Temperature acclimation of net photosynthesis and its underlying component processes in four tropical tree species

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Received June 22, 2021; accepted January 11, 2022; handling Editor Marilyn Ball

The effect of temperature change on leaf physiology has been extensively studied in temperate trees and to some extent in boreal and tropical tree species. While increased temperature typically stimulates leaf CO\textsubscript{2} assimilation and tree growth in high-altitude ecosystems, tropical species are often negatively affected. These trees may operate close to their temperature optima and have a limited thermal acclimation capacity due to low seasonal and historical variation in temperature. To test this hypothesis, we studied the extent to which the temperature sensitivities of leaf photosynthesis and respiration acclimate to growth temperature in four common African tropical tree species. Tree seedlings native to different altitudes and therefore adapted to different growth temperatures were cultivated at three different temperatures in climate-controlled chambers. We estimated the acclimation capacity of the temperature sensitivities of light-saturated net photosynthesis, the maximum rates of Rubisco carboxylation ($V_{\text{cmax}}$) and thylakoid electron transport ($J$), and dark respiration. Leaf thylakoid membrane lipid composition, nitrogen content and leaf mass per area were also analyzed. Our results showed that photosynthesis in tropical tree species acclimated to higher growth temperatures, but that this was weakest in the species originating from the coolest climate. The temperature optimum of $J$ acclimated significantly in three species and variation in $J$ was linked to changes in the thylakoid membrane lipid composition. For $V_{\text{cmax}}$, there was only evidence of significant acclimation of optimal temperature in the lowest elevation species. Respiration acclimated to maintain homeostasis at growth temperature in all four species. Our results suggest that the lowest elevation species is better physiologically adapted to acclimate to high growth temperatures than the highest elevation species, indicating a potential shift in competitive balance and tree community composition to the disadvantage of montane tree species in a warmer world.

**Keywords**: climate change, $J_{\text{max}}$, $V_{\text{cmax}}$, warming.

**Introduction**

Increasing temperatures experienced by plants in a time of rapid climate change may have severe impacts on tropical ecosystems. Plant species in the humid tropical zone are generally expected to have a low ability to acclimate to changes in temperature since they do not experience seasonality in the same way as temperate and boreal species do (Janzen 1967, Way and Oren 2010, Feeley et al. 2012). In climates with pronounced seasons, plants have to be able to change their physiology and biochemistry over the course of a few weeks or months, and to later change back again. The physiological acclimation capacity of trees has mostly been studied in temperate, and to a certain extent also in boreal, regions (e.g., Tjoelker et al. 1999, Gunderson et al. 2000, Campbell et al. 2007, Kattge and Knorr 2007, Way and Sage 2008, Dillaway and Kruger 2010, Benomar et al. 2019). However, data on tropical trees are scarce
(Dusenge and Way 2017) despite tropical forests containing half of the carbon in the biomass on land (Saugier et al. 2001) and more than three-quarters of all terrestrial species (Barlow et al. 2018). Negative effects of warming on tropical forests could have large consequences for tree community composition and biodiversity (Feeley et al. 2011, Esquivel-Muelbert et al. 2019, Feeley et al. 2020), as well as forest capacity to store carbon and thereby mitigate ongoing climate change (Clark 2004, Lewis et al. 2004, Hubau et al. 2020).

Increasing temperatures have a range of implications for plant functioning, productivity and survival. Physiologically, higher temperatures alter the reaction rates of both photosynthesis and respiration, e.g., by changing the fluidity of membranes (Los and Murata 2004) and the activity and stability of enzymes like Rubisco and Rubisco activase (Sage and Kubien 2007, Yamori et al. 2014). Studies on photosynthetic acclimation in tropical trees conducted this far have mostly been based on measured responses of net photosynthesis ($A_n$), with contrasting results. In an in situ study, $A_n$ decreased in high-temperature grown plants (Doughty 2011). Studies using potted plants showed both decreased (Cheesman and Winter 2013b) and increased $A_n$ (Li et al. 2020a) in response to elevated growth temperature. Some studies showed a lack of acclimation capacity of the optimum temperature ($T_{opt}$) of $A_n$ (Ferrar et al. 1989, Sheu and Lin 1999, Smith and Dukes 2017, Crous et al. 2018) while other studies showed significant acclimation (Read 1990, Kositsup et al. 2009, Slot and Winter 2017b), ranging from slight upward shifts in $T_{opt}$ to complete acclimation. These shifts in $T_{opt}$ could be accompanied by an increase (Kositsup et al. 2009) or by a decrease (Slot and Winter 2017b) in absolute values of $A_n$ at $T_{opt}$. In two tropical forests in Panama, as well as in a common garden in Rwanda, community average $T_{opt}$ was remarkably similar across species and almost identical to mean maximum daytime air temperature (Vårhammar et al. 2015, Slot and Winter 2017a). The shape of the temperature response curve in tropical tree species has been shown to exhibit a sharper peak shape than for temperate species, meaning that high $A_n$ is maintained over a narrower temperature span (Cunningham and Read 2002).

The differences among these observations may be due to several processes contributing to the realized $A_n$ temperature responses. Both of the two major rate-limiting steps in photosynthesis—the maximum velocities of CO$_2$ fixation by Rubisco ($V_{cmax}$) and electron transport capacity by the thylakoid electron transport chain ($J_{max}$)—are temperature dependent. Moreover, values of $A_n$ are also affected by leaf respiration ($R$) and stomatal conductance ($g_s$). Measuring $A_n$ alone does not separate between these different processes and how, or if, they acclimate. For better mechanistic understanding of photosynthetic thermal acclimation, it is therefore advisable to study the different processes that together determine $A_n$, i.e., $V_{cmax}$, $J_{max}$, $R$ and $g_s$ (Lin et al. 2012, Dusenge and Way 2017).

So far, only three studies have explicitly reported instantaneous temperature responses of $V_{cmax}$ and $J_{max}$ for tropical trees. Vårhammar et al. (2015) compared the temperature responses of species with different climatic backgrounds. However, acclimation could not be assessed as the study was conducted in a common garden with the same environmental conditions. A second study (Crous et al. 2018) investigated the temperature responses of $V_{cmax}$ and $J_{max}$ in two Eucalyptus species acclimated to different temperatures in a glasshouse experiment. The measured range for the individuals of tropical provenances however did not cover $T_{opt}$. A third study (Dusenge et al. 2021) showed a slight, but nonsignificant shift in $T_{opt}$ of $V_{cmax}$ and $J_{max}$ of two Rwandan tropical tree species in response to increased growth temperature.

Leaf dark respiration ($R_{dark}$) in tropical trees has been shown to be a highly adjustable trait. Rates at a reference temperature are lower in warm-grown compared with cool-grown trees (Cheesman and Winter, 2013a, 2013b, Slot et al. 2014, Drake et al. 2015, Slot and Winter 2018, Mujawamariya et al. 2020). Across biomes, there is usually a partial acclimation (Atkin et al. 2015, Slot and Kitajima 2015, Smith and Dukes 2017), with decreased $R_{dark}$ at a common temperature, but increased $R_{dark}$ at $T_{growth}$ with warming. This has also been reported for tropical tree species (Slot et al. 2014). More often, though, it has been found that $R_{dark}$ is equal at all $T_{growth}$—so-called homeostasis (Slot and Winter 2018, Mujawamariya et al. 2020)—or that even more extreme reductions in $R_{dark}$ with warming lead to a decrease in $R_{dark}$ with $T_{growth}$ (Cheesman and Winter 2013a, Mujawamariya et al. 2020).

We herein report results from an experiment on whether and how much tropical trees acclimate $A_n$ and its underlying processes ($V_{cmax}$, $J$, $R$ and $g_s$) to an increase in growth temperature in four native Rwandan tree species selected to cover a range of climatic origins. We hypothesized that (i) both $A_n$ and photosynthetic capacity ($V_{cmax}$ and $J$) exhibit thermal acclimation to warming, but it is partial and weaker than observed for temperate and boreal species; (ii) $R_{dark}$ downregulates in plants grown at high temperature, such that rates at prevailing growth temperature do not significantly differ among treatments; and (iii) the species originating from the warmest climate show a larger heat acclimation capacity of photosynthesis than the species from the coolest climate.

**Materials and methods**

**Plant material**

We selected four widely distributed African tropical tree species: *Maesa lanceolata*, Forssk. (Mla), *Ficus thoningii*, Blume (Fth), *Croton megalocarpus*, Hutch. (Cme) and *Markhamia lutea*, (Benth.) K. Schum. (former *M. plathycaul*, Mlu). They differ in climatic origin and, to some extent, successional strategy (Table 1). The altitudinal distribution of the species was
Table 1. Species taxonomy, native elevation and temperature range and successional strategy. Species are ordered according to the lowest altitude at which they are reported in Rwanda.

| Species                  | Short | Family        | Altitudinal range (m) | Mean air T (°C) | Successional strategy |
|--------------------------|-------|---------------|-----------------------|-----------------|-----------------------|
| Maesa lanceolata         | Mla   | Primulaceae   | 1350–3000             | 12–21           | Early                 |
| Ficus thonningii         | Fth   | Moraceae      | 1000–2300             | 16–22           | Early and late        |
| Croton megalocarpus      | Cme   | Euphorbiaceae | 950–2400              | 15–23           | Early                 |
| Markhamia lutea          | Mlu   | Bignoniaceae  | 950–1800              | 18–23           | Early and late        |

1From two Rwandan floras; 950 m is the lowest elevation in Rwanda and given if no minimum elevation is provided. Species occur outside Rwanda, but African tropical floras have been excluded since latitudes may be much higher.
2Ranges correspond to the alitudinal range, data from the years 1970–2000.
3Species occurs in both early and late-successional stands.

retrieved from Fischer and Killmann (2008) and Bloesch et al. (2009). For each species, we report the extreme low and the extreme high elevation value stated. We also report the annual mean air temperatures at these elevations (Table 1), extrapolated from climate (Fick and Hijmans 2017) and remote sensing databases (US Geological Survey 2021).

Mlu is a montane species, which typically occurs in stands at the early successional stage. It produces seeds that are eaten and dispersed by a variety of bird and monkey species (Graham et al. 1995). Fth is a mid- to high-altitude species that can occur in both early- and late-successional stands. Its leaves and twigs are a food source for many different mammal species (World Agroforestry Centre 2021a), and its fruits are also eaten by a range of bird species (Kirika et al. 2008). Cme is a mid-altitude species, but can also occur in montane forests up to 2400 m. It is an early successional species. Its seeds are used as poultry feed due to their high content of protein and leaves are used as mulch in agriculture because of their high levels of nitrogen and phosphorus content (World Agroforestry Centre 2021b). Mlu has the lowest altitudinal range in this study, though very close to Cme. It occurs in both early- and late-successional stands and has been shown to be an important food source for black-and-white colobus, Colobus guereza (Onderdonk and Chapman 2000). All four species are used in traditional medicine and in forestry for their timber (Sindambiwe et al. 1996, Lacroix et al. 2009, Aliyu et al. 2010, Dangarendembi et al. 2013).

Plant cultivation and treatments

The seeds for this experiment were collected in research plantations at Rubona research station, Huye district, Rwanda (−2°28′54.9″ S, 29°46′00.1″ E, Cme and Mlu) and in the nearby Ruhande Arboretum (−2°36′49.9″ S, 29°45′24.7″ E, Mla). Fth plants were grown from cuttings of six different trees in Rubona. The genetic origin of the mother plants may though be different. We grew seedlings in planting soil (peat with perlite) in 0.2-l pots at a common temperature in growing rooms in Gothenburg, Sweden. Depending on the rate of growth, they were transplanted to 1-l pots (all four species) and later to 2-l pots (Fth and Mlu). At harvest, plant dry mass was 2–40 g only (Figure S1 available as Supplementary data at Tree Physiology Online), so pot size should not have been limiting plant growth much. They were well-watered (thrice a week) and fertilized (once a week) with nitrogen, phosphorus, potassium (NPK ratio 5:1:4) and the most essential minerals (B, Cu, Fe, Mn, Mo, Zn and Mg) throughout the experiment. When at least four leaves were fully developed (~3–4 months after germination or plantation of cuttings), six individuals of each species were randomly assigned to each of three treatments: 20, 25 and 30 °C growth temperature during daytime, with 5 °C lower nighttime temperatures in climate-controlled chambers (Percival Scientific, Perry, IA, USA). In the following, Tgrowth values refer to daytime temperatures in all text and figures except in the context of dark respiration where nighttime temperatures are more relevant. Photosynthetic photon flux density (PPFD) was 400 μmol m−2 s−1 from 6:00 to 18:00 h and light was otherwise off. This radiation level is lower than the 600–800 μmol m−2 s−1 daytime PPFD found in the origin forests of these species (Dusenge et al. 2021), but not unrealistically low since values of 400 μmol m−2 s−1 are common under cloudy conditions or at lower sun angles. Ventilation was installed to the climate-controlled chambers to ensure stable ambient CO2 concentrations. Temperature and relative air humidity in the chambers was measured every 30 min. Vapor pressure deficit was calculated to be on average 0.76, 1.53 and 1.34 kPa at the three different growth temperatures (20, 25 and 30 °C, respectively). The reason for rather similar vapor pressure deficits in the 25 and 30 °C treatments was the richer irrigation in 30 °C chambers, with a water mirror covering the trays onto which pots were placed for longer time after each irrigation. The 20 and 25 °C treatments cover the range of mean annual day temperatures at the lowest altitude at which the four species are reported in Rwanda (21–23 °C; Table 1) while the 25 and 30 °C treatments are equivalent to future climate change scenarios. A 5 °C difference between day and night temperatures was chosen since the diurnal variation in temperature is 4–5 °C in the origin forests in Rwanda (Mujawamariya et al. 2020). The difference between the coolest and warmest months in the origin forests is only 2 °C (Dusenge et al. 2021), and this seasonal variation is not represented in this experiment.
Gas exchange measurements

When all 18 trees of one species had developed new leaves under chamber treatment conditions, leaf gas exchange was measured on one attached leaf per plant using two LI6400 instruments equipped with the 6-cm² standard leaf chamber and the red-blue light source (LI-COR, Lincoln, NE, USA). The PPFD was set to 400 μmol m⁻² s⁻¹, which equaled the daytime PPFD inside the growth chambers and was near light saturation according to light response measurements made for each species. We measured photosynthetic responses to leaf intercellular CO₂ concentration (c_li) (so-called A–C_i curves) at leaf temperatures in 5 °C intervals from 15 to 40 °C, starting at the lowest temperatures. Measurements were taken at 12 concentrations of CO₂ of the air entering the leaf chamber: 415, 60, 120, 200, 300, 415, 700, 1000, 1350, 1700, 2000 and again 415 μmol mol⁻¹. At high temperatures, air humidity was increased by connecting a LI610 dew point generator (LI-COR) to the LI6400. The air temperature of the room where measurements were made was increased alongside leaf temperature up to ∼35 °C and the LI610 was set at a temperature a few degrees below room temperature to avoid condensation. Nevertheless, stomatal closure responses were unavoidable and in some leaves, A–C_i curves could not be reliably used for V_cmax and J determination at the highest temperatures due to low g_s (≤0.02 mol m⁻² s⁻¹). In the figures relating to photosynthesis, we only show data with n > 2, apart from Cme and Mla at T_growth 20 °C and T_leaf 40 °C, where n = 2.

In addition to A–C_i curves, we also measured steady-state g_s and R_d. Acclimated g_s was recorded after waiting 30 min at 25 °C and at respective growth temperature (if different) for each plant. VPD during the g_s measurements increased across species from 1.0 at 20 °C and 1.5 at 25 °C to 2.0 at 30 °C. Leaf R_d measurements were conducted on neighboring leaves after 30 min dark acclimation at a CO₂ concentration (C_a) of 415 μmol mol⁻¹. It was measured in 5 °C steps between 15 and 30 °C.

Photosynthesis

The parameters V_cmax and J were estimated from the A–C_i curves using the Farquhar et al. (1980) biochemical model of C₃ photosynthesis with updated temperature response parameters for Rubisco kinetics (Bernacchi et al. 2001). The model, which assumes that photosynthesis is limited by either V_cmax or J, was fitted to data using the least squares method. Apparent values of V_cmax and J, parameterized based on C_i, are reported. The equation for carboxylation-limited photosynthesis (A_c; rate limiting at low C_i) is:

\[ A_c = V_{cmax} \left( \frac{C_i - \Gamma^*_s}{C_i + K_c \left( 1 + \frac{O}{K_o} \right)} \right) - R_d, \]  

(1)

where \( \Gamma^*_s \) is the CO₂ concentration at which the carboxylation reaction of Rubisco equals the oxygenation reaction, \( K_c \) and \( K_o \) are the Michaelis–Menten constants of Rubisco for carboxylation and oxygenation, respectively, \( O \) is the partial pressure of oxygen inside the leaf and \( R_d \) is respiration in light.

At high C_i, photosynthesis is limited by J (A_j; J produces energy to regenerate RuBP, ribulose1, 5-bisphosphate, in the Calvin cycle) and is calculated as:

\[ A_j = \frac{J \left( C_i - \Gamma^*_s \right)}{4C_i + 8\Gamma^*_s} - R_d. \]  

(2)

We determined J instead of J_max since measurements were made at PPFD of 400 μmol mol⁻¹. R_d in Eqs. (1) and (2) was allowed to vary along with V_cmax or J in the fitting. All determined values of V_cmax and J (from all six trees per species and treatment) were used to fit a peaked Arrhenius function (Medlyn et al. 2002):

\[ f(T_k) = k_{opt} \frac{H_d \exp \left( H_a(T_k - T_{opt})/T_kRT_{opt} \right)}{H_d - H_a \left( 1 - \exp \left( H_d(T_k - T_{opt})/T_kRT_{opt} \right) \right)}, \]  

(3)

where \( k_{opt} \) is the value of J or V_cmax at T_opt, \( H_d \) is the deactivation energy determining the rate of decrease of the function above the optimum, and \( H_a \) is the activation energy determining the rate of initial exponential increase of the function below the optimum. Values of T_opt, k_{opt} and H_d were fitted while H_a was assumed constant at 200 kJ mol⁻¹ to avoid over-parameterization (Medlyn et al. 2002).

Photosynthesis at a common C_i of 290 μmol mol⁻¹ (i.e., 70% of ambient air CO₂) was estimated from the fitted A–C_i curves to isolate biochemical influences on photosynthesis from stomatal limitations. Temperature response functions of A_h at a C_a of 415 μmol mol⁻¹ (the first measurement of each A–C_i curve) and at a constant C_i of 290 μmol mol⁻¹ were fitted to a second-order equation (Säll and Pettersson 1994, Gunderson et al. 2000):

\[ A_h(T) = A_{opt} - b(T - T_{opt})^2, \]  

(4)

where A_h(T) is A_h at a given air temperature (T), A_{opt} is A_h at T_{opt} and b is a constant that determines the width of the peak of the curve.

As an indicator for water-use efficiency, we calculated g_1 (inversely related to intrinsic water-use efficiency) from the model for optimal stomatal conductance by Medlyn et al. (2011):

\[ g_s = g_0 + 1.6 \left( 1 + \frac{g_1}{\sqrt{D}} \right) \frac{A_h}{C_a}, \]  

(5)

where g_0 is the g_s (for water vapor) at zero A_h, 1.6 is the ratio of the diffusivities of water vapor and CO₂, and D is the leaf-to-air water vapor pressure deficit. The g_0 was assumed to be zero since values are often small (Medlyn et al. 2011).
Respiration
The temperature response function of \( R \) was fitted for each leaf by determining the \( R_{\text{dark}} \) value at the lowest measured temperature, 15 °C (\( R_{15} \)), and the \( Q_{10} \) value (the proportional change in respiration per 10 °C change in \( T_{\text{leaf}} \); Tjoelker et al. 1999):

\[
R_{\text{dark}(T)} = R_{15} * Q_{10}^{(T-15)/10}, \tag{6}
\]

where \( R_{\text{dark}(T)} \) is respiration in the dark at a certain measurement temperature in the range 15–30 °C (\( T \)).

Leaf traits, thylakoid membrane lipids and nitrogen content
After gas exchange measurements, the leaves were sampled for the determination of leaf mass per area (LMA) and nitrogen content. For this, 6–8 20 mm diameter leaf discs from the central part of both sides of the leaf (avoiding the mid-vein) were taken, when possible. For small leaves (\( F_{\text{th}} \)), an 8-mm diameter puncher was used instead. Half of the leaf discs were dried and weighed to determine LMA. The bulk leaf material (except the midvein) was dried and ground in a ball mill. Thereafter, nitrogen content was measured by dry combustion using an element analyzer (EA 1108; Fison Instruments, Rodano, Italy).

The remaining half of the discs were immediately frozen in liquid nitrogen and transferred to a −80 °C freezer. Thylakoid lipids were extracted from these samples using a modified Bligh and Dyer lipid extraction protocol (Kourtchenko et al. 2007). The thylakoid lipid composition was determined using liquid chromatography coupled to tandem mass spectrometry using an Agilent 1200 LC system coupled to either an Agilent 6470 Triple Quadrupole mass spectrometer or/and Agilent 6410 triple quadrupole mass spectrometer. The lipids were separated using a C30 column and a solvent gradient as previously described (Nilsson et al. 2019). The thylakoid lipid species were monitored using the transitions and settings previously described (Nilsson et al. 2014). The total amount of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), phosphatidylglycerol (PG), sulfoquinovosyldiacylglycerol (SQDG) was quantified as a sum of the species for each lipid class.

The tree seedlings were harvested after the measurements and dry mass of stems, leaves and roots were determined. For \( F_{\text{th}} \), only new plant parts were included in the biomass determination, not the cuttings.

Statistics
We tested assumptions for two-way ANOVAs on gas exchange variables, biomass and lipid content of the thylakoid membrane. Since most variables had heterogeneous variances, we subsequently conducted species-specific one-way ANOVAs for all parameters but respiration, which fulfilled assumptions (two-way ANOVA, followed by Tukey). If variances before one-way ANOVA were still heterogeneous (i.e., between treatments), or residuals were non-normally distributed, we conducted non-parametric Kruskal–Wallis tests (see footnotes in Table 2 and Table S1 available as Supplementary data at Tree Physiology Online).

Significant differences between optimum temperatures were determined as follows: since Eqs (3) and (4) were fitted to data pooled for each growth temperature and species, we conducted three pairwise treatment comparisons and accounted for multiple comparisons by choosing a \( P \) limit of 0.017 to determine significance. This means that the probability of obtaining at least one significant difference by pure chance will be 5% (\( 1 - (1 - 0.017)^3 \)). Species means were therefore regarded significantly different if the following relationship between mean (\( \bar{x} \)) and standard error of the mean (SE) values was true:

\[
| x_1 - x_2 | - 2.39 \sqrt{SE_1^2 + SE_2^2} > 0. \tag{7}
\]

\( A-C_1 \) curves were fit in Microsoft Excel, all other analyses were performed in R 4.0.0 (R Core Team 2020).

Results
Net photosynthesis and underlying component processes
All four species showed some flexibility in \( A_{\text{h}} \) and/or its underlying processes under varying growth temperatures. The photosynthetic performance at high growth temperature markedly improved with the species origin temperature, being best for the lowest elevation species and worst for the highest elevation species. In the figures, species are therefore ordered according to decreasing lowest elevation of their native central African distribution: \( M_{\text{la}}, F_{\text{th}}, C_{\text{me}} \) and \( M_{\text{lu}} \).

All species had a higher \( T_{\text{opt}} \) of \( A_{\text{h}} \) at ambient \( C_{\text{O}_2} \) concentration (\( A_{\text{C}_{415}} \)) in the warmer treatments, but it was only statistically significant in the highest elevation species, \( M_{\text{la}} \), and the lowest elevation species, \( M_{\text{lu}} \) (Figure 1). Averaged across all species, \( T_{\text{opt}} \) acclimated to 0.41 °C per 1 °C warming (Figure 2). The performance advantage of cool-grown plants at low \( T_{\text{leaf}} \) was particularly pronounced in the highest elevation species, \( M_{\text{la}} \), which exhibited increased \( A_{\text{C}_{415}} \) at low \( T_{\text{leaf}} \) in the 20 °C treatment (Figure 1a). The lowest elevation species, \( M_{\text{lu}} \), had significant treatment (\( T_{\text{growth}} \)) differences in \( A_{\text{C}_{415}} \) at both low and high \( T_{\text{leaf}} \). This was the species that benefitted most from higher growth temperature, with 30 °C-grown \( M_{\text{lu}} \) plants exhibiting strong stimulation of \( A_{\text{C}_{415}} \) at high \( T_{\text{leaf}} \) compared with plants grown at 20 and 25 °C (Figure 1d). The two intermediate-elevation species were also intermediate in their responses to warming. The warming response of \( A_{\text{C}_{415}} \) measured at respective \( T_{\text{growth}} \) differed between species. There was a significant decrease in \( A_{\text{C}_{415}} \) at \( T_{\text{growth}} \) with warming in the highest elevation species \( M_{\text{la}} \), no significant change in \( C_{\text{me}} \) and \( F_{\text{th}} \), and an increase in the lowest elevation species \( M_{\text{lu}} \) (Table 2).

Photosynthesis at the ambient \( C_{\text{O}_2} \) concentration in the air is determined by the photosynthetic performance itself, but also
Table 2. \(P\)- and \(F\)-values of one-way ANOVA (and \(P\)- and \(x^2\)-values in case of non-parametric Kruskal–Wallis tests for data with non-normal distribution or heterogeneous variances) for the effect of growth temperature on parameters derived from gas exchange measurements\(^1\) and on relative amount of thylakoid lipid classes and average amount of double bonds in the lipid classes in the four different species (short names explained in Table 1). Significant (\(P < 0.05\)) and near-significant (\(0.05 < P \leq 0.10\)) values are in bold and regular font style, respectively. ns, not significant.

| Variables                  | Mla          | F/\(x^2\) | \(P\) | Fth         | F/\(x^2\) | \(P\) | Cme          | F/\(x^2\) | \(P\) | Mlu          | F/\(x^2\) | \(P\) |
|----------------------------|--------------|-----------|-------|-------------|-----------|-------|--------------|-----------|-------|--------------|-----------|-------|
| \(A_{C415} at T_{growth}\) | 7.52\(^2\)   | 0.023\(^3\) | ns    | ns          | ns        | 3.89  | 0.043        |
| \(A_{C290} at T_{growth}\) | ns\(^2\)     | ns        | ns    | ns          | ns        | 5.02  | 0.021        |
| \(g_s at T_{growth}\)     | 61.94        | <0.001    | ns    | ns          | ns        | 3.58  | 0.054        | 5.30      | 0.018 |
| \(g_s at T_{25}\)         | ns           | ns        | ns    | ns          | ns        | 17.76 | <0.001       |
| \(g_1 at T_{growth}\)     | 38.30        | <0.001    | ns    | ns          | ns        | 8.28  | 0.004        |
| \(g_1 at T_{25}\)         | ns           | ns        | ns    | ns          | ns        | 19.83 | <0.001       |
| \(C/C_s at T_{growth}\)   | 62.94        | <0.001    | ns    | ns          | ns        | 5.70  | 0.014        | 2.87      | 0.088 |
| \(C/C_s at T_{25}\)       | ns           | 3.29      | 0.067 | ns          | ns        | 26.91 | <0.001       |
| \(V_{\text{cmax}}\)       | ns\(^2\)     | ns        | ns    | ns          | ns        | 10.93 | 0.001        |
| \(J_25/V_{\text{cmax}}\)  | 14.48        | <0.001    | ns    | ns          | ns        | 6.44  | 0.010        | 20.55     | <0.001|
| Biomass                    | 4.75         | 0.025     | 6.19  | 0.024       | 10.98     | 0.001 | 8.18         | 0.004     |
| Root/shoot                 | ns           | ns        | ns    | 3.28        | ns        | 0.068 |
| LMA                        | ns\(^2\)     | ns        | ns    | ns          | ns        |       |              |
| Nitrogen per area          | ns           | 10.65     | 0.001 | ns          | ns        | 4.72  | 0.034        |
| %MGDG                      | 12.28\(^2\)  | 0.002\(^2\) | ns    | ns          | ns        | 11.14 | 0.001        |
| %DGDG                      | 11.49        | 0.001     | ns    | ns          | ns        | 9.58  | 0.002        |
| %PG                        | 7.10         | 0.008     | ns    | ns          | ns        |       |              |
| %SQDG                      | ns           | ns        | ns\(^2\) | ns\(^2\) | ns\(^2\) |       |              |
| ADB MGDG                   | 10.34\(^2\)  | 0.006\(^2\) | ns    | ns          | ns        | 3.75  | 0.052        | 25.84     | <0.001|
| ADB DGDG                   | 16.59        | <0.001    | ns    | ns          | ns        | 8.80  | 0.012\(^2\) | 12.32\(^2\) | 0.002\(^2\) |
| ADB PG                     | 97.53        | <0.001    | ns    | 18.02\(^2\) | <0.001   | 11.09 | 0.001        |
| ADB SQDG                   | 16.99        | <0.001    | 16.22 | <0.001      | 11.20     | 0.001 | 6.53         | 0.009     |

\(^1\)The parameters are: net photosynthesis at ambient CO\(_2\) and at growth temperature (\(A_{C415} at T_{growth}\)) and at a CO\(_2\) concentration of 290 \(\mu\)mol mol\(^{-1}\) (\(A_{C290} at T_{growth}\)), stomatal conductance at growth temperature (\(g_s at T_{growth}\)) and at 25 °C (\(g_s at T_{25}\)), the \(g_1\) parameter (see Eq. (5)) at growth temperature (\(g_1 at T_{growth}\)), and at 25 °C (\(g_1 at T_{25}\)), the ratio of internal leaf CO\(_2\) to ambient air CO\(_2\) at growth temperature (\(C/C_s at T_{growth}\)), and at 25 °C (\(C/C_s at T_{25}\)), \(V_{\text{cmax}}\), \(J\) and dark respiration at 25 °C (\(V_{\text{cmax}}\), \(J_25\) and \(R_{25}\)), the change in respiration per 10 °C change in measurement temperature (\(Q_{10}\)), total dry biomass (Biomass), the ratio of root dry biomass to shoot dry biomass (Root/shoot) and leaf mass per unit area (LMA).

\(^2\)Results (\(x^2\) and \(P\)-values) for non-parametric Kruskal–Wallis tests.

The specific importance of \(A_n\) can better be seen using a common CO\(_2\) concentration of 290 \(\mu\)mol mol\(^{-1}\) (70% of CO\(_2\) concentration in the air) in the leaf intercellular spaces (\(A_{C290}\)). \(T_{\text{opt}}\) of \(A_{C290}\) was also higher in the warmer treatments (Figure 2), but this was only significant in the high-elevation species Mla. Across all species, the change in \(T_{\text{opt}}\) was on average 0.50 °C per 1 °C increase in \(T_{\text{growth}}\) (Figure 3). The \(A_{C290}\) results of the two intermediate species did not differ a lot from those on \(A_{C415}\). The lowest elevation species Mlu showed a higher magnitude of \(A_{C290}\) across all \(T_{\text{leaf}}\) in cold-grown compared with warm-grown plants. For \(A_{C290}\) at \(T_{\text{growth}}\), the three higher altitude species showed no significant change with warming, while it decreased with warming in Mlu. The large shift in \(T_{\text{opt}}\) in the latter species was nonsignificant due to a high variation and uncertainty for plants grown at a daytime temperature of 30 °C.

Stomatal conductance at \(T_{\text{growth}}\) significantly decreased in Mla and increased in Mlu by more than 50% with warming (Figure 4a). The change in \(A_{C415} at T_{\text{growth}}\) in Mla (decrease) and Mlu (increase, Figure 1a and d) was thus not caused by acclimation of photosynthetic capacity (\(A_{C290}\), Figure 3) but rather by acclimation of \(g_s\) to growth under high temperatures. Similar patterns were seen for \(g_1\) at \(T_{\text{growth}}\) (Figure 4c), the parameter of the combined stomatal conductance photosynthesis model that is inversely related to intrinsic water-use efficiency and the \(C_i/C_s\) ratio (though not significant in Mlu, Figure 4e). Response patterns at a measurement temperature of 25 °C were similar to those daytime at \(T_{\text{growth}}\), except that Mlu did not show any difference among temperature treatments (Figure 4b, d and f).
Figure 1. Temperature dependence of net photosynthesis at light saturation and ambient CO₂ in the tree species *Maesa lanceolata* (a), *Ficus thonningii* (b), *Croton megalocarpus* (c) and *Markhamia lutea* (d) grown at daytime temperatures of 20, 25 or 30 °C. Data points are means ± SE. Large points indicate the values at the growth temperature of each treatment. The top part of each panel shows the estimated optimum temperatures. Asterisks denote significant differences among growth temperatures tested at each leaf temperature ($P \leq 0.05$) and between optimal temperatures ($P \leq 0.017$). Statistical results are provided in Table 2 and Table S2 (available as Supplementary data at Tree Physiology Online).

plants (Figure 5). For some species and treatments (*Cme* at 25 °C; *Cme*, *Fth* and *Mlu* at 30 °C), $T_{opt}$ was estimated above the measured $T_{leaf}$ range. The shift in $T_{opt}$ across all four species, and excluding estimates above the measurement range, was on average 0.24 °C per 1 °C increase in $T_{growth}$ (Figure 2). The capacity for carboxylation at 25 °C ($V_{cmax25}$) remained unchanged with warming in three species and decreased only in *Mlu* (Table 2, Figure S2 available as Supplementary data at Tree Physiology Online).

The $T_{opt}$ of photosynthetic electron transport ($J$) increased significantly under higher $T_{growth}$ in all species except *Mlu* (again due to a high variation in the 30 °C treatment, Figure 6, $P = 0.033$). The shift in $T_{opt}$ was on average 0.33 °C per 1 °C increase in $T_{growth}$ (Figure 2). Absolute values of $J$ at 25 °C ($J_{25}$) showed that *Cme* and *Mlu* significantly reduced $J$ at 25 °C with $T_{growth}$ (Table 2, Figure S2 available as Supplementary data at Tree Physiology Online).

We determined whether $V_{cmax}$ or $J$ was limiting $A_{Ca415}$ for all $A$–$C_{i}$ curves using Eqs (1) and (2). The species *Mla* and *Cme* were most often limited by $V_{cmax}$ at the lowest $T_{growth}$ and got more often limited by $J$ at higher $T_{growth}$. The species *Fth* was at nearly all $T_{growth}$ and $T_{leaf}$ limited by $V_{cmax}$. The species *Mlu*, on the other hand, was most often limited by $J$ at all $T_{growth}$, but especially in 30 °C-grown plants.

**Thylakoid membrane lipid compositions**

Analysis of the thylakoid lipids revealed that both the lipid class composition, as well as the average amount of double bonds in each class, was affected by growth temperature. The degree of lipid unsaturation decreased with increasing $T_{growth}$ in almost
all species and lipid classes (Figure 7, Table 2). Mla and Mlu downregulated the amount of double bonds (ADB) in all four thylakoid lipid classes. Cme also downregulated ADB of DGDG, PG and SQDG. The species Fth, on the other hand, decreased ADB in only SQDG (Figure 7h). Linear regression showed that the average ADB of all lipid classes correlated with Topt of J (P < 0.05, data not shown). The amount of MGD in relation to other thylakoid lipids increased with warming in Mla and Mlu while the proportion of DGDG and PG decreased. The effect was particularly strong in Mla. Linear regression showed that J25 significantly increased with an increased ratio of MGDG/DGDG across all species (P = 0.033).

Leaf respiration

Dark respiration at a leaf temperature of 25 °C (R25) differed significantly between species (P < 0.001) and Tgrowth. (P < 0.001). It was higher in Fth than in all other species and decreased from nighttime Tgrowth of 15–25 °C as well as from 20 to 25 °C significantly across species (Figure 8). On average, R25 decreased by 35–56% with a 10 °C increase in Tgrowth. The decrease of R25 did not significantly differ between species. Dark respiration at nighttime Tgrowth was constant across treatments, indicating full acclimation, or homeostasis, of leaf Rdark (striped bars in Figure 8). While Rdark at a given Tleaf decreased with increasing Tgrowth, the shape of the temperature response curves stayed the same. This is shown by the Q10 values (the proportional change in respiration per 10 °C change in Tleaf), which did not significantly differ between treatments or species. Across all treatments and species, Q10 averaged 2.3 with an SE of 0.2.

Plant biomass and leaf traits

The total biomass of all species was affected by Tgrowth (Table 2). Mla acquired less biomass in the warmest Tgrowth than at colder temperatures, while the other three species acquired more biomass in warmer temperatures (Figure S1 available as Supplementary data at Tree Physiology Online). In Cme and Mlu, there was a reduction between 25 and 30 °C treatments. This is in accordance with gas exchange data, showing that An at Tgrowth decreased in Mla only (Figure 1). The ratio of root dry biomass to leaf and shoot dry biomass (Root/shoot) as well as LMA did not change with Tgrowth in any species. Leaf nitrogen on an area basis decreased significantly with high Tgrowth in Cme, but did not change in the other species (Figure S1 available as Supplementary data at Tree Physiology Online).

Discussion

Our results highlight how differently tropical tree species respond to altered growth temperature. The montane species Mla showed decreased performance at high temperatures due to a decrease in gs, while the lowest elevation species Mlu showed improved performance reflected in An and biomass increase (Table 3). The intermediate-elevation species were also intermediate in their responses to warming. Moreover, the contributions to the overall responses by acclimation of different component processes (Vcmax, J, gs and Rdark) were highly species-specific. One of the most important outcomes of this study is therefore that photosynthetic acclimation cannot be thoroughly understood (or predicted) by looking at An responses only. In the following, we will discuss the different component processes of photosynthesis in the four species.

This is, to the best of our knowledge, the second study to explicitly estimate the long-term acclimation capacity of Topt for both Vcmax and J in tropical tree species. A previous study on Rwandan tropical trees grown in the field reported that shifts in Topt of Vcmax and Jmax in response to increased growth temperature were too low to be significant (Dusenge et al. 2021). Another study explored short-term temperature responses of Vcmax and Jmax in plants acclimated to different temperature treatments for 1 week and found significant Topt shifts in Vcmax and Jmax (Smith and Dukes 2017). Tropical species showed a shift in Topt of Jmax of 0.12 °C per 1 °C change in Tgrowth, but the value for Vcmax was not reported. Otherwise, previous studies have determined Topt acclimation for An only. A global meta-analysis with mostly data for temperate species found that thermal acclimation of An reaches usually ~0.5 °C per 1 °C
change in $T_{\text{growth}}$ in C$_3$ species (Yamori et al. 2014). Our results suggest that changes in $T_{\text{opt}}$ of $A_n$ in tropical tree species may be in the same range (0.41 °C across species in this study) but that there is high variability between species. Changes in $T_{\text{opt}}$ of $V_{\text{cmax}}$ and $J$ were overall a bit lower (0.24 and 0.33 °C, respectively) than for $A_n$. The observed shift in $T_{\text{opt}}$ of $V_{\text{cmax}}$ is similar to previous results on tropical trees, but the shift in $T_{\text{opt}}$ of $J$ is stronger in our study (Smith and Dukes 2017, Dusenge et al. 2021). These shifts also showed high variability across species, roughly aligning with, but not fully explaining the $T_{\text{opt}}$ shifts in $A_n$, which were also influenced by responses of $g_s$. Other types of physiological responses that were not analyzed in this study, like, for example, mesophyll conductance, may also have the shift in $T_{\text{opt}}$ (Warren 2007, Li et al. 2020b).

Light-saturated photosynthesis is usually limited by $V_{\text{cmax}}$ in trees (De Kauwe et al. 2015), including tropical species (Vårhammar et al. 2015). Our study is in agreement with this, except for Mlu, which was mostly limited by $J$. Under field conditions with varying radiation, photosynthesis will constantly change between $V_{\text{cmax}}$ and $J$ limitation, with leaves allocating resources between the two processes to optimize overall carbon assimilation (Niinemets et al. 2004, Quebeman and Ramirez 2016). It is therefore important to understand thermal acclimation of Rubisco carboxylation as well as the mechanisms underlying shifts in the electron transport capacity of thylakoid membranes to assess how warming affects $A_n$.

The degree of flexibility in $T_{\text{opt}}$ of $J$ can be explained by changes in the unsaturation of the thylakoid membranes. Increased saturation keeps the membranes stable at higher temperatures and allows for a change in $T_{\text{opt}}$ of $J$ (electron transport) across these membranes. Thylakoid membrane lipid unsaturation was in general lower in the 30 °C treatment than in the 20 °C treatment, with few exceptions (Figure 7). The decrease in unsaturation was mainly due to a decrease in the most highly unsaturated species of the galactolipids.

Figure 3. Temperature dependence of net photosynthesis at light saturation and a common leaf intercellular CO$_2$ concentration (C$_i$) of 290 p.p.m. in the tree species Maesa lanceolata (a), Ficus thonningii (b), Croton megalocarpus (c) and Markhamia lutea (d). Asterisks denote significant differences among growth temperatures tested at each leaf temperature ($P \leq 0.05$) and between optimal temperatures ($P \leq 0.017$).
Tropical tree temperature acclimation

Figure 4. Stomatal conductance ($g_s; a, b$), the slope parameter of the stomatal-photosynthesis model ($g_1$, see Eq. (5); $c, d$) and the ratio of leaf intercellular to ambient air CO$_2$ concentration ($C_i/C_a; e, f$) at growth temperature (left-hand panels) and 25 °C (right-hand panels) in the tree species Maesa lanceolata ($Mla$), Ficus thonningii ($Fth$), Croton megalocarpus ($Cme$) and Markhamia lutea ($Mlu$) grown at daytime temperatures of 20, 25 or 30 °C. Data points are means ± SE and asterisks denote significant differences ($P \leq 0.05$) among treatments.

Figure 5. Temperature dependence of the maximum rate of Rubisco carboxylation ($V_{cmax}$) at light saturation is expressed relative to the value at 25 °C in the tree species Maesa lanceolata ($a$), Ficus thonningii ($b$), Croton megalocarpus ($c$) and Markhamia lutea ($d$). Figure explanations as for Figure 1, but without testing for differences in magnitude of the dependent variable. The $T_{opt}$ for Croton megalocarpus and Markhamia lutea (not visible in figure) were estimated at 45.3 and 44.1 °C, respectively, with an SE of 5.5 and 2.0. Asterisks denote significant differences among growth temperatures tested at each leaf temperature ($P \leq 0.05$) and between optimal temperatures ($P \leq 0.017$).

Table 3. Results overview: arrows represent upward or downward acclimation of the respective process with $T_{growth}$. Colors represent positive (blue) and negative (red) effects of high $T_{growth}$ on plant performance. The thick and narrow arrows represents a significant ($<0.05$) or near-significant ($0.05 < P \leq 0.10$) shift (in $T_{opt}$, significant values have $P < 0.017$ due to multiple comparisons).

| Mla | Fth | Cme | Mlu |
|-----|-----|-----|-----|
| $T_{opt}$ of $V_{cmax}$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ |
| $T_{opt}$ of $J$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ |
| $g_s$ at $T_{growth}$ | $\downarrow$ | $\downarrow$ | $\downarrow$ | $\downarrow$ |
| $R_{25}$ | $\downarrow$ | $\downarrow$ | $\downarrow$ | $\downarrow$ |
| ADB | $\downarrow$ | $\downarrow$ | $\downarrow$ | $\downarrow$ |
| $A_{415}$ at $T_{growth}$ | $\downarrow$ | $\downarrow$ | $\downarrow$ | $\downarrow$ |
| Total biomass | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ |
Figure 6. Temperature dependence of photosynthetic electron transport (J) at light saturation expressed relative to the value at 25 °C in the tree species *Maesa lanceolata* (a), *Ficus thonningii* (b), *Croton megalocarpus* (c) and *Markhamia lutea* (d) grown at daytime temperatures of 20, 25 or 30 °C. Figure explanations as for Figure 1, but without testing for differences in the magnitude of the dependent variable. Asterisks denote \( P \leq 0.1 \). The \( T_{\text{opt}} \) for *Markhamia lutea* (not visible in figure) was estimated at 46.9 °C. Asterisks denote significant differences among growth temperatures tested at each leaf temperature (\( P \leq 0.05 \)) and between optimal temperatures (\( P \leq 0.017 \)).

There are several reasons why an increase in MGDG with warming may be beneficial. It has been shown that small heat shock proteins prefer to bind to the non-bilayer forming lipids, such as MGDG, rather than for instance, PG and DGDG (Balogi et al. 2005). Another possibility is that the change in MGDG/DGDG shows, rather than a change in membrane composition, a change in the ratio of grana to stroma thylakoids. Grana thylakoids are higher in MGDG than stroma thylakoids (Gounaris et al. 1983) and they are richer in photosystem II (PSII), which is the more heat-labile of the two photosystems (Routaboul et al. 2011). It may therefore be beneficial for plants to increase the amount of grana thylakoids (and thus MGDG) with warming.

Temperature-induced changes in the MGDG/DGDG ratio (both increase and decrease) have been recorded before, but it is commonly a decrease in the ratio that is associated with thermotolerance, not an increase (Chen et al. 2006, Wang and Lin 2006, Su et al. 2009, González-Cruz and Pastenes 2012, Zhang et al. 2018). Increasing thermotolerance with the amount of DGDG has been suggested to be related to this lipid class’ role in stabilizing extrinsic proteins in the oxygen-evolving complex (González-Cruz and Pastenes 2012). In our study, the highest elevation species instead significantly decreased in DGDG (Mla) with \( T_{\text{growth}} \) and it is noteworthy that this is the species that showed a decrease in photosynthesis and biomass with temperature (Figure 1a, Figure S2a available as Supplementary data at *Tree Physiology* Online). However, the decrease in photosynthesis was not due to reduced photosynthetic capacity, but decreased \( g_s \) (Figure 4a), so an influence of DGDG is unlikely. The exact link between decreased unsaturation, increased MGDG content and the acclimation of the electron transport chain to higher growth temperature remains to be understood. Nevertheless, both phenomena were well linked to a higher ability of temperature acclimation of photosynthetic electron transport.

The decrease in \( A_n \) at high \( T_{\text{growth}} \) in the high-altitude species *Mla* was caused by decreased \( g_s \) with warming.
Tropical tree temperature acclimation

Figure 8. Dark respiration ($R_{\text{dark}}$) measured at 15, 20, 25 and 30 °C in the trees species *Maesa lanceolata* (a), *Ficus thonningii* (b), *Croton megalocarpus* (c) and *Markhamia lutea* (d) grown at 15, 20 or 25 °C (nighttime temperatures, 5 °C lower than daytime temperatures). Dark respiration at 25 °C differed significantly between temperature treatment as well as between species ($P < 0.001$), with no significant interaction between these factors. Dark respiration at growth temperature did not significantly differ between temperature treatments. Bars are means ± SE ($n = 6$). Striped bars represent measurements at nighttime growth temperature in each treatment.

(Figure 4a and b), rather than by decreased photosynthetic capacity, as shown by $A_{C290}$ (Figure 3a). This confirms the importance of considering stomatal responses when interpreting $A_n$ responses. However, few studies have investigated the response of $g_s$ to warming in tropical species, as well as in other biomes (Locke et al. 2013, Way and Yamori 2014). A study on tropical tree seedlings showed that $g_s$ at growth temperature decreased with warming, but did not differ at a common temperature (Slot and Winter 2017b). This is generally similar to the temperature response observed here in *Mla*, *Fth* and *Cme* (Figure 4). *Mlu*, however, increased $g_s$ measured at $T_{\text{growth}}$ as well as at 25 °C. Water was not a limiting factor in the current study, but since water availability will most likely decrease under future warming, the *Mlu* response to increase $g_s$ at higher $T_{\text{growth}}$ may turn out not to be as good a strategy as it was in this experiment.

A global meta-analysis including five tropical studies suggested that $R_{\text{dark}}$ at varying $T_{\text{growth}}$ commonly acclimates in tropical tree and liana species, but only partially (Slot and Kitajima 2015). On the contrary, a recent field study showed complete or even over-compensatory acclimation in 16 African tropical tree species (Mujawamariya et al. 2020). That study included the four species described here and found homeostasis in *Mlu* and *Cme* across all sites and over-compensation in *Mla* and *Fth* at the warmest site. In our study, $R_{\text{dark}}$ at respective $T_{\text{growth}}$ did not change with warming in any species (Figure 8). A possible reason for larger down-regulation in $R_{\text{dark}}$ in *Mla* and *Fth* in the field study compared with this well-watered climate chamber study is that heat-induced reductions in photosynthesis may be larger under field conditions. Acclimation in $R_{\text{dark}}$ has been shown to be dependent on $A_n$, such that $R_{\text{dark}}$ acclimation depends on substrate availability and, thus, changes in $A_n$ (Dusenge et al. 2019).

Conclusions

We investigated temperature acclimation of photosynthesis and its underlying processes in four Rwandan tree species.

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*Figure 8. Dark respiration ($R_{\text{dark}}$) measured at 15, 20, 25 and 30 °C in the trees species *Maesa lanceolata* (a), *Ficus thonningii* (b), *Croton megalocarpus* (c) and *Markhamia lutea* (d) grown at 15, 20 or 25 °C (nighttime temperatures, 5 °C lower than daytime temperatures). Dark respiration at 25 °C differed significantly between temperature treatment as well as between species ($P < 0.001$), with no significant interaction between these factors. Dark respiration at growth temperature did not significantly differ between temperature treatments. Bars are means ± SE ($n = 6$). Striped bars represent measurements at nighttime growth temperature in each treatment.*
The growth temperatures in this experiment represent natural conditions in Rwandan forests—including Nyungwe national park, the largest remaining montane tropical forest in Africa—as well as conditions that may be expected with climate change in this region in the next century. We found that the $T_{opt}$ of $A_n$ and its underlying processes is able to acclimate, but acclimation was only partial. Dark respiration was found to acclimate strongly and reach homeostasis, as has been found for tropical species before. We also show that $g_s$ is key to realize potential photosynthesis. In our experiment, the montane species Mla showed a decrease in $g_s$ in response to warming alone which is, likely to be aggravated in a climate that is also drier. On the contrary, the lowest elevation species Mlu increased $g_s$ at higher growth temperature. This increased the performance of Mlu in the warm treatments, but may not be a viable strategy in a climate that is also drier.

Our results support what has been found in South American tropical forests along elevation gradients: that warming can give a competitive advantage to low-elevation warm-adapted species and lead to thermophilization of tree communities (Duque et al. 2015, Fadrique et al. 2018). Poor physiological acclimation of native high-elevation species may contribute to this trend and put them at risk in future tropical climates. Research on these species is therefore a matter of urgency in order to gain more knowledge about highland ecosystems in the tropics, with all their valuable functions.

Supplementary data
Supplementary data for this article are available at Tree Physiology Online.

Acknowledgments
We are grateful to the Rwanda Agriculture and Animal Resources Development Board (RAB) for the supply of germplasm. We would also like to thank Louise C. Andersen for constructive comments on the manuscript and Mirindi E. Dusenge for helpful discussions about data analysis and interpretation.

Conflict of interest
None declared.

Funding
This work was funded by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas; 2015-1458) and the Swedish Research Council (VR; 2015-03338).

Authors’ contributions
M.W., J.U. and G.W. planned and designed the study, B.N. assisted as expert on tree traits and choice, M.W., L.T., G.W. and M.X.A. performed the experiments, M.W., J.U., M.X.A., L.T. and G.W. analyzed and interpreted the data, M.W. and J.U. wrote the manuscript and M.W., J.U., M.X.A., L.T., B.N. and G.W. revised the manuscript.

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