. However, as we highlight in our results, there is considerable variability both within and between taxonomic groups with respect to how small trees may alter their traits and their resulting drought tolerance strategy.

This study highlights the importance of forest structural changes in controlling the traits of what are likely to be the next generation of trees growing up during prolonged drought stress. We show that, relative to large trees, small trees have a larger capacity to acclimate their hydraulic systems to increases in light availability following drought-induced mortality of large canopy-dominant trees. Our results suggest that small trees are able to acclimate the hydraulic conductance and leaf area to sapwood area ratio despite experiencing prolonged soil moisture stress, which resulted here in lower leaf water potentials and greater PLC. Also, our results demonstrate that there is a consistent and larger shift in the plant hydraulic strategy of saplings relative to large trees across most of Amazonia's hyper-abundant taxonomic groups. Whilst we find adjustment of traits in response to the drought treatment, it remains unknown whether all small trees community can respond in the same way or only the long-term drought surviving trees. In this way, a key uncertainty that remains to be answered are relates to the long-term development of these trees. Assuming these small trees continue to develop under the experimental drought stressed conditions, it would be of interest to know if the trajectory of change in hydraulic traits we observe can be sufficient to increase the hydraulic resistance to drought of these trees as they approach full size.

Ultimately, continued acclimation of hydraulic systems throughout a the lifespan of a tree may allow a more drought-resilient ecosystem to develop following the negative impacts of drought on pre-existing larger trees. Therefore, even the current generation of trees showing huge mortality rates, the next generation might be followed by a new stable community composed of those small trees that can adapt to drought. This implies that for prediction of the future of tropical ecosystems function we needs to consider trait adjustment in the future forest instead currently forest.

Data and Materials Availability

We are in the process of making this data publically available through the main funding bodies data centre, NERC EIDC (https://eidc.ac.uk/), if accepted the data will be fully publically available on a link we will provide.

Supplementary Data

Supplementary tables

Table S1. Linear mixed effect model analysis of stress indicator variables from small tress.

Table S2. Linear mixed effect model analysis of hydraulic traits from small tress.

Table S3 Numbers of individuals for small and large tree in each treatment (TFE an Control) and mean and standard deviation of all variables measured.

Table S4. Statistics from the NMDS modelling shown in Figure 3.

Table S5 Results of linear effect models of size (Size vs TFE) on the stress indicator variables on Control plot.

Table S6 Results of linear effect models of size (Size vs TFE) on the hydraulic traits variables on TFE plot.

Table S7 Results of linear effect models of size (Size vs TFE) and genus on the stress indicator and hydraulic variables on TFE plot.

Supplementary figures

Figure S1. Height and diameter in each treatment (TFE vs. Control) and by genus for the most common small tree genera in this study (9 genera)

Figure S2. Soil water content during 2016 in the Throughfall Exclusion Experiment plot and in the Control plot at 10 cm and at 100 cm.

Figure S3. Comparison between the small trees and large trees from the throughfall exclusion (TFE) and Control plots from grouping all 9 genera available within the large and small tree groupings.

Conflict of interest

The authors have no conflict of interest to declare.

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Authors' Contributions

ALG collected and compiled the data alongside LR, PRLB, IC, PBC, PG, LVF, DDV, JASJ, DCB and ACLdC. LR designed the study with MM, ACLdC, PM, ALG and RO. ALG, MM, PRLB and LR performed the statistical analysis and ALG, LR and RO wrote the paper, all authors substantially contributed to revisions.

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Figure 1 Stress indicators and hydraulic traits for small trees (1-10 cm DBH) measured in dry season oct/2017 on the Control plot and through-fall exclusion (TFE).

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Tables

Table 1 – Mean and standard deviation from small trees: P_{50} - xylem embolism resistance (MPa); P_{88} - xylem embolism resistance (MPa); G_{min} – minimum stomatal conductance (mol m^{-2} s^{-1}); K_s – maximum hydraulic specific conductivity (kg m^{-2} s^{-1} MPa^{-1}); K_{sl} - maximum hydraulic leaf-specific conductivity (kg m^{-2} s^{-1} MPa^{-1}); A_L:A_{SW} – leaf to sapwood area ratio (m^2 m^{-2}); W_D- Woody density; Ψ_{pd} - predawn water potential (MPa); Ψ_{md} - midday water potential (MPa); HSM – branch hydraulic safety margin to P_{50} (MPa); PLC – native dry season percentage loss of conductivity (%), separated by genus and treatment.

		Hydraulic traits										
Genus (n individuals)	Treatment	P_{50}	P_{88}	G_{min}	K_s	K_{sl}	$A_L:A_{SW}$	W_D	Ψ_{pd}	Ψ_{md}	HSM	PLC
***Eschweilera* (3)**	**Control**	-2.91±0.07	-5.08±0.31	0.028±0.023	1.12±0.19	0.57±0.30	112.07±32.41	0.73±0.12	-0.32±0.23	-1.68±0.24	1.13±0.32	49.14±4.9
***Eschweilera* (3)**	**TFE**	-3.66±2.01	-6.32±3.80	0.026±0.019	2.80±2.62	4.71±6.12	92.12±65.44	0.59±0.09	-0.52±0.24	-1.87±0.26	1.89±2.28	9.22±4.36
***Inga* (4)**	**Control**	-4.60±1.63	-7.84±3.10	0.02±0.014	2.3±1.43	1.56±0.85	84.59±47.51	0.64±0.18	-0.37±0.26	-1.51±0.57	3.09±2.07	11.33±10.19
***Inga* (3)**	**TFE**	-3.48±0.58	-6.22±1.62	0.02±0.006	4.56±1.68	1.93±0.73	160.81±58.68	0.63±0.08	-0.39±0.22	-1.35±0.78	2.13±0.4	19.28±13.21
***Licania* (4)**	**Control**	-5.28±1.98	-9.62±4.40	0.025±0.014	0.15±0.04	0.12±0.07	66.15±24.41	0.76±0.062	-0.25±0.07	-1.65±0.85	3.62±2.75	38.29±28.52
***Licania*(4)**	**TFE**	-6.18±1.59	-9.07±1.77	0.024±0.02	2.17±2.19	0.37±0.40	104.90±45.99	0.761±0.014	-0.85±0.81	-1.388±0.78	5.183±1.70	68.667±28.13
***Mouriri* (3)**	**Control**	-4.77±0.54	-7.69±1.31	0.025±0.017	0.62±0.05	0.22±0.20	154.33±59.51	0.867±0.003	-0.24±0.09	-0.943±0.08	3.829±0.48	58.031±27.65
***Mouriri* (3)**	**TFE**	-5.55±0.74	-7.35±2.18	0.077±0.022	3.63±3.03	1.32±0.88	143.30±92.13	0.751±0.17	-1.07±1.32	-2.583±0.95	2.972±0.77	60.769±15.83
***Ocotea* (3)**	**Control**	-3.59±1.49	-8.72±2.63	0.007±0.003	1.63±0.81	0.84±0.17	125.38±54.22	0.638±0.05	-0.36±0.4	-0.6±0.364	2.994±1.26	36.718±18.42
***Ocotea* (3)**	**TFE**	-5.04±2.08	-8.61±4.88	0.03±0.024	1.58±0.66	0.60±0.46	84.83±32.64	0.68±0.13	-1.44±1.17	-2.41±0.81	2.62±2.45	65.27±24.19
***Protium* (5)**	**Control**	-2.30±0.71	-4.16±2.40	0.017±0.01	1.68±0.94	0.75±0.41	78.60±6.37	0.74±0.07	-0.31±0.3	-1.23±0.31	1.07±0.78	54.73±17.02
***Protium* (3)**	**TFE**	-3.64±1.47	-5.65±0.73	0.013±0.01	1.10±0.07	0.44±0.07	90.57±17.71	0.72±0.049	-0.48±0.16	-1.00±0.24	2.55±1.73	49.74±11.94
***Swartzia* (3)**	**Control**	-3.17±1.28	-5.98±1.89	0.06±0.04	1.67±0.26	0.78±0.55	72.45±18.20	0.73±0.02	-0.23±0.12	-1.57±0.16	1.60±1.36	59.73±9.94
***Swartzia* (3)**	**TFE**	-4.34±0.57	-6.94±0.06	0.06±0.02	2.78±0.51	0.89±0.54	210.45±67.51	0.72±0.005	-0.79±0.48	-2.36±0.09	1.98±0.66	49.13±8.57
***Tetragastris* (5)**	**Control**	-2.31±1.48	-4.34±1.81	0.03±0.01	2.22±1.66	2.29±3.12	83.86±59.38	0.64±0.05	-0.28±0.13	-1.06±0.58	1.25±1.31	22.12±15.60
***Tetragastris* (3)**	**TFE**	-4.36±1.19	-6.52±2.90	0.016±0.01	1.33±0.62	1.04±0.45	88.10±34.28	0.58±0.04	-1.43±0.40	-2.44±0.13	1.92±1.06	43.24±6.33
***Vouacapoa* (3)**	**Control**	-3.57±0.13	-5.37±1.45	0.015±0.003	1.00±0.16	0.95±0.64	56.71±22.69	0.69±0.13	-0.39±0.18	-1.59±0.19	1.97±0.31	43.78±11.37
***Vouacapoa* (3)**	**TFE**	-2.22±0.79	-3.54±1.63	0.012±0.004	0.83±0.51	0.67±0.78	229.76±101.26	0.70±0.01	-0.77±0.35	-2.07±0.24	0.15±0.72	33.24±19.67

Table 2 Results of linear mixed effect models of plot (Control versus TFE) on the stress indicators and hydraulic traits for small trees (1-10 cm DBH) measured in dry season (Oct/2017) on the Control plot and through-fall exclusion TFE. WD - wood density; AL:ASW - leaf to sapwood area ratio; P50 - xylem embolism resistance; P88 - xylem embolism resistance; Gmin - minimum stomatal conductance; Ks - maximum hydraulic specific conductivity; Ksl - maximum hydraulic leaf -specific conductivity; Ψpd - predawn water potential; Ψmd - midday water potential. HSM - branch hydraulic safety margin to P50; PLC - native dry season percentage loss of conductivity. The table shows the least square means for the Control and TFE, and the random genus effects (see analysis section in Methods for details). The first row of each trait gives the mean and second row gives one standard error for the fixed effects and the 95% confidence interval for genus-level random effects. Traits for which plot had a significant effect, and species for which the random effects were different from zero, are marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) and ns (non-significant).

	Plot-level Coefficients		**Genus-level Coefficients**								
Variable	**Control**	**TFE**	**Eschweilera**	**Inga**	**Licania**	**Mouriri**	**Ocotea**	**Protium**	**Swartzia**	**Tetragastris**	**Vouacapoa**
P_{50}	3.56 (-4.12/-2.99)	-4.26 (-5.761/-2.79) ns	-3.29 (-4.67/-1.91) ***	0.83 (-2.56/0.90)	2.38 (-4.11/-0.65) **	1.87 (-3.65/-0.09) *	-1.03 (-2.81/0.75)	0.60 (-1.13/2.33)	-0.35 (-2.20/1.50)	0.21 (-1.48/1.90)	0.39 (-1.39/2.17)
P_{88}	6.49 (-7.45/-5.42)	-6.58 (-9.34/-4.00) ns	-5.71 (-8.18/-3.24) ***	1.44 (-4.54/1.65)	3.69 (-6.78/-0.59) *	-1.82 (-5.01/1.37)	-2.96 (-6.15/0.22)	1.11 (-1.98/4.21)	-0.66 (-3.97/2.65)	0.54 (-2.48/3.57)	1.25 (-1.94/4.43)
G_{min}	0.027 (0.02/0.03)	0.030 (0.008/0.053) ns	0.03 (0.01/0.04) ***	0.01 (-0.03/0.01)	0.00 (-0.02/0.02)	0.03 (0.01/0.05) **	-0.01 (-0.03/0.01)	-0.01 (-0.03/0.01)	0.04 (0.02/0.06) ***	0.00 (-0.02/0.02)	-0.01 (-0.03/0.01)

K_s	1.46 (0.95/2.00)	2.32 (0.98/3.64) *	1.79 (0.46/3.13) **	1.48 (-0.28/3.23)	-0.49 (-2.24/1.26)	0.34 (-1.47/2.15)	-0.19 (-2.00/1.63)	-0.33 (-2.03/1.38)	0.33 (-1.56/2.22)	0.17 (-1.58/1.92)	-0.88 (-2.69/0.93)
K_{sl}	1.00 (0.45/1.55)	1.22 (0.21/2.58) ns	2.23 (0.86/3.61) **	0.51 (-2.31/1.29)	-1.96 (-3.76/-0.17)	-1.45 (-3.31/0.40)	-1.51 (-3.37/0.35)	-1.59 (-3.34/0.16)	-1.41 (-3.35/0.54)	-0.29 (-2.09/1.50)	-1.42 (-3.28/0.44)
$A_L:A_{SW}$	90.77 (69.76/110.74)	131.70 (81.14/183.50) **	104.09 (48.95/159.23) ***	13.17 (-59.03/85.37)	-18.57 (-88.86/51.73)	44.73 (-29.93/119.39)	1.02 (-73.64/75.68)	-21.00 (-91.29/49.29)	23.56 (-54.42/101.54)	-18.64 (-88.93/51.65)	39.15 (-35.52/113.81)
W_D	0.71 (0.68/0.75)	0.68 (0.59/0.78) ns	0.68 (0.60/0.75) ***	0.03 (-0.14/0.07)	0.09 (-0.01/0.19)	0.13 (0.03/0.24) **	-0.01 (-0.12/0.10)	0.06 (-0.04/0.16)	0.05 (-0.06/0.16)	-0.05 (-0.15/0.06)	0.03 (-0.08/0.13)
Ψ_{pd}	0.31 (0.14/0.48)	0.86 (0.63/1.52) ***	-0.42 (-0.05/0.90)	0.04 (-0.69/0.61)	0.13 (-0.50/0.76)	0.24 (-0.44/0.91)	0.48 (-0.19/1.15)	-0.03 (-0.66/0.60)	0.03 (-0.67/0.74)	0.29 (-0.34/0.92)	0.17 (-0.51/0.84)
Ψ_{md}	1.29 (1.09/1.54)	1.93 (1.50/2.19) **	-1.78 (1.19/2.37) ***	0.34 (-1.14/0.46)	-0.26 (-1.03/0.52)	-0.02 (-0.85/0.81)	-0.27 (-1.10/0.56)	-0.63 (-1.41/0.14)	0.11 (-0.76/0.98)	-0.20 (-0.98/0.57)	0.05 (-0.78/0.88)
HSM	2.25 (1.64/2.86)	2.35 (0.74/3.96) ns	1.52 (0.05/2.99) *	1.16 (-0.69/3.01)	2.77 (0.93/4.62) **	1.88 (-0.02/3.78)	1.29 (-0.61/3.19)	-0.02 (-1.87/1.83)	0.24 (-1.74/2.21)	-0.02 (-1.82/1.79)	-0.46 (-2.36/1.45)
PLC	41.315 (32.38/49.21)	45.82 (24.14/67.78) ns	33.18 (15.62/50.74) ***	18.44 (-41.43/4.56)	22.47 (-0.52/45.47)	26.22 (2.44/50.00) *	17.82 (-5.96/41.60)	19.68 (-2.70/42.07)	22.31 (-2.52/47.15)	-4.01 (-27.79/19.77)	5.33 (-18.45/29.11)

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Table 3. Results of linear mixed effect models of size (Large versus Small) on the stress indicators and hydraulic traits for small trees (1-10 cm DBH) and large trees (>20 cm DBH) measured in dry season (Oct/2017) on the Control plot and through-fall exclusion TFE. WD - wood density; AL:ASW - leaf to sapwood area ratio; P50 - xylem embolism resistance; P88 - xylem embolism resistance; Gmin - minimum stomatal conductance; Ks - maximum hydraulic specific conductivity; Ksl - maximum hydraulic leaf -specific conductivity; Ψpd - predawn water potential; Ψmd - midday water potential. HSM - branch hydraulic safety margin to P50; PLC - native dry season percentage loss of conductivity. The table shows the least square means for the Control and TFE, and the random genus effects (see analysis section in Methods for details). The first row of each trait gives the mean and second row gives one standard error for the fixed effects and the 95% confidence interval for genus-level random effects. Traits for which plot had a significant effect, and species for which the random effects were different from zero, are marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) and ns (non-significant).

Plot-level Coefficients			**Genus-level Coefficients**				
Variable	**Large**	**Small**	**Eschweilera**	**Inga**	**Licania**	**Protium**	**Swartzia**
P_{50}	-2.66 (-3.24/-2.08)	-4.06 (-5.20/-2.93) ***	-2.654 (-3.382/-1.926)***	-0.824 (-1.798/0.150) ns	-1.526 (-2.540/-0.513) **	0.014 (-0.985/1.013) ns	-0.593 (-1.592/0.406) ns
P_{88}	-4.83 (-5.87/-3.80)	-7.08 (-9.18/-5.02))***	-4.83 (-6.16/ -3.49) ***	-1.35 (-3.13/0.44) ns	-2.53 (-4.39/ -0.67) **	0.21 (-1.62 /2.04) ns	-1.02 (-2.85 /0.81) ns
G_{min}	0.08 (0.06/0.09)	0.02 (0.007/0.06) ***	0.07482 (0.053/0.09) ***	-0.01930 (-0.049/0.010)ns	-0.02935 (-0.05941/0.00071)*	-0.03583 (-0.066/-0.005) *	0.01851 (-0.011/0.048) ns
K_s	4.60 (2.20/5.58)	2.10 (1.09/4.33))***	3.88 (2.59/5.16) ***	1.98 (0.28/3.68) *	-2.81 (-4.69/ -0.94) **	-0.90 (-2.64/0.83) ns	-0.30 (-2.11/1.52) ns
K_{sl}	6.13 (5.06/7.19)	5.00 (3.09/6.89) *	4.35 (3.42/5.28) ***	0.90 (-0.41/2.21)	1.55 (0.24/2.86) **	1.56 (0.29/2.83) **	2.98 (1.69/4.28)***
W_D	0.70 (0.61/0.70)	0.60 (0.52/0.67) ***	0.63 (0.59/0.67) ***	0.01 (-0.04/0.06) ns	0.05 (0.00/0.11)*	-0.05 (-0.11/0.00)*	0.05 (-0.01/0.10) ns
Ψ_{pd}	-0.44 (-0.50/-0.38)	-0.44 (-0.61/-0.28)ns	-0.48 (-0.59/-0.36) ***	0.04 (-0.12/0.20) ns	0.09 (-0.07/0.25) ns	-0.05 (-0.21/0.11) ns	0.08 (-0.08/0.24) ns
Ψ_{md}	-1.75 (-2.04/-1.46)	-1.50 (-1.98/-1.02)**	-1.85 (-2.07/-1.63) ***	0.17 (-0.12 to 0.47) ns	0.54 (0.24/0.84) ***	0.41 (0.11/0.71) ***	-0.23 (-0.54/0.08) ns
HSM	0.90	2.70	0.92	0.97	1.96	0.21	0.27

	(1.09/2.32)	(1.32/3.91)***	(0.10/1.74) **	(-0.13/2.08) ns	(0.82/3.11) ***	(-0.92/1.34) ns	(-0.85/1.40) ns
PLC	19.50 (8.91/30.75)	42.03 (23.13/60.94)***	19.19 (8.87/29.50) ***	-4.71 (-18.36/8.93) ns	12.90(-2.14/27.94) ns	20.93 (7.02/34.84) **	16.15 (1.56/ 30.74) *

Figures

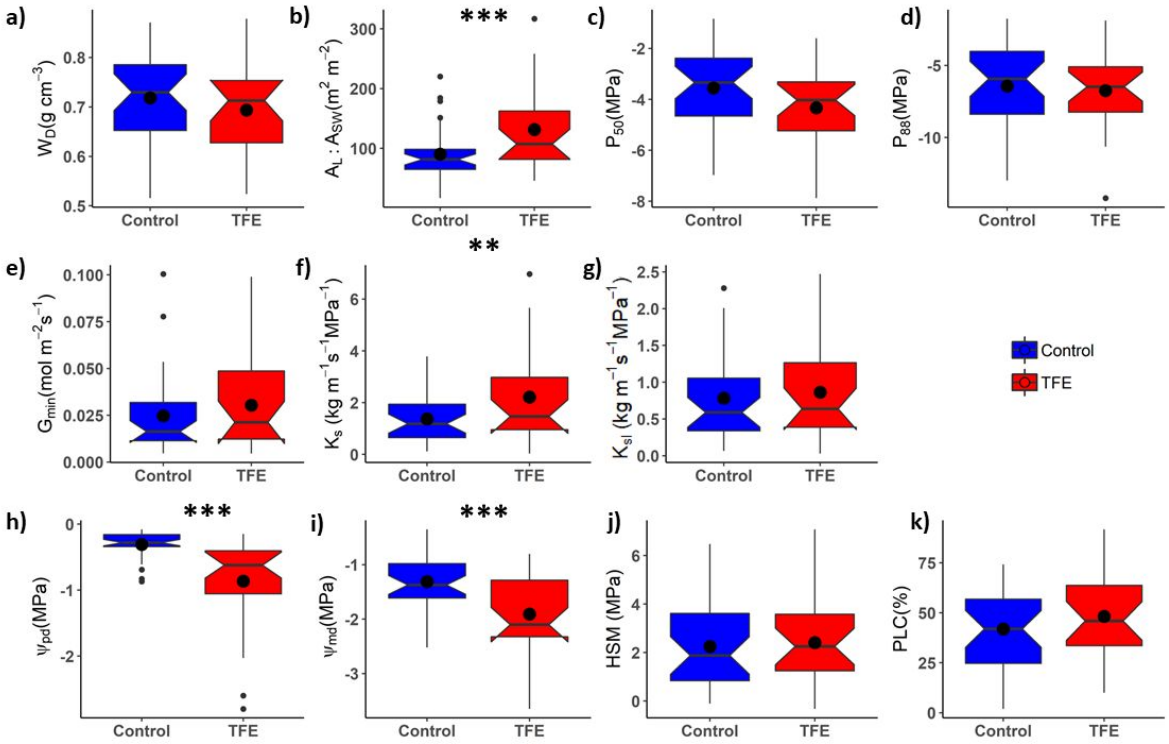
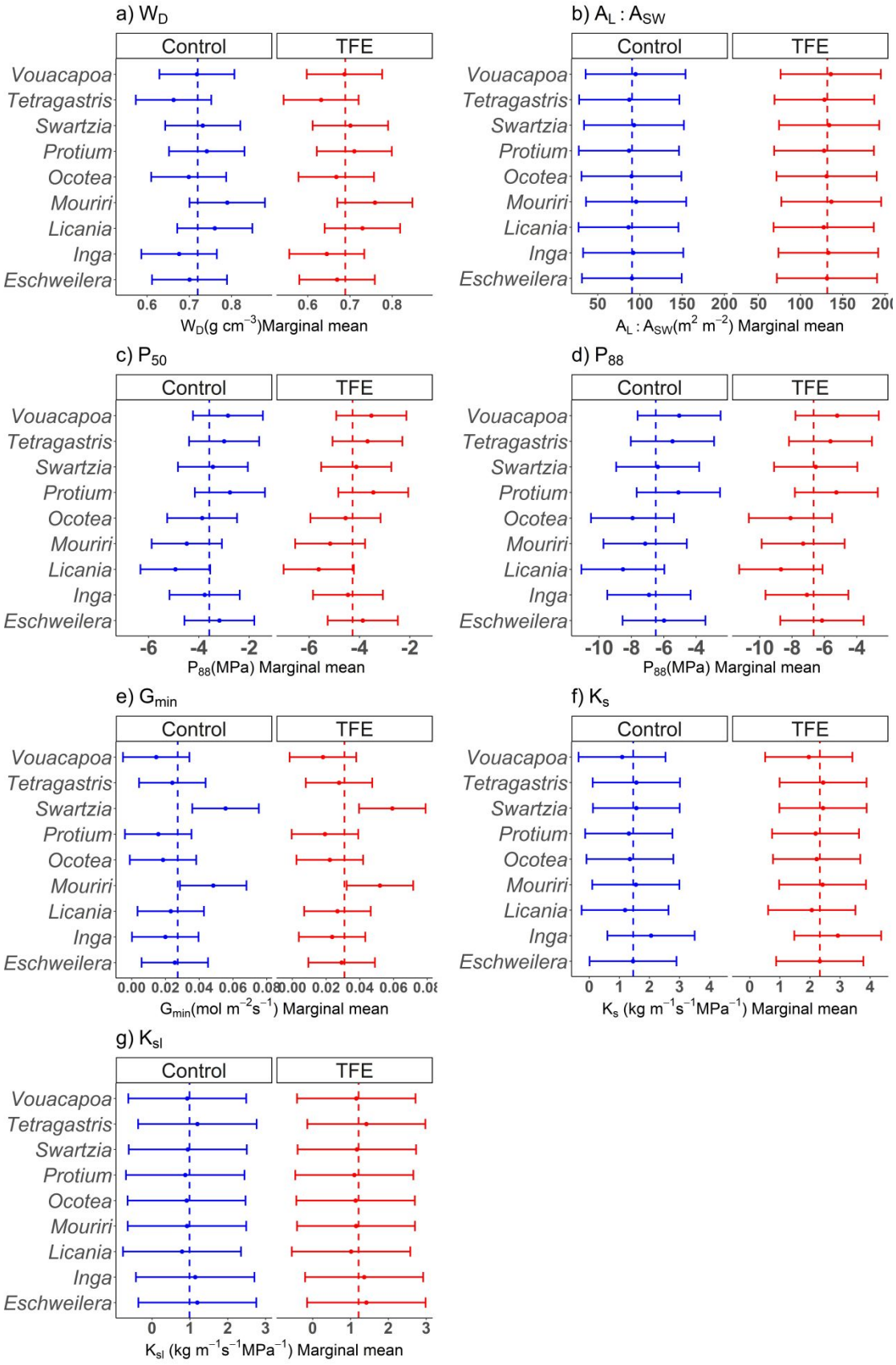


Figure 1 Stress indicators and hydraulic traits for small trees (1-10 cm DBH) measured in dry season oct/2017 on the Control plot (blue) and through-fall exclusion (TFE, red). a) W_D – wood density b) A_L:A_{SW} - leaf to sapwood area ratio c) P_{50} - xylem embolism resistance; d) P_{88} - xylem embolism resistance; e) G_{min} – minimum stomatal conductance; f) K_s – maximum hydraulic specific conductivity; g) K_{sl} - maximum hydraulic leaf -specific conductivity; h) Ψ_{pd} - predawn water potential; i) Ψ_{md} - midday water potential. j) HSM – branch hydraulic safety margin to P_{50}; l) PLC – native dry season percentage loss of conductivity. The boxes represent quartiles 1 and 3, the central line indicates the median and the black points the mean of each treatment. Whiskers are either maximum value or 1.5 interquartile range above quartile 3, if outliers are present and notches represents a confidence interval around the median represented by central line. Traits for which plot had a significant effect are marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). P-values are from mixed effects analysis (see Table 2 for models and analysis section in Methods



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1040 **Figure 2** Hydraulic traits by genus for small trees (1-10 cm DBH) surviving after 15 years of
1041 throughfall exclusion (TFE – red) and the Control plot (blue). a) W_D - wood density b) A_L:A_{SW} - leaf
1042 to sapwood area ratio c) P_{50} - xylem embolism resistance; d) P_{88} - xylem embolism resistance; e)

G_{min} - minimum stomatal conductance; f) K_s - maximum hydraulic specific conductivity; g) K_{sl} - maximum hydraulic leaf -specific conductivity. The vertical dashed coloured lines represent the marginal fixed effects for plot. The points represent random effects plus fixed effect mean by genus and the horizontal lines represents standard error for each genus (see Table 2 for models and analysis section in Methods).

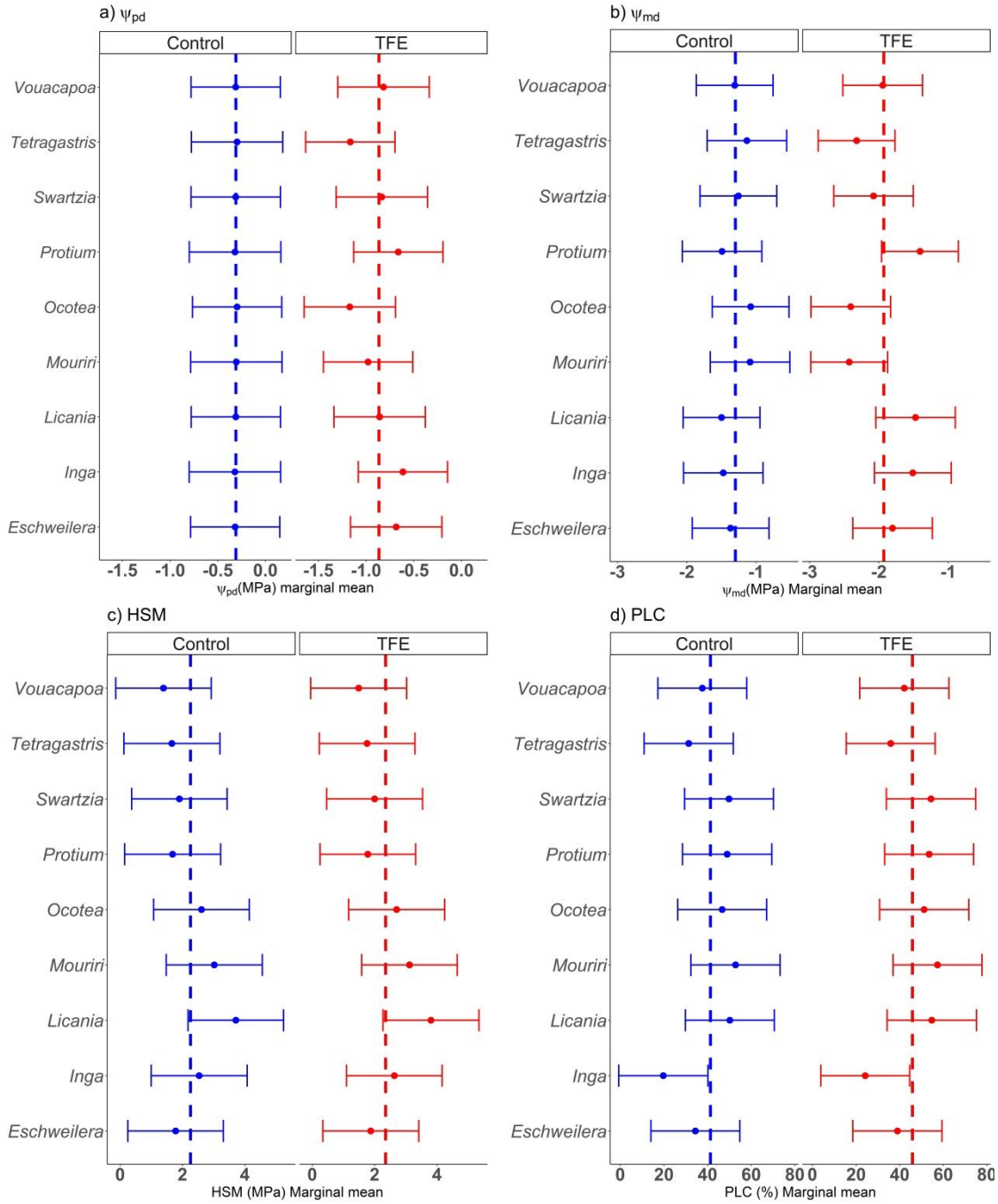

Figure 3 Drought stress indicators considered by genus for small trees (1-10 cm DBH) surviving after 15 years of throughfall exclusion (TFE – red) and the Control plot (blue). a) Ψ_{pd} - predawn water potential; b) Ψ_{md} - midday water potential. c) HSM – branch hydraulic safety margin to P_{50}; d) PLC – native dry season percentage loss of conductivity. The vertical dashed lines represents marginal fixed effects for plot, the points represents random effects plus fixed effect mean by genus and the horizontal lines represents standard error by genus (see Table 2 for models and analysis section in Methods).

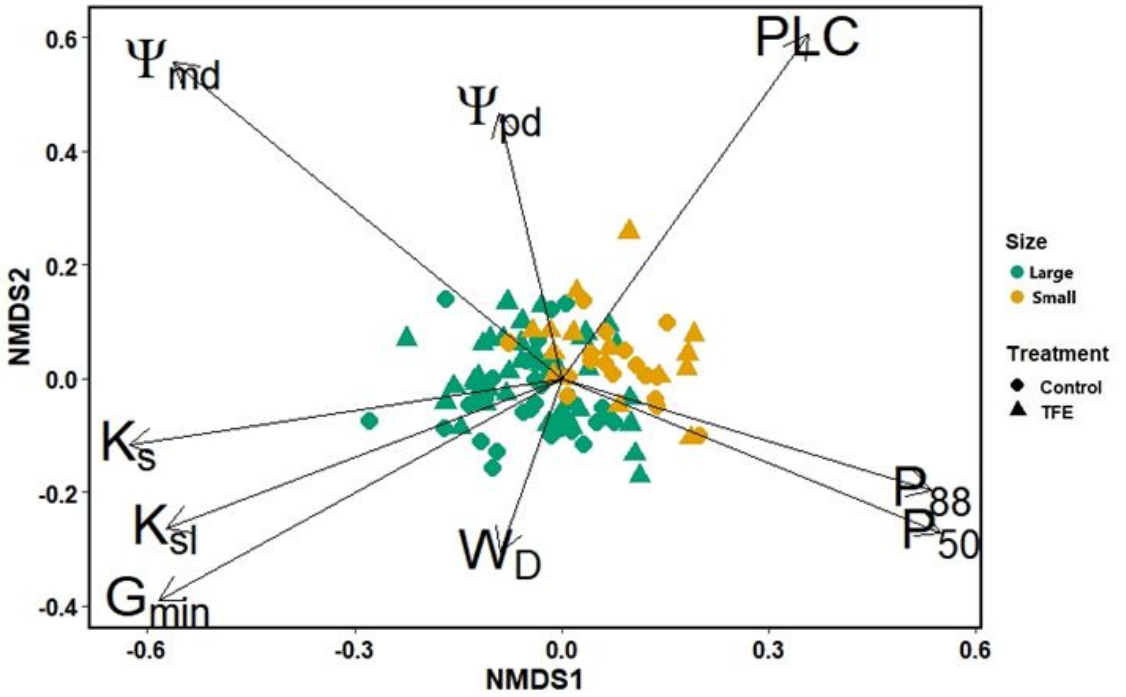

Figure 4 Non-metric multidimensional scaling (NMDS) of drought stress indicators and hydraulic traits. Ordination showing multidimensional space filled by small (yellow) and large (green) trees indicating distinct hydraulic ecological strategies (MANOVA; $P < 0.05$) between trees from the TFE and Control. Hydraulic traits represented by arrows (Arrow length represent predictor "strength"). Dots represent individuals in Control and triangles individuals in TFE treatment. The green colour represents large trees and yellow represents small trees.

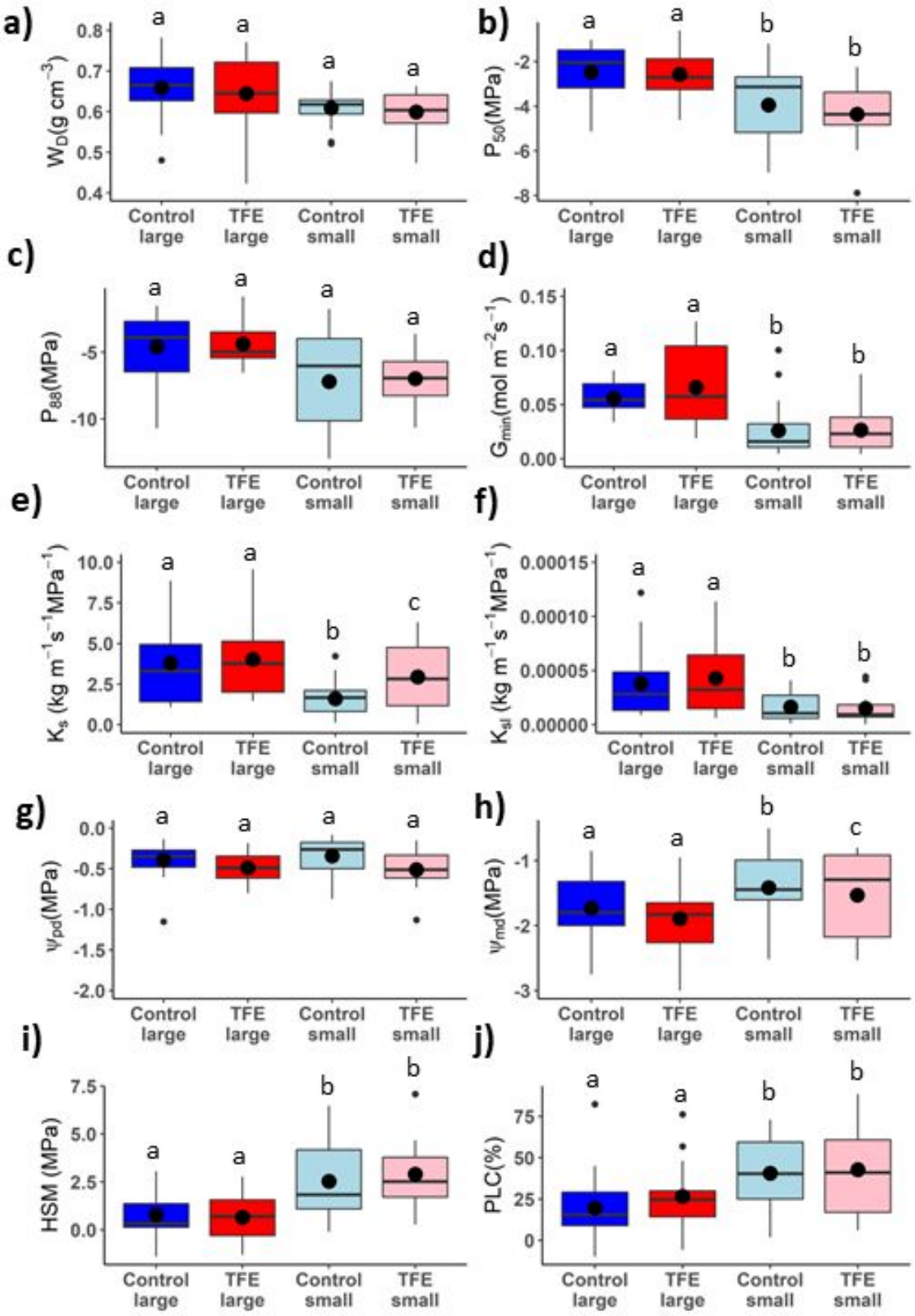

Figure 5 Comparison between small trees and large trees from the throughfall exclusion (TFE) and Control plots. a) W_D – wood density; b) P_{50} - xylem embolism resistance; c) P_{88} - xylem embolism resistance; d) G_{min} – minimum stomatal conductance; e) K_s – maximum hydraulic specific conductivity; f) K_{sl} - maximum hydraulic leaf -specific conductivity; g) Ψ_{pd} - predawn water potential; h) Ψ_{md} midday water potential; i) HSM – branch hydraulic safety margin to P_{50}; j) PLC –

native dry season percentage loss of conductivity. The boxes represent quartiles 1 and 3, the central line indicates the median and the black points the mean of each treatment. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present. Different letters indicate significant differences within each graph, $p<0.05$.

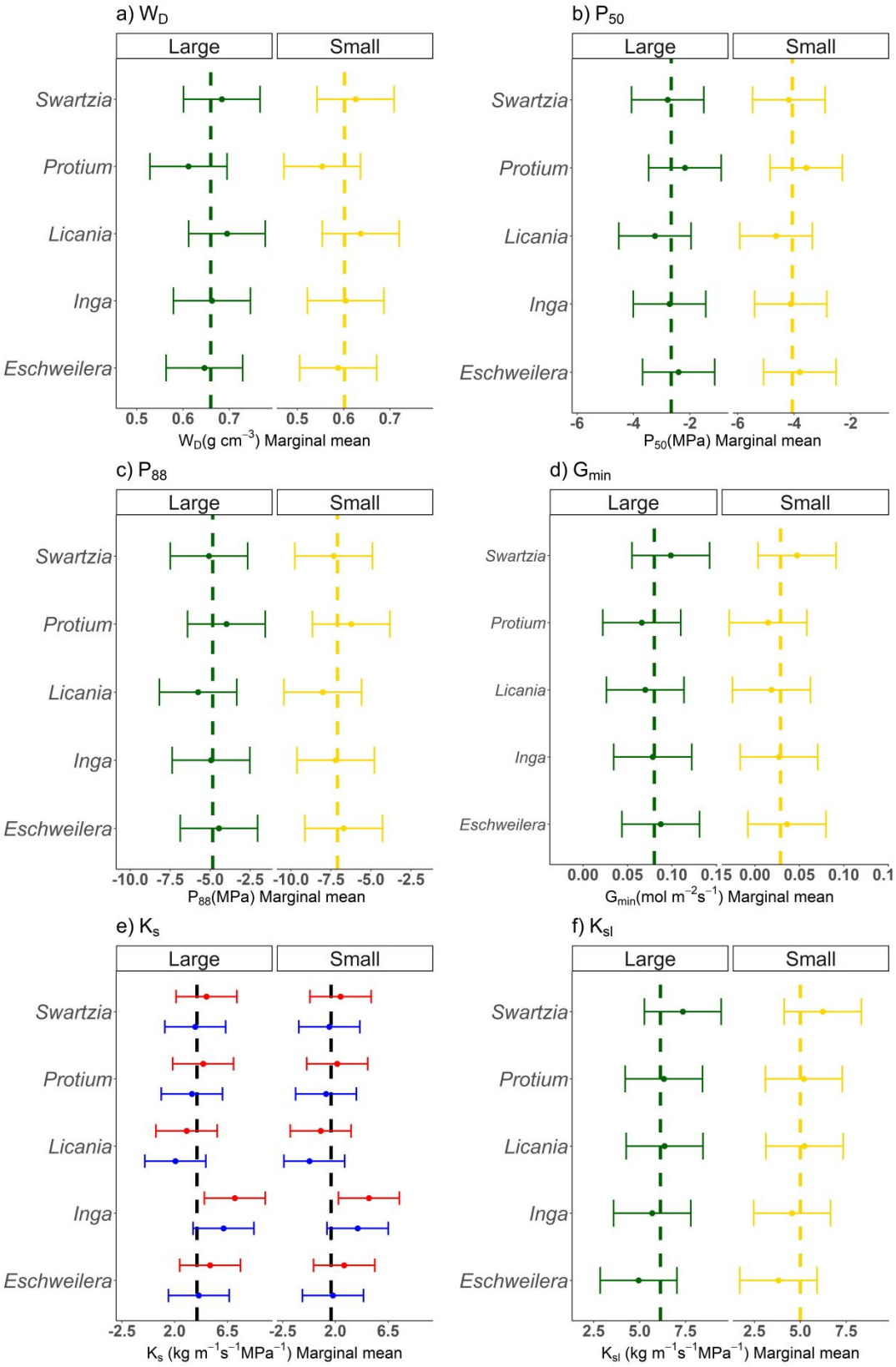

Figure 6 Hydraulic traits comparison between small trees and large trees from throughfall exclusion (TFE) and Control plot. a) W_D – wood density; b) P_{50} - xylem embolism resistance; c) P_{88} - xylem embolism resistance; d) G_{min} – minimum stomatal conductance; e) K_s – maximum hydraulic specific conductivity; f) K_{sl} - maximum hydraulic leaf-specific conductivity. The vertical dashed lines represents marginal fixed effect mean, green vertical lines represents large trees and yellow vertical lines, the points represents random plus fixed effect mean by each level (by genus) and the horizontal lines represents standard error by each random effects level. The blue and red in horizontal lines represents Control and TFE plot, respectively and are show when a significant plot effect was found. All points and lines represent genus in each treatment (see Table 3 for models and analysis section in Methods).

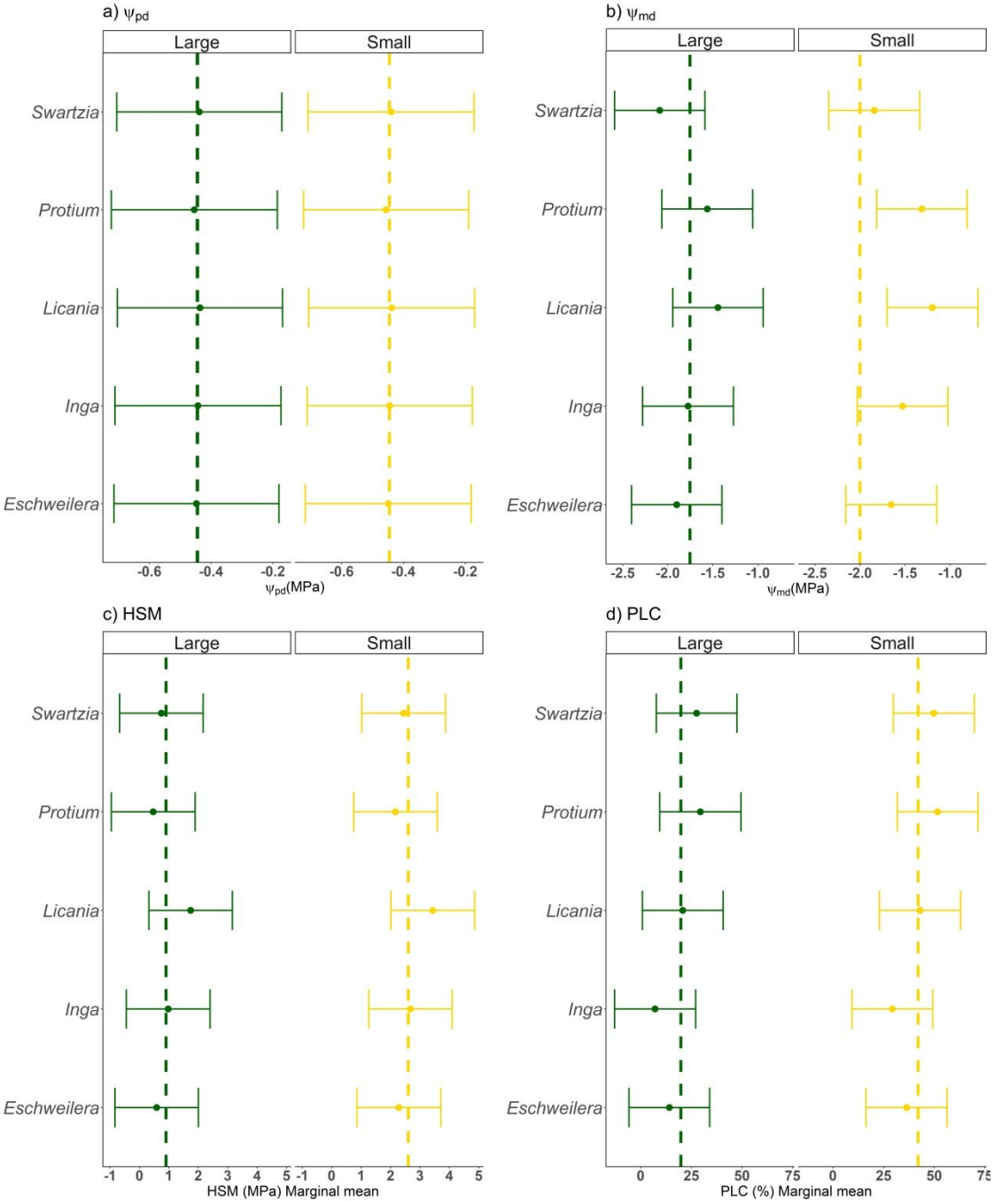

Figure 7 Drought stress indicators comparison between small trees and large trees from throughfall exclusion (TFE) and Control plot. a) Ψpd - predawn water potential; b) Ψmd - midday

water potential. c) HSM – branch hydraulic safety margin to P50; d) PLC – native dry season percentage loss of conductivity. The vertical dashed lines represents marginal fixed effect mean, the points represents random effects plus fixed effect mean by each level (by genus) and the horizontal lines represents standard error by each random effects level. All points and lines represent genus in each treatment. P-values are from mixed effects analysis (see Table 3 for models and analysis section in Methods).

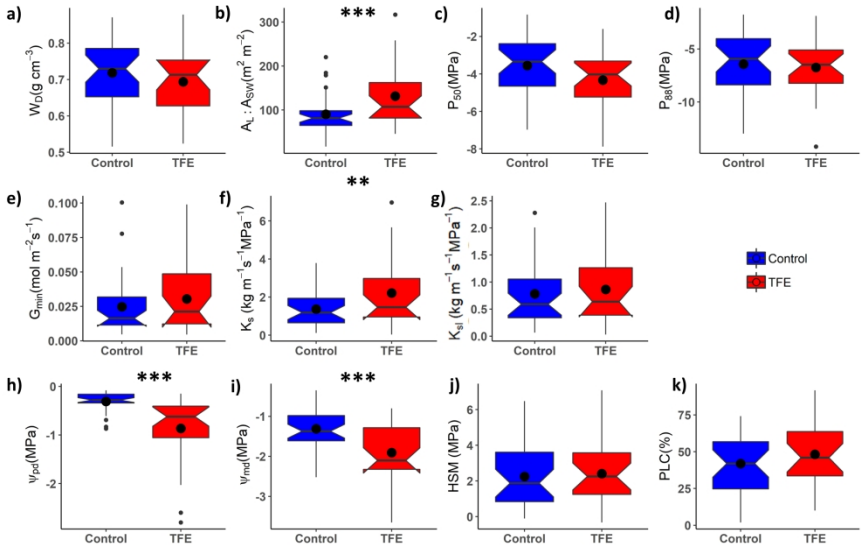

Figure 1 Stress indicators and hydraulic traits for small trees (1-10 cm DBH) measured in dry season oct/2017 on the Control plot (blue) and through-fall exclusion (TFE, red). a) WD – wood density b) AL:ASW - leaf to sapwood area ratio c) P50 - xylem embolism resistance; d) P88 - xylem embolism resistance; e) Gmin – minimum stomatal conductance; f) Ks – maximum hydraulic specific conductivity; g) Ksl - maximum hydraulic leaf -specific conductivity; h) Ψpd - predawn water potential; i) Ψmd - midday water potential. j) HSM – branch hydraulic safety margin to P50; l) PLC – native dry season percentage loss of conductivity. The boxes represent quartiles 1 and 3, the central line indicates the median and the black points the mean of each treatment. Whiskers are either maximum value or 1.5 interquartile range above quartile 3, if outliers are present and notches represents a confidence interval around the median represented by central line. Traits for which plot had a significant effect are marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). P-values are from mixed effects analysis (see Table 2 for models and analysis section in Methods.

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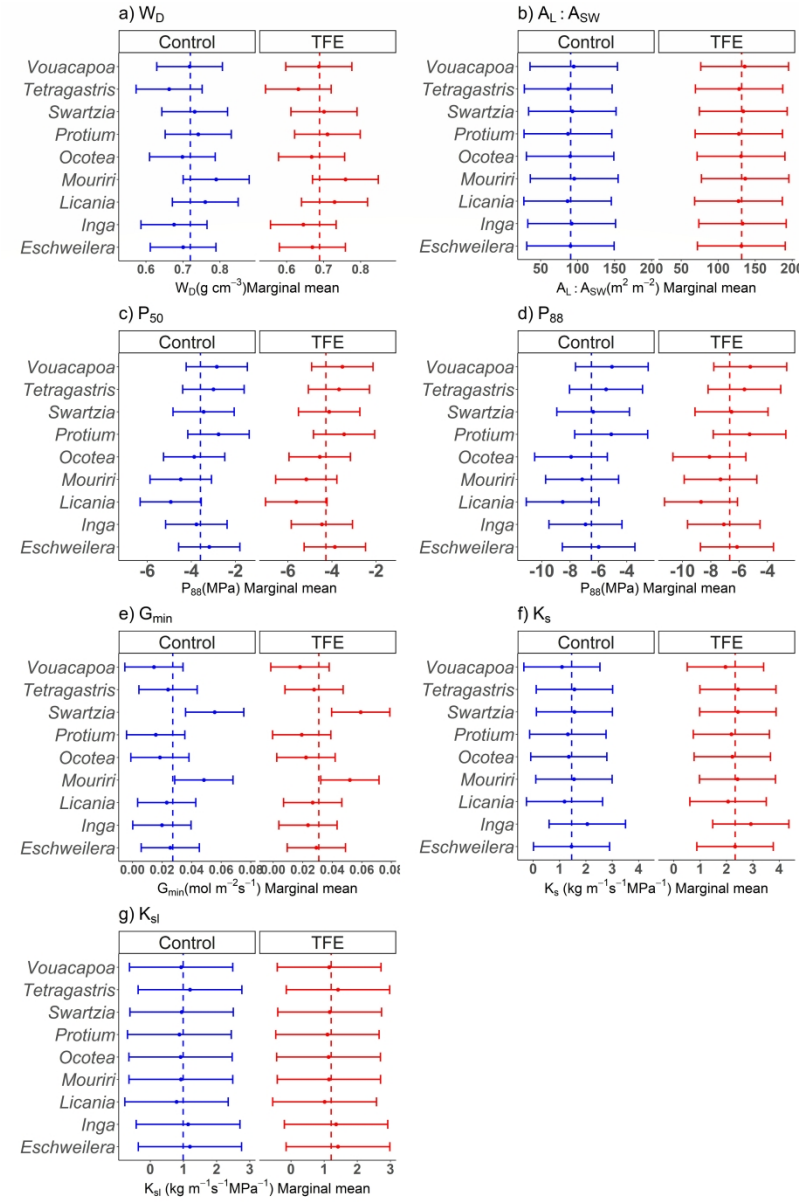

Figure 2 Hydraulic traits by genus for small trees (1-10 cm DBH) surviving after 15 years of throughfall exclusion (TFE – red) and the Control plot (blue). a) WD - wood density b) AL:ASW - leaf to sapwood area ratio c) P50 - xylem embolism resistance; d) P88 - xylem embolism resistance; e) Gmin - minimum stomatal conductance; f) Ks - maximum hydraulic specific conductivity; g) Ksl - maximum hydraulic leaf -specific conductivity. The vertical dashed coloured lines represent the marginal fixed effects for plot. The points represent random effects plus fixed effect mean by genus and the horizontal lines represents standard error for each genus (see Table 2 for models and analysis section in Methods).

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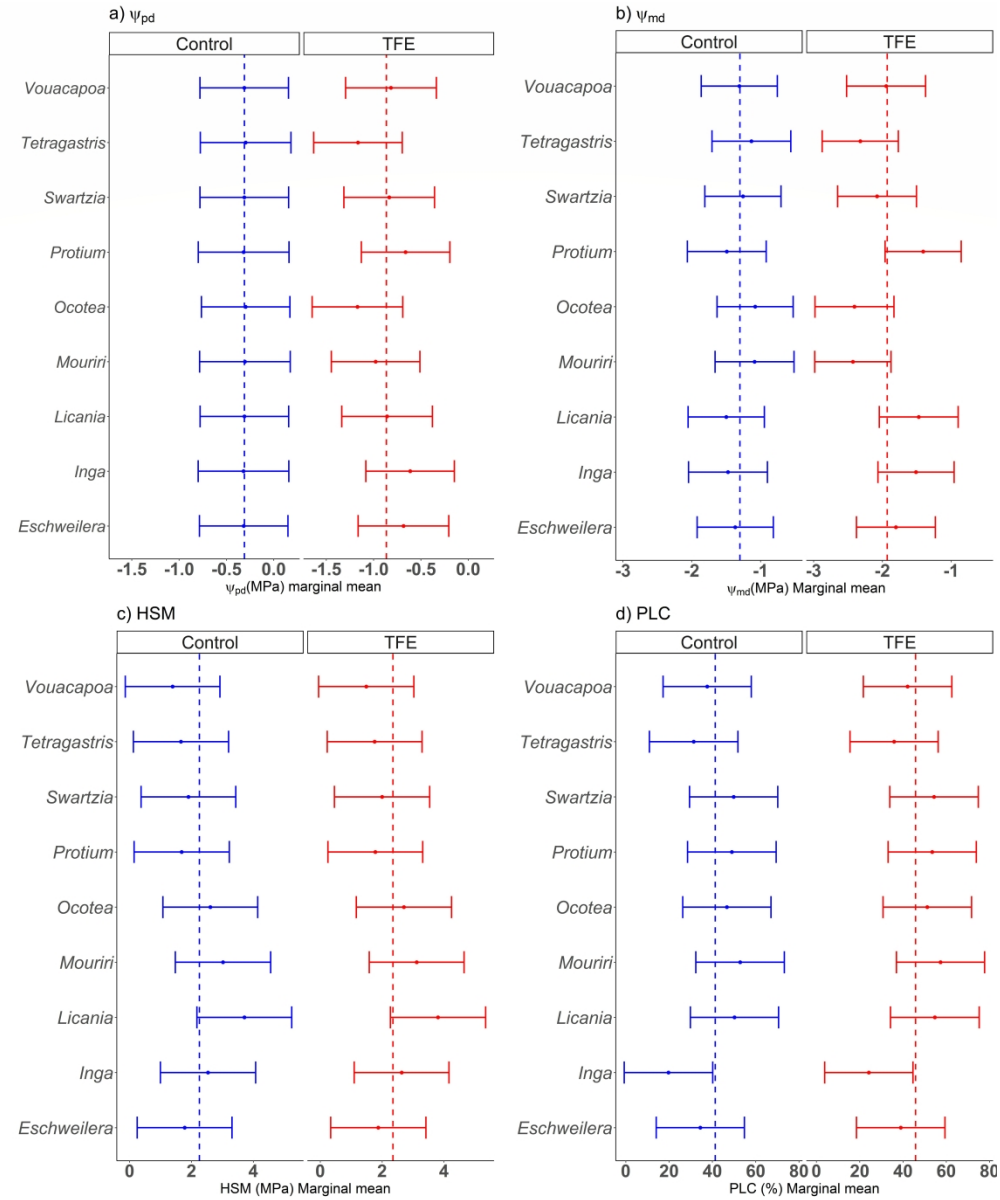

Figure 3 Drought stress indicators considered by genus for small trees (1-10 cm DBH) surviving after 15 years of throughfall exclusion (TFE – red) and the Control plot (blue). a) Ψpd - predawn water potential; b) Ψmd - midday water potential. c) HSM – branch hydraulic safety margin to P50; d) PLC – native dry season percentage loss of conductivity. The vertical dashed lines represents marginal fixed effects for plot, the points represents random effects plus fixed effect mean by genus and the horizontal lines represents standard error by genus (see Table 2 for models and analysis section in Methods).

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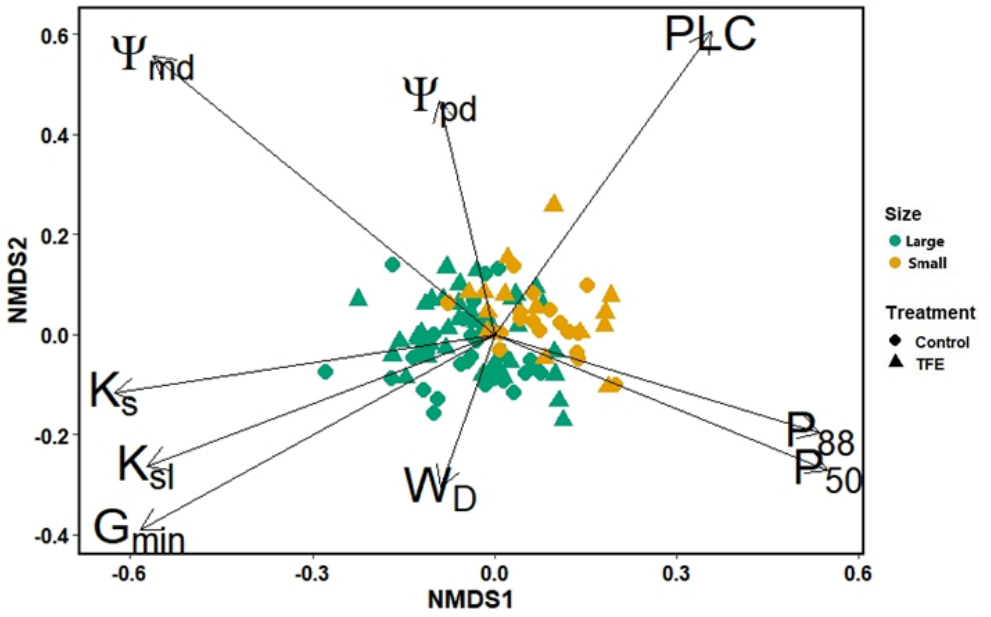

Figure 4 Non-metric multidimensional scaling (NMDS) of drought stress indicators and hydraulic traits. Ordination showing multidimensional space filled by small (yellow) and large (green) trees indicating distinct hydraulic ecological strategies (MANOVA; $P < 0.05$) between trees from the TFE and Control. Hydraulic traits represented by arrows (Arrow length represent predictor "strength"). Dots represent individuals in Control and triangles individuals in TFE treatment. The green colour represents large trees and yellow represents small trees.

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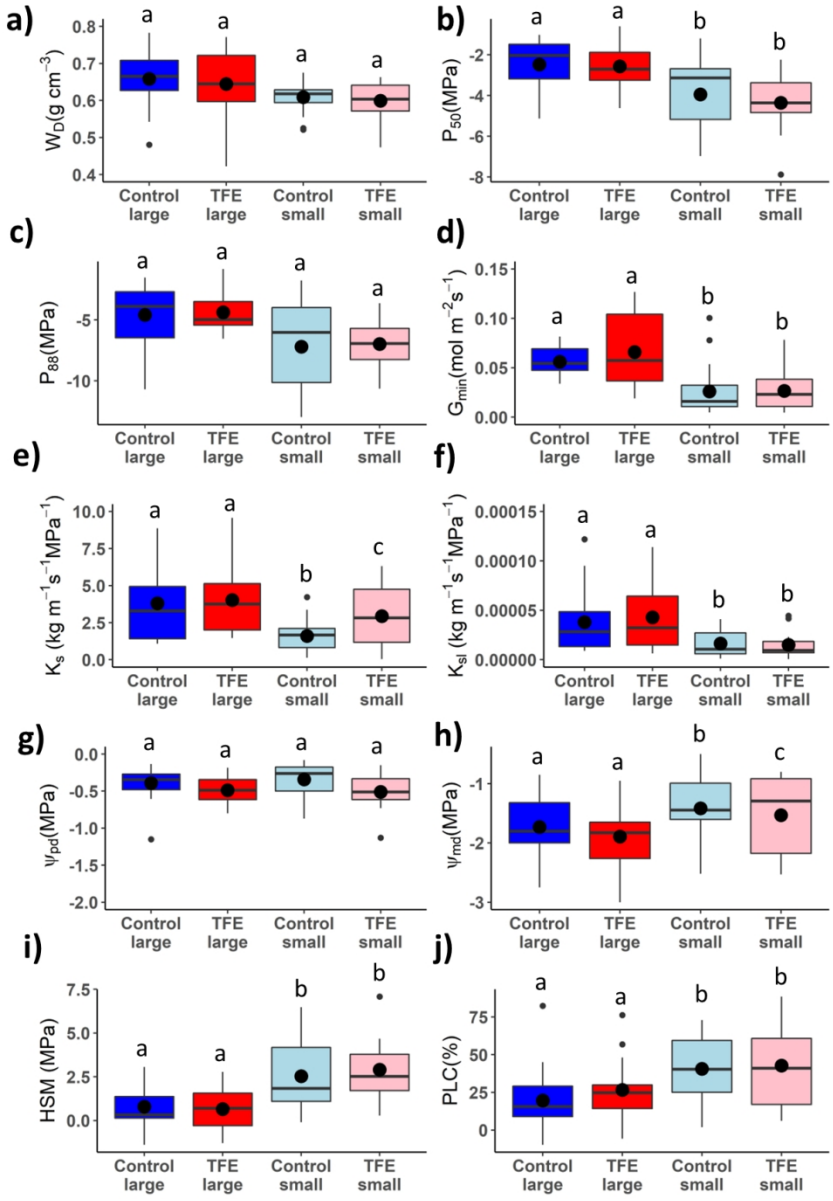

Figure 5 Comparison between small trees and large trees from the throughfall exclusion (TFE) and Control plots. a) WD – wood density; b) P50 - xylem embolism resistance; c) P88 - xylem embolism resistance; d) Gmin – minimum stomatal conductance; e) Ks – maximum hydraulic specific conductivity; f) Ksl - maximum hydraulic leaf -specific conductivity; g) Ψpd - predawn water potential; h) Ψmd midday water potential; i) HSM – branch hydraulic safety margin to P50; j) PLC – native dry season percentage loss of conductivity. The boxes represent quartiles 1 and 3, the central line indicates the median and the black points the mean of each treatment. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present. Different letters indicate significant differences within each graph, $p<0.05$.

321x456mm (96 x 96 DPI)

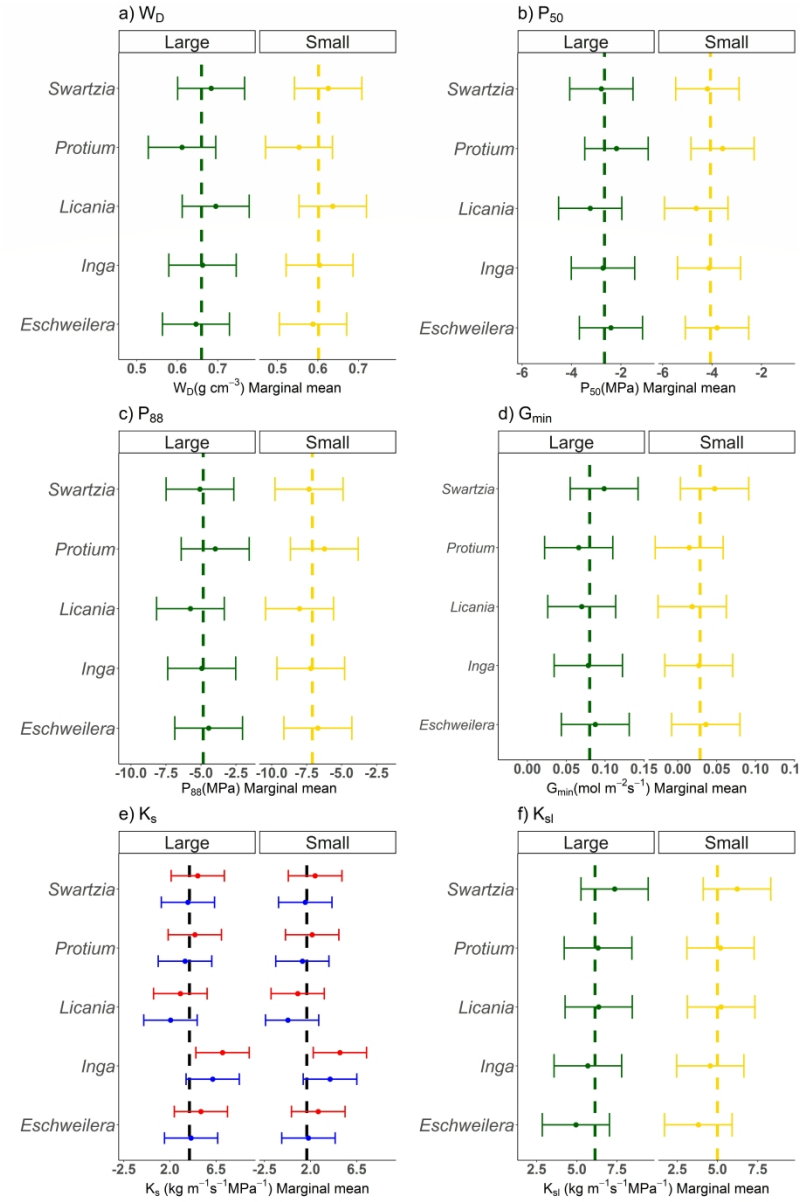

Figure 6 Hydraulic traits comparison between small trees and large trees from throughfall exclusion (TFE) and Control plot. a) WD – wood density; b) P50 - xylem embolism resistance; c) P88 - xylem embolism resistance; d) Gmin – minimum stomatal conductance; e) Ks – maximum hydraulic specific conductivity; f) Ksl - maximum hydraulic leaf-specific conductivity. The vertical dashed lines represents marginal fixed effect mean, green vertical lines represents large trees and yellow vertical lines, the points represents random plus fixed effect mean by each level (by genus) and the horizontal lines represents standard error by each random effects level. The blue and red in horizontal lines represents Control and TFE plot, respectively and are show when a significant plot effect was found. All points and lines represent genus in each treatment (see Table 3 for models and analysis section in Methods).

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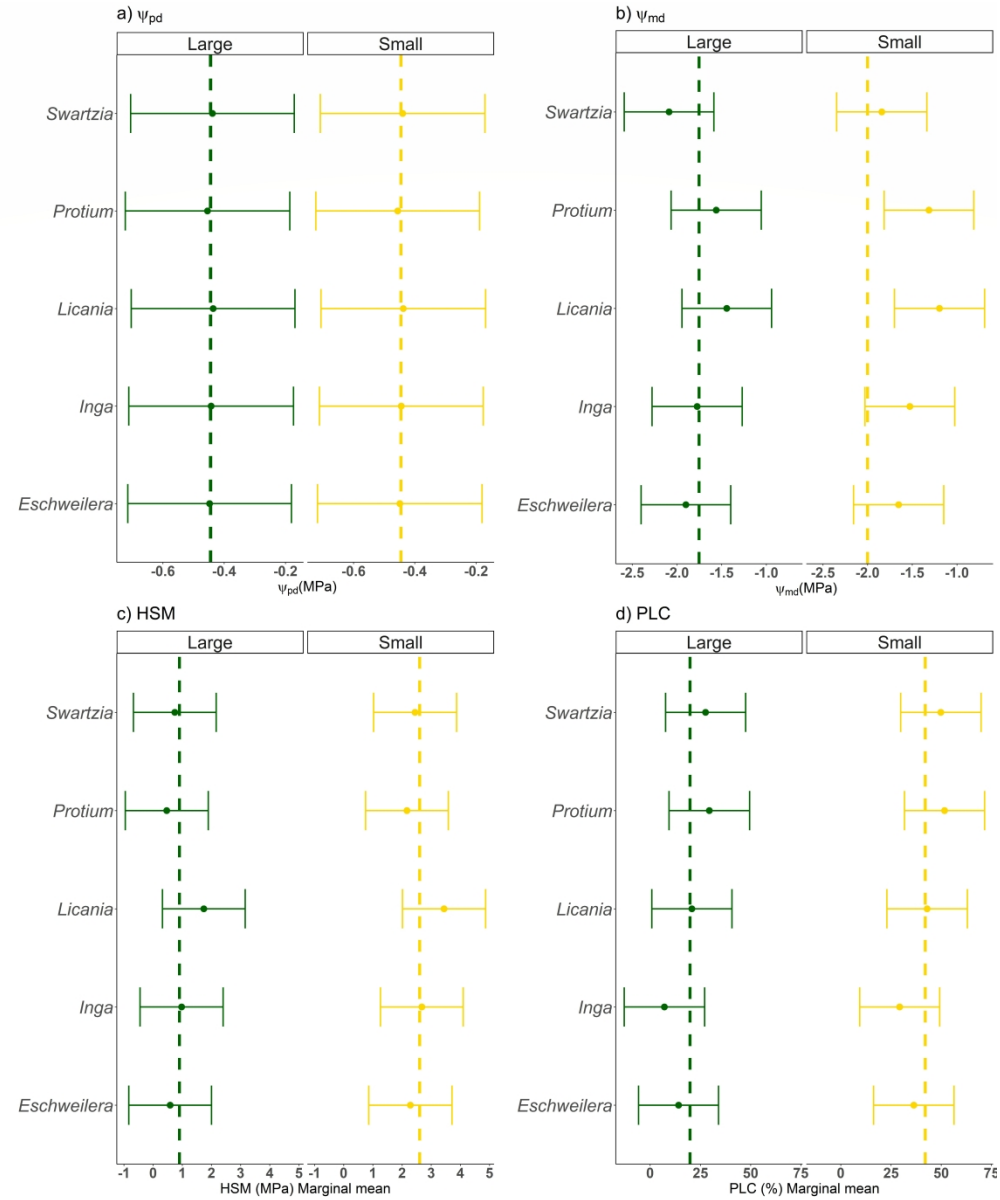

Figure 7 Drought stress indicators comparison between small trees and large trees from throughfall exclusion (TFE) and Control plot. a) Ψpd - predawn water potential; b) Ψmd - midday water potential. c) HSM – branch hydraulic safety margin to P50; d) PLC – native dry season percentage loss of conductivity. The vertical dashed lines represents marginal fixed effect mean, the points represents random effects plus fixed effect mean by each level (by genus) and the horizontal lines represents standard error by each random effects level. All points and lines represent genus in each treatment. P-values are from mixed effects analysis (see Table 3 for models and analysis section in Methods).

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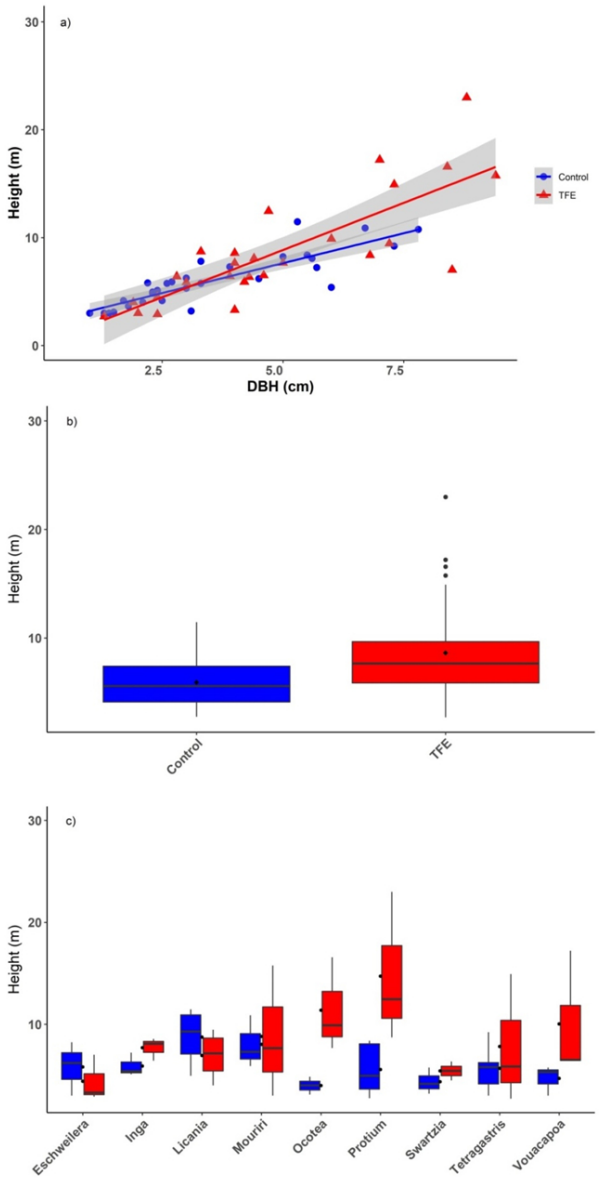

Figure S1. Height and diameter in each treatment (TFE vs. Control) and by genus for the most common small tree genera in this study (9 genera). a)The significant relationship between diameter (DBH) and Height by treatment ($F=48.61$; $R^2=0.72$; p-value<0.001), b) Height of Small trees by treatment c) Height of Small trees by genus. The box represents quartiles 1 and 3, with the central line indicating the median. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present.

228x341mm (96 x 96 DPI)

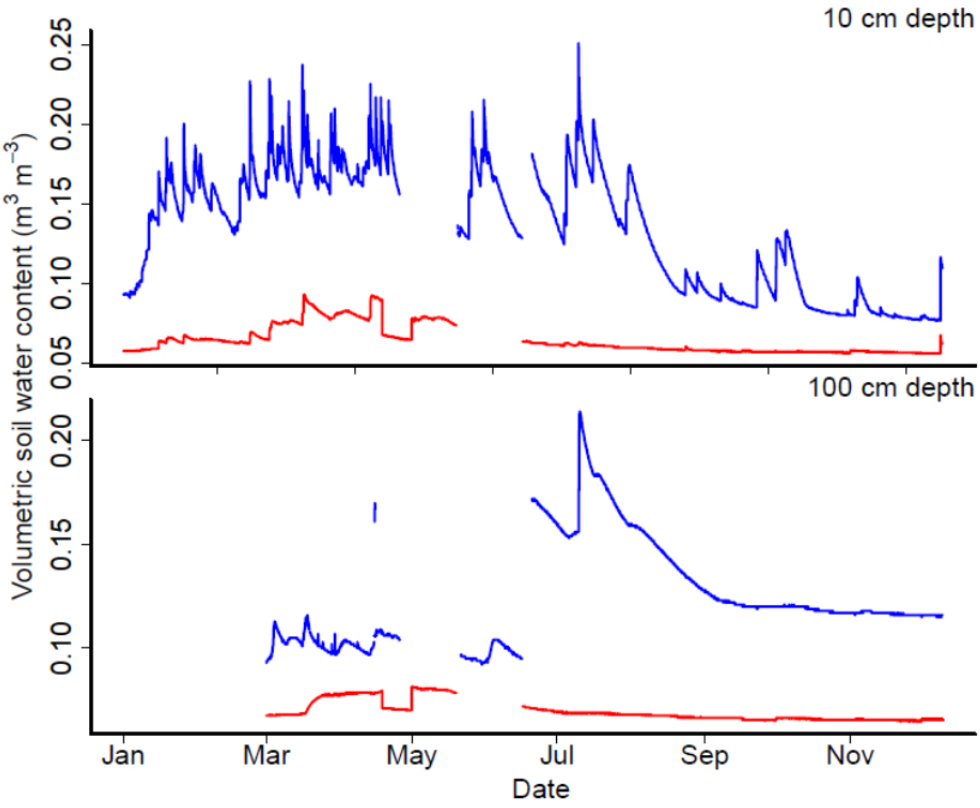

Figure S2. Soil water content during 2016 in the Throughfall Exclusion Experiment plot (red) and in the Control plot (blue) at 10 cm and at 100 cm depth adapted from Bittencourt et al. 2020. The TFE had a mean reduction, in relation to Control, of 48% and 56% in soil water content at 10 cm and 100 cm depth, respectively. Data are missing for periods when sensors failed.

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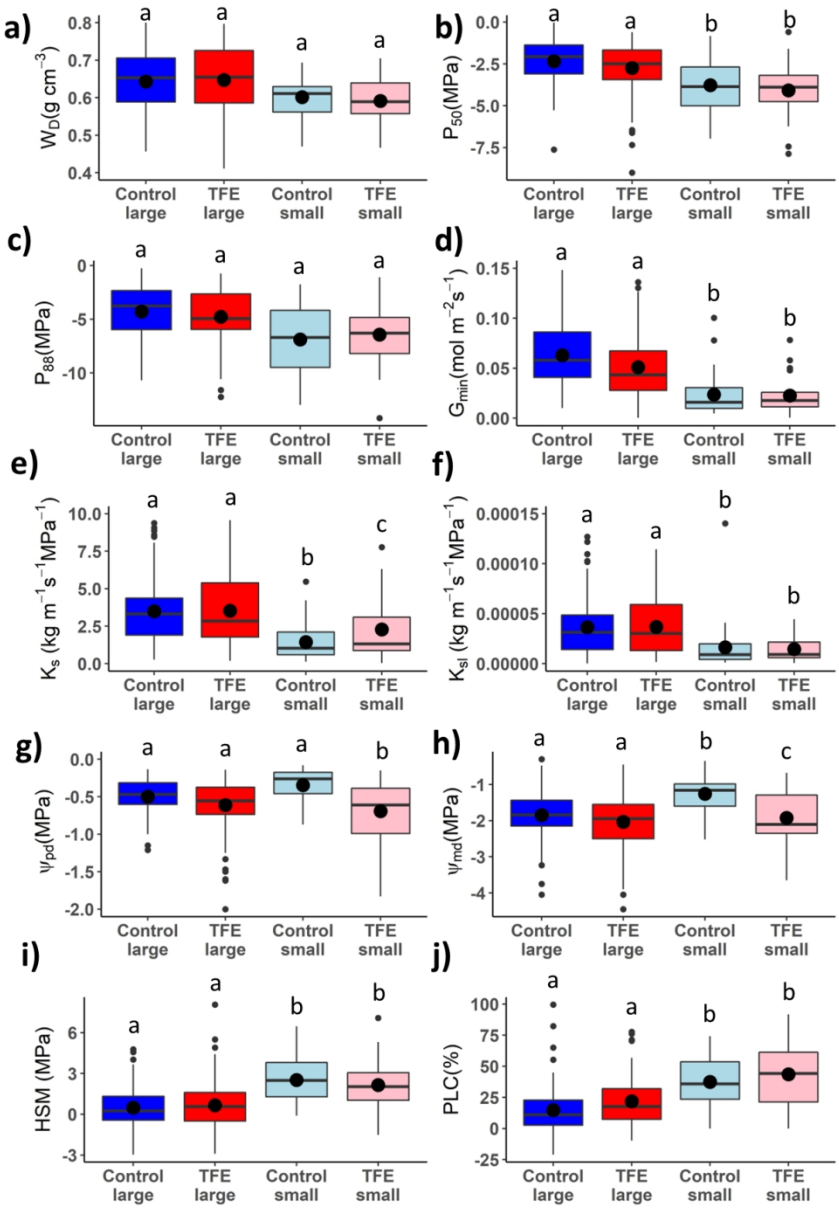

Figure S3- Comparison between the small trees and large trees from the throughfall exclusion (TFE) and Control plots from grouping all 9 genera available within the large and small tree groupings. Note that four of these genera were not present in both of the large and small tree groupings, and were thus excluded from Figure 3. a) WD – wood density; b) P50 - xylem embolism resistance (MPa); c) P88 - xylem embolism resistance; d) Gmin – minimum stomatal conductance; e) Ks – maximum hydraulic specific conductivity; f) Ksl - maximum hydraulic leaf -specific conductivity; g) Ψpd - predawn water potential; h) Ψmd midday water potential; i) PLC – native dry season percentage loss of conductivity; j) HSM – hydraulic safety margin to P50. The box represents quartiles 1 and 3, with the central line indicating the median. Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when outliers are present. Different letter indicant significant differences, $p<0.001$.

325x456mm (96 x 96 DPI)