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

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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 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.

KEYWORDS
Amazon rainforest, drought, embolism resistance, hydraulic traits, plant functional diversity, throughfall exclusion, tree size, tropical forest

1 | 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, Anten, Schieving, & Zuidema, 2016). The Amazon forest is predicted to become warmer and drier over the coming decades (Duffy, Brando, Asner, & Field, 2015; Lopes, Chiang, Thompson, & Dracup, 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 till 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, Costa, et al., 2015), 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 (Christoffersen et al., 2016; Eller et al., 2018) 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 and 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 2 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 that 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 4 months of TFE in Malaysia (Inoue et al., 2017), leaf turgor loss point had decreased, however, as photosynthesis was reduced, this also implies that 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 2 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, Lobo-do-Vale, 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. Hydraulic adjustments, such as 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, may confer greater drought tolerance (Cruiziat, Cochard, & Améglio, 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, 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, Costa, et al. (2015) found that resistance to embolism decreases as trees get taller. If correct, these results imply that there are: (a) height-related trade-offs, such as changes in hydraulic efficiency, constraining tree hydraulic safety; and/or (b) large trees overcompensate 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 downregulate embolism resistance; or (c) 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 (Bennett, McDowell, Allen, & Anderson-Teixeira, 2015; McDowell & Allen, 2015; Olson et al., 2018; Rowland, Costa, et al., 2015). 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, Lobo-do-Vale, et al., 2015) found no interaction between tree size, embolism resistance and a drought treatment. However, Rowland, Costa, et al. (2015) were only able to study six 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, predawn 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 data set, 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, Costa, et al. (2015), 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 data set acquired in this study.

2 | METHODS

2.1 | 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's annual rainfall varies between 2,000 and 2,500 mm with a dry season (<120 mm monthly rainfall) from July to December. A TFE experiment was started in 2002, whereby 50% of canopy throughfall is excluded by a plastic panel structure installed at a height of 1–2 m 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 TFE 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) and Rowland, Lobo-do-Vale, et al. (2015). In 2016, the TFE caused a mean reduction of 48% and 56% of soil water content at 10 and 100 cm depth, respectively, in the TFE plot compared to the control plot (Figure 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 Table S1). For each individual, we collected two branches 1.5–2.5 m long from the top of the canopy. The branches were from fourth to sixth order, counting from the leaves. We collected one set of branches before sunrise (04:00–06:00 hr) and used them for measures of embolism resistance and predawn leaf water potential. We collected a second set of branches at midday (11:30–13:30 hr) 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 (WD) 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 min 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.

2.2 | 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.

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

We measured WD on woody sections which were about 40–80 mm in length and 4–7 mm in diameter cut from the branch. We debarked samples, immersed them in water for 24 hr to rehydrate and measured saturated volume using the water displacement method (Pérez-Harguindeguy et al., 2013). We then oven-dried the samples at 60°C for 48 hr 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 ImageJ software (version 1.6.0_20; Schneider, Rasband, & Eliceiri, 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 min in the dark, using an infrared gas analyzer (Li-COR 6400). 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 data set presented in Rowland et al. (under revision) and further leaf gas exchange measurement details can be found therein.

2.4 | Hydraulic efficiency and native embolism

We calculated maximum hydraulic-specific conductivity (K_s) as a measure of xylem hydraulic efficiency and maximum leaf-specific conductivity (K_{ls}) as a measure of leaf water supply capacity. We used the native percentage loss of conductivity of the collected branches (PLC_{nat}) as a measure of native embolism. To estimate these variables, we measured branch xylem hydraulic conductivity before and after flushing to remove emboli. We also measured the leaf area distal to the sample. We used the 1.5–2.5 m long branches collected at midday to measure hydraulic conductivity. We cut 10–15 cm long segments from each branch base underwater and let them rehydrate for 15 min to release tension and avoid artefacts (Venturas, Mackinnon, Jacobsen, & Pratt, 2015). Subsequently, to relax the tension in the branch we cut 1–1.5 m 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 embolized vessels were present in the measured sample. Maximum vessel length, measured on a subsample of 17 branches was 32.7 ± 15.2 cm (55.5 cm maximum), confirming 2 m long branches were sufficient to avoid open vessel artefacts. All samples used for hydraulic measurements were: first- or second-order branches, between 30–55 mm in length and 3–5 mm in diameter and recut underwater with a sharp razor blade before connecting to the apparatus, to ensure all vessels were open at both ends. We measured flow using the pressure drop over a capillary method in an hydraulic apparatus (Espino & Schenk, 2011; Pereira & Mazzafera, 2012; Sperry, Donnelly, & Tyree, 1988), where a capillary of known conductance is connected in series with the sample, and the samples were flushed to remove emboli and estimate maximum conductance (Martin-StPaul et al., 2014). We calculated PLC_{nat} as the ratio of K_{snat} to K_s multiplied by 100. We calculated K_{ls} as the sample hydraulic conductivity (i.e. sample conductance times sample length) after flushing divided by the leaf area distal to the measured sample.

2.5 | 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

TABLE 1 Summary of Caxiuanã hydraulic traits and status values for each genus and separately for Control and throughfall exclusion (TFE) plots. Values presented are mean ± *SD*. Diameter values are maximum and minimum tree diameter at 1.5 m height. Total sample size (i.e. 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	14–34	−1.9 ± 0.38	−3.9 ± 1.1	3.3 ± 3.3	0.23 ± 0.23	13,000 ± 3,900	0.068 ± 0.017	0.61 ± 0.13	−0.91 ± 0.35	−2.2 ± 0.74	−0.083 ± 0.48	20 ± 16
	TFE	16–32	−3.1 ± NA	−4.9 ± NA	0.97 ± 0.97	0.13 ± 0.14	7,500 ± 1,500	0.081 ± 0.025	0.59 ± 0.11	−0.56 ± 0.35	−1.9 ± 0.67	0.49 ± NA	34 ± 26
Eschweilera	Control	17–42	−2.3 ± 1.1	−4.2 ± 2.1	4.1 ± 2.4	0.46 ± 0.37	11,000 ± 5,600	0.089 ± 0.058	0.64 ± 0.073	−0.42 ± 0.11	−1.7 ± 0.39	0.75 ± 1	7.6 ± 2
	TFE	13–30	−2.6 ± 1.1	−4.9 ± 2.3	5.1 ± 1.9	0.49 ± 0.13	11,000 ± 5,400	0.076 ± 0.038	0.65 ± 0.08	−0.58 ± 0.14	−2.1 ± 0.45	0.65 ± 0.96	18 ± 13
Inga	Control	15–39	−2.6 ± 0.98	−4.4 ± 1	6.7 ± 1.9	0.87 ± 0.57	12,000 ± 6,300	0.11 ± 0.052	0.71 ± 0.069	−0.38 ± 0.16	−1.9 ± 0.37	0.82 ± 1.3	14 ± 11
	TFE	13–56	−2.3 ± 1.2	−4 ± 1.9	6.8 ± 3.3	0.66 ± 0.33	15,000 ± 7,100	0.06 ± 0.037	0.67 ± 0.12	−0.44 ± 0.14	−2 ± 0.2	0.37 ± 1.2	18 ± 6.1
Licania	Control	10–28	−2 ± 0.73	−3.5 ± 2	0.86 ± 0.51	0.068 ± 0.03	14,000 ± 3,600	0.057 ± 0.024	0.73 ± 0.031	−0.29 ± 0.11	−1.3 ± 0.27	0.79 ± 0.66	26 ± 11
	TFE	11–28	−3.2 ± 0.51	−6.3 ± 0.93	0.81 ± 0.85	0.083 ± 0.051	10,000 ± 4,800	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	18–63	−1.2 ± 0.63	−2.3 ± 1.2	3.6 ± 0.41	0.35 ± 0.15	13,000 ± 8,800	0.096 ± 0.058	0.6 ± 0.044	−0.52 ± 0.16	−2.4 ± 0.88	−2.3 ± 0.99	11 ± 6.9
	TFE	14–24	−1.1 ± NA	−1.9 ± NA	4.4 ± 1.3	0.2 ± 0.13	11,000 ± 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	12–42	−2 ± 0.89	−3.8 ± 2.2	2.5 ± 0.88	0.24 ± 0.078	11,000 ± 5,000	0.079 ± 0.042	0.71 ± 0.066	−0.52 ± 0.16	−1.5 ± 0.21	0.53 ± 0.91	19 ± 7.5
	TFE	13–41	−1.3 ± 0.87	−3.4 ± 3.5	2.5 ± 1.4	0.32 ± 0.13	11,000 ± 4,200	0.052 ± 0.022	0.68 ± 0.061	−0.61 ± 0.23	−1.4 ± 0.27	−0.19 ± 0.83	21 ± 17
Pouteria	Control	12–59	−2.2 ± 1.2	−4.6 ± 2.8	2.9 ± 1.2	0.52 ± 0.4	7,600 ± 3,900	0.092 ± 0.049	0.75 ± 0.12	−0.63 ± 0.2	−2.2 ± 0.48	−0.073 ± 1.2	19 ± 12
	TFE	10–52	−1.4 ± 0.49	−2.4 ± 1.4	2.3 ± 0.74	0.25 ± 0.21	11,000 ± 4,100	0.067 ± 0.029	0.7 ± 0.088	−0.92 ± 0.32	−2.7 ± 0.82	−1.5 ± 0.74	18 ± 15
Protium	Control	11–38	−2.3 ± 0.72	−5.6 ± 1.7	3.4 ± 1.7	0.36 ± 0.15	9,900 ± 4,100	0.056 ± 0.027	0.57 ± 0.099	−0.65 ± 0.32	−1.5 ± 0.44	0.46 ± 0.63	17 ± 9.6
	TFE	16–38	−2.7 ± 0.78	−4.4 ± 1.4	4.3 ± 1.7	0.55 ± 0.19	8,200 ± 3,700	0.058 ± 0.03	0.56 ± 0.098	−0.62 ± 0.17	−1.7 ± 0.67	0.91 ± 1.2	41 ± 21
Swartzia	Control	22–68	−3.1 ± 1.6	−5.8 ± 2.8	5.2 ± 3.1	0.49 ± 0.26	11,000 ± 3,600	0.11 ± 0.077	0.67 ± 0.038	−0.3 ± 0.067	−2.1 ± 0.52	1.1 ± 1.6	21 ± 16
	TFE	10–64	−3.1 ± 1.1	−5.3 ± 1.2	3.1 ± 1.6	0.37 ± 0.2	9,400 ± 5,700	0.094 ± 0.057	0.72 ± 0.067	−0.46 ± 0.19	−2.2 ± 0.41	0.78 ± 1.4	33 ± 11
Syzygiopsis	Control	14–52	−1.4 ± 0.75	−2.2 ± 1	2.8 ± 0.87	0.4 ± 0.24	8,000 ± 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	24–59	−1.5 ± 0.74	−3.3 ± 1.9	2.8 ± 0.69	0.47 ± 0.058	6,000 ± 730	0.067 ± NA	0.55 ± 0.021	−1 ± 0.6	−2.7 ± 0.72	−1.2 ± 1.5	34 ± NA
Virola	Control	27–48	−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	20–35	−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	12–56	−3 ± 0.59	−5.7 ± 1.9	2.4 ± 2.2	0.27 ± 0.34	9,500 ± 5,400	0.088 ± 0.027	0.65 ± 0.093	−0.45 ± 0.11	−2.1 ± 0.73	0.38 ± 1.1	14 ± 8.9
	TFE	15–57	−2.6 ± 1.6	−4.4 ± 3.2	3.5 ± 2.5	0.44 ± 0.4	9,800 ± 3,700	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

Note: P50 and P88—xylem embolism resistance, xylem water potential leading to 50% and 88% of loss in water transport capacity (MPa); K_s—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—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 (MPa); Ψ_{md}—midday water potential (MPa); HSMP50—hydraulic safety margin to P50 (MPa); PLC—native dry season percentage loss of conductivity (%).

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 min; details in Methods S1 and Bittencourt, Pereira, & Oliveira, 2018). We dehydrated branches using the bench dehydration method. Before each air discharge measurement, we bagged branches for 1 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:

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

2.6 | Data analysis

To test our hypotheses, we evaluated the significance of plot, diameter (a proxy of tree height and canopy exposure—see Figure 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: (a) the model intercept; (b) the relationship between the independent variable and tree diameter; and (c) 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 (AIC) 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 retested Rowland, Costa, et al. (2015) relationship between tree size and P50 by analysing our individual data set alone (30 samples Control and 32 samples TFE), considering only the genera in common with Rowland, Costa, et al. (2015), and then in combination with the Rowland, Costa, et al. (2015) data set (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, Costa, et al. (2015). It should be noted that here we use the pneumatic method for determining P50, whereas Rowland, Costa, et al. (2015) 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 (Figure S3). Finally, to test the effect that species composition of our and the Rowland, Costa, et al. (2015) 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 data set (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, Bates, DebRoy, & Sarkar, 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, Ieno, Walker, Saveliev, and Smith (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 <.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 data set using base package functions for linear models and 'influence.ME' (Nieuwenhuis, te Grotenhuis, & Pelzer, 2012) package functions for mixed effect models. The embolism resistance data set is smaller than the other data sets, 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 .05 and marginally significant when it was between .05 and .10.

3 | RESULTS

3.1 | TFE effects on tree hydraulics

Drought stress caused by 15 years of TFE had limited effect on hydraulic traits (Figure 1; Table 1). Xylem embolism resistance (P50 and P88), K_s, K_{ls}, LS and 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 ($p = .011$), with a decrease of 0.007 mol m^{-2} s^{-1} (Figure 1f; all coefficients are presented in Table S3).

The TFE manipulation had a highly significant effect on all hydraulic status variables (Figure 2), except on hydraulic safety margin (HSMP50; Table 2). TFE caused a decrease in peak dry season Ψ_{md} of −0.19 MPa ($p = .02$) with a high intrageneric variability (*SD* of 0.38 MPa for random genus effect on intercept and an average increase in percentage loss of conductance of 8.3% (PLC; $p = .001$; see coefficients in Table S3). Predawn water potential (Ψ_{pd}) was affected by the TFE with a reduction of −0.18 MPa ($p < .001$), equal to a 35% decrease relative to control, which was further modulated by random genus-specific effects ($p < .001$; Figure 3; see also the following paragraph), leading to a TFE maximum effect of reducing predawn water potential by 0.52 MPa in *Micropholis*.

3.2 | 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 hydraulic traits (Table 2). For K_s, the effect of increasing diameter was genus-dependent, as indicated by a significant random genus effect on diameter ($p < .001$; 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 (Figure 4a; Table S3). For K_{ls}, stem diameter was significant

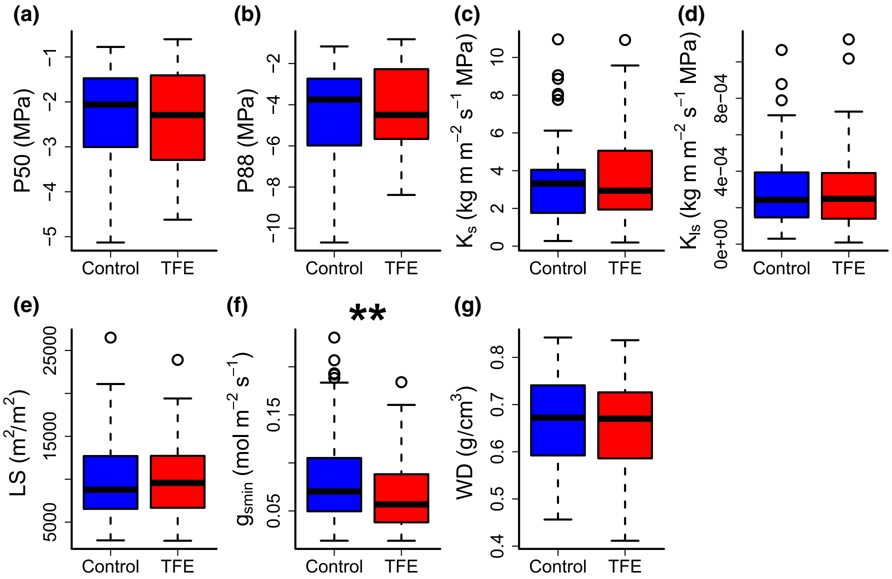

FIGURE 1 Hydraulic trait responses on trees surviving after 15 years of throughfall exclusion (TFE) in Caxiuanã. (a, b) P50 and P88—xylem embolism resistance (MPa); (c) K_s—maximum hydraulic-specific conductivity (kg m m^{-2} s^{-1} MPa^{-1}); (d) K_{ls}—maximum hydraulic leaf-specific conductivity (kg m m^{-2} s^{-1} MPa^{-1}); (e) LS—leaf to sapwood area ratio (m^2/m^2); (f) g_{smin}—minimum stomatal conductance (mol m^{-2} s^{-1}); (g) WD—wood density (g/cm^3). We consider changes in structural hydraulic traits (i.e. plot effect models) as plasticity in response to drought. The box represents quartiles 1 and 3, with the central line indicating the median. Whiskers are either of maximum value or 1.5 interquartile range above the quartile 3, when outliers are present. Traits for which plot had a significant effect are marked with *($p < .05$), **($p < .01$) and ***($p < .001$). *p*-values are from mixed effects analysis (see Table 2 for models and Section 2.6) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Results of linear mixed effect models of plot (Control vs. TFE) and tree diameter effects on hydraulic traits and hydraulic status. The combination of the tested random effects is the genus effect on intercept only, and/or on plot and diameter slopes (see Section 2.6 for details). Values for fixed effects are fitted parameter ± *SE*; 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 the 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*			.16
	P88	−4.22 ± 0.21						0
	K_s	3.31 ± 0.48			2.29***		0.065***	.53
	K_{ls}	0.21 ± 0.06		0.005 ± 0.001**	0.11**			.26
	LS	10,266 ± 439.6						0
	g_{smin}	0.083 ± 0.005	−0.018 ± 0.007**					.043
	WD	0.65 ± 0.02			0.06***			.36
Status	Ψ_{pd}	−0.52 ± 0.05	−0.18 ± 0.07***		0.13***	0.19***		.49
	Ψ_{md}	−1.88 ± 0.12	−0.2 ± 0.09*		0.38***			.35
	HSMP50	0.14 ± 0.24			0.71***			.26
	PLC	16.2 ± 2.3	8.3 ± 2.5**		5.0*			.2

Note: P50—xylem embolism resistance (MPa); Ψ_{pd}—predawn water potential (MPa); Ψ_{md}—midday water potential (MPa); HSMP50—hydraulic safety margin to P50 (MPa); PLC—native dry season percentage loss of conductivity (%); K_s—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—leaf to sapwood area ratio (m^2/m^2); g_{smin}—minimum stomatal conductance (mol m^{-2} s^{-1}); WD—wood density (g/cm^3).

*$p < .05$; **$p < .01$; ***$p < .001$.

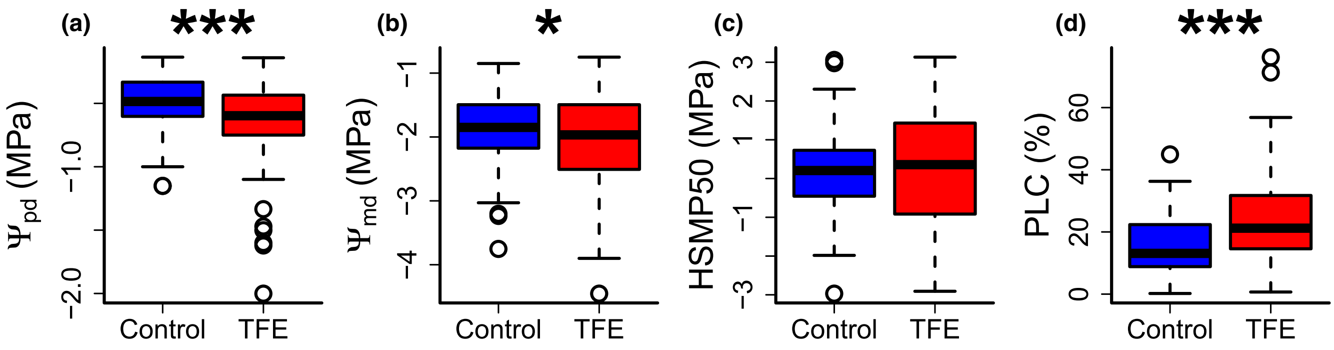

FIGURE 2 Hydraulic status of trees surviving after 15 years of throughfall exclusion (TFE) in the peak of the dry season in Caxiuanã. (a) Ψ_{pd}—predawn water potential (MPa); (b) Ψ_{md}—midday water potential (MPa); (c) HSMP50—hydraulic safety margin to P50; (d) PLC—native dry season percentage loss of conductivity (% maximum conductance). We consider homoeostasis 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 *($p < .05$), **($p < .01$) and ***($p < .001$). *p*-values are from mixed effects analysis (see Table 2 for models and Section 2.6) [Colour figure can be viewed at wileyonlinelibrary.com]

(Table 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 (Figure 4b). The random genus effect on the diameter–P50 relationship was significant ($p = .035$; Table S2; Figure 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 ($p = .29$, Table S2) but the interaction effect between diameter and plot was marginally significant ($p = .066$; Table S2). We found no evidence of any interaction of plot and tree stem diameter with hydraulic traits or with hydraulic status (Table 2; Table S2).

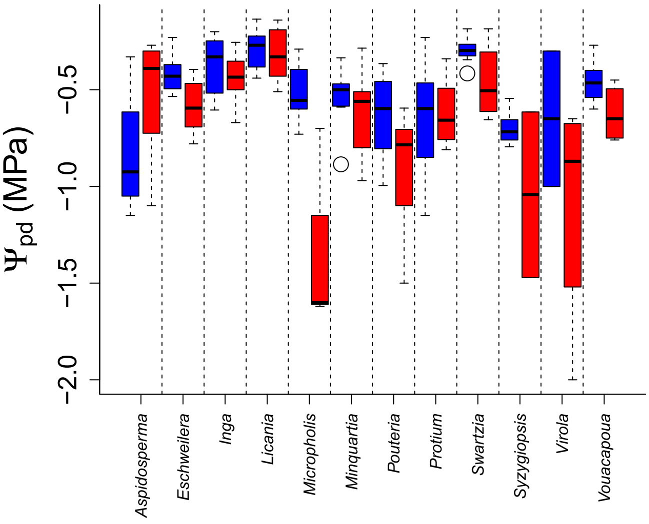

FIGURE 3 Throughfall exclusion (TFE) effect on predawn water potential of the studied genus (Ψ_{pd}), showing the genus-specific variation in Ψ_{pd} response to TFE. TFE had both a fixed effect of reducing Ψ_{pd} and a random effect (genus-specific effect) on Ψ_{pd} (see Table 1). Red and blue boxplots are data from the TFE plot and control plots respectively. The box represents quartiles 1 and 3, with the central line indicating the mean. Whiskers are either maximum value or 1.5× interquartile range above the quartile 3, when outliers are present [Colour figure can be viewed at wileyonlinelibrary.com]

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

Most of the hydraulic traits and hydraulic status variables 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 (Figure 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 an MEM for Ψ_{pd}, with plot as a fixed variable, it had an *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.

3.4 | Revisiting Rowland et al. (2015)

Rowland, Costa, et al. (2015) presented the first data set of P50 for Amazon trees at this site (reproduced in Figure 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, Costa, et al. (2015), 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, Costa, et al. (2015), and the interaction between genus and diameter was also significant (p = .046; Figure 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

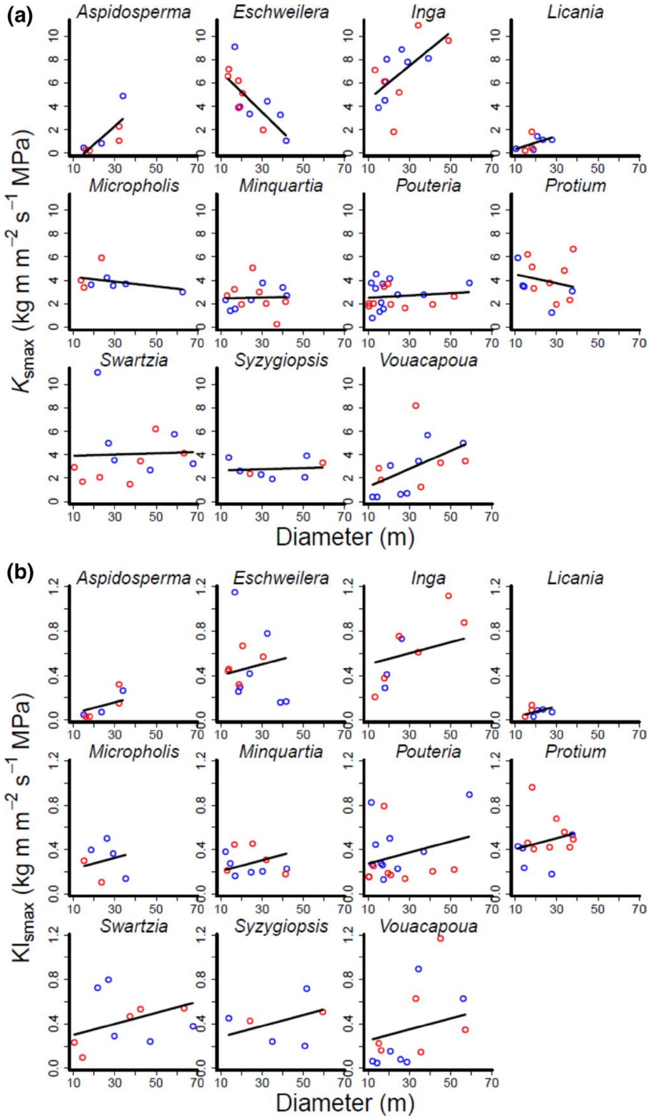

FIGURE 4 Diameter effects on xylem-specific conductivity (K_s; a) and leaf-specific conductivity (K_{ls}; b) for each studied genus. Fitted lines show the fitted mixed effects model (see Table 1 and Section 3), modified according to the effect of the random variable (genus) on either the intercept or the slope of the relationship of diameter to the independent variable (K_s or K_{ls}). For K_s, the interaction between diameter and genus random effect is significant (p < .001), resulting in different slopes for each genus. For K_{ls}, the interaction is significant (p = .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 throughfall exclusion plots respectively [Colour figure can be viewed at wileyonlinelibrary.com]

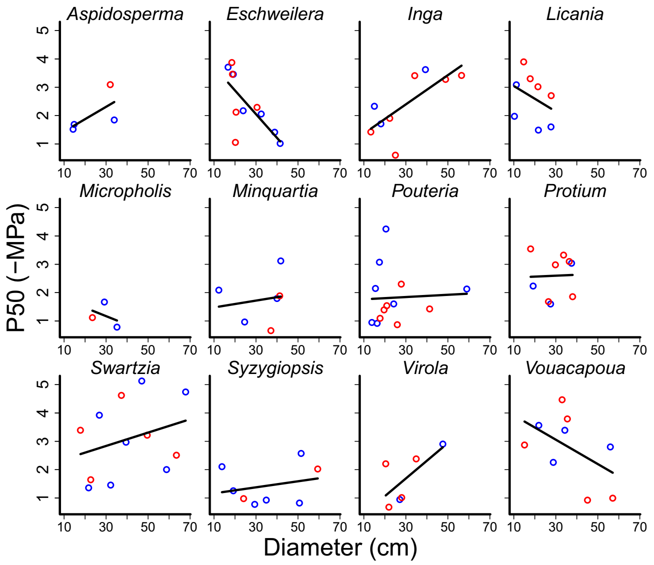

FIGURE 5 Diameter effects on embolism resistance (P50; presented as positive values in −MPa) for each studied genus. We modelled the variables using linear models with diameter, genus and their interaction as fixed effects. The model is marginally significant (*p* = .066). The fitted line is the fitted, marginally significant model, for each genus. Blue and red points are data from Control and throughfall exclusion plots respectively (there was no plot effect on P50 so data were pooled) [Colour figure can be viewed at wileyonlinelibrary.com]

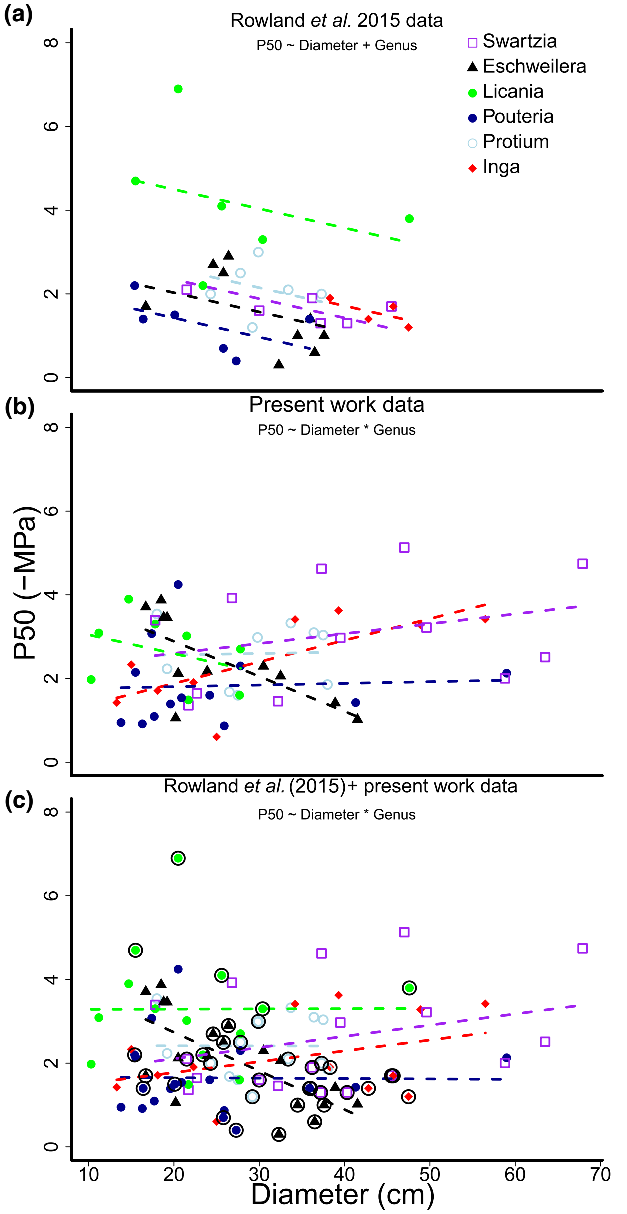

FIGURE 6 Relationship between embolism resistance (P50) and tree diameter using the same subset of genera as analysed in Rowland, Costa, et al. (2015). (a) The original data and analysis of Rowland, Costa, et al. (2015). (b) The data from this work only. (c) The combined data sets from (a) and (b). Linear 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, Costa, et al. (2015), we display the *Y*-axis as the negative of P50 (−P50). The same figure and analysis is presented in Figure 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, Costa, et al. (2015) [Colour figure can be viewed at wileyonlinelibrary.com]

or an almost negligible diameter effect (Figure 6). When the two data sets are combined, the same pattern remains, with P50 being significantly affected by the interaction between diameter and genus and a slightly more significant diameter effect (*p* = .023; Figure 6c). However, after removal of a single genus with a strong positive relationship between P50 and diameter (*Eschweilera*) from the combined data sets, both the diameter interaction with genus and the diameter effect disappear (*p* = .62 and .14; Figure S4c). The same occurs if *Eschweilera* is removed from the non-combined data sets (Figure S4a,b).

The results from the linear fixed effect models using the Rowland, Costa, et al. (2015) subset of the data are partially consistent with the results for this study is full data set with all 12 genera, whereby a diameter interaction with genus is found to be marginally significant (*p* = .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 data set (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 (Figure 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 data set, which also strongly influenced whether P50 increased, decreased or did not change with increasing tree size (Figure S5). On the other hand, neither the present results nor those of Rowland, Costa, et al. (2015) show any significant plot (i.e. treatment) effects on P50.

4 | DISCUSSION

We analysed the effects of a long-term (>15 years) TFE 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, Costa, et al., 2015), which should have reduced overall intertree competition for soil water, soil water content is significantly lower in the TFE plot relative to the control plot (Figure 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 (Figure 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 et al., 2010; Rowland, Costa, et al., 2015).

4.1 | 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, Costa, et al., 2015) 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 the 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, Williams, Lourdes Ruivo, Costa, & Meir, 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, Costa, et al., 2015). The higher PLC values in trees in the TFE are likely a consequence of the observed more negative Ψ_{pd} and Ψ_{md} (Figure 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, Anderegg, Berry, & Field, 2014; Gaylord, Kolb, & McDowell, 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), PLC 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 HSMP50 to 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).

4.2 | 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

TABLE 3 Predicted percentage of individuals with PLC > 50% and PLC > 88% in the Control and throughfall exclusion experiment (TFE) plots in the peak of the dry season in Caxiuanã. Predictions are quantiles with PLC > 50% and 88% of the percentage loss of conductivity (PLC) data distribution fitted with a normal variable (PLC ~ *N*(μ, *SD*)). The μ (mean) parameter is the genus mean from the linear mixed effects model fitted to PLC data (i.e. fixed intercept plus fitted random coefficient for the genus; see Table 2; Table S2) with or without the TFE effect added. The *SD* (standard deviation) parameter is either assumed to be equal to all groups (*SD* of all data set; 'Constant *SD*') or to be taxon-specific (*SD* of each group)

	Constant *SD*				Group-specific *SD*			
	PLC > 50%		PLC > 88%		PLC > 50%		PLC > 88%	
Genus	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

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, Pereira, & Oliveira, 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 (Figure 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 (Bittencourt et al., 2016; Cruiziat et al., 2002), which may change for other purposes in relation to tree size.

We found no evidence of a significant size × plot interaction in structural hydraulic traits. This contrasts with findings of leaf physiological traits measured on the same trees where the responses to the drought were modulated by crown exposure to light, and thus tree height (Rowland et al., under revision). 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, Tohver, Ray, Moutinho, & Cardinot, 2007; Phillips et al., 2010). In effect, we could not detect any interaction between tree size and TFE treatment on response hydraulic traits, suggesting that 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 data set 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 that occuring with *Eschweilera*, observed size dependent mortality may be reflecting taxon-specific patterns, rather than a general community-level pattern.

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

Rowland, Costa, et al. (2015) used data from six genera at our study site to demonstrate that embolism resistance (P50) decreases as tree size increases. This was the first data set 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 (Ambrose et al., 2009; Domec, Warren, Meinzer, & Lachenbruch, 2009; Olson et al., 2018; Prendin et al., 2018). Rowland, Costa, et al. (2015) 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 using a different protocol for hydraulic measurements (Figure 6), but found 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 (Figure S4). The data for *Eschweilera* show a strong decrease in embolism resistance with increasing diameter (Figure 4). This is relevant more generally as *Eschweilera coriacea*, one of our sampled species, represents one of the hyperdominant trees across the Amazon accounting 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, Costa, et al. (2015), our full data set 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 data set, 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 (Figure 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 (Figure S5). By sequentially removing one or two genera from our full data set, we obtained a significant relationship of embolism resistance with tree size 30%–50% of the time respectively. These results indicate that decreases in embolism resistance with tree size are highly dependent on the combination of the 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, 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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CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

P.R.L.B., R.S.O., M.M., P.M. and L.R. conceived the research ideas, developed the project and wrote the manuscript. P.M. and A.C.L.D. conceived and performed experiment. L.A.G., I.C., B.P.C., D.B., S.S.V., L.V.F., A.R., A.A.R., J.A.S.J., L.R. and P.R.L.B. contributed to data collection and all authors contributed to manuscript preparation.

DATA AVAILABILITY STATEMENT

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 the UK NERC independent fellowship grant NE/N014022/1.

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REFERENCES

Adams, H. D., Zeppel, M. J. B., Anderegg, W. R. L., Hartmann, H., Landhäusser, S. M., Tissue, D. T., … McDowell, N. G. (2017). A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature Ecology & Evolution*, *1*(9), 1285–1291. https://doi.org/10.1038/s41559-017-0248-x

Ambrose, A. R., Sillett, S. C., & Dawson, T. E. (2009). Effects of tree height on branch hydraulics, leaf structure and gas exchange in California redwoods. *Plant, Cell & Environment*, *32*(7), 743–757. https://doi.org/10.1111/j.1365-3040.2009.01950.x

Anderegg, W. R. L., Anderegg, L. D. L., Berry, J. A., & Field, C. B. (2014). Loss of whole-tree hydraulic conductance during severe drought and multi-year forest die-off. *Oecologia*, *175*(1), 11–23. https://doi.org/10.1007/s00442-013-2875-5

Awad, H., Barigah, T., Badel, E., Cochard, H., & Herbette, S. (2010). Poplar vulnerability to xylem cavitation acclimates to drier soil conditions. *Physiologia Plantarum*. https://doi.org/10.1111/j.1399-3054.2010.01367.x

Barros, F. D. V., Bittencourt, P. R. L., Brum, M., Restrepo-Coupe, N., Pereira, L., Teodoro, G. S., … Oliveira, R. S. (2019). Hydraulic traits explain differential responses of Amazonian forests to the 2015 El Niño-induced drought. *New Phytologist*, *223*(3), 1253–1266. https://doi.org/10.1111/nph.15909

Barton, K. (2016). MuMIn: Multi-model inference. Retrieved from https://cran.r-project.org/web/packages/MuMIn/index.html

Beikircher, B., & Mayr, S. (2009). Intraspecific differences in drought tolerance and acclimation in hydraulics of *Ligustrum vulgare* and *Viburnum lantana*. *Tree Physiology*, *29*(6), 765–775. https://doi.org/10.1093/treephys/tpp018

Bennett, A. C., McDowell, N. G., Allen, C. D., & Anderson-Teixeira, K. J. (2015). Larger trees suffer most during drought in forests worldwide. *Nature Plants*, *1*(10), 15139. https://doi.org/10.1038/nplants.2015.139

Binks, O., Meir, P., Rowland, L., Costa, A. C. L., Vasconcelos, S. S., Oliveira, A. A. R., … Mencuccini, M. (2016). Plasticity in leaf-level water relations of tropical rainforest trees in response to experimental drought. *New Phytologist*, *211*(2), 477–488. https://doi.org/10.1111/nph.13927

Binks, O., Mencuccini, M., Rowland, L., Costa, A. C. L., Carvalho, C. J. R., Bittencourt, P., … Meir, P. (2019). Foliar water uptake in Amazonian trees: Evidence and consequences. *Global Change Biology*, *25*(8), 2678–2690. https://doi.org/10.1111/gcb.14666

Bittencourt, P. R., Pereira, L., & Oliveira, R. S. (2016). On xylem hydraulic efficiencies, wood space-use and the safety–efficiency tradeoff. *New Phytologist*, *211*(4), 1152–1155. https://doi.org/10.1111/nph.14044

Bittencourt, P., Pereira, L., & Oliveira, R. (2018). Pneumatic method to measure plant xylem embolism. *BIO-PROTOCOL*, *8*(20). https://doi.org/10.21769/BioProtoc.3059

Brodribb, T. J., & Cochard, H. (2009). Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiology*, *149*(1), 575–584. https://doi.org/10.1104/pp.108.129783

Brum, M., Vadeboncoeur, M. A., Ivanov, V., Asbjornsen, H., Saleska, S., Alves, L. F., … Oliveira, R. S. (2019). Hydrological niche segregation defines forest structure and drought tolerance strategies in a seasonal Amazon forest. *Journal of Ecology*, *107*(1), 318–333. https://doi.org/10.1111/1365-2745.13022

Choat, B., Brodribb, T. J., Brodersen, C. R., Duursma, R. A., López, R., & Medlyn, B. E. (2018). Triggers of tree mortality under drought. *Nature*, *558*(7711), 531–539. https://doi.org/10.1038/s41586-018-0240-x

Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., … Zanne, A. E. (2012). Global convergence in the vulnerability of forests to drought. *Nature*, *491*, 752–755. https://doi.org/10.1038/nature11688

Christoffersen, B. O., Gloor, M., Fauset, S., Fyllas, N. M., Galbraith, D. R., Baker, T. R., … Meir, P. (2016). Linking hydraulic traits to tropical forest function in a size-structured and trait-driven model (TFS vol 1-Hydro). *Geoscientific Model Development Discussions*, *1–60*, https://doi.org/10.5194/gmd-2016-128

Ciemer, C., Boers, N., Hirota, M., Kurths, J., Müller-Hansen, F., Oliveira, R. S., & Winkelmann, R. (2019). Higher resilience to climatic disturbances in tropical vegetation exposed to more variable rainfall. *Nature Geoscience*, *12*(3), 174–179. https://doi.org/10.1038/s41561-019-0312-z

Corlett, R. T. (2016). The impacts of droughts in tropical forests. *Trends in Plant Science*, *21*(7), 584–593. https://doi.org/10.1016/j.tplants.2016.02.003

Cruiziat, P., Cochard, H., & Améglio, T. (2002). Hydraulic architecture of trees: Main concepts and results. *Annals of Forest Science*, *59*(7), 723–752. https://doi.org/10.1051/forest:2002060

da Costa, A. C. L., Galbraith, D., Almeida, S., Portela, B. T. T., da Costa, M., de Athaydes Silva Junior, J., … Meir, P. (2010). Effect of 7 yr of experimental drought on vegetation dynamics and biomass storage of an eastern Amazonian rainforest. *New Phytologist*, *187*(3), 579–591. https://doi.org/10.1111/j.1469-8137.2010.03309.x

da Costa, A. C. L., Metcalfe, D. B., Doughty, C. E., de Oliveira, A. A. R., Neto, G. F. C., da Costa, M. C., … Malhi, Y. (2014). Ecosystem respiration and net primary productivity after 8–10 years of experimental through-fall reduction in an eastern Amazon forest. *Plant Ecology & Diversity*, *7*(1–2), 7–24. https://doi.org/10.1080/17550874.2013.798366

Dayer, S., Peña, J. P., Gindro, K., Torregrosa, L., Voinesco, F., Martínez, L., … Zufferey, V. (2017). Changes in leaf stomatal conductance, petiole hydraulics and vessel morphology in grapevine (*Vitis vinifera* cv. Chasselas) under different light and irrigation regimes. *Functional Plant Biology*, *44*(7), 679. https://doi.org/10.1071/FP16041

Deckmyn, G., Evans, S. P., & Randle, T. J. (2006). Refined pipe theory for mechanistic modeling of wood development. *Tree Physiology*, *26*(6), 703–717. https://doi.org/10.1093/treephys/26.6.703

Delzon, S. (2015). New insight into leaf drought tolerance. *Functional Ecology*, *29*, 1247–1249. https://doi.org/10.1111/1365-2435.12500

Domec, J.-C., Warren, J. M., Meinzer, F. C., & Lachenbruch, B. (2009). Safety factors for xylem failure by implosion and air-seeding within roots, trunks and branches of young and old conifer trees. *IAWA Journal*, *30*(2), 101–120. https://doi.org/10.1163/22941932-90000207

Duffy, P. B., Brando, P., Asner, G. P., & Field, C. B. (2015). Projections of future meteorological drought and wet periods in the Amazon. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(43), 13172–13177. https://doi.org/10.1073/pnas.1421010112

Egea, G., González-real, M. M., Baille, A., Nortes, P. A., Conesa, M. R., & Ruiz-salleres, I. (2012). Effects of water stress on irradiance acclimation of leaf traits in almond trees. *Tree Physiology*, *32*(4), 450–463. https://doi.org/10.1093/treephys/tps016

Eller, C. B., de V Barros, F., Bittencourt, P. R. L., Rowland, L., Mencuccini, M., & Oliveira, R. S. (2018). Xylem hydraulic safety and construction costs determine tropical tree growth: Tree growth vs hydraulic safety trade-off. *Plant, Cell & Environment*, *41*(3), 548–562. https://doi.org/10.1111/pce.13106

Espino, S., & Schenk, H. J. (2011). Mind the bubbles: Achieving stable measurements of maximum hydraulic conductivity through woody plant samples. *Journal of Experimental Botany*, *62*(3), 1119–1132. https://doi.org/10.1093/jxb/erq338

Esquivel-Muelbert, A., Baker, T. R., Dexter, K. G., Lewis, S. L., ter Steege, H., Lopez-Gonzalez, G., … Phillips, O. L. (2017). Seasonal drought limits tree species across the Neotropics. *Ecography*, *40*(5), 618–629. https://doi.org/10.1111/ecog.01904

Fauset, S., Johnson, M. O., Gloor, M., Baker, T. R., Monteagudo, M. A., Brienen, R. J. W., … Phillips, O. L. (2015). Hyperdominance in Amazonian forest carbon cycling. *Nature Communications*, *6*, 6857. https://doi.org/10.1038/ncomms7857

Fisher, R. A., Williams, M., da Costa, A. L., Malhi, Y., da Costa, R. F., Almeida, S., & Meir, P. (2007). The response of an Eastern Amazonian rain forest to drought stress: Results and modelling analyses from a throughfall exclusion experiment. *Global Change Biology*, *13*(11), 2361–2378. https://doi.org/10.1111/j.1365-2486.2007.01417.x

Fisher, R. A., Williams, M., de Lourdes Ruivo, M., de Costa, A. L., & Meir, P. (2008). Evaluating climatic and soil water controls on evapotranspiration at two Amazonian rainforest sites. *Agricultural and Forest Meteorology*, *148*(6–7), 850–861. https://doi.org/10.1016/j.agrformet.2007.12.001

Galbraith, D., Levy, P. E., Sitch, S., Huntingford, C., Cox, P., Williams, M., & Meir, P. (2010). Multiple mechanisms of Amazonian forest biomass losses in three dynamic global vegetation models under climate change. *New Phytologist*, *187*(3), 647–665. https://doi.org/10.1111/j.1469-8137.2010.03350.x

Gaylord, M. L., Kolb, T. E., & McDowell, N. G. (2015). Mechanisms of piñon pine mortality after severe drought: A retrospective study of mature trees. *Tree Physiology*, *35*(8), 806–816. https://doi.org/10.1093/treephys/tpv038

Inoue, Y., Ichie, T., Kenzo, T., Yoneyama, A., Kumagai, T., & Nakashizuka, T. (2017). Effects of rainfall exclusion on leaf gas exchange traits and osmotic adjustment in mature canopy trees of *Dryobalanops aromatica* (Dipterocarpaceae) in a Malaysian tropical rain forest. *Tree Physiology*, *37*(10), 1301–1311. https://doi.org/10.1093/treephys/tpx053

Kumagai, T., Kuraji, K., Noguchi, H., Tanaka, Y., Tanaka, K., & Suzuki, M. (2001). Vertical profiles of environmental factors within tropical rainforest, Lambir Hills National Park, Sarawak, Malaysia. *Journal of Forest Research*, *6*(4), 257–264. https://doi.org/10.1007/BF02762466

Li, X., Blackman, C. J., Rymer, P. D., Quintans, D., Duursma, R. A., Choat, B., … Tissue, D. T. (2018). Xylem embolism measured retrospectively is linked to canopy dieback in natural populations of *Eucalyptus piperita* following drought. *Tree Physiology*, *38*(8), 1193–1199. https://doi.org/10.1093/treephys/tpy052

Lopes, A. V., Chiang, J. C. H., Thompson, S. A., & Dracup, J. A. (2016). Trend and uncertainty in spatial-temporal patterns of hydrological droughts in the Amazon basin: Hydrological droughts in the Amazon. *Geophysical Research Letters*, *43*(7), 3307–3316. https://doi.org/10.1002/2016GL067738

Malhi, Y., Aragao, L. E. O. C., Galbraith, D., Huntingford, C., Fisher, R., Zelazowski, P., … Meir, P. (2009). Exploring the likelihood and mechanism of a climate-change-induced dieback of the Amazon rainforest. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(49), 20610–20615. https://doi.org/10.1073/pnas.0804619106

Marengo, J. A., Souza, C. M., Thonicke, K., Burton, C., Halladay, K., Betts, R. A., … Soares, W. R. (2018). Changes in climate and land use over the Amazon region: Current and future variability and trends. *Frontiers in Earth Science*, *6*, https://doi.org/10.3389/feart.2018.00228

Martin-StPaul, N. K., Longepierre, D., Huc, R., Delzon, S., Burlett, R., Joffre, R., … Cochard, H. (2014). How reliable are methods to assess xylem vulnerability to cavitation? The issue of 'open vessel' artifact in oaks. *Tree Physiology*, *34*(8), 894–905. https://doi.org/10.1093/treephys/tpu059

Maseda, P. H., & Fernandez, R. J. (2006). Stay wet or else: Three ways in which plants can adjust hydraulically to their environment. *Journal of Experimental Botany*, *57*(15), 3963–3977. https://doi.org/10.1093/jxb/erl127

McDowell, N. G., & Allen, C. D. (2015). Darcy's law predicts widespread forest mortality under climate warming. *Nature Climate Change*, *5*(7), 669–672. https://doi.org/10.1038/nclimate2641

Meinzer, F. C., & McCulloh, K. A. (2013). Xylem recovery from drought-induced embolism: Where is the hydraulic point of no return? *Tree Physiology*, *33*(4), 331–334. https://doi.org/10.1093/treephys/tpt022

Meir, P., Mencuccini, M., Binks, O., da Costa, A. L., Ferreira, L., & Rowland, L. (2018). Short-term effects of drought on tropical forest do not fully predict impacts of repeated or long-term drought: Gas exchange versus growth. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1760), 20170311. https://doi.org/10.1098/rstb.2017.0311

Meir, P., Wood, T. E., Galbraith, D. R., Brando, P. M., Da Costa, A. C. L., Rowland, L., & Ferreira, L. V. (2015). Threshold responses to soil moisture deficit by trees and soil in tropical rain forests: Insights from field experiments. *BioScience*, *65*(9), 882–892. https://doi.org/10.1093/biosci/biv107

Nepstad, D. C., Tohver, I. M., Ray, D., Moutinho, P., & Cardinot, G. (2007). Mortality of large trees and lianas following experimental drought in an Amazon forest. *Ecology*, *88*(9), 2259–2269. https://doi.org/10.1890/06-1046.1

Nieuwenhuis, R., te Grotenhuis, M., & Pelzer, B. (2012). Influence.ME: Tools for detecting influential data in mixed effects models. *R Journal*, *4*(2), 38. https://doi.org/10.32614/rj-2012-011

Olson, M. E., Soriano, D., Rosell, J. A., Anfodillo, T., Donoghue, M. J., Edwards, E. J., … Méndez-Alonzo, R. (2018). Plant height and hydraulic vulnerability to drought and cold. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(29), 7551–7556. https://doi.org/10.1073/pnas.1721728115

Pammenter, N. W., & Vander Willigen, C. (1998). A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiology*, *18*(8–9), 589–593. https://doi.org/10.1093/treephys/18.8-9.589

Pereira, L., Bittencourt, P. R. L., Oliveira, R. S., Junior, M. B. M., Barros, F. V., Ribeiro, R. V., & Mazzafera, P. (2016). Plant pneumatics: Stem air flow is related to embolism – New perspectives on methods in plant hydraulics. *New Phytologist*, *211*(1), 357–370. https://doi.org/10.1111/nph.13905

Pereira, L., & Mazzafera, P. (2012). A low cost apparatus for measuring the xylem hydraulic conductance in plants. *Bragantia*, *71*(4), 583–587. https://doi.org/10.1590/S0006-87052013005000006

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., … Cornelissen, J. H. C. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, *61*(3), 167. https://doi.org/10.1071/BT12225

Phillips, O. L., van der Heijden, G., Lewis, S. L., López-González, G., Aragão, L. E. O. C., Lloyd, J., … Vilanova, E. (2010). Drought-mortality relationships for tropical forests. *New Phytologist*, *187*(3), 631–646. https://doi.org/10.1111/j.1469-8137.2010.03359.x

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2014). *Nlme: Linear and nonlinear mixed effects models*. R package version 3.1-118. Retrieved from http://CRAN.R-project.org/package=nlme

Prendin, A. L., Mayr, S., Beikircher, B., von Arx, G., & Petit, G. (2018). Xylem anatomical adjustments prioritize hydraulic efficiency over safety as Norway spruce trees grow taller. *Tree Physiology*, *38*(8), 1088–1097. https://doi.org/10.1093/treephys/tpy065

R Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rowland, L., da Costa, A., Oliveira, R., Bittencourt, P., Giles, A., Coughlin, I., … Meir, P. (under revision). The response of carbon assimilation and storage to long-term drought in tropical trees is dependent on light availability. *Functional Ecology*.

Rowland, L., da Costa, A. C. L., Galbraith, D. R., Oliveira, R. S., Binks, O. J., Oliveira, A. A. R., … Meir, P. (2015). Death from drought in tropical forests is triggered by hydraulics not carbon starvation. *Nature*, *528*(7580), 119–122. https://doi.org/10.1038/nature15539

Rowland, L., Lobo-do-Vale, R. L., Christoffersen, B. O., Melém, E. A., Kruijt, B., Vasconcelos, S. S., … Meir, P. (2015). After more than a decade of soil moisture deficit, tropical rainforest trees maintain photosynthetic capacity, despite increased leaf respiration. *Global Change Biology*, *21*(12), 4662–4672. https://doi.org/10.1111/gcb.13035

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Scholz, F. G., Bucci, S. J., Goldstein, G., Meinzer, F. C., Franco, A. C., & Miralles-Wilhelm, F. (2007). Biophysical properties and functional significance of stem water storage tissues in Neotropical savanna trees. *Plant, Cell & Environment*, *30*(2), 236–248. https://doi.org/10.1111/j.1365-3040.2006.01623.x

Schuldt, B., Leuschner, C., Horna, V., Moser, G., Köhler, M., van Straaten, O., & Barus, H. (2011). Change in hydraulic properties and leaf traits in a tall rainforest tree species subjected to long-term throughfall exclusion in the perhumid tropics. *Biogeosciences*, *8*(8), 2179–2194. https://doi.org/10.5194/bg-8-2179-2011

Smith, N. G., & Dukes, J. S. (2013). Plant respiration and photosynthesis in global-scale models: Incorporating acclimation to temperature and CO_2. *Global Change Biology*, *19*(1), 45–63. https://doi.org/10.1111/j.1365-2486.2012.02797.x

Sperry, J. S., Donnelly, J. R., & Tyree, M. T. (1988). A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell and Environment*, *11*(1), 35–40. https://doi.org/10.1111/j.1365-3040.1988.tb01774.x

Sperry, J. S., & Love, D. M. (2015). What plant hydraulics can tell us about responses to climate-change droughts. *New Phytologist*, *207*(1), 14–27. https://doi.org/10.1111/nph.13354

Sterck, F., Anten, N. P. R., Schieving, F., & Zuidema, P. A. (2016). Trait acclimation mitigates mortality risks of tropical canopy trees under global warming. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.00607

ter Steege, H., Pitman, N. C. A., Sabatier, D., Baraloto, C., Salomao, R. P., Guevara, J. E., … Silman, M. R. (2013). Hyperdominance in the Amazonian tree flora. *Science*, *342*(6156), 1243092. https://doi.org/10.1126/science.1243092

Thomas, R., Lello, J., Medeiros, R., Pollard, A., Robinson, P., Seward, A., … Vaughan, I. (2017). *Data analysis with R statistical software: A guidebook for scientists*. Cardiff, UK: Eco-Explore.

Tng, D. Y. P., Apgaua, D. M. G., Ishida, Y. F., Mencuccini, M., Lloyd, J., Laurance, W. F., & Laurance, S. G. W. (2018). Rainforest trees respond to drought by modifying their hydraulic architecture. *Ecology and Evolution*. https://doi.org/10.1002/ece3.4601

Tomasella, M., Beikircher, B., Häberle, K.-H., Hesse, B., Kallenbach, C., Matyssek, R., & Mayr, S. (2018). Acclimation of branch and leaf hydraulics in adult *Fagus sylvatica* and *Picea abies* in a forest through-fall exclusion experiment. *Tree Physiology*, *38*(2), 198–211. https://doi.org/10.1093/treephys/tpx140

Urli, M., Porte, A. J., Cochard, H., Guengant, Y., Burlett, R., & Delzon, S. (2013). Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. *Tree Physiology*, *33*(7), 672–683. https://doi.org/10.1093/treephys/tpt030

Venturas, M. D., Mackinnon, E. D., Jacobsen, A. L., & Pratt, R. B. (2015). Excising stem samples underwater at native tension does not induce xylem cavitation: No evidence for a tension-cutting artefact. *Plant, Cell & Environment*, *38*(6), 1060–1068. https://doi.org/10.1111/pce.12461

Way, D. A., & Yamori, W. (2014). Thermal acclimation of photosynthesis: On the importance of adjusting our definitions and accounting for thermal acclimation of respiration. *Photosynthesis Research*, *119*(1–2), 89–100. https://doi.org/10.1007/s11120-013-9873-7

Yue, X., Zuo, X., Yu, Q., Xu, C., Lv, P., Zhang, J., … Smith, M. D. (2019). Response of plant functional traits of *Leymus chinensis* to extreme drought in Inner Mongolia grasslands. *Plant Ecology*, *220*(2), 141–149. https://doi.org/10.1007/s11258-018-0887-2

Zach, A., Schuldt, B., Brix, S., Horna, V., Culmsee, H., & Leuschner, C. (2010). Vessel diameter and xylem hydraulic conductivity increase with tree height in tropical rainforest trees in Sulawesi, Indonesia. *Flora – Morphology, Distribution, Functional Ecology of Plants*, *205*(8), 506–512. https://doi.org/10.1016/j.flora.2009.12.008

Zhang, Y. A., Lamarque, L. J., Torres-Ruiz, J. M., Schuldt, B., Karimi, Z., Li, S., … Jansen, S. (2018). Testing the plant pneumatic method to estimate xylem embolism resistance in stems of temperate trees. *Tree Physiology*, *38*(7), 1016–1025. https://doi.org/10.1093/treephys/tpy015

Zhou, S.-X., Medlyn, B. E., & Prentice, I. C. (2016). Long-term water stress leads to acclimation of drought sensitivity of photosynthetic capacity in xeric but not riparian *Eucalyptus* species. *Annals of Botany*, *117*(1), 133–144. https://doi.org/10.1093/aob/mcv161

Zuur, A., Ieno, E., Walker, N., Saveliev, A., & Smith, G. (2009). *Mixed effects models and extensions in ecology with R*. New York, NY: Springer Verlag.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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