Limited thermal acclimation of photosynthesis in tropical montane tree species

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Abstract

The temperature sensitivity of physiological processes and growth of tropical trees remains a key uncertainty in predicting how tropical forests will adjust to future climates. In particular, our knowledge regarding warming responses of photosynthesis, and its underlying biochemical mechanisms, is very limited. We grew seedlings of two tropical montane rainforest tree species, the early-successional species *Harungana montana* and the late-successional species *Syzygium guineense*, at three different sites along an elevation gradient, differing by 6.8°C in daytime ambient air temperature. Their physiological and growth performance was investigated at each site. The optimum temperature of net photosynthesis (T\text{optA}) did not significantly increase in warm-grown trees in either species. Similarly, the thermal optima (T\text{optV} and T\text{optJ}) and activation energies (E\text{aV} and E\text{aJ}) of maximum Rubisco carboxylation capacity (V\text{cmax}) and maximum electron transport rate (J\text{max}) were largely unaffected by warming. However, V\text{cmax}, J\text{max} and foliar dark respiration (R\text{d}) at 25°C were significantly reduced by warming in both species, and this decline was partly associated with concomitant reduction in total leaf nitrogen content. The ratio of J\text{max}/V\text{cmax} decreased with increasing leaf temperature for both species, but the ratio at 25°C was constant across sites. Furthermore, in *H. montana*, stomatal conductance at 25°C remained constant across the different temperature treatments, while in *S. guineense* it increased with
The temperature sensitivity of photosynthesis in tropical tree species remains a significant uncertainty in predicting global forest productivity and climate feedbacks in a warmer climate (Booth et al., 2012; Mercado et al., 2018; Smith & Dukes, 2013). Global climate models project a warming of 1–1.5 and 4–5°C for the forested tropical region during the present century under the best- and worst-case climate change scenarios, respectively, compared to the period 1986–2005 (IPCC, 2013). This degree of warming is small compared to changes expected for higher latitudes, and which may thus lead to the conclusion that tropical forests are at lower risk in future climates. However, tropical forests have experienced stable thermal regimes both on short (e.g., seasonal) and geological timescales (Trewin, 2014). It has therefore been hypothesized that these forests have a narrow thermal niche with limited capacity to acclimate (Janzen, 1967). It has also been shown that tropical plants are currently operating at temperatures that are close to their upper thermal limits (Lloyd & Farquhar, 2008; Sentinella et al., 2020). Recent findings show that both community- and ecosystem-scale photosynthetic thermal optima are close to the ambient air temperatures currently experienced by tropical trees (Huang et al., 2019; Slot & Winter, 2017a; Tan et al., 2017), suggesting that they will have to thermally acclimate photosynthesis to avoid operating at supra-optimal temperatures in future climates. However, few studies have assessed the capacity of photosynthetic thermal acclimation in tropical species (Booth et al., 2012; Mercado et al., 2018). Tropical highland or montane tree species may be particularly sensitive to warming, as recently seen in Andean studies along elevation gradients which show declines in the relative abundance of these species within their historical habitat during recent decades (Duque et al., 2015; Fadrique et al., 2018). This decline of highland species in their native habitat is followed by the colonization of species initially from lower and warmer elevation, in a process termed “thermophlization” (Duque et al., 2015).

Thermal acclimation of net photosynthesis ($A_n$) typically results in an upward shift in the thermal optimum ($T_{\text{optA}}$) of the instantaneous temperature response of $A_n$ (Gunderson et al., 2009; Slot & Winter, 2017b; Way, 2019; Way & Yamori, 2014; Yamori et al., 2014). In most species, photosynthetic acclimation to warming results in an increase of the $T_{\text{optA}}$ by about 0.43–0.62°C per 1°C of warming (Berry & Björkman, 1980; Kumarathunge et al., 2019; Way, 2019; Way & Yamori, 2014). However, this estimate of the thermal sensitivity of $T_{\text{optA}}$ is heavily based on studies on temperate and boreal species (Berry & Björkman, 1980; Kumarathunge et al., 2019; Sendall et al., 2015; Way & Yamori, 2014). Of the relatively few studies on tropical trees investigating responses of $T_{\text{optA}}$ to elevated growth temperatures, some report significant upward shifts in $T_{\text{optA}}$ with warming (Cunningham & Read, 2003; Kositsup et al., 2009; Read, 1990; Slot & Winter, 2017b), while others do not (Carter et al., 2020; Crous et al., 2018).

In temperate and boreal trees, adjustments in the shape of the instantaneous temperature response of net photosynthesis and its $T_{\text{optA}}$ have been shown to be linked to changes in temperature sensitivity of underlying process components such as thermal optimum and activation energies of the maximum Rubisco carboxylation capacity of Rubisco, $V_{\text{cmax}}$ ($T_{\text{optV}}$ and $E_v$), and the maximum electron transport rate, $J_{\text{max}}$ ($T_{\text{optJ}}$ and $E_J$), and the ratio of $J_{\text{max}}$ to $V_{\text{cmax}}$, $U_{\text{max}}/V_{\text{cmax}}$; Dusenge et al., 2020; Kumarathunge et al., 2019; Slot & Winter, 2017b; Stefanski et al., 2019; Way, 2019; Yamaguchi et al., 2016). So far in tropical tree species, shifts in $T_{\text{optA}}$ with warming were seen to coincide with decreases in the ratio of $J_{\text{max}}$ to $V_{\text{cmax}}$ (Kositsup et al., 2009; Slot & Winter, 2017b), probably reflecting within-leaf resource allocation since photosynthesis is commonly more carboxylation limited at higher temperatures (Smith & Keenan, 2020). The $T_{\text{optV}}$, $T_{\text{optJ}}$, $E_v$, $E_J$ and $J_{\text{max}}/V_{\text{cmax}}$ are also important parameters in dynamic global vegetation models when representing net photosynthesis and its thermal acclimation (Kumarathunge et al., 2019; Mercado et al., 2018). To date, only a very few studies have investigated thermal acclimation of the temperature sensitivities of $V_{\text{cmax}}$ and $J_{\text{max}}$ in tropical trees (Crous et al., 2018; Fauset et al., 2019; Kositsup et al., 2009; Smith & Dukes, 2017). However, none of them was able to accurately determine $T_{\text{optV}}$ and $T_{\text{optJ}}$. Clearly, more studies that investigate the effect of warming on the temperature sensitivities of $V_{\text{cmax}}$ and $J_{\text{max}}$ and their relative importance to shifts in $T_{\text{optA}}$ are needed in tropical trees, and particularly in highland species.

In addition to shifts in $T_{\text{optA}}$, acclimation of photosynthesis to warming also affects the magnitude of photosynthetic rates. Shifts in the magnitude of $A_n$ at the new, warmer growth conditions are
largely driven by changes in the achieved rates of $V_{cmax}$, $J_{max}$ and stomatal conductance ($g_d$; Crous et al., 2018; Dusenge et al., 2020; Scafaro et al., 2017; Way & Sage, 2008). Meta-analyses, dominated by studies on boreal and temperate tree species, have showed that $V_{cmax}$ and $J_{max}$ rates measured at a common leaf temperature of 25°C ($V_{cmax25}$ and $J_{max25}$) are largely unaltered by warming (Kattge et al., 2009; Kumarathunge et al., 2019; Way & Oren, 2010). Across individual studies, some did not find any effect of warming on either $V_{cmax25}$ or $J_{max25}$ (e.g., Bermudez et al., 2020; Carter et al., 2020; Fauset et al., 2019; Lamba et al., 2018; Stefanski et al., 2019), while others observed significant declines (e.g., Dusenge et al., 2015, 2020; Way & Sage, 2008), or increases (e.g., Crous et al., 2013) in warm-grown plants. Therefore, there is no consistent acclimation response of photosynthetic capacity to warming. The few data available on tropical trees show that $V_{cmax25}$ and $J_{max25}$ were not impacted by warming (Scafaro et al., 2017; Crous et al., 2018; Fauset et al., 2019; but see Kositsup et al., 2009).

Although $g_d$ decreases as a response to the short-term increases in the vapor pressure deficit (VPD) that are typically associated with increasing temperature (Grossiord et al., 2020; López et al., 2021; Oren et al., 1999), $g_d$ may also acclimate to long-term warming. In different tree species, $g_d$ increased (Marchin et al., 2016), decreased (Dusenge et al., 2020; Fauset et al., 2019; Lamba et al., 2018) or remained unchanged (Drake et al., 2015; Dusenge et al., 2020) with growth at elevated temperatures. The few studies that explored the warming effect on $g_d$ in tropical tree species showed that $g_d$ tends to be reduced in warm-grown trees when measured at growth conditions (Drake et al., 2015; Fauset et al., 2019). In tropical tree species, $g_d$ often imposes strong limitations on $A_{n}$ (Doughty & Goulden, 2008; Tan et al., 2017), and may thus partly underlie observed progressive reductions in tropical carbon sink strength and tree growth during recent decades (Hubau et al., 2020; McDowell et al., 2018; Sullivan et al., 2020). Thus, further studies on thermal adjustments of both photosynthetic capacity and $g_d$ in tropical tree species grown in field conditions are critically needed to improve our understanding of how warming may affect net CO$_2$ assimilation, tree growth and plant-climate feedbacks of tropical forests in future climates (Mercado et al., 2018).

Foliar respiration ($R_f$), which uses carbohydrates from photosynthesis as substrate, supply energy for maintenance of leaf functions and allocation of carbohydrates to other parts of the tree for growth (O’Leary et al., 2019). It is thus a key determinant of forest growth and carbon storage in future warmer climates. Although foliar respiration increases near exponentially with short-term increase in temperature (Atkin & Tjoelker, 2003; O’Sullivan et al., 2013; Smith & Dukes, 2017), it is commonly found that respiration acclimates and is lower at a given temperature in warm-grown trees (e.g., Reich et al., 2016; Slot & Kitajima, 2015; Zhu et al., 2020). This thermal acclimation of respiration has also been observed in trees from the tropics (Cheesman & Winter, 2013a, 2013b; Drake et al., 2015; Scafaro et al., 2017; Slot et al., 2014; Slot & Winter, 2017b, 2018; Smith & Dukes, 2017). A recent study from the same elevation gradient experiment used in this study (Rwanda-TREE), but with 16 montane tropical tree species freely rooted in the soil, reported that thermal acclimation of leaf respiration at the warmer, lower-elevation sites led to complete or even over-compensation of respiration (Mujawamariya et al., 2020). Altogether, these findings suggest that foliar respiration readily acclimates to warming, and may have little constraint on carbon availability for tropical tree growth in future climates.

The least-cost theory of photosynthesis posits that in any given environment, $A_{n}$ is optimized at the lowest cost, mainly associated with nitrogen (N) and water uptake (Prentice et al., 2014; Smith et al., 2019; Wright et al., 2003). Therefore, the biochemical demand for (i.e., Rubisco carboxylation and associated N costs) and supply of carbon (via stomatal conductance) are generally balanced such that a given net CO$_2$ assimilation rate is achieved at the lowest water loss in any environment (Prentice et al., 2014). Furthermore, based on optimality theory of photosynthetic capacity, $V_{cmax}$ measured at a given common temperature should be lower in plants grown in warmer growth conditions compared to their cool-grown counterparts (Smith & Keenan, 2020; Wang et al., 2020). This is because enzymatic reactions are faster at high temperatures; therefore, warm-grown plants can achieve optimal net CO$_2$ assimilation rates with a relatively lower photosynthetic enzyme content (Smith & Keenan, 2020; Yamori et al., 2014). Since both Rubisco carboxylation and electron transport rates, and the contents of the enzymes that regulate each process (Rubisco and chlorophyll pigments) are co-regulated (Lu et al., 2020; Maire et al., 2012; Wullschleger, 1993), then warm acclimated plants should consequently reduce both $V_{cmax}$ and $J_{max}$. Since respiration is involved in providing energy for protein turn over, which is the largest factor that explains variation in leaf $R_f$, then thermal acclimation of respiration should closely follow that of photosynthetic capacity (Wang et al., 2020). Therefore, with reduced metabolic leaf N content, then leaf respiration should also be suppressed in warm-grown plants (Wang et al., 2020).

With the goal of improving our understanding of how tropical trees respond to a warmer climate, we assessed the thermal acclimation capacity of net photosynthesis and its component processes in two tropical montane tree species grown at three different sites along an elevational gradient in the Rwanda-TREE (Tropical Elevation Experiment) project. We further evaluated to what extent these leaf physiological processes were linked to changes in leaf nitrogen status and tree growth responses at the different sites. Based on previous research and proposed theories, the following four hypothesized predictions were tested:

$H_1$: The $T_{opt}$ of $A_{net}$, $V_{cmax}$ and $J_{max}$ increases in a warmer climate, but the acclimation is only partial (i.e., approximately 0.5°C per 1°C of warming).

$H_2$: Based on both least-cost and photosynthetic optimality theories, $g_d$ and photosynthetic capacity (i.e., $V_{cmax}$ and $J_{max}$) at a common temperature decrease with warming.

$H_3$: Leaf $R_f$ rates measured at a common leaf temperature shows strong downward acclimation to warming.

$H_4$: Thermal acclimation of photosynthetic capacity and leaf respiration are tightly correlated, and linked to leaf N.
MATERIALS AND METHODS

2.1 | Experimental sites

This study was conducted within the Rwanda-TREE project (www.rwandatree.com) established at three different experimental plantation sites along an elevation gradient in Rwanda, central Africa, set up to represent a possible climate change scenario. The sites were high-elevation Sigira (2400 m a.s.l., S2°30′54″; E29°28′30″; hereafter denoted “HE”), mid-elevation Rubona (1600 m a.s.l., S2°28′30″; E29°46′49″; hereafter denoted “ME”) and low-elevation Ibanda-Makera (1300 m a.s.l., S2°6′31″; E30°51′16″E; hereafter denoted “LE”). Identical multi-species plantations, each composed of 1800 trees representing 20 native species, were established in January 2018 at the sites. HE is considered as the control site as most species used in this experiment naturally grow in the neighbouring montane rainforest, named Nyungwe National Park. Further details of the experiment can be found in Mujawamariya et al. (2020). In this study, we report findings from a parallel study using the same sites and plant material as in the main experiment, but in which eight species were grown in 11-litre pots using the soil from the Sigira site at all three sites, to eliminate soil as a possible confounding factor. The soil of the main plot, from where the soil to the pots were taken, was classified as an Ultisol with clay texture with pH (KCl) = 3.3 ± 0.13 (mean ± SD); bulk density = 0.99 ± 0.13 g cm−3; Organic C = 3.8 ± 0.6%; NH4+ and NO3− = 39 ± 11 g m−3 and available p = 18 ± 4 g m−3.

2.2 | Environmental data

During the 1-year growing period (1st February 2018 to 31st January 2019) for the trees used in this study, weather parameters were recorded every 30 min at all sites. The HE site had an annual mean average air temperature of 15.1°C while both the ME and LE sites were about 5°C warmer (20.0 and 20.4°C, respectively) compared to the HE site. However, the diurnal temperature variation differs between ME and LE, with the latter site having warmer days and cooler nights. The mean daytime (~12 h) air temperatures were 17, 22.4 and 23.8°C and mean monthly maximum air temperatures were 23.8, 28.6 and 31.4°C for HE, ME and LE, respectively. Nighttime is coolest at HE (13.2°C), but between the two lower-elevation sites, the LE site (16.6°C) is 0.9°C cooler than the ME site (17.5°C). The mean air temperature of the coldest and the warmest month differed depending on site between 1.8 and 2.1°C on diurnal basis and 2.7 and 3.0°C on daytime basis. The daytime (16.2, 21.9 and 23.6°C, for HE, ME, and LE, respectively) and nighttime (13.2, 17.6, 17.7°C, for HE, ME, and LE, respectively) temperatures during the 4-month period (1st February to 25th May) preceding and during the gas exchange measurements campaign did not differ very much from the corresponding temperatures for the 1-year growing period (provided above). The mean daytime air temperatures were 16.2, 21.9 and 23.6°C, while the nighttime air temperatures were 13.2, 17.6, 17.7°C, for HE, ME and LE, respectively. However, the mean monthly maximum air temperatures for the 4-month period were relatively cooler compared to the 1-year period temperatures at each site. The mean monthly maximum air temperatures for the 4-month period were 21.5, 27 and 29.6°C for HE, ME and LE, respectively.

The relative distribution of precipitation over the year is similar across all three sites where the highest rainfall period is March–May followed by the major dry period in June–August. However, the sites differ in total annual precipitation. From February to January 2018/19, HE, ME and LE received 2100, 1576 and 1046 mm in total precipitation, respectively. Throughout the experiment, the daytime vapor pressure deficit (VPD, kPa) increased from HE site to the two lower-elevation sites, with a mean value of 0.88, 1.31, 1.39 kPa for HE, ME and LE, respectively, representing on average a 49%-58% higher daytime air VPD in warmer, lower-elevation sites compared to the cool, high-elevation site. Due to high cloud cover at the HE site, the mean daytime photosynthetic photon flux density (PPFD) is lower (660 µmol m−2 s−1) compared to the lower-elevation sites which received on average 807 and 760 µmol m−2 s−1 ME and LE, respectively, during 2018.

2.3 | Plant material

The two species used in this study, early-successional Harungana montana (Spirlet) and late-successional Syzygium guineense (Wild.) DC, were grown from seeds collected in Nyungwe National Park and propagated in a nursery established at the ME site. Both species are abundant tree species in Nyungwe (Nyirambangutse et al., 2017; Ziegler et al., 2020). After seedlings had established roots (with a height of 43.4 ± 1.4 and 48.8 ± 1.6 cm, mean ± SE, for S. guineense and H. montana, respectively), they were transferred to 11-liter pots, and eight pots of each species were randomly assigned to each of the three experimental sites in mid-January 2018. At the time of plantation, the total dry mass of the S. guineense and H. montana seedlings were 7.2 ± 2.7 and 8.0 ± 1.9 g, respectively, based on initial harvests of eight seedlings per species, randomly selected from the same populations as the potted seedlings. Seedlings were then grown at the sites for 1 year until harvest in January 2019. The pots were buried in the ground to avoid unnatural diurnal soil temperature variation. Seedlings were fertilized during nursery cultivation but not afterwards. However, no major nutrient or pot limitations during the experiment were likely indicated by the continued growth until the end of the study (Figure S1). Throughout the study, seedlings were watered to maintain a moist growth medium throughout the experiment. Since the three sites differ in total annual precipitation, watering was also done a bit differently across sites. At the high-elevation site (HE), which receives the highest total annual precipitation (see details above), the watering was done twice a week, while at both the intermediate (ME) and lowest (LE), which receive relatively lower total precipitation, the watering was done every day.
On each watering day, each plant was given 2 L of water in the early evening.

2.4 | Gas exchange measurements

Leaf gas exchange measurements were conducted between 23rd April 2018 and 25th May 2018, 3–4 months after placing pots at the experimental sites, using the portable photosynthesis systems (Li-Cor 6400 XT, Li-Cor Inc.). On both instruments, the 6400-88 expanded temperature control kit was installed on IRGA sensor head to expand the temperature control. One healthy (i.e., green without visible damage from herbivores) leaf from each of four to six seedlings of each species at each site were measured. These leaves were developed under the new growth conditions at each site. Light-saturated net CO₂ assimilation rates (Aₛ) were measured at varying intercellular CO₂ concentrations (Cᵢ), producing A–Cᵢ curves. These A–Cᵢ curves were measured at a PPFD (photosynthetic photon flux density) of 1800 μmol photons m⁻² s⁻¹, air flow rate of 400 μmol s⁻¹ and at five leaf temperatures (19, 25, 30, 35 and 40°C). The target relative humidity in the leaf cuvette was between 60% and 70%. However, at 35 and 40°C reached, the leaf was given 10–15 min to acclimate before starting an ambient temperature in the field. After the target leaf temperature was reached, the leaf was given 10–15 min to acclimate before starting an addition of approximately 10 ml of water in the soda lime column. Therefore, the VPD in the leaf cuvette increased with the measuring leaf temperature (Figure S2). Only the leaf temperature in the cuvette was controlled; therefore, the rest of the plant was exposed to ambient temperature in the field. After the target leaf temperature was reached, the leaf was given 10–15 min to acclimate before starting an A–Cᵢ. The A–Cᵢ curve was started once gas exchange was stable at a reference CO₂ concentration of 400 μmol mol⁻¹; CO₂ concentrations were then changed sequentially to 250, 125, 50, 400, 600, 800, 1200, 1600 and 2000 and 400 μmol mol⁻¹. Due to the difficulty of reaching 35 and 40°C on cooler days, particularly in HE, and also of maintaining a reasonable minimum gₛ (considered to be 0.025 mol H₂O m⁻² s⁻¹ in this study, also see Vårhammar et al., 2015) at these leaf temperatures, there were fewer replicates with reliable Vᵢₐₕₑₐₜ and Jₘₚₐₓ data (two to four instead of five) at 35 and 40°C.

During A–Cᵢ curve measurements, a neighboring leaf, right next to the one being measured, was covered by aluminum foil for at least 30 min to be measured for dark-adapted respiration (Rₒ). At the end of the A–Cᵢ curves, the light source in the leaf cuvette was turned off, the reference CO₂ concentration was set to 400 μmol mol⁻¹, and the air flow rate was decreased to 250 μmol s⁻¹ before clamping on to the neighbouring leaf without exposing it to daylight. After reaching stability, three dark respiration points with a 10-s interval were taken at a leaf temperature of 25°C. These three points were averaged to get one value of Rₒ for each measured leaf. These three Rₒ measurements for each leaf did not differ by more than 0.1 μmol m⁻² s⁻¹ in any leaf. Empty chamber measurements (i.e., with no leaf in the Li-6400 XT leaf cuvette) were also collected at the end of each A–Cᵢ curve (i.e., empty A–Cᵢ curve) and leaf respiration measurements. These measurements were used to correct for any instrument noise prior to data analyses.

2.5 | Parameterization of photosynthesis models

The C₃ photosynthesis model developed by Farquhar et al. (1980) was used to estimate Vᵢₐₕₑₐₜ and Jₘₚₐₓ from the A–Cᵢ curves, using the “litacis” function and the “bilinear” fitting method from the “plantecphys” R package (Duursma, 2015) in R version 3.6.3 (R Core Team, 2020). We maintained the default temperature dependencies of the CO₂ compensation point in the absence of mitochondrial respiration (Γᵢ) and the Michaelis–Menten constants for CO₂ and O₂ (Kᵢ and Kₒ) from Bernacchi et al. (2001). Apparent values of Vᵢₐₕₑₐₜ and Jₘₚₐₓ parameterized based on intercellular CO₂ concentrations (Cᵢ) are reported. Values of Jₘₚₐₓ are reported only when the Jₘₚₐₓ-limited photosynthesis (Aₒ) part of the A–Cᵢ curve had at least two data points, or from one single data point if Cᵢ >1000 μmol m⁻² s⁻¹ and/or Aₒ was at least 10% lower than the Vᵢₐₕₑₐₜ-limited photosynthesis (Aᵢ) predicted at the Cᵢ value of that point. Based on these criteria, we could not parameterize Jₘₚₐₓ for 14 out of 146 total fitted A–Cᵢ curves. The triose phosphate use (TPU) was also fitted, but Aᵣ was not visibly TPU-limited in any of the A–Cᵢ curves.

The first Aₒ data point at a reference CO₂ concentration of 400 μmol mol⁻¹ was retrieved from each A–Cᵢ curve measurement, and the temperature response of Aᵢ was fitted using a nonlinear quadratic function (Battaglia et al., 1996):

\[
Aᵢ(T) = Aᵢopt - b \left( T - Tᵢopt \right)^2,
\]

where Aᵢ(T) is the Aᵢ (μmol m⁻² s⁻¹) at a given air temperature T (°C) and Aᵢopt is the Aᵢ at the optimum temperature (Tᵢopt).

To estimate to what extent stomatal conductance and light respiration (Rₒ day) may have influenced the shifts in Tᵢopt we re-calculated net photosynthesis (Aᵢ₂₈₇) and gross photosynthesis (i.e., including Rₒ day; Aᵢ₂₈₇) at a common Cᵢ of 287 μmol mol⁻¹ (i.e., with C/Cᵢ ratio of 0.7 considering an ambient atmospheric CO₂ concentration of 410 ppm) using parameterized Vᵢₐₕₑₐₜ, Jₘₚₐₓ and Rₒ day (only for Aᵢ₂₈₇) in the following equations:

\[
Aᵢ = \frac{Vᵢₐₕₑₐₜ \left( Cᵢ - Γᵢ^+ \right)}{\left( Cᵢ + Kᵢ \left( 1 + \frac{Aᵢ}{Kₒ} \right) \right)} - Rₒ day,
\]

where O is the intercellular concentrations of O₂, Kᵢ and Kₒ are the Michaelis–Menten coefficients of Rubisco activity for CO₂ and O₂, respectively, and Γᵢ is the CO₂ compensation point in the absence of mitochondrial respiration. Values at 25°C and temperature sensitivities of Γᵢ, Kᵢ and Kₒ were taken from Bernacchi et al. (2001). To account for differences in air pressure from different sites (caused by differences in altitude), Cᵢ and O concentrations were converted in unit pascal.

\[
Aᵢ = \left( \frac{Jₘₚₐₓ}{4} \right) \times \frac{\left( Cᵢ - Γᵢ^+ \right)}{\left( Cᵢ + 2Γᵢ^+ \right)} - Rₒ day,
\]

Aᵢ₂₈₇ and Aᵢ₂₈₇ were considered as the minimum of Aᵢ and Aᵢ. Using Equation (1), TᵢoptAᵢ₂₈₇ and TᵢoptAᵢ₂₈₇ were estimated.
The temperature responses of $V_{\text{cmax}}$ and $J_{\text{max}}$ were fitted using the following peaked Arrhenius function from (Medlyn et al., 2002):

$$f(T) = k_{\text{opt}} \exp \left( \frac{E_a}{R(T - T_{\text{opt}})} \right) \left( 1 - \exp \left( \frac{H(T - T_{\text{opt}})}{R(T_{\text{opt}})} \right) \right),$$

(4)

where $k_{\text{opt}}$ is the process rate (i.e., $V_{\text{cmax}}$ or $J_{\text{max}}$: $\mu$mol m$^{-2}$ s$^{-1}$) at the optimum temperature, $T_{\text{opt}}$ is expressed in degrees Kelvin ($T_{\text{optV}}$ or $T_{\text{optJ}}$; °K), $H$ (kJ mol$^{-1}$) is the activation energy term that describes the decline in enzyme activity at supra-optimal temperature, $E_a$ (kJ mol$^{-1}$) is the activation energy term that describes the initial exponential increase in enzyme activity with increasing temperature, $R$ is the universal gas constant (8.314 J mol$^{-1}$ K$^{-1}$) and $T_0$ is a given temperature in degrees Kelvin. The value of $H$ was fixed at 200 kJ mol$^{-1}$ to avoid over-parameterization (Dreyer et al., 2001; Medlyn et al., 2002).

To calculate the magnitude of the shifts of $T_{\text{optV}}$, $T_{\text{optJ}}$ and $T_{\text{optA}}$ in response to increased growth temperatures, these parameters were regressed against daytime growth temperature for the 4-month period preceding and during gas exchange measurements campaign.

### 2.6 Leaf structural and chemical analyses and plant biomass

After gas exchange measurements, measured leaves were collected and discs of known area were taken using a puncher and dried at 70°C until they reached a constant weight. The leaf dry mass per unit leaf area (LMA) was subsequently determined. The dry discs were ground to a fine powder which was analyzed for leaf N content using an elemental gas analyzer (EA 1108; Fison Instruments).

After 1 year, all eight trees per species and site combination were harvested and separated into roots, stem and leaf components. Roots were separated from soil by washing them with clean water, and a sieve was used to retain all the fine roots after washing. All harvested components were dried at 70°C to a constant weight and thereafter weighed separately to determine dry mass for each component and individual tree.

### 2.7 Statistical analyses

Since for many of the individual leaves it was difficult to parameterize Equation (1) and in a few cases Equation (4), we fit these two equations on data pooled across each species and site combination. Then, to test whether the fitted parameters from these two equations significantly differed among sites, we used the Welch’s t test (see details of the equations for the test in the supplementary material) by making three separate tests with each test comparing two sites (Table 1). To reduce the type I error among these multiple pairwise comparisons, two sites were judged to differ at a $p$-value threshold of 0.01 (i.e., if $p < 0.01$). Other measured traits for each species were analyzed by a one-way ANOVA with site as the main factor and a significant difference was assessed at a $p$-value threshold of 0.05 ($p < 0.05$; Table 2). When a significant site main effect was detected, we used post-hoc Tukey’s significance tests to evaluate differences among sites using emmeans (Lenth, 2021) and multcomp (Hothorn et al., 2008) R packages. To test whether increasing growth temperatures affected the temperature response of $V_{\text{cmax}}$, $J_{\text{max}}$, $A_0$ and $A_\infty$ for each species, we used a one-way repeated-measures ANOVA (Table 3) using a mixed-effects analysis approach with lme function from the nlme R package (Pinheiro et al., 2019) and with method set to the maximum likelihood (ML; Field et al., 2012). In this analysis, the leaf temperature and site were the main effects and each individual tree (across different leaf temperatures) as a random factor. All the data in figures and tables are reported as means ± SE. All analyses were performed in R version 3.6.3 (R Core Team, 2020).

### 3 RESULTS

#### 3.1 Leaf nitrogen and morphological traits

In *H. montana*, leaf nitrogen content on an area basis (leaf $N_a$) was significantly lower (37%) in seedlings grown at both the ME and LE sites compared to their counterparts grown at the HE site (2.3 g m$^{-2}$; Tables 2 and 4). In *S. guineense*, leaf $N_a$ was significantly different across sites, where leaf $N_a$ was 4% and 21% lower at LE and ME sites, respectively, compared to seedlings grown at HE (2.3 g m$^{-2}$; Tables 2 and 4). Site differences for leaf $N_m$ were similar to those for $N_a$ in both species since leaf mass per unit leaf area (LMA) was constant across sites in both species (Tables 2 and 4).

#### 3.2 Warming effect on temperature sensitivity parameters of underlying biochemical processes of net photosynthesis

The shape of the temperature response curve of neither the maximum carboxylation rates of Rubisco ($V_{\text{cmax}}$) nor the maximum electron transport rates ($J_{\text{max}}$) was significantly altered by rising growth temperature in either species (Figure 1; Table 3). In *H. montana*, the thermal optimum of $V_{\text{cmax}}$ ($T_{\text{optV}}$) was not significantly different in seedlings across the three sites, despite being 1.8 ± 1.8 °C and 3.6 ± 1.8°C higher in seedlings at LE and ME sites, respectively, than in seedlings grown at the coolest, HE site (Tables 1 and 4). In *S. guineense*, $T_{\text{optV}}$ was also not significantly different in seedlings across the three sites, although $T_{\text{optV}}$ was on average 2.3 ± 1.8°C higher in seedlings grown at LE site compared to seedlings grown at both ME and HE sites (Tables 1 and 4). These small and statistically non-significant shifts in $T_{\text{optV}}$ at lower compared to the highest elevation sites represent a 0.35 and 0.22 °C increase in daytime mean growth temperature for *H. montana* and *S. guineense*, respectively (Figure S3). Unlike *S. guineense* in which the thermal optimum of $J_{\text{max}}$ ($T_{\text{optJ}}$) was similar across the three sites, in *H. montana*, $T_{\text{optJ}}$ significantly differed among sites, being unexpectedly 4°C
lower at LE compared to ME, while the $T_{\text{opt}}$ of the HE was intermediate and not significantly different from the other two sites (Tables 1 and 4). The shift in $T_{\text{opt}}$ in *H. montana* was 0.16°C per 1°C of warming, averaged for ME and LE (Figure S1). The activation energy of $V_{\text{cmax}} (E_aV)$ and $J_{\text{max}} (E_aJ)$ did not significantly differ in seedlings of either species across the three sites (Tables 1 and 4). Although mean values $E_aV$ showed a progressive decline towards lower-elevation and warmer sites, this change was not significant as there was large variation in the values for this parameter (Tables 1 and 4). For both species, the ratio of $J_{\text{max}}$ to $V_{\text{cmax}}$ decreased with increasing leaf

---

**TABLE 1** Statistical outputs of the Welch t test on the temperature sensitivity parameters of net photosynthesis and photosynthetic capacity

<table>
<thead>
<tr>
<th>Species</th>
<th>HE</th>
<th>ME</th>
<th>LE</th>
<th>HE</th>
<th>ME</th>
<th>LE</th>
<th>HE</th>
<th>ME</th>
<th>LE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{optA}}$</td>
<td></td>
<td></td>
<td></td>
<td>df</td>
<td>T-value</td>
<td>df</td>
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<td>T-value</td>
</tr>
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<td>5</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME</td>
<td></td>
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<td></td>
<td>8</td>
<td>0.2</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>LE</td>
<td>8</td>
<td>0.2</td>
<td></td>
<td>8</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{\text{optAg287}}$</td>
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<td></td>
<td></td>
<td>6</td>
<td>1</td>
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<td>HE</td>
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<td>8</td>
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<td>ME</td>
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<td>0.6</td>
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<td></td>
<td>8</td>
<td>0.3</td>
<td></td>
<td></td>
<td>5</td>
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<tr>
<td>LE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Bold numbers represent p-value less than 0.01 ($p < 0.01$). HE, High elevation; ME, Intermediate elevation; LE, Low elevation; df, degrees of freedom; maximum carboxylation rates of Rubisco at the thermal optimum ($V_{\text{cmaxOpt}}, \mu\text{mol m}^{-2} \text{s}^{-1}$); maximum electron transport rate at the thermal optimum ($J_{\text{maxOpt}}, \mu\text{mol m}^{-2} \text{s}^{-1}$); thermal optimum of $V_{\text{cmax}} (T_{\text{optV}}, \circ C)$; thermal optimum of $J_{\text{max}} (T_{\text{optJ}}, \circ C)$; activation energy of $V_{\text{cmax}} (E_aV)$; activation energy of $J_{\text{max}} (E_aJ)$; thermal optimum of net CO$_2$ assimilation ($T_{\text{optA}}, \circ C$); thermal optimum of net CO$_2$ assimilation at a common intercellular CO$_2$ concentration ($C_i$) of 287 µmol mol$^{-1}$ ($T_{\text{optA287}}, \circ C$); thermal optimum of gross CO$_2$ assimilation at a common $C_i$ of 287 µmol mol$^{-1}$ ($T_{\text{optAg287}}, \circ C$).
temperature, although the slopes differed among sites in both species. In H. montana, \( J_{\text{max}}/V_{\text{cmax}} \) decreased more strongly at LE and HE compared to ME, while in S. guineense \( J_{\text{max}}/V_{\text{cmax}} \) decreased more strongly at HE compared to other two sites (Figure 1e,f).

### 3.3 Warming effects on rate of net photosynthesis and its thermal optimum

The responses of net photosynthesis (\( A_n \)) to short-term leaf temperature variation exhibited different site differences between the two species (Figure 2a,b; Table 3). At each site and species, \( A_n \) was relatively constant between 18 and 30°C and declined above this temperature range. However, in H. montana, \( A_n \) was substantially higher between 18 and 30°C at HE compared to ME and LE sites (both of which had similar \( A_n \) rates in this leaf temperature range), but dropped drastically at leaf temperatures above 30°C to net CO₂ assimilation rates comparable to the other two sites (Figure 2a; Table 3). In contrast, in S. guineense, \( A_n \) rates decreased at leaf temperatures above 30°C, but \( A_n \) rates were similar among sites throughout the entire measuring temperature range (Figure 2b; Table 3). Decreases in \( A_n \) above 30°C in both species were largely driven by stomatal closure (Figure 2c,d) associated with high VPD in the leaf chamber at these high leaf temperatures (Figure 2c,d; Figures S2 and S4). Furthermore, the thermal optimum of \( A_n \) did not significantly shift in response to warmer growth conditions at lower-elevation sites in either species (Tables 1 and 4). In H. montana, the non-significant shifts in the thermal optimum of \( A_n \) (\( T_{\text{opt}} \)) were 3.2 and 1.9°C higher in seedlings grown at ME and LE sites, respectively, compared to those grown at cool, HE site. In S. guineense, the corresponding shifts were only 0.4 and 1.4°C (Tables 1 and 4). These small shifts in \( T_{\text{opt}} \) represent an average change of 0.34 and 0.16°C per 1°C of warming for H. montana and S. guineense, respectively (Figure S3).

Since the shift in \( T_{\text{opt}} \) with a rise in growth temperature is also affected by the simultaneous adjustments in stomatal conductance and leaf respiration (in addition to biochemical components), we explored further to what extent these two processes may have impacted the shifts in \( T_{\text{opt}} \). The thermal optimum estimated from \( A_n \) at a common intercellular CO₂ concentration (\( C_i \)) of 287 (\( T_{\text{optA287}} \)) was not significantly different from the corresponding \( T_{\text{opt}} \) value within each site in either species (Tables 1 and 4), suggesting that stomatal conductance did not control the thermal optimum of \( A_n \) across different sites. This was due to a similar response of \( g_s \) to increasing leaf-to-air vapour pressure deficit inside the leaf chamber (VPD,) as measurement temperature rises across all sites and in either species (Figure 2a,b; Figures S2 and S4; Table 3). Similarly, the thermal optimum of gross photosynthesis at a common \( C_i \) of

---

**Table 2** Summary report of ANOVA showing \( F \)-values and \( p \)-values for effect of site on chemical, morphological, photosynthetic capacity and plant biomass in Harungana montana and Syzygium guineense

<table>
<thead>
<tr>
<th>Variable</th>
<th>( \text{df}_{\text{Site}} )</th>
<th>( \text{df}_{\text{Res}} )</th>
<th>( F )-value</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Harungana montana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N_{\text{n}} )</td>
<td>2</td>
<td>21</td>
<td>40.1</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>( N_{\text{s}} )</td>
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<td>21</td>
<td>27.3</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>LMA</td>
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<td>21</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>( V_{\text{cmax25}} )</td>
<td>2</td>
<td>14</td>
<td>30.7</td>
<td>&lt;0.00001</td>
</tr>
<tr>
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<td>36.7</td>
<td>&lt;0.00001</td>
</tr>
<tr>
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<td>13</td>
<td>0.4</td>
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</tr>
<tr>
<td>( S_{\text{25}} )</td>
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<td>0.6</td>
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<tr>
<td>( A_{\text{n25}} )</td>
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<td>8.3</td>
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</tr>
<tr>
<td><strong>Total biomass</strong></td>
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<td>&lt;0.00001</td>
</tr>
<tr>
<td><strong>Root biomass ratio</strong></td>
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<td>0.6</td>
</tr>
<tr>
<td><strong>Stem biomass ratio</strong></td>
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<tr>
<td><strong>Leaf biomass ratio</strong></td>
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<tr>
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<td><strong>Total roots biomass</strong></td>
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</tbody>
</table>

**Note:** Bold numbers represent \( p \)-value less than 0.05 (\( p < 0.05 \)). Total leaf nitrogen expressed on area (\( N_{\text{n}}, \text{g m}^{-2} \)) and dry mass (\( N_{\text{s}}, \text{g g}^{-1} \)) basis; leaf mass per unit area (LMA, \( \text{g m}^{-2} \)); maximum carboxylation rates of Rubisco (\( V_{\text{cmax25}}, \text{µmol m}^{-2} \text{s}^{-1} \)) and maximum electron transport rate (\( J_{\text{max25}}, \text{µmol m}^{-2} \text{s}^{-1} \)) measured at 25°C; the ratio of \( J_{\text{max25}} \) to \( V_{\text{cmax25}} \) (\( J_{\text{max25}}/V_{\text{cmax25}} \)); stomatal conductance (\( g_{\text{s25}}, \text{µmol H}_{2}\text{O m}^{-2} \text{s}^{-1} \)), net CO₂ assimilation rate (\( A_{\text{n25}}, \text{µmol m}^{-2} \text{s}^{-1} \)) and foliar dark respiration rate (\( R_{\text{d25}}, \text{µmol CO}_2 \text{ mol}^{-2} \text{s}^{-1} \)) measured at 25°C; total biomass (\( g \)); fractions of total biomass allocated to roots (Root biomass ratio), stem (Stem biomass ratio), leaves (Leaf biomass ratio), total leaf biomass (\( g \)), total stem biomass (\( g \)) and total roots biomass (\( g \)); df: degrees of freedom.
TABLE 3 Summary report of the repeated-measures ANOVA for the temperature response curves of *Harungana montana* and *Syzygium guineense*, showing F-values and p-values for effects of leaf temperature ($T_{lcl}$), site and their interaction

<table>
<thead>
<tr>
<th></th>
<th>df num</th>
<th>df den</th>
<th>Harungana montana</th>
<th>Syzygium guineense</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F-value</td>
<td>p-value</td>
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<td>59</td>
<td>54.8</td>
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<tr>
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<td></td>
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<td>14</td>
<td>0.7</td>
</tr>
<tr>
<td>$J_{maxRel}$</td>
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<td>0.06</td>
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<td>Site</td>
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<td>13</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>$T_{leaf}$</td>
<td>2</td>
<td>49</td>
<td>2.0</td>
</tr>
<tr>
<td>$J_{max}/V_{cmax}$</td>
<td>$T_{leaf}$</td>
<td>1</td>
<td>49</td>
<td>256</td>
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<td></td>
<td>$T_{leaf}$</td>
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<td>5.2</td>
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<td>$T_{leaf}$</td>
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<td></td>
<td>$T_{leaf}$</td>
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<td>59</td>
<td>9.5</td>
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</table>

Note: Bold numbers represent p-value less than 0.05 (p < 0.05). Traits analyzed were as follows: relative maximum carboxylation rate of Rubisco ($V_{cmaxRel}$); maximum electron transport rate ($J_{maxRel}$); stomatal conductance ($g_s$; mmol H$_2$O m$^{-2}$ s$^{-1}$); net CO$_2$ assimilation rate ($A_n$; µmol m$^{-2}$ s$^{-1}$); df: degrees of freedom.

TABLE 4 Chemical, morphological, and photosynthetic traits measured in *Harungana montana* and *Syzygium guineense* seedlings grown at the three sites

<table>
<thead>
<tr>
<th></th>
<th>Harungana montana</th>
<th>Syzygium guineense</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HE</td>
<td>ME</td>
</tr>
<tr>
<td>$N_{nj}$</td>
<td>23.8 ± 1.2$^a$</td>
<td>13.9 ± 0.1$^b$</td>
</tr>
<tr>
<td>$N_{n}$</td>
<td>2.3 ± 0.1$^a$</td>
<td>1.5 ± 0.1$^b$</td>
</tr>
<tr>
<td>LMA</td>
<td>96.9 ± 3.1$^a$</td>
<td>108.2 ± 7.2$^a$</td>
</tr>
<tr>
<td>$Y_{cmaxOpt}$</td>
<td>89.4 ± 4.4$^a$</td>
<td>62.4 ± 3.8$^b$</td>
</tr>
<tr>
<td>$J_{maxOpt}$</td>
<td>152.4 ± 9.4$^a$</td>
<td>95.5 ± 6.4$^b$</td>
</tr>
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<td>$T_{optTV}$</td>
<td>32.8 ± 0.9$^a$</td>
<td>36.4 ± 1.4$^a$</td>
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<td>$E_{fV}$</td>
<td>76.9 ± 21.7$^a$</td>
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</tr>
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<td>30.8 ± 1.2$^a$</td>
<td>33.3 ± 1.1$^a$</td>
</tr>
<tr>
<td>$E_{fJ}$</td>
<td>25.0 ± 10.9$^a$</td>
<td>32.9 ± 13.7$^a$</td>
</tr>
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<td>24.0 ± 2.3$^a$</td>
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<td>23.7 ± 1.04$^a$</td>
<td>24.1 ± 2.4$^a$</td>
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<tr>
<td>$T_{optAg287}$</td>
<td>23.7 ± 1.2$^a$</td>
<td>23.8 ± 2.9$^a$</td>
</tr>
</tbody>
</table>

Note: Letters after each value represent group comparisons across the three sites (HE High elevation, ME intermediate elevation, LE low elevation) generated from a Tukey post-hoc test (p < 0.05). Total leaf nitrogen expressed on area (N$_{nj}$ g m$^{-2}$) and dry mass (N$_{n}$ mg g$^{-1}$) basis; leaf mass per unit area (LMA, g m$^{-2}$); maximum carboxylation rates of Rubisco at the thermal optimum ($V_{cmaxOpt}$; µmol m$^{-2}$ s$^{-1}$); maximum electron transport rate at the thermal optimum ($J_{maxOpt}$; µmol m$^{-2}$ s$^{-1}$); thermal optimum of $V_{cmax}$ ($T_{optC}$); thermal optimum of $J_{max}$ ($T_{optJ}$); activation energy of $V_{cmax}$ ($E_{fV}$); activation energy of $J_{max}$ ($E_{fJ}$); thermal optimum of net CO$_2$ assimilation ($T_{optA}$); thermal optimum of net CO$_2$ assimilation at a common intercellular CO$_2$ concentration of 287 µmol mol$^{-1}$ ($T_{optAg287}$); thermal optimum of gross CO$_2$ assimilation at a common C of 287 µmol mol$^{-1}$ ($T_{optAg287}$); Means ± SE, n = 4–8.

287 ppm ($T_{optAg287}$) did not significantly differ from $T_{optA}$ at either site and in any of the species (Tables 1 and 4). However, $T_{optAg287}$ seemed lower than $T_{optA}$ at the LE site for either species (Tables 1 and 4), implying that day respiration may have played a role in photosynthetic thermal acclimation in seedlings at the warmest site to some extent.
3.4 | Warming effect on stomatal conductance

Stomatal conductance decreased with increasing leaf temperature and was not significantly different among the three sites across the measuring temperature range of 18–40°C in either species (Figure 2c,d; Table 3). However, when assessed at a standard leaf temperature of 25°C ($g_{s25}$), $g_{s25}$ in S. guineense was 25% and 50% higher in seedlings at ME and LE sites, respectively, compared to those at HE site (Figure 3b; Table 2). In H. montana, $g_{s25}$ was not significantly different between sites (Figure 3a; Table 2).

3.5 | Warming effect on photosynthetic capacity rates

Photosynthetic capacity at a common leaf temperature of 25°C (i.e., $V_{cmax25}$ and $J_{max25}$) was significantly affected by increased growth temperature in both species (Figure 4a–d). In H. montana, $V_{cmax25}$ decreased by 45% and 48% in seedlings grown at the ME and LE sites, respectively, compared to those grown at the HE site (Figure 4a; Table 2). Similarly, $J_{max25}$ was 49% lower in seedlings grown at both ME and LE sites than those grown at HE site (Figure 4c; Table 2). In S. guineense, $V_{cmax25}$ and $J_{max25}$ were also reduced, but to a lesser extent compared to H. montana, decreasing by 20%–25% at both ME and LE sites compared to HE site (Figure 4b,d; Table 2). For both species, the ratio of $J_{max25}$ to $V_{cmax25}$ ($J_{max25}/V_{cmax25}$) was constant across all three sites (Figure 4e,f; Table 2).

The photosynthetic capacity at the thermal optimum was also generally reduced by warming in both species. In H. montana, $V_{cmax}$ at the thermal optimum ($V_{cmaxOpt}$) was 30% and 43% lower in seedlings grown at the ME and LE sites, respectively, compared to seedlings grown at the HE site (Tables 1 and 4). The corresponding values for $J_{max}$ at the thermal optimum ($J_{maxOpt}$) were 37% and 45% (Tables 1 and 4). In S. guineense, the $V_{cmaxOpt}$ also significantly decreased by 22% and 25% in seedlings grown at ME and LE sites, respectively, compared to seedlings grown at HE site (Tables 1 and 4). However, the $J_{maxOpt}$ was not significantly different between sites (Tables 1 and 4).

3.6 | Warming effect on leaf dark respiration

Leaf dark respiration at a common leaf temperature of 25°C ($R_{d25}$) acclimated to warming in both species (Figure 5; Table 2). In H. montana, $R_{d25}$ was 37% and 53% lower in seedlings grown at the ME and LE sites, respectively, compared to seedlings grown at the HE site (Figure 5a; Table 2). In S. guineense, $R_{d25}$ was on average 32% lower at both lower-elevation sites compared to HE site (Figure 5b; Table 2). When data were pooled across sites within each species, $R_{d25}$ was positively related to $V_{cmax25}$ and $J_{max25}$ (Figure 6; Table S1), suggesting a coordinated thermal acclimation between respiration and photosynthesis.

3.7 | Warming effect on plant biomass and allocation

Plant biomass was differently affected by increased growth temperature in the two species. In H. montana, the dry biomass was 11% and 184% higher in seedlings grown at the ME and LE sites, respectively, compared to seedlings grown at the HE site (Figure 7a; Table 2). Moreover, the dry biomass of leaves, stem and roots increased by the same order of magnitude at warmer sites (Figure S5; Table 2). However, the proportion of biomass allocated to roots (31%),
stem (20%) and leaves (49%) did not differ significantly across sites (Figure 7c,e,g). By contrast, total biomass in *H. montana* was similar across all three sites (Figure 7b; Table 2), but the allocation to roots increased at warmer sites (Figure 7d) while allocation to leaves and stem did not significantly differ between sites (Figure 7f,h; Figure S5).

**4 | DISCUSSION**

In this study, we report findings from the first comprehensive field study investigating the instantaneous temperature responses of photosynthesis and how they acclimate to long-term warming in tropical tree species. Trees of two montane species with contrasting successional strategies were grown in the field in 11-liter pots with similar soil, along an elevation gradient in the Rwanda-TREE project. Surprisingly, the optimal temperature of $V_{\text{cmax}}$, $J_{\text{max}}$, and $A_n$ did not significantly increase with warming in any species. However, both photosynthetic capacity ($V_{\text{cmax}}$, $J_{\text{max}}$) and leaf respiration ($R_d$) significantly decreased in a coordinated manner at warmer sites. Plant biomass was strongly increased by warming in *H. montana* seedlings while it was constant across sites in *S. guineense*.

**4.1 | Effect of growth temperature on photosynthetic temperature sensitivity parameters**

Surprisingly, despite a 7.4°C increase in growth temperature (considering the 4-month period preceding and during the gas exchange
FIGURE 4 Photosynthetic capacity in Harungana montana (a, c, e) and Syzygium guineense (b, d, f) grown at different sites in Rwanda-TREE. (a, b) Maximum carboxylation rate of Rubisco ($V_{\text{cmax25}}$, $\mu$mol m$^{-2}$ s$^{-1}$), (c, d) maximum electron transport rate ($J_{\text{max25}}$, $\mu$mol m$^{-2}$ s$^{-1}$) and (e, f) ratio of $J_{\text{max25}}$ to $V_{\text{cmax25}}$ ($J_{\text{max25}}/V_{\text{cmax25}}$) at measured at 25°C. Colors represent different sites (high-elevation Sigira, HE = white; intermediate-elevation Rubona, ME = grey; low-elevation Makera, LE = black). Different letters on bars represent differences across the three sites (Tukey post-hoc test, $p < 0.05$). Means ± SE. $n = 4–6$.

FIGURE 5 Leaf dark respiration measured at 25°C ($R_{25}$, $\mu$mol m$^{-2}$ s$^{-1}$) in Harungana montana (a) and Syzygium guineense (b) grown at different sites in Rwanda-TREE. Colors represent different sites (high-elevation Sigira, HE = white; intermediate-elevation Rubona, ME = grey; low-elevation Makera, LE = black). Different letters on bars represent differences across the three sites (Tukey post-hoc test, $p < 0.05$). Means ± SE. $n = 4–6$.
measurements campaign), $T_{\text{opt}}$ did not significantly increase in either species in our study (Table 1), suggesting a limited capacity of photosynthesis to acclimate to warmer growth temperatures in these tropical montane rainforests tree species. The statistically non-significant shifts in $T_{\text{opt}}$ were on average 0.34–0.16°C per 1°C for *H. montana* and *S. guineense*, respectively (Figure S3). This range was comparable to the non-significant shifts in thermal optimum of $V_{\text{cmax}}$ ($T_{\text{opt}V}$; 0.22 and 0.35°C per 1°C of warming for *S. guineense* and *H. montana*, respectively), indicating that possible shifts in $T_{\text{opt}}$ are controlled biochemically by thermal acclimation of Rubisco carboxylation. The shift in $T_{\text{opt}}$ in this study is lower than the value previously observed in a tropical lowland study (0.47°C per 1°C; Slot & Winter, 2017b), and in global meta-analyses dominated by temperate and boreal tree species (0.43–0.62°C; Kumarathunge et al., 2019; Yamori et al., 2014). Therefore, based on our data, we conclude that thermal acclimation of $T_{\text{opt}V}$ is either lacking or weaker than in most previous studies, which mostly included temperate and boreal tree species. Our first hypothesis of partial $T_{\text{opt}}$ acclimation is therefore neither completely supported nor rejected. Although it is possible that the temperature sensitivity of net photosynthesis across sites may have been potentially affected by differences in leaf N, there is currently little support for this effect (Tarvainen et al., 2017).

### 4.2 Effect of growth temperature on stomatal conductance

In our study, $g_s$ at a given leaf temperature increased with warming, particularly in the leaf temperature range between 25 and 35°C, but this increase was significant in *S. guineense* only (Figures 2 and 3). In the short term, $g_s$ typically decreases with the increasing VPD accompanying an increase in temperature (Figures S2 and S4; Grossiord et al., 2020; López et al., 2021; Oren et al., 1999). The decrease acts to prevent water loss in high-VPD air, but simultaneously restricts the CO$_2$ supply for photosynthesis (Farquhar & Sharkey, 1982). At our experimental sites, the two warmer, lower-elevation sites had on average 49–58% higher daytime air VPD compared to the cool, high-elevation site. However, trees growing in a warm climate with high VPD may acclimate their water uptake, transport system and stomatal regulation to increase their $g_s$ at a given VPD (Marchin et al., 2016). This happened in our study, at least for *S. guineense*, which increased both $g_s$ and allocation of biomass to roots in seedlings grown at lower elevations with relatively warmer and drier conditions (Figures 3b and 7d). This response contrasts the least-cost theory (Prentice et al., 2014) and our second hypothesis. Clearly, additional studies that explore long-term responses of $g_s$ to high temperature and VPD are needed (Grossiord et al., 2020; López et al., 2021; Marchin et al., 2016; Oren et al., 1999). Such knowledge is critical since the response of $g_s$ to global change factors has been identified as an important factor that will dictate climate change responses of terrestrial productivity, hydrology and energy balance (Cernusak et al., 2019; Grossiord et al., 2020), particularly for tropical forests (Doughty & Goulden, 2008; McDowell et al., 2018; Tan et al., 2017).

### 4.3 Effect of growth temperature on photosynthetic capacity

In both species, photosynthetic capacity measured at a common leaf temperature of 25°C (i.e., $V_{\text{cmax}25}$ and $J_{\text{max}25}$) decreased substantially at increased growth temperature (Figure 4). The decrease in photosynthetic capacity was largely driven by reduced leaf N with warming (also previously observed in Crous et al., 2018; Dusenge et al., 2020; Scafaro et al., 2017; Way & Sage, 2008), but the effect remained also after normalization for differences in leaf N (Figure S6; Table S2). These results suggest that the decreased photosynthetic capacity at warmer sites was caused by both decline in total leaf N.
Bars represent differences across the three sites (Tukey post-hoc). Thermal acclimation of \( V_{c_{\text{max}}25} \) (Wang et al., 2020) increased leaf nutrient demand should be reduced with an extended number of species (16 early- and late-successional species) freely rooted in the soil (Mujawamariya et al., 2020). These values are in good agreement with observations for \( S.\ guineense \), where \( V_{c_{\text{max}}25} \) was decreased by 23% at both lower-elevation sites. However, in \( H.\ montana \), \( V_{c_{\text{max}}25} \) decreased by 45% and 48% at the ME and LE, respectively. These large reductions in \( H.\ montana \) may partly reflect some pot limitation, with this species showing strongly increased growth at the two warmer sites, in contrast to \( S.\ guineense \) (Figure 7). Although being in the direction predicted by optimality theory, our findings contrast to previous studies on tropical species which did not find any significant effect of warming on basal rates of photosynthetic capacity (Carter et al., 2020; Crous et al., 2018; Scafaro et al., 2017).

We did not see any reduction of the \( J_{\text{max25}}/V_{c_{\text{max25}}} \) ratio with warming (Figure 4), which is otherwise commonly reported in several individual studies and meta-analyses (e.g., Bermudez et al., 2020; Dusenge et al., 2015, 2020; Kattge et al., 2009; Kumarakthunge et al., 2019; Stefanski et al., 2019). However, similar to our findings, there was also no warming-induced decrease in \( J_{\text{max}}/V_{c_{\text{max}}} \) ratio in other previous studies with tropical trees (Crous et al., 2018; Scafaro et al., 2017). Decreases in \( J_{\text{max}}/V_{c_{\text{max}}} \) may occur as a mechanism that offset increasing carboxylation limitation of photosynthesis under warm conditions as \( g_s \) declines and photorespiration increases. In our study, the constant \( J_{\text{max}}/V_{c_{\text{max}}} \) may be explained by increased CO\(_2\) supply via observed warming-induced increase in stomatal conductance at 25°C (observed in both species but significant only in \( S.\ guineense \); Figure 5a,b). This may have stimulated carboxylation with no further need to alter resource allocation between the two biochemical processes. Our results therefore indicate that whether decreased or maintained \( J_{\text{max25}}/V_{c_{\text{max25}}} \) is optimal in warmer conditions depends on the response of \( g_s \) and its influence on \( C_t \).

### 4.4 Thermal acclimation of foliar dark respiration

In our study, \( R_{d25} \) was lower in warm-grown seedlings compared to cool-grown counterparts in both species, consistent with our third hypothesis (Figure 5). These results are also consistent with those from several previous studies on tropical trees (Slot & Kitajima, 2015; Slot et al., 2014; Slot & Winter, 2018; Smith & Dukes, 2017; Zhu et al., 2020). Thermal acclimation of leaf \( R_{d25} \) was stronger in \( H.\ montana \) than in \( S.\ guineense \). This species difference in thermal acclimation of \( R_d \) may be linked to the stronger warming-induced decline in both leaf N (as also seen in Crous et al., 2017; Dusenge et al., 2020) and photosynthetic capacity in \( H.\ montana \) compared to \( S.\ guineense \) (Table 2; Figure 4a–d). Strong thermal acclimation of \( R_d \) was also observed in a companion study from the same experimental sites but with an extended number of species (16 early- and late-successional species) freely rooted in the soil (Mujawamariya et al., 2020).

Values of \( R_{d25} \) were positively related to both \( V_{c_{\text{max25}}} \) and \( J_{\text{max25}} \) (Figure 6), suggesting that thermal acclimation of \( R_d \) was coordinated with thermal acclimation of photosynthetic capacity, consistent with our fourth hypothesis. These findings agree with the optimality theory of photosynthetic capacity recently proposed by Wang et al. (2020), which demonstrated that thermal acclimation of
$R_d$ follow closely that of $V_{c,max}$ to maintain optimal photosynthesis with efficient use of resources in a given environment. This thermal acclimation in leaf respiration can, in turn, be achieved via several biochemical processes including reductions in Cytochrome C Oxidase (COX) content, a key central respiratory protein (Rashid et al., 2019), decreases in mitochondrial density (Armstrong et al., 2006) or changes in some intermediates of glycolysis and tricarboxylic acid cycle (Rashid et al., 2020). The strong correlation between thermal acclimation of leaf respiration and photosynthesis likely reflects both the dependence of respiration on substrate from photosynthesis (Dusenge et al., 2019; Mujawamariya et al., 2020) and the reduced energy required to maintain less, but more efficient leaf proteins (particularly Rubisco) in warm-grown plants (Wang et al., 2020).

4.5 Effect of growth temperature on tree growth and biomass allocation

Growth and allocation of dry biomass to different plant organs (roots, stem and leaves) responded differently to warming between the two species (Figure 7). In $H. montana$ (early-successional and fast-growing species), the total dry biomass significantly increased at the warmer sites, while in $S. guineense$ (late-successional and slow-growing species), it remained constant across all sites. With respect to tree biomass components, for $H. montana$, dry biomass of leaf, stem and roots also increased at warmers sites, but with no changes in biomass allocation. In contrast, for $S. guineense$, only the dry biomass of roots increased at warmers sites, while the dry biomass of both leaves and stems was largely constant across all sites (Figure S5). Contrasts in tree growth responses to warming between the two species may be linked to differences in their responses of both leaf physiology and leaf production.

In $H. montana$, increases in leaf biomass with warming may have been the main driver of higher total biomass at warmer sites, despite concomitant declines in net photosynthesis. Net CO$_2$ assimilation decreased by 31%–34% in both lower sites compared to the high-elevation site (Figure S7). The $R_{d,955}$ decreased even more strongly by 37 and 53% at the mid and low elevation, respectively (Figure 5). Again, assuming a $Q_{10}$ value of 2.3, this $R_d$ downregulation resulted in constant and lower $R_d$ at average nighttime growth temperatures at ME and LE sites, respectively; that is, complete or over-compensatory thermal acclimation. Increased growth at warmer sites in $H. montana$ may, therefore, be attributed to two linked processes. First, a greatly increased leaf biomass (Figure S5) and likely also increased total canopy leaf N (Figure S8) in a warmer climate may have resulted in increased canopy carbon fixation in spite of lower leaf level rates. Second, if a similarly strong downregulation of respiration was present in plant parts other than upper-canopy leaves, the strong downregulation in respiration at the warmest site (i.e., lower rates at ambient night temperature) may have contributed to increased carbon availability for tree growth with warming.

By contrast, $S. guineense$ maintained similar net CO$_2$ assimilation rates across the three sites (Figure S7). In addition, leaf respiration at 25°C decreased by 32% at the two lower-elevation sites. Assuming a $Q_{10}$ value of respiration of 2.3 (Atkin & Tjoelker, 2003; Slot & Winter, 2017b; Weerasinghe et al., 2014), this implies approximately homeostasis in $R_d$ at mean nighttime growth temperatures (Mujawamariya et al., 2020). Therefore, $S. guineense$ may have maintained relatively similar $A_d$ and $R_d$ rates across all three sites, and when combined with similar sites leaf biomass, it largely resulted in constant growth across all sites.

Overall, our findings indicate that montane fast-growing and early-successional species (pioneer) will respond more positively to warming compared to slow-growing and late-successional counterparts (climax). $H. montana$ (a shade intolerant, early-successional and a fast-growing species) is common in secondary forests that are formed either naturally after creation of canopy gaps within an old forest (e.g., landslides, tree falls) or after human disturbances (e.g., forest fires, mining). The environmental conditions within these gaps are characterized by higher light and temperature variability compared to understory environments (Lebrija-Trejos et al., 2011), where species such as $S. guineense$ (shade-tolerant, late-successional and slow-growing species) commonly regenerate (Nyirambangutse et al., 2017). Growth in $H. montana$ may, therefore, be more responsive to increased temperature when grown at warmer, lower-elevation sites, where radiation is also higher due to lower cloud cover (Gliniars et al., 2013; Mujawamariya et al., 2020). In addition, physiological plasticity in early-successional species in response to warming, which has been indicated in some studies (Slot & Winter, 2017b, 2018), may also be an important factor. In our study, this physiological plasticity was observed in leaf respiration (Figure 5). In contrast, late-successional $S. guineense$ may be less stimulated by increased growth temperatures. Our findings are in line with results from some previous controlled experimental studies, but with tropical lowland species, which showed that early-successional species tend to respond more positively to warming compared to late-successional species (Cheesman & Winter, 2013a, 2013b). However, a recent controlled experimental study on tropical lowland tree species did not find any effects of warming across nine early- and late-successional species (Slot & Winter, 2018). More experimental tropical studies, especially in field settings and with a larger species representation from each functional group, are still needed to draw a firm conclusion to whether early- and fast-growing versus late-successional and slow-growing species respond differently to warming.

5 CONCLUSIONS

Our study demonstrates that photosynthetic thermal acclimation is limited in tropical montane tree species. In both species, the thermal optima of net photosynthesis and underlying biochemical processes ($V_{c,max}$ and $J_{max}$) did not significantly shift in response to higher growth temperature. However, photosynthetic capacity and leaf dark respiration synchronously acclimated by decreasing with increasing
growth temperatures. Growth was stimulated by warming in the montane early-successional and fast-growing species but not in the late-successional and slow-growing species correlating with species differences in total leaf biomass. Our findings suggest that dynamic global vegetation models (DGVMs) should not generalize the partial thermal acclimation of the optimum of net photosynthesis derived from meta-analyses dominated by temperate and boreal species for tropical montane rainforest tree species. Moreover, DGVMs should also consider the tight coordination in thermal acclimation of leaf respiration and photosynthetic capacity when predicting feedback between terrestrial vegetation and climate.

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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