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

3 **Running title: Hydraulic drought acclimation of Amazonian trees**

4

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- 25 **Key words:** Acclimation, drought, hydraulic traits, tropical tree, tropical forest, embolism
- 26 resistance, hydraulic safety, hydraulic efficiency

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28 Abstract

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

47

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

50 Introduction

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

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

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

93 To date, only eight throughfall exclusion experiments (TFE experiments) have been
94 implemented in tropical forests with reductions in soil moisture imposed for a year or more
95 (Meir *et al.*, 2015). In a TFE experiment in Sulawesi, after two years of drought, Schuldts *et al.*
96 (2011) found a reduction in tree hydraulic efficiency, which they suggest might have led
97 to a reduction in tree growth. This may suggest the hydraulic systems of tropical trees can
98 respond plastically to drought, but that full acclimation to maintain hydraulic performance
99 and growth did not occur. In another Asian tropical forest, after four months of TFE in

100 Malaysia (Inoue *et al.*, 2017), leaf turgor loss point had decreased, however, as
101 photosynthesis was reduced, this also implies substantial acclimation did not occur.
102 Observations in a TFE experiment in Australia have also shown plastic responses in leaf and
103 wood anatomical properties linked to hydraulic safety after two years of TFE-imposed
104 drought, yet similarly no evidence was reported to suggest this led to substantial acclimation
105 (Tng *et al.*, 2018). In the world's only long-running tropical TFE (15+ years of TFE), in an
106 Amazonian forest, limited plasticity was found in leaf level anatomical and water relations
107 traits (Binks *et al.*, 2016) and none in embolism resistance, a key trait controlling hydraulic
108 safety (Rowland *et al.*, 2015).. Studying drought responses over longer time periods is
109 valuable, particularly given that there is likely to be variation in the responses and types of
110 adjustments occurring from annual to decadal time periods (da Costa *et al.*, 2014; Meir *et al.*,
111 2018).

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

123

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

138 Constraints or trade-offs in hydraulic traits related to tree height or architecture may
139 be greater in larger trees, which are close to their maximum height. This may limit their
140 capabilities to acclimate to drought, potentially explaining why larger trees are more
141 susceptible to drought-induced mortality in tropical forests and other biomes (McDowell &
142 Allen, 2015; Bennett *et al.*, 2015; Rowland *et al.*, 2015a; Olson *et al.*, 2018). Drought
143 experiments in tropical forests have however rarely focussed on tree size effects. The only
144 existing study investigating how drought responses change with tree size (Rowland *et al.*,
145 2015) found no interaction between tree size, embolism resistance and a drought treatment.
146 However, Rowland *et al.* (2015a) were only able to study 6 genera in forest plots containing
147 94 genera and they only focused on trees which were fully sunlit at their canopy tops. To test
148 more fully the capacity of tropical forest trees to adjust their hydraulic system to size changes

149 and to drought, further work is required on a larger number of taxa, spanning multiple tree
150 size classes and canopy positions.

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

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

174 moisture deficit, such that the capacity to acclimate to soil moisture deficit
175 decreases with tree size.

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

180 **Methods**

181 *Site and plant material*

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

195 During the peak of the September-October 2016 dry season, we sampled 161 trees
196 from 12 genera (36 species), 85 from the control plot and 76 from the TFE, with diameters
197 ranging from 14 to 68 cm at 1.5 m height (sampling details in Table 1 and S1). For each
198 individual, we collected two branches 1.5 - 2.5 meters long from the top of the canopy. The
199 branches were fourth to sixth order, counting from the leaves. We collected one set of
200 branches before sunrise (0400 to 0600 hours) and used them for measures of embolism
201 resistance and predawn leaf water potential. We collected a second set of branches at midday
202 (1130 to 1330 hours) and used these for measures of midday leaf water potential, native
203 embolism, leaf-to-sapwood area, xylem and leaf specific conductivity, minimum leaf

204 conductance and wood density measurements. Immediately after collection, branches were
205 bagged in thick black plastic sacks with moist paper to humidify internal air and limit leaf
206 transpiration. Branches were transported 100 m from the plots to measure leaf water
207 potential, and for the remaining measurements the branches were transported for 30 minutes
208 to a laboratory. Each day, branches were collected from 6 to 10 different individuals. In all
209 the branches measured, heartwood was absent and pith area was either absent or negligible.

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

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

<i>Swartzia</i>	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
<i>Syzygiopsis</i>	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
<i>Virola</i>	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
<i>Vouacapoua</i>	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

213

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

219 *Predawn and midday water potential*

220 We measured leaf water potential (Ψ) in the field immediately after collection using a
221 pressure chamber (Model 1505, PMS; 0.05 MPa resolution). For each tree we measured
222 water potential of two leaves, or three leaves if the first two measures differed by more than
223 0.2 MPa for predawn and 0.4 MPa for midday measurements.

224

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

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

231 We determined leaf to sapwood area ratio (LS), on all branches by measuring leaf
232 area and calculating sapwood area from two diameter measurements of the debarked basal
233 part of the branch using precision calipers. We measured leaf area by scanning all leaves on
234 the branch and quantifying their area using Image J software (version 1.6.0_20; Schneider *et*
235 *al.*, 2012). We calculated LS as the total branch leaf area divided by its basal sapwood area.

236 For minimum leaf conductance (g_{min}) we used the leaf conductance to water vapour
237 measured on the abaxial surface of leaves kept 30 minutes in the dark, using an infrared gas
238 analyzer (Li-COR 6400, USA). All leaves measured were adult, undamaged, upper canopy
239 leaves. g_{min} is likely a combination of stomatal conductance due to leakage from
240 partially closed stomata and cuticular conductance. g_{min} is part of the dataset presented in
241 Rowland *et al.* (submitted) and further leaf gas exchange measurement details can be found
242 therein.

243

244 *Hydraulic efficiency and native embolism*

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

268

269 *Embolism resistance and hydraulic safety*

270 As an index of xylem embolism resistance, we used P50 and P88, the xylem water
271 potentials where, respectively, 50% and 88% of hydraulic conductivity is lost (Choat *et al.*
272 2012). We also used P50 to calculate the hydraulic safety margin - the difference between
273 P50 and Ψ_{md} , an index of tree hydraulic safety. We measured the xylem embolism resistance
274 of each branch using the pneumatic method (Pereira *et al.*, 2016; Zhang *et al.*, 2018). With
275 this method the loss of hydraulic conductance is estimated from the increase in air volume
276 inside the wood caused by embolism formation, as the branch dehydrates. Air volume is
277 estimated from the air discharge from the cut end of the branch into a vacuum reservoir (~50
278 kPa absolute pressure) of known volume during a given amount of time (2.5 minutes; details
279 in Methods S1 and Bittencourt *et al.*, 2018). We dehydrated branches using the bench
280 dehydration method. Before each air discharge measurement, we bagged branches for one
281 hour for leaf and wood xylem water potential to equilibrate. Directly after the air discharge
282 was measured, we estimated wood xylem water potential by measuring the leaf water
283 potential of one or two leaves. Drought embolism resistance is then given by the increase in
284 air discharge (PAD – percentage air discharge) with decreasing xylem water potential for
285 each tree. To calculate P50, we pooled together the data for the 2-3 branch replicates from the
286 same tree and fitted a sigmoid curve to the data, where P50 and slope (a) are the fitted
287 parameters (Pammenter & Vander Willigen, 1998) and P88 is predicted from the fitted
288 model:

$$289 \text{ PAD} = 100 / (1 + \exp(a(\Psi - P50)))$$

290

291 *Data analysis*

292 To test our hypotheses, we evaluated the significance of plot, diameter (a proxy of
293 tree height and canopy exposure - see Fig. S5 for tree size and canopy exposure relationships
294 based on the tree light score estimates for both plots), genus and their interactions as variables
295 affecting structural and response hydraulic traits. We used linear mixed effect models with
296 plot, diameter and their interaction treated as fixed effects and we tested the random effect of
297 genus on the intercept of the model and the slope of the independent variable with the fixed
298 model terms (i.e. plot and diameter). We started with a full fixed and random effect model of
299 plot, diameter and their interaction and tested the significance of the random effect by
300 removing it and evaluating if the model significantly worsened. We tested sequentially for the
301 random effect of genus on: 1) the model intercept; 2) the relationship between the
302 independent variable and tree diameter and; 3) on the relationship between the independent
303 variable and plot;. The genus effect on plot without effect on intercept produces the same fit
304 as genus effect on plot and intercept, as plot is a factorial term, so this model was not tested.
305 When more than one random effect format was significant, we chose the simplest random
306 effect (i.e. intercept effect only), unless the Akaike Information Criterion of the more
307 complex model was at least 2 units lower than the simpler model. After testing the random
308 effects, we tested the fixed effects by first removing the interaction term and testing if this
309 significantly worsened the model and after this using the same approach with the additive
310 terms. If no random effect was significant, we changed to a fixed effect model (R base
311 package “lm” function) and analysed fixed effects in the same way. To be confident in our
312 capacity to evaluate plasticity/acclimation we also repeated the above analysis at a species
313 level, instead of genus level. Analysing the data in this way leads to a less balanced sampling
314 design and a reduction in the replicates available for comparison between the plots, due to
315 high tropical forest diversity. However, if a comparison of the genus level and species level
316 analysis delivers the same results it confers confidence in our capacity to demonstrate either

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

319 We also re-tested Rowland *et al.*'s (2015a) relationship between tree size and P50 by
320 analysing our individual dataset alone (30 samples Control and 32 samples TFE, considering
321 only the genera in common with Rowland *et al.* (2015a), and then in combination with the
322 Rowland *et al.* (2015a) dataset (48 samples Control and 51 samples TFE) using fixed effect
323 models. We use a fixed-effect model for consistency with the analysis carried out in Rowland
324 *et al.* (2015a). It should be noted that here we use the pneumatic method for determining P50,
325 whereas Rowland *et al.* (2015a) used the air-injection method and fitted a Weibull, not a
326 sigmoidal function. Despite the different methods, we find a correlation of 0.95 between P50
327 estimated from Weibull and Sigmoid functions for our data (Fig. S2). Finally, to test the
328 effect that species composition of our and the Rowland *et al.* (2015a) data set has on the P50-
329 tree size relationships and to verify the sensitivity of the results (p-value) to the combination
330 of genera used, we performed a taxon-sensitivity analysis by deleting one, two, three and four
331 genera at a time from the full dataset (all 12 genera in this study) for all possible genera
332 combinations (793 total combinations tested).

333 We used the R programming environment and statistical packages (version 3.3.0; R
334 Core Team 2016) for all data processing and analysis. We fitted linear mixed effect models
335 with “lme” function of the nlme package (Pinheiro *et al.* 2014) and linear fixed effect models
336 with the “lm” function (base packages). We used marginal and conditional pseudo-r² to
337 quantify mixed models relative goodness of fit (function “r.squaredGLMM” of the MuMIn
338 package; Barton 2016). We followed the guidelines of Zuur *et al.* (2009) and Thomas *et al.*
339 (2017) for assessing significance of model terms and validating model assumptions. For
340 mixed effect model terms, we fitted models with restricted maximum likelihood and
341 calculated a log-likelihood ratio to test if the removal of a term significantly affected the

342 quality of the model in relation to the more complex model in which it was nested. For fixed
343 effect models, we used the same approach but with an F-test between the model with and the
344 model without the removed variable. We used the “anova” function (stats package, with
345 mixed effect model methods from nlme package) for log-likelihood ratio and F tests. If the p
346 value of the test was less than 0.05, we assumed that the model without the variable was
347 significantly worse than the model with the variable included. The final model with only
348 significant terms was refitted with maximum likelihood and we validated model assumptions
349 using diagnostic plots for normality and homogeneity of residuals and collinearity of
350 predictors. To identify influential observations, we calculated Cook’s distance and dfbeta for
351 each dataset using base package functions for linear models and “influence.ME”
352 (Nieuwenhios *et al.* 2012) package functions for mixed effect models. The embolism
353 resistance dataset is smaller than the other datasets, as we could not process all collected
354 samples (Table 1). No hydraulic traits are presented for *Virola* as hydraulic conductivity
355 clearly decreased with the duration of the measurement, producing negative percentage loss
356 of conductance, likely due to the abundance of latex being exuded from the cut end of the
357 sample. Whenever we had more than one replicate per tree, as for leaf water potential, we
358 used the tree-level mean of the replicates. We considered significant the probability of the
359 tested hypothesis versus the null hypothesis (p-value) being lower than 0.05 and marginally
360 significant when it was between 0.05 and 0.10. All data will be made available at the NERC
361 Centre for Environmental Data Analysis (<https://nerc.ukri.org/research/sites/data/>).

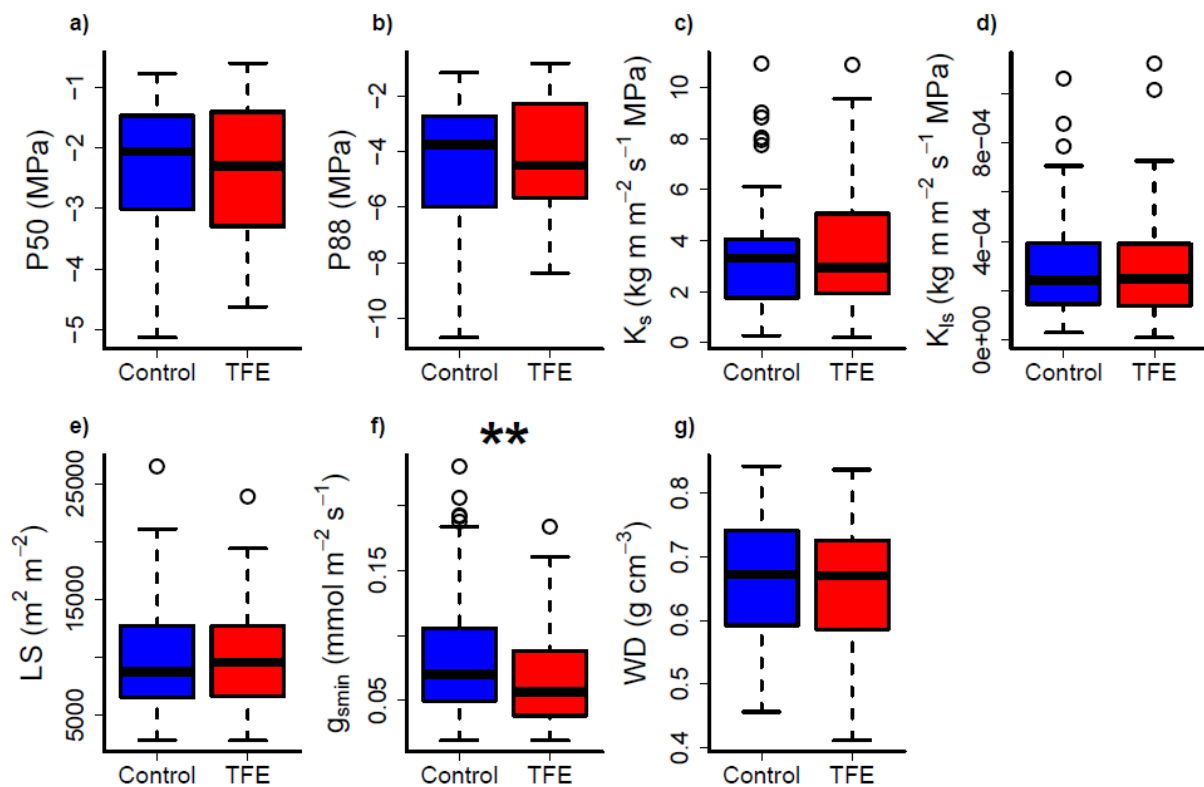
362 **Results**

363 *Throughfall exclusion effects on tree hydraulics*

364 Drought stress caused by 15 years of throughfall exclusion (TFE) had limited effect
365 on hydraulic traits (Fig. 1; Table 1) Xylem embolism resistance (P50 and P88), specific

366 conductivity (K_s), leaf specific conductivity (K_{ls}), leaf to sapwood area (LS) and wood
367 density (WD) did not change in response to the TFE manipulation (Table 2; Table S2).
368 Minimum stomatal conductance (g_{smin}) was the only trait which adjusted in response to the
369 TFE ($p = 0.011$), with a decrease of $0.007 \text{ mmol m}^{-2} \text{ s}^{-1}$ (Fig. 1f; all coefficients are presented
370 in Table S3).

371



372

373 Figure 1. Hydraulic trait responses on trees surviving after 15 years of throughfall exclusion
 374 (TFE) in Caxiuanã. a-b) P50 and P88 - xylem embolism resistance (MPa); c) K_s – maximum
 375 hydraulic specific conductivity ($\text{kg m m}^{-2} \text{s}^{-1} \text{MPa}$); e) K_{ls} - maximum hydraulic leaf -specific
 376 conductivity ($\text{kg m m}^{-2} \text{s}^{-1} \text{MPa}$); e) LS – leaf to sapwood area ratio ($\text{m}^2 \text{m}^{-2}$); f) g_{smin} –
 377 minimum stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$); g) WD – wood density (g cm^{-3}). We consider
 378 changes in structural hydraulic traits (i.e. plot effect models) as plasticity in response to
 379 drought. The box represents quartiles 1 and 3, with the central line indicating the median.
 380 Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when
 381 outliers are present. Traits for which plot had a significant effect are marked with * ($p <$
 382 0.05), ** ($p < 0.01$) and *** ($p < 0.001$). P-values are from mixed effects analysis (see Table
 383 2 for models and analysis section in Methods).

384

385 Table 2. Results of linear mixed effect models of plot (Control versus TFE) and tree diameter effects on hydraulic traits and hydraulic state
 386 variables. The combination of the tested random effects is genus effect on intercept only, and/or on plot and diameter slopes (see analysis section
 387 in Methods for details). Values for fixed effects are fitted parameter \pm standard error; values for random effects are standard deviation of the
 388 normal distribution from where coefficients were fitted. R^2 is the full model coefficient of determination (conditional pseudo- R^2). Random
 389 effects notation are: 1|genus is a random genus effect on intercept; diameter|genus or plot|genus indicates a random intercept effect plus a
 390 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.
 391 experimental treatment) is a two level factor (Control and TFE), with Control as the reference level. Blank cells indicate that the effect is non-
 392 significant.

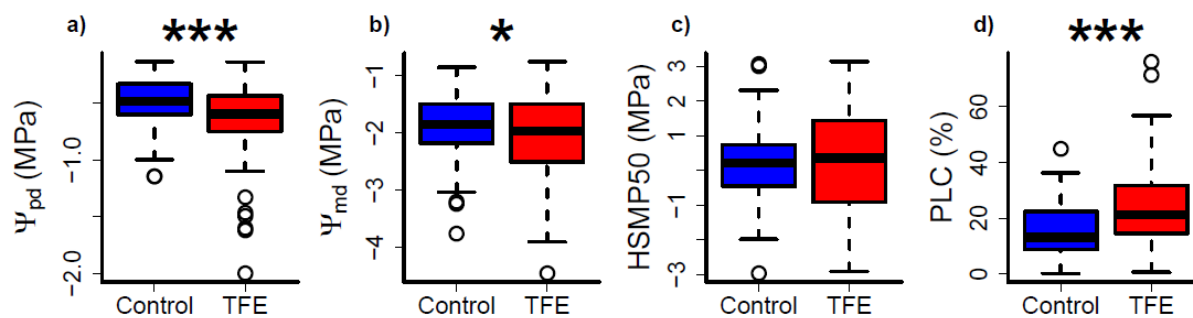
	Fixed effect			Random effects			R^2
	Intercept	Plot	Diameter	Genus	Plot Genus	Diameter Genus	
P50	-2.21 \pm 0.16			0.44*			0.16
P88	-4.22 \pm 0.21						0
Traits	K_s	3.31 \pm 0.48		2.29***		0.065***	0.53
	K_{ls}	0.21 \pm 0.06		0.11**			0.26
	LS	10266 \pm 439.6					0
	g_{smin}	0.083 \pm 0.005	-0.018 \pm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

	WD	0.65 ± 0.02		0.06***	0.36	
State variables	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

393 P50 - xylem embolism resistance (MPa); Ψ_{pd} - predawn water potential (MPa); Ψ_{md} - midday water potential (MPa); HSMP50 – hydraulic
 394 safety margin to P50 (MPa); PLC – native dry season percentage loss of conductivity (%); K_s – maximum hydraulic specific conductivity (kg m
 395 $m^{-2} s^{-1} MPa^{-1}$); 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} –
 396 minimum stomatal conductance ($mmol m^{-2} s^{-1}$); WD – wood density ($g cm^{-3}$).

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

398 The TFE manipulation had a highly significant effect on all hydraulic state variables
399 (Fig. 2), except on hydraulic safety margin (HSMP50; Table 2). TFE caused a decrease in
400 peak dry season Ψ_{md} of -0.19 MPa ($p = 0.02$) with a high intra-generic variability (SD of
401 0.38 MPa for random genus effect on intercept and an average increase in percentage loss of
402 conductance of 8.3% (PLC; $p = 0.001$; see coefficients in Table S3). Predawn water potential
403 (Ψ_{pd}) was affected by the TFE with a reduction of -0.18 MPa ($p < 0.001$), equal to a 35%
404 decrease relative to control, which was further modulated by random genus-specific effects (p
405 < 0.001 ; Figure 3; see also the following paragraph), leading to a TFE maximum effect of
406 reducing predawn water potential by 0.52 MPa in *Micropholis*.

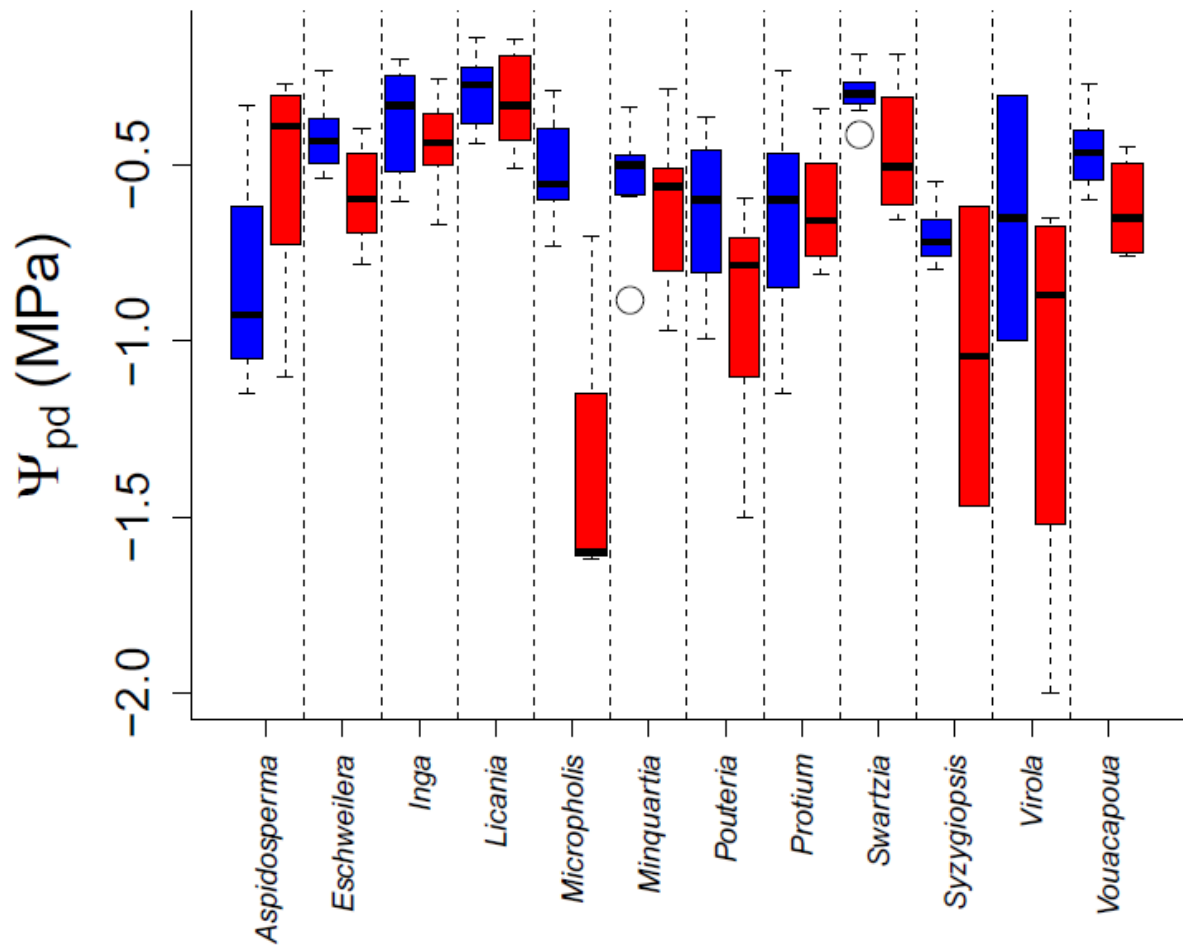


407

408 Figure 2. Hydraulic state variables of trees surviving after 15 years of throughfall exclusion
 409 (TFE) in the peak of the dry season in Caxiuanã. a) Ψ_{pd} - predawn water potential (MPa); b)
 410 Ψ_{md} midday water potential (MPa); c) HSMP50 – hydraulic safety margin to P50; d) PLC –
 411 native dry season percentage loss of conductivity (% maximum conductance). We consider
 412 homeostasis of hydraulic state variables (i.e. no plot effect) as an indicator of hydraulic
 413 acclimation. The box represents quartiles 1 and 3, with the central line indicating the median.
 414 Whiskers are either maximum value or 1.5 interquartile range above the quartile 3, when
 415 outliers are present. Traits for which plot (i.e. TFE treatment) had a significant effect are
 416 marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). P-values are from mixed effects
 417 analysis (see Table 2 for models and analysis section in Methods).

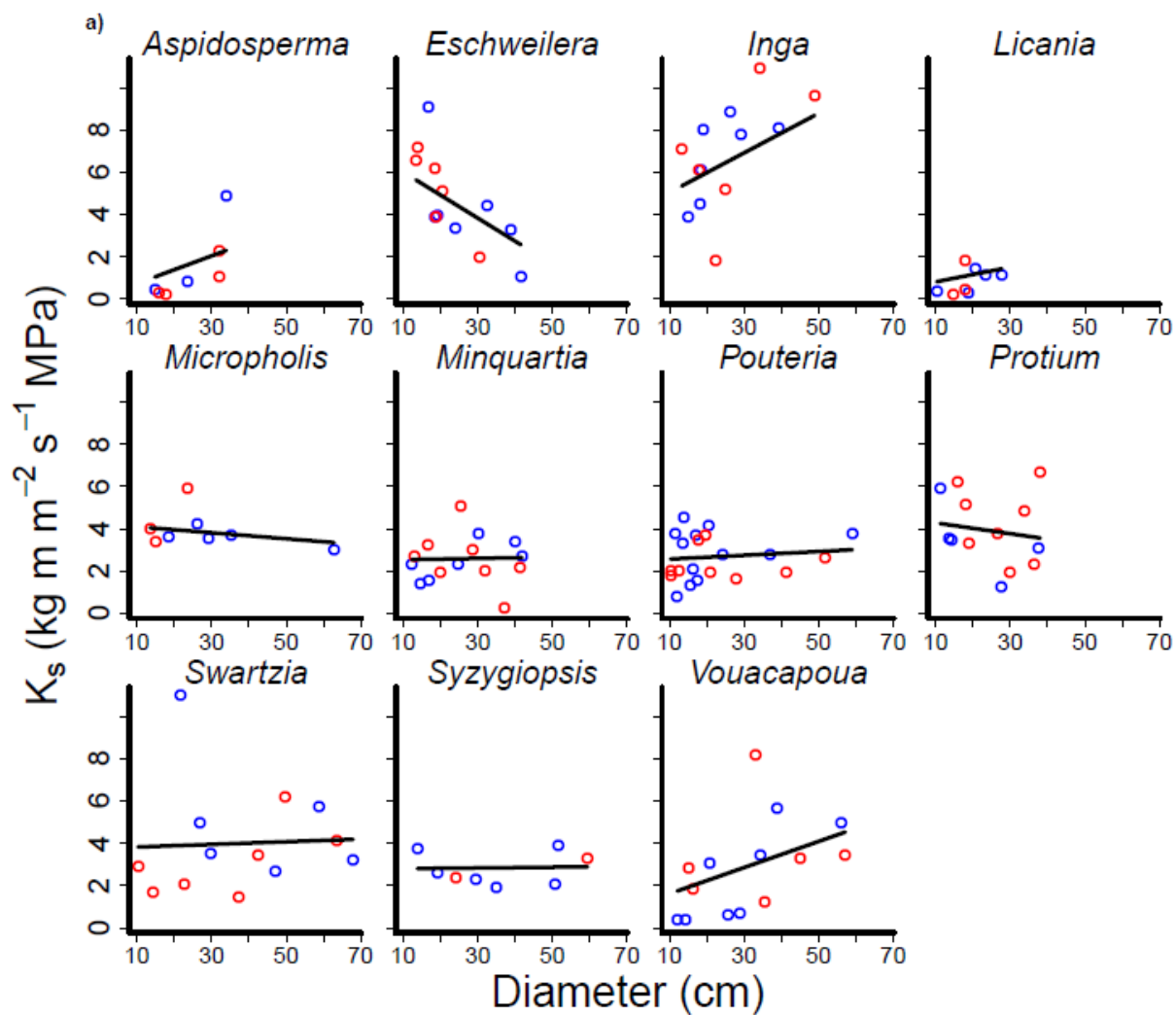
418 *Tree size effects on hydraulic traits and interaction with TFE*

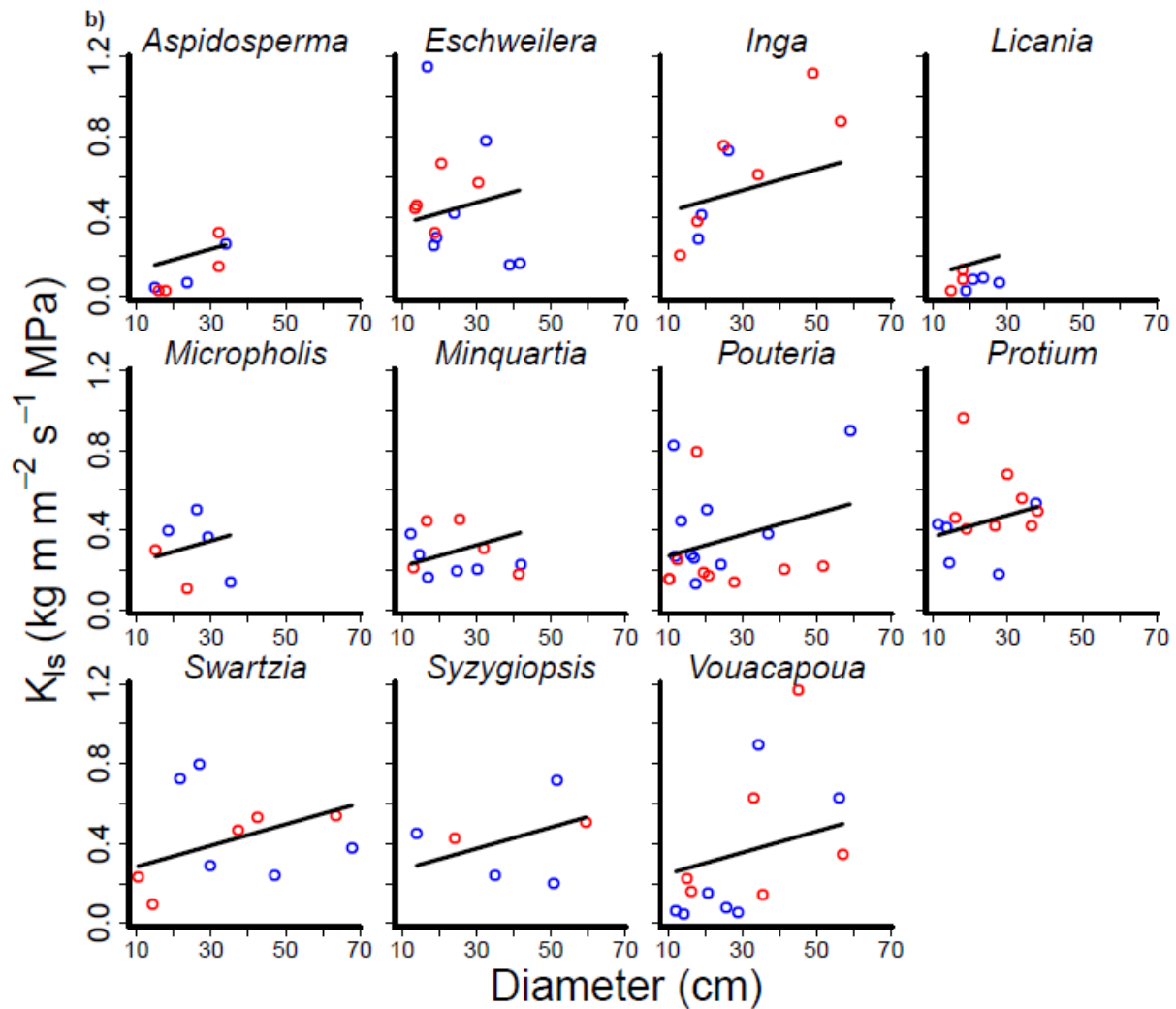
419 Tree stem diameter did not affect hydraulic state variables and only affected K_s and
420 K_{ls} among the hydraulic traits (Table 2). For K_s , the effect of increasing diameter was genus-
421 dependent, as indicated by a significant random genus effect on diameter ($p < 0.001$; Figure
422 4a; Table 2; Table S2). The effect of diameter on K_s was positive for *Inga*, *Aspidosperma* and
423 *Vouacapoua* while it was negative for *Eschweilera* and close to zero for the other genera
424 (Fig. 4a and Table S3). For K_{ls} , stem diameter was significant (Tables 1) however, contrary to
425 K_s , the effect was not genus dependent (no random genus effect on diameter- K_{ls} relationship),
426 and K_{ls} showed a consistent increase with stem diameter (Fig. 4b). The random genus effect
427 on the diameter-P50 relationship was significant ($p = 0.035$; Table S2; Fig. 5), but was not
428 the most parsimonious model (AIC of 316 against an AIC of 315.8 for the random intercept
429 model only). There was no fixed diameter effect on P50 ($p = 0.29$, Table S2) but the
430 interaction effect between diameter and plot was marginally significant ($p = 0.066$; Table S2).
431 We found no evidence of any interaction of plot and tree stem diameter hydraulic traits or
432 hydraulic state variables (Table 2 and Table S2).



433

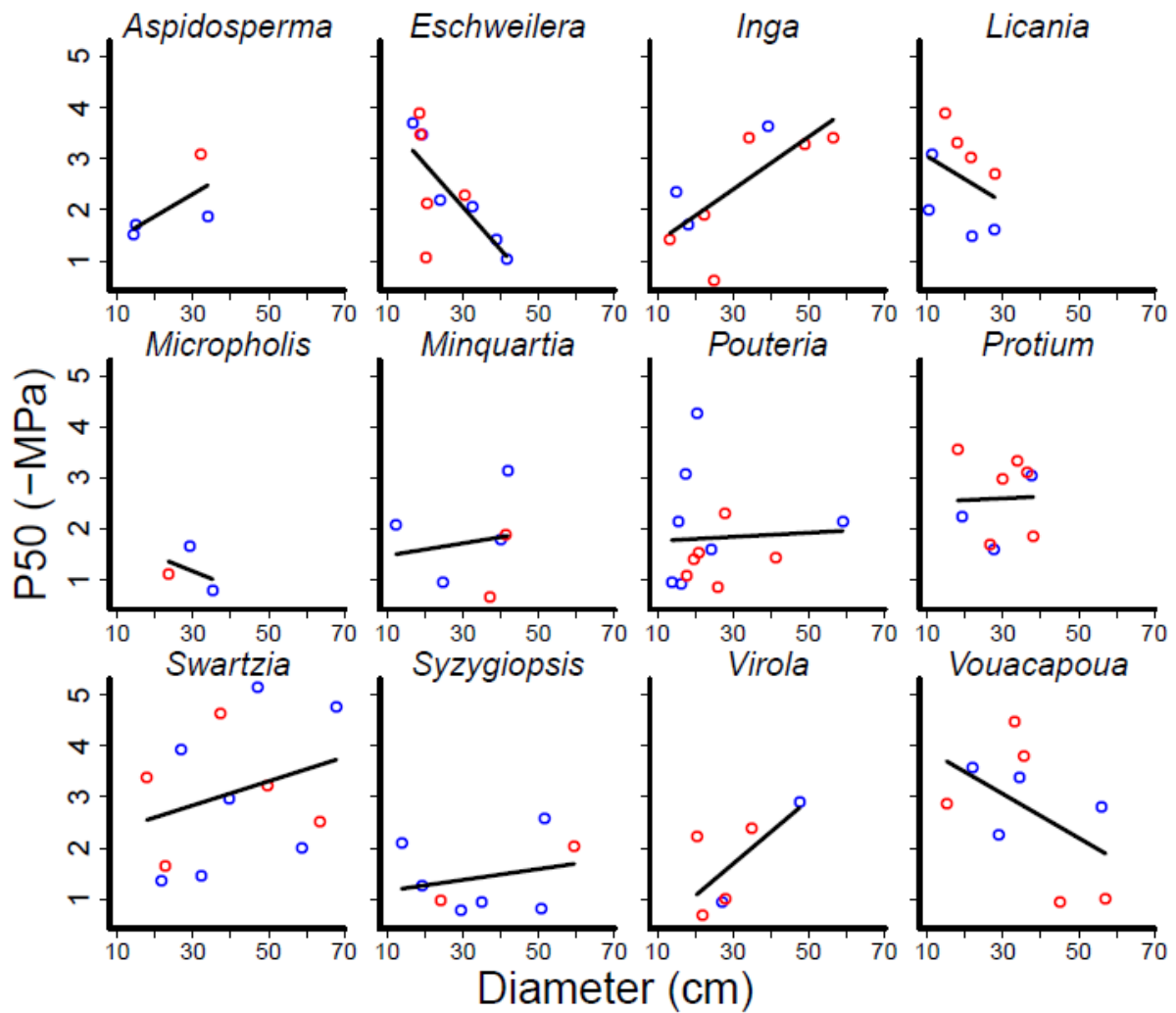
434 Figure 3. Throughfall exclusion (TFE) effect on predawn water potential of the studied genus
 435 (Ψ_{pd}), showing the genus specific variation in Ψ_{pd} response to TFE. TFE had both a fixed
 436 effect of reducing Ψ_{pd} and a random effect (genus specific effect) on Ψ_{pd} (see Table 1). Red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





441

442 Figure 4. Diameter effects on xylem specific conductivity (K_s ; a) and leaf specific
 443 conductivity (K_{ls} ; b) for each studied genus. Fitted lines show the fitted mixed effects model
 444 (see Table 1 and Results section), modified according to the effect of the random variable
 445 (genus) on either the intercept or the slope of the relationship of diameter to the independent
 446 variable (K_s or K_{ls}). For K_s , the interaction between diameter and random effect, genus is
 447 significant ($p < 0.001$), resulting in different slopes for each genus. For K_{ls} , the interaction is
 448 significant ($p = 0.02$) but is not the most parsimonious model (AIC of 40.2 against an AIC of
 449 38.6 for genus effect on intercept only, see Table S2), resulting in vertical shifts of otherwise
 450 parallel lines. Blue and red points are data from Control and TFE plots, respectively.



451

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

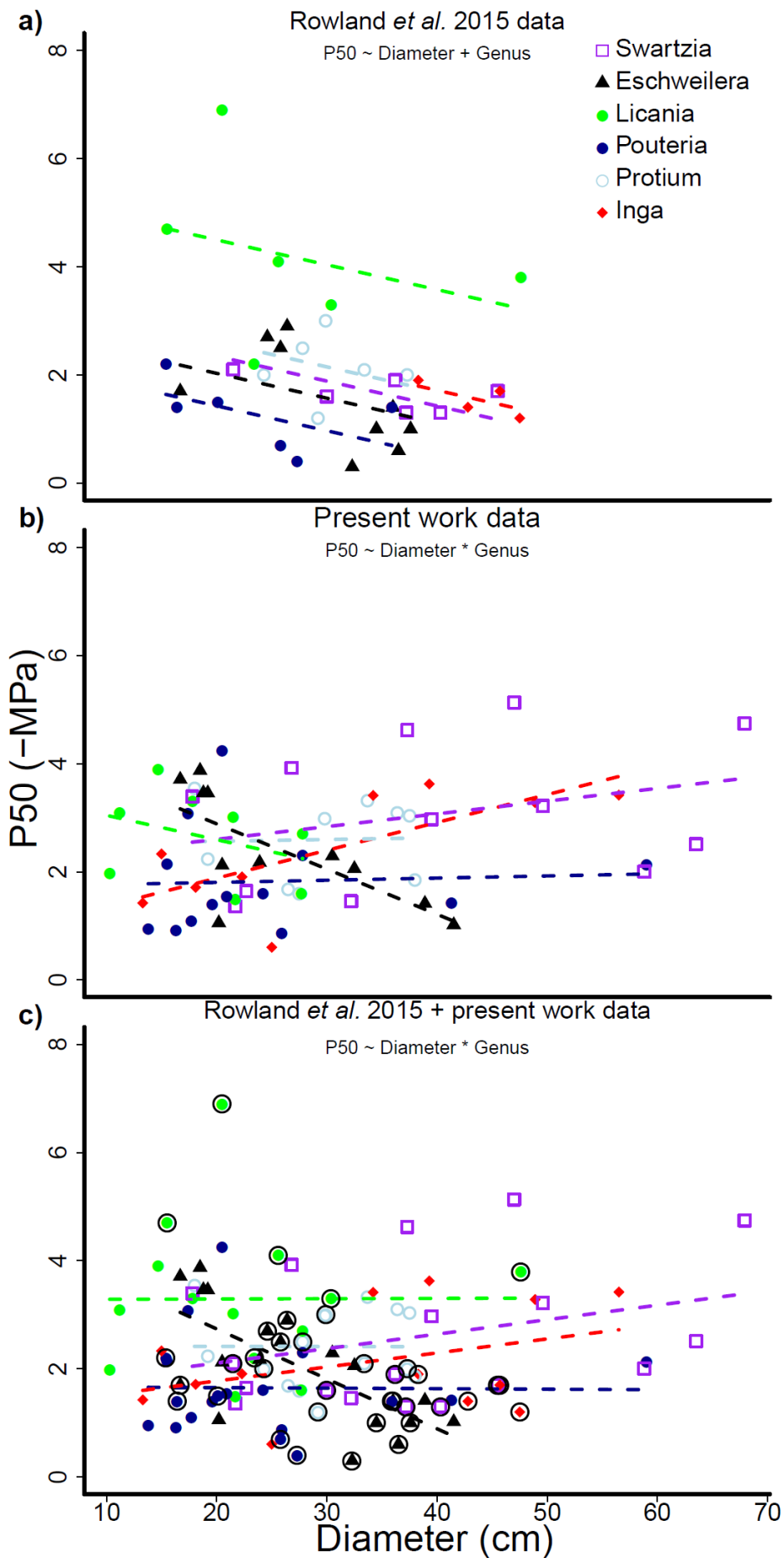
458 *Taxonomic effects on the hydraulic system and their interactions with drought and tree size*

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

472 *Revisiting Rowland et al. (2015)*

473 Rowland *et al.* (2015a) presented the first dataset of P50 for Amazon trees at this site
474 (reproduced in Fig.6a). We provide here a comparison of the two analyses. Using fixed effect
475 models, when we analysed the same subset of six genera as analysed by Rowland *et al.*
476 (2015a), our data also show a significant effect of diameter on P50. However, in our work,
477 the slope was positive, and not negative as in Rowland *et al.* (2015a), and the interaction
478 between genus and diameter was also significant ($p = 0.046$, Fig. 6b). While *Eschweilera*
479 presents a strong increase in P50 (less negative values) with diameter, the other genera
480 showed either a weak decrease in P50 with diameter or an almost negligible diameter effect
481 (Fig. 6). When the two datasets are combined, the same pattern remains, with P50 being

482 significantly affected by the interaction between diameter and genus and a slightly more
483 significant diameter effect ($p = 0.023$; Fig. 6c). However, after removal of a single genus
484 with a strong positive relationship between P50 and diameter (*Eschweilera*) from the
485 combined datasets, both the diameter interaction with genus and the diameter effect disappear
486 ($p = 0.62$ and $p = 0.14$; Fig. S3c). The same occurs if *Eschweilera* is removed from the non-
487 combined datasets (Fig. S3a and b).



489 Figure 6. Relationship between embolism resistance (P50) and tree diameter using the same
490 subset of genera as analysed in Rowland *et al.* (2015a). a) The original data and analysis of
491 Rowland *et al.* (2015a). b) The data from this work only; and c) the combined datasets from
492 a) and b). Linear fixed-effect models were used in the analysis and the dashed lines are the
493 fitted model for each genus. In a), diameter and genus were significant while the interaction
494 term was not. In both b) and c) genus, diameter and their interaction are significant. The
495 circled data points in c) indicate the 2015 data. As done in Rowland *et al.* (2015a), we display
496 the Y-axis as the negative of P50 (-P50). The same figure and analysis is presented in Fig. S3
497 after removing data for *Eschweilera*, the genus with an increase in -P50 with diameter. Note:
498 P50 values are presented as -MPa, as in (Rowland *et al.*, 2015a).

499 The results from the linear fixed effect models using the Rowland et al (2015a) subset
500 of the data are partially consistent with the results for this study is full dataset with all 12
501 genera, whereby a diameter interaction with genus is found to be marginally significant ($p =$
502 0.066; Table S2), but this is not the most parsimonious model (AIC of 316 for the random
503 genus-diameter interaction against an AIC of 315.8 for the simpler genus intercept effect).
504 We reanalysed the full dataset (all 12 genera in this study) removing one, two, three and four
505 genera at a time, for all possible genus combinations (793 total combinations tested), to verify
506 the sensitivity of the results to the combination of genera used. When removing only one
507 genus at a time, P50 was significantly affected by diameter with a genus interaction 33.3% of
508 the times and marginally significantly affected 75% of the times. A similar pattern was
509 observed when two or more genera were removed simultaneously (Fig. S4). This further
510 supports our finding that the influence of tree diameter on P50 is strongly dependent on the
511 taxonomic identity of the trees in the dataset, which also strongly influenced whether P50
512 increased, decreased or did not change with increasing tree size (Fig. S3). On the other hand,
513 neither the present results nor those of Rowland *et al.* (2015a) show any significant plot (i.e.
514 treatment) effects on P50.

515 Discussion

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

534

535 *Plasticity and acclimation to drought in Amazonian trees*

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

539 safety were observed in a TFE experiment located in tropical Australian rainforest (Tng *et al.*,
540 2018).

541 Our data indicate that in tropical trees exposed to prolonged soil moisture stress, neither the
542 traits associated with hydraulic safety nor those associated with hydraulic efficiency adjust to
543 enable acclimation. It is possible that other, unmeasured, traits may be influencing tree
544 hydraulic status, for example changes in stomatal control and rooting depths. However, the
545 significantly lower leaf water potential and greater PLC in the TFE trees suggest other traits,
546 as with our measured traits, are not adjusting sufficiently to allow the hydraulic system of the
547 droughted trees to acclimate. These patterns which we observe at genus level remain
548 unchanged when the data are analysed at species level, providing confidence that acclimation
549 did not occur.

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

561 The higher values for PLC in the TFE trees relative to the Control trees, is consistent
562 with previous studies at this site which suggested that hydraulic impairment is likely to play a
563 significant role in the much higher levels of drought-induced mortality observed in the TFE
564 (Rowland *et al.*, 2015a). The higher PLC values in trees in the TFE are likely a consequence
565 of the observed more negative Ψ_{pd} and Ψ_{md} (Fig. 2). The mean PLC of the Control trees

566 during the peak dry season was 16.2%, whereas it was 24.5% on the TFE. Extrapolating from
567 our PLC results, on average 4.1% of the TFE trees have PLC > 50% while only 1% of
568 Control trees cross this threshold during the peak dry season (Table 3). However, when
569 genus-specific effects are considered, the percentage of trees in the TFE crossing the
570 threshold can be as high as 10.1% for *Protium* trees and as low as 2.1% for *Eschweilera* trees.
571 Increased PLC under drought is a distinct signal related to tree mortality (Anderegg *et al.*,
572 2014; Gaylord *et al.*, 2015; Li *et al.*, 2018). While there is no consensus on what PLC level
573 marks the point of no return from hydraulic failure (Brodribb & Cochard, 2009; Urli *et al.*,
574 2013), PLCs levels above 50-60 % are very likely to be lethal to trees (Adams *et al.*, 2017).
575 We note that we did not see a change in the HSMP50 state variable to support the observed
576 changes in LWP and PLC. However, we suggest this is because HSMP50 was determined
577 much more by P50 (correlation coefficient of -0.87) than by Ψ_{md} (correlation coefficient of
578 0.61).

579 The large variations in PLC which we observed among different genera also confirms
580 previous observations that drought-induced mortality is more likely for specific groups of
581 taxa (Esquivel-Muelbert *et al.*, 2017) . According to our models it is unlikely that any
582 individual tree surviving in the TFE crosses the PLC > 88% threshold in a normal year, such
583 as when we made our measurements. This suggests that either trees which crossed this
584 threshold have already died, as such PLC rates are likely to be unsustainable (Adams *et al.*,
585 2017; Meinzer & McCulloh, 2013), or that such a threshold is only crossed when there is a
586 particularly intense or long atmospheric drought occurring alongside the soil moisture deficit
587 caused by the TFE treatment. However, it is also possible that our PLC estimates are an
588 underestimate as refilling in small branches and leaves, likely related to foliar water uptake
589 may also occur (Binks *et al.*, 2019).

590 Table 3. Predicted percentage of individuals with PLC > 50% and PLC > 88% in the Control
 591 and Throughfall Exclusion Experiment (TFE) plots in the peak of the dry season in Caxiuanã.
 592 Predictions are quantiles with PLC > 50% and 88% of the PLC data distribution fitted with a
 593 normal variable (PLC ~ N(μ , SD)). The μ (mean) parameter is the genus mean from the
 594 linear mixed effects model fitted to PLC data (i.e. fixed intercept plus fitted random
 595 coefficient for the genus; see Table 2 and S2) with or without the TFE effect added. The SD
 596 (standard deviation) parameter is either assumed to be equal to all groups (SD of all dataset;
 597 “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
<i>Aspidosperma</i>	1.7	5.9	0	0	3.4	18.7	0	0.9
<i>Eschweilera</i>	0.5	2.1	0	0	0	1.2	0	0
<i>Inga</i>	0.6	2.7	0	0	0	0	0	0
<i>Licania</i>	1.5	5.5	0	0	0.3	0	0	0
<i>Micropholis</i>	0.4	1.7	0	0	0	0	0	0
<i>Minquartia</i>	0.8	3.4	0	0	0	5.5	0	0
<i>Pouteria</i>	0.9	3.6	0	0	0.3	3.7	0	0
<i>Protium</i>	3.3	10.1	0	0	0.3	19	0	0.4
<i>Swartzia</i>	2.2	7.5	0	0	3	3.2	0	0
<i>Syzygiopsis</i>	1.1	4.3	0	0	0	NA	0	NA
<i>Vouacapoua</i>	0.7	3	0	0	0	0.2	0	0

598

599

600 *Size-related changes in hydraulic plasticity*

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

612 Hydraulic efficiency and leaf water supply efficiency (K_s and K_{ls}) were the traits with
613 the greatest plasticity associated with tree size. Increases in K_s are expected as trees grow
614 taller. To cope with increasing resistance to water flow and drier atmospheric conditions with
615 increased height, trees may make changes such as increasing the efficiency of water transport
616 or the investment in xylem tissue, (Bittencourt *et al.*, 2016; Deckmyn, Evans, & Randle,
617 2006). Vessel diameter, and consequently K_s of the apex and stem of trees also typically
618 increases with tree height, allowing K_s to increase (Olson *et al.*, 2018). Although our data do
619 show that K_s changes with tree height, the direction and degree of this change varies
620 substantially among genera (Fig. 3a). *Inga* and *Eschweilera* had the greatest change in K_s
621 with stem diameter, however in opposite directions. The other genera showed either very
622 limited or no plasticity with tree size. These results may be a consequence of K_s being a

623 function of multiple structural and anatomical properties (Cruziat *et al.*, 2002; Bittencourt *et*
624 *al.*, 2016), which may change for other purposes in relation to tree size.

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

640

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

642 Rowland *et al.* (2015a) used data from six genera at our study site to demonstrate that
643 embolism resistance (P50) decreases as tree size increases. This was the first dataset of its
644 kind for mature Amazonian tropical trees. The limited data available for temperate trees is
645 inconclusive on direction and strength of this P50 – size relationship (Domec *et al.*, 2009;
646 Ambrose *et al.*, 2009; Prendin *et al.*, 2018, Olson *et al.*, 2018). Rowland *et al.*, (2015a)

647 presented these results as evidence that hydraulic failure acts as a trigger of drought-induced
648 mortality, with a higher mortality risk in taller drought-stressed trees (which had P50 values
649 closer to zero). By subsetting our data to include the same genera, we were able to replicate
650 this result using different individuals of the same genus and using a different protocol for
651 hydraulic measurements (Fig. 6), but finding an overall inverse relationship between tree
652 diameter and P50. However, when data for *Eschweilera* were removed from the analysis, the
653 relationship between tree size and P50 disappeared (Fig. S3). The data for *Eschweilera* show
654 a strong decrease in embolism resistance with increasing diameter (Fig. 4). This is relevant
655 more generally as *Eschweilera coriacea*, one of our sampled species, represents one of the
656 hyper-dominant trees across the Amazon. It accounts for 5.1% of Amazon trees and 5.5% of
657 Amazon biomass (Fauset *et al.*, 2015; ter Steege *et al.*, 2013), but which we demonstrate is
658 highly sensitive to drought when it reaches full stature.

659 Compared to Rowland *et al.* (2015a), our full dataset is comprised of a much larger
660 number of samples, many more tree species and a wider diameter range, including shaded, or
661 partially shaded trees. Using this much larger dataset, we found no evidence of a general
662 decrease in embolism resistance (increasing P50) with tree size. However, we did find
663 evidence of a marginal tree size effect on P50 interacting with genus identity (Fig. 5),
664 suggesting that changes in embolism resistance with tree size exist, but are highly dependent
665 on tree taxonomic identity. Critically, the strength of the P50-tree size relationship was
666 strongly affected by the subset of data used (Fig. S4). By sequentially removing one or two
667 genera from our full dataset, we obtained a significant relationship of embolism resistance
668 with tree size 30% to 50% of the time, respectively. These results indicate that decreases in
669 embolism resistance with tree size are highly dependent on the combination of genera
670 analysed and, at least based on our extensive sampling at this site, particularly on the

671 presence of *Eschweilera* (which has a strong P50-size relationship). Future studies conducted
672 in highly diverse systems should incorporate taxon-sensitivity analyses.

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

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700 **Author Contributions**

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

705

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