



# 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<sub>2</sub> 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<sub>2</sub> concentration of the atmosphere surrounding the leaf;  $C_i$  – intercellular CO<sub>2</sub> concentration;  $g_s$  – stomatal conductance;  $g_{sc}$  – stomatal conductance to CO<sub>2</sub>;  $g_{tc}$  – total conductance to CO<sub>2</sub>;  $J_{max}$  – 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> mixer controlling the incoming (reference) CO<sub>2</sub> concentration at approximately ambient concentration (410 μmol mol<sup>-1</sup>). 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<sup>-2</sup> s<sup>-1</sup>, 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<sup>-1</sup> to a minimum of 200 μmol s<sup>-1</sup> 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<sub>2</sub> response ( $P-C_i$ ) curves at ambient (~31°C) and high (~38°C) temperatures. Photosynthesis was first measured at 410 μmol(CO<sub>2</sub>) mol<sup>-1</sup>, after which values were recorded at 13 additional CO<sub>2</sub> concentrations, first below ambient CO<sub>2</sub> concentrations, after which the curve was completed through step-wise increases above ambient levels to a maximum of 1,800 μmol mol<sup>-1</sup>. 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<sub>2</sub> concentration ( $C_i$ ) using the *fitaci* function from the *Plantecophys* package in *R* (Duursma 2015). The chloroplast CO<sub>2</sub> concentration is lower than  $C_i$  because of mesophyll resistance to CO<sub>2</sub> 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– $T_{\text{leaf}}$  relations, and the selected data).

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

$$P_N = P_{\text{opt}} - b \times (T_{\text{leaf}} - T_{\text{opt}})^2 \quad (1)$$

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  $C_i/C_a$  ratio at each target VPD.

We further calculated the stomatal slope parameter  $g_1$  by Medlyn *et al.* (2011) as the slope of  $g_s$  vs.  $1.6 \times P_N / (C_a \times \sqrt{\text{VPD}})$ , where  $C_a$  is the  $\text{CO}_2$  concentration of the atmosphere surrounding the leaf. The intercept,  $g_0$ , was also recorded.  $g_1$  represents the carbon cost of water supply and is inversely related to water-use efficiency. As such, VPD sensitivity might scale with  $g_1$ , and  $g_1$  might predict species differences in VPD effects on photosynthesis.

The relative limitations placed on  $\text{CO}_2$  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):

$$L_s = \frac{g_{\text{tc}} \cdot \partial P_N / \partial C_i}{g_{\text{tc}} + \partial P_N / \partial C_i} \quad (2)$$

$$L_b = \frac{g_{\text{tc}}}{g_{\text{tc}} + \partial P_N / \partial C_i} \quad (3)$$

where  $g_{\text{tc}}$  is the total conductance to  $\text{CO}_2$ ,  $g_{\text{sc}}$  is the stomatal conductance to  $\text{CO}_2$ , and  $\partial P_N / \partial C_i$  is the slope of the  $P-C_i$  curves calculated over a range of 50–100 ppm  $\text{CO}_2$  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  $\geq 80\%$  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.

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

Photosynthesis correlated positively with the  $C_i/C_a$  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  $C_i/C_a$  across all VPD levels with the lowest  $C_i/C_a$  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

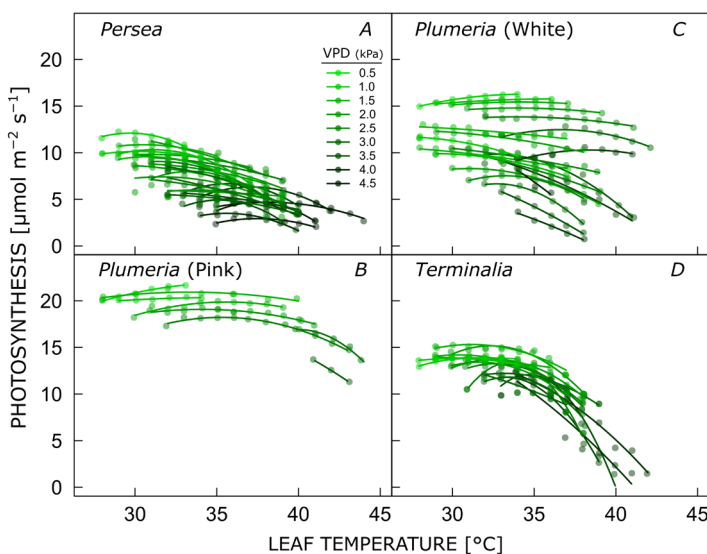


Fig. 1. Leaf-level temperature responses of photosynthesis at different controlled levels of vapor pressure deficit (VPD) for three species, including two varieties of *Plumeria rubra*. Temperature-response curves are fitted at the leaf level using Eq. 1.

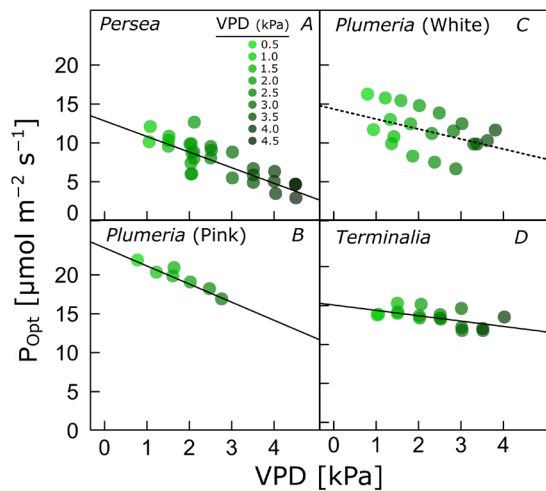


Fig. 2. Photosynthetic  $\text{CO}_2$  uptake at optimum temperature ( $P_{\text{opt}}$ ) plotted against the vapor pressure deficit (VPD) at which the parameters were determined, for three species, including two varieties of *Plumeria rubra*, for which leaf-level temperature response curves at different 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  $g_1$ , proportional to the marginal water cost of carbon gain, varied considerably among species, ranging from  $2.1 \text{ kPa}^{0.5}$  in *Persea* to  $7.4 \text{ kPa}^{0.5}$  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. 3C,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. 3D,H). These patterns represented the extremes across all trees measured (Fig. 3; Fig. 4S, supplement).

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

**Relative limitations to photosynthesis by  $\text{CO}_2$  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  $\sim 35^\circ\text{C}$ , 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 ( $V_{\text{cmax}}$ ) and RuBP regeneration ( $J_{\text{max}}$ )  $\pm$  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,  $g_1$ .

Species	$T_{\text{leaf}} [^\circ\text{C}]$	$V_{\text{cmax}} [\mu\text{mol m}^{-2} \text{s}^{-1}]$	$J_{\text{max}} [\mu\text{mol m}^{-2} \text{s}^{-1}]$	$J_{\text{max}}/V_{\text{cmax}}$	$L_s [\%]$	$L_b [\%]$	$g_1 [\text{kPa}^{0.5}]$
<i>Persea americana</i>	31.0	$69 \pm 3$	$73 \pm 6$	1.07	53.6	45.7	2.1
	38.0	$162 \pm 70$	$45 \pm 10$	0.38	57.6	41.8	
<i>Plumeria rubra</i> (pink variety)	31.0	$105 \pm 9$	$118 \pm 1$	1.13	29.7	68.7	4.9
	38.0	$192 \pm 8$	$157 \pm 27$	0.82	38.0	60.8	
<i>Plumeria rubra</i> (white variety)	31.0	$112 \pm 4$	$125 \pm 7$	1.12	31.2	67.3	7.4
	38.0	$189 \pm 18$	$148 \pm 6$	0.78	38.8	60.0	
<i>Terminalia catappa</i>	31.0	$80 \pm 3$	$106 \pm 1$	1.32	31.9	66.9	4.2
	38.0	$153 \pm 7$	$126 \pm 8$	0.83	44.0	55.0	

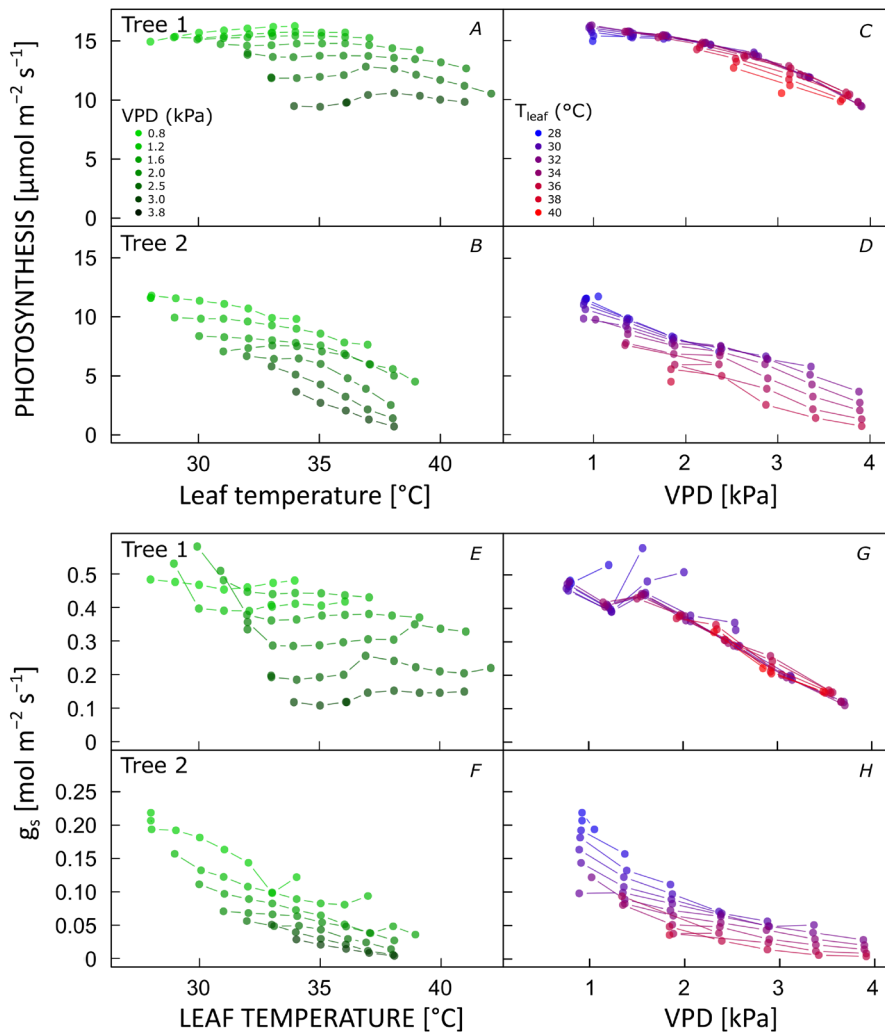


Fig. 3. Temperature and vapor pressure deficit (VPD) responses of photosynthesis (A–D) and stomatal conductance ( $g_s$ ) (E–H) of two different *Plumeria rubra* (white) trees.

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  $\geq 80\%$  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  $\geq 35^\circ\text{C}$ , 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  $T_{\text{opt}}$ . 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  $\text{CO}_2$  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.

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