Amazonian trees have limited capacity to acclimate plant hydraulic properties in response to long-term drought

Running title: Hydraulic drought acclimation of Amazonian trees

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Abstract

The fate of tropical forests under future climate change is dependent on the capacity of their trees to adjust to drier conditions. The capacity of trees to withstand drought is likely to be determined by traits associated with their hydraulic systems. However, data on whether tropical trees can adjust hydraulic traits when experiencing drought remain rare. We measured plant hydraulic traits (e.g. hydraulic conductivity and embolism resistance) and plant hydraulic system status (e.g. leaf water potential, native embolism and safety margin) on >150 trees from 12 genera (36 species) and spanning a stem size range from 14 to 68 cm diameter at breast height (DBH) at the world's only long-running tropical forest drought experiment. Hydraulic traits showed no adjustment following 15 years of experimentally imposed moisture deficit. This failure to adjust resulted in these drought-stressed trees experiencing significantly lower leaf water potentials, and higher, but variable, levels of native embolism in the branches. This result suggests that hydraulic damage caused by elevated levels of embolism is likely to be one of the key drivers of drought-induced mortality following long-term soil moisture deficit. We demonstrate that some hydraulic traits changed with tree size, however, the direction and magnitude of the change was controlled by taxonomic identity. Our results suggest that Amazonian trees, both small and large, have limited capacity to acclimate their hydraulic systems to future droughts, potentially making them more at risk of drought-induced mortality.

Key-words: hydraulic traits; throughfall exclusion; Amazon rainforest; drought; embolism resistance; tropical forest, plant functional diversity; tree size.

Introduction

The responses of forested ecosystems to global changes in climate will partly depend on the capacity of forest trees to acclimate to new environmental conditions (Corlett, 2016; Galbraith *et al.*, 2010; Smith & Dukes, 2013; Sterck *et al.*, 2016). The Amazon forest is predicted to become warmer and drier over the coming decades (Duffy *et al.*, 2015; Lopes *et al.* 2016; Marengo *et al.*, 2018). This is likely to influence species composition, forest cover and the strength of the carbon sink (Malhi *et al.*, 2009; Phillips *et al.*, 2010), unless trees can fully or partially acclimate to survive and maintain function in the new conditions they face (Sterck *et al.*, 2016). Currently however, there is limited knowledge about the plasticity of traits in Amazonian trees and therefore their capacity to acclimate functionally to new environmental conditions, particularly drought. The few drought experiments constructed to date in tropical rainforests show some tree traits are plastic and respond to drought (Binks *et al.*, 2016; Schuldt *et al.*, 2011; Tng *et al.*, 2018). However, to our knowledge no studies exist which test the potential of tropical trees to adjust their hydraulic system to long-term drought, including traits indicating both hydraulic safety and efficiency (Meir *et al.*, 2018). As tree mortality is likely to be linked to the failure of a plant in controlling its hydraulic system status (Choat *et al.*, 2018; McDowell & Allen, 2015; Rowland *et al.*, 2015a), understanding the adjustment capacity of these traits will be vital for predicting future responses of tropical rainforests to changes in climate, particularly given the natural longevity of their constituent trees.

The plant hydraulic system is tightly linked to its water and carbon metabolism (Eller *et al.*, 2018; Christoffersen *et al.*, 2016) and therefore it is likely that plants require adjustments in their hydraulic system to maintain a positive carbon balance in the face of climate change. Several studies have shown plastic responses (variations in phenotype expression in response to environmental change) of leaf physiology and plant architecture to

experimental or natural drought (Ambrose, Sillett, & Dawson, 2009; Dayer *et al.*, 2017; Egea *et al.*, 2012; Prendin, Mayr, Beikircher, von Arx, & Petit, 2018; Yue *et al.*, 2019). Some studies also report plastic responses in hydraulic traits to short-term drought, related to both hydraulic safety and hydraulic efficiency (Awad, Barigah, Badel, Cochard, & Herbette, 2010; Beikircher & Mayr, 2009; Prendin *et al.*, 2018; Tomasella *et al.*, 2018). However, whether this plasticity can positively influence plant function (e.g. water stress status, photosynthesis, growth or reproduction) and can lead to partial or full acclimation in function is often hard to determine. As a working hypothesis, we define acclimation as a functional adjustment (which may be physiological, anatomical, or morphological) to maintain or enhance performance in response to new environmental conditions, similar to Way & Yamori (2014). Research on hydraulic acclimation in tropical trees in response to drought is limited as most drought experiments have been short-term (<2 years), performed on saplings and/or in greenhouses and amongst these very few have included measurements of plant hydraulic traits or have been located in tropical rainforest environments, with the drought treatment imposed at hectare-scale affecting hundreds of trees together. Consequently, the capacity of trees to acclimate to drought, that is to maintain the same performance under drought when compared to non-droughted conditions, remains highly uncertain, particularly in tropical rainforest environments.

To date, only eight throughfall exclusion experiments (TFE experiments) have been implemented in tropical forests with reductions in soil moisture imposed for a year or more (Meir *et al.*, 2015). In a TFE experiment in Sulawesi, after two years of drought, Schuldt *et al.* (2011) found a reduction in tree hydraulic efficiency, which they suggest might have led to a reduction in tree growth. This may suggest the hydraulic systems of tropical trees can respond plastically to drought, but that full acclimation to maintain hydraulic performance and growth did not occur. In another Asian tropical forest, after four months of TFE in

Malaysia (Inoue *et al.*, 2017), leaf turgor loss point had decreased, however, as photosynthesis was reduced, this also implies substantial acclimation did not occur. Observations in a TFE experiment in Australia have also shown plastic responses in leaf and wood anatomical properties linked to hydraulic safety after two years of TFE-imposed drought, yet similarly no evidence was reported to suggest this led to substantial acclimation (Tng *et al.*, 2018). In the world's only long-running tropical TFE (15+ years of TFE), in an Amazonian forest, limited plasticity was found in leaf level anatomical and water relations traits (Binks *et al.*, 2016) and none in embolism resistance, a key trait controlling hydraulic safety (Rowland *et al.*, 2015). Studying drought responses over longer time periods is valuable, particularly given that there is likely to be variation in the responses and types of adjustments occurring from annual to decadal time periods (da Costa *et al.*, 2014; Meir *et al.*, 2018).

Acclimation of a tree's hydraulic system to drought requires adjustments in one or more key traits to maintain a tree's hydraulic status. The hydraulic status can be evaluated using hydraulic status variables, such as leaf water potential, hydraulic safety margins or percentage loss of conductivity in the xylem tissue. These adjustments may confer greater drought tolerance, for example by increasing xylem resistance to embolism or a greater capacity to supply water to the leaves when water becomes available, which can occur, as a consequence by increasing hydraulic conductivity or reducing leaf area to sapwood area ratio's. (Cruiziat *et al.* 2002; Maseda & Fernandez, 2006; Sperry & Love, 2015). Other adjustments, such as root system expansion or higher stomatal control, can increase the capacity to avoid drought by allowing trees to access deeper, wetter soils, or to decouple from the atmosphere (Delzon *et al.* 2015).

Similar processes of acclimation may also be expected as a tree grows. As a tree gets taller it can be exposed to greater drought stress as it is exposed to higher radiation fluxes, a

more desiccating atmosphere and strong winds, alongside having lower leaf water potential as a consequence of a longer hydraulic path from root to leaf (Kumagai *et al.*, 2001). Consequently, it should be advantageous for a tree to adjust its hydraulic traits to become more drought tolerant as it becomes taller. There is evidence of increased hydraulic efficiency with height for tropical trees (Zach *et al.*, 2010), however Rowland *et al.* (2015a) found that resistance to embolism decreases as trees get taller. If correct, these results imply that there are: i) height-related trade-offs, such as changes in hydraulic efficiency, constraining tree hydraulic safety; and/or ii) large trees over-compensate for the drier canopy environment by having larger water storage, capacitance and/or deeper roots and/or better water loss control (Brum *et al.*, 2019), allowing them to down-regulate embolism resistance; or iii) larger trees are undergoing damage to their hydraulic system, lowering their hydraulic safety (e.g. weaker pit membranes, due to repeated damage) (Scholz *et al.*, 2007).

Constraints or trade-offs in hydraulic traits related to tree height or architecture may be greater in larger trees, which are close to their maximum height. This may limit their capabilities to acclimate to drought, potentially explaining why larger trees are more susceptible to drought-induced mortality in tropical forests and other biomes (McDowell & Allen, 2015; Bennett *et al.*, 2015; Rowland *et al.*, 2015a; Olson *et al.*, 2018). Drought experiments in tropical forests have however rarely focussed on tree size effects. The only existing study investigating how drought responses change with tree size (Rowland *et al.*, 2015) found no interaction between tree size, embolism resistance and a drought treatment. However, Rowland *et al.* (2015a) were only able to study 6 genera in forest plots containing 94 genera and they only focused on trees which were fully sunlit at their canopy tops. To test more fully the capacity of tropical forest trees to adjust their hydraulic system to size changes and to drought, further work is required on a larger number of taxa, spanning multiple tree size classes and canopy positions.

In this study we test the capacity of tropical trees from varying canopy positions to adjust their hydraulic systems in response to 15 years of experimental TFE and associated substantial soil moisture deficit. We present measurements of hydraulic traits and hydraulic status variables (i.e. indicators of the status of a plant is water transport system) linked to safety and efficiency on >150 individuals of 12 genera, including: resistance to embolism (xylem water potential causing 50 % and 88% of loss in water transport capacity - P50 and P88), stem and leaf specific conductivity, leaf to xylem area ratios and leaf minimum conductance to water vapour (hydraulic traits); and native embolism, pre-dawn and midday leaf water potential and hydraulic safety margins (hydraulic status). This combination of hydraulic traits - the mechanistic traits determining a tree's hydraulic functioning - and hydraulic status variables - the status of a tree's hydraulic system during functioning - allow us to examine both plasticity (adjustment in hydraulic traits) and acclimation (maintenance of hydraulic status) following changes in the environment. With this dataset, we test the following hypotheses:

1) Tropical trees can acclimate to prolonged soil moisture deficit, by adjusting key hydraulic traits, to maintain the same plant water status as nearby, cognate, but non-droughted trees.
2) Tropical trees acclimate to the negative impacts of long-water transport pathways and greater exposure to drier atmospheric conditions imposed by increasing tree height by adjusting key plant hydraulic traits, resulting in different sized trees having similar water status.
3) Adjustments in hydraulic traits in response to prolonged soil moisture deficit are modulated by tree size. We predict that tree size interacts with long-term soil moisture deficit, such that the capacity to acclimate to soil moisture deficit decreases with tree size.

Additionally, we replicate the analyses of Rowland *et al.* (2015a), which were made at the same experimental site, testing whether embolism resistance changes with tree size and across drought-stressed and non-drought-stressed trees, but here we use the much more extensive sample size and larger dataset acquired in this study.

Methods

Site and plant material

Our study site is a tropical lowland rainforest located in the Caxiuanã National Forest, state of Pará, north-east Brazil (1°43′S, 51°27 W). The site annual rainfall is 2000-2500mm with a dry season (< 120 mm monthly rainfall) from July to December. A throughfall exclusion (TFE) experiment was started in 2002, whereby 50% of canopy throughfall is excluded by a plastic panel structure installed at 1-2m height over a 1 ha area. A 1-2 m trench was dug in the perimeter of the TFE plot to prevent lateral infiltration of water and all litterfall falling in the panels is redistributed manually to the soil. The TFE plot is accompanied by a 1 ha control plot, with a similar perimeter trench but where no throughfall exclusion has taken place. Both plots have been monitored continuously since 2001; detailed information on the experiment can be found in (da Costa *et al.*, 2010; Fisher *et al.*, 2007; Meir *et al.*, 2015; Rowland *et al.*, 2015b) . In 2016, the TFE caused a mean reduction of 48% and 56% of soil water content at 10 cm and 100 cm depth, respectively, in the TFE plot compared to the control plot (Fig. S1).

During the peak of the September-October 2016 dry season, we sampled 161 trees from 12 genera (36 species), 85 from the control plot and 76 from the TFE, with diameters ranging from 14 to 68 cm at 1.5 m height (sampling details in Table 1 and S1). For each individual, we collected two branches 1.5 - 2.5 meters long from the top of the canopy. The branches were fourth to sixth order, counting from the leaves. We collected one set of branches before sunrise (0400 to 0600 hours) and used them for measures of embolism resistance and predawn leaf water potential. We collected a second set of branches at midday (1130 to 1330 hours) and used these for measures of 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 limit leaf transpiration. Branches were transported 100 m from the plots to measure leaf water potential, and for the remaining measurements the branches were transported for 30 minutes to a laboratory. Each day, branches were collected from 6 to 10 different individuals. In all the branches measured, heartwood was absent and pith area was either absent or negligible.

206 Table 1. Summary of Caxiuanã hydraulic traits and status values for each genus and separately for Control and Throughfall Exclusion (TFE)
207 plots. Values presented are mean ± SD. Diameter values are maximum and minimum tree diameter at 1.5 m height. Total sample size (i.e.,
208 number of individual trees sampled for each trait) is given at the bottom of the table for each trait.

			Hydraulic traits							Hydraulic status			
Genus	Plot	Diameter	P50	P88	K_s	K_{ls}	LS	g_{smin}	WD	Ψ_{pd}	Ψ_{md}	HSMP50	PLC
Aspidosperma	Control	14 to 34	-1.9 ± 0.38	-3.9 ± 1.1	3.3 ± 3.3	0.23 ± 0.23	13000 ± 3900	0.068 ± 0.017	0.61 ± 0.13	-0.91 ± 0.35	-2.2 ± 0.74	-0.083 ± 0.48	20 ± 16
	TFE	16 to 32	-3.1 ± NA	-4.9 ± NA	0.97 ± 0.97	0.13 ± 0.14	7500 ± 1500	0.081 ± 0.025	0.59 ± 0.11	-0.56 ± 0.35	-1.9 ± 0.67	0.49 ± NA	34 ± 26
Eschweilera	Control	17 to 42	-2.3 ± 1.1	-4.2 ± 2.1	4.1 ± 2.4	0.46 ± 0.37	11000 ± 5600	0.089 ± 0.058	0.64 ± 0.073	-0.42 ± 0.11	-1.7 ± 0.39	0.75 ± 1	7.6 ± 2
	TFE	13 to 30	-2.6 ± 1.1	-4.9 ± 2.3	5.1 ± 1.9	0.49 ± 0.13	11000 ± 5400	0.076 ± 0.038	0.65 ± 0.08	-0.58 ± 0.14	-2.1 ± 0.45	0.65 ± 0.96	18 ± 13
Inga	Control	15 to 39	-2.6 ± 0.98	-4.4 ± 1	6.7 ± 1.9	0.87 ± 0.57	12000 ± 6300	0.11 ± 0.052	0.71 ± 0.069	-0.38 ± 0.16	-1.9 ± 0.37	0.82 ± 1.3	14 ± 11
	TFE	13 to 56	-2.3 ± 1.2	-4 ± 1.9	6.8 ± 3.3	0.66 ± 0.33	15000 ± 7100	0.06 ± 0.037	0.67 ± 0.12	-0.44 ± 0.14	-2 ± 0.2	0.37 ± 1.2	18 ± 6.1
Licania	Control	10 to 28	-2 ± 0.73	-3.5 ± 2	0.86 ± 0.51	0.068 ± 0.03	14000 ± 3600	0.057 ± 0.024	0.73 ± 0.031	-0.29 ± 0.11	-1.3 ± 0.27	0.79 ± 0.66	26 ± 11
	TFE	11 to 28	-3.2 ± 0.51	-6.3 ± 0.93	0.81 ± 0.85	0.083 ± 0.051	10000 ± 4800	0.061 ± 0.035	0.72 ± 0.056	-0.32 ± 0.15	-1.1 ± 0.29	2.1 ± 0.69	23 ± 1.9
Micropholis	Control	18 to 63	-1.2 ± 0.63	-2.3 ± 1.2	3.6 ± 0.41	0.35 ± 0.15	13000 ± 8800	0.096 ± 0.058	0.6 ± 0.044	-0.52 ± 0.16	-2.4 ± 0.88	-2.3 ± 0.99	11 ± 6.9
	TFE	14 to 24	-1.1 ± NA	-1.9 ± NA	4.4 ± 1.3	0.2 ± 0.13	11000 ± NA	0.034 ± 0.0067	0.61 ± 0.062	-1.3 ± 0.53	-2.2 ± 0.46	-0.53 ± NA	6.2 ± 8.7
Minquartia	Control	12 to 42	-2 ± 0.89	-3.8 ± 2.2	2.5 ± 0.88	0.24 ± 0.078	11000 ± 5000	0.079 ± 0.042	0.71 ± 0.066	-0.52 ± 0.16	-1.5 ± 0.21	0.53 ± 0.91	19 ± 7.5
	TFE	13 to 41	-1.3 ± 0.87	-3.4 ± 3.5	2.5 ± 1.4	0.32 ± 0.13	11000 ± 4200	0.052 ± 0.022	0.68 ± 0.061	-0.61 ± 0.23	-1.4 ± 0.27	-0.19 ± 0.83	21 ± 17
Pouteria	Control	12 to 59	-2.2 ± 1.2	-4.6 ± 2.8	2.9 ± 1.2	0.52 ± 0.4	7600 ± 3900	0.092 ± 0.049	0.75 ± 0.12	-0.63 ± 0.2	-2.2 ± 0.48	-0.073 ± 1.2	19 ± 12
	TFE	10 to 52	-1.4 ± 0.49	-2.4 ± 1.4	2.3 ± 0.74	0.25 ± 0.21	11000 ± 4100	0.067 ± 0.029	0.7 ± 0.088	-0.92 ± 0.32	-2.7 ± 0.82	-1.5 ± 0.74	18 ± 15
Protium	Control	11 to 38	-2.3 ± 0.72	-5.6 ± 1.7	3.4 ± 1.7	0.36 ± 0.15	9900 ± 4100	0.056 ± 0.027	0.57 ± 0.099	-0.65 ± 0.32	-1.5 ± 0.44	0.46 ± 0.63	17 ± 9.6
	TFE	16 to 38	-2.7 ± 0.78	-4.4 ± 1.4	4.3 ± 1.7	0.55 ± 0.19	8200 ± 3700	0.058 ± 0.03	0.56 ± 0.098	-0.62 ± 0.17	-1.7 ± 0.67	0.91 ± 1.2	41 ± 21

Swartzia	Control	22 to 68	-3.1 ± 1.6	-5.8 ± 2.8	5.2 ± 3.1	0.49 ± 0.26	11000 ± 3600	0.11 ± 0.077	0.67 ± 0.038	-0.3 ± 0.067	-2.1 ± 0.52	1.1 ± 1.6	21 ± 16
	TFE	10 to 64	-3.1 ± 1.1	-5.3 ± 1.2	3.1 ± 1.6	0.37 ± 0.2	9400 ± 5700	0.094 ± 0.057	0.72 ± 0.067	-0.46 ± 0.19	-2.2 ± 0.41	0.78 ± 1.4	33 ± 11
Syzygiopsis	Control	14 to 52	-1.4 ± 0.75	-2.2 ± 1	2.8 ± 0.87	0.4 ± 0.24	8000 ± 1900	0.057 ± 0.025	0.55 ± 0.039	-0.7 ± 0.089	-1.8 ± 0.19	-0.38 ± 0.78	15 ± 5.1
	TFE	24 to 59	-1.5 ± 0.74	-3.3 ± 1.9	2.8 ± 0.69	0.47 ± 0.058	6000 ± 730	0.067 ± NA	0.55 ± 0.021	-1 ± 0.6	-2.7 ± 0.72	-1.2 ± 1.5	34 ± NA
Virola	Control	27 to 48	-1.9 ± 1.4	-4.4 ± 3.2	NA	NA	NA	0.047 ± 0.039	0.49 ± NA	-0.65 ± 0.49	-2.4 ± 0.21	-0.42 ± 1.6	NA
	TFE	20 to 35	-1.6 ± 0.85	-3.3 ± 1.6	NA	NA	NA	0.05 ± 0.01	0.5 ± 0.056	-1.1 ± 0.63	-2.5 ± 0.53	-0.95 ± 1.2	NA
Vouacapoua	Control	12 to 56	-3 ± 0.59	-5.7 ± 1.9	2.4 ± 2.2	0.27 ± 0.34	9500 ± 5400	0.088 ± 0.027	0.65 ± 0.093	-0.45 ± 0.11	-2.1 ± 0.73	0.38 ± 1.1	14 ± 8.9
	TFE	15 to 57	-2.6 ± 1.6	-4.4 ± 3.2	3.5 ± 2.5	0.44 ± 0.4	9800 ± 3700	0.064 ± 0.032	0.68 ± 0.11	-0.62 ± 0.14	-2.8 ± 0.79	-0.14 ± 2.3	21 ± 9.5
Total samples			99	99	135	135	135	150	140	159	159	99	135

209

210 P50 and P88- xylem embolism resistance, xylem water potential leading to 50% and 88% of loss in water transport capacity (MPa); K_s –
211 maximum hydraulic specific conductivity (kg m m^{-2} s^{-1} MPa^{-1}); K_{ls} - maximum hydraulic leaf specific conductivity (kg m m^{-2} s^{-1} MPa^{-1}); LS –
212 leaf to sapwood area (m^2 m^{-2}); g_{smin} – minimum stomatal conductance (mol m^{-2} s^{-1}); WD – wood density (g cm^{-3}); Ψ_{pd} - predawn water potential
213 (MPa); Ψ_{md} – midday water potential (MPa); HSMP50 – hydraulic safety margin to P50 (MPa); PLC – native dry season percentage loss of
214 conductivity (%).

Predawn and midday water potential

We measured leaf water potential (Ψ) in the field immediately after collection using a pressure chamber (Model 1505, PMS; 0.05 MPa resolution). For each tree we measured water potential of two leaves, or three leaves if the first two measures differed by more than 0.2 MPa for predawn and 0.4 MPa for midday measurements.

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

We measured wood density on woody sections 40 to 80 mm long and 4 to 7 mm diameter cut from the branch. We debarked samples, immersed them in water for 24 hours to rehydrate and measured saturated volume using the water displacement method (Pérez-Harguindeguy *et al.*, 2013). We then oven dried the samples at 60ºC for 48 hours and measured their dry weight with a precision scale.

We determined leaf to sapwood area ratio (LS), 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 calipers. 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 LS as the total branch leaf area divided by its basal sapwood area.

For minimum leaf conductance (g_{smin}) 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 analyzer (Li-COR 6400, USA). All leaves measured were adult, undamaged, upper canopy leaves leaves. g_{smin} is likely a combination of stomatal conductance due to leakage from partially closed stomata and cuticular conductance. g_{smin} is part of the dataset presented in Rowland *et al*. (submitted) and further leaf gas exchange measurement details can be found therein.

239

240 *Hydraulic efficiency and native embolism*

241 We calculated maximum hydraulic specific conductivity (K_s) as a measure of xylem
242 hydraulic efficiency and maximum leaf specific conductivity (K_{ls}) as a measure of leaf water
243 supply capacity. We used the native percentage loss of conductivity of the collected branches
244 (PLC_{nat}) as a measure of native embolism. To estimate these variables, we measured branch
245 xylem hydraulic conductivity before and after flushing to remove emboli. We also measured
246 the leaf area distal to the sample. We used the 1.5 to 2.5 m long branches collected at midday
247 to measure hydraulic conductivity. We cut 10-15 cm long segments from each branch base
248 underwater and let them rehydrate for 15 min to release tension and avoid artefacts (Venturas
249 *et al.*, 2015). Subsequently, to relax the tension in the branch we cut 1-1.5 m of branch from
250 base to leaves underwater, in steps of ~15 cm, and used the distal end of the branch for
251 hydraulic measurements, to ensure no artificially embolised vessels were present in the
252 measured sample. Maximum vessel length, measured on a subsample of 17 branches was
253 32.7 ± 15.2 cm (55.5 cm maximum), confirming 2 m long branches were sufficient to avoid
254 open vessel artefacts. All samples used for hydraulic measurements were first or second order
255 branches, were between 30-55 mm in length and 3-5 mm diameter and were recut underwater
256 with a sharp razor blade before connecting to the apparatus, to ensure all vessels were open at
257 both ends. We measured flow using the pressure drop over a capillary method in an hydraulic
258 apparatus (Sperry *et al.*, 1988; Espino & Schenk, 2011; Pereira & Mazzafera, 2012), where a
259 capillary of known conductance is connected in series with the sample, and flushing samples
260 to removeemboli and estimate maximum conductance (Martin-StPaul *et al.*, 2014). We
261 calculated PLC_{nat} as the ratio of K_{snat} to K_s multiplied by 100. We calculated K_{ls} as sample
262 hydraulic conductivity (i.e. sample conductance times sample length) after flushing divided
263 by the leaf area distal to the measured sample.

Embolism resistance and hydraulic safety

As an index of xylem embolism resistance, we used P50 and P88, the xylem water potentials where, respectively, 50% and 88% of hydraulic conductivity is lost (Choat *et al.* 2012). We also used P50 to calculate the hydraulic safety margin - the difference between P50 and Ψ_{md}, an index of tree hydraulic safety. 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; details in Methods S1 and Bittencourt *et al.*, 2018). We dehydrated branches using the bench dehydration method. Before each air discharge measurement, we bagged branches 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 or two leaves. Drought embolism resistance is then given by the increase in air discharge (PAD – percentage air discharge) with decreasing xylem water potential for each tree. To calculate P50, we pooled together the data for the 2-3 branch replicates from the same tree and fitted a sigmoid curve to the data, where P50 and slope (a) are the fitted parameters (Pammenter & Vander Willigen, 1998) and P88 is predicted from the fitted model:

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

Data analysis

To test our hypotheses, we evaluated the significance of plot, diameter (a proxy of tree height and canopy exposure - see Fig. S2 for tree size and canopy exposure relationships based on the tree light score estimates for both plots), genus and their interactions as variables affecting hydraulic traits and hydraulic status. We used linear mixed effect models with plot, diameter and their interaction treated as fixed effects and we tested the random effect of genus on the intercept of the model and the slope of the independent variable with the fixed model terms (i.e. plot and diameter). We started with a full fixed and random effect model of plot, diameter and their interaction and tested the significance of the random effect by removing it and evaluating if the model significantly worsened. We tested sequentially for the random effect of genus on: 1) the model intercept; 2) the relationship between the independent variable and tree diameter and; 3) on the relationship between the independent variable and plot. The genus effect on plot without effect on intercept produces the same fit as genus effect on plot and intercept, as plot is a factorial term, so this model was not tested. When more than one random effect format was significant, we chose the simplest random effect (i.e. intercept effect only), unless the Akaike Information Criterion of the more complex model was at least 2 units lower than the simpler model. After testing the random effects, we tested the fixed effects by first removing the interaction term and testing if this significantly worsened the model and after this using the same approach with the additive terms. If no random effect was significant, we changed to a fixed effect model (R base package "lm" function) and analysed fixed effects in the same way. To be confident in our capacity to evaluate plasticity/acclimation we also repeated the above analysis at a species level, instead of genus level. Analysing the data in this way leads to a less balanced sampling design and a reduction in the replicates available for comparison between the plots, due to high tropical forest diversity. However, if a comparison of the genus level and species level analysis delivers the same results it confers confidence in our capacity to demonstrate either

full, partial or no drought acclimation through combining a more statistically robust data-set at the genus-level, with a less robust, but more scale-appropriate analysis at the species level.

We also re-tested Rowland *et al.*'s (2015a) relationship between tree size and P50 by analysing our individual dataset alone (30 samples Control and 32 samples TFE, considering only the genera in common with Rowland *et al.* (2015a), and then in combination with the Rowland *et al.* (2015a) dataset (48 samples Control and 51 samples TFE) using fixed effect models. We use a fixed-effect model for consistency with the analysis carried out in Rowland *et al.* (2015a). It should be noted that here we use the pneumatic method for determining P50, whereas Rowland *et al.* (2015a) used the air-injection method and fitted a Weibull, not a sigmoidal function. Despite the different methods, we find a correlation of 0.95 between P50 estimated from Weibull and Sigmoid functions for our data (Fig. S3). Finally, to test the effect that species composition of our and the Rowland *et al.* (2015a) data set has on the P50-tree size relationships and to verify the sensitivity of the results (p-value) to the combination of genera used, we performed a taxon-sensitivity analysis by deleting one, two, three and four genera at a time from the full dataset (all 12 genera in this study) for all possible genera combinations (793 total combinations tested).

We used the R programming environment and statistical packages (version 3.3.0; R Core Team 2016) for all data processing and analysis. We fitted linear mixed effect models with "lme" function of the nlme package (Pinheiro *et al.* 2014) and linear fixed effect models with the "lm" function (base packages). We used marginal and conditional pseudo-r^2 to quantify mixed models relative goodness of fit (function "r.squaredGLMM" of the MuMIn package; Barton 2016). We followed the guidelines of Zuur *et al.* (2009) and Thomas *et al.* (2017) for assessing significance of model terms and validating model assumptions. For mixed effect model terms, we fitted models with restricted maximum likelihood and calculated a log-likelihood ratio to test if the removal of a term significantly affected the

quality of the model in relation to the more complex model in which it was nested. For fixed effect models, we used the same approach but with an F-test between the model with and the model without the removed variable. We used the "anova" function (stats package, with mixed effect model methods from nlme package) for log-likelihood ratio and F tests. If the p value of the test was less than 0.05, we assumed that the model without the variable was significantly worse than the model with the variable included. The final model with only significant terms was refitted with maximum likelihood and we validated model assumptions using diagnostic plots for normality and homogeneity of residuals and collinearity of predictors. To identify influential observations, we calculated Cook's distance and dfbeta for each dataset using base package functions for linear models and "influence.ME" (Nieuwenhios *et al.* 2012) package functions for mixed effect models. The embolism resistance dataset is smaller than the other datasets, as we could not process all collected samples (Table 1). No hydraulic conductivity data is presented for *Virola* as it clearly decreased with the duration of the measurement, producing negative percentage loss of conductance, likely due to the abundance of latex being exuded from the cut end of the sample. Whenever we had more than one replicate per tree, as for leaf water potential, we used the tree-level mean of the replicates. We considered significant the probability of the tested hypothesis versus the null hypothesis (p-value) being lower than 0.05 and marginally significant when it was between 0.05 and 0.10.

Results

Throughfall exclusion effects on tree hydraulics

Drought stress caused by 15 years of throughfall exclusion (TFE) had limited effect on hydraulic traits (Fig. 1; Table 1) Xylem embolism resistance (P50 and P88), specific conductivity (K_s), leaf specific conductivity (K_{ls}), leaf to sapwood area (LS) and wood density (WD) did not change in response to the TFE manipulation (Table 2; Table S2). Minimum stomatal conductance (g_{smin}) was the only trait which adjusted in response to the TFE ($p = 0.011$), with a decrease of 0.007 mol m^{-2} s^{-1} (Fig. 1f; all coefficients are presented in Table S3).

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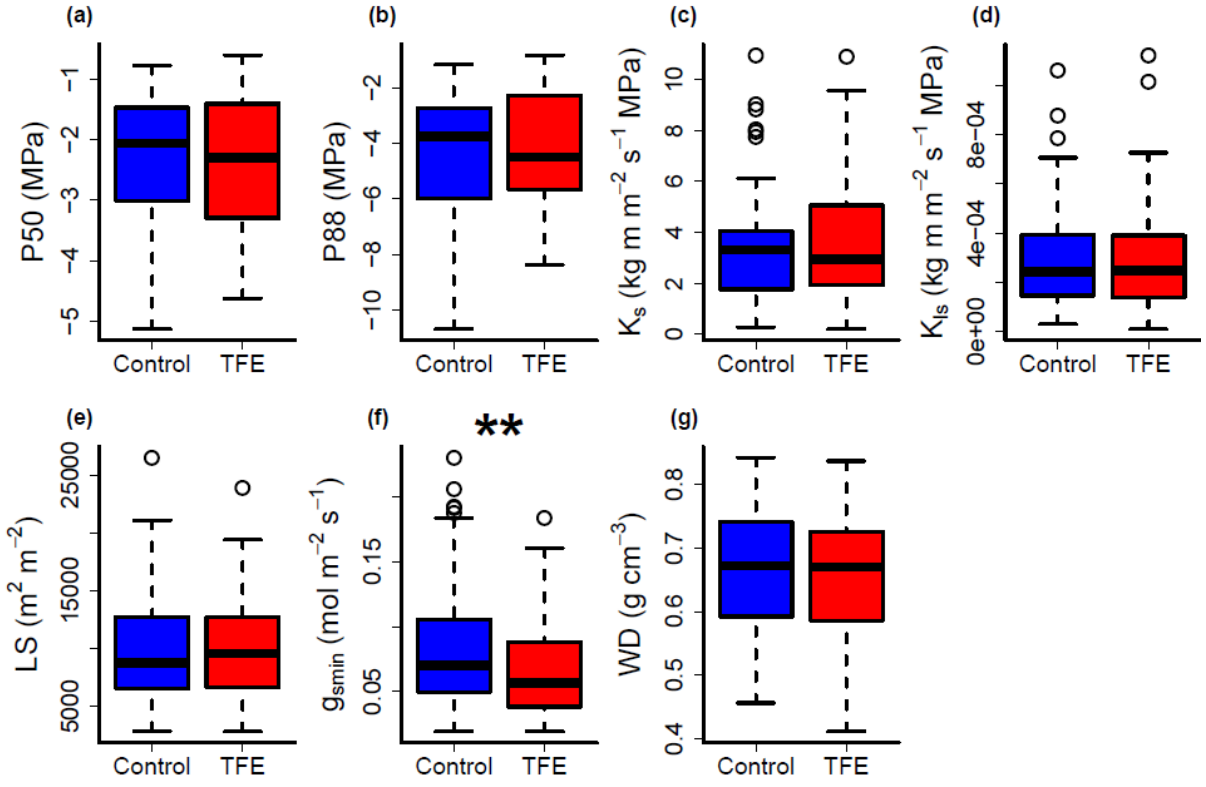


368

369 Figure 1. Hydraulic trait responses on trees surviving after 15 years of throughfall exclusion
370 (TFE) in Caxiuanã. a-b) P50 and P88 - xylem embolism resistance (MPa); c) K_s – maximum
371 hydraulic specific conductivity (kg m m^{-2} s^{-1} MPa); e) K_{ls} - maximum hydraulic leaf -specific
372 conductivity (kg m m^{-2} s^{-1} MPa);; e) LS – leaf to sapwood area ratio (m^2 m$^{-2)}$; f) g_{smin} –
373 minimum stomatal conductance (mol m^{-2} s^{-1}); g) WD – wood density (g cm^{-3}). We consider
374 changes in structural hydraulic traits (i.e. plot effect models) as plasticity in response to
375 drought. The box represents quartiles 1 and 3, with the central line indicating the median.
376 Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when
377 outliers are present. 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
378 2 for models and analysis section in Methods).

380

Table 2. Results of linear mixed effect models of plot (Control versus TFE) and tree diameter effects on hydraulic traits and hydraulic status. The combination of the tested random effects is genus effect on intercept only, and/or on plot and diameter slopes (see analysis section in Methods for details). Values for fixed effects are fitted parameter ± standard error; values for random effects are standard deviation of the normal distribution from where coefficients were fitted. R^2 is the full model coefficient of determination (conditional pseudo-R^2). Random effects notation are: 1|genus is a random genus effect on intercept; diameter|genus or plot|genus indicates a random intercept effect plus a random genus effect on diameter or plot term (i.e. an interaction term of genus modelled as a random variable with plot or diameter). Plot (i.e. experimental treatment) is a two level factor (Control and TFE), with Control as the reference level. Blank cells indicate that the effect is non-significant.

		Fixed effect			Random effects			
		Intercept	Plot	Diameter	Genus	Plot|Genus	Diameter|Genus	R^2
Traits	P50	-2.21 ± 0.16			0.44*			0.16
	P88	-4.22 ± 0.21						0
	K_s	3.31 ± 0.48			2.29***		0.065***	0.53
	K_{ls}	0.21 ± 0.06		0.005 ± 0.001 **	0.11**			0.26
	LS	10266 ± 439.6						0
	g_{smin}	0.083 ± 0.005	-0.018 ± 0.007**					0.043
	WD	0.65 ± 0.02			0.06***			0.36

Status	Ψ_{pd}	-0.52 ± 0.05	-0.18 ± 0.07***	0.13***	0.19***	0.49
	Ψ_{md}	-1.88 ± 0.12	-0.2 ± 0.09 *	0.38***		0.35
	HSMP50	0.14 ± 0.24		0.71***		0.26
	PLC	16.2 ± 2.3	8.3 ± 2.5**	5.0 *		0.2

388 P50 - xylem embolism resistance (MPa); Ψ_{pd} - predawn water potential (MPa); Ψ_{md} - midday water potential (MPa); HSMP50 – hydraulic
389 safety margin to P50 (MPa); PLC – native dry season percentage loss of conductivity (%); K_s – maximum hydraulic specific conductivity (kg m
390 m^{-2} s^{-1} MPa^{-1}); K_{ls} - maximum hydraulic leaf-specific conductivity (kg m m^{-2} s^{-1} MPa^{-1}); LS – leaf to sapwood area ratio (m^2 m^{-2}); g_{smin} –
391 minimum stomatal conductance (mol m^{-2} s^{-1}); WD – wood density (g cm^{-3}).

392 * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

393 The TFE manipulation had a highly significant effect on all hydraulic status variables
394 (Fig. 2), except on hydraulic safety margin (HSMP50; Table 2). TFE caused a decrease in
395 peak dry season Ψ_{md} of – 0.19 MPa ($p = 0.02$) with a high intra-generic variability (SD of
396 0.38 MPa for random genus effect on intercept and an average increase in percentage loss of
397 conductance of 8.3% (PLC; $p = 0.001$; see coefficients in Table S3). Predawn water potential
398 (Ψ_{pd}) was affected by the TFE with a reduction of – 0.18 MPa ($p < 0.001$), equal to a 35%
399 decrease relative to control, which was further modulated by random genus-specific effects (p
400 < 0.001; Figure 3; see also the following paragraph), leading to a TFE maximum effect of
401 reducing predawn water potential by 0.52 MPa in *Micropholis*.

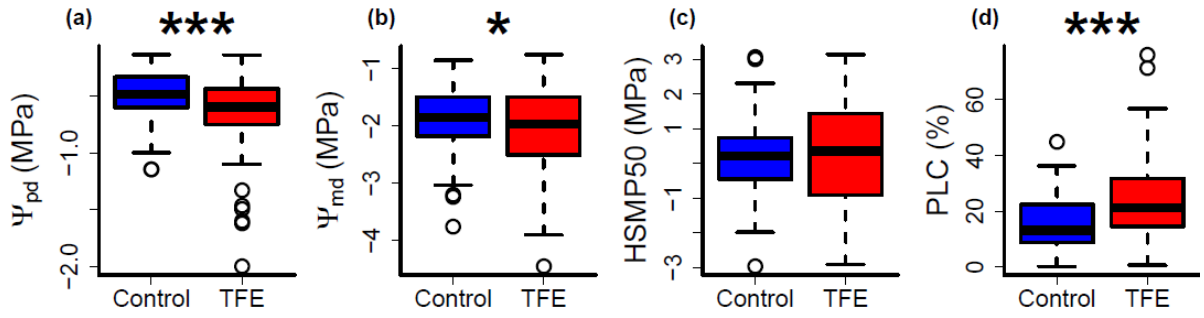
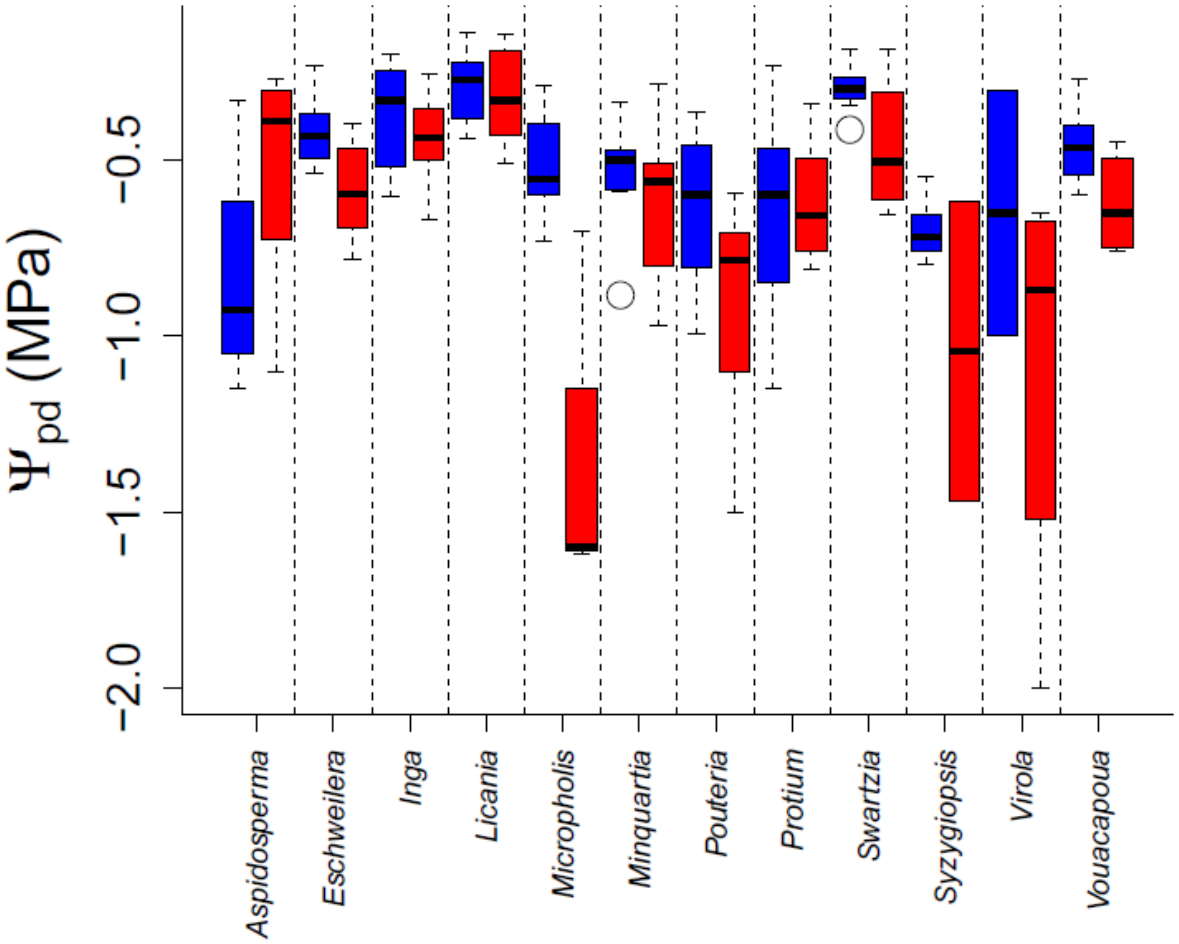


Figure 2. Hydraulic status of trees surviving after 15 years of throughfall exclusion (TFE) in the peak of the dry season in Caxiuanã. a) Ψ_{pd} - predawn water potential (MPa); b) Ψ_{md} midday water potential (MPa); c) HSMP50 – hydraulic safety margin to P50; d) PLC – native dry season percentage loss of conductivity (% maximum conductance). We consider homeostasis of hydraulic status (i.e. no plot effect) as an indicator of hydraulic acclimation. 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. Traits for which plot (i.e. TFE treatment) 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).

Tree size effects on hydraulic traits and interaction with TFE

Tree stem diameter did not affect hydraulic status and only affected K_s and K_{ls} among the hydraulictraits (Table 2). For K_s, the effect of increasing diameter was genus-dependent, as indicated by a significant random genus effect on diameter ($p < 0.001$; Figure 4a; Table 2; Table S2). The effect of diameter on K_s was positive for *Inga*, *Aspidosperma* and *Vouacapoua* while it was negative for *Eschweilera* and close to zero for the other genera (Fig. 4a and Table S3). For K_{ls}, stem diameter was significant (Tables 1) however, contrary to K_s, the effect was not genus dependent (no random genus effect on diameter-K_{ls} relationship), and K_{ls} showed a consistent increase with stem diameter (Fig. 4b). The random genus effect on the diameter-P50 relationship was significant ($p = 0.035$; Table S2; Fig. 5), but was not the most parsimonious model (AIC of 316 against an AIC of 315.8 for the random intercept model only). There was no fixed diameter effect on P50 ($p = 0.29$, Table S2) but the interaction effect between diameter and plot was marginally significant ($p = 0.066$; Table S2). We found no evidence of any interaction of plot and tree stem diameter with hydraulic traits or with hydraulic status (Table 2 and Table S2).



428

429 Figure 3. Throughfall exclusion (TFE) effect on predawn water potential of the studied genus
430 (Ψ_{pd}), showing the genus specific variation in Ψ_{pd} response to TFE. TFE had both a fixed
431 effect of reducing Ψ_{pd} and a random effect (genus specific effect) on Ψ_{pd} (see Table 1). Red
432 and blue boxplots are data from the TFE plot and control plots, respectively. The box
433 represents quartiles 1 and 3, with the central line indicating the mean. Whiskers are either
434 maximum value or 1.5 x interquartile range above the quartile 3, when outliers are present.

435

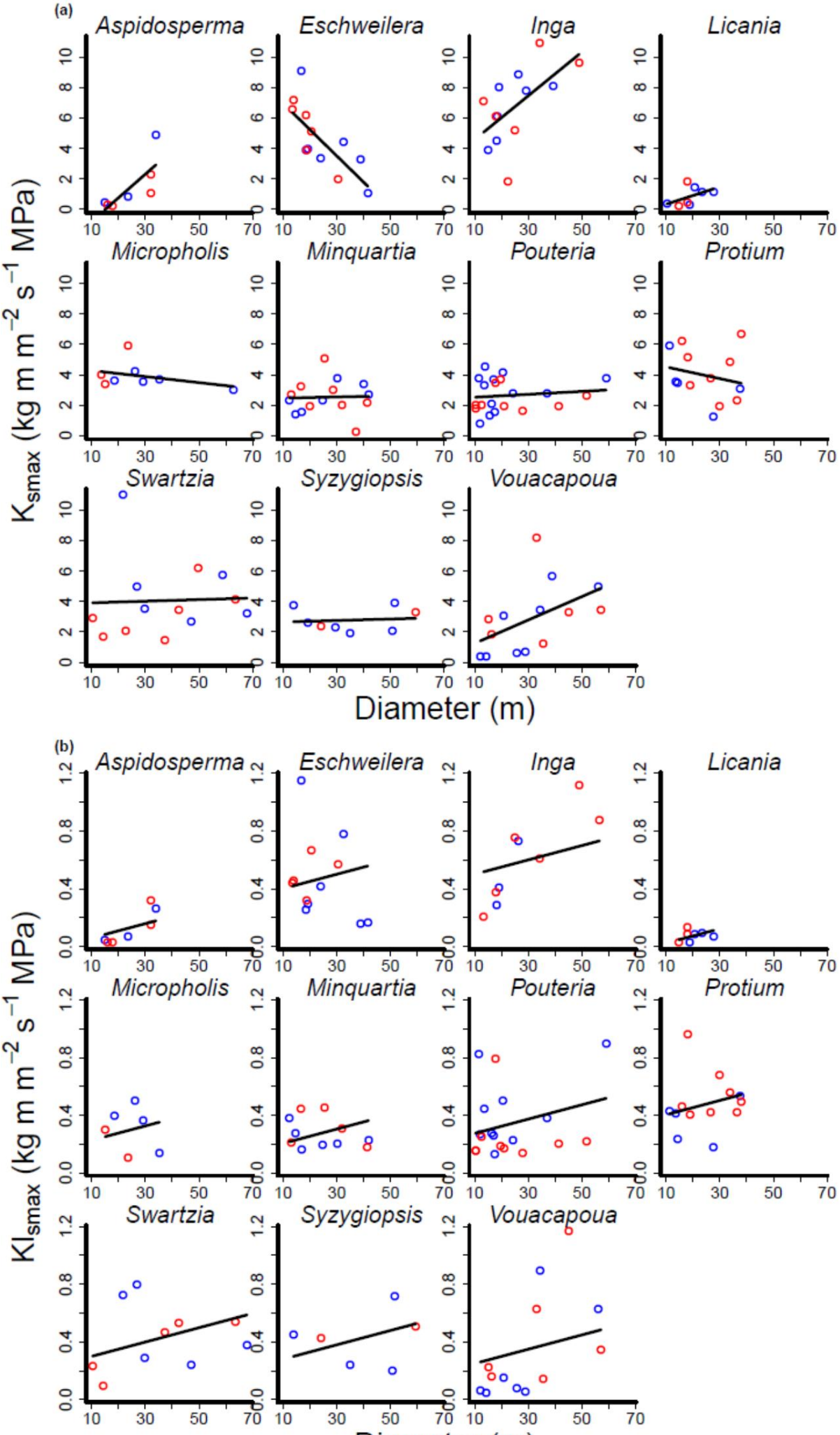
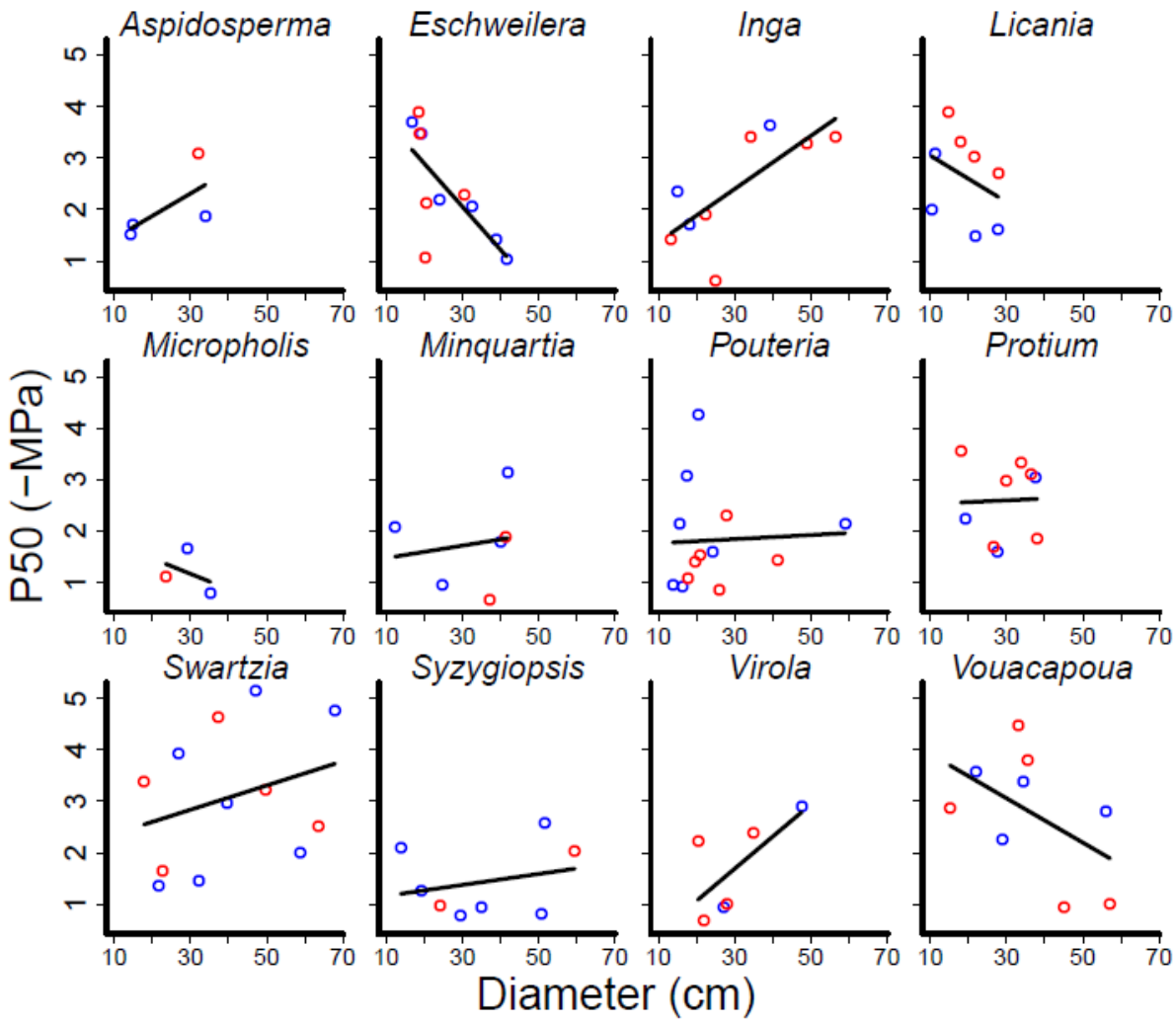

(a)
Aspidosperma
Eschweilera
Inga
Licania
Micropholis
Minquartia
Pouteria
Protium
Swartzia
Syzygiopsis
Vouacapoua
K_{smax} (kg m m^{-2} s^{-1} MPa)
Diameter (m)
(b)
Aspidosperma
Eschweilera
Inga
Licania
Micropholis
Minquartia
Pouteria
Protium
Swartzia
Syzygiopsis
Vouacapoua
Kl_{smax} (kg m m^{-2} s^{-1} MPa)

Figure 4. Diameter effects on xylem specific conductivity (K_s; a) and leaf specific conductivity (Kl_s; b) for each studied genus. Fitted lines show the fitted mixed effects model (see Table 1 and Results section), modified according to the effect of the random varrable (genus) on either the intercept or the slope of the relationship of diameter to the independent variable (K_s or Kl_s). For K_s, the interaction between diameter and random effect, genus is significant ($p < 0.001$), resulting in different slopes for each genus. For K_{ls}, the interaction is significant ($p = 0.02$) but is not the most parsimonious model (AIC of 40.2 against an AIC of 38.6 for genus effect on intercept only, see Table S2), resulting in vertical shifts of otherwise parallel lines. Blue and red points are data from Control and TFE plots, respectively.



446

447 Figure 5. Diameter effects on embolism resistance (P50; presented as positive values in –
448 MPa) for each studied genus. We modelled the variables using linear models with diameter,
449 genus and their interaction as fixed effects. The model is marginally significant ($p = 0.066$).
450 The fitted line is the fitted, marginally significant model, for each genus. Blue and red points
451 are data from Control and TFE plots, respectively (there was no plot effect on P50 so data
452 were pooled).

Taxonomic effects on the hydraulic system and their interactions with drought and tree size

Most of the hydraulic traits and hydraulic statusvariables we measured varied significantly by genus (Table 2). The form of the taxonomic effect was, except for Ψ_{pd} and K_s, an additive change in intercept with no effect on the plot response (Table 2). Taxonomic effects on K_s were shown in the previous section. For Ψ_{pd}, genus-specific effects were evident on the TFE (Fig. 3). Certain genera demonstrated substantially greater reductions in Ψ_{pd} in response to the TFE than others, for example *Micropholis* had a mean decline of -0.43 MPa on the TFE. When genus was included as a random effect in a MEM for Ψ_{pd}, with plot as a fixed variable, it had a SD of 0.19 MPa on the plot-Ψ_{pd} relationship (see interaction coefficients in Table S3). We could not detect any taxonomic effect on LS, which had a large variability, or on g_{smin} and P88. When we analysed the data at species level, all the above patterns remained unchanged (Table S4), except for K_s, whose random species effect on the diameter slope could not be detected, and midday water potential, where a random species effect on the plot effect was now detectable.

Revisiting Rowland et al. (2015)

Rowland *et al.* (2015a) presented the first dataset of P50 for Amazon trees at this site (reproduced in Fig.6a). We provide here a comparison of the two analyses. Using fixed effect models, when we analysed the same subset of six genera as analysed by Rowland *et al.* (2015a), our data also show a significant effect of diameter on P50. However, in our work, the slope was positive, and not negative as in Rowland *et al.* (2015a), and the interaction between genus and diameter was also significant ($p = 0.046$, Fig. 6b). While *Eschweilera* presents a strong increase in P50 (less negative values) with diameter, the other genera showed either a weak decrease in P50 with diameter or an almost negligible diameter effect (Fig. 6). When the two datasets are combined, the same pattern remains, with P50 being

477 significantly affected by the interaction between diameter and genus and a slightly more
478 significant diameter effect (p = 0 .023; Fig. 6c). However, after removal of a single genus
479 with a strong positive relationship between P50 and diameter (*Eschweilera*) from the
480 combined datasets, both the diameter interaction with genus and the diameter effect disappear
481 (p = 0.62 and p = 0.14; Fig. S4c). The same occurs if *Eschweilera* is removed from the non-
482 combined datasets (Fig. S4a and b).

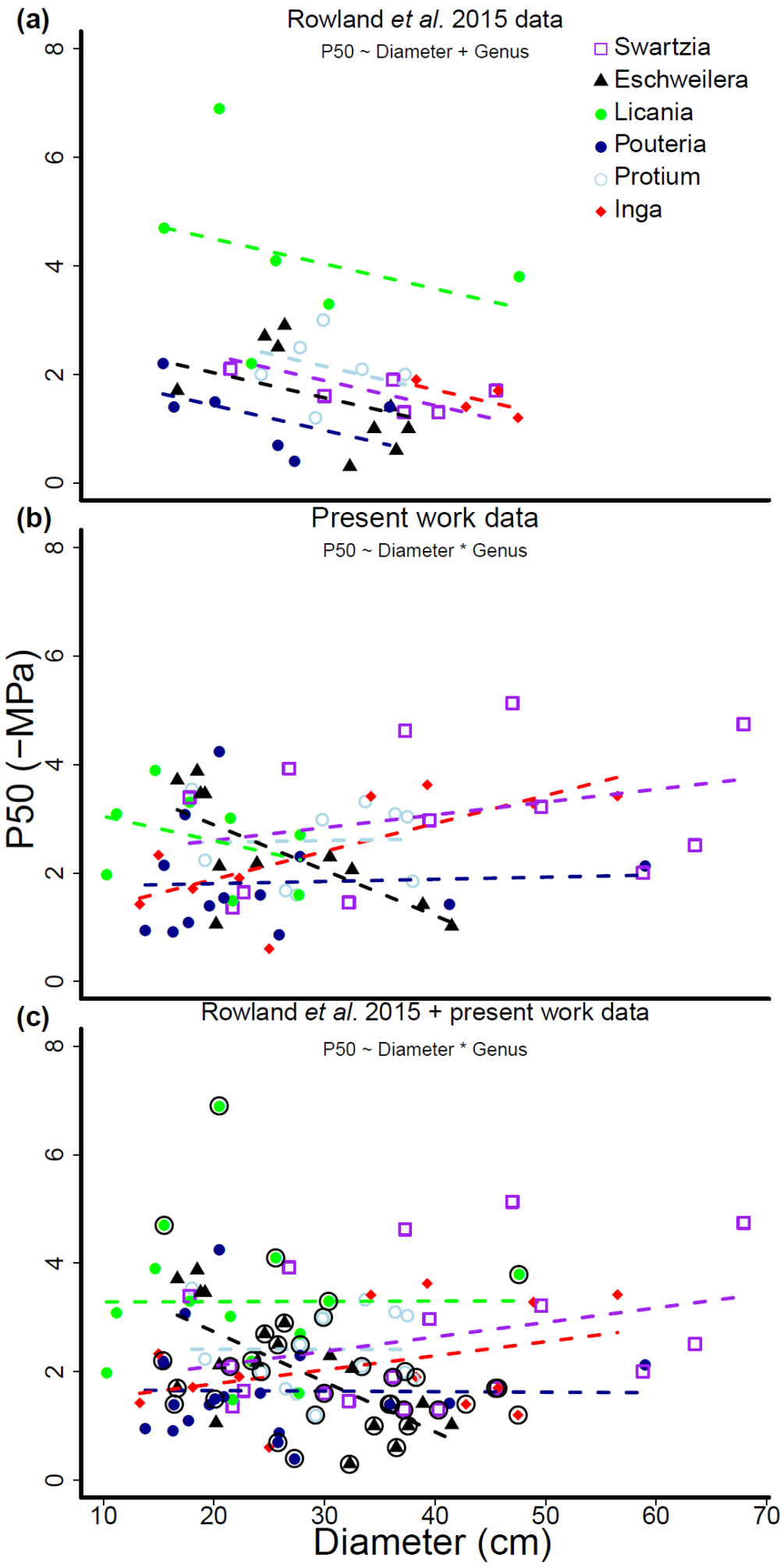

(a)
Rowland et al. 2015 data
P50 ~ Diameter + Genus
Swartzia
Eschweilera
Licania
Pouteria
Protium
Inga
(b)
Present work data
P50 ~ Diameter * Genus
P50 (−MPa)
(c)
Rowland et al. 2015 + present work data
P50 ~ Diameter * Genus
0
2
4
6
8
10
20
30
40
50
60
70
Diameter (cm)

Figure 6. Relationship between embolism resistance (P50) and tree diameter using the same subset of genera as analysed in Rowland *et al.* (2015a). a) The original data and analysis of Rowland *et al.* (2015a). b) The data from this work only; and c) the combined datasets from a) and b). Linear fixed-effect models were used in the analysis and the dashed lines are the fitted model for each genus. In a), diameter and genus were significant while the interaction term was not. In both b) and c) genus, diameter and their interaction are significant. The circled data points in c) indicate the 2015 data. As done in Rowland *et al.* (2015a), we display the Y-axis as the negative of P50 (-P50). The same figure and analysis is presented in Fig. S4 after removing data for *Eschweilera*, the genus with an increase in -P50 with diameter. Note: P50 values are presented as –MPa, as in (Rowland *et al.*, 2015a).

The results from the linear fixed effect models using the Rowland et al (2015a) subset of the data are partially consistent with the results for this study is full dataset with all 12 genera, whereby a diameter interaction with genus is found to be marginally significant ($p = 0.066$; Table S2), but this is not the most parsimonious model (AIC of 316 for the random genus-diameter interaction against an AIC of 315.8 for the simpler genus intercept effect). We reanalysed the full dataset (all 12 genera in this study) removing one, two, three and four genera at a time, for all possible genus combinations (793 total combinations tested), to verify the sensitivity of the results to the combination of genera used. When removing only one genus at a time, P50 was significantly affected by diameter with a genus interaction 33.3% of the times and marginally significantly affected 75% of the times. A similar pattern was observed when two or more genera were removed simultaneously (Fig. S5). This further supports our finding that the influence of tree diameter on P50 is strongly dependent on the taxonomic identity of the trees in the dataset, which also strongly influenced whether P50 increased, decreased or did not change with increasing tree size (Fig. S5). On the other hand, neither the present results nor those of Rowland *et al.* (2015a) show any significant plot (i.e. treatment) effects on P50.

Discussion

We analysed the effects of a long-term (>15 years) throughfall exclusion experiment (TFE experiment) on key hydraulic traits and hydraulic status variables, which indicate the hydraulic stress being experienced by the tree across its water transport system. Despite the high mortality rates of the largest TFE trees (da Costa et al 2010; Rowland *et al.*, 2015a), which should have reduced overall inter-tree competition for soil water, soil water content is significantly lower in the TFE plot relative to the control plot (Fig. S1). This indicates that competition for water is still high amongst surviving trees and reduced competition following mortality events has not alleviated soil water stress, which is demonstrated by the observed lower Ψ_{pd} and Ψ_{md} and greater PLC in the droughted trees (Fig. 2). Furthermore, no plasticity was observed in key hydraulic traits related to hydraulic safety (P50 and P88) and hydraulic efficiency (K_s, K_{ls}) in the droughted trees relative to the control, suggesting that tropical trees may not be able to acclimate their hydraulic systems to long-term drought in order to maintain the same water status as non-droughted trees, and thus avoid damage to their hydraulic systems. The high variability in the extent of native embolism and in tissue water potential among genera supports the hypothesis that some tree groups on the TFE suffer far greater hydraulic stress than others, which is likely to play a substantial role in triggering the extensive, genus dependent, drought-induced mortality observed on the plot (da Costa 2010; Rowland *et al.*, 2015a).

Plasticity and acclimation to drought in Amazonian trees

Tropical forest drought experiments have reported decreases in hydraulic efficiency (Schuldt *et al.*, 2011), or no change in embolism resistance (Rowland *et al.*, 2015a) in comparison to non-droughted, control forest trees. However, shifts in anatomical traits related to hydraulic

safety were observed in a TFE experiment located in tropical Australian rainforest (Tng *et al.*, 2018). Our data indicate that in tropical trees exposed to prolonged soil moisture stress, neither the traits associated with hydraulic safety nor those associated with hydraulic efficiency adjust to enable acclimation. It is possible that other, unmeasured, traits may be influencing tree hydraulic status, for example changes in stomatal control and rooting depths. However, the significantly lower leaf water potential and greater PLC in the TFE trees suggest other traits, as with our measured traits, are not adjusting sufficiently to allow the hydraulic system of the droughted trees to acclimate. These patterns which we observe at genus level remain unchanged when the data are analysed at species level, providing confidence that acclimation did not occur.

Forest resistance and resilience to drought are likely to be mediated by medium and long-term precipitation variability (Barros *et al.*, 2019; Ciemer *et al.*, 2019), which themselves influence species distribution patterns at different scales across the Amazon (Esquivel-Muelbert *et al.*, 2017). Our study site, like much of Amazônia, experiences relatively small seasonal changes in water availability (Fisher *et al.*, 2008) and it is possible that species in this region have not evolved significant organ-level plasticity in response to variability in moisture stress. The capacity of trees to acclimate to drought stress may be linked to the existing and historical drought stress they experience within an environment (Zhou, Medlyn, & Prentice, 2016). Therefore, despite a certain degree of seasonality, the lack of strong rainfall variation, or history of sufficient interannual variance in rainfall at our study site, may ultimately be responsible for the observed lack of capacity to acclimate.

The higher values for PLC in the TFE trees relative to the Control trees, is consistent with previous studies at this site which suggested that hydraulic impairment is likely to play a significant role in the much higher levels of drought-induced mortality observed in the TFE (Rowland *et al.*, 2015a). The higher PLC values in trees in the TFE are likely a consequence

of the observed more negative Ψ_{pd} and Ψ_{md} (Fig. 2). The mean PLC of the Control trees during the peak dry season was 16.2%, whereas it was 24.5% on the TFE. Extrapolating from our PLC results, on average 4.1% of the TFE trees have PLC > 50% while only 1% of Control trees cross this threshold during the peak dry season (Table 3). However, when genus-specific effects are considered, the percentage of trees in the TFE crossing the threshold can be as high as 10.1% for *Protium* trees and as low as 2.1% for *Eschweilera* trees. Increased PLC under drought is a distinct signal related to tree mortality (Anderegg *et al.*, 2014; Gaylord *et al.*, 2015; Li *et al.*, 2018). While there is no consensus on what PLC level marks the point of no return from hydraulic failure (Brodribb & Cochard, 2009; Urli *et al.*, 2013), PLCs levels above 50-60 % are very likely to be lethal to trees (Adams *et al.*, 2017). We note that we did not see a change in the HSMP50to support the observed changes in Ψ and PLC. However, we suggest this is because HSMP50 was determined much more by P50 (correlation coefficient of -0.87) than by Ψ_{md} (correlation coefficient of 0.61).

The large variations in PLC which we observed among different genera also confirms previous observations that drought-induced mortality is more likely for specific groups of taxa (Esquivel-Muelbert *et al.*, 2017) . According to our models it is unlikely that any individual tree surviving in the TFE crosses the PLC > 88% threshold in a normal year, such as when we made our measurements. This suggests that either trees which crossed this threshold have already died, as such PLC rates are likely to be unsustainable (Adams *et al.*, 2017; Meinzer & McCulloh, 2013), or that such a threshold is only crossed when there is a particularly intense or long atmospheric drought occurring alongside the soil moisture deficit caused by the TFE treatment. However, it is also possible that our PLC estimates are an underestimate as refilling in small branches and leaves, likely related to foliar water uptake may also occur (Binks *et al.*, 2019).

583 Table 3. Predicted percentage of individuals with PLC > 50% and PLC > 88% in the Control
584 and Throughfall Exclusion Experiment (TFE) plots in the peak of the dry season in Caxiuanã.
585 Predictions are quantiles with PLC > 50% and 88% of the PLC data distribution fitted with a
586 normal variable (PLC ~ N(μ, SD)). The μ (mean) parameter is the genus mean from the
587 linear mixed effects model fitted to PLC data (i.e. fixed intercept plus fitted random
588 coefficient for the genus; see Table 2 and S2) with or without the TFE effect added. The SD
589 (standard deviation) parameter is either assumed to be equal to all groups (SD of all dataset;
590 "Constant SD") or to be taxon-specific (SD of each group).

Genus	Constant SD				Group-specific SD			
	PLC > 50%		PLC > 88%		PLC > 50%		PLC > 88%	
	Control	TFE	Control	TFE	Control	TFE	Control	TFE
All together	1	4.1	0	0	0.1	6.4	0	0
Aspidosperma	1.7	5.9	0	0	3.4	18.7	0	0.9
Eschweilera	0.5	2.1	0	0	0	1.2	0	0
Inga	0.6	2.7	0	0	0	0	0	0
Licania	1.5	5.5	0	0	0.3	0	0	0
Micropholis	0.4	1.7	0	0	0	0	0	0
Minquartia	0.8	3.4	0	0	0	5.5	0	0
Pouteria	0.9	3.6	0	0	0.3	3.7	0	0
Protium	3.3	10.1	0	0	0.3	19	0	0.4
Swartzia	2.2	7.5	0	0	3	3.2	0	0
Syzygiopsis	1.1	4.3	0	0	0	NA	0	NA
Vouacapoua	0.7	3	0	0	0	0.2	0	0

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Size-related changes in hydraulic plasticity

Given the limited observed plasticity in hydraulic traits between the TFE and Control, we evaluated how these traits change with tree size and tree canopy exposure, as taller are more exposed to higher radiation loads and drier atmospheric conditions. Our results demonstrate plastic responses of some hydraulic traits as trees increase in size, but these responses varied significantly among genera. Despite these variations, we find no general relationship between our hydraulic status variables and tree size across all taxa. This may suggest that other, unmeasured traits, such as whole tree water storage, may be playing a greater role in allowing these trees to adjust to the high radiation load and drier atmosphere that is experienced higher up in the canopy, particularly during dry seasons, as discussed above. Variations in unmeasured traits may also be influencing some of the varying size-hydraulic trait relationships we observe among taxa.

Hydraulic efficiency and leaf water supply efficiency (K_s and K_{ls}) were the traits with the greatest plasticity associated with tree size. Increases in K_s are expected as trees grow taller. To cope with increasing resistance to water flow and drier atmospheric conditions with increased height, trees may make changes such as increasing the efficiency of water transport or the investment in xylem tissue, (Bittencourt *et al.*, 2016; Deckmyn, Evans, & Randle, 2006). Vessel diameter, and consequently K_s of the apex and stem of trees also typically increases with tree height, allowing K_s to increase(Olson *et al.*, 2018). Although our data do show that K_s changes with tree height, the direction and degree of this change varies substantially among genera (Fig. 3a). *Inga* and *Eschweilera* had the greatest change in K_s with stem diameter, however in opposite directions. The other genera showed either very limited or no plasticity with tree size. These results may be a consequence of K_s being a function of multiple structural and anatomical properties (Cruiziat *et al.*, 2002; Bittencourt *et al.*, 2016), which may change for other purposes in relation to tree size.

We found no evidence of a significant size x plot interaction in structural hydraulic traits. This contrasts with findings of leaf physiological traits measured on these same trees where the responses to the drought were modulated by crown exposure to light, and thus tree height (Rowland *et al.*, in review). Overall, our results suggest that the hydraulic traits we measured are unlikely to be directly causing the differential drought-induced mortality observed between small and large trees (da Costa *et al.*, 2010; Nepstad *et al.*, 2007; Phillips *et al.*, 2010). In effect, we could not detect any interaction between tree size and TFE treatment on response hydraulic traits, suggesting the surviving small and large trees in the TFE are being equally (negatively) affected by the imposed soil moisture deficit. Either size-dependent drought effects are related to mechanisms not studied or not captured in our dataset or they are not reflected in surviving trees. However, we do note that embolism resistance may contain a genus-dependent interaction with tree size (see next section). If a taxon that becomes less embolism resistant with increasing size has a large biomass or high abundance, such as occurs with *Eschweilera*, observed size dependent mortality may be reflecting taxon-specific patterns, rather than a general community-level pattern.

Does embolism resistance change with tree size? Revisiting Rowland et al. (2015)

Rowland *et al.* (2015a) used data from six genera at our study site to demonstrate that embolism resistance (P50) decreases as tree size increases. This was the first dataset of its kind for mature Amazonian tropical trees. The limited data available for temperate trees is inconclusive on direction and strength of this P50 – size relationship (Domec *et al.*, 2009; Ambrose *et al.*, 2009; Prendin *et al.*, 2018, Olson *et al.*, 2018). Rowland *et al.*, (2015a) presented these results as evidence that hydraulic failure acts as a trigger of drought-induced mortality, with a higher mortality risk in taller drought-stressed trees (which had P50 values

closer to zero). By subsetting our data to include the same genera, we were able to replicate this result using different individuals of the same genus and using a different protocol for hydraulic measurements (Fig. 6), but finding an overall inverse relationship between tree diameter and P50. However, when data for *Eschweilera* were removed from the analysis, the relationship between tree size and P50 disappeared (Fig. S4). The data for *Eschweilera* show a strong decrease in embolism resistance with increasing diameter (Fig. 4). This is relevant more generally as *Eschweilera coriacea*, one of our sampled species, represents one of the hyper-dominant trees across the Amazon. It accounts for 5.1% of Amazon trees and 5.5% of Amazon biomass (Fauset *et al.*, 2015; ter Steege *et al.*, 2013), but which we demonstrate is highly sensitive to drought when it reaches full stature.

Compared to Rowland *et al.* (2015a), our full dataset is comprised of a much larger number of samples, many more tree species and a wider diameter range, including shaded, or partially shaded trees. Using this much larger dataset, we found no evidence of a general decrease in embolism resistance (increasing P50) with tree size. However, we did find evidence of a marginal tree size effect on P50 interacting with genus identity (Fig. 5), suggesting that changes in embolism resistance with tree size exist, but are highly dependent on tree taxonomic identity. Critically, the strength of the P50-tree size relationship was strongly affected by the subset of data used (Fig. S5). By sequentially removing one or two genera from our full dataset, we obtained a significant relationship of embolism resistance with tree size 30% to 50% of the time, respectively. These results indicate that decreases in embolism resistance with tree size are highly dependent on the combination of genera analysed and, at least based on our extensive sampling at this site, particularly on the presence of *Eschweilera* (which has a strong P50-size relationship). Future studies conducted in highly diverse systems should incorporate taxon-sensitivity analyses.

In summary, we tested whether hydraulic traits in Amazon rainforest trees can acclimate to prolonged soil moisture deficit, and if this adjustment varies with tree size. We found low plasticity in hydraulic traits in response to prolonged soil drought. This prevented acclimation in water use from occurring and led to higher levels of hydraulic impairment in the xylem of some of the droughted trees, suggesting hydraulic impairment is likely to contribute directly to the drought-induced mortality observed at this site for some genera. In contrast, we observed some plasticity in hydraulic traits with tree size, but found the plasticity to be heavily genus-dependent, possibly related to mechanisms allowing acclimation to the drier atmospheric environment that a tree's canopy experiences as it grows taller. This study provides new insights into how Amazon rainforest trees may respond to future climate changes, and suggests overall that their capacity to acclimate may be low. However, critically, we also show that taxonomic diversity is likely to play an important and complex role in determining forest-wide hydraulic strategies, acclimation potential and trait relationships, leading to taxon dependent impacts of climate changes and, possible changes in forest composition.

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Author Contributions

PRLB, RSO, MM, PM and LR conceived the research ideas, developed the project and wrote the manuscript. PM and ACLD conceived of and run the experiment. LAG, IC, BPC, DB, SSV, LVF, AR, AAR, JASJ, LR and PRLB contributed to data collection and all authors contributed to manuscript preparation.

Data accessibility

The data that support the findings of this study will be openly available in 2021 at the NERC Centre for Environmental Data Analysis (http://eidc.ceh.ac.uk/) with all data collection funded by UK NERC independent fellowship grant NE/N014022/1.

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