In situ temperature relationships of biochemical and stomatal controls of photosynthesis in four lowland tropical tree species

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Abstract

Net photosynthetic carbon uptake of Panamanian lowland tropical forest species is typically optimal at 30–32°C. The processes responsible for the decrease in photosynthesis at higher temperatures are not fully understood for tropical trees. We determined temperature responses of maximum rates of RuBP-carboxylation ($V_{\text{CMax}}$) and RuBP-regeneration ($J_{\text{Max}}$), stomatal conductance ($G_s$) and respiration in the light ($R_{\text{Light}}$) in situ for four lowland tropical tree species in Panama. $G_s$ had the lowest temperature optimum ($T_{\text{Opt}}$), similar to that of net photosynthesis, and photosynthesis became increasingly limited by stomatal conductance as temperature increased. $J_{\text{Max}}$ peaked at 34–37°C and $V_{\text{CMax}}$ ~2°C above that, except in the late-successional species *Calophyllum longifolium*, in which both peaked at ~33°C. $R_{\text{Light}}$ significantly increased with increasing temperature, but simulations with a photosynthesis model indicated that this had only a small effect on net photosynthesis. We found no evidence for Rubisco-activase limitation of photosynthesis. $T_{\text{Opt}}$ of $V_{\text{CMax}}$ and $J_{\text{Max}}$ fell within the observed in situ leaf temperature range, but our study nonetheless suggests that net photosynthesis of tropical trees is more strongly influenced by the indirect effects of high temperature—e.g., through elevated vapor pressure deficit and resulting decreases in stomatal conductance—than by direct temperature effects on photosynthetic biochemistry and respiration.

Keywords: Climate change, $J_{\text{Max}}$, Global warming, Photosynthetic temperature response, Plant functional types, $R_{\text{Light}}$, Stomatal conductance, Tropical forest, $V_{\text{CMax}}$, VPD
Photosynthetic carbon uptake in tropical forests decreases at high temperature. To investigate the mechanisms underlying this decrease we analyzed the temperature sensitivities of biochemical and stomatal controls over net photosynthesis for four lowland tropical tree species in Panama. While net photosynthesis and stomatal conductance peaked near current ambient temperatures, biochemical control factors VCMax and JMax peaked at much higher temperatures. This, combined with model simulations, suggests that the decreased carbon uptake at high temperatures is caused stomatal closure, e.g., in response to increased vapor pressure deficit, and not by a direct temperature effect on the biochemical machinery of photosynthesis.

**Introduction**

Because of the importance of tropical forests to the global carbon cycle (Pan *et al.* 2011, 2013; Schimel *et al.* 2015) there is a need to improve their representation in Earth system models (e.g., Norby *et al.* 2017). More specifically, a better understanding is needed of the effects of temperature on the carbon exchange properties of tropical forest trees. A recent survey showed that the temperature optimum for net photosynthesis (TOpt) converged on mean daytime temperatures across 42 tropical tree and liana species in Panama (Slot & Winter 2017a), consistent with observations of stand-level photosynthesis across tropical sites (Tan *et al.* 2017). Observations of decreasing net carbon uptake above current ambient temperatures raise questions about how close to a high-temperature threshold tropical forests operate (Doughty & Goulden 2008). Above TOpt both stomatal and biochemical factors become increasingly limiting to net photosynthesis (Sage & Kubien 2007), but biochemical parameters were not rigorously assessed in the survey of Slot & Winter (2017a). Such an assessment, together with information on stomatal conductance will be essential to identify the mechanisms determining the temperature-responses of net carbon uptake by tropical forest trees.
The temperature response of photosynthesis of fully-illuminated leaves is controlled by photosynthetic biochemistry, stomatal conductance, and the rate of respiration in the light (Lin et al. 2012). At current ambient atmospheric [CO₂] the dominant biochemical limitation at suboptimal temperatures is triose phosphate utilization (TPU), a limitation of inorganic phosphate for photophosphorylation caused by low rates of starch and sucrose synthesis (Sage & Kubien 2007). At higher temperatures the maximum rate of RuBP-carboxylation ($V_{C_{\text{Max}}}$) and the maximum rate of RuBP-regeneration ($J_{\text{Max}}$)—generally assumed to reflect photosynthetic electron transport—become limiting, with de-activation of Rubisco caused by heat-sensitive Rubisco activase probably also playing a role at supraoptimal temperatures (Crafts-Brandner & Salvucci 2000; Sage & Kubien 2007). $V_{C_{\text{Max}}}$ and $J_{\text{Max}}$ are commonly assumed to either increase in an exponential fashion with increasing temperature, or to show a peaked temperature response (Medlyn et al. 2002). Stomatal conductance ($G_s$) has a peaked temperature response, which reflects the balance between a positive direct effect of temperature on stomatal opening, and a negative indirect effect, as leaf-to-air vapor pressure deficit (VPD) increases with increasing temperature causing $G_s$ to decrease (Peak & Mott 2011). Non-photorespiratory mitochondrial respiration in the light ($R_{\text{Light}}$) increases near-exponentially with temperature over ecologically relevant temperature ranges (Way et al. 2015), and may significantly lower net photosynthesis rates at high temperature (Way & Yamori 2014). Although reasonably well understood conceptually, these temperature-response parameters are not sufficiently quantified for tropical forest species, and the temperature response of tropical vegetation remains a key uncertainty in terrestrial carbon modeling (e.g., Ahlström et al. 2012; Cox et al. 2013; Huntingford et al. 2013; Rowland et al. 2015).
To improve the representation of tropical forests in Earth system models, the relationships of $V_{C_{\text{Max}}}$ and $J_{\text{Max}}$ with leaf nutrients have recently been determined for a large number of tropical forest species (Bahar et al. 2017; Norby et al. 2017). Bahar et al. (2017) reported that $V_{C_{\text{Max}}}$ and $J_{\text{Max}}$ at 25°C decreased with increasing mean annual temperature along an elevation gradient, but local temperature responses were not determined at any site. Lack of such data may have consequences for model performance. For example, Rowland et al. (2015) found that when simulating photosynthesis in tropical forests the uncertainty in, and the difference across, sophisticated vegetation models increased with temperature, in part because the models used different temperature optima for $V_{C_{\text{Max}}}$. $T_{\text{Opt}}$ values ranged from 30°C in the SPA model (Williams 1996) to 41°C in ED2 (Medvigy et al. 2009), and $V_{C_{\text{Max}}}$ at $T_{\text{Opt}}$ varied by >50% across the models. Variation in estimates of $V_{C_{\text{Max}}}$ (and $J_{\text{Max}}$) at a set temperature can be reduced by determining their relationship with foliar nutrient content (Bahar et al. 2017; Norby et al. 2017), but empirical data on temperature responses of $V_{C_{\text{Max}}}$ and $J_{\text{Max}}$ will be critical to constrain the temperature relationships and improve the representation of photosynthesis in Earth system models (Rogers et al. 2017).

We measured $V_{C_{\text{Max}}}$, $J_{\text{Max}}$, $G_{s}$, and $R_{\text{Light}}$ on free-growing saplings and trees of four ecologically-contrasting lowland tropical tree species across a range of leaf temperatures to determine the temperature optima and temperature-sensitivities of these parameters, and to evaluate species differences. In addition, we determined whether the temperature sensitivity of Rubisco activase influenced photosynthetic performance over ecologically relevant temperature ranges. Photorespiration ($R_{p}$), the oxygenation of Rubisco that leads to a loss of CO$_2$, represents another biochemical limitation of net carbon gain that increases with rising temperature (Brooks & Farquhar 1985), but this was not studied, as the available non-destructive methods for estimating $R_{p}$ require accurate assessment of mesophyll conductance currently not available for our target species. Based on previous measurements of the
temperature response of tree and liana net photosynthesis (Slot & Winter 2017a) and dark respiration (Slot et al. 2013, 2014) in Panama, we hypothesized that the process rates would be higher in early-successional species than in late-successional species, but that the thermal optima and the rate of increase in $R_{\text{Light}}$ with measurement temperature would be similar across species. To avoid the risk of measurement artifacts caused by excising branches (Santiago & Mulkey 2003) or by artificial growth conditions, all measurements were made on attached leaves in situ.

**Materials and Methods**

*Plant material*

Four common tree species, with one or more field-grown individuals with accessible leaves, were selected at the Santa Cruz Experimental Field Facility of the Smithsonian Tropical Research Institute in Gamboa, Panama (Table 1). Two of these were early-successional species and two were late-successional, enabling us to evaluate potential differences between plant functional types. The late-successional trees grew on the forest edge, receiving full solar radiation from mid-morning till mid-afternoon; the early-successional species were more permanently sun exposed. Soils at the site are classified as Alfisols. Mean annual temperature is 26.9°C, with mean daily minima and maxima of 23.7°C and 32.6°C, respectively. Annual rainfall averages 2058 mm, >80% of which falls between May and December (data from 2014–2016, meteorological data collected on site). *Ficus insipida* Willd. is an early-successional species that is most common in young or disturbed forests; *Lagerstroemia speciosa* (L.) Pers. is an introduced ornamental tree that is associated with open habitats and secondary forest in its native range in south Asia; *Calophyllum longifolium* Willd. is a late-successional species that is widespread in mature forests; and *Garcinia madruno* (Kunth)
Hammel is a late-successional species associated with wet forests. The three native species are all broadly distributed throughout tropical South and Central America. All trees were planted; the *L. speciosa* trees ~25 years ago, the others in early 2014, when they were ~1 year old.

**[TABLE 1]**

*A-C* curve measurements

*A-C* curves of trees are often measured on detached branches (e.g., Bahar *et al.* 2017), but branch excision may lead to changes in hydraulic properties, especially in latex-producing species, where the latex clogs the xylem upon excision (Santiago & Mulkey 2003). Lowered stomatal conductance may lead to truncated response curves, complicating parameter estimation. Because three of the four species in the current study produce latex (Table 1), we measured all parameters of interest on intact, attached leaves *in situ*. In the field, low leaf temperatures are rare in the afternoon, so testing the degree of hysteresis of gas-exchange parameters was not feasible. However, by measuring *A-C* curves at high temperature both before and after solar noon (Fig. S1), we made sure that our results do not simply reflect diurnal patterns, but indeed represent temperature responses.

Between November 2016 and February 2017 we measured *A-C* curves on sun-exposed leaves over as wide a temperature range as possible (see Table 1), making use of ambient temperature changes and controlling the block temperature of the cuvette using the Peltier cooling/heating capacity of the LI-6400 portable photosynthesis system (LI-COR Biosciences, Lincoln, Nebraska, USA). Measurements continued into the dry season, and
when the soil started to dry in February, plants were watered twice a week. There were no
trends in gas-exchange parameters with progression of the dry season.

Repeated measurements of the same leaf may result in stomatal closure independent
of temperature or VPD, so we measured each leaf only once, thereby generating temperature-
response curves not at the leaf level but at the species level (as in Slot & Winter 2017a).
Measurements were made on leaves between 0.7 and 1.7 m above the ground. Each species
was measured at a range of temperatures on five to eight days. The light intensity was set to a
pre-determined saturation level of 1500 µmol m⁻² s⁻¹. At high measurement temperature the
incoming air was humidified by moistening the soda lime in the column through which
ambient air entered the LI-6400. Light-saturated photosynthesis rates were determined at ≥13
CO₂ concentrations between 50 and 1800 ppm, using the built-in CO₂ mixer of the LI-6400 to
control CO₂ concentrations, maintaining a flow rate of 500 µmol s⁻¹. Leaf temperature during
measurement was monitored with a nickel-chromium thermocouple, attached abaxially to the
leaf inside the cuvette. After equilibration at 400 ppm CO₂, photosynthesis was generally
recorded at 50, 100, 200, 300, 400, 450, 500, 550, 625, 700, 900, 1250 and 1800 ppm CO₂ of
the incoming air. At leaf temperatures >~33°C stomatal conductance often dropped gradually
during the measurements, leading to declining rather than stabilizing photosynthesis rates
during the equilibration period. When this was observed the measurements were discontinued
and a new leaf was studied, because A-Ci curves based on non-steady state measurements do
not yield reliable estimates of V₉₉ and J₉₉. Despite humidifying the incoming air to reduce
the VPD at high leaf temperature, obtaining meaningful response curves at temperatures
>35°C was challenging. The relationship between temperature and relative humidity
experienced by leaves inside the cuvette was similar to that of the ambient air to which leaves
were exposed to in situ while not being measured (Fig. S2), and this relationship was similar
across species (Fig. S3).
Concentration gradients between the leaf cuvette and the atmosphere can cause CO2 to diffuse into, or out of the cuvette, which is then erroneously assigned to the photosynthetic flux. We therefore quantified the diffusion errors by measuring CO2-response curves for dead leaves, and corrected photosynthesis and C\textsubscript{i} values accordingly. Leaves were killed by submersion in boiling water (Flexas et al. 2007), for 2–10 minutes, depending on species, and CO2-response curves were measured at 2–4 target temperatures between 26 and 36°C. This protocol was repeated after replacing the gaskets of the leaf cuvette.

*Light respiration*

Respiration in the light was estimated at different leaf temperatures according to Laisk (1977). In brief, CO2 response curves were measured in the low CO2 range (for most leaves we used 45, 60, 75, 100, 125 and 200 ppm) at several different light levels (600, 200, and 100 µmol m\textsuperscript{-2} s\textsuperscript{-1}); the intersection point of the curves at different light levels indicates Γ\textsubscript{*}, the CO2 compensation point in the absence of respiration (apart from photorespiration). R\textsubscript{Light} is the CO2 exchange rate associated with Γ\textsubscript{*}. When photosynthesis at 200 ppm CO2 deviated from the linear part of the curve, this point was omitted to avoid introducing errors (Walker & Ort 2015). For all leaves for which the three linear fits of diffusion-corrected net photosynthesis versus C\textsubscript{i} intersected in one place, Γ\textsubscript{*} was calculated as:

\[
\Gamma^* = \frac{\text{Intercept}_{600} - \text{Intercept}_{100}}{\text{Slope}_{100} - \text{Slope}_{600}},
\]  

(Eqn 1)

where the subscripts refer to the irradiance level, and the slopes and intercepts describe the linear fits of the CO2 response curves at those irradiance levels. From these data species-specific relationships between Γ\textsuperscript{*} and temperature and between R\textsubscript{Light} and temperature were determined.
Leaf chemical and morphological traits

For each species three leaves were collected, leaf area was determined with an LI-3100 leaf area meter (LI-COR), and leaves were dried at 70°C until stable mass and weighed to determine leaf mass per area (LMA). Leaf nitrogen (N) content was measured using a Thermo Flash EA1112 analyzer (Waltham, MA, USA).

Leaf temperature monitoring

To characterize the thermal environment of sun-exposed leaves of each species, we measured leaf temperatures with fine-wire copper-constantan thermocouple wires attached abaxially with Transpor™ surgical tape (3M, Maplewood, MN, USA) to 4–7 leaves per species, logging the data every minute to a CR10X datalogger (Campbell Scientific, Logan, UT, USA). Each species was monitored for ~5 days in the early dry season.

Parameter calculation

CO₂-diffusion errors were independent of leaf temperature, but not identical across species. We therefore used species-specific, but temperature-independent error corrections to adjust photosynthesis and C₅ prior to A-C₅ curve fitting (Fig. 1).

\[ V_{\text{C} \text{Max}} \text{ and } J_{\text{Max}} \text{ were calculated from the corrected A-C}_5 \text{ curves according to the Farquhar, von Caemmerer and Berry model (FvCB model; Farquhar et al. 1980; von Caemmerer & Farquhar 1981) with the ‘fitaci’ function from the ‘plantecophys’ package (Duursma 2015) in R version 3.3.2 (R Development Core Team, 2016). The ‘fitaci’ function} \]
uses the temperature dependencies of the CO₂ compensation point (Γ*) and the Michaelis-Menten constants of Rubisco activity for CO₂ and O₂ from Bernacchi et al. (2001). Visual evaluation of the curves revealed no evidence for TPU limitation (see, e.g., Fig. 1) and hence TPU limitation was not considered in the calculations. At current ambient [CO₂] TPU is indeed unlikely to limit photosynthesis, given the high ambient temperatures in the lowland tropics (e.g., Sage & Kubien 2007). The thus calculated V_CMax and J_Max values slightly underestimate the chloroplastic rates, as mesophyll conductance (G_m, the diffusion limitation of CO₂ transfer from the substomatal cavity to the site of carboxylation in the chloroplast) was assumed to be infinite. G_m can be estimated from gas exchange data alone in the absence of carbon isotope fractionation or chlorophyll fluorescence measurements, but this approach is not highly recommended because it requires a priori assignation of data points to different limitations (RuBP-carboxylation, RuBP-regeneration, TPU); the results can be challenging to interpret as estimates can be affected by changes in V_CMax; and it assumes that G_m is stable across a wide range of [CO₂] (Pons et al. 2009). Furthermore, the temperature response of G_m is highly species-specific (von Caemmerer & Evans 2015) and we did not want to make assumptions about G_m that could influence the calculated temperature dependence of V_CMax and J_Max. R_Light was not measured on the same leaves as the A-Ci curves, but using the species-specific temperature-response curves of R_Light we calculated an R_Light value for every measurement temperature as model input. Similarly, Γ* estimates were calculated for each temperature from the temperature-response curves of Γ* determined with the Laisk method.
Temperature responses of $V_{CMax}$ and $J_{Max}$

For the analysis of the temperature relationships of photosynthetic parameters, data collected on different trees and on different days were pooled for each species. On each measurement day both low and high temperature measurements were made to avoid systematic bias in the temperature-response curves fitted through the pooled data.

The relationships of $V_{CMax}$ and $J_{Max}$ with leaf temperature in Kelvin ($T_k$) were fitted according to Medlyn et al. (2002) as:

$$ f(T_k) = k_{Opt} \times \frac{\left( \frac{H_a \times (T_k - T_{Opt})}{T_{Opt}} \right)}{H_d \times \left( 1 - e^{-\left( \frac{H_a \times (T_k - T_{Opt})}{T_{Opt}} \right)} \right)} \quad \text{(Eqn 2)} $$

where $k_{Opt}$ is $V_{CMax}$ or $J_{Max}$ at $T_{Opt}$ in Kelvin, $H_a$ describes the exponential rise of the curve before $T_{Opt}$, equivalent to the activation energy in an Arrhenius function, $H_d$ is the ‘de-activation energy’, reflecting the rate of decrease above $T_{Opt}$, and $R$ is the universal gas constant (8.314 J K$^{-1}$ mol$^{-1}$). In Medlyn et al. (2002) and many later publications, $H_d$ was fixed at 200 kJ mol$^{-1}$. We estimated all four parameters, including $H_d$, but for comparison also fitted the curves with $H_d$ fixed at 200 kJ mol$^{-1}$. Obtaining robust parameter estimates for complex models such as Eqn 2 requires more independent observations than are typically collected when re-measuring individual leaves. By combining leaves that were each measured only once we avoided overfitting and had enough independent data points per species to estimate all parameters of the temperature response curves.
Temperature-responses of all parameters were also fitted with a much simpler equation developed for $J_{\text{Max}}$ by June et al. (2004):

$$\text{k} (T_c) = k_{\text{Opt}} \times e^{-\frac{(T_k - T_{\text{Opt}})^2}{\Omega}}$$  

(Eqn 3)

where $\Omega$ is the temperature difference between $T_{\text{Opt}}$ and the temperature at which $k$ is reduced to $e^{-1}$ (~37%) of its value at $T_{\text{Opt}}$; it describes the ‘sharpness’ of the peak of the symmetrical curve.

Net photosynthesis at 400 ppm ($A_{400}$) was extracted from each $A$-$C_i$ curve, and $A_{400}$ as a function of leaf temperature was fitted with Eqn 3 to determine $T_{\text{Opt}}$ of $A_{400}$. These steps were repeated for photosynthesis at 300 ppm CO$_2$ ($A_{300}$), 600 ppm ($A_{600}$), and at 900 ppm ($A_{900}$).

**Stomatal limitation**

Stomatal limitation of net photosynthesis (‘$l$’) was calculated following Farquhar & Sharkey (1982) by comparing observed rates of photosynthesis with rates expected at infinite stomatal conductance:

$$l = 1 - \frac{A_{\text{Observed}}}{A_{(\text{infinite } G_s)}}$$  

(Eqn 4)

where $A_{(\text{infinite } G_s)}$ is calculated from the FvCB model by setting $C_i$ to equal the CO$_2$ concentration in the cuvette ($C_a$) in Eqn 5:
where \( A_{\text{gross}} \) equals light-saturated photosynthesis + \( R_{\text{Light}} \), and \( K_c \) is the Michaelis–Menten constant of Rubisco activity for CO\(_2\).

**Rubisco activase effects on photosynthesis**

We assessed the effect of Rubisco activase on net photosynthesis using the method described in Sage *et al.* (2008). Declining activity of Rubisco activase at high temperature may reduce the activation state of Rubisco and thereby limit photosynthesis (Crafts-Brandner & Salvucci 2000). However, to sustain high activity, Rubisco activase requires ample ATP supply, and if the electron transport rate (i.e., \( J_{\text{Max}} \)) is reduced at high temperature, the resulting reduction in ATP supply could inhibit Rubisco activase. To determine whether Rubisco activase limits net photosynthesis at high temperature independent of decreasing \( J_{\text{Max}} \), we determined the effect of temperature on Rubisco activase at low [CO\(_2\)], where electron transport does not limit photosynthesis. We assessed the role of Rubisco activase by comparing measurements of the initial slopes of A-C\(_i\) curves—i.e., at low [CO\(_2\)] where the ATP supply is non-limiting to Rubisco activase—with slope estimates that were derived from a model that assumes that Rubisco is fully-activated. The method thus consists of testing this model against observations, and by extension, testing the assumptions in the model. The initial slope was modeled as:

\[
\text{Initial slope} = \frac{V_{\text{Chmax}}}{(\Gamma^* + K_c \times (1 + \frac{O}{K_o}))} \quad \text{(Eqn 6)}
\]

where \( K_c \) and \( K_o \) are the Michaelis–Menten constants of Rubisco activity for CO\(_2\) and O\(_2\), respectively, and O is the oxygen concentration in the chloroplast stroma. \( \Gamma^* \) was estimated.
for each temperature as before, O was assumed to equal 210 mbar, and \( K_c \) and \( K_o \) were taken from Bernacchi et al. (2001). Measurements of the initial slope for each leaf—simple linear regressions—used all datapoints with \( C_i < 200 \mu \text{mol mol CO}_2 \). When measured slopes are lower than modeled slopes, the assumption of fully-activated Rubisco is invalidated and de-activation of Rubisco is implied. By plotting the initial slopes against leaf temperature, the temperature at which Rubisco activase limitation occurs can be determined.

**Statistical analyses**

All non-linear curve fitting was done with non-linear least squares analyses using the ‘nls’ function in the ‘stats’ package in R. Differences in temperature-response parameters among species and between \( V_{C_{\text{Max}}} \) and \( J_{\text{Max}} \) were determined by checking for overlap of the 95% confidence intervals. Comparisons between early-successional and late-successional species were made with t-tests. Simple linear regressions were analyzed with the ‘lm’ function in R. To identify the best predictors of net photosynthesis rate across different temperature ranges, we used the subset selection method for multiple regression (Miller 2002) from the ‘Leaps’ package, which identifies the best combination of predictors for any subset size from all possible combinations of those predictors, using \( r^2 \), adjusted \( r^2 \), and Mallows’ \( C_p \) (Mallows 1973) as selection criteria. To verify the implications of different thermal optima and temperature sensitivities of biochemical, stomatal, and respiratory limitations for net photosynthesis rates we simulated net photosynthesis with the FvCB model using the ‘Photosyn’ function from the ‘plantecophys’ package (Duursma 2015). In these simulations we used all measured parameter values as input and compared the modeled photosynthesis rates with rates that were obtained in simulations in which \( V_{C_{\text{Max}}} \), \( J_{\text{Max}} \), \( G_s \), or \( R_{\text{Light}} \) were
increased by 50%, either individually, or in combinations. All analyses were performed in R, version 3.3.2.

Results

A total of 149 A-C$_4$ curves were included in the final analyses (Table 1) after 29 curves were discarded because they could not be fitted with the standard iterative method of the ‘fitaci’ function, or because visual inspection indicated that they were of poor quality. Early-successional species _L. speciosa_ had the lowest LMA while late-successional _C. longifolium_ had the highest LMA (Table 1). These species also had the smallest and largest diffusion error, respectively (Fig. 1a). The species-specific diffusion error correction that we applied to the raw data resulted in a considerable reduction of V$_{C_{\text{Max}}}$ from an average of 5% in _F. insipida_ and _L. speciosa_ to 13% in _G. madruno_. The reduction in J$_{\text{Max}}$ ranged from 7% in _L. speciosa_ to 14% in _G. madruno_. The percent reduction in process rates was inversely proportional to absolute V$_{C_{\text{Max}}}$ and J$_{\text{Max}}$ values (Fig. 1b). Despite these systematic changes in parameter estimates, T$_{\text{Opt}}$ for these parameters was not affected by error correction (data not shown).

Temperature response of biochemical parameters

**Maximum rate of RuBP-carboxylation, V$_{C_{\text{Max}}}$**

T$_{\text{Opt}}$ of V$_{C_{\text{Max}}}$ calculated with Eqn 2 with four free parameters, ranged from 32.9°C in _C. longifolium_ to 39.7°C in _L. speciosa_ (Table 2). T$_{\text{Opt}}$ was highest in _L. speciosa_ but did not differ significantly among the other species (they had overlapping 95%-confidence intervals; Fig. 2). V$_{C_{\text{Max}}}$ at T$_{\text{Opt}}$ was higher in early-successional species than in late-successional
species; especially *G. madruno* had much lower values than all other species (Table 2). Early-
successional species also had higher leaf N content than late-successional species (*P* = 0.003,  
t-test, Table 2), but a positive correlation between leaf N content and *V*<sub>CMax</sub> at *T*<sub>Opt</sub> was not  
significant (*P* = 0.16). The activation energy (H<sub>a</sub>) of *V*<sub>CMax</sub> was reasonably well constrained  
around 75 kJ mol<sup>-1</sup> for three of the species, but H<sub>a</sub> was 350 ± 303 kJ mol<sup>-1</sup> for *C. longifolium*.  
The de-activation energy, H<sub>d</sub>, differed enormously among species, and its estimates were  
highly uncertain (Table 2). When fixing H<sub>d</sub> to 200 kJ mol<sup>-1</sup>, *T*<sub>Opt</sub> of *V*<sub>CMax</sub> was moderately  
higher in all species (Table 2; Fig. 2) while *V*<sub>CMax</sub> at *T*<sub>Opt</sub> tended to be lower. Fitting the  
symmetric curves of Eqn 3 yielded fairly similar parameter estimates as the model with fixed  
H<sub>d</sub> (Table 2), but Eqn 3 tended to overestimate *V*<sub>CMax</sub> above *T*<sub>Opt</sub>.  

When using an approximation of *V*<sub>CMax</sub> (indicated as $\bar{V}_{CMax}$) by estimating it with the  
one-point method (e.g., De Kauwe *et al.* 2016) *T*<sub>Opt</sub> was similar to *T*<sub>Opt</sub> of measured *V*<sub>CMax</sub>.  
However, $\bar{V}_{CMax}$ tended to be lower than *V*<sub>CMax</sub>, and this discrepancy increased with  
ingcreasing leaf temperature (Fig. S4).

**Maximum rate of RuBP-regeneration, J<sub>Max</sub>**

The optimum temperature of J<sub>Max</sub> was more constrained across species than that of *V*<sub>CMax</sub>,  
with all values falling between 33.5°C and 37.5°C (Table 2). *T*<sub>Opt</sub> of J<sub>Max</sub> appeared lower than  
*T*<sub>Opt</sub> of *V*<sub>CMax</sub> in three of the four species, but this difference was significant only for *L. speciosa* (Fig. 2). J<sub>Max</sub> at *T*<sub>Opt</sub> was highest in the early-successional species and lowest in *G. madruno*. There was a marginally significant positive correlation between J<sub>Max</sub> at *T*<sub>Opt</sub> and  
leaf N content (*P* = 0.09, $r^2$ = 0.83). H<sub>a</sub> and H<sub>d</sub> of J<sub>Max</sub> were not well constrained, and while  
H<sub>a</sub> was higher, H<sub>d</sub> was lower in the late-successional species compared to the early-
successional species. Fixing H<sub>d</sub> to 200 kJ mol<sup>-1</sup> decreased both *T*<sub>Opt</sub> of J<sub>Max</sub> and J<sub>Max</sub> at *T*<sub>Opt</sub> in
all species except *G. madruno* (Table 2). \( T_{\text{Opt}} \) and \( J_{\text{Max}} \) at \( T_{\text{Opt}} \) estimated with Eqn 3 yielded comparable results to those estimated with Eqn 2. The ratio of \( J_{\text{Max}} \) to \( V_{\text{CMax}} \) at \( T_{\text{Opt}} \) was lowest in the early-successional *L. speciosa* (0.65–0.75, depending on the method used to fit the temperature-response curves) and highest in the late-successional *G. madruno* (1.12–1.15) (Table 2). Because \( V_{\text{CMax}} \) is underestimated a bit more than \( J_{\text{Max}} \) when infinite \( G_m \) is assumed in the calculation (e.g., Sun *et al.* 2014), the \( J_{\text{Max}} \) to \( V_{\text{CMax}} \) ratio will have been slightly overestimated compared to the chloroplastic ratio.

**[TABLE 2]**

*Rubisco activase*

Initial slopes of measured \( A-C_i \) curves did not differ significantly from slopes that were modeled with the assumption of fully-activated Rubisco. This is especially clear when comparing the smooth fitted curve representing the modeled slopes in Fig. 3 with the measured slopes averaged across 2°C leaf temperature bins; even at the highest temperature the measured values do not drop significantly below the modeled values, suggesting that over the leaf temperature range used in the current study Rubisco-activase limitation was not a significant factor affecting net photosynthesis.
Temperature responses of stomatal conductance and respiration in the light

\( T_{\text{Opt}} \) of stomatal conductance at 400 ppm CO\(_2\), determined with Eqn 3, was on average 30.3 ± 1.7°C, and thus considerably lower than \( T_{\text{Opt}} \) of the biochemical parameters, except in \( C. \) longifolium (Fig. 2). Species differed in \( G_s \) at \( T_{\text{Opt}} \), with late-successional species \( G. \) madruno having average rates of 0.15 mol m\(^{-2}\) s\(^{-1}\), while early-successional species \( L. \) speciosa and \( F. \) insipida had rates of 0.41 and 0.50 mol m\(^{-2}\) s\(^{-1}\), respectively. In \( G. \) madruno \( T_{\text{Opt}} \) was outside the measured range, and its estimate was highly uncertain (Fig. 2).

\( R_{\text{Light}} \) increased with temperature in an exponential fashion (Fig. 2). The rate of increase was significantly higher in the early-successional than the late-successional species \((P = 0.02; t\)-test\), but this difference did not translate into systematically higher \( R_{\text{Light}} : A_{\text{Gross}} \) ratios in early-successional species at high temperatures. The \( R_{\text{Light}} : A_{\text{Gross}} \) ratio was highest for \( G. \) madruno, with average values ranging from 0.05 at 30°C to 0.10 at 37°C, compared to 0.01–0.02 at 30°C and 0.06–0.09 at 37°C for all other species.

Net photosynthesis, [\( \text{CO}_2 \)], stomatal limitation, and leaf temperatures

Net photosynthesis at 400 ppm CO\(_2\) (\( A_{400} \)) peaked between 30.0 and 32.3°C (Table 3), which roughly corresponds to the most commonly experienced daytime leaf temperatures (Fig. 4). \( T_{\text{Opt}} \) of net photosynthesis and the rate of net photosynthesis at \( T_{\text{Opt}} \) both increased with increasing measurement [\( \text{CO}_2 \)], with rates more than doubling between 300 and 900 ppm CO\(_2\), and \( T_{\text{Opt}} \) increasing by on average 2.2°C over the same CO\(_2\) range (Table 3; Fig. 5).
As $A_{400}$ decreased above $T_{\text{Opt}}$, stomatal limitation of photosynthesis significantly increased (Fig. 6). *C. longifolium*, the species with the lowest temperature optima of $V_{\text{CMax}}$ and $J_{\text{Max}}$, was the only species that did not display a significant increase in stomatal limitation with increasing temperature. Consistent with this, $A_{400}$ scaled significantly with $C_i : C_a$ ratios in all species except *C. longifolium* (slope significantly greater than zero with $P < 0.001$ in the early-successional species and $P = 0.05$ in *G. madruno*), indicating that reduction in photosynthesis in all species except *C. longifolium* was associated with a draw-down of CO$_2$ in the substomatal cavities, and thus with increased stomatal resistance (Fig. S3).

In situ leaf temperatures tended to be higher in the late-successional species, possibly as a result of their lower stomatal conductance, but because leaf temperature is strongly influenced by solar irradiance incident on the leaf surface (e.g., Rey-Sánchez et al. 2016) and irradiance was not monitored at the leaf level, this relationship between leaf temperature and stomatal conductance could not be confirmed. Stomatal conductance and associated transpirational cooling did, however, appear to play a role in determining the frequency distribution of leaf temperatures in all species. Because the transition of leaf temperatures associated with indirect light to direct light is rapid, the frequency distributions of temperatures of sun leaves were almost bimodal (Fig. 4), with the first peak—corresponding with mean daytime air temperature—associated with predominantly indirect light, near-maximum stomatal aperture, and transpirational cooling, and the second peak associated with full sun exposure, closed stomata and minimal transpirational cooling.
Net photosynthesis above $T_{\text{Opt}}$

$A_{400}$ did not correlate significantly with $V_{\text{CMax}}$ in the early-successional species regardless of the measurement temperature range, while significant positive correlations were found for the late-successional species at almost all temperatures (Fig. 7a). In all species except *C. longifolium* the steepness of the relationship increased with increasing measurement temperature range, and at higher temperatures the relationships became marginally significant for the early-successional species ($P < 0.1$). All species showed significant positive correlations between $A_{400}$ and $J_{\text{Max}}$, and in the early-successional species the steepness of this relationship increased with increasing temperature (Fig. 7b). In *L. speciosa* this correlation was only significant $> 35^\circ\text{C}$. $A_{400}$ correlated with stomatal conductance in all species, and the strength of the correlation and the steepness of the regression slopes were mostly independent of temperature (Fig. 7c). $A_{400}$ correlated negatively with respiration in the light, and this relationship generally got steeper at higher temperatures (Fig. 7d).

Stomatal conductance was the strongest single predictor for net photosynthesis when considering the full dataset, with $r^2$ values ranging from 0.44 in *G. madruno* to 0.78 in *C. longifolium*. As temperature approached $T_{\text{Opt}}$ of $J_{\text{Max}}$, $J_{\text{Max}}$ became the strongest predictor of net photosynthesis (Fig. 8). In *C. longifolium* $J_{\text{Max}}$ was equally important as $G_s$ at temperature $>31^\circ\text{C}$ ($r^2 = 0.8$ for both), suggesting an earlier transition into becoming electron-transport limited than in the other species. The best two-predictor model for $A_{400}$ always included $G_s$ and $J_{\text{Max}}$.

Simulations with the FvCB model showed that increasing measured $G_s$ by 50% stimulated modeled net photosynthesis rates, and consistent with Fig. 6, this stimulation significantly increased with increasing leaf temperature (Fig. S5-V). Reducing $R_{\text{Light}}$ had a positive effect on net photosynthesis that increased significantly with increasing leaf
temperature in all species, but photosynthesis was stimulated by at most \( \sim 6\% \) (Fig. S5-V).

The simulations suggest that there is no overall positive correlation between leaf temperature and the stimulation of photosynthesis with an increase in \( J_{\text{Max}} \). In fact, in \( L. \ speciosa \), the stimulating effect that increased \( J_{\text{Max}} \) had on photosynthesis decreased with increasing temperature. However, this decrease occurred only below \( T_{\text{Opt}} \) of \( J_{\text{Max}} \) and increasing \( J_{\text{Max}} \) above \( T_{\text{Opt}} \) did stimulate photosynthesis in all species except \( G. \ madruno \), but with only a few datapoints above \( T_{\text{Opt}} \) this stimulation was not always significant. In \( G. \ madruno \)—the species with the lowest \( V_{\text{C}_{\text{Max}}} \) values and with fairly flat temperature-response curves (Fig. 2)—there was a temperature-independent stimulation of photosynthesis of 25–30\% with 50\% increase in \( V_{\text{C}_{\text{Max}}} \) (Fig. S5-V). Increasing both \( G_s \) and \( J_{\text{Max}} \) increased the stimulation in net photosynthesis beyond that of single-parameter increase except in \( G. \ madruno \), for which an increase in \( G_s \) and \( V_{\text{C}_{\text{Max}}} \) had the most stimulating effect on photosynthesis of any combination (data not shown). In \( C. \ longifolium \), in which \( T_{\text{Opt}} \) values of \( V_{\text{C}_{\text{Max}}} \), \( J_{\text{Max}} \), and \( G_s \) were very similar, increasing \( G_s \) and \( V_{\text{C}_{\text{Max}}} \), or increasing \( V_{\text{C}_{\text{Max}}} \) and \( J_{\text{Max}} \) stimulated photosynthesis as much as increasing \( G_s \) and \( J_{\text{Max}} \).

**Discussion**

Consistent with previous observations (Doughty & Goulden 2008; Slot \textit{et al.} 2016; Slot & Winter 2017a) net photosynthesis of all measured species peaked near ambient daytime temperature, at about 30–32°C, supporting our hypothesis that the optimum temperature would not differ between early- and late-successional species. The temperature-responses of underlying parameters did, however, differ considerably among species (Fig. 8), but there were some general patterns. The temperature optima for RuBP-carboxylation and RuBP-regeneration were generally in the 35–40°C range, while stomatal conductance peaked at
much lower temperatures. Stomatal limitation of net photosynthesis increased with temperature and appeared to be controlling the rates of net photosynthesis for much of the measured temperature range. The increasingly strong and increasingly positive correlation between net photosynthesis and J_max at high leaf temperatures suggests that photosynthesis experiences increasing limitation by photosynthetic electron transport capacity as well, although the temperature at which this happens differs among species. We found no evidence for Rubisco-activase limitation, and R_Light was not a major limitation to net photosynthesis at high temperatures. The inhibition of respiration by light, as inferred from the Laik method, has recently been disputed (Farquhar & Busch 2017), but our model simulations show that changes in R_Light have very minimal effects on net photosynthesis rates, so even if we underestimated R_Light this is unlikely to change our conclusions. While there is uncertainty about the optimal temperatures of processes of photosynthetic biochemistry in vegetation models (Rowland et al. 2015), our results suggest that for most of the ecologically relevant temperature range in the lowland tropics, constraining parameter estimates for stomatal conductance will be of greater importance for reducing uncertainty in simulations of biosphere-atmosphere carbon exchange in tropical forests, than constraining the optima of biochemical parameters.

*Temperature optima of biochemical parameters*

We were able to identify T_opt values for V_Cmax and J_max without the need to extrapolate beyond the measurement range, as has often been necessary in previous studies (e.g. Scafaro et al. 2017; some species in Vårhammar et al. 2015). Nonetheless, in *L. speciosa* the position of T_opt was driven by very few high-temperature datapoints. Our *in situ* approach avoided potential artifacts caused by excision of latex-producing branches (Santiago & Mulkey 2003),
or by artificial conditions of potted plants in greenhouses conditions, but it limited our ability to study leaves at very high temperature. Several previous studies were able to determine $V_{\text{CMax}}$ and $J_{\text{Max}}$ up to 40°C by acclimatizing whole seedlings to elevated temperatures (e.g., Dreyer et al. 2001; Aspinwall et al. 2017), while plants in the field temperatures typically close their stomata when reaching temperatures in the mid-to-high 30’s (M. Slot, personal observation). While more measurements at high temperature would improve the accuracy of the $T_{\text{Opt}}$ estimates for *L. speciosa*, $T_{\text{Opt}}$ of the other species was more confidently determined to be in the 33–37°C range. These values are not substantially higher than those of cool-climate species (Kattge & Knorr 2007; Vårhammar et al. 2015) even though Kattge & Knorr (2007) reported a positive correlation between growth temperature and $T_{\text{Opt}}$ of $V_{\text{CMax}}$ and $J_{\text{Max}}$. The temperature optima we determined fall within the range of values used in the models that Rowland et al. (2015) compared, and they fall within the observed leaf temperature range (Fig. 4). Nonetheless, biochemical limitations to net photosynthesis appear to play a secondary role compared to stomatal limitation for most of the ambient temperature range.

**Stomatal conductance as key limitation of net photosynthesis**

The optimum temperature was lower for stomatal conductance than for the biochemical parameters $V_{\text{CMax}}$ and $J_{\text{Max}}$ in three of the four species; stomatal conductance was the strongest correlate of net photosynthesis; increasing stomatal conductance above measured values in model simulations consistently stimulated net photosynthesis; this stimulation of modeled photosynthesis increased with temperature in all species, more so than stimulating other parameters did (Fig. S5-V); and stomatal limitation parameter ‘l’ significantly increased with temperature, except in *C. longifolium*. The comparatively low optimum temperatures of
$J_{\text{Max}}$ and $V_{\text{CMax}}$ and early onset of $J_{\text{Max}}$ as strongest correlate of net photosynthesis in $C.\text{longifolium}$ suggests a greater degree of co-limitation of photosynthesis by stomatal conductance and biochemical parameters at high temperature in this species.

Strong declines in stomatal conductance above $T_{\text{Opt}}$ in previous in situ measurements of tropical trees suggested that stomatal conductance is the major limiting factor of net photosynthesis at supra-optimal temperatures (Slot & Winter 2017a). The current analysis confirms that the decrease in photosynthesis above $T_{\text{Opt}}$ is primarily driven by the indirect effect of temperature—through VPD and stomatal conductance—not by the direct effect of temperature on photosynthetic biochemistry or respiratory metabolism. This is further illustrated by the similarity in the curves of photosynthesis versus leaf temperature and photosynthesis versus VPD, a similarity that is driven by the strong effect of leaf temperature on VPD (Figs. S3, S6). At higher temperatures electron transport rates do decrease (Fig. 8; Wise et al. 2004; Sage & Kubien 2007; Vårhammar et al. 2015), but the effect of this on net photosynthesis for field-grown tropical forest trees is probably small. A-C$_{i}$-based studies systematically underestimate the importance of stomatal closure for photosynthesis at high temperature, because of the inherent selection bias favoring leaves with open stomata to obtain meaningful A-C$_{i}$ curves. In most leaves of the trees studied here, including $C.\text{longifolium}$, stomata closed at temperatures in the mid-to-high 30°C’s. If these leaves would be taken into consideration, an even stronger stomatal limitation would be inferred. Even among the measured leaves stomatal conductance had already decreased by 30–50% relative to its maximum when temperatures reach $T_{\text{Opt}}$ of $J_{\text{Max}}$, so it is likely that even at supra-optimal temperatures for $J_{\text{Max}}$ CO$_{2}$ reaching the chloroplasts is a much stronger limitation than electron transport rate. The fact that by the time leaves in the field reach temperatures supra-optimal for $J_{\text{Max}}$, the stomata typically are fully closed, renders potential electron transport limitations largely irrelevant under field conditions. Our results for tropical trees, based on a
thorough study of attached leaves in situ, are consistent with model simulations at the leaf- and biome level (Lloyd & Farquhar 2008; Rowland et al. 2015) and with observations at the stand level (Wu et al. 2016; Tan et al. 2017).

Although net photosynthesis of these tropical trees is clearly limited by stomatal conductance at high temperatures, mesophyll conductance ($G_m$), may also impose a significant limitation. However, despite progress in our mechanistic understanding of $G_m$ and its regulation (e.g., von Caemmerer & Evans 2015; Qui et al. 2017; Xiao & Zhu 2017), the temperature response of $G_m$ remains poorly resolved and appears to be highly species-specific (Flexas et al. 2008; von Caemmerer & Evans 2015). Furthermore, most Earth system models do not yet include $G_m$ (Sun et al. 2014; Rogers et al. 2017). Generally, $G_m$ either increases and then plateaus at high temperature, or it starts decreasing above 30 to 35°C (reviewed in Flexas et al. 2008 and Qiu et al. 2017). Data for tropical forest plants are rare. $G_m$ of the one tropical tree species in von Caemmerer & Evans (2015) peaked at ~35°C, while Pons & Welschen (2003) showed a marginal decline in $G_m$ between 28 and 38°C for *Eperua grandiflora*, another tropical tree species. Clearly, more measurements would be required to establish the contribution of $G_m$ to the decline in net photosynthesis above current ambient temperatures in tropical forest species.

Species differences and sampling challenges in hyper-diverse tropical forests

The highest temperature optima for $V_{C_{\text{Max}}}$ and $J_{\text{Max}}$ were found in *L. speciosa*, a species native to south Asia, where maximum temperatures are higher than in Panama. The lowest optima were found in *C. longifolium*, a Neotropical late-successional species. Interestingly, $T_{\text{Opt}}$ of net photosynthesis was almost identical for these two species (Table 3). Although well-replicated at the leaf-level, species in the current study were not represented by many
individuals. Nonetheless, the apparent species differences in the regulation of temperature responses of photosynthesis are disconcerting given the species richness of tropical forests and the desire for a simple, mechanistic understanding of how tropical species at large behave at high temperature, for the sake of modeling climate change effects on tropical vegetation.

While some species differences were consistent with species’ functional groups (e.g., greater temperature sensitivity of $R_{\text{Light}}$, and lower activation energy of $V_{\text{CMax}}$ in early-successional species than in late-successional species), others were not. Identifying commonality in control processes will be instrumental for reducing the uncertainty of ecosystem models, but determining biochemical parameters and $R_{\text{Light}}$ is labor-intensive, and tropical species richness is high. We assessed the utility of estimating $V_{\text{CMax}}$ with the one-point method when studying temperature relations (S4) and while we have greater confidence in the absolute values of $V_{\text{CMax}}$ determined from A-C$_{i}$ curves than in estimates derived from the one-point method (see Fig. S4), the one-point method is very rapid. When assessing the effects of high temperature, progressive stomatal closure during A-C$_{i}$ curve measurements may compromise the quality of the curves. Measurements after quick equilibration at ambient CO$_{2}$, or even rapid non-equilibration A-C$_{i}$ measurements (Stinziano et al. 2017) may increase the chances of obtaining useful high temperature data and enable the assessment of a larger number of species and individuals per species.

Concluding remarks and outlook

Net photosynthesis of lowland tropical trees peaks close to ambient daytime temperature, despite some species differences in the temperature responses of the factors controlling net photosynthesis. The indirect effect of temperature—through VPD and stomatal conductance—is consistently a stronger limitation of net photosynthesis than the direct effect
of temperature on biochemical process rates, especially when considering that many leaves with very low stomatal conductance were excluded from A-C, curve measurements. These results stress the importance of parameterizing stomatal properties in relation to temperature, VPD, and soil hydraulic conditions to better simulate the carbon dynamics of tropical forest trees in the face of atmospheric and climate change. And although challenging experimentally, disentangling temperature effects from VPD effects on stomatal conductance will be particularly valuable for understanding and modeling photosynthesis in a changing climate (Peak & Mott 2011).

As temperature continues to rise, thermal acclimation will likely increase the optimum temperature of photosynthesis (Slot & Winter 2017b). Rising atmospheric [CO₂] will further increase the temperature optimum by lowering photorespiration (Fig. 5; Cernusak et al. 2013). On the other hand, lower stomatal conductance associated with elevated CO₂ may reduce transpirational cooling and increase leaf temperatures. At higher [CO₂] TPU may also become limiting (Sage & Kubien 2007), particularly because low phosphorus availability in tropical forests (e.g., Vitousek et al. 2010) may enhance TPU limitation (Ellsworth et al. 2015). Given that differences in thermal optima of biochemical control factors of photosynthesis exist among species, an important challenge will be to describe the long-term effects of climate change in terms of changes in the stomatal and biochemical properties of tropical tree species, and to identify general patterns across species to facilitate predictions of long-term changes across the tropics.

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Supporting Materials

S1. Mean leaf temperatures during A-C_i curves measured at different times during the day.

S2. Relative humidity as a function of temperature inside the leaf cuvette compared with that of the ambient air.

S3. Species comparison of leaf temperature effects on environmental and physiological traits associated with atmospheric moisture demand.

S4. Comparison of V_{C_max} determined from A-C_i curves and estimated with the one-point method.

S5. Exploring limitations to net photosynthesis with the Farquhar-von Caemmerer-Berry photosynthesis model

Figure 1. Illustration of the species-specific, [CO$_2$]-dependent error in photosynthesis measurements caused by CO$_2$ diffusion into and out of the cuvette (a), and the effects of this error for the calculation of V$_{C\text{Max}}$ and J$_{\text{Max}}$ in a species with high rates of photosynthesis (b, Ficus insipida) and a species with low rates of photosynthesis (c, Garcinia madruno).

Photosynthesis data were corrected prior to analyses of derived parameters.

*L. speciosa*: $y = -3.0 \times 10^{-7} x^2 + 0.0015 x - 0.717 \quad (r^2=0.97)$

*F. insipida*: $y = -4.6 \times 10^{-7} x^2 + 0.0022 x - 1.051 \quad (r^2=0.97)$

*C. longifolium*: $y = -4.7 \times 10^{-7} x^2 + 0.0022 x - 0.914 \quad (r^2=0.98)$

*G. madruno*: $y = -4.8 \times 10^{-7} x^2 + 0.0021 x - 0.862 \quad (r^2=0.98)$
Figure 2. Temperature relationships of the maximum rates of RuBP-carboxylation ($V_{C_{Max}}$, a) and RuBP-regeneration ($J_{Max}$, b), stomatal conductance ($G_s$, c), net photosynthesis ($A_{Net}$, d) and respiration in the light ($R_{Light}$, e) for four lowland tropical tree species. Temperature responses of $V_{C_{Max}}$ and $J_{Max}$ are fitted with Eqn 2 with four free parameters (solid lines) and with the de-activation energy $H_d$ set to 200 kJ mol$^{-1}$ (dashed lines). $G_s$ and $A_{Net}$ are fitted with Eqn 3 and for $R_{Light}$ exponential fits are shown. Vertical dotted lines and pink bars indicate temperature optima ($T_{Opt}$) calculated with Eqn 4 with four free parameters and their 95% confidence intervals. $T_{Opt}$ for $V_{C_{Max}}$ and $J_{Max}$ fitted with $H_d = 200$ kJ mol$^{-1}$ can be found in Table 2.
Figure 3. Initial slopes of A-C\textsubscript{i} curves as a function of leaf temperature in four lowland tropical tree species. Light gray symbols are all the measured slopes, dark gray symbols are plotted as means ± SD per 2°C leaf temperature bin; smooth solid red lines represent the modeled initial slopes calculated with Eqn 6.
Figure 4. Frequency distributions of daytime (7.00–18.00) leaf temperatures of sun-exposed leaves of four tropical tree species. For each species multiple leaves were monitored abaxially for several days, and temperatures were logged at 1–minute intervals.
Figure 5. Net photosynthesis of four lowland tropical tree species as a function of leaf temperature at a range of measurement CO₂ concentrations. Curves were fitted according to Eqn 3; parameter values derived from these curves can be found in Table 3. Dashed arrows indicate the shift in the maximum photosynthetic rate with increasing measurement CO₂ concentration.
Figure 6. Stomatal limitation of net photosynthesis measured at 400 ppm CO₂ calculated with Eqn 4 and its response to temperature in four lowland tropical tree species presented on a scale from 0—observed photosynthesis equals the hypothetical rate associated with infinite stomatal conductance—to 1—complete suppression of photosynthesis by low stomatal conductance. Solid lines indicate significant trends ($P < 0.01$).
Figure 7. Net photosynthesis measured at 400 ppm CO$_2$ in relation to $V_{\text{CMax}}$ (a), $J_{\text{Max}}$ (b), stomatal conductance (c), and respiration in the light (d) for four lowland tropical tree species, with regression lines fitted for different measurement temperature ranges. Solid lines indicate significant fits with $P < 0.05$; dashed lines indicate non-significant fits.
Figure 8. Fitted curves of maximum RuBP-carboxylation rate ($V_{C_{\text{Max}}}$, green), RuBP-regeneration rate ($J_{\text{Max}}$, blue), stomatal conductance ($G_s$, red; scaled on the secondary y-axis), and net photosynthesis ($A_{\text{Net}}$, black) versus leaf temperature for four lowland tropical tree species. Vertical dotted lines indicate $T_{\text{Opt}}$ of $A_{\text{Net}}$. Horizontal bars indicate measured temperature ranges over which $V_{C_{\text{Max}}}$ (green), $J_{\text{Max}}$ (blue), and $G_s$ (red) are decreasing. Arrows indicate the temperature ranges over which $G_s$ (red) or $J_{\text{Max}}$ (blue) is the strongest single predictor of $A_{\text{Net}}$. $V_{C_{\text{Max}}}$ and $J_{\text{Max}}$ were fitted with Eqn 2 (with 4 free parameters); $A_{\text{Net}}$ and $G_s$ were fitted with Eqn 3.
Table 1. Tree species studied and their characteristics, including whether or not they are latex-producing; the number and estimated mean height ($\bar{h}$) and age of the individuals; the number of A-C$_i$ curves included in the final analyses, and the temperature range ($T_{\text{Leaf range}}$) over which they were measured.

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Species</th>
<th>Family</th>
<th>Latex</th>
<th>Trees</th>
<th>$\bar{h}$</th>
<th>Age</th>
<th>Curves</th>
<th>$T_{\text{Leaf range}}$</th>
<th>LMA (g m$^{-2}$)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early successional</td>
<td><em>Ficus insipida</em> Willd.</td>
<td>Moraceae</td>
<td>Y</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>34</td>
<td>26.8 – 38.4</td>
<td>109 ± 7</td>
<td>2.5 ± 0.3</td>
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<tr>
<td></td>
<td><em>Lagerstroemia speciosa</em> (L.) Pers.</td>
<td>Lythraceae</td>
<td>N</td>
<td>2</td>
<td>8</td>
<td>25</td>
<td>47</td>
<td>27.9 – 40.8</td>
<td>87 ± 9</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Late successional</td>
<td><em>Calophyllum longifolium</em> Willd.</td>
<td>Clusiaceae</td>
<td>Y</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>28</td>
<td>27.9 – 37.8</td>
<td>175 ± 4</td>
<td>1.0 ± 0.0</td>
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<tr>
<td></td>
<td><em>Garcinia madruno</em> (Kunth) Hammel</td>
<td>Clusiaceae</td>
<td>Y</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>40</td>
<td>29.6 – 38.5</td>
<td>136 ± 10</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

Leaf mass per unit leaf area (LMA) and leaf nitrogen (N) content (± SD) were determined for three leaves per species.
Table 2. Temperature response traits of biochemical controls over photosynthesis estimated with different models for four lowland tropical tree species

<table>
<thead>
<tr>
<th>Species</th>
<th>V&lt;sub&gt;CMAX&lt;/sub&gt;</th>
<th>J&lt;sub&gt;MAX&lt;/sub&gt;</th>
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<tr>
<td></td>
<td>T&lt;sub&gt;Opt&lt;/sub&gt;</td>
<td>V&lt;sub&gt;CMAX&lt;/sub&gt; (T&lt;sub&gt;Opt&lt;/sub&gt;)</td>
</tr>
<tr>
<td></td>
<td>(°C)</td>
<td>(µmol m&lt;sup&gt;–2&lt;/sup&gt;s&lt;sup&gt;–1&lt;/sup&gt;)</td>
</tr>
<tr>
<td>F. insipida</td>
<td>M&lt;sub&gt;4&lt;/sub&gt;</td>
<td>36.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>M&lt;sub&gt;3&lt;/sub&gt;</td>
<td>36.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>35.0 ± 0.8</td>
</tr>
<tr>
<td>L. speciosa</td>
<td>M&lt;sub&gt;4&lt;/sub&gt;</td>
<td>39.7 ± 0.3</td>
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<td>40.3 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>40.3 ± 2.1</td>
</tr>
<tr>
<td>C. longifolium</td>
<td>M&lt;sub&gt;4&lt;/sub&gt;</td>
<td>32.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>M&lt;sub&gt;3&lt;/sub&gt;</td>
<td>33.5 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>33.5 ± 0.6</td>
</tr>
<tr>
<td>G. madruno</td>
<td>M&lt;sub&gt;4&lt;/sub&gt;</td>
<td>37.1 ± 0.8</td>
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<tr>
<td></td>
<td>M&lt;sub&gt;3&lt;/sub&gt;</td>
<td>38.9 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>38.6 ± 3.7</td>
</tr>
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</table>

Parameter estimates ± SEM were determined through optimization of Eqn 2 allowing all four parameters to value (M<sub>4</sub>), by setting H<sub>d</sub> in Eqn 2 to 200 kJ mol<sup>–1</sup> (three free parameters, M<sub>3</sub>), or by using Eqn 3 (J). Shown are the optimum temperature (T<sub>Opt</sub>), the rates at optimum temperature [V<sub>CMAX</sub> (T<sub>Opt</sub>) and J<sub>MAX</sub> (T<sub>Opt</sub>)], and for M<sub>3</sub> and M<sub>4</sub> the activation energy H<sub>a</sub> and the de-activation energy H<sub>d</sub> for the maximum rate of RuBP-carboxylation (V<sub>CMAX</sub>) and the maximum rate of RuBP-regeneration (J<sub>MAX</sub>).

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Table 3. Temperature response traits of net photosynthesis measured at different CO₂ concentrations for four lowland tropical tree species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Parameter estimates</th>
<th>A₃₀₀</th>
<th>A₄₀₀</th>
<th>A₆₀₀</th>
<th>A₉₀₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T_{Opt}</td>
<td>A₃₀₀ (T_{Opt})</td>
<td>A₄₀₀ (T_{Opt})</td>
<td>A₆₀₀ (T_{Opt})</td>
<td>A₉₀₀ (T_{Opt})</td>
</tr>
<tr>
<td></td>
<td>(°C)</td>
<td>(µmol m⁻² s⁻¹)</td>
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</tr>
<tr>
<td><em>F. insipida</em></td>
<td>29.3 ± 0.7</td>
<td>16.1 ± 0.6</td>
<td>21.6 ± 0.6</td>
<td>30.8 ± 0.6</td>
<td>30.0 ± 1.1</td>
</tr>
<tr>
<td><em>L. speciosa</em></td>
<td>31.4 ± 0.7</td>
<td>14.6 ± 0.5</td>
<td>19.7 ± 0.4</td>
<td>33.2 ± 0.8</td>
<td>25.0 ± 1.0</td>
</tr>
<tr>
<td><em>C. longifolium</em></td>
<td>31.7 ± 0.5</td>
<td>11.9 ± 0.7</td>
<td>16.2 ± 0.8</td>
<td>32.1 ± 0.5</td>
<td>22.3 ± 1.2</td>
</tr>
<tr>
<td><em>G. madruno</em></td>
<td>30.5 ± 2.5</td>
<td>5.4 ± 0.3</td>
<td>7.2 ± 0.4</td>
<td>10.6 ± 0.5</td>
<td>32.5 ± 0.8</td>
</tr>
</tbody>
</table>

through optimization of Eqn 3, the resulting curves of which are shown in Fig. 5. Shown are the optimum temperature for net photosynthesis (T_{Opt}), and the rate of photosynthesis at optimum temperature for measurements at 300, 400, 600, and 900 ppm CO₂. Subscripts in the parameter names indicate the CO₂ concentration of the measurements.