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

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

3

4 Running title: *Leaf energy balance of Atlantic forest trees*

5

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22

23 **Summary Statement**

24 In the first study of leaf energy balance in tropical montane forests, we observed

25 current leaf temperature patterns in the Atlantic forest, Brazil, and assessed whether

26 and why patterns may vary among species. We found large leaf-to-air temperature
27 differences that were influenced strongly by radiation, and differences in leaf
28 temperature between two species due to variation in leaf width and stomatal
29 conductance. We highlight the importance of leaf functional traits for leaf
30 thermoregulation, and also note that the high radiation levels which occur in montane
31 forests may exacerbate the threat from increasing air temperatures.

32

33 **Abstract**

34

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

51 which occur in montane forests may exacerbate the threat from increasing air
52 temperatures.

53

54 **Key-words:** leaf temperature, tropical forest, montane, leaf width, stomatal
55 conductance, transpiration, radiation, functional traits, boundary layer, energy
56 balance.

57

58 **Introduction**

59

60 The surface temperature of the Earth is increasing (Rahmstorf, Foster, & Cahill, 2017)
61 and set to continue increasing into the future (Collins *et al.*, 2013). The majority of
62 tropical forests show a trend of increasing air temperature over the past 35 years
63 which is particularly strong in South America with recent increases up to 0.5°C per
64 decade (Supplementary Figure. S1). Temperature extremes are also increasing and are
65 predicted to increase in the coming century (Coumou & Robinson, 2013). Whilst it is
66 known that temperature influences plant functioning, the response of plants to
67 increasing temperature and variation between species is a major uncertainty (Teskey
68 *et al.*, 2015). Tropical forests are particularly important in this regard as they are a
69 considerable store of terrestrial carbon (Pan *et al.*, 2011), potentially already function
70 near their maximum temperature (Doughty & Goulden, 2008) and given their location
71 cannot be replaced by species from lower latitudes. The biodiversity of tropical
72 montane forests, which house many endemic species with restricted ranges, may be at
73 particular risk from higher temperatures due to limits on upslope migration, especially
74 for tree species in forests occurring on mountaintops (Phillips, 1997). Modelling
75 studies suggest increasing temperatures are also likely to have a negative effect on

76 tropical forest vegetation carbon, however the extent of projected impact varies
77 greatly between different models (Galbraith *et al.*, 2010; Huntingford *et al.*, 2013) as
78 do the physiological mechanisms behind the declines (Galbraith *et al.*, 2010).
79
80 Temperature can impact plant physiology both directly, by influencing rates of
81 photosynthesis and respiration, and indirectly by altering the ambient vapour pressure
82 deficit (D) (Lloyd & Farquhar, 2008). D increases with an increase in air temperature
83 (T_A) if relative humidity (h) stays constant, and stomatal conductance (g_s) typically
84 declines with increasing D (Leuning, 1995) to avoid excessive water loss. The
85 reduction in g_s with D has the consequence of reduced CO_2 concentration within the
86 leaf. The photosynthetic optima of plants are typically near the mean maximum
87 ambient temperature, showing the acclimation of plants to their environment (Slot &
88 Winter, 2017). The temperature of the leaf tissue itself is relevant temperature for the
89 control of leaf physiological processes, rather than T_A . High leaf temperatures (T_L) can
90 induce damage to photosynthetic machinery; above *c.* 35°C thylakoid membranes
91 have been observed to structurally change (Gounaris, Brain, Quinn, & Williams,
92 1983; Gounaris, Brain, Quinn, & Williams, 1984) and above 40°C photosystem II
93 (PSII) may become deactivated and the electron transport rate reduced (Allakhverdiev
94 *et al.*, 2008). Chlorophyll fluorescence parameters to assess heat tolerance of PSII
95 show critical temperature thresholds in the region of 45 – 60 °C, with significant
96 variation between species (O’Sullivan *et al.*, 2017; Sastry & Barua, 2017).
97 Irreversible thermal damage to photosynthetic machinery has been observed to occur
98 at 52 °C in a tropical species (Krause *et al.*, 2010).
99

100 Extremes of microclimate can lead to leaf temperatures that are markedly different
101 from T_A . For example, leaf temperatures up to 10 °C above air temperatures when
102 leaves were brightly lit have been observed in the Amazon (Doughty & Goulden
103 2008) and in Panama (Rey-Sanchez, Slot, Posada, & Kitajima, 2016), and Slot,
104 Garcia, & Winter, (2016) found leaf temperatures of a *Ficus insipida* regularly
105 exceeded 40°C and even reached 48 °C during a three week period in Panama. Yet,
106 despite these striking patterns of leaf temperatures, and the on-going and anticipated
107 increases in air temperatures, there are few datasets examining fluctuations of leaf
108 temperatures *in situ* in tropical forests, and, to our knowledge, none in tropical
109 montane forests.

110

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

124 through changes in angle and/or orientation to increase or decrease the amount of
125 radiation received.

126

127 Leaf structural traits (leaf mass per area (LMA) and leaf dry matter content (LDMC))
128 and stomatal conductance (g_s) influence the time required for leaf temperature to
129 change following a change in the environment (the thermal time constant (τ), Jones,
130 1992). Leaves with a long τ will show smaller temperature changes in a fluctuating
131 environment, maintaining the leaf temperature closer to the mean air temperature than
132 a leaf with a small τ which will track fluctuation in air temperature (Michaletz *et al.*,
133 2015).

134

135 Given the diversity of leaf structures and physiology observed within and among
136 tropical forest species (e.g. variation in leaf(let) area over 5 orders of magnitude for a
137 large sample of tropical species, Wright *et al.*, 2017), it is possible that there will be
138 diversity in leaf strategies with regard to temperature (Michaletz *et al.*, 2015). This
139 means that the impacts of potential future environmental changes may vary between
140 species even within a single biome. Future combined atmospheric changes of
141 increasing CO₂ and increasing T_A could be particularly important for T_L , as plants tend
142 to respond to increasing CO₂ by reducing g_s (Way, Oren, & Kroner, 2015). Reducing
143 g_s decreases water use, but also has the consequence of increasing leaf temperature
144 (Drake, González-Meler, & Long, 1997; Barker *et al.*, 2005) and can lead to
145 premature leaf senescence under heat-wave conditions (Warren, Norby, &
146 Wullschleger, 2011). Increases in T_A could be particularly important under fluctuating
147 and extreme conditions (e.g. heat-waves), increasing the occurrence of leaves
148 reaching or exceeding threshold temperatures resulting in leaf damage.

149

150 We present an observational study of leaf temperatures in a highly threatened tropical
151 forest region – the Atlantic forest, among the most diverse and threatened of
152 biodiversity hotspots (Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000;
153 Colombo & Joly, 2010). Our mountaintop study site is home to many endemic
154 species. Humans have exploited the Atlantic forest for 500 years resulting in a highly
155 fragmented landscape (Joly, Metzger, & Tabarelli, 2014) that reduces possibilities for
156 species migration. Hence, a greater understanding of forests in this region is of great
157 interest given their high threat level. We focus here on determining and understanding
158 interspecific differences in leaf temperatures caused by differences in leaf traits. Our
159 approach aims to begin to reveal whether or not trees are likely to be able to cope with
160 future conditions, and the extent to which species identity is likely to be important.
161 This is a step towards an understanding of the resilience of tropical trees and is part of
162 a broader effort to assess the effects of stressors on remaining Atlantic forests and
163 their ability to recover.

164

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

172 i) What are the current patterns of leaf temperature of the Atlantic forest species
173 *Alchornea*, *Miconia* and *Guapira* under fluctuating microclimatic conditions?

- 174 ii) Are there differences in leaf thermoregulation between the species?
175 iii) To what extent do leaf traits (width, stomatal conductance) and microclimate
176 (radiation, T_A , D , wind speed) determine leaf temperatures?
177

178 **Materials and Methods**

179

180 *Study Site*

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

192

193 *Microclimate measurements*

194 A narrow 27 m high tower was used for access to the canopy and microclimate
195 measurements (T_A , photosynthetically active radiation (PAR), relative humidity (h)
196 and wind speed (U)) were collected to detail the microclimate vertical profile (Fig. 1).
197 As the tower is just 30 cm wide and tree branches are within arms reach of the tower
198 (see Supplementary Figure S2d), we consider that the presence of the tower likely has

199 only minimal influence on the microclimate of the sampled leaves. From 18 m above
200 the ground, at the height of the highest leaves adjacent to the tower, 16 PAR sensors
201 were suspended from the tower at *c.* 1 m intervals, with an additional sensor
202 positioned at 25 m above the ground. Sensors were made following Fielder &
203 Comeau (2000) using gallium arsine phosphide (GaAsP) photodiodes (G1118,
204 Hamamatsu, Japan) and calibrated against a LI-COR 190 quantum sensor (LI-COR
205 Inc., Nebraska, USA). PAR sensors were positioned on plastic supports in the
206 horizontal plane. In addition, 7 thermistors (107, Campbell Scientific, Utah, USA) to
207 measure T_A were deployed in radiation screens spread through the vertical profile
208 (heights 1.5, 5, 7.5, 10, 12.5, 15 and 18 m, Fig. 2). PAR and T_A data were measured
209 and recorded at 10 s intervals using two CR800 data loggers with AM 16/32
210 multiplexers (Campbell Scientific, Utah, USA). Four data-logging h sensors (RHT10,
211 Extech, Massachusetts, USA) measured and recorded at 1 min intervals at heights 2,
212 8, 12.5 and 18 m. Four sonic anemometers (Sonicwind) measured U every 0.5 s at
213 heights of 1.5, 6.5, 11.5 and 25 m, and 10 s averages were produced for each height.
214 U for leaves positioned above 11.5 m height was linearly interpolated between the 25
215 m and 11.5 m measurement.

216

217 Vapour pressure deficit (D) was calculated from T_A and h (Campbell & Norman,
218 1998),

219

$$220 \quad D = e_{sat} \cdot (1 - h) \quad (1)$$

$$221 \quad e_{sat} = a \cdot \exp \cdot \left(\frac{bT_A}{T_A + c} \right) \quad (2)$$

222

223 where T_A is in °C, h is relative humidity (as a proportion), e_{sat} is saturating vapour
224 pressure in kPa and a , b and c are constants ($a = 0.611$, $b = 17.502$, $c = 240.97$).

225

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

230

231 *Sampled species*

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

240

241 *Leaf temperature measurements*

242 To measure leaf-to-air temperature differences (ΔT , also called ‘leaf temperature
243 excess’ in the literature) we followed the two-junction thermocouple design of
244 Singsaas & Sharkey (1998). This approach has the advantage of more accurately
245 measuring ΔT than performing measurements of absolute T_L and T_A separately. Two
246 long (15 cm length) constantan fine wires (0.07 mm diameter) were soldered to either
247 end of a short (3 cm length) copper fine wire (0.07 mm diameter). These

248 thermocouples produce a voltage proportional to the temperature difference between
249 the junctions. Thermocouples were individually calibrated by inserting one junction
250 into sand in a temperature controlled dry bath (TCBD-02, Cleaver Scientific, UK)
251 with the second junction in the air *c.* 2 cm above the bath. The temperature of the bath
252 and the air were each measured with two thermistors. Four different temperature
253 differences between the bath and air were produced ($\sim 0, 5, 10$ and 12°C). Data for the
254 calibration were selected during periods with a constant dry bath temperature (i.e.
255 excluding periods when the bath was heating up or cooling down).
256
257 Between 30 September and 3 October 2016 thermocouples were installed on 10
258 *Alchornea* leaves, 9 *Miconia* leaves and 4 *Guapira* leaves. Selected leaves were fully
259 expanded and mature, but not senescent, within reach from the canopy tower, and
260 spread through the vertical profile (Fig. 1). The thermocouple junction to measure leaf
261 temperature was secured to the abaxial mesophyll surface (avoiding any large veins)
262 near to the midrib using surgical tape (Transpore, 3M, Minnesota, USA). The second
263 junction was suspended in the air *c.* 2 cm below the leaf. Additional cabling was
264 cable-tied to a twig near to the leaf (or the petiole in the case of *Miconia*) and to the
265 tower. This attachment procedure enabled the majority of thermocouples to remain
266 attached to leaves during wind and rain (see Supplementary Figure S2 for
267 photographs of the equipment installation). The petioles of two leaves, both of
268 *Miconia*, snapped during the monitoring period. Supplementary Table S1 gives details
269 of the sampled leaves. ΔT was measured and recorded at 10 s intervals using a CR800
270 data logger with AM 16/32 multiplexer (Campbell Scientific, Utah, USA) until 11
271 October 2016.
272

273 *Spot measurements*

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

297

298 *Leaf trait measurements*

299 All sampled leaves were collected and stored in moist plastic bags for 24 hours before
300 fully rehydrating and measuring structural traits in the lab at the Instituto de Botânica,
301 São Paulo. Measurements were performed of leaf thickness (mm) with a digital
302 calliper, leaf area (cm²) with leaf area meter (LI-3100, LI-COR, Nebraska, USA), leaf
303 mass (g), leaf width (cm), and leaf length (cm). Petioles were removed before
304 measurements. Subsequently, leaves were dried at 70 °C, and dry weight measured.
305 These measurements were used to calculate leaf mass per area (LMA, g m⁻²) and leaf
306 dry matter content (LDMC, g g⁻¹). For *Guapira* the sample size for leaf traits was six
307 (rather than four as for leaf temperature).

308

309 *Leaf Energy Balance*

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

321

322
$$\Delta T_e = T_L - T_A = \frac{r_{b,HR}(r_{b,W}+r_{l,W})\gamma R_{ni}}{\rho_a c_{pa}[\gamma(r_{b,W}+r_{l,W})+sr_{b,HR}]} - \frac{r_{b,HR}D}{\gamma(r_{b,W}+r_{l,W})+sr_{b,HR}} \quad (3)$$

323

324 where T_L and T_A are the leaf and air temperatures respectively ($^{\circ}\text{C}$), R_{ni} is the net
325 isotropic radiation (W m^{-2} , assuming the sky temperature is equal to T_A measured at
326 the nearest T_A sensor to the leaf and sky emissivity of 0.97), γ is the psychrometric
327 constant (Pa K^{-1}), $r_{b,HR}$ is the boundary layer resistance to heat and radiation and $r_{b,W}$
328 and $r_{l,W}$ are the boundary layer and leaf resistances to water respectively (all
329 resistances in s m^{-1}), ρ_a is the density of dry air (kg m^{-3}), c_{pa} is the specific heat
330 capacity of dry air ($1012 \text{ J kg}^{-1} \text{ K}^{-1}$), s is the slope of relationship between temperature
331 and saturated vapour pressure evaluated at T_A , and D is the vapour pressure deficit
332 (Pa).

333

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

339

$$340 \quad g_{b,H} = 0.0105(U/W)^{0.5} \quad (4)$$

341

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

343

344 The thermal time constant (τ , s) was defined following Michaletz *et al.* (2016) as

345

$$346 \quad \tau = \varphi \cdot LMA \cdot \left(\frac{c_{pw}}{LDMC \cdot H} + \frac{c_{pd} - c_{pw}}{H} \right) \quad (5)$$

347

348 where ϕ , the ratio of projected to total leaf area, is 0.5 for flat leaves, LMA is in units
 349 kg m^{-2} , c_{pw} is the specific heat capacity of water ($4181 \text{ J kg}^{-1} \text{ K}^{-1}$), c_{pd} is the specific
 350 heat capacity of dry leaf matter ($\text{J kg}^{-1} \text{ K}^{-1}$). c_{pd} varies by species and here we use
 351 $2814 \text{ J kg}^{-1} \text{ K}^{-1}$, the mean of seven tropical tree species from Jayalakshmy & Philip,
 352 (2010). H is a heat transfer coefficient ($\text{W m}^{-2} \text{ K}^{-1}$) accounting for convection,
 353 radiation and transpiration (Michaletz *et al.*, 2016)

354

$$355 \quad H = \rho_a c_{pa} (g_{b,H} + g_{b,R} + g_s s / \gamma) \quad (6)$$

356

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

360

361 *Leaf boundary layer resistance*

362 Initial estimations of the leaf energy balance using equation 3 showed that when ΔT_e
 363 was evaluated at low wind speeds ($< 0.5 \text{ m s}^{-1}$) the values were overestimated
 364 compared to the observed ΔT . Using equations 4 and S5 to estimate the boundary
 365 layer resistance to water ($r_{b,W}$), there is a steep increase in $r_{b,W}$ below wind speeds of
 366 0.5 m s^{-1} (Supplementary Figure S3). To test if these high resistances were supported
 367 by the data, we solved the leaf energy balance equation for $r_{b,W}$ and estimated $r_{b,W}$
 368 using the observations of ΔT (see Appendix 2). Plotted against wind speed, the
 369 estimated $r_{b,W}$ was lower than predicted by equations 4 and S5 at low wind speeds
 370 (Supplementary Figure S3). Hence, we re-parameterised constants from equation 4
 371 using the $r_{b,W}$ estimated from the leaf energy balance and observed wind speed and
 372 leaf width (see Appendix 2). Parameter estimation was performed separately for

373 *Miconia* and *Alchornea* (there was not sufficient data for parameter estimation of
374 *Guapira*) using non-linear least squares (R function nls).

375

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

392

393 *Data Analysis*

394 Linear mixed effects models with leaf as a random factor were used for all statistical
395 analyses including repeated measurements of the same leaf using the R package nlme
396 (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2017). R^2 for mixed effects
397 models are given using either the marginal-pseudo- R^2 that accounts for fixed factors

398 only or conditional pseudo- R^2 (Nakagawa & Schielzeth, 2013). The marginal-pseudo-
399 R^2 is used unless otherwise stated, and R^2 values were calculated using the function
400 provided in the R package MuMIn (Bartoń, 2016). Statistical analyses comparing
401 between species using single values for each leaf used ANOVA for three species
402 comparisons and t-test for two species comparisons.

403

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

408

409 To compare leaf temperatures under comparable microclimate conditions data was
410 first selected for 20 min periods where ΔT_e matched measured ΔT to ensure
411 microclimate variables are representative of the leaf surface, as for *Leaf boundary*
412 *layer resistance* (see above) but using the species-level parameterization of $r_{b,w}$ to
413 estimate ΔT_e . The selected dataset was then subsetted according the microclimate
414 (PAR, T_A and U) for each leaf. We produced subsets of ΔT under low PAR and T_A
415 (PAR 50-150 $\mu\text{mol m}^{-2} \text{s}^{-1}$, T_A 13-15 °C), medium PAR and T_A (PAR 50-150 $\mu\text{mol m}^{-2}$
416 s^{-1} , T_A 13-15 °C), high PAR and T_A (PAR 1000-1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, T_A 18-20 °C), and
417 very high PAR (PAR 1600-1900 $\mu\text{mol m}^{-2} \text{s}^{-1}$, T_A 18-20 °C), all at wind speed 0.5 –
418 1.5 m s^{-1} . Differences in ΔT between species for each microclimate were evaluated
419 with linear mixed effects models with leaf as a random factor.

420

421 **Results**

422

423 *Validation of thermocouple data*

424 T_L based on ΔT measured with thermocouples and T_A measured with thermistors ($T_L =$
425 $\Delta T + T_A$) was highly correlated with T_L as measured by the IR thermometer (Pearson's
426 correlation coefficient for each leaf 0.60 - 0.99, where T_L was measured with IR
427 thermometer on the abaxial leaf surface). The slopes of linear regression lines forced
428 through zero were significantly different from 1 for only three leaves, where the
429 thermocouples slightly underestimated T_L by up to 9 % (Supplementary Figure S5).
430 Overall, the close agreement between the two measurement methods gives confidence
431 in the thermocouple data.

432

433 *Microclimate during the monitoring period*

434 Microclimate during the monitoring period is shown in Supplementary Figure S6 and
435 for a single sunny day in Fig. 2. The first seven days (1 – 7 Oct) were predominately
436 overcast with low PAR, high h and low D , with some sunny periods on 6 and 7 Oct.
437 Subsequently, two days (8 and 9 Oct) had longer bright periods. The final day of data
438 collection (10 Oct) was again overcast. Throughout the period lower canopy levels
439 received substantially less PAR and experienced lower D . However, on sunny days,
440 high PAR levels and higher D extended throughout the vertical profile (e.g. 8 October
441 2016, Fig. 2). Mean daytime T_A at the top of the canopy was 15.0°C, with a maximum
442 T_A of 22.1°C recorded on 8 October 2016. Mean nighttime T_A was 12.4°C, and was
443 lowest preceding sunny days. Mean windspeed above the canopy (at 25 m) was $1.0 \pm$
444 0.7 m s^{-1} , and $0.26 \pm 0.18 \text{ m s}^{-1}$ within the canopy (averaged over all sample heights
445 1.5, 6.5 and 11.5 m).

446

447 *Patterns of leaf temperature*

448 Leaves occasionally reached much higher temperatures than the ambient air, over 10
449 °C above T_A . The maximum T_L for each leaf observed over the monitoring period
450 ranged from 22.5-37.2 °C and was above 35 °C for 5 of the 23 leaves. *Miconia* leaves
451 had significantly higher maximum temperatures than *Alchornea* leaves (Table 1).
452 Daytime mean T_L showed less variation than maximum T_L between leaves and species
453 (Table 1, Supplementary Figure S7). Leaves of *Guapira*, which were all at the bottom
454 of the canopy, had lower maximum and mean T_L (not tested for significance due to
455 low sample size). Distributions of T_L show positive skew (Supplementary Figure S7)
456 which was significantly higher for leaves of *Miconia* than *Alchornea* (Table 1),
457 showing that they reached high temperatures more often than *Alchornea*. During
458 daylight hours ΔT was positive for leaves of *Alchornea* and *Miconia*, and was close to
459 zero for all *Guapira* leaves (Supplementary Figure S8, Table 1). The maximum ΔT
460 observed was 18.3 °C, again recorded from *Miconia* leaf M1. As for T_L the
461 distributions of ΔT were positively skewed, with significantly higher skewness for
462 *Miconia* than *Alchornea* (Table 1). Minimum daytime ΔT was significantly lower for
463 *Alchornea* than *Miconia* leaves (Table 1). Night-time ΔT was typically negative but
464 close to zero, and was significantly lower for *Alchornea* than *Miconia* (Table 1).
465
466 T_A set a rough minimum bound on T_L (Fig. 3), with many excursions above T_A due to
467 high radiation (see section *Leaf Energy Balance*) and a small number of excursions
468 below T_A , likely occurring when leaf surfaces were wet during/after rain or in fog. T_L
469 excursions above T_A occurred more often for leaves positioned higher in the canopy.
470
471 *Leaf temperatures under specific microclimates*

472 We compared leaf temperatures under specific microclimatic conditions. Under low
473 light and temperature conditions leaves of *Guapira* were significantly colder than
474 those of *Alchornea* and *Miconia* (Fig. 4a); there was no significant difference in ΔT
475 between the latter two species. Under medium light and temperature conditions there
476 again was no significant difference in ΔT between *Alchornea* and *Miconia* (Fig. 4b);
477 *Guapira* leaves did not experience these or brighter light conditions due to their
478 position in the understory. Under high light and temperature conditions ΔT was
479 significantly higher for *Miconia* than *Alchornea* (Fig. 4c). Under the highest light
480 conditions analysed ΔT was again higher for *Miconia* than *Alchornea*, however the
481 difference was not quite significant (Fig. 4d).

482

483 *Thermal trait variation between species*

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

490

491 Structural leaf traits with importance for thermoregulation also varied between
492 species (Table S1, Fig. 6). *Miconia* leaves were significantly wider, larger and had
493 higher LMA than both *Alchornea* and *Guapira* (Fig. 6a-c). LDMC significantly
494 differed between all species and was highest for *Miconia* ($0.42 \pm 0.013 \text{ g g}^{-1}$)
495 followed by *Alchornea* ($0.37 \pm 0.016 \text{ g g}^{-1}$) and finally *Guapira* ($0.20 \pm 0.022 \text{ g g}^{-1}$)
496 (ANOVA $F = 38.8$, $p < 0.0001$, and Tukey post-hoc test).

497

498 The thermal time constant (τ) ranged over two orders of magnitude from 9 - 350 s
499 (Fig. 7a) and varied significantly between species (linear mixed effects model, $F =$
500 48.1, $df = 20$, $p < 0.0001$). τ for *Guapira* were significantly longer and more varied
501 (mean \pm sd 155.4 ± 84.0) than both *Alchornea* (mean \pm sd 276.5 ± 11.1) and *Miconia*
502 (mean \pm sd 46.4 ± 14.4). τ decreased with increasing g_s and was particularly high
503 under very low g_s (Fig. 7b). For a given g_s , τ increased in the order *Alchornea* <
504 *Miconia* < *Guapira* (Figure 7b). These differences were driven by the leaf structural
505 traits LMA and LDMC (Supplementary Figure S9). When estimated using a fixed
506 LMA value the differences between *Alchornea* and *Miconia* are lost (Supplementary
507 Figure S9b) showing that the higher LMA of *Miconia* increases τ . When estimated
508 using a fixed LDMC value the *Guapira* values collapse into line with *Alchornea* (the
509 two species have similar LMA) (Supplementary Figure S9c) showing that the lower
510 LDMC of *Guapira* increases τ .

511

512 *Leaf energy balance and drivers of ΔT and T_L*

513 Leaf-to-air temperature difference estimated from leaf energy balance (ΔT_e) using the
514 spot measurements matched observed ΔT well, but with some underestimation at
515 higher ΔT (Fig. 8), showing that our data adequately parameterized the leaf energy
516 balance for instances when leaf surface PAR and g_s were measured. To investigate the
517 drivers of ΔT with the larger dataset of continuous ΔT and microclimate
518 measurements, the dataset was restricted to periods where predicted ΔT_e matched
519 observed ΔT , as for the analysis of ΔT under specific microclimate conditions. This is
520 to ensure we are using appropriate values of PAR which was not measured at the leaf
521 surface in the continuous dataset. Both observed ΔT and ΔT_e increase with PAR (Fig.

522 9), a pattern repeated when T_L and T_{Le} (leaf temperature estimated from energy
523 balance) were analysed (Supplementary Figure S13). The slope of the relationship
524 between leaf temperature variables and PAR were different between *Miconia* and
525 *Alchornea*, where *Miconia* has higher ΔT and T_L for a given PAR (Fig. 10,
526 Supplementary Figure S13). Whilst the absolute values of ΔT_e and T_{Le} are somewhat
527 higher than the observations, the differences between the species are maintained in the
528 energy balance estimations. Relationships between ΔT and ΔT_e and other
529 microclimate variables (T_A , D , U) were much weaker than for PAR with all R^2 values
530 below 0.3 (Supplementary Figures S10-S12), while T_L and T_{Le} were strongly related
531 to T_A and D with R^2 values above 0.7 (Fig. S14-S16).

532

533 To determine what causes the differences between ΔT of *Miconia* and *Alchornea*, we
534 applied traits (leaf width, stomatal conductance strategy) of *Miconia* sequentially to
535 *Alchornea* and re-estimated ΔT_e using the observed microclimate data. As shown in
536 Supplementary Figure 17, applying the larger leaf width of *Miconia* acts to increase
537 the *Alchornea* ΔT_e for a given PAR, almost to the extent that it matches the high ΔT_e
538 of *Miconia*. If the higher intercept of the $g_s \sim D$ relationship for *Miconia* is applied, the
539 *Alchornea* ΔT_e for a given PAR decreases. In contrast, if the steeper $g_s \sim D$ slope for
540 *Miconia* is applied, the *Alchornea* ΔT_e for a given PAR increases. The effect is not as
541 strong as the effect of leaf width (Supplementary Figure 17). Applying both the
542 *Miconia* intercept and slope results in an intermediate *Alchornea* ΔT_e for a given
543 PAR, slightly higher than for *Alchornea* with its original parameterization. If all
544 *Miconia* traits are applied (leaf width and stomatal conductance strategy) *Alchornea*
545 ΔT_e for a given PAR increases to a greater extent than for any trait alone, and even
546 exceeds the ΔT_e of *Miconia*. This is likely due to the higher D that the highest six

547 *Alchornea* leaves are exposed to due to their position above the *Miconia* leaves (Fig.
548 1).

549

550 **Discussion**

551

552 *Current leaf temperature patterns and the role of microclimate*

553

554 Despite the low T_A (maximum 22 °C) occurring during the study period we observed
555 leaf temperatures over 30 °C. Whilst few datasets are available on field-measured leaf
556 temperatures of tropical trees, the maximum ΔT we observed (18.3 °C) is somewhat
557 higher than those previously reported (e.g. 13.9 °C for a range of Proteaceae species
558 in Australia (Leigh, Sevanto, Close, & Nicotra, 2017), 10 °C for five species in
559 Panama (Rey-Sánchez *et al.*, 2016) and in the Amazon (Doughty & Goulden, 2008), 7
560 °C for three species in tropical China (Dong, Prentice, Harrison, Song, & Zhang,
561 2017)). This could be due to high sampling frequency used in this study (every 10 s)
562 compared to others (every 2 minutes in Rey-Sánchez *et al.*, 2016, half hourly in Dong
563 *et al.*, 2017 and unspecified in Leigh *et al.*, 2017) as at high frequency extreme values
564 are more likely to be recorded, though Doughty & Goulden (2008) used a 1 s
565 sampling frequency. It could also be due to the light conditions and study species
566 measured (discussed below). The highest T_L observed (37.2 °C) is less than those
567 reported by others under higher ambient air temperatures (e.g. 45 °C - Doughty &
568 Goulden, 2008; 48 °C – Slot *et al.*, 2016, Krause *et al.*, 2010). This work supports the
569 view that ambient air temperatures cannot necessarily be used as a proxy for leaf
570 temperature in physiological models as they are not necessarily equal (Michaletz *et*

571 *al.*, 2016; Rey-Sánchez *et al.*, 2016), and that vegetation models should be tested for
572 their ability to reproduce patterns of ΔT (Dong *et al.*, 2017).

573

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

593

594 Within the range of conditions during the study period, radiation was the most
595 important microclimate variable for determining ΔT (Fig. 9, Supplementary Fig. S10-

596 S12). This has also been shown in other studies (e.g. Doughty & Goulden, 2008; Rey-
597 Sánchez *et al.*, 2016) and is understood biophysically (Jones, 1992). For absolute T_L ,
598 PAR, T_A , and D were all important (Supplementary Fig. S13-S16), though the
599 strength of the D effect is likely due at least in part to its co-variation with T_A . Recent
600 work has shown the occurrence of a ‘crossover’ T_A at 25-28 °C (Michaletz *et al.*,
601 2016, Dong *et al.*, 2017). The crossover temperature is the T_A at which $\Delta T=0$ and
602 above which ΔT is negative. We found no evidence of a crossover T_A , likely due to
603 the relatively low T_A during our study. The light levels observed in the study were
604 high, occasionally exceeding 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. We consider the light levels recorded
605 in the profile to be accurate as they were highly comparable to an independent dataset
606 from a weather station mounted at 27 m on the same tower (Figure S18). The values
607 are higher than the PAR observed in similar studies from lowland forests with typical
608 maximum PAR of 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Rey-Sánchez *et al.*, 2016; Doughty & Goulden,
609 2008). Again, measurement frequency may be important here for recording extreme
610 instantaneous values. In fact, this maximum quantity of PAR is equivalent to more
611 radiation than the solar constant (incoming light at the top of the atmosphere, 1.353
612 kW m^{-2}) which is possible in mountains when light is reflected from clouds
613 (Stoutjesdijk & Barkman, 2014). Incoming radiation increases by 8 % for every 1000
614 m increase in elevation (Blumthaler, Ambach, & Ellinger, 1997). Montane forests are
615 therefore likely to experience higher maximum radiation loads than lowland forest, as
616 has been measured at this site (Rosado, Joly, Burgess, Oliveira, & Aidar, 2016).
617 Given the importance of radiation for T_L , trees at high elevation may have greater risk
618 of hitting damaging T_L thresholds if air temperatures increase with climate change. At
619 this specific site, in addition to increased radiation, D also increases with elevation
620 and trees show more conservative water use (Rosado *et al.*, 2016) which will further

621 influence leaf temperatures. Mountaintop species are already considered to be more
622 greatly threatened than lowland species by increased temperatures as there is no
623 cooler place for species to move to. The high radiation load increasing leaf
624 temperatures may exacerbate this problem.

625

626 As microclimate is a key driver of leaf temperature, it is important to consider the
627 vertical gradient in microclimate (Supplementary Figure S6). We found that all
628 microclimate variables displayed vertical gradients, especially during sunny days
629 when the differences between the top and bottom of the canopy exceeded $5\text{ }^{\circ}\text{C } T_A$,
630 $2200\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}\text{ PAR}$ and $1.3\text{ kPa } D$. The difference in T_A leads to a larger
631 difference in T_L than the values of ΔT we typically found (Table 1). Whilst vertical
632 gradients of PAR are often accounted for in vegetation models, often the gradients of
633 other key variables are not considered, which would lead to error in quantification of
634 leaf temperatures below the canopy top.

635

636 *Differences in leaf thermoregulation between species*

637

638 We found striking differences in leaf temperature patterns between species that were
639 attributable to differing leaf traits. *Miconia* leaves more commonly experienced high
640 ΔT excursions than *Alchornea*, with higher skew in T_L and ΔT distributions, higher
641 maximum ΔT and lower minimum ΔT (Table 1). Leaf temperatures of *Miconia* were
642 consistently higher than *Alchornea* when controlling for microclimate between
643 measurements, and significantly so during high light conditions (Fig. 4). The
644 differences increased with increasing thermal stress (higher PAR , T_A and D). The lack
645 of significance at the highest PAR/T_A subset tested is likely due to low data

646 availability and higher PAR measurement errors at high PAR. As PAR was not
647 measured directly at the leaf surface it was difficult to ensure correspondence between
648 PAR as measured by the nearest sensor and received at the leaf surface; this is more
649 problematic under direct light conditions where leaf angle, orientation, sun angle and
650 within canopy shading greatly impact leaf surface PAR. We recommend all studies of
651 leaf temperature attempt to measure PAR at the leaf surface despite the higher efforts
652 required.

653

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

668

669 The study species only showed differing relationships between PAR, and T_L and ΔT ,
670 with similar responses to other microclimatic variables (Fig. 9, Supplementary

671 Figures S10-S16). This shows that it is the consequences for input solar energy that
672 varies between the species, rather than differing mechanisms in response to T_A . It is
673 not to say that other microclimatic variables are not important for T_L or ΔT , but that
674 the response of T_L and ΔT to other variables is the same for the two species, at least
675 under the measurement conditions.

676

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

686

687 Leaf temperatures of the subcanopy *Guapira* tree were consistently similar to air
688 temperatures and showed little variation (Table 1) likely due to the canopy position
689 receiving very little light (Fig. 1). However, when the data were subsetted for low
690 PAR conditions only, leaves of *Guapira* still showed a lower ΔT than the two other
691 species (Fig. 4a). This could be due to the narrower leaf width of *Guapira* leaves (Fig.
692 6), though the width is not significantly different from *Alchornea*. It could also be due
693 to the unusual leaf angles displayed by the *Guapira* leaves that were hanging near
694 vertically (Table S1, Figure 6e) which would limit the amount of light received and
695 result in over estimates of the light environment from using a horizontally orientated

696 sensor. Another potential contributor is the long τ values estimated, as T_L is expected
697 to vary less when τ is long (Ball, Cowan, & Farquhar, 1988). The long τ for *Guapira*
698 leaves were a result of the combined low g_s and low LDMC (Fig. 7, Supplementary
699 Figure S9); because water has a higher specific heat capacity than dry leaf matter, the
700 higher water content of *Guapira* leaves causes a longer τ (Vogel, 2009).

701

702 *Towards a better understanding of tropical leaf temperature behaviour*

703

704 The link between functional traits and leaf thermoregulation has been highlighted in
705 recent work (Michaletz *et al.*, 2015; 2016). Here, we provide field-based evidence for
706 this link in the most detailed study of leaf energy balance in tropical montane forests
707 to date, and include variation in water-use as a key component. The traits that we find
708 important (leaf width, g_s at high D , LDMC) may possibly connect other axes of plant
709 functional variation (Reich, 2014) – the leaf economics spectrum (Wright *et al.*, 2004)
710 and plant hydraulics. Species that are able to maintain transpiration under high
711 thermal stress conditions (high T_A , PAR, D) will require water to supply the
712 transpiration stream from an efficient hydraulic system or from high water
713 capacitance to avoid hydraulic failure. Avoiding extremes of T_L and maintaining open
714 stomata will then have the benefit of keeping T_L closer to the temperature optima of
715 photosynthesis, maintaining a CO₂ supply, and all this while PAR is high to drive a
716 high photosynthetic rate (Ball *et al.*, 1988). Conversely, lower transpiration under
717 high thermal stress conditions will prevent excessive water loss and therefore avoid
718 risk of hydraulic failure through xylem embolism, but increase risk of the leaf
719 reaching a damaging high temperature threshold. Critical thresholds of photosynthetic
720 activity vary by species (O’Sullivan *et al.*, 2017). A recent study of critical thresholds

721 of 41 co-occurring tropical species found that variation was related to the leaf
722 economics spectrum (Wright *et al.*, 2004), with high LMA species showing higher
723 temperature tolerance (Sastry & Barau, 2017). *Miconia* has significantly higher LMA
724 than *Alchornea* (Fig. 6), and it would be parsimonious if it also displays a higher
725 critical temperature for damage to photosynthetic machinery. In summary, we
726 hypothesise that trees at the ‘slow’ end of the life-history spectrum (Reich, 2014) are
727 likely to reach higher leaf temperatures, have lower g_s and photosynthesis under high
728 thermal stress conditions, lower risk of hydraulic failure, and a higher threshold for
729 thermal damage, with the converse true of ‘fast’ species.

730

731 If we are to understand the implications of climate change for tropical forests it will
732 be crucial to understand mechanisms of leaf thermoregulation and how this varies
733 between species. We have based our findings on only a small, if detailed, dataset.
734 There are very few comparable datasets available for tropical forests. More datasets
735 exploring the full energy balance of tropical leaves from multiple sites with varying
736 climatologies, and ideally over extended time periods, would certainly aid this.
737 Beyond understanding current patterns of leaf temperatures, it is also necessary to
738 understand the response of energy balance parameters to high T_A and CO_2 . For
739 example, herbarium data for an Australian shrub species showed a reduction in leaf
740 width over the last century (Guerin, Wen, & Lowe, 2012) which could mitigate
741 increases in T_L due to increased T_A . Conversely, declines in g_s are a common response
742 of tree species to increased CO_2 which, while potentially reducing water-use, could
743 lead to higher T_L (e.g. Barker *et al.*, 2005; Warren *et al.*, 2011). However, the extent
744 of reductions in g_s under elevated CO_2 varies with species (Way *et al.*, 2015). In a
745 study of seedlings of 10 tropical species, Cernusak *et al.* (2011) found reductions in g_s

746 in all species in response to elevated CO₂, but the reductions were larger for species
747 with high g_s in ambient conditions. Warming may also cause changes in g_s ; results
748 from warming experiments show a variety of responses – increases, decreases, no
749 change (Way *et al.* 2015) - and a recent meta-analysis found decreases in stomatal
750 density with higher T_A in trees but not in herbs (Yan, Zhong, & Shangguan, 2017). If
751 trees do indeed decrease g_s under higher growth temperatures this could result in
752 further leaf warming beyond T_A increases, but only if transpiration declines as well as
753 g_s , which is not certain given the expected rise in D with increased T_A . Our
754 understanding of the effects of combined CO₂ and warming is even more limited. If
755 both cause a decline in g_s separately, would the combined effect be additive leading to
756 even greater reductions? The limited experimental data does not paint a clear picture
757 (Way *et al.*, 2015). A final question is whether leaves that reach higher temperatures
758 are better adapted to cope with high temperatures and therefore increasing T_L would
759 be less consequential than for low temperature species, or does the fact that leaf
760 temperatures are already high mean that high-temperature species are more at risk?
761

762 *Conclusions*

763 In this study we made detailed measurements of leaf energy balance for three tree
764 species in the montane Atlantic forest, Brazil. Our results show surprising high leaf-
765 to-air temperature differences given the relatively low air temperatures, which we
766 attribute to the high light conditions during the study. The higher radiation levels
767 occurring at high elevations may contribute to the risks of climate change to tropical
768 montane forests. We find differences in leaf thermoregulation between leaves of
769 *Alchornea* and *Miconia*, which is attributable to lower transpiration under high
770 thermal stress conditions for *Miconia* due to its wider leaves and stronger reduction of

771 g_s with increasing D . Leaf energy balance modelling can be a powerful tool to
772 understand variation between species in leaf thermoregulation which will be
773 necessary to model the impact of climate change on leaf physiology.

774

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789

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1020 **Table 1.** Leaf temperature variables for three species. n = number of leaves measured
 1021 for each tree.

	Mean \pm SD			<i>p</i> *
	<i>Alchornea</i> (n=10)	<i>Miconia</i> (n=9)	<i>Guapira</i> (n=4)	
Daytime minimum T_L ($^{\circ}$ C)	7.71 \pm 0.63	8.51 \pm 1.55	8.13 \pm 0.59	0.18
Daytime mean T_L ($^{\circ}$ C)	15.98 \pm 0.47	16.14 \pm 0.72	14.6 \pm 0.01	0.6
Daytime maximum T_L ($^{\circ}$ C)	30.56 \pm 3.6	34.63 \pm 2.63	23.33 \pm 1.22	0.012
Daytime T_L skewness	0.84 \pm 0.43	1.38 \pm 0.27	0.56 \pm 0.27	0.005
Daytime minimum ΔT ($^{\circ}$ C)	-3.72 \pm 1.36	-2.16 \pm 0.75	-5.07 \pm 2.50	0.007
Daytime mean ΔT ($^{\circ}$ C)	0.79 \pm 0.40	0.84 \pm 0.43	0.007 \pm 0.001	0.8
Daytime maximum ΔT ($^{\circ}$ C)	11.27 \pm 4.15	14.23 \pm 2.71	3.28 \pm 1.08	0.08
Daytime ΔT skewness	2.51 \pm 1.32	4.56 \pm 1.63	0.73 \pm 5.86	0.009
Night-time mean ΔT ($^{\circ}$ C)	-0.13 \pm 0.07	-0.06 \pm 0.03	-0.008 \pm 0.01	0.02

1022 * *p*-value from t-tests comparing *Alchornea* and *Miconia*.

1023

1024 **Table 2.** ANOVA table for the linear mixed effects model of *D*, species, and their
 1025 interaction on g_s . Leaf is included as random intercept.

	Numerator DF	Denominator DF	F	p
Intercept	1	167	1097.0	< 0.0001
<i>D</i>	1	167	18.7	< 0.0001
Species	2	20	40.0	< 0.0001
<i>D</i> :Species interaction	2	167	6.6	0.0018

1026

1027 **Figure legends**

1028

1029 **Figure 1.** Schematic of field data collection showing positions of microclimate

1030 measurements and leaves sampled for temperature.

1031

1032 **Figure 2.** Time-series of microclimate and leaf-to-air temperature difference on 8

1033 October 2016. a) Photosynthetically active radiation, b) air temperature, c) vapour

1034 pressure deficit, d) leaf-to-air temperature difference for leaves of *Alchornea* (A6),

1035 *Miconia* (M1) and *Guapira* (G3). Colours refer to measurement heights.

1036

1037 **Figure 3.** Leaf (T_L) and air (T_A) temperatures measured over 10 days for a) *Alchornea*

1038 (10 leaves), b) *Miconia* (10 leaves) and c) *Guapira* (4 leaves). Colour denotes leaf

1039 height. Grey line – $y=x$. Each data point is single measure of a single leaf taken from

1040 the thermocouple time series recorded every 10 s.

1041

1042 **Figure 4.** Leaf to air temperature difference (ΔT) variation between species and

1043 microclimatic conditions. Vertical lines show the mean value for the species.

1044 Significance values are shown testing for differences between species (linear mixed

1045 effects model with leaf as a random factor) under four different microclimates. PAR –

1046 photosynthetically active radiation, T_A – air temperature.

1047

1048 **Figure 5.** Relationship between stomatal conductance and vapour pressure deficit,

1049 and variation between species. Equations for each species – *Alchornea*: $g_s = 325.1 (\pm$

1050 $22.4) - 68.8 (\pm 22.6) D$; *Guapira*: $g_s = 185.4 (\pm 36.4) - 212.7 (\pm 22.6) D$; *Miconia*:

1051 $401.6 (\pm 31.0) - 189.8 (\pm 35.4) D$ (errors are SE).

1052

1053 **Figure 6.** Variation in leaf structural traits between species. Letters represent
1054 significantly different groups calculated using ANOVA and Tukey post-hoc tests.

1055

1056 **Figure 7.** Distributions of thermal time constants estimated for each species (a), and
1057 the relationship between stomatal conductance and the thermal time constant (b).

1058

1059 **Figure 8.** Leaf to air temperature difference (ΔT) from observations and energy
1060 balance estimations. Grey dash – $y=x$ line, grey solid – linear regression line for all
1061 data, regression slope = 0.72 ± 0.03 SE, intercept -0.41 ± 0.07 SE, $F = 725.8$, $df =$
1062 185 , $p < 0.0001$, $R^2 = 0.80$.

1063

1064 **Figure 9.** Relationships between PAR and observed ΔT (a, b) and estimated ΔT_e (c, d)
1065 for *Alchornea* (a, c) and *Miconia* (b, d). Solid line – modelled relationship for the
1066 plotted species, dash line – modelled relationship for the alternative species.

1067 Statistical models are linear mixed effects model with leaf as a random factor. R^2 is
1068 the marginal pseudo R^2 . To account for uneven sampling with respect to PAR data
1069 was subsampled for 1000 points in $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ bins for points below $1000 \mu\text{mol}$
1070 $\text{m}^{-2} \text{s}^{-1}$.

1071