The upper temperature limits of leaf function in tropical trees from a wet tropical forest

Thesis submitted towards the partial fulfilment of BS-MS dual degree programme

By

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Certificate

This is to certify that this dissertation entitled “The upper temperature limits of leaf function in tropical trees from a wet tropical forest” towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Vikhyath Premugh at the Indian Institute of Science Education and Research (IISER), Pune under the supervision of Dr. Deepak Barua, Associate Professor, Department of Biology, IISER Pune and at the National Centre for Biological Sciences (NCBS), Bangalore under the supervision of Dr. Mahesh Sankaran, Associate Professor G, NCBS Bangalore during the academic year 2019-2020.

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Declaration

I hereby declare that the matter in the report entitled “The upper temperature limits of leaf function in tropical trees from a wet tropical forest” are the result of the work carried out by me at the Department of Biology, Indian Institute of Science Education and Research (IISER), Pune under the supervision of Dr. Deepak Barua and at the National Centre for Biological Sciences (NCBS), Bangalore under the supervision of Dr. Mahesh Sankaran and the same has not been submitted elsewhere for any other degree.

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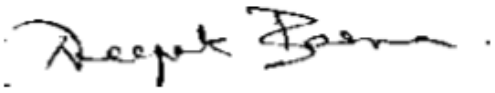

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Abstract

With the increase in high temperature incidences due to global warming, it has become very important to understand the thermotolerance of plant species. In this study, the thermotolerance and leaf traits of 41 forest species and 14 agroforestry species from a wet tropical forest in the central Western Ghats were measured and their thermal time constants and leaf temperatures calculated. The thermal time constant, τ, determines a leaf's resistance to external environmental fluxes and the leaf temperature (T_{leaf}) is an estimate of the temperature a leaf actually experiences in a given ambient temperature. Around half of the measured species showed a negative thermal safety margin (difference between thermotolerance limit, T50, and estimated high value for T_{leaf}) suggesting that these forests are at great risk from warming events and may experience huge losses to biodiversity. Contrary to expectations, the thermotolerance of the plants did not show any relation to any of the functional traits (leaf mass per area, leaf dry matter content), morphological traits (leaf area, effective leaf width, normalised difference in margin complexity and perimeter/area), leaf temperature (T_{leaf}) or thermal time constant (τ). Among the functional traits, LDMC showed stronger negative correlation with T_{leaf} and τ than LMA, suggesting that plants with greater carbon investment to their leaves might be at an advantage in future warming events while being less resistant to sudden environmental fluxes. The positive correlation between T_{leaf} and τ hints to a trade-off between the ability to maintain leaf temperatures and resistance to sudden environmental changes. Comparison of the thermotolerance of species which occur in both the northern and central Western Ghats did not show any significant difference between their thermotolerance between the two sites.

List of Figures

Acknowledgements

I would like to thank Dr. Deepak Barua for his continued guidance and support. I would thank Dr. Mahesh Sankaran for letting me use his field station and its associated facilities. I would like to thank Mr. H. V. Raghavendra and Mr. Balu Hegde for their aid in sample collection and species identification. I offer my gratitude to Mr. Himanshu Lagachu for the training and his aid during the first month of my field work. Finally, I would like to thank all my fellow lab members in Barua Lab for their support.

Introduction

Temperature is an important environmental factor which limits the geographical distribution of a species (Araújo et al., 2013). In plants, exposure to temperatures above their tolerance limits has detrimental effects on photosynthesis and respiration, could cause tissue damage, and might lead to reduced growth and mortality (Berry and Björkman, 1980; Hüve et al., 2011; O'Sullivan et al., 2013; Teskey et al., 2015). Furthermore, it is expected that the duration and frequency of extreme temperature events would increase due to global warming (IPCC, 2014). This could lead to more species being exposed to temperature above their tolerance limits. This is of great concern in the tropics as tropical species already live in temperatures close to their thermotolerance limits (Deutsh et al., 2008; Doughty and Goulden, 2009; O'Sullivan et al., 2017).

In a previous study which examined the thermotolerance of plant species occurring in a variety of locations throughout the world, it was observed that a 20°C increase in maximum habitat temperature along with latitude corresponded to only an increase of 8°C in average thermotolerance (O'Sullivan et al. 2017). In contrast, the variation of thermotolerance among coexisting species ranges from 10°C to 20°C (O'Sullivan et al. 2017; Lösch 1980; Gauslaa 1984; Knight and Ackerly 2003; Weng and Lai 2005; Neuner and Buchner 2012). This could be due to coexisting species experiencing different leaf temperatures due to microclimate variations, canopy position, thermoregulation, leaf habit, etc. Due to the consistent covariance of thermal protective traits with leaf mass per area (LMA) it has been predicted that LMA would be positively related to thermotolerance (Curtis et al., 2012). However, the studies which checked for such a relation showed mixed results with some

finding the predicted positive relation (Knight and Ackerly 2003; Gallagher 2014; Sastry and Barua 2017) and others not finding any relationship at all (Zhang et al. 2012; O'Sullivan et al. 2017). Since LMA and LDMC (leaf dry matter content) are key functional traits associated with a plant's resource utilisation strategy (Michaletz et al., 2016), understanding how these traits relate to thermotolerance would help us make more accurate predictions about the type of plants likely to survive future warming events.

One of the limitations of many of the previous studies on thermotolerance is that they compare plant thermal limits to habitat temperatures. This might not give an accurate picture on the impacts of thermotolerance as plants have the ability to regulate their leaf temperatures (Michaletz et al., 2015). It is possible to use the leaf energy budget equation to estimate leaf temperature (T_{leaf}) for a given air temperature and environmental conditions (Michaletz et al., 2015; Jones 2013). Plants which show lower T_{leaf} at high ambient temperatures could be expected to have lower thermotolerance as they are less likely to encounter extremely high leaf temperatures. Another important measure of leaf thermal response is the thermal time constant, τ (Tau), which determines how rapidly leaves respond to changes in surface energy fluxes with leaves having smaller τ responding more rapidly than those with higher values for it (Michaletz et al., 2015). Leaves with higher τ could be expected to have lower thermotolerance as they would be exposed to a smaller range of temperatures than those with a smaller τ .Three other leaf morphological traits, namely leaf area (LA), normalized difference in margin complexity (NDMC) and effective leaf width, have been implicated in the leaf temperature response by influencing the thickness of the leaves' air boundary layer (Leigh, 2017). As higher LA

and effective leaf width would lead to a larger boundary layer and NDMC acts to decrease the thickness of the boundary layer, we could expect LA and effective leaf width to be positively related to τ and NDMC to be inversely related. Another implication of the influence of leaf size and shape is that smaller or more dissected leaves would have smaller leaf to air temperature difference and would be at an advantage in extremely hot conditions (Leigh et al., 2017). In this study, we decided to include an additional measure of margin complexity which is the ratio of leaf margin perimeter to projected leaf area (perimeter/area) as it was difficult to measure NDMC for the larger compound leaves. We expect perimeter/area to be highly related to NDMC and to show similar interactions with τ and T_{leaf} as NDMC.

In this study, thermotolerance was measured as the temperature at which the dark-adapted chlorophyll a fluorescence or F_v/F_m reduces to half its value at room temperature. This value is referred to as T_{50} and is a commonly used measure of thermotolerance (Björkman and Demmig 1987; Havaux et al. 1991; Barua et al. 2003).

The data acquired during the course of this study was used along with thermotolerance data from the Northern Western Ghats which was available in the lab to try and achieve the following objectives:

1. To understand the variation in thermotolerance among coexisting species.
2. To estimate T_{leaf} and use it to derive a thermal safety margin for the species under consideration.
3. To understand the relations between T_{50}, leaf functional traits, leaf morphological traits, T_{leaf} and τ.
4. To check for inter-site variation in thermotolerance.

Materials and Methods

Study site

This study was conducted in forests in the Hosagadde village near Sirsi, Karnataka, India (14.4783°N, 74.7573°E, altitude 667m) which is a part of the Central Western Ghats. The study site includes a tropical evergreen forest and an agroforestry plantation which receive an annual rainfall of 6,457mm (http://wp.lancs.ac.uk/basin-network/home-page/map/smart-watersheds-western-ghats). The wet season in Sirsi lasts 4.4 months from late May to early October and the remaining 7.6 months constitute the dry season (https://weatherspark.com/y/107840/Average-Weather-in-Sirsi-India-Year-Round#Sections-Summary). April is the warmest month of the year with the temperature averaging 27.5°C. Daily maximum temperatures higher than 40°C occasionally occur in Sirsi.

The additional thermotolerance data was from Bhimasankar, Pune, India (19.1320° N, 73.5540° E, altitude 900m), a part of the Northern Western Ghats. The wet season in Bhimasankar is in the months of June to September leading to an annual rainfall of around 3000 mm.

Sample collection and hydration

Leaves were collected from six mature individuals each from forty-one species of forest trees and an additional fourteen species from the agroforestry plantation. Collection occurred from 27th September 2019 to 7th December 2019. Samples were collected between 2:30pm and 6:00pm as stem cuttings from the upper canopy with mature, sun-exposed leaves whenever possible. The stem cuttings were deposited in labelled Ziploc bags with a wad of wet tissue paper in it to prevent drying. After collection, the samples were dipped in a plastic, water-filled

container with the cut end dipped in water. The dipped end was cut again underwater to remove any possibilities of air bubbles within the stem. The plastic container containing the sample was the placed in a Ziploc bag and covered with a black garbage bag for overnight hydration.

Thermotolerance assay

After overnight hydration, the leaves were collected from the stem cuttings and leaf discs were prepared from the leaves using a cork borer of 0.8 cm diameter. The discs were placed in a packet made with muslin cloth and sandwiched between two layers of aluminum foil and placed in a small Ziploc bag with some wet tissue inside. The bags were then placed between a wire mesh folded in half and then immersed in a water bath (Julabo, Model F25, Seelbach, Germany). For each replicate discs were exposed to the following temperatures: 40°C, 45°C, 47.5°C, 50°C, 52.5°C and 55°C for 30 minutes each. For control, leaf discs were placed in water without heating for 30 minutes. The leaf discs, after heat treatment were stored in petri plates lined with wet tissue paper for 24 hours. The leaf discs were then dark adapted for 30 minutes in packets made by sandwiching two layers of black felt cloth between two layers of black chart paper. The F_v/F_m was then measured using a PAM 2500 fluorometer (Walz, Effeltrich, Germany).

Leaf traits

In order to calculate leaf weights, six water saturated leaves from each individual plant were weighed on the same day as the thermotolerance assay in order to determine their fresh weights. The same leaves were then scanned using CanoScan Lide 110 and the area was measured from these scanned images later using ImageJ. The dry weights for the

leaves were measured after at least 48 hours in a hot air oven at 70°C. Leaf mass per area (LMA) was calculated as the ratio of dry weights to leaf area and leaf dry matter content (LDMC) was calculated as the ratio of dry weight to fresh weight. In the case of *Areca catechu*, 10 leaf discs from each individual were used for LMA and LDMC measurements as the whole leaves were too big to handle. NDMC was measured as the difference between leaf margin perimeter and the perimeter of the smallest convex polygon that could fit around the leaf (convex hull) divided by the sum of leaf margin perimeter and convex hull perimeter. Perimeter by area is calculated as the leaf margin perimeter divided by the projected leaf area. Effective leaf width was calculated as the diameter of the largest circle that could fit inside the margins of the leaf. NDMC, perimeter/area, and effective leaf width were measured using MATLAB 2018a.

T_{leaf}, τ and thermal safety margin

The calculation of T_{leaf} is based on the steady state leaf energy budget theory (Jones, 2014). T_{leaf} and τ were calculated using the formulae described in Michaletz et al., 2015. Due to the difficulty in measuring the actual dimensions of the leaf for *Areca catechu*, an effective leaf width of 6cm was used as an estimate for that species. See Appendix Table 2. For the different formulae and constants used to calculate T_{leaf} and τ.

For calculating Tleaf, two temperatures and two sets of environmental conditions were used. A high temperature of 38℃ was considered as an extreme temperature value which the plants in the study site could be expected to experience. In contrast to this, a low temperature of 30℃ was also considered as an average temperature which the plants normally experience. In order to get an estimated extreme for leaf temperatures, other parameters related to cooling, namely wind speed,

stomatal conductance, and relative humidity were adjusted to minimise cooling and were considered as “low cooling” parameters. An estimate of normal conditions of cooling parameters were also considered forming the “high cooling” group of parameters (see Appendix Table 3). For the purpose of correlations and estimation of thermal safety margin, T_{leaf} corresponding to low cooling/ high temperature were used. This is because thermal safety margin and correlations in require an estimated extreme of high temperature.

The thermal safety margin was calculated as $T_{50} - T_{leaf}$ for each species.

Data analysis

From the F_v/F_m values acquired from the thermotolerance assay, temperature response curves (F_v/F_m vs temperature) were generated using the R package ‘drc’ by fitting a four-parameter logistic curve to the F_v/F_m values. The temperature of control was considered to be 25℃ for the purpose of generating the temperature response curves.The temperature at which the F_v/F_m values reduced to 50% was regarded as the T_{50} (R version R-3.6.2).

Pearson’s correlation tests were conducted between the different traits using a correlation matrix from the ‘PerformanceAnalytics’ package in R. Prior to performing the correlation analysis, the different traits were tested for normality using a Shapiro test in base R. Where the traits deviated from normality, the following corrections were made: LA and perimeter/area were log10 transformed, and NDMC and Tau were square-root transformed.

To check for any difference in T_{50} for individuals of species which occur both in Bhimashankar and Sirsi, a paired t-test was performed using R.

Results

The F_v/F_m of most species started dropping from around 45°C and dropped to zero by around 52.5°C. There wasn't any significant decrease in F_v/F_m from 25°C to 40°C (Figure1). Among the forest species, the T_{50} ranged from a low of 46.51°C for *Celtis timorensis* to a high of 51.74°C for *Holigarna arnottiana* (Figure 2). For the agroforestry species, T_{50} ranged from a low of 46.00°C for *Theobroma cacao* to a high of 50.45°C for *Areca catechu* (Figure3).

Among the forest species, the thermal safety margin was distributed evenly with 20 out of 41 species having a positive value and the others being negative. *Macaranga peltate* had the lowest thermal safety margin of -6.31°C and *Catunaregam spinosa* had the highest value of 3.07°C (Figure 4). Among the agroforestry species, *Theobroma cacao* had the lowest thermal safety margin of -6.13°C and *Syzygium aromaticum* had the highest value of 1.91°C (Figure 5).

T_{leaf} showed an interesting distribution with it showing high values and great spread when the cooling is low. At high cooling, the distribution is much narrower. Within a cooling level, T_{leaf} was higher with higher T_{air} (Figure 6).

Within the cross-correlation matrix, T50 didn't show any relation with the other leaf functional traits, morphological traits, T_{leaf} or τ. As expected, the functional traits (LMA and LDMC) were strongly related to each other. Even though the morphological traits were strongly correlated with each other, the direction of correlation for some of the traits were unexpected. Effective leaf width showed the expected positive correlation with LA and the expected negative correlation with perimeter/area but was negatively correlated with NDMC. Contrary to

expectations, NDMC and perimeter/area were shown to be inversely related (Figure 7). Between LMA and LDMC, LDMC showed stronger relations with T_{leaf} and τ. LDMC was negatively correlated with τ while LMA showed a weak positive correlation (Figure 8). Effective leaf width showed strong positive relations with both T_{leaf} and τ (Figure 9). T_{leaf} and τ were positively correlated with each other (Figure 10).

A paired t-test between the thermotolerance of individuals of species which occur both in Northern and Central Western Ghats did not show any significant difference in thermotolerance between the two sites.

Discussion

The T_{50} values of all species ranged from 46°C to around 52°C. These values are more informative when compared against the estimated T_{leaf}. The thermal safety margin of the species ranged from -6°C to around 3°C for forest species and from around -6°C to 2°C for agroforestry species. 20 out of the 41 forest species showed a positive thermal safety margin while 8 out of 14 agroforestry species showed positive values. Since a T_{air} of 38°C was used for the T_{leaf} calculation and temperatures of 40°C and above are already being reported in Sirsi, we can conclude that a large number of species are already living in temperatures above their tolerance limits. One limitation of this analysis however is that one forest species and a majority of agroforestry species (See appendix table 1) are shaded in their habitats and since leaf temperature is largely influenced by incident radiation (R), these species can be expected to have much higher thermal safety margins than that reported in this study. Another limitation is that the T_{50} measurements were made post rains during the cooler months of September to December. It is possible that the species may acclimate to have higher T_{50} during the hot season when they actually experience extremely high temperatures. A repeat of this study conducted in the hot season could provide more useful data regarding the thermal safety margins for these species.

The estimated T_{leaf} is largely dependent on the parameters used for its calculation. An 8°C difference in T_{air} lead to around 5°C difference in average T_{leaf}. When leaves are allowed to cool, the average temperature is much lower (approx. 10°C lower) than when they aren't for the same air temperatures. The much broader spread of T_{leaf} values under low cooling conditions mean that the difference in experienced leaf

temperatures will be much more pronounced under conditions such as low wind speed, low stomatal conductance and high relative humidity.

From the correlation analysis, it was quite surprising that T_{50} was not related to any of the leaf traits, T_{leaf} or Tau. It is possible that something other than the traits considered in this study might be driving the variation in T_{50}s. Further evaluation of microclimate variation, leaf habit, canopy position of species, etc might provide clues as to what drives the variation of thermotolerance in this region. As expected, the leaf functional traits were strongly related to each other. Both LMA and LDMC were related to Tau. This is not entirely unexpected as LMA and LDMC are already variables used in the calculation of Tau. The positive relation of Tau to LMA and negative relation to LDMC means that Tau, while related to both leaf functional traits may not be linked to the slow-fast resource acquisition spectrum (Wright et al., 2004). T_{leaf} on the other hand showed a weak negative relation to LDMC suggesting that T_{leaf} might be linked to the leaf economics spectrum with plants following a conservative resource acquisition strategy experiencing lower leaf temperatures than species following an acquisitive strategy.

All the leaf morphological traits seem to be driven by leaf area. NDMC and perimeter/area were both negatively correlated despite both being measures of margin complexity. This is likely because perimeter/area is not a normalised measure and is bound by mathematics to be more driven by area than by perimeter, leading to the negative relation between log (LA) and perimeter/area. NDMC on the other hand seems to be an adaptation of large-leaved plants to reduce leaf temperature, causing the positive relation between log (LA) and NDMC. In Leigh et al., 2017, it was seen that NDMC was related to lower leaf temperature only in large leaved plants. Effective leaf width is strongly, positively

related to log (LA) and is a more direct contributor to T_{leaf} and Tau due to it being an important variable in calculating the boundary layer conductance which is then used in both T_{leaf} and Tau calculation. Because of this, effective leaf width can be seen to be strongly related to both T_{leaf} and Tau. In effect, it could be observed that larger leaves both experience higher temperatures and cool (and heat) slower than smaller leaves.

The strong positive relation between T_{leaf} and Tau implies that leaves which experience higher temperatures are also more resilient to sudden temperature changes as a result of environmental fluxes. This suggests a possible trade-off between better cooling which would benefit plants growing under high environmental temperatures and better resilience to temperature change which would benefit plants which experience short-term temperature peaks.

While there wasn't a significant difference in the means of T_{50} of plants occurring in both Bhimashankar and Sirsi, it could be seen that the T_{50}s are different for the species in the different habitats. While most species showed a higher T_{50} in Sirsi than in Bhimashankar, three of the thirteen species showed a lower value in Sirsi. Bhimashankar and Sirsi differ largely in rainfall with Sirsi being considerably wetter. Since most of the species had more thermotolerant individuals in Sirsi than in Bhimashankar, it is possible that thermotolerance might be linked to the difference in water availability between the two habitats.

Figures

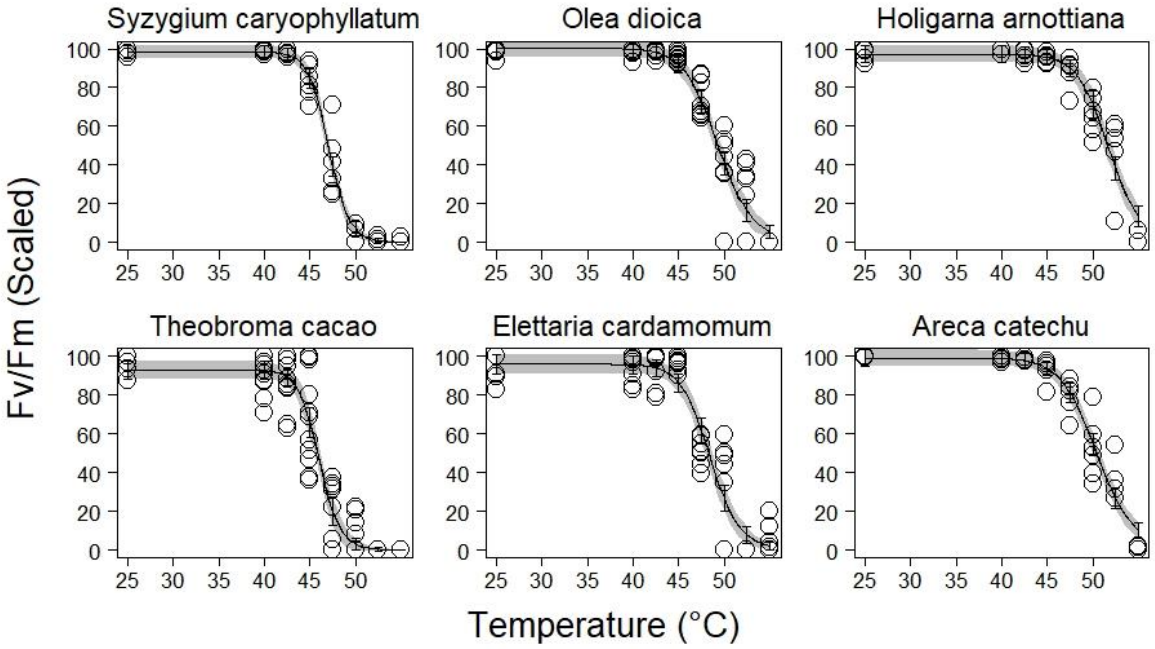

Figure 1: Representative temperature response curves. The first row contains temperature response curves for forest species and the second row contains temperature response curves for agroforestry species. The x-axis is temperature in °C and the y-axis is F_v/F_m scaled to a maximum of 100.

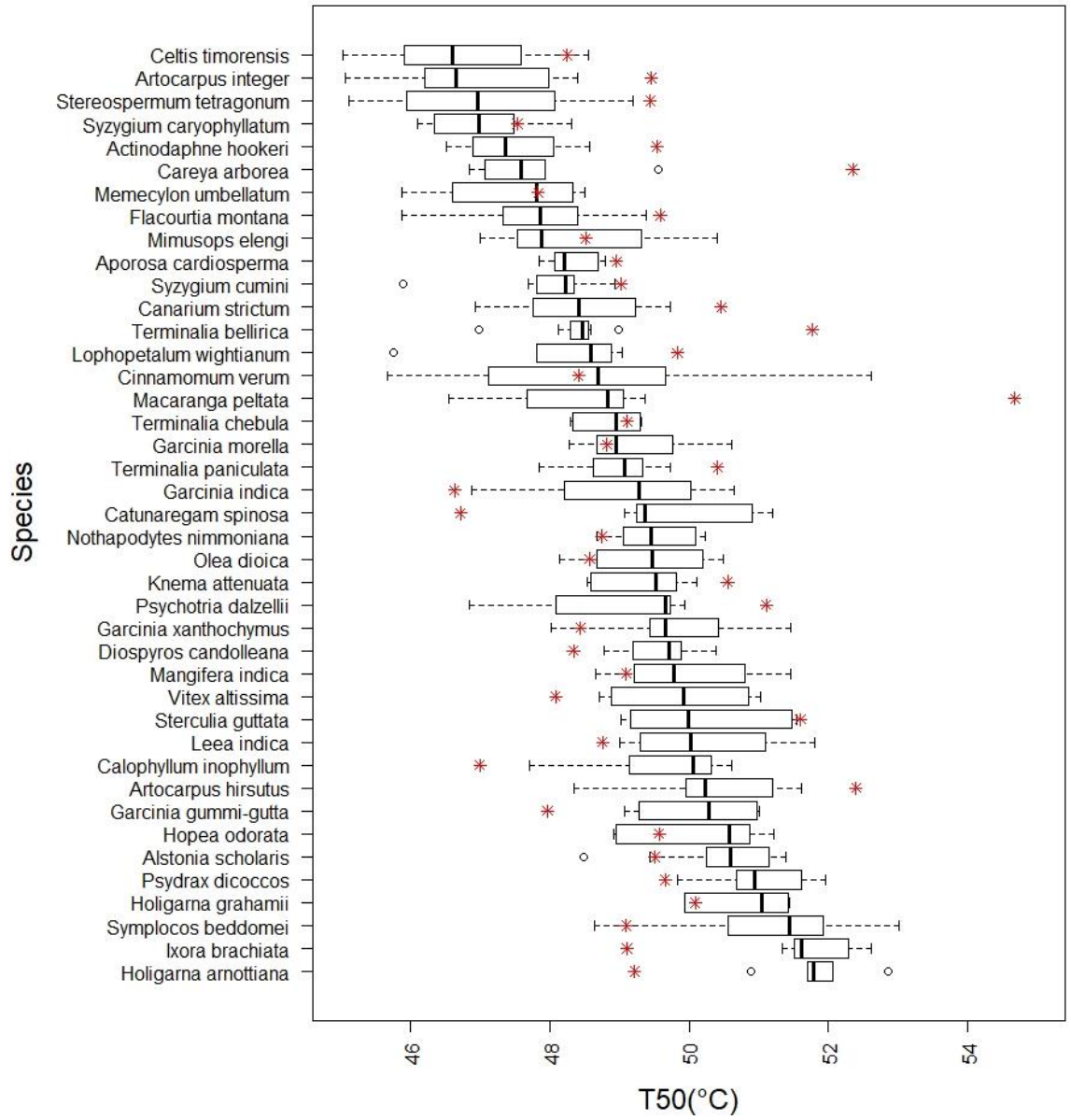

Figure 2: T50 values of forest species. Each box plot represents the spread of T50 values of a species plotted at the individual level. The red marks represent the high temperature/ low cooling value of T_{leaf} for that species. The x-axis represents temperature (T_{50} and T_{leaf}) in °C and the y axis lists the name of the species corresponding to each box plot.

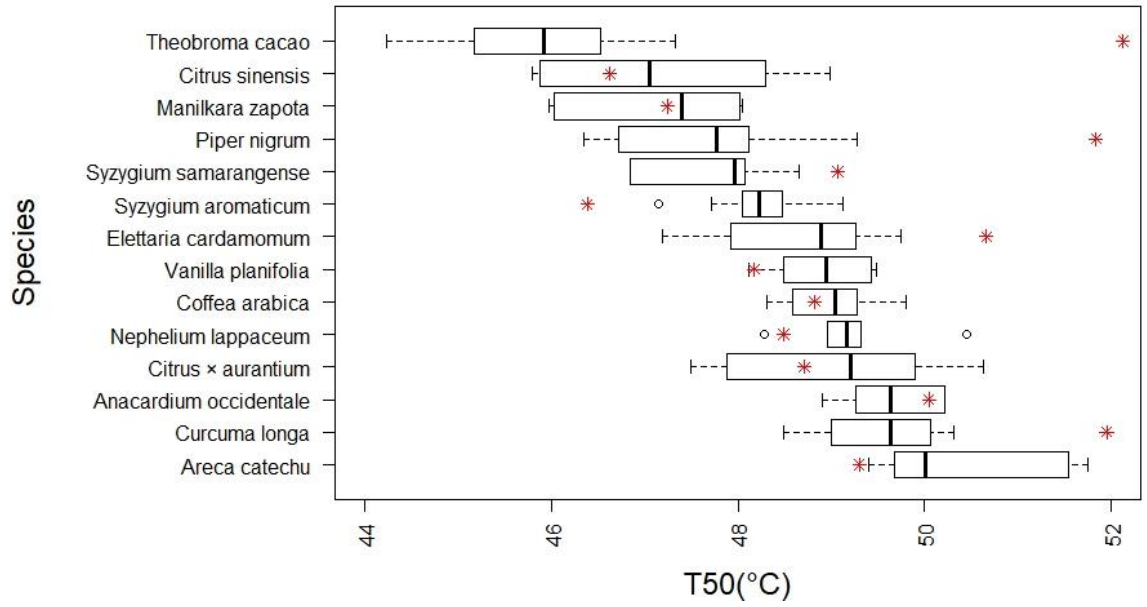

Figure 3: T50 values of agroforestry species. Each box plot represents the spread of T50 values of a species plotted at the individual level. The red marks represent the high temperature/ low cooling value of T_{leaf} for that species. The x-axis represents temperature (T_{50} and T_{leaf}) in °C and the y axis lists the name of the species corresponding to each box plot.

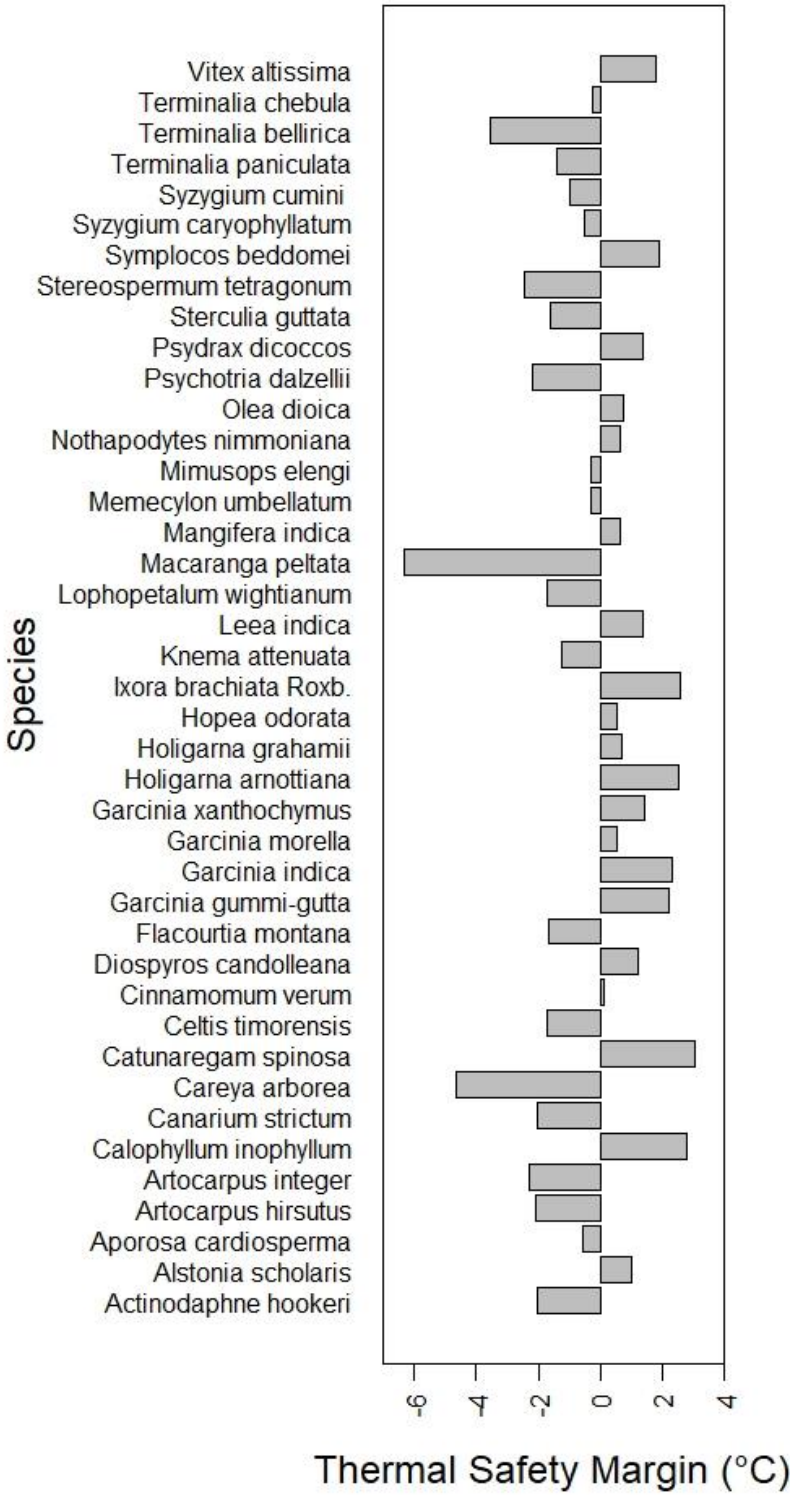

Figure 4: Thermal safety margins of forest species. Each bar represents the thermal safety margin for a species. The x-axis is the thermal safety margin in °C and the y axis lists the species names corresponding to each bar.

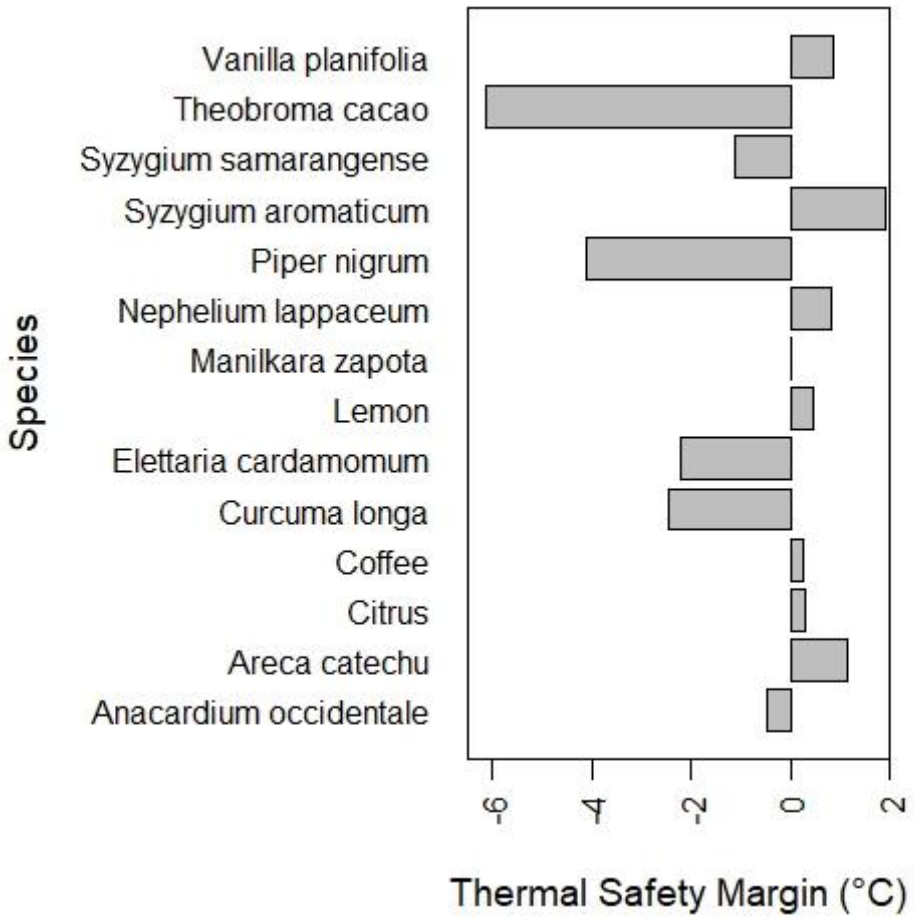

Figure 5: Thermal safety margins of Agroforestry species. Each bar represents the thermal safety margin for a species. The x-axis is the thermal safety margin in °C and the y axis lists the species names corresponding to each bar.

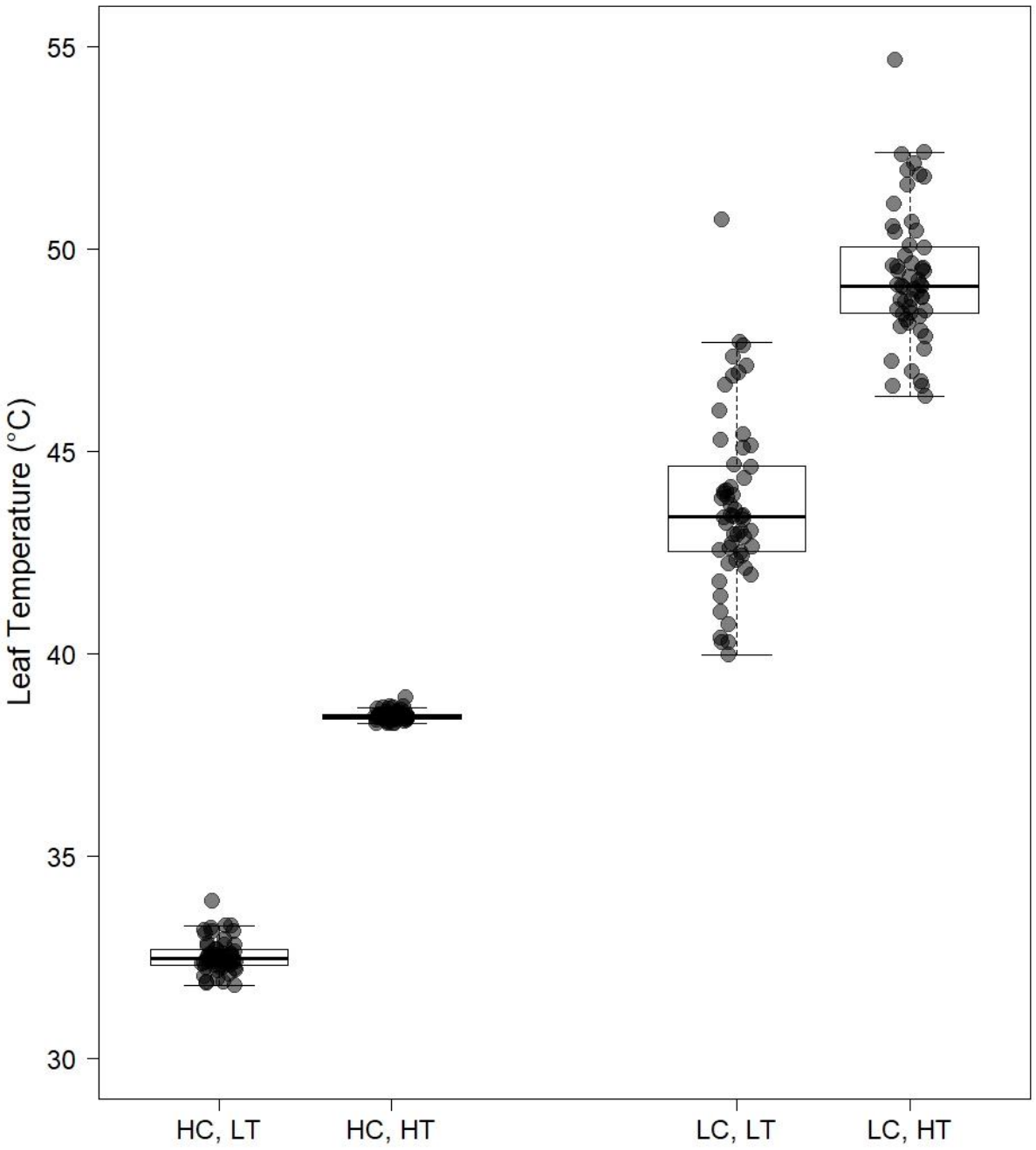

Figure 6: Box-plots showing the distribution of leaf temperatures at different environmental conditions. Each box plot from left to right shows the distribution of leaf temperatures plotted at the species level for the following conditions respectively: - high cooling/ low T_{air} (HC, LT), high cooling/ high T_{air} (HC, HT), low cooling/ low T_{air} (LC, LT), low cooling/ high T_{air} (LC, HT). Each point represents the leaf temperature estimate for a single species. The y-axis is the leaf temperature in °C.

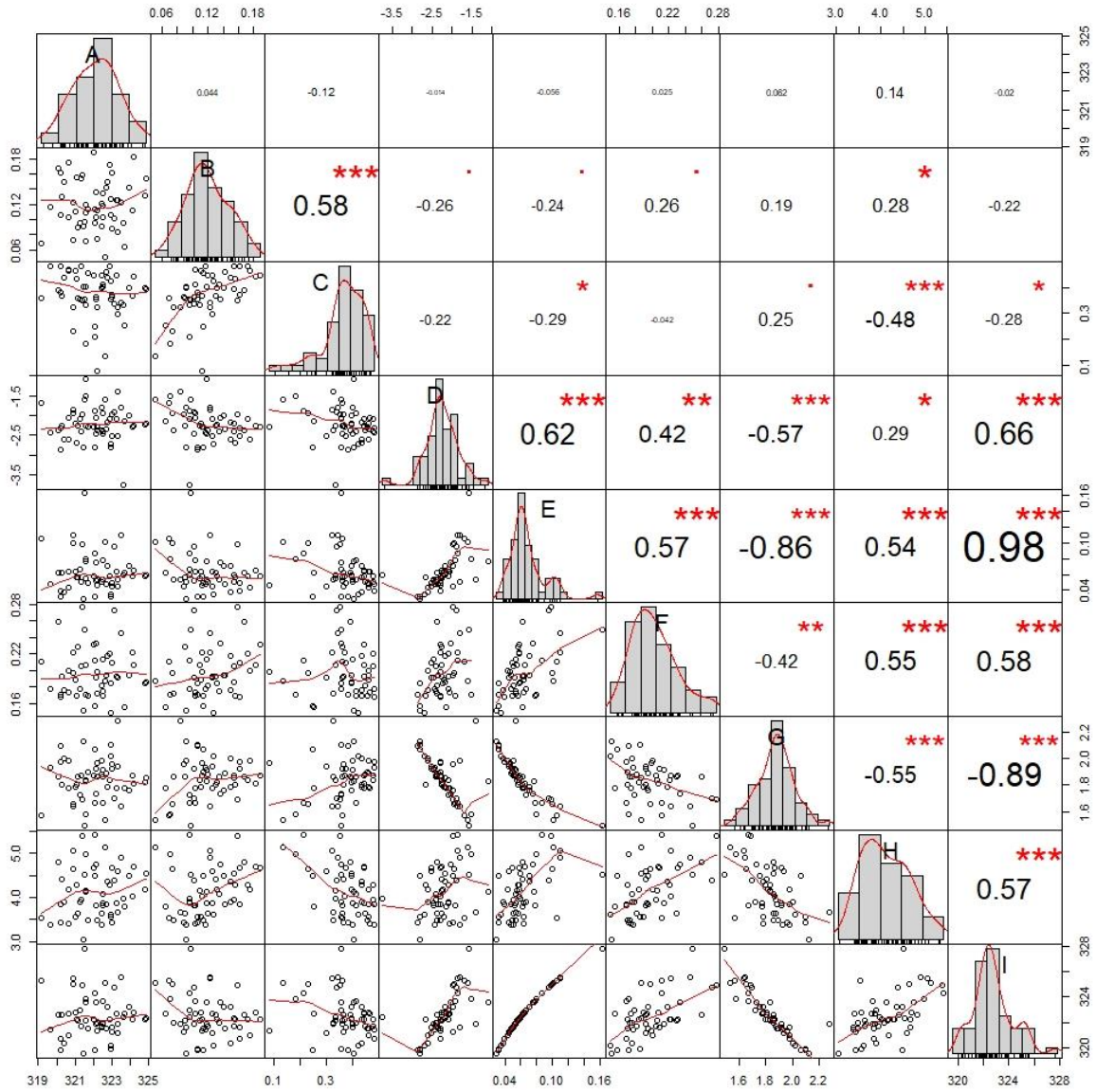

Figure 7: Cross-correlation matrix showing relations between leaf traits. All traits are presented in their SI units. The alphabetical labels along the diagonal represent the following: A- T50, B- LMA, C- LDMC, D- Log(LA), E- Effective leaf width, F- $NDMC^{0.5}$, G- Log(Perimeter/Area), H- $\tau^{.5}$, I- T_{leaf}. The diagonal shows the frequency distribution for each trait. The upper diagonal region lists the Pearson's correlation coefficient for each relation as well as the significance level. The lower diagonal region presents the scatterplot showing the relations. The T_{leaf} presented in this figure is for the conditions of high temperature/ low cooling.

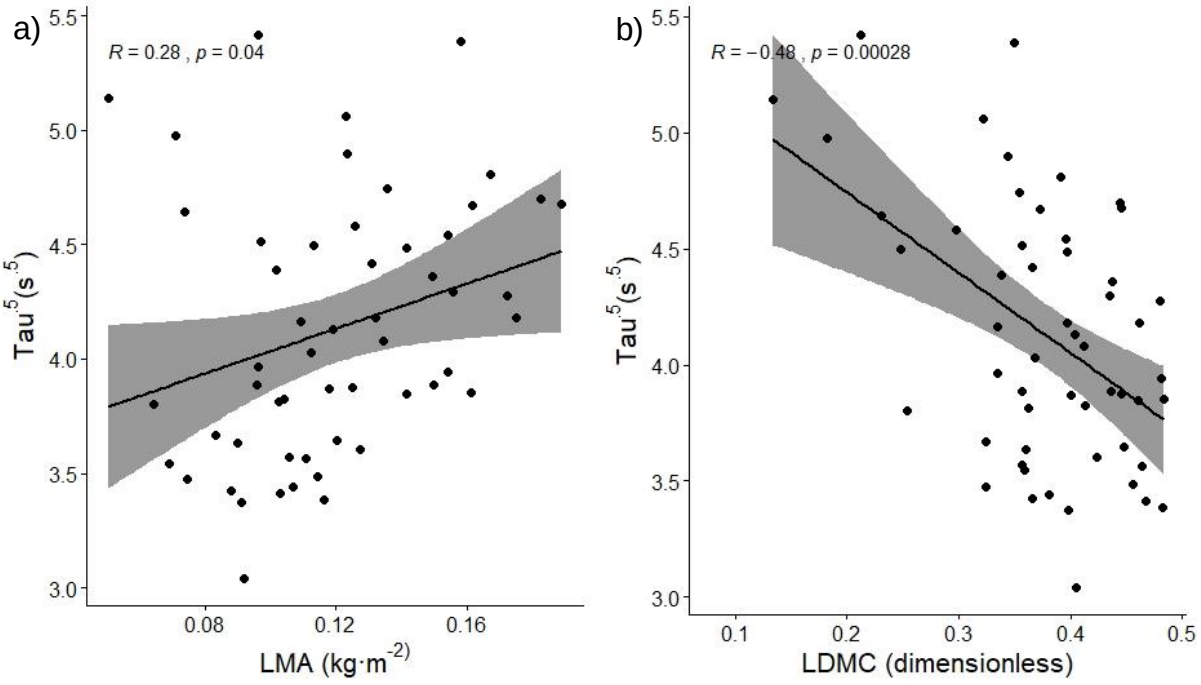

Figure 8: Scatterplots showing the correlation between a: LMA and $Tau^{.5}$, and b: LDMC and $Tau^{.5}$. R is the Pearson's correlation coefficient and p is the p-value. $Tau^{.5}$ is expressed in the units of $s^{.5}$, LMA in $kg{\cdot}m^{-2}$, and LDMC is dimensionless.

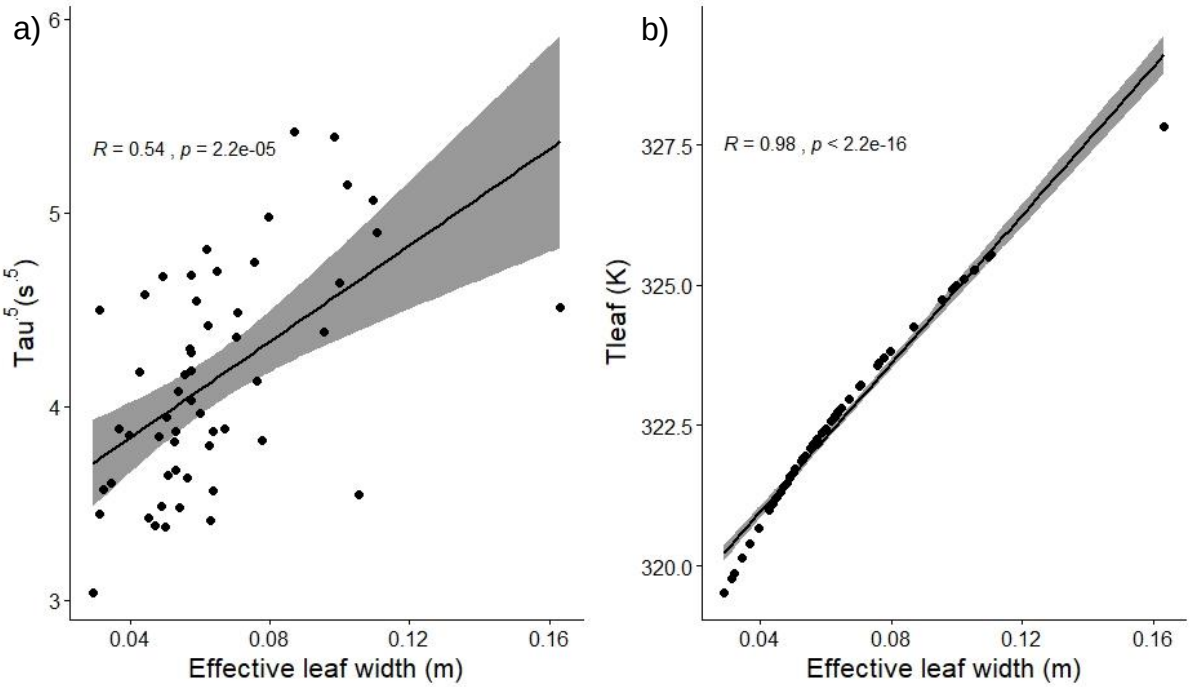

Figure 9: Scatterplots showing the correlation between a: Effective leaf width and $Tau^{.5}$, and b: Effective leaf width and T_{leaf}. R is the Pearson's correlation coefficient and p is the p-value. $Tau^{.5}$ is expressed in the units of $s^{.5}$, Effective leaf width in m, and T_{leaf} is in K.

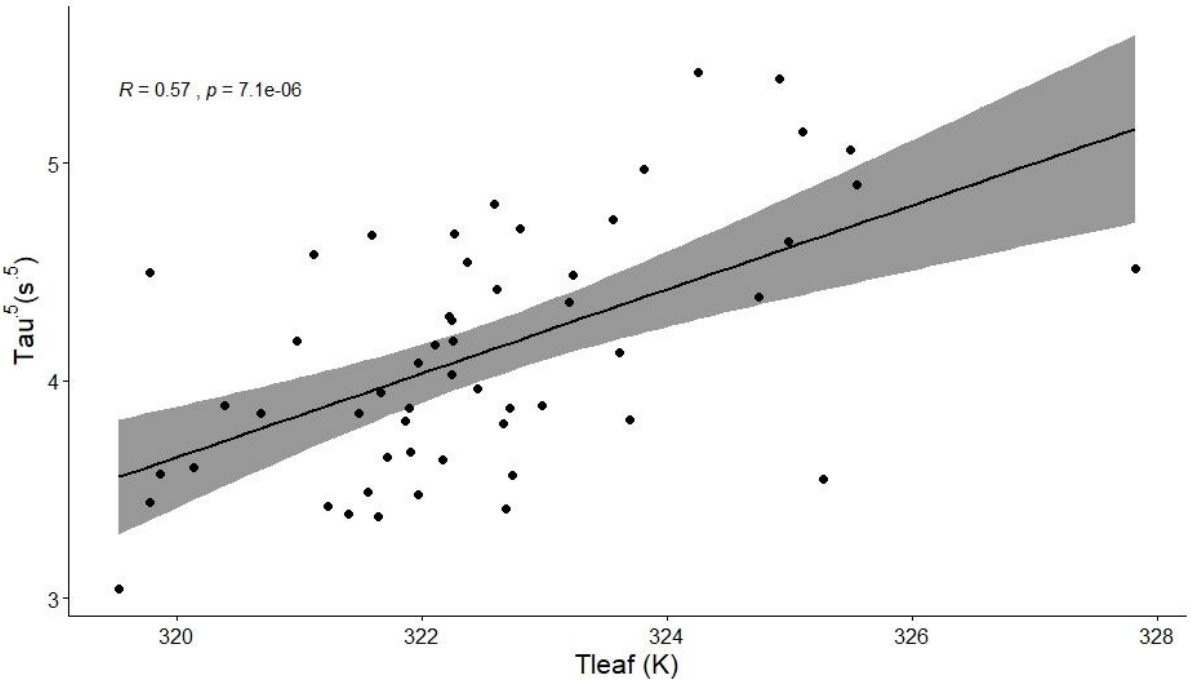

Figure 10: Scatterplot showing the correlation between T_{leaf} and $Tau^{.5}$. R is the Pearson's correlation coefficient and p is the p-value. $Tau^{.5}$ is expressed in the units of $s^{.5}$, and T_{leaf} is in K.

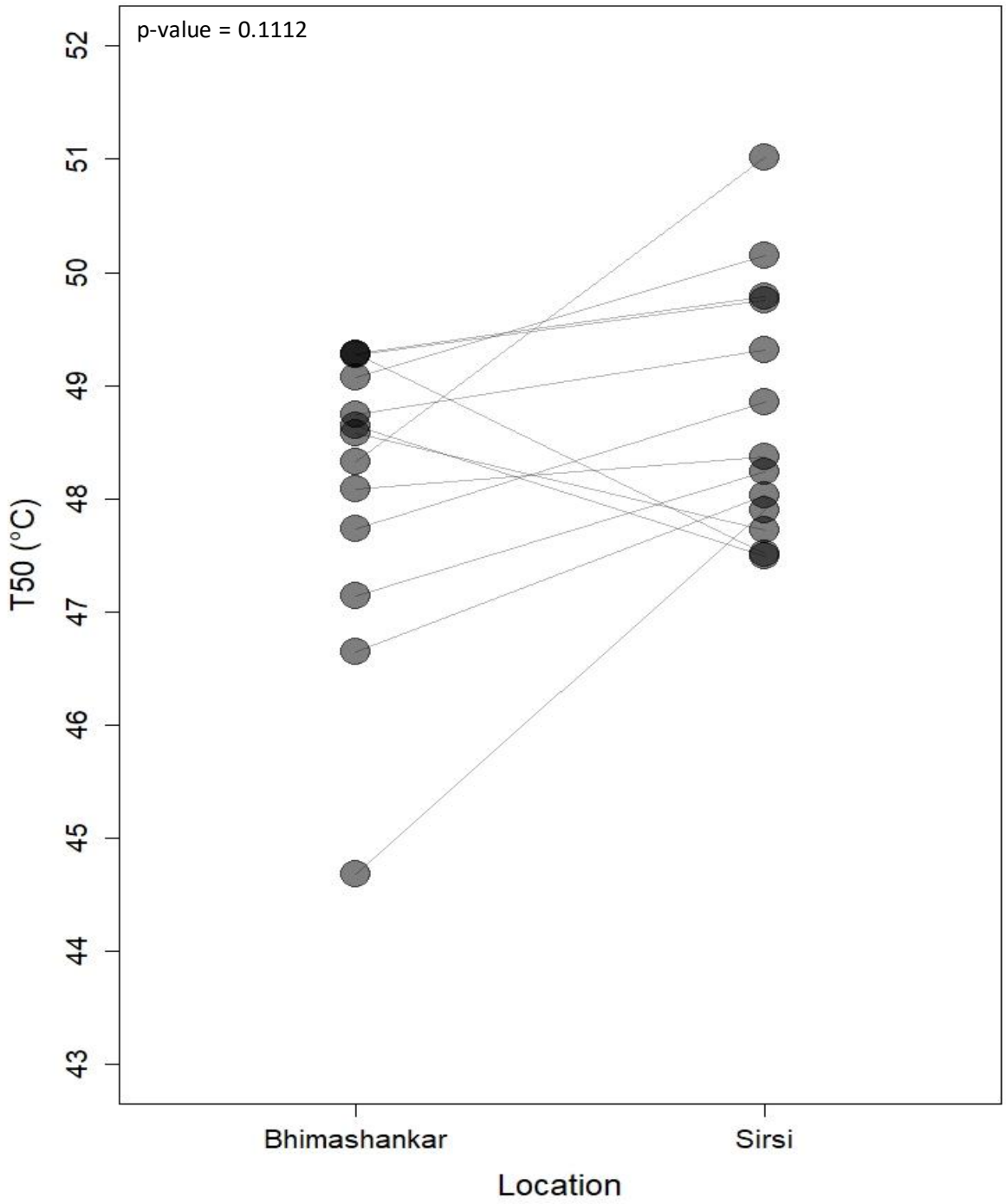

Figure 11: Variation of T50 between individuals of the same species living in different habitats. The x-axis is the location (Bhimashankar or Sirsi and the y-axis is the T_{50} in °C. Each point represents the T_{50} of a species at that habitat. The points for the same species in the two locations are joined by a line. The reported p-value was determined by conducting a paired t-test.

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Appendix

Appendix 1: List of species. For the species marked with '*', the accepted name is not resolved in "The **Plant List** (2010). Version 1". Under leaf habit, "E" refers to evergreen plants and "D" refers to deciduous plants. Under canopy, "Sun" refers to canopies that are sun exposed while "Shade" refers to canopies that are shaded.

Accepted name	**Family**	**Leaf Habit**	**Growth form**	**Canopy**
	Forest Species			
Actinodaphne hookeri Meisn.	*Lauraceae*	E	Tree	Sun
Aporosa cardiosperma (Gaertn.) Merr.	*Phyllanthaceae*	E	Tree	Sun
Alstonia scholaris (L.) R. Br.	*Apocynaceae*	E	Tree	Sun
Artocarpus hirsutus Lam.	*Moraceae*	E	Tree	Sun
Artocarpus integer (Thunb.) Merr.	*Moraceae*	E	Tree	Sun
Calophyllum inophyllum L.	*Clusiaceae*	E	Tree	Sun
Canarium strictum Roxb.	*Burseraceae*	E	Tree	Sun
Psydrax dicoccos Gaertn.	*Rubiaceae*	E	Tree	Sun
Careya arborea Roxb.	*Lecythidaceae*	D	Tree	Sun
Cinnamomum verum J.Presl	*Lauraceae*	E	Tree	Sun
Celtis timorensis Span.	*Cannabaceae*		Tree	Sun
Diospyros candolleana Wight	*Ebenaceae*	E	Tree	Sun
Flacourtia montana J.Graham	*Salicaceae*	D	Tree	Sun
Garcinia morella (Gaertn.) Desr.	*Clusiaceae*	E	Tree	Sun
Garcinia xanthochymus Hook.f. ex T.Anderson	*Clusiaceae*	E	Tree	Sun
Garcinia gummi-gutta (L.) Roxb.	*Clusiaceae*	E	Tree	Sun
Holigarna arnottiana Hook.f.	*Anacardiaceae*	E	Tree	Sun
Holigarna grahamii (Wight) Kurz	*Anacardiaceae*	E	Tree	Sun
Hopea odorata Roxb.	*Dipterocarpaceae*	E	Tree	Sun
Ixora brachiata Roxb.	*Rubiaceae*	E	Tree	Sun
*Knema attenuate**		E	Tree	Sun
Garcinia indica (Thouars) Choisy	*Clusiaceae*	E	Tree	Sun
Leea indica (Burm. f.) Merr.	*Vitaceae*	E	Shrub	Sun
*Lophopetalum wightianum**		E	Tree	Sun
Macaranga peltata (Roxb.) Müll.Arg.	*Euphorbiaceae*	D	Tree	Sun
Mangifera indica L.	*Anacardiaceae*	E	Tree	Sun
Nothapodytes nimmoniana (J.Graham) Mabb.	*Icacinaceae*	E	Tree	Sun
*Memecylon umbellatum**		E	Tree	Sun
Mimusops elengi L.	*Sapotaceae*	E	Tree	Sun
Olea dioica Roxb.	*Oleaceae*	E	Tree	Sun
Psychotria dalzellii Hook.f.	*Rubiaceae*	E	Shrub	Shade

Catunaregam spinosa (Thunb.) Tirveng.	*Rubiaceae*	D	Tree	Sun
*Sterculia guttata**		D	Tree	Sun
Stereospermum tetragonum DC.	*Bignoniaceae*		Tree	Sun
*Symplocos beddomei**			Tree	Sun
Syzygium caryophyllatum (L.) Alston	*Myrtaceae*		Tree	Sun
Syzygium cumini (L.) Skeels	*Myrtaceae*	E	Tree	Sun
Terminalia bellirica (Gaertn.) Roxb.	*Combretaceae*	D	Tree	Sun
Terminalia chebula Retz.	*Combretaceae*	D	Tree	Sun
*Terminalia paniculate**		D	Tree	Sun
Vitex altissima L.f.	*Lamiaceae*	D	Tree	Sun
	Agroforestry Species			
Areca catechu L.	*Arecaceae*	E	Palm	Sun
Elettaria cardamomum (L.) Maton	*Zingiberaceae*		Geophyte	Shade
Anacardium occidentale L.	*Anacardiaceae*	E	Tree	Sun
Manilkara zapota (L.) P.Royen	*Sapotaceae*	E	Tree	Sun
Citrus × *aurantium* L.	*Rutaceae*	E	Tree	Shade
Syzygium aromaticum (L.) Merr. & L.M.Perry	*Myrtaceae*	E	Tree	Sun
Theobroma cacao L.	*Malvaceae*	E	Tree	Shade
Coffea arabica L.	*Rubiaceae*	E	Shrub	Shade
Citrus sinensis (L.) Osbeck	*Rutaceae*	E	Tree	Shade
Piper nigrum L.	*Piperaceae*	E	Vine	Shade
Nephelium lappaceum L.	*Sapindaceae*	E	Tree	Sun
Syzygium samarangense (Blume) Merr. & L.M.Perry	*Myrtaceae*	E	Tree	Shade
Curcuma longa L.	*Zingiberaceae*	E	Geophyte	Shade
Vanilla planifolia Jacks. ex Andrews	*Orchidaceae*	E	Epiphyte	Shade

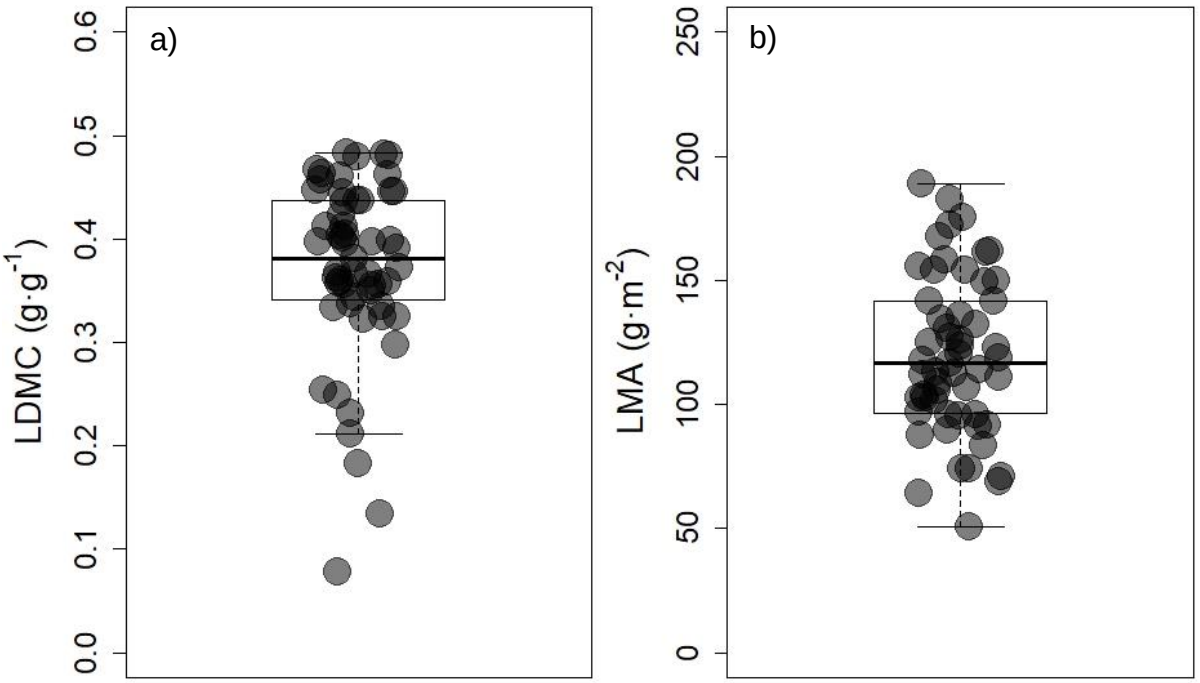
Appendix 2: Formulae and values of constants used to calculate leaf temperatures (T_{leaf}) and thermal time constant (τ).

Symbol	Formulae / Value (If available)	Units	Description
R	300	$W \cdot m^{-2}$	Incoming radiation
RH	See Appendix 3	%	Relative humidity
U	See Appendix 3	$m \cdot s^{-1}$	Wind speed
g_s	See Appendix 3	$m \cdot s^{-1}$	Stomatal conductance
L		m	Effective leaf width
c_p	1010	$J \cdot kg^{-1} \cdot K$	Specific heat capacity of air
ε	0.98	dimensionless	Emissivity constant
σ	5.670367×10^{-8}	$W \cdot m^{-2} \cdot K^{-4}$	Stephan-Boltzman constant
φ	0.5 for flat leaves	dimensionless	Ratio of projected to total leaf area
T_{air}	See Appendix 3	°C	Air temperature
T_K	$T_{air} + 273.15$	K	Air temperature in Kelvin
ρ_s	$1.2897 - (\mathrm{T_{air}} \times 0.0049)$	$kg \cdot m^{-3}$	Density of saturated air
ρ_a	$1.2898 - (T_{air} \times 0.0041)$	$kg \cdot m^{-3}$	Density of dry air
ρ	$\rho_a - \left((\rho_a - \rho_s) \times \frac{RH}{100}\right)$	$kg \cdot m^{-3}$	Density of humid air
s	$133.322 \times 4098 \times 4.584 \times \frac{2.71828182846^{\frac{17.27 \times T_{air}}{237.3 + T_{air}}}}{(\mathrm{T_{air}} + 237.3)^2}$	$kPa \cdot °C^{-1}$	Slope of the saturation vapour pressure curve versus temperature
γ	$64.904 + (0.0644 \times \mathrm{T_{air}})$	Pa·K-1	Psychrometric constant

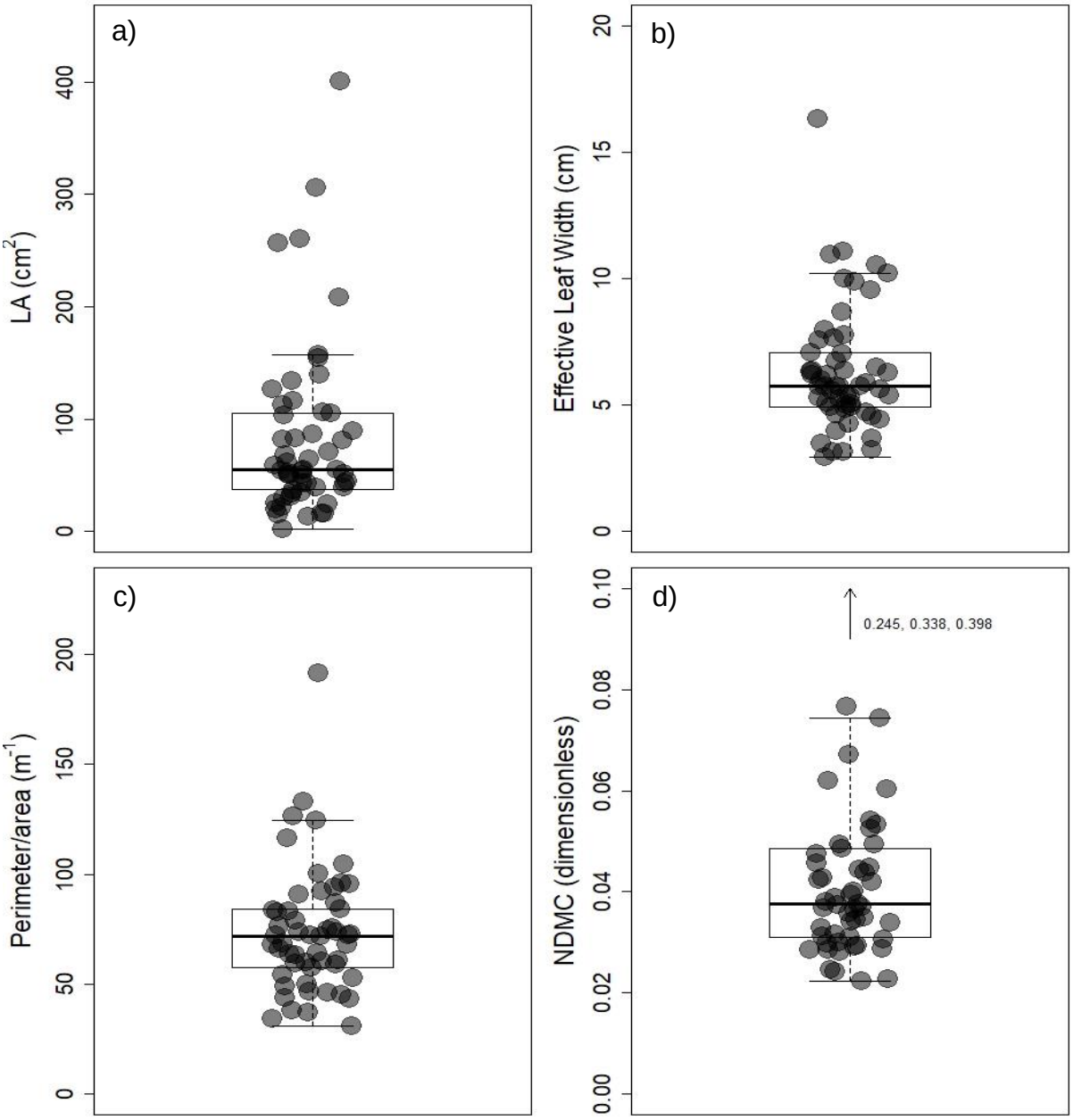
SVP	$610.7 \times 10^{\frac{7.5 \times T_{air}}{237.3+T_{air}}}$	Pa	Saturation vapour pressure
VPD	$\left(1\text{-}\frac{RH}{100}\right) \times SVP$	Pa	Vapour pressure deficit
Rni	$R\text{-}\left(4 \times \varepsilon \times \sigma \times T_K{}^3 \times \left(T_K\text{-}(45 + 273.15)\right)\right)$	$W \cdot m^{-2}$	Net isothermal radiation
g_r	$\frac{4 \times \varepsilon \times \sigma \times T_K{}^3}{\rho_a \times c_p}$	$m \cdot s^{-1}$	Boundary layer conductance to radiation
g_h	$0.007 \times \sqrt{\frac{U}{L}}$	$m \cdot s^{-1}$	boundary layer conductance to heat
g_w	$\frac{g_s \times g_h}{g_{s+}g_h}$	$m \cdot s^{-1}$	
H	$\rho_a \times c_p \times \left(g_h + g_r + \frac{g_w \times s}{\gamma}\right)$	$W \cdot m^{-2} \cdot K^{-1}$	Heat transfer coefficient
LMA		$kg \cdot m^{-2}$	Leaf mass per area
LDMC		dimensionless	Leaf dry matter content
τ	$\varphi \times LMA \times \left(\frac{4181}{LDMC \times H} + \frac{2814\text{-}4181}{H}\right)$	s	Thermal time constant
n_1	$Rni \times \gamma \times \frac{g_h}{g_w}$		
d_1	$\rho \times c_p \times g_h \times \left(s + \gamma \times \frac{g_h}{g_w}\right)$		
d_2	$s + \gamma \times \frac{g_h}{g_w}$		
T_{leaf}	$T_K + \frac{n_1}{d_1}\text{-}\frac{VPD}{d_2}$	K	Leaf temperature

Appendix 3: Parameters used for calculating the leaf temperature (T_{leaf}) under different air temperatures (T_{air}) and cooling parameters (RH, U, and Gs), and the thermal time constant of cooling (τ).

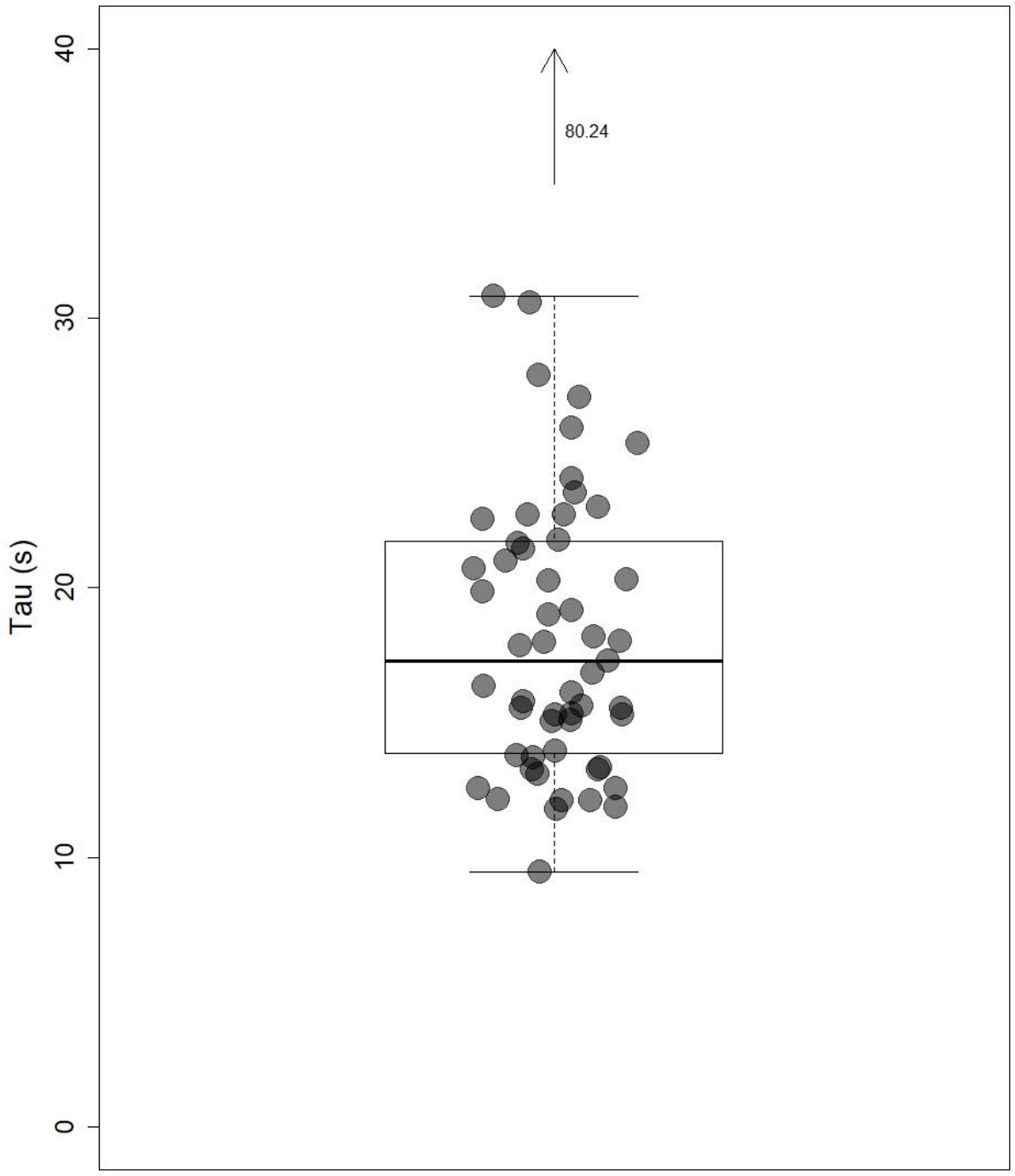
Parameters	T_{leaf} (low cooling/ high T_{air})	T_{leaf} (low cooling/ low T_{air})	T_{leaf} (high cooling/ high T_{air})	T_{leaf} (high cooling/ low T_{air})	τ
T_{air} (°C)	38	30	38	30	30
Relative humidity, RH (%)	75	75	25	25	75
Wind speed, U ($m \cdot s^{-1}$)	0.5	0.5	3	3	0.5
Stomatal conductance, g_s ($m \cdot s^{-1}$)	0.001	0.001	0.004	0.004	0.001



Appendix 4: Box-plots showing distribution of leaf functional traits at species level. Panel a represents the distribution of LDMC and panel b represents the distribution of LMA. Each point represents the average LDMC (for the first plot) or LMA (for the second plot) for a single species. LDMC is presented in $g \cdot g^{-1}$ and LMA in $g \cdot m^{-2}$.



Appendix 5: Box-plots showing the distribution of leaf morphological traits at species level. Panel a represents the distribution of LA, panel b represents the distribution of effective leaf width, panel c represents the distribution of perimeter/area, and panel d represents the distribution of NDMC. Each point represents the average LA, effective leaf width, perimeter/area or NDMC for a single species. LA is presented in cm^2, effective leaf width in cm, perimeter/area in m^{-1}, and NDMC is dimensionless.



Appendix 6: Box-plot showing the distribution of Tau at species level. The y-axis is τ in seconds (s). Each point represents the τ for a single species.