Photosynthetic plasticity of a tropical tree species, *Tabebuia rosea*, in response to elevated temperature and [CO₂]

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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) CO₂. We measured temperature responses of the maximum rates of ribulose 1,5-bisphosphate carboxylation (V_{C_{Max}}), 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_{C_{Max}}, J_{Max} and P_{Net} were 1–5°C higher in treatment than in control plants, while photosynthetic capacity (V_{C_{Max}}, 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 vapour 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.

Keywords
acclimation, climate change, global warming, J_{Max} photosynthetic temperature response, stomatal conductance, tropical forest, V_{C_{Max}}, VPD

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 night-time 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 (Kositsup 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 CO₂ concentrations ([CO₂]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. CO₂ as the substrate for photosynthesis directly stimulates rates of carbon uptake. Higher [CO₂]atm also suppresses carbon loss associated with photorespiration, the result of oxygenation of Rubisco (e.g., Ainsworth & Rogers, 2007; Long, Ainsworth, Rogers, & Ort, 2004). Photorespiration increases with temperature, so when photorespiration is suppressed by elevated [CO₂]atm plants can achieve a higher optimum temperature of photosynthesis than at ambient [CO₂]atm (Berry & Björkman, 1980; Long, 1991). The decrease in Rubisco limitation of photosynthesis under elevated CO₂ conditions means that net photosynthesis is increasingly limited by the maximum photosynthetic electron transport rate (Jmax), 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 (Hikosaka, Ishikawa, Borjigidaij Muller, & Onoda, 2006; Sage & Kubien, 2007). In the long term, elevated [CO₂]atm may cause a reduction in both the maximum capacity of RuBP carboxylation (VcMax) and RuBP regeneration (Jmax). VcMax might decrease as a result of lower investment in Rubisco when high [CO₂]atm reduces carbon limitation of photosynthesis and optimization requires proportionally greater investment in electron transport (Ainsworth & Rogers, 2007). VcMax and Jmax 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 CO₂ 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 and 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 (Topt) 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 (Topt) towards higher values may or may not be accompanied by increases in Popt, the rate of photosynthesis at Topt (Berry & Björkman, 1980; Slot, Garcia, & Winter, 2016; Way & Yamori, 2014). 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 VcMax and JMax are required, including their activation energies, deactivation energies, entropy factors (ΔS, sensu Medlyn et al., 2002), and their temperature optima (Mercado et al., 2018; Stinziano, Way, & Bauerle, 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 (Lombardozzi, Bonan, Smith, Dukes, & Fisher, 2015; Mercado et al., 2018; Smith & Dukes, 2013; Smith, Malyshov, Shevlakova, Kattge, & Dukes, 2016), and limited experimental data are available on the combined effects of warming and elevated [CO₂] that can provide mechanistic foundations for modelling acclimation (Way, Oren, & Kroner, 2015), particularly for tropical plants. To address thermal acclimation of photosynthesis, several modelling studies have capitalized on the clear trend of a decreasing Jmax/VcMax ratio with acclimation to higher growth temperatures, as synthesized by Kattge and Knorr (2007), and more recently confirmed by Smith and Dukes (2018) (e.g., Lombardozzi et al., 2015; Mercado et al., 2018; Smith et al., 2016). However, [CO₂]atm may affect the Jmax/VcMax ratio independent of temperature. While acclimation to warming consistently decreases the Jmax/VcMax ratio, increased [CO₂]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 VcMax. Fauset et al. (2019) found that the Jmax/VcMax ratio of the tropical tree species Alchornea glandulosa decreased with increasing growth temperature, but increased with elevated [CO₂], such that Jmax/VcMax for plants grown at 800 ppm CO₂ was higher at 35°C than that of control plants at 30°C. The same pattern of opposing effects of warming and elevated CO₂ on Jmax/VcMax was found in the boreal tree species Larix laricina, and to a lesser extent in Picea mariana (Dusenge, Madhavji, & Way, 2020). The utility of Jmax/VcMax changes to model photosynthetic acclimation may thus be limited when both temperature and CO₂ 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 sensitivities of VcMax, Jmax, 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 vapour 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 limitations by Jmax in tropical montane species in Rwanda. Growth at elevated CO₂ generally results in decreases in stomatal conductance (Ainsworth & Rogers, 2007; Saxe, Ellsworth, & Heath, 1998), 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 (Dusenge et al., 2020; Marchin, Broadhead, Bostic, Dunn, & Hoffmann, 2016; Wu et al., 2018). 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 CO₂ 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 (a) acclimation would result in higher temperature optima for photosynthetic parameters, and in lower respiration rates at a set temperature; (b) long-term acclimation (of newly developed leaves) would be stronger than short-term acclimation (of pre-existing leaves); and (c) stomatal limitation of photosynthesis would increase under treatment conditions of warming and elevated CO₂. 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.

2 | MATERIALS AND METHODS

2.1 | Plant material and growth conditions

*T. 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-l tree pots (TP1124R, Stuewe & Sons, Tangent, Oregon) 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). Five plants were placed in a temperature- and CO₂ controlled 6 m-diameter geodesic dome (SolarDomes Industries Limited, Nursling, UK) maintained at current local ambient temperature and ~420 ppm CO₂; five other plants were placed in a second dome maintained at ambient temperature + 4°C and ~800 ppm CO₂. 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 3,101, 21.3 Amp, Modine Manufacturing Company, Racine, WI; treatment dome). The domes operated as semi-closed systems, with enough ventilation to maintain the ambient dome at ~420 ppm CO₂ from a cylinder that was injected at a small, but constant flow rate to maintain the treatment dome at ~800 ppm CO₂ 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) CO₂ 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 1 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 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 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). 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 1,500 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 CO₂ concentrations between 50 and 1950 ppm, using the built-in CO₂ mixer of the LI-6400XT to control CO₂ 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 [CO₂] in the dome, photosynthesis was recorded at 50, 100, 200, 300, 400, 475, 550, 675, 800, 950, 1,200, 1,500, and 1,950 ppm CO₂ 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 CO₂ concentration.

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-Cᵣ curves had been collected for control plants, and 46 curves for treatment plants. After 1 week at their new condition, we started re-measuring the same plants (and the same leaves). The post-transition measurements yielded another 46 A-Cᵣ curves for control plants transferred to treatment conditions and 44 curves for treatment plants transferred to control conditions.

Before calculating Vₖmax and Jₖmax, we corrected photosynthesis rates and Cᵣ values for diffusion errors associated with CO₂ concentration gradients between the leaf cuvette and the atmosphere. To do so, we measured CO₂-response curves in both domes for leaves that were killed by submersion in boiling water for ~5 min. 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 Vₖmax and Jₖmax 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 [CO₂] of the measurement domes to minimize diffusion-related measurement errors. The airflow rate was set to 250 μmol s⁻¹. Respiration rates were logged for 2 min and the logged values 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 cm² of the standard cuvette of the LI-6400XT). The temperature sensitivity of respiration was expressed as the Q₁₀, calculated as:

\[ Q_{10} = e^{(10 \times b)} \]

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

\[ \ln(R_{\text{Dark}}) = a + b \times T_{\text{Leaf}} \]

From this, we calculated respiration rates at a set temperature of 30°C (\( R_{\text{Opt}} \)) and at the mean night-time temperatures of the two domes.

2.4 Parameter estimation

\( V_{\text{CMax}} \) and \( J_{\text{Max}} \) were calculated from the diffusion-error-corrected A-Cᵣ 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 (\( g_{\text{m}} \), representing the ease of CO₂ transfer from the substomatal cavity to the site of carboxylation in the chloroplast) for this species, \( g_{\text{m}} \) was assumed to be infinite. Therefore, the calculated \( V_{\text{CMax}} \) and \( J_{\text{Max}} \) values slightly underestimate the chloroplastic rates. For \( R_{\text{Light}}, \Gamma^* \) and additional parameters of the FvCB model, we used default estimates from the ‘fitaci’ function that are derived from for example, Bernacchi, Singasas, Pimentel, Portis Jr, and Long (2001) and Medlyn et al. (2002).

2.5 Temperature responses of photosynthesis parameters

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

\[ f(T_k) = k_{\text{Opt}} \times \frac{H_k \times e^{\left(\frac{\Delta H_k}{T_k - T_{\text{Opt}}}\right)}}{H_k - H_\Delta \times \left(1 - e^{\left(\frac{\Delta H_k}{T_k - T_{\text{Opt}}}\right)}\right)} \]

where temperatures are in Kelvin, \( T_k \) is leaf temperature, \( k_{\text{Opt}} \) is \( V_{\text{CMax}} \) or \( J_{\text{Max}} \) at \( T_{\text{Opt}} \), \( H_k \) represents the activation energy – it describes the exponential rise of the curve before \( T_{\text{Opt}} - H_\Delta \) is the ‘de-activation energy’, reflecting the rate of decrease above \( T_{\text{Opt}} \), 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 \( \Delta S \) (sensu Medlyn et al., 2002), which is related to \( T_{\text{Opt}} \) as:
and thus:

\[
\Delta S = \frac{H_0}{T_{\text{Opt}}} + R \ln \left( \frac{H_2}{H_0 - H_2} \right) 
\]

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 were not amenable to a random effects model. Figure S1 shows leaf–pooled measurements made on different leaves within each treatment.

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, and Edwards (2010) as:

\[
P_{400} \text{ or } P_{800} = P_{\text{Opt}} - b \times (T_k - T_{\text{Opt}})^2 
\]

where \(P_{\text{Opt}}\) is the rate of net photosynthesis at 400 or 800 ppm CO\(_2\) at optimum temperature \(T_{\text{Opt}}\), 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 Equation 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 analysed with Equation (6). These \(C_i\) values correspond with the mean \(C_i\) associated with measurement CO\(_2\) concentrations of 400 and 800 ppm, respectively. By analysing 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).

2.6 | Stomatal limitation

The degree of stomatal limitation of net photosynthesis \((I)\) was calculated following Farquhar and 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:

\[
I = 1 - \frac{P_{\text{Observed}}}{P_{\text{Infinitely high} g_s}} 
\]

\(P_{\text{Infinitely high} g_s}\) is calculated from the FvCB model by setting \(C_i\) in Equation (7) to equal \(C_o\), the observed CO\(_2\) concentration in the cuvette:

\[
V_{\text{CMAX}} = \frac{P_{\text{Gross}} \times (C_i + K_c)}{C_i - I^*} 
\]

\(P_{\text{Gross}}\) equals light-saturated photosynthesis plus \(R_{\text{Light}}\), where \(R_{\text{Light}}\) is assumed to be equal to 1.5\% of \(V_{\text{CMAX}}\); \(K_c\) 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, and Kubien (2008). In this approach, measurements of the initial slopes of A-Ci curves are compared with estimates of these slopes that are based on the FvCB model that implicitly assumes that Rubisco is fully activated in Equation (9) below; lower values for the measured than the modelled slopes indicate Rubisco inactivation. By 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 \(i.e., J_{\text{MAX}}\) 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 \(C_i < 200 \mu\text{mol} \text{ mol CO}_2\). The initial slopes were modelled as:

\[
\text{Initial slope} = \frac{V_{\text{CMAX}}}{\left(R^* + K_c \times \left(1 + \frac{O}{O^*}\right)^c\right)} 
\]

\(K_c\) 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 \(R^*\). \(K_c\) 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 four leaves, of similar age and sun exposure as the leaves used for gas-exchange measurements – were collected prior to the switch in conditions, and more at the end of the experiment. Leaf area was measured with an LI-3100C leaf area metre (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). To determine stomatal density, epidermal impressions were taken with the nail varnish method from three leaves per treatment. T. rosea is a 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 \( T_{\text{Opt}} \) of \( P_{\text{CO2}} \), \( P_{\text{B00}} \), \( V_{\text{CMax}} \) and \( J_{\text{Max}} \), and of the activation energies of treatment and control plants. Stomatal acclimation was assessed by comparing \( T_{\text{Opt}} \) of \( P_{\text{CO2}} \) and \( P_{270} \) (and \( P_{\text{B00}} \) and \( P_{200} \)), and by analysing 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):

\[
\text{Acclim}_{\text{SetTemp}} = \frac{R_{\text{Control at 30°C}}}{R_{\text{Treatment at 30°C}}} \tag{10}
\]

\( \text{Acclim}_{\text{SetTemp}} > 1.0 \) indicates thermal acclimation. The homeostasis method determines the degree of homeostasis:

\[
\text{Acclim}_{\text{Homeo}} = \frac{R_{\text{Control at } T_{\text{Control}}}}{R_{\text{Treatment at } T_{\text{Treatment}}}} \tag{11}
\]

When \( \text{Acclim}_{\text{Homeo}} \approx 1.0 \) respiration rates are homeostatic across conditions and respiration has fully acclimated; when \( \text{Acclim}_{\text{Homeo}} < 1.0 \), acclimation, if any, is imperfect.

2.10 | Curve fitting and statistical analyses

We fit Equations (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 Equations (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 \( k_{\text{Opt}}, P_{\text{Opt}}, H_{s} \), and \( T_{\text{Opt}} \). 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 \( H_{s} \) parameter produced bi-modal posterior distributions of other model parameters (e.g., \( T_{\text{Opt}} \) and so \( H_{s} \) 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 warm-up, and a subsequent 4,000 iterations during sampling. \( \Delta S \) was calculated from \( T_{\text{Opt}} \) and \( H_{s} \) estimates for each iteration with Equation 5. Models were checked to ensure convergence (\( R^{2} \sim 1 \)), 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 \( J_{\text{Max}} \) to \( V_{\text{CMax}} \) ratio, and the VPD response of stomatal conductance were determined with ANCOVA, with temperature and VPD 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 \( A_{c} \) 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, \( df = 6, t = 2.3, p = .059 \)) 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, \( df = 3, t = 6.5, p = .007 \)). 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, \( df = 6, t = 6.4, p < .001 \)). Transferring control plants to treatment conditions significantly increased LMA (paired two-sample t test, \( df = 3, t = 6.7, p = .007 \)), 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

Net photosynthesis at 400 ppm CO\(_{2}\) (\( P_{\text{CO2}} \)) peaked at 32.2 °C in treatment plants, which was almost 5 °C higher than \( T_{\text{Opt}} \) in control plants.

| TABLE 1 | Leaf mass per area (LMA), concentrations of leaf nitrogen (N) and carbon (C), and nitrogen concentration per unit leaf area (\( N_{\text{Area}} \)) 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 (\( \rightarrow \text{Treatment} \)) and vice versa (\( \text{Treatment} \rightarrow \text{Control} \)) before being re-measured after \( >7 \) days |
|-------------------|-----------------|-----------------|-----------------|-----------------|
| **Treatment**     | **LMA (g m\(^{-2}\))** | **N (mg g\(^{-1}\))** | **C (mg g\(^{-1}\))** | **\( N_{\text{Area}} (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        |
| Control           | 81.1 ± 6.2      | 20.2 ± 4.2      | 452 ± 12        | 165 ± 31        |
| Treatment         | 83.2 ± 6.3      | 18.4 ± 1.1      | 453 ± 59        | 153 ± 18        |
(Figure 1), with limited overlap of the credible intervals (see Figure 2 for the full posterior distributions of $T_{\text{Opt}}$ with highlighted 50, 90 and 99% credible intervals, and Table 2 for 5–95 percentiles of the posterior distribution). Median $P_{400}$ at $T_{\text{Opt}}$ was lower for the treatment plants than for the controls (Table 2). Transfer of control plants to treatment conditions increased $T_{\text{Opt}}$ of $P_{400}$ from 27.3 to 31.2°C (Figure 2) and decreased $P_{400}$ at $T_{\text{Opt}}$ by 41% to 11.3 μmol m$^{-2}$ s$^{-1}$ (Table 2). Transfer of treatment plants to control conditions decreased $T_{\text{Opt}}$ from 32.2 to 28.9°C (Figure 2) with minimal change in $P_{400}$ at $T_{\text{Opt}}$ (Table 2). When fitting the data with the peaked Arrhenius function (Equation (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 $T_{\text{Opt}}$ of plants transferred to warmer and cooler conditions, respectively (Figures 2 and 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

In treatment plants, the optimum temperature of $V_{\text{CMax}}$ was 43.3°C, which was 1.4°C higher than in control plants (Figure 3) with moderate overlap in credible intervals (Figure 2; Table 3). Transferring plants from control to treatment conditions increased $T_{\text{Opt}}$ of $V_{\text{CMax}}$ by 0.8°C, and the posterior distribution overlapped with that of both control and treatment plants (Figure 2). The transferred plants had lower $V_{\text{CMax}}$ at $T_{\text{Opt}}$ (Table 3). Plants transferred from treatment to control conditions reduced $T_{\text{Opt}}$ by 1.5°C, while $V_{\text{CMax}}$ at $T_{\text{Opt}}$ 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 $\Delta S$ of $V_{\text{CMax}}$ tended to be lower in treatment than in control plants, and when conditions were switched $\Delta S$ 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).

$J_{\text{Max}}$ peaked at ~2.0°C lower temperatures than $V_{\text{CMax}}$ (Table 3). Differences in $T_{\text{Opt}}$ of $J_{\text{Max}}$ 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 (Figure 2; Table 3). $J_{\text{Max}}$ at $T_{\text{Opt}}$ 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$P_{400}$</th>
<th>$P_{800}$</th>
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<tbody>
<tr>
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<td>5%</td>
<td>50%</td>
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<tr>
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<td>31.2</td>
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<tr>
<td>Treatment</td>
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<td>32.2</td>
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<tr>
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<td>29.0</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$P_{400}$ at $T_{\text{Opt}}$ (μmol m$^{-2}$ s$^{-1}$)</th>
<th>$P_{800}$ at $T_{\text{Opt}}$ (μmol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>19.1</td>
</tr>
<tr>
<td>Treatment</td>
<td>10.1</td>
<td>11.3</td>
</tr>
<tr>
<td>Treatment</td>
<td>13.3</td>
<td>14.4</td>
</tr>
<tr>
<td>Control</td>
<td>13.7</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Note: Shown are the 5, 50 and 95% quantiles of the posterior distribution of the optimum temperature ($T_{\text{Opt}}$) (a), the rates at optimum temperature (b), and the $b$ parameter (c).
were transferred to control conditions (Table 3). \( H_a \) and \( \Delta S \) of \( J_{\text{Max}} \) did not differ systematically among treatments and had overlapping 90% credible intervals (Table 3).

The \( J_{\text{Max}} \) to \( V_{\text{CMax}} \) ratio decreased with increasing measurement temperature in control and treatment plants by on average 21% between 30 and 40 °C (Figure 4). The mean \( J_{\text{Max}} \) to \( V_{\text{CMax}} \) ratio was lower in treatment than in control plants (ANCOVA with Tukey post hoc test, \( t = -2.76, p = .032 \)) and decreased when control plants were transferred to treatment conditions (\( t = -5.67, p < .001 \); Figure 4).

### 3.4 Stomatal conductance and stomatal limitation

Stomatal conductance did not exhibit strong temperature responses (Figure 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 Figure 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 Figure 4). Transfer to treatment conditions reduced stomatal conductance by ~43% (ANCOVA with Tukey post hoc test, \( t = -3.58, p = .003 \)), whereas transfer from treatment to control conditions did not change conductance (Figure 5). Consistent with the weak temperature response of stomatal conductance, stomatal limitation did not increase with increasing measurement temperature (Figure 6). Below 35 °C, stomatal limitation was higher in control plants transferred to treatment conditions than in control conditions (two-tailed \( t \) test, \( df = 26, t = 3.3, p = .003 \)), consistent with their reduced stomatal conductance (Figure 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 \( P_{800} \). \( T_{\text{Opt}} \) of \( P_{270} \) was moderately higher than that of \( P_{400} \); in particular for control plants and treatment plants moved to control conditions, but the 90% credible intervals overlapped; \( T_{\text{Opt}} \) differences between \( P_{505} \) and \( P_{800} \) were much smaller (Figure S2). These results suggest that stomatal conductance had some influence on \( T_{\text{Opt}} \) at ambient but not elevated measurement CO₂.

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**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 [CO₂] 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 Equation (3) with MCMC sampler (4,000 iterations, 100 random samples are plotted). \( T_{\text{Opt}} \) 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 \( T_{\text{Opt}} \). [Colour figure can be viewed at wileyonlinelibrary.com]
The $C_\text{a}/C_\text{c}$ ratio was not systematically affected by treatment conditions, and there were no short-term temperature response patterns in $C_\text{a}/C_\text{c}$ (Figure 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 modelled initial slopes appeared to diverge when leaf temperatures exceeded 42°C (Figure 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 (Figure S4).

### 3.6 Respiration

Dark respiration of treatment plants increased less steeply than that of control plants (Figure 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 (Equations 10 and 11) indicated limited acclimation; $\text{Acclim}_{\text{setTemp}}$ 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 night-time temperatures (26.5 vs. 29.5°C) treatment plants respired considerably more than controls ($\text{Acclim}_{\text{homeo}}$ was 0.63 – or 0.66 when calculated for mean daytime temperatures). Transfer from control to treatment caused a marginally significant decrease in $Q_{10}$ (paired two-sample t test, $t = 2.4$, $p = .08$), while the transfer from treatment to control conditions did not lead to a significant change of $Q_{10}$ (Table 4). $\text{Acclim}_{\text{setTemp}}$ and $\text{Acclim}_{\text{homeo}}$ 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 the treatment plants resulted in lower respiration rates at temperatures >35°C (Figure 7).

### 4 DISCUSSION

The tropical tree species $T.\text{rosea}$ showed clear signs of photosynthetic acclimation to elevated temperature and CO$_2$: treatment plants had moderately higher thermal optima for photosynthetic parameters $V_{\text{Cmax}}$ and $J_{\text{Max}}$ and downregulated respiration rates at high temperature, thereby improving photosynthetic performance at treatment conditions.
conditions and supporting our hypothesis of acclimation. Higher thermal optima of net photosynthesis were observed regardless of measurement CO2 concentration, indicating that the shift in the optimum temperature was not solely due to suppression of photorespiration by elevated CO2 in the treatment dome. The observed reduction in $V_{\text{Cmax}}$ in treatment plants is consistent with acclimation to elevated CO2. However, reduced $V_{\text{Cmax}}$ has also been observed as a result of warming alone – for example, in (sub-) tropical Eucalyptus provenances (Crous et al., 2018) – suggesting that the observed decrease in $V_{\text{Cmax}}$ can be related to increased CO2, increased temperature, or a combination of the two. Indeed, in the tropical tree species A. glandulosa neither warming nor elevated CO2 significantly reduced $V_{\text{Cmax}}$, but the combination of the two did (Fauset et al., 2019). In contrast to common observations in studies in which only CO2 is increased (e.g., Ainsworth & Rogers, 2007; Klein & Ramon, 2019), stomatal conductance and stomatal density were not reduced in our combined warming and CO2 enrichment experiment, suggesting that the CO2 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 $\text{T_{Opt}}$, as the shifts in $\text{T_{Opt}}$ 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 Rubisco activase limitation. Collectively, these results indicate that T. rosea plants exhibited strong acclimation to the combined effects of 4°C warming and doubling of CO2. Acclimation appeared to be underpinned by changes in the biochemical parameters, particularly in shifts in $\text{T_{Opt}}$ of $V_{\text{Cmax}}$ associated with adjustment of $\Delta S$.

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 parameters, and to more thoroughly probe the uncertainty around the estimated treatment effects. This is valuable for difficult-to-estimate model parameters such as $\text{T_{Opt}}$. Uncertainty of $\text{T_{Opt}}$ 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 $\text{T_{Opt}}$ of $P_{400}$ were left skewed, because of the small number of observations below $\text{T_{Opt}}$.  

![Figure 4](https://wileyonelibrary.com)
and those of \( V_{\text{CMax}} \) tended to be right skewed (Figure 2). Even with few data points above \( T_{\text{Opt}} \) of \( V_{\text{CMax}} \) and \( J_{\text{Max}} \), 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 \( \text{CO}_2 \) 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., 2021), especially in association with El Niño events (Rifai, Li, & Malhi, 2019). Physiological processes in tropical trees are 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 are consistent with acclimation of photosynthesis to higher temperatures (Slot & Winter, 2017a; Way & Yamori, 2014; Yamori, Hikosaka, & Way, 2014). Furthermore, \( T_{\text{Opt}} \) of \( V_{\text{CMax}} \) and \( J_{\text{Max}} \) were moderately higher under treatment than control conditions, consistent with observed warming effects on other ecosystems (Kattge & Knorr, 2007). However, at 400 ppm, \( T_{\text{Opt}} \) was almost 5°C higher in treatment plants that experienced ~5°C higher daytime temperatures than in controls, while \( T_{\text{Opt}} \) commonly only increases by ~0.4°C per degree increase in growth temperature at ambient \( \text{CO}_2 \) (Slot & Winter, 2017a; Yamori et al., 2014). \( T_{\text{Opt}} \) of net photosynthesis also increases when temperature responses are measured at elevated \( \text{CO}_2 \) because the beneficial effect that elevated \( \text{CO}_2 \) has on reducing photorespiration is greater at higher than at lower leaf temperatures (Brooks & Farquhar, 1985). In the current study, \( T_{\text{Opt}} \) increased regardless of measurement \( \text{CO}_2 \) (Figure 1), but higher \( C_i \) in the treatment plants can account for reduced photorespiration and higher \( T_{\text{Opt}} \). The difference in \( T_{\text{Opt}} \) between control and treatment plants thus reflects a response to the combination of elevated temperature and elevated \( \text{CO}_2 \), consistent with observations of additive effects of warming and elevated \( \text{CO}_2 \) on \( T_{\text{Opt}} \) of two boreal tree species (Dusenge et al., 2020).

Prolonged exposure to elevated \( \text{CO}_2 \) can result in feedback inhibition of photosynthesis if more carbohydrates are synthesized than are exported to sink organs (e.g., Neales & Incoll, 1968). Accumulation
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 $V_{C_{\text{Max}}}$ alone cannot account for reduced net photosynthesis. $J_{\text{Max}}$ was, however, also reduced, as is commonly observed with plants grown at elevated CO₂ (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 $T_{\text{Opt}}$ and the $J_{\text{Max}}/V_{C_{\text{Max}}}$ ratio has long been known. Here, we found weak and non-significant relationships, both for $J_{\text{Max}}/V_{C_{\text{Max}}}$ at 30°C and $J_{\text{Max}}/V_{C_{\text{Max}}}$ at their respective $T_{\text{Opt}}$ (Figure S5). Changes in the activation energy ($H_a$ in Equation 3) have been found to play an important role in acclimation in some studies (e.g., Dusenge et al., 2020; Hikosaka et al., 2006; Kumarathunge et al., 2019), whereas Kattge and Knorr (2007) found that $\Delta S$, the entropy term of the instantaneous temperature response, was significantly impacted by plant growth temperature. In the current study, $T_{\text{Opt}}$ of $P_{400}$ and $P_{800}$ scaled with $\Delta S$ of $V_{C_{\text{Max}}}$ and with $T_{\text{Opt}}$ of $V_{C_{\text{Max}}}$ (Figure S5). In contrast, $T_{\text{Opt}}$ 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 $\Delta S$ 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 $T_{\text{Opt}}$ of $V_{C_{\text{Max}}}$. 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 $\Delta S$ estimates, although not very well constrained, correlated much better with $T_{\text{Opt}}$ of $P_{400}$ and $P_{800}$ than the $J_{\text{Max}}/V_{C_{\text{Max}}}$ ratio, lending support to Kattge and 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 (Hernández et al., 2020; Slot & Winter, 2017b, 2017c). The relatively small role of stomatal 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 & Ainsworth, 2004), presumably due to the challenges of water delivery.

**Figure 6** Stomatal limitation of net photosynthesis determined with Equation (7) in relation to temperature in *Tabebuia rosea* plants grown at ambient (control) and at elevated temperature and [CO₂] (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.
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 (Smith et al., 2020; Tan et al., 2017).

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 $T_{\text{opt}}$ of photosynthesis at 400 ppm CO$_2$, as illustrated by moderately higher $T_{\text{opt}}$ 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, and 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 and 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 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 (Figure 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 CO2 in which Medlyn et al. (2001) also observed that the relationship between conductance and VPD was not affected by growth at elevated CO2. 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 CO2.

4.3 Weak thermal acclimation response of respiration

Consistent with most previous studies, dark respiration increased exponentially, leading to high rates of CO2 loss at high temperature. Treatment plants had lower temperature sensitivity of respiration (indicative of Type 1 acclimation, Atkin & Tjoelker, 2003; Slot & Kitajima, 2015). Nonetheless, thermal acclimation of respiration was moderate, with limited adjustment of AcclimTtest and no indication of homeostasis. While growth at elevated temperature tends to reduce respiration (Atkin & Tjoelker, 2003; Slot & Kitajima, 2015), growth at elevated CO2 tends 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 CO2, and consistent with Dusenge et al. (2020), the responses to temperature were seemingly stronger than the responses to CO2.

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 CO2 manipulations (e.g., Cheesman & Winter, 2013; Fauset et al., 2019; Kositsup et al., 2009; Lovelock, Winter, Mersits, & Popp, 1998; Slot & Winter, 2017a, 2018; Winter, Garcia, Gottsberger, & Popp, 2001; Winter & Virgo, 1998), 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.

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 were 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 CO2 concentrations, plants of the early successional tree species T. 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 conflict of interest.

AUTHOR CONTRIBUTIONS

Martijn Slot, Klaus Winter: Designed the study. Martijn Slot: Performed the measurements. Martijn Slot, Sami W. Rifai: Analysed the data and made the figures. Martijn Slot: Wrote the paper, with significant input from Sami W. Rifai and Klaus Winter.

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 as a zip file in the online supplementary materials (Appendix 1).

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REFERENCES


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

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