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Vapor-pressure-deficit-controlled temperature response of photosynthesis in tropical trees

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Abstract

Rising temperatures can affect stomatal and nonstomatal control over photosynthesis, through stomatal closure in response to increasing vapor pressure deficit (VPD), and biochemical limitations, respectively. To explore the independent effects of temperature and VPD, we conducted leaf-level temperature-response measurements while controlling VPD on three tropical tree species. Photosynthesis and stomatal conductance consistently decreased with increasing VPD, whereas photosynthesis typically responded weakly to changes in temperature when a stable VPD was maintained during measurements, resulting in wide parabolic temperature-response curves. We have shown that the negative effect of temperature on photosynthesis in tropical forests across ecologically important temperature ranges does not stem from direct warming effects on biochemical processes but from the indirect effect of warming, through changes in VPD. Understanding the acclimation potential of tropical trees to elevated VPD will be critical to anticipate the consequences of global warming for tropical forests.

Keywords: climate change; photosynthesis; stomatal conductance; temperature response; tropical forest; vapor pressure deficit.

Introduction

Tropical forests are responsible for one-third of the global terrestrial primary production (Beer *et al.* 2010) but are currently experiencing reduced growth rates and increased mortality due to atmospheric and climate change (Sullivan *et al.* 2020, Gora and Esquivel-Muelbert 2021). The gross primary productivity of tropical forests results from a delicate balance of large fluxes of CO_2 exchanged between the tropical biosphere and the atmosphere, where both fluxes are strongly affected by environmental factors such as sunlight, temperature, and humidity. Rising temperatures and their effects on tropical forest growth and gross primary productivity have interested tropical plant scientists for decades (*e.g.*, Clark *et al.* 2003, 2010, 2013; Corlett 2011, Pau *et al.* 2018, Sullivan *et al.* 2020). In recent years, vapor pressure deficit (VPD) has emerged as a potentially decisive environmental variable that may significantly affect the physiology of tropical forests (Rowland *et al.* 2015, Slot and Winter 2017b, Smith *et al.* 2020). A better understanding of the mechanisms underlying photosynthetic responses to increasing VPD is critical for improving our ability to predict the future of tropical forests.

Highlights

- Vapor pressure deficit limits tropical tree photosynthesis
- Photosynthesis and stomatal conductance decrease as vapor pressure deficit rises
- Warming impacts photosynthesis *via* vapor pressure deficit in tropical trees

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Abbreviations: C_a – CO_2 concentration of the atmosphere surrounding the leaf; C_i – intercellular CO_2 concentration; g_s – stomatal conductance; g_{sc} – stomatal conductance to CO_2; g_{tc} – total conductance to CO_2; J_{max} – maximum rate of RuBP regeneration; L_b – relative limitation to P_N by biochemical capacity; L_s – relative limitation to P_N by stomatal diffusion; P_N – net photosynthesis; P_{Nmax} – light-saturated net photosynthetic rate; P_{opt} – photosynthetic rate at the optimum temperature; T_{leaf} – leaf temperature; T_{opt} – optimum temperature; V_{cmax} – maximum rate of RuBP carboxylation; VPD – vapor pressure deficit.

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The saturating vapor pressure of the atmosphere increases exponentially with temperature, so without an increase in atmospheric water vapor content, VPD, the difference between the saturation pressure and actual vapor pressure, increases with increasing temperature. Hence as global temperatures rise, the VPD increases (Barkhordarian *et al.* 2019). High VPD induces stomatal closure, minimizing water loss but reducing CO_2 uptake and leaf cooling. High VPD associated with climate change has been discussed as a major contributor to recently observed drought-induced plant mortality in several studies (Breshears *et al.* 2013, Eamus *et al.* 2013, Stovall *et al.* 2019, Grossiord *et al.* 2020, Hammond *et al.* 2022). Furthermore, by limiting cell turgor pressure, high VPD has been recently implicated in reducing stem growth in tropical forest trees (Peters *et al.* 2023).

Separating the direct effects of elevated temperatures on photosynthetic CO_2 uptake from indirect effects through changes in VPD is challenging due to the strong correlation between both factors in natural environments (Grossiord *et al.* 2020, Mills *et al.* 2024). Comparison of Gross Ecosystem Productivity between low VPD conditions at the Biosphere 2 experimental facility in Arizona with forests in Brazil and Mexico in which VPD increased with temperature suggested that stomatal responses to VPD drove the decrease in stand-level photosynthetic carbon uptake (Smith *et al.* 2020). Likewise, statistical separation of VPD and temperature effects on photosynthesis in tropical forest canopies showed that stomatal sensitivity to rising VPD lowered the estimated temperature optimum by ~4°C (Slot *et al.* 2024). These studies suggest that across leaves and plants, those that experience higher temperatures have lower stomatal conductance and lower rates of photosynthesis. What remains unknown, however, is how consistent this pattern is across and within species when individual leaves undergo gradual warming.

To explore the independent short-term effects of VPD and temperature on photosynthetic CO_2 uptake of tropical trees, we experimentally controlled VPD across ranges of temperatures during leaf-level photosynthesis measurements on three tropical tree species in central Panama, using the enhanced humidity control feature of the *LI-6800* photosynthesis system (*LI-COR*, Lincoln, NE, USA). We hypothesized that VPD consistently drives the apparent temperature response of photosynthesis in tropical forest trees.

Materials and methods

Plant material: Three tree species were examined in Panama City, Panama (8.9824°N, 79.5199°W), characterized by a seasonally dry climate with mean annual temperature (MAT) of 25.9°C, mean annual precipitation (MAP) of ~1,900 mm, and a distinct 4-month dry season (Paton 2020): *Persea americana* Mill., *Plumeria rubra* L. (white and pink varieties), and *Terminalia catappa* L. In the following, species will be referred to only by their genus name. The species were selected based on their proximity to one another in the same soil and microclimate, and the accessibility of sun-exposed branches of reproductively mature trees. We prioritized the number of temperature and VPD conditions measured across multiple species, over replication at the species level. Measurements were taken *in situ* on free-growing (*i.e.*, not potted) trees from March to May 2022.

VPD-controlled temperature response measurements: All measurements were taken with an *LI-6800* portable photosynthesis system (*Li-Cor*, Lincoln, NE, USA) with a CO_2 mixer controlling the incoming (reference) CO_2 concentration at approximately ambient concentration (410 μmol mol^{-1}). For each species, we first measured 1–3 light-response curves to determine a saturating and non-photoinhibitory level of photosynthetically active radiation (PAR). Based on these light-response curves, we set the PAR level for all three species to 1,500 μmol(quantum) m^{-2} s^{-1}, provided by 90% red and 10% blue LED lights in the leaf gas-exchange chamber.

To determine light-saturated net photosynthesis (P_{Nmax}) over a range of different temperatures while maintaining a constant VPD, we used the humidifying column of the *LI-6800* with water-saturated *Stuttgarter Masse*, which enabled the maintenance of a low VPD at high temperature. There are, however, limits to the temperature range over which a given VPD can be maintained, due to the risk of condensation at low temperatures, and an insufficient capacity of the humidifying column to maintain low VPD at high temperatures. To extend the humidity control, the airflow rate was reduced from 600 μmol s^{-1} to a minimum of 200 μmol s^{-1} at high temperatures, when necessary. *See* Fig. 1S (*supplement*) for the target VPD levels and associated leaf temperature (T_{leaf}) ranges, and the data selected for further analysis. At each target VPD level, P_{Nmax} was measured at a series of temperatures sequentially on the same leaf. At each temperature, we waited for stabilization of stomatal conductance (g_s) and P_{Nmax} before recording values, typically after 5–15 min, but occasionally exceeding 20 min.

We determined the effect of temperature on the maximum rate of RuBP carboxylation (V_{cmax}) and maximum rate of RuBP regeneration (J_{max}) by measuring CO_2 response (P–C_i) curves at ambient (~31°C) and high (~38°C) temperatures. Photosynthesis was first measured at 410 μmol(CO_2) mol^{-1}, after which values were recorded at 13 additional CO_2 concentrations, first below ambient CO_2 concentrations, after which the curve was completed through step-wise increases above ambient levels to a maximum of 1,800 μmol mol^{-1}. For each species (variety), two curves were measured at each temperature. V_{cmax} and J_{max} were estimated from the relationship between P_{Nmax} and substomatal CO_2 concentration (C_i) using the *fitaci* function from the *Plantecophys* package in *R* (Duursma 2015). The chloroplast CO_2 concentration is lower than C_i because of mesophyll resistance to CO_2 transport, and as a result, V_{cmax} derived from P–C_i curves is underestimated (Niinemets *et al.* 2009). Reliable mesophyll conductance measurements are, however, challenging at best (Leverett and Kromdijk 2024), and doing so in the field was not feasible in this project.

Data analyses: Even with extended humidity control and reduced flow rates, it was not always possible to maintain VPD within a narrow range of the target as temperature increased. We restricted the analyses to cases where VPD was maintained within 0.5 kPa of the target (*see* Fig. 1S for the VPD–T_{leaf} relations, and the selected data).

For each target VPD, a temperature-response curve was fitted using Eq. 1 (Gunderson *et al.* 2010) to estimate T_{opt} (optimum temperature) and P_{opt} (photosynthetic rate at the optimum temperature) for each replicate leaf, as:

$$P_{\text{N}} = P_{\text{opt}} - \text{b} \times \left(T_{\text{leaf}} - T_{\text{opt}}\right)^2 \tag{1}$$

where b is a constant that is proportional to the width of the curve. The curves were fitted with nonlinear least squares regression analyses, using the '*nls_multstart*' function in the '*nls.multstart*' package (Padfield and Matheson 2018) in *R* (R Development Core Team 2021). To test for stomatal limitation of photosynthesis, we plotted photosynthesis against the $C_{\text{i}}/C_{\text{a}}$ ratio at each target VPD.

We further calculated the stomatal slope parameter g1 by Medlyn *et al.* (2011) as the slope of g_{s} *vs.* $1.6 \times P_{\text{N}}/\left(C_{\text{a}} \times \sqrt{\text{VPD}}\right)$, where C_{a} is the CO_2 concentration of the atmosphere surrounding the leaf. The intercept, g0, was also recorded. g1 represents the carbon cost of water supply and is inversely related to water-use efficiency. As such, VPD sensitivity might scale with g1, and g1 might predict species differences in VPD effects on photosynthesis.

The relative limitations placed on CO_2 uptake by the stomatal diffusion (L_{s}) and the biochemical capacity (L_{b}) were estimated from the P–C_{i} response curves according to the approach of Grassi and Magnani (2005):

$$L_{\text{s}} = \frac{\frac{g_{\text{tc}}}{g_{\text{sc}}} \cdot \partial P_{\text{N}}/\partial C_{\text{i}}}{g_{\text{tc}} + \partial P_{\text{N}}/\partial C_{\text{i}}} \tag{2}$$

$$L_{\text{b}} = \frac{g_{\text{tc}}}{g_{\text{tc}} + \partial P_{\text{N}}/\partial C_{\text{i}}} \tag{3}$$

where g_{tc} is the total conductance to CO_2, g_{sc} is the stomatal conductance to CO_2, and $\partial P_{\text{N}}/\partial C_{\text{i}}$ is the slope of the *P–Ci* curves calculated over a range of 50–100 ppm CO_2 as suggested by Tomás *et al.* (2013).

All data were analyzed using *R version 4.1.2* (R Development Core Team 2021).

Results

Leaf level measurements with VPD control: When VPD was controlled experimentally in the cuvette of the *LI-6800* (*i.e.*, when different levels of VPD were maintained over certain temperature ranges), leaf-level temperature response curves of P_{N} were typically shallow and P_{N} only approached zero at very high temperatures when VPD was also high, *e.g.*, at 38°C in *Plumeria* (white) and 40°C in *Terminalia*, both at 4.0 kPa VPD (Fig. 1). On average, photosynthesis could be maintained at ≥80% of the maximum rate across a 14°C range from 25.2 to 39.2°C; for measurements <3.0 kPa VPD the range was even wider, going from 23.4 to 39.6°C.

P_{opt} decreased significantly with increasing VPD for all species, by 2.0, 2.3, 1.3, and 0.7 µmol m^{-2} s^{-1} kPa^{-1} for *Persea*, *Plumeria* (pink), *Plumeria* (white), and *Terminalia*, respectively (when only including curves for which P_{opt} could be reliably estimated with $P<0.05$) (Fig. 2). While the rate of photosynthesis decreased with increasing VPD, there was a tendency for T_{opt} to increase, by on average 1.8°C per kPa VPD (Fig. 2S, *supplement*). The increase in T_{opt} was significant with $P<0.05$ in *Persea.*

Photosynthesis correlated positively with the $C_{\text{i}}/C_{\text{a}}$ ratio at almost all VPD levels in *Plumeria* (white) and *Terminalia*, without a systematic change in the slope (Fig. 3S, *supplement*), indicating that decreases in photosynthesis at any given VPD level were associated with increasing stomatal limitations. The strong correlation between P_{N} and $C_{\text{i}}/C_{\text{a}}$ across all VPD levels with the lowest $C_{\text{i}}/C_{\text{a}}$ ratios was observed at the highest VPD in both species. In contrast, in *Persea*, there was a small positive relationship at most VPD levels consistent with

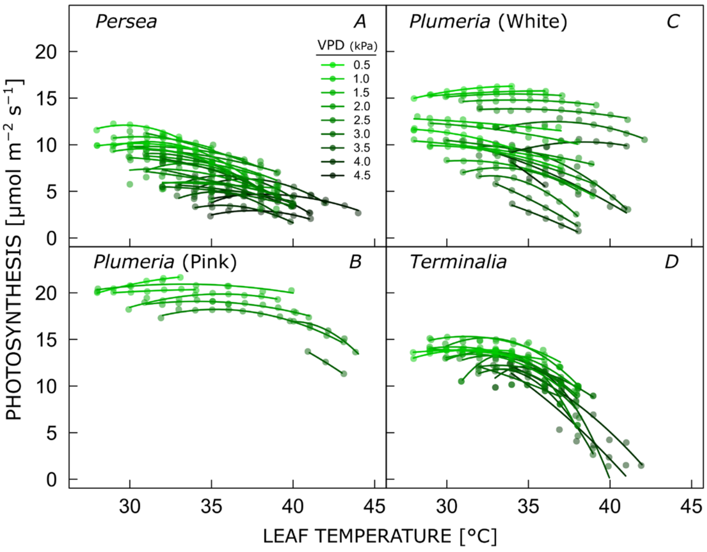

Fig. 1. Leaf-level temperature responses of photosynthesis at different controlled levels of vapor pressure deficit (VPD) for three species, including two varieties of *Plumeria rubra*. Temperature-response curves are fitted at the leaf level using Eq. 1.

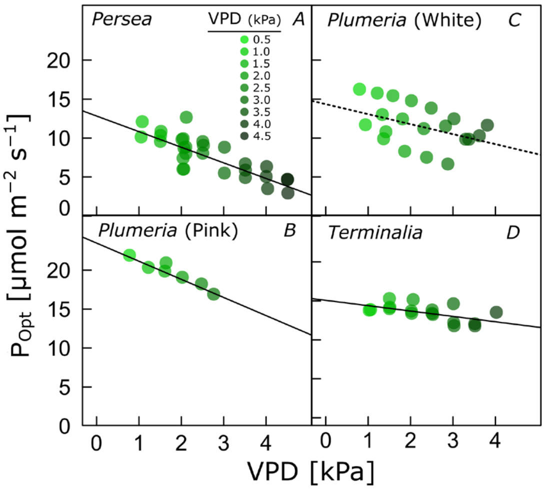

Fig. 2. Photosynthetic CO_2 uptake at optimum temperature (P_{opt}) plotted against the vapor pressure deficit (VPD) at which the parameters were determined, for three species, including two varieties of *Plumeria rubra*, for which leaf-level temperature response curves at different VPD were measured. *Solid* and *dotted lines* indicate linear regressions with $P<0.05$ and $P>0.05$, respectively.

stomatal limitations, but while photosynthesis decreased with increasing VPD, there was no parallel decrease in C_i/C_a. Finally, in *Plumeria* (pink), no consistent patterns were found, possibly due to a smaller sample size for this species. The stomatal slope parameter g1, proportional to the marginal water cost of carbon gain, varied considerably among species, ranging from 2.1 kPa$^{0.5}$ in *Persea* to 7.4 kPa$^{0.5}$ in *Plumeria* (white) (Table 1).

Temperature and VPD responses were not consistent among individuals of the same species (Fig. 3), despite growing close to one another, in the same soil, hydraulic, and light conditions. For example, whereas *Plumeria* (white) tree 1 was able to maintain high P_N and g_s with increasing temperature when VPD was controlled, in tree 2, P_N and g_s decreased with increasing temperature despite maintaining a stable VPD. The VPD response of P_N and g_s was almost completely independent of temperature in tree 1. Only at the very highest temperatures, a moderate reduction in the elevation of the curve (Fig. 3*C*,*G*) was observed. In tree 2, the slopes of the VPD responses were independent of temperature, but the elevation of the curves decreased with increasing temperature across the entire temperature range (Fig. 3*D*,*H*). These patterns represented the extremes across all trees measured (Fig. 3; Fig. 4S, *supplement*).

Biochemical parameters: Between 31 and 38°C, V_{cmax} increased in all species by an average of 94%, from an average of 91 to 174 µmol m^{-2} s^{-1}, respectively (Table 1). J_{max} of *Persea* decreased by 39% between 31 and 38°C, while J_{max} of the remaining species increased by, on average, 24%, from an average of 116 µmol m^{-2} s^{-1} at 31°C to 144 µmol m^{-2} s^{-1} at 38°C. The J_{max}/V_{cmax} ratio at 38°C was lower than at 31°C in all species, by on average 40%.

Relative limitations to photosynthesis by CO_2 diffusion: The relative limitation to photosynthesis by stomatal diffusion (L_s) increased in all species by an average of 24% (range: 7.5–37.9%) between 31 and 38°C while the limitation by biochemical capacity (L_b) decreased by an average of 12% (8.5–17.8%; Table 1). At both temperatures, the L_b values were consistently higher than the L_s values for each species except *Persea*, where the reverse was the case.

Discussion

We have presented experimental support for our hypothesis that the closure of stomata in response to rising vapor pressure deficit (VPD) is the primary driver of the short-term temperature response of photosynthesis of tropical trees. When controlling VPD, photosynthesis exhibited relatively weak responses to temperature, especially below ~35°C, although exact patterns varied among species and individual trees. Across ecologically-relevant temperature ranges the photosynthetic response of sun-exposed tropical forest trees to temperature is thus predominantly governed by the response of stomatal conductance to VPD. Here we showed that temperature manipulation at the leaf

Table 1. Biochemical parameters measured at local ambient (31°C) and elevated (38°C) temperatures, and water-use parameters of the species for which photosynthesis was measured with VPD control. Maximum rates of RuBP carboxylation (V_{cmax}) and RuBP regeneration (J_{max}) ± SEM ($n = 2$), the mean temperature during the measurements, their ratio, the relative limitation to P_N by stomatal diffusion (L_s) and biochemical capacity (L_b), and the stomatal slope parameter, g1.

Species	T_{leaf} [°C]	V_{cmax} [µmol m^{-2} s^{-1}]	J_{max} [µmol m^{-2} s^{-1}]	J_{max}/V_{cmax}	L_s [%]	L_b [%]	g1 [kPa$^{0.5}$]
Persea americana	31.0	69 ± 3	73 ± 6	1.07	53.6	45.7	2.1
	38.0	162 ± 70	45 ± 10	0.38	57.6	41.8	
Plumeria rubra	31.0	105 ± 9	118 ± 1	1.13	29.7	68.7	4.9
(pink variety)	38.0	192 ± 8	157 ± 27	0.82	38.0	60.8	
Plumeria rubra	31.0	112 ± 4	125 ± 7	1.12	31.2	67.3	7.4
(white variety)	38.0	189 ± 18	148 ± 6	0.78	38.8	60.0	
Terminalia catappa	31.0	80 ± 3	106 ± 1	1.32	31.9	66.9	4.2
	38.0	153 ± 7	126 ± 8	0.83	44.0	55.0	

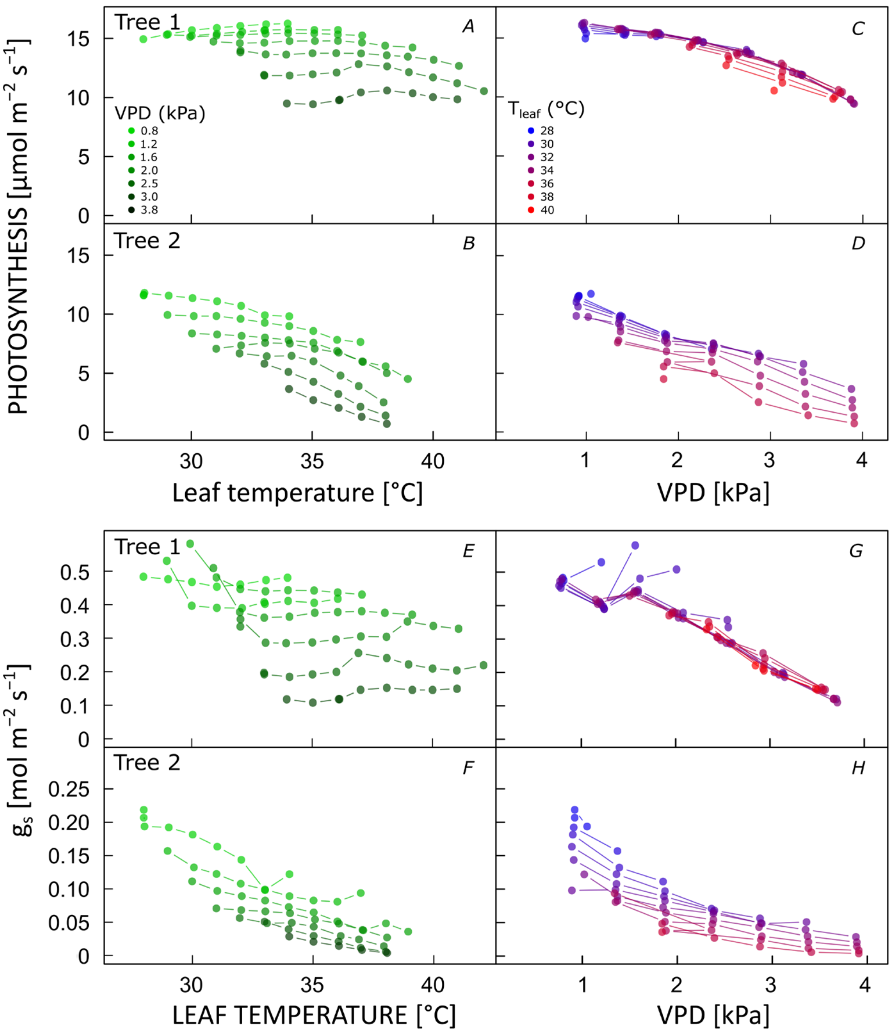

Fig. 3. Temperature and vapor pressure deficit (VPD) responses of photosynthesis (*A–D*) and stomatal conductance (g_s) (*E–H*) of two different *Plumeria rubra* (white) trees.

level yields this same pattern as observed from pooling measurements across canopy leaves (Slot *et al.* 2024).

Temperature responses and cumulative heat effects: The extended humidity control of the *LI-6800* enabled us to measure photosynthesis across a wide enough temperature range to estimate T_{opt} and P_{opt} at each target VPD. The decrease in P_{opt} with increasing VPD was consistent with our hypothesis, but the increasing T_{opt} was contrary to expectations (*e.g.*, Kumarathunge *et al.* 2020, Slot *et al.* 2024). However, firstly, when studying P_N–temperature relationships under controlled VPD conditions, temperature-response curves were relatively flat in the temperature range around T_{opt}, so T_{opt} becomes a somewhat notional concept, with *P* frequently at ≥80% of P_{opt} across the entire measurement range. Secondly, the T_{opt} increase was observed when restricting the analysis to T_{opt} values that could be estimated with confidence; T_{opt} values outside the measured temperature range were not included, because their estimates were poorly constrained. With increasing VPD, the measured temperature ranges shifted up. For example, in *Persea*, photosynthesis was measured between 28 and 34°C when VPD was 1.0 kPa, but between 35 and 44°C when VPD was 4.5 kPa (*see* Fig. 1S), and the T_{opt} values within those ranges also increased with increasing VPD (*see* Fig. 2S). Thus, despite the extended humidity control of the *LI-6800*, the VPD control approach has its limits, as the risk of condensation and lack of humidity set limits to the minimum and maximum temperatures achievable at a given target VPD, respectively. Even with experimental control of VPD, temperature, and VPD thus still covaried across the full dataset, *i.e.*, our dataset did not include measurements at high temperature and low VPD or low temperature and high VPD (Fig. 1S).

Despite VPD control, increasing temperature frequently decreased photosynthesis, resulting in negative temperature-response slopes at most VPD levels. The decrease in photosynthesis at a given VPD tended to be accompanied by decreasing C_i/C_a, suggesting that photosynthesis was limited by stomatal conductance. The effect of temperature on stomatal conductance independent of VPD has been less explored (Grossiord *et al.* 2020, Mills *et al.* 2024), and the mechanism is still a subject of debate (Buckley 2019). Some authors report increasing stomatal conductance with increasing temperature at a constant VPD (Fredeen and Sage 1999, Mott and Peak 2010, Urban *et al*. 2017, Mills *et al*. 2024). Sadok *et al*. (2021) suggest that such an increase in stomatal conductance due to an increase in temperature might be associated with increased hydraulic conductivity as the viscosity of water decreases, and because the transmembrane water movement enabled by aquaporins increases with increasing temperature. In contrast, and in line with some of the temperature responses in the current study, Eamus *et al*. (2008) reported that at a constant VPD of 2.1 kPa, stomatal conductance of *Eucalyptus haemastoma* leaves declined as temperature increased from 18 to 38°C. They argue that increased cuticular transpiration at high temperatures causes a reduction in water supply to guard cells, thereby decreasing guard cell turgor and stomatal aperture.

Cuticular conductance varies widely among species (Schuster *et al*. 2017, Duursma *et al*. 2019), and so does its temperature sensitivity (Riederer 2006, Bueno *et al*. 2019, Slot *et al*. 2021). However, the stomatal response to temperature also varies within species (Fig. 3). Intraspecific variation in cuticle conductance has been linked to growth conditions (Bueno *et al*. 2020) and the presence of leaf endophytes (Arnold and Engelbrecht 2007), neither of which are expected to vary enough among neighboring trees to cause significant differences in cuticular water loss in the current study. Besides potential genetic differences, which can be substantial within species (Alonso-Blanco *et al*. 2009), it thus remains unclear what explains the stomatal temperature response at fixed VPD and its variation across and within species.

Differences in temperature responses of photosynthesis and stomatal conductance may also be affected by differences in mesophyll conductance and its temperature response (*e.g.*, von Caemmerer and Evans 2015). There is some evidence for intraspecific variation in mesophyll conductance, but only across a species distribution range (Peguero-Pina *et al*. 2017), and not between neighboring individuals. To evaluate the relative importance of inter and intraspecific variation in controls over the temperature response of photosynthesis, greater species-level replication is required.

The temperature-response curves of photosynthesis shown in Fig. 1 might be influenced by cumulative heat effects. Under natural conditions, leaf temperatures are highly dynamic (*e.g.*, Vogel 2009, Fauset *et al*. 2018) and even forest canopy leaves in the tropics are unlikely to experience sustained high temperatures. For example, five-minute averages of leaf temperature monitored at a semi-deciduous forest in Panama rarely exceed 35°C (Rey-Sánchez *et al*. 2016). In contrast, in our experiments, individual leaves were sequentially exposed to a series of increasing high temperatures, including four or more temperatures at ≥35°C, and measurements were not taken until g_s and *P* had stabilized at each target temperature. Increased heat exposure duration could increase cuticular water loss, and/or lead to changes in the expression of aquaporins and heat shock proteins (*e.g.*, Araújo *et al*. 2019). This cumulative heat exposure might have contributed to VPD-independent changes in stomatal conductance and photosynthesis during leaf-level temperature responses. Given the dynamic nature of leaf temperatures within forest canopies, parameters estimated from exposing leaves to a series of increasing high temperatures might need validation to confirm the behavior of canopy leaves.

Persea americana was the only species for which J_{max} was lower at 38°C than at 31°C, suggesting that reduced electron transport rate capacity may have limited photosynthesis at high temperatures, consistent with a recent model by Scafaro *et al*. (2023), in which Rubisco activation state and the electron transport capacity were identified as the key drivers of the decrease in photosynthesis above T_{opt}. In this species, growing alongside the other species in the same soil and microclimate, measurements at high VPD levels were not associated with greater stomatal limitation and a decrease in C_i/C_a. The decrease in photosynthesis with VPD being independent of C_i/C_a might reflect the higher temperature ranges across which high VPD values could be maintained (Fig. 1S), and the associated decrease of electron transport capacity in this species.

Long-term effects of rising temperature and VPD: Our study investigated the effect of VPD on the short-term temperature response of photosynthesis. In response to ongoing climate change, over time, forest trees may exhibit acclimation or adaptation responses to rising temperature and VPD that differ from the short-term response (Berry and Björkman 1980, Hikosaka *et al*. 2006, Kumarathunge *et al*. 2019, Crous *et al*. 2022). The differential response between the long- and short-term has been shown to vary between tropical (*see* Slot and Winter 2017c) and temperate forests (*see* Marchin *et al*. 2016 and Schönbeck *et al*. 2022). While experiments have been conducted on the acclimation of temperate forest tree species to warming with VPD manipulation (Marchin *et al*. 2016, Dusenge *et al*. 2021, Schönbeck *et al*. 2022), similar experiments are rare in the tropics (Middleby *et al*. 2024). Growing tropical forest species under a range of temperature and VPD conditions is clearly warranted.

Concluding remarks: Photosynthetic carbon uptake by tropical forests is a critical regulator of the earth's climate, especially in the context of anthropogenic climate change (Malhi *et al*. 2008). The stabilizing influence of tropical forests is threatened by rising temperatures and associated increases in VPD (Tan *et al*. 2017, Smith *et al*. 2020). Model predictions could be fine-tuned with improved mechanistic understanding of the independent roles of temperature and VPD in affecting the photosynthetic CO_2 uptake of tropical trees. It is well established that photosynthesis decreases above a temperature optimum that corresponds roughly to local mean temperatures (Slot and Winter 2017a, Tan *et al*. 2017, Huang *et al*. 2019, Kumarathunge *et al*. 2019), but the processes responsible for this decrease are not yet entirely clear (Slot and Winter 2016, 2017b; Scafaro *et al*. 2023). We showed here that independent of temperature, VPD adversely affects the photosynthesis of tropical trees. Conversely, photosynthesis exhibited relatively weak responses to temperature when controlling VPD, especially at moderate temperatures.

High VPD can result in water-deficit stress in plants, and lead to decreased vegetation productivity through reduced stomatal conductance and thus photosynthesis (Yuan *et al*. 2019, Gharun *et al*. 2020, Grossiord *et al*. 2020, Schönbeck *et al*. 2022). It is currently unknown to what extent the photosynthesis of tropical vegetation can acclimate to changes in VPD, so more experimental data on the long-term effects of high VPD and temperature on tropical tree photosynthesis are needed.

References

Alonso-Blanco C., Aarts M.G.M., Bentsink L. *et al*.: What has natural variation taught us about plant development, physiology, and adaptation? – Plant Cell **21**: 1877-1896, 2009.

Araújo M., Ferreira de Oliveira J.M.P., Santos C. *et al*.: Responses of olive plants exposed to different irrigation treatments in combination with heat shock: physiological and molecular mechanisms during exposure and recovery. – Planta **249**: 1583-1598, 2019.

Arnold A.E., Engelbrecht B.M.J.: Fungal endophytes nearly double minimum leaf conductance in seedlings of a neotropical tree species. – J. Trop. Ecol. **23**: 369-372, 2007.

Barkhordarian A., Saatchi S.S., Behrangi A. *et al*.: A recent systematic increase in vapor pressure deficit over tropical South America. – Sci. Rep.-UK **9**: 15331, 2019.

Beer C., Reichstein M., Tomelleri E. *et al*.: Terrestrial gross carbon dioxide uptake: global distribution and covariation with climate. – Science **329**: 834-838, 2010.

Berry J., Björkman O.: Photosynthetic response and adaptation to temperature in higher plants. – Annu. Rev. Plant Physiol. **31**: 491-543, 1980.

Breshears D.D., Adams H.D., Eamus D. *et al*.: The critical amplifying role of increasing atmospheric moisture demand on tree mortality and associated regional die-off. – Front. Plant Sci. **4**: 266, 2013.

Buckley T.N.: How do stomata respond to water status? – New Phytol. **224**: 21-36, 2019.

Bueno A., Alfarhan A., Arand K. *et al*.: Effects of temperature on the cuticular transpiration barrier of two desert plants with water-spender and water-saver strategies. – J. Exp. Bot. **70**: 1613-1625, 2019.

Bueno A., Sancho-Knapik D., Gil-Pelegrín E. *et al*.: Cuticular wax coverage and its transpiration barrier properties in *Quercus coccifera* L. leaves: does the environment matter? – Tree Physiol. **40**: 827-840, 2020.

Clark D.A., Clark D.B., Oberbauer S.F.: Field-quantified responses of tropical rainforest aboveground productivity to increasing CO_2 and climatic stress, 1997–2009. – J. Geophys. Res.: Biogeo. **118**: 783-794, 2013.

Clark D.A., Piper S.C., Keeling C.D., Clark D.B.: Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984–2000. – PNAS **100**: 5852-5857, 2003.

Clark D.B., Clark D.A., Oberbauer S.F.: Annual wood production in a tropical rain forest in NE Costa Rica linked to climatic variation but not to increasing CO_2. – Glob. Change Biol. **16**: 747-759, 2010.

Corlett R.T.: Impacts of warming on tropical lowland rainforests. – Trends Ecol. Evol. **26**: 606-613, 2011.

Crous K.Y., Uddling J., De Kauwe M.G.: Temperature responses of photosynthesis and respiration in evergreen trees from boreal to tropical latitudes. – New Phytol. **234**: 353-374, 2022.

Dusenge M.E., Wittemann M., Mujawamariya M. *et al*.: Limited thermal acclimation of photosynthesis in tropical montane tree species. – Glob. Change Biol. **27**: 4860-4878, 2021.

Duursma R.A.: Plantecophys – an R package for analysing and modelling leaf gas exchange data. – PLoS ONE **10**: e0143346, 2015.

Duursma R.A., Blackman C.J., Lopez R. *et al*.: On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. – New Phytol. **221**: 693-705, 2019.

Eamus D., Boulain N., Cleverly J., Breshears D.D.: Global change-type drought-induced tree mortality: vapor pressure deficit is more important than temperature per se in causing decline in tree health. – Ecol. Evol. **3**: 2711-2729, 2013.

Eamus D., Taylor D.T., Macinnis-Ng C.M.O. *et al*.: Comparing model predictions and experimental data for the response of stomatal conductance and guard cell turgor to manipulations of cuticular conductance, leaf-to-air vapour pressure difference and temperature: feedback mechanisms are able to account for all observations. – Plant Cell Environ. **31**: 269-277, 2008.

Fauset S., Freitas H.C., Galbraith D.R. *et al*.: Differences in leaf thermoregulation and water use strategies between three co-occurring Atlantic forest tree species. – Plant Cell Environ. **41**: 1618-1631, 2018.

Fredeen A.L., Sage R.F.: Temperature and humidity effects on branchlet gas exchange in white spruce: an explanation for the increase in transpiration with branchlet temperature. – Trees-Struct. Funct. **14**: 161-168, 1999.

Gharun M., Hörtnagl L., Paul-Limoges E. *et al*.: Physiological response of Swiss ecosystems to 2018 drought across plant types and elevation. – Philos. T. Roy. Soc. B **375**: 20190521, 2020.

Gora E.M., Esquivel-Muelbert A.: Implications of size-dependent tree mortality for tropical forest carbon dynamics. – Nat. Plants **7**: 384-391, 2021.

Grassi G., Magnani F.: Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. – Plant Cell Environ. **28**: 834-849, 2005.

Grossiord C., Buckley T.N., Cernusak L.A. *et al*.: Plant responses to rising vapor pressure deficit. – New Phytol. **226**: 1550-1566, 2020.

Gunderson C.A., O'Hara K.H., Campion C.M. *et al*.: Thermal plasticity of photosynthesis: the role of acclimation in forest responses to a warming climate. – Glob. Change Biol. **16**: 2272-2286, 2010.

Hammond W.M., Williams A.P., Abatzoglou J.T. *et al*.: Global field observations of tree die-off reveal hotter-drought fingerprint for Earth's forests. – Nat. Commun. **13**: 1761, 2022.

Hikosaka K., Ishikawa K., Borjigidai A. *et al*.: Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. – J. Exp. Bot. **57**: 291-302, 2006.

Huang M., Piao S., Ciais P. *et al*.: Air temperature optima of vegetation productivity across global biomes. – Nat. Ecol. Evol. 3: 772-779, 2019.

Kumarathunge D.P., Drake J.E., Tjoelker M.G. *et al*.: The temperature optima for tree seedling photosynthesis and growth depend on water inputs. – Glob. Change Biol. **26**: 2544-2560, 2020.

Kumarathunge D.P., Medlyn B.E., Drake J.E. *et al*.: Acclimation and adaptation components of the temperature dependence of plant photosynthesis at the global scale. – New Phytol. **222**: 768-784, 2019.

Leverett A., Kromdijk J.: The long and tortuous path towards improving photosynthesis by engineering elevated mesophyll conductance. – Plant Cell Environ. **47**: 3411-3427, 2024.

Malhi Y., Roberts J.T., Betts R.A. *et al*.: Climate change, deforestation, and the fate of the Amazon. – Science **319**: 169-172, 2008.

Marchin R.M., Broadhead A.A., Bostic L.E. *et al*.: Stomatal acclimation to vapour pressure deficit doubles transpiration of small tree seedlings with warming. – Plant Cell Environ. **39**: 2221-2234, 2016.

Medlyn B.E., Duursma R.A., Eamus D. *et al*.: Reconciling the optimal and empirical approaches to modelling stomatal conductance. – Glob. Change Biol. **17**: 2134-2144, 2011.

Middleby K.B., Cheesman A.W., Cernusak L.A.: Impacts of elevated temperature and vapour pressure deficit on leaf gas exchange and plant growth across six tropical rainforest tree species. – New Phytol. **243**: 648-661, 2024.

Mills C., Bartlett M.K., Buckley T.N.: The poorly-explored stomatal response to temperature at constant evaporative demand. – Plant Cell Environ. **47**: 3428-3446, 2024.

Mott K.A., Peak D.: Stomatal responses to humidity and temperature in darkness. – Plant Cell Environ. **33**: 1084-1090, 2010.

Niinemets Ü., Díaz-Espejo A., Flexas J. *et al*.: Importance of mesophyll diffusion conductance in estimation of plant photosynthesis in the field. – J. Exp. Bot. **60**: 2271-2282, 2009.

Padfield D., Matheson G.: Package 'nls.multstart'. Robust non-linear regression using AIC scores, 2018. *Available at*: https://cran.r-project.org/package=nls.multstart.

Paton S.: Yearly Reports_Parque Natural Metropolitano Crane. Smithsonian Tropical Research Institute, Dataset, 2020.

Pau S., Detto M., Kim Y., Still C.J.: Tropical forest temperature thresholds for gross primary productivity. – Ecosphere **9**: e02311, 2018.

Peguero-Pina J.J., Aranda I., Cano F.J. *et al*.: The role of mesophyll conductance in oak photosynthesis: among- and within-species variability. – In: Gil-Pelegrín E., Peguero-Pina J., Sancho-Knapik D. (ed.): Oaks Physiological Ecology. Exploring the Functional Diversity of Genus *Quercus* L. Tree Physiology. Vol. 7. Pp. 303-325. Springer, Cham 2017.

Peters R.L., Kaewmano A., Fu P.-L: *et al*.: High vapour pressure deficit enhances turgor limitation of stem growth in an Asian tropical rainforest tree. – Plant Cell Environ. **46**: 2747-2762, 2023.

R Development Core Team: R: A language and environment for statistical computing. Version 4.1.2. R Foundation for Statistical Computing, Vienna 2021. *Available at*: https://www.r-project.org/.

Rey-Sánchez A.C., Slot M., Posada J.M., Kitajima K.: Spatial and seasonal variation in leaf temperature within the canopy of a tropical forest. – Clim. Res. **71**: 75-89, 2016.

Riederer M.: Thermodynamics of the water permeability of plant cuticles: characterization of the polar pathway. – J. Exp. Bot. **57**: 2937-2942, 2006.

Rowland L., Harper A., Christoffersen B.O. *et al*.: Modelling climate change responses in tropical forests: similar productivity estimates across five models, but different mechanisms and responses. – Geosci. Model Dev. **8**: 1097-1110, 2015.

Sadok W., Lopez J.R., Smith K.P.: Transpiration increases under high-temperature stress: Potential mechanisms, trade-offs and prospects for crop resilience in a warming world. – Plant Cell Environ. **44**: 2102-2116, 2021.

Scafaro A.P., Posch B.C., Evans J.R. *et al*.: Rubisco deactivation and chloroplast electron transport rates co-limit photosynthesis above optimal leaf temperature in terrestrial plants. – Nature Commun. **14**: 2820, 2023.

Schönbeck L.C., Schuler P., Lehmann M.M. *et al*.: Increasing temperature and vapour pressure deficit lead to hydraulic damages in the absence of soil drought. – Plant Cell Environ. **45**: 3275-3289, 2022.

Schuster A.-C., Burghardt M., Riederer M.: The ecophysiology of leaf cuticular transpiration: are cuticular water permeabilities adapted to ecological conditions? – J. Exp. Bot. **68**: 5271-5279, 2017.

Slot M., Nardwattanawong T., Hernández G.G. *et al*.: Large differences in leaf cuticle conductance and its temperature response among 24 tropical tree species from across a rainfall gradient. – New Phytol. **232**: 1618-1631, 2021.

Slot M., Rifai S.W., Eze C.E., Winter K.: The stomatal response to vapor pressure deficit drives the apparent temperature response of photosynthesis in tropical forests. – New Phytol., 2024.

Slot M., Winter K.: The effects of rising temperature on the ecophysiology of tropical forest trees. – In: Goldstein G., Santiago L.S. (ed.): Tropical Tree Physiology. Tree Physiology. Vol. 6. Pp. 385-412. Springer, Cham 2016.

Slot M., Winter K.: *In situ* temperature response of photosynthesis of 42 tree and liana species in the canopy of two Panamanian lowland tropical forests with contrasting rainfall regimes. – New Phytol. **214**: 1103-1117, 2017a.

Slot M., Winter K.: *In situ* temperature relationships of biochemical and stomatal controls of photosynthesis in four lowland tropical tree species. – Plant Cell Environ. **40**: 3055-3068, 2017b.

Slot M., Winter K.: Photosynthetic acclimation to warming in tropical forest tree seedlings. – J. Exp. Bot. **68**: 2275-2284, 2017c.

Smith M.N., Taylor T.C., van Haren J. *et al*.: Empirical evidence for resilience of tropical forest photosynthesis in a warmer world. – Nat. Plants **6**: 1225-1230, 2020.

Stovall A.E.L., Shugart H., Yang X.: Tree height explains mortality risk during an intense drought. – Nat. Commun. **10**: 4385, 2019.

Sullivan M.J.P., Lewis S.L., Affum-Baffoe K. *et al*.: Long-term thermal sensitivity of Earth's tropical forests. – Science **368**: 869-874, 2020.

Tan Z.-H., Zeng J., Zhang Y.-J. *et al*.: Optimum air temperature for tropical forest photosynthesis: mechanisms involved and implications for climate warming. – Environ. Res. Lett. **12**: 054022, 2017.

Tomás M., Flexas J., Copolovici L. *et al*.: Importance of leaf anatomy in determining mesophyll diffusion conductance to CO_2 across species: quantitative limitations and scaling up by models. – J. Exp. Bot. **64**: 2269-2281, 2013.

Urban J., Ingwers M., McGuire M.A., Teskey R.O.: Stomatal conductance increases with rising temperature. – Plant Signal. Behav. **12**: e1356534, 2017.

Vogel S.: Leaves in the lowest and highest winds: temperature, force and shape. – New Phytol. 183: 13-26, 2009.

von Caemmerer S., Evans J.R.: Temperature responses of mesophyll conductance differ greatly between species. – Plant Cell Environ. **38**: 629-637, 2015.

Yuan W., Zheng Y., Piao S. *et al*.: Increased atmospheric vapor pressure deficit reduces global vegetation growth. – Sci. Adv. **5**: eaax1396, 2019.