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Experimental warming across a tropical forest canopy height gradient reveals minimal photosynthetic and respiratory acclimation

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Abstract

Tropical forest canopies cycle vast amounts of carbon, yet we still have a limited understanding of how these critical ecosystems will respond to climate warming. We implemented in situ leaf-level + 3°C experimental warming from the understory to the upper canopy of two Puerto Rican tropical tree species, *Guarea guidonia* and *Ocotea sintenisii*. After approximately 1 month of continuous warming, we assessed adjustments in photosynthesis, chlorophyll fluorescence, stomatal conductance, leaf traits and foliar respiration. Warming did not alter net photosynthetic temperature response for either species; however, the optimum temperature of *Ocotea* understory leaf photosynthetic electron transport shifted upward. There was no *Ocotea* respiratory treatment effect, while *Guarea* respiratory temperature sensitivity (Q_{10}) was down-regulated in heated leaves. The optimum temperatures for photosynthesis (T_{opt}) decreased 3–5°C from understory to the highest canopy position, perhaps due to upper canopy stomatal conductance limitations. *Guarea* upper canopy T_{opt} was similar to the mean daytime temperatures, while *Ocotea* canopy leaves often operated above T_{opt}. With minimal acclimation to warmer temperatures in the upper canopy, further warming could put these forests at risk of reduced CO_2 uptake, which could weaken the overall carbon sink strength of this tropical forest.

KEYWORDS
electron transport, experimental leaf warming, photosynthesis, respiration, stomatal conductance, thermal acclimation

1 | INTRODUCTION

While only a fraction of Earth's surface, tropical forests are major components of Earth's carbon cycle (Pan et al., 2013). At the same time, tropical regions are predicted to reach temperatures outside of their historical climate norms more quickly than other biomes (Diffenbaugh & Scherer, 2011; Hoegh-Guldberg et al., 2018; Mora et al., 2013). Tropical forest canopies often exceed their photosynthetic thermal thresholds (Doughty & Goulden, 2008; Mau et al., 2018), and rising temperatures may reduce overall tropical forest carbon dioxide (CO_2) uptake (Brienen et al., 2015; Clark et al., 2010; Malhi et al., 2009). In addition, the narrow temperature ranges that tropical forests experience suggest that, compared to temperate forests, tropical plants may have lower thermal plasticity and a lower capability to adjust to climate warming (Cunningham & Read, 2002; Cunningham & Read, 2003; Janzen, 1967).

The balance between plant photosynthesis and respiration plays a crucial role in controlling Earth's atmospheric carbon fluxes (Liu et al., 2015); therefore, understanding how these processes respond to increasing temperature is necessary to accurately predict effects of

the future climate (Dusenge et al., 2019; Luo, 2007; Smith & Dukes, 2013). Photosynthesis, or carbon dioxide (CO_2) uptake, has a peaked response to temperature, where net photosynthesis increases to a maximum (A_{opt}), then declines after the optimum temperature (T_{opt}) is reached (Berry & Bjorkman, 1980). Respiration, or CO_2 release, also has a peaked temperature response (reviewed in Atkin et al., 2005). However, respiratory function drops at temperatures much higher than those of net photosynthesis (Atkin et al., 2005; Heskel et al., 2016; Kumarathunge et al., 2019; O'Sullivan et al., 2013). Because respiration continues to increase with moderately high temperatures, whereas photosynthesis declines, if these two processes are not able to physiologically adjust to conserve CO_2 balance under warmer temperatures, we could see systems shift towards a greater net loss of greenhouse gases to the atmosphere.

Warmer temperatures can reduce photosynthesis both indirectly and directly, and plants have a variety of mechanisms to mitigate these effects. High temperatures can indirectly reduce carbon assimilation through declined rates of stomatal conductance (Campbell & Norman, 1998; Farquhar & Sharkey, 1982). High temperatures can directly reduce net photosynthesis either by increasing thylakoid membrane permeability, which impairs electron transport function (Bukhov et al., 1999; Zhang et al., 2009), or by decreasing the capacity for Rubisco CO_2 fixation (Hikosaka et al., 2006; Medlyn et al., 2002; Salvucci & Crafts-Brandner, 2004; Salvucci et al., 2001; Wang & Portis, 1992). Warmer growth temperatures can also alter resource allocation, potentially resulting in measurable changes to leaf morphology, such as higher (Slot et al., 2014) or, more often, lower (Poorter et al., 2009; Scafaro et al., 2017; Yamori et al., 2005) leaf mass per area (LMA). To counteract negative effects of warming on carbon uptake, plants can make physiological adjustments that enhance or maintain performance at higher temperatures; that is, they can thermally acclimate.

Way and Yamori (2014) defined photosynthetic acclimation as an up-regulation, or constructive adjustment that increases photosynthesis relative to unacclimated plants at a new growth temperature. Therefore, while plants can either increase or decrease photosynthetic performance at warmer temperatures, we use 'acclimation' to refer to up-regulations of photosynthesis (e.g., an increase in T_{opt} and/or A_{opt}) at higher growth temperatures. Despite the important role that tropical forest canopies play in global carbon uptake, few studies investigate their photosynthetic thermal acclimation potential (Cavaleri et al., 2015; Doughty, 2011; Dusenge & Way, 2017; Slot et al., 2014). Experiments in controlled environments suggest that tropical plants can make constructive alterations to photosynthesis under warmer temperatures (Slot & Winter, 2017b; Slot & Winter, 2018; Smith & Dukes, 2017), but not always (Cheesman & Winter, 2013; Slot & Winter, 2018). Our recent in situ forest warming experiment of two tropical understory shrubs in the same stand as this study revealed evidence of photosynthetic acclimation with warming in one tropical shrub species, while the other species experienced declines in C uptake with warmer temperature (Carter et al., 2020). A tropical canopy leaf warming study found that warming individual leaves an average of $+2^{\circ}C$ above ambient temperature could cause damage to the photosynthetic apparatus, leading to reduced rates of photosynthesis (Doughty, 2011). Similarly, studies on a broadleaf Mediterranean species (Aspinwall et al., 2017) and temperate broadleaf *Eucalyptus* species (Crous et al., 2013) suggest that evergreen trees experience reduced rates of photosynthesis at warmer temperatures. While the thermal acclimation potential of T_{opt} within any forest canopy vertical gradient has rarely been investigated (but see Carter & Cavaleri, 2018), upper canopies may have a higher ability to acclimate than lower canopy and understory leaves. The optimum temperature of photosynthetic electron transport can acclimate to higher irradiance (Niinemets et al., 1999; Niinemets & Valladares, 2004), and exposed leaves can have higher heat tolerances than shaded leaves (Slot et al., 2019), suggesting that upper canopy leaves may have a greater thermal acclimation capacity.

Thermal acclimation of respiration at higher growth temperatures occurs through overall declined CO_2 release (i.e., down-regulation), either due to reduced basal respiration rates or reduced sensitivity to temperature (Atkin & Tjoelker, 2003). Evidence suggests that, overall, tropical plant respiration will acclimate to climate warming (Aspinwall et al., 2016; Mujawamariya et al., 2020; Slot et al., 2014; Slot & Kitajima, 2015; Way & Oren, 2010). However, our in situ experimental warming study of tropical forest shrubs at the same site found no evidence of respiratory acclimation after 8 months of whole-plant warming (Carter et al., 2020). It is even less understood how respiratory acclimation can vary with height. Foliar respiration at a standardized temperature increases with increasing canopy height in tropical forests (Asao et al., 2015; Cavaleri et al., 2008; Meir et al., 2001; Weerasinghe et al., 2014). However, the relationship between respiratory temperature sensitivity (Q_{10}; the multiplicative increase in respiration for every $+10^{\circ}C$) and height is less understood. The few studies in forest canopies suggest that respiratory temperature sensitivity is constant or increases across canopy heights (Cavaleri et al., 2008; Griffin et al., 2002; Turnbull et al., 2003; Weerasinghe et al., 2014). One study investigating seasonal temperature acclimation found no vertical variation in foliar respiratory acclimation throughout the temperate forest canopy (Araki et al., 2017). Considered together, these studies suggest that tropical forest respiration is capable of acclimation, and acclimation may be consistent across the height gradient.

In temperate forests, studies have implemented in situ canopy warming using open top chambers around individual branches (Yamaguchi et al., 2016), heated branch cables (Nakamura et al., 2010), infrared heaters installed in the canopy (Nakamura et al., 2016) and whole-forest system open-top chambers (Hanson et al., 2017). However, many of these whole-canopy or even branch-level warming methodologies used in temperate forests are cost prohibitive in a tropical forest, where canopies can reach more than 50 m in height (Feldpausch et al., 2010; Pan et al., 2013). Leaf-level warming, on the other hand is relatively inexpensive, yet can provide valuable mechanistic insight into physiological thermal responses (Cavaleri et al., 2015). To date, two studies have implemented leaf-level warming in tropical forest upper canopies (Doughty, 2011; Slot et al., 2014), and one study did so across multiple canopy heights of a temperate

deciduous forest (Carter & Cavaleri, 2018). Leaf-level warming provides an opportunity to investigate vertical gradients of physiological responses to temperature, a critical gap in our current understanding of forest responses to the changing climate.

We used leaf-level experimental warming to investigate the physiological thermal acclimation potential across vertical canopy transects of two Puerto Rican tree species. In addition, we assessed underlying mechanisms of temperature responses, including stomatal conductance, chlorophyll fluorescence and leaf traits. This work represents the first mature canopy warming experiment in any ecosystem to investigate both photosynthetic and respiratory acclimation. In addition, this is the first study to investigate physiological thermal acclimation across a canopy height gradient in a tropical forest. Our overall hypotheses were: (1) photosynthesis will up-regulate (i.e., acclimate) in response to leaf-level warming, and the positive response will be stronger higher in the canopy, where more extreme climate variations already occur; (2) respiration will down-regulate (i.e., acclimate) to experimental leaf warming; however, acclimation response will be uniform throughout the canopy gradient.

2 | METHODS

2.1 | Study site

This experiment was conducted on a 20.1 m canopy access tower (UpRight Inc., Dublin, Ireland) built at the Tropical Responses to Altered Climate Experiment (TRACE) site, within the Luquillo Experimental Forest in northeastern Puerto Rico (18°18′N, 65°50′W). Mean annual precipitation during the 2 years prior to experimental warming was 2,271 mm, and mean annual temperature was 24°C (Harris et al., 2012). The forest is classified as a tropical wet forest with low seasonal variation in precipitation and temperature. The site is located on Ultisol soils (Scatena, 1989) at 100 m elevation. In 2016, the secondary growth forest had a basal area of 39 m^2 ha^{-1} and a stand density of 3,100 trees ha^{-1} (Carter et al., 2020). The most abundant canopy tree species in the stand at the time of the study were *Prestoea montana*, *Syzgium jambos*, *Ocotea leucoxylon* and *Casearia arborea*.

2.2 | Leaf-level warming

Physiological thermal acclimation was assessed by installing leaf-level warming devices across the vertical canopy gradient. We heated leaves of two species accessible from the canopy access tower, *Guarea guidonia* and *Ocotea sintenisii* (Mez) Alain (synonym: *Nectandra turbacensis [Kunth] Nees*). *Guarea* is a shade tolerant species and *Ocotea* has been classified as shade intolerant (Adame et al., 2014). Two to four leaves per species were successfully heated at each canopy height for a total of approximately 30 heated/control pairs (Table S1). Most leaves were heated for 23–29 days, but due to logistical challenges one leaf was heated for 16 days, one was heated 18 days and one leaf was heated for 33 days (Table S1). To avoid the interactive effects of carbon importation that occurs in developing leaves (Turgeon, 2006), all leaves selected for warming were fully developed at the time of warming initiation. *Guarea* was accessible at 1.8, 9.0, 10.8, 12.6, 14.4 and 16.2 m height and *Ocotea* was accessible from 1.8, 14.4, 16.2, 18.0 and 19.8 m (Table S1). Separate plants were used for each 1.8 m (understory) heated and control leaf pairs except for one 1.8 m *Guarea* leaf pair, which were chosen from the same plant. Only one *Ocotea* tree was accessible from the tower; therefore, all measurements from 14.4 to 19.8 m were from the same plant. Two *Guarea* trees were accessible from the canopy access tower; one accessible from 9.0 to 10.8 m, and the other accessible from 10.8 to 16.2 m. Light environment was measured using diffuse non-interceptance (DIFN%), or the fraction of radiation transmitted through the canopy, using two plant canopy analysers (LAI-2000 and LAI-2200, Li-COR Inc.). DIFN was measured from all four sides of the tower at each height on 6 July 2017 and 8 August 2017.

Each heated leaf was maintained +3°C higher than a paired control leaf. Heated leaf temperatures were controlled by turning a relay module (SSR-25 DA, Fotek Controls Co.), and thus a heating pad (100 watt 120VAC, 24 100 k Kat's Five Star Manufacturing Group Inc.), off when the heated leaf temperature was more than 3°C higher than the control leaf. Temperatures were monitored every 15 s and averaged over 2 min using thermocouples (TT-T-30 SLE[ROHS], OMEGA Engineering Inc.) wired in a multiplexor (AM25T; Campbell Scientific Inc.), and recorded with a datalogger (CR1000; Campbell Scientific Inc.). Heating pads were attached to a galvanized steel wire frame positioned 8–16 cm underneath the leaf. The metal frame was attached to a sturdy branch, allowing the heater to experience the same movement as the leaf (Figure 1). Heated leaves received a similar light environment to their associated control leaves and were continuously heated 24 h/day (for further methodological details, see Carter & Cavaleri, 2018).

FIGURE 1 Examples of leaf heaters in the upper canopy of *Ocotea sintenisii*

For each heated and control leaf, we measured net photosynthetic temperature response, stomatal conductance, photosynthetic electron transport, photosystem II function (F_v/F_m) and night-time respiratory responses to temperature. We additionally measured leaf traits: nitrogen per unit leaf area (N_{area}), nitrogen per unit leaf mass (N_{mass}), carbon to nitrogen ratio (*C:N*), leaf chlorophyll, leaf mass per area (LMA), leaf area and percent leaf water content. Four heated leaves were removed from the net photosynthetic and photosynthetic electron transport data analysis because they had negative values, likely due to heating damage to the leaf or petiole (two *Ocotea* at 18 m and one at 19.8 m, one *Guarea* at 1.8 m). The *Ocotea* leaf at 19.8 m was not included in any data analysis because the petiole broke, and there were no other viable heated leaves. Leaf heaters were turned off prior to gas exchange measurements. Photosynthesis was measured immediately and respiration was measured the following night whenever possible. Due to inclement weather, *Guarea* heaters at 10.8 m were off for 36 h prior to respiration measurement, and *Ocotea* heaters at 16.2 m were off for 48 and 60 h before photosynthesis and respiration was measured, respectively.

2.3 | Photosynthesis and stomatal conductance

Photosynthetic-temperature response curves were constructed at 25, 27, 30, 33, 35, 37, and 40°C using an LI6400XT infrared gas analyser with fluorometer attachment, enabling the simultaneous measurements of gas exchange and photosynthetic electron transport rate (ETR) from a 2 cm^2 area (6400–044, Li-COR Inc.). Response curves took 1.0–1.5 h each, and were primarily conducted between 8:30 a.m.-12 p.m.; and never later than 3:30 p.m. Measurements were conducted at only 1–2 adjacent canopy heights throughout any given day. Prior to measuring net photosynthesis (A_{net}) and ETR, each leaf was light-acclimated to saturating light for at least 20 min; after which, temperature response curves were measured. Saturating light was determined for each canopy height using preliminary light response curves (*data not shown*; 800 PPFD for 0–12.6 m and 1,000 PPFD for 14.4–19.8 m). Temperature was controlled using a water jacket (Expanded Temperature Control Kit 6,400–88, Li-COR Inc.), which used gravity to cycle hot or cold water from thermoses (Mau et al., 2018). CO_2 was controlled at 400 ppm. Vapour pressure deficit (VPD) inside of the chamber was consistent between species and canopy height; however, VPD varied during measurements from 1.5 to 3.5 kPa from 25 to 40°C. Flow rate was controlled between 200 and 400 μmol s^{-1} to maintain as low VPD as possible at high temperatures.

The optimum temperature of A_{net} (T_{opt}) was determined by fitting A_{net} response to temperature to the curve derived from June et al. (2004):

$$A(T) = A_{opt} \times e^{-\left(\frac{T_{leaf} - T_{opt}}{\Omega}\right)^2} \quad (1)$$

where A_{opt} is the net assimilation rate at the optimum temperature (T_{opt}) and Ω is the photosynthetic thermal niche or the difference in temperature between T_{opt} and the temperature where A_{net} declines to 37% of its rate at T_{opt}, in other words, the width of the curve's peak. Two *Guarea* Ω were negative; therefore, thermal niche was corrected to the absolute value. We were unable to fit Equation (1) to four of the 59 (27 heated, 32 control) as the model did not converge. For these measurements, three *Ocotea* in the middle and upper canopy, and one *Guarea* in the understory, we estimated T_{opt} as the temperature at the maximum recorded point and A_{opt} was the rate of photosynthesis at this point.

Stomatal conductance (g_s) had a negative linear response to temperature. We therefore extracted the intercepts (β_{0_gs-T}) and slopes (β_{1_gs-T}) and of the $gs - T_{leaf}$ relationship for each measured photosynthetic-temperature curve. We calculated the slope parameter of the stomatal-photosynthesis model (g_1) using Medlyn et al. (2011):

$$g_s = 1.6\left(1 + \frac{g_1}{\sqrt{VPD}}\right)\frac{A_{net}}{C_s} \quad (2)$$

where VPD is the vapour pressure deficit at the leaf surface and C_s is the CO_2 concentration of the air surrounding the leaf. g_1 ($kPa^{-0.5}$) is the slope parameter, which provides a normalized variable that incorporates VPD and is inversely related to water-use efficiency (Medlyn et al., 2017).

The optimum temperature of photosynthetic electron transport (T_{optETR}) was extracted by fitting ETR to the Equation (1) and replacing ETR for A_{net}, where ETR_{opt} is the electron transport rate at T_{optETR}. ETR for each leaf was corrected for absorption using the chlorophyll–absorption relationship described in Bauerle et al. (2004):

$$\text{Absorptance} = 89.2 - 56.8\, e^{-0.0723 * Chl} \quad (3)$$

where Chl is chlorophyll content. Chlorophyll content was measured subsequent to the assimilation/ fluorescence measurements using a chlorophyll content meter (CCM-200+, OPTI-SCIENCES). One *Ocotea* control leaf in the understory had T_{opt} that was overestimated at 73°C, a value that is biologically unlikely. These data were removed for ETR data analysis only. Dark-adapted maximum chlorophyll fluorescence (F_v/F_m), which assesses Photosystem II function, was measured on the heated and control leaves predawn directly after R_d measurements (described below) using a handheld portable fluorometer (FluorPen FP Max, Photo Systems Instruments).

2.4 | Respiration

Dark respiration (R_d) was measured on the same leaves as photosynthesis and was assessed predawn (1:30 a.m.–5:30 a.m.), the night after photosynthesis measurements were collected. There were six instances where *Ocotea* leaves broke in between the photosynthesis and respiration measurements: two control leaves from 18.0 m, one control from 19.8 m, one control from 16.2 m and two heated from 18.0 m. When this occurred for a control leaf, we measured a leaf on the same stem in a similar cohort, and when this occurred for a heated leaf, we measured a leaf that was positioned very close to the heater that received residual heat.

R_d measurements were conducted using an LI6400 fitted with an aluminium foil-wrapped dark 6400-05 conifer chamber and 6400-088 expanded temperature kit (Li-COR Inc). The conifer chamber provides space for higher leaf area, providing more accurate detections of R_d. Each leaf was allowed to stabilize to the chamber conditions for at least 5 min prior measurement. Respiratory temperature curves were constructed by measuring the rate of respiration at 25, 30, 35, 37, 40°C. CO_2 was controlled at 400 ppm, and flow was controlled at 400 μmol s^{-1}. After all measurements were completed, all 62 leaves (30 heated, 32 control) were collected and scanned using a desktop scanner (EPSON Stylus NX420). Leaf scans were analysed for leaf area using ImageJ v.1.50 image analysis software. Each R_d measurement was corrected for calculated leaf area.

Each respiratory response curve was fitted to the nonlinear equation:

$$R_d = \beta_0 \times \exp(T_{leaf} \times \beta_1) \quad (4)$$

where R_d is the respiration rate (μmol m^{-2} s^{-1}) at T_{leaf} and β_0 and β_1 are model parameters. The β_1 parameter is then used to calculate the multiplicative change in respiration rate with every 10°C:

$$Q_{10} = \exp(10 \times \beta_1) \quad (5)$$

Respiration at 25°C (R_{25}) is estimated by substituting 25 for T_{leaf} in Equation (4).

R:A Ratio

The ratio between R_{25} and photosynthesis at 25°C (*R:A*) was calculated by dividing R_{25} by A_{25}. A_{25} was calculated by plugging in $T_{leaf} = 25$ and the already calculated A_{opt}, T_{opt} and Ω terms into Equation (1) and solving for A_{net}. For the three curves that did not fit Equation (1), A_{net} at the measured 25°C was used as A_{25}.

2.5 | Leaf traits

Directly after respiration measurements, measured leaves were detached and stored in a refrigerator no more than 36 h prior to being weighed for fresh mass and scanned for leaf area using a desktop scanner. Samples were then dried in a 60°C degree oven for at least 48 h before collecting dry mass. Dried leaf samples were ground to a powder using a ball mill (SPEX SamplePrep 8000M Mixer/Mill) and analysed for nitrogen and carbon content with an elemental analyser (Elemental Americas). Leaf mass per area (LMA) was calculated as dry mass (g) divided by leaf area (cm^2). Percent leaf water content (% LWC) was calculated as fresh mass (g) minus dry mass (g), divided by the fresh mass (g) and multiplied by 100. *N* per leaf area (N_{area}) was calculated by multiplying *N* (g g^{-1}) by LMA.

2.6 | Data analysis

Warming device efficacy was evaluated by investigating the instances of temperature spiking in the heated leaves across canopy height. Heated leaf maximum temperature ($T_{leafMAX}$) and the frequency of heated leaves exceeding control leaves by 10°C or more (ΔT > 10°C) were calculated to assess spiking. Effects of height, species and the interaction between height and species on temperature spiking were investigated using analysis of covariance (ANCOVA) procedures. Datapoints were removed if: a heater malfunctioned, we had to begin warming a different leaf due to leaf damage, or temperatures were well outside of realistic ranges (e.g., T_{leaf} < 0°C).

We investigated the height response of daytime mean temperatures ($T_{leafMEAN}$) and daily maximum temperatures ($T_{leafMAX}$) of control leaves using linear regressions. We then compared control $T_{leafMEAN}$ and $T_{leafMAX}$ to T_{opt} and T_{optETR} at the highest canopy positions of each species (*Ocotea* upper 18.0–19.8 m; *Guarea* mid 14.4–16.2 m) using Student's *t*-test to assess whether and how frequently leaf temperatures exceeded T_{opt} values.

Photosynthetic parameters (T_{opt}, A_{opt}, A_{25}, Ω, T_{optETR} and ETR_{opt}), chlorophyll fluorescence (F_v/F_m), respiratory parameters (R_{25} Q_{10} and *R:A*), stomatal conductance parameters (β_{0_gs-T} and β_{1_gs-T}) and leaf traits (leaf area, LMA, %LWC, N_{mass}, N_{area} and *C:N*) were all analysed for effects of treatment, canopy height and the interaction between treatment and canopy height using mixed effects models with tree as the random intercept. Canopy height was analysed as a continuous variable except for the stomatal sensitivity analysis (see below). All parameters were analysed in response to treatment and log-transformed DIFN (light) using mixed-effects models. Log-transformed light response to canopy height was investigated using a linear model, and light at the top of the canopy (19.8 m) was assumed 100% DIFN. To further investigate stomatal sensitivity across the canopy gradient, the canopy was split into four categories of 'canopy class': understory (0–1.5 m), lower canopy (9–12.6 m), mid canopy (14.4–16.2 m) and upper canopy (18–19.8 m). A mixed-effects model was run for each species investigating stomatal conductance response to temperature, canopy class and the interaction between temperature and canopy class. Pairwise comparison of canopy class slopes and intercepts were conducted using 'emmeans' package (Lenth, 2019) in R. Mixed-effects models were also used to describe g_1 response to treatment and the second order polynomial transformation of leaf temperature. Mixed-effects models were analysed using the 'lme' function in the package 'nlme' (Pinheiro et al., 2018), and all data analyses were conducted using R statistical software 3.5.0 (R Core Team, 2018).

3 | RESULTS

3.1 | Warming device and environmental conditions

Overall, our warming apparatus effectively heated leaves +3°C above control leaves (Figure 2). Instances of temperature spikes, where the differences between heated and control leaf temperature were greater than 10°C (ΔT > 10°C), occurred less than 1% of the time (Table S2; Figure S1a). *Ocotea* had a higher frequency of temperature spikes than *Guarea* (Table S2). All heated *Ocotea* leaves had four or

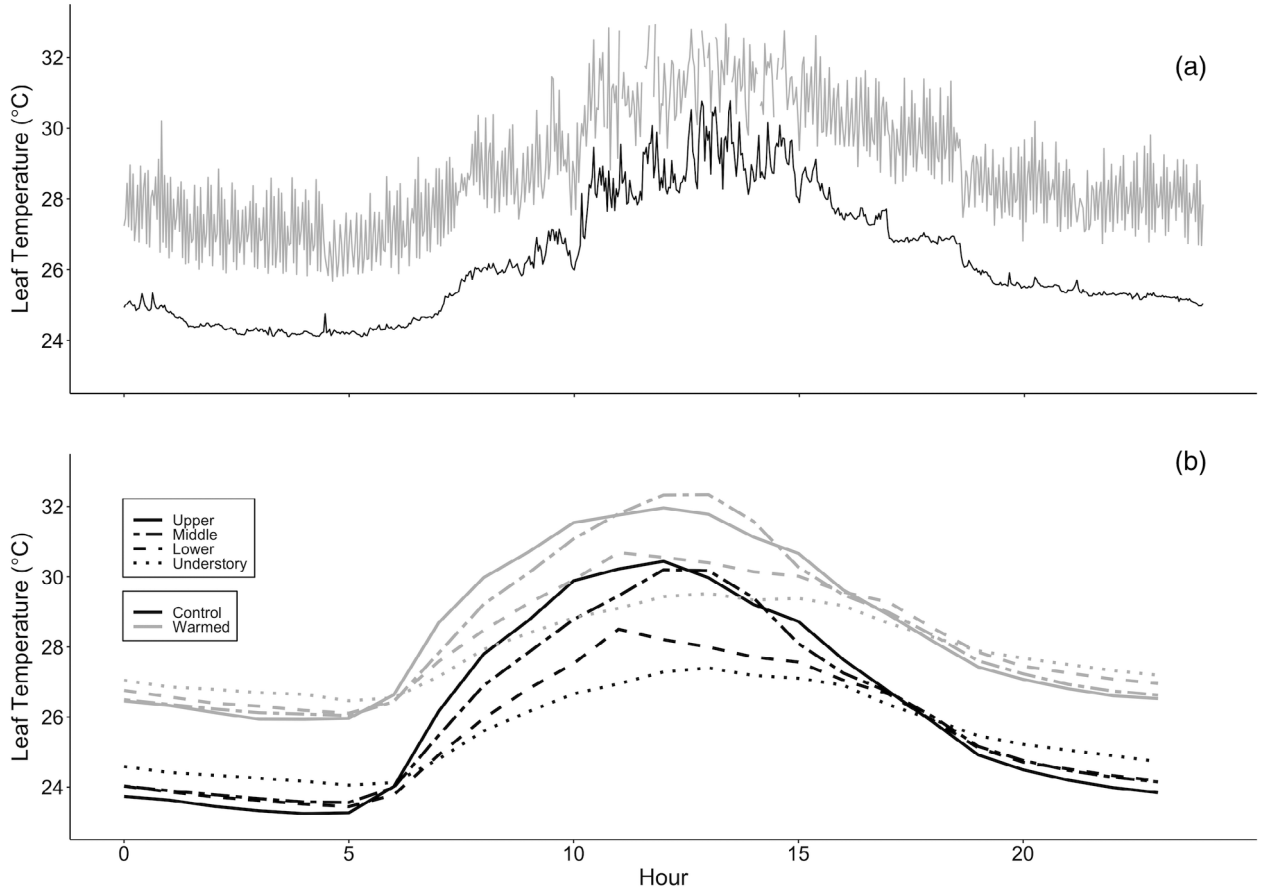

FIGURE 2 Performance of leaf-level warming. (a) 24 hours of heating for one control and associated heated leaf. Example leaf was *Guarea guidonia* 14.4 m height on July 22, 2017 and (b) mean heated and control leaf temperature at the four canopy positions for *G. guidonia* and *Ocotea sintenisii* combined (30 heated and 32 control leaves). Control leaves are represented by black lines and heated lines are represented by grey lines. Upper canopy is depicted by solid lines, mid canopy is depicted by dot-dash lines, lower canopy is depicted by dashed lines, and understory is depicted by dotted lines

more instances of temperature spiking; whereas, temperature spiking occurred in 10 of the 17 heated *Guarea* leaves. *Ocotea* also had significantly higher heated leaf maximum temperatures (38–46°C) in the mid and upper canopies compared to *Guarea* (38–39°C at mid canopy; Table S2, Figure S1b).

Control leaf daily daytime mean temperatures ($T_{leafMEAN}$) varied across the canopy vertical gradient by only 1–3°C, while maximum ($T_{leafMAX}$) temperatures spanned 6–8°C. *Guarea* daytime $T_{leafMEAN}$ ranged from 26.8 to 28.2°C from the understory to the mid canopy (the tallest canopy layer for *Guarea*) ($p < 0.001$; $R^2 = 0.20$; Figure 3a). *Ocotea* $T_{leafMEAN}$ increased from 26.2 to 29.0°C from the understory to the upper canopy ($p = 0.001$; $R^2 = 0.04$; Figure 3b). *Guarea* $T_{leafMAX}$ ranged from 30.5 to 36.7°C ($p < 0.001$; $R^2 = 0.20$; Figure 3a), and *Ocotea* $T_{leafMAX}$ ranged from 31.3 to 39.1°C ($p < 0.001$; $R^2 = 0.16$; Figure 3b). In addition, there was a temperature spike in *Ocotea* mid canopy where $T_{leafMAX}$ daily mean was 40.9°C at 14.4 m (Figure 3b). Natural log-transformed light increased with increasing canopy height (Figure 4; ln[DIFN] = 1.96 + 0.10Height; $p < 0.001$, Adj $R^2 = 0.262$).

3.2 | Gas exchange responses to experimental warming

Neither species showed evidence of net photosynthetic acclimation after 4 weeks of leaf-level warming. Neither *Guarea* nor *Ocotea* showed significant treatment or treatment × height interaction effects for T_{opt}, A_{opt} or Ω (Table 1; Figure 5). These results suggest that these species did not acclimate through up-regulation of temperature response parameters, nor did they show stress responses of warming through declined rates of net photosynthesis in response to leaf-level warming. We also found no treatment or treatment × height interaction effects for the slope (β_{1_gs-T}) or intercept (β_{0_gs-T}) of g_s response to temperature for either species (Table 1; Figure S2), suggesting that g_s did not change with warming.

Unlike the results for net photosynthesis, we did find evidence of electron transport rate acclimation for *Ocotea* only, and only in the understory. Neither *Guarea* nor *Ocotea* showed significant treatment effects for optimum temperature of electron transport

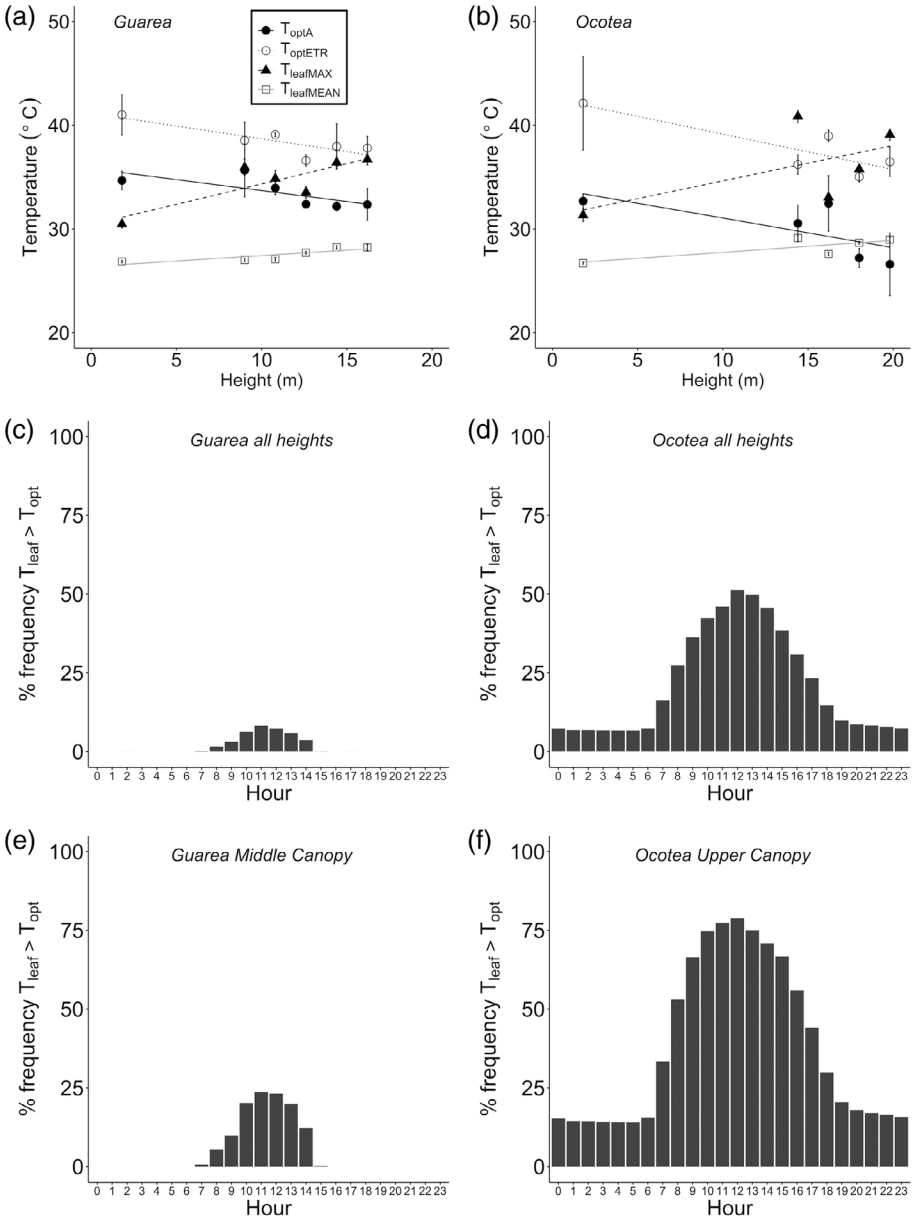

FIGURE 3 Relationship between leaf temperatures and optimum temperatures throughout the canopy. Control leaf optimum temperatures of net photosynthesis (T_{opt}, filled circle, solid black line) and photosynthetic electron transport (T_{optETR}, open circle, dotted black line) compared to the mean daily maximum leaf temperature ($T_{leafMAX}$, filled triangle, dashed black line) and mean daily daytime leaf temperature ($T_{leafMEAN}$, empty square, solid gray line) for (a) *Guarea guidonia* and (b) *Ocotea sintenisii*. Percentage of recorded instances where T_{leaf} exceeds T_{opt} for (c) all canopy heights combined for *Guarea*, (d) all canopy heights for *Ocotea*, (e) *Guarea* middle canopy only and (f) *Ocotea* upper canopy only. $T_{leafMAX}$ and $T_{leafMEAN}$ were calculated as a daily mean for each species at each canopy position. Data are shown for control leaves only. Error bars represent SEM. *Guarea* $T_{leafMEAN}$ equation is $T_{leafMEAN} = 25.39\ (0.14) + 0.03(0.01)$Height, *Guarea* $T_{leafMAX}$ equation is $T_{leafMAX} = 30.96(0.69) + 0.36\ (0.06)$Height, *Ocotea* $T_{leafMEAN}$ equation is $T_{leafMEAN} = 25.55(0.15) + 0.03(0.01)$ Height and *Ocotea* $T_{leafMAX}$ equation is $T_{leafMAX} = 31.43(0.76) + 0.31(0.05)$ Height. Equation values given in parentheses represent SEM. Equations for T_{opt} and T_{optETR} are given in Table 1

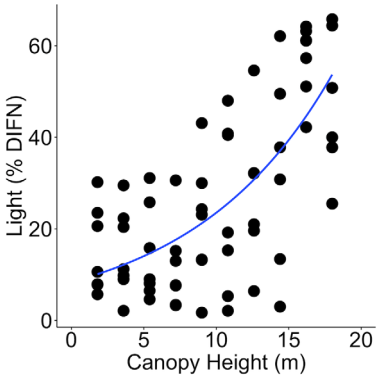

FIGURE 4 Light environment response to canopy height. Regression equation: DIFN = 4.59 × $\exp^{(0.15 \times \text{height})}$ [Colour figure can be viewed at wileyonlinelibrary.com]

rate (T_{optETR}) (Table 1; Figure 6a,b). *Guarea* also showed no significant treatment × height or treatment × light interaction, suggesting that warming did not affect T_{optETR} (Figure 6a). There was a significant treatment × height and treatment × light interaction for *Ocotea* that was largely driven by the understory leaves (Figure 6b). The heated leaves had a steeper T_{optETR} slope with height and light, where T_{optETR} was approximately 5°C greater for heated than control leaves in the understory only. There were no significant treatment or treatment × height interactions for either species' ETR_{opt} (Table 1, Figure 6c,d), or for *Guarea* ETR thermal niche (Ω) (Figure 6e). There was a significant treatment × height interaction for *Ocotea* ETR Ω (Table 1), where the heated leaves had wider temperature response curves than control leaves in the understory but, as with T_{optETR}, the difference diminished as canopy height and light increased (Figure 6f; Table S4).

TABLE 1 Mixed effects model results and model coefficients ± SE for leaf physiological response to treatment (heated or control), height and the interaction between treatment and height. Individual tree is the model random effect

Mixed effects model						Model coefficients			
Species	Physiological parameter	df	Treatment *p*-value	Height *p*-value	Tmt × Ht *p*-value	Control y-intercept	Control slope	Heated y-intercept	Heated slope
Guarea	T_{opt}	1,21	0.658	0.063	0.317	35.43 ± 1.23	−0.17 ± 0.14	1.91 ± 1.67	−0.15 ± 0.14
	A_{opt}	1,21	0.291	**0.033**	0.230	6.82 ± 1.04	0.08 ± 0.09	−2.60 ± 1.58	0.17 ± 0.14
	A_{25}	1,21	0.500	**0.003**	0.575	3.47 ± 0.93	0.18 ± 0.08	−1.27 ± 1.41	0.07 ± 0.13
	Ω	1,21	0.662	0.421	0.219	12.35 ± 1.83	0.29 ± 0.19	2.92 ± 3.00	−0.33 ± 0.26
	T_{optETR}	1,19	0.703	**0.009**	0.569	41.18 ± 1.40	−0.25 ± 0.12	1.43 ± 2.54	−0.12 ± 0.21
	ETR_{opt}	1,19	0.317	**0.006**	0.443	54.50 ± 10.92	2.01 ± 0.96	−25.08 ± 19.87	1.30 ± 1.65
	ETR Ω	1,19	0.407	0.932	0.587	19.07 ± 1.65	0.05 ± 0.15	0.64 ± 2.88	−0.13 ± 0.24
	F_v/F_m	1,22	0.246	0.128	0.076	0.80 ± 0.02	$1.29 \times 10^{-3} \pm 1.73 \times 10^{-3}$	−0.03 ± 0.02	$2.50 \times 10^{-3} \pm 1.34 \times 10^{-3}$
	β_{1_gs-T}	1,21	0.746	0.053	0.777	$1.83 \times 10^{-3} \pm 2.38 \times 10^{-3}$	$-4.59 \times 10^{-4} \pm 2.33 \times 10^{-4}$	$-3.13 \times 10^{-4} \pm 38.93 \times 10^{-4}$	$0.93 \times 10^{-4} \pm 3.22 \times 10^{-4}$
	β_{0_gs-T}	1,21	0.658	0.062	0.841	$-1.70 \times 10^{-2} \pm 8.95 \times 10^{-2}$	$1.63 \times 10^{-2} \pm 0.87 \times 10^{-2}$	$-0.55 \times 10^{-2} \pm 14.80 \times 10^{-2}$	$-0.25 \times 10^{-2} \pm 1.25 \times 10^{-2}$
	R:*A*	1,21	0.146	**0.003**	0.644	$1.83 \times 10^{-2} \pm 1.55 \times 10^{-2}$	$0.30 \times 10^{-2} \pm 0.14 \times 10^{-2}$	$0.34 \times 10^{-2} \pm 2.35 \times 10^{-2}$	$0.09 \times 10^{-2} \pm 0.21 \times 10^{-2}$
	Q_{10}	1,22	0.052	0.941	0.467	2.16 ± 0.13	$0.56 \times 10^{-2} \pm 1.19 \times 10^{-2}$	−0.30 ± 0.19	$1.24 \times 10^{-2} \pm 1.68 \times 10^{-2}$
	R_{25}	1,22	0.569	**<0.001**	0.756	$1.46 \times 10^{-2} \pm 6.95 \times 10^{-2}$	$2.45 \times 10^{-2} \pm 0.63 \times 10^{-2}$	$-0.30 \times 10^{-2} \pm 9.89 \times 10^{-2}$	$0.28 \times 10^{-2} \pm 0.89 \times 10^{-2}$
Species	Physiological parameter	df	Treatment *p*-value	Height *p*-value	Tmt × Ht *p*-value	Control y-intercept	Control slope	Heated y-intercept	Heated slope
Ocotea	T_{opt}	1,18	0.704	**0.007**	0.804	33.87 ± 2.02	−0.31 ± 0.13	−0.48 ± 2.87	0.05 ± 0.19
	A_{opt}	1,18	0.512	**0.009**	0.930	4.90 ± 1.86	0.27 ± 0.12	−0.28 ± 2.65	−0.02 ± 0.18
	A_{25}	1.18	0.485	**0.008**	0.940	4.14 ± 1.96	0.28 ± 0.13	−0.37 ± 2.79	−0.01 ± 0.18
	Ω	1,15	0.275	0.857	0.944	22.94 ± 4.01	−0.02 ± 0.26	−2.72 ± 5.73	−0.03 ± 0.40
	T_{optETR}	1,18	0.767	**0.018**	**0.002**	41.17 ± 2.43	−0.17 ± 0.18	8.42 ± 2.43	−0.55 ± 015
	ETR_{opt}	1,18	0.281	0.242	0.983	58.59 ± 20.28	1.62 ± 1.55	−8.64 ± 23.66	0.03 ± 1.49
	ETR Ω	1,18	0.608	0.966	**0.018**	20.02 ± 3.98	0.40 ± 0.30	10.47 ± 4.67	−0.76 ± 0.29
	F_v/F_m	1,21	0.130	0.567	0.473	0.76 ± 0.03	$1.96 \times 10^{-3} \pm 2.12 \times 10^{-3}$	$0.12 \times 10^{-2} \pm 4.72 \times 10^{-2}$	$-2.23 \times 10^{-3} \pm 3.06 \times 10^{-3}$
	β_{1_gs-T}	1,18	0.241	**0.003**	0.632	$-1.48 \times 10^{-3} \pm 1.73 \times 10^{-3}$	$-3.20 \times 10^{-4} \pm 1.11 \times 10^{-4}$	$-0.89 \times 10^{-4} \pm 24.55 \times 10^{-4}$	$0.79 \times 10^{-4} \pm 1.63 \times 10^{-4}$
	β_{0_gs-T}	1,18	0.230	**0.003**	0.705	0.11 ± 0.07	$1.27 \times 10^{-2} \pm 0.46 \times 10^{-2}$	$-0.75 \times 10^{-2} \pm 10.24 \times 10^{-2}$	$-2.61 \times 10^{-3} \pm 6.78 \times 10^{-3}$
	R:*A*	1,18	0.618	**0.014**	0.961	$1.14 \times 10^{-2} \pm 1.59 \times 10^{-2}$	$0.21 \times 10^{-2} \pm 0.10 \times 10^{-2}$	$0.83 \times 10^{-2} \pm 2.26 \times 10^{-2}$	$-7.40 \times 10^{-5} \pm 149.36 \times 10^{-5}$
	Q_{10}	1,20	0.622	0.147	0.437	2.19 ± 0.18	$-2.57 \times 10^{-2} \pm 1.51 \times 10^{-2}$	−0.13 ± 0.21	$-2.02 \times 10^{-2} \pm 1.29 \times 10^{-2}$
	R_{25}	1,20	0.797	**0.001**	0.830	$-0.12 \times 10^{-2} \pm 10.89 \times 10^{-2}$	$2.48 \times 10^{-2} \pm 0.70 \times 10^{-2}$	$2.32 \times 10^{-2} \pm 15.44 \times 10^{-2}$	$-0.22 \times 10^{-2} \pm 01.00 \times 10^{-2}$

Note: Bold *p*-values indicate significance at $\alpha < 0.05$ level. df presents the numerator and denominator degrees of freedom. Physiological parameters are: the optimum temperature of net photosynthesis (T_{opt}), the rate of photosynthesis at T_{opt} (A_{opt}), the width of the net photosynthetic response curve (Ω), the optimum temperature of photosynthetic electron transport (T_{optETR}), the rate of electron transport at T_{optETR} (ETR_{opt}), the width of the electron transport temperature response curve (ETR Ω), maximum chlorophyll fluorescence (F_v/F_m), the slope of stomatal conductance response to temperature (β_{1_gs-T}), the intercept of stomatal conductance response to temperature (β_{0_gs-T}), the ratio of net photosynthesis and leaf respiration at 25°C (*R*:*A*), temperature sensitivity of leaf dark respiration response to temperature (Q_{10}) and the rate of respiration at 25°C (R_{25}).

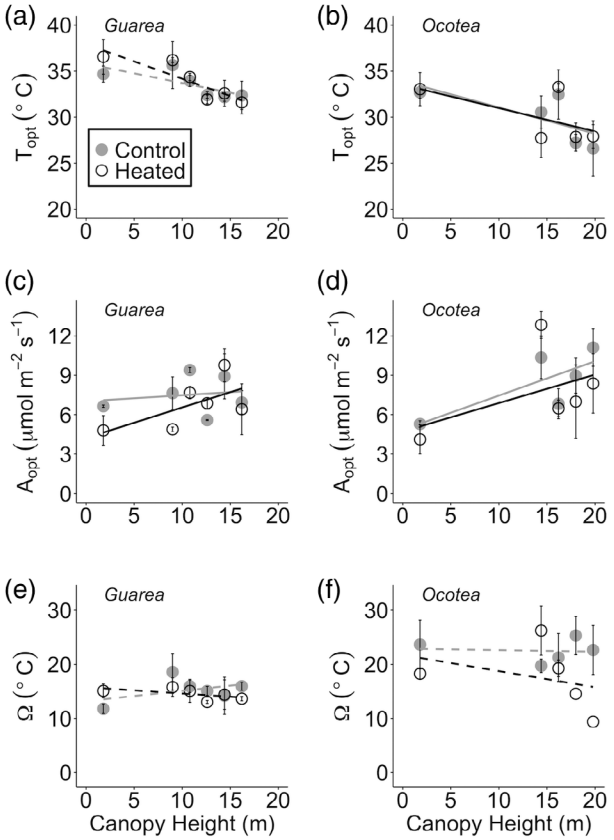

FIGURE 5 Net Photosynthetic temperature response parameter trends with canopy height. (a) *Guarea guidonia* optimum temperature for net photosynthesis (T_{opt}), (b) *Ocotea sintenisii* T_{opt}, (c) *Guarea* rate of net photosynthesis at T_{opt} (A_{opt}), (d) *Ocotea* A_{opt} (e) *Guarea* photosynthetic thermal niche (Ω), and (f) *Ocotea* Ω response to canopy height for heated (black open circles) and control (gray closed circles) leaves. Linear regressions were fit individually for heated (black) and control (gray) leaves. Significance of height response for heated and control plots are represented by solid (significant response) or dashed (non-significant response) lines (Table 1)

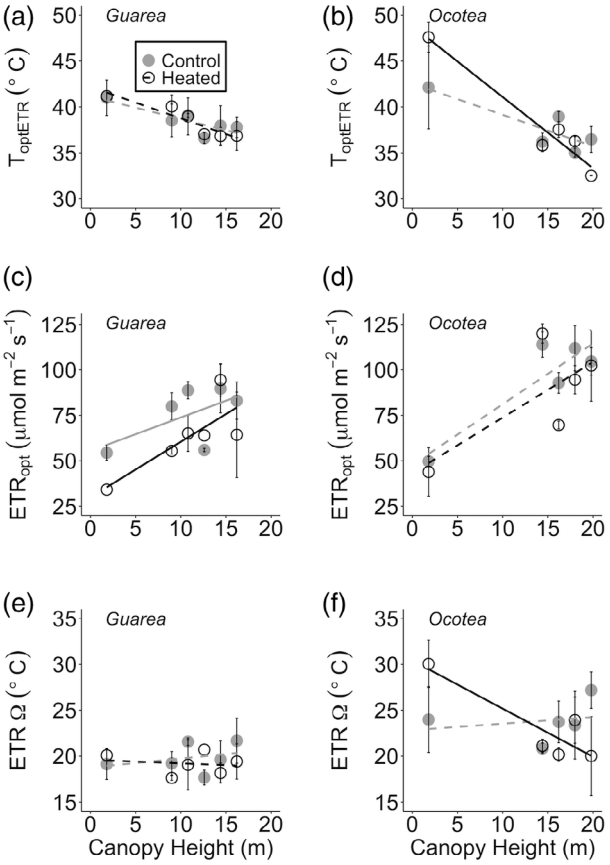

FIGURE 6 Photosynthetic electron transport temperature response parameter responses to canopy height. (a) *Guarea guidonia* optimum temperature for photosynthetic electron transport (T_{optETR}), (b) *Ocotea sintenisii* T_{optETR}, (c) *Guarea* rate of net photosynthesis at T_{optETR} (ETR_{opt}), (d) *Ocotea* ETR_{opt}, (e) *Guarea* electron transport thermal niche (ETR Ω) and (f) *Ocotea* ETR Ω response to canopy height for heated (black open circles) and control (gray closed circles) leaves. Linear regressions were fit individually for heated (black) and control (gray) leaves. Significance of height response for heated and control plots are represented by solid (significant response) or dashed (non-significant response) lines (Table 1)

Neither species showed evidence of Photosystem II stress after 1 month of warming, and there was no change in PSII stress with canopy height. There were no F_v/F_m treatment or treatment × height interactions for either *Guarea* or *Ocotea* (Table 1). Similarly, F_v/F_m did not change with canopy height for either species (Table 1).

We found evidence of respiratory thermal acclimation for *Guarea* only. Heated *Guarea* leaves showed a marginally significant treatment effect, indicating a down-regulation of Q_{10} compared to the control leaves ($p = 0.052$; Table 1; Figure 7a). The lack of treatment × height interaction suggests that the treatment effect was consistent across canopy height. *Ocotea* had no significant treatment or treatment × height interaction effects for Q_{10} (Table 1; Figure 7b). Neither species had significant treatment or interaction effects for either R_{25} or the ratio between respiration and photosynthesis at 25°C (*R*:*A*; Table 1; Figure 7c–f).

3.3 | Gas exchange responses to height and light

Net photosynthesis increased with canopy height for both of our study species and, surprisingly, the optimum temperature for net photosynthesis declined as canopy height increased. *Guarea* T_{opt} decreased with increasing canopy height (marginally significant, at $p = 0.063$), and *Ocotea* T_{opt} significantly declined with height ($p = 0.007$, Table 1). *Guarea* T_{opt} ranged from 33.1°C in the understory to 28.0°C in the mid canopy (Figure 5a), and *Ocotea* T_{opt} ranged from 35.5°C in the understory to 32.7°C in the upper canopy (Figure 5b). Both species' A_{opt} and A_{25} increased with canopy height, but *Ocotea* A_{opt} increased more rapidly, almost doubling from the understory to the upper canopy (*Guarea* $p = 0.094$ and *Ocotea* $p = 0.025$; Table 1, Figure 5c,d). T_{opt} similarly declined with increasing log-transformed light. Neither species' A_{opt} responded to light environments (Table S4); however, *Guarea* A_{25} increased with increasing light ($p = 0.019$).

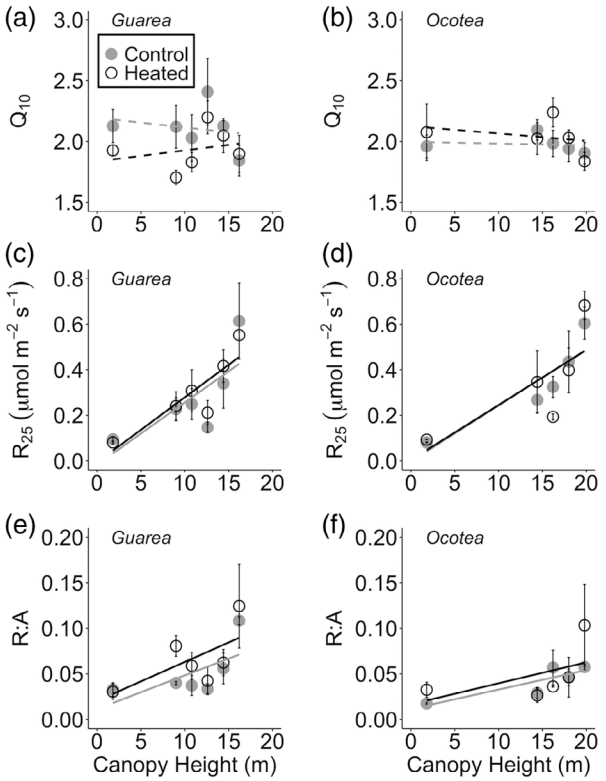

FIGURE 7 Leaf respiration temperature response parameter responses to canopy height. (a) *Guarea guidonia* respiratory increase with every 10°C increase in leaf temperature (Q_{10}), (b) *Ocotea sintenisii* Q_{10}, (c) *Guarea* rate of leaf respiration at 25°C (R_{25}), (d) *Ocotea* R_{25} (e) *Guarea* ratio between R_{25} and photosynthesis at 25°C (*R:A*) and (f) *Ocotea R:A* response to canopy height for heated (black open circles) and control (gray closed circles) leaves. Linear regressions were fit individually for heated (black) and control (gray) leaves. Significance of height response for heated and control plots are represented by solid (significant response) or dashed (non-significant response) lines (Table 1)

Showing a similar pattern as net photosynthesis, the optimum temperature of photosynthetic electron transport also declined, and the rate of election transport at the optimum temperature increased with increasing canopy height for both species. Both *Guarea* and *Ocotea* had a significant height and light effects for T_{optETR} (Table 1, Table S4; Figure 6a,b). *Guarea* T_{optETR} decreased from 41.1 to 37.3°C from understory to mid canopy. As described previously, *Ocotea* heated and control leaves had differing responses to canopy height (Figure 6b). *Guarea* and *Ocotea* ETR_{opt} increased with rising canopy height (Table 1; Figure 6c,d). *Guarea* ETR_{opt} increased with increasing log-transformed light, while *Ocotea* ETR_{opt} did not (Table S4).

We investigated how close to thermal physiological thresholds these trees were operating by comparing maximum leaf temperatures to optimal photosynthetic temperatures. While maximum leaf temperatures did not exceed photosynthetic temperature optima in the understory or lower canopy, they approached temperature optima of electron transport and exceeded net photosynthetic temperature optima in the mid and upper canopy. *Guarea* mid canopy T_{opt} (Student's *t*-test $p = 0.001$) and T_{optETR} ($p = 0.001$) were higher than their associated daily daytime mean control leaf temperatures ($T_{leafMEAN}$; Figure 3a). In the uppermost leaves measured for *Guarea* (~16 m), T_{opt} values were lower than $T_{leafMAX}$ ($p < 0.001$ Figure 3a), while T_{optETR} was no different than $T_{leafMAX}$ ($p = 0.352$; Figure 3a). *Ocotea* T_{opt} did not differ from $T_{leafMEAN}$ ($p = 0.543$; Figure 3b) but T_{optETR} was higher than $T_{leafMEAN}$ in the upper canopy ($p < 0.001$). Patterns were similar for *Ocotea*, where upper canopy T_{opt} values were lower than $T_{leafMAX}$ ($p < 0.001$; Figure 3b), while T_{optETR} was similar to $T_{leafMAX}$ ($p = 0.140$).

We further investigated how often control treatment T_{leaf} exceeded control T_{opt} throughout the day. For all canopy positions combined, *Guarea* T_{leaf} did not exceed T_{opt} more than 10% of the time (Figure 3c), while *Ocotea* showed $T_{leaf} > T_{opt}$ more than 20% of the time for daylight hours (8 a.m.–6 p.m.), and more than 50% of the time from 12 to 1 p.m. (Figure 3d). In the mid canopy, of *Guarea*, showed $T_{leaf} > T_{opt}$ only occurred >20% of the time from 10 a.m. to 1 p.m. *Ocotea* upper canopy T_{leaf} exceeded $T_{opt} > 50\%$ of the time during most daylight hours (8 a.m.–5 p.m.), and $T_{leaf} > T_{opt}$ more than 70% of the time from 10 a.m. to 3 p.m.

Temperature sensitivity of g_s increased with height for both species. The $g_s - T_{leaf}$ response slope (β_{1_gs-T}) became steeper and more negative with increasing canopy height and log-transformed light (Table 1, Table S4; Figure S2a,b). To further quantify temperature responses of g_s within the canopy, we separated the canopy into four distinct positions. $T_{leaf} \times$ height class interactions were significant (*Guarea* $p < 0.001$; *Ocotea* $p = 0.002$), and post hoc pairwise comparisons showed that mid canopy *Guarea* (the highest canopy position for this species) had a steeper, more negative slope than both the lower canopy ($p < 0.001$) and understory ($p = 0.005$; Figure 8a). *Guarea* $g_s - T_{leaf}$ intercepts were higher in the mid canopy compared to the lower canopy ($p = 0.001$), but there were no differences between mid and understory ($p = 0.236$) or lower canopy and understory ($p = 0.236$; Figure 8a). *O. sintenisii* upper canopy $g_s - T_{leaf}$ slope was more negative than in the understory ($p = 0.001$), but there was no difference between mid-canopy and understory ($p = 0.142$) or mid and upper canopy ($p = 0.126$; Figure 8b). The *Ocotea* understory had a lower $g_s - T_{leaf}$ intercept than both the upper ($p < 0.001$) and mid ($p < 0.001$) canopies), while the upper and mid canopy did not differ from each other ($p = 0.989$; Figure 8b). These results suggest that, even though maximum g_s increased throughout the canopy, stomatal sensitivity to temperature increased with increasing canopy height.

The stomatal-photosynthesis model parameter, g_1, showed similar responses between treatments, except for *Guarea* understory where heated leaves showed overall higher g_1 than control leaves. g_1 showed a polynomial response to temperature for both species at all canopy positions, where g_1 initially declined, then increased at high temperatures (Figure S3; Table S3). This suggests that, in most cases, water-use efficiency initially increased with rising leaf temperatures but began decreasing as temperature reached 33–42°C.

The respiration-temperature response slope was constant across the vertical canopy gradient for both species; however, respiration rates at 25°C and the ratio of respiration to photosynthesis both

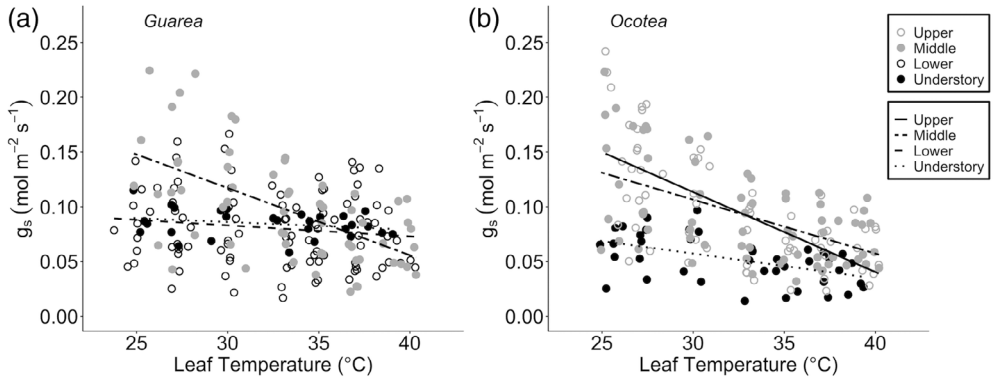

FIGURE 8 Stomatal conductance response to leaf temperature at different canopy positions. Stomatal conductance response to leaf temperature for (a) *Guarea guidonia* and (b) *Ocotea sintenisii*. Canopy positions shown for *Guarea* are understory (0–1.5 m; black filled circle), lower canopy (9–12.6 m; black open circle), mid canopy (14.4–16.2 m; gray open circle). Canopy positions shown for *Ocotea* are the understory, mid and upper canopy (18–19.8 m; gray open circle). Lines depict regression lines for the understory (dotted), lower canopy (dashed), mid (dot-dashed) and upper (solid) canopies

increased with height for both species. The height and light effects on Q_{10} were not significant for either species (Table 1, Table S4, Figure 7a,b). R_{25} and *R:A* had positive relationships with canopy height and light for both species (Table 1, Table S4, Figure 7c–f).

3.4 | Leaf traits

The warming treatment did not affect any measured leaf trait for either species. Neither species' leaf area significantly varied across the canopy height gradient (Table 2, Figure S4a,b). However, *LMA* increased from 35.3 to 100.2 g^{-1} cm^2 for *Guarea* and 60.8 to 147.3 g^{-1} cm^2 for *Ocotea* throughout the height gradient (Table 2; Figure S4c,d). Neither species showed significant treatment effects or height × treatment interactions for leaf area or *LMA* (Table 2). Both species' LMA and leaf area responded similarly to the light environment as with canopy height (Table S5).

Percent leaf water content (%LWC) declined with height for both species, and neither species responded to the warming treatment. Neither *Guarea* nor *Ocotea* showed a treatment or treatment × height interaction for %LWC (Table 2). From the understory to the highest portion of the canopy measured, *Guarea* %LWC declined from 76.7% to 60.3% and *Ocotea* declined from 65.8% to 40.4% (Table 2; Figure S4e,f). Both species' %LWC also declined with increasing log-transformed light (Table S5).

Leaf nitrogen was not affected by warming; however, area-basis nitrogen (N_{area}) increased with canopy height for both species, and mass-basis nitrogen (N_{mass}) decreased throughout the height gradient for *Ocotea*. N_{area} ranged from 1.1 g m^{-2} to 3.0 g m^{-2} for *Guarea* (Table 2; Figure S5a) and from 1.3 to 2.7 g m^{-2} for *Ocotea* (Figure S5b). *Guarea* N_{mass} showed no response to canopy height (Table 2; Figure S5c), while *Ocotea* N_{mass} declined from 21.8 mg g^{-1} in the understory to 18.6 mg g^{-1} in the upper canopy (Figure S5d). The carbon to nitrogen ratio (*C:N*) followed the trends of N_{mass}, where *Guarea C:N* was not correlated with canopy height and *Ocotea C:N* increased from 21.6 g g^{-1} in the understory to 27.2 g g^{-1} in the upper canopy (Figure S5e,f). *Guarea* N_{area} increased with increasing log-transformed light, while *Ocotea* did not (Table S5). *Guarea* N_{mass} did not respond to light, while *C:N* marginally increased as light increased (Table S5). *Ocotea* N_{mass} decreased with increasing log-transformed light, while *C:N* increased (Table S5).

4 | DISCUSSION

4.1 | Evidence for electron transport acclimation in Ocotea, but no net photosynthetic acclimation in either species

We hypothesized that photosynthetic acclimation would occur higher in the canopy where leaf temperatures are both warmer and more variable, but not in the lower canopy layers. In contrast to our expectations, we found evidence for acclimation of electron transport in the understory only for *Ocotea* and no net photosynthetic acclimation at any canopy position (Figures 5 and 6b). Since net photosynthesis showed no acclimation, electron transport may not be the limiting photosynthetic process for *Ocotea*. One study in Brazil's Amazon rain forest that heated individual canopy leaves found reduced photosynthesis with leaf-level warming (Doughty, 2011). While Doughty (2011) assessed acclimation using point measurements of photosynthesis at two temperatures instead of response curves, the results do suggest that net photosynthesis did not fully acclimate in the canopy leaves. In our study, *Guarea* understory heated leaves trended towards a higher g_1 (Figure S3e), or essentially lower water-use efficiency, suggesting that climate warming might actually increase leaf water loss per carbon gain. While our experimental warming device did provide consistent temperatures for our heated leaves, many of the heated leaves experienced occasional temperature spiking throughout the experiment, often in the early morning. The instances of spiking could have inhibited acclimation if metabolic machinery was damaged;

however, the lack of F_v/F_m treatment effect suggests that PSII was not stressed. Recent studies have found that tropical seedlings can photosynthetically acclimate to higher growth temperatures (Slot & Winter, 2017b; Smith & Dukes, 2017), which could explain why we found T_{optETR} acclimation only in *Ocotea*, immature, understory plants. Thus, ontogeny may play a role in thermal plasticity. Younger, smaller trees are more reliant on immediate carbon assimilation than larger trees because they have not yet built up their non-structural carbohydrate reserves (Niinemets, 2010; Sala & Hoch, 2009); therefore, younger plants may need to acclimate more quickly to maintain carbon uptake.

Light is also a stronger limiting resource in the understory compared to the upper canopy; therefore, T_{optETR} acclimation could have occurred in the understory to preserve light utilization in light-limited plants. Smith and Dukes (2017) found that tropical seedling acclimation was more likely to occur in processes associated with the light reactions of photosynthesis (e.g., electron transport), as opposed to processes associated with the carbon reactions of photosynthesis (e.g., Rubisco carboxylation). In contrast, Hernández et al. (2020) recently found no T_{opt} differences in ETR between tropical tree sun and shade leaves, suggesting no temperature acclimation. Electron transport acclimation can occur through stabilization of the thylakoid membrane (Havaux, 1996; Neta-Sharir et al., 2005) or implementation of cyclic electron transport (Havaux, 1996; Schrader et al., 2004), which allows for maintained adenosine triphosphate (ATP) phosphorylation when photosystem II function has declined Schrader et al. (2004). Tropical forests often have dense canopies and our understory plants receive less light than higher in the canopy (Figure 4); therefore, acclimation of the photosynthetic processes associated with light capture and electron transport may provide an advantage. Our results of electron transport acclimation in the understory support this hypothesis for one of our study species; however, we did not investigate acclimation of Rubisco carboxylation and cannot rule out the possibility of carboxylation adjustments.

4.2 | Evidence for respiratory acclimation in Guarea only

We expected respiration to acclimate throughout the canopy, and we found partial support for our hypothesis in that respiration acclimated via a down-regulation of Q_{10} for *Guarea*, but not *Ocotea*. Meta-analyses conducted globally (Slot & Kitajima, 2015) and experimental studies across the tropics (Aspinwall et al., 2016; Cheesman & Winter, 2013; Drake et al., 2015; Slot et al., 2014; Slot & Winter, 2018; Smith & Dukes, 2017) suggest that plant respiration will acclimate to warmer temperatures via down-regulation of respiration; however, we found no respiratory acclimation of understory shrubs at the TRACE study site after 8 months of whole-plant experimental warming (Carter et al., 2020). The only other leaf-level warming study in a tropical forest canopy found tropical leaf respiration to acclimate within 7 days of experimental warming (Slot et al., 2014). Even when photosynthesis does not systematically acclimate, tropical leaf respiration often does (Cheesman & Winter, 2013; Slot & Winter, 2018). We only found acclimation in one of our study species (Figure 7), suggesting that there may be different respiratory acclimation potential among species.

Leaf maturity could have been a factor determining these species' respiratory acclimation responses. Leaves that were already fully developed prior to initiating a new growth temperature can have a lower acclimation capacity (Atkin et al., 2000; Loveys et al., 2003; Turnbull et al., 1993). This could have limited the acclimation potential of in our study species and may explain why we did not detect *Ocotea* respiratory acclimation. *Guarea* acclimated through a lower Q_{10} in the heated leaves and Q_{10} acclimation is more likely to occur with fully mature leaves (Atkin et al., 2005; Atkin & Tjoelker, 2003). Q_{10} acclimation is often associated with either lower substrate availability (due to decreased sugar production from photosynthesis) or adenylate control (where excess ATP production leads to a down-regulation of respiration) (Amthor & Baldocchi, 2001; Aspinwall et al., 2016; Atkin & Tjoelker, 2003). Therefore, *Guarea* Q_{10} may have acclimated because net photosynthesis did not, potentially limiting substrate availability. Even with the lack of net photosynthetic and respiratory acclimation in *Ocotea* and lack of net photosynthetic acclimation in *Guarea*, we did not see any warming effect on *R:A* (Figure 7e,f), which suggests that the balance between these two processes was not disrupted due to warming.

4.3 | Stomatal conductance temperature sensitivity limits upper canopy photosynthesis

Stomatal conductance (g_s) is one of the primary processes that limit photosynthesis as VPD increases above T_{opt} (Lin et al., 2012; Lloyd & Farquhar, 2008), and may be the most important photosynthetic thermal limitation in tropical trees (Slot & Winter, 2017a; Smith et al., 2020). Fauset et al. (2019) recently found that plants grown in warming chambers shifted from a positive g_s relationship to increasing VPD in ambient conditions to a negative relationship after exposure to the warming treatment. In contrast, Slot and Winter (2017b) found no difference in g_s between seedlings grown at different temperatures. We did not find that experimental warming altered the slope or intercept of the g_s response to temperature for either study species (Table 1); however, we did find greater stomatal sensitivity to temperature in the upper canopy compared to the lower canopy leaves (Figure 8), which may be driving the decline in T_{opt} as canopy height increased (Figure 5a,b). Leaves in the upper canopy have a higher capacity for g_s in order to support the higher photosynthetic capacity experienced by the upper canopy leaves (Buckley, 2005; Kenzo et al., 2015), but high stomatal thermal sensitivity may make these upper canopy leaves more vulnerable to stomatal limitations to CO_2 assimilation under warmer conditions.

4.4 | Leaf temperature and proximity to T_{opt}

Few studies have investigated how T_{opt} varies across a vertical forest canopy gradient (Carter & Cavaleri, 2018; Mau et al., 2018). The only

TABLE 2 Mixed effects model results of leaf trait response to treatment, height and the interaction between treatment and height, where individual tree is the random variable

		Mixed effects model				Equation			
Species	**Leaf trait**	**df**	**Treatment**	**Height**	**Tmt × Ht**	**Controly-intercept**	**Control slope**	**Heatedy-intercept**	**Heated slope**
Guarea	Leaf area	1, 22	0.270	0.283	0.430	63.69 ± 10.28	−0.79 ± 1.17	13.97 ± 12.11	−0.82 ± 1.02
	LMA	1, 21	0.404	**< 0.001**	0.785	25.38 ± 5.66	3.80 ± 0.56	−0.39 ± 8.24	0.20 ± 0.72
	%LWC	1, 21	0.690	**< 0.001**	0.619	79.71 ± 1.65	−1.07 ± 0.16	−1.17 ± 2.27	0.11 ± 0.21
	N_{area}	1, 21	0.739	**< 0.001**	0.846	0.78 ± 0.16	0.13 ± 0.01	$4.38 \times 10^{-3} \pm 24.95 \times 10^{-3}$	$-4.35 \times 10^{-3} \pm 22.09 \times 10^{-3}$
	N_{mass}	1, 22	0.185	0.472	0.996	31.85 ± 1.89	0.12 ± 0.20	−1.48 ± 2.65	$-0.13 \times 10^{-3} \pm 23.16 \times 10^{-3}$
	C:N	1, 22	0.206	0.642	0.923	14.40 ± 0.96	$3.50 \times 10^{-2} \pm 10.08 \times 10^{-2}$	0.59 ± 1.56	$1.16 \times 10^{-2} \pm 11.86 \times 10^{-2}$
Ocotea	Leaf area	1, 21	0.985	0.983	0.223	37.05 ± 10.18	0.56 ± 0.78	16.79 ± 14.45	−1.14 ± 0.91
	LMA	1, 21	0.383	**< 0.001**	0.396	47.98 ± 8.63	4.84 ± 0.55	8.60 ± 12.24	−0.69 ± 0.79
	%LWC	1, 21	0.224	**< 0.001**	0.421	69.08 ± 4.45	−1.37 ± 0.28	−2.40 ± 6.32	0.34 ± 0.41
	N_{area}	1, 21	0.279	**< 0.001**	0.439	1.16 ± 0.17	$8.23 \times 10^{-2} \pm 1.08 \times 10^{-2}$	0.12 ± 0.24	$-1.23 \times 10^{-2} \pm 1.56 \times 10^{-2}$
	N_{mass}	1, 21	0.644	**0.002**	0.778	22.65 ± 0.98	−0.17 ± 0.06	−0.74 ± 1.39	0.03 ± 0.09
	C:N	1, 22	0.465	**<0.001**	0.856	20.20 ± 1.35	0.29 ± 0.09	1.16 ± 1.92	−0.03 ± 0.12

Note: Bold *p*-values indicate significance at $\alpha < 0.05$ level. df presents the numerator and denominator degrees of freedom. Leaf mass per area, LMA; percent leaf water content, % LWC; nitrogen per unit leaf area, N_{area}; nitrogen per unit leaf mass, N_{mass}; leaf carbon to nitrogen ratio, *C:N*.

tropical study to do so found T_{opt} to be constant with height; however, only mid and upper canopy were measured, not understory foliage (Mau et al., 2018). We found T_{opt} to decrease with increasing canopy height (Figure 5a,b) and, notably, the upper canopy experienced maximum temperatures well above T_{opt} (Figure 3a,b). In addition, *Ocotea* leaf temperatures often exceeded T_{opt} in the upper canopy (Figure 3f), suggesting that these leaves were often operating above their photosynthetic thermal thresholds. Leaf temperatures are hottest during midday (Figure 2), when plants are often experiencing mid-day depression due to stomatal closure (Pons & Welschen, 2003; Zotz et al., 1995). These results support other studies that found that tropical species (Crous et al., 2018; Vårhammar et al., 2015) and, in particular, tropical forest upper canopies (Doughty & Goulden, 2008; Mau et al., 2018) may be operating above their photosynthetic thermal optimums, or even approaching temperatures that induce long-term tissue damage (Krause et al., 2010). We found maximum leaf temperatures to exceed T_{opt} in the highest canopy position of both of our study species; although more frequently in the *Ocotea* upper canopy. This is particularly important, as the sunlit upper canopies of closed canopy forests can cycle more carbon than all other shaded canopy layers combined (He et al., 2018). Our study species' T_{optETR} also decreased with increasing canopy height. A global study by Kumarathunge et al. (2019) showed that lower ratios of electron transport per Rubisco carboxylation actually *increase* the net photosynthetic optimum temperature (T_{opt}). Although we did not measure Rubisco carboxylation rates, lower net photosynthetic T_{opt} would suggest that, even with lower T_{optETR} in the upper canopy, the ratio between these key photosynthetic processes were likely either steady or Rubisco carboxylation decreased at a faster rate than electron transport as canopy height increased.

4.5 | Leaf thermoregulation varies with canopy height

Thermoregulation strategies can differ across a canopy transect; for example, megathermy occurs when T_{leaf} exceeds T_{air}, and poikilothermy is when $T_{leaf} = T_{air}$ (Cavaleri, 2020; Michaletz et al., 2016). A concurrent study found this canopy to have two different modes of thermoregulation, where the upper layer only is largely megathermic, while the rest of the shaded foliage is poikilothermic (Miller et al., 2021). While leaf area can affect thermoregulation, where smaller leaves have thinner boundary layers and thus greater evaporative cooling via transpiration (Fauset et al., 2018), we found little variation in leaf area with height (Table 2). The overall higher maximum g_s higher in the canopy likely drives the greater stomatal temperature sensitivity of the middle and upper canopy, which is consistent with a study investigating tropical tree sun and shade leaves (Hernández et al., 2020). The low g_s at high temperature suggests that these leaves do not have high transpiration and, therefore, low thermoregulation at high temperatures. Potentially low transpirational cooling, combined with higher irradiance (Figure 4), helps explain why we see megathermy and temperatures above photosynthetic optima in the uppermost canopy (Miller et al., 2021). In addition, g_1 declined until temperatures reached values from 33 to 42°C, after which g_1 increased (Figure S3). This suggests that water-use efficiency initially increases and then declines at high temperatures, providing further evidence that high leaf temperatures did not initiate transpirational cooling. As such, if T_{opt} is not acclimating to warmer temperatures, the upper canopy leaves could be pushed towards a lower capability for carbon uptake (Kramer et al., 2020; Pau et al., 2018) with large implications for the biosphere's carbon uptake and exchange with the atmosphere.

4.6 | Functional relationships in the canopy

Our study design allowed us to explore relationships of gas exchange and leaf traits across a tropical forest canopy gradient, and we found A_{opt} and A_{25} to increase with canopy height. Higher photosynthesis in the upper canopy is commonly found in tropical forests (Kosugi et al., 2012; Mau et al., 2018; Weerasinghe et al., 2014), and is attributed to acclimation to a higher light environment (Meir et al., 2002; Pearcy, 1987; Rozendaal et al., 2006). Photosynthesis rates, however, did not systematically increase with higher light, except for *Guarea* A_{25} (Table S4). High light variability at each canopy level could have caused light to be a less consistent predictor of photosynthesis for these species (Figure 4). Other factors, such as microclimate (Bond et al., 1999; EliµsÏ et al., 1989), nitrogen allocation (Crous et al., 2018; Crous et al., 2021; Medlyn et al., 1999), leaf morphology (Niinemets, 1999) and leaf age (Crous et al., 2021) might better describe our observed photosynthetic rates than light alone (Bond et al., 1999; Niinemets & Valladares, 2004).

R:A increased with increasing canopy height (Figure 7e,f), a relationship found in other tropical forests (Cavaleri et al., 2008; Weerasinghe et al., 2014). *Guarea R:A* was approximately 4 times higher and *Ocotea R:A* was approximately 3 times higher in the upper canopy than the understory. The increased *R:A* with height may provide further evidence that the upper canopy leaves are at risk carbon balance disruption. Q_{10} was consistent with height suggesting that, even though there was Q_{10} acclimation to experimental warming in *Guarea*, respiration does not systematically acclimate to compensate for the warmer temperatures that already exist in the upper canopies.

While experimental warming did not alter any of our measured leaf traits, we found various levels of height-related plasticity. As shown in many studies previously (Cavaleri et al., 2010; Coble & Cavaleri, 2015; Coble et al., 2017; Kenzo et al., 2015; Turnbull et al., 2003; Weerasinghe et al., 2014), N_{area} rose with increasing height (Figure S5a,b), which can be explained through the simultaneous increase in LMA (Figure S4c,d). LMA is strongly controlled by light and hydrostatic stress across vertical canopy gradients (Coble et al., 2017; Coble & Cavaleri, 2014), but is more strongly controlled by height than light in tropical canopies (Cavaleri et al., 2010). N_{mass} is generally constant throughout the canopy, and shows more interspecies than height variation (Niinemets et al., 2015). *Ocotea* N_{mass} slightly decreased with increasing canopy height (Figure S5c,d), while

C:N rose with increasing height. Higher *C:N* indicates higher investments in structure (Niinemets & Kull, 1998; Niinemets et al., 1998), which can conserve tissue water potential (Niinemets et al., 2015; Niinemets & Kull, 1998), and both species experienced lower % leaf water content as canopy height rose (Figure S5e,f). Therefore, perhaps the higher C:N in the upper canopy makes *Ocotea* is less vulnerable to upper canopy water stress.

5 | CONCLUSION

This study showed limited physiological acclimation of tropical tree leaves to in situ leaf-level warming. Contrary to our expectations, net photosynthesis did not acclimate at any canopy height. Foliar respiration only acclimated for one of our study species, and respiratory acclimation did not depend on height. Understanding how thermal acclimation may vary across a height gradient will better inform models predicting CO_2 exchange at the canopy, ecosystem, or global scale, as the shaded portion of the canopy may play an increasingly important role in forest carbon uptake with rising temperatures (Chen et al., 2012; He et al., 2018). Upper canopy stomatal conductance was particularly sensitive to increasing temperature, and the decreasing photosynthetic optimal temperatures with canopy height suggests that photosynthesis was limited by stomatal conductance in the upper canopy. Uppermost canopy leaves were operating beyond their thermal optimum frequently, especially during mid-day. Because neither of these species were able to positively adjust either their optimum temperature or their photosynthetic rates with experimental warming, climate warming may push these two species even further beyond their operating temperatures. If this trend is conserved across tropical species, tropical forest CO_2 uptake could decline with continued warming.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Forest Service Research Data Archive: https://doi.org/10.2737/RDS-2021-0053

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SUPPORTING INFORMATION

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