Photosynthetic plasticity of a tropical tree species, *Tabebuia rosea*, in response to elevated temperature and [CO$_2$]

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Running head: photosynthetic acclimation to climate change

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ABSTRACT

Atmospheric and climate change will expose tropical forests to conditions they have not experienced in millions of years. To better understand the consequences of this change we studied photosynthetic acclimation of the neotropical tree species *Tabebuia rosea* to combined 4°C warming and twice-ambient (800 ppm) CO_2. We measured temperature responses of the maximum rates of ribulose 1,5-bisphosphate carboxylation (V_{CMax}), photosynthetic electron transport (J_{Max}), net photosynthesis (P_{Net}), and stomatal conductance (g_s), and fitted the data using a probabilistic Bayesian approach. To evaluate short-term acclimation plants were then switched between treatment and control conditions and re-measured after 1–2 weeks. Consistent with acclimation, the optimum temperatures (T_{Opt}) for V_{CMax}, J_{Max} and P_{Net} were 1–5°C higher in treatment than in control plants, while photosynthetic capacity (V_{CMax}, J_{Max}, and P_{Net} at T_{Opt}) was 8–25% lower. Likewise, moving control plants to treatment conditions moderately increased temperature optima and decreased photosynthetic capacity. Stomatal density and sensitivity to leaf-to-air vapor pressure deficit were not affected by growth conditions, and treatment plants did not exhibit stronger stomatal limitations. Collectively, these results illustrate the strong photosynthetic plasticity of this tropical tree species as even fully-developed leaves of saplings transferred to extreme conditions partially acclimated.

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1 | INTRODUCTION

Tropical forest species are believed to be particularly sensitive to global warming, as they are adapted to conditions of limited seasonal temperature variation. Furthermore, temperatures in the lowland tropics are already the highest known to support closed-canopy forest, and distances to cooler refugial areas can be large (Wright, Muller-Landau & Schipper 2009). Tropical species will therefore need to acclimate to changing conditions for tropical forests to continue to have a mitigating effect on anthropogenic climate change.

Despite millions of years of thermal stability in the tropics, tropical trees do have the capacity to acclimate to warming. For example, experimental nighttime warming results in down-regulation of respiratory carbon loss from leaves (Cheesman & Winter 2013; Slot et al. 2014; Slot & Winter 2017a, 2018), consistent with thermal acclimation (Atkin & Tjoelker 2003); under elevated growth temperature the optimum temperature for photosynthetic carbon uptake shifts towards the new, higher growth temperature (Kotisup et al. 2009; Slot & Winter 2017a); and growth rates do not necessarily decrease under either nighttime warming or daytime warming (Cheesman & Winter 2013; Scafaro et al. 2017; Slot & Winter 2018). Thus, despite differences among species, the comparative thermal stability of the tropics has not deprived tropical species of the physiological plasticity that enables them to acclimate to moderate warming.

As plants are confronted with rising temperatures, atmospheric CO_2 concentrations ($[CO_2]_{atm}$) are also increasing, and in the short term this has both direct and indirect effects on photosynthetic carbon uptake and its response to temperature. CO_2 as the substrate for photosynthesis directly stimulates rates of carbon uptake. Higher $[CO_2]_{atm}$ also suppresses carbon

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loss associated with photorespiration, the result of oxygenation of Rubisco (e.g., Long, Ainsworth, Rogers & Ort. 2004; Ainsworth & Rogers 2007). Photorespiration increases with temperature, so when photorespiration is suppressed by elevated $[CO_2]_{atm}$ plants can achieve a higher optimum temperature of photosynthesis than at ambient $[CO_2]_{atm}$ (Berry & Björkman 1980; Long 1991). The decrease in Rubisco limitation of photosynthesis under elevated CO_2 conditions means that net photosynthesis is increasingly limited by the maximum photosynthetic electron transport rate (J_{Max}), reflecting the maximum rate of ribulose 1,5-bisphosphate (RuBP) regeneration (Sage & Kubien 2007). This affects the temperature response of net photosynthesis because electron transport limited-photosynthesis has a higher temperature optimum than Rubisco limited photosynthesis (Sage & Kubien 2007; Hikosaka, Ishikawa, Borjigidai, Muller & Onoda 2006). In the long term, elevated $[CO_2]_{atm}$ may cause a reduction in both the maximum capacity of RuBP carboxylation (V_{CMax}) and RuBP regeneration (J_{Max}). V_{CMax} might decrease as a result of lower investment in Rubisco when high $[CO_2]_{atm}$ reduces carbon limitation of photosynthesis and optimization requires proportionally greater investment in electron transport (Ainsworth & Rogers 2007). V_{CMax} and J_{Max} might also decrease if leaf nitrogen (N) concentrations decrease (Medlyn et al. 1999) as a result of N dilution by rapid growth (Luo, Field & Mooney 1994), or as a result of progressive soil N limitation (Luo et al. 2004; Warren, Jensen, Medlyn, Norby & Tissue 2015). Changes in leaf N in plants grown at elevated CO_2 could directly affect the thermal acclimation capacity of plants, as there appears to be an important role for N allocation to Rubisco—an N-rich enzyme—during acclimation to warming (Scafaro et al. 2017).

To assess thermal acclimation of photosynthesis, the short-term temperature response of the photosynthetic parameters is compared between warmed and control plants. Berry &

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Björkman (1980), summarizing previous research, showed that photosynthesis peaked at higher temperatures in plants acclimated to warmer conditions than in cool grown plants. A recent global meta-analysis showed that across sites, the optimum temperature for net photosynthesis (T_{Opt}) scaled with growth temperature, and this pattern could be explained by thermal acclimation; adaptation—expressed as inherent differences based on source populations—was of lesser importance (Kumarathunge et al. 2019), stressing the importance of physiological plasticity. A shift in the optimum temperature (T_{Opt}) towards higher values may or may not be accompanied by increases in P_{Opt}, the rate of photosynthesis at T_{Opt} (Berry & Björkman 1980; Way & Yamori 2014; Slot, Garcia & Winter 2016). Vegetation models are highly sensitive to the formulation of temperature responses (Booth et al. 2012). To inform such models about acclimation, temperature response parameters of V_{CMax} and J_{Max} are required, including their activation energies, de- activation energies, entropy factors (ΔS, sensu Medlyn et al. 2002), and their temperature optima (Stinziano, Way & Bauerle 2018; Mercado et al. 2018). Because of the inherent non-linearity of temperature responses and Jensen's inequality, implementation of acclimation parameters would be most meaningful if the parameterization reflected the true diversity of these parameter values, rather than single averages.

Acclimation processes are currently not well represented in most dynamic global vegetation models and earth system models (Smith & Dukes, 2013; Smith, Malyshev, Shevliakova, Kattge & Dukes. 2016; Lombardozzi, Bonan, Smith, Dukes & Fisher. 2015; Mercado et al. 2018), and limited experimental data is available on the combined effects of warming and elevated [CO_2] that can provide mechanistic foundations for modeling acclimation (Way, Oren & Kroner 2015), particularly for tropical plants. To address thermal acclimation of photosynthesis several modeling studies have capitalized on the clear trend of a decreasing J_{Max}/V_{CMax} ratio with

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acclimation to higher growth temperatures, as synthesized by Kattge & Knorr (2007), and more recently confirmed by Smith & Dukes (2018) (e.g., Lombardozzi et al. 2015; Smith et al. 2016; Mercado et al. 2018). However, $[CO_2]_{atm}$ may affect the J_{Max}/V_{CMax} ratio independent of temperature. While acclimation to warming consistently decreases the J_{Max}/V_{CMax} ratio, increased $[CO_2]_{atm}$ may either increase the ratio (e.g. meta-analysis by Ainsworth & Rogers 2007), or not affect it (e.g., meta-analysis by Medlyn et al. 1999). These differences may be related to nutrient supply and /or to source-sink relationships (Arp 1991; Sage 1994), as nutrient- or sink limitation may cause proportionally greater reduction in V_{CMax}. Fauset et al. (2019) found that the J_{Max}/V_{CMax} ratio of the tropical tree species *Alchornea glandulosa* decreased with increasing growth temperature, but increased with elevated $[CO_2]$, such that J_{Max}/V_{CMax} for plants grown at 800 ppm CO_2 was higher at 35°C than that of control plants at 30°C. The same pattern of opposing effects of warming and elevated CO_2 on J_{Max}/V_{CMax} was found in the boreal tree species *Larix laricina*, and to a lesser extent in *Picea mariana* (Dusenge et al. 2020). The utility of J_{Max}/V_{CMax} changes to model photosynthetic acclimation may thus be limited when both temperature and CO_2 increase, and additional information on photosynthetic parameters is needed.

The short-term temperature response of net photosynthesis can be controlled by different factors, including the temperature sensitivies of V_{CMax}, J_{Max}, and respiration in the light, and by stomatal conductance (Lin, Medlyn & Ellsworth 2012). We have shown that the temperature response of net photosynthesis of field-grown lowland tropical trees is largely controlled by decreases in stomatal conductance as the leaf-to-air vapor pressure deficit (VPD) increases with increasing measurement temperature (Slot & Winter 2017b, 2017c; Hernández, Winter & Slot 2020; see also Smith *et al*. 2020), whereas Vårhammar et al. (2015) reported significant

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limitations by J_{Max} in tropical montane species in Rwanda. Growth at elevated CO_2 generally results in decreases in stomatal conductance (Saxe, Ellsworth, & Heath 1998; Ainsworth & Rogers 2007), potentially increasing stomatal control over net photosynthesis. However, upregulation of stomatal conductance at a given VPD during acclimation to elevated temperature and VPD has also been observed in some species (Marchin, Broadhead, Bostic, Dunn & Hoffmann 2016; Wu et al. 2018; Dusenge, Madhavji & Way 2020). Knowing which process limits photosynthetic carbon fixation is important to inform vegetation models and to better predict how environmental change will impact photosynthetic carbon uptake of tropical forest trees.

Here we report on an experiment with the neotropical tree species *Tabebuia rosea* (Bertol.) Bertero ex A.DC. (Bignoniaceae), grown under combined warming and elevated CO_2 conditions. We evaluated the capacity for acclimation of photosynthesis and respiration. Specifically, we examined leaves developed under treatment conditions ("long-term acclimation"), and of pre-existing leaves of plants transferred from control to treatment conditions and vice versa ("short-term acclimation"). We hypothesized that (i) acclimation would result in higher temperature optima for photosynthetic parameters, and in lower respiration rates at a set temperature; (ii) long-term acclimation (of newly-developed leaves) would be stronger than short-term acclimation (of pre-existing leaves); and (iii) stomatal limitation of photosynthesis would increase under treatment conditions of warming and elevated CO_2. Nonlinear models characterizing the temperature responses for each treatment were fitted using a probabilistic Bayesian approach. This approach enabled us to present the parameters of interest in the form of a probability distribution of values, to better reflect the range of potential parameter values.

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2 | MATERIALS AND METHODS

2.1 Plant material and growth conditions

Tabebuia rosea is a common tree species throughout its native range in central America and northern South America where it occurs in dry, moist, and wet forests, in plantations, gardens and farms, and as a common ornamental roadside tree (Condit, Pérez & Daguerre 2010). It is a light-demanding species that forms sparse canopies with minimal self-shading. Seeds of *T. rosea* were collected near Panama City, Republic of Panama, and germinated in trays with MiracleGrow® potting soil. After germination the seedlings were transferred to individual 61 cm tall, 30.3-liter tree pots (TP1124R, Stuewe & Sons, Tangent, OR) filled with 80:20 ratio of local clay-textured top soil and river sand. After 1 month in these pots, all plants were supplemented with 15:9:12 Osmocote Plus very slow-release (8−9 months) N:P:K fertilizer (Scotts-Sierra, Maryville, OH, USA). Five plants were placed in a temperature- and CO_2 controlled 6 m-diameter geodesic dome (Solardomes Industries Limited, Nursling, UK) maintained at current local ambient temperature and ~420 ppm CO_2; five other plants were placed in a second dome maintained at ambient temperature +4°C and ~800 ppm CO_2. By the time measurements were started, 8 weeks after the pots were moved into the domes, the plants were about 8 months old, 120 cm tall and contained at least five opposite pairs of compound leaves—the first leaves after germination to emerge are simple. Dome temperatures were controlled by split AC units and passive warming (control dome) and heaters (HER 75B 3101, 21.3 Amp, Modine Manufacturing Company, Racine, WI, USA) (treatment dome). The domes operated as semi-closed systems, with enough ventilation to maintain the ambient dome at ~420 ppm. CO_2 from a cylinder was injected at a small, but constant flow rate to maintain the treatment dome at ~800 ppm CO_2

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during daytime hours. For the duration of the experiment air temperature was on average 4.0°C higher in the treatment dome than in the control dome (3°C at night—between 6 p.m. and 6 a.m.—and 5°C during the day) and mean daily relative humidity (± SD) was 74 ± 7% in the treatment dome and 85 ± 4% in the control dome. Daily mean (± SD) CO_2 concentration in the treatment dome was 809 ± 71 ppm during the month of photosynthesis measurements.

After gas exchange measurements were made on the plants grown under these contrasting conditions and samples were collected to assess leaf morphological and chemical traits (see below), the plants were switched between the domes, and after one week the same leaves were measured again. Re-measuring the plants took 9 and 7 days for the plants moved to control and to treatment conditions, respectively, resulting in an average short-term acclimation duration of 11 days. On each day measurements were taken over a range of temperatures, thereby minimizing the risk of introducing bias in the temperature responses based on the number of days since conditions were switched. The experiment was conducted at the Santa Cruz Experimental Field Facility of the Smithsonian Tropical Research Institute in Gamboa, Republic of Panama, where the average 24-hour mean temperature and the average maximum temperature equal 26.9°C and 32.6°C, respectively.

2.2 | A-C_i curve measurements

Between 31 August and 14 September 2017, we measured A-C_i curves on sun-exposed leaves of *T. rosea* saplings over a wide, but realistic leaf temperature range (i.e., no temperatures lower than minimum daytime temperatures were included to avoid cold stress responses in these lowland tropical plants). In the control dome the measurement leaf temperature ranged from 26.5

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to 43.3°C; in the treatment dome the range was 28.0–44.6°C. Target temperatures varied based on the leaf temperatures reached and the requirements to fill the gaps in the curves. Dome temperatures were maintained as close as possible to target leaf temperatures to minimize the temperature differential between the measured leaf and the rest of the plant. The block temperature of the leaf cuvette was controlled using the Peltier cooling/heating capacity of the LI-6400XT portable photosynthesis system (LI-COR Biosciences, Lincoln, Nebraska, USA). Leaf temperature always exceeded air temperature because of the heat generated by the light source; hence the lowest measurement temperature was higher than the lowest ambient daytime air temperature.

Fully-expanded mature sun-exposed leaves that had developed under the growth conditions in the dome were selected from the second-or third most recently emerged leaf pair and each leaf was measured 2–18 times (average 12, median 14). Measurements were made on leaves between 0.5 and 1.0 m above ground level. A-C_i curves were measured at a pre-determined light saturation level of 1500 μmol m^{-2} s^{-1} that was provided by the red:blue LED light source of the LI-6400XT. Light-saturated photosynthesis rates were determined at ≥13 CO_2 concentrations between 50 and 1950 ppm, using the built-in CO_2 mixer of the LI-6400XT to control CO_2 concentrations of the incoming air, which was delivered at a flow rate of 500 μmol s^{-1}. Leaf temperature during measurements was monitored abaxially with a Type E thermocouple inside the leaf cuvette. After equilibration at ambient [CO_2] in the dome, photosynthesis was recorded at 50, 100, 200, 300, 400, 475, 550, 675, 800, 950, 1200, 1500 and 1950 ppm CO_2 of the incoming air. Measurements were discontinued whenever a precipitous decline in stomatal conductance was observed and photosynthesis rates failed to stabilize at a given target CO_2 concentration.

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After completing these measurements of plants at their respective growth conditions, control plants were moved to the treatment dome, and treatment plants to the control dome. At this point 46 useable A-C_i curves had been collected for control plants, and 46 curves for treatment plants. After one week at their new condition, we started re-measuring the same plants (and the same leaves). The post-transition measurements yielded another 46 A-C_i curves for control plants transferred to treatment conditions and 44 curves for treatment plants transferred to control conditions.

Before calculating V_{CMax} and J_{Max}, we corrected photosynthesis rates and C_i values for diffusion errors associated with CO_2 concentration gradients between the leaf cuvette and the atmosphere. To do so, we measured CO_2-response curves in both domes for leaves that were killed by submersion in boiling water for ~5 minutes. These measurements were taken at 30°C, as previous tests showed no temperature effect on the diffusion error (Slot & Winter 2017b). Applying the correction changed V_{CMax} and J_{Max} estimates by 5 and 7% (median), respectively, but had no effect on the temperature responses of these parameters.

2.3 | Dark respiration

We assessed mitochondrial respiration in darkness, as there are inconsistencies among the different methods used to estimate respiration in the light (Way et al. 2019). At ~5 a.m. we covered leaves with thin aluminum foil to keep them darkened after dawn, and measured dark respiration rates at several different temperatures during the morning with the LI-6400XT. Dome temperatures were set to match the target leaf temperatures. Measurements were made at the [CO_2] of the measurement domes to minimize diffusion-related measurement errors. The air flow rate was set to 250 μmol s^{-1}. Respiration rates were logged for 2 minutes and the logged values

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were averaged prior to further analyses to reduce the effects of random fluctuations on the respiration estimates caused by the relatively low signal-to-noise ratio when measuring low flux rates on a small leaf area (e.g., the 6 cm^2 of the standard cuvette of the LI-6400XT). The temperature sensitivity of respiration was expressed as the Q_{10}, calculated as:

$$Q_{10} = e^{(10 \times b)}, \hspace{4cm} \text{Eqn. 1}$$

where b is the slope of the natural log-transformed respiration rates versus leaf temperature:

$$\ln(R_{Dark}) = a + b \times T_{Leaf} \hspace{4cm} \text{Eqn. 2}$$

From this, we calculated respiration rates at a set temperature of 30°C (R_{30}) and at the mean nighttime temperatures of the two domes.

2.4 | Parameter estimation

V_{CMax} and J_{Max} were calculated from the diffusion-error-corrected A-C$_i$ curves with the 'fitaci' function from the 'plantecophys' package (Duursma 2015) in R version 3.5 (R Development Core Team, 2018), which uses the Farquhar, von Caemmerer, and Berry (FvCB) model (Farquhar, von Caemmerer & Berry 1980; von Caemmerer & Farquhar 1981). There was no sign of triose-phosphate utilization limitation so this was not considered in the calculations. In the absence of reliable, temperature-dependent estimates of mesophyll conductance (g_m, representing the ease of CO_2 transfer from the substomatal cavity to the site of carboxylation in the chloroplast) for this species, g_m was assumed to be infinite. Therefore, the calculated V_{CMax} and J_{Max} values slightly underestimate the chloroplastic rates. For R_{Light}, Γ^* and additional parameters

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of the FvCB model we used default estimates from the 'fitaci' function that are derived from

e.g., Bernacchi, Singsaas, Pimentel, Portis Jr & Long (2001) and Medlyn et al. (2002).

2.5 | Temperature responses of photosynthesis parameters

The temperature responses of V_{CMax} and J_{Max} were fitted with a peaked Arrhenius function according to Medlyn et al. (2002) as:

$$f(T_k) = k_{Opt} \times \frac{H_d \times e^{\left(\frac{H_a \times (T_k - T_{Opt})}{T_k \times R \times T_{Opt}}\right)}}{H_d - H_a \times \left(1 - e^{\left(\frac{H_d \times (T_k - T_{Opt})}{T_k \times R \times T_{Opt}}\right)}\right)} \qquad \text{Eqn. 3}$$

where temperatures are in Kelvin, T_k is leaf temperature, k_{Opt} is V_{CMax} or J_{Max} at T_{Opt}, H_a represents the activation energy—it describes the exponential rise of the curve before T_{Opt}— H_d is the 'de-activation energy', reflecting the rate of decrease above T_{Opt}, and R is the universal gas constant (8.314 J K^{-1} mol^{-1}). Table S1 shows how this equation was used to estimate parameters for all four treatment categories. We also calculated the entropy parameter ΔS (sensu Medlyn et al. 2002), which is related to T_{Opt} as:

$$T_{Opt} = \frac{H_d}{\Delta S - R \ln\left(\frac{H_a}{H_d - H_a}\right)}$$

Eqn. 4

and thus:

$$\Delta S = \frac{H_d}{T_{Opt}} + R \ln\left(\frac{H_a}{H_d - H_a}\right)$$

Eqn. 5

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To improve our ability to obtain robust parameter estimates we pooled measurements made on different leaves within each treatment—the experimental design and the number of leaf-level replicates was not amenable to a random effects model. Fig. S1 shows leaf-level curves in comparison to pooled-data curves.

Net photosynthesis at the ambient conditions of control and treatment domes, that is, at about 400 ppm (P_{400}) and at 800 ppm (P_{800}), respectively, were extracted from each A-Ci curve, and P_{400} and P_{800} were fitted with a parabolic function following Gunderson, O'Hara, Campion, Walker & Edwards (2010) as:

$$P_{400} \text{ or } P_{800} = P_{Opt} - b \times \left(T_k - T_{Opt}\right)^2 \qquad \text{Eqn. 6}$$

where P_{Opt} is the rate of net photosynthesis at 400 or 800 ppm CO_2 at optimum temperature T_{Opt}, and b is a shape parameter that is inversely proportional to the width of the parabolic curve. The full equation used for estimating parameters for all four treatment categories is shown in Table S1. Net photosynthesis was also fitted with Eqn 3, for which the results are presented in Table S3.

In addition, for each curve the net photosynthesis rate at C_i of 270 (P_{270}) and 505 ppm (P_{505}) were calculated and their temperature responses were analyzed with Eqn. 6. These C_i values correspond with the mean C_i associated with measurement CO_2 concentrations of 400 and 800 ppm, respectively. By analyzing the temperature response of photosynthesis at a given C_i, the temperature and associated VPD effects on stomatal conductance are accounted for, and hence, comparison of the parameters obtained from the P_{400} and P_{270} (and P_{800} and P_{505}) can reveal the role of stomatal conductance in determining the temperature optimum of photosynthesis (Kumarathunge et al. 2019).

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2.6 | Stomatal limitation

The degree of stomatal limitation of net photosynthesis (*l*) was calculated following Farquhar &

Sharkey (1982). In this approach the observed photosynthesis rate is compared to what the rate

would be if stomatal conductance (g_s) would be infinite:

$$l = 1 - \frac{P_{Observed}}{P_{(infinite\ g_S)}}$$ Eqn. 7

$P_{(infinite\ gs)}$ is calculated from the FvCB model by setting C_i in Eqn 7 to equal C_a, the observed

CO_2 concentration in the cuvette:

$$\square_{\square\square\square\square} = \frac{P_{\square\square\square\square\square} \times (\square_\square + \square_\square)}{\square_\square - \square^*}$$ Eqn. 8

P_{gross} equals light-saturated photosynthesis plus R_{Light}, where R_{Light} is assumed to be equal to

1.5% of V_{CMax}; K_c is the Michaelis–Menten constant of Rubisco activity for CO_2 taken from

Bernacchi et al. (2001).

2.7 | Activation state of Rubisco

We evaluated whether photosynthesis at high temperature was limited by the activation state of

Rubisco using the method described in Sage, Way & Kubien (2008). In this approach,

measurements of the initial slopes of A-C_i curves are compared with estimates of these slopes

that are based on the FvCB model that implicitly assumes that Rubisco is fully-activated in Eqn 9

below; lower values for the measured than the modeled slopes indicate Rubisco inactivation. By

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comparing the model against observations, the assumption in the model of fully-activated

Rubisco is tested. Rubisco activase requires sufficient ATP to sustain high activity, and if the

electron transport rate (i.e., J_{Max}) is reduced at high temperature, the resulting reduction in ATP

supply could inhibit Rubisco activase. Therefore, the temperature effect on Rubisco activase is

assessed at low $[CO_2]$, where electron transport is not limiting.

The measured initial slopes for each A-Ci curve were determined with linear regressions

using all data points for which $C_i < 200$ µmol mol CO_2. The initial slopes were modeled as:

$$\square\square\square\square\square\square\square \ \square\square\square\square\square = \frac{\square_{\square\square\square\square}}{\left(\square^* + \square_\square \times \left(1 + \frac{\square}{\square_\square}\right)\right)}$$

Eqn. 9

K_c and K_o are the Michaelis–Menten constants of Rubisco activity for CO_2 and O_2, respectively,

and O is the oxygen concentration in the chloroplast stroma. O_2 was assumed to equal 210 mbar,

and Γ^*, K_c and K_o were taken from Bernacchi et al. (2001).

2.8 | Leaf chemical and morphological traits

For each treatment four leaflets—one from each of three leaves, of similar age and sun exposure

as the leaves used for gas-exchange measurements—were collected prior to the switch in

conditions, and three more at the end of the experiment. Leaf area was measured with an LI-

3100C leaf area meter (LI-COR), and leaves were dried at 70°C and weighed to determine leaf

mass per area (LMA). Leaf nitrogen (N) content was measured using a Thermo Flash EA1112

analyzer (Waltham, MA, USA). To determine stomatal density epidermal impressions were

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taken with the nail varnish method from three leaves per treatment. *T. rosea* is hypostomatous, so only stomatal impressions of the abaxial sides—three per leaf—were counted.

2.9 | Acclimation analyses

To evaluate acclimation of photosynthetic parameters we compared posterior distributions of T_{Opt} of P_{400}, P_{800}, V_{CMax}, and J_{Max}, and of the activation energies of treatment and control plants. Stomatal acclimation was assessed by comparing T_{Opt} of P_{400} and P_{270} (and P_{800} and P_{505}), and by analyzing changes in the C_i/ C_a ratio. We further compared the relationship between g_s and VPD between treatments (see below). Acclimation of respiration was determined with the set temperature method and the homeostasis method (Atkin, Bruhn & Tjoelker 2005; Slot & Kitajima 2015): The set temperature method compares respiration (R) of control and treatment leaves at a set temperature (here, 30°C):

$$Acclim_{SetTemp} = \frac{R_{Control} \text{ at } 30°C}{R_{Treatment} \text{ at } 30°C}$$

Eqn. 10

$Acclim_{SetTemp} > 1.0$ indicates thermal acclimation. The homeostasis method determines the degree of homeostasis:

$$Acclim_{Homeo} = \frac{R_{Control} \text{ at } T_{Control}}{R_{Treatment} \text{ at } T_{Treatment}}$$

Eqn. 11

When $Acclim_{Homeo} \approx 1.0$ respiration rates are homeostatic across conditions and respiration has fully acclimated; when $Acclim_{Homeo} < 1.0$, acclimation, if any, is imperfect.

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2.10 | Curve fitting and statistical analyses

We fit Eqns 2, 3 and 6 using a Bayesian framework with the MCMC sampler Stan using the R libraries 'rstan' (Stan Development team 2018) and 'brms' (Bürkner 2018) with R version 3.5, which facilitated a more thorough exploration of the uncertainty of the parameter estimates. The full models used for Eqns. 2, 3 and 6 contained terms for all treatments; control parameters were estimated as the basis, with parameters for treatment plants, plants transferred from control to treatment conditions, and from treatment to control conditions being estimated as deviations from the controls (see Table S1). Informed priors (Table S2) were used to constrain k_{Opt}, P_{Opt}, H_a, and T_{Opt}. These priors were based on literature (e.g. Medlyn et al., 2002; Slot & Winter 2017b), selected to be realistic (i.e. only positive values), and were refined to avoid multimodal posterior fits. Attempts at estimation of the H_d parameter produced bi-modal posterior distributions of other model parameters (e.g. T_{Opt}) and so H_d was fixed at 200 kJ mol^{-1} following the example of Medlyn et al. (2002). Each model was fit using four chains with 2000 iterations during warmup, and a subsequent 4000 iterations during sampling. ΔS was calculated from T_{Opt} and $\hat{H}_a$ estimates for each iteration with Eqn 5. Models were checked to ensure convergence ($\hat{R}\sim$ 1), posterior distributions were unimodal, and that posterior predictive checks could approximate the distribution of the response variable. Comparisons of plant-level parameters between control, treatment, and transfer effect plants were made by comparing the distribution of credible intervals between groups.

Treatment effects on the temperature response of the J_{Max} to V_{CMax} ratio, and the VPD response of stomatal conductance were determined with ANCOVA, with temperature and VPD

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as the respective covariate. To visualize treatment effects on the VPD response of stomatal conductance and the temperature responses of stomatal conductance and the initial A-C_i slopes used to assess Rubisco activase status, temperature responses were fitted with generalized additive models using cubic regression splines fit with restricted maximum likelihood in the 'mgcv' package (Wood 2017) for R, and 95% confidence intervals were approximated by plotting curves ± 2 standard errors.

3 | RESULTS

3.1 | Leaf morphology and chemistry

Leaf nitrogen content was marginally lower (two-tailed t-test, df = 6, t = 2.3, $P = 0.059$) in treatment plants (20.2 ± 4.2 mg g^{-1}, mean ± SD) than in control plants (29.6 ± 6.8 mg g^{-1}). Leaf nitrogen content decreased by 39% to 18.7 ± 3.8 mg g^{-1} when control plants were transferred to treatment conditions (paired two-sample t-test, df = 3, t = 6.5, $P = 0.007$). The moderate decrease in leaf nitrogen in plants transferred from treatment to control conditions (Table 1) was not significant. LMA was significantly greater in treatment than in control plants (two-tailed t-test, df = 6, t = 6.4, $P < 0.001$). Transferring control plants to treatment conditions significantly increased LMA (paired two-sample t-test, df= 3, t = −6.7, $P = 0.007$), while plants transferred from treatment to control conditions did not show any change in LMA (Table 1). Stomatal density was similar in control (260 ± 34 mm^{-2}) and treatment leaves (245 ± 30 mm^{-2}).

3.2 | Net photosynthesis

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Net photosynthesis at 400 ppm CO_2 (P_{400}) peaked at 32.2°C in treatment plants, which was almost 5°C higher than T_{Opt} in control plants (Fig. 1), with limited overlap of the credible intervals (See Fig. 2 for the full posterior distributions of T_{Opt} with highlighted 50, 90, and 99% credible intervals, and Table 2 for 10–90 percentiles of the posterior distribution). Median P_{400} at T_{Opt} was lower for the treatment plants than for the controls (Table 2). Transfer of control plants to treatment conditions increased T_{Opt} of P_{400} from 27.3 to 31.2°C (Fig. 2) and decreased P_{400} at T_{Opt} by 41% to 11.3 µmol m^{-2} s^{-1} (Table 2). Transfer of treatment plants to control conditions decreased T_{Opt} from 32.2 to 28.9°C (Fig. 2) with minimal change in P_{400} at T_{Opt} (Table 2). When fitting the data with the peaked Arrhenius function (Eqn 3), the activation energy (H_a) was higher in treatment plants than in control plants, and transferring plants resulted in adjustments in H_a towards the values of plants grown under the conditions plants were transferred to, while not quite reaching those values (Table S3). Estimates of H_a tended to have right-skewed posterior distributions.

The optimum temperature of photosynthesis at 800 ppm (P_{800}) differed by 3.6°C between control and treatment plants, with moderate upward and downward adjustments in T_{Opt} of plants transferred to warmer and cooler conditions, respectively (Figs. 2, 3; Table 2). Control plants achieved higher rates of P_{800} than treatment plants, and P_{800} was reduced by 35% in control plants transferred to treatment conditions (Table 2). H_a of P_{800} was higher in treatment plants than in control plots (Table S3). P_{800} of treatment plants was considerably higher than P_{400} of controls, indicating higher net photosynthesis rates at their respective conditions.

3.3 | Temperature response of biochemical parameters

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In treatment plants the optimum temperature of V_{CMax} was 43.3°C, which was 1.4°C higher than in control plants (Fig. 3) with moderate overlap in credible intervals (Fig. 2; Table 3). Transferring plants from control to treatment conditions increased T_{Opt} of V_{CMax} by 0.8°C, and the posterior distribution overlapped with that of both control and treatment plants (Fig. 2). The transferred plants had lower V_{CMax} at T_{Opt} (Table 3). Plants transferred from treatment to control conditions reduced T_{Opt} by 1.5°C, while V_{CMax} at T_{Opt} was similar before and after the transfer. The activation energy H_a averaged 76.9 kJ mol^{-1} across treatments, without notable differences between control and treatment plants (Table 3). The median entropy factor ΔS of V_{CMax} tended to be lower in treatment than in control plants, and when conditions were switched ΔS decreased in plants moved to treatment conditions and increased in the plants moved to control conditions; however, the 90% credible intervals of all treatment groups overlapped (Table 3).

J_{Max} peaked at ~2.0°C lower temperatures than V_{CMax} (Table 3). Differences in T_{Opt} of J_{Max} across treatments were small, with treatment plants 1.2°C higher than control plants, and upward and downward shifts of ≤1.6°C following transfer to treatment and control conditions respectively (Fig. 2; Table 3). J_{Max} at T_{Opt} was lower in treatment than in control plants and decreased when control plants were transferred to treatment conditions (i.e., 90% credible intervals did not overlap), but did not change much when treatment plants were transferred to control conditions (Table 3). H_a and ΔS of J_{Max} did not differ systematically among treatments and had overlapping 90% credible intervals (Table 3).

The J_{Max} to V_{CMax} ratio decreased with increasing measurement temperature in control and treatment plants by on average 21% between 30 and 40°C (Fig. 4). The mean J_{Max} to V_{CMax} ratio was lower in treatment than in control plants (ANCOVA with Tukey post hoc test, t =

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−2.76, P = 0.032) and decreased when control plants were transferred to treatment conditions (t = −5.67, P < 0.001) (Fig. 4).

3.4 | Stomatal conductance and stomatal limitation

Stomatal conductance did not exhibit strong temperature responses (Fig. 5). While conductance tended to be higher in control plants at low temperatures, this pattern reversed at higher temperatures, as illustrated by diverging temperature trends in Fig. 5. Stomatal conductance decreased in response to increasing VPD, with conductance at low VPD being significantly higher in controls (as indicated by non-overlapping confidence intervals in Fig. 4). Transfer to treatment conditions reduced stomatal conductance by ~43% (ANCOVA with Tukey post hoc test, t = −3.58, P = 0.003), whereas transfer from treatment to control conditions did not change conductance (Fig. 5). Consistent with the weak temperature response of stomatal conductance, stomatal limitation did not increase with increasing measurement temperature (Fig. 6). Below 35°C stomatal limitation was higher in control plants transferred to treatment conditions than in control conditions (two-tailed t-test, df = 26, t = 3.3, P = 0.003), consistent with their reduced stomatal conductance (Fig. 5). Temperature responses of photosynthesis at fixed C_i of 270 ppm were comparable to those of P_{400}, and curves with C_i of 505 were similar to curves of P_{800}. T_{Opt} of P_{270} was moderately higher than that of P_{400}, particularly for control plants and treatment plants moved to control conditions, but the 90% credible intervals overlapped; T_{Opt} differences between P_{505} and P_{800} were much smaller (Fig. S2). These results suggest that stomatal conductance had some influence on T_{Opt} at ambient but not elevated measurement CO_2.

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The C_i/C_a ratio was not systematically affected by treatment conditions, and there were no short-term temperature response patterns in C_i/C_a (Fig. S3).

3.5 | Rubisco activase

There were no clear indications of photosynthesis being limited by Rubisco activase in either control or treatment plants. The temperature trends of observed and modeled initial slopes appeared to diverge when leaf temperatures exceeded 42°C (Fig. S4), but only in control plants transferred to treatment conditions was there a clear separation of the curves and their 95% confidence intervals within the measured temperature range. Treatment plants transferred to control conditions experienced moderate Rubisco activase limitation at most temperatures (Fig. S4).

3.6 | Respiration

Dark respiration of treatment plants increased less steeply than that of control plants (Fig. 7), resulting in a lower Q_{10} (Table 4; non-overlapping 90% credible interval). Respiration rates at a set temperature of 30°C were not different between control and treatment plants, nor did switching the conditions affect the plants' R_{30} (Table 4). Moving control plants to treatment conditions decreased Q_{10} (Table 4). The acclimation metrics (Eqn. 10 and 11) indicated limited acclimation; $Acclim_{SetTemp}$ was 0.86 (Table 4), indicating that respiration at 30°C was higher in treatment plants relative to control plants. When compared at their respective mean nighttime temperatures (26.5 vs 29.5°C) treatment plants respired considerably more than controls ($Acclim_{Homeo}$ was 0.63—or 0.66 when calculated for mean daytime temperatures). Transfer from

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control to treatment caused a marginally significant decrease in Q_{10} (paired two-sample t-test, t = 2.4, P = 0.08), while the transfer from treatment to control conditions did not lead to a significant change of Q_{10} (Table 4). $Acclim_{SetTemp}$ and $Acclim_{Homeo}$ were both lower following short-term transfer than when comparing plants at their growth conditions (Table 4). Notwithstanding the acclimation metrics, the lower Q_{10} values in treatment plants resulted in lower respiration rates at temperatures >35°C (Fig. 7).

4 | DISCUSSION

The tropical tree species *Tabebuia rosea* showed clear signs of photosynthetic acclimation to elevated temperature and CO_2: treatment plants had moderately higher thermal optima for photosynthetic parameters V_{CMax} and J_{Max}, and downregulated respiration rates at high temperature, thereby improving photosynthetic performance at treatment conditions and supporting our hypothesis of acclimation. Higher thermal optima of net photosynthesis were observed regardless of measurement CO_2 concentration, indicating that the shift in the optimum temperature was not solely due to suppression of photorespiration by elevated CO_2 in the treatment dome. The observed reduction in V_{CMax} in treatment plants is consistent with acclimation to elevated CO_2. However, reduced V_{CMax} has also been observed as a result of warming alone—e.g. in (sub-) tropical *Eucalyptus* provenances (Crous et al. 2018)—suggesting that the observed decrease in V_{CMax} can be related to increased CO_2, increased temperature, or a combination of the two. Indeed, in the tropical tree species *A. glandulosa* neither warming nor elevated CO_2 significantly reduced V_{CMax}, but the combination of the two did (Fauset et al. 2018)., In contrast to common observations in studies in which only CO_2 is increased (e.g.,

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Ainsworth & Rogers 2007; Klein & Ramon 2019), stomatal conductance and stomatal density were not reduced in our combined warming and CO_2 enrichment experiment, suggesting that the CO_2 effect may be temperature dependent.

As hypothesized, net photosynthesis of leaves developed under control conditions and transferred to treatment conditions for 1–2 weeks, did not acclimate to the same extent as leaves that had developed under treatment conditions, as indicated by smaller shifts in temperature optima, and greater overlap in the credible intervals of parameter estimates. We did not find support for our hypothesis that stomatal conductance would exert stronger control over net photosynthesis under treatment conditions; plants were not more inhibited by low stomatal conductance under treatment conditions than under control conditions. Accounting for stomatal conductance by examining temperature responses at a fixed C_i showed that stomatal conductance had only a small influence on T_{Opt}, as the shifts in T_{Opt} were comparable for P_{270} and P_{505} as they were for P_{400} and P_{800}. The activation of Rubisco was not limiting net photosynthesis under growth conditions, neither in control nor treatment plants, but transferred plants exhibited signs of Rubsico activase limitation. Collectively, these results indicate that *T. rosea* plants exhibited strong acclimation to the combined effects of 4°C warming and doubling of CO_2. Acclimation appeared to be underpinned by changes in the biochemical parameters, particularly in shifts in T_{Opt} of V_{CMax} associated with adjustment of ΔS.

Our Bayesian approach for fitting temperature response curves was motivated by complications associated with fitting these nonlinear models with ordinary least square regression. With five saplings per treatment the plant-level replication in our study was modest, and small sample size may constrain the ability to accurately identify treatment effects. The Bayesian approach enabled us to more reliably estimate how treatments affected the key

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parameters, and to more thoroughly probe the uncertainty around the estimated treatment effects. This is valuable for difficult-to-estimate model parameters such as T_{Opt}: Uncertainty of T_{Opt} can be asymmetric, especially when constrained by the lower or upper limits of temperature control of leaf cuvettes of commercially available portable photosynthesis systems. For example, the posterior distributions of T_{Opt} of P_{400} were left skewed, because of the small number of observations below T_{Opt}, and those of V_{CMax} tended to be right skewed (Fig. 2). Even with few data points above T_{Opt} of V_{CMax} and J_{Max}, its estimates were relatively well constrained with the help of informed priors, with the 10% and 90% percentiles separated by on average only 2.3°C.

4.1 | Acclimation of photosynthesis to extreme atmospheric and climate change conditions

Experimental research on photosynthetic responses to elevated CO_2 has focused on economically important species and on temperate and boreal ecosystems, leaving a knowledge gap in the tropics (Leakey, Bishop & Ainsworth 2012) where high temperatures are increasingly common (Tiwari et al. 2020), especially in association with El Niño events (Rifai, Li & Malhi 2019). Physiological processes in tropical trees are obviously the same as those for temperate vegetation, but temperature regimes in the tropics have long been relatively stable, and current increases of the already high ambient temperatures in the tropics may result in different thermal sensitivities than in non-tropical species (Corlett 2011).

The observed shifts in the short-term temperature response of net photosynthesis towards a higher optimum temperature under treatment conditions is consistent with acclimation of photosynthesis to higher temperatures (Yamori, Hikosaka & Way 2014; Way & Yamori 2014; Slot & Winter 2017a). Furthermore, T_{Opt} of V_{CMax} and J_{Max} were moderately higher under

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treatment than control conditions, consistent with observed warming effects on other ecosystems (Kattge & Knorr 2007). However, at 400 ppm T_{Opt} was almost 5°C higher in treatment plants that experienced ~5°C higher daytime temperatures than in controls, while T_{Opt} commonly only increases by ~0.4°C per degree increase in growth temperature at ambient CO_2 (Yamori et al. 2014; Slot & Winter 2017a). T_{Opt} of net photosynthesis also increases when temperature responses are measured at elevated CO_2 because the beneficial effect that elevated CO_2 has on reducing photorespiration is greater at higher than at lower leaf temperatures (Brooks & Farquhar 1985). In the current study T_{Opt} increased regardless of measurement CO_2 (Fig. 1), but higher C_i in the treatment plants can account for reduced photorespiration and higher T_{Opt}. The difference in T_{Opt} between control and treatment plants thus reflects a response to the combination of elevated temperature and elevated CO_2, consistent with observations of additive effects of warming and elevated CO_2 on T_{Opt} of two boreal tree species (Dusenge et al. 2020).

Prolonged exposure to elevated CO_2 can result in feedback inhibition of photosynthesis if more carbohydrates are synthesized than are exported to sink organs (e.g. Neales & Incoll, 1968). Accumulation of carbohydrates can then lead to reduced levels of Rubisco protein, and a reduction in V_{CMax} (Moore, Cheng, Sims & Seemann 1999). V_{CMax} indeed tended to be lower in treatment than control plants across much of the ecologically relevant temperature range (Fig. 3), as were net photosynthesis and leaf nitrogen content. Furthermore, higher LMA in treatment plants may indicate accumulation of carbohydrates. Photosynthesis measured at 800 ppm—outside the carboxylation limited range of C_i—was reduced as much as at 400 ppm in treatment plants, so while there may have been feedback inhibition, lower V_{CMax} alone cannot account for reduced net photosynthesis. J_{Max} was, however, also reduced, as is commonly observed with

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plants grown at elevated CO_2 (Ainsworth & Rogers 2007), probably related to the reduced

nitrogen content in leaves of treatment plants (Table 1).

The mechanisms underlying thermal acclimation are not fully understood, but a

relationship between T_{Opt} and the J_{Max}/V_{CMax} ratio has long been known. Here we found weak

and non-significant relationships, both for J_{Max}/V_{CMax} at 30°C and J_{Max}/V_{CMax} at their respective

T_{Opt} (Fig. S5). Changes in the activation energy (H_a, in Eqn 3) have been found to play an

important role in acclimation in some studies (e.g. Hikosaka et al. 2006; Kumarathunge et al.

2019; Dusenge et al. 2020), whereas Kattge & Knorr (2007) found that ΔS, the entropy term of

the instantaneous temperature response, was significantly impacted by plant growth temperature

. In the current study T_{Opt} of P_{400} and P_{800} scaled with ΔS of V_{CMax} and with T_{Opt} of V_{CMax} (Fig.

S5). In contrast, T_{Opt} of P_{400} and P_{800} did not correlate significantly with H_a of either of the

biochemical parameters. An issue with the use of ΔS is that it is strongly influenced by the value

of H_d (Stinziano et al. 2018), and H_d can generally not be reliably estimated due to the lack of

data points above T_{Opt} of V_{CMax}. Even when using informed priors we were not able to estimate

H_d reliably and had to use a fixed value of 200 kJ mol^{-1}. The resulting ΔS estimates, although not

very well-constrained, correlated much better with T_{Opt} of P_{400} and P_{800} than the J_{Max} / V_{CMax}

ratio, lending support to Kattge & Knorr (2007).

4.2 | Stomatal conductance

Previous studies in Panama suggested an important role for stomatal conductance in controlling

the short-term temperature response of photosynthesis in naturally growing lowland tropical

trees (Slot & Winter 2017b,c; Hernández et al. 2020). The relatively small role of stomatal

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limitation in the control over the temperature response of photosynthesis in the current study on well-watered potted saplings suggests that stomata in tall trees are more sensitive to changes in VPD (as shown e.g. for Mediterranean oaks by Mediavilla & Escudero 2004), presumably due to the challenges of water delivery to the canopy. Stomatal limitations in response to VPD may therefore be underestimated when scaling results from studies such as the current one to tropical forests at large. Indeed, stomatal response to increased VPD has been shown to underpin the high temperature decrease of net ecosystem exchange across tropical forest sites (Tan et al. 2017; Smith et al. 2020).

Stomatal conductance was not strongly affected by the elevated CO_2 and warming treatment in the current study and there was no obvious effect of the moderately lower relative humidity in the treatment dome. Stomatal limitation had only a small effect on T_{Opt} of photosynthesis at 400 ppm CO_2, as illustrated by moderately higher T_{Opt} values of photosynthesis at a fixed C_i—consistent with global observations by Kumarathunge et al. (2019). Previous work found reductions (Cernusak et al. 2011) or no effect (Fauset et al. 2019) on stomatal conductance when tropical species were grown at elevated CO_2. Berryman, Eamus & Duff, (1994) showed that the tropical tree species *Maranthes corymbosa* Blume exhibited both a short-term, reversible reduction in stomatal conductance in response to elevated CO_2—regulated by stomatal aperture—and a long-term, non-reversible reduction associated with decreased stomatal density.

The response of stomatal conductance of tropical trees exposed to elevated temperature is also variable. Significant reductions were reported following ~2°C warming of leaves in the field (Doughty 2011) and 1.5°C warming of greenhouse-grown *A. glandulosa*, while Slot & Winter (2017a) did not find clear patterns for potted seedlings of three tropical tree species across a 10°C growth temperature range. Given the variation in observed patterns, the response of

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stomatal conductance to a combination of elevated CO_2 and temperature is highly uncertain. Fauset et al. (2019) found a stronger reduction in stomatal conductance in *A. glandulosa* due to 1.5°C warming than due to doubling of CO_2, resulting in reduced stomatal conductance in warmed plants at elevated CO_2. In the current study on well-watered plants under +4°C warming and double ambient CO_2, stomatal conductance did not acclimate. Stomatal conductance was slightly reduced in treatment plants relative to control plants, but only at low temperatures (Fig. 5). Furthermore, there was no acclimation of the relationship of stomatal conductance with VPD, which is consistent with a meta-analysis on stomatal responses to elevated CO_2 in which Medlyn et al. (2001) also observed that the relationship between conductance and VPD was not affected by growth at elevated CO_2. In our study, control plants transferred to treatment conditions did have lower stomatal conductance than treatment plants. This suggests that the decrease in conductance is of a transient nature, and that leaves developed under treatment conditions have more similar properties to control leaves than pre-existing leaves exposed to treatment conditions. Nonetheless, treatment and control plants had similar stomatal densities, possibly the result of opposing effects of warming and elevated CO_2.

4.3 | Weak thermal acclimation response of respiration

Consistent with most previous studies, dark respiration increased exponentially, leading to high rates of CO_2 loss at high temperature. Treatment plants had lower temperature sensitivity of respiration (indicative of Type I acclimation, Atkin & Tjoelker 2003; Slot & Kitajima 2015). Nonetheless, thermal acclimation of respiration was moderate, with limited adjustment of $Acclim_{Tset}$ and no indication of homeostasis. While growth at elevated temperature tends to reduce respiration (Atkin & Tjoelker 2003; Slot & Kitajima 2015), growth at elevated CO_2 tends

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to increase respiration (Leakey et al. 2009). As with stomatal responses, the patterns we observed represent the balance of responses to elevated temperature and doubling of CO_2, and consistent with Dusenge et al. (2020), the responses to temperature were seemingly stronger than the responses to CO_2.

4.4 | Short term versus long term acclimation

In the absence of large-scale manipulative experiments with mature trees in the tropics, studies of climate change effects rely on seedling or sapling temperature and/or CO_2 manipulations (e.g. Winter & Virgo 1998; Winter, Garcia, Gottsberger. & Popp 2001; Lovelock, Winter, Mersits & Popp 1998; Kotisup et al. 2009; Cheesman & Winter 2013; Slot & Winter 2017a, 2018; Fauset et al. 2019), or on manipulation of individual leaves or branches of mature trees (Körner & Würth 1996; Lovelock, Virgo, Popp & Winter 1999; Doughty 2011; Slot et al. 2014, but see Smith et al. 2020). Inferring long-term acclimation potential from manipulated pre-existing canopy leaves is challenging because newly developed tissue tends to exhibit stronger acclimation responses than pre-existing leaves. This was confirmed in our study; stronger acclimation was accomplished when plants were measured at their growth conditions than when fully-formed leaves were re-measured after the plants were switched between treatments. Acclimation of newly developed tissues is more relevant for long-term predictions and simulations.

4.5 | Conclusions

Nonlinear models are notoriously difficult to fit when sample sizes are limited, or if multiple solutions can be found. Fitting the temperature response models presented in this study through a probabilistic Bayesian MCMC approach allowed us to constrain parameters to plausible

biological ranges, and to determine probabilistic estimates of model parameters when data was sparse at the leaf temperature extremes. These analyses show that despite tropical vegetation having experienced millions of years of relative stability in terms of temperature, there is no lack of physiological plasticity to effectively respond to changes in mean temperature. In the presence of double-ambient CO_2 concentrations, plants of the early-successional tree species *Tabebuia rosea* can adjust to 4°C warming thereby largely preventing adverse effects on carbon gain. Even short-term exposure to such extreme conditions results in partial acclimation.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

AUTHOR CONTRIBUTIONS

MS and KW designed the study. MS performed the measurements. MS and SWR analyzed the data and made the figures. MS wrote the paper, with significant input from SWR and KW.

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DATA AVAILABILITY STATEMENT

The data and code used to fit temperature response models and to generate the figures are available in a publicly accessible repository maintained by SWR at https://github.com/sw-rifai/Tabebuia_rosea_thermal_co2_acclim and are included in a zip file attached to the online supplementary materials.

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Supporting information

Table S1. Full equations used for Bayesian curve fitting of temperature responses.

Table S2. Parameter priors, their distribution, and allowed ranges for Bayesian curve fitting of temperature responses of photosynthesis and respiration.

Table S3. Peaked Arrhenius temperature response traits of P_{400} and P_{800}

Fig. S1. Leaf-level vs treatment-level temperature responses of V_{CMax} and J_{Max}.

Fig. S2. Temperature responses of photosynthesis as fixed C_i of 270 and 505 ppm CO_2.

Fig. S3. C_i/C_a ratio in relation to leaf temperature.

Fig. S4. Rubisco activation state—Initial slopes of A-C_i curves as a function of leaf temperature

Fig. S5. Optimum temperature of net photosynthesis in relation to ΔS, h_a, and T_{Opt} of V_{CMax} and J_{Max}, and J_{Max}/V_{CMax}.

Code&Data.zip: Code and data for curve fitting and generating all figures:

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Figure legends

Figure 1. The temperature response of photosynthesis at 400 ppm CO_2 (P_{400}; left-hand panels) and at 800 ppm CO_2 (P_{800}; right hand panels) of *Tabebuia rosea* plants, as affected by growth conditions. Panels a and b show the effect of growth at elevated temperature and $[CO_2]$ relative to control plants grown at ambient conditions; effects of short-term transfer from control to treatment (c, d), and from treatment to control conditions (e, f) are also shown. Curves were fitted with Eqn 6 with MCMC sampler (4000 iterations, 100 random samples are plotted). T_{Opt} is indicated in solid lines for control (blue) and treatment (red) plants; dashed lines indicate the 5 and 95% percentiles of the credible interval of T_{Opt}.

Figure 2. Posterior distributions of T_{Opt} of P_{400} (a), P_{800} (b), V_{CMax} (c), and J_{Max} (d), with highlighted 50, 90, and 99% credible intervals.

Figure 3. Maximum RuBP carboxylation capacity (left-hand panels) and maximum electron transport rate (right-hand panels) in relation to leaf temperature in *Tabebuia rosea* plants. Panels a and b show the effect of growth at elevated temperature and $[CO_2]$ relative to control plants grown at ambient conditions; effects of short-term transfer from control to treatment (c, d), and from treatment to control conditions (e, f) are also shown. Curves were fitted with Eqn 3 with MCMC sampler (4000 iterations, 100 random samples are plotted). T_{Opt} is indicated in solid lines for control (blue) and treatment (red) plants; dashed lines indicate the 5 and 95% percentiles of the credible interval of T_{Opt}.

Figure 4. J_{Max} to V_{CMax} ratio in relation to leaf temperature in *Tabebuia rosea* plants grown at ambient conditions and plants grown at elevated temperature and $[CO_2]$ conditions (a). Also shown are J_{Max} to V_{CMax} ratios of control plants before and after they were transferred to

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treatment conditions (b) and of treatment plants before and after they were transferred to control conditions (c).

Figure 5. Stomatal conductance (g_s) in relation to leaf temperature (left-hand panels) and leaf-to-air vapor pressure deficit (VPD) (right-hand panels) in *Tabebuia rosea* plants grown at ambient conditions and plants grown at elevated temperature and [CO_2] conditions (top). Shaded areas represent the 95% confidence intervals of fitted temperature trends. Also shown are relationships of control plants before and after they were transferred to treatment conditions (center) and of treatment plants before and after they were transferred to control conditions (bottom).

Figure 6. Stomatal limitation of net photosynthesis determined with Eqn 7 in relation to temperature in *Tabebuia rosea* plants grown at ambient (control) and at elevated temperature and [CO_2] (left-hand panels), and plants that were transferred from control to treatment and from treatment to control conditions (right-hand panels). Complete suppression of photosynthesis by low stomatal conductance would yield an *l* of 1.0, whereas 0 indicates that measured photosynthesis is identical to what it would be under infinite stomatal conductance.

Figure 7. Dark respiration rates in relation to leaf temperature in *Tabebuia rosea* plants grown at ambient conditions and plants grown at elevated temperature and [CO_2] conditions (a). Also shown are respiration rates of control plants before and after they were transferred to treatment conditions (b) and of treatment plants before and after they were transferred to control conditions (c) Curves were fitted to the data pooled across 4–5 plants per treatment with Eqn 2 with a MCMC sampler (4000 iterations, 100 random samples of which are plotted).

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Table 1. Leaf mass per area (LMA), concentrations of leaf nitrogen (N) and carbon (C), and nitrogen concentration per unit leaf area (N_{Area}) for *Tabebuia rosea* plants (n = 4) grown at ambient (Control) and elevated temperature and CO_2 concentration (Treatment), and then transferred from control to treatment (→Treatment) conditions and *vice versa* (→Control) before being re-measured after >7 days.

Treatment	**LMA**		N		C		N_{Area}	
	$(g\ m^{-2})$		$(mg\ g^{-1})$		$(mg\ g^{-1})$		$(mg\ m^{-2})$	
Control	54.6 ± 5.6		29.6 ± 6.8		465 ± 5		159 ± 27	
→Treatment	81.1 ± 7.4		18.0 ± 4.0		452 ± 5		146 ± 35	
Treatment	81.1 ± 6.2		20.2 ± 4.2		452 ± 12		165 ± 31	
→Control	83.2 ± 6.3		18.4 ± 1.1		453 ± 59		153 ± 18	

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Table 2. Temperature response traits of P_{400} and P_{800} for *Tabebuia rose*a plants grown at ambient (Control) and elevated temperature and CO_2 concentration (Treatment), and then transferred between treatments and re-measured after 7–16 days. Curves were fitted with Eqn 6 (see Table S1 for details). Shown are the 5%, 50%, and 95% quantiles of the posterior distribution of the optimum temperature (T_{Opt}) (a), the rates at optimum temperature (b), and the b parameter (c).

	Treatment	P_{400}			P_{800}		
		5%	50%	95%	5%	50%	95%
		T_{Opt} (°C)			T_{Opt} (°C)		
(a)	Control	23.7	27.5	30.5	33.0	34.4	35.7
	→Treatment	28.7	31.2	33.9	33.5	35.8	38.0
	Treatment	30.0	32.2	35.0	35.9	38.0	40.2
	→Control	26.6	29.0	31.4	33.0	35.3	37.6
		P_{400} at T_{Opt} (µmol m^{-2} s^{-1})			P_{800} at T_{Opt} (µmol m^{-2} s^{-1})		
(b)	Control	18.3	19.1	20.0	31.1	32.2	33.3
	→Treatment	10.1	11.3	12.6	19.5	20.9	22.3
	Treatment	13.3	14.4	15.5	26.0	27.2	28.5
	→Control	13.7	15.0	16.4	25.2	26.6	28.0
		b			b		
(c)	Control	0.02	0.03	0.04	0.04	0.06	0.08

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Table 3. Temperature response traits of V_{CMax} and J_{Max} for *Tabebuia rosea* plants grown at ambient (Control) and elevated temperature and CO_2 concentration (Treatment), and then transferred between treatments and re-measured after 7–16 days. Curves were fitted with Eqn 3 (see Table S1 for details). Shown are the 5%, 50%, and 95% percentiles of the posterior distribution of the optimum temperature (T_{Opt}) (a), the rates at optimum temperature (b), the activation energy (H_a)(c), and the ΔS parameter (d).

		V_{CMax}			J_{Max}		
	Treatment	**5%**	**50%**	**95%**	**5%**	**50%**	**95%**
(a)		**T_{Opt} (°C)**			**T_{Opt} (°C)**		
	Control	40.7	41.9	43.3	39.0	39.9	41.1
	→Treatment	41.0	42.8	44.6	39.4	41.1	42.9
	Treatment	41.7	43.3	45.1	39.6	41.1	42.7
	→Control	40.3	41.8	43.4	38.2	39.5	40.8
(b)		**V_{CMax} at T_{Opt} (µmol m^{-2} s^{-1})**			**J_{Max} at T_{Opt} (µmol m^{-2} s^{-1})**		
	Control	240.5	252.2	265.3	280.9	291.6	302.1
	→Treatment	184.6	202.1	219.5	175.4	189.5	204.4
	Treatment	213.3	230.6	249.6	223.4	238.0	253.3
	→Control	207.7	224.3	239.7	212.0	226.8	241.4
(c)		**H_a (kJ mol^{-1})**			**H_a (kJ mol^{-1})**		
	Control	62.0	75.7	90.5	46.2	58.9	72.4
	→Treatment	60.0	79.1	96.9	38.1	55.9	76.3
	Treatment	59.2	77.3	96.0	41.7	60.9	79.2
	→Control	57.2	76.6	96.0	39.5	58.4	77.9
(d)		**ΔS (J mol^{-1} °K^{-1})**			**ΔS (J mol^{-1} °K^{-1})**		
	Control	625.8	630.8	635.0	627.1	631.5	635.6
	→Treatment	623.0	629.5	634.9	621.3	628.5	634.8
	Treatment	622.0	628.1	633.7	623.4	629.4	634.8
	→Control	625.3	630.9	636.1	626.2	632.1	637.2

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Table 4. Leaf dark respiration rates at 30°C (R_{30}) and the Q_{10}, the temperature sensitivity of respiration between ~30 and 40°C, for *Tabebuia rosea* plants (n=4) grown at ambient (Control) and elevated temperature and CO_2 concentration (Treatment), and then transferred between treatments before being re-measured after >7 days (a). Shown are the 5%, 50%, and 95% quantiles of the posterior distribution of R_{30} and Q_{10}. (b) presents acclimation parameters $Acclim_{SetTemp}$ (Eqn. 10), calculated from median rates of R_{30} (i.e., 50% quantiles), and $Acclim_{Homeo}$ (Eqn. 11), based on median rates of respiration at mean nighttime temperatures at control (26.5°C) and treatment (29.5°C) conditions.

(a)	R_{30} (μmol m^{-2} s^{-1})				Q_{10}		
	5%	**50%**	**95%**		**5%**	**50%**	**95%**
Control	0.9	1.0	1.1		2.3	2.7	3.2
→Treat	1.1	1.2	1.3		1.6	1.9	2.2
Treatment	1.0	1.2	1.3		1.5	1.8	2.2
→Control	1.2	1.3	1.5		1.3	1.6	1.9
(b)	**$Acclim_{SetTemp}$**			**$Acclim_{Homeo}$**			
Control vs Treatment	0.86			0.63			
→Treat	0.83			0.60			
→Control	0.87			0.91			

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Y-axis label: Accepted Annotation / Acceptoral Annotation

X-axis label: Leaf temperature (°C)

(a) Control
400 ppm

(b) Control→Treatment
800 ppm

(c) Treatment
800 ppm

(d) Treatment→Control
400 ppm