Differences in leaf thermoregulation and water-use strategies between three co-occurring Atlantic forest tree species

Running title: Leaf energy balance of Atlantic forest trees

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Summary Statement

In the first study of leaf energy balance in tropical montane forests, we observed current leaf temperature patterns in the Atlantic forest, Brazil, and assessed whether
and why patterns may vary among species. We found large leaf-to-air temperature differences that were influenced strongly by radiation, and differences in leaf temperature between two species due to variation in leaf width and stomatal conductance. We highlight the importance of leaf functional traits for leaf thermoregulation, and also note that the high radiation levels which occur in montane forests may exacerbate the threat from increasing air temperatures.

Abstract

Given anticipated climate changes, it is crucial to understand controls on leaf temperatures including variation between species in diverse ecosystems. In the first study of leaf energy balance in tropical montane forests, we observed current leaf temperature patterns on three tree species in the Atlantic forest, Brazil, over a 10-day period, and assessed whether and why patterns may vary among species. We found large leaf-to-air temperature differences (maximum 18.3°C) and high leaf temperatures (over 35°C) despite much lower air temperatures (maximum 22°C). Leaf-to-air temperature differences were influenced strongly by radiation, while leaf temperatures were also influenced by air temperature. Leaf energy balance modelling informed by our measurements showed that observed differences in leaf temperature between two species were due to variation in leaf width and stomatal conductance. The results suggest a trade-off between water-use and leaf thermoregulation; *Miconia cabussu* has more conservative water-use compared to *Alchornea triplinervia* due to lower transpiration under high vapour pressure deficit, with the consequence of higher leaf temperatures under thermal stress conditions. We highlight the importance of leaf functional traits for leaf thermoregulation, and also note that the high radiation levels
which occur in montane forests may exacerbate the threat from increasing air

temperatures.

**Key-words:** leaf temperature, tropical forest, montane, leaf width, stomatal

conductance, transpiration, radiation, functional traits, boundary layer, energy

balance.

Introduction

The surface temperature of the Earth is increasing (Rahmstorf, Foster, & Cahill, 2017)

and set to continue increasing into the future (Collins *et al.*, 2013). The majority of

tropical forests show a trend of increasing air temperature over the past 35 years

which is particularly strong in South America with recent increases up to 0.5°C per

decade (Supplementary Figure. S1). Temperature extremes are also increasing and are

predicted to increase in the coming century (Coumou & Robinson, 2013). Whilst it is

known that temperature influences plant functioning, the response of plants to

increasing temperature and variation between species is a major uncertainty (Teskey

*et al.*, 2015). Tropical forests are particularly important in this regard as they are a

considerable store of terrestrial carbon (Pan *et al.*, 2011), potentially already function

near their maximum temperature (Doughty & Goulden, 2008) and given their location

cannot be replaced by species from lower latitudes. The biodiversity of tropical

montane forests, which house many endemic species with restricted ranges, may be at

particular risk from higher temperatures due to limits on upslope migration, especially

for tree species in forests occurring on mountaintops (Phillips, 1997). Modelling

studies suggest increasing temperatures are also likely to have a negative effect on
tropical forest vegetation carbon, however the extent of projected impact varies
greatly between different models (Galbraith et al., 2010; Huntingford et al., 2013) as
do the physiological mechanisms behind the declines (Galbraith et al., 2010).

Temperature can impact plant physiology both directly, by influencing rates of
photosynthesis and respiration, and indirectly by altering the ambient vapour pressure
deficit ($D$) (Lloyd & Farquhar, 2008). $D$ increases with an increase in air temperature
($T_a$) if relative humidity ($h$) stays constant, and stomatal conductance ($g_s$) typically
decreases with increasing $D$ (Leuning, 1995) to avoid excessive water loss. The
reduction in $g_s$ with $D$ has the consequence of reduced CO$_2$ concentration within the
leaf. The photosynthetic optima of plants are typically near the mean maximum
ambient temperature, showing the acclimation of plants to their environment (Slot &
Winter, 2017). The temperature of the leaf tissue itself is relevant temperature for the
control of leaf physiological processes, rather than $T_a$. High leaf temperatures ($T_L$) can
induce damage to photosynthetic machinery; above c. 35°C thylakoid membranes
have been observed to structurally change (Gounaris, Brain, Quinn, & Williams,
1983; Gounaris, Brain, Quinn, & Williams, 1984) and above 40°C photosystem II
(PSII) may become deactivated and the electron transport rate reduced (Allakhverdiev
et al., 2008). Chlorophyll fluorescence parameters to assess heat tolerance of PSII
show critical temperature thresholds in the region of 45 – 60 °C, with significant
variation between species (O’Sullivan et al., 2017; Sastry & Barua, 2017).
Irreversible thermal damage to photosynthetic machinery has been observed to occur
at 52 °C in a tropical species (Krause et al., 2010).
Extremes of microclimate can lead to leaf temperatures that are markedly different from $T_a$. For example, leaf temperatures up to 10 °C above air temperatures when leaves were brightly lit have been observed in the Amazon (Doughty & Goulden 2008) and in Panama (Rey-Sanchez, Slot, Posada, & Kitajima, 2016), and Slot, Garcia, & Winter, (2016) found leaf temperatures of a *Ficus insipida* regularly exceeded 40°C and even reached 48 °C during a three week period in Panama. Yet, despite these striking patterns of leaf temperatures, and the on-going and anticipated increases in air temperatures, there are few datasets examining fluctuations of leaf temperatures *in situ* in tropical forests, and, to our knowledge, none in tropical montane forests.

Leaf energy balance theory can be used to address the drivers of $T_L$ in a mechanistic approach (e.g. Michaletz *et al.* 2016). Developed from the Penman energy balance approach to evapotranspiration (Penman, 1948), the leaf energy balance equation (see Materials and Methods, equation 2) estimates the leaf-to-air temperature difference ($\Delta T$) for given microclimatic and leaf-specific variables (Jones, 1992). The leaf energy balance shows that $\Delta T$ is dependent on the net energy provided (or lost) by radiation and the energy lost through transpiration. The effects of these fluxes on $\Delta T$ depend on leaf shape and physiology through the boundary layer and stomatal resistances to water transport. Stomatal resistance is dependent on stomatal activity and boundary layer resistance increases with leaf width (see Materials and Methods). Hence, whilst $\Delta T$ is strongly influenced by microclimatic conditions (in particular radiation and $D$), leaf traits (width and stomatal conductance) can also play a role in regulation of leaf temperature. In addition, leaves can alter their physical position
through changes in angle and/or orientation to increase or decrease the amount of radiation received.

Leaf structural traits (leaf mass per area (LMA) and leaf dry matter content (LDMC)) and stomatal conductance ($g_s$) influence the time required for leaf temperature to change following a change in the environment (the thermal time constant ($\tau$), Jones, 1992). Leaves with a long $\tau$ will show smaller temperature changes in a fluctuating environment, maintaining the leaf temperature closer to the mean air temperature than a leaf with a small $\tau$ which will track fluctuation in air temperature (Michaletz et al., 2015).

Given the diversity of leaf structures and physiology observed within and among tropical forest species (e.g. variation in leaf(let) area over 5 orders of magnitude for a large sample of tropical species, Wright et al., 2017), it is possible that there will be diversity in leaf strategies with regard to temperature (Michaletz et al., 2015). This means that the impacts of potential future environmental changes may vary between species even within a single biome. Future combined atmospheric changes of increasing CO$_2$ and increasing $T_A$ could be particularly important for $T_L$, as plants tend to respond to increasing CO$_2$ by reducing $g_s$ (Way, Oren, & Kroner, 2015). Reducing $g_s$ decreases water use, but also has the consequence of increasing leaf temperature (Drake, González-Meler, & Long, 1997; Barker et al., 2005) and can lead to premature leaf senescence under heat-wave conditions (Warren, Norby, & Wullschleger, 2011). Increases in $T_A$ could be particularly important under fluctuating and extreme conditions (e.g. heat-waves), increasing the occurrence of leaves reaching or exceeding threshold temperatures resulting in leaf damage.
We present an observational study of leaf temperatures in a highly threatened tropical forest region – the Atlantic forest, among the most diverse and threatened of biodiversity hotspots (Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000; Colombo & Joly, 2010). Our mountaintop study site is home to many endemic species. Humans have exploited the Atlantic forest for 500 years resulting in a highly fragmented landscape (Joly, Metzger, & Tabarelli, 2014) that reduces possibilities for species migration. Hence, a greater understanding of forests in this region is of great interest given their high threat level. We focus here on determining and understanding interspecific differences in leaf temperatures caused by differences in leaf traits. Our approach aims to begin to reveal whether or not trees are likely to be able to cope with future conditions, and the extent to which species identity is likely to be important. This is a step towards an understanding of the resilience of tropical trees and is part of a broader effort to assess the effects of stressors on remaining Atlantic forests and their ability to recover.

We used a narrow canopy tower to access leaves of three trees each of different species (*Alchornea triplinervia* (Spreng.) Mull. Arg. (Euphorbiaceae), *Miconia cabussu* Hoehne (Melastomataceae) and *Guapira opposita* (Vell.) Reitz (Nyctaginaceae), hereafter referred to by genus only). We monitored leaf temperature and microclimate relevant to leaf energy balance over a period of 10 days, and quantified the stomatal behaviour and structural leaf traits of the sample trees. With this dataset we aim to answer the following questions:

i) What are the current patterns of leaf temperature of the Atlantic forest species *Alchornea, Miconia* and *Guapira* under fluctuating microclimatic conditions?
ii) Are there differences in leaf thermoregulation between the species?

iii) To what extent do leaf traits (width, stomatal conductance) and microclimate (radiation, $T_A$, $D$, wind speed) determine leaf temperatures?

**Materials and Methods**

**Study Site**

The field study was carried out in the Serra do Mar State Park, São Paulo state, Brazil. The park is home to the largest contiguous patch of Atlantic forest remaining, running along a steep coastal mountain range. The study site (23.3254 S, 45.0938 W) is located within a 1 ha permanent plot at 1000 m elevation. The vegetation is mid-successional secondary forest, regenerating from clear felling for charcoal before the establishment of the park in 1977 (Marchiori, Rocha, Tamashiro, & Aidar, 2016). The forest is classified as montane moist dense forest (Vieira *et al.*, 2011), mean annual precipitation is 2300 mm with a dry season in July and August, mean annual temperature is 17°C (Joly *et al.*, 2012) and fog occurs frequently (Rosado, Oliveira, & Aidar, 2010). Canopy height of emergent trees reaches 30 m. Data collection was carried out between 1 – 10 October 2016.

**Microclimate measurements**

A narrow 27 m high tower was used for access to the canopy and microclimate measurements ($T_A$, photosynthetically active radiation (PAR), relative humidity ($h$) and wind speed ($U$)) were collected to detail the microclimate vertical profile (Fig. 1). As the tower is just 30 cm wide and tree branches are within arms reach of the tower (see Supplementary Figure S2d), we consider that the presence of the tower likely has
only minimal influence on the microclimate of the sampled leaves. From 18 m above
the ground, at the height of the highest leaves adjacent to the tower, 16 PAR sensors
were suspended from the tower at c. 1 m intervals, with an additional sensor
positioned at 25 m above the ground. Sensors were made following Fielder &
Comeau (2000) using gallium arsine phosphide (GaAsP) photodiodes (G1118,
Hamamatsu, Japan) and calibrated against a LI-COR 190 quantum sensor (LI-COR
Inc., Nebraska, USA). PAR sensors were positioned on plastic supports in the
horizontal plane. In addition, 7 thermistors (107, Campbell Scientific, Utah, USA) to
measure $T_a$ were deployed in radiation screens spread through the vertical profile
(heights 1.5, 5, 7.5, 10, 12.5, 15 and 18 m, Fig. 2). PAR and $T_a$ data were measured
and recorded at 10 s intervals using two CR800 data loggers with AM 16/32
multiplexers (Campbell Scientific, Utah, USA). Four data-logging $h$ sensors (RHT10,
Extech, Massachusetts, USA) measured and recorded at 1 min intervals at heights 2,
8, 12.5 and 18 m. Four sonic anemometers (Sonicwind) measured $U$ every 0.5 s at
heights of 1.5, 6.5, 11.5 and 25 m, and 10 s averages were produced for each height.
$U$ for leaves positioned above 11.5 m height was linearly interpolated between the 25
m and 11.5 m measurement.

Vapour pressure deficit ($D$) was calculated from $T_a$ and $h$ (Campbell & Norman,
1998),

\[
D = e_{sat} \cdot (1 - h) \tag{1}
\]

\[
e_{sat} = a \cdot \exp\left(\frac{b T_a}{T_a + c}\right) \tag{2}
\]
where $T_A$ is in °C, $h$ is relative humidity (as a proportion), $e_{sat}$ is saturating vapour pressure in kPa and $a$, $b$ and $c$ are constants ($a = 0.611$, $b = 17.502$, $c = 240.97$).

Due to a sensor fault, $h$ was available only from 5 – 10 October 2016. To estimate $D$ within the profile for measurement days prior to this, we estimated $h$ within the profile based on the observed relationship between $T_A$ and $h$ at the four measurement heights ($R^2 0.76 – 0.87$) from the available data collected over six days.

**Sampled species**

Both *Alchornea* and *Miconia* are overstorey species, while *Guapira* is found in the subcanopy (Guilherme, Morellato, & Assis, 2004), and the species are ranked second, fifth and sixth, respectively, in terms of abundance in the plot (Marchiori et al., 2016). All species are early successional (Marchiori et al., 2016) and are found in nearby old-growth forest. *Alchornea* and *Guapira* are present at lower elevations in the park (Joly et al., 2012; Rosado, Oliveira, Joly, Aidar, & Burgess, 2012). The species cover a range of leaf sizes; *Guapira* has the smallest leaves (notophyll), followed by *Alchornea* (mesophyll), with the largest leaves for *Miconia* (platyphyll).

**Leaf temperature measurements**

To measure leaf-to-air temperature differences ($\Delta T$, also called ‘leaf temperature excess’ in the literature) we followed the two-junction thermocouple design of Singsaas & Sharkey (1998). This approach has the advantage of more accurately measuring $\Delta T$ than performing measurements of absolute $T_L$ and $T_A$ separately. Two long (15 cm length) constantan fine wires (0.07 mm diameter) were soldered to either end of a short (3 cm length) copper fine wire (0.07 mm diameter). These
thermocouples produce a voltage proportional to the temperature difference between
the junctions. Thermocouples were individually calibrated by inserting one junction
into sand in a temperature controlled dry bath (TCBD-02, Cleaver Scientific, UK)
with the second junction in the air c. 2 cm above the bath. The temperature of the bath
and the air were each measured with two thermistors. Four different temperature
differences between the bath and air were produced (~0, 5, 10 and 12°C). Data for the
calibration were selected during periods with a constant dry bath temperature (i.e.
excluding periods when the bath was heating up or cooling down).

Between 30 September and 3 October 2016 thermocouples were installed on 10
Alchornea leaves, 9 Miconia leaves and 4 Guapira leaves. Selected leaves were fully
expanded and mature, but not senescent, within reach from the canopy tower, and
spread through the vertical profile (Fig. 1). The thermocouple junction to measure leaf
temperature was secured to the abaxial mesophyll surface (avoiding any large veins)
near to the midrib using surgical tape (Transpore, 3M, Minnesota, USA). The second
junction was suspended in the air c. 2 cm below the leaf. Additional cabling was
cable-tied to a twig near to the leaf (or the petiole in the case of Miconia) and to the
tower. This attachment procedure enabled the majority of thermocouples to remain
attached to leaves during wind and rain (see Supplementary Figure S2 for
photographs of the equipment installation). The petioles of two leaves, both of
Miconia, snapped during the monitoring period. Supplementary Table S1 gives details
of the sampled leaves. $\Delta T$ was measured and recorded at 10 s intervals using a CR800
data logger with AM 16/32 multiplexer (Campbell Scientific, Utah, USA) until 11
October 2016.
Spot measurements

In addition to continuous measurements of $\Delta T$ from the thermocouples, instantaneous spot measurements were made of leaf temperature using an infra-red (IR) thermometer (62MAX+, Fluke, Washington, USA) on the adaxial and abaxial leaf surfaces, PAR at the adaxial leaf surface (accounting for leaf angle and orientation) with a quantum sensor (LightScout, Spectrum Technologies, Illinois, USA) and $g_s$ using a porometer (SC-1, Decagon Devices, Washington, USA). These measurements were made in order to i) validate the thermocouple data against an independent $T_L$ measurement, ii) compare PAR received at the leaf surface with that measured from the tower, and iii) investigate variation in $g_s$ between species and over time. Spot measurements were collected during daylight hours throughout the day on 6 days between 4 – 10 October 2016. Stomatal conductance measurements could only be performed when the leaf surface was dry. Hence, fewer spot measurements of $g_s$ were collected (213 in total, on average $c.2$ measurements per leaf per day) compared with leaf temperature on adaxial surface (785 in total, on average $c.6$ measurements per leaf per day), leaf temperature on abaxial surface (398 in total, on average $c.3$ measurements per leaf per day) and PAR (350 in total, on average $c.3$ measurements per leaf per day). Measurements of $g_s$ with the SC-1 porometer are completed in 30 s, and as the response of stomata to a change in the environment is on the order of minutes (e.g. Vialet-Chabrand et al., 2017) we assume that the leaf $g_s$ will not have changed due to the altered microclimate of the porometer chamber within the measurement interval. Leaf angle (angle to the horizontal) was measured sporadically (minimum 5 measurements per leaf) using a clinometer (Suunto, Finland). No spot measurements were carried out during the night.
Leaf trait measurements

All sampled leaves were collected and stored in moist plastic bags for 24 hours before fully rehydrating and measuring structural traits in the lab at the Instituto de Botânica, São Paulo. Measurements were performed of leaf thickness (mm) with a digital calliper, leaf area (cm$^2$) with leaf area meter (LI-3100, LI-COR, Nebraska, USA), leaf mass (g), leaf width (cm), and leaf length (cm). Petioles were removed before measurements. Subsequently, leaves were dried at 70 °C, and dry weight measured. These measurements were used to calculate leaf mass per area (LMA, g m$^{-2}$) and leaf dry matter content (LDMC, g g$^{-1}$). For Guapira the sample size for leaf traits was six (rather than four as for leaf temperature).

Leaf Energy Balance

With input of measured microclimate, stomatal conductance and leaf width the leaf energy balance (equation 3, Jones, 1992) can be estimated to predict the leaf-to-air temperature difference ($\Delta T_e$, °C). It is important to note that the leaf energy balance assumes no leaf heat storage and that the leaf energy balance is considered to be in a steady state. $\Delta T_e$ was estimated from spot measurements to test if $\Delta T_e$ matched observations of $\Delta T$ when leaf surface PAR and $g_s$ were directly measured, and from the continuous microclimate data with $g_s$ estimated from the observed species-specific relationships between $g_s$ and $D$ in order to assess the influence of microclimate and leaf specific variables on leaf temperatures using a large dataset. As the Guapira leaves were not exposed to a large range of microclimates due their position in the understorey, we only consider $\Delta T_e$ of Miconia and Alchornea in the latter analysis.

\[
\Delta T_e = T_L - T_A = \frac{r_{b,HR}(r_{b,W}+r_{L,W})r_{R,li}}{\rho_a c_p a T_a (r_{b,W}+r_{L,W}) + \frac{r_{b,HR}D}{T_a(r_{b,W}+r_{L,W})+\frac{r_{b,HR}}{ST_{b,HR}}}} - \frac{r_{b,HR}D}{T_a(r_{b,W}+r_{L,W})+\frac{r_{b,HR}}{ST_{b,HR}}} \tag{3}
\]
where $T_L$ and $T_A$ are the leaf and air temperatures respectively ($^\circ$C), $R_{ni}$ is the net isotropic radiation (W m$^{-2}$, assuming the sky temperature is equal to $T_A$ measured at the nearest $T_A$ sensor to the leaf and sky emissivity of 0.97), $\gamma$ is the psychrometric constant (Pa K$^{-1}$), $r_{b,HR}$ is the boundary layer resistance to heat and radiation and $r_{b,W}$ and $r_{l,W}$ are the boundary layer and leaf resistances to water respectively (all resistances in s m$^{-1}$), $\rho_a$ is the density of dry air (kg m$^{-3}$), $c_{pa}$ is the specific heat capacity of dry air (1012 J kg$^{-1}$ K$^{-1}$), $s$ is the slope of relationship between temperature and saturated vapour pressure evaluated at $T_A$, and $D$ is the vapour pressure deficit (Pa).

Leaf traits ($g_s$ and leaf width) are included in equation 3 through the leaf and boundary layer resistances. Leaf resistance to water, $r_{l,W}$, is the inverse of $g_s$, Boundary layer conductance to heat or water, $g_{b,H}$, which is included in the determination of both $r_{b,HR}$ and $r_{b,W}$ which are both used in equation 3, is dependent on leaf width ($W$, m) and wind speed ($U$, m s$^{-1}$)

$$g_{b,H} = 0.0105\left(\frac{U}{W}\right)^{0.5}$$ (4)

Further details on the estimation of leaf energy balance are given in Appendix 1.

The thermal time constant ($\tau$, s) was defined following Michaletz et al. (2016) as

$$\tau = \varphi \cdot LMA \cdot \left(\frac{c_{pw}}{LDMC \cdot H} + \frac{c_{pd} - c_{pw}}{H}\right)$$ (5)
where $\phi$, the ratio of projected to total leaf area, is 0.5 for flat leaves, LMA is in units kg m$^{-2}$, $c_{pw}$ is the specific heat capacity of water (4181 J kg$^{-1}$ K$^{-1}$), $c_{pd}$ is the specific heat capacity of dry leaf matter (J kg$^{-1}$ K$^{-1}$). $c_{pd}$ varies by species and here we use 2814 J kg$^{-1}$ K$^{-1}$, the mean of seven tropical tree species from Jayalakshmy & Philip, (2010). $H$ is a heat transfer coefficient (W m$^{-2}$ K$^{-1}$) accounting for convection, radiation and transpiration (Michaletz et al., 2016)

$$H = \rho_\alpha c_{pa}(g_{b,H} + g_{b,R} + g_s s/\gamma)$$  \hspace{1cm} (6)

where $g_{b,H}$ and $g_{b,R}$ are the boundary layer conductance to heat and radiation respectively (both m s$^{-1}$, see Appendix 1). $\tau$ varies over time due to its dependence on $g_s$ and boundary layer resistance, and was estimated from spot measurements.

Leaf boundary layer resistance

Initial estimations of the leaf energy balance using equation 3 showed that when $\Delta T_e$ was evaluated at low wind speeds ($< 0.5$ m s$^{-1}$) the values were overestimated compared to the observed $\Delta T$. Using equations 4 and S5 to estimate the boundary layer resistance to water ($r_{b,w}$), there is a steep increase in $r_{b,w}$ below wind speeds of 0.5 m s$^{-1}$ (Supplementary Figure S3). To test if these high resistances were supported by the data, we solved the leaf energy balance equation for $r_{b,w}$ and estimated $r_{b,w}$ using the observations of $\Delta T$ (see Appendix 2). Plotted against wind speed, the estimated $r_{b,w}$ was lower than predicted by equations 4 and S5 at low wind speeds (Supplementary Figure S3). Hence, we re-parameterised constants from equation 4 using the $r_{b,w}$ estimated from the leaf energy balance and observed wind speed and leaf width (see Appendix 2). Parameter estimation was performed separately for
Miconia and Alchornea (there was not sufficient data for parameter estimation of Guapira) using non-linear least squares (R function nls).

In order to have accurate estimates of $r_{b,w}$ from the energy balance, it is essential that all microclimate inputs are correct. PAR was measured at various points from the tower (Fig. 1). Examination of the spot measurement data showed that PAR measured by the nearest sensor suspended from the tower (maximum 1 m distance from leaf) occasionally strongly overestimated or underestimated leaf surface PAR (Supplementary Figure S4) as they are not measured at precisely the same location, angle or orientation, and PAR shows high spatial variability. To select only data where PAR measured from the tower appropriately represented PAR at the leaf surface, the daytime data was split into twenty minute periods and $\Delta T_e$ estimated for every 10 s datapoint. Linear regression was then used to identify periods where $\Delta T_e$ matched measured $\Delta T$, selecting only periods where the slope of the relationship between $\Delta T_e$ and $\Delta T$ was $1 \pm 0.3$ and the intercept was $\pm 2 \, ^\circ C$. Based on this selection procedure we identified 20% of the dataset (c. 150,000 data points) considered to have representative PAR measurements. This approach does not entirely eliminate noise from the dataset as within the 20 min period there can still be some erroneous data-points.

Data Analysis

Linear mixed effects models with leaf as a random factor were used for all statistical analyses including repeated measurements of the same leaf using the R package nlme (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2017). $R^2$ for mixed effects models are given using either the marginal-pseudo-$R^2$ that accounts for fixed factors...
only or conditional pseudo-$R^2$ (Nakagawa & Schielzeth, 2013). The marginal-pseudo-$R^2$ is used unless otherwise stated, and $R^2$ values were calculated using the function provided in the R package MuMIn (Bartoń, 2016). Statistical analyses comparing between species using single values for each leaf used ANOVA for three species comparisons and t-test for two species comparisons.

Relationships between $g_s$ and $D$ were analysed for each species using a linear mixed effects model with leaf as a random factor. The relationships produced were used to estimate a time-series of $g_s$ for each leaf based on $D$. The intercept of the $g_s \sim D$ relationship was thus leaf specific, and the slope species specific.

To compare leaf temperatures under comparable microclimate conditions data was first selected for 20 min periods where $\Delta T_e$ matched measured $\Delta T$ to ensure microclimate variables are representative of the leaf surface, as for *Leaf boundary layer resistance* (see above) but using the species-level parameterization of $r_{bl,W}$ to estimate $\Delta T_e$. The selected dataset was then subsetted according the microclimate (PAR, $T_A$ and $U$) for each leaf. We produced subsets of $\Delta T$ under low PAR and $T_A$ ($\text{PAR} 50-150\,\text{μmol m}^{-2}\text{s}^{-1}, T_A 13-15\,\text{°C}$), medium PAR and $T_A$ ($\text{PAR} 50-150\,\text{μmol m}^{-2}\text{s}^{-1}, T_A 18-20\,\text{°C}$), and very high PAR ($\text{PAR} 1600-1900\,\text{μmol m}^{-2}\text{s}^{-1}, T_A 18-20\,\text{°C}$), all at wind speed $0.5-1.5\,\text{m s}^{-1}$. Differences in $\Delta T$ between species for each microclimate were evaluated with linear mixed effects models with leaf as a random factor.

**Results**
Validation of thermocouple data

$T_L$ based on $\Delta T$ measured with thermocouples and $T_A$ measured with thermistors ($T_L = \Delta T + T_A$) was highly correlated with $T_L$ as measured by the IR thermometer (Pearson’s correlation coefficient for each leaf 0.60 - 0.99, where $T_L$ was measured with IR thermometer on the abaxial leaf surface). The slopes of linear regression lines forced through zero were significantly different from 1 for only three leaves, where the thermocouples slightly underestimated $T_L$ by up to 9 % (Supplementary Figure S5).

Overall, the close agreement between the two measurement methods gives confidence in the thermocouple data.

Microclimate during the monitoring period

Microclimate during the monitoring period is shown in Supplementary Figure S6 and for a single sunny day in Fig. 2. The first seven days (1 – 7 Oct) were predominately overcast with low PAR, high $h$ and low $D$, with some sunny periods on 6 and 7 Oct. Subsequently, two days (8 and 9 Oct) had longer bright periods. The final day of data collection (10 Oct) was again overcast. Throughout the period lower canopy levels received substantially less PAR and experienced lower $D$. However, on sunny days, high PAR levels and higher $D$ extended throughout the vertical profile (e.g. 8 October 2016, Fig. 2). Mean daytime $T_A$ at the top of the canopy was 15.0°C, with a maximum $T_A$ of 22.1°C recorded on 8 October 2016. Mean nighttime $T_A$ was 12.4°C, and was lowest preceding sunny days. Mean windspeed above the canopy (at 25 m) was 1.0 ± 0.7 m s$^{-1}$, and 0.26 ± 0.18 m s$^{-1}$ within the canopy (averaged over all sample heights 1.5, 6.5 and 11.5 m).

Patterns of leaf temperature
Leaves occasionally reached much higher temperatures than the ambient air, over 10 °C above $T_A$. The maximum $T_L$ for each leaf observed over the monitoring period ranged from 22.5-37.2 °C and was above 35 °C for 5 of the 23 leaves. *Miconia* leaves had significantly higher maximum temperatures than *Alchornea* leaves (Table 1). Daytime mean $T_L$ showed less variation than maximum $T_L$ between leaves and species (Table 1, Supplementary Figure S7). Leaves of *Guapira*, which were all at the bottom of the canopy, had lower maximum and mean $T_L$ (not tested for significance due to low sample size). Distributions of $T_L$ show positive skew (Supplementary Figure S7) which was significantly higher for leaves of *Miconia* than *Alchornea* (Table 1), showing that they reached high temperatures more often than *Alchornea*. During daylight hours $\Delta T$ was positive for leaves of *Alchornea* and *Miconia*, and was close to zero for all *Guapira* leaves (Supplementary Figure S8, Table 1). The maximum $\Delta T$ observed was 18.3 °C, again recorded from *Miconia* leaf M1. As for $T_L$ the distributions of $\Delta T$ were positively skewed, with significantly higher skewness for *Miconia* than *Alchornea* (Table 1). Minimum daytime $\Delta T$ was significantly lower for *Alchornea* than *Miconia* leaves (Table 1). Night-time $\Delta T$ was typically negative but close to zero, and was significantly lower for *Alchornea* than *Miconia* (Table 1).

$T_A$ set a rough minimum bound on $T_L$ (Fig. 3), with many excursions above $T_A$ due to high radiation (see section *Leaf Energy Balance*) and a small number of excursions below $T_A$, likely occurring when leaf surfaces were wet during/after rain or in fog. $T_L$ excursions above $T_A$ occurred more often for leaves positioned higher in the canopy.

*Leaf temperatures under specific microclimates*
We compared leaf temperatures under specific microclimatic conditions. Under low light and temperature conditions leaves of *Guapira* were significantly colder than those of *Alchornea* and *Miconia* (Fig. 4a); there was no significant difference in $\Delta T$ between the latter two species. Under medium light and temperature conditions there again was no significant difference in $\Delta T$ between *Alchornea* and *Miconia* (Fig. 4b); *Guapira* leaves did not experience these or brighter light conditions due to their position in the understory. Under high light and temperature conditions $\Delta T$ was significantly higher for *Miconia* than *Alchornea* (Fig. 4c). Under the highest light conditions analysed $\Delta T$ was again higher for *Miconia* than *Alchornea*, however the difference was not quite significant (Fig. 4d).

**Thermal trait variation between species**

Stomatal conductance ($g_s$) significantly declined with increasing $D$, and the relation varied significantly between species (Fig. 5, Table 2). At low $D$, $g_s$ was highest for *Miconia* and lowest for *Guapira*. *Miconia* showed a significantly stronger negative relationship between $g_s$ and $D$ than *Alchornea*, hence at higher values of $D$, *Miconia* leaves had lower $g_s$. Conditional $R^2$ for the overall mixed model including the random factor for leaf was 0.49.

Structural leaf traits with importance for thermoregulation also varied between species (Table S1, Fig. 6). *Miconia* leaves were significantly wider, larger and had higher LMA than both *Alchornea* and *Guapira* (Fig. 6a-c). LDMC significantly differed between all species and was highest for *Miconia* ($0.42 \pm 0.013 \text{ g g}^{-1}$) followed by *Alchornea* ($0.37 \pm 0.016 \text{ g g}^{-1}$) and finally *Guapira* ($0.20 \pm 0.022 \text{ g g}^{-1}$) (ANOVA $F = 38.8$, $p < 0.0001$, and Tukey post-hoc test).
The thermal time constant (τ) ranged over two orders of magnitude from 9 - 350 s (Fig. 7a) and varied significantly between species (linear mixed effects model, F = 48.1, df = 20, p < 0.0001). τ for Guapira were significantly longer and more varied (mean ± sd 155.4 ± 84.0) than both Alchornea (mean ± sd 276.5 ± 11.1) and Miconia (mean ± sd 46.4 ± 14.4). τ decreased with increasing gs and was particularly high under very low gs (Fig. 7b). For a given gs, τ increased in the order Alchornea < Miconia < Guapira (Figure 7b). These differences were driven by the leaf structural traits LMA and LDMC (Supplementary Figure S9). When estimated using a fixed LMA value the differences between Alchornea and Miconia are lost (Supplementary Figure S9b) showing that the higher LMA of Miconia increases τ. When estimated using a fixed LDMC value the Guapira values collapse into line with Alchornea (the two species have similar LMA) (Supplementary Figure S9c) showing that the lower LDMC of Guapira increases τ.

Leaf energy balance and drivers of ΔT and T_L

Leaf-to-air temperature difference estimated from leaf energy balance (ΔT_L) using the spot measurements matched observed ΔT well, but with some underestimation at higher ΔT (Fig. 8), showing that our data adequately parameterized the leaf energy balance for instances when leaf surface PAR and gs were measured. To investigate the drivers of ΔT with the larger dataset of continuous ΔT and microclimate measurements, the dataset was restricted to periods where predicted ΔT_L matched observed ΔT, as for the analysis of ΔT under specific microclimate conditions. This is to ensure we are using appropriate values of PAR which was not measured at the leaf surface in the continuous dataset. Both observed ΔT and ΔT_L increase with PAR (Fig. 8).
a pattern repeated when $T_L$ and $T_{Le}$ (leaf temperature estimated from energy balance) were analysed (Supplementary Figure S13). The slope of the relationship between leaf temperature variables and PAR were different between *Miconia* and *Alchornea*, where *Miconia* has higher $AT$ and $T_L$ for a given PAR (Fig. 10, Supplementary Figure S13). Whilst the absolute values of $AT_e$ and $T_{Le}$ are somewhat higher than the observations, the differences between the species are maintained in the energy balance estimations. Relationships between $AT$ and $AT_e$ and other microclimate variables ($T_A$, $D$, $U$) were much weaker than for PAR with all $R^2$ values below 0.3 (Supplementary Figures S10-S12), while $T_L$ and $T_{Le}$ were strongly related to $T_A$ and $D$ with $R^2$ values above 0.7 (Fig. S14-S16).

To determine what causes the differences between $AT$ of *Miconia* and *Alchornea*, we applied traits (leaf width, stomatal conductance strategy) of *Miconia* sequentially to *Alchornea* and re-estimated $AT_e$ using the observed microclimate data. As shown in Supplementary Figure 17, applying the larger leaf width of *Miconia* acts to increase the *Alchornea* $AT_e$ for a given PAR, almost to the extent that it matches the high $AT_e$ of *Miconia*. If the higher intercept of the $g_s$–$D$ relationship for *Miconia* is applied, the *Alchornea* $AT_e$ for a given PAR decreases. In contrast, if the steeper $g_s$–$D$ slope for *Miconia* is applied, the *Alchornea* $AT_e$ for a given PAR increases. The effect is not as strong as the effect of leaf width (Supplementary Figure 17). Applying both the *Miconia* intercept and slope results in an intermediate *Alchornea* $AT_e$ for a given PAR, slightly higher than for *Alchornea* with its original parameterization. If all *Miconia* traits are applied (leaf width and stomatal conductance strategy) *Alchornea* $AT_e$ for a given PAR increases to a greater extent than for any trait alone, and even exceeds the $AT_e$ of *Miconia*. This is likely due to the higher $D$ that the highest six
Alchornea leaves are exposed to due to their position above the Miconia leaves (Fig. 1).

**Discussion**

*Current leaf temperature patterns and the role of microclimate*

Despite the low $T_A$ (maximum 22 °C) occurring during the study period we observed leaf temperatures over 30 °C. Whilst few datasets are available on field-measured leaf temperatures of tropical trees, the maximum $\Delta T$ we observed (18.3 °C) is somewhat higher than those previously reported (e.g. 13.9 °C for a range of Proteaceae species in Australia (Leigh, Sevanto, Close, & Nicotra, 2017), 10 °C for five species in Panama (Rey-Sánchez et al., 2016) and in the Amazon (Doughty & Goulden, 2008), 7 °C for three species in tropical China (Dong, Prentice, Harrison, Song, & Zhang, 2017)). This could be due to high sampling frequency used in this study (every 10 s) compared to others (every 2 minutes in Rey-Sánchez et al., 2016, half hourly in Dong et al., 2017 and unspecified in Leigh et al., 2017) as at high frequency extreme values are more likely to be recorded, though Doughty & Goulden (2008) used a 1 s sampling frequency. It could also be due to the light conditions and study species measured (discussed below). The highest $T_L$ observed (37.2 °C) is less than those reported by others under higher ambient air temperatures (e.g. 45 °C - Doughty & Goulden, 2008; 48 °C – Slot et al., 2016, Krause et al., 2010). This work supports the view that ambient air temperatures cannot necessarily be used as a proxy for leaf temperature in physiological models as they are not necessarily equal (Michaletz et
al., 2016; Rey-Sánchez et al., 2016), and that vegetation models should be tested for
their ability to reproduce patterns of ΔT (Dong et al., 2017).

The distributions of $T_L$ and ΔT collected over the ten-day period were significantly
skewed (Supplementary Fig. S7, S8, Table 2). This is because under the predominant
microclimatic conditions of relatively low PAR and $D$, ΔT is low (< 1 °C) and $T_L$ is
similar to $T_A$. However, due to fluctuating conditions – especially PAR which alters
rapidly with cloud movements and wind, and varies with sun angle, leaf angle and
orientation - large increases in ΔT occur. The duration of high ΔT excursions depends
on how long the microclimate is sustained. The extent of high ΔT excursions is
important because during high leaf temperatures beyond the photosynthetic
temperature optima primary productivity will reduce carbon gain and very high leaf
temperatures can cause irreversible thermal damage (e.g. above 50 – 53 °C for a
Panamanian tree species, Krause et al., 2010). Our data suggest that, at least during
our measurement period, tree leaves at this Atlantic forest site are not approaching
thresholds of irreversible damage, but do reach temperatures known to affect
thylakoid membrane structure (35 °C, Gounaris et al., 1983; Gounaris et al., 1984)
and reduce electron transport rates (40 °C, Allakhverdiev et al., 2008) although
critical temperatures of PSII activity are known to vary among species (O’Sullivan et
al., 2017; Sastry & Barua, 2017). While we do not know the photosynthetic
temperature optima of these trees, it is likely that the higher leaf temperatures reached
were supra-optimal for photosynthesis despite the low air temperatures.

Within the range of conditions during the study period, radiation was the most
important microclimate variable for determining ΔT (Fig. 9, Supplementary Fig. S10-
This has also been shown in other studies (e.g. Doughty & Goulden, 2008; Rey-Sánchez et al., 2016) and is understood biophysically (Jones, 1992). For absolute $T_L$, PAR, $T_A$, and $D$ were all important (Supplementary Fig. S13-S16), though the strength of the $D$ effect is likely due at least in part to its co-variation with $T_A$. Recent work has shown the occurrence of a ‘crossover’ $T_A$ at 25-28 °C (Michaletz et al., 2016, Dong et al., 2017). The crossover temperature is the $T_A$ at which $\Delta T=0$ and above which $\Delta T$ is negative. We found no evidence of a crossover $T_A$, likely due to the relatively low $T_A$ during our study. The light levels observed in the study were high, occasionally exceeding 3000 μmol m$^{-2}$ s$^{-1}$. We consider the light levels recorded in the profile to be accurate as they were highly comparable to an independent dataset from a weather station mounted at 27 m on the same tower (Figure S18). The values are higher than the PAR observed in similar studies from lowland forests with typical maximum PAR of 2000 μmol m$^{-2}$ s$^{-1}$ (Rey-Sánchez et al., 2016; Doughty & Goulden, 2008). Again, measurement frequency may be important here for recording extreme instantaneous values. In fact, this maximum quantity of PAR is equivalent to more radiation than the solar constant (incoming light at the top of the atmosphere, 1.353 kW m$^{-2}$) which is possible in mountains when light is reflected from clouds (Stoutjesdijk & Barkman, 2014). Incoming radiation increases by 8 % for every 1000 m increase in elevation (Blumthaler, Ambach, & Ellinger, 1997). Montane forests are therefore likely to experience higher maximum radiation loads than lowland forest, as has been measured at this site (Rosado, Joly, Burgess, Oliveira, & Aidar, 2016). Given the importance of radiation for $T_L$, trees at high elevation may have greater risk of hitting damaging $T_L$ thresholds if air temperatures increase with climate change. At this specific site, in additional to increased radiation, $D$ also increases with elevation and trees show more conservative water use (Rosado et al., 2016) which will further
influence leaf temperatures. Mountaintop species are already considered to be more
greatly threatened than lowland species by increased temperatures as there is no
cooler place for species to move to. The high radiation load increasing leaf
temperatures may exacerbate this problem.

As microclimate is a key driver of leaf temperature, it is important to consider the
vertical gradient in microclimate (Supplementary Figure S6). We found that all
microclimate variables displayed vertical gradients, especially during sunny days
when the differences between the top and bottom of the canopy exceeded 5 °C $T_A$,
2200 μmol m$^{-2}$ s$^{-1}$ PAR and 1.3 kPa $D$. The difference in $T_A$ leads to a larger
difference in $T_L$ than the values of $\Delta T$ we typically found (Table 1). Whilst vertical
gradients of PAR are often accounted for in vegetation models, often the gradients of
other key variables are not considered, which would lead to error in quantification of
leaf temperatures below the canopy top.

Differences in leaf thermoregulation between species

We found striking differences in leaf temperature patterns between species that were
attributable to differing leaf traits. *Miconia* leaves more commonly experienced high
$\Delta T$ excursions than *Alchornea*, with higher skew in $T_L$ and $\Delta T$ distributions, higher
maximum $\Delta T$ and lower minimum $\Delta T$ (Table 1). Leaf temperatures of *Miconia* were
consistently higher than *Alchornea* when controlling for microclimate between
measurements, and significantly so during high light conditions (Fig. 4). The
differences increased with increasing thermal stress (higher PAR, $T_A$ and $D$). The lack
of significance at the highest PAR/$T_A$ subset tested is likely due to low data
availability and higher PAR measurement errors at high PAR. As PAR was not measured directly at the leaf surface it was difficult to ensure correspondence between PAR as measured by the nearest sensor and received at the leaf surface; this is more problematic under direct light conditions where leaf angle, orientation, sun angle and within canopy shading greatly impact leaf surface PAR. We recommend all studies of leaf temperature attempt to measure PAR at the leaf surface despite the higher efforts required.

The higher leaf temperatures displayed by *Miconia* can be accounted for by lower transpirational cooling due to two reasons. Firstly, the wider leaf width increases boundary layer resistance, which lowers the evaporation from stomatal pores. Secondly, *Miconia* leaves showed a strong negative relationship between $g_s$ and $D$ which lowers transpiration under conditions of high thermal stress (as high $D$ typically occurs concurrently with high PAR and $T_A$). Using the leaf energy balance equation we find that the physical difference in leaf width is the dominant factor in producing the variation in $\Delta T$ between *Miconia* and *Alchornea* (Supplementary Figure. 17). *Miconia* leaves get hotter than *Alchornea* leaves and hence may have a higher risk of thermal damage. However, this increased heating may come with a water-use advantage, as, under high $D$ conditions, transpiration rates per leaf area will be lower for *Miconia* than *Alchornea*. This could reduce the risk of xylem cavitation under water stress conditions. Differing thermoregulation strategies of trees likely arise in combination with trade-offs in terms of water use.

The study species only showed differing relationships between PAR, and $T_L$ and $\Delta T$, with similar responses to other microclimatic variables (Fig. 9, Supplementary
Figures S10-S16). This shows that it is the consequences for input solar energy that varies between the species, rather than differing mechanisms in response to $T_A$. It is not to say that other microclimatic variables are not important for $T_L$ or $\Delta T$, but that the response of $T_L$ and $\Delta T$ to other variables is the same for the two species, at least under the measurement conditions.

Night-time $\Delta T$ were consistently negative for all species. However, $\Delta T$ of *Alchornea* leaves were more negative than the other species (Table 1). The cause may be that many of the sampled *Alchornea* leaves were at the outer canopy and therefore heat radiation to space may be more effective for them due to the lack of obstacles (other leaves or canopies), resulting in greater cooling. Another factor may be that transpiration is maintained at night in this species more so than *Miconia* and *Guapira*. Observations from Rosado *et al.* (2012) do show night-time transpiration occurring for *Alchornea* trees at this site, but *Alchornea* did not show higher transpiration than other measured species.

Leaf temperatures of the subcanopy *Guapira* tree were consistently similar to air temperatures and showed little variation (Table 1) likely due to the canopy position receiving very little light (Fig. 1). However, when the data were subsetted for low PAR conditions only, leaves of *Guapira* still showed a lower $\Delta T$ than the two other species (Fig. 4a). This could be due to the narrower leaf width of *Guapira* leaves (Fig. 6), though the width is not significantly different from *Alchornea*. It could also be due to the unusual leaf angles displayed by the *Guapira* leaves that were hanging near vertically (Table S1, Figure 6e) which would limit the amount of light received and result in over estimates of the light environment from using a horizontally orientated
sensor. Another potential contributor is the long $\tau$ values estimated, as $T_L$ is expected to vary less when $\tau$ is long (Ball, Cowan, & Farquhar, 1988). The long $\tau$ for Guapira leaves were a result of the combined low $g_s$ and low LDMC (Fig. 7, Supplementary Figure S9); because water has a higher specific heat capacity than dry leaf matter, the higher water content of Guapira leaves causes a longer $\tau$ (Vogel, 2009).

Towards a better understanding of tropical leaf temperature behaviour

The link between functional traits and leaf thermoregulation has been highlighted in recent work (Michaletz et al., 2015; 2016). Here, we provide field-based evidence for this link in the most detailed study of leaf energy balance in tropical montane forests to date, and include variation in water-use as a key component. The traits that we find important (leaf width, $g_s$ at high $D$, LDMC) may possibly connect other axes of plant functional variation (Reich, 2014) – the leaf economics spectrum (Wright et al., 2004) and plant hydraulics. Species that are able to maintain transpiration under high thermal stress conditions (high $T_A$, PAR, $D$) will require water to supply the transpiration stream from an efficient hydraulic system or from high water capacitance to avoid hydraulic failure. Avoiding extremes of $T_L$ and maintaining open stomata will then have the benefit of keeping $T_L$ closer to the temperature optima of photosynthesis, maintaining a CO$_2$ supply, and all this while PAR is high to drive a high photosynthetic rate (Ball et al., 1988). Conversely, lower transpiration under high thermal stress conditions will prevent excessive water loss and therefore avoid risk of hydraulic failure through xylem embolism, but increase risk of the leaf reaching a damaging high temperature threshold. Critical thresholds of photosynthetic activity vary by species (O’Sullivan et al., 2017). A recent study of critical thresholds
of 41 co-occurring tropical species found that variation was related to the leaf economics spectrum (Wright et al., 2004), with high LMA species showing higher temperature tolerance (Sastry & Barau, 2017). *Miconia* has significantly higher LMA than *Alchornea* (Fig. 6), and it would be parsimonious if it also displays a higher critical temperature for damage to photosynthetic machinery. In summary, we hypothesise that trees at the ‘slow’ end of the life-history spectrum (Reich, 2014) are likely to reach higher leaf temperatures, have lower $g_s$ and photosynthesis under high thermal stress conditions, lower risk of hydraulic failure, and a higher threshold for thermal damage, with the converse true of ‘fast’ species.

If we are to understand the implications of climate change for tropical forests it will be crucial to understand mechanisms of leaf thermoregulation and how this varies between species. We have based our findings on only a small, if detailed, dataset. There are very few comparable datasets available for tropical forests. More datasets exploring the full energy balance of tropical leaves from multiple sites with varying climatologies, and ideally over extended time periods, would certainly aid this. Beyond understanding current patterns of leaf temperatures, it is also necessary to understand the response of energy balance parameters to high $T_A$ and CO$_2$. For example, herbarium data for an Australian shrub species showed a reduction in leaf width over the last century (Guerin, Wen, & Lowe, 2012) which could mitigate increases in $T_L$ due to increased $T_A$. Conversely, declines in $g_s$ are a common response of tree species to increased CO$_2$ which, while potentially reducing water-use, could lead to higher $T_L$ (e.g. Barker et al., 2005; Warren et al., 2011). However, the extent of reductions in $g_s$ under elevated CO$_2$ varies with species (Way et al., 2015). In a study of seedlings of 10 tropical species, Cernusak et al. (2011) found reductions in $g_s$
in all species in response to elevated CO₂, but the reductions were larger for species
with high gs in ambient conditions. Warming may also cause changes in gs; results
from warming experiments show a variety of responses – increases, decreases, no
change (Way et al. 2015) - and a recent meta-analysis found decreases in stomatal
density with higher TA in trees but not in herbs (Yan, Zhong, & Shangguan, 2017). If
trees do indeed decrease gs under higher growth temperatures this could result in
further leaf warming beyond TA increases, but only if transpiration declines as well as
gs, which is not certain given the expected rise in D with increased TA. Our
understanding of the effects of combined CO₂ and warming is even more limited. If
both cause a decline in gs separately, would the combined effect be additive leading to
even greater reductions? The limited experimental data does not paint a clear picture
(Way et al., 2015). A final question is whether leaves that reach higher temperatures
are better adapted to cope with high temperatures and therefore increasing TL would
be less consequential than for low temperature species, or does the fact that leaf
temperatures are already high mean that high-temperature species are more at risk?

Conclusions

In this study we made detailed measurements of leaf energy balance for three tree
species in the montane Atlantic forest, Brazil. Our results show surprising high leaf-
to-air temperature differences given the relatively low air temperatures, which we
attribute to the high light conditions during the study. The higher radiation levels
occurring at high elevations may contribute to the risks of climate change to tropical
montane forests. We find differences in leaf thermoregulation between leaves of
Alchornea and Miconia, which is attributable to lower transpiration under high
thermal stress conditions for Miconia due to its wider leaves and stronger reduction of
$g_s$ with increasing $D$. Leaf energy balance modelling can be a powerful tool to understand variation between species in leaf thermoregulation which will be necessary to model the impact of climate change on leaf physiology.

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Table 1. Leaf temperature variables for three species. n = number of leaves measured for each tree.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Alchornea (n=10)</th>
<th>Miconia (n=9)</th>
<th>Guapira (n=4)</th>
<th>p*</th>
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<tbody>
<tr>
<td>Daytime minimum T_L (°C)</td>
<td>7.71 ± 0.63</td>
<td>8.51 ± 1.55</td>
<td>8.13 ± 0.59</td>
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<tr>
<td>Daytime mean T_L (°C)</td>
<td>15.98 ± 0.47</td>
<td>16.14 ± 0.72</td>
<td>14.6 ± 0.01</td>
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<td>Daytime maximum T_L (°C)</td>
<td>30.56 ± 3.6</td>
<td>34.63 ± 2.63</td>
<td>23.33 ± 1.22</td>
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<td>Daytime T_L skewness</td>
<td>0.84 ± 0.43</td>
<td>1.38 ± 0.27</td>
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<tr>
<td>Daytime minimum ΔT (°C)</td>
<td>-3.72 ± 1.36</td>
<td>-2.16 ± 0.75</td>
<td>-5.07 ± 2.50</td>
<td>0.007</td>
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<tr>
<td>Daytime mean ΔT (°C)</td>
<td>0.79 ± 0.40</td>
<td>0.84 ± 0.43</td>
<td>0.007 ± 0.001</td>
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<td>Daytime maximum ΔT (°C)</td>
<td>11.27 ± 4.15</td>
<td>14.23 ± 2.71</td>
<td>3.28 ± 1.08</td>
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<tr>
<td>Daytime ΔT skewness</td>
<td>2.51 ± 1.32</td>
<td>4.56 ± 1.63</td>
<td>0.73 ± 5.86</td>
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<tr>
<td>Night-time mean ΔT (°C)</td>
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<td>-0.06 ± 0.03</td>
<td>-0.008 ± 0.01</td>
<td>0.02</td>
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* p-value from t-tests comparing Alchornea and Miconia.
Table 2. ANOVA table for the linear mixed effects model of $D$, species, and their interaction on $g_s$. Leaf is included as random intercept.

<table>
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<tr>
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<tr>
<td>$D$</td>
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<td>$D$:Species</td>
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Figure legends

Figure 1. Schematic of field data collection showing positions of microclimate measurements and leaves sampled for temperature.

Figure 2. Time-series of microclimate and leaf-to-air temperature difference on 8 October 2016. a) Photosynthetically active radiation, b) air temperature, c) vapour pressure deficit, d) leaf-to-air temperature difference for leaves of *Alchornea* (A6), *Miconia* (M1) and *Guapira* (G3). Colours refer to measurement heights.

Figure 3. Leaf ($T_L$) and air ($T_A$) temperatures measured over 10 days for a) Alchornea (10 leaves), b) Miconia (10 leaves) and c) Guapira (4 leaves). Colour denotes leaf height. Grey line – $y=x$. Each data point is single measure of a single leaf taken from the thermocouple time series recorded every 10 s.

Figure 4. Leaf to air temperature difference ($\Delta T$) variation between species and microclimatic conditions. Vertical lines show the mean value for the species. Significance values are shown testing for differences between species (linear mixed effects model with leaf as a random factor) under four different microclimates. PAR – photosynthetically active radiation, $T_A$ – air temperature.

Figure 5. Relationship between stomatal conductance and vapour pressure deficit, and variation between species. Equations for each species – Alchornea: $g_s = 325.1 \pm 22.4 - 68.8 \pm 22.6 D$; Guapira: $g_s = 185.4 \pm 36.4 - 212.7 \pm 22.6 D$; Miconia: $g_s = 401.6 \pm 31.0 - 189.8 \pm 35.4 D$ (errors are SE).
Figure 6. Variation in leaf structural traits between species. Letters represent significantly different groups calculated using ANOVA and Tukey post-hoc tests.

Figure 7. Distributions of thermal time constants estimated for each species (a), and the relationship between stomatal conductance and the thermal time constant (b).

Figure 8. Leaf to air temperature difference ($\Delta T$) from observations and energy balance estimations. Grey dash – $y=x$ line, grey solid – linear regression line for all data, regression slope = 0.72 ± 0.03 SE, intercept -0.41 ± 0.07 SE, $F = 725.8$, df = 185, $p < 0.0001$, $R^2 = 0.80$.

Figure 9. Relationships between PAR and observed $\Delta T$ (a, b) and estimated $\Delta T_e$ (c, d) for *Alchornea* (a, c) and *Miconia* (b, d). Solid line – modelled relationship for the plotted species, dash line – modelled relationship for the alternative species. Statistical models are linear mixed effects model with leaf as a random factor. $R^2$ is the marginal pseudo $R^2$. To account for uneven sampling with respect to PAR data was subsampled for 1000 points in 250 $\mu$mol m$^{-2}$ s$^{-1}$ bins for points below 1000 $\mu$mol m$^{-2}$ s$^{-1}$.