Photosynthetic temperature responses of tree species in Rwanda: evidence of pronounced negative effects of high temperature in montane rainforest climax species

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Abstract
The sensitivity of photosynthetic metabolism to temperature has been identified as a key uncertainty for projecting the magnitude of the terrestrial feedback on future climate change. While temperature responses of photosynthetic capacities have been comparatively well investigated in temperate species, the responses of tropical tree species remain unexplored. We compared the responses of seedlings of native cold-adapted tropical montane rainforest tree species with those of exotic warm-adapted plantation species, all growing in an intermediate temperature common garden in Rwanda. Leaf gas exchange responses to carbon dioxide (CO2) at different temperatures (20-40°C) were used to assess the temperature responses of biochemical photosynthetic capacities. Analyses revealed a lower optimum temperature for photosynthetic electron transport rates than for Rubisco carboxylation rates, along with lower electron transport optima in the native cold-adapted than in the exotic warm-adapted species. The photosynthetic optimum temperatures were generally exceeded by daytime peak leaf temperatures, in particular in the native montane rainforest climax species. This study thus provides evidence of pronounced negative effects of high temperature in tropical trees and indicates high susceptibility of montane rainforest climax species to future global warming.

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Photosynthetic temperature responses of tree species in Rwanda: evidence of pronounced negative effects of high temperature in montane rainforest climax species

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Summary

• The sensitivity of photosynthetic metabolism to temperature has been identified as a key uncertainty for projecting the magnitude of the terrestrial feedback on future climate change. While temperature responses of photosynthetic capacities have been comparatively well investigated in temperate species, the responses of tropical tree species remain unexplored.
• We compared the responses of seedlings of native cold-adapted tropical montane rainforest tree species with those of exotic warm-adapted plantation species, all growing in an intermediate temperature common garden in Rwanda. Leaf gas exchange responses to carbon dioxide (CO2) at different temperatures (20–40°C) were used to assess the temperature responses of biochemical photosynthetic capacities.
• Analyses revealed a lower optimum temperature for photosynthetic electron transport rates than for Rubisco carboxylation rates, along with lower electron transport optima in the native cold-adapted than in the exotic warm-adapted species. The photosynthetic optimum temperatures were generally exceeded by daytime peak leaf temperatures, in particular in the native montane rainforest climax species.
• This study thus provides evidence of pronounced negative effects of high temperature in tropical trees and indicates high susceptibility of montane rainforest climax species to future global warming.

Introduction

Observations have unequivocally demonstrated increasing global surface air temperatures (Hartmann et al., 2013), and global climate change models project a continuation of temperature increase over the coming century, irrespective of the chosen emission scenario (IPCC, 2013; Burrows et al., 2014). As temperature is one of the most important environmental factors controlling physiological processes (Hughes, 2000; Poethig, 2003; Root et al., 2003; Hegland et al., 2009), increased temperature is expected to have significant effects on the fitness of all living organisms.

Improving the understanding of the effect of temperature on terrestrial plant species is particularly important, as most migrate far more slowly than would be necessary to remain in a suitable climate under mid- and high-range rates of global warming (IPCC, 2013, 2014). Furthermore, terrestrial vegetation has important biogeochemical, hydrological and biophysical interactions with the atmosphere and its responses thus affect both local and global climate (Denman et al., 2007; Bonan, 2008). In particular, it is paramount to understand the responses of plant primary production to climate change in order to project terrestrial feedbacks on the carbon cycle along with the potential of the terrestrial biosphere to be either mitigative or promotive to further global warming (Cox et al., 2000; Cao et al., 2001; Bonan, 2008). The sensitivity of photosynthetic metabolism to temperature has been identified as the most important uncertainty with respect to projections of the magnitude of the terrestrial feedback on future climate change, highlighting the need for a better understanding of plant photosynthetic responses to high temperature (Booth et al., 2012).

In global vegetation models, photosynthesis of terrestrial plants is modelled using the well-established biochemical model of photosynthesis developed by Farquhar et al. (1980; Sellers et al., 1997; Pitman, 2003; Prentice et al., 2007). This model requires parameters of two photosynthetic capacities: the maximum carboxylation rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; Vcmax) and the maximum rate of electron transport (Jmax), necessary to regenerate ribulose-1,5-bisphosphate. The maximum rates of photosynthetic carboxylation and electron transport depend on multiple factors, of which the most important include light (Carswell et al., 2000; Kenzo et al., 2006),
nutrient availability (particularly nitrogen and phosphorus; Kattge et al., 2009; Domingues et al., 2010; Mercado et al., 2011) and intercellular carbon dioxide (CO$_2$) concentration ($C_i$), which is dependent on stomatal conductance ($g_s$; Farquhar & Sharkey, 1982), as well as temperature (Kattge & Knorr, 2007). Increasing temperature causes $J_{max}$ and $V_{cmax}$ to rise to a maximum followed by a rapid decrease at supraoptimal temperatures (Berry & Björkman, 1980; Medlyn et al., 2002a). Electron transport is considered to be particularly sensitive to high temperatures, because of its greater dependence on membrane stability (Murakami et al., 2000; Sage & Kubien, 2007). Thus, the temperature at which the maximum rates occur, the optimum temperature ($T_{opt}$), is often found to be significantly lower for $J_{max}$ than for $V_{cmax}$ (Walcroft et al., 1997; Dreyer et al., 2001; Medlyn et al., 2002a).

In temperate tree species, the $T_{opt}$ values for $V_{cmax}$, $J_{max}$ and leaf net photosynthesis ($A_n$) correlate with the prevailing temperature regimes (Battaglia et al., 1996; Dreyer et al., 2001; Medlyn et al., 2002a; Kattge & Knorr, 2007; Gunderson et al., 2010). Furthermore, plant photosynthesis has been shown to acclimate to prevailing growth temperature by shifting its $T_{opt}$, particularly that of $J_{max}$ towards the temperature of the ambient air if subjected to warming (Battaglia et al., 1996; Cunningham & Read, 2002; Kirschbaum, 2004; Hikosaka et al., 2006; Gunderson et al., 2010). Photosynthetic thermal acclimation abilities have also been demonstrated in studies taking advantage of temperature differences with season (Battaglia et al., 1996; Gunderson et al., 2000; Medlyn et al., 2002a; Crous et al., 2013), altitude (Cavieres et al., 2000; Zhang et al., 2007) and provenance (Hill et al., 1988; Gunderson et al., 2000; Cunningham & Read, 2002; Medlyn et al., 2002b).

While the photosynthetic temperature responses of temperate tree species are comparatively well studied, the responses of tropical tree species remain unexplored (Medlyn et al., 2002a; Kattge & Knorr, 2007; Gunderson et al., 2010). This is unfortunate, as tropical forests are inhabited by over half of all plant and animal species on Earth, represent one-fourth of the global terrestrial carbon storage and are responsible for at least one-third of the global primary production (Denman et al., 2007; Bonan, 2008; Beer et al., 2010; Zhou et al., 2013). Tropical forests play a crucial role in regulating both regional and global climate (Lewis, 2006), and better understanding of the effect of increased temperature on tropical forests is therefore paramount (Denman et al., 2007). Most studies investigating the photosynthetic temperature responses of tropical tree species have focused on responses of $A_n$ and not the underlying biochemical mechanisms (i.e. $J_{max}$ and $V_{cmax}$). They predominantly focus on species and ecosystems in South America, primarily Amazonia (Rada et al., 1996; Clark et al., 2003; Wittich et al., 2012; Cheesman & Winter, 2013), South East Asia (Mori et al., 1990; Ishida et al., 1999) and Australia (Pearcy, 1987; Read, 1990; Cunningham & Read, 2002), whereas to date, no temperature response assessments have been conducted on tropical African trees. These studies have indicated that tropical trees may have a more narrow optimum temperature range, with $A_n$ quickly declining as temperatures become suboptimal or supraoptimal, compared with temperate and boreal species. This is believed to be a consequence of adaptation to a more stable climate and may imply that tropical species are more sensitive to future temperature increases (Berry & Björkman, 1980; Read, 1990; Battaglia et al., 1996; Cunningham & Read, 2002; Way & Oren, 2010). Observations of considerable declines in growth rates of tropical trees resulting from only subtle increases in air temperature (Clark et al., 2003; Feeley et al., 2007; Doughty & Goulden, 2008; Way & Oren, 2010) further support this hypothesis. Tropical forests are thus suspected of being close to a thermal threshold, above which CO$_2$ uptake is strongly reduced (Doughty & Goulden, 2008).

This study aimed to improve the limited understanding of temperature responses of tropical primary production by providing the first temperature response assessments of photosynthetic capacities (i.e. $J_{max}$ and $V_{cmax}$) in tropical tree species. This was achieved by examining leaf gas exchange responses to CO$_2$ at different temperatures (20–40°C) in three native cold-adapted, tropical montane rainforest species and three common exotic warm-adapted plantation species, in an intermediate temperature common garden in Rwanda. We hypothesized that: (1) $J_{max}$ is more sensitive to high temperature than $V_{cmax}$ as has been found in temperate and boreal tree species. (2) Cold-adapted native montane rainforest species have lower photosynthetic optimum temperatures (i.e. $T_{opt}$ for $J_{max}$, $V_{cmax}$ and $A_n$) than warm-adapted exotic plantation species, as a consequence of adaptation to the species’ climate of origin, which is not erased by acclimation to the common garden conditions. (3) The optimum temperatures of photosynthesis are commonly exceeded in the native tropical species growing in the common garden, but not in the exotic plantation species, demonstrating limited acclimation ability and high sensitivity to future global warming.

Along with increased temperatures, climate change also includes continued increases in atmospheric CO$_2$ concentrations ($C_{a}$; IPCC, 2013, 2014). The expected CO$_2$ rises are predicted to increase the optimum temperature of photosynthesis, such that the negative effects of increased temperature will be mitigated (Long, 1991; Cao et al., 2001; Kirschbaum, 2004; Lloyd & Farquhar, 2008). A fourth hypothesis explores the temperature sensitivity under higher atmospheric CO$_2$ concentration conditions, according to the photosynthesis model parameterized for the investigated species: (4) in a moderate climate change scenario for year 2100 (IPCC, 2013, 2014), a 50% increase in atmospheric CO$_2$ concentration cancels the negative effects of 3°C warming such that heat-induced reductions in photosynthesis remain similar compared with today.

Materials and Methods

Study site and plant material

The study was carried out on seedlings cultivated in the Rwasave nursery, located on the edge of the Ruhande Arboretum (Rwanda; 2°36’S, 29°44’E; c. 1640 m above sea level (asl)) and surrounded by vegetation in all directions. At a meteorological station c. 2 km from the arboretum (1765 m asl; Nsabimana et al., 2009), the average day and night air temperatures at 7.5 m
above ground were 20.8 and 17.1°C, respectively, the average relative humidity was 74% and annual rainfall was 1231 mm during 2006–2013. The climate of the region is tropical humid and the difference in mean temperature between the warmest and coldest months is 1.5°C. The rainfall is bimodal with most rain in March–May and light occurrence in September–December, separated by a major drought period in June–August and a moderately dry period in January–February. Maximum temperatures and the average diurnal temperature range at the meteorological station are provided in Table 1.

Daytime air temperature (Model TinyTag Plus 2; Gemini Data Loggers Ltd, Chichester, UK; placed inside self-ventilating radiation shields) at 1.8 m above ground in the nursery was similar to that at 1.8 m above ground at the meteorological station, but predawn temperature was c. 3°C lower at the lower location of the nursery. The vertical variation in air temperature was considerable, with the temperatures at 1.8 and 0.4 m above ground being 2°C and 4°C higher compared with the temperature at 7.5 m in the nursery.

The taxa selected (Table 2) were either native tropical montane rainforest tree species or silviculturally important exotics, planted in the Ruhande Arboretum one to two generations ago. The native species included two climax taxa from the Meliaceae family, *Carapa grandiflora* Sprague and *Entandrophragma excelsum* (Dawe & Sprague) Sprague, along with one pioneer species from the Rosaceae family, *Hagenia abyssinica* (Bruce) J. F. Gmelin. *Carapa grandiflora* is a dominant species in transitional Afromontane rainforest and has an altitudinal distribution ranging between 1600 and 2500 m asl (White, 1983; Fischer & Killmann, 2008; Bloesch et al., 2009). *Entandrophragma excelsum* is a dominant species in Afromontane rainforest and occurs between 1500 and 2100 m asl (White, 1983; Fischer & Killmann, 2008; Bloesch et al., 2009). The plant material of the three native species originates from the Nyungwe national park montane rainforest (Rwanda; 2°15’–2°55’S, 29°00’–29°30’E; 1500–2950 m asl). At a meteorological station located at Uwinka (2°28’43’’S, 29°12’00’’E; 2465 m asl; Nsabimana, 2009), the average day and night air temperatures at 7.5 m above ground were 15.7 and 13.5°C, respectively, the relative humidity was 81%, and annual rainfall was 1879 mm during 2007–2013. At another meteorological station located at 1935 m asl in Nyungwe, the annual mean temperature was 1.5°C higher than at Uwinka. Maximum temperatures and average diurnal temperature range experienced at Uwinka are provided in Table 1.

Three common exotic plantation taxa were selected to represent warm-adapted exotics (Table 2): two Myrtaceae species, *Eucalyptus microcorys* F. Muell and *Eucalyptus maidenii* F. Muell, along with a deciduous pioneer species from the Meliaceae family, *Cedrela serrata* Royal. *Eucalyptus maidenii* occurs in temperate southern coastal New South Wales and Victoria, Australia (Hill, 1991), whereas *E. microcorys* is distributed in warmer subtropical coastal areas of New South Wales and Queensland, Australia (Hill, 1991). *Cedrela serrata* is known to occur from Central to South East Asia (Orwa et al., 2009). Maximum temperatures and average diurnal temperature range experienced by the exotic species in their known native distributions are provided in Table 1.

The seedlings were cultivated in pots containing clay soil from the surrounding area (ISAR, 2011) and were irrigated twice daily with water sourced from the local creek. The pots were placed in monospecific plots, some partially shaded from the sun by basic shade houses. Seedling dimensions are provided in Table 2.

**Gas exchange measurements**

Gas exchange measurements were completed on two seedlings per day during July and August 2011. One fully developed sun leaf with a healthy appearance (i.e. normal colouration and no

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**Table 1** Temperature parameters (°C) for Nyungwe montane rainforest, the common garden of Ruhande Arboretum, and the known native distribution of three exotic tree species.

| Air temperature parameter | Nyungwe montane rainforest¹ | Common garden² | Eucalyptus microcorys³ | Eucalyptus maidenii³ | Cedrela serrata⁴,⁵ |
|---------------------------|-----------------------------|----------------|----------------------|---------------------|-------------------|
| Mean diurnal range⁶       | 2.2                         | 3.7            | 11.5                 | 12.0                | 10.7              |
| Mean daily maximum of warmest month⁷ | 19.2           | 24.8            | 26.9                 | 24.3                | n/a               |
| Mean monthly maximum of warmest month⁸ | 22.1           | 27.5            | 33.1                 | 32.6                | 29.6              |
| Mean annual maximum⁹      | 22.6                        | 27.8            | 40.8                 | 40.4                | n/a               |

Data for the native species refers to footnotes 1 and 2 and for the exotic species to footnotes 3–9. Values for the exotic species are based on climate extracted for coordinates where species observations have been recorded (*Eucalyptus*: 5598 records, 1940–2012; *Cedrela serrata*: nine records, dates not available). n/a, data not available.

¹Measured at Uwinka meteorological station at 2465 m above sea level (asl) during 2007–2013.
²Measured at a nearby (c. 2 km away) meteorological station at 1765 m asl during 2006–2013.
³ALA (2014).
⁴GBIF (2014).
⁵Hijmans et al. (2005).
⁶Mean diurnal range (average of years 1950–2000; °C).
⁷Daily maximum of warmest month (average of years 1927–1995; °C).
⁸Monthly maximum of warmest month (average of years 1957–2004; °C).
⁹Annual maximum (average of years 1957–2004; °C).
visible damage) was measured on each seedling and five to six seedlings per species were investigated. The species to be measured each day was selected randomly and different monospecific seedlings per species were investigated. The species to be measured for some replicates. As a result, the temperature range for the native species $E. excelsum$ for the exotic species $E. maidenii$ was probably a minor concern, as lacks of discernible peaks within the measured temperature range for half of the species introduced a comparatively large uncertainty regarding the $V_{cmax}$ high temperature responses (see the 'Photosynthetic temperature responses' subsection).

### Parameterization of photosynthesis models

The photosynthesis model by Farquhar et al. (1980), with modifications of photosynthetic temperature dependences (Bernacchi et al., 2001; Medlyn et al., 2002a), was used to parameterize the photosynthetic capacities $V_{cmax}$ and $J_{max}$ as well as the nonphotosynthetic respiratory CO2 release in the light ($R_d$), from $A-C$ curve data using the least-square method. The rates of $V_{cmax}$-limited photosynthesis ($A_t$) and $J_{max}$-limited photosynthesis ($A_j$) were estimated using Eqs 1 and 2:

$$ A_t = (1 - \frac{\Gamma_*}{C_i}) \cdot \frac{V_{cmax} C_i}{C_i + K(1 + \frac{Q}{\Gamma})} - R_d $$  \hspace{1cm} Eqn 1

$$ A_j = J - \frac{C_i}{4C_i + 8\Gamma} - R_d $$  \hspace{1cm} Eqn 2

(\(K_c\) and \(K_o\), Michaelis–Menten constants for CO2 and O2, respectively; \(\Gamma_*\), the CO2 concentration at which the carboxylation reaction of Rubisco equals the oxygenation reaction.) Values

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**Table 2** Information on species native distribution, average plant height, leaf size (taken as $0.7 \times$ length $\times$ width), leaf mass per unit area, leaf nitrogen content per unit area ($N_s$) and mass ($P_s$) and leaf phosphorus content per unit area ($P_m$) and mass ($P_m$)

| Species                  | Native distribution             | Plant height (m) | Leaf size (cm$^2$) | Leaf mass per unit area (g m$^{-2}$) | $N_s$ (g m$^{-2}$) | $P_s$ (mg m$^{-2}$) | $N_m$ (%) | $P_m$ (%) |
|--------------------------|---------------------------------|------------------|-------------------|-------------------------------------|-------------------|---------------------|-----------|-----------|
| Carapa grandiflora$^{1,2,3}$ | East African montane forest endemic | 0.85 ± 0.04 | 85.9 ± 6.6A | 81.4 ± 3.4A | 2.14 ± 0.09A | 106 ± 10AB | 2.63 ± 0.08A | 0.13 ± 0.01A |
| Entandrophragma excelsum$^{3,4}$ | Central East African forest endemic | 0.23 ± 0.02 | 35.7 ± 4.3B | 55.5 ± 2.08C | 1.33 ± 0.04B | 172 ± 14A | 2.39 ± 0.10A | 0.31 ± 0.03B |
| Hagenia abyssinica$^{2,3}$ | South-east Asia | 0.39 ± 0.05 | 24.0 ± 2.9B | 44.4 ± 2.8C | 1.58 ± 0.11B | 86.4 ± 17B | 3.56 ± 0.14B | 0.20 ± 0.04AB |
| Cedrela serrata$^6$ | East Australia; NSW and VIC | 0.82 ± 0.03 | 19.2 ± 1.6B | 56.8 ± 2.9BC | 2.18 ± 0.20A | 96.2 ± 12AB | 3.82 ± 0.24B | 0.17 ± 0.02A |
| Eucalyptus maidenii$^6$ | East Australia; NSW and QLD | 0.89 ± 0.06 | 60.2 ± 9.3C | 65.5 ± 1.5B | 2.26 ± 0.11A | 82.5 ± 7.9B | 3.45 ± 0.16B | 0.13 ± 0.01A |
| Eucalyptus microcorys$^6$ | *** | 15.7 ± 1.1B | 67.6 ± 8.0AB | 1.83 ± 0.18AB | 145 ± 42AB | 2.80 ± 0.20A | 0.20 ± 0.05AB |

Species investigated include native tropical montane species (Carapa grandiflora, Entandrophragma excelsum and Hagenia abyssinica) and exotic plantation species (Cedrela serrata, Eucalyptus maidenii and Eucalyptus microcorys). Values represent mean ± SE. The significance of results from ANOVA (species comparison) and Student’s t-test (native vs exotic species) is reported as: ns, $P > 0.05$; **, $P < 0.01$; ****, $P < 0.001$. The same capital letter indicates no significant difference between species according to Tukey’s post hoc test.

$^1$Kenfack (2011). $^2$Blosch et al. (2009). $^3$Fischer & Killmann (2008). $^4$Lemmens (2008). $^5$Orwa et al. (2009). $^6$Brooker & Klenig (2006).
(at 25°C) and temperature sensitivities of $\Gamma^*$, $K_C$ and $K_a$ were taken from Bernacchi et al. (2001). Values of $R_d$ are not reported because $A$–$C_i$ curve based estimates of $R_d$ are subject to rather large uncertainty.

Leaf mesophyll conductance to CO$_2$ was not estimated and therefore apparent $V_{c,max}$ and $J_{max}$ values were determined, based on $C_i$ rather than the CO$_2$ concentration at the chloroplast. The uncertainty of the values of curvature of the light response (0.9) and quantum yield of electron transport (0.3 mol electrons mol$^{-1}$ photons) used when calculating $J_{max}$ from actual electron transport ($j$) has only a slight effect on the estimated value of $J_{max}$ (Medlyn et al., 2002a). The only a priori restriction placed on the $V_{c,max}$ and $J_{max}$ determination through $A$–$C_i$ fitting was that data points with $C_i$ below 100 µmol mol$^{-1}$ were forced to be $V_{c,max}$-limited. Triose phosphate use limitation of photosynthesis, which mainly occurs at high $C_i$ and low temperature (Sage, 2002), was not observed in the $A$–$C_i$ curves of the present study (data not shown). As a consequence of low $g_s$ at higher temperatures, the $C_i$ did not reach values high enough for $A_n$ to be limited by the rate of electron transport in some $A$–$C_i$ curves. Determination of $J_{max}$ required that $A_n$ was clearly limited by $j$ in at least one data point, for which the value predicted by electron transport limitation was >10% lower than that predicted by carboxylation limitation (according to data at lower $C_i$). Inclusion of $J_{max}$ data also required that there were at least two $J_{max}$ values for each species and temperature combination. As a result, $J_{max}$ data were unavailable for 20 of the 150 $A$–$C_i$ curves obtained; mainly values at high temperatures in the exotic species $C. serrata$ and the native species $C. grandiflora$ and $E. excelsum$.

The temperature responses of photosynthetic capacities were determined by regressing $V_{c,max}$ and $J_{max}$ against measured $T_{leaf}$ using a peaked Arrhenius equation (Eqn 3), where a deactivation term accounts for the negative effects at higher temperatures (Medlyn et al., 2002a):

$$f(T_k) = \frac{H_d \exp \left( \frac{H_a (T_k - T_{opt})}{R T_{opt}} \right)}{H_d - H_a \left(1 - \exp \left( \frac{H_a (T_k - T_{opt})}{R T_{opt}} \right) \right)}$$

Eqn 3

($H_a$, the activation energy (kJ mol$^{-1}$); $T_{opt}$, optimum temperature (°K); $k_{opt}$, the value of $J_{max}$ or $V_{c,max}$ at $T_{opt}$ (µmol m$^{-2}$ s$^{-1}$); $H_d$, the deactivation energy (kJ mol$^{-1}$); $T_k$, the measured leaf temperature (°K); $R$, the universal gas constant (8.314 J mol$^{-1}$ K$^{-1}$).) By fitting the observed data to Eqn 3, $H_a$, $T_{opt}$, $k_{opt}$ and $H_d$ could be estimated. However, the peaked Arrhenius equation is overparameterized if all four parameters are allowed to vary (Dreyer et al., 2001; Medlyn et al., 2002a; Kattneg & Knorr, 2007) and, in such a case, data may be insufficient to reliably estimate all parameters. Therefore, following the method of previous studies on temperate and boreal species, $H_d$ was held at a constant 200 kJ mol$^{-1}$ for all species (Medlyn et al., 2002a; Kattneg & Knorr, 2007). In an analysis where $H_d$ was also allowed to vary, it was only significantly different from 200 kJ mol$^{-1}$ in one case ($V_{c,max}$ in $E. maidenii$).

As all replicates did not have data covering the entire temperature range, Eqn 3 was fitted to data pooled for each species. Fitted parameters were considered to significantly differ between two species if $P \leq 0.01$, that is, if the following relationship between mean values ($x$) and SE was true:

$$x_1 - x_2 - 2.58 \sqrt{SE_1^2 + SE_2^2} > 0$$

Eqn 4

While the probability of obtaining at least one significant difference by pure chance is 14% ($1 - 0.99^{15}$) when making 15 pairwise comparisons, the probability of obtaining at least two significant differences (as found here; Table 3) is just 2%.

Net photosynthesis temperature responses

To estimate biochemical limitations of $A_n$ at varying temperatures, the Farquhar et al. (1980) photosynthesis model parameters from each $A$–$C_i$ curve were used to calculate $A_n$ at a common $C_i$ and saturating photosynthetic photon flux density (1800 µmol m$^{-2}$ s$^{-1}$), for each leaf replicate and measurement temperature. The intercellular to ambient CO$_2$ concentration ratio ($C_i/C_a$) was assumed to be 0.7. As the global ambient CO$_2$ concentration at the time of measurement was 389 µmol mol$^{-1}$ (Thoning et al., 2014), the common $C_i$ was set to a constant value of 272 µmol mol$^{-1}$. In an additional analysis to assess the effect of 50% elevated CO$_2$ concentration on photosynthetic temperature optimum, $A_n$ was calculated at a common $C_i$ of 408 µmol mol$^{-1}$.

In order to also account for stomatal limitations of $A_n$ at increasing temperature, a third analysis was conducted in which the decrease in $C_i$ with increasing temperature and leaf-to-air vapour pressure deficit (VPD) was calculated according to a coupled stomatal-photosynthesis model assuming optimal stomatal behaviour ($A_n^{opt}$; Medlyn et al., 2011). The atmospheric vapour pressure was held constant at 1.6 kPa (the mean predawn value from the nearby meteorological station, which varied little over the year and remained fairly constant during the day). The $C_i$ concentrations calculated with this stomatal behaviour model were, for each 5°C increase between 20 and 40°C, 284, 253, 229, 209 and 192 µmol mol$^{-1}$. For a ±50% atmospheric CO$_2$ concentration scenario, the corresponding values were 427, 380, 344, 314 and 287 µmol mol$^{-1}$.

The temperature responses of $A_n$ at $C_i$ of 272 µmol mol$^{-1}$ ($A_{n272}$) and $C_i$ of 408 µmol mol$^{-1}$ ($A_{n408}$) were parameterized for each leaf replicate using nonlinear regression of a second-order equation (Säll & Pettersson, 1994; Battaglia et al., 1996; Gunderson et al., 2010), where $A_n(T)$ is the $A_n$ (µmol m$^{-2}$ s$^{-1}$) at a given air temperature $T$ (°C) and $A_{n^{opt}}$ is the $A_n$ at the optimum temperature ($T_{opt}$):

$$A_n(T) = A_{n^{opt}} - b(T - T_{opt})^2$$

Eqn 5

Eqn 5 was fitted to $A_{n272}$ and $A_{n408}$ data for individual leaves, and the significance of differences in $T_{opt}$ among species and provenances (i.e. cold-adapted vs warm-adapted) was determined.
Leaf traits and energy balance

Leaf size (length and width) was recorded with a ruler to the nearest millimetre. A hole punch (13 mm diameter) was used to collect discs from each leaf, avoiding major veins, in order to determine leaf mass per unit area (LMA) and leaf nitrogen and phosphorus content expressed per unit area ($N_a$ and $P_a$, respectively) and mass ($N_m$ and $P_m$, respectively). The collected leaf material was oven-dried at 70°C for at least 48 h before dry mass was recorded. The discs were milled using a ball mill with stainless steel grinding jars (Model MM 301; Retsch, Haan, Germany). Leaf nitrogen content was determined using an elemental analyser (EA 1108; Fison Instruments, Rodano, Italy). Leaf phosphorus content was determined by extracting and oxidating leaf phosphorus into phosphate (Valderrama, 1981), followed by determining phosphate content through spectrophotometry (Ames, 1966). Eighteen leaf samples were analysed for phosphorus content using a different method, inductively coupled plasma mass spectrometry (ICP-MS; Basic Suite 1VE1; ACME Analytical Laboratories, Vancouver, BC, Canada), in order to calibrate the spectrophotometric method.

Data on $g_s$ (at 25°C) and leaf dimensions, together with micrometeorological data collected at 7.5 m above ground from the local meteorological station, were used to model the annual mean leaf temperature (modelled $T_{leaf}$) of horizontal leaves at the hottest hour of the day (15:00 h) for each species. As a result of the large variation in vertical air temperature, the micrometeorological input data for the $T_{leaf}$ modelling were corrected for the 3°C higher temperature at canopy height (c. 1.0 m) and the corresponding vertical difference in VPD (assuming no variation in absolute air humidity). The horizontal wind speed at canopy height was estimated by assuming a logarithmic wind profile (Campbell & Norman, 1998). The micrometeorological data used in the energy balance calculations included mean annual air temperature (26.0°C; 23.0°C at 7.5 m), wind speed (0.45 m s$^{-1}$; 1.52 m s$^{-1}$), incoming radiation (381 W m$^{-2}$) and VPD (2.12 kPa; 1.36 kPa at 7.5 m) at 15:00 h. In an additional analysis, $T_{leaf}$ was estimated based on mean daytime (06:30–18:30 h) environmental conditions at canopy height (air temperature 23.9°C; wind speed 0.35 m s$^{-1}$; incoming radiation 285 W m$^{-2}$; VPD 1.36 kPa). Leaf energy balance equations were taken from Campbell & Norman (1998), with particular attention being paid to expressing variables on a relevant leaf area basis (i.e. there was often no discernible peak).

Leaf temperatures were also measured with infrared thermometers (Model IR-66; CEM, Shenzhen, China) on seedlings of all species except the exotic species E. microcyus. These were measured with infrared thermometers (Model IR-66; CEM, Shenzhen, China) on seedlings of all species except the exotic species E. microcyus. These values can be found in Table 3.
measurements were conducted on similarly sized plants growing in the nursery in March 2014, under sunny conditions in the early afternoon (13:00–15:00 h) of four days. On each day, \( T_{\text{leaf}} \) was measured on 20–30 leaves per species, selecting leaves with a horizontal leaf angle.

**Results**

**Photosynthetic temperature responses**

Values of \( J_{\text{max}} \) and \( V_{\text{cmax}} \) at 25°C differed among all six species (Table 3; Fig. 1: \( J_{\text{max}} \); \( P < 0.01 \); \( V_{\text{cmax}} \); \( P < 0.01 \)), but all had a similar \( J_{\text{max}} \) to \( V_{\text{cmax}} \) ratio (\( J : V \); mean 1.75 ± 0.07 SE), except for \( H. \) abyssinica which had a mean ratio of 2.29 ± 0.14 SE (\( P < 0.001 \)). The native species, \( E. \) excelsum and \( C. \) grandiflora, had the lowest values of both \( J_{\text{max}} \) and \( V_{\text{cmax}} \) (Table 3; Fig. 1). Additionally, \( g_s \) at 25°C differed significantly among species (\( P < 0.01 \); Table 3), in a similar pattern as for photosynthetic capacities. Comparisons between native tropical montane species and the exotic plantation species demonstrated that native species had significantly lower values of \( V_{\text{cmax}} \) (\( P = 0.04 \)) and higher \( J : V \) ratios than exotic species (\( P = 0.01 \)), while \( J_{\text{max}} \) and \( g_s \) did not differ between these two groups (Table 3).

Both \( J_{\text{max}} \) and \( V_{\text{cmax}} \) at 25°C had significant positive relationships with leaf nitrogen content, expressed on both an area (\( N \)) and a mass basis (\( N_{\text{in}} \); \( P < 0.04 \); Fig. 2); however, stronger relationships were found for \( N_{\text{in}} \) (Fig. 2). Leaf nitrogen content also differed significantly among species (\( P < 0.01 \); Table 2), where higher \( N_{\text{in}} \) was found in \( C. \) serrata, \( E. \) maidenii and \( H. \) abyssinica, and higher \( N_{\text{in}} \) was found in \( C. \) serrata, \( E. \) maidenii and \( C. \) grandiflora. The species also differed in LMA (\( P < 0.01 \); Table 2), with the highest values found in \( C. \) grandiflora and the lowest in \( H. \) abyssinica. Neither LMA nor leaf nutrient content significantly differed between native and exotic taxa (Table 2).

The leaf phosphorus content also differed among species (\( P < 0.01 \)), but not between native and exotic taxa (Table 2). The response of \( J_{\text{max}} \) and \( V_{\text{cmax}} \) at 25°C to leaf phosphorus content varied among species, on both a mass (\( P_{\text{in}} \)) and an area basis (\( P_{\text{in}} \); data not shown). The data could therefore not be pooled, as for the response to leaf nitrogen content, and data replication was deemed insufficient to conclude that there were any effects of leaf phosphorus content on photosynthetic capacities at an individual species level.

The activation energy (\( H_\text{a} \)) of \( V_{\text{cmax}} \) and \( J_{\text{max}} \) did not significantly differ among species, but \( H_\text{a} \) was generally higher for \( V_{\text{cmax}} \) than for \( J_{\text{max}} \) (paired t-test: \( P = 0.002 \)). \( H_\text{a} \) for \( V_{\text{cmax}} \) was also significantly higher in the native than in the exotic species (\( P = 0.04 \); Table 3), while \( H_\text{a} \) for \( J_{\text{max}} \) did not differ between the two groups.

Values of \( T_{\text{opt}} \) for \( V_{\text{cmax}} \) and \( J_{\text{max}} \) differed among species and ranged between 29.3 and 38.3°C and between 34.6 and 41.9°C, respectively (Table 3; Fig. 1). Values of \( T_{\text{opt}} \) were lower for \( J_{\text{max}} \) than for \( V_{\text{cmax}} \) in native species, but not in exotics (Table 3; Fig. 1). Native species also had significantly lower \( T_{\text{opt}} \) for \( J_{\text{max}} \) than exotic species (\( P < 0.01 \)), whereas \( T_{\text{opt}} \) for \( V_{\text{cmax}} \) did not differ (Table 3).

For \( J_{\text{max}} \) the temperature response curves had clear discernible peaks within the measured temperature range, particularly for the three native species (Fig. 1a). Similarly, clear peaks within the temperature responses for \( V_{\text{cmax}} \) could be produced for \( E. \) excelsum, \( H. \) abyssinica and \( C. \) serrata. However, the lack of clear peaks causes uncertainty in the \( T_{\text{opt}} \) estimates of \( V_{\text{cmax}} \) for the remaining three species, particularly \( C. \) grandiflora, for which measurements only occurred up to 35°C (Fig. 1b).

Three different parameters of \( A_{\text{n}} \) were determined: \( A_{\text{n}} \) at a constant \( C_i : C_{\text{n}} \) ratio of 0.7 (\( A_{n272} \)); \( A_{\text{n}} \) at a constant \( C_i : C_{\text{n}} \) ratio of 0.7 in an atmosphere with 50% higher CO₂ concentration (\( A_{n408} \)) and \( A_{\text{n}} \) at a \( C_i : C_{\text{n}} \) ratio that decreased with increasing temperature, as predicted by an optimal stomatal behaviour model (\( A_{nT} \); Medlyn et al., 2011; Fig. 3). The optimum temperatures of \( A_{n272} \) and \( A_{n408} \) did not significantly differ among species or between native and exotic species (Table 3). The average \( T_{\text{opt}} \) values for \( A_{n272} \) and \( A_{n408} \) across species were 26.3°C (± 0.85

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**Fig. 1** Responses of (a) maximum electron transport rate (\( J_{\text{max}} \)) and (b) maximum rate of Rubisco carboxylation (\( V_{\text{cmax}} \)) to temperature. Species include native tropical montane species (closed symbols and solid lines) and exotic species (open symbols and dashed lines). Error bars indicate ± SE. Lines represent the fitted peaked Arrhenius equations.
SE) and 29.0°C (±0.75 SE), respectively (Figs 3a, b, 4). The \( A_{nT} \) at ambient atmospheric CO\(_2\) concentration was negatively affected by temperatures above 20–25°C in all species and its \( T_{opt} \) could thus not be confidently estimated (Fig. 3c). This demonstrates that, when stomatal responses are also considered, photosynthetic optima drastically decrease. For a +50% atmospheric CO\(_2\) concentration scenario, \( A_{nT} \) peaked at 24–27°C (Fig. 3d).

In a sensitivity analysis, we assessed the influence of a 6% down-regulation in \( V_{cmax} \) under elevated CO\(_2\); a typical response of trees to growth in elevated CO\(_2\) (Ainsworth & Long, 2005). Such down-regulation had only a minor effect on \( T_{opt} \) of \( A_{n408} \), which was increased from 29.0 to 29.5°C (±1.2 SE) across all species (Supporting Information Fig. S1).

Leaf temperatures in relation to photosynthetic optimum temperatures

Leaf width, length and \( g_s \) (Table 2) along with micrometeorological parameters were used to model the annual mean \( T_{leaf} \) of the six species at the hottest hour (15:00 h) in the common garden. Modelled \( T_{leaf} \) values were considerably higher for species with large leaves and low \( g_s \) as compared with species with smaller leaves and/or higher \( g_s \) (Fig. 4; Table 2). Consequently, \( C. grandiflora \) and \( E. excelsum \) (large leaves, low \( g_s \)) were found to have the highest modelled \( T_{leaf} \) and \( E. microcorys \) (small leaves, high \( g_s \)) had the lowest, while the other species had intermediate values of leaf size, \( g_s \) and modelled \( T_{leaf} \). Field measurements collected at 15:00 h on four days in March 2014 confirmed the pattern of differences in modelled \( T_{leaf} \) among species (Fig. 4). During these observations, air temperature at the meteorological station was 25.3°C; that is, somewhat above the mean annual air temperature at 15:00 h of 23.0°C. Estimates of \( T_{leaf} \) based on average daytime (06:30–18:30 h) environmental conditions were 1–2°C lower than the estimates for 15:00 h (Fig. 4).

As the optimum temperature of photosynthesis (\( J_{max}, V_{cmax}, A_{n272} \) and \( A_{n408} \)) refers to leaf temperature, the results were compared with modelled and observed \( T_{leaf} \) values in order to determine acclimation capacity to the temperature of the common garden and potential sensitivity to global warming. Daytime mean and peak \( T_{leaf} \) greatly exceeded the \( T_{opt} \) for \( J_{max} \) in the native species \( C. grandiflora \) and \( E. excelsum \) (Fig. 4). In \( H. abyssinica \) and \( C. serrata \), values of \( T_{leaf} \) and \( T_{opt} \) for \( J_{max} \) were similar, while the two \( Eucalyptus \) species had considerably lower \( T_{leaf} \) than \( T_{opt} \) for \( J_{max} \). Daytime mean and peak \( T_{leaf} \) values were similar to \( T_{opt} \) for \( V_{cmax} \) in \( C. grandiflora \), \( E. excelsum \) and \( C. serrata \) but lower than \( T_{opt} \) for \( V_{cmax} \) in the other three species (Fig. 4). As for \( J_{max} \), the difference between optimal temperatures of photosynthetic capacities and \( T_{leaf} \) was largest (c. 10°C) for the two \( Eucalyptus \) species.

The optimal temperature of \( A_n \) at a \( C_i \) of 272 µmol mol\(^{-1}\) was 26.3°C averaged across all six species (±0.85 SE), and thus...
significantly lower than daytime mean and peak $T_{\text{leaf}}$ in all species (Fig. 4; Table 3). Increasing the CO$_2$ concentration by 50% increased the average optimum temperature of $A_n$ by 2.7 °C, from 26.3 to 29 °C, across all six species (Fig. 4; Table 3). The $T_{\text{opt}}$ for $A_n$ however, remained significantly below $T_{\text{leaf}}$ in all species except $E. microcorys$. The exceedance of $T_{\text{opt}}$ for both $A_n272$ and $A_n408$ by $T_{\text{leaf}}$ was largest in the two climax montane rainforest tree species $C. grandiflora$ and $E. excelsum$.

Accounting also for stomatal responses to increasing VPD as temperature rises, peak daytime $T_{\text{leaf}}$ exceeded the $T_{\text{opt}}$ for $A_n$ in all species under ambient CO$_2$ concentrations (Figs 3c, 4). For a +50% atmospheric CO$_2$ concentration scenario, $T_{\text{leaf}}$ exceeded the $T_{\text{opt}}$ for $A_n$ in all species except $E. microcorys$ (Figs 3d, 4).

**Discussion**

The overall objective of this study was to improve the limited understanding of temperature responses of photosynthesis in tropical tree species. This study has provided the first temperature response assessments of photosynthesis in tropical African montane trees and, to the best of our knowledge, the first estimates of temperature responses of photosynthetic capacities (i.e. $J_{\text{max}}$ and $V_{\text{cmax}}$) in tropical tree species.

Hypothesis (1) predicted that $J_{\text{max}}$ would be more sensitive to increased temperature than $V_{\text{cmax}}$, which was confirmed for the tropical montane species, with $T_{\text{opt}}$ being 5–7°C lower for $J_{\text{max}}$ than for $V_{\text{cmax}}$ (Table 3). This finding conforms to the concept of a greater dependence of electron transport than of Rubisco carboxylation on thylakoid membrane stability and, thus, higher sensitivity to high temperatures (Murakami et al., 2000; Sage & Kubien, 2007). It is also consistent with findings of previous studies on temperate and boreal species (Dreyer et al., 2001; Medlyn et al., 2002a; Kattge & Knorr, 2007). In contrast to the native montane rainforest species, the exotic plantation species had similarly high $T_{\text{opt}}$ for both $J_{\text{max}}$ and $V_{\text{cmax}}$ (Table 3). The $T_{\text{opt}}$ values for $J_{\text{max}}$ in the exotic species were in the upper range of those reported previously (Kattge & Knorr, 2007), indicating that the photosynthesis of these species is well adapted to high temperatures.

Hypothesis (2) predicted that the native montane species would have lower optimum temperatures for photosynthesis than the exotic plantation species, as a consequence of being adapted to a cooler climate combined with expected lower acclimation ability (Cunningham & Read, 2002; Way & Oren, 2010). This was confirmed for $J_{\text{max}}$ but not for $V_{\text{cmax}}$ (Table 3; Figs 1, 4), suggesting that thylakoid electron transport capacity does not readily acclimate to the environmental conditions of the common garden in the native montane species. This implies that there are genetically controlled differences in photosynthetic temperature responses between the native and exotic species of this study, relating to their adaptations to a colder or warmer origin.
The apparent contradiction of native and exotic species having different $T_{\text{opt}}$ for $J_{\text{max}}$ but not for $A_n$ at a common $C_i$ ($A_{n272}$ and $A_{n408}$) is caused by $A_n$ at CO$_2$ concentrations of 272 and 408 μmol mol$^{-1}$ typically being $V_{\text{max}}$ limited. The $T_{\text{opt}}$ for $V_{\text{max}}$ was not significantly different between native and exotic species (Table 3).

Hypothesis (3) predicted that limited acclimation ability of the native montane rainforest species would cause their estimated photosynthetic optimum temperatures to be frequently surpassed by their leaf temperatures, whereas the exotic plantation species, adapted to a warmer and more variable climate (Table 1), would not be as susceptible to high temperatures and thus would be better acclimated to the common garden conditions. This hypothesis was confirmed by the finding of very large (ter acclimated to the common garden conditions. This hypothesis adapted to a warmer and more variable climate (Table 1), would photosynthetic optimum temperatures to be frequently surpassed native montane rainforest species would cause their estimated leaf energy balance (i.e. leaf size and $g_s$) when evaluating plant sensitivity to air temperature and global warming. Different species grown together may have similar physiological temperature responses but still experience very different degrees of heat stress as a result of differences in their leaf energy budgets.

Our results demonstrate that photosynthesis of native montane rainforest climax tree species (i.e. C. grandiflora and E. excelsum) grown in a c. 5°C warmer habitat (Table 1) regularly operates at supraoptimal temperatures with respect to biochemical limitations (i.e. $A_{n272}$; Fig. 4), and that this situation is worsened if also considering stomatal limitations ($A_{n15}$; Fig. 3c). Our results thus support earlier suggestions that the commonly observed midday dips in photosynthesis are linked to supraoptimal temperatures and that tropical trees may be especially susceptible to warming (Clark et al., 2003; Clark, 2004; Doughty & Goulden, 2008). The finding of tropical climax species being particularly sensitive to high temperature is also in line with evidence from investigations of warming effects on the growth of tropical South American seedlings. For example, Chesman & Winter (2013) have demonstrated a stronger negative effect of warming on growth in seedlings of tropical climax species than in those of tropical pioneer species. While not conclusive or directly applicable to mature forest, this suggests that warmer ambient temperatures have the potential to threaten seedling growth and recruitment and potentially migration and survival rates of tropical montane rainforest climax species (Medjibe et al., 2014; Zhu et al., 2014). This may also affect not only canopy species, but co-occurring biota which may not be able to persist in nonclimax vegetation communities (Poulsen et al., 2011).

Hypothesis (4), predicting that a moderate climate change scenario (+50% increased atmospheric CO$_2$ concentration and +3°C) would have minimal effects on heat-induced reductions in photosynthesis, was corroborated by the finding that 50% increased atmospheric CO$_2$ concentration increased the optimum temperature of $A_n$ by 2.7°C across all species (Table 3). This finding of balancing effects of projected increases in CO$_2$ and temperature on the $T_{\text{opt}}$ exceedances of photosynthesis in tropical trees is in line with findings by Lloyd & Farquhar (2008). While heat-induced reductions in the photosynthesis of a given species may be unaltered by concurrently rising atmospheric CO$_2$ and temperature, global warming will probably affect the competitive balance between tropical tree species with different magnitudes of optimum temperature.

Fig. 4 Leaf temperatures in relation to photosynthetic optimum temperatures. Leaf temperatures are observations from four clear days ($T_{\text{Lobs}}$; red; 28.3°C air temperature at 1 m above ground) or modelled estimates based on mean annual weather data ($T_{\text{Lmod}}$; orange; upper values for 15:00 h weather; lower values for mean 06:30–18:00 h weather) for the native tropical montane species Carapa grandiflora, Entandrophragma excelsum and Hagenia abyssinica and the exotic plantation species Cedrela serrata, Eucalyptus maidenii and Eucalyptus microcorys. Also shown are optimum temperature ($T_{\text{opt}}$) of maximum electron transport rate ($J_{\text{max}}$; yellow) and maximum Rubisco carboxylation rate ($V_{\text{max}}$; blue). Error bars indicate ± SE. Horizontal lines indicate the optimum temperature for net photosynthesis at a constant internal CO$_2$ of 272 ($T_{\text{opt}}$ for $A_{n272}$; solid line; 26.3°C) and 408 μmol mol$^{-1}$ ($T_{\text{opt}}$ for $A_{n408}$; dashed line; 29°C).
exceedances. Our results (Fig. 4) as well findings in earlier experiments (Cheesman & Winter, 2013; Zhu et al., 2014) indicate that such effects would be to the disadvantage of montane rainforest climax species, which may be outcompeted by species exhibiting smaller heat-induced reductions in photosynthesis; that is, pioneer species or species adapted to a warmer climate.

In conclusion, results presented here demonstrate that the photosynthesis of seedlings of cool-adapted montane rainforest climax tree species is very sensitive to high temperature. The leaf temperatures of sunlit foliage at the hottest hour of the day in these species (c. 40°C) greatly exceeded the photosynthetic optimum temperatures, as a result of low transpiratory cooling (i.e. low gₛ) and inefficient heat dissipation (i.e. large leaf size). Furthermore, montane rainforest tree species had lower optimum temperatures for Jₘₐₓ compared with warm-adapted exotic plantation species, indicating that they do not readily acclimate to the c. 5°C warmer conditions in the common garden. Our results suggest that montane rainforest climax species may be particularly sensitive to future global warming and highlight the urgent need for more research on thermal responses of photosynthesis (considering biochemical and stomatal limitations as well as leaf energy balance) in tropical trees in order to better assess their sensitivity to global warming.

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**Supporting Information**

Additional supporting information may be found in the online version of this article.

**Fig. S1** Temperature responses of net photosynthesis at an elevated atmospheric [CO$_2$] of 408 μmol mol$^{-1}$ ($A_{408}$) using 6% lower $V_{c,max}$ values.

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