ORIGINAL ARTICLE

Small tropical forest trees have a greater capacity to adjust carbon metabolism to long-term drought than large canopy trees

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Abstract

The response of small understory trees to long-term drought is vital in determining the future composition, carbon stocks and dynamics of tropical forests. Long-term drought is, however, also likely to expose understory trees to increased light availability driven by drought-induced mortality. Relatively little is known about the potential for understory trees to adjust their physiology to both decreasing water and increasing light availability. We analysed data on maximum photosynthetic capacity (J_{max}, V_{cmax}), leaf respiration (R_{leaf}), leaf mass per area (LMA), leaf thickness and leaf nitrogen and phosphorus concentrations from 66 small trees across 12 common genera at the world's longest running tropical rainfall exclusion experiment and compared responses to those from 61 surviving canopy trees. Small trees increased J_{max}, V_{cmax}, R_{leaf} and LMA (71, 29, 32, 15% respectively) in response to the drought treatment, but leaf thickness and leaf nutrient concentrations did not change. Small trees were significantly more responsive than large canopy trees to the drought treatment, suggesting greater phenotypic plasticity and resilience to prolonged drought,

although differences among taxa were observed. Our results highlight that small tropical trees have greater capacity to respond to ecosystem level changes and have the potential to regenerate resilient forests following future droughts.

KEYWORDS

drought, leaf respiration, light, ontogeny, photosynthesis, through-fall exclusion experiment, tropical forest, understory

1 | INTRODUCTION

Climate change can simultaneously affect multiple environmental variables across ecosystems globally (IPCC, 2019). However, little is known about how trees respond to multiple environmental shifts, especially in tropical forests (Bonal, Burban, Stahl, Wagner, & Herault, 2016; Niinemets, 2010). Both episodic and sustained droughts have been shown to cause mortality of large canopy trees in Amazonia (Bennett, McDowell, Allen, & Anderson-Teixeira, 2015; Meir et al., 2015; Nepstad, Tohver, Ray, Moutinho, & Cardinot, 2007; Phillips et al., 2010; Rowland, da Costa, et al., 2015), resulting in canopy openings and changes in the understory environment (Chazdon & Fetcher, 1984; Brown, 2009; Pfeifer et al., 2016; Rowland et al. under review). Small understory trees must therefore respond to concurrent reductions in soil moisture availability and increased light availability to survive, compete and grow under drought conditions. The ability of these small trees to adjust their physiology in response to multiple environmental shifts could be critical for predicting the future of tropical forests.

If canopy trees are susceptible to mortality during drought under future climates, it becomes important to understand the response of understory trees to drought conditions within higher light environments, and concomitant changes in air temperature and VPD. However, most of our current knowledge of tropical tree drought responses is focused on either large canopy trees or seedlings (e.g., Feldpausch et al., 2016; Nepstad et al., 2007; O'Brien, Leuzinger, Philipson, Tay, & Hector, 2014; O'Brien, Reynolds, Ong, & Hector, 2017; L. Poorter & Hayashida-Oliver, 2000; Rowland, Lobo-do-Vale, et al., 2015; Schuldt et al., 2011). Some studies have investigated the impacts of short-term drought events on tropical understory trees (e.g., Newbery, Lingenfelder, Poltz, Ong, & Ridsdale, 2011; Phillips et al., 2010), but, to our knowledge, no studies to date have investigated the effects of prolonged drought in tropical forests on understorey trees also exposed to elevated light conditions. This represents a gap in our knowledge as understory trees tend to have very different resource requirements to adult trees and seedlings (Sterck, Markesteijn, Toledo, Schieving, & Poorter, 2014). If we are to fully predict the fate of tropical forests and improve climate predictions from the latest generation of demography-based vegetation models, it is critical to understand the physiological responses of understory trees in drought-affected forests (Fisher et al., 2018; Moorcroft, Hurtt, & Pacala, 2001; Moore, Zhu, Huntingford, & Cox, 2018; Smith, Prentice, & Sykes, 2008).

Differences in physiology, microenvironments and resource requirements will likely lead to different drought responses in small understory trees compared with large canopy trees (Kitajima & Poorter, 2008). Small trees tend to be particularly sensitive to reductions in soil moisture availability (Kitajima, Cordero, & Wright, 2013; Quevedo-Rojas, Garcia-Nunez, Jerez-Rico, Jaimez, & Schwarzkopf, 2018; Ruger, Wirth, Wright, & Condit, 2012), because of smaller total carbon reserves (Hartmann et al., 2018) and shallower rooting depths (Brum et al., 2018; Stahl et al., 2013). However, vulnerability to drought may actually be lower in small trees, as carbon reserves per unit biomass may be greater (Hartmann et al., 2018). An ability to maintain a positive balance between carbon assimilation and use will ultimately be critical for these small trees to survive and avoid carbon starvation under drought conditions (McDowell et al., 2018; O'Brien et al., 2014). The adjustment of functional traits related to carbon metabolism, including maximum photosynthetic capacity (J_{max} and V_{cmax}) and leaf respiration (R_{leaf}), is important for buffering long-term reductions in soil moisture availability. Under drought stress, some trees have been shown to reduce photosynthetic capacity because of drought-induced impairment or for nutrient re-allocation for stress repair (Damour, Vandame, & Urban, 2008; Damour, Vandame, & Urban, 2009), while others have been shown to maintain photosynthetic capacity to optimize carbon assimilation during wetter periods (Rowland, Lobo-do-Vale, et al., 2015). In response to this, R_{leaf} can increase under drought conditions to support stress-related repair or support osmoregulation (Rowland, Lobo-do-Vale, et al., 2015), however perhaps more typically R_{leaf} has been found to decrease during drought stress in response to reduced photosynthesis (Atkin & Macherel, 2009; Ayub, Smith, Tissue, & Atkin, 2011). The extent of plasticity in these carbon metabolic traits could determine the likelihood of small trees surviving long periods of reduced soil moisture availability. However, these traits are not simply controlled by drought stress, but are also highly sensitive to light availability (Atkin et al., 2015; Hasper et al., 2017; H. Poorter, Niinemets, Poorter, Wright, & Villar, 2009). Consequently, the light environment of a tree should also be considered when understanding responses to drought.

Tropical forests display strong vertical gradients in light availability with small understory trees generally adapted to shade conditions relying on diffuse light and unpredictable, fleeting sunflecks for the majority of their carbon assimilation (Chazdon & Pearcy, 1991; Leakey, Press, & Scholes, 2003). Under normal conditions, tropical trees are able to acclimate their leaf physiology to the steep gradient in irradiance experienced through the vertical profile of the canopy, with photosynthetic capacity and leaf mass per area (LMA) increasing with

light availability (Cavaleri, Oberbauer, Clark, Clark, & Ryan, 2010; Domingues et al., 2010; Meir et al., 2002; H. Poorter et al., 2009). Trees modify their leaf physiology to optimize the balance between carbon gain and carbon and water loss, in order to maximize growth, reproduction and competitiveness. Both large and small understory trees in tropical forests have been shown to be highly responsive and plastic to changes in their light environment (Kitajima et al., 2013; Quevedo-Rojas et al., 2018), suggesting light is likely to be the most limiting factor for photosynthesis in intact tropical forests. However, if another factor, such as water availability, also becomes limiting, these trees may no longer be able to acclimate to high light levels, potentially leading to negative impacts of increasing light on growth and survival. Increases in light availability under drought conditions may result in excessive photon flux density, elevated leaf temperatures and elevated VPD, inducing photoinhibition (Kamaluddin & Grace, 1992; Krause, Virgo, & Winter, 1995; Mulkey & Pearcy, 1992), prolonged stomatal closure (Reynolds-Henne et al., 2010) and xylem embolism. To avoid these negative consequences of concurrent high light and drought stress, small understory trees may need to modify their physiology in different ways compared to canopy trees.

Insights from the same eastern Amazon throughfall exclusion experiment used in this study showed the responses of large trees to drought were indeed influenced by the light environment (Rowland et al. under review). Large trees that had also experienced increased canopy exposure following mortality events, experienced reductions in photosynthetic capacity under drought conditions, while those that did not maintained photosynthetic capacity (Rowland et al. under review). However, large trees will have experienced relatively minor shifts in their light environment when compared to small understory trees. These larger trees may also be more exposed to hydraulic stress from the drought, which may limit their ability to respond positively to elevated light (Bittencourt et al., 2020), although they may already be acclimated to higher VPD conditions at the top of the canopy so may not experience hydraulic stress from sudden shifts in VPD that will occur in the understory. Studying how small understory trees adjust their leaf physiology to concurrent shifts in water and light availability and how these responses differ from large canopy trees may also give new insights into the potential for forest regeneration following drought events.

Here, we use data from a 15-year tropical forest drought experiment located in eastern Amazonia, to test how long-term drought affects carbon metabolism and leaf morphology in small understory trees. By comparing data on maximum photosynthetic capacity (J_{max} and V_{cmax}), leaf dark respiration (R_{leaf}), leaf mass per area and leaf thickness from 66 small understory trees (1–10 cm DBH) against those from 61 surviving canopy trees across 12 genera between a throughfall exclusion (TFE) experiment and neighbouring control plot, we test the following hypotheses:

1. Small understory trees respond to canopy openings following long-term drought stress by increasing photosynthetic capacity (J_{max} and V_{cmax}) in response to elevated light availability.
2. Small understory trees increase leaf dark respiration and LMA in response to long-term drought stress.

3. Leaf physiological traits (J_{max}, V_{cmax}, R_{leaf} and LMA) are more responsive to reduced soil moisture availability and canopy openings following prolonged soil moisture deficit in small understory trees than large trees.

2 | METHODS

2.1 | Study site

This study was carried out at the world's longest running TFE experiment in Caxiuanã National Forest Reserve, Para, Brazil (1°43'S, 51°27'W). The experiment is located in seasonally dry *terra firme* forest with an annual precipitation of 2,000–2,500 mm. Here, a pronounced dry season occurs between June and November, where average precipitation drops to <100 mm per month. The experiment consists of two plots: the TFE plot (1 ha) where 50% of incoming canopy throughfall has been excluded using clear plastic panels at 1–2 m height since 2002, and a neighbouring control plot (1 ha) located <50 m from the TFE. The two plots were both trenched around the perimeter to a depth of 1–2 m to minimize horizontal throughflow. Both plots have been continuously maintained and monitored since 2001. For experimental details, see (da Costa et al., 2010; Fisher et al., 2007; Meir et al., 2018; Rowland, Lobo-do-Vale, et al., 2015). Reduced soil moisture availability on the TFE plot compared with the control plot has been shown previously with lower mean pre-dawn leaf water potentials on the TFE plot (Figure S1 in Data S1) (Bittencourt et al., 2020).

2.2 | Sampling

From August to September 2017, we sampled 66 small trees (1–10 cm diameter at breast height; 1.3 m DBH; 2.7–23.0 m height) across the two plots: 30 from the TFE and 36 from the control. We selected individuals from 12 of the most common genera within the two plots (*Duguetia, Eschweilera, Inga, Iryanthera, Licania, Manilkara, Minquartia, Ocotea, Protium, Tetragastris, Swartzia, Vouacapoa*) in accordance with a corresponding study on large tree carbon metabolism and storage (Table S1a in Data S1; Rowland et al. under review). All species represent canopy or emergent trees when mature. In order to minimize edge effects within the plots, we sampled trees located within one quarter of each 1 ha plot (i.e., 0.25 ha), with all trees located at least 20 m from the plot perimeter. For each genus, individual tree selection was designed to cover a range of sizes from 1 to 10 cm DBH. Two people independently assessed the relative canopy position of each tree and assigned it as either shaded or in a canopy gap according to whether the leaves experienced vertical shading or not. All canopy position assessments were recorded at approximately the same time each day, and the presence of leaves directly above the target tree were used to minimize bias from different sun positions at the time of measurement.

We selected an additional 61 large trees (>20 cm DBH: range 20.2–67.9 cm) from a parallel study across eight corresponding genera

(*Eschweilera, Inga, Licania, Manilkara, Minquartia, Protium, Swartzia, Tetragastris*) to compare responses of large trees against a subset of 48 small trees for the same eight genera (Table S1b in Data S1). For more details about large tree sampling, see Rowland et al. (under review).

2.3 | Gas exchange measurements

During the peak dry season (August–September 2017), we collected a branch of approximately 1 m length from the top of the crown of each tree, using pole pruners. For the majority of the trees (>95%), the branches were cut between 09:00 and 10:00 hours, but on some occasions branches were cut between 10:00 and 13:00. Once harvested, branches were immediately placed in water and were cut twice underwater to restore water supply to the leaves (Domingues et al., 2010). The branches were subsequently left to stabilize in full sunlight for a minimum of 30 min. Following stabilization, we selected non-senescing, fully formed leaves to be measured using two cross-calibrated portable photosynthesis systems (LI-6400XT and LI-6800, LI-COR, Nebraska, USA). For each tree, we measured one leaf for estimates of photosynthetic capacity and one neighbouring leaf for dark-adapted leaf respiration (R_{leaf}).

We performed photosynthetic CO_2 response curves (A-C_i) to estimate maximum photosynthetic capacity. We placed a leaf within the leaf chamber of a portable photosynthesis system and measured net photosynthetic assimilation (A) and leaf internal carbon dioxide (CO_2) concentration (C_i). We generated A-C_i curves by manipulating CO_2 concentrations within the leaf chamber (400, 200, 75, 400, 800, 1,200, 2000 ppm), while providing a photosynthetic active radiation (*PAR*) of 1,500 µmol m^{-2} s^{-1}, a temperature of 28°C and a relative humidity of 60–70%. Light response curves carried out on a subset of the samples ($n = 8$) ensured 1,500 µmol m^{-2} s^{-1} *PAR* represented saturating light conditions for the leaves (data not shown). In order to maintain data quality, A-C_i curves were aborted if stomatal conductance (g_s) dropped below 0.03 mol m^{-2} s^{-1} (following Rowland, Lobo-do-Vale, et al., 2015). No difference between measurements of photosynthetic capacity or R_{leaf} on cut versus uncut branches have been found in previous studies at this site (Rowland, Lobo-do-Vale, et al., 2015), suggesting our measurements reflect true values of in situ leaves and differences between the two plot treatments. We waited for steady-state conditions within the leaf chamber to be reached before any gas exchange measurements were recorded. Using these data, we estimated the maximum rate of electron transport (J_{max}) and the maximum rate of carboxylation (V_{cmax}) standardized to 25°C following the equations from the C_3 photosynthesis model of Farquhar, von Caemmerer, and Berry (1980) as in Sharkey, Bernacchi, Farquhar, and Singsaas (2007) using the *optim* function from the *stats* package in R statistical software (R Core Team, 2013). Ten trees were removed from our sample where A-C_i curves could not be fitted.

Dark-adapted leaf respiration (R_{leaf}) was measured on a leaf adjacent to that used to measure maximum photosynthetic capacity. Leaves were wrapped in aluminium foil for a minimum of 30 min before the portable photosynthesis system was used to measure R_{leaf}. We maintained stable leaf chamber conditions of 400 ppm CO_2 concentration, 0 µmol m^{-2} s^{-1} *PAR* and 28°C for all respiration measurements. Following stabilization within the leaf chamber, three measurements of respiration were recorded at 5 s intervals, then standardised to 25°C using a Q10 value of 2.2 following Rowland, Lobo-do-Vale, et al. (2015) and a mean value was calculated.

2.4 | Leaf morphological traits

After completing leaf gas exchange measurements, we removed each of the leaves used to measure A-C_i curves and R_{leaf} from the branch and placed them in a sealed airtight plastic zip-lock bag. Moist cotton wool was placed in the bag to maintain high levels of humidity and prevent any water loss from the leaf. We scanned leaves using a flatbed scanner (CanoScan LiDE 120, Canon Inc., Tokyo, Japan) and calculated the area of each leaf using ImageJ software (Schneider, Rasband, & Eliceiri, 2012). Leaves were then placed in an oven to dry for 24 hr at 70°C, to constant mass. We measured dry leaf mass using a precision balance and calculated leaf mass per area (LMA, g m^{-2}) by dividing dry leaf mass by leaf area. We used LMA from the leaves used to measure A-C_i curves and R_{leaf} to estimate maximum photosynthetic capacity and R_{leaf}, respectively, on a mass basis. We measured leaf thickness at three different points on fully hydrated leaves using digital callipers avoiding any major veins and calculated the mean. We averaged the LMA and thickness for the A-C_i and R_{leaf} leaves to generate an estimate for the overall branch.

2.5 | Leaf nutrient analyses

We collected an additional sample, depending on leaf size, of 3–20 leaves adjacent to the leaves used for the gas exchange measurements to quantify leaf nitrogen and phosphorus concentrations ($[N]_{leaf}$, $[P]_{leaf}$). Following collection, leaves were dried in an oven for 24 hr at 70°C to constant mass before being combined with the leaves used for gas exchange measurements. Major veins were then removed and the remaining foliar matter was ground to a fine powder using a ball mill. Nitrogen concentrations were measured using the semi-micro Kjeldahl method (Malavolta, 1997), while phosphorus concentrations were measured using a Femto 600+ Spectrophotometer using the ammonium metavandate method (Malavolta, 1997). All analyses were tested against laboratory standards. We divided the gas exchange parameters on a mass basis by leaf nutrient concentrations to estimate leaf photosynthetic and respiratory nutrient use efficiency.

2.6 | Data analysis

2.6.1 | Small tree physiological responses

We used linear mixed effects models, using the package *lme4* (Bates, Mächler, Bolker, & Walker, 2014), to test for treatment (TFE vs. control) and taxonomic effects on leaf gas exchange, morphological, nutrient content and nutrient-use efficiency traits in small trees

(n = 66). Taxonomy was included as a random intercept effect in our models, while the TFE treatment was incorporated as a fixed effect. We tested for a genus and a species nested within genus taxonomic effect by comparing the full linear mixed effects model to a generalized least squares model, following Zuur, Ieno, Walker, Saveliev, and Smith (2009) using the *nlme* package (Pinheiro et al., 2012). When genus was not significant, linear models were used to test the significance of the fixed effects. For each trait response variable, the treatment effect was tested by comparing the Akaike Information Criterion corrected for sample size (AICc) between models using restricted maximum likelihood in the package *MuMIn* (Bartón, 2018).

Since traits may be coordinated, we additionally used standardised major axis regression (SMA) to test the effect of the TFE trait trade-offs. We tested for differences in the slopes and intercepts of the bivariate trait–trait relationships between small trees on the different treatments using a Wald test in the package *smatr* (Warton, Duursma, Falster, & Taskinen, 2012). We investigated relationships between the gas exchange measurements and leaf morphological variables and the relationship between J_{max} and V_{cmax}.

2.6.2 | Small and large tree comparisons

We tested for differences in individual tree-level responses to the TFE treatment for large canopy (n = 61) and small understory trees (n = 48), for the same set of species and genera in which data were available (Table S1b in Data S1). We used linear mixed effect models to test the effect of the TFE treatment, tree size (large vs small), canopy shading (canopy gap vs fully shaded) and the interactions between treatment and tree size, and between treatment and canopy shading on leaf gas exchange and morphological traits. We also tested for taxonomic effects by including genus and species nested within genus as random effect variables and comparing to a generalized least squares model, following the same protocol used for small tree analyses (Bartón, 2018). Within this paper, all data represent the mean and associated errors denote *SE*s of the mean. All data analyses were undertaken on individual tree-level data in R (R 3.5.1, R Core Team, 2018).

3 | RESULTS

3.1 | Physiological responses to long-term drought in small understory trees

We found a significant positive effect of the TFE, relative to the control plot, on both mean J_{max} (71.1%; $\Delta 14.18 \pm 2.65$ µ mol m^{-2} s^{-1}, $p < .001$; Figure 1a) and V_{cmax} (29.2%; $\Delta 3.99 \pm 1.40$ µ mol m^{-2} s^{-1}, $p < .01$; Figure 1b) standardised to 25°C. J_{max} ranged from 7.11 to 41.00 µ mol m^{-2} s^{-1} in the control trees compared with 15.94 to 68.93 µ mol m^{-2} s^{-1} in the TFE trees, while V_{cmax} ranged from 7.84 to 26.12 µ mol m^{-2} s^{-1} in the control and 11.52 to 39.19 µ mol m^{-2} s^{-1} in the TFE. We found a 32.2% increase in 25°C standardised leaf dark respiration (R_{leaf}) on the TFE plot compared to the control plot

($\Delta 0.12 \pm 0.06$ µ mol m^{-2} s^{-1}, $p = .045$; Figure 1c), with values ranging from 0.11–0.95 µ mol m^{-2} s^{-1} on the control and 0.06–1.49 µ mol m^{-2} s^{-1} on the TFE. LMA was 10.68 ± 4.04 g m^{-2} (15.1%) higher for small trees found on the TFE plot ($p < .01$; Figure 1d), but mean leaf thickness did not significantly differ between the two treatments (Table 1). We found no significant differences in mean leaf nitrogen and phosphorus concentrations ($[N]_{leaf}$ and $[P]_{leaf}$) on a mass-basis between the control and TFE treatments (Figure 1e,f, Table 1). We found a significant effect of genus on the intercept for all of the traits except J_{max} and R_{leaf}, but no significant species-nested-within-genus effect for any traits (Table S2 in Data S1).
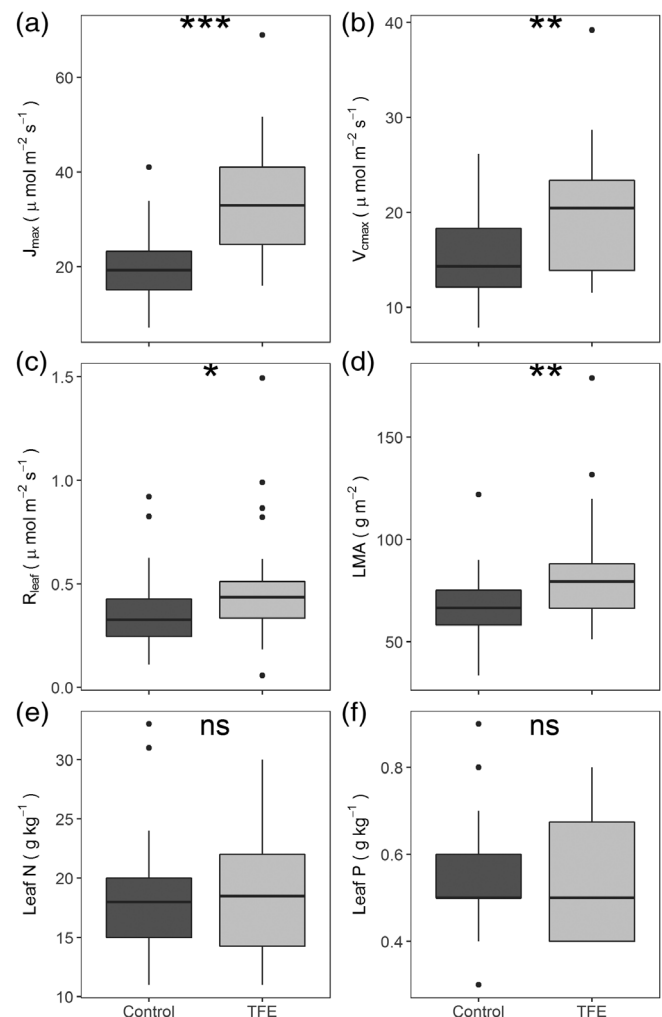

FIGURE 1 Boxplots showing how maximum photosynthetic capacity (J_{max}: [a]; V_{cmax}: [b]), leaf dark respiration (R_{leaf}: [c]), leaf mass per area (LMA: [d]), leaf nitrogen ($[N]_{leaf}$; e) and leaf phosphorus ($[P]_{leaf}$; f) differed between the control and TFE plot for small understory trees (1–10 cm DBH). Asterisks represent significant differences from linear mixed effect model tests at different p-values (ns: non-significant; *$p < .05$; **$p < .01$; ***$p < .001$). See Table S2 in Data S1 for full model details. Boxes represent the interquartile range with a horizontal line for the median and the whiskers represent 1.5*interquartile range or the maximum and minimum point. Dots represent points outside the extent of the whiskers

![Plant, Cell & Environment]

TABLE 1 Parameter estimates for the minimal adequate models explaining J_{max}, V_{cmax}, R_{leaf}, leaf mass per area (LMA) and leaf thickness for large (>20 cm DBH; $n = 61$) and small (1–10 cm DBH; $n = 48$) trees

Trait	Intercept	Canopy position (shaded)	Plot (TFE)	Tree size (small)	Size: Canopy interaction (TFE: Shaded)	Size: Plot interaction (TFE: Small)	Genus intercept	Fixed effect (marginal) R^2	Total (conditional) R^2
J_{max}	***	***	*	***	–	***	*	0.526	0.596
	55.68 ± 2.93	−14.90 ± 3.29	−7.52 ± 3.01	−18.82 ± 3.84	–	18.88 ± 4.56	20.28 ± 4.50		
V_{cmax}	**	**	ns	***	–	**	**	0.336	0.482
	29.96 ± 1.83	−5.82 ± 1.89	−3.27 ± 1.72	−7.59 ± 2.18	–	6.69 ± 2.58	10.70 ± 3.27		
R_{leaf}		–	ns	***	–	–	–	0.188	0.188
	0.63 ± 0.04		0.09 ± 0.05	−0.23 ± 0.05					
LMA		–	*	***	–	–	***	0.267	0.527
	103.91 ± 6.81		8.19 ± 4.10	−30.66 ± 4.60			266.90 ± 16.34		
Leaf thickness	0.18 ± 0.01	–	–	–	–	–	** 0.0005 ± 0.022	0	0.232

Note: The intercept represents the trait value when the fixed effects are zero: large control trees that are not shaded. Canopy position represents the difference when a tree is shaded, plot represents the difference between the TFE treatment compared to the control and tree size represents the difference for small trees compared to large trees. Coefficient estimates ± the SE are presented for each fixed effect and refer to the differences between the factor levels indicated in brackets for each column and the overall intercept. The genus intercept is given whenever the genus included as a random intercept effect improved the model fit. Species nested within genus was not significant for any traits, so is not included here. Random effect variance for genus ± SD is presented. Total (conditional) R^2 represents the total variation explained by the model and is partitioned into the variation explained by the fixed effects (marginal R^2) and fixed plus random-effects (conditional R^2). Asterisks represent the significance level of each variable: ns $p > .05$; *$p < .05$; **$p < .01$; ***$p < .001$. Dashes represent variables that were not retained in the minimal adequate model but were tested in the initial fully saturated model. See Methods and Table S3 in Data S1 for more details about model selection.

Using standardised major axis (SMA) regression to compare differences in the relationships between J_{max}, V_{cmax}, R_{leaf}, and LMA across the two experimental plots, we found no significant trait–trait relationships, except for J_{max}-V_{cmax} (Table S3 in Data S1). An increase in the log_{10}-transformed electron transport rates relative to carboxylation on the TFE treatment compared with the control was observed (TFE = 1.72 ± 0.07, Control = 1.36 ± 0.08, Wald = 8.503, df = 1, p = 0.004; Figure 2), but no difference in the slope of the relationship (p = 0.757).

We found no relationship between J_{max}, V_{cmax} or R_{leaf} with $[N]_{leaf}$ or $[P]_{leaf}$ on either plot (Table S3 in Data S1). However, the TFE trees significantly increased J_{max} on a mass basis per unit N_{leaf} and unit P_{leaf} relative to the control (Figure 3). J_{max} increased per unit nitrogen and unit phosphorus in the TFE trees by 0.006 ± 0.002 µmol g^{-1} s^{-1} and 0.227 ± 0.068 µmol g^{-1} s^{-1}, respectively (J_{max}/N_{leaf}: p = 0.018; J_{max}/P_{leaf}: p < .01; Figure 3). Overall, neither V_{cmax} nor R_{leaf} increased per unit N_{leaf} or P_{leaf} in the TFE trees relative to the control (Figure 3).

3.2 | Comparison of responses to long-term drought between large canopy and small understory trees

We compared the responses of leaf traits in large (>20 cm DBH) and small (1–10 cm DBH) trees to the TFE treatment and to canopy
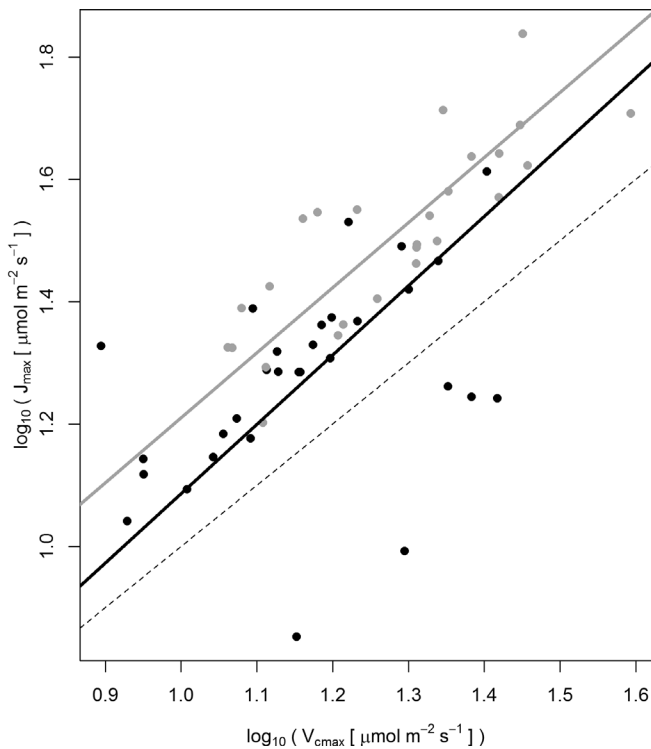
shading using linear mixed models. We found a significant effect of tree size and the TFE treatment on J_{max}, V_{cmax}, R_{leaf}, and LMA, except for a non-significant effect of the TFE treatment on V_{cmax} and R_{leaf} (Figure 4; Table 1). J_{max}, V_{cmax}, and R_{leaf} were, respectively, 33.8% (Δ18.82 ± 3.01 µmol m^{-2} s^{-1}), 25.3% (Δ7.59 ± 2.18 µmol m^{-2} s^{-1}) and 36.5% (Δ0.23 ± 0.05 µmol m^{-2} s^{-1}) lower in small trees after accounting for canopy shading in the models. Canopy shading had a significantly negative effect on J_{max} and V_{cmax}, but not R_{leaf} or LMA, with maximum photosynthetic capacity higher for trees positioned in the canopy or a canopy gap compared to trees shaded by the canopy (Table 1). In addition, a significant interaction between the TFE treatment and tree size for J_{max} and V_{cmax} was found, showing the response of maximum photosynthetic capacity to the TFE treatment
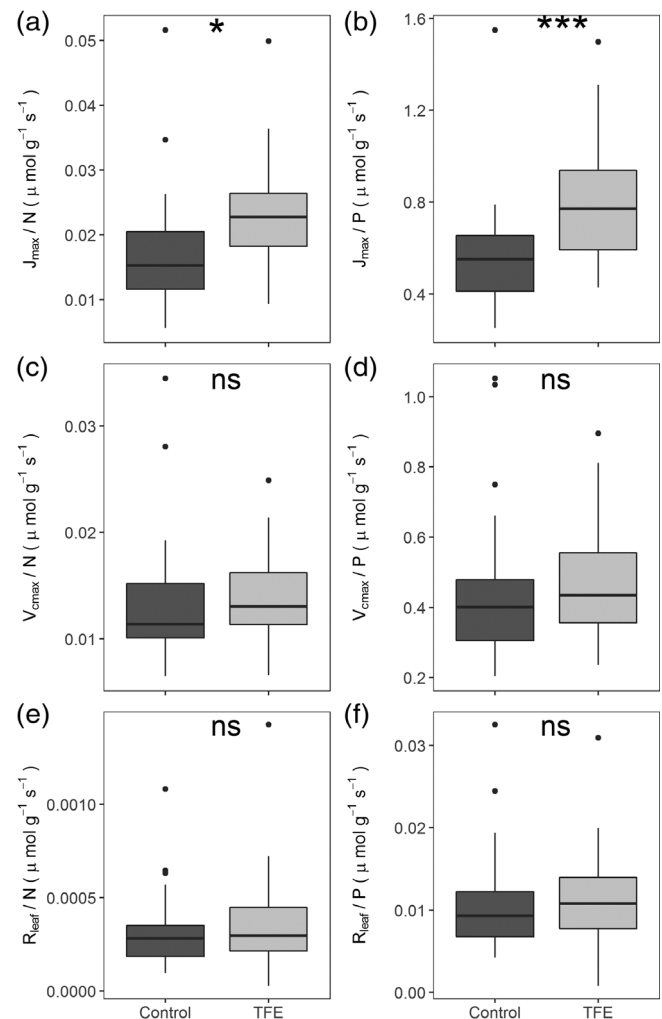

FIGURE 3 Boxplots showing how J_{max} (a,b), V_{cmax} (c,d) and R_{leaf} (e,f) nutrient use efficiency (NUE) changed between the control and TFE treatment for nitrogen (a,c,e) and phosphorus (b,d,f). NUE was calculated by dividing gas exchange parameters on a mass basis by leaf nutrient concentrations. Asterisks represent significant differences from linear mixed effects models between the two treatments (ns: non-significant; *p < .05; **p < .01; ***p < .001). Boxes represent the interquartile range with a horizontal line for the median and the whiskers represent 1.5*interquartile range or the maximum and minimum point. Dots represent points outside the extent of 1.5*interquartile range
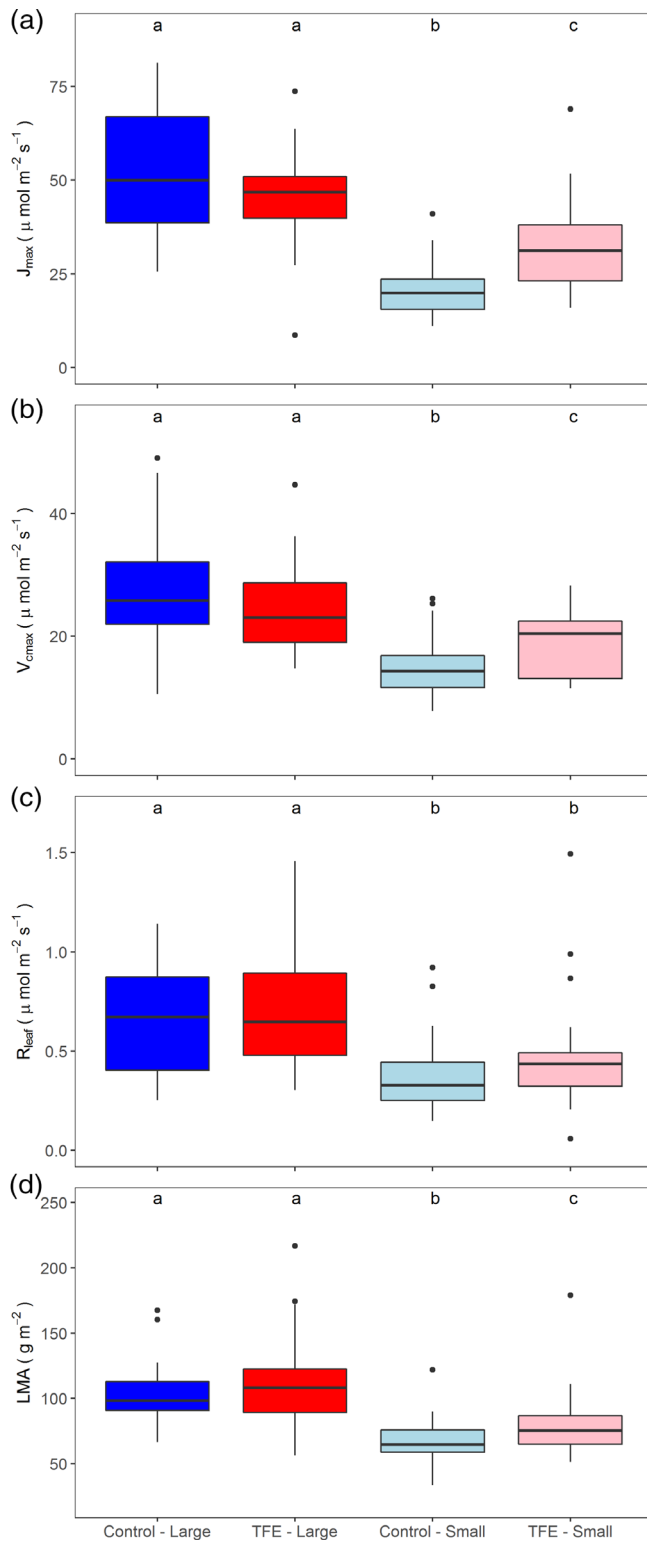

FIGURE 2 Standardised Major Axis (SMA) regression between J_{max} and V_{cmax} on a log_{10} scale for small understory trees (1–10 cm DBH) on the control plot (black) and the TFE plot (grey). The dashed line represents a 1:1 ratio. A significant difference in the elevation (p < .01) and a shift in the data (p < .001) between the treatments were found, but no significant difference between the slopes was found (p = .757)

FIGURE 4 Boxplots showing how J_{max} (a), V_{cmax} (b), R_{leaf} (c) and leaf mass per area (LMA; d) change between the control (blue) and TFE (red) treatments for large (>20 cm DBH; red and dark blue) and small (1–10 cm DBH; pink and light blue) trees. Identical letters represent categories where there is no significant difference ($p > .05$) from pairwise Wilcoxon rank-sum tests. Boxes represent the interquartile range with a horizontal line for the median and the whiskers represent 1.5*interquartile range or the maximum and minimum point. Dots represent points outside the extent of 1.5*interquartile range

to be dependent on tree size after accounting for canopy shading. The TFE effect on small tree J_{max} and V_{cmax} was reduced compared to large trees by 100.3% ($\Delta 18.88 \pm 4.56$ µmol m^{-2} s^{-1}) and 88.1% ($\Delta 6.69 \pm 2.58$ µmol m^{-2} s^{-1}), respectively. We found no significant effect of canopy shading, TFE treatment or tree size on leaf thickness. A significant genus effect was present for all leaf traits except R_{leaf}, showing these leaf traits are not solely determined by the environment, but also vary with taxonomy. For example, *Inga* and *Swartzia* had the highest J_{max} and V_{cmax}, respectively, while *Protium* had the lowest J_{max} and V_{cmax} of the genera studied, according to the intercepts in our mixed effect models. See Table 1 and Table S4 in Data S1 for effect sizes, *SEs*, significance and R^2 values.

When the above results were repeated separately for the five most common genera in our dataset, we discovered the effect of tree size and the TFE treatment varied for different genera (Figure 5). We show *Eschweilera* and *Swartzia* to have a positive response to the TFE treatment in small trees for J_{max}, with *Swartzia* having the largest (positive) response for V_{cmax} (Figure 5). In contrast our data show *Inga* and *Protium* to exhibit little response to the TFE treatment in small trees for all traits. Different genera also exhibit different degrees of variation in their leaf morphology, showing that the responses to the TFE treatment vary by taxa (Figure 5). Despite relatively small sample sizes, our data indicate similar or greater intraspecific than interspecific variation in leaf traits of small trees, especially photosynthetic capacity on the TFE, with the *SD* in trait values within species exceeding the *SD* in species-level means on the TFE in 8/9, 6/9, 4/10, and 2/10 species for J_{max}, V_{cmax}, R_{leaf} and LMA respectively (Table S5 in Data S1).

4 | DISCUSSION

We demonstrate that small understory trees (1–10 cm DBH) are more responsive than large canopy trees (>20 cm DBH) to prolonged drought conditions. Canopy openings driven by drought-induced mortality of large trees (Rowland, da Costa, et al., 2015) have allowed small understory trees to increase photosynthetic capacity (J_{max} & V_{cmax}), leaf respiration (R_{leaf}) and LMA in spite of reduced soil moisture availability and no changes in leaf nutrient concentrations (Figure 1 & Figures S1 and S2 in Data S1). These small understory trees show greater capacity to adjust their leaf physiology than large canopy trees following 15 years of through-fall exclusion (TFE; Figure 4), suggesting responses to drought are dependent on tree size. Measured values of J_{max}, V_{cmax}, R_{leaf} and LMA in this study remained lower in small trees compared to large trees for both treatments, but values for small understory trees under the TFE treatment approach those of large trees for J_{max} and V_{cmax} after accounting for canopy position (canopy gap vs shaded understory) in our statistical models (Table 1). This indicates small trees are sufficiently plastic to increase photosynthetic capacity with higher light availability, even under drought conditions.

Maximum photosynthetic capacity was highly responsive to the TFE treatment in small understory trees with a 71% increase in the maximum rate of electron transfer (J_{max}) and a 29% increase in the
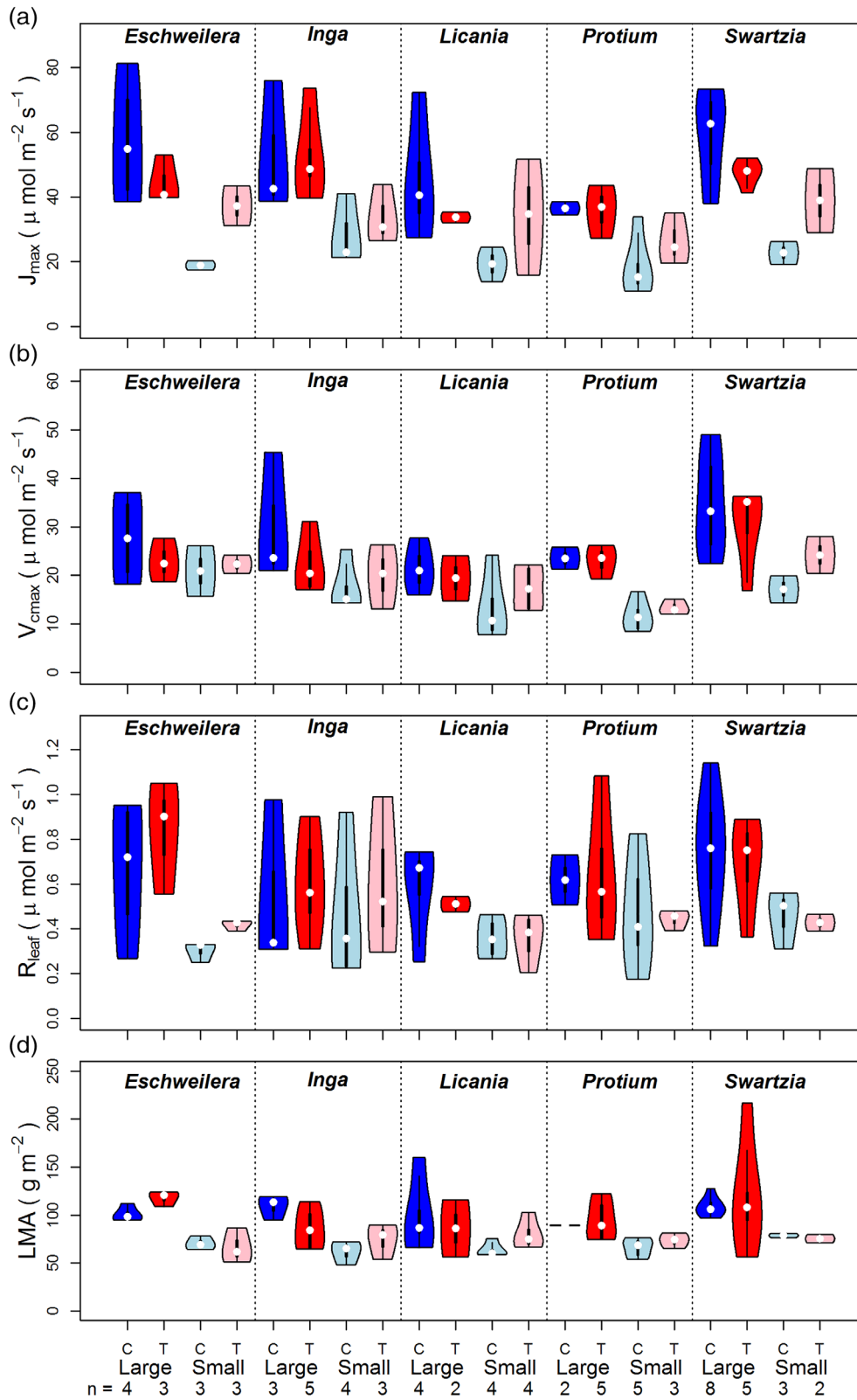
FIGURE 5 Violin plots comparing J_{max} (a), V_{cmax} (b), R_{leaf} (c) and leaf mass per area (LMA; [d]) between the Control (C) and TFE (T) treatment for large (>20 cm DBH) and small (1–10 cm DBH) trees across the five most common genera: *Eschweilera*, *Inga*, *Licania*, *Protium*, *Swartzia*. All genera with $n \geq 2$ for each of the four categories are presented. White dots represent the median, thick grey lines represent the interquartile range and thin grey lines represent the mean ± 1.5 *SEs* [Colour figure can be viewed at wileyonlinelibrary.com]

maximum rate of carboxylation (V_{cmax}; Figure 1). The increase in light availability in the droughted forest and the release from extreme light limitation is likely to be driving these physiological adjustments as light represents an important control on both traits (Hasper et al., 2017). Typically, very little light penetrates the canopy to the

understory in tropical forests because of the high leaf area index characteristic of tropical rainforests (Asner, Scurlock, & Hicke, 2003). With the opening of the canopy, increased light availability may mean understory trees are no longer dependent on sunflecks for the majority of their photosynthesis (Chazdon & Pearcy, 1991; Leakey

et al., 2003). As a consequence, the magnitude of the change in the light environment may be sufficiently great that light acts as a strong selection pressure on photosynthetic capacity in these small trees, even when exposed to long-term drought conditions.

The greater plasticity of J_{max} compared to V_{cmax} and resultant increase in the J_{max} / V_{cmax} ratio on the TFE plot (Figure 2) is highly indicative of a response to light, as the electron transfer reactions are directly involved in capturing light energy (Farquhar et al., 1980; Sharkey et al., 2007). A similar shift in the ratio in tropical montane cloud forests compared to lowland tropical forests has been attributed to lower total daily light availability caused by cloud cover, but with periods of intermittent intense light (van de Weg, Meir, Grace, & Ramos, 2012). Following the mortality of canopy trees, the understory may experience similar sporadic periods of intense light, as a consequence of increased sunfleck occurrence and duration. A larger upregulation of J_{max} compared to V_{cmax} may allow these trees to maximize light capture during these prolonged sunflecks, with carboxylation reactions occurring subsequently (Pearcy, 1990). The division of the light dependent and light independent reactions may allow small understory trees to increase overall carbon assimilation while avoiding the additional maintenance costs of elevated V_{cmax}.

We show small trees can increase J_{max}, but not V_{cmax}, without additional leaf nitrogen or phosphorus (Figure 1), most likely by increasing nutrient use efficiency (Figure 3). This may occur via a potential re-allocation of nitrogen and phosphorus to optimize photosynthetic capacity (Hasper et al., 2017; Mo et al., 2019). The carboxylation reactions have greater nutrient demand for enzymes, such as RuBisCO, compared to those in the electron transport chain (Evans, 1989; Raven, 2013; Xu et al., 2012). This may allow J_{max} to be more plastic and responsive to changes in light availability than V_{cmax}, without increasing leaf nutrient concentrations. Overall, the reduction in light limitation of photosynthesis in understory trees we observe here could facilitate increased wet-season growth rates as observed previously at this experiment in larger understory trees (Metcalfe et al., 2010; Rowland, da Costa, et al., 2015). Consequently, the ability of small understory trees to respond to light while under soil moisture deficit may allow them to regenerate and recover biomass faster than currently predicted by dynamic vegetation models (Fisher et al., 2007).

The ability of small understory trees to respond to increased light availability in the face of drought, increased leaf temperatures and elevated VPD may have various explanations. Firstly, it is possible that despite the reduced soil moisture concentrations (Figure S1 in Data S1), our small trees are less stressed than the adult trees by the drought treatment. This could be a consequence of reduced competition for water following the mortality of large trees (Rowland, da Costa, et al., 2015), or a result of physiological adjustments to their hydraulic architecture or stomatal conductance. Small trees may be able to avoid drought stress by having stronger stomatal regulation or greater resistance to xylem embolism (Anderegg et al., 2018; Bittencourt et al., 2020). Reduced physiological and architectural constraints also allow small trees to explore more trait combinations than

canopy trees that may allow them to be more effective at resisting drought (Damián, Fornoni, Domínguez, Boege, & Baltzer, 2017; Reed, Schindler, & Waples, 2011). Alternatively, small trees may be able to avoid drought stress by reducing non-maintenance related metabolic activity, such as growth, in the dry season when drought conditions are most pronounced, and maximizing growth during the wet season when the soil moisture deficit is reduced. This hypothesis is consistent with previous observations of high wet season and lower dry season diameter growth rates in the trees of smaller size classes on the TFE relative to the control (Metcalfe et al., 2010; Rowland, da Costa, et al., 2015). Whichever mechanism small trees use, our results suggest that these trees are likely to be able to minimize the impact of the drought, relative to larger trees, in order to facilitate the upregulation of photosynthetic capacity and a release from extreme light limitation.

In addition to upregulation of maximum photosynthetic capacity, we found R_{leaf} to increase in small trees following long-term drought by 32% (Figure 1). These elevated respiratory rates likely reflect the additional maintenance costs of higher photosynthetic capacity under elevated light conditions (Atkin et al., 2015). However, we do not find a direct relationship between R_{leaf} and V_{cmax} or J_{max} in these trees (Table S3 in Data S1), suggesting elevated R_{leaf} is not simply a consequence of increased photosynthetic capacity. Instead, elevated R_{leaf} may reflect a response to increased stress under reduced water availability, potentially representing a means of drought resistance through increasing carbon metabolism for maintenance respiration (Rowland et al., 2018). Under water limitation, particularly when combined with increased irradiance, leaves can accumulate harmful reactive oxygen species (ROS) that cause cell damage (Wang & Vanlerberghe, 2013). An upregulation of respiration can provide a mechanism for leaves to purge these harmful ROS and avoid damage to the photosynthetic metabolic machinery (Atkin & Macherel, 2009). These negative effects of ROS are likely to be strongest during the dry season when water availability is particularly low. Here, small understory trees may have increased their R_{leaf} in the dry season to avoid drought stress and facilitate responses to increased light availability. It must also be acknowledged that our results may be influenced by changes in canopy temperature profiles between the plots, with a smaller expected vertical gradient in temperature in the more open canopy of the TFE plot, but measuring this was beyond the scope of this study. We do find large intra-generic variations in R_{leaf} and low explanatory power of the TFE treatment in our statistical models (Table S2 in Data S1), suggesting this trait is not simply influenced by long-term drought, but also by a range of other unaccounted for factors that are likely species-specific.

An increase in light availability in the understory of the droughted forest is also likely to be the main explanation for the 15% increase in LMA in small trees on the TFE we observe here. LMA is most strongly controlled by light (H. Poorter et al., 2009), as leaves invest more in proteins that catalyse photosynthesis and produce more carbohydrates under elevated light conditions (Niinemets, Kull, & Tenhunen, 1998; H. Poorter et al., 2009). Higher concentrations of these high molecular weight metabolites will increase the density of

cells and hence LMA. However, it should be noted that elevated LMA may also occur in response to reduced water availability, as a water conservation strategy (H. Poorter et al., 2009; Wright et al., 2004). Unlike photosynthetic capacity and R_{leaf}, LMA has been shown to increase in large trees at the same experiment via increased investment in spongy mesophyll for water storage (Rowland, Lobo-do-Vale, et al., 2015; Binks et al., 2016; Rowland et al. under review), indicating increased LMA in small trees may also be directly associated to lower water availability in the TFE.

We show small trees were more responsive to prolonged drought conditions than large canopy trees, suggesting small trees experience different selection pressures or possess a greater ability to respond to ecosystem level changes in the physical environment. Maximum photosynthetic capacity of small trees increased in response to the TFE treatment – the exact opposite to the response of large trees to the TFE once differences in canopy position have been accounted for in our models (Table 1). In fact, only canopy shading prevents small trees from matching the capacity of large trees. Under prolonged drought, small trees experience changes to both their light and water availability, whereas larger canopy trees will predominantly only experience substantial shifts in water availability. The different responses to prolonged drought suggest small trees are responding most strongly to changes in their light environment, while large trees are responding to reductions in soil moisture availability. These shifts may reflect changes in the most limiting resource from the understory to the top canopy, with understory trees strongly light limited and canopy trees strongly water or nutrient limited (Chazdon & Fetcher, 1984; Sterck & Schieving, 2011).

Despite a general trend of physiological adjustments to prolonged drought conditions in small understory trees, our results clearly demonstrate that different genera have different abilities to respond to changes in their physical environment. Small *Eschweilera* and *Swartzia* trees displayed the greatest directional change in their traits in response to the TFE, as represented by the increase in carbon metabolism trait values (J_{max} & V_{cmax}; Figure 5). In contrast small *Inga* and *Protium* trees showed very low directional trait variability in response to the TFE. The capacity of a tree to change its traits and potentially acclimate to new environmental conditions is likely to provide a competitive advantage under unstable environmental conditions (Lusk, Wright, & Reich, 2003; Pattison, Goldstein, & Ares, 1998), allowing some species to outcompete others. The large positive responses of small *Eschweilera* trees to drought observed here may allow it to maintain its hyperdominance in the Amazon (ter Steege et al. 2013), while species that show limited responses, for example, *Protium*, may become less dominant in future communities. However, it should be noted that we show large intrageneric variation in the responses of trees of all sizes (Figure 5), suggesting taxonomy may not be the only critical factor determining responses to drought-induced changes to the physical environment. Moreover, we focus here on leaf-level physiology and do not measure architectural (e.g., rooting depth) or stomatal responses that may facilitate adaptive responses in genera with low leaf-level plasticity. Further studies investigating recruitment following long-term drought are required to fully understand how taxonomic dominance may change in the future.

5 | CONCLUSION

In this study, we highlight the important role of forest structural changes and light in determining physiological responses to long-term drought. Small trees (<10 cm DBH) relative to large trees (>20 cm DBH) display sufficient phenotypic plasticity in leaf morphology and carbon metabolism traits to allow them to respond to increases in light availability despite long-term drought. The ability of small trees to increase their photosynthetic capacity may facilitate increased growth and consequently partial recovery of forest aboveground biomass following earlier drought-induced mortality of large trees (Rowland, da Costa, et al., 2015). This capacity of small trees to show positive responses to ecosystem-level changes in water and light availability could ultimately allow a more resilient forest to establish and potentially moderate the negative impacts of climate change on the forest ecosystem.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

D.B., L.R., M.M., R.O. and P.M. designed the data collection, A.C.L.D. and P.M. designed the drought experiment, D.B., L.R., A.C.L.D., P.R.L.B., I.C., P.B.C., T.F.D., R.C.M., L.V.F., and O.A.A.R. all contributed to data collection and all authors contributed to writing the manuscript.

DATA AVAILABILITY STATEMENT

Data will be deposited in DRYAD on acceptance of the manuscript and prior to publication.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.