# Thermal safety margins of plant leaves across biomes under a heatwave

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## HIGHLIGHTS

- It is urgent to know thermal safety of plants across biomes under climate warming.
- Thermal safety margin (TSM) of PSII was used to measure thermal safety of plants.
- TSM increased from hot to cool forests, and savanna forest is vulnerable under heatwave.
- Thermotolerances of leaves showed opposite trends with TSMs across biomes.
- The maximum leaf temperature instead of thermotolerance can predict TSM of plant leaves.

## GRAPHICAL ABSTRACT
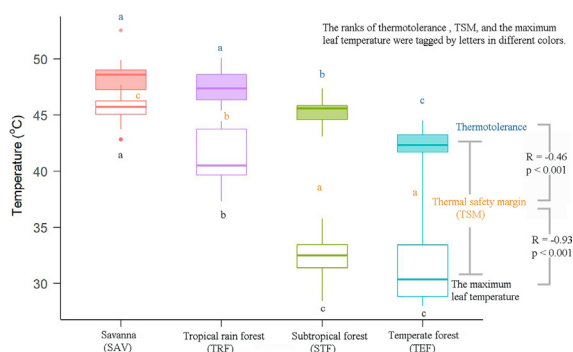


## ARTICLE INFO

## ABSTRACT

Climate change has great impacts on forest ecosystems, especially with the increasing frequency of heatwaves. Thermal safety margin (TSM) calculated by the difference between body temperature and thermotolerance threshold is useful to predict thermal safety of organisms. It has been widely used for animals, whereas has rarely been reported for plants. Besides, most of the previous studies used only thermotolerance to estimate thermal safety or used thermotolerance and air temperature (Ta) to calculate TSM. However, leaf temperature (Tl) is the real "body" temperature of plant leaves. Tl decoupling from Ta might induce large error in TSM. Here, we investigated TSM of photosystem II (thermotolerance of PSII – the maximum Tl) of dominant canopy plants in four forests from tropical to temperate biomes during a heatwave, and compared the TSMs calculated by Tl (TSM.Tl) and Ta (TSM.Ta) respectively. Also, thermal related leaf traits were investigated. The results showed that both TSM.Tl and TSM.Ta decreased from the cool forests to the hot forests. TSM.Tl was highly correlated with the maximum leaf temperature (Tlmax), while had an opposite trend with thermotolerance across biomes. Thus, Tlmax instead of thermotolerance can be used to evaluate TSM. The maximum Ta (Tamax), Tlmax and leaf traits explained 68% of the variance of thermotolerance in a random forest model, where Tamax and Tlmax explained 62%. TSM.Ta could not distinguish thermal safety differences between co-occurring species. The overestimation of TSM by TSM.Ta increased from the tropical to the temperate forest, and increased with Tl within biome. Therefore, it is not recommended to use TSM.Ta in cold forests. The present study enriches the dataset of photosynthetic TSMs across biomes, proposes using Tlmax to estimate TSMs of leaves, and highlights the risk of hot dry forest during heatwaves.

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## 1. Introduction

Global warming has caused an increase in the frequency and intensity of extreme weather, especially droughts and heatwaves (Alexander et al., 2006; Hansen et al., 2012). Globally, it is projected that heatwaves will be quadruple by 2040 (Coumou and Robinson, 2013). Heatwave events have caused severe reduction in forest and agriculture productivity (Ciais et al., 2005; Tatarinov et al., 2016), and large scale tree mortality (Allen et al., 2015; Allen et al., 2010; Chaste et al., 2019). High temperature associated with drought will reduce net photosynthesis by suppressing assimilation rates and promoting respiration (Teskey et al., 2015), and even damage photosynthetic components (Havaux, 1993). Measurements in a Brazil tropical forest showed a rapid decrease in leaf photosynthesis above 37.5 °C (Doughty and Goulden, 2008). Accurately evaluating how plants will be affected by climate change is important to predict species change in plant communities and protect natural resources.

Although thermotolerance is an ability of plants to survive under high temperature, it is not enough to assess security of plants under hot stress, i.e. thermal safety. A leaf with low thermotolerance might control leaf temperature well below its thermal limit by physical and physiological cooling (Lin et al., 2017), while a highly thermotolerant species might experience high leaf temperatures. With regard to this, thermal safety margin (TSM) which is defined as the difference between body temperature (leaf temperature for plants) and critical temperatures that represent threshold for function or lethality was proposed to assess thermal safety (Gunderson and Stillman, 2015). TSM has been widely applied in animal studies (Denny and Dowd, 2012; McArley et al., 2017; Pincebourde and Casas, 2019; Sunday et al., 2014; Vinagre et al., 2019), but its application in plants is lacking. Recently, it has begun to catch attention of researchers. There are reports of TSM of plants at particular sites (Araújo et al., 2021; Leon-Garcia and Lasso, 2019), across latitude gradients (O'Sullivan et al., 2017), and in common gardens (Ahrens et al., 2021; Cook et al., 2021; Curtis et al., 2016; Perez and Feeley, 2020; Sastry and Barua, 2017). However, TSM at one site cannot reveal TSM patterns across biomes. Plants growing in common gardens might have acclimated to local environment, thus had different TSMs from those in natural environments. In addition, most of the studies used air temperature instead of leaf temperature when calculating TSM (Curtis et al., 2016; O'Sullivan et al., 2017; Sastry and Barua, 2017).

Leaf temperature is the real "body temperature" for leaf metabolic processes, influencing leaf carbon economics (Michaletz et al., 2016; Michaletz et al., 2015). It can exceed air temperature up to 15 °C (Ackerly and Stuart, 2009), and varies across species and environment (Cook et al., 2021; Leuzinger and Körner, 2007). Even under the same environment, leaf temperature can be very different, depending on leaf physical and physiological traits (Fauset et al., 2018; Lin et al., 2017). Michaletz et al. (2015) demonstrated limited homeothermy of plants based on energy budget. Both site measurements and isotope analysis showed that leaf temperatures were more stable than air temperatures across biomes (Dong et al., 2017; Song et al., 2011). However, we still have no information of how the decoupling of leaf temperature and air temperature influences the difference between TSM calculated by leaf temperature (TSM.Tl) and air temperature (TSM.Ta) across biomes.

Leaf traits including morphological, optical, and physiological traits all have great impacts on leaf temperature (Gates, 2003). Convective cooling can be enhanced by reducing leaf size (Okajima et al., 2012; Smith, 1978); compound or dissected leaves have advantage of heat exchange by increasing the contact edge with air (Stokes et al., 2006); high water content or leaf mass area (LMA) can prolong leaf thermal time constant and delay leaf warming (Leigh et al., 2012; Smith, 1978); high reflectivity reduces radiation loads on leaves; and transpiration is efficient to cool leaves (Crawford et al., 2012; Lin et al., 2017). Leaf temperature is the direct micro-environment for leaf function, thus thermotolerance should acclimate to leaf temperature. Accordingly, leaf traits might affect thermotolerance through the impact on leaf temperature.

Aside of leaf temperature, thermotolerance is another important parameter in TSM calculation. Photosystem II is sensitive to temperature, and its thermotolerance can be quantified by heat induced change of chlorophyll fluorescence parameters: the initial fluorescence ($F_o$) or the ratio of variable to maximum fluorescence ($F_v/F_m$) (Baker, 2008). The critical temperature ($T_{crit}$) of the intersection of lines extrapolated from the slow and fast rise portion of the temperature-dependent fluorescence response ($F_o$-T curve), indicating the start point of the collapse of Photosystem II (Knight and Ackerly, 2002). The temperatures leading to 50% reduction in $F_v/F_m$ ratios is defined as $T_{50}$ (Knight and Ackerly, 2003; Krause et al., 2010). The two fluorescence parameters are positively correlated (Krause et al., 2010; Lancastera and Humphreys, 2020). Most studies of thermotolerance across biomes used $T_{crit}$, because $F_o$ can be continuously monitored in a heating bath with the same samples (Dahl et al., 2019; Knight and Ackerly, 2003; Lancastera and Humphreys, 2020; O'Sullivan et al., 2017; Song et al., 2011; Zhu et al., 2018). However, the measurement of $F_v/F_m$ needs new samples at each temperature gradient, and the results vary with the exposure time at the target temperature. Therefore, we used $T_{crit}$ in the present research. Some unified trends have been found for thermotolerances of plants: e.g. species from warmer habitats are inherently higher in thermotolerance; thermotolerance acclimates to growth temperature (Zhu et al., 2018); and plants from dry habitat are more thermotolerant than plants from wet habitat (Curtis et al., 2016; Knight and Ackerly, 2003). However, whether the maximum leaf temperatures vary proportionally to thermotolerance across biomes is unknown, thus global thermotolerance patterns corresponding to TSM are still unclear.

In the present study, we investigated TSM and leaf traits of plants in four forests across biomes (savanna, tropical rain forest, subtropical broad-leaved forest, temperate mixed forest) with contrasting precipitation and temperature (Table 1). Using these data, we (1) compare the patterns of thermotolerance and TSM across biomes to test the hypothesis that more thermotolerant plants are safer (higher TSM) than the plants with low thermotolerance; (2) evaluate thermal risk of plants across biomes, and test the hypothesis that the plants in hot dry forest are more risk than the plants in hot wet and cool forests, and (3) assess the difference between TSM.Tl and TSM.Ta to provide suggestions on the method of TSM measurement.

## 2. Materials and methods

### 2.1. Study sites and species selection

Four forests with contrasting temperature, precipitation and plant communities across biomes were selected in Yunnan province, south western China: savanna (SAV), tropical rain forest (TRF), subtropical broad-leaved forest (STF), and temperate mixed forest (TEF). Four dominant upper canopy species and three individuals for each species were chosen in each forest, according to the rank of richness in the upper canopy within reach. They covered all the species of emergent trees in TRF, all the canopy species in TEF, and the most important canopy species in STF and SAV. Healthy, sun-exposed, and fully mature leaves in the upper canopy were sampled for temperature and leaf traits measurement. We accessed to the tall canopy using canopy cranes at TRF and STF, and using ladders in SAV and TEF. Detailed information of the forests and species are given in Tables 1 and 2. All field measurements were conducted at the end of dry season in 2019 from May 13 to May 16 at TRF, May 19 to May 23 at STF, May 25 to May 28 at SAV, June 4 to June 7 at TEF. This period was the most severe heatwave in recent 10 years, which was widely spread in Yunnan province (Figs. S1 & S2).

### 2.2. Temperature measurement

Temperatures were measured by Type-T thermocouples (TT-T-30-SLE-1000, OMEGA, USA; diameter = 0.25 mm). To avoid thermocouples falling from leaves, we hung them on the adaxial surfaces of leaves and

**Table 1**
Site information.

| Forest | Abbreviation | Location | Elevation (m) | MTamax (°C) | MAP (mm) | Canopy height (m) |
|---|---|---|---|---|---|---|
| Savanna | SAV | 23°28′N, 102°10′E | 481 | 45.9 | 733 | 4–6 |
| Tropical rain forest | TRF | 21°22′N, 101°34′E | 704 | 38.4 | 1415 | >50 |
| Subtropical broad-leaved forest | STF | 24°32′N, 101°02′E | 2501 | 29.9 | 1931 | 25–30 |
| Temperate mixed forest | TEF | 27°00′N, 100°13′E | 3240 | 28.1 | 1300 | 25–30 |

MTamax, the maximum air temperature above the canopy in 2019, averaged by all the measure points on the canopy; MAP, mean annual precipitation.

fixed the tips with heat-conducting glue (Fig. S3). The glue can strengthen the attachment and block direct irradiation on the sensor head. We compared our method with the traditional method (using tape to attach thermocouples on the abaxial sides of leaves) for four species with $t$-test. Four leaves of each species in one individual were measured. Two of the species had slightly higher leaf temperatures on abaxial sides than on adaxial sides at noon ($p < 0.005$), while no significant differences were found for the other two species (Fig. S4). It demonstrated that the impact of direct sunshine on leaf temperature was not significant. To simulate the extreme drought situation, we selected 2 leaves with similar size, age and orientation beside the leaves with temperature measurements, put Vaseline on the abaxial side of the leaves to stop transpiration (all the leaves are hypostomatous), and recorded their temperatures (Tn) with the same type of thermocouples. Air temperatures were simultaneously measured by the same type of thermocouples near the leaves with temperature measurements, avoiding direct solar radiation. For each individual, we measured air temperature at one point (Ta), temperatures of 4 control sun leaves (Tl) and 2 Vaseline leaves (Tn).

All the temperatures were continuously recorded by data logger (UX120-04, HOBO, USA) at one-minute interval from May 13 to May 16 at TRF, May 19 to May 23 at STF, May 25 to May 28 at SAV, June 4 to June 7 at TEF in 2019. On each day, we extracted the 10 highest leaf temperatures, and took the minimum as Tlmax for that day. The maximum Tlmax among all these days was Tlmax for each individual. With this method, we confirmed the temperature which was equal and higher than Tlmax lasted for at least 10 min (assuming one record lasted for one minute).

### 2.3. Thermotolerance measurement

Thermotolerance was measured with PlanTherm PT100 (PSI, Czekh). The measurement of thermotolerance was based on the response of initial chlorophyll fluorescence to temperature ($F_o$-T curve) (Schreiber and Berry, 1977). Three sun leaves similar to the leaves with temperature measurements were sampled from each individual in the morning, dark adapted for at least half an hour in plastic bags

with wet tissue paper inside to prevent water loss. Leaves were rinsed with deionized water, cut rectangular segments (2 cm long) from the middle of the leaf avoiding main veins. Leaf segments were immersed into 5 ml deionized water in a cuvette, then set the temperature increasing rate at 2 °C·min$^{-1}$ from 25 °C to 70 °C. This heating rate is recommended by PSI company. We also compared $T_{crit}$ measured under different heating rates (1 °C·min$^{-1}$ and 2 °C·min$^{-1}$) with two species and three leaves for each species. Paired $t$-test showed no significant differences, and the mean difference between $T_{crit}$ under the two heating rates was −0.395 °C. A magnetic stirrer bar was put in the water bath to achieve uniform heating. $T_{crit}$ was calculated by the intersection of lines extrapolated from the slow and fast rise portion of the temperature-dependent fluorescence response (Knight and Ackerly, 2002).

### 2.4. Leaf traits measurement

We selected leaf traits which might have impact on leaf temperature, including morphological traits, optical traits, material properties, anatomical traits, and physiological traits (Table 3).

Leaves with similar size, age and orientation to the leaves with temperature measurement were collected. Eight to ten leaves for each individual were scanned using a flatbed-scan scanner. Leaf area (Area), leaf perimeter (P), perimeter/area ratio (P/A), leaf length (Length), and leaf width (Width) were analyzed by ImageJ 1.52q based on the scanned image. Optical properties of leaf reflectivity (Ref), transmissivity

### 2.3. Thermotolerance measurement

**Table 2**
Species information.

| Forest | Species name | Life form | Leaf type |
|---|---|---|---|
| Savanna | *Bauhinia brachycarpa* | Deciduous | Broad leaf |
| | *Lannea coromandelica* | Deciduous | Broad leaf |
| | *Polyalthia cerasoides* | Deciduous | Broad leaf |
| | *Woodfordia fruticosa* | Deciduous | Broad leaf |
| Tropical rain forest | *Duabanga grandiflora* | Evergreen | Broad leaf |
| | *Parashorea chinensis* | Evergreen | Broad leaf |
| | *Pometia pinnata* | Evergreen | Broad leaf |
| | *Semecarpus reticulata* | Evergreen | Broad leaf |
| Subtropical broad-leaved forest | *Castanopsis rufescens* | Evergreen | Broad leaf |
| | *Lithocarpus xylocarpus* | Evergreen | Broad leaf |
| | *Machilus yunnanensis* | Evergreen | Broad leaf |
| | *Schima noronhae* | Evergreen | Broad leaf |
| Temperate mixed forest | *Pinus yunnanensis* | Evergreen | Needle leaf |
| | *Populus rotundifolia* | Deciduous | Broad leaf |
| | *Quercus pannosa* | Evergreen | Broad leaf |
| | *Rhododendron decorum* | Evergreen | Broad leaf |

**Table 3**
The investigated traits.

| Class | Leaf traits | Abbreviation |
|---|---|---|
| Morphological trait | Leaf area | Area (cm$^2$) |
| | Perimeter | P (cm) |
| | Leaf length | Length (cm) |
| | Leaf width | Width (cm) |
| | Length/width | L/W |
| | The ratio of perimeter to area | P/A (cm$^{-1}$) |
| Optical trait | Reflectivity | Ref (%) |
| | Absorptivity | Abs (%) |
| | Transmissivity | Trans (%) |
| | Greenness | Greenness |
| Material property | Leaf fresh mass density | Density.f (g cm$^{-3}$) |
| | Leaf dry mass density | Density.d (g cm$^{-3}$) |
| | Water content | WC (%) |
| | Leaf mass per area | LMA (cm$^{-2}$ g$^{-1}$) |
| Anatomical trait | Leaf thickness | Thickness (µm) |
| | Thickness of upper epidermis | Thickness_up (µm) |
| | Thickness of lower epidermis | Thickness_low (µm) |
| | Thickness of spongy tissue | Thickness_spongy (µm) |
| | Thickness of palisade tissue | Thickness_ palisade (µm) |
| | Leaf vein density | Vein density (mm$^{-1}$) |
| | Stomata size | St.size (µm) |
| | Stomata density | St.density (No mm$^{-2}$) |
| | Stomata size × stomata density | SPI (mm$^{-1}$) |
| Physiological trait | The maximum photosynthesis rate | Trmax (mmol m$^{-2}$ s$^{-1}$) |
| | The maximum transpiration rate | Amax (µmol m$^{-2}$ s$^{-1}$) |
| | The maximum stomata conductance | Cmax (mol m$^{-2}$ s$^{-1}$) |
| Temperature trait | The maximum leaf temperature | Tlmax (°C) |
| | The maximum air temperature | Tamax (°C) |

(Trans), and absorptivity (Abs) were measured by spectrometer (USB2000, Ocean Optics, USA), using 10 leaves for each individual. These leaves were also used to measure greenness which is proportional to the amount of chlorophyll present in leaves by chlorophyll meter (SPAD-502, Minolta, Japan). Three to ten leaves of each individual (more blades for small leaves) were collected in the morning and stored in sealed plastic bags with moist paper inside for leaf density and water content (WC) measurements. They were weighed soon after harvesting, and used water displacement to get leaf volumes, then oven dried under 80 °C to constant weight. Leaf density was calculated by the ratio of leaf mass (both fresh and dry density) to leaf volume (Perez-Harguindeguy et al., 2013). Water content was calculated by the ratio of weight difference between fresh and dry leaves to the dry mass (Perez-Harguindeguy et al., 2013). Histological technique of Paraffin-fixing (Biosystems, 2021) was used to make cross-sections for the measurements of leaf thickness (Thickness), the thickness of upper and lower epidermis (Thickness_up, Thickness_low), palisade mesophyll (Thickness_palisade) and spongy mesophyll (Thickness_spongy) (4 leaves for each individual). All the anatomical sections were photographed under a microscope (Leica Microsystems Vertrieb GmbH, Wetzlar, Germany), and then analyzed with ImageJ. Stomatal density and size were measured using paradermal sections. Paradermal sections were cut from the middle part of leaf avoiding main veins and boiled in water for 10–15 min, then immersed in a 1:1 mixture of 30% $H_2O_2$ and acetic glacial aqueous solution until they became soft and disintegrated. The needle leaves for stomata sections were bleached with 1:1 of $HNO_3$, and $H_2O$ in saturated $KClO_3$. The samples for vein density analysis were bleached with 5% NaOH until they become transparent. Stain leaves for 15 min in 1% safranin diluted with ethanol. All the sections were mounted on slides and photographed under a Leica DM2500 light microscope. Vein density was calculated by measuring total length of veins in the image and divided this number by the image area.

Diurnal transpiration rate, photosynthesis rate and stomatal conductance were measured by Portable Photosynthesis System (LI-6400, LI-COR, USA) for two sunny days at each forest, then we combined the two days measurements into one diurnal curve. Three leaves adjacent to the leaves with temperature measurement were selected for each individual. All leaves were measured one by one alternately from morning to afternoon, the start and end time were dependent on solar radiation and the availability of canopy crane at each forest (SAV: 8:00–17:00; TRF: 9:20–14:40; STF: 9:30–16:30; TEF: 8:30–17:40).

### 2.5. Thermal safety margin

Thermal safety margin was calculated based on $T_{crit}$ to represent thermal safety of photosynthetic system II.

$$TSM = T_{crit} - T \tag{1}$$

In formula (1), three different metrics of temperature (T) were used to assess the impact of different assumptions of body temperature on TSM. For TSM.Tl, T was the maximum leaf temperature of the individual (Tlmax). For TSM.Ta, T was the maximum air temperature beside the individual (Tamax). For TSM.MTa, T was the maximum canopy air temperature of each forest.

### 2.6. Data analysis

#### 2.6.1. Comparison of TSM and $T_{crit}$ across and within biomes

The difference of TSM and $T_{crit}$ across and within biomes was analyzed by multiple comparison of least significant difference (LSD) (Steel et al., 1997). p-Value < 0.05 was considered as significant difference.

#### 2.6.2. Impact factors on TSM.Tl

TSM.Tl is determined by two parameters — $T_{crit}$ and Tlmax. We constructed a mixed effects model by setting TSM.Tl as the response variable, $T_{crit}$ and Tlmax as the fixed effects, and species nested in forest as random effects. The contribution of $T_{crit}$ and Tlmax to the variance of TSM.Tl was analyzed using function "partR2" in R package "partR2" (Stoffel et al., 2021).

#### 2.6.3. Impact factors on $T_{crit}$

Impact factors included 27 leaf traits related to leaf temperature (Table 3) and Tlmax. Random forest was used to find the important leaf traits playing important roles in explaining variation of thermotolerance (Breiman, 2001). This model corrects data overfitting, and allows non-linear relationships and collinear variables (Breiman, 2001). We calculated variation explained rate by setting the number of variables randomly sampled as candidates at each split (mtry) from 1 to 28 (the number of variables minus 1), and got the highest variation explained rate when mtry = 20. OOB error converged at number of trees to grow (ntree) = 400. Therefore, we fit the random forest model with mtry = 20 and ntree = 400, then used node purity values to inform the importance of each predictor (Breiman, 2001). Mixed-effects model was also used to confirm the results. Species nest in forest was set as random effect, the important leaf traits selected by random forest were fixed effects and $T_{crit}$ was the response variable. The contribution of fixed effects to the variance of $T_{crit}$ was analyzed using function "partR2" in R package "partR2" (Martin A. Stoffel et al., 2021).

#### 2.6.4. Impact factors on the difference between TSM.Tl and TSM.Ta (TSM.Ta-TSM.Tl)

Repeated Measures Correlation in R package "rmcorr" (Bakdash and Marusich, 2017) was used to calculate the correlation between temperature traits and TSM.Ta - TSM.Tl by setting forest as subject. Temperature traits included maximum leaf temperature (Tlmax), and maximum air temperature (Tamax).

All the analyses were performed using R 3.6.1 (Team, 2019).

## 3. Results

### 3.1. Patterns of thermal safety margin (TSM) across and within biomes

Site mean TSM.Tl ranged from 3.0 ± 0.5 °C at SAV to 12.8 ± 0.9 °C at STF. The rank of TSM calculated by the maximum air temperature around each individual (TSM.Ta) and the maximum leaf temperature of each individual (TSM.Tl) across biomes followed the same trend: STF = TEF > TRF > SAV, and TSM calculated by the maximum air temperature at each forest (TSM.MTa) followed STF > TEF > TRF > SAV. The patterns within biomes were different (Fig. 1a). TSM.Tl, TSM.Ta and TSM.MTa had positive correlations at SAV, TSM.Tl and TSM.Ta were positively correlated at TRF, while TSM.Ta and TSM.MTa were positively correlated at STF and TEF.

No negative TSM.Tl was found for control leaves. However, *Woodfodia fruticosa* and *Bauhinia brachycarpa* presented negative and zero TSM respectively, when transpiration was blocked. All the Vaseline leaves of these two species dried and dropped at the end of the experiment due to the extreme temperature conditions.

### 3.2. The patterns of $T_{crit}$ across and within biomes

$T_{crit}$ is one of the two parameters in TSM calculation. Site mean $T_{crit}$ range from 42.5 ± 0.6 °C in TEF to 48.5 ± 0.5 °C in SAV, and followed the pattern: SAV = TRF > STF > TEF (Fig. 1b). $T_{crit}$ was linearly and negatively correlated with TSM.Tl (Pearson correlation coefficient = −0.46, p-value < 0.001) across biomes. There were no significant correlations between $T_{crit}$ and TSM.Tl within biomes, except for a positive correlation at SAV (Pearson correlation coefficient = 0.70, p-value = 0.01). The random forest model showed that Tlmax and Tamax were
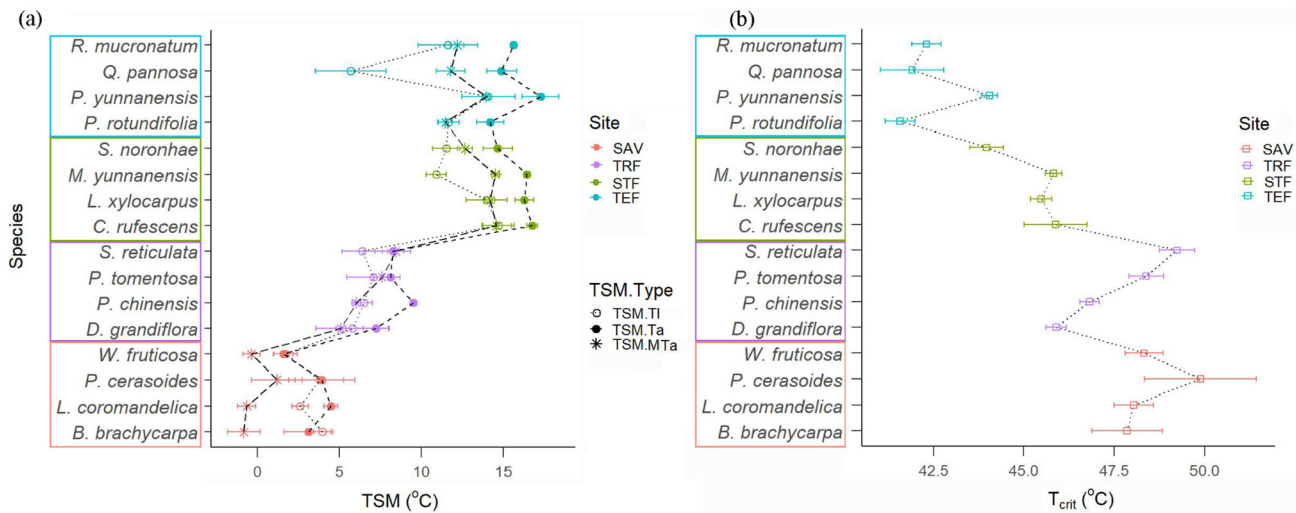
**Fig. 1.** The patterns of (a) thermal safety margin (TSM) and (b) thermotolerance ($T_{crit}$). TSM.Ta, TSM calculated by the maximum air temperature of each individual; TSM.Tl, TSM calculated by the maximum leaf temperature of each individual; TSM.MTa, TSM calculated by the maximum air temperature at each forest. SAV, savanna; TRF, tropical rain forest; STF, subtropical broad-leaved forest; TEF, temperate mixed forest.

the most important traits to explain the variance of $T_{crit}$ (Table S1). All leaf traits and the maximum air temperature explained 68% of the variance of $T_{crit}$, among them, Tlmax and Tamax explained 62%. Considering of the high correlation between Tlmax and Tamax, we used Tamax and Tlmax as a fixed effect respectively, and species nested in forest as random forest in mixed effects model. The model used Tamax as fixed effect gave conditional $R^2 = 0.798$ and marginal $R^2 = 0.571$, and the model used Tlmax as fixed effect gave conditional $R^2 = 0.799$ and marginal $R^2 = 0.131$. However, the Pearson correlations between Tamax (or Tlmax) and $T_{crit}$ were not significant within biomes except for Tamax and $T_{crit}$ at TRF. Therefore, $T_{crit}$ increased with environmental temperature across biomes; while the positive relationships between $T_{crit}$ and environmental temperature within biomes was not confirmed.

### 3.3. Patterns of leaf temperature across and within biomes

Leaf temperature is another parameter in TSM calculation. It linearly increased with air temperature, however, the increasing slope increased from the hot to the cold forest. Except for the SAV species, all the other species had significant higher Tlmax than Tamax (Fig. 2a). During daytime, leaves had strong cooling effects to reduce Tl close to Ta at SAV, while Tl was much higher than Ta for most of the time at TEF. Thus,

the difference between Tl and Ta increased from the hot to the cold forest (Fig. 2a). The variances of Tlmax between species were within 8 °C in SAV, STF and TRF, and reached to 12.2 °C in TEF, while the variances of Tamax between species within all biomes were lower than 5.5 °C.

Control leaf temperatures were all below $T_{crit}$ except for two savanna species *L. coromandelica* and *W. fruticosa*, and their temperatures exceeded $T_{crit}$ for less than 1 min. When transpiration was blocked, leaf temperature increased, and the increase extent was highest for SAV species (Fig. 2b). Vaseline leaf temperature of all savanna species and one subtropical species exceeded $T_{crit}$. Among them, the overheating time of the two savanna species *B. brachycarpa* and *W. fruticosa* exceeded 10 min (10.9 ± 7.3 min and 39.2 ± 11.6 min respectively).

### 3.4. Factors affecting TSM.Tl

TSM.Tl was calculated using Tlmax and $T_{crit}$. Compared with $T_{crit}$, Tlmax were highly variable. The range of $T_{crit}$ across biomes was 12.4 °C, while the range of Tlmax was much higher (19.7 °C). In the mixed effects model, marginal $R^2$ contributed by Tlmax was 87.1%, while marginal $R^2$ contributed by $T_{crit}$ was 0%. Pearson correlation coefficient between TSM.Tl and Tlmax was −0.93. TSM.Tl can be predicted by
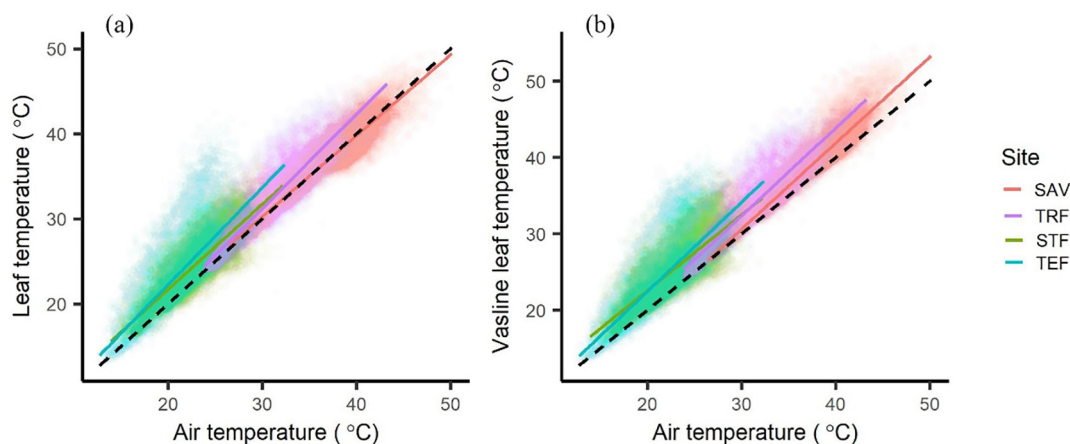


**Fig. 2.** The relationships between (a) leaf temperature and air temperature; and (b) Vaseline leaf temperature and air temperature during daytime (9:00– 17:00). SAV, savanna; TRF, tropical rain forest; STF, subtropical broad-leaved forest; TEF, temperate mixed forest. Dash line is the regression line of y = x.
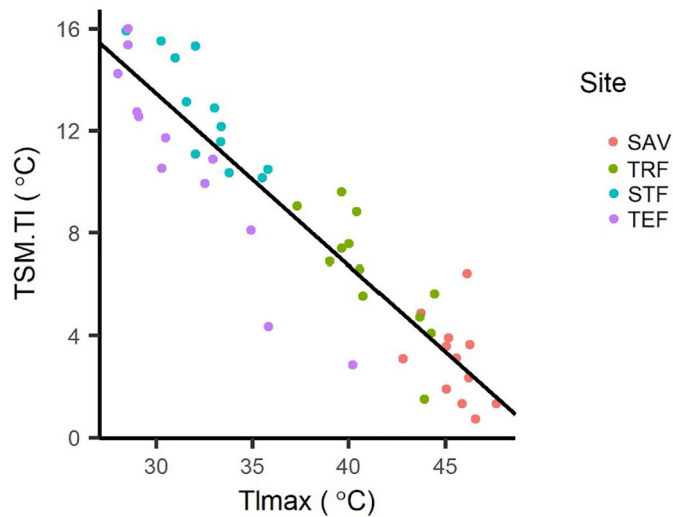
**Fig. 3.** The relationship between thermal safety margin (TSM) calculated by the maximum leaf temperature (TSM.Tl) and the maximum leaf temperature (Tlmax). The regression line can be modeled by TSM.Tl = $-0.672 \times$ Tlmax + 33.581 ($R^2 = 0.85$, p-value $< 0.001$).

Tlmax by the model TSM.Tl = $-0.672 \times$ Tlmax + 33.581 ($R^2 = 0.85$, p-value $< 0.001$), and 83% of the residuals were within 1.5 °C (Fig. 3).

### 3.5. Factors affecting the difference between TSM.Tl and TSM.Ta (TSM.Ta − TSM.Tl)

TSM.Ta − TSM.Tl increased from $0.3 \pm 0.9$ °C at SAV, $1.8 \pm 1.1$ °C at TRF, and $3.2 \pm 1.1$ °C at STF, to $4.7 \pm 2.1$ °C at TEF. Repeated measures correlation revealed that Tlmax (cor 0.87, p-value $< 0.001$) had the highest correlation with TSM.Ta − TSM.Tl. TSM.Ta − TSM.Tl linearly increase with Tlmax, but the intercept increased from SAV to TEF (Fig. 4).

## 4. Discussion

### 4.1. General patterns of TSM and $T_{crit}$

Patterns of TSM (regardless of the temperature metrics used) followed similar trends with previous studies across biomes: TSM decreased from the cool to the hot forests (Curtis et al., 2016; O'Sullivan et al., 2017). During the heatwave, TSMs of savanna species were the
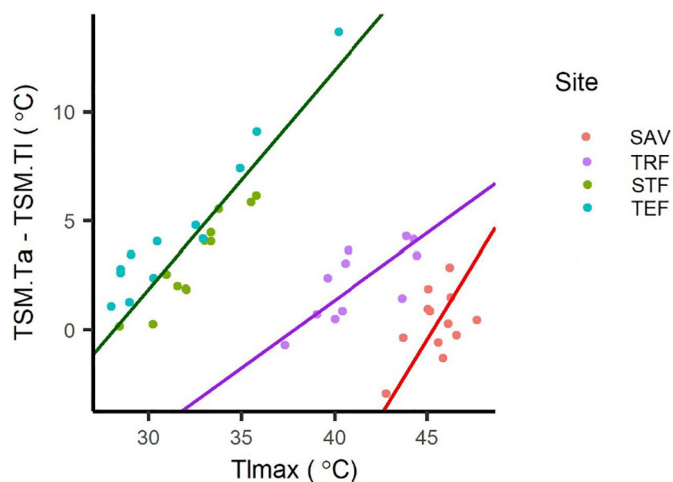


**Fig. 4.** The relationships between thermal safety margin (TSM) calculated by the maximum air temperature (TSM.Ta) and the maximum leaf temperature (TSM.Tl) of individuals respectively and the maximum leaf temperature (Tlmax). SAV, savanna; TRF, tropical rain forest; STF, subtropical broad-leaved forest; TEF, temperate mixed forest.

lowest, indicating that their photosynthetic systems were more at risk under heat stress. Especially when transpiration was suppressed, leaves of some species in SAV were dried and dropped at the end of the experiment. A global study also demonstrated that woody productivity in the hottest forests among 590 permanent plots across the tropics were more sensitive to temperature than at cooler sites (Sullivan et al., 2020). The risks of species disappear and reduction of carbon stock in tropical hot forests under heatwave requires high attention (Gallagher et al., 2019; Tiwari et al., 2020).

The pattern and values of $T_{crit}$ in the present study were comparable with $T_{crit}$ of other studies using the same methodology (O'Sullivan et al., 2017; Zhu et al., 2018). $T_{crit}$ values were lower than $T_{50}$ measured using $F_v/F_m$-T method (Perez et al., 2020). Random forest model showed that Tlmax and Tamax can explain 62% of the variance of $T_{crit}$. This indicated the important influence of environmental temperature on $T_{crit}$. However, the correlations between $T_{crit}$ and Tlmax or Tamax were not significant within all the biomes, which might due to the large difference of the range of Tamax between across and within biomes: the range of Tamax across biomes was 21.6 °C, while the maximum range of Tamax within biomes were below 5.1 °C. In addition, other traits might have stronger impacts on $T_{crit}$ than Tamax within biomes. Some leaf traits have been reported having relationships with $T_{crit}$, e.g. LMA (Gallagher, 2014; Sastry et al., 2018), leaf carbon assimilation (Perez et al., 2020), and leaf chemical composition (Zhu et al., 2018). Thus, more samples and more leaf traits should be investigated to clarify the main effects on $T_{crit}$ within biomes.

### 4.2. Contrary pattern between TSM and thermotolerance

Traditionally, the plants with higher thermotolerance are considered to be more resilient to heat stress (Wahid et al., 2007). However, the calculation of TSM (formula (1)) showed that $T_{crit}$ is negatively related to TSM. Because TSM decreases with Tlmax, while $T_{crit}$ increases with Tlmax. Our results also demonstrated the contrary pattern between TSM and thermotolerance across biomes. The SAV species have high thermotolerance, however, they are more vulnerable to heat damage than the TEF and STF species which have low thermotolerance and low leaf temperature. Notably, the negative relationship between TSM.Tl and $T_{crit}$ was only found across biomes, and their relationships within biomes differed with forests. We found a positive correlation between TSM.Tl and $T_{crit}$ at SAV, but no significant relationships between them at other forests; while a study of 19 plant species in Fairchild Tropical Botanic garden found negative correlation between thermotolerance and TSM.Tl (Perez and Feeley, 2020). Accordingly, thermotolerance alone cannot be used to estimate thermal safety of plant leaves.

### 4.3. How to detect vulnerable species under heat stress

Our results demonstrated that evaluating thermal safety of plants based on leaf physical traits is not reliable. TSM was determined by $T_{crit}$ and the maximum leaf temperature (Tlmax). $T_{crit}$ increased with Tlmax, whereas only by around one third of a degree per degree increase in Tlmax. A previous study also reported that $T_{crit}$ ranged around 8 °C from arctic to equatorial sites compared with 20 °C ranged in mean maximum daily temperature of the warmest month (O'Sullivan et al., 2017). As a result, the variance of TSM was mainly determined by Tlmax. The calculation of TSM requires the measurements of $T_{crit}$ and Tlmax simultaneously, which costs time and cannot be done in situ. Considering the high correlation between leaf temperature and TSM, leaf temperature is an efficient substitute to estimate thermal safety of leaves. Thermal camera can quickly and remotely measure temperature of multiple leaves, thus instantly evaluating thermal safety of leaves in the field.

The method to determine Tlmax has great impact on TSM. The damage of high temperature on leaves is determined by both the threshold

of temperature and the exposure time. If leaf temperature exceeding $T_{crit}$ lasted for a few seconds, it might not damage the leaf. Previous research usually used 15 min to treat leaves under water bath when measure the response of $F_v/F_m$ to temperature (Curtis et al., 2014; Krause et al., 2010). In the present study, we observed leaves died when leaf temperature exceeding $T_{crit}$ for more than 10 min in one day. Therefore, the calculation of Tlmax in TSM should consider its duration time.

*4.4. Can we use air temperature to measure TSM?*

The present research systematically compared TSM calculated by leaf temperature and air temperature of canopy plants across and within biomes using in situ measurements. Generally, both leaf temperature and air temperature based TSM produced similar rank of TSMs across biomes, however TSM.Ta overestimates TSM especially at cool biomes (Fig. 4), because the differences between Tl and Ta increased from hot biomes to cold biomes (Fig. 2). Within each biome, TSM.Ta - TSM.Tl increased with leaf temperature (Fig. 4). Hence, it will cause large errors if TSM.Ta was applied in cool biomes and for species with high leaf temperatures within biomes.

## 5. Conclusion

TSM is important to predict thermal safety of organisms under global warming (Sunday et al., 2014). Our results suggest using leaf temperature instead of thermotolerance to evaluate thermal safety of plants. In this way, thermal safety can be instantly and remotely measured by infrared camera in the field. This will greatly improve the detection of threatened species under heat stress. The present research assesses the differences of TSM.Tl and TSM.Ta across and within biomes, which is helpful to evaluate the reliability of previous studies of TSM based on air temperature. Our results are valuable for understanding the impact of heat stress on vegetation, and can be applied in forest management.

## CRediT authorship contribution statement

**Nawatbhrist Kitudom:** Investigation, Writing – original draft. **Sophie Fauset:** Investigation, Writing – review & editing. **Yingying Zhou:** Investigation. **Zexin Fan:** Resources. **Murong Li:** Investigation. **Mingjian He:** Investigation. **Shubin Zhang:** Investigation. **Kun Xu:** Resources. **Hua Lin:** Conceptualization, Methodology, Writing – review & editing, Investigation, Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability statement

Primary data are stored at figshare, https://10.6084/m9.figshare.14038478.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150416.

## References

Ackerly, D.D., Stuart, S.A., 2009. Physiological ecology: plants. In: Levin, S.A. (Ed.), The Princeton Guide to Ecology. Princeton University Press, New Jersey, USA.

Ahrens, C.W., Challis, A., Byrne, M., Leigh, A., Nicotra, A.B., Tissue, D., et al., 2021. Repeated extreme heatwaves result in higher leaf thermal tolerances and greater safety margins. New Phytol. https://doi.org/10.1111/nph.17640.

Alexander, L.V., Zhang, X., Peterson, T.C., Caesar, J., Gleason, B., Tank, A.M.G.K., et al., 2006. Global observed changes in daily climate extremes of temperature and precipitation. J. Geophys. Res.-Atmos. 111, D006290. https://doi.org/10.1029/2005jd006290.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., et al., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manag. 259, 660–684. https://doi.org/10.1016/j.foreco.2009.09.001.

Allen, C.D., Breshears, D.D., McDowell, N.G., 2015. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere 6, 1–55. https://doi.org/10.1890/Es15-00203.1.

Araújo, I., Marimon, B.S., Scalon, M.C., Fauset, S., Junior, B.M., Tiwari, R., et al., 2021. Trees at the Amazonia-Cerrado transition are approaching high temperature thresholds. Environ. Res. Lett. 16, 034047. https://doi.org/10.1088/1748-9326/Abe3b9.

Bakdash, J.Z., Marusich, L.R., 2017. Repeated measures correlation. Front. Psychol. 8, 456. https://doi.org/10.3389/fpsyg.2017.00456.

Baker, N.R., 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59, 89–113.

Biosystems, L, 2021. In: Rolls, G. (Ed.), An Introduction to Specimen Processing . https://www.leicabiosystems.com/knowledge-pathway/an-introduction-to-specimen-processing.

Breiman, L., 2001. Random forests. Mach. Learn. 45, 5–32. https://doi.org/10.1023/A:1010933404324.

Chaste, E., Girardin, M.P., Kaplan, J.O., Bergeron, Y., Hely, C., 2019. Increases in heat-induced tree mortality could drive reductions of biomass resources in Canada's managed boreal forest. Landsc. Ecol. 34, 403–426. https://doi.org/10.1007/s10980-019-00780-4.

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., et al., 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437, 529–533. https://doi.org/10.1038/nature03972.

Cook, A.M., Berry, N., Milner, K.V., Leigh, A., 2021. Water availability influences thermal safety margins for leaves. Funct. Ecol. 1–11. https://doi.org/10.1111/1365-2435.13868.

Coumou, D., Robinson, A., 2013. Historic and future increase in the global land area affected by monthly heat extremes. Environ. Res. Lett. 8, 034018. https://doi.org/10.1088/1748-9326/8/3/034018.

Crawford, A.J., McLachlan, D.H., Hetherington, A.M., Franklin, K.A., 2012. High temperature exposure increases plant cooling capacity. Curr. Biol. 22, R396–R397. https://doi.org/10.1016/j.cub.2012.03.044.

Curtis, E.M., Knight, C.A., Petrou, K., Leigh, A., 2014. A comparative analysis of photosynthetic recovery from thermal stress: a desert plant case study. Oecologia 175, 1051–1061. https://doi.org/10.1007/s00442-014-2988-5.

Curtis, E.M., Gollan, J., Murray, B.R., Leigh, A., 2016. Native microhabitats better predict tolerance to warming than latitudinal macro-climatic variables in arid-zone plants. J. Biogeogr. 43, 1156–1165. https://doi.org/10.1111/jbi.12713.

Dahl, J.E., Bertrand, M., Pierre, A., Curtit, B., Pillard, C., Tasiemski, A., et al., 2019. Thermal tolerance patterns of a carabid beetle sampled along invasion and altitudinal gradients at a sub-Antarctic island. J. Therm. Biol. 86. https://doi.org/10.1016/j.jtherbio.2019.102447.

Denny, M.W., Dowd, W.W., 2012. Biophysics, environmental stochasticity, and the evolution of thermal safety margins in intertidal limpets. J. Exp. Biol. 215, 934–947. https://doi.org/10.1242/jeb.058958.

Dong, N., Prentice, I.C., Harrison, S.P., Song, Q.H., Zhang, Y.P., 2017. Biophysical homoeostasis of leaf temperature: a neglected process for vegetation and land-surface modelling. Glob. Ecol. Biogeogr. 26, 998–1007. https://doi.org/10.1111/geb.12614.

Doughty, C.E., Goulden, M.L., 2008. Are tropical forests near a high temperature threshold? J. Geophys. Res.-Biogeosci. 113, G00B07. https://doi.org/10.1029/2007jg000632.

Fauset, S., Freitas, H.C., Galbraith, D.R., Sullivan, M.J.P., Aidar, M.P.M., Joly, C.A., et al., 2018. Differences in leaf thermoregulation and water use strategies between three co-

occurring Atlantic forest tree species. Plant Cell Environ. 41, 1618–1631. https://doi.org/10.1111/pce.13208.

Gallagher, D.W., 2014. Photosynthetic thermal tolerance and recovery to short duration temperature stress in desert and montane plants: a comparative study. Science in Biology. Master. California Polytechnic State University, California.

Gallagher, R.V., Allen, S., Wright, I.J., 2019. Safety margins and adaptive capacity of vegetation to climate change. Sci. Rep-Uk. 9, 8241. https://doi.org/10.1038/S41598-019-44483-X.

Gates, D.M., 2003. Biophysical ecology. Dover Publications, New York, USA.

Gunderson, A.R., Stillman, J.H., 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc. R. Soc. B-Biol. Sci. 282, 20150401. https://doi.org/10.1098/Rspb.2015.0401.

Hansen, J., Sato, M., Ruedy, R., 2012. Perception of climate change. Proc. Natl. Acad. Sci. USA 109, E2415–E2423. https://doi.org/10.1073/pnas.1205276109.

Havaux, M., 1993. Characterization of thermal-damage to the photosynthetic electron-transport system in potato leaves. Plant Sci. 94, 19–33. https://doi.org/10.1016/0168-9452(93)90003-I.

Knight, C.A., Ackerly, D.D., 2002. An ecological and evolutionary analysis of photosynthetic thermotolerance using the temperature-dependent increase in fluorescence. Oecologia 130, 505–514.

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 Phytol. 160, 337–347. https://doi.org/10.1046/j.1469-8137.2003.00880.x.

Krause, G.H., Winter, K., Krause, B., Jahns, P., Garcia, M., Aranda, J., et al., 2010. High-temperature tolerance of a tropical tree, Ficus insipida: methodological reassessment and climate change considerations. Funct. Plant Biol. 37, 890–900.

Lancastera, L.T., Humphreys, A.M., 2020. Global variation in the thermal tolerances of plants. Proc. Natl. Acad. Sci. USA 117, 201918162. https://doi.org/10.1073/pnas.1918162117.

Leigh, A., Sevanto, S., Ball, M.C., Close, J.D., Ellsworth, D.S., Knight, C.A., et al., 2012. Do thick leaves avoid thermal damage in critically low wind speeds. New Phytol. 194, 477–487.

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, e0224218. https://doi.org/10.1371/journal.pone.0224218.

Leuzinger, S., Körner, C., 2007. Tree species diversity affects canopy leaf temperatures in a mature temperate forest. Agric. For. Meteorol. 146, 29–37. https://doi.org/10.1016/j.agrformet.2007.05.007.

Lin, H., Chen, Y.J., Zhang, H.L., Fu, P.L., Fan, Z.X., 2017. Stronger cooling effects of transpiration and leaf physical traits of plants from a hot dry habitat than from a hot wet habitat. Funct. Ecol. 31, 2202–2211. https://doi.org/10.1111/1365-2435.12923.

McArley, T.J., Hickey, A.J.R., Herbert, N.A., 2017. Chronic warm exposure impairs growth performance and reduces thermal safety margins in the common triplefin fish (Forsterygion lapillum). J. Exp. Biol. 220, 3527–3535. https://doi.org/10.1242/jeb.162099.

Michaletz, S.T., Weiser, M.D., Zhou, J.Z., Kaspari, M., Helliker, B.R., Enquist, B.J., 2015. Plant thermoregulation: energetics, trait-environment interactions, and carbon economics. Trends Ecol. Evol. 30, 714–724. https://doi.org/10.1016/j.tree.2015.09.006.

Michaletz, S.T., Weiser, M.D., McDowell, N.G., Zhou, J.Z., Kaspari, M., Helliker, B.R., et al., 2016. The energetic and carbon economic origins of leaf thermoregulation. Nat. Plants 2, 16129. https://doi.org/10.1038/Nplants.2016.147.

Okajima, Y., Taneda, H., Noguchi, K., Terashima, I., 2012. Optimum leaf size predicted by a novel leaf energy balance model incorporating dependencies of photosynthesis on light and temperature. Ecol. Res. 27, 333–346. https://doi.org/10.1007/s11284-011-0905-5.

O'Sullivan, O.S., Heskel, M.A., Reich, P.B., Tjoelker, M.G., Weerasinghe, L.K., Penillard, A., et al., 2017. Thermal limits of leaf metabolism across biomes. Glob. Chang. Biol. 23, 209–223. https://doi.org/10.1111/gcb.13477.

Perez, T.M., Feeley, K.J., 2020. Photosynthetic heat tolerances and extreme leaf temperatures. Funct. Ecol. 34, 2236–2245. https://doi.org/10.1111/1365-2435.13658.

Perez, T.M., Socha, A., Tserej, O., Feeley, K.J., 2020. Photosystem II heat tolerances characterize thermal generalists and the upper limit of carbon assimilation. Plant Cell Environ. 2021, 1–10. https://doi.org/10.1111/pce.13990.

Perez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., et al., 2013. New handbook for standardised measurement of plant functional traits worldwide. Aust. J. Bot. 61.

Pincebourde, S., Casas, J., 2019. Narrow safety margin in the phyllosphere during thermal extremes. Proc. Natl. Acad. Sci. USA 116, 5588–5596. https://doi.org/10.1073/pnas.1815828116.

Sastry, A., Barua, D., 2017. Leaf thermotolerance in tropical trees from a seasonally dry climate varies along the slow-fast resource acquisition spectrum. Sci. Rep-Uk. 7, 11246. https://doi.org/10.1038/s41598-017-11343-5.

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, plx070. https://doi.org/10.1038/s41598-017-11343-5.

Schreiber, U., Berry, J.A., 1977. Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of photosynthetic apparatus. Planta 136, 233–238. https://doi.org/10.1007/BF00385990.

Smith, W.K., 1978. Temperatures of desert plants - another perspective on adaptability of leaf size. Science 201, 614–616. https://doi.org/10.1126/science.201.4356.614.

Song, X., Barbour, M.M., Saurer, M., Helliker, B.R., 2011. Examining the large-scale convergence of photosynthesis-weighted tree leaf temperatures through stable oxygen isotope analysis of multiple data sets. New Phytol. 192, 912–924. https://doi.org/10.1111/j.1469-8137.2011.03851.x.

Steel, R.G.D., Torrie, J.H., Dickey, D.A., 1997. Principles and Procedures of Statistic: A Biometrical Approach. McGraw-Hill, New York.

Stoffel, Martin A., Nakagawa, Shinichi, Schielzeth, H., 2021. partR2: partitioning R2 in generalized linear mixed models. Peer J. 9, e11414.

Stokes, V.J., Morecroft, M.D., Morison, J.I.L., 2006. Boundary layer conductance for contrasting leaf shapes in a deciduous broadleaved forest canopy. Agric. For. Meteorol. 139, 40–54. https://doi.org/10.1016/j.agrformet.2006.05.011.

Sullivan, M.J.P., Lewis, S.L., Affum-Baffoe, K., Castilho, C., Costa, F., Sanchez, A.C., et al., 2020. Long-term thermal sensitivity of earth's tropical forests. Science 368, 869–874. https://doi.org/10.1126/science.aaw7578.

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T., et al., 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc. Natl. Acad. Sci. USA 111, 5610–5615. https://doi.org/10.1073/pnas.1316145111.

Tatarinov, F., Rotenberg, E., Maseyk, K., Ogee, J., Klein, T., Yakir, D., 2016. Resilience to seasonal heat wave episodes in a Mediterranean pine forest. New Phytol. 210, 485–496. https://doi.org/10.1111/nph.13791.

Team, R.D.C., 2019. R: A Language and Environment for Statistical Computing.

Teskey, R., Wertin, T., Bauweraerts, I., Ameye, M., McGuire, M.A., Steppe, K., 2015. Responses of tree species to heat waves and extreme heat events. Plant Cell Environ. 38, 1699–1712. https://doi.org/10.1111/pce.12417.

Tiwari, R., Gloor, E., da Cruz, W.J.A., Marimon, B.S., Marimon, B., Reis, S.M., et al., 2020. Photosynthetic quantum efficiency in south-eastern Amazonian trees may be already affected by climate change. Plant Cell Environ. 13770. https://doi.org/10.1111/pce.13770.

Vinagre, C., Dias, M., Cereja, R., Abreu-Afonso, F., Flores, A.A.V., Mendonca, V., 2019. Upper thermal limits and warming safety margins of coastal marine species - indicator baseline for future reference. Ecol. Indic. 102, 644–649. https://doi.org/10.1016/j.ecolind.2019.03.030.

Wahid, A., Gelani, S., Ashraf, M., Foolad, M.R., 2007. Heat tolerance in plants: an overview. Environ. Exp. Bot. 61, 199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011.

Zhu, L.L., Bloomfield, K.J., Hocart, C.H., Egerton, J.J.G., O'Sullivan, O.S., Penillard, A., et al., 2018. Plasticity of photosynthetic heat tolerance in plants adapted to thermally contrasting biomes. Plant Cell Environ. 41, 1251–1262. https://doi.org/10.1111/pce.13133.