

# Variation in thermotolerance in herbaceous plants: differences between plant functional types and relationship with leaf functional traits

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



By

Urmi Poddar

Reg. no. 20131072

Biology department

IISER, Pune

Under supervision of

Dr. Deepak Barua

Biology department

IISER, Pune

## Certificate

This is to certify that this dissertation entitled “Variation in thermotolerance in herbaceous plants: differences between plant functional types and relationship with leaf functional traits” 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 Urmi Poddar at the Indian Institute of Science Education and Research (IISER), Pune under the supervision of Dr. Deepak Barua, associate professor, Biology department, IISER Pune, during the academic year 2017-2018.



Signature of Supervisor

Dr. Deepak Barua

Associate Professor

IISER Pune



Signature of Student

Urmi Poddar

BS-MS student

IISER Pune

## Declaration

I hereby declare that the matter embodied in the report entitled “Variation in thermotolerance in herbaceous plants: differences between plant functional types and relationship with leaf functional traits” are the results 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 the same has not been submitted elsewhere for any other degree.



Signature of Student

Urmi Poddar

BS-MS student

IISER Pune



Signature of Supervisor

Dr. Deepak Barua

Associate Professor

IISER Pune

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## **Abstract**

Tropical species are expected to be particularly negatively affected by the temperature increases associated with climate change. However, not much is known about the thermotolerance of tropical herbaceous plants, even though this knowledge is required for predicting their sensitivity to future warming. In this study, the thermotolerance of tropical herbaceous plants of Northern Western Ghats was measured, in order to understand how this trait varies between plant functional types, and whether it is correlated with leaf mass per area (LMA) and leaf dry matter content (LDMC). Different plant functional types showed differences in thermotolerance, with geophytes and graminoids showing higher heat tolerance than forbs. A positive correlation with LMA was also found. As LMA and functional type are related to the ecological functions of plants, these results have important implications for future climate-change induced alterations in herbaceous plant community composition and consequently, in ecological processes influenced by plants, especially carbon sink strength of vegetation.

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## Introduction

Global surface temperatures are expected to rise by 1°C- 4°C by the end of the century, along with an increase in the number and intensity of heat-waves (IPCC, 2014). Substantial warming has already been observed in the past few decades (IPCC, 2014). As a result, many organisms will be, or are already being, exposed to unprecedentedly high temperatures. Animal species may be able to find some respite from heat stress by seeking favourable micro-habitats (Sunday et al., 2014), but plants, being sessile, cannot do so. Thus, they will be exposed to high temperatures, which can have several detrimental effects (Teskey et al., 2015), like disruption of respiration (Huve et al., 2011; O'Sullivan et al., 2013) and photosynthesis (Berry and Björkman, 1980; Huve et al., 2011), and permanent leaf tissue damage (Huve et al., 2011; O'Sullivan et al., 2013), to name a few. Therefore, it is important for plants to possess sufficient thermotolerance, or an ability to tolerate high temperatures. However, not much is known about tropical plant thermotolerance, especially for plants from the Indian subcontinent, and this, even though tropical organisms are considered to be particularly sensitive to climate change. This sensitivity can be ascribed to the fact that tropical species are adapted to the relatively small temperature fluctuations in the tropics, and hence tend to have narrower temperature tolerance ranges (Janzen, 1967). They have also been found to show lower thermal safety margins, that is, lower differences between organismal thermotolerance values and the highest temperatures in their habitats (Deutsch et al., 2008; Doughty and Goulden, 2009). Even though equatorial plants, on average, have higher thermotolerance than temperate plants, this difference is only of 8°C, while habitat temperature differs by 20°C over the same latitudinal range (O'Sullivan et al., 2016). As a result, the thermotolerance of temperate species is sufficiently higher than their current and predicted future habitat temperatures, but the same is not true for tropical and sub-tropical species. Moreover, since plants play important roles in terrestrial ecosystems, especially as primary producers and carbon sinks, therefore it is important to understand their susceptibility to climate change. For these reasons, the thermotolerance of tropical herbaceous (non-woody) species from the Northern Western Ghats was studied in this project.



Previous work from this group has focused on thermotolerance in tropical woody species of Northern Western Ghats (Sastry and Barua, 2017; Sastry et al., 2017; Mohan, 2017). Therefore, studying thermotolerance in herbaceous species would help in gaining a more comprehensive understanding of plant thermotolerance in this region. Previous studies on herbaceous species indicate that these plants show a wide variation in thermotolerance, with values ranging from 45°C to 64°C (Larcher, 2003). Region, functional type/life-form and seasonal changes are some of the factors correlated with this variation (Larcher, 2003; Gurvich et al., 2002; Jameson, 1961; Weng and Lai, 2005). However, herbaceous plants usually have low values of thermotolerance, especially compared to woody species (Weng and Lai, 2005; Jameson, 1961; Gauslaa, 1984). This could make tropical herbaceous species even more vulnerable to climate change. This is another reason why it is important to examine their thermotolerance.

An unexplained pattern in many studies that have examined plant thermotolerance is that the variation in thermotolerance within co-occurring plant species from the same site is quite high, especially when compared to the variation in this trait from higher latitudes to the equator (O'Sullivan et al., 2016). Within a site, the difference between the thermotolerance values of the most and least heat-tolerant species sampled can be up to 10°C - 20°C (O'Sullivan et al., 2016; Weng and Lai, 2005; Gauslaa, 1984). Effect of differences in micro-habitat on thermotolerance is one of the possible explanations for this large within-site variation (Curtis et al., 2016). Another possible explanation is that thermotolerance varies with leaf functional traits and differs with plant functional types, as these are representative of different ecological strategies used by plants. This hypothesis was investigated in this study.

Leaf functional traits are important structural, morphological or physiological leaf traits that are closely related to plant fitness. Leaf mass per area (LMA) and leaf dry matter content (LDMC) are two important leaf functional traits that are reflective on the carbon investment in a leaf. These traits are good predictors of plant resource-acquisition and resource-use strategies (Wright et al., 2005; Wilson et al., 1999). Moreover, the community-level distribution of these traits have recently be used to in trait-based

ecology approaches to estimate ecosystem properties like net primary productivity, litter decomposition rate, and total soil carbon and nitrogen (Garnier et al., 2004). LMA is especially important, as it is positively correlated with stress tolerance and leaf life span, but negatively correlated with growth rate and photosynthetic rate (Wright et al., 2005). This trait is expected to be positively correlated with thermotolerance, based on its relationship with traits that influence leaf temperature (Curtis et al., 2012). Such a correlation would imply that due to climate change, plant communities would be dominated by slow-growing, high LMA species in future. This could result in a fall in primary productivity and total carbon uptake by plants. However, previous research on the LMA-thermotolerance relationship has shown mixed results. Some studies have indeed found a positive correlation (Sastry and Barua, 2017; Mohan, 2017; Gallagar, 2014; Knight and Ackerly, 2003), but others have found no correlation (O'Sullivan et al., 2016; Zhang et al., 2012) or even a negative correlation (Godoy et al., 2011). LDMC is also expected to be positively correlated with thermotolerance.

Plant functional types (PFTs) are groups of species which show similarities in their effects on ecosystem processes and their responses to environmental changes (Walker 1992; Noble and Gitay, 1996). From the definition, it follows that species from the same PFTs would be expected to respond in a similar manner to climate change and global warming. If this is indeed the case, then climate change could affect ecosystem processes which are governed or influenced by the more heat sensitive PFTs.

Plants can be broadly classified into woody and herbaceous functional types. Woody species can be further sub-classified into trees, shrubs and lianas, while forbs, graminoids and geophytes are the sub-classifications of herbaceous species (table 1). Based on the time taken to complete their life cycle, the latter can also be divided into annuals and perennials (table 1). Herbaceous species are expected to show lower thermotolerance than woody species, as they have higher growth rates and shorter life-spans (Salguero-Gómez et al., 2015; Lambers and Poorter, 1992), which tend to be correlated with lower stress tolerance and lower resilience to climatic variability (Lambers and Porter, 1992; Morris et al., 2008). There is some evidence which supports this prediction (Weng and Lai, 2005; Gauslaa, 1984; Jameson, 1961), but the opposite

has also been reported (Larcher, 2003; Weng and Lai, 2005; Gurvich et al., 2002). Moreover, most of this evidence is from the sub-tropics or temperate and alpine regions.

Within herbaceous plants, the thermotolerance of graminoids and geophytes is expected to be higher than that of forbs. This expectation is based on the fact that geophytes tend to have longer life-spans than most other herbaceous species, while C4 grasses, which dominate graminoid communities in the tropics (Ehleringer, 1978), have been shown to be highly thermotolerant (Larcher, 2003; Gurvich et al., 2002, Weng and Lai 2005). Previous research on sub-tropical and temperate species supports the above-mentioned hypothesis on the relative thermotolerance of forbs and graminoids (Larcher, 2003; Gurvich et al., 2002), but to the best of my knowledge, no studies have compared this trait in geophytes to that in the other two herbaceous functional types. I also hypothesize that perennials will have a higher thermotolerance than annuals, as they have slower growth rates (Lambers and Poorter, 1992) and longer life-spans (by definition) compared to annuals. Studies on desert plants, turfgrasses and ryegrass cultivars support this hypothesis (Downton et al., 1984; Yang et al., 2014; Richardson, 2004).

Thermotolerance has been measured using various methods like electrolyte leakage (Anderson et al., 1990; Gurvich et al., 2002), photosynthetic gas exchange (Berry and Björkman; 1980), visual leaf damage (Gauslaa, 1984; Buchner and Neuner, 2003; Buchner et al., 2017), chlorophyll fluorescence (Downton et al., 1984; Weng and Lai, 2005; Valladares and Pearcy, 1997) etc. This study estimated the same by looking at the change in dark-adapted chlorophyll *a* fluorescence, or  $F_v/F_m$ , with temperature. This is one of the most common methods for measuring thermotolerance (Valladares and Pearcy, 1997; Yamasaki, 2002; Krause, 2010; Buchner et al., 2017), and is considered to be a good indicator of photosynthetic and organismal heat tolerance (Havaux et al., 1991; Barua et al., 2003).  $F_v/F_m$  is a measure of the efficiency of PSII photochemistry (Maxwell and Johnson, 2000), which one of the most thermosensitive processes in leaves (Berry and Björkman, 1980; Havaux et al., 1991). Thus, a drop in  $F_v/F_m$  with temperature is a sign of heat-sensitivity. In this study, the temperature at which  $F_v/F_m$

fell to half its value at room temperature was called  $T_{50}$  and was considered the measure of the upper thermal tolerance limits of plant species.

Thus, this study aims to measure the  $T_{50}$  values of tropical herbaceous plant species in order to understand their susceptibility to future warming. It also tries to investigate whether thermotolerance is correlated with leaf functional traits, whether it differs with different plant functional types, and how the thermotolerance of herbaceous species compares with that of woody species from the same region. This can help in predicting how changes in the plant community due to climate change will affect various ecosystem functions and processes which are influenced by herbaceous plants.

## **Materials and methods**

### *Study sites and sample collection*

Fifty-five species were sampled from three sites in the Northern Western Ghats: a) Vetal Tekdi Biodiversity Park, Pune (18°31'58"N, 73°48'46"E); b) Chalkewadi, Near Kaas Plateau, Satara (17°35'34"N, 73°49'38"E); c) open site near Nigdale village, Bhimashankar wildlife sanctuary (19° 4'23"N, 73°33'13"E). Rainfall in this region is highly seasonal and occurs in the form of monsoon rains (mid-June to early October). The wet monsoon season is preceded by a hot, dry season, and is followed by a cool dry season. During this time of the year, the vegetation of these sites is dominated by ephemeral herbs (Joshi and Kumbhojkar, 1997; Lekhak and Yadav, 2012; Rahangdale and Rahangdale, 2017). Most of the plants in these herb dominated communities germinate after the onset of the rains, around the mid to late June, and flower towards the end of the rains, around mid to late October. Sampling was carried out from mid-August to early October (late monsoon). For all species, we selected plants which were more or less fully grown and well established.

Around fourteen leaves each were collected from most individuals sampled. In case of smaller plants with fewer leaves per individual, fourteen leaves were collected from multiple individuals, which were considered to represent one replicate. In this way, 5-6 replicates were sampled per species. After collection, samples were transported to the

lab and placed in water-filled containers, which were then stored overnight in sealed plastic bags. This allowed the samples to water saturate. Assays and measurements were started the next morning.

#### *Thermotolerance assay*

Eight leaves per replicate were used for this assay. With the help of a cork borer, leaf discs of 0.8 cm diameter were punched out from the leaves. In case of thinner leaves with width < 0.8 cm, leaf sections of the same length were punched, while leaves/leaflets with length and width less than 0.8 cm were used whole. These discs/sections were sandwiched between two layers of muslin cloth and aluminum foil and were placed in a plastic bag. This bag was then sealed and immersed in a temperature controlled water bath (Julabo, Model F25, Seelbach, Germany). In this way, leaf discs were exposed to seven different temperatures (25°C, 35°C, 40°C, 45°C, 47.5°C, 50°C or 52.5°C) for 30 minutes each (As in Sastry and Barua, 2017).

After heating, discs/sections were placed on petri plates with moist tissue paper, which were then stored in sealed plastic bags. Approximately 24 hours after temperature treatment, discs/sections were dark-adapted, followed by measurement of  $F_v/F_m$ . Dark-adaptation was carried out by placing the discs/sections between layers of opaque cloth and paper for 30 minutes.  $F_v/F_m$  was measured using a PAM 2500 fluorometer (Walz, Effeltrich, Germany). For each individual or replicate,  $F_v/F_m$  was also measured for a whole leaf which had not been subjected to temperature treatment. These leaves were considered as controls.

#### *Measurement of Leaf functional traits*

For most replicate individuals, leaf functional traits were measured for six leaves. Water saturated leaves were scanned with a CanoScan Lide 110 scanner (Canon, Hanoi, Vietnam). Then a disc/section of 0.8 cm diameter/length was punched out from each leaf and the leaves and sections were weighed separately. After weighing, the leaves were dried in a hot-air oven at 70°C for at least 48 hours. Leaves were weighed again after drying. Area was calculated from the scanned images using the software ImageJ

(Version 1.51, ImageJ, USA). LMA was calculated as the ratio of leaf dry weight to leaf area, while LDMC was calculated as the ratio of leaf dry weight to fresh weight.

### *Statistical analyses*

Temperature response curves (TRCs), or curves of  $F_v/F_m$  vs. temperature, were generated in order to estimate thermotolerance. This was done by fitting a four parameter logistic curve to  $F_v/F_m$  values, using the R package 'drc'. The lower asymptote of these curves was set to zero.  $T_{50}$  was calculated as the temperature at which  $F_v/F_m$  was half the value of the upper asymptote.  $F_v/F_m$  values of all replicates of a species were pooled to generate a species level TRC and to calculate a species level  $T_{50}$  value. These species level values were used for further analyses.

Using an ANOVA of species nested in functional type, the effect of PFT and species on  $T_{50}$  values was assessed. Similar ANOVAs were performed for LMA, LDMC and leaf area. The correlation between leaf functional traits and thermotolerance was studied with the help of Pearson's correlation tests. The effect of plant functional type on the relationship between thermotolerance and leaf functional traits was examined by performing ANCOVAs (which tested for equality of slopes and intercepts). This effect was also visually assessed by fitting SMA regressions to the data. Perennial graminoids and geophytes were excluded from the ANCOVAs and SMA regressions due to small sample sizes in these groups. For comparing the thermotolerance of herbaceous and woody species, data from previous studies on woody species of this region was used (Sastry and Barua, 2017; Mohan, 2017).

Temperature data from Pune was used as a representative for habitat temperatures of sampled species. The highest temperatures recorded in the past decade for each day of the year, henceforth called absolute maximum temperatures, were used. Further, based on the calculations of Zelazowski et al. (2011), the vulnerability of sampled species to future warming was estimated. These calculations predict that temperatures in the tropics will increase by 3°C – 6°C by 2100. Therefore 3°C and 6°C were added to the current habitat temperatures to represent future temperatures. These were then compared to  $T_{50}$  values.

Other than ANOVAs, which were done in Statistica (Version 10, Statsoft, Tulsa, OK, USA), all statistical tests were carried out in R (version 3.4.0). The R package 'smatr' was used for the SMA regressions, and the package 'mcr' for Pearson's correlation tests. All other analyses was done using the base package.

## Results

The temperature response curves showed that  $F_v/F_m$  of most species fell to zero at between 47.5°C to 52.5°C. However, the curves of some sensitive species reached zero at lower temperatures, while a few extremely heat tolerant species were able to maintain a non-zero  $F_v/F_m$  value even at 52.5°C. The decline in  $F_v/F_m$  from control values started at lower temperatures for less thermotolerant species, as compared to more thermotolerant species (fig. 1). There were significant differences in the thermotolerance between species (table 2). The  $T_{50}$  values of sampled species ranged from 40.78°C in *Senecio bombayensis* (an annual forb) to 48.65°C in *Cyperus sp.* (a perennial graminoid), with an average of 44.33°C.

Thermotolerance was also found to vary with plant functional type (table 2). Perennial graminoids were the most heat tolerant, followed by geophytes and annual graminoids, and then by perennial forbs (fig. 2). Annual forbs were the least heat tolerant. The mean thermotolerance of forbs, graminoids and geophytes was 43.55°C, 45.11°C and 45.55°C, respectively.

All the leaf functional traits examined were significantly different between species (table 3). A positive correlation between thermotolerance and LMA was found, but there was no significant correlation of thermotolerance with LDMC (fig. 3). Thermotolerance was also uncorrelated with leaf area (data not shown). Different functional types had similar slopes of their  $T_{50}$ -LMA relationships and  $T_{50}$ -LDMC relationships, but the elevations of these relationships differed with functional type (table 4, table 5, fig. 4).

Herbaceous species in this study showed lower thermotolerance than what has been observed for woody species from this region (fig. S1), the mean of the latter being 47.74°C. On pooling the data for herbaceous and woody species, a significant positive

correlation between LMA and  $T_{50}$ , and a significant negative correlation between LDMC and  $T_{50}$ , was seen (fig. S2). Performing an ANCOVA and SMA regressions showed that herbaceous and woody plants differed in the slopes of their LMA- $T_{50}$  relationships (fig. S3, table S2). Similar analyses for the LDMC- $T_{50}$  relationships showed that the two groups had similar slopes, but different elevations (fig. S3, table S3).

On comparing with habitat temperatures, thermotolerance of all species was found to be greater than the highest temperature recorded during the growing season (fig. 5), which is 35.9°C. Even after adding 3°C to the current temperatures,  $T_{50}$  was higher than the maximum habitat temperatures (during growing season) by several degrees Celcius. However, on adding 6°C to current temperatures, 5 species were considered to be at risk due to climate change, as their  $T_{50}$  values were equal to or less than maximum temperatures (during growing season). These species were *Senecio bombayensis*, *Smithia hirsuta*, *Impatiens lawii*, *Impatiens tomentosa* and *Neanotis lancifolia*, all of which are annual forbs. Six other species had  $T_{50}$  values which were only slightly higher (<1°C higher) than future maximum temperatures (current temperatures + 6°C).

## Discussion

The  $T_{50}$  values of the 55 tropical herbaceous species sampled in this study ranged from 40.78°C to 48.65°C, and this trait was found to differ with plant functional type. A positive correlation with the key leaf functional trait, LMA was also seen, though no correlation with LDMC was found. The thermotolerance values observed in this study are similar to what has been reported for tropical herbs in another study, which also used a PSII based measure of thermotolerance (Weng and Lai, 2005). However, a slightly larger range of values was reported in the latter.

The relative thermotolerance of different plant functional types was as hypothesized. Perennials showed higher heat tolerance than annuals, and graminoids and geophytes showed higher heat tolerance than forbs. Annuals tend to have higher photosynthetic rates and growth rates as compared to perennials (Lambers and Poorter, 1992). Similarly, forbs, on average, show higher photosynthetic rates than graminoids (Reich et



al., 2007). The leaf litter of forbs also tends to decomposes faster than that of graminoids (Cornwell et al., 2008). Likewise, geophytes are also expected have lower photosynthetic and litter decomposition rates, as they showed higher LMA, which is correlated with lower photosynthetic rate and slower decomposition (Cornwell et al., 2008, Wright et al. 2005). Due to these reasons, a shift in tropical herbaceous plant communities towards graminoid and geophyte dominance could reduce plant productivity and decomposition rates, and thus change nutrient cycles. Moreover, graminoids tend to maintain higher canopy temperatures than forbs (Gersony et al., 2017). As a result, arthropods (and other organisms living among herbaceous vegetation) could experience higher microhabitat temperatures in a graminoid-dominated community. However, it should be noted that substantial variation was also seen within each PFT, with overlap between the thermotolerance ranges of different PFTs. This implies that at least some fraction of species belonging to the heat sensitive groups may be able to tolerate a warmer climate. Nevertheless, it is not clear whether this will prevent significant future changes in the relative abundance of different PFTs (by increase in abundance of more heat tolerant members of a particular PFT to compensate for the loss of heat sensitive members).

The relative thermotolerance of herbaceous and woody species was also as predicted, with herbaceous species showing lower thermotolerance than woody species. However, some perennial graminoids and geophytes had  $T_{50}$  values comparable to that of woody plants. This could explain why some studies have reported the thermotolerance of herbaceous plants, especially graminoids, to be comparable to, or even higher than that of woody plants (Larcher, 2003; Weng and Lai, 2005; Gurvich et al., 2002).

The  $T_{50}$ -LMA correlation found in this study implies that slow-growing species with low productivity are less susceptible to future warming (Wright et al., 2005). Thus climate change could reduce the average productivity and the amount of carbon dioxide taken up by tropical herbaceous plant communities. This in turn could increase the concentration of atmospheric carbon dioxide, thus worsening climate change.

When the data for woody species and herbaceous species was combined,  $T_{50}$  was found to be positively correlated with LMA and negatively correlated with LDMC. This is

surprising, as both LMA and LDMC represent carbon investment in leaves and are considered to be similar predictors of plant ecological strategies (Wilson et al., 1999). Further, the  $T_{50}$ -LMA relationship for herbaceous plants was steeper than that for woody plants. The latter group tends to have higher values of thermotolerance and LMA. This, along with a visual assessment of the data, suggests that LMA may have a saturating relationship with thermotolerance. Thus, based on the range of LMA of sampled species, one may or may not find a correlation between thermotolerance and LMA. This could explain why some researchers have found this correlation (Sastry and Barua, 2017; Mohan, 2017; Gallagar, 2014; Knight and Ackerly, 2003) and while others have failed to find it (O'Sullivan et al., 2016; Zhang et al., 2012). However, the range of LMA in another study on tropical plants which failed to find this correlation (Zhang et al., 2012), lies within the range in this study, which weakens the above hypothesis.

The species studied here grow during the cool monsoon season and do not experience the hot summer, but their thermotolerance was much higher than the current maximum temperatures during monsoon (and even higher than the summer maximum for most species). Nevertheless, under the worst-case scenario of future warming (current temperatures + 6°C), 5 species were classified as 'at-risk' (thermotolerance  $\leq$  predicted habitat temperature), and the thermotolerance of 6 more species was  $<1^\circ\text{C}$  higher than the future maximum temperature. Moreover, these estimates may be conservative, as leaf temperatures can be higher than the ambient temperature, sometimes by as much as  $10^\circ\text{C} - 20^\circ\text{C}$  (Stoutjesdijk, 1970).

It should, however, be noted that leaf temperature can also be lower than air temperature, though usually by only a few degrees Celcius (Stoutjesdijk, 1970). Thus, by reducing leaf temperature, a plant may be able to tolerate air temperatures higher than its thermotolerance value. Transpirational cooling is one mechanism by which this can be achieved (Stoutjesdijk, 1970). This makes it harder to compare air temperatures with thermotolerance values measured with a leaf temperature-based assay. Plants can also increase their heat tolerance through acclimation, as has been demonstrated by many researchers (Downton et al., 1984; Gauslaa, 1984; Buchner and Neuner, 2003; Buchner et al., 2017). If species with lower (basal) thermotolerance have a greater

ability to acclimate and/or cool their leaves, then they may be able to compensate for their low basal thermotolerance. Moreover, heritable intra-specific variation in thermotolerance, if present, may allow heat-sensitive species to adapt to a warmer climate. Therefore, these alternative heat tolerance strategies should be studied in order to make better predictions of plant susceptibility to climate change. Another question that requires investigation is the effect of various biotic and abiotic factors, like herbivory, light, changes in CO<sub>2</sub> concentration etc., on plant thermotolerance and heat sensitivity. The relationship between photosynthetic thermotolerance and whole organism thermotolerance also needs to be better understood.

## **Conclusions**

The large with-in site variation in thermotolerance, which has been reported here and in many other studies, can be at least partially attributed to differences between PFTs and correlation of thermotolerance with LMA. This has important implications for the effects of a warming climate on the plant community and ecological processes influenced by plants. In particular, both LMA and PFTs are related to photosynthetic rate, suggesting that heat tolerant species tend to have lower photosynthetic rates. This indicates that changes in plant community composition due to future warming would not be random, but would lean towards a decrease average plant productivity and carbon sequestration potential of vegetation. Other likely consequences of the differential sensitivity of PFTs to climate change include future changes in decomposition rates and microhabitat temperatures for animals living among vegetation. However, alternative heat tolerance strategies and the relationship between photosynthetic and whole organism heat sensitivity need to be further studied in order to make better predictions.

## Tables and figures

**Table 1:** Definition of terms associated with plant functional types

Functional type	Definition
<b>Ecophysiology based classification:</b>	
Herbaceous plant	Plants which do not have any woody above-ground stems (Du Rietz, 1931)
Forb	An herbaceous flowering plant which is neither a graminoid nor a geophyte, usually with broad leaves (Box, 1981)
Graminoid	An herbaceous flowering plant with narrow grass-like leaves. Includes grasses, sedges and rushes (Box, 1981; Warming and Vahl, 1909)
Geophyte	An herbaceous flowering plant possessing underground storage organs (Raunkiaer, 1907)
Woody plant	Plants whose above-ground shoots have lignified (woody) stems (Du Rietz, 1931)
Tree	A perennial woody plant with a well-defined main stem (trunk) which lacks branches in the lower parts (Du Rietz, 1931)
Shrub	A short perennial woody plant which lacks a well-defined trunk. Instead, the main stem branches from its basal part (Du Rietz, 1931)
Liana	A woody vine which climbs on trees, rocks and other structures (Du Rietz, 1931)
<b>Life-span based classification:</b>	
Annual	A plant which completes its life-cycle within a year (Warming and Vahl, 1909)
Perennial	A plant which lives for more than two years (Warming and Vahl, 1909)

**Table 2:** Variation in thermotolerance with species and plant functional type (PFT). Results of a nested ANOVA examining the effect of species and PFT on  $T_{50}$ . P-values marked with an asterisk (\*) are significant ( $\alpha = 0.05$ ).

Source	dF	SS	MS	F	p
Species (in PFT)	50	533.0	10.7	16.5	<0.0001*
PFT	4	304.4	76.1	117.4	<0.0001*
Error	223	144.5	0.6		

**Table 3:** Variation in leaf functional traits with species and plant functional type (PFT). Results of nested ANOVAs examining the effect of species and PFT on a) leaf mass per area (LMA), b) leaf dry matter content (LDMC) and c) leaf area. P-values marked with an asterisk (\*) are significant ( $\alpha = 0.05$ ).

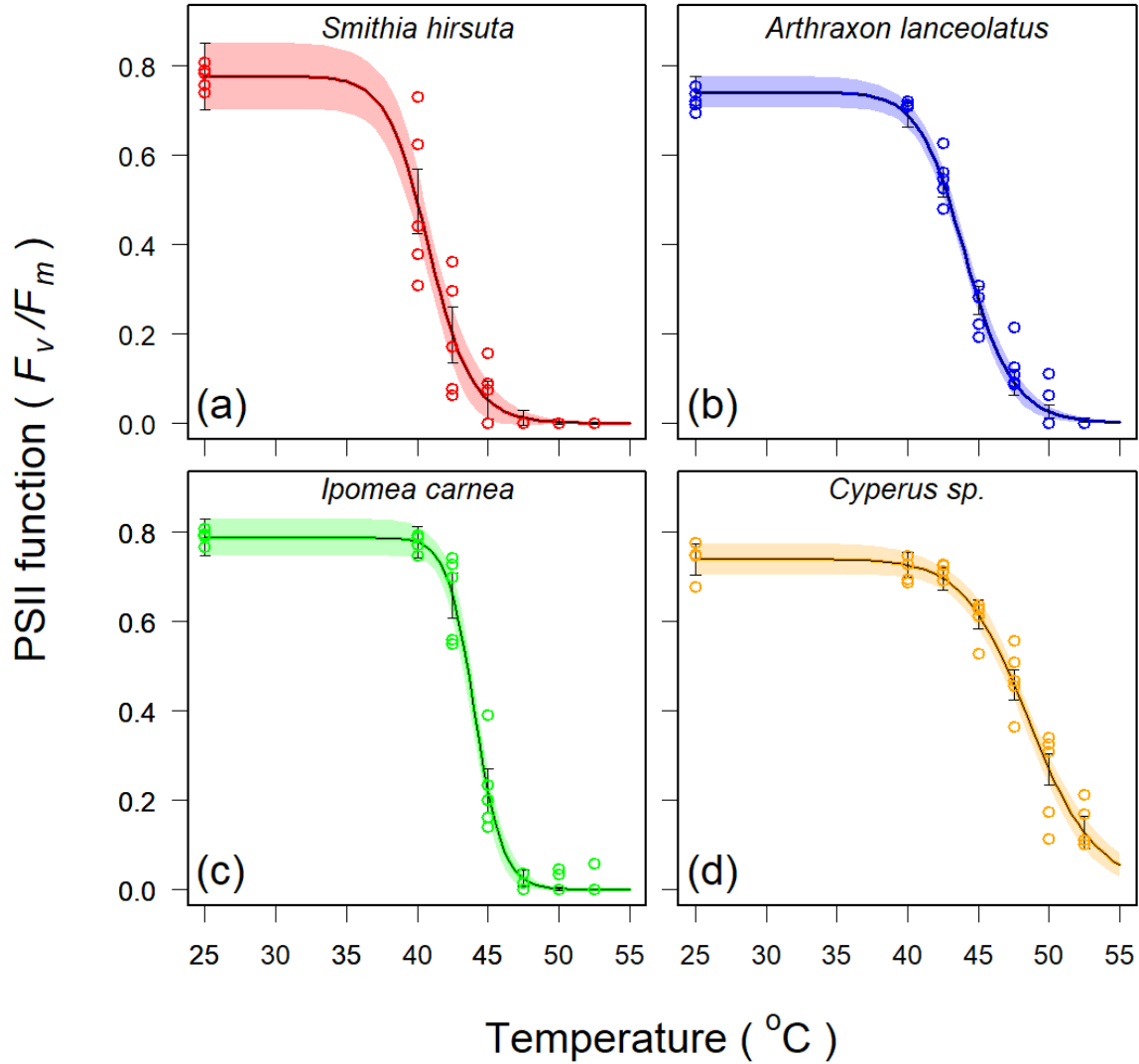
Source	dF	SS	MS	F	p
<b>a) LMA</b>					
Species (in PFT)	50	89755.9	1795.1	56.21	<0.0001*
PFT	4	17101.2	4275.3	133.88	<0.0001*
Error	222	7089.4	31.9		
<b>b) LDMC</b>					
Species (in PFT)	50	776254	194063	249.14	<0.0001*
PFT	4	1155421	23108	29.67	<0.0001*
Error	223	173703	779		
<b>c) Leaf area</b>					
Species (in PFT)	50	122236.3	2444.73	17.5517	<0.0001*
PFT	4	22935.6	5733.91	41.1661	<0.0001*
Error	222	30921.8	139.29		

**Table 4:** Effect of plant functional type on the relationship between thermotolerance and leaf mass per area (LMA). Results of ANCOVAs testing for a) equality of slopes and b) equality of intercepts between T<sub>50</sub>-LMA relationships of different PFTs. P-values marked with an asterisk (\*) are significant ( $\alpha = 0.05$ ).

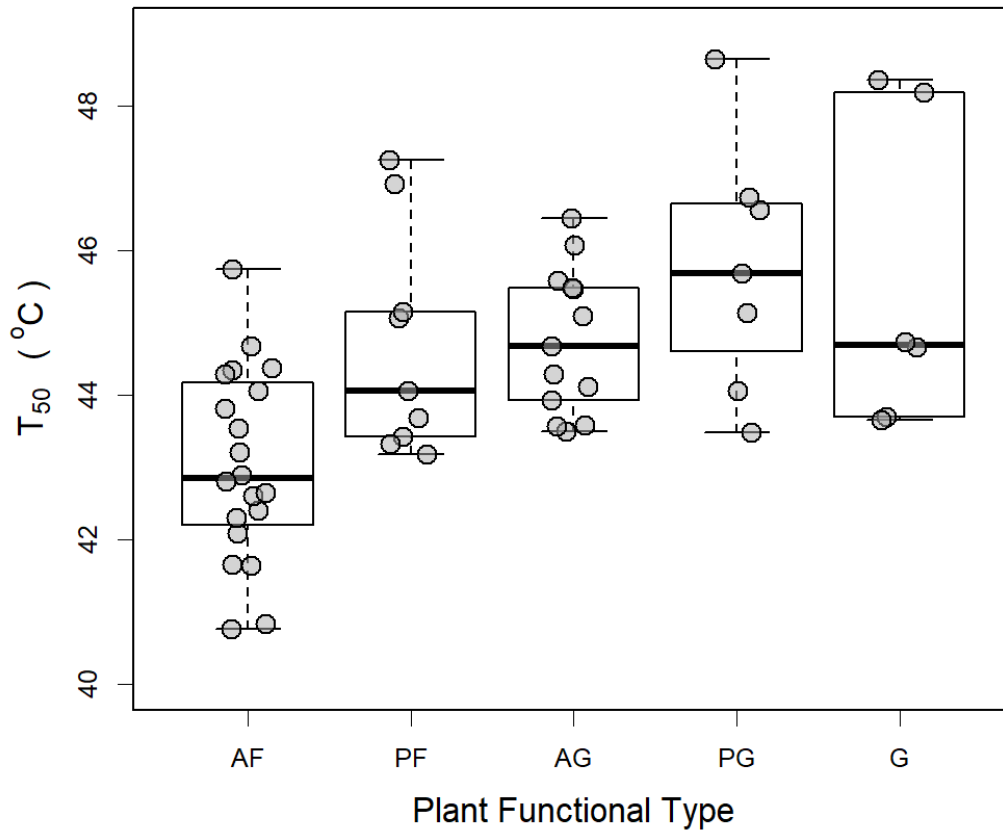
Source	dF	SS	MS	F	p
<b>a) equality of slopes</b>					
LMA	1	9.35	9.351	6.516	0.0148*
PFT	1	25.99	25.992	18.113	0.0001*
LMA x PFT	1	4.04	4.043	2.817	0.1014
error	38	54.53	1.435		
<b>b) equality of intercepts</b>					
LMA	1	9.35	9.351	6.226	0.0169*
PFT	1	25.99	25.992	17.306	0.0002*
error	39	58.57	1.502		

**Table 5:** Effect of plant functional type on the relationship between thermotolerance and leaf dry matter content (LDMC). Results of ANCOVAs testing for a) equality of slopes and b) equality of intercepts between T<sub>50</sub>-LDMC relationships of different PFTs. P-values marked with an asterisk (\*) are significant ( $\alpha = 0.05$ ).

Source	dF	SS	MS	F	p
<b>a) equality of slopes</b>					
LDMC	1	3.13	3.135	1.799	0.1878
PFT	1	22.54	22.541	12.935	0.0009*
LDMC x PFT	1	2.02	2.019	1.159	0.2885
error	38	66.22	1.743		
<b>b) equality of intercepts</b>					
LDMC	1	3.13	3.135	1.792	0.1884
PFT	1	22.54	22.541	12.882	0.0009*
error	39	68.24	1.75		

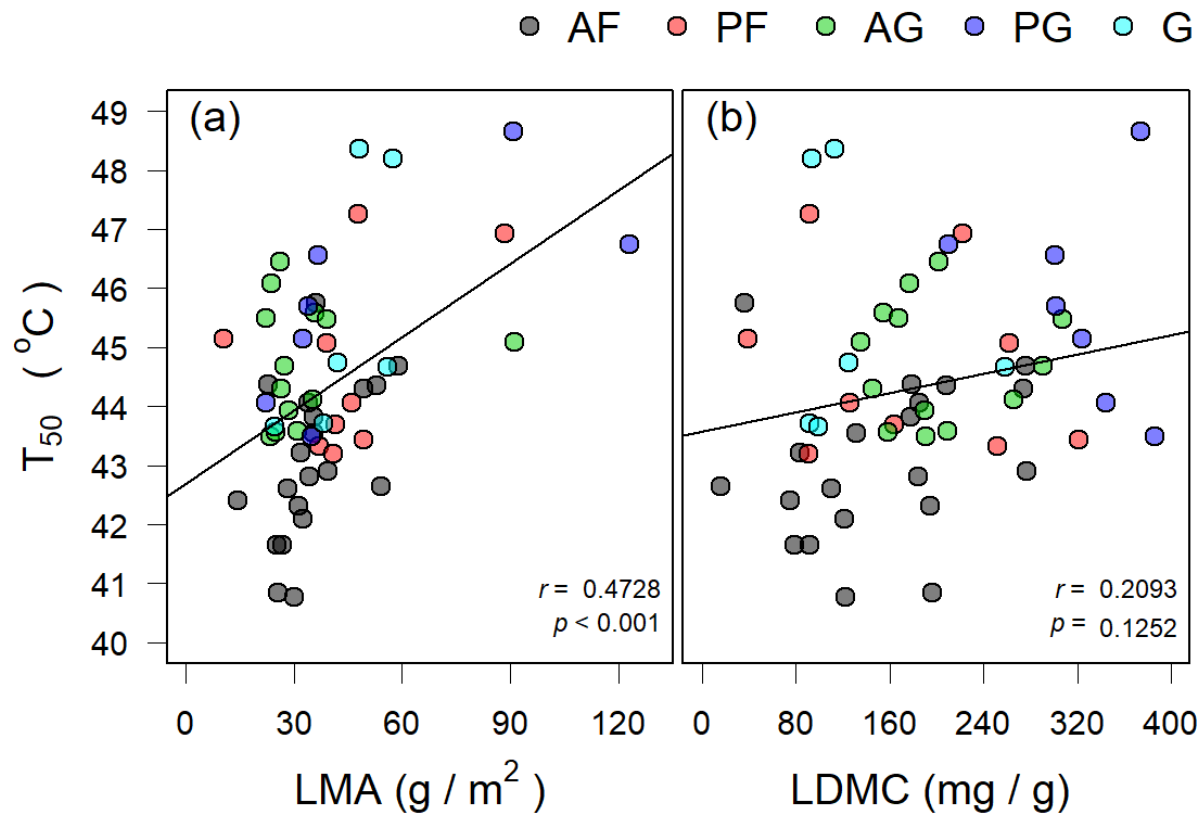


**Figure 1:** Representative temperature response curves (TRCs). a) TRC of *Smithia hirsuta*, a species with low thermotolerance, b) TRC of *Arthraxon lanceolatus*, a species with intermediate thermotolerance, c) TRC of *Ipomea carnea*, another species with intermediate thermotolerance, and d) TRC of *Cyperus sp.*, a species with high thermotolerance. The x-axis shows leaf temperature and y-axis shows  $F_v/F_m$ , the maximum quantum yield of PSII photochemistry. Error bars represent 95% confidence intervals.

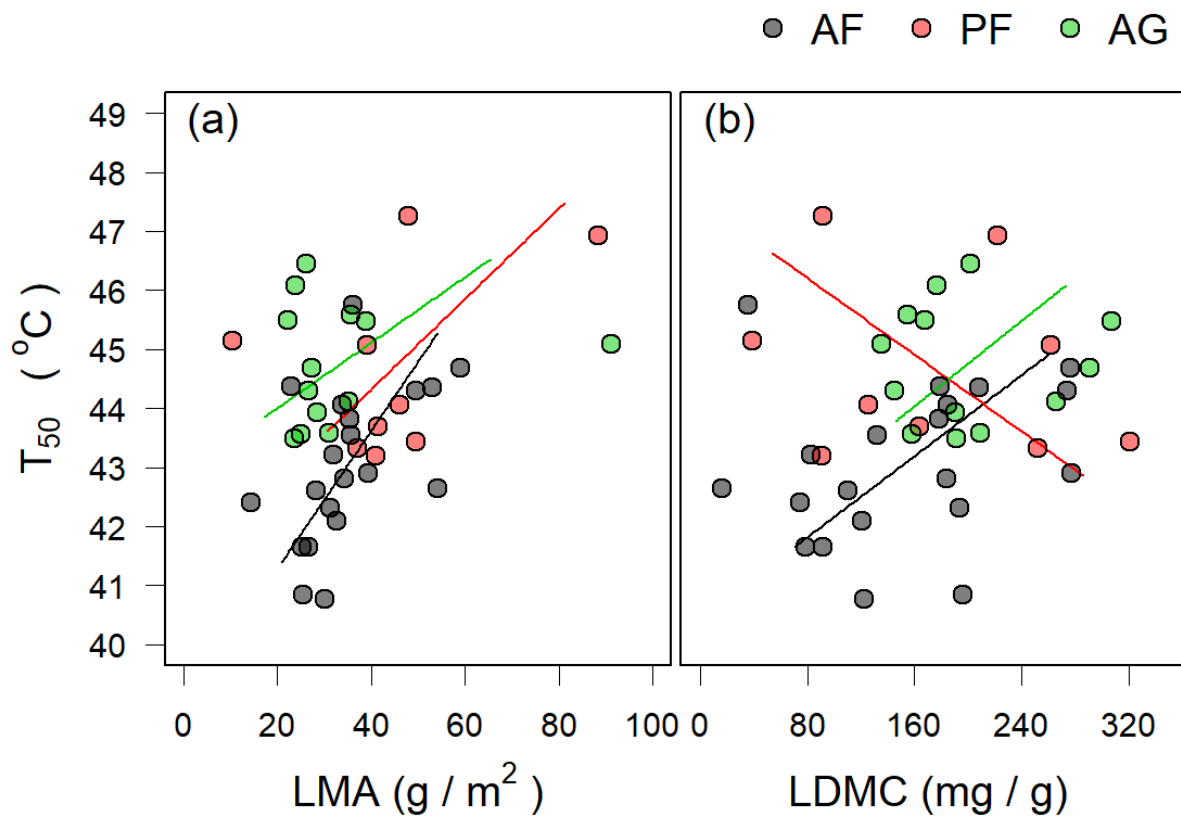


**Figure 2:** Variation in thermotolerance with plant functional type. Each boxplot represents the distribution of  $T_{50}$  values for the respective functional type. AF – annual forbs, PF – perennial forbs, AG – annual graminoids, PG – perennial graminoids, G – geophytes. Number of species in each group is as follows: AF – 20, PF – 9, AG – 13, PG – 7, G – 6.

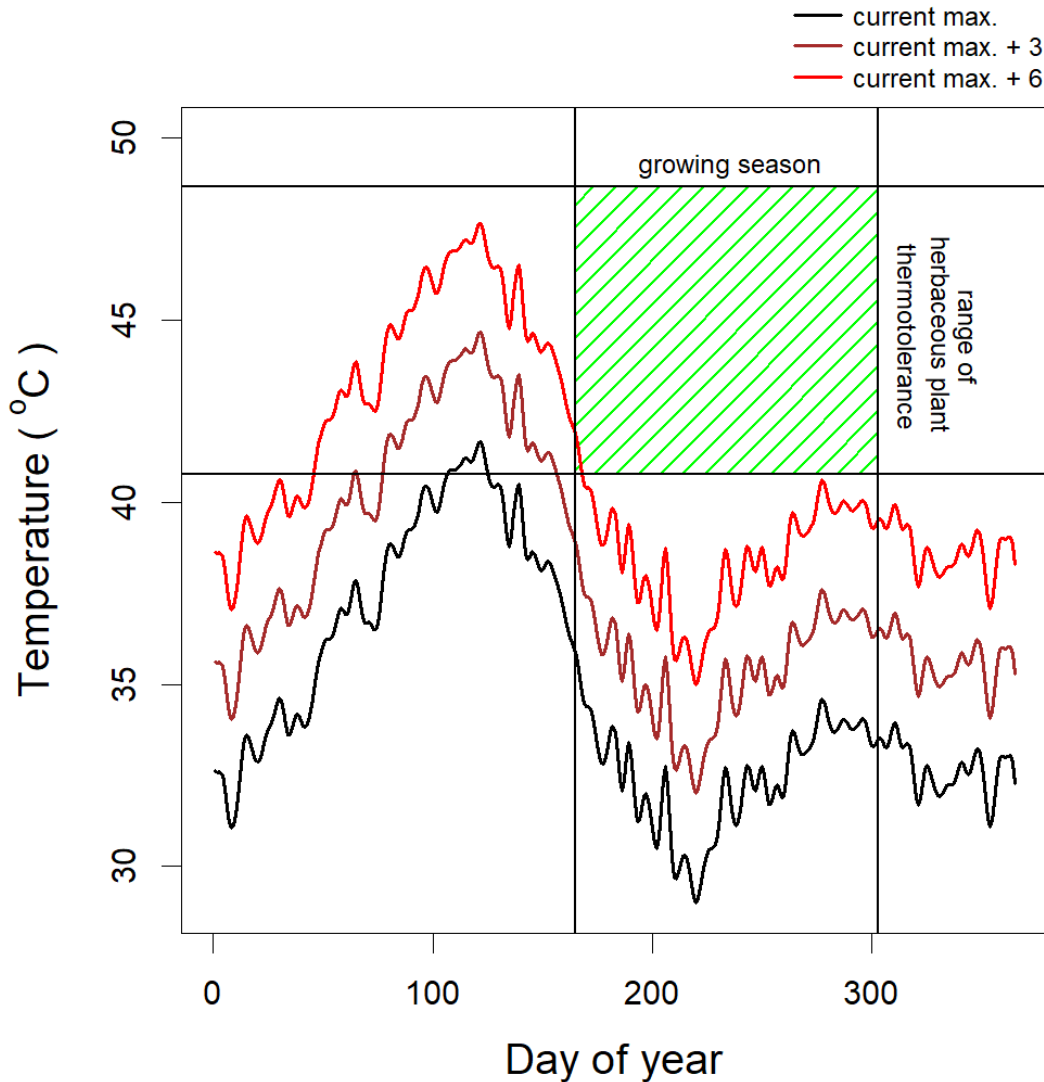




**Figure 3:** Relationship of  $T_{50}$ , with a) leaf mass per area (LMA) and b) leaf dry matter content (LDMC). Each data point represents a species, with its colour representing the PFT to which that species belongs. AF – annual forbs, PF – perennial forbs, AG – annual graminoids, PG – perennial graminoids, G – geophytes. Number of species in each group is as follows: AF – 20, PF – 9, AG – 13, PG – 7, G – 6.



**Figure 4:** Relationship of  $T_{50}$ , with a) leaf mass per area (LMA) and b) leaf dry matter content (LDMC) for different functional types. SMA regressions used for fitting slopes. Each data point represents a species, with its colour representing the PFT to which that species belongs. AF – annual forbs, PF – perennial forbs, AG – annual graminoids. Number of species in each group is as follows: AF – 20, PF – 9, AG – 13.



**Figure 5:** Comparison of herbaceous plant thermotolerance with current and future habitat temperature. Absolute maximum temperatures recorded in Pune during the 2004 – 2014 period is used as an estimate of habitat temperature. Future temperatures calculated from the predictions of Zelazowski et al. (2011). The shaded region shows the overlap between the range of  $T_{50}$  values observed in this study and the growing season of sampled species. Overlap between the shaded region and the future habitat temperature curve (current max. + 6) indicates that future maximum temperatures can be higher than the  $T_{50}$  values of some species.

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## Appendix

**Table S1:** List of sampled species. A/P refers to annual/perennial. Site abbreviations: B – Bhimashankar, C – Chalkewadi, V – Vetal. Habitat abbreviations: T – terrestrial, Aq – aquatic, SAq – semi aquatic. Reference abbreviations: 1 - Rahangdale & Rahangdale, 2017; 2 - Joshi & Kumbhojkar, 1997; 3 - Nerlekar & Kulkarni, 2015; 4 – floras, correspondence with Prena Agarwal (lab member); 5 –personal observations of Ashish Nerlekar (former lab member).

S. No.	Species	N	Site	PFT	A/P	Habitat	Ref.
1	<i>Adelocaryum coelestinum</i> (Lindl.) Brand	5	B	forb	P	T	1
2	<i>Apluda mutica</i> L.	5	V	graminoid	P	T	2
3	<i>Arthraxon lanceolatus</i> (Roxb.) Hochst.	5	V	graminoid	A	T	3
4	<i>Arthraxon</i> sp.	5	C	graminoid	A	T	4
5	<i>Blainvillea acmella</i> (L.) Philipson	5	V	forb	A	T	2
6	<i>Blumea lacera</i> (Burm.f.) DC.	5	B	forb	A	T	1
7	<i>Boerhavia diffusa</i> L.	5	V	forb	P	T	2
8	<i>Bothriochloa pertusa</i> (L.) A.Camus	5	V	graminoid	P	T	2
9	<i>Caesulia axillaris</i> Roxb.	5	V	forb	A	Aq/SAq	2
10	<i>Canscora diffusa</i> (Vahl) R.Br. ex Roem. & Schult	5	C	forb	A	T	4
11	<i>Cajanus</i> sp.	5	V	forb	P	T	5
12	<i>Chrysopogon fulvus</i> (Spreng.) Chiov.	5	V	graminoid	P	T	2
13	<i>Chlorophytum tuberosum</i> (Roxb.) Baker	6	V	geophyte	P	T	2
14	<i>Cyanotis fasciculata</i> (B.Heyne ex Roth) Schult. & Schult.f.	5	V	forb	A	T	2
15	<i>Cyperus</i> sp.	5	V	graminoid	P	SAq	2
16	<i>Digitaria ciliaris</i> (Retz.) Koeler	5	V	graminoid	A	T	2
17	<i>Drimia indica</i> (Roxb.) Jessop	5	V	geophyte	P	T	2
18	<i>Eleusine indica</i> (L.) Gaertn.	5	V	graminoid	A	T	2
19	<i>Eriocaulon sedgwickii</i> Fyson	6	C	forb	A	SAq	4
20	<i>Eragrostis uniolooides</i> (Retz.) Nees ex Steud.	5	V	graminoid	A	T	5
21	<i>Euphorbia heterophylla</i> L.	5	V	forb	A	T	2
22	<i>Evolvulus alsinoides</i> (L.) L.	5	V	forb	P	T	2
23	<i>Glyphochloa forficulata</i> (C.E.C. Fisch.) Clayton	5	B	graminoid	A	T	1
24	<i>Hackelochloa granularis</i> (L.) Kuntze	6	V	graminoid	A	T	2

**Table S1**, continued.

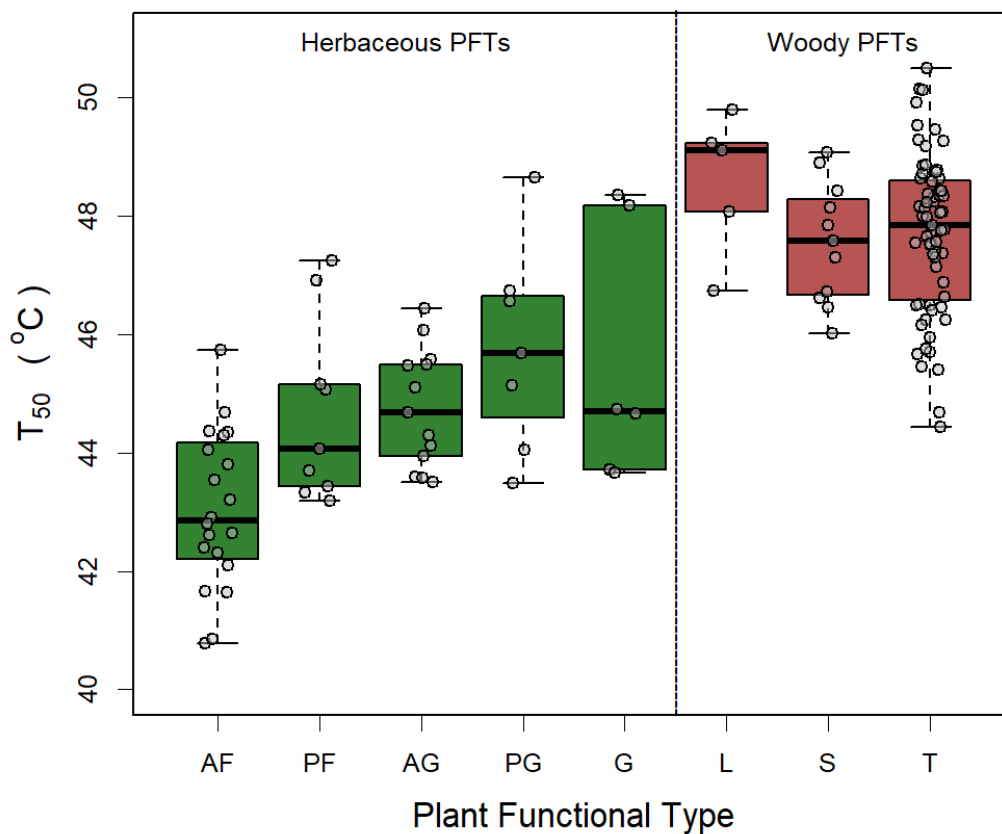
<b>S. No.</b>	<b>Species</b>	<b>N</b>	<b>Site</b>	<b>PFT</b>	<b>A/P</b>	<b>Habitat</b>	<b>Ref.</b>
25	<i>Habenaria heyneana</i> Lindl.	5	C	geophyte	P	T	4
26	<i>Heteropogon contortus</i> (L.) P.Beauv. ex Roem. & Schult.	5	V	graminoid	P	T	2
27	<i>Impatiens lawii</i> Hook.f. & Thomson	5	C	forb	A	T	4
28	<i>Impatiens oppositifolia</i> L.	3	B	forb	A	T	1
29	<i>Impatiens tomentosa</i> B.Heyne	5	C	forb	A	T	4
30	<i>Indigofera cordifolia</i> Roth	5	V	forb	A	T	2
31	<i>Indigofera dalzellii</i> T.Cooke	5	C	geophyte	P	T	4
32	<i>Ipomoea carnea</i> Jacq.	5	V	forb	P	Aq/SAq	2
33	<i>Isachne lisboae</i> Hook.f.	5	C	graminoid	A	T	4
34	<i>Jansenella griffithiana</i> (C.Muell.) Bor	5	C	graminoid	A	T	4
35	<i>Justicia prostrata</i> Schltld. ex Nees	5	V	forb	P	T	2
36	<i>Lepidagathis cristata</i> Willd.	5	V	forb	P	T	2
37	<i>Melanocenchris jacquemontii</i> Jaub. & Spach	5	V	graminoid	A	T	2
38	<i>Murdannia lanuginosa</i> (Wall. ex C.B.Clarke) G.Brückn.	5	C	geophyte	P	T	4
39	<i>Murdannia simplex</i> (Vahl) Brenan	5	C	forb	P	T	4
40	<i>Neanotis lancifolia</i> (Hook.f.) W.H.Lewis	5	C	forb	A	T	4
41	<i>Nymphoides cristata</i> (Roxb.) Kuntze	5	V	forb	P	Aq	2
42	<i>Paspalum canarae</i> (Steud.) Veldkamp	5	C	graminoid	A	T	4
43	<i>Peristylus densus</i> (Lindl.) Santapau & Kapadia	5	C	geophyte	P	T	4
44	<i>Polytrias indica</i> (Houtt.) Veldkamp	8	C	graminoid	A	T	4
45	<i>Pulicaria wightiana</i> (DC.) C.B.Clarke	6	V	forb	A	T	2
46	<i>Rotala mexicana</i> Schltld. & Cham.	5	C	forb	A	SAq	4
47	<i>Senecio bombayensis</i> N.P. Balakr.	3	B	forb	A	T	1
48	<i>Senna tora</i> (L.) Roxb.	5	V	forb	A	T	2
49	<i>Setaria pumila</i> (Poir.) Roem. & Schult.	5	V	graminoid	A	T	2
50	<i>Smithia hirsuta</i> Dalzell	5	C	forb	A	T	4
51	<i>Spermacoce pusilla</i> Wall.	5	V	forb	A	T	2
52	<i>Trichodesma indicum</i> (L.) Lehm.	5	V	forb	A	T	2
53	<i>Tripogon jacquemontii</i> Stapf	5	V	graminoid	P	T	2
54	<i>Typha domingensis</i> Pers.	5	V	graminoid	P	Aq/SAq	2
55	<i>Zornia gibbosa</i> Span.	5	V	forb	A	T	2

**Table S2:** Effect of presence/absence of woody tissues on the relationship between thermotolerance and leaf mass per area (LMA). Results of an ANCOVA examining the equality of slopes of LMA-T<sub>50</sub> relationships of herbaceous and woody species. H/W refers to the effect of whether a species is herbaceous or woody. P-values marked with an asterisk (\*) are significant ( $\alpha = 0.05$ ).

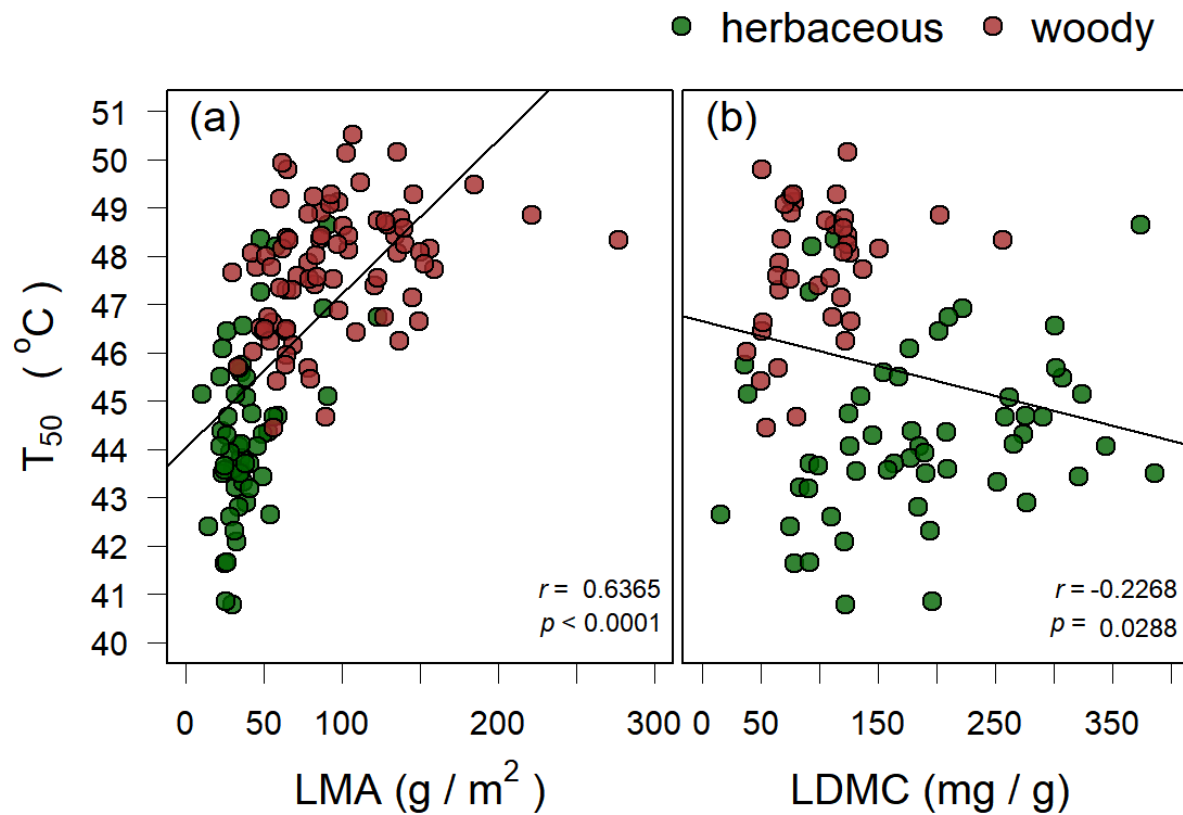
Source	dF	SS	MS	F	p
LMA	1	273.61	273.61	144.57	<0.0001*
H/W	1	137.48	137.48	72.663	<0.0001*
LMA x H/W	1	18.13	18.13	9.579	0.0024*
error	130	246.06	1.89		

**Table S3:** Effect of presence/absence of woody tissues on the relationship between thermotolerance and leaf dry matter content (LDMC). Results of ANCOVAs testing for a) equality of slopes and b) equality of intercepts of LDMC-T<sub>50</sub> relationships of herbaceous and woody plants. H/W refers to the effect of whether a species is herbaceous or woody. P-values marked with an asterisk (\*) are significant ( $\alpha = 0.05$ ).

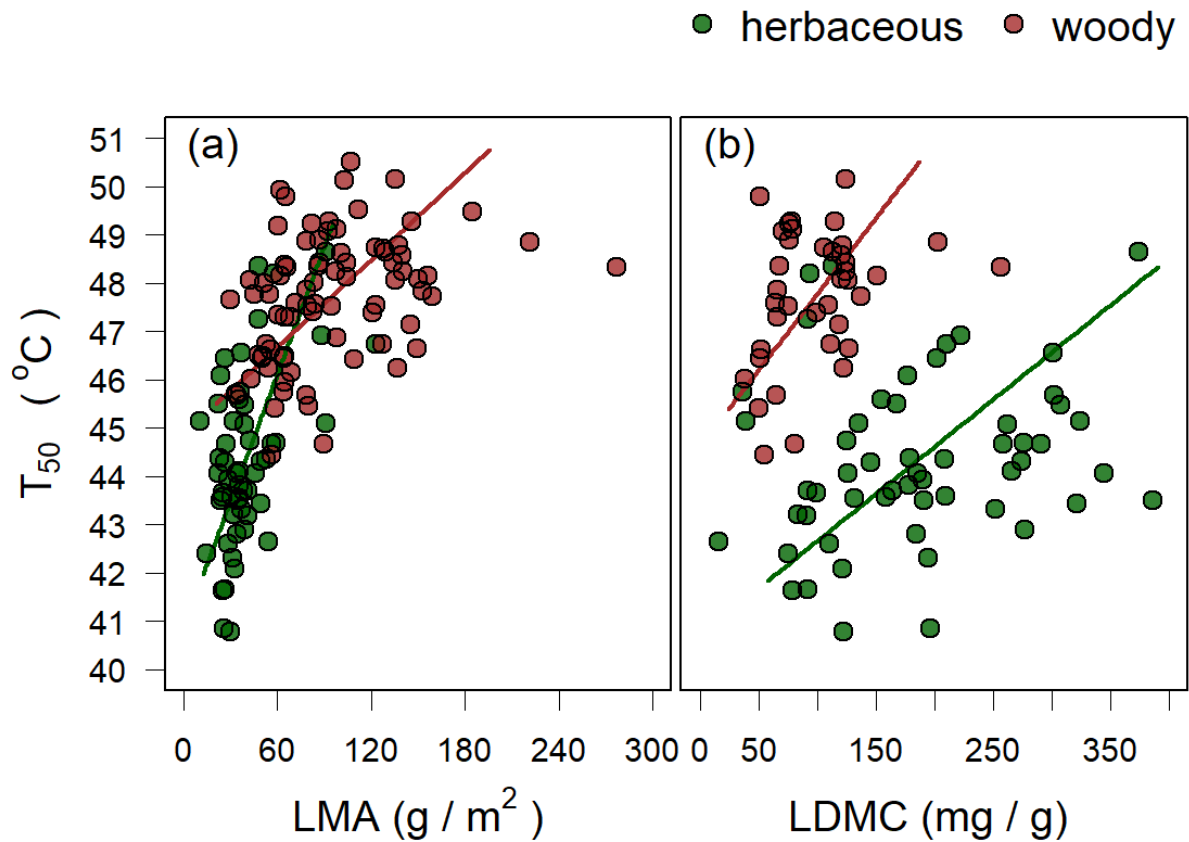
Source	dF	SS	MS	F	p
<b>a) equality of slopes</b>					
LDMC	1	25.69	25.69	10.316	0.0018*
H/W	1	250.37	250.37	100.538	<0.0001*
LDMC x H/W	1	1.91	1.91	0.766	0.3837
Error	89	221.64	2.49		
<b>b) equality of intercepts</b>					
LDMC	1	3.13	3.135	1.792	0.1884
H/W	1	22.54	22.541	12.882	0.0009*
error	39	68.24	1.75		



**Figure S1:** Variation in thermotolerance with woody and herbaceous plant functional types. Each boxplot represents the distribution of  $T_{50}$  values for the respective PFT. Colour of each boxplot represents whether the respective PFT is herbaceous or woody. AF – annual forbs, PF – perennial forbs, AG – annual graminoids, PG – perennial graminoids, G – geophytes, L – lianas, S – shrubs, T – trees. Sample sizes are as follows: AF – 20, PF – 9, AG – 13, PG – 7, G – 6, L – 6, S – 11, T – 63.



**Figure S2:** Relationship between leaf functional traits and thermotolerance. Data for herbaceous and woody species has been pooled. Each data point represents a species, with its colour representing whether the respective species is herbaceous or woody. a) LMA -  $T_{50}$  relationship and b) LDMC -  $T_{50}$  relationship. Sample sizes are as follows: a) herbaceous – 55, woody – 79, b) herbaceous – 55, woody – 38.



**Figure S3:** Relationship between leaf functional traits and thermotolerance for herbaceous species and woody species. SMA regressions used for fitting slopes. Each data point represents a species, with its colour representing whether the respective species is herbaceous or woody. a) LMA -  $T_{50}$  relationship and b) LDMC -  $T_{50}$  relationship. Sample sizes are as follows: a) herbaceous – 55, woody – 79, b) herbaceous – 55, woody – 38.