Received: 2 January 2017 | Accepted: 26 May 2017

DOI: 10.1111/1365-2435.12923

RESEARCH ARTICLE

Functional Ecology BRITISH ECOLOGICAL SOCIETY

Stronger cooling effects of transpiration and leaf physical traits of plants from a hot dry habitat than from a hot wet habitat

Hua Lin[1] | Yajun Chen[1] | Houlei Zhang[2] | Peili Fu[1] | Zexin Fan[1]

[1]Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan, China

[2]Division of New Energy Science and Engineering, Nanjing University of Science & Technology, Nanjing, Jiangsu, China

Correspondence
Hua Lin
Email: lh@xtbg.ac.cn

Funding information
National Natural Science Foundation of China, Grant/Award Number: 31200307; Applied Fundamental Research Program of Yunnan Province, Grant/Award Number: 2013FB078; CAS President's International Fellowship Initiative, Grant/Award Number: 2016VBA036

Handling Editor: Jenny Watling

Abstract

1. Leaf temperature exerts an important impact on the microenvironment and physiological processes of leaves. Plants from different habitats have different strategies to regulate leaf temperature. The relative importance of physical traits and transpiration for leaf temperature regulation in the hot habitat is still unclear.
2. We investigated 22 leaf physical traits, transpiration, and thermal properties of 38 canopy species of seedlings in a greenhouse, including 18 dominant species from a hot wet habitat (HW) and 20 dominant species from a hot dry habitat (HD). To separate the impact of transpiration and leaf physical traits on leaf temperature, we measured the diurnal courses of leaf temperatures with and without transpiration. The temperature of a reference leaf beside each individual was measured simultaneously to render temperatures comparable.
3. Generally, the species from HD showed lower leaf temperatures than the species from HW under the same conditions. Both transpiration capacity and cooling effect of leaf physical traits were stronger for the plants from HD. Active transpiration provides a suitable thermal environment for photosynthesis, while xeromorphic leaves can dampen heat stress when transpiration is suppressed. Higher vein density and stomatal pore area index (SPI) facilitated higher transpiration capacity of the plants from HD. Meanwhile, shorter leaves and thinner lower epidermis of the plants from HD were more efficient in heat transfer, although relationships were much weaker than the synergic effect of all the physical traits.
4. Our results confirmed that transpiration and leaf physical traits provided double insurance for avoiding overheating, particularly for plant from HD. We emphasize that transpiration is a more effective way to cool leaves than physical traits when water is sufficient, which may be an important adaptation for plant from HD where rainfall is sporadic. Our results provide further insight into the relationship between physical traits and transpiration for the regulation of leaf temperature, and the co-evolution of gas exchange and thermal regulation of leaves.

KEYWORDS
hot dry, hot wet, leaf temperature, temperature regulation, thermal effect, transpiration capacity

Paper previously published as Standard Paper.

1 | INTRODUCTION

Leaf temperature is a proxy for the energy balance between the leaf interior and the ambient environment (Campbell & Norman, 1998; Gates, 2003). It influences enzyme activity and the metabolic processes of leaves. Incoming solar radiation absorbed by a leaf is partly used for biochemical reactions (e.g. photosynthesis), but a larger proportion is converted to the thermal energy of leaves. Leaf temperature may be very different from the air temperature, and varies between species owing to the physical and physiological differences in plants (Martin, Hinckley, Meinzer, & Sprugel, 1999; Stokes, Morecroft, & Morison, 2006). For example, the leaf temperature of pineapple was 7.6°C higher than the air temperature when exposed to direct sunlight, while the leaf temperature of papaya was lower than the air temperature under the same conditions (Noffinger, 1961).

Leaf temperature is predominately determined by ambient temperature, but also regulated by leaf physical traits and transpiration (Defraeye, Verboven, Ho, & Nicolai, 2013; Lambers, Chapin, & Pons, 1998; Scheffers et al., 2016). When plants are exposed to hot conditions, they can reduce the amount of accepted radiation through reflection and movement and can dissipate excessive heat via radiation emission, heat convection, and transpiration. However, the relative contribution of each of these processes differs greatly in different habitats or between different plant groups. For the plants in arid habitats, physical adjustments are commonly believed to be the main means by which plants achieve heat dissipation, while the contribution of transpiration to leaf cooling is often ignored (Ansari & Loomis, 1959; Lambers et al., 1998). For example, some plants from arid habitats have often evolved leaf hairs that reflect radiation (Lang & Schindler, 1994; Lang, Schindler, & Lichtenthaler, 1995; Ye et al., 2011), smaller leaves for better heat convection (Schuepp, 1993; Yates, Verboom, Rebelo, & Cramer, 2010), have developed thorn-like or succulent leaves to minimize transpiration. However, some arid habitat species use another strategy that they rapidly fix carbon in the short period when water is available (Tomlinson et al., 2013), during which they may have high transpiration. In this case, transpiration cooling is beneficial for achieving high rates of photosynthesis.

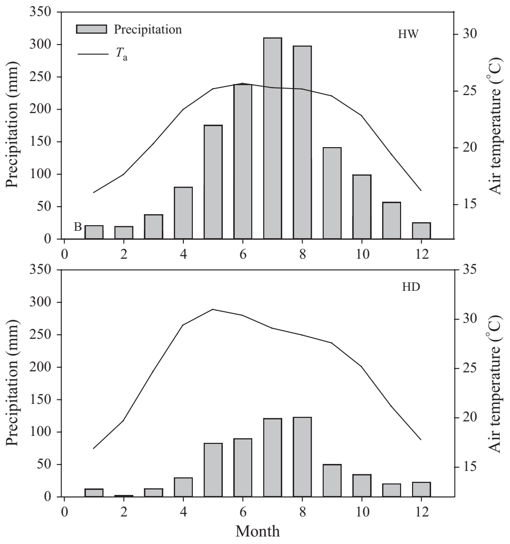

FIGURE 1 Monthly total precipitation and average air temperature in the hot wet habitat (HW) and the hot dry habitat (HD)

The relationship between leaf physical traits and leaf temperature can be simulated theoretically (Gates, 2003), tested in wind tunnels, or using replica leaves (Leigh et al., 2012; Sinclair, 1970; Vogel, 2009). However, the interaction between traits makes it difficult to measure the thermal impact of physical traits and transpiration separately for real leaves. Some previous studies only analysed the effects of one or several leaf physical traits on leaf temperature (Monteiro, Blanusa, Verhoef, Hadley, & Cameron, 2016), while there are many physical traits that could be associated with leaf temperature. In addition, the thermal effects of all the physical traits may differ from the individual contribution of a trait.

In this study, we planted seedlings of 38 canopy species originating from contrasting hydrothermal habitats in a greenhouse, including 18 dominant species from a tropical seasonal rainforest (HW) and 20 dominant species from a hot dry valley (HD) in southwestern China. We measured 22 leaf physical traits, transpiration, and the thermal properties of all the species. To separate the thermal effects of transpiration and physical traits, we monitored the diurnal temperatures of controlled leaves and vaselined leaves (transpiration prohibited) using a thermal camera. The temperature of the reference leaf beside each individual was measured simultaneously to exclude differences in thermal environment between measurements and individuals. The aim of our study was to: (1) assess the temperature differences between the plants from HD and HW in a greenhouse environment; (2) assess the relative importance of transpiration and leaf physical traits in leaf temperature regulation; (3) determine leaf temperature regulation strategies for the plants from HW and HD.

2 | MATERIALS AND METHODS

2.1 | Site and study species

We collected the first-year germinant seedlings of 18 dominant canopy species from the tropical seasonal rainforest in Xishuangbanna (HW, 21°57′41″N, 101°12′0″E, 750 m a.s.l.) and the first-year seedlings of 20 dominant canopy species from a hot dry valley in Yuanjiang, China (HD, 23°28′40″N, 102°10′56″E, 481 m a.s.l.). All HW species were trees, and HD species were trees, shrubs, and lianas (see Table S1). These sites are 200 km apart in the Yunnan province, southwestern China. All seedlings (four individuals for each species) were planted in separate pots (height: 32 cm, diameter: 30 cm) in a greenhouse located 8 km away from the tropical seasonal rainforest. The annual mean temperature at the HW site was 19.9°C and the average maximum air temperature was 34.6°C. Annual precipitation averaged 1,557 mm/year, 85% of which occurred during the rainy season (May–October) (Cao, Zou, Warren, & Zhu, 2006). The annual mean temperature at the HD site was 24.9°C, while the maximum air temperature reached 42°C. The average precipitation was 666.4 mm/year with most precipitation occurring from May to October (Figure 1).

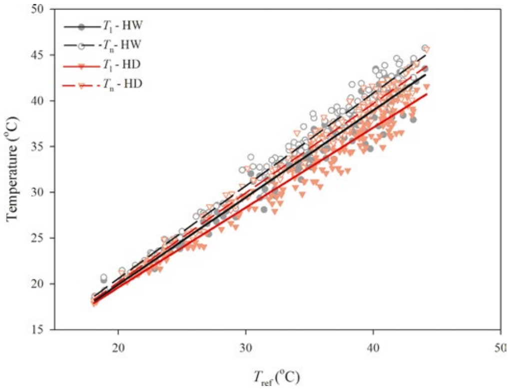

FIGURE 2 Regression relationships of temperatures of controlled leaves (T_l) and vaselined leaves (T_n) against reference leaf temperatures (T_{ref}). Fitted regression lines from ANCOVA are indicated as $T_l = 0.91 + 0.95T_{ref}$ ($R^2 = .98$, $n = 162$) for controlled leaves from the hot wet habitat (HW), $T_n = 0.31 + 1.013T_{ref}$ ($R^2 = .98$, $n = 162$) for leaves from HW without transpiration, $T_l = 2.24 + 0.87T_{ref}$ ($R^2 = .96$, $n = 180$) for controlled leaves from the hot dry habitat (HD), $T_n = 0.70 + 0.98T_{ref}$ ($R^2 = .98$, $n = 180$) for leaves from HD without transpiration. The slopes of all the regression lines were significantly different except for the regression slope of T_l against T_{ref} of the plants from HW and T_n against T_{ref} of the plants from HD

2.2 | Meteorological measurements

All the measurements were carried out in the third year of planting. During the experiment, we exposed the plants under a transparent roof. Solar radiation (R, measured by S-LIB-M003, HOBO, USA), photosynthetically active radiation (PAR, measured by S-LIA-M003, HOBO, USA), air temperature, relative humidity (T_a and RH, measured by S-THB-M002, HOBO, USA), and barometric pressure (Pressure, measured by S-BPA-CM10, HOBO, USA) were measured every 5 s, and 5 mins averages were saved on a data logger. Monitoring was carried out from 15 to 20 April 2015. It was sunny on 15 to 17 April, while 18 to 20 April were cloudy in the afternoon. Wind was negligible in the greenhouse. Detailed information regarding the weather is presented in Figure S2.

2.3 | Leaf temperature and transpiration measurements

The analysis of anatomy showed that all the studied plants only had stomata on the abaxial surfaces of leaves, and it has been shown that transpiration can be reduced 95%–98% by a thin coating of Vaseline (Curtis, 1936), so we evenly vaselined the abaxial surface of 2–3 leaves for each individual to suppress transpiration. The adaxial side of leaf was controlled to minimize changes to the emissivity and reflectance of each leaf. Leaf temperature was measured with an infrared camera (T420bx, Flir, USA), which records 7.5–13 μm infrared spectrum with a thermal sensitivity of 0.045°C. The emissivity of leaf was set to 0.95 (Aerts et al., 2004). Transpiration was measured with a portable gas exchange system (LI-6400; Li-Cor Inc., Lincoln, NE, USA), with the exception of five species from HD with tiny leaves.

All plants were watered in the evening prior to the day measurement. Thermal and transpiration measurements were carried out every 1 hr from 8:30 to 16:30. We were not able to measure all 38 species on the same day, and hence the measurement environment varied from day to day. To standardize temperature, we put a unified elliptical iron leaf (length: 15 cm, width: 9.4 cm, thickness: 0.5 mm) beside each individual as a reference. The iron leaf was coated with a white paper on the adaxial side to enhance emissivity similar to real leaves. For each individual, we measured the temperatures of two controlled leaves (T_l), two to three vaselined leaves (T_n) and the reference leaf (T_{ref}). Meanwhile, we measured the transpiration of one of the two controlled leaves on each individual.

2.4 | Leaf traits

All the controlled leaves used for transpiration measurements were harvested for analyses of leaf physical traits. Leaf area and perimeter were measured via a flatbed-scanned image or digital photograph (for large leaves) using ImageJ. Hand-cut transverse sections were taken from middle-lamina sections, avoiding the midribs. These sections were cleared in commercial bleach (49.9% g/L sodium hypochlorite, 12% g/L sodium hydroxide), rinsed thoroughly, and stained with 1% safranin solution. The thickness of the adaxial epidermis, palisade, sponge mesophylls, the abaxial epidermis and lamina were measured with a Leica DM2500 light microscope (Leica Microsystems Vertrieb GmbH, Wetzlar, Germany). Leaf vein density was measured using paradermal sections. Paradermal sections were cut from the middle part of the leaf and boiled in a 5% NaOH aqueous solution until they became soft and disintegrated. Subsequently, they were removed and rinsed carefully in clean water. The size and density of stomata were measured using a razor to slice the abaxial epidermis. All the sections were mounted on slides and observed under a Leica DM2500 microscope. The values for each leaf were averaged from five fields of the same laminar section. Leaf hair was classified into three categories according to the density of the hair. Ten to twenty leaves were collected for each individual to measure fresh weights, then oven dried at 80°C to constant weight. Leaf density was calculated by the fresh weight divided by the product of leaf area and leaf thickness. The water content of each leaf was calculated by the ratio of the difference in fresh weight and dry weight to fresh weight. Reflectance, transmittance and absorbance were measured using a spectrometer (USB2000, Ocean Optics, USA) at wavelength from 400 to 700 nm (the abbreviate names of leaf traits are summarized in Table 1).

2.5 | Data analysis

Because T_l and T_n varied with T_a, and we were not able to make all the measurements simultaneously, and this change in T_a could potentially have impact on the comparison of T_l and T_n between species. To solve this problem, we compared the increasing rates of T_l and T_n with T_{ref}, (i.e. slope$_l$: dT_l/dT_{ref} and slope$_n$: dT_n/dT_{ref}, instead of comparing

TABLE 1 Abbreviation names of leaf traits and the statistic results of the differences between the leaf traits of the two groups

No.	Full name	Unit	Abbreviation	Significance
1	Area	cm^2	Area	*
2	Perimeter	mm	Perimeter	*
3	Perimeter/ Area	\	P/A	*
4	Length	cm	Length	**
5	Width	cm	Width	
6	The radio of length to width	\	L/W	*
7	Maximum evapotranspiration	$mmol\ m^{-2}\ s^{-1}$	E_{max}	*
8	Maximum stomatal conductance	$mol\ H_2O\ m^{-2}\ s^{-1}$	g_{smax}	*
9	Upper epidermis thickness	μm	UE	
10	Lower epidermis thickness	μm	LE	
11	Palisade thickness	μm	*P*	
12	Sponge thickness	μm	*S*	
13	Thickness ratio of sponge to palisade	\	S/P	*
14	Leaf thickness	μm	*T*	
15	Hair	\	*H*	
16	Stomata density	$No\ mm^{-2}$	SD	
17	Stomata size	μm	SS	
18	Stomatal pore area index	No mm	SPI	*
19	Vein density	$\mu m\ mm^{-2}$	VD	*
20	Water content	%	WC	
21	Specific leaf area	$cm\ g^{-1}$	SLA	
22	Leaf density	$kg\ m^{-2}$	Density	
23	Reflectance	%	Reflectance	*
24	Transmittance	%	Transmittance	*
25	Absorbance	%	Absorbance	*
26	Temperature of the controlled leaf	°C	T_l	*
27	Temperature of Vasline-coated leaf	°C	T_n	
28	Temperature of the reference leaf	°C	T_{ref}	
29	Increasing slope of T_l	\	$Slope_l$	**
30	Increasing slope of T_n	\	$Slope_n$	*

*$p < .05$, **$p < .01$

T_l and T_n directly. We used the measured maximum transpiration rate (E_{max}) to represent transpiration capacity. The temperature differences between the vaselined leaves and the reference leaves $|T_n - T_{ref}|$ were induced by physical differences, and the temperature differences between the controlled leaves and vaselined leaves $|T_n - T_l|$ were determined by transpiration. The contribution of transpiration to temperature regulation was therefore calculated as:

$$\frac{T_n - T_l}{|T_n - T_l| + \left|T_n - T_{ref}\right|} \times 100 \tag{1}$$

and the contribution of physical traits was calculated as:

$$\frac{T_n - T_{ref}}{|T_n - T_l| + \left|T_n - T_{ref}\right|} \times 100 \tag{2}$$

The effects of each leaf physical traits on T_n were further analysed by multiple linear regression model. We firstly classified the physical traits into three groups: shape related traits (area, perimeter, length, width, L/W, and P /A), thickness related traits (UE, *P*, *S*, S/P, LE, SLA and *T*), material property related traits (WC and density), and optical traits (reflectance, transmittance, and absorbance). The variables that were not normally distributed were log transformed. We excluded the highly correlated traits (correlation coefficient >0.7) within each group according to the correlation between traits and slope_n. We regressed the remaining traits against slope_n, and used Akaike's information criterion (AIC) to select the best model. The independent variables in the final model were considered to exert impacts on T_n.

Aerodynamic boundary layer resistance r_a (s/m) was calculated as:

$$r_a = \frac{\rho c_p (T_l - T_a)}{R_n - \lambda E} \quad (3)$$

where ρ is air density (kg/m^3), c_p is specific heat of air at constant pressure (kJ kg^{-1}°C^{-1}). E is transpiration (kg m^{-2} s^{-1}), λ is latent heat of evaporation of water (kJ/kg). R_n (w/m^2) is net radiation which was estimated by energy balance:

$$R_n = L_{in} - L_{out} + S_{in} - S_{out} \quad (4)$$

Incoming longwave radiation (L_{in}, w/m^2) was estimated from the air temperature, using Swinbank formula (Monteith & Unsworth, 2008). Outgoing longwave radiation (L_{out}, w/m^2) was calculated by Stephan-Bolzman law, assuming emissivity was 0.95. Incoming shortwave radiation (S_{in}, w/m^2) was measured from the pyranometer data. Outgoing shortwave radiation (S_{out}, w/m^2) was calculated by S_{in} × reflectance. Total leaf resistance (r_t, s/m) was calculated as:

$$r_t = \frac{\rho c_p (e_l - e_a)}{\lambda E \gamma} \quad (5)$$

γ is the psychrometric constant (kPa/°C). Then stomata conductance (r_s, s/m) can be calculated as:

$$r_s = r_t - r_a \quad (6)$$

The decoupling coefficient was a measure of the degree of stomatal control of transpiration. It ranges from 0 to 1. Stomatal control of transpiration grows progressively weaker as Ω approaches 1. It is calculated according to McNaughton and Jarvis (1983):

$$\Omega = \frac{s/\gamma + 2}{s/\gamma + 2 + r_s/r_a} \quad (7)$$

where s is the slope of the saturated vapour pressure versus temperature curve (kPa/°C) and γ is the psychrometer constant (kPa/°C).

3 | RESULTS

3.1 | Comparison of leaf temperatures between species from HW and HD

Leaf temperature increased linearly with T_{ref}, and the increasing rates were different between the plants from HW and HD ($p < .01$). The increasing rates of T_l and T_n of the plants from HW were significantly higher than the plants from HD (Figure 2; $p < .001$). The plants from HW therefore showed higher T_l and T_n than the species from HD under identical conditions. The magnitude of the temperature difference between the two groups of plant leaves amplified with increasing T_{ref} (Figure 2).

Generally, the extent of cooling induced by transpiration was larger for the plants from HD than for the plants from HW. In comparison with the reference leaves, the leaf physical traits of the plants from HW showed warming effect, especially at noon. Leaf temperatures of the plants from HD were close to T_{ref} when transpiration was suppressed (Figure 3).

3.2 | Impact of transpiration and leaf physical traits on leaf temperature

Transpiration contributed more to the temperature difference between the controlled leaves and reference leaves than leaf physical traits for both of the two groups of plants, and more notably in the plants from HD (Figure 4).

Transpiration rates and stomatal conductance were significantly different between the plants from HW and HD at all time points except for 15:30 (Figure 5a,b). The average maximum transpiration rate of plants from HD was 1.5 times that of plants from HW. The average transpiration rate peaked at 10:30 for the plants from HW, while the rate peaked 1 hr later for plants from HD (Figure 5a). Stomatal conductances were significantly higher in the plants from HD than the plants from HW at most time points, especially in the late morning and at noon (Figure 5b). Excluding transpiration, the plants from HW had significantly higher leaf aerodynamic resistances than the plants from HD at 9:30 and 11:30 (Figure 5c). Ω showed similar diurnal pattern with g_s, except for a mild peak value of plants from HW at 10:30. Ninety one percent of Ω was lower than 0.5. Eighteen percent of species from

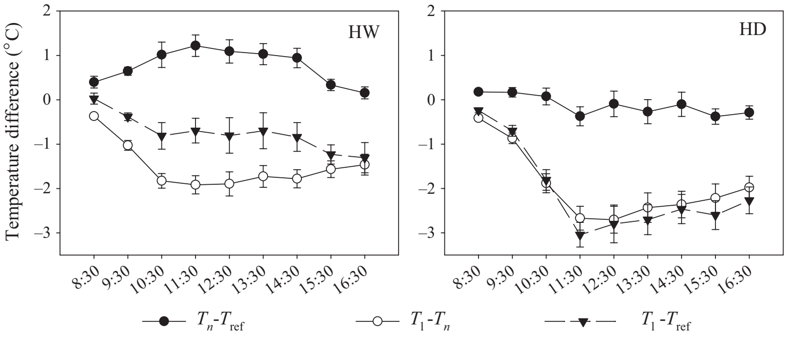

FIGURE 3 Diurnal temperature differences compared with the reference leaves for the plants from hot wet habitat (HW, n = 18) and hot dry habitat (HD, n = 20). T_l is the temperature of the controlled leaf, T_n is the temperature of the vaselined leaf, and T_{ref} is the temperature of the reference leaf. The data are mean ± SE

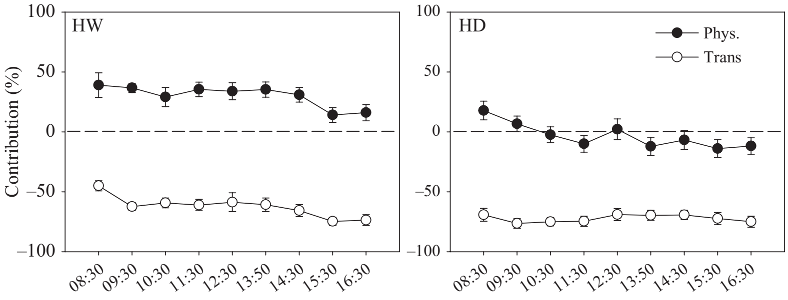

FIGURE 4 Diurnal courses of the relative contributions of leaf physical traits (phys.) and transpiration (trans) to the temperature differences between the controlled leaves and reference leaves for the plants from hot wet habitat (HW, *n* = 18) and hot dry habitat (HD, *n* = 20). The data are mean ± *SE*

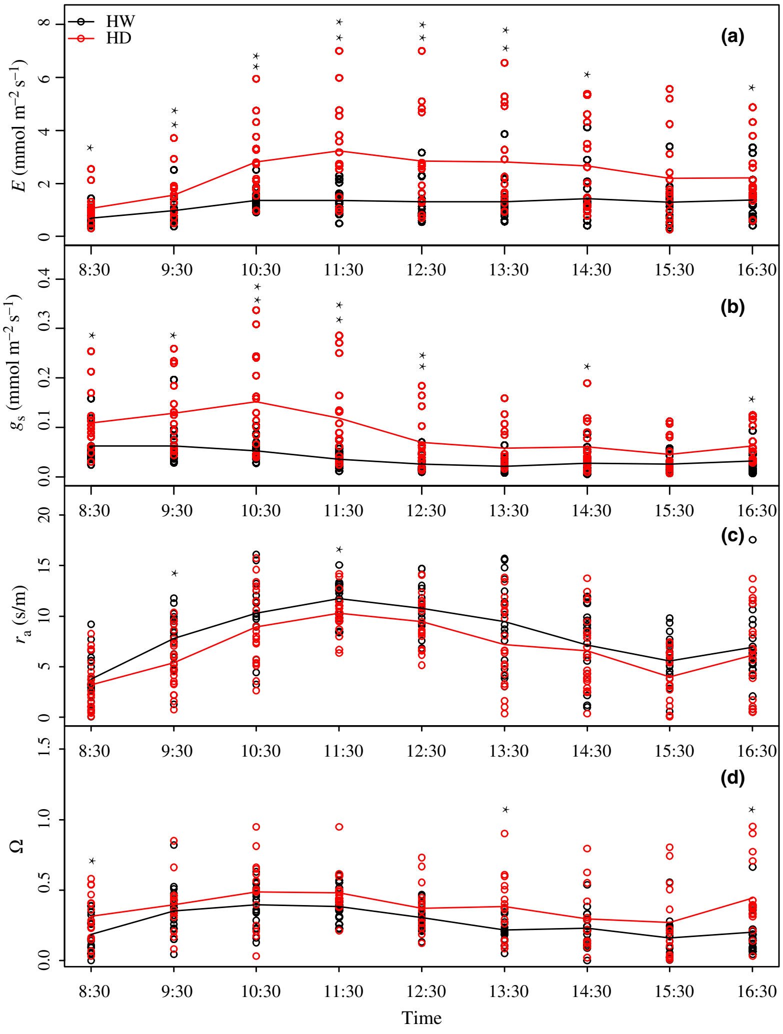

FIGURE 5 Diurnal course of (a) transpiration rates (E), (b) stomatal conductances (g_s), (c) aerodynamic resistances (r_a) of the leaves without transpiration, and (d) decoupling coefficients (Ω). HW: plants from hot wet habitat, *n* = 18; HD: plants from hot dry habitat, *n* = 15. **p* < .05; ***p* < .01

HW had Ω higher than 0.4, while this percentage was much higher for species from HD (34%) (Figure 5d).

Of the 33 species for which transpiration measurements were made, 19 species (9 species from HW and 10 species from HD) showed positive linear relationship between $T_n - T_l$ and transpiration rates. The extents of cooling ranged from 0.61°C to 2.12°C per unit of transpiration (mmol m^{-2} s^{-1}). Forty five percent of the species from HW had slopes higher than 1 compared with 25% for the species from HD.

The leaves of HD species showed xeromorphic properties. The shape and thickness related traits (area, perimeter, *L*, L/W and S/P) were significantly larger in the plants from HW in comparison to the plants from HD. The ratio of the perimeter to area of plants from HW was significantly smaller than in the plants from HD. Transpiration-related traits (E_{max}, SPI, and vein density) were significantly higher in the plants from HD than in the plants from HW. The plants from HD absorbed less radiation as a result of higher reflectance and transmittance in comparison to the plants from HW (Figure 6; Table 1). Of all

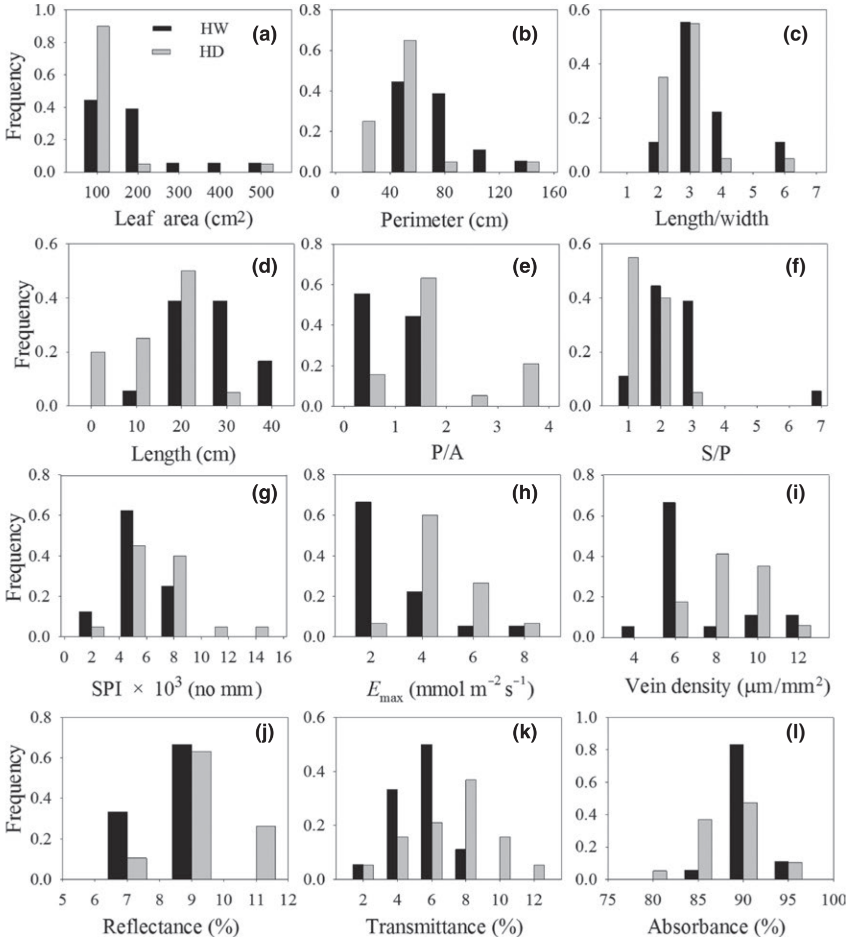

FIGURE 6 Histograms of leaf traits that were significantly different between the plants from the hot wet habitat (HW) and the hot dry habitat (HD). P/A: perimeter/ area; S/P: thickness of sponge tissue/thickness of palisade tissue; SPI: stomatal pore area index; E_{max}: maximum evapotranspiration rate

the physical traits, leaf length and the thickness of lower epidermis had significantly positive impacts on T_n.

4 | DISCUSSION

Our results confirmed that the plants from HD dissipated heat more efficiently than the plants from HW via higher rates of transpiration and synergistic thermal effects of leaf physical traits. Thus, the plants from HD showed lower leaf temperatures than the plants from HW under identical environmental conditions. Moreover, transpiration contributed more to leaf cooling than physical traits when there was no water limitation.

Leaf cooling via transpiration are commonly believed to be greatly inhibited due to frequent soil water shortage (Monteith & Unsworth, 2013). An extreme example is the crassulacean acid metabolism (CAM) plants, mostly evolved with succulent structure, the transpiration related traits were degraded to save water. However, our results showed that there was an increase in transpiration capacity and allied traits including vein density, SPI, and maximum stomatal conductances in the plants from HD. Decoupling coefficients also showed transpiration of HW species were more restricted by stomatal conductances than those of HD species. Although the cooling efficiency of transpiration (cooling extent per unit of transpiration) was higher for plants from HW, transpiration contributed more to leaf cooling due to the higher transpiration rates of plants from HD. Increasing stomatal conductances in response to high temperature have been reported in desert plants (Althawadi & Grace, 1986; Schulze, Lange, Kappen, Buschbom, & Evenari, 1973). Taken together, these results revealed that HD plants performed a wider range of stomatal regulation and were more efficient in water transport than HW plants. Plants reduce stomatal opening under drought to save water, thereby reducing CO_2 exchange. In this case, leaf cooling mainly relies on physical traits. Some species even shed leaves in the dry season. To compensate for the reduction in carbon synthesis during the dry season, plants need an efficient system to fix carbon when water is available. Larger vein density, SPI, and stomatal conductance enable a suitable leaf temperature and high photosynthesis (Hill, Guerin, Hill, & Watling, 2014). Transpiration, in turn, dominates leaf cooling. The hot dry valley in Yuanjiang, China has a shorter growing season than the tropical seasonal rainforest, and most of the HD species are deciduous (Zhang, 2007). Moreover, the temperature is very high in the hot dry valley (Figure 1) with a peak air temperature greater than 40°C even during the rainy season. It has been reported that the heat tolerance temperatures of those plants from HD ranged from 44°C to 47°C (Zhang, Poorter, Hao, & Cao, 2012), thus, photosystems II of these plants cease to function optimally if the temperature differences between the leaves and air exceeded 4°C. Accordingly, 19 out of 20 HD species in this study would be threatened in the absence of transpiration; while this number would be reduced to eight out of twenty, with the use of transpiration. Hence, transpiration provides a safe microenvironment for photosynthesis in plants from HD when water is available. Field measurements also showed that most species in the hot dry valley had higher photosynthesis capacity and stomatal conductances than HW plants during the growing season (Chen, 2014; Hill et al., 2014; Zhu, 2010). Many studies reported that high vein density was often associated with higher drought resistance (Blackman, Brodribb, & Jordan, 2010; Nardini, Peda, & La Rocca, 2012; Scoffoni, Rawls, McKown, Cochard, & Sack, 2011; Sommerville, Gimeno, & Ball, 2010). High temperature can also increase stomatal conductances (Althawadi & Grace, 1986) and transpiration capacity (Crawford, McLachlan, Hetherington, & Franklin, 2012). Therefore, the hot and dry environment can improve the vigour of water transferring structures of leaves.

Leaf physical traits exerts impacts on leaf temperature through the influence on heat transfer, storage and received radiation (Gates, 2003; Monteith & Unsworth, 2008). The leaf physical traits of the plants from HD were more efficient in leaf cooling than the plants from HW as a result of their xerophytic characteristics (Figures 2–4). Plants from HD had smaller leaves than plants from HW. Generally, smaller leaves are more coupled to air temperature owing to smaller aerodynamic resistance (Figure 5c) which have been demonstrated by previous studies (Fetcher, 1981; Leuzinger & Korner, 2007; Nobel, 1974). However, the effect of leaf size could be lower in the field due to wind (Defraeye et al., 2013). Although the transpiration capacity in the species from HD is higher than in the species from HW, they are not likely to lose more water via transpiration even when water is sufficient because of the smaller total leaf area. Therefore, small leaves are not only beneficial for heat convection but also for water saving. Higher P/A in plants from HD than plants from HW provided longer edges for heat convection. Plants from HD also received less radiation via higher reflectance and transmittance. There have no enough evidences for the thermal effect of the abaxial epidermis. We speculate that it functions as an insulation layer for the leaf and extends the vapor path from stomata to air, so there is a positive correlation between its thickness and leaf temperature.

Generally, we did not detect a high correlation between any one specific physical trait and leaf temperature, because leaf temperature was affected by multiple traits. According to previous studies, the impact of leaf physical traits on leaf temperature maybe positive, e.g. leaf size (Fetcher, 1981; Yates et al., 2010), negative, e.g. leaf thickness (Leigh et al., 2012), reflectance and water content (Schymanski, Or, & Zwieniecki, 2013), and transferable depending on the status, e.g. leaf hair (Parkhurst, 1976; Ripley, Pammenter, & Smith, 1999; Woodman & Fernandes, 1991; Wuensche, 1970; Ye et al., 2011). The interaction of all the positive and negative effects might weaken the thermal effect of one specific trait in the statistic analysis. For example, in this study, most of the leaves with high water content were large leaves (e.g. *Myristica yunnanensis*, *Horsfieldia tetratepala*, and *Horsfieldia pandurifolia*), and thus the negative effect of heat capacity on leaf temperature was muted by the positive thermal impact of leaf size. Likewise, it is difficult to isolate the thermal effect of one specific leaf trait when a study involves multiple species with diverse leaf traits. In this situation, the thermal effect of the combination of all the physical traits is more meaningful. Thus, our methods are especially useful for comparing the thermal effects between plant groups. On the other hand, the range of leaf traits can also have an impact on the analysis of the thermal effects of leaf traits, as some leaf traits may exert a limited influence on leaf temperature within a certain range. For example, increasing water content by 50% would not significantly reduce leaf temperature, while a 20-fold increase in leaf water content (e.g. from 0.05 to 1 kg/m^2), could reduce peak leaf temperatures by 5°C (Schymanski et al., 2013). In addition, leaf movement also have influence on leaf temperature (Levizou & Kyparissis, 2016). For example, in the present study, *Bauhinia brachycarpa* folded their leaves at noon, and *Tarenna depauperata* curled its leaves when there was water stress. Deep root systems of the HD species are also beneficial for water gain. It was reported that the length ratio and biomass ratio of above-ground to below-ground reached to one for most of the species in the Yuanjiang valley (Zhang, 2007). To avoid further heat damage by drought, some plants shed leaves or stay dormancy during dry season.

Although physical and transpiration cooling can be solved theoretically or experimentally using heat balance (Kitano & Eguchi, 1989; Leuning & Foster, 1990; Martin et al., 1999; Monteith & Unsworth, 2013), replica leaves (Daudet, Le Roux, Sinoquet, & Adam, 1999; Daudet, Silvestre, Ferreira, Valancogne, & Pradelle, 1998; Katsoulas, Baille, & Kittas, 2007) and numeric simulation (Roth-Nebelsick, 2001; Roy, Vidal, Fargues, & Boulard, 2008), there is still challenge of separating the convective processes of heat and vapour, and accurate calculation of aerodynamic resistance under changing environment. Furthermore, heterogeneous boundary conditions render experimental and theoretical analysis difficult (Defraeye et al., 2013). In the present study, the thermal effects of the suite of physical traits, were reflected by $T_n - T_{\mathrm{ref}}$ as a whole, where only three temperatures (T_l, T_n, T_{ref}) were needed to accurately separate the cooling effects of physical traits and transpiration. We call this method as three-temperature method. Note that our model is different from the three-temperature model proposed by Qiu et al. (2002) who used T_l, T_n, and T_a to estimate transpiration.

Studying the strategies of leaf temperature regulation in different plant groups can improve our understanding of leaf design and the adaptation of plants to various environments. Our results demonstrate that a hot and dry environment could enhance the cooling capacity of plants via increasing transpiration capacity and synergic physical traits. The xeromorphic syndrome of the leaves can alleviate the heat stress to a certain extent when transpiration is suppressed. When water is sufficient, physical traits and transpiration provide double protection in avoiding heat damage and enable high photosynthesis at a suitable temperature. Stomatal indices and leaf veins always couple together to determine gas exchange characteristics of leaves (Brodribb, Feild, & Sack, 2010). Thus, the development of hydraulic structure should follow the highest transpiration rates of the plants rather than the average rates (Brodribb, McAdam, & Carins Murphy, 2017). The adaptation of plants to hot and dry environment conditions perfectly reflected the coordinated evolution of transpiration capacity, stomatal indices, and leaf temperature regulation.

5 | CONCLUSIONS

Leaf temperature can differ between plant groups even under identical environment. Compared with the plants from HW, the plants from HD developed higher leaf vein densities, and larger SPIs to facilitate greater transpiration, while their leaf physical traits, most notably the shorter leaf and thinner lower epidermis dissipate heat more efficiently. Transpiration is a predominant way of leaf cooling both for the plants from HD and HW. Our results provide further insight into the relationship between leaf physical traits and transpiration in leaf temperature regulation, and the co-evolution of gas exchange and thermal regulation of leaves.

ACKNOWLEDGEMENTS

This study was funded by the National Natural Science Foundation of China (NSFC, 31200307), the Applied Fundamental Research Program of Yunnan Province (2013FB078) and the CAS President's International Fellowship Initiative, 2016VBA036. We thank the Yuanjiang Savanna Ecosystem Research Station, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences and the Xishuangbanna Station for Tropical Rainforest Ecosystem Studies for the support of fieldwork and data management. We thank Hong Ma, Dongbo Chen and Huaxin He for field assistance, Professor Ram Oren, Professor Richard Corlett and Professor Kyle Thomlinson for the comments on this manuscript.

AUTHOR'S CONTRIBUTIONS

H.L. conceived the ideas and designed methodology. H.L. and Y.C. collected the data. H.L., and H.Z. analysed the data. H.L. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Species descriptions and leaf traits list: uploaded as online Supporting information. Leaf traits data: Dryad Digital Repository https://doi.org/10.5061/dryad.g179n (Lin, Chen, Zhang, Fu, & Fan, 2017).

REFERENCES

Aerts, R., Wagendorp, T., November, E., Behailu, M., Deckers, J., & Muys, B. (2004). Ecosystem thermal buffer capacity as an indicator of the restoration status of protected areas in the northern Ethiopian highlands. *Restoration Ecology*, *12*, 586–596.

Althawadi, A. M., & Grace, J. (1986). Water use by the desert Cucurbit *Citrullus colocynthis* (L.) Schrad. *Oecologia*, *70*, 475–480.

Ansari, A. Q., & Loomis, W. E. (1959). Leaf temperatures. *American Journal of Botany*, *46*, 713–717.

Blackman, C. J., Brodribb, T. J., & Jordan, G. J. (2010). Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytologist*, *188*, 1113–1123.

Brodribb, T. J., Feild, T. S., & Sack, L. (2010). Viewing leaf structure and evolution from a hydraulic perspective. *Functional Plant Biology*, *37*, 488–498.

Brodribb, T. J., McAdam, S. A., & Carins Murphy, M. R. (2017). Xylem and stomata, coordinated through time and space. *Plant Cell & Environent*, *40*, 872–880.

Campbell, G. S., & Norman, J. M. (1998). *Introduction to environmental biophysics*, 2nd edn. New York, NY: Springer.

Cao, M., Zou, X. M., Warren, M., & Zhu, H. (2006). Tropical forests of Xishuangbanna, China. *Biotropica*, *38*, 306–309.

Chen, Y. J. (2014). The water-use characteristics of tropical lianas and the comparison with co-occurring trees. PhD Thesis, Graduate University of Chinese Academy of Sciences, China.

Crawford, A. J., McLachlan, D. H., Hetherington, A. M., & Franklin, K. A. (2012). High temperature exposure increases plant cooling capacity. *Current Biology*, *22*, R396–R397.

Curtis, O. F. (1936). Leaf temperatures and the cooling of leaves by radiation. *Plant Physiology*, *11*, 343–364.

Daudet, F. A., Le Roux, X., Sinoquet, H., & Adam, B. (1999). Wind speed and leaf boundary layer conductance variation within tree crown – Consequences on leaf-to-atmosphere coupling and tree functions. *Agricultural and Forest Meteorology*, *97*, 171–185.

Daudet, F. A., Silvestre, J., Ferreira, M. I., Valancogne, C., & Pradelle, F. (1998). Leaf boundary layer conductance in a vineyard in Portugal. *Agricultural and Forest Meteorology*, *89*, 255–267.

Defraeye, T., Verboven, P., Ho, Q. T., & Nicolai, B. (2013). Convective heat and mass exchange predictions at leaf surfaces: Applications, methods and perspectives. *Computers and Electronics in Agriculture*, *96*, 180–201.

Fetcher, N. (1981). Leaf size and leaf temperature in tropical vines. *American Naturalist*, *117*, 1011–1014.

Gates, D. M. (2003). *Biophysical ecology*. Mineola, NY: Dover Publications.

Hill, K. E., Guerin, G. R., Hill, R. S., & Watling, J. R. (2014). Temperature influences stomatal density and maximum potential water loss through stomata of *Dodonaea viscosa* subsp. *angustissima* along a latitude gradient in southern Australia. *Australian Journal of Botany*, *62*, 657–665.

Katsoulas, N., Baille, A., & Kittas, C. (2007). Leaf boundary layer conductance in ventilated greenhouses: An experimental approach. *Agricultural and Forest Meteorology*, *144*, 180–192.

Kitano, M., & Eguchi, H. (1989). Dynamic analysis of stomatal responses by an improved method of leaf heat-balance. *Environmental and Experimental Botany*, *29*, 175–185.

Lambers, H., Chapin III, F. S., & Pons, T. (Eds.) (1998) Leaf energy budgets: Effects of radiation and temperature. In *Plant physiological Ecology* (pp. 210–229). New York, NY: Springer.

Lang, M., & Schindler, C. (1994). The effect of leaf-hairs on blue and red fluorescence emission and on zeaxanthin cycle performance of *Senecio Medley* L. *Journal of Plant Physiology*, *144*, 680–685.

Lang, M., Schindler, C., & Lichtenthaler, H. K. (1995). Function of leaf hairs and leaf waxes in the protection of the photosynthetic apparatus against photoinhibition. In P. Mathis (Ed.), *Proceedings of the Xth International Photosynthesis Congress* (pp. 3413–3416). Montpellier, France: Springer.

Leigh, A., Sevanto, S., Ball, M. C., Close, J. D., Ellsworth, D. S., Knight, C. A., … Vogel, S. (2012). Do thick leaves avoid thermal damage in critically low wind speeds? *New Phytologist*, *194*, 477–487.

Leuning, R., & Foster, I. J. (1990). Estimation of transpiration by single trees – Comparison of a ventilated chamber, leaf energy budgets and a combination equation. *Agricultural and Forest Meteorology*, *51*, 63–86.

Leuzinger, S., & Korner, C. (2007). Tree species diversity affects canopy leaf temperatures in a mature temperate forest. *Agricultural and Forest Meteorology*, *146*, 29–37.

Levizou, E., & Kyparissis, A. (2016). A novel pattern of leaf movement: The case of *Capparis spinosa* L. *Tree Physiology*, *36*, 1117–1126.

Lin, H., Chen, Y., Zhang, H., Fu, P., & Fan, Z. (2017). Data from: Stronger cooling effects of transpiration and morphology of the plants from a hot dry habitat than from a hot wet habitat. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.g179n

Martin, T. A., Hinckley, T. M., Meinzer, F. C., & Sprugel, D. G. (1999). Boundary layer conductance, leaf temperature and transpiration of *Abies amabilis* branches. *Tree Physiology*, *19*, 435–443.

McNaughton, K. G., & Jarvis, P. G. (1983). Predicting effects of vegetation changes on transpiration and evaporation. In T. T. Kozlowski (Ed.), *Water deficits and plant growth* (Vol. V, pp. 1–48). New York, NY: Academic Press.

Monteiro, M. V., Blanusa, T., Verhoef, A., Hadley, P., & Cameron, R. W. F. (2016). Relative importance of transpiration rate and leaf morphological traits for the regulation of leaf temperature. *Australian Journal of Botany*, *64*, 32–44.

Monteith, J. L., & Unsworth, M. H. (2008). *Principles of environmental physics*, 3rd edn. Boston, FL: Elsevier, Amsterdam.

Monteith, J. L., & Unsworth, M. H. (2013). *Principles of environmental physics: Plants, animals, and the atmosphere*, 4th edn. Boston, FL; Amsterdam: Elsevier/Academic Press.

Nardini, A., Peda, G., & La Rocca, N. (2012). Trade-offs between leaf hydraulic capacity and drought vulnerability: Morpho-anatomical bases, carbon costs and ecological consequences. *New Phytologist*, *196*, 788–798.

Nobel, P. S. (1974). *Introduction to biophysical plant physiology*. San Francisco, CA: W. H Freeman.

Noffinger, T. L. (1961). Leaf and air temperature under Hawaii conditions. *Pacific Science*, *XV*, 304–306.

Parkhurst, D. F. (1976). Effects of *Verbascum thapsus* leaf hairs on heat and mass-transfer – Reassessment. *New Phytologist*, *76*, 453–457.

Qiu, G. Y., Miyamoto, K., Sase, S., Gao, Y., Shi, P. J., & Yano, T. (2002). Comparison of the three-temperature model and conventional models for estimating transpiration. *Jarq-Japan Agricultural Research Quarterly*, *36*, 73–82.

Ripley, B. S., Pammenter, N. W., & Smith, V. R. (1999). Function of leaf hairs revisited: The hair layer on leaves *Arctotheca populifolia* reduces photoinhibition, but leads to higher leaf temperatures caused by lower transpiration rates. *Journal of Plant Physiology*, *155*, 78–85.

Roth-Nebelsick, A. (2001). Computer-based analysis of steady-state and transient heat transfer of small-sized leaves by free and mixed convection. *Plant Cell & Environment*, *24*, 631–640.

Roy, J. C., Vidal, C., Fargues, J., & Boulard, T. (2008). CFD based determination of temperature and humidity at leaf surface. *Computers and Electronics in Agriculture*, *61*, 201–212.

Scheffers, B. R., Edwards, D. P., Macdonald, S., Senior, R. A., Andriamahohatra, L. R., Roslan, N., … Williams, S. E. (2016). Extreme thermal heterogeneity in structurally complex tropical rain forests. *Biotropica*, *49*, 35–44.

Schuepp, P. H. (1993). Tansley Review No. 59. Leaf boundary layers. *New Phytologist*, *125*, 477–507.

Schulze, E. D., Lange, O. L., Kappen, L., Buschbom, U., & Evenari, M. (1973). Stomatal responses to changes in temperature at Increasing water stress. *Planta*, *110*, 29–42.

Schymanski, S. J., Or, D., & Zwieniecki, M. (2013). Stomatal control and leaf thermal and hydraulic capacitances under rapid environmental fluctuations. *PLoS ONE*, *8*, e54231.

Scoffoni, C., Rawls, M., McKown, A., Cochard, H., & Sack, L. (2011). Decline of leaf hydraulic conductance with dehydration: Relationship to leaf size and venation architecture. *Plant Physiology*, *156*, 832–843.

Sinclair, R. (1970). Convective heat transfer from narrow leaves. *Australian Journal of Biological Sciences*, *23*, 309–322.

Sommerville, K. E., Gimeno, T. E., & Ball, M. C. (2010). Primary nerve (vein) density influences spatial heterogeneity of photosynthetic response to drought in two *Acacia* species. *Functional Plant Biology*, *37*, 840–848.

Stokes, V. J., Morecroft, M. D., & Morison, J. I. L. (2006). Boundary layer conductance for contrasting leaf shapes in a deciduous broadleaved forest canopy. *Agricultural and Forest Meteorology*, *139*, 40–54.

Tomlinson, K. W., Poorter, L., Sterck, F. J., Borghetti, F., Ward, D., Bie, S., & Langevelde, F. V. (2013). Leaf adaptations of evergreen and deciduous trees of semi-arid and humid savannas on three continents. *Journal of Ecology*, *101*, 430–440.

Vogel, S. (2009). Leaves in the lowest and highest winds: Temperature, force and shape. *New Phytologist*, *183*, 13–26.

Woodman, R. L., & Fernandes, G. W. (1991). Differential mechanical defense - herbivory, evapotranspiration, and leaf-hairs. *Oikos*, *60*, 11–19.

Wuensche, J. E. (1970). The effect of leaf hairs of *Verbascum thapsus* on leaf energy exchange. *New Phytologist*, *69*, 65–73.

Yates, M. J., Verboom, G. A., Rebelo, A. G., & Cramer, M. D. (2010). Ecophysiological significance of leaf size variation in Proteaceae from the Cape Floristic Region. *Functional Ecology*, *24*, 485–492.

Ye, C. Q., Li, M. Z., Hu, J. P., Cheng, Q. F., Jiang, L., & Song, Y. L. (2011). Highly reflective superhydrophobic white coating inspired by poplar leaf hairs toward an effective “cool roof”. *Energy & Environmental Science*, *4*, 3364–3367.

Zhang, J. L. (2007) Phenology, leaf structure and function and seasonal variation in photosynthesis of woody plants in a dry-hot valley of Yuanjiang, southwestern China. PhD Thesis, Graduate University of Chinese Academy of Sciences, China.

Zhang, J. L., Poorter, L., Hao, G. Y., & Cao, K. F. (2012). Photosynthetic thermotolerance of woody savanna species in China is correlated with leaf life span. *Annals of Botany*, *110*, 1027–1033.

Zhu, S. D. (2010) Comparison of leaf traits, hydraulic architecture and photosynthesis between lianas and trees in tropical seasonal rain forests. PhD Thesis, Graduate University of Chinese Academy of Sciences, China.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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