

# Leaf heat tolerance of 147 tropical forest species varies with elevation and leaf functional traits, but not with phylogeny

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## Abstract

Exceeding thermal thresholds causes irreversible damage and ultimately loss of leaves. The lowland tropics are among the warmest forested biomes, but little is known about heat tolerance of tropical forest plants. We surveyed leaf heat tolerance of sun-exposed leaves from 147 tropical lowland and pre-montane forest species by determining the temperatures at which potential photosystem II efficiency based on chlorophyll *a* fluorescence started to decrease ( $T_{\text{crit}}$ ) and had decreased by 50% ( $T_{50}$ ).  $T_{\text{crit}}$  averaged 46.7°C (5th–95th percentile: 43.5°C–49.7°C) and  $T_{50}$  averaged 49.9°C (47.8°C–52.5°C). Heat tolerance partially adjusted to site temperature;  $T_{\text{crit}}$  and  $T_{50}$  decreased with elevation by 0.40°C and 0.26°C per 100 m, respectively, while mean annual temperature decreased by 0.63°C per 100 m. The phylogenetic signal in heat tolerance was weak, suggesting that heat tolerance is more strongly controlled by environment than by evolutionary legacies.  $T_{\text{crit}}$  increased with the estimated thermal time constant of the leaves, indicating that species with thermally buffered leaves maintain higher heat tolerance. Among lowland species,  $T_{50}$  increased with leaf mass per area, suggesting that in species with structurally more costly leaves the risk of leaf loss during hot spells is reduced. These results provide insight in variation in heat tolerance at local and regional scales.

## KEYWORDS

chlorophyll *a* fluorescence, climate thresholds, functional traits, global warming, heat tolerance, phylogenetics, thermoregulation, tropical forest

## 1 | INTRODUCTION

The effect of global environmental change on life on earth often exhibits threshold dynamics in which performance steeply and irreversibly drops off above or below a critical threshold (Scheffer, 2009). One example is the heat tolerance of ectotherms: beyond a critical temperature threshold ( $T_{\text{crit}}$ ) performance rapidly declines, eventually resulting in irreversible damage typically only a few degrees above  $T_{\text{crit}}$ , as shown in heterotrophs (e.g., Duarte et al., 2012; Franken, Huizinga, Ellers, & Berg, 2018; García-Robledo, Kuprewicz, Staines, Erwin, & Kress, 2016), as well as in autotrophs such as phytoplankton (Padfield et al., 2017) and terrestrial plants (e.g., Knight &

Ackerly, 2003; Krause et al., 2010; Sachs, 1864; Sapper, 1935). To better anticipate the consequences of rising temperatures for ectotherms it will be important to understand variation in thermal thresholds among co-occurring species within a community and among different communities. Because of the dependence of virtually all organisms on primary producers, understanding thermal thresholds of photosynthesizing organisms is particularly important.

More than 150 years ago, Sachs (1864) reported that plants in his university's botanical garden could withstand exposure to air temperatures up to 50°C without leaf damage, but that 51°C or higher temperatures killed the leaves. Later studies reported more variation in heat tolerance, with both lower and higher values being observed in

the early 20th century (Sapper, 1935), but it remained challenging to identify general patterns underlying this variation. In recent years, leaf heat tolerance has received renewed interest as ongoing global warming and more frequent and intense heatwaves may push ecosystems past their critical thermal thresholds (Feeley et al., 2020; Geange et al., 2021; Lancaster & Humphreys, 2020; Perez & Feeley, 2020, 2021). Recent studies have shown that heat tolerance increases from the poles toward the tropics (O'Sullivan et al., 2017) and from high elevation to low elevation (Feeley, Martinez-Villa, et al., 2020), in parallel with increasing temperatures under which plants develop. While consistent, the increase in thermal thresholds along these gradients is moderate: 0.38 °C per °C increase in mean maximum temperature of the warmest month from poles to the tropics (O'Sullivan et al., 2017), and <0.1°C per °C mean annual temperature from high to low elevation (Feeley, Martinez-Villa, et al., 2020). Furthermore, at each latitude and elevation heat tolerance varies considerably among species, and the mechanisms underlying this variation have not been explored in detail.

Variation in heat tolerance among co-occurring species may represent functional or ecological differences in micro-climate adaptation among species. For example, Sapper (1935) already showed that sun species have higher heat tolerance than shade species, and even within species, heat tolerance tends to be moderately higher in sun-exposed leaves than in shade leaves (Curtis, Knight, & Leigh, 2019; Slot, Krause, Krause, Hernández, & Winter, 2019). Within site variation may also reflect different evolutionary histories of plants. Dick, Lewis, Maslin, and Bermingham (2013) reported that many common Neotropical tree species have emerged long enough ago to have previously experienced climatological conditions not unlike those predicted for the end of the current century. As such, Dick et al. (2013) proposed, these survivors of past extreme climates may be more likely to tolerate high temperatures than species in contemporary Neotropical forests that have emerged more recently under relatively cooler conditions. Neotropical forests have existed for at least ~58 million years (Wing et al., 2009) and are characterized not only by high species diversity, but also by great diversity at higher taxonomic levels. For example, on 50 ha of forest in central Panama >300 species of woody plants with >1 cm stem diameter have been identified, belonging to >60 families, including ancient families such as Fabaceae and Lecythidaceae and families of more recent origins such as Chrysobalanaceae and Apocynaceae. If heat tolerance is conserved, within site variation of temperature sensitivity, if observed, may thus also be related to the evolutionary history of species.

Tropical forest trees routinely experience high temperatures when exposed to direct irradiance, especially during moments of low wind speed, with leaf temperatures exceeding air temperature by as much as 10°C–18°C (Doughty & Goulden, 2008; Fauset et al., 2018; Rey-Sanchez, Slot, Posada, & Kitajima, 2016). Leaf traits can influence both the magnitude and rate of leaf heating (Jones, 2013; Tserej & Feeley, 2021). For example, greater leaf width and leaf size are generally associated with a thicker leaf boundary layer and greater decoupling of leaf and air temperatures (Jones, 2013). Indeed, Fauset et al. (2018) parameterized a leaf energy balance model for tropical

montane species and found that leaf width was the most important leaf morphological driver of species differences in the leaf-to-air temperature differential. Multiple leaf traits can also be combined into composite traits that characterize the dynamics of leaf temperatures. For example, the thermal time constant ( $\tau$ ; s) quantifies the thermal stability of a leaf, that is, how rapidly leaf temperature responds to temporal variation in microclimate (Michaletz et al., 2015, 2016). The short  $\tau$  of relatively small and thin leaves indicates that they heat up and cool down quickly, so that leaf temperatures essentially track changes in microclimate variables, including very high temperatures when in full sun. By contrast, leaves with a comparatively large thermal mass and a long  $\tau$  are buffered against fluctuations in microclimate, so that leaf temperatures lag behind changes in microclimate variables (Michaletz et al., 2015, 2016; Tserej & Feeley, 2021). Leaf temperature can also be affected by transpirational cooling, especially in hot and dry environments (Lin et al., 2017), and thus stomatal conductance is another potentially relevant leaf trait. However, because of the prevalence of mid-day stomatal depression (Goulden et al., 2004; Kosugi et al., 2008; Zotz, Harris, Königer, & Winter, 1995), the highest leaf temperatures are experienced when stomata are closed, and maximum stomatal conductance is therefore unlikely to correlate with maximum temperatures across species (but see Perez & Feeley, 2020). Furthermore, a sensitivity analysis revealed that variation in stomatal conductance had virtually no effect on the thermal time constant  $\tau$  (Michaletz et al., 2016).

One would expect a strong selective advantage of high heat tolerance in species that have traits that cause them to experience high maximum temperatures. Indeed, heat tolerance correlated positively with maximum recorded leaf temperatures across 13 tree species measured in a botanical garden in Florida (Perez & Feeley, 2020). High heat tolerance would likewise seem advantageous for species with traits that result in high temperatures being maintained for long periods of time. The rapid response to warming of short  $\tau$  species should predispose them to experiencing higher maximum temperatures, whereas the slow-cooling long  $\tau$  species maintain high temperatures longer once they are reached. The photosynthetic capacity of species with short  $\tau$  peaks at higher ambient temperatures than for species with long  $\tau$ , suggesting that species with short  $\tau$  are better acclimated to higher temperatures than long  $\tau$  species (Michaletz et al., 2016). Whether short  $\tau$  species also have higher heat tolerances under field conditions has not yet been tested.

Leaves that are structurally relatively costly to produce need to last long enough for the plant to offset the investment, and therefore tend to be better protected against biotic (Coley, 1987) and abiotic stressors (Nardini, Pedà, & Rocca, 2012). Correspondingly, high heat tolerance of such leaves would be expected. This has indeed been suggested for plants experiencing distinct temperature seasonality, for which heat tolerance correlated with leaf mass per area (LMA) (e.g., Knight & Ackerly, 2003; Sastry & Barua, 2017), a correlate of leaf lifespan and construction costs (Wright et al., 2004). Whether this pattern is maintained in more thermally stable ecosystems such as tropical forests remains to be tested.

We measured leaf heat tolerance across a functionally and phylogenetically diverse set of 147 tropical species from lowland and pre-montane forest sites in Panama. We focused primarily on trees, as key components of forest ecosystems, but we also included a small number of lianas, shrubs, and forbs. With these data we tested the following hypotheses:

1. Heat tolerance will be greater in lowland than in pre-montane sites, consistent with previously observed relationships between heat tolerance and growth temperature. Based on previous observations we expect this difference to be smaller than the difference in growth temperature. Over a 20°C growing season temperature range from the arctic to the tropics, mean heat tolerance increased by only 9°C (O'Sullivan et al., 2017), and over a 17°C mean annual temperature range across a tropical elevation gradient, Feeley, Martinez-Villa, et al. (2020) reported an increase in heat tolerance of less than 2°C. These observations suggest that upper temperature thresholds are fairly high relative to mean air temperatures in cool conditions, and increase only moderately along gradients in growth temperature.
2. Heat tolerance will be phylogenetically structured and lineages that can be considered survivors of past hot epochs will have higher heat tolerance than species or lineages that emerged later and are naïve to such conditions.
3. Heat tolerance will increase with traits that enable leaves to reach high temperature extremes, such that species that quickly heat up will have higher heat tolerance than species that are more buffered against high temperatures. This would be consistent with Perez and Feeley (2020) and with the higher temperature optimum for photosynthesis of short  $\tau$  species (Michaletz et al., 2016). Alternatively, heat tolerance may be greater in thermally buffered species because they

cool down more slowly and thus maintain stable temperatures for longer. Heat tolerance may differ with duration of exposure; when individual leaves are warmed gradually over a wide temperature range (commonly by 1°C per min) estimated heat tolerance is often relatively low, suggesting a negative effect of the cumulative heat exposure (Krause et al., 2010). If the negative impact of sustained high temperatures is greater than that of higher, but shorter peak temperatures, heat tolerance might correlate positively with  $\tau$  across species.

4. Species that produce structurally expensive leaves will have higher heat tolerance than species with “cheap” leaves that can be readily replaced. High investment in leaves that are relatively thick and dense (high LMA), or with high leaf dry matter content (LDMC), is associated with higher leaf longevity (Wright et al., 2004), and thus with greater likelihood of the leaves experiencing temperature extremes during their lifetime.

## 2 | MATERIALS AND METHODS

### 2.1 | Site information

Samples were collected from plants growing in six regions in the Republic of Panama (Table 1; Figure S1). We extracted mean, minimum, and maximum monthly temperatures for each study site from WorldClim 2.0 (Fick & Hijmans, 2017), which provides spatially interpolated monthly climate variables with an accuracy of  $\sim 1$  km<sup>2</sup>. For the lowland sites additional climate data were available from the Physical Monitoring Program of the Smithsonian Tropical Research Institute, and from the Panama Canal Authority (Table S1). The WorldClim data correlated very well with the monitored data ( $R^2 = 0.9$ , Table S1)

**TABLE 1** Geographic and temperature data for the sample sites

Region	Site	Lat. (°N)	Long. (°W)	Elevation (m asl) <sup>a</sup>	$T_{\text{Min}}$ (°C)	MAT (°C)	$T_{\text{Max}}$ (°C)	#spp
Panama City	Ancón   Albrook   Clayton   Parque natural Metropolitano	9.9637	79.5458	18 <sup>b</sup>   6   14   30	22.8	27.0	31.6	37
Parque Nacional Soberanía	Gamboa	9.1240	79.6965	44	22.6	26.6	30.7	44
	Barro Colorado Island	9.1165	79.6965	54	22.5	26.5	30.7	3
Colón	Parque Nacional San Lorenzo	9.3640	79.9593	62	22.8	26.4	30.1	20
	Punta Galeta	9.4008	79.8697	2	23.2	26.9	30.6	6
	Santa Rita Arriba	9.3395	79.6792	200	21.8	25.8	29.6	5
Panama Oeste	Parque Nacional Altos de Campana	8.6875	79.9875	526	20.1	24.4	29.3	18
	Arraiján	8.9394	79.6423	129	22.2	26.3	30.8	7
	Cerro los Cañones	8.9236	80.0358	300	21.7	26.0	30.5	1
Coclé	Penonomé	8.5503	80.3547	99	22.2	26.9	32.3	3
Chepo district	Cerro Jefe	9.2319	79.3954	750	18.2	22.1	26.4	12
	Cerro Azul	9.2347	79.3514	650	18.8	22.8	27.1	2
	Carretera Llano-Cartí	9.3000	78.9875	434	20.8	25.5	28.5	2

Note:  $T_{\text{Min}}$  is the mean minimum temperature of the coolest month, MAT is mean annual temperature and  $T_{\text{Max}}$  is the mean maximum temperature of the warmest month.

<sup>a</sup>Combination of elevation recorded at study sites and WorldClim altitude data.

<sup>b</sup>Weighted average of species sampled across a 7–66 m elevation range in Ancon. Analyses use elevation from all sample locations in Ancon.

and for consistency we used WorldClim data for all sites in the analyses.

## 2.2 | Species selection and sample collection

We collected samples from 147 tropical forest species from 54 families from lowlands up to pre-montane forests in the Republic of Panama between February 2019 and January 2020. In addition, we included leaves from two species of *Agave* cultivated at the Smithsonian Tropical Research Institute's Santa Cruz plant growth facilities in Gamboa, and an invasive grass common at forest edges. In Panama there is no evidence for seasonal changes in heat tolerance for the species for which this has been tested (Krause et al., 2010) as temperature seasonality is minimal. Figure S2 shows measured heat tolerance parameters through the collection period, showing no indication of seasonal patterns. Species included gymnosperms (5) but mostly consisted of angiosperms (145)—primarily trees (129), but also palms (2), lianas and vines (5), large woody shrubs (5), large forbs (3), and a grass (1) (Table S2). For *Acacia mangium*, we measured phyllodes instead of leaves, as this species does not produce true leaves beyond the sapling stage. The majority of the species are native to Panama (134), but several common non-native species were included (16), most of them ornamentals.

Sun-exposed outer canopy leaves were collected using (pole) pruners, where possible from multiple individuals ( $n = 1-4$ , mean 1.5). For each species vouchers were collected and deposited at the herbarium of the University of Panama (PMA). At Parque Nacional San Lorenzo, canopy leaves were accessed with the aid of a construction crane maintained by the Smithsonian Tropical Research Institute. CAM (crassulacean acid metabolism) plants were collected in the afternoon of sunny days to make sure that leaf acid content was low, as vacuolar release of these acids from fully acidified tissues during sample preparation can exacerbate leaf damage and significantly lower estimates of heat tolerance (Krause, Winter, Krause, & Virgo, 2016). All other species were collected in the morning to reduce the risk of sampling heat-stressed or photoinhibited leaves—this included the facultative CAM species *Clusia minor* and *C. pratensis* that were sampled during the wet season when expressing  $C_3$ . Branches were enclosed in large opaque plastic bags with moist tissue paper until processed in the laboratory in Panama City on the same day.

Because we hypothesized an environmental effect on heat tolerance, and most species in tropical forests are locally rare, we prioritized sampling more species to represent the breadth of species diversity, over increasing species-level replication within sites by restricting our study to species that are locally abundant.

## 2.3 | Chlorophyll a fluorescence protocol

There are many approaches to determining heat tolerance (see Geange et al., 2021). Our approach was based on the protocol that

Krause et al. (2010) developed to study leaf heat tolerance of (Panamanian) tropical trees. The protocol yields results that are very similar to those obtained in classical necrosis tests (Krause et al., 2010), and is now commonly used (e.g., Feeley, Martinez-Villa, et al., 2020; Leon-Garcia & Lasso, 2019; Perez & Feeley, 2020, 2021; Slot et al., 2019; Tiwari et al., 2021). We measured  $F_v/F_m$  on leaf disks 24 hr after they were incubated for 15 min in a temperature-controlled water bath, using 8–12 incubation temperatures between 44°C and 54°C (where necessary 58°C), with a minimum of five leaf disks at each temperature. When multiple individuals were sampled for a species, they were pooled, and at each temperature a random set of leaf discs was used. On average 48 leaves were used per species (median = 50). Leaf surfaces were first cleaned with distilled water. Leaf disks (typically 2 cm diameter, or smaller when leaves were narrow) were wrapped into strips of miracloth (Calbiochem, La Jolla, CA)—to avoid hypoxic conditions—and then put into individual small zip-lock bags with small glass rods at the bottom. The bags were placed in preheated water baths (Lauda RM6/RMS circulating water bath or Lauda Alpha immersion thermostat; Analytical Instruments, LLC, Golden Valley, MN). After 15 min the miracloth was removed and the disks transferred to moist tissue paper in petri dishes. Samples were allowed to recover for 24 hr at low ( $<10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) photosynthetically active radiation to ensure that the reductions in fluorescence yield were irreversible and not transient in nature. After dark adaptation for 15 min we then measured the initial Chl *a* fluorescence emission ( $F_0$ ), maximum fluorescence ( $F_m$ ), and recorded the ratio of variable ( $F_m - F_0$ ) to maximum fluorescence ( $F_v/F_m$ ). Measurements were made with a PAM-2000 and a mini-PAM fluorometer (Walz GmbH, Effeltrich, Germany).

## 2.4 | Heat tolerance parameters

We calculated three temperature-response parameters of  $F_v/F_m$ :  $T_{\text{crit}}$ , the critical temperature beyond which  $F_v/F_m$  declines and leaves are irreversibly heat damaged;  $T_{50}$ , the temperature at which PSII functionality is reduced by 50%; and *b*, the steepness of the decrease in  $F_v/F_m$  around  $T_{50}$ .  $T_{50}$  and *b* were determined by fitting the data with Equation (1) as:

$$F_v/F_m = \frac{F_v/F_{m,\text{Max}}}{1 + e^{b \times (T_{\text{Leaf}} - T_{50})}}, \quad (1)$$

where  $F_v/F_{m,\text{Max}}$  is the upper horizontal asymptote representing  $F_v/F_m$  associated with healthy, non-stressed leaves, and  $T_{\text{Leaf}}$  is the incubation temperature (°C) of the leaf disks. Curves were fitted with the “nls\_multstart” function from the “nls.multstart” package (Padfield & Matheson, 2018) in R version 3.6.1 (R Core Team, 2019). The curves thus generated enabled us to produce species-level estimates of  $T_{50}$ , *b*, and  $F_v/F_{m,\text{Max}}$ , with their standard errors.  $T_{\text{crit}}$  was calculated as the temperature at which a line describing the slope of the  $F_v/F_m$  decline at  $T_{50}$  intersected with a horizontal line indicating  $F_v/F_{m,\text{Max}}$ .

## 2.5 | Functional traits

We measured leaf traits on at least five leaves per species. Fresh mass was measured on fully hydrated leaves. Leaf length and width were measured with digital callipers or rulers, and leaf lamina thickness was recorded on at least three positions from the apical, median and basal section for each leaf using a 293-348-30 Digimatic micrometer (Mitutoyo, Kawasaki, Japan). We measured leaf area with a LI-3100C area meter (LI-COR, Lincoln, NE). Dry mass was determined after drying the leaves at 70°C until constant weight. From these measurements we calculated leaf mass per area (LMA, kg m<sup>-2</sup>), leaf dry matter content (LDMC, g g<sup>-1</sup>), and leaf density (leaf dry mass per unit leaf volume). To address hypothesis 3, we estimated leaf thermal time constant  $\tau$  (s) from first principles following Michaletz et al. (2015), using species-level mean values of LMA, LDMC, leaf area, and leaf width; mean annual temperature derived from WorldClim2; and wind speed set to 2 m s<sup>-1</sup>, as:

$$\tau = \varphi LMA \left[ \frac{c_{p,w}}{LDMC \cdot h} + \frac{c_{p,d} - c_{p,w}}{h} \right], \quad (2)$$

where  $\varphi$  (dimensionless) is the ratio of projected-to-total leaf area,  $c_{p,d}$  (J kg<sup>-1</sup> °C<sup>-1</sup>) is the specific heat capacity of dry leaf matter taken from Phillips, Sastry, and Morrow (1983), and  $c_{p,w}$  (J kg<sup>-1</sup> °C<sup>-1</sup>)\*\*\*\* is the specific heat capacity of water. The heat transfer coefficient  $h$  (W m<sup>-2</sup> °C<sup>-1</sup>) was calculated as  $h = \rho_a c_{p,a} g_h$ , where  $\rho_a$  (kg m<sup>-3</sup>) is air density,  $c_{p,a}$  (J kg<sup>-1</sup> °C<sup>-1</sup>) is the specific heat capacity of air at a constant pressure, and  $g_h$  is the heat conductance.  $g_h$  (mm s<sup>-1</sup>) was calculated as  $g_h = 1.5 \times 6.62 \times \sqrt{U/w}$ , where 1.5 is a factor to account for outdoor turbulence (Jones, 2013),  $U$  is the wind velocity (m s<sup>-1</sup>), and  $w$  is the leaf width (m). The effects of radiation and transpiration were not included as these data were not available for all species. This permits us, however, to quantify how variation in the measured morphological traits alone causes variation in thermal time constants.

To test hypothesis 4, we used LMA and LDMC as proxies for leaf construction costs (Wright et al., 2004). Functional trait data were obtained for 132 species—for some species insufficient healthy leaves were available. For species with compound leaves we used traits collected on leaflets for further analyses. All functional traits except LDMC and leaf density were natural-log transformed to improve normality of the data.

## 2.6 | Phylogenetic and statistical analyses

We constructed a phylogenetic tree of all identified species ( $n = 144$ ) with the V.PhyloMaker package (Jin & Qian, 2019) in R, which generates phylogenies for vascular plants using two mega-trees as a backbone (Smith & Brown, 2018; Zanne et al., 2014). Trait variation was mapped onto the phylogeny using the Phytools package (Revell, 2012), which was also used to test for phylogenetic signals in heat tolerance and other leaf traits. Phylogenetic signals were

evaluated with Blomberg's K parameter (Blomberg, Garland Jr, & Ives, 2003) and with Pagel's  $\lambda$  (Pagel, 1999).

Effects of elevation and site temperature on heat tolerance parameters were analyzed using ordinary least squares (OLS) regression. We focused our analyses on plants with C<sub>3</sub> photosynthesis—species with C<sub>4</sub> ( $n = 1$ ) and CAM ( $n = 3$ ) photosynthesis were only sampled at one site. The effects of elevation for species measured at two different sites was determined with linear regression, weighted by the elevation difference between sites. This approach reduced the weight of species measured at two sites of similar elevation, as elevation is unlikely to explain heat tolerance differences in these cases. Within species, site differences in  $T_{50}$  values were evaluated by comparing their 95% confidence intervals.

To test how heat tolerance correlated with specific traits associated with thermoregulation (H3) and leaf construction costs (H4), we used OLS regression; phylogenetically controlled multiple regression analyses were not required because there was no significant phylogenetic signal in heat tolerance parameters (see below), even if there were phylogenetic patterns in the traits themselves (Revell, 2010). These multiple regression analyses were restricted to species with C<sub>3</sub> photosynthesis for which trait data were available. In addition, we used the “dredge” function from the MuMIn package (Barton, 2020) to rank all possible models for  $T_{50}$  and  $T_{crit}$  as a function of elevation and leaf traits according to AIC.

## 3 | RESULTS

$F_v/F_{m,Max}$  was >0.7 for all species, indicating that the leaves used in this study were not photoinhibited prior to heat treatments—only in *Podocarpus guatemalensis* grown in Gamboa  $F_v/F_{m,Max}$  was moderately lower, at 0.63.  $T_{50}$  of the tropical forest species averaged  $49.9 \pm 1.4^\circ\text{C}$  (mean  $\pm$  SD) and ranged from  $45.8^\circ\text{C}$  in *Hedyosmum bonplandianum*, a montane forest tree, to  $53.7^\circ\text{C}$  in *Rhizophora mangle*, the pan-tropical red mangrove (Table S2), with 90% of observations falling between  $47.8^\circ\text{C}$  and  $52.5^\circ\text{C}$ . Higher values were found in non-forest CAM plants, with  $T_{50}$  of  $55^\circ\text{C}$  in *Agave americana*. Within ecosystems there was also a considerable range in  $T_{50}$  values: among the 117 lowland species, values ranged from  $47.7^\circ\text{C}$  in *Luehea speciosa* to  $53.5^\circ\text{C}$  in *Cecropia longipes*.  $T_{50}$  of the phyllodes of *Acacia mangium* was similar to that of true leaves of other lowland tree species:  $49.7 \pm 0.3^\circ\text{C}$  (mean  $\pm$  SEM).  $T_{crit}$  likewise varied considerably, from  $42.0^\circ\text{C}$  in *H. bonplandianum* to  $50.9^\circ\text{C}$  in the palm *Dypsis lutescens*, with an average of  $46.7 \pm 1.9^\circ\text{C}$  and 90% of observations falling between  $43.5^\circ\text{C}$  and  $49.7^\circ\text{C}$ . Values of  $T_{50}$  were more narrowly distributed around the overall mean than  $T_{crit}$  values (Figure S3).  $T_{50}$  and  $T_{crit}$  were highly correlated ( $F_{1,150} = 223.1$ ,  $p < .001$ ,  $r^2 = 0.60$ ).  $T_{50}$  was on average  $3.2^\circ\text{C}$  higher than  $T_{crit}$ .  $F_v/F_m$  declined more steeply around  $T_{50}$  in species in which the decline started at a higher temperature, that is, the distance between  $T_{crit}$  and  $T_{50}$  decreased as  $T_{crit}$  increased. This was indicated by a decrease in the b parameter of Equation (1)—b becoming more negative—with increasing  $T_{crit}$  ( $F_{1,150} = 62.8$ ,  $p < .001$ ,  $r^2 = 0.29$ ).



### 3.1 | Effects of elevation and site temperature on heat tolerance and leaf traits

$T_{50}$  and  $T_{crit}$  both decreased significantly with elevation ( $F_{1,150} = 35.4$  and  $44.0$ , for  $T_{50}$  and  $T_{crit}$ , respectively,  $p < .001$ ) (Figure 1, Table S2). Including CAM and  $C_4$  plants in the analysis did not change the slopes, but decreased the variance explained—all CAM and  $C_4$  plants were collected from lowland sites and heat tolerance was higher in CAM plants, and lower in the  $C_4$  plant than in  $C_3$  plants.  $T_{50}$  and  $T_{crit}$  decreased by  $0.26^\circ\text{C}$  and  $0.38^\circ\text{C}$  per 100 m, respectively. The lapse rate across the study sites was  $0.63^\circ\text{C}$  decrease in mean annual temperature (MAT) per 100 m increase in elevation.

Heat tolerance increased significantly with MAT ( $F_{1,150} = 30.3$  and  $36.5$ , for  $T_{50}$  and  $T_{crit}$ , respectively,  $p < .001$ ) (Figure 1). For every  $1^\circ\text{C}$  increase in MAT,  $T_{50}$  increased by  $0.41^\circ\text{C}$  and  $T_{crit}$  increased by  $0.59^\circ\text{C}$ .  $T_{50}$  and  $T_{crit}$  also correlated significantly with the mean maximum temperature of the warmest month and with the mean minimum temperature of the coldest month (Figure S4). The b parameter, the steepness of the decline in  $F_v/F_m$  around  $T_{50}$ , significantly decreased with elevation and increased with MAT, so  $F_v/F_m$  declined less steeply in cooler, higher elevation species than in lowland species. However, elevation and MAT only explained a small amount of variance in b ( $r^2 \leq 0.05$ ).

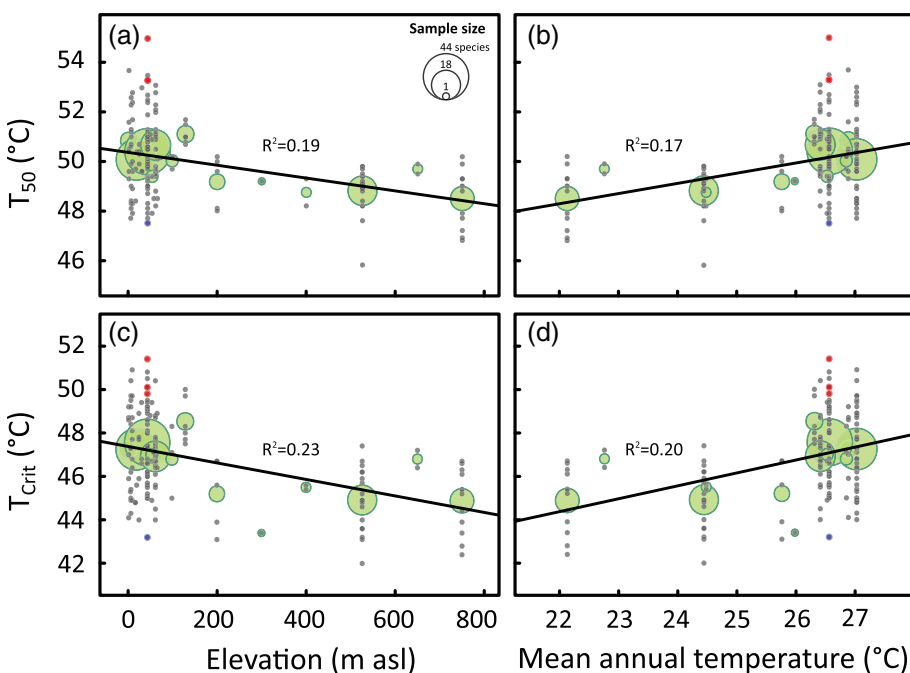
Consistent with the patterns across species, within species there was a tendency for heat tolerance to decrease with elevation and increase with site temperature (irrespective of whether minimum, mean, or maximum) (Figure 2). Among lowland sites (between 0 and 200 m elevation, with MAT differences  $<1^\circ\text{C}$ ) patterns in heat tolerance with elevation and MAT were not consistent and the confidence intervals of the two values tended to overlap (Figure 2).  $T_{50}$  and  $T_{crit}$  decreased with elevation by  $0.4^\circ\text{C}$  and  $0.7^\circ\text{C}$  per 100 m, respectively, based on a regression weighted by elevation difference between sites.

$T_{50}$  and  $T_{crit}$  increased with site temperature by  $0.9^\circ\text{C}$  and  $1.5^\circ\text{C}$  per  $^\circ\text{C}$  MAT, respectively.

To enable comparison of heat tolerance across the phylogeny and among plant categories we standardized  $T_{50}$  and  $T_{crit}$  to sea-level values using the trendlines in Figure 1. Standardized  $T_{50}$  and  $T_{crit}$  were not significantly different between native and non-native species, or between evergreen and deciduous species (Figure S5). Gymnosperms tended to have moderately higher  $T_{50}$  than angiosperms ( $p < .1$ , two-sample  $t$ -test) because of the high heat tolerance of three *Zamia* species, but the sample size of gymnosperm species ( $n = 5$ ) was insufficient to draw meaningful biological conclusions from these apparent differences. For similar reasons, cycads (i.e., *Zamia* species in our study), had higher  $T_{50}$  than lianas ( $n = 6$ ), shrubs (6), and trees (134), and higher  $T_{crit}$  than lianas (Figure S5).

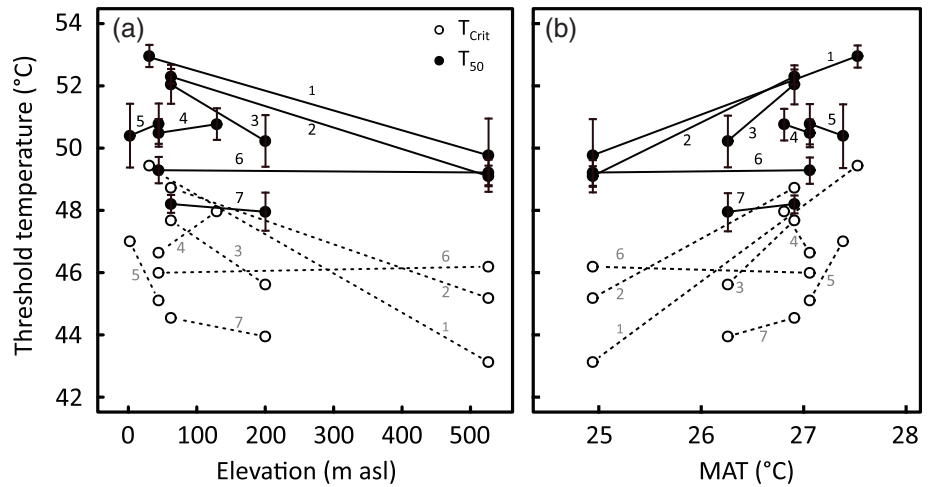
### 3.2 | Other traits

LMA moderately increased with elevation ( $F_{1,127} = 8.2$ ,  $p = .005$ ,  $r^2 = 0.06$ ), as did leaf thickness ( $F_{1,127} = 6.9$ ,  $p = .009$ ,  $r^2 = 0.05$ ): Including the CAM plants in the analysis—all lowland plants with very high LMA and thick leaves—rendered these relationships non-significant. Leaf size decreased with elevation ( $F_{1,132} = 9.8$ ,  $p = .002$ ,  $r^2 = 0.07$ ), also when the CAM plants were included. As expected, opposite patterns were found with increasing site temperature. Other functional traits (density, LDMC, and leaf length: width ratio) did not change significantly with elevation. Likewise, there was no change in the thermal time constant ( $\tau$ ) with elevation. Across  $C_3$  species,  $\tau$  ranged from 3.8 to 82 s, with 90% of the values between 7 and 43 s. CAM plants fell on the extremes of the distributions of most functional traits,  $\tau$ , and heat tolerance, but there were insufficient CAM or  $C_4$  plants to develop separate models for species with different



**FIGURE 1** Thermal thresholds  $T_{50}$  (a, b) and  $T_{crit}$  (c, d) as a function of elevation and mean annual temperature across 150 tropical species sampled from the 13 sites listed in Table 1. Site mean values are indicated in green, with symbol size proportional to sample size. Values for CAM plants and  $C_4$  plants are indicated in red and blue, respectively. Lines and  $r^2$  values indicate linear regressions for  $C_3$  plants only: (a)  $T_{50} = 50.4 - 0.0026 \times \text{Elevation (m asl)}$ . (b)  $T_{50} = 39.2 + 0.41 \times \text{MAT}$ . (c)  $T_{crit} = 47.4 - 0.0038 \times \text{Elevation}$ . (d)  $T_{crit} = 31.3 + 0.60 \times \text{MAT}$  [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**FIGURE 2** Thermal thresholds  $T_{50}$  and  $T_{Crit}$  in relation to elevation for species that were measured at two contrasting elevations. Error bars indicate 95% confidence intervals approximated as  $T_{50} \pm 1.96 \times$  standard error of  $T_{50}$ . 1. *Posoqueria latifolia*, 2. *Aspidosperma spruceanum*, 3. *Protium panamense*, 4. *Ceiba pentandra*, 5. *Coccoloba uvifera*, 6. *Podocarpus guatemalensis*, 7. *Guatteria lucens*



photosynthetic pathways. Analyses of trait correlations therefore focused on  $C_3$  plants alone.

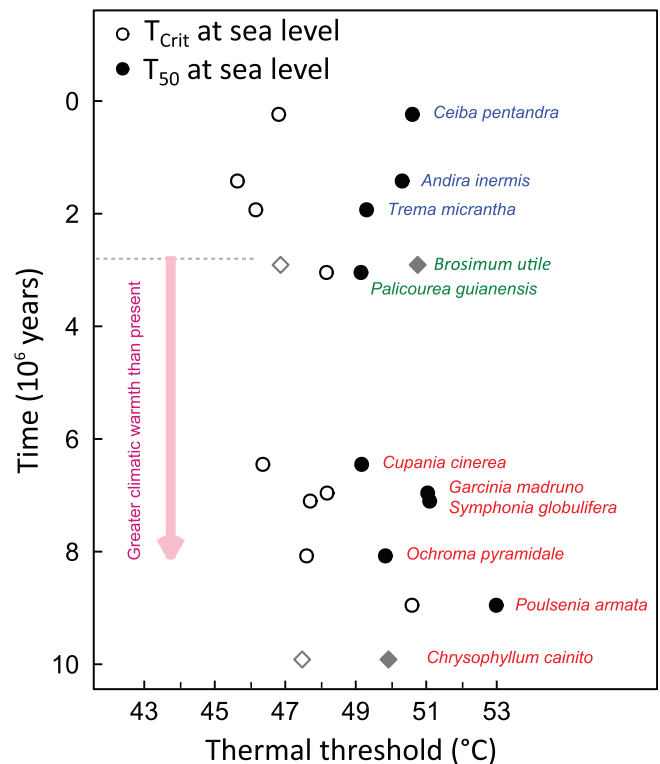
### 3.3 | Phylogenetic and evolutionary patterns

#### 3.3.1 | Heat tolerance versus taxon age

Heat tolerance was not related to the age of species estimated in Dick et al. (2013) (Figure 3). Linear regressions between age and  $T_{50}$  and between age and  $T_{Crit}$  were not significant ( $p > .05$  for both), suggesting no gradual trends. Likewise, species with warmer (Miocene) origins did not have systematically higher heat tolerance than species that emerged in the cooler recent (Pleistocene) past (two-sample  $t$ -test.  $T_{50}$ :  $t = -1.4$ ,  $p = .20$ .  $T_{Crit}$ :  $t = -2.1$ ,  $p = .09$ ). Standardizing  $T_{50}$  and  $T_{Crit}$  to sea level values did not change these results. These results were based on heat tolerance of nine of the 12 species in Dick et al. (2013). Adding *Chrysophyllum cainito* and *Brosimum utile*, sister species of *C. argenteum* and *B. guianense*, respectively, in Dick et al. (2013), did not change the results. We also tested for trends of heat tolerance over longer timescales, using family age estimates from Wikström, Savolainen, and Chase (2001), who presented estimates for 29 of the families (representing 96 of the species) in the current study. As was the case for the data in Figure 3, there were no trends in heat tolerance with family ages (Figure S6).

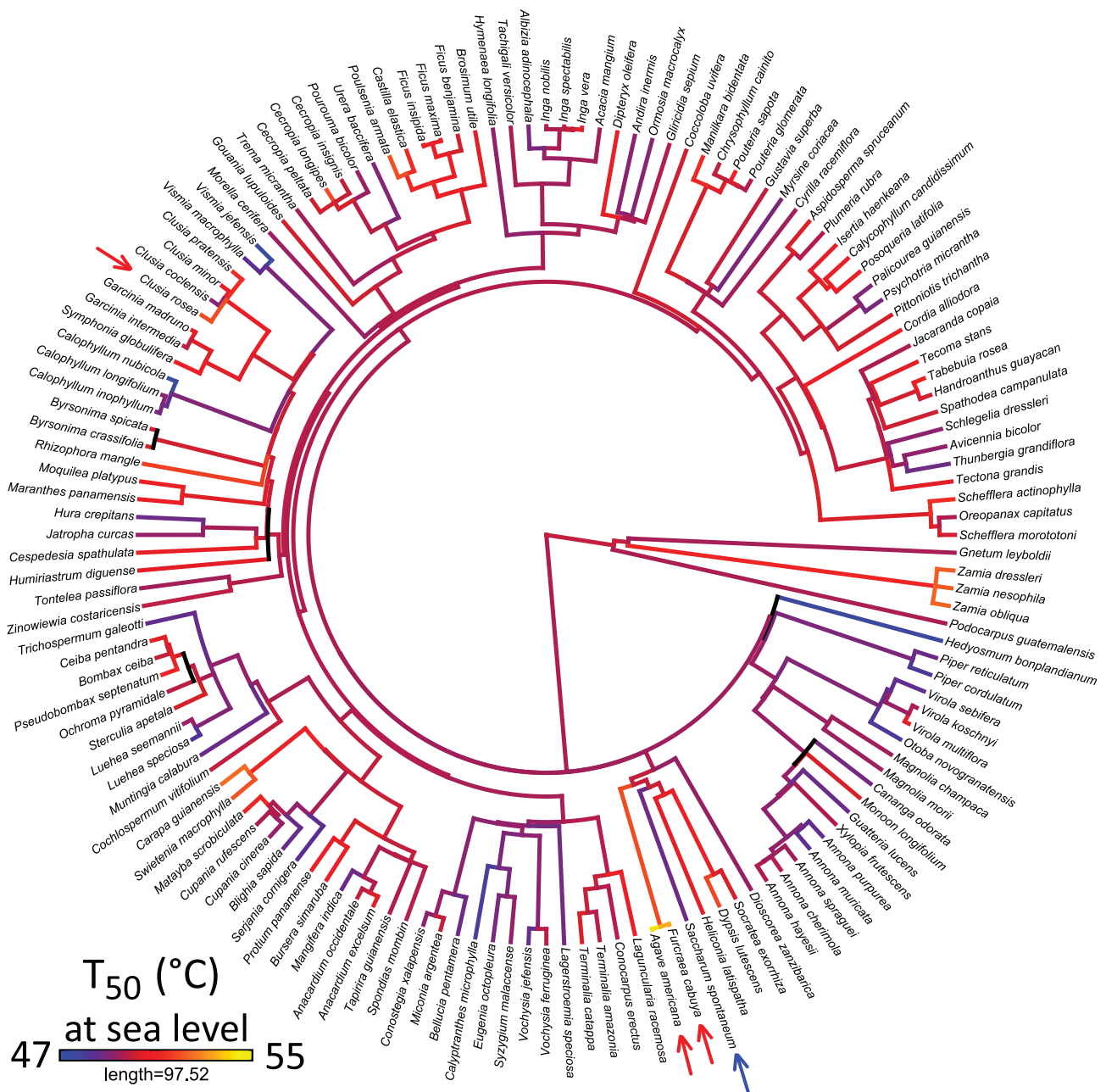
#### 3.3.2 | Phylogenetic patterns and trait correlations

Figure 4 shows the phylogenetic tree of the 144 identified species in our dataset, colour coded by  $T_{50}$  standardized to sea level using the linear relationship with elevation shown in Figure 1. There are some distinct clusters of related species with similar trait values, such as the high  $T_{50}$  values of the species of *Zamia*, the relatively high heat tolerance of the Moraceae (*Ficus* spp., *Castilla elastica*, *Poulsenia armata*, and *Brosimum utile*), and the relative low heat tolerance of the Annonaceae. However, the phylogenetic signal on  $T_{50}$  was not statistically significant with  $\alpha$  of 0.05 (Blomdahl's  $K = 0.11$ ,  $p = .08$ ; Pagel's



**FIGURE 3** Relationship between thermal thresholds  $T_{50}$  and  $T_{Crit}$  and species “ages.” Grey diamonds show data for *Brosimum utile* and *Chrysophyllum cainito*, sister species of *B. guianense*, and *C. argenteum* dated in Dick et al. (2013), respectively. Species names in blue represent Pleistocene; green, Pliocene; red, Miocene [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

$\lambda = 0.53$ ,  $p = .06$ ), suggesting that across the phylogenetic tree related pairs of species were not significantly more similar to each other than random pairs.  $T_{Crit}$  likewise did not show a significant phylogenetic signal ( $K = 0.11$ ,  $p = .06$ ;  $\lambda = 0.16$ ,  $p = .99$ . Figure S7). In fact, the species with the highest and lowest  $T_{Crit}$  values among native lowland trees both belong to the Urticaceae:  $44.0^\circ\text{C}$  for *Pouroma bicolor*, and  $50.8^\circ\text{C}$  for *Cecropia longipes*.



**FIGURE 4** Phylogenetic tree of study species with branches colour coded by the value of  $T_{50}$  standardized to sea level. Red and blue arrows indicate CAM-exhibiting species and  $C_4$  species, respectively. Black nodes represent polytomies [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

The leaf functional traits all had phylogenetic signals, with the exception of Blomdahl's  $K$  for leaf length.  $\tau$  was also phylogenetically structured ( $K = 0.18$ ,  $p = .018$ ;  $\lambda = 0.90$ ,  $p < .001$ ).

### 3.4 | Heat tolerance in relation to leaf thermoregulation

Regression models of  $T_{50}$  and  $T_{\text{Crit}}$  were consistently better (based on AIC and  $R^2_{\text{adj}}$ ) when the predictors included elevation rather than any of the temperature metrics ( $T_{\text{Min}}$ , MAT, or  $T_{\text{Max}}$ ), presumably because

the temperature metrics are derived from spatially interpolated model data while elevation was recorded. Because temperature and elevation were tightly correlated ( $R^2 = 0.96$  for MAT~Elevation), we did not include both in the same models. We tested the effects of various leaf traits associated with thermoregulation on heat tolerance. Leaf width did not correlate with  $T_{50}$  but had a significant effect on  $T_{\text{Crit}}$  when elevation was included in the model ( $R^2_{\text{adj}} = 0.24$ ,  $F_{2,119} = 20.3$ ,  $p < .001$ ). Leaf width effect,  $p = .046$ ) (Table S3). Leaf area and elevation combined also yielded a good model for  $T_{\text{Crit}}$  ( $R^2_{\text{adj}} = 0.25$ ,  $F_{2,119} = 21.6$ ,  $p < .001$ ), as did leaf length and elevation ( $F_{2,119} = 23.6$ ,  $p < .001$ ,  $R^2_{\text{adj}} = 0.27$ ). Species with a longer  $\tau$ , that is, species with



leaves that take longer to heat and cool, had higher  $T_{\text{Crit}}$  when accounting for elevation ( $F_{2,119} = 20.7$ ,  $p < .001$ ,  $R^2_{\text{adj}} = 0.25$ ), without an interaction effect of elevation— $T_{\text{Crit}}$  was greater at low than at high elevation (two-sample  $t$ -test,  $t = 7.41$ ,  $p < .001$ ), whereas  $\tau$  did not differ between sites (Figure 5). When substituting MAT for elevation  $\tau$  also significantly affected  $T_{\text{Crit}}$  ( $F_{2,119} = 18.7$ ,  $p < .001$ ,  $R^2_{\text{adj}} = 0.23$ ) (Table S3).  $T_{50}$  and  $b$  were not affected by  $\tau$ , and elevation did not interact significantly with  $\tau$ 's relationship with heat tolerance traits.

### 3.5 | Heat tolerance in relation to leaf construction costs

For  $T_{50}$  the best, most parsimonious model included elevation, LMA and their interaction, with an adjusted  $R^2$  of 0.29 ( $F_{3,118} = 17.7$ ,  $p < .001$ ). The significant interaction term arose from a strong positive effect of LMA on  $T_{50}$  in lowland species that was not observed among mid-elevation and pre-montane species (Figure 6). LDMC did not have a significant effect on  $T_{50}$  ( $p > .1$ ). For  $T_{\text{Crit}}$ , neither LMA nor LDMC were significant predictors. LDMC had a weak, but significant effect on  $b$ , when elevation was included in the model ( $F_{2,119} = 4.7$ ,  $p < .01$ ,  $R^2_{\text{adj}} = 0.06$ ), with species with greater LDMC showing steeper declines in  $F_v/F_m$  around  $T_{50}$ . LMA had a moderate, but statistically significant effect on  $b$  ( $F_{2,118} = 7.5$ ,  $p = .007$ ,  $R^2_{\text{adj}} = 0.05$ ).

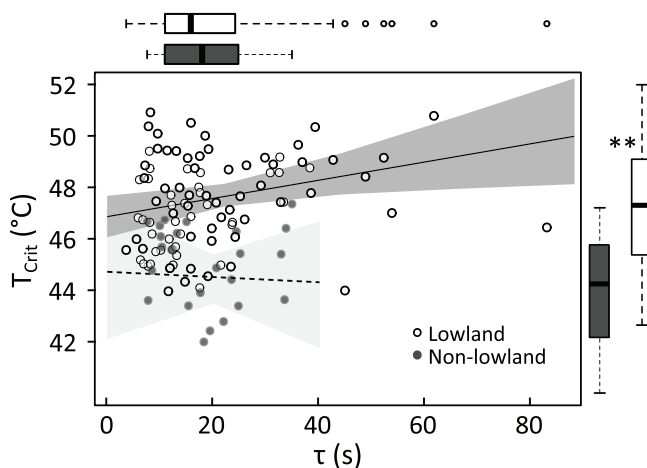
## 4 | DISCUSSION

We evaluated variation in heat tolerance of sun-exposed leaves among tropical forest plants in Panama and show that irreversible

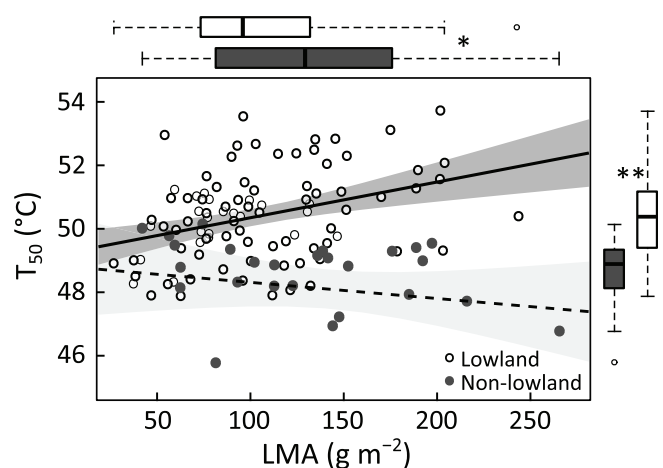
damage to leaves starts at temperatures about 10°C–15°C above current mean maximum air temperatures of the warmest month, but only a few degrees above the highest leaf temperatures measured in the field (Doughty & Goulden, 2008; Krause et al., 2010; Rey-Sanchez et al., 2016; Slot, Garcia, & Winter, 2016). Heat tolerance of plants with  $C_3$  photosynthesis was lower than that of the CAM species *Agave americana* and *Furcraea cabuya*, but had wide ranges nonetheless, with  $T_{\text{Crit}}$  being more variable than  $T_{50}$ . As hypothesized, interspecific variation in heat tolerance was underpinned, in part, by differences in elevation and associated ambient temperatures across sites, and related to differences in leaf functional traits associated with thermoregulation and leaf construction costs within sites, particularly among lowland species, while phylogeny played a smaller role.

### 4.1 | Heat tolerance decreases with elevation

The decrease in heat tolerance with elevation and associated change in temperature is not a function of species turnover and was observed both across and within species. This trend is consistent with patterns along an elevation gradient in Colombia (Feeley, Martinez-Villa, et al., 2020), and with trends in heat tolerance with mean annual or mean growing season temperature across latitudes (O'Sullivan et al., 2017; Zhu et al., 2018). The fact that elevation was a better predictor of heat tolerance than MAT or the mean maximum temperature of the warmest month may reflect greater accuracy of elevation than of temperature data, as the WorldClim temperature data is spatially gridded at  $\sim 1 \text{ km}^2$  resolution and thus less accurate than measurements at the collection sites—detailed micrometeorological data can be expected to improve our understanding of the effects of growth



**FIGURE 5** Leaf critical temperature ( $T_{\text{Crit}}$ ) as a function of the estimated thermal time constant ( $\tau$ ) and elevation. Solid and dashed lines indicate significant and non-significant correlations for lowland and non-lowland data, respectively. Shaded areas indicate 95% confidence intervals of the predictions. Boxplots illustrate lowland (open bars) versus non-lowland (mid-elevation and pre-montane) sites (closed bars). \*\*  $p < .001$



**FIGURE 6**  $T_{50}$  as a function of leaf mass per area (LMA) and elevation. Solid and dashed lines indicate significant and non-significant correlations for lowland and non-lowland data, respectively. Shaded areas indicate 95% confidence intervals of the predictions. Boxplots illustrate lowland (open bars) versus non-lowland (mid-elevation and pre-montane) sites (closed bars). \*  $p < .05$ , \*\*  $p < .01$

environment on heat tolerance.  $T_{50}$  increased by 1.6°C from a mean of 48.5°C at Cerro Jefe to 50.1°C in Panama City, while MAT increased by 4.9°C from 22.1°C to 27.0°C, with an average increase across sites of 0.4°C per °C MAT. Thus, while there is a clear adjustment to temperature along this gradient, the thermal safety margin based on MAT—that is, the difference between MAT and  $T_{50}$ —decreases with increasing growth temperature and is smallest in the already relatively hot lowlands.

The 0.4°C increase in  $T_{50}$  per °C change in MAT was comparable to the 0.38°C increase in  $T_{50}$  of  $F_0$  rise per °C mean temperature of the warmest month in the global dataset by O'Sullivan et al. (2017). However, along the tropical elevation gradient studied by Feeley, Martinez-Villa, et al. (2020),  $T_{50}$  increased by only 0.08°C per °C MAT. One possible reason for the strong response of heat tolerance to local temperature compared to Feeley, Martinez-Villa, et al. (2020) is the relatively narrow ranges in environmental conditions in the current study. Heat tolerance can increase with drought, cold, and other stressors (Kappen, 1964; Ladjal, Epron, & Ducrey, 2000; Sapper, 1935; Sastry, Guha, & Barua, 2018), as mild conditioning to other stressors may increase antioxidant scavenging (Gill & Tuteja, 2010) that would also benefit heat tolerance.  $T_{50}$  of the warmest site in Feeley, Martinez-Villa, et al. (2020) was comparable to that observed in the current study, so it is the cooler sites in Feeley et al. that have relatively high heat tolerance. The coolest site in Feeley, Martinez-Villa, et al. (2020) is at almost 3,000 m asl. At such elevation UV radiation can be strong, potentially causing cellular damage against which antioxidant activity can provide protection (Foyer, Lelandais, & Kunert, 1994). At another high-UV site in Colombia,  $T_{50}$  values averaged 49.2°C (Leon-Garcia & Lasso, 2019), despite mean annual temperatures of <10°C, supporting the notion that high heat tolerance is not solely associated with high growing temperatures. The growing conditions at our pre-montane sites are favourable for most plants, and the elevation gradient represents a temperature gradient not confounded by gradients in other stressors such as UV radiation, cold, or drought.

Of the seven species collected at multiple sites, three were collected both in lowland and in pre-montane forest. Of these, two showed the expected pattern in heat tolerance with elevation and temperature; *Aspidosperma spruceanum*—primarily a lowland species—and *Posoqueria latifolia*—a species widely distributed across lowland and pre-montane sites. The species that did not show any trends was *Podocarpus guatemalensis*, a gymnosperm species of lower montane regions. This species maintained comparatively low heat tolerance when it was growing under warm lowland conditions and had the lowest  $F_v/F_m$  of control plants among all plants included in the study. This is consistent with observations that while heat tolerance can be downregulated in response to (seasonal) low temperature (e.g., Sastry & Barua, 2017; Zhu et al., 2018), there is limited evidence for the upregulation of heat tolerance in response to warming (but see Drake et al., 2018), especially of tropical species (Krause et al., 2010; Krause, Cheesman, Winter, Krause, & Virgo, 2013).

## 4.2 | Limited phylogenetic patterns in physiologically plastic traits

Our study included species belonging to families that differ widely in their earliest occurrence in evolutionary history, and therefore in climate regimes they have experienced throughout their existence. For example, Fabaceae have existed in the Neotropics for at least 58 million years (Wing et al., 2009) and their relative abundance did not decrease during the Paleocene-Eocene Thermal Maximum (~56 Ma) when temperatures in the tropics rapidly rose by 3°C–5°C (Jaramillo et al., 2010). We found no evidence, however, that Fabaceae or other relatively old families have greater heat tolerance and are therefore better equipped for end-of-century conditions than taxa that emerged more recently. Knight and Ackerly (2003) found that congeneric species of Californian coastal and desert ecosystems had very similar  $T_{50}$  when grown in a common environment, despite maximum temperatures being 10°C greater in the desert than in the coastal region. The lack of convergent adaptation of heat tolerance suggested high plasticity in heat tolerance instead (Knight & Ackerly, 2003). Across the phylogenetic tree there were some distinct clusters, indicating similarity in heat tolerance among sister species; overall the phylogenetic signal in heat tolerance was, however, weak. Species-level replication was limited in the current study, and if intraspecific variation would be significant, greater replication could potentially slightly change heat tolerance estimates and therefore affect the phylogenetic analysis. Nonetheless, the absence of strong phylogenetic patterns in heat tolerance is in accordance with other recent studies (Lancaster & Humphreys, 2020; Perez & Feeley, 2021). Furthermore, the predominance of plasticity over evolutionary legacies is consistent with the plasticity in the temperature relations of other aspects of metabolism in trees from the aseasonal lowland tropics, such as the convergence of the optimum temperature for photosynthesis on local mean temperatures in diverse forest communities (Slot & Winter, 2017a), and acclimation of photosynthesis (Slot & Winter, 2017b) and leaf respiration (Cheesman & Winter, 2013; Slot et al., 2014) to experimental warming.

## 4.3 | Large-leaved species and slow-cooling species have higher heat tolerance

Leaf width is an important driver of leaf temperature (Fauset et al., 2018; Leigh, Sevanto, Close, & Nicotra, 2017), as is overall leaf size (Leigh et al., 2017), with wide and large leaves warming up more than narrow and small ones under similar conditions.  $T_{crit}$  increased with both leaf width and leaf size when elevation was accounted for. This supports the idea that species with a thick boundary layer that are more likely to experience high temperatures should have higher tolerance to those temperatures. Indeed, Perez and Feeley (2020) recently showed that  $T_{50}$  correlated positively with the highest measured leaf temperature among 19 species in the Fairchild Tropical Botanical Garden in Florida.

Heat tolerance—specifically  $T_{\text{Crit}}$ , the temperature associated with the onset of thermal damage—increases with the estimated thermal time constant  $\tau$ , while it was hypothesized to decrease. Species with short  $\tau$  can rapidly heat up, so they were expected to have a selective advantage of high heat tolerance. However, they also cool down again quickly, so while they may experience higher temperatures, these high temperatures are unlikely to be sustained for long periods of time. The protocol we used to determine heat tolerance involves 15-min exposure to set temperatures, and it is the exposure to 15 min of  $T_{\text{Crit}}$  that causes the initiation of irreversible heat damage. Species with long  $\tau$  are more buffered against temperature fluctuations and thus more likely to experience a temperature for 15 min continuously than species with short  $\tau$ , even if the peak temperatures are lower than those short- $\tau$  species experience. We could not assess whether long  $\tau$  leaves in the current study indeed experienced more sustained high temperatures in situ and short  $\tau$  leaves higher but shorter peak temperatures, nor whether large-leaved species were consistently warmer than small-leaved species, as high-resolution, long-term monitoring of leaf temperatures of 147 tropical forest species was not feasible, although knowledge of micrometeorology and leaf temperatures will be critical in furthering our understanding of how heat tolerance varies across environments. New developments in wireless sensor networks and infrared thermometry provide avenues for detailed monitoring of canopy microclimates and leaf temperatures in the near future (e.g., Egea, Padilla-Díaz, Martínez-Guanter, Fernández, & Pérez-Ruiz, 2017; Jin, Wang, Jiang, & Chen, 2018; Webster, Westoby, Rutter, & Jonas, 2018), to determine variation in the thermal safety margins across species, and their underlying morphological and physiological traits.

#### 4.4 | Heat tolerance in relation to leaf construction costs

$T_{50}$  scaled with LMA, supporting the hypothesis that heat tolerance increases with increasing construction costs of the leaves. This relationship was strong among lowland species, but the (considerably smaller) variation in  $T_{50}$  among mid-elevation and pre-montane species was driven by other, unidentified factors. Previous links between heat tolerance and leaf cost traits LMA and LDMC were obtained at sites with considerable temperature seasonality (e.g., Knight & Ackerly, 2003; Sastry & Barua, 2017). In the current study temperature seasonality was minimal, with the difference in mean temperature between the hottest and the coolest month  $\leq 2.0^\circ\text{C}$  at all sites, including the lowland sites. In these lowland forests LMA correlates strongly with leaf lifespan (Kitajima & Poorter, 2010). While greater leaf lifespan does not significantly increase the likelihood of exposure to higher air temperature, leaf temperature may be considerably higher in the dry season, when incoming solar radiation at the canopy level is greater (Rey-Sanchez et al., 2016). Increased heat tolerance may thus be advantageous for leaves that persist through the dry season, and this may be more important in lowland species with smaller thermal safety margins than in comparatively cool higher-elevation

species. Evergreen species did not have higher heat tolerance than deciduous species in a subset of 99 species for which this information was known (Figure S5), but more detailed information on leaf phenology may be required to evaluate the effects of potentially high dry season leaf temperatures.

#### 4.5 | Heat tolerance metrics, their relations, and identifying large-scale patterns

Tiwari et al. (2021) recently showed that the higher  $T_{\text{Crit}}$  was among seven Amazonian tree species, the steeper the decrease in  $F_v/F_m$  above  $T_{\text{Crit}}$ . We found the same pattern across the 147 species in our study. As  $T_{\text{Crit}}$  increased in our study, the  $b$  parameter became more negative ( $r^2 = 0.29$ ).  $T_{50}$  of lowland tropical species appears to be close to a hard, upper limit, as suggested by the fact that growth at elevated temperature does not significantly increase  $T_{50}$  (Krause et al., 2010, 2013). Indeed,  $T_{50}$  of tropical plants tends to be less variable than  $T_{\text{Crit}}$  (Tiwari et al., 2021; Perez & Feeley, 2020; current study). This suggests that when plants can prevent irreversible damage up to a higher temperature (i.e., have a higher  $T_{\text{Crit}}$ ) the decrease in  $F_v/F_m$  is necessarily steeper. This also means that across a temperature gradient,  $T_{\text{Crit}}$  increases more steeply with temperature than  $T_{50}$ . Interspecific variation in  $b$  also explains why  $T_{50}$  and  $T_{\text{Crit}}$ , although correlated, did not scale equally with other leaf traits. For example, we found a significant relationship between  $T_{50}$  and LMA among lowland plants, but not between  $T_{\text{Crit}}$  and LMA, because  $b$  correlated with LMA in such a way that the slope of the  $T_{\text{Crit}}$ -LMA relationship was reduced relative to the  $T_{50}$ -LMA slope.

In 1935, Sapper (1935) wrote that since the pioneering work by Sachs (1864) various studies had been conducted to investigate plant heat tolerance, but that systematic comparisons are complicated by the all-too-different methodologies used. This remains true 85 years later (Geange et al., 2021). A recent study (Lancaster & Humphreys, 2020) compiled heat tolerance data from across the plant kingdom, combining metrics with fundamentally different meanings, such as  $T_{50}$  of  $F_v/F_m$  as defined in the current study (using data from, e.g., Slot et al., 2019),  $T_{50}$  of  $F_0$  rise (e.g., Zhu et al., 2018),  $T_{\text{Crit}}$  of  $F_0$  rise (Marias, Meinzer, Woodruff, & McCulloh, 2016),  $T_{\text{Max}}$  of dark respiration (e.g., O'Sullivan et al., 2017), and heat tolerance based on electrolyte leakage (e.g., Nobel & Smith, 1983). Identifying reliable, general patterns of thermal tolerance across environmental gradients thus remains challenging, and more studies are needed using consistent methodology (Geange et al., 2021). Here, we studied >110 lowland species—the largest dataset on heat tolerance of lowland tropical forest plants to date—and almost 40 mid-elevation to pre-montane species, measured with a commonly used protocol that was tested against the classical leaf necrosis assay (Krause et al., 2010). We show that ~30% of the heat tolerance variation across a phylogenetically and functionally diverse range of species could be explained by elevation and LMA alone. Site elevation is readily available, and LMA is one of the best represented traits in the TRY database (Kattge et al., 2020), making this a useful predictor, at least for the species-

rich and understudied lowland tropics—at higher elevation neither LMA, nor indeed any of the tested traits was a strong predictor of heat tolerance. Remaining variation is likely explained in part by variation in microclimate, which has been shown to have strong effects on maximum leaf temperatures (Fauset et al., 2018), and heat tolerance (Curtis, Gollan, Murray, & Leigh, 2016; Perez & Feeley, 2020); differences in preconditioning to mild stressors that caused upregulation in antioxidant activity; and variation in other aspects that may protect leaves against heat damage, such as the capacity for isoprene emission, which is associated with higher maximum temperature for carbon fixation among tropical forest species (Taylor, Smith, Slot, & Feeley, 2019).

Tropical forests are sensitive to rising temperatures. Warming decreases net carbon uptake (Clark, Piper, Keeling, & Clark, 2003; Sullivan et al., 2020), results in shifts in species composition (e.g., Fadrique et al., 2018; Feeley, Bravo-Avila, Fadrique, Perez, & Zuleta, 2020), and exacerbates climate extremes (Rifai, Li, & Malhi, 2019) and the risk of associated mortality. Due to the increased frequency of extreme events  $T_{crit}$  may already be exceeded occasionally in forests in the southern Amazon (Tiwari et al., 2021). We showed that lowland forests in Panama are operating closer to their critical upper temperature thresholds than pre-montane forests, but that in both ecosystems species differ considerably in their proximity to critical thresholds. To properly determine thermal safety margins detailed information is needed on variation in leaf temperature dynamics among co-occurring species, and predicting future safety margins further requires understanding of the plasticity of heat tolerance; upregulation of heat tolerance appears limited, but this needs to be tested for more tropical forest species.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHORS CONTRIBUTIONS

Martijn Slot and Klaus Winter designed the study; Daniela Cala, Martijn Slot, Jorge Aranda and Aurelio Virgo collected the samples; Jorge Aranda and Aurelio Virgo identified species, helped locate target species, and provided logistical support in the field and lab; Daniela Cala measured fluorescence and leaf traits; Martijn Slot and Daniela Cala performed the analyses; Sean T. Michaletz calculated thermal time constants and interpreted their results; Martijn Slot wrote the

manuscript with contributions of Daniela Cala, Klaus Winter, and Sean T. Michaletz.

## DATA AVAILABILITY STATEMENT

All species-level heat tolerance data are presented in the Table S2. Raw chlorophyll fluorescence data will be made available on FigShare, doi: 10.25573/data.14183045.

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## REFERENCES

- Barton, K. (2020). MuMIn: Multi-Model Inference. R package version 1.43.17. Retrieved from <https://CRAN.R-project.org/package=MuMIn>
- Blomberg, S. P., Garland, T., Jr., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, 57(4), 717–745.
- Cheesman, A. W., & Winter, K. (2013). Elevated night-time temperatures increase growth in seedlings of two tropical pioneer tree species. *New Phytologist*, 197(4), 1185–1192.
- Clark, D. A., Piper, S. C., Keeling, C. D., & Clark, D. B. (2003). Tropical rain forest tree growth and atmospheric carbon dynamics linked to inter-annual temperature variation during 1984–2000. *Proceedings of the National Academy of Sciences*, 100(10), 5852–5857.
- Coley, P. D. (1987). Interspecific variation in plant anti-herbivore properties: The role of habitat quality and rate of disturbance. *New Phytologist*, 106(1), 251–263.
- Curtis, E. M., Gollan, J., Murray, B. R., & Leigh, A. (2016). Native microhabitats better predict tolerance to warming than latitudinal macroclimatic variables in arid-zone plants. *Journal of Biogeography*, 43(6), 1156–1165.
- Curtis, E. M., Knight, C. A., & Leigh, A. (2019). Intracanalopy adjustment of leaf-level thermal tolerance is associated with microclimatic variation across the canopy of a desert tree (*Acacia papyrocarpa*). *Oecologia*, 189(1), 37–46.
- Dick, C. W., Lewis, S. L., Maslin, M., & Bermingham, E. (2013). Neogene origins and implied warmth tolerance of Amazon tree species. *Ecology and Evolution*, 3(1), 162–169.
- Doughty, C. E., & Goulden, M. L. (2008). Are tropical forests near a high temperature threshold? *Journal of Geophysical Research - Biogeosciences*, 113(G1).
- Drake, J. E., Tjoelker, M. G., Vårhammar, A., Medlyn, B. E., Reich, P. B., Leigh, A., ... Crous, K. Y. (2018). Trees tolerate an extreme heatwave via sustained transpirational cooling and increased leaf thermal tolerance. *Global Change Biology*, 24(6), 2390–2402.
- Duarte, H., Tejedo, M., Katzenberger, M., Marangoni, F., Baldo, D., Beltrán, J. F., ... Gonzalez-Voyer, A. (2012). Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Global Change Biology*, 18(2), 412–421.
- Egea, G., Padilla-Díaz, C. M., Martínez-Guanter, J., Fernández, J. E., & Pérez-Ruiz, M. (2017). Assessing a crop water stress index derived from aerial thermal imaging and infrared thermometry in super-high density olive orchards. *Agricultural Water Management*, 187, 210–221.
- Fadrique, B., Báez, S., Duque, Á., Malizia, A., Blundo, C., Carilla, J., ... Feeley, K. J. (2018). Widespread but heterogeneous responses of Andean forests to climate change. *Nature*, 564(7735), 207–212.
- Fauset, S., Freitas, H. C., Galbraith, D. R., Sullivan, M. J., Aidar, M. P., Joly, C. A., ... Gloor, M. U. (2018). Differences in leaf thermoregulation and water use strategies between three co-occurring Atlantic forest tree species. *Plant, Cell & Environment*, 41(7), 1618–1631.



- Feeley, K. J., Bravo-Avila, C., Fadrique, B., Perez, T. M., & Zuleta, D. (2020). Climate-driven changes in the composition of New World plant communities. *Nature Climate Change*, 10(10), 1062.
- Feeley, K. J., Martinez-Villa, J., Perez, T. M., Silva Duque, A., Triviño Gonzalez, D., & Duque, A. (2020). The thermal tolerances, distributions, and performances of tropical montane tree species. *Frontiers in Forest and Global Change*, 3, 25.
- Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37(12), 4302–4315.
- Foyer, C. H., Lelandais, M., & Kunert, K. J. (1994). Photooxidative stress in plants. *Physiologia Plantarum*, 92(4), 696–717.
- Franken, O., Huizinga, M., Ellers, J., & Berg, M. P. (2018). Heated communities: Large inter- and intraspecific variation in heat tolerance across trophic levels of a soil arthropod community. *Oecologia*, 186(2), 311–322.
- García-Robledo, C., Kuprewicz, E. K., Staines, C. L., Erwin, T. L., & Kress, W. J. (2016). Limited tolerance by insects to high temperatures across tropical elevational gradients and the implications of global warming for extinction. *Proceedings of the National Academy of Sciences*, 113(3), 680–685.
- Geange, S. R., Arnold, P. A., Catling, A. A., Coast, O., Cook, A. M., Gowland, K. M., ... Zhu, L. (2021). The thermal tolerance of photosynthetic tissues: A global systematic review and agenda for future research. *New Phytologist*, 229(5), 2497–2513.
- Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48(12), 909–930.
- Goulden, M. L., Miller, S. D., da Rocha, H. R., Menton, M. C., de Freitas, H. C., Figueira, M., & de Sousa, C. A. D. (2004). Diel and seasonal patterns of tropical forest CO<sub>2</sub> exchange. *Ecological Applications*, 14(4), 42–54.
- Jaramillo, C., Ochoa, D., Contreras, L., Pagani, M., Carvajal-Ortiz, H., Pratt, L. M., ... Rodriguez, G. (2010). Effects of rapid global warming at the Paleocene-Eocene boundary on neotropical vegetation. *Science*, 330(6006), 957–961.
- Jin, J., Wang, Y., Jiang, H., & Chen, X. (2018). Evaluation of microclimatic detection by a wireless sensor network in forest ecosystems. *Scientific Reports*, 8(1), 1–9.
- Jin, Y., & Qian, H. (2019). V. PhyloMaker: An R package that can generate very large phylogenies for vascular plants. *Ecography*, 42(8), 1353–1359.
- Jones, H. G. (2013). *Plants and microclimate: A quantitative approach to environmental plant physiology* (3rd ed.). Cambridge, England: Cambridge University Press.
- Kappen, L. (1964). Untersuchungen über den Jahreslauf der Frost-, Hitze- und Austrocknungsresistenz von Sporophyten einheimischer Polypodiaceen (Filicinae). *Flora*, 155(1), 123–166.
- Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., ... Wirth, C. (2020). TRY plant trait database—enhanced coverage and open access. *Global Change Biology*, 26(1), 119–188.
- Kitajima, K., & Poorter, L. (2010). Tissue-level leaf toughness, but not lamina thickness, predicts sapling leaf lifespan and shade tolerance of tropical tree species. *New Phytologist*, 186(3), 708–721.
- Knight, C. A., & Ackerly, D. D. (2003). Evolution and plasticity of photosynthetic thermal tolerance, specific leaf area and leaf size: Congeneric species from desert and coastal environments. *New Phytologist*, 160(2), 337–347.
- Kosugi, Y., Takahashi, S., Ohkubo, S., Matsuo, N., Tani, M., Mitani, T., ... Nik, A. R. (2008). CO<sub>2</sub> exchange of a tropical rainforest at Pasoh in peninsular Malaysia. *Agricultural and Forest Meteorology*, 148(3), 439–452.
- Krause, G. H., Cheesman, A. W., Winter, K., Krause, B., & Virgo, A. (2013). Thermal tolerance, net CO<sub>2</sub> exchange and growth of a tropical tree species, *Ficus insipida*, cultivated at elevated daytime and nighttime temperatures. *Journal of Plant Physiology*, 170(9), 822–827.
- Krause, G. H., Winter, K., Krause, B., Jahns, P., García, M., Aranda, J., & Virgo, A. (2010). High-temperature tolerance of a tropical tree, *Ficus insipida*: Methodological reassessment and climate change considerations. *Functional Plant Biology*, 37(9), 890–900.
- Krause, G. H., Winter, K., Krause, B., & Virgo, A. (2016). Protection by light against heat stress in leaves of tropical crassulacean acid metabolism plants containing high acid levels. *Functional Plant Biology*, 43(11), 1061–1069.
- Ladjal, M., Epron, D., & Ducrey, M. (2000). Effects of drought preconditioning on thermotolerance of photosystemII and susceptibility of photosynthesis to heat stress in cedar seedlings. *Tree Physiology*, 20(18), 1235–1241.
- Lancaster, L. T., & Humphreys, A. M. (2020). Global variation in the thermal tolerances of plants. *Proceedings of the National Academy of Sciences*, 117(24), 13580–13587.
- Leigh, A., Sevanto, S., Close, J. D., & Nicotra, A. B. (2017). The influence of leaf size and shape on leaf thermal dynamics: Does theory hold up under natural conditions? *Plant, Cell & Environment*, 40(2), 237–248.
- Leon-Garcia, I. V., & Lasso, E. (2019). High heat tolerance in plants from the Andean highlands: Implications for paramos in a warmer world. *PLoS One*, 14(11), e0224218.
- Lin, H., Chen, Y., Zhang, H., Fu, P., & Fan, Z. (2017). Stronger cooling effects of transpiration and leaf physical traits of plants from a hot dry habitat than from a hot wet habitat. *Functional Ecology*, 31(12), 2202–2211.
- Marias, D. E., Meinzer, F. C., Woodruff, D. R., & McCulloh, K. A. (2016). Thermotolerance and heat stress responses of Douglas-fir and ponderosa pine seedling populations from contrasting climates. *Tree Physiology*, 37(3), 301–315.
- Michaletz, S. T., Weiser, M. D., McDowell, N. G., Zhou, J., Kaspari, M., Helliker, B. R., & Enquist, B. J. (2016). The energetic and carbon economic origins of leaf thermoregulation. *Nature Plants*, 2(9), 16129.
- Michaletz, S. T., Weiser, M. D., Zhou, J., Kaspari, M., Helliker, B. R., & Enquist, B. J. (2015). Plant thermoregulation: Energetics, trait-environment interactions, and carbon economics. *Trends in Ecology & Evolution*, 30(12), 714–724.
- Nardini, A., Pedà, G., & Rocca, N. L. (2012). Trade-offs between leaf hydraulic capacity and drought vulnerability: Morpho-anatomical bases, carbon costs and ecological consequences. *New Phytologist*, 196(3), 788–798.
- Nobel, P. S., & Smith, S. D. (1983). High and low temperature tolerances and their relationships to distribution of agaves. *Plant, Cell & Environment*, 6(9), 711–719.
- O'Sullivan, O. S., Heskell, M. A., Reich, P. B., Tjoelker, M. G., Weerasinghe, L. K., Penillard, A., ... Atkin, O. K. (2017). Thermal limits of leaf metabolism across biomes. *Global Change Biology*, 23(1), 209–223.
- Padfield, D., Lowe, C., Buckling, A., French-Constant, R., Student Research Team, Jennings, S., ... Yvon-Durocher, G. (2017). Metabolic compensation constrains the temperature dependence of gross primary production. *Ecology Letters*, 20(10), 1250–1260.
- Padfield, D., & Matheson, G. (2018). nls.multstart: Robust Non-Linear Regression using AIC Scores. R package version 1.0.0. Retrieved from <https://CRAN.R-project.org/package=nls.multstart>
- Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, 401(6756), 877–884.
- Perez, T. M., & Feeley, K. J. (2020). Photosynthetic heat tolerances and extreme leaf temperatures. *Functional Ecology*, 34(11), 2236–2245.
- Perez, T. M., & Feeley, K. J. (2021). Weak phylogenetic and climatic signals in plant heat tolerance. *Journal of Biogeography*, 48(1), 91–100.
- Phillips, J. R., Sastry, S. K., & Morrow, C. T. (1983). Prediction of sprinkler off-times during frost protection of apple buds: A heat transfer model. *Transactions of the ASME*, 26(5), 1430–1434.
- R Core Team. (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing Retrieved from <https://www.R-project.org/>



- Revell, L. J. (2010). Phylogenetic signal and linear regression on species data. *Methods in Ecology and Evolution*, 1(4), 319–329.
- Revell, L. J. (2012). Phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3(2), 217–223.
- Rey-Sanchez, C., Slot, M., Posada, J. M., & Kitajima, K. (2016). Spatial and seasonal variation of leaf temperature within the canopy of a tropical forest. *Climate Research*, 71(1), 75–89.
- Rifai, S. W., Li, S., & Malhi, Y. (2019). Coupling of El Niño events and long-term warming leads to pervasive climate extremes in the terrestrial tropics. *Environmental Research Letters*, 14(10), 105002.
- Sachs, J. (1864). Über die obere Temperaturgränze der Vegetation. *Flora*, 47, 5–12 24–29, 33–39, 65–75.
- Sapper, I. (1935). Versuche zur Hitzeresistenz der Pflanzen. *Planta*, 23(4), 518–556.
- Sastry, A., & Barua, D. (2017). Leaf thermotolerance in tropical trees from a seasonally dry climate varies along the slow-fast resource acquisition spectrum. *Scientific Reports*, 7(1), 11246.
- Sastry, A., Guha, A., & Barua, D. (2018). Leaf thermotolerance in dry tropical forest tree species: Relationships with leaf traits and effects of drought. *AoB Plants*, 10(1), plx070.
- Scheffer, M. (2009). *Critical transitions in nature and society* (Vol. 16). Princeton, NJ: Princeton University Press.
- Slot, M., Garcia, M. N., & Winter, K. (2016). Temperature response of CO<sub>2</sub> exchange in three tropical tree species. *Functional Plant Biology*, 43(5), 468–478.
- Slot, M., Krause, G. H., Krause, B., Hernández, G. G., & Winter, K. (2019). Photosynthetic heat tolerance of shade and sun leaves of three tropical tree species. *Photosynthesis Research*, 141(1), 119–130.
- Slot, M., Rey-Sánchez, C., Gerber, S., Lichstein, J. W., Winter, K., & Kitajima, K. (2014). Thermal acclimation of leaf respiration of tropical trees and lianas: Response to experimental canopy warming, and consequences for tropical forest carbon balance. *Global Change Biology*, 20(9), 2915–2926.
- Slot, M., & Winter, K. (2017a). In situ temperature response of photosynthesis of 42 tree and liana species in the canopy of two Panamanian lowland tropical forests with contrasting rainfall regime. *New Phytologist*, 214(3), 1103–1117.
- Slot, M., & Winter, K. (2017b). Photosynthetic acclimation to warming in tropical forest tree seedlings. *Journal of Experimental Botany*, 68(9), 2275–2284.
- Smith, S. A., & Brown, J. W. (2018). Constructing a broadly inclusive seed plant phylogeny. *American Journal of Botany*, 105(3), 302–314.
- Sullivan, M. J., Lewis, S. L., Affum-Baffoe, K., Castilho, C., Costa, F., Sanchez, A. C., ... Phillips, O. L. (2020). Long-term thermal sensitivity of Earth's tropical forests. *Science*, 368(6493), 869–874.
- Taylor, T. C., Smith, M. N., Slot, M., & Feeley, K. J. (2019). The capacity to emit isoprene differentiates the photosynthetic temperature responses of tropical plant species. *Plant, Cell & Environment*, 42(8), 2448–2457.
- Tiwari, R., Gloor, E., da Cruz, W. J. A., Schwantes Marimon, B., Marimon-Junior, B. H., Reis, S. M., ... Galbraith, D. (2021). Photosynthetic quantum efficiency in south-eastern Amazonian trees may be already affected by climate change. *Plant, Cell & Environment* In press. <https://doi.org/10.1111/pce.13770>
- Tserej, O., & Feeley, K. J. (2021). Variation in leaf temperatures of tropical and subtropical trees are related to leaf thermoregulatory traits and not geographic distributions. *Biotropica* In press. <https://doi.org/10.1111/btp.12919>
- Webster, C., Westoby, M., Rutter, N., & Jonas, T. (2018). Three-dimensional thermal characterization of forest canopies using UAV photogrammetry. *Remote Sensing of Environment*, 209, 835–847.
- Wikström, N., Savolainen, V., & Chase, M. W. (2001). Evolution of the angiosperms: Calibrating the family tree. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268(1482), 2211–2220.
- Wing, S. L., Herrera, F., Jaramillo, C. A., Gómez-Navarro, C., Wilf, P., & Labandeira, C. C. (2009). Late Paleocene fossils from the Cerrejón formation, Colombia, are the earliest record of Neotropical rainforest. *Proceedings of the National Academy of Sciences*, 106(44), 18627–18632.
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., ... Villar, R. (2004). The world-wide leaf economics spectrum. *Nature*, 428, 821–827.
- Zanne, A. E., Tank, D. C., Cornwell, W. K., Eastman, J. M., Smith, S. A., FitzJohn, R. G., ... Royer, D. L. (2014). Three keys to the radiation of angiosperms into freezing environments. *Nature*, 506(7486), 89–92.
- Zhu, L., Bloomfield, K. J., Hocart, C. H., Egerton, J. J., O'Sullivan, O. S., Penillard, A., ... Atkin, O. K. (2018). Plasticity of photosynthetic heat tolerance in plants adapted to thermally contrasting biomes. *Plant, Cell & Environment*, 41(6), 1251–1262.
- Zotz, G., Harris, G., Königer, M., & Winter, K. (1995). High rates of photosynthesis in the tropical pioneer tree, *Ficus insipida* Willd. *Flora*, 190(3), 265–272.

## SUPPORTING INFORMATION

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

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