Research paper

Similar temperature dependence of photosynthetic parameters in sun and shade leaves of three tropical tree species

Georgia G. Hernández [ID] [1,2], Klaus Winter[1] and Martijn Slot [ID] [1,3]

[1]Smithsonian Tropical Research Institute, Apartado Postal 0843-03092, Panama, Republic of Panama; [2]Present address: Department of Ecology & Evolutionary Biology, University of Connecticut, 75 North Eagleville Rd, Unit 3043, Storrs, CT 06269, USA [3]Corresponding author (martijnslot78@gmail.com);

Photosynthetic carbon uptake by tropical forests is of critical importance in regulating the earth's climate, but rising temperatures threaten this stabilizing influence of tropical forests. Most research on how temperature affects photosynthesis focuses on fully sun-exposed leaves, and little is known about shade leaves, even though shade leaves greatly outnumber sun leaves in lowland tropical forests. We measured temperature responses of light-saturated photosynthesis, stomatal conductance, and the biochemical parameters V_{CMax} (maximum rate of RuBP carboxylation) and J_{Max} (maximum rate of RuBP regeneration, or electron transport) on sun and shade leaves of mature tropical trees of three species in Panama. As expected, biochemical capacities and stomatal conductance were much lower in shade than in sun leaves, leading to lower net photosynthesis rates. However, the key temperature response traits of these parameters—the optimum temperature (T_{Opt}) and the activation energy—did not differ systematically between sun and shade leaves. Consistency in the J_{Max} to V_{CMax} ratio further suggested that shade leaves are not acclimated to lower temperatures. For both sun and shade leaves, stomatal conductance had the lowest temperature optimum (∼25 °C), followed by net photosynthesis (∼30 °C), J_{Max} (∼34 °C) and V_{CMax} (∼38 °C). Stomatal conductance of sun leaves decreased more strongly with increasing vapor pressure deficit than that of shade leaves. Consistent with this, modeled stomatal limitation of photosynthesis increased with increasing temperature in sun but not shade leaves. Collectively, these results suggest that modeling photosynthetic carbon uptake in multi-layered canopies does not require independent parameterization of the temperature responses of the biochemical controls over photosynthesis of sun and shade leaves. Nonetheless, to improve the representation of the shade fraction of carbon uptake dynamics in tropical forests, better understanding of stomatal sensitivity of shade leaves to temperature and vapor pressure deficit will be required.

Keywords: climate change, J_{Max}, shade leaves, stomatal conductance, temperature, tropical forest, vapor pressure deficit, V_{CMax}, VPD.

Introduction

Tropical forests are responsible for one-third of the global terrestrial primary production (Beer et al. 2010), but this flux is sensitive to changes in temperature (Clark et al. 2003, Feeley et al. 2007). As temperatures rise, the net carbon uptake by tropical forest decreases (Clark 2004, Clark et al. 2010, Doughty and Goulden 2008, Feng et al. 2018). This can have

major consequences for the role of tropical forests in mitigating the effects of anthropogenic carbon emissions, because tropical forests could change from carbon sinks to carbon sources during the current century (Cox et al. 2000, Clark 2004, Cavaleri et al. 2015). Tropical forests appear to operate close to, or even above their high-temperature threshold (Doughty and Goulden 2008, Mau et al. 2018), so more detailed observations and

experimental studies are needed, both to determine current and to accurately predict future constraints on photosynthetic carbon fixation in tropical forests.

Leaf traits, including photosynthetic parameters, are predominantly measured on sun-exposed leaves. Sun leaves are metabolically more active than shade leaves, and measurements on sun leaves can readily be compared across species/studies without requiring further standardization of the light environment. However, forests typically maintain a leaf area index (LAI, projected one-sided leaf area per unit ground area) of 5 m^2 m^{-2} (Asner et al. 2003), and in lowland tropical forests, the LAI is often higher still (e.g., 6.0 m^2 m^{-2} in Costa Rica; Clark et al. 2008). This means that the LAI of shade leaves is about three times greater than the LAI of sunlit leaves, if we assume spherical leaf orientation (Bonan 2015). Collectively, these often-ignored shade leaves contribute significantly to carbon uptake and dynamics in the forest; currently estimated to account for more than 40% of total terrestrial gross primary productivity, the relative importance of shade leaves is expected to increase with ongoing climate change as elevated [CO_2] may support higher LAI (He et al. 2018).

For sun leaves of tropical forest trees, photosynthetic carbon uptake peaks very close to mean daytime air temperature (Slot and Winter 2017a, Doughty and Goulden 2008). To simulate the short-term temperature sensitivity of photosynthesis in coupled climate–vegetation models, information is required on the temperature responses of the processes underlying the temperature response of net photosynthesis (Mercado et al. 2018), including biochemistry, stomatal conductance and respiration in the light (Lin et al. 2012). Detailed measurements of temperature relations of biochemical parameters such as V_{CMax} (maximum RuBP carboxylation rate) and J_{Max} (maximum RuBP regeneration rate, assumed to reflect the electron transport rate), stomatal conductance and respiration in the light of sun leaves of four tropical tree species suggest that the decrease in photosynthesis above the optimum temperature (T_{Opt}) is mainly driven by decreasing stomatal conductance, rather than by negative high-temperature effects on photosynthetic biochemistry (Slot and Winter 2017c). This decrease of stomatal conductance is caused by increases in leaf-to-air vapor pressure deficit associated with an increase in leaf temperature (Lin et al. 2012, Slot and Winter 2017c). At high temperature, photosynthesis can also become inhibited by a reduced activation state of Rubisco caused by the thermally labile Rubisco activase enzyme (Feller et al. 1998, Crafts-Brandner and Salvucci 2000, Yamori et al. 2006). For sun leaves of tropical trees Slot and Winter (2017c) did not find evidence for Rubisco activase limitation of net photosynthesis at temperatures up to 38–40 °C, but growth temperature can affect how the Rubsico activation state changes with temperature (Yamori et al. 2006). How these observations translate to potential differences in controls over

the temperature responses of photosynthesis of sun and shade leaves is currently not known.

Shade leaves have lower photosynthetic capacities, respiration rates and stomatal conductance than sun leaves (Boardman 1977, Björkman 1981, Givnish 1988), but little is known about the temperature sensitivities of these processes in shade leaves. Shade leaves generally experience lower maximum temperatures and smaller temperature ranges than sun leaves (Rey-Sánchez et al. 2016, Fauset et al. 2018), which may affect the temperature sensitivities and the optimum temperatures of various metabolic processes. In *Populus deltoides*, the temperature sensitivity of dark respiration was significantly higher in the shaded lower canopy than in the sun-exposed upper canopy (Griffin et al. 2002), which may reflect acclimation to cooler conditions (Atkin and Tjoelker 2003, Slot and Kitajima 2015). The temperature sensitivity of electron transport was also higher in shaded, cooler parts of the canopy of two temperate tree species, as indicated by lower activation energy of J_{Max} (Niinemets et al. 1999). For net photosynthesis in contrast, lower temperature sensitivity has been reported for shade leaves of grapevine compared with sun leaves (Greer and Weedon 2012). These studies suggest that temperature sensitivities might differ depending on light environments, but without data on tropical trees, we cannot evaluate systematic patterns in temperature sensitivities in these high LAI forests.

Thermal acclimation of photosynthesis results in a shift of the optimum temperature towards the growth temperature (Way and Yamori 2014, Slot and Winter 2017b), suggesting that net photosynthesis may peak at lower temperatures in shade than in sun leaves. Indeed, Mau et al. (2018) reported a moderate increase in T_{Opt} of photosynthesis with increasing light availability through the canopies of three tropical forest species in Puerto Rico. Whether the underlying biochemical (V_{CMax} and J_{Max}) and stomatal processes differ for tropical sun and shade leaves has not yet been studied.

Here, we tested the hypothesis that the temperature responses of biochemical and stomatal parameters differ between sun and shade leaves of three tropical tree species. We hypothesized that T_{Opt_JMax} and T_{Opt_VCMax} would be lower in shade than in sun leaves consistent with acclimation of the biochemical parameters to lower temperatures (Kattge and Knorr 2007). We also hypothesized that the temperature sensitivity of photosynthetic parameters, expressed by their activation energies, would differ between sun and shade leaves. We further examined whether the temperature response of shade leaves is largely controlled by stomatal conductance—as has been shown for sun leaves (e.g., Slot and Winter 2017c)— or whether lower investment in photosynthetic capacity in shade leaves may result in increased biochemical limitation of photosynthesis at high temperatures.

Materials and methods

Species and growth conditions

We selected mature individuals of three tropical tree species for which sun and shade leaves within the same tree could be accessed from the ground. The trees grew at the Santa Cruz Experimental Field Facilities of the Smithsonian Tropical Research Institute (STRI) in Gamboa: 9.1165° N, 79.6965° W and at the Tupper Center of STRI in Panama City: 8.9824° N, 79.5199° W, Republic of Panama (Table 1). From each species we sampled one mature, free-standing tree, the outer-canopy leaves of which were fully sun-exposed for most of the day. Shade leaves were selected from the same trees, from branches deeper inside the crown nearer the stem, where light levels were 5–20% of that experienced by sun leaves (based on repeated measurements with an LI-190R quantum sensor (LI-COR Biosciences, Lincoln, NE, USA) attached to a LI-189 handheld Quantum/Radiometer/Photometer (LI-COR) on sunny and overcast days).

Calophyllum inophyllum L. (Calophyllaceae) is a slow-growing tree native to the eastern tropical region around the Indian ocean, from East Africa, coastal India, to Malaysia and Australia, where it occurs mainly in the coast, while elsewhere in the tropics, it is cultivated as a street tree (Woodson and Schery 1980). *Ficus insipida* Willd. (Moraceae) is a widespread neotropical tree that ranges from northwestern México to Paraguay (Woodson and Schery 1980) and is characteristic for young, secondary forest, where it reaches high growth rates. *Gliricidia sepium* (Jacq.) Steud. (Fabaceae) is a neotropical tree distributed from México to Colombia (Woodson and Schery 1980) that is associated with fencerows and other human habitation. *Gliricidia sepium* grows rapidly during early ontogeny, with lower growth rates during later stages of development (Hughes 1987).

Mean annual temperature is 26.9 °C at both sites, with mean daily maxima of 32.2 and 32.6 °C in Panama City and Gamboa, respectively. The maximum photosynthetic photon flux density (PPFD) at the study sites commonly exceeds 2000 μmol m^{-2} s^{-1}. Annual rainfall is approximately 1900 mm in Panama City and 2250 mm in Gamboa, with >80% of the rain falling in the wet season between April and December, although the rainy season extended to the end of January in the year of the current study. The natural vegetation of both sites is classified as tropical moist forest according to the Holdridge Life Zones (Holdridge and Grenke 1971), and soils are relatively fertile (B.L. Turner, personal communication).

A–C$_i$ curve measurement protocol

We measured curves of net assimilation rate vs the CO$_2$ concentration in the substomatal cavities (A–C$_i$ curves) A–C$_i$ curves on fully expanded sun and shade leaves, between October 2017 and January 2018. We used the LI-6400XT and LI-6800 portable photosynthesis systems

Table 1. Three tropical tree species studied, their diameter at breast height (DBH), height and location of the studied trees, the number of A–C$_i$ curves included in the analyses from sun and shaded parts of the canopy, the temperature range over which the curves were measured, LMA, and nitrogen content (N) on a mass and area basis (means ± SD; $n = 5$).

Species	Family	DBH[1] (cm)	Height[1] (m)	Site	Exposition	Curves	T$_{leaf}$ range (°C)	LMA (g m^{-2})	N (%)	N (g m^{-2})
Calophyllum inophyllum L.	Calophyllaceae	29.5	7.5	Panama City	Sun	51	27.5–39.0	133.0 ± 9.5	1.83 ± 0.02	2.42 ± 0.14
					Shade	64	27.0–40.0	92.1 ± 7.3	1.79 ± 0.15	1.65 ± 0.18
Ficus insipida Willd.	Moraceae	39.0	9.0	Gamboa	Sun	34	26.8–38.4	109.0 ± 6.6	2.50 ± 0.28	2.71 ± 0.18
					Shade	49	27.0–39.0	81.3 ± 13.1	2.16 ± 0.23	1.76 ± 0.28
Gliricidia sepium Kunth.	Fabaceae	37.0	7.0	Panama City	Sun	30	27.0–38.0	78.0 ± 6.2	3.49 ± 0.19	2.72 ± 0.31
					Shade	49	27.0–38.3	50.4 ± 4.2	3.12 ± 0.28	1.57 ± 0.16

*Measured in 2019.

(LI-COR). In total, 277 A–C_i curves, measured between 26.8 and 40.0 °C, were analyzed (Table 1). Sun and shade leaves were selected at similar heights (∼1.5–2.0 m) from the outer and inner canopy, respectively. The light level in the cuvette was set to 1500 µmol m^{-2} s^{-1} for sun leaves and 1000 µmol m^{-2} s^{-1} for shade leaves. Measurements of light-response curves confirmed that these light levels saturated photosynthesis in the respective environments for all species. We attempted to measure the same leaves repeatedly across a temperature range of ≥10 °C to minimize the influence of leaf-to-leaf variation when constructing temperature response curves, but this was not always possible. We therefore focused our analyses on curves that were constructed from pooled measurements at a given light environment and report leaf-level curves in the online supplementary materials (Table S2, Figure S3). Leaf temperature was controlled using the Peltier heater/cooler of the cuvette and was further aided by diurnal changes in air temperature. It was technically not feasible to control leaf temperatures during in situ measurements to the extent that we could test for hysteresis effects, but measurements were taken across a fairly wide temperature range both before and after solar noon (Figure S1). Relative humidity in the cuvette during the measurement was maintained between 50 and 80%; when target leaf temperatures exceeded 38 °C, relative humidity occasionally dropped below 50%. When the leaf was clamped into the cuvette we waited for photosynthesis to equilibrate at 400 p.p.m., and then we recorded photosynthesis at 40, 100, 200, 300, 400, 475, 550, 675, 800, 950, 1200, 1500, 1950 p.p.m. CO_2 of the air entering the cuvette. When stomatal conductance dropped during the measurements (e.g., due to high temperature, high VPD, or high CO_2 concentration), causing photosynthesis rates to decline continuously instead of stabilizing, we stopped the measurements and moved to another leaf. Whenever the same leaf was studied consecutively, we waited 15–20 min for stomatal conductance to re-equilibrate before starting the next curve. This was especially necessary when high temperatures and high [CO_2] resulted in low stomatal conductance at the end of the previous curve.

Diffusion of CO_2 between the cuvette and the surrounding air due to concentration gradients can lead to erroneous estimations of A and C_i. Therefore we estimated the diffusion error for both the LI-6400XT and LI-6800 as described in Flexas et al. (2007) by measuring CO_2-response curves on leaves that were killed by submersion in boiling water for 2–15 min (depending on species). We estimated the diffusion error for each species and treatment (sun and shade leaves) at 30 °C. Previous analysis of such diffusion errors suggested that the error is not temperature dependent (Slot and Winter 2017c).

A–C_i curve analysis

V_{CMax} and J_{Max} were calculated from A–C_i curves fitted to the diffusion-error-corrected data. Curves were fitted with the 'fitaci'

function from the 'plantecophys' package in R (Duursma 2015), which uses the Farquhar–von Caemmerer–Berry model (FvCB) (Farquhar et al. 1980, Farquhar and von Caemmerer 1982). We also used the Excel macro of Sharkey et al. (2007) on a set of detailed A–C_i curves with >20 CO_2 concentrations to determine the temperature dependence of mesophyll conductance (g_m, CO_2 transfer conductance from the intercellular airspaces of the leaf into the chloroplast). In this curve-fitting approach, g_m is estimated along with V_{CMax}, J_{Max}, triose phosphate utilization and day respiration (Sharkey 2016). However, we did not find any trend or consistent pattern of g_m with temperature that we could apply systematically to all data. Therefore, V_{CMax} and J_{Max} values reported here are slight underestimates, because they were calculated assuming infinite mesophyll conductance.

A recent study has called into question the assumption of the FvCB model of saturated vapor pressure inside the substomatal cavities and showed that violation of this assumption can influence estimates of stomatal conductance and C_i in a VPD-dependent manner (Cernusak et al. 2018). In the absence of data on substomatal vapor pressures in the current study, we had no grounds for applying corrections. Nonetheless, we explored the potential effects of violation of the assumption of vapor pressure saturation would have on the temperature response parameters for V_{CMax} through changes in C_i and g_s (see Notes S1 and Figure S2, available as Supplementary Data at Tree Physiology Online).

Temperature response

The temperature responses of V_{CMax} and J_{Max} were fitted according to Medlyn et al. (2002) as:

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

where k_{Opt} is the value of V_{CMax} or J_{Max} at T_{Opt}; H_a represents the rate of exponential increase of the function below the optimum equivalent to the activation energy; H_d describes the rate of decrease of the function above the optimum; T_{Opt} is the optimum temperature; R is the universal gas constant (8.314 J K^{-1} mol^{-1}); and T_k is the leaf temperature in Kelvin. H_d is hard to estimate when there are few observations above T_{Opt}. We therefore fixed H_d to 200 kJ mol^{-1} as done by Medlyn et al. (2002) and others, and estimated the other three parameters using least squares optimization (see below). Results of analyses in which H_d was allowed to vary are shown in Table S1.

We extracted net photosynthesis (A_{Net}) at 400 p.p.m. CO_2 from A–C_i curves and fitted its temperature response with a much simpler equation from June et al. (2004) to estimate T_{Opt},

using

$$A(T) = A_{Opt} \times e^{-\left(\frac{T_{Leaf} - T_{Opt}}{\Omega}\right)^2} \tag{2}$$

where Ω indicates the steepness of the peak of the curve and as such reflects the photosynthetic thermal niche. It is the difference in temperature between T_{Opt} and the temperature at which A equals 37% of its value at T_{Opt} (i.e., 2Ω represents the width of the temperature range over which $A > 37\%$ of its maximum). A_{Opt} is the rate of photosynthesis at T_{Opt}.

Stomatal limitation

We calculated stomatal limitation of net photosynthesis (*l*) according to Farquhar and Sharkey 1982) as:

$$l = 1 - \frac{A_{Observed}}{A_{(infinite\ g_s)}} \tag{3}$$

where $A_{(infinite\ g_s)}$ is calculated from the FvCB model by setting C_i to C_a (CO_2 concentration in the cuvette) in Eq. (4):

$$V_{CMax} = \frac{A_{gross}\ (C_i + K_c)}{C_i - \Gamma^*} \tag{4}$$

A_{gross} equals light-saturated photosynthesis + respiration in the light, where respiration in the light was estimated as $0.015 \times V_{CMax}$; K_c (Michaelis–Menten constants for CO_2) and Γ^* (CO_2 compensation point in the absence of dark respiration) were taken from Bernacchi et al. (2001).

Rubisco activase effect

Rubisco activase limitation was assessed under conditions where electron transport and associated ATP supply do not limit Rubisco activation and photosynthesis, by comparing measurements and modeled values of the initial slope of A–C_i curves at CO_2 concentrations lower than 200 p.p.m. The initial slope was modeled using the method described in Sage et al. (2008) as follows:

$$\text{Initial slope} = \frac{V_{CMax}}{\left(\Gamma^* + K_c \times \left(1 + \frac{O}{K_o}\right)\right)} \tag{5}$$

K_o (Michaelis–Menten constant for O_2), K_c, Γ^*, T_k as before were obtained from Bernacchi et al. (2001), and O is the O_2 concentration, assumed to be 210 mbar. The model assumes fully activated Rubisco. When the observed slopes are lower than the modeled slopes this assumption of Rubisco activation must have been violated, suggesting that Rubisco activase functioning is compromised at the temperature at which this model–observation discrepancy is observed.

Leaf chemical and morphological traits

To characterize sun and shade leaves for each species, we harvested three to five leaves at each light level at which A–C_i curves were measured. Leaf area was determined with a LI-3100C leaf area meter (LI-COR), leaves were dried at 65 °C and weighed, and leaf mass per area (LMA) was determined. Leaf nitrogen (N) content was measured on powered oven-dried samples with a Thermo Flash EA1112 analyzer (Waltham, MA, USA).

Data analyses

We used the 'nls_multstart' function in the 'nls.multstart' package version 1.0.0. (Padfield and Matheson 2018) for all nonlinear least squares regression analyses, i.e., to fit Eqs (1) and (2). 95% confidence intervals of key parameters were determined from bootstrapping based on 10,000 bootstrap iterations, using the 'bootstrap' function in the 'modelr' package version 0.1.1 (Wickham 2017). Light-level and species differences in LMA and leaf N content were determined with Scheirer-Ray-Hare tests from the 'rcompanion' package. All analyses were performed in R, version 3.5.0 (R Development Core Team 2018).

Results

Temperature response of photosynthetic parameters

The leaves selected to represent the sun-exposed and shaded parts of the canopies were morphologically characteristic of sun and shade leaves, respectively, with significantly lower leaf mass per area ($df = 2$, $H = 6.0$, $P = 0.015$), moderately lower N concentration and consequently, significantly lower leaf N per unit leaf area in shade than in sun leaves (Table 1) ($df = 2$, $H = 16.2$, $P < 0.001$).

The temperature responses of the parameters calculated from the A–C_i curves are shown in Figure 1. Temperature response parameters were determined from measurements pooled across multiple leaves; for comparison, leaf-level parameter estimates are shown in Table S2 and Figure S3. V_{CMax} and J_{Max} were consistently higher in sun than in shade leaves (Table 2; Figure 2), but the shapes of the temperature response curves were similar within species. In *F. insipida*, $T_{Opt}\_V_{CMax}$ of both sun and shade leaves fell within the measured leaf temperature range. In *C. inophyllum* and *G. sepium* $T_{Opt}\_V_{CMax}$ exceeded the highest measurement temperature. As a result, $T_{Opt}\_V_{CMax}$ and the deactivation energy H_d could not be estimated with high confidence in *C. inophyllum* and *G. sepium*, so the fact that the modeled value of $T_{Opt}\_V_{CMax}$ was on average 1.9 °C higher in sun than in shade leaves (Table 2) did not reflect the data particularly well (Figure 1), and the similarity in T_{Opt} of V_{CMax} and J_{Max} between sun and shade leaves was more striking than the difference. This is further illustrated by the overlapping 95% confidence intervals of the parameters in Figure 2.

J_{Max} tended to peak at lower temperatures than V_{CMax} for all the treatments and species (Figures 1 and 2), with an average $T_{Opt}\_J_{Max}$ across species and treatment of 34.1 °C, compared with 38.1 °C for $T_{Opt}\_V_{CMax}$, when the poorly constrained
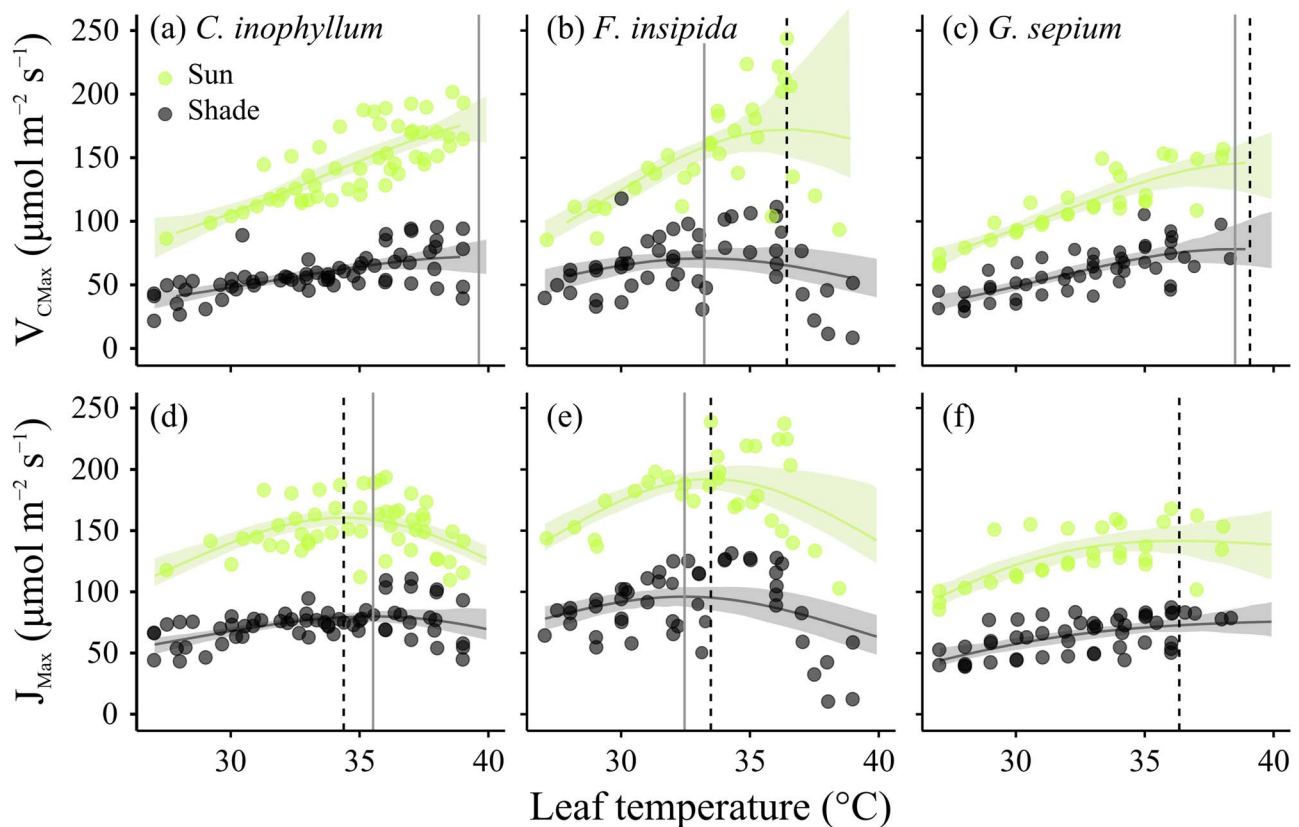
Figure 1. Maximum rates of RuBP carboxylation (V_{CMax}, a–c) and –regeneration (J_{Max}, d–f) in relation to leaf temperature in sun (light green symbols) and shade (gray symbols) leaves of three tropical tree species. Temperature responses were fitted with Eq. (1) (solid lines) with the deactivation energy H_d set to 200 kJ mol^{-1}. Shaded areas indicate the 95% confidence bands around the predictions of the fitted curves; vertical dashed and solid lines represent T_{Opt} for sun and shade leaves, respectively.

Table 2. Temperature response parameters ($\pm$ SEM) of the maximum rate of RuBP-carboxylation (V_{CMax}) and the maximum rate of RuBP-regeneration (J_{Max}) and the J_{Max} to V_{CMax} ratio at 30 °C in sun and shade leaves of three tropical tree species.

Species	Exposure	V_{CMax}			J_{Max}			$J_{Max.30}/V_{CMax.30}$
		T_{Opt} (°C)	V_{CMax} at T_{Opt} (µmol m^{-2} s^{-1})	H_a (kJ mol^{-1})	T_{Opt} (°C)	J_{Max} at T_{Opt} (µmol m^{-2} s^{-1})	H_a (kJ mol^{-1})	
Calophyllum	Sun	41.6[1] $\pm$ 3.9	182.5 $\pm$ 25.4	59.7 $\pm$ 21.0	34.0 $\pm$ 0.6	160.6 $\pm$ 3.6	81.3 $\pm$ 60.4	1.34
inophyllum	Shade	39.6 $\pm$ 2.6	72.2 $\pm$ 5.5	59.6 $\pm$ 20.9	35.1 $\pm$ 0.9	79.9 $\pm$ 2.2	57.7 $\pm$ 22.7	1.44
Ficus insipida	Sun	36.4 $\pm$ 1.4	172.2 $\pm$ 9.4	116.0 $\pm$ 98.4	33.1 $\pm$ 1.0	192.1 $\pm$ 5.8	94.3 $\pm$ 89.4	1.42
	Shade	33.2 $\pm$ 2.1	70.9 $\pm$ 4.0	92.8 $\pm$ 218.4	32.1 $\pm$ 1.4	96.0 $\pm$ 5.4	80.6 $\pm$ 161.3	1.42
Gliricidia	Sun	39.1[1] $\pm$ 2.1	146.0 $\pm$ 11.3	72.7 $\pm$ 20.0	36.0 $\pm$ 4.2	141.6 $\pm$ 5.3	184.5 $\pm$ 27.2	1.33
sepium	Shade	38.5[1] $\pm$ 2.4	78.2 $\pm$ 7.5	91.2 $\pm$ 38.4	[2]	77.7 $\pm$ 21.6	200.0 $\pm$ 4.7	1.59

Parameters were determined with Eq. (1), setting the deactivation energy (H_d) to 200 kJ mol^{-1} (leaving three parameters to be estimated). Shown are the optimum temperatures (T_{Opt}), maximum values of V_{CMax} and J_{Max} and their activation energy (H_a).
[1] Estimate falls marginally outside the measured temperature range.
[2] No meaningful estimate

estimate for shade leaves of *G. sepium* was not included. Across species the activation energy (H_a) of V_{CMax} ranged from 59.7 to 116 kJ mol^{-1} in sun leaves, and from 59.6 to 92.8 kJ mol^{-1} in shade leaves, without systematic differences between them (Table 2). H_a of J_{Max} ranged from 81.3 to 184.5 kJ mol^{-1} in sun leaves and from 57.7 to 200.0 kJ mol^{-1} in shade leaves. As for V_{CMax}, H_a of J_{Max} did not differ between sun and shade

leaves. The J_{Max} to V_{CMax} ratio at a set temperature of 30 °C was moderately higher in shade leaves of two of the three species (Table 2), but there was no overall significant difference ($P = 0.26$, paired t-test).

There was no systematic pattern when comparing T_{Opt} of net photosynthesis and stomatal conductance between sun and shade leaves (Figure 3). The average T_{Opt} of net photosynthesis
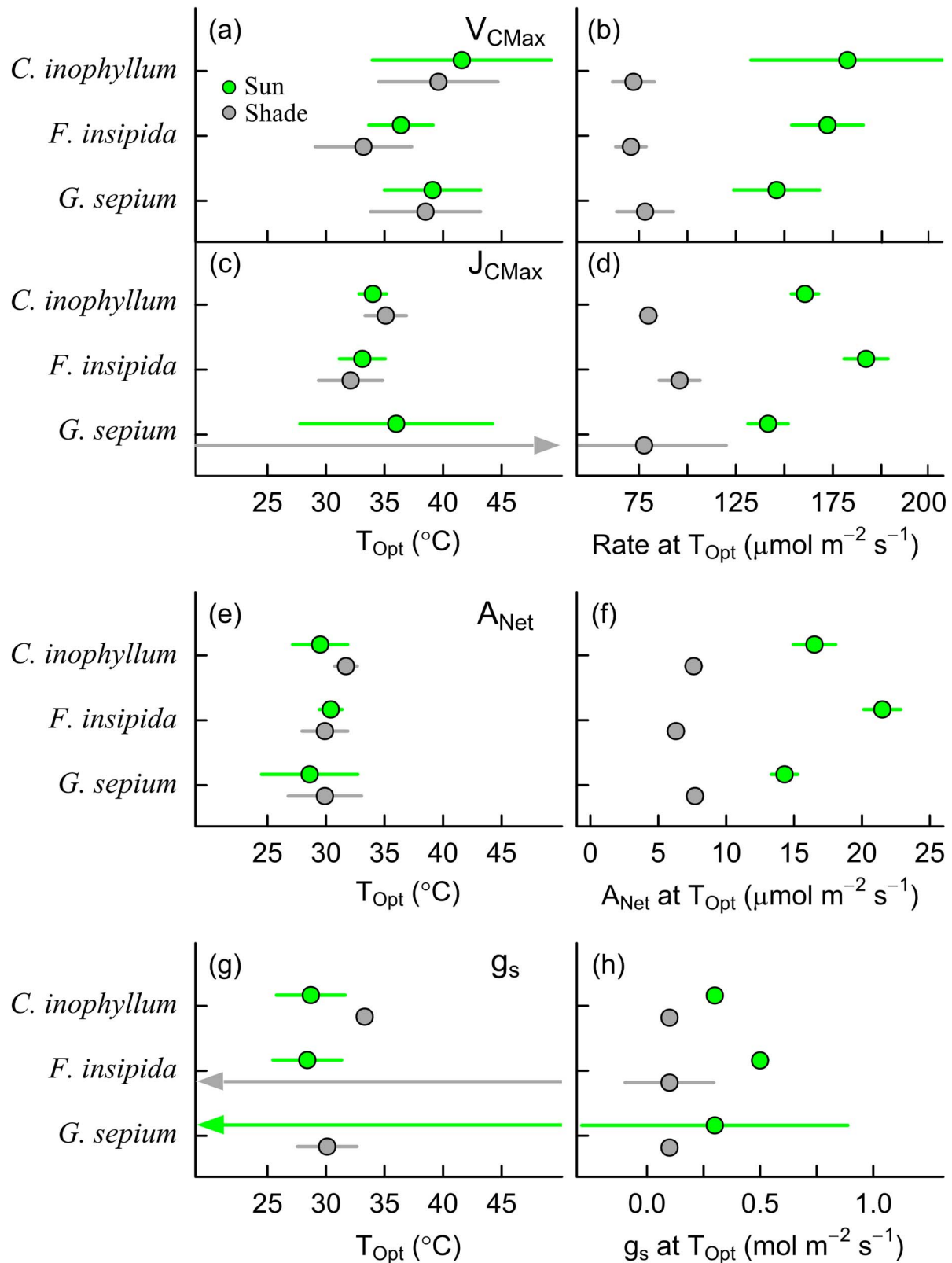
Figure 2. Temperature optima (left panels) and process rates (right panels) of V_{CMax} (a and b), J_{Max} (c and d), A_{Net} (e and f) and g_s (g and h) of sun (light green symbols) and shade leaves (gray symbols) of three tropical tree species. The 95% confidence intervals are constructed from standard errors of the parameters, assuming normal distribution of the parameter estimates. For confidence intervals that span the width of the panel, arrows indicate where T_{Opt} is relative to displayed temperature range.
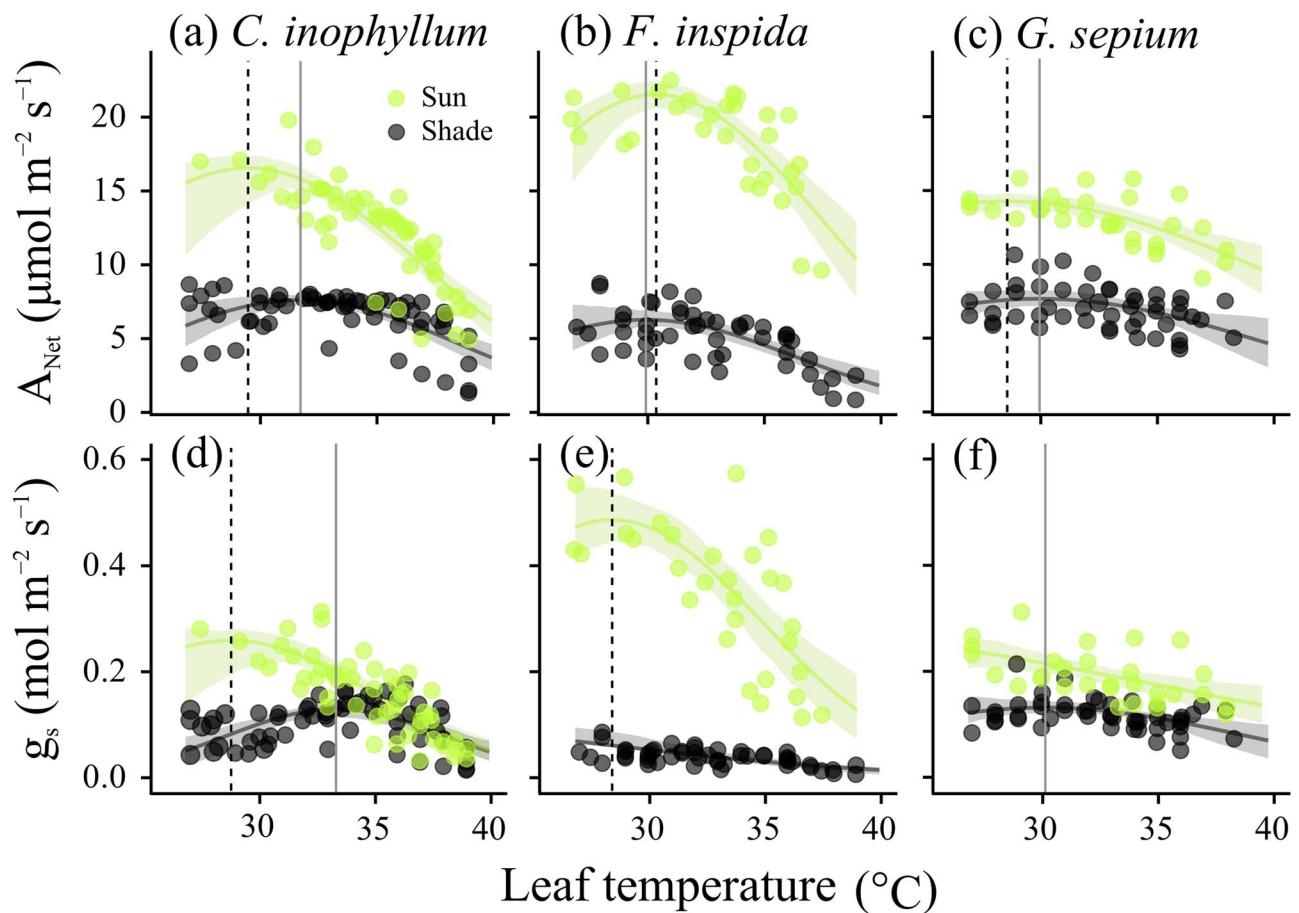
Figure 3. Temperature relationships of net photosynthesis at 400 p.p.m. CO_2 (A_{Net}) and stomatal conductance (g_s) in sun (light green symbols) and shade (gray symbols) leaves of three tropical tree species. Shaded areas indicate the 95%-confidence bands around the predictions of the fitted curves; solid lines are the fitted temperature response; vertical dashed and solid lines represent T_{Opt} for sun and shade leaves, respectively.

at 400 p.p.m. ($\pm$ SD) was 29.5 $\pm$ 0.9 °C for sun leaves and 30.5 $\pm$ 1.0 °C for shade leaves, and T_{Opt} did not differ between sun and shade leaves for any of the three species (Table 3, Table S3). Stomatal conductance tended to decrease with increasing temperature across most of the studied temperature range (Figure 3). For stomatal conductance, there were very few data points below T_{Opt}, and the T_{Opt} estimates were not well constrained (Table S4). Ω of net photosynthesis tended to be lower in shade leaves than in sun leaves, indicating moderately narrower photosynthetic thermal niche; Ω values for stomatal conductance did not differ systematically and were less well constrained (Table 3, Table S3, Table S4).

Controls over photosynthesis

As temperature increased, stomatal conductance and net photosynthesis peaked first, then J_{Max}, and finally V_{CMax} reached its optimum (Figures 2 and 4), with the latter often being outside the measurement range. Correlations between V_{CMax} and net photosynthesis tended to be positive for shade leaves, in contrast to sun leaves, in which net photosynthesis tended to decrease with increasing V_{CMax} (Figure S4), because for sun

leaves the majority of measurements were taken at temperatures between $T_{Opt}\_A_{Net}$ and $T_{Opt}\_V_{CMax}$. Net photosynthesis had a significant positive relationship with J_{Max} in sun leaves of all species and in *C. inophyllum*, this correlation was significant in the shade as well (Figure S4). Net photosynthesis significantly correlated with stomatal conductance for all species and light exposures in a log-linear fashion (Figure S4).

In sun leaves, stomatal limitation (calculated with Eq. (3)) increased more strongly with temperature than in shade leaves (Figure 5), and for two of the species, the increase was significant at $\alpha = 0.01$. Nonetheless, in both sun and shade leaves net photosynthesis decreased significantly as stomatal conductance decreased with increasing temperature and the associated rise in VPD (Table S3, Figure 6). The negative correlation between stomatal conductance and VPD was steeper in sun than in shade leaves (Figure 6), and this difference remained when adjusting for inherent differences in maximum conductance between sun and shade leaves by scaling stomatal conductance for all groups from 0 to 1 (data not shown). When temperature increased, C_i/C_a moderately, but significantly increased in all groups, except in shade leaves of *G. sepium* (Figure S5).

Table 3. Parameters (± SEM) estimated for net photosynthesis and stomatal conductance in response to temperature, in sun and shade leaves for three tropical tree species.

Species	Exposure	Net photosynthesis			Stomatal conductance		
		T_{Opt} (°C)	Ω	A_{Opt} (μmol m^{-2} s^{-1})	T_{Opt} (°C)	Ω	g_{s_Opt} (mol m^{-2} s^{-1})
Calophyllum	Sun	29.5 ± 1.2	10.5 ± 1.5	**16.5 ± 0.8**	28.7 ± 1.5	8.8 ± 1.5	**0.3 ± 0.0**
inophyllum	Shade	31.7 ± 0.5	9.7 ± 1.1	**7.6 ± 0.3**	33.3 ± 0.3	6.7 ± 0.7	**0.1 ± 0.0**
Ficus insipida	Sun	30.4 ± 0.5	10.0 ± 1.3	**21.5 ± 0.7**	28.4 ± 1.5	9.1 ± 2.1	**0.5 ± 0.0**
	Shade	29.9 ± 1.0	9.0 ± 1.2	**6.3 ± 0.3**	17.4 ± 20.6	15.9 ± 11.4	**0.1 ± 0.1**
Gliricidia sepium	Sun	28.6 ± 2.1	17.5 ± 5.0	**14.3 ± 0.5**	15.0 ± 52.8	28.6 ± 44.6	0.3 ± 0.3
	Shade	29.9 ± 1.6	13.9 ± 4.2	**7.7 ± 0.3**	30.1 ± 1.3	12.1 ± 3.3	0.1 ± 0.0

Parameters ± SEM were determined with Eq. (2). The displayed parameters are the optimum temperature (T_{Opt}), the steepness of the peak of the curve (Ω) and photosynthesis and stomatal conductance at 400 p.p.m. CO_2 at the optimum temperature (A_{Opt} and g_{s_Opt}, respectively). Bold numbers are for those parameters for which confidence intervals do not overlap.
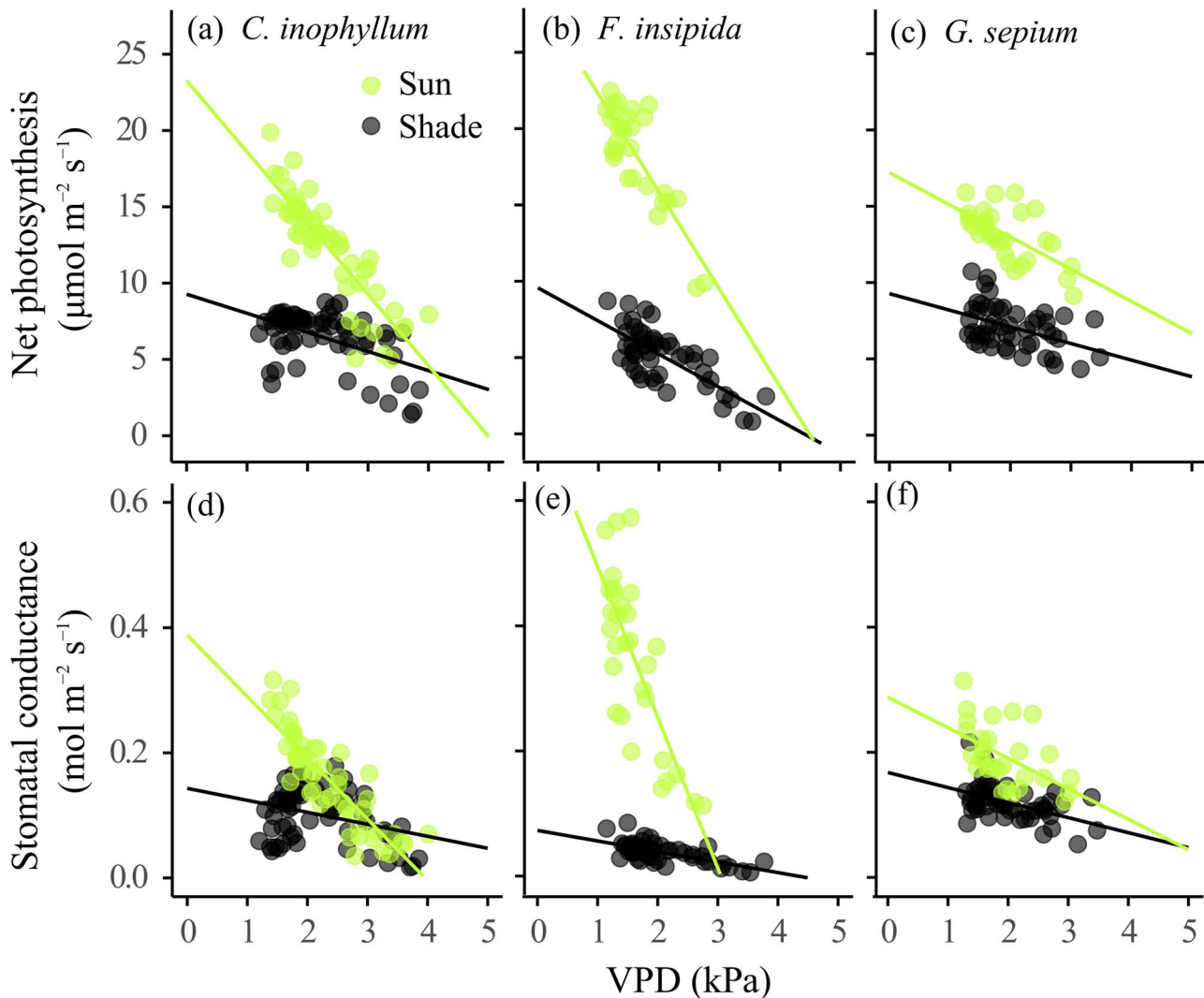

Figure 4. Temperature response of fitted curves for stomatal conductance (g_s, black), net photosynthesis at 400 p.p.m. CO_2 (A_{Net}, green), and maximum rates of RuBP carboxylation (V_{CMax}, red) and regeneration (J_{Max}, blue). Net photosynthesis and g_s were fitted with Eq. (2); V_{CMax} and J_{Max} were fitted with Eq. (1). Solid and dashed lines represent sun and shade leaves, respectively.
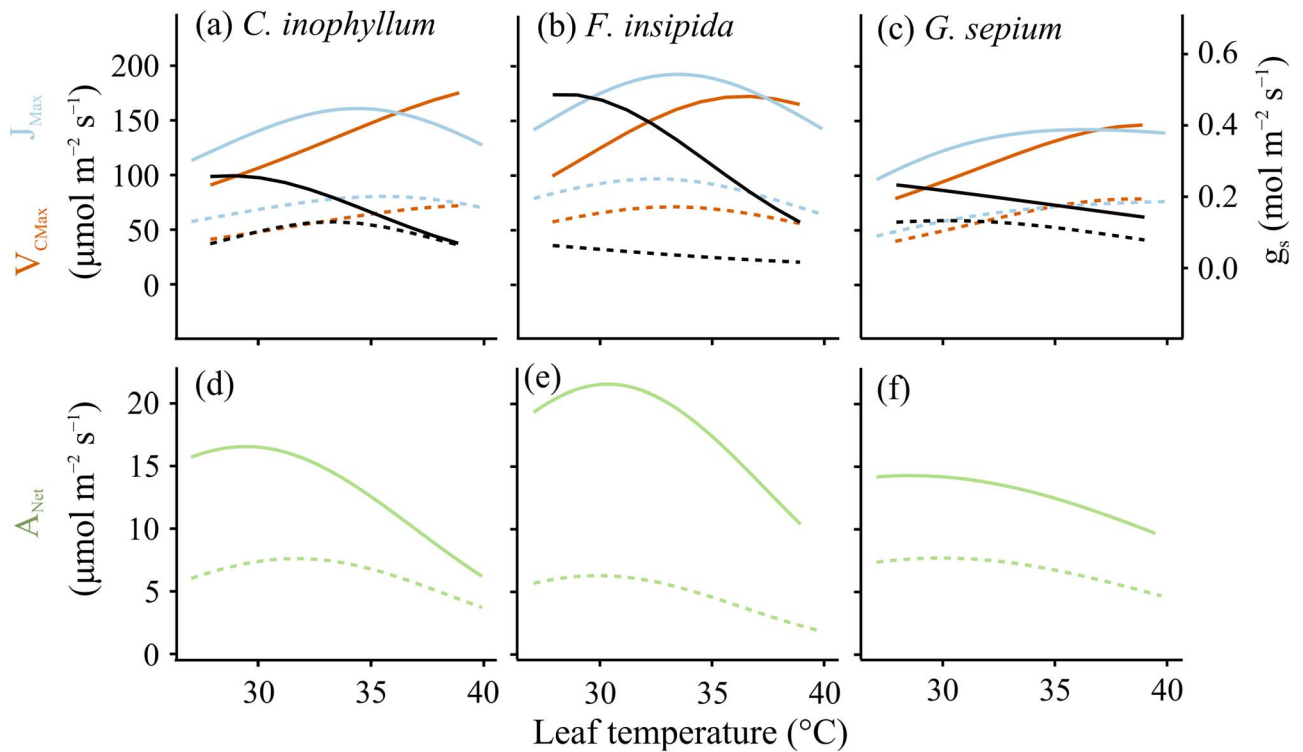
Figure 5. Stomatal limitation at 400 p.p.m. CO_2 and its temperature response for sun (light green symbols) and shade (gray symbols) leaves of three tropical tree species. Stomatal limitation, calculated with Eq. (3), ranges from 0 (when the observed photosynthesis equals the hypothetical rate associated with infinite stomatal conductance) to 1 (when stomatal closure completely suppresses photosynthesis). Solid regression lines represent significant linear regressions ($P < 0.01$); dashed lines are not significant ($P > 0.05$).
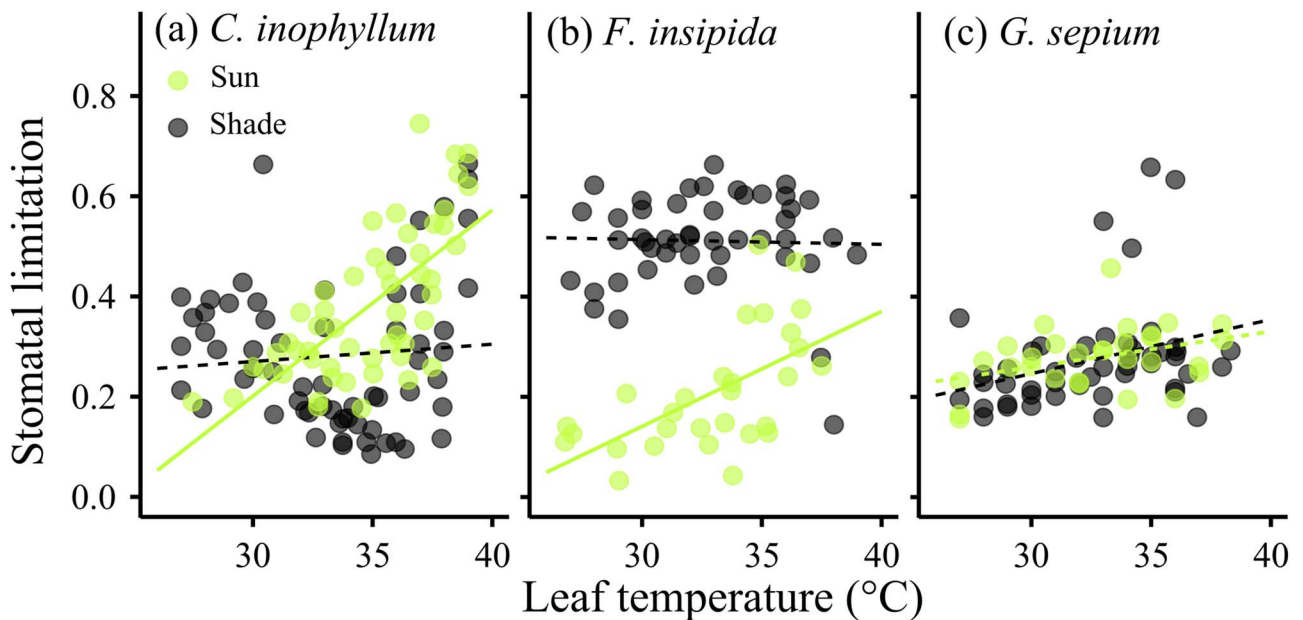

Figure 6. Relationships between net photosynthesis at 400 p.p.m. CO_2 (a–c) and leaf-to-air vapor pressure deficit (VPD), and between stomatal conductance at 400 p.p.m. CO_2 and VPD (d–f), for sun leaves (light green symbols) and shade leaves (gray symbols) of three tropical tree species. Solid lines indicate significant linear regressions ($P < 0.01$).

Initial slopes of measured A–C_i curves did not differ from modeled slopes that assumed fully activated Rubisco (Figure S6). Over the range of temperatures used in this study Rubisco-activase limitation did not affect net photosynthesis; even at high temperatures the measured values did not drop under the modeled curve.

Discussion

The aim of this study was to compare the temperature responses of photosynthetic parameters between sun and shade leaves of tropical trees and to determine whether coupled climate–vegetation models may need to assign different temperature response parameters to sun and shade leaves when simulating carbon fluxes from multi-layered forests. We hypothesized that shade leaves would exhibit characteristics of cool-acclimated leaves and therefore have a lower optimum temperature of V_{CMax} and J_{Max}. However, the optimal temperatures—where they could be estimated with confidence–were not significantly different from those of sun leaves; only the biochemical process rates were lower for shade leaves. Shade leaves did not have lower temperature sensitivity either, as their activation energies for V_{CMax} and J_{Max} were in the same range as those of sun leaves. Consistent with previous studies, net photosynthesis peaked close to ambient temperature (Doughty and Goulden 2008, Slot et al. 2016, Slot and Winter 2017a, 2017c, Mau et al. 2018), but the temperature optimum of net photosynthesis did not differ between sun and shade leaves, suggesting that shade leaves were not acclimated to the lower temperatures they typically experience in the sub-canopy. The decrease in net photosynthesis above T_{Opt} was associated with decreasing stomatal conductance as VPD increased with increasing temperature, particularly in sun leaves. In contrast to sun leaves, stomatal limitation of shade leaves did not increase with temperature; nonetheless, even at the highest temperatures there was no evidence for photosynthesis being limited by Rubisco activation. Collectively, these results suggest that modeling photosynthetic carbon uptake in multi-layered canopies does not require independent parameterization of the temperature responses of the biochemical controls over photosynthesis of sun and shade leaves. However, the apparent difference in sensitivity of stomatal conductance to VPD in shade leaves compared with sun leaves requires further study.

Biochemical parameters

The temperature optima for both V_{CMax} and J_{Max} fall within the range of what has been reported in other studies of fully illuminated leaves of forbs and both temperate and tropical trees (from 33–41 °C and 30–38 °C for V_{CMax} and J_{Max}, respectively) (Medlyn et al. 2002, Kattge and Knorr 2007, Vårhammar et al. 2015, Slot and Winter 2017c). In the current study there

was a tendency for lower T_{Opt_VCMax} values for shade leaves, but this difference was not significant. T_{Opt_JMax} showed no differences, suggesting limited or no thermal acclimation of these parameters to cooler conditions in the shade. Lack of statistically significant differences might result from uncertainty in the estimates of T_{Opt} as T_{Opt} tends to be poorly constrained when it is close to, or above the highest measured temperature, as it often does for V_{CMax} and J_{Max} (e.g., Medlyn et al. 2002, Vårhammar et al. 2015, Scafaro et al. 2017, Slot and Winter 2017c). Therefore, we should also evaluate other indicators of acclimation than T_{Opt} alone. There is evidence of acclimation of net photosynthesis to growth temperature in tropical tree species (Scafaro et al. 2017, Slot and Winter 2017b), but how such acclimation is achieved in terms of changes in the temperature response of the controlling parameters is not well understood. However, as in temperate species (Kattge and Knorr 2007, Smith and Dukes 2018), the J_{Max} to V_{CMax} ratio at a set temperature tends to decrease with increasing growth temperature (Slot and Winter 2017b, 2018, Mercado et al. 2018; but see Scafaro et al. 2017). In the current study, the J_{Max} to V_{CMax} ratio at a set temperature of 30 °C did not differ significantly between shade and sun leaves or species, consistent with the absence of biochemical acclimation to different temperature regimes in sun and shade leaves.

It seems disadvantageous for the photosynthetic machinery of sun and shade leaves not to be acclimated to the different temperature regimes they experience. However, sun-exposed outer canopy leaves do tend to have higher maximum heat tolerance than shade leaves (Slot et al. 2019). When exposed to full sunlight, these sun leaves typically show strong mid-day stomatal depressions (Zotz et al. 1995, Cernusak et al. 2013) leading to very high leaf temperatures, up to 48 °C (Krause et al. 2010) in the absence of transpirational cooling (Gates 1968), necessitating acclimation to the higher maximum temperatures to prevent irreversible leaf damage. If stomata of sun leaves commonly are fully closed at the hottest time of the day, there is little to gain in terms of photosynthetic carbon uptake from biochemical acclimation to such higher mid-day and afternoon temperatures. Photosynthesis of shade leaves may in fact operate at higher temperatures than sun leaves if a less pronounced mid-day stomatal depression associated with lower VPD in the understory (e.g., Jifon and Syvertsen 2003) enables shade leaves to remain active through the afternoon.

The lack of sun–shade differences in T_{Opt} of net photosynthesis is also consistent with observations of stand-level photosynthesis in tropical forests peaking close to mean daytime air temperature (Doughty and Goulden 2008, Tan et al. 2017) or canopy temperatures (Pau et al. 2018), as is the case at the leaf level for sun leaves (Slot and Winter 2017a); if T_{Opt} of shade leaves was considerably lower than that of sun leaves, stand-level photosynthesis would have peaked below T_{Opt} of sun leaves.

Consistent with Slot and Winter (2017c), there was no evidence for Rubisco activase limitation of net photosynthesis. Maximum measurement temperatures in the current study were higher than those in Slot and Winter (2017c), but did not exceed 40 °C. Crafts-Brandner and Salvucci (2000) reported an optimum temperature of 42 °C for the activity of Rubisco activase isolated from *Nicotiana rusticum* L. (tobacco), a warm climate species, and Scafaro et al. (2016) found peak activity at 42 °C for *Oryza australiensis* Domin, a wild rice species with thermally stable Rubisco activase ortholog (as opposed to 36 °C in *Oryza sativa* L.). Cool-acclimated shade leaves might be expected to have lower thermal limits to Rubisco activation, if the temperature sensitivity of the Rubisco activation state (the balance between Rubisco activation and deactivation) acclimates to growth temperature. Rubisco activation acclimation has been shown e.g., for spinach grown at temperature regimes that differed by 15 °C (Yamori et al. 2006). However, others have not found such adjustments despite a 10 °C growth temperature difference (Haldimann and Feller 2005), so the small sun-shade temperature contrast in the current study is unlikely to have yielded detectable acclimation of Rubisco activase that would lead to lower thermal limits in shade leaves.

Stomatal conductance

Stomatal conductance (g_s) tended to decrease across most of the temperature range, and as a result, estimates of the optimum temperature of g_s were not well constrained. Nonetheless, g_s clearly peaked at lower temperatures than the biochemical parameters V_{CMax} and J_{Max}, which is consistent with studies that have shown g_s to be the parameter to affect photosynthesis of field-grown tropical trees the most at temperatures above T_{Opt} of net photosynthesis (Slot and Winter 2017c) and to be key to the response of tropical forest carbon dynamics in a changing climate (Lloyd and Farquhar 2008). The steeper decrease in g_s of sun leaves above T_{Opt} is underpinned by a greater sensitivity of g_s of sun leaves to increasing atmospheric water demand as VPD increases from 1 to 4 kPa (see Figure 6). Despite a tendency for increasing stomatal limitation with temperature—especially in sun leaves—the C_i/C_a ratio increased. If stomatal conductance limits photosynthesis more with increasing temperature than biochemical parameters do, one might expect the C_i/C_a ratio to decrease, as internal CO_2 would get depleted through biochemical fixation. However, increasing respiration rates may account for the rise in C_i/C_a, especially in metabolically more active sun leaves. Together, these results suggest that increases in temperature and associated increases in VPD may have a stronger, negative impact on the carbon uptake of sun leaves than of that of shade leaves. Tropical forests appear to operate close to a high-temperature threshold (Doughty and Goulden 2008, Mau et al. 2018) and beyond the optimum VPD (Rowland et al. 2015). Sun leaves in particular may experience steep declines in g_s and associated loss of

potential carbon gain in a warming environment, even when operating below the temperature optimum of V_{CMax} and J_{Max}. Consequently, the relative importance of shade leaves for whole-canopy carbon uptake may increase with global warming and associated increases in VPD (He et al. 2018) if sun leaves are increasingly constrained by stomatal limitations (this study) and by heat damage (Slot et al. 2019).

Species differ in how tightly stomata regulate plant water status, with isohydric species exhibiting strong stomatal control, while anisohydric species have less responsive stomata that are kept open until much lower leaf water potentials are reached. Temperate species with high photosynthetic capacity and g_s are more anisohydric, while species with low photosynthetic capacity and g_s are more isohydric (Meinzer et al. 2017). Sun leaves of *F. insipida* had the highest photosynthesis rates and g_s, but also the steepest decrease of g_s with increasing VPD. More research is required to establish whether stomatal responses to VPD vary systematically across species in a manner akin to species sorting along the isohydry-anisohydry spectrum.

For both sun and shade leaves, the results reported here are biased towards those leaves for which stomatal conductance was maintained at sufficiently high levels to allow for $A–C_i$ curves to be fitted. The bias may have been higher in shade leaves, which have very low g_s compared with sun leaves (Figure 2; Boardman 1977, Allen and Pearcy 2000, Sefcik et al. 2006) and are more sensitive to being handled during measurements (G.G. Hernández, personal observation), leading to more $A–C_i$ measurements being aborted in shade leaves as g_s approached zero. To confirm whether sun and shade leaves indeed differ in their VPD sensitivity studies are required with greater environmental control than in the current, field-based study.

Conclusions

To improve simulations of global climate change, more leaf- and canopy-scale measurements are still needed to validate the accuracy of ecosystem simulations (Rowland et al. 2015). Niinemets et al. (1999) concluded for temperate forest that when modeling whole-canopy photosynthesis it is not appropriate to use a single J_{Max}. Several models distinguish between the sun and shade fraction of the forest canopy (e.g., Bonan et al. 2014, Jiang and Ryu 2016, He et al. 2018). Our results suggest that photosynthesis of sun and shade leaves have similar temperature response characteristics, requiring no separate treatment of the two leaf types when modeling ecosystem photosynthesis in relation to temperature. Nonetheless, the apparent difference in stomatal sensitivity to VPD and stomatal limitation of photosynthesis with increasing temperature suggests that different mechanisms control the temperature responses in sun and shade leaves. Tan et al. (2017) showed that stomatal processes have an overriding importance in determining T_{Opt} at the stand level, which integrates the temperature responses

of sun and shade leaves. Studies at the leaf level also have found that differences in T_{Opt} of net photosynthesis and in photosynthetic temperature responses are not primarily due to biochemical factors but rather due to stomatal responses to VPD (Lloyd and Farquhar 2008, Lin et al. 2012, Rowland et al. 2015, Slot and Winter 2017c). To better understand how concurrent atmospheric and climate changes affect the 40% of GPP that shade leaves represent globally today (He et al. 2018), sun and shade leaves of a larger number of tropical tree species need to be compared. Such a larger dataset together with temperature–VPD studies under tightly controlled laboratory conditions would provide means to identify the mechanistic understanding of sun-shade differences needed to improve representation of tropical forests in the terrestrial global carbon uptake.

Supplementary Data

Supplementary Data can be found at *Tree Physiology* online.

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Conflict of interest statement

None declared.

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Authors' contributions

M.S. and K.W. designed the study, G.G.H. and M.S. collected the data, performed the analyses and wrote the manuscript, and K.W. made substantial contributions to the writing.

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