

2022-02

Thermal safety margins of plant leaves across biomes under a heatwave

Kitudom, N

<http://hdl.handle.net/10026.1/20029>

10.1016/j.scitotenv.2021.150416

Science of The Total Environment

Elsevier BV

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

1 Thermal safety margins of plant leaves across biomes

2 Kitodom Nawatbhrist^{1,5}, Fauset Sophie^{1,4}, Zhou Yingying^{1,5}, Fan Zexin^{1,2,3}, Li

3 Murong^{1,6}, He Mingjian^{1,6}, Zhang Shubin¹, Xu kun⁷, Lin Hua^{1,2*}

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

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

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

10 ⁴School of Geography, Earth and Environmental Sciences, University of Plymouth,
11 Plymouth, UK.

12 ⁵University of Chinese Academy of Sciences, Beijing 100049, China

13 ⁶College of Biology and Chemistry, Puer University, Puer, Yunnan 665000, China.

14 ⁷Yunnan 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

18
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% H₂O₂ 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 HNO₃, and H₂O in saturated KClO₃. 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_{crit} across and within biomes**

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

283 ± 0.6 °C in TEF to 48.5 ± 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.Tl
285 (Pearson correlation coefficient = -0.46, p-value < 0.001) across biomes. There were
286 no significant correlations between T_{crit} and TSM.Tl 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, Tlmax and Tamax
290 explained 62%. Considering of the high correlation between Tlmax and Tamax, we
291 used Tamax and Tlmax as a fixed effect respectively, and species nested in site as
292 random forest in mixed effects model. The model used Tamax as fixed effect gave
293 conditional $R^2 = 0.798$ and marginal $R^2 = 0.571$, and the model used Tlmax as fixed
294 effect gave conditional $R^2 = 0.799$ and marginal $R^2 = 0.131$. However, the Pearson
295 correlations between Tamax (or Tlmax) and T_{crit} were not significant within biomes
296 except for Tamax 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 Tlmax
303 than Tamax (Fig. 2a). During daytime, leaves had strong cooling effects to reduce Tl
304 close to Ta at SAV, while Tl was much higher than Ta 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_{crit} except for two savanna species *L.*
310 *coromandelica* and *W. fruticosa*, and their temperatures exceeded T_{crit} 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_{crit}. Among them, the
314 overheating time of the two savanna species *B. brachycarpa* and *W. fruticosa*
315 exceeded 10 minutes (10.9 ± 7.3 minutes and 39.2 ± 11.6 minutes respectively).

316 **3.4 Factors affecting TSM.Tl**

317 TSM.Tl were calculated using Tlmax and T_{crit}. Compared with T_{crit}, Tlmax were
318 highly variable. The range of T_{crit} across biomes was 12.4 °C, while the range of
319 Tlmax were much higher (19.7 °C). In the mixed effects model, marginal R²
320 contributed by Tlmax was 87.1%, while marginal R² contributed by T_{crit} 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 $TSM.Tl = -0.672 \times Tlmax + 33.581$ (R² = 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°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_{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_{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_{crit} of other studies using the same
346 methodology (O'Sullivan et al., 2017; Zhu et al., 2018). T_{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_{lmax} and T_{amax} can explain 62% of the variance of T_{crit} . This indicated
349 the important influence of micro-environment on T_{crit} . However, the correlations
350 between T_{crit} and T_{lmax} or T_{amax} were not confirmed within biomes. The range of
351 T_{amax} across biomes was 21.6 °C, while the maximum range of T_{amax} within
352 biomes were below 5.1 °C. Therefore, environmental temperature can explain more
353 than 60% of the variance of T_{crit} across biomes, while leaf traits might be the main
354 explanation on T_{crit} variance within biomes. There are some leaf traits have been
355 reported having relationships with T_{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_{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.Tl and T_{crit} was only found across
366 biomes, and their relationships within biomes are uncertain. We found positive
367 correlation between TSM.Tl and T_{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.Tl (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

414 **Acknowledgements**

415 Thanks for the support of National Forest Ecosystem Research Station at
416 Xishuangbanna, National Forest Ecosystem Research Station at Ailaoshan, Yuanjiang
417 Savanna Ecosystem Research Station, Xishuangbanna Tropical Botanical Garden,
418 Chinese Academy of Sciences, Yunnan Lijiang Forest Ecosystem National
419 Observation and Research Station, and the central laboratory of Xishuangbanna
420 Tropical Botanical Garden, Chinese Academy of Sciences.

421 **Funding**

422 This study was funded by the National Natural Science Foundation of China (grant
423 number 31870386), the CAS 135 Program (grant number 2017XTBG-F01), Chinese
424 Academy of Sciences President's International Fellowship Initiative (grant number
425 2016VBA036), and the National Natural Science Foundation of China (NSCF-TRF
426 project, grant number 4186114401).

427 **Data availability statement**

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

429

430 **References**

- 431 Ackerly DD, Stuart SA. Physiological Ecology: Plants. In: Levin SA, editor. The
432 Princeton Guide to Ecology. Princeton University Press, New Jersey, USA,
433 2009.
- 434 Alexander LV, Zhang X, Peterson TC, Caesar J, Gleason B, Tank AMGK, et al.
435 Global observed changes in daily climate extremes of temperature and
436 precipitation. *Journal of Geophysical Research-Atmospheres* 2006; 111:
437 D006290.
- 438 Allen CD, Breshears DD, McDowell NG. On underestimation of global vulnerability
439 to tree mortality and forest die-off from hotter drought in the Anthropocene.
440 *Ecosphere* 2015; 6: 1-55.
- 441 Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, et
442 al. A global overview of drought and heat-induced tree mortality reveals

443 emerging climate change risks for forests. *Forest Ecology and Management*
444 2010; 259: 660-684.

445 Araújo I, Marimon BS, Scalon MC, Fauset S, Junior BM, Tiwari R, et al. Trees at the
446 Amazonia-Cerrado transition are approaching high temperature thresholds.
447 *Environmental Research Letters* 2021; 16: 034047.

448 Bakdash JZ, Marusich LR. Repeated measures correlation. *Frontiers in Psychology*
449 2017; 8: 456.

450 Baker NR. Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo. The annual
451 review of plant biology 2008; 59: 89-113.

452 Biosystems L. An Introduction to Specimen Processing. In: Rolls G, editor, 2021.

453 Breiman L. Random forests. *Machine Learning* 2001; 45: 5-32.

454 Chaste E, Girardin MP, Kaplan JO, Bergeron Y, Hely C. Increases in heat-induced tree
455 mortality could drive reductions of biomass resources in Canada's managed
456 boreal forest. *Landscape Ecology* 2019; 34: 403-426.

457 Ciais P, Reichstein M, Viovy N, Granier A, Ogee J, Allard V, et al. Europe-wide
458 reduction in primary productivity caused by the heat and drought in 2003.
459 *Nature* 2005; 437: 529-533.

460 Coumou D, Robinson A. Historic and future increase in the global land area affected
461 by monthly heat extremes. *Environmental Research Letters* 2013; 8: 034018.

462 Crawford AJ, McLachlan DH, Hetherington AM, Franklin KA. High temperature
463 exposure increases plant cooling capacity. *Current Biology* 2012; 22: R396-
464 R397.

465 Curtis EM, Gollan J, Murray BR, Leigh A. Native microhabitats better predict
466 tolerance to warming than latitudinal macro-climatic variables in arid-zone
467 plants. *Journal of Biogeography* 2016; 43: 1156-1165.

468 Curtis EM, Knight CA, Petrou K, Leigh A. A comparative analysis of photosynthetic
469 recovery from thermal stress: a desert plant case study *Oecologia* 2014; 176:
470 1213-1213.

471 Dahl JE, Bertrand M, Pierre A, Curtit B, Pillard C, Tasiemski A, et al. Thermal
472 tolerance patterns of a carabid beetle sampled along invasion and altitudinal
473 gradients at a sub-Antarctic island. *Journal of Thermal Biology* 2019; 86:
474 102447.

475 Denny MW, Dowd WW. Biophysics, environmental stochasticity, and the evolution of
476 thermal safety margins in intertidal limpets. *Journal of Experimental Biology*
477 2012; 215: 934-947.

478 Dong N, Prentice IC, Harrison SP, Song QH, Zhang YP. Biophysical homeostasis of
479 leaf temperature: A neglected process for vegetation and land-surface
480 modelling. *Global Ecology and Biogeography* 2017; 26: 998-1007.

481 Doughty CE, Goulden ML. Are tropical forests near a high temperature threshold?
482 *Journal of Geophysical Research-Biogeosciences* 2008; 113: G00B07.

483 Fauset S, Freitas HC, Galbraith DR, Sullivan MJP, Aidar MPM, Joly CA, et al.
484 Differences in leaf thermoregulation and water use strategies between three
485 co-occurring Atlantic forest tree species. *Plant Cell and Environment* 2018; 41:
486 1618-1631.

487 Gallagher DW. Photosynthetic thermal tolerance and recovery to short duration
488 temperature stress in desert and montane plants: A comparative study. *Science*
489 *in Biology*. Master. California Polytechnic State University, California, 2014.
490 Gallagher RV, Allen S, Wright IJ. Safety margins and adaptive capacity of vegetation
491 to climate change. *Scientific Reports* 2019; 9: 8241.
492 Gates DM. *Biophysical Ecology*. New York, USA: Dover Publications, 2003.
493 Gunderson AR, Stillman JH. Plasticity in thermal tolerance has limited potential to
494 buffer ectotherms from global warming. *Proceedings of the Royal Society B-*
495 *Biological Sciences* 2015; 282: 20150401.
496 Hansen J, Sato M, Ruedy R. Perception of climate change. *Proceedings of the*
497 *National Academy of Sciences of the United States of America* 2012; 109:
498 E2415-E2423.
499 Havaux M. Characterization of thermal-damage to the photosynthetic electron-
500 transport system in potato leaves. *Plant Science* 1993; 94: 19-33.
501 Knight CA, Ackerly DD. An ecological and evolutionary analysis of photosynthetic
502 thermotolerance using the temperature-dependent increase in fluorescence.
503 *Oecologia* 2002; 130: 505-514.
504 Knight CA, Ackerly DD. Evolution and plasticity of photosynthetic thermal tolerance,
505 specific leaf area and leaf size: congeneric species from desert and coastal
506 environments. *New Phytologist* 2003; 160: 337-347.
507 Krause GH, Winter K, Krause B, Jahns P, Garcia M, Aranda J, et al. High-temperature
508 tolerance of a tropical tree, *Ficus insipida*: methodological reassessment and
509 climate change considerations. *Functional Plant Biology* 2010; 37: 890-900.
510 Lancaster LT, Humphreys AM. Global variation in the thermal tolerances of plants.
511 *Proceedings of the National Academy of Sciences of the United States of*
512 *America* 2020; 117: 201918162.
513 Leigh A, Sevanto S, Ball MC, Close JD, Ellsworth DS, Knight CA, et al. Do thick
514 leaves avoid thermal damage in critically low wind speeds. *New Phytologist*
515 2012; 194: 477-487.
516 Leon-Garcia IV, Lasso E. High heat tolerance in plants from the Andean highlands:
517 Implications for paramos in a warmer world. *PLoS One* 2019; 14: e0224218.
518 Leuzinger S, Körner C. Tree species diversity affects canopy leaf temperatures in a
519 mature temperate forest. *Agricultural and Forest Meteorology* 2007; 146: 29-
520 37.
521 Lin H, Chen YJ, Zhang HL, Fu PL, Fan ZX. Stronger cooling effects of transpiration
522 and leaf physical traits of plants from a hot dry habitat than from a hot wet
523 habitat. *Functional Ecology* 2017; 31: 2202-2211.
524 Martin A. Stoffel, Shinichi Nakagawa, Schielzeth H. partR2: Partitioning R2 in
525 generalized linear mixed models. *Peer J* 2021; 9: e11414.
526 McArley TJ, Hickey AJR, Herbert NA. Chronic warm exposure impairs growth
527 performance and reduces thermal safety margins in the common triplefin fish
528 (*Forsterygion lapillum*). *Journal of Experimental Biology* 2017; 220: 3527-
529 3535.
530 Michaletz ST, Weiser MD, McDowell NG, Zhou JZ, Kaspari M, Helliker BR, et al.

531 The energetic and carbon economic origins of leaf thermoregulation. *Nature*
532 *Plants* 2016; 2: 16129.

533 Michaletz ST, Weiser MD, Zhou JZ, Kaspari M, Helliker BR, Enquist BJ. Plant
534 thermoregulation: Energetics, trait-environment interactions, and carbon
535 economics. *Trends in Ecology & Evolution* 2015; 30: 714-724.

536 O'Sullivan OS, Heskell MA, Reich PB, Tjoelker MG, Weerasinghe LK, Penillard A, et
537 al. Thermal limits of leaf metabolism across biomes. *Global Change Biology*
538 2017; 23: 209-223.

539 Okajima Y, Taneda H, Noguchi K, Terashima I. Optimum leaf size predicted by a
540 novel leaf energy balance model incorporating dependencies of photosynthesis
541 on light and temperature. *Ecological Research* 2012; 27: 333-346.

542 Perez-Harguindeguy N, Diaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, et al.
543 New handbook for standardised measurement of plant functional traits
544 worldwide. *Australian Journal of Botany* 2013; 61:167-234.

545 Perez TM, Feeley KJ. Photosynthetic heat tolerances and extreme leaf temperatures.
546 *Functional Ecology* 2020; 34: 2236-2245.

547 Perez TM, Socha A, Tserej O, Feeley KJ. Photosystem II heat tolerances characterize
548 thermal generalists and the upper limit of carbon assimilation. *Plant & Cell*
549 *Environment* 2020; 44: 2321-2330.

550 Pincebourde S, Casas J. Narrow safety margin in the phyllosphere during thermal
551 extremes. *Proceedings of the National Academy of Sciences of the United*
552 *States of America* 2019; 116: 5588-5596.

553 Sastry A, Barua D. Leaf thermotolerance in tropical trees from a seasonally dry
554 climate varies along the slow-fast resource acquisition spectrum. *Scientific*
555 *Reports* 2017; 7: 11246.

556 Sastry A, Guha A, Barua D. Leaf thermotolerance in dry tropical forest tree species:
557 relationships with leaf traits and effects of drought. *Aob Plants* 2018; 10:
558 plx070.

559 Schreiber U, Berry JA. Heat-induced changes of chlorophyll fluorescence in intact
560 leaves correlated with damage of photosynthetic apparatus. *Planta* 1977; 136:
561 233-238.

562 Smith WK. Temperatures of desert plants - another perspective on adaptability of leaf
563 size. *Science* 1978; 201: 614-616.

564 Song X, Barbour MM, Saurer M, Helliker BR. Examining the large-scale
565 convergence of photosynthesis-weighted tree leaf temperatures through stable
566 oxygen isotope analysis of multiple data sets. *New Phytologist* 2011; 192:
567 912-924.

568 Stokes VJ, Morecroft MD, Morison JIL. Boundary layer conductance for contrasting
569 leaf shapes in a deciduous broadleaved forest canopy. *Agriculture and Forest*
570 *Meteorology* 2006; 139: 40-54.

571 Sullivan MJP, Lewis SL, Affum-Baffoe K, Castilho C, Costa F, Sanchez AC, et al.
572 Long-term thermal sensitivity of earth's tropical forests. *Science* 2020; 368:
573 869-874.

574 Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, et al.

575 Thermal-safety margins and the necessity of thermoregulatory behavior across
576 latitude and elevation. *Proceedings of the National Academy of Sciences of*
577 *the United States of America* 2014; 111: 5610-5615.

578 Tatarinov F, Rotenberg E, Maseyk K, Ogee J, Klein T, Yakir D. Resilience to seasonal
579 heat wave episodes in a Mediterranean pine forest. *New Phytologist* 2016; 210:
580 485-496.

581 Team RDC. *R: A Language and Environment for Statistical Computing*. 2019.

582 Teskey R, Wertin T, Bauweraerts I, Ameye M, McGuire MA, Steppe K. Responses of
583 tree species to heat waves and extreme heat events. *Plant Cell and*
584 *Environment* 2015; 38: 1699-1712.

585 Tiwari R, Gloor E, da Cruz WJA, Marimon BS, Marimon B, Reis SM, et al.
586 Photosynthetic quantum efficiency in south-eastern Amazonian trees may be
587 already affected by climate change. *Plant Cell and Environment* 2020: 13770.

588 Vinagre C, Dias M, Cereja R, Abreu-Afonso F, Flores AAV, Mendonca V. Upper
589 thermal limits and warming safety margins of coastal marine species -
590 Indicator baseline for future reference. *Ecological Indicators* 2019; 102: 644-
591 649.

592 Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: An overview.
593 *Environmental and Experimental Botany* 2007; 61: 199-223.

594 Zhu LL, Bloomfield KJ, Hocart CH, Egerton JGG, O'Sullivan OS, Penillard A, et al.
595 Plasticity of photosynthetic heat tolerance in plants adapted to thermally
596 contrasting biomes. *Plant Cell and Environment* 2018; 41: 1251-1262.

597
598
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