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

Kitudom, N

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1 Thermal safety margins of plant leaves across biomes

2 Kitodom Nawatbhrist<sup>1,5</sup>, Fauset Sophie<sup>1,4</sup>, Zhou Yingying<sup>1,5</sup>, Fan Zexin<sup>1,2,3</sup>, Li

3 Murong<sup>1,6</sup>, He Mingjian<sup>1,6</sup>, Zhang Shubin<sup>1</sup>, Xu kun<sup>7</sup>, Lin Hua<sup>1,2\*</sup>

4 <sup>1</sup>Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical  
5 Garden, Chinese Academy of Sciences, Mengla, Yunnan 666303, China.

6 <sup>2</sup>Center of Plant Ecology, Core Botanical Gardens, Chinese Academy of Sciences,  
7 Xishuangbanna 666303, China.

8 <sup>3</sup>Ailaoshan Station of Subtropical Forest Ecosystem Studies, Xishuangbanna Tropical  
9 Botanical Garden, Chinese Academy of Sciences, Jingdong, Yunnan 676209, China.

10 <sup>4</sup>School of Geography, Earth and Environmental Sciences, University of Plymouth,  
11 Plymouth, UK.

12 <sup>5</sup>University of Chinese Academy of Sciences, Beijing 100049, China

13 <sup>6</sup>College of Biology and Chemistry, Puer University, Puer, Yunnan 665000, China.

14 <sup>7</sup>Yunnan Lijiang Forest Ecosystem National Observation and Research Station,  
15 Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan  
16 650201, China

17 \*Corresponding author, E-mail: lh@xtbg.ac.cn

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19 Running title: Thermal safety margins of plants across biomes

20

21 **Abstract**

22 Climate change has great impacts on forest ecosystems, especially with the increasing  
23 frequency of heatwaves. Thermal safety margin (TSM) calculated by the difference  
24 between body temperature and thermotolerance threshold is useful to predict thermal  
25 safety of organisms. It has been widely used for animals, whereas has rarely been  
26 reported for plants. Besides, most of the previous studies used only thermotolerance to  
27 estimate thermal safety; or used thermotolerance and air temperature ( $T_a$ ) to calculate  
28 TSM. However, leaf temperature ( $T_l$ ) is the real body temperature of plant leaves. The  
29 departure of  $T_l$  from  $T_a$  might induce large error in TSM. Here, we investigated TSM  
30 of photosystem II (thermotolerance of PSII – the maximum  $T_l$ ) of dominant canopy  
31 plants in four forests from tropical to temperate biomes during a heatwave, and  
32 compared the TSMs calculated by  $T_l$  (TSM. $T_l$ ) and  $T_a$  (TSM. $T_a$ ) respectively. Also,  
33 thermal related leaf traits were investigated. The results showed that both TSM. $T_l$  and  
34 TSM. $T_a$  decreased from the cool forests to the hot forests. TSM. $T_l$  was highly  
35 correlated with the maximum leaf temperature ( $T_{lmax}$ ), while had an opposite trend  
36 with thermotolerance across biomes. Thus,  $T_{lmax}$  instead of thermotolerance can be  
37 used to evaluate TSM. The maximum  $T_a$  ( $T_{amax}$ ) and  $T_{lmax}$  explained 68% of the  
38 variance of thermotolerance in a random forest model, while other leaf traits including  
39 morphological, optical, material properties, anatomic and physiological traits only  
40 explained 6%. TSM. $T_a$  cannot distinguish thermal safety differences between co-  
41 occurring species. The overestimation of TSM by TSM. $T_a$  increased from the tropical  
42 to the temperate forest, and increased with  $T_l$  within biome. The present study

43 enriches the dataset of photosynthetic TSMs across biomes, proposes using  $T_{\max}$  to  
44 estimate TSMs of leaves, and highlights the risk of hot dry forest during heatwaves.  
45 The results also stress that it is not proper to use  $TSM.Ta$  in cold forests.

46

47 **Keywords :** photosynthetic heat tolerance, thermal environment, heatwave, heat  
48 stress, thermal stability

49

## 50 **1. Introduction**

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

63         Although thermotolerance is an ability of plants to survive under high  
64 temperature, it is not enough to assess thermal safety of plants. A leaf with low

65 thermotolerance might control leaf temperature well below its thermal limit by  
66 physical and physiological cooling (Lin et al., 2017), while a highly thermotolerant  
67 species might experience high leaf temperature. With regard to this, thermal safety  
68 margin (TSM) which is defined as the difference between body temperature (leaf  
69 temperature for plants) and critical temperatures that represent threshold for function  
70 or lethality was proposed to assess thermal safety (Gunderson and Stillman, 2015).  
71 TSM has been widely applied in animal studies (Denny and Dowd, 2012; McArley et  
72 al., 2017; Pincebourde and Casas, 2019; Sunday et al., 2014; Vinagre et al., 2019), but  
73 its application in plants is lacking. Recently, it has begun to catch attention of  
74 researchers. There are reports of TSM of plants at particular sites (Araújo et al., 2021;  
75 Leon-Garcia and Lasso, 2019), across latitude gradients (O'Sullivan et al., 2017), and  
76 in common gardens (Curtis et al., 2016; Perez and Feeley, 2020; Sastry and Barua,  
77 2017). However, TSM at one site cannot reveal TSM patterns across biomes. Plants  
78 growing in common gardens might have acclimated to local environment, thus had  
79 different TSMs from those in natural environments. In addition, most of the studies  
80 used air temperature instead of leaf temperature when calculating TSM (Curtis et al.,  
81 2016; O'Sullivan et al., 2017; Sastry and Barua, 2017).

82         Leaf temperature is the real “body temperature” for leaf metabolic processes,  
83 influencing leaf carbon economics (Michaletz et al., 2016; Michaletz et al., 2015). It  
84 can departure from air temperature up to 15 °C (Ackerly and Stuart, 2009), and varies  
85 across species and environment (Leuzinger and Körner, 2007). Even under the same  
86 environment, leaf temperature can be very different, depending on leaf physical and

87 physiological traits (Fauset et al., 2018; Lin et al., 2017). Michaletz et al. (2015) have  
88 demonstrated limited homeothermy of plants based on energy budget. Both site  
89 measurements and isotope analysis showed that leaf temperatures were more stable  
90 than air temperatures across biomes (Dong et al., 2017; Song et al., 2011). However,  
91 we still have no information of how the decoupling of leaf temperature and air  
92 temperature influences the difference between TSM calculated by leaf temperature  
93 (TSM.Tl) and air temperature (TSM.Ta) across biomes. Leaf traits including  
94 morphological traits, optical traits, physiological traits all have great impacts on leaf  
95 temperature (Gates, 2003). Convective cooling can be enhanced by reducing leaf size  
96 (Okajima et al., 2012; Smith, 1978); compound or dissected leaves have advantage of  
97 heat exchange by increasing the contact edge with air (Stokes et al., 2006); high water  
98 content or leaf mass area (LMA) can prolong leaf thermal time constant and delay leaf  
99 warming (Leigh et al., 2012; Smith, 1978); high reflectivity reduces radiation loads on  
100 leaves; and transpiration is efficient to cool leaves (Crawford et al., 2012; Lin et al.,  
101 2017). Leaf temperature is the direct micro-environment for leaf function, thus  
102 thermotolerance should acclimate to leaf temperature. Accordingly, leaf traits might  
103 affect thermotolerance through the impact on leaf temperature.

104       Except for leaf temperature, thermotolerance is another important parameter  
105 in TSM calculation. Photosystem II is sensitive to temperature, and its  
106 thermotolerance can be quantified by heat induced change of chlorophyll fluorescence  
107 parameters: the initial fluorescence ( $F_o$ ) or the ratio of variable to maximum  
108 fluorescence ( $F_v/F_m$ ) (Baker, 2008). The critical temperature of the intersection of

109 lines extrapolated from the slow and fast rise portion of the temperature-dependent  
110 fluorescence response ( $F_o$ -T curve) indicates the start point of the collapse of  
111 Photosystem II (Knight and Ackerly, 2002). The temperatures leading to 50%  
112 reduction in  $F_v/F_m$  ratios was defined as  $T_{50}$  (Knight and Ackerly, 2003; Krause et al.,  
113 2010). The two fluorescence parameters are positively correlated (Krause et al., 2010;  
114 Lancaster and Humphreys, 2020). Most studies of thermotolerance across biomes  
115 used  $T_{crit}$ , because  $F_o$  can be continuously monitored in a heating bath with the same  
116 samples (Dahl et al., 2019; Knight and Ackerly, 2003; Lancaster and Humphreys,  
117 2020; O'Sullivan et al., 2017; Song et al., 2011; Zhu et al., 2018). However, the  
118 measurement of  $F_v/F_m$  needs new samples at each temperature gradient, and the  
119 results vary with the exposure time at the target temperature. Therefore, we used  $T_{crit}$   
120 in the present research. Some unified trends have been found for thermotolerances of  
121 plants: e.g. species from warmer habitats are inherently higher in thermotolerance;  
122 thermotolerance acclimates to growth temperature (Zhu et al., 2018); and plants from  
123 dry habitat are more thermotolerant than plants from wet habitat (Curtis et al., 2016;  
124 Knight and Ackerly, 2003). However, we don't know whether the maximum leaf  
125 temperatures vary proportionately to thermotolerance across biomes, thus how global  
126 thermotolerance patterns corresponding to TSM are still unclear. In the present study,  
127 we investigated TSM and leaf traits of plants in four forests across biomes (savanna,  
128 tropical rain forest, subtropical broad-leaved forest, temperate mixed forest) along  
129 precipitation and temperature gradients. Aim to (1) compare the patterns of  
130 thermotolerance and TSM across biomes, (2) evaluate thermal risk of plants across

131 biomes, and (3) assess the difference between TSM.Tl and TSM.Ta.

132

## 133 **2. Materials and Methods**

134 **2.1 Study sites and species selection.** Four forests with contrasting temperature and  
135 precipitation gradients across biomes were selected in Yunnan province, south western  
136 China: savanna (SAV), tropical rain forest (TRF), subtropical broad-leaved forest  
137 (STF), temperate mixed forest (TEF). Four dominant upper canopy species and three  
138 individuals for each species were chosen in each forest, considering both richness in  
139 the upper canopy and reachability. They covered all the species of emergent trees in  
140 TRF, all the canopy species in TEF, and the most important canopy species in STF  
141 and SAV. Healthy, sun-exposed, and fully mature leaves in the upper canopy were  
142 sampled for temperature and leaf traits measurement. We accessed to the tall canopy  
143 using canopy cranes at TRF and STF, and using ladders in SAV and TEF. Detailed  
144 information of the sites and species were given in Table 1 and 2. All field  
145 measurements were conducted at the end of dry season in 2019 from May 13 ~ May  
146 16 at TRF, May 19 ~ May 23 at STF, May 25~ May 28 at SAV, June 4 ~ June 7 at TEF.  
147 This period was the most severe heatwave in recent 10 years, which was widely  
148 spread in Yunnan province (Fig S1).

149 **2.2 Temperature measurement.** Temperatures were measured by Type-T  
150 thermocouples (TT-T-30-SLE-1000, OMEGA, USA; diameter = 0.25 mm). To avoid  
151 thermocouples falling from leaves, we hung them on the adaxial surfaces of leaves  
152 and fixed the tips with heat-conducting glue (Fig S2). The glue can strengthen the  
153 attachment and block direct irradiation on the sensor head. We compared our method



154 with the traditional method (using tape to attach thermocouples on the abaxial sides of  
155 leaves) for four species. Two of the species had slightly higher leaf temperatures on  
156 abaxial sides than on adaxial sides at noon, while no significant differences were  
157 found for the other two species (Fig S3). It demonstrated that the impact of direct  
158 sunshine on leaf temperature was not significant. To simulate the extreme drought  
159 situation, we selected 2 leaves with similar size, age and orientation beside the leaves  
160 with temperature measurements, put Vaseline on the abaxial side of the leaves to stop  
161 transpiration (all the leaves are hypostomatous), and recorded their temperatures ( $T_n$ )  
162 with the same type of thermocouples. Air temperatures were simultaneously measured  
163 by the same type of thermocouples near the leaves with temperature measurements,  
164 avoiding direct solar radiation. For each individual, we measured one air temperature  
165 ( $T_a$ ), temperatures of 4 sun leaves ( $T_l$ ) and 2 Vaseline leaves ( $T_n$ ). All the  
166 temperatures were continuously recorded by data logger (UX120-04, HOBO, USA) at  
167 one-minute interval from May 13 ~ May 16 at TRF, May 19 ~ May 23 at STF, May  
168 25~ May 28 at SAV, June 4 ~ June 7 at TEF in 2019. On each day, we extracted the  
169 intervals that describe the hottest leaf temperatures for at least 10 minutes; these could  
170 be several intervals that sum to 10 minutes or a single interval that spans 10 minutes.  
171 We took the minimum temperature recorded in these intervals as  $T_{lmax}$  for that day.  
172 The maximum  $T_{lmax}$  during these days was  $T_{lmax}$  for each individual. With this  
173 method, we confirmed the temperature which was equal or higher than  $T_{lmax}$  lasted  
174 for at least 10 minutes.

175 **2.3 Thermotolerance measurement.** Thermotolerance was measured with

176 PlanTherm PT100 (PT100, Czech). The measurement of thermotolerance was based  
177 on the response of initial chlorophyll fluorescence to temperature ( $F_o$ -T curve)  
178 (Schreiber and Berry, 1977). Three sun leaves similar with the leaves with  
179 temperature measurements were sampled from each individual in the morning, dark  
180 adapted for at least half an hour in plastic bags with wet tissue paper inside to prevent  
181 water loss. Leaves were rinsed with deionized water, cut rectangular segments (2 cm  
182 long) from the middle of the leaf avoiding main veins. Leaf segments were immersed  
183 into 5 ml deionized water in a cuvette, then set the temperature increasing rate at 2 °C  
184 /min from 25 °C - 70 °C. A magnetic stirrer bar was put in the water bath to achieve  
185 uniform heating.  $T_{crit}$  is calculated by the intersection of lines extrapolated from the  
186 slow and fast rise portion of the temperature-dependent fluorescence response (Knight  
187 and Ackerly, 2002)

188 **2.4 Leaf traits measurement.** We selected leaf traits which might have impact on  
189 leaf temperature, including morphological traits, optical traits, material properties,  
190 anatomical traits, and physiological traits (Table 3).

191 The leaves of similar size, age and orientation to the leaves with temperature  
192 measurement were collected. Eight to ten leaves for each individual were scanned  
193 using a flatbed-scan scanner. Leaf area (Area), leaf perimeter (P), perimeter/area ratio  
194 (P/A), leaf length (Length), and leaf width (Width) were analyzed by ImageJ 1.52q  
195 based on the scanned image. Optical properties of leaf reflectivity (Ref),  
196 transmissivity (Trans), and absorptivity (Abs)) were measured by spectrometer  
197 (USB2000, Ocean Optics, USA), using 10 leaves for each individual. These leaves

198 were also used to measure greenness which is proportional to the amount of  
199 chlorophyll present in leaves by chlorophyll meter (SPAD-502, Minolta, Japan).  
200 Three to ten leaves of each individual (more blades for small leaves) were collected in  
201 the morning and stored in the sealed plastic bags with moist paper inside for density  
202 and water content (WC) measurements. They were weighed soon after harvesting, and  
203 used water displacement to get leaf volumes, then oven dried under 80 °C to constant  
204 weight. Leaf density was calculated by the ratio of leaf mass (both fresh and dry  
205 density) to leaf volume. Water content was calculated by the ratio of weight difference  
206 between fresh and dry leaves to the dry mass (Perez-Harguindeguy et al., 2013).  
207 Histological technique of Paraffin-fixing (Biosystems, 2021) was used to make cross-  
208 sections for the measurements of leaf thickness (Thickness), the thickness of upper  
209 and lower epidermis (Thickness\_up, Thickness\_low), palisade mesophyll (Thickness\_  
210 palisade) and spongy mesophyll (Thickness\_spongy) (4 leaves for each individual).  
211 All the anatomical sections were photographed under a microscope (Leica  
212 Microsystems Vertrieb GmbH, Wetzlar, Germany), and then analyzed with ImageJ.  
213 Stomatal density and size were measured using paradermal sections. Paradermal  
214 sections were cut from the middle part of leaf avoiding main veins and boiled in water  
215 for 10-15 min, then immersed in a 1:1 mixture of 30% H<sub>2</sub>O<sub>2</sub> and acetic glacial  
216 aqueous solution until they became soft and disintegrated. The needle leaves for  
217 stomata sections were bleached with 1:1 of HNO<sub>3</sub>, and H<sub>2</sub>O in saturated KClO<sub>3</sub>. The  
218 samples for vein density analysis were bleached with 5% NaOH until they become  
219 transparent. Stain leaves for 15 min in 1% safranin in ethanol. All the sections were

220 mounted on slides and photographed under a Leica DM2500 light microscope.  
221 Measure total length of veins in the image and divide this number by the image area  
222 to get vein density.

223 Diurnal transpiration rate, photosynthesis rate and stomatal conductance were  
224 measured by Portable Photosynthesis System (LI-6400, LI-COR, USA) for two sunny  
225 days at each site, then we combined the two days measurements into one diurnal  
226 curve. Three leaves adjacent to the leaves with temperature measurement were  
227 selected for each individual. All the leaves were measured one by one alternately from  
228 morning to afternoon, the start and end time were dependent on solar radiation and the  
229 availability of canopy crane at each forest (SAV: 8:00~17:00; TRF: 9:20~14:40; STF:  
230 9:30~16:30; TEF: 8:30 ~ 17:40).

231 **2.5 Thermal safety margin.** Thermal safety margin was calculated based on  $T_{crit}$  to  
232 represent thermal safety of photosynthetic system II.

$$233 \quad TSM = T_{crit} - T \quad (1)$$

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

239 **2.6 Data analysis**

240 **Comparison of TSM and  $T_{crit}$  across and within biomes.** The difference of TSM  
241 and  $T_{crit}$  across and within biomes were analyzed by multiple comparison of least  
242 significant difference (LSD). P-value < 0.05 was considered as significant difference.

243 **Impact factors on TSM.TI.** TSM.TI is determined by two parameters —  $T_{crit}$  and  
244  $T_{lmax}$ . We constructed a mixed effects model by setting TSM.TI as the response  
245 variable,  $T_{crit}$  and  $T_{lmax}$  as the fixed effects, and species nested in site as random  
246 effects. The contribution of  $T_{crit}$  and  $T_{lmax}$  to the variance of TSM.TI were analyzed  
247 by partR2 in R package “partR2” (Martin A. Stoffel et al., 2021).

248 **Impact factors on  $T_{crit}$ .** Impact factors including 27 leaf traits which might have  
249 relationships with leaf temperature (Table 3) and  $T_{lmax}$ . Random forest (Breiman,  
250 2001) was used to find the important leaf traits playing strong roles in explaining  
251 variation of thermotolerance. This model corrects data overfitting, and allows non-  
252 linear relationships and colinear variables (Breiman, 2001). We calculated variation  
253 explained rate by setting number of variables randomly sampled as candidates at each  
254 split (mtry) from 1 to 27 (the number of variables minus 1), and got the highest  
255 variation explained rate when mtry = 20. OOB error converged at number of trees to  
256 grow (ntree) = 400. Therefore, we fit the random forest model with mtry = 20 and  
257 ntree = 400, then used node purity values to inform the importance of each predictor.  
258 Mixed-effects model was also used to confirm the results. Species nest in site was set  
259 as random effect, the important leaf traits selected by random forest were fixed effects  
260 and  $T_{crit}$  was the response variable.

261 **Impact factors on the difference between TSM.Tl and TSM.Ta (TSM.Ta-**  
262 **TSM.Tl).** Repeated Measures Correlation in R package “rmcorr” (Bakdash and  
263 Marusich, 2017) was used to calculate the correlation between temperature traits and  
264 TSM.Ta - TSM.Tl by setting site as subject. Temperature traits included maximum  
265 leaf temperature (Tlmax), maximum air temperature (Tamax).

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

### 267 **3. Results**

#### 268 **3.1 Patterns of thermal safety margin (TSM) across and within biomes**

269 Site mean TSM.Tl ranged from  $3.0 \pm 0.5^\circ\text{C}$  at SAV to  $12.8 \pm 0.9^\circ\text{C}$  at STF. The rank  
270 of TSM calculated by the maximum air temperature of each individual (TSM.Ta) and  
271 the maximum leaf temperature of each individual (TSM.Tl) across biomes followed  
272 the same trend:  $\text{STF} = \text{TEF} > \text{TRF} > \text{SAV}$ , and TSM calculated by the maximum air  
273 temperature at each forest (TSM.MTa) followed  $\text{STF} > \text{TEF} > \text{TRF} > \text{SAV}$ . The  
274 patterns within biomes were different. TSM.Tl, TSM.Ta and TSM.MTa had positive  
275 correlations at SAV, TSM.Tl and TSM.Ta were positively correlated at TRF, while  
276 TSM.Ta and TSM.MTa were positively correlated at STF and TEF (Fig. 1a).

277 No negative TSM.Tl was found for normal leaves. However, *Woodfodia*  
278 *fruticosa* and *Bauhinia brachycarpa* presented negative and zero TSM respectively,  
279 when transpiration was blocked. All the Vaseline leaves of these two species were  
280 dried and dropped at the end of the experiment.

#### 281 **3.2 The patterns of $T_{\text{crit}}$ across and within biomes**

282  $T_{\text{crit}}$  is one of the two parameters in TSM calculation. Site mean  $T_{\text{crit}}$  range from 42.5

283  $\pm 0.6$  °C in TEF to  $48.5 \pm 0.5$  °C in SAV, and followed the pattern: SAV = TRF >  
284 STF > TEF (Fig. 1b).  $T_{crit}$  was linearly and negatively correlated with TSM.TI  
285 (Pearson correlation coefficient = -0.46, p-value < 0.001) across biomes. There were  
286 no significant correlations between  $T_{crit}$  and TSM.TI within biomes, except for  
287 positive correlation at SAV (Pearson correlation coefficient = 0.70, p-value = 0.01).  
288 The random forest model including all the leaf traits and the maximum air  
289 temperature explained 68% of the variance of  $T_{crit}$ , among them,  $T_{lmax}$  and  $T_{amax}$   
290 explained 62%. Considering of the high correlation between  $T_{lmax}$  and  $T_{amax}$ , we  
291 used  $T_{amax}$  and  $T_{lmax}$  as a fixed effect respectively, and species nested in site as  
292 random forest in mixed effects model. The model used  $T_{amax}$  as fixed effect gave  
293 conditional  $R^2 = 0.798$  and marginal  $R^2 = 0.571$ , and the model used  $T_{lmax}$  as fixed  
294 effect gave conditional  $R^2 = 0.799$  and marginal  $R^2 = 0.131$ . However, the Pearson  
295 correlations between  $T_{amax}$  (or  $T_{lmax}$ ) and  $T_{crit}$  were not significant within biomes  
296 except for  $T_{amax}$  and  $T_{crit}$  at TRF. Therefore,  $T_{crit}$  increased with environmental  
297 temperature across biomes; while the positive relationships between  $T_{crit}$  and  
298 environmental temperature within biomes was not confirmed.

### 299 **3.3 Patterns of leaf temperature across and within biomes**

300 Leaf temperature is another parameter in TSM calculation. It linearly increased with  
301 air temperature, however, the increasing slope increased from the hot to the cold  
302 forest. Except for the SAV species, all the other species had significant higher  $T_{lmax}$   
303 than  $T_{amax}$  (Fig. 2a). During daytime, leaves had strong cooling effects to reduce TI  
304 close to  $T_a$  at SAV, while TI was much higher than  $T_a$  for most of the time at TEF.

305 Thus, the difference between Tl and Ta increased from the hot to the cold forest (Fig.  
306 2a). The variances of Tlmax between species were within 8 °C in SAV, STF and TRF,  
307 reached to 12.2 °C in TEF, while the variances of Tamax between species within  
308 biomes were all lower than 5.5 °C.

309 Normal leaf temperatures were all below T<sub>crit</sub> except for two savanna species *L.*  
310 *coromandelica* and *W. fruticosa*, and their temperatures exceeded T<sub>crit</sub> for less than  
311 one minute. When transpiration was blocked, leaf temperature increased, and the  
312 increase extent was highest for SAV species (Fig. 2b). Vaseline leaf temperature of all  
313 savanna species and one subtropical species exceeded T<sub>crit</sub>. Among them, the  
314 overheating time of the two savanna species *B. brachycarpa* and *W. fruticosa*  
315 exceeded 10 minutes ( $10.9 \pm 7.3$  minutes and  $39.2 \pm 11.6$  minutes respectively).

#### 316 **3.4 Factors affecting TSM.Tl**

317 TSM.Tl were calculated using Tlmax and T<sub>crit</sub>. Compared with T<sub>crit</sub>, Tlmax were  
318 highly variable. The range of T<sub>crit</sub> across biomes was 12.4 °C, while the range of  
319 Tlmax were much higher (19.7 °C). In the mixed effects model, marginal R<sup>2</sup>  
320 contributed by Tlmax was 87.1%, while marginal R<sup>2</sup> contributed by T<sub>crit</sub> was 0%.  
321 Pearson correlation coefficient between TSM.Tl and Tlmax was -0.93. TSM.Tl can be  
322 predicted by Tlmax by the model  $TSM.Tl = -0.672 \times Tlmax + 33.581$  (R<sup>2</sup> = 0.85, p-  
323 value < 0.001), and 83% of the residuals were within 1.5 °C (Fig. 3).

#### 324 **3.5 Factors affecting the difference between TSM.Tl and TSM.Ta (TSM.Ta- 325 TSM.Tl)**



326 TSM.Ta - TSM.Tl increased from  $0.3 \pm 0.9^{\circ}\text{C}$  at SAV,  $1.8 \pm 1.1^{\circ}\text{C}$  at TRF, and  $3.2 \pm$   
327  $1.1^{\circ}\text{C}$  at STF, to  $4.7 \pm 2.1^{\circ}\text{C}$  at TEF. Repeated measures correlation revealed that  
328 Tlmax (cor 0.87, p-value < 0.001) had the highest correlation with TSM.Ta - TSM.Tl.  
329 TSM.Ta - TSM.Tl linearly increase with Tlmax, but the intercept increased from SAV  
330 to TEF (Fig. 4).

331

## 332 **4. Discussion**

333 **4.1 General patterns of TSM and  $T_{\text{crit}}$ .** Patterns of TSM (regardless of the  
334 temperature metrics used) followed similar trends with previous studies across biomes:  
335 TSM decreased from the cool forests to the hot forests (Curtis et al., 2016; O'Sullivan  
336 et al., 2017). During the heatwave, TSMs of savanna species were the lowest,  
337 indicating that their photosynthetic systems were more dangerous under heat stress.  
338 Especially when transpiration was suppressed, leaves of some species in SAV were  
339 dried and dropped at the end of the experiment. A global study also demonstrated that  
340 woody productivity in the hottest forests among 590 permanent plots across the  
341 tropics are more sensitive to temperature than at cooler sites (Sullivan et al., 2020).  
342 The risks of species disappear and reduction of carbon stock in tropical hot forests  
343 under heatwave requires high attention (Gallagher et al., 2019; Tiwari et al., 2020).

344 Site mean  $T_{\text{crit}}$  ranged from  $42.5^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$  at TEF to  $48.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  at SAV.  
345 The pattern and values were comparable with  $T_{\text{crit}}$  of other studies using the same  
346 methodology (O'Sullivan et al., 2017; Zhu et al., 2018).  $T_{\text{crit}}$  values were lower than  
347  $T_{50}$  measured using  $F_v/F_m-T$  method (Perez et al., 2020). Random forest model

348 showed that  $T_{\text{max}}$  and  $T_{\text{max}}$  can explain 62% of the variance of  $T_{\text{crit}}$ . This indicated  
349 the important influence of micro-environment on  $T_{\text{crit}}$ . However, the correlations  
350 between  $T_{\text{crit}}$  and  $T_{\text{max}}$  or  $T_{\text{max}}$  were not confirmed within biomes. The range of  
351  $T_{\text{max}}$  across biomes was 21.6 °C, while the maximum range of  $T_{\text{max}}$  within  
352 biomes were below 5.1 °C. Therefore, environmental temperature can explain more  
353 than 60% of the variance of  $T_{\text{crit}}$  across biomes, while leaf traits might be the main  
354 explanation on  $T_{\text{crit}}$  variance within biomes. There are some leaf traits have been  
355 reported having relationships with  $T_{\text{crit}}$ , e.g. LMA (Gallagher, 2014; Sastry et al.,  
356 2018), leaf carbon assimilation (Perez et al., 2020), and leaf chemical composition  
357 (Zhu et al., 2018). Thus, more samples should be collected to confirm which leaf traits  
358 are more related to  $T_{\text{crit}}$  within biomes.

#### 359 **4.2 Contrary pattern between TSM and thermotolerance.**

360 Traditionally, the plants with higher thermotolerance are considered to be more  
361 resilient to heat stress (Wahid et al., 2007). However, our results showed that TSMs  
362 might be negatively related to thermotolerance. For example, the SAV species have  
363 high thermotolerance, however, they are more vulnerable to heat damage than the  
364 TEF and STF species which have low thermotolerance while low leaf temperature.  
365 Notably, the negative relationship between TSM.TI and  $T_{\text{crit}}$  was only found across  
366 biomes, and their relationships within biomes are uncertain. We found positive  
367 correlation between TSM.TI and  $T_{\text{crit}}$  at SAV, but no significant relationships between  
368 them at other forests; while the study of 19 plant species in Fairchild Tropical Botanic  
369 garden found negative correlation between thermotolerance and TSM.TI (Perez and

370 Feeley, 2020). Accordingly, thermotolerance cannot be used to estimate thermal safety  
371 of plant leaves.

### 372 **4.3 How to detect vulnerable species under heat stress**

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

385 The method to determine  $T_{lmax}$  has great impact on TSM. The damage of high  
386 temperature on leaves is determined by both the threshold of temperature and the  
387 exposure time. If leaf temperature exceeding  $T_{crit}$  lasted for a few seconds, it could not  
388 damage leaf. Previous research usually used 15 minutes to treat leaves under water  
389 bath when measure the response of  $F_v/F_m$  to temperature (Curtis et al., 2014; Krause  
390 et al., 2010). In the present study, we observed leaves died when leaf temperature  
391 exceeding  $T_{crit}$  for more than 10 minutes in one day. Therefore, the calculation of

392 Tlmax in TSM should consider its duration time.

#### 393 **4.4 Can we use air temperature to measure TSM?**

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

#### 403 **5. Conclusion**

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

413

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427 **Data availability statement**

428 Primary data are stored at figshare, [https:// 10.6084/m9.figshare.14038478](https://10.6084/m9.figshare.14038478).

429

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597  
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599

600 Figure legend

601

602 Figure 1 The patterns of (a) thermal safety margin (TSM) and (b) thermotolerance  
603 ( $T_{crit}$ ). TSM.Ta, TSM calculated by the maximum air temperature of each individual;  
604 TSM.Tl, TSM calculated by the maximum leaf temperature of each individual;  
605 TSM.MTa, TSM calculated by the maximum air temperature at each forest. SAV,  
606 savanna; TRF, tropical rain forest; STF, subtropical broad-leaved forest; TEF,  
607 temperate mixed forest.

608

609 Figure 2 The relationships between (a) leaf temperature and air temperature; and (b)  
610 Vasline leaf temperature and air temperature during daytime (9:00 ~ 17:00). SAV,  
611 savanna; TRF, tropical rain forest; STF, subtropical broad-leaved forest; TEF,  
612 temperate mixed forest. Dash line is the regression line of  $y = x$ .

613

614 Figure 3 The relationship between TSM.Tl and  $T_{lmax}$ . The regression line can be  
615 modeled by  $TSM.Tl = -0.672 \times T_{lmax} + 33.581$  ( $R^2 = 0.85$ ,  $p\text{-value} < 0.001$ ).

616

617 Figure 4 The relationships between TSM calculated by the maximum air temperature  
618 and the maximum leaf temperature of individuals respectively (TSM.Ta - TSM.Tl)  
619 and the maximum leaf temperature ( $T_{lmax}$ ). SAV, savanna; TRF, tropical rain forest;  
620 STF, subtropical broad-leaved forest; TEF, temperate mixed forest.

621