

# The stomatal response to vapor pressure deficit drives the apparent temperature response of photosynthesis in tropical forests

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Received: 7 September 2023  
Accepted: 18 April 2024

New Phytologist (2024)  
doi: 10.1111/nph.19806

**Key words:** climate change, Panama, photosynthesis, stomatal conductance, temperature response,  $T_{opt}$ , tropical forest, vapor pressure deficit.

## Summary

- As temperature rises, net carbon uptake in tropical forests decreases, but the underlying mechanisms are not well understood. High temperatures can limit photosynthesis directly, for example by reducing biochemical capacity, or indirectly through rising vapor pressure deficit (VPD) causing stomatal closure.
- To explore the independent effects of temperature and VPD on photosynthesis we analyzed photosynthesis data from the upper canopies of two tropical forests in Panama with Generalized Additive Models.
- Stomatal conductance and photosynthesis consistently decreased with increasing VPD, and statistically accounting for VPD increased the optimum temperature of photosynthesis ( $T_{opt}$ ) of trees from a VPD-confounded apparent  $T_{opt}$  of c. 30–31°C to a VPD-independent  $T_{opt}$  of c. 33–36°C, while for lianas no VPD-independent  $T_{opt}$  was reached within the measured temperature range. Trees and lianas exhibited similar temperature and VPD responses in both forests, despite 1500 mm difference in mean annual rainfall.
- Over ecologically relevant temperature ranges, photosynthesis in tropical forests is largely limited by *indirect* effects of warming, through changes in VPD, not by *direct* warming effects of photosynthetic biochemistry. Failing to account for VPD when determining  $T_{opt}$  misattributes the underlying causal mechanism and thereby hinders the advancement of mechanistic understanding of global warming effects on tropical forest carbon dynamics.

## Introduction

The varied effects of global warming on tropical forests are not yet fully understood, but several studies have reported a trend of decreasing net carbon uptake in recent decades (Feeley *et al.*, 2007; Brienen *et al.*, 2015; Hubau *et al.*, 2020) that may be underpinned by rising temperatures (Clark *et al.*, 2003; Sullivan *et al.*, 2020). A significant reduction in carbon uptake and storage in tropical forests will have major consequences for the global carbon cycle (Anderegg *et al.*, 2015), as tropical forests account for one third of annual gross primary productivity of the world's forests (Beer *et al.*, 2010; Pan *et al.*, 2013). A more precise understanding of the mechanisms underlying these trends will be critical for improving predictions of tropical forest change in the 21<sup>st</sup> century.

How might warming decrease net carbon uptake and growth of tropical trees? One hypothesis is that warming increases carbon loss from mitochondrial respiration, as respiration increases exponentially with temperature (at least in the short term), leaving less carbon to support growth. DA Clark *et al.* (2003, 2013) and DB Clark *et al.* (2010) reported a negative correlation between

nighttime temperature and annual diameter increment of tropical trees in Costa Rica, suggesting that nighttime processes such as respiration were driving the growth reduction in warm years. However, elevated respiration under warm conditions can also be associated with increased growth rates (Cheesman & Winter, 2013), as despite the net carbon loss, respiration is essential for biosynthetic processes (Penning de Vries *et al.*, 1974). Furthermore, if respiration acclimates to elevated growth temperature, less carbon is lost at high temperature than without acclimation (Atkin & Tjoelker, 2003). Thermal acclimation of root and stem respiration has not been studied extensively, but leaf respiration typically acclimates to warming, including in plants in the tropics (Slot & Kitajima, 2015). This suggests that in addition to possible increases in respiration rates, other mechanisms might contribute to the observed changes in growth of tropical forest trees.

For example, tropical tree growth also correlates negatively with maximum daytime temperatures (Vlam *et al.*, 2014; Sullivan *et al.*, 2020). High daytime temperature may impact plant growth both directly and indirectly. Heat can cause leaf necrosis and tissue loss (e.g. Sachs, 1864), and although there is no evidence of large-scale heat-induced leaf damage in tropical forests under

current temperature regimes, this might change as tropical regions continue to warm (Doughty *et al.*, 2023). High temperature may also reduce photosynthesis rates directly by limiting the biochemical capacity for photosynthesis, *for example* by reducing the maximum rate of RuBP carboxylation ( $V_{\text{cmax}}$ ), or the maximum rate of RuBP regeneration linked to the photosynthetic electron transport rate ( $J_{\text{max}}$ ) (Sage & Kubien, 2007; Vårhammar *et al.*, 2015; Crous *et al.*, 2022; Scafaro *et al.*, 2023). At high temperatures, net photosynthesis of plants with  $C_3$  photosynthesis (i.e. virtually all tropical tree species) also decreases as a result of increased photorespiration, because as temperature rises Rubisco increasingly fixes  $O_2$  instead of  $CO_2$ . Eventually, Rubisco deactivation can limit photosynthesis because Rubisco activase is thermally labile (Crafts-Brandner & Salvucci, 2000; Sage & Kubien, 2007; Scafaro *et al.*, 2023). The optimum temperature ( $T_{\text{opt}}$ ) for light-saturated photosynthesis tends to correspond to growth temperature (Huang *et al.*, 2019; Kumarathunge *et al.*, 2019), *c.* 30–32°C in lowland tropical forests (Slot & Winter, 2017a), and yet, the biochemical inhibitions of photosynthesis directly caused by high temperature are typically not observed until temperatures exceed *c.* 35–36°C (Slot & Winter, 2017b). Scafaro *et al.* (2023) recently argued that photosynthesis above  $T_{\text{opt}}$  is limited by Rubisco deactivation and the maximum electron transport rate. However, their model overestimated the temperature at which photosynthesis decreased in their tropical plant dataset as stomatal conductance limited  $CO_2$  supply above  $T_{\text{opt}}$  and their model did not explicitly account for stomatal effects. Instead of direct effects of temperature on the biochemical controls over photosynthesis, indirect effects of warming might thus be critical in driving the decrease in carbon uptake in tropical plants when the optimum temperature is exceeded by a few degrees.

Without a change in water content of the air, rising temperatures cause the vapor pressure deficit (VPD) of the air to increase, a process that has already been observed across tropical South America (Barkhordarian *et al.*, 2019). This atmospheric drying, an indirect effect of high temperature, increases rates of tree mortality (Adams *et al.*, 2009; Breshears *et al.*, 2013; Bauman *et al.*, 2022; Hammond *et al.*, 2022), and reduces global vegetation growth (Yuan *et al.*, 2019), including in the wet tropics (Rifai *et al.*, 2018). High VPD negatively affects photosynthesis because stomata close to reduce water loss (Grossiord *et al.*, 2020). Simulations with a theoretical model suggest that photosynthesis of tropical vegetation is more strongly affected by VPD than by temperature *per se* (Lloyd & Farquhar, 2008), but because temperature and VPD inherently covary, disentangling the direct and indirect effects of temperature on photosynthesis of tropical trees remains challenging.

To better understand the long-term effects of elevated temperature and VPD on tropical forests, we first need to understand how temperature and VPD affect photosynthesis of tropical vegetation in the short term, that is from minutes to hours as conditions change during the day. We analyzed a large dataset of nearly 4000 light-saturated photosynthesis measurements collected over a wide range of leaf temperatures in the upper canopy of two tropical forests on opposite ends of a steep rainfall gradient in Panama (associated with Slot & Winter, 2017a) with

Generalized Additive Models. This enabled us to statistically account for direct temperature effects on photosynthesis (e.g. associated with changes in biochemical process rates and photorespiration) and indirect temperature effects (associated with stomatal responses to changes in VPD), and infer the independent effects of temperature and VPD on photosynthesis.

Our main questions were: (1) Can rising VPD explain the short-term response of photosynthesis to increasing temperature in tropical forests? If this is the case, then VPD confounds the apparent temperature response of photosynthesis and needs to be accounted for when the effects of temperature *per se* are of interest. And if it does, (2) Over what temperature range is the stomatal response to VPD the primary factor explaining the decrease of photosynthesis with increasing temperature? And finally, (3) Do temperature responses of photosynthesis differ between wet and dry forests due to different water use characteristics of the local species? We hypothesized that the stomatal response to VPD drives the decrease in photosynthesis up to *c.* 35–36°C, the average temperature above which  $V_{\text{cmax}}$  and  $J_{\text{max}}$  of tropical tree species in Panama start to decrease (Slot & Winter, 2017b). Above this temperature, the decrease in photosynthesis is expected to become independent of VPD and driven by biochemical limitations instead. We further hypothesized that the VPD control over net photosynthesis will be stronger in species from the drier site, as forests with longer dry seasons are thought to be more isohydric than wet forests (Konings & Gentine, 2017), and an efficient water use strategy would involve early stomatal closure with increasing VPD (Grossiord *et al.*, 2020).

## Materials and Methods

### Description of dataset

To disentangle temperature and VPD effects on photosynthesis, we used the dataset from Slot & Winter (2017a), which is publicly available from the Smithsonian Institution repository (<https://repository.si.edu/handle/10088/30581>). This dataset consists of 3941 measurements of light-saturated photosynthesis rates collected between February and May 2016. Fully expanded, sun-exposed leaves of 42 species of tree and liana representing 31 families were measured in the upper canopy at two tropical forests in central Panama that differ in annual rainfall and dry season intensity. Parque Natural Metropolitano (hereafter, Parque Metropolitano), where 12 tree species and 9 liana species were studied, is a seasonally dry forest with mean annual temperature (MAT) of 25.9°C, mean annual precipitation (MAP) of *c.* 1900 mm, and a distinct 4-month dry season (Paton, 2020a). Bosque Protector San Lorenzo (hereafter, San Lorenzo, 18 tree species, 5 liana species) is a moist tropical forest with MAT of 25.3°C and MAP of 3400 mm and a shorter, less-pronounced dry season (Paton, 2020b). Average maximum temperatures are *c.* 1°C higher at Parque Metropolitano (30.8°C) than at San Lorenzo (29.9°C) (data from 1997 to 2015).

Details of the dataset can be found in Slot & Winter (2017a). In brief: upper canopies were accessed using construction cranes and photosynthesis was measured with an LI-6400XT portable

photosynthesis system (Li-Cor Environmental Sciences, Lincoln, NE, USA) at  $c. 390 \mu\text{mol mol}^{-1} \text{CO}_2$  and  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  red: blue LED radiation over, on average, a  $12.7^\circ\text{C}$  leaf temperature ( $T_{\text{leaf}}$ ) range, with average minimum and maximum leaf temperatures  $T_{\text{leaf}}$  of  $26.3^\circ\text{C}$  and  $39.0^\circ\text{C}$ , respectively. For each species, 41–245 leaves were measured (median = 79).  $T_{\text{leaf}}$  was measured abaxially with a Type E (Nickel–Chromium) thermocouple connected to the leaf cuvette and used to calculate leaf-to-air VPD. To maximize the temperature range, we used a combination of the diel change of ambient temperature and moderate manipulations of the block-temperature of the leaf cuvette. Datapoints were removed if stomatal conductance exceeded  $0.8 \text{ mol m}^{-2} \text{ s}^{-1}$  in the early morning when photosynthesis was still low, as these values were almost certainly erroneous and probably associated with leaves not having been effectively dried before measurements.

## Data analyses

**General approach** Accurate estimation of the photosynthetic and stomatal conductance responses to increasing leaf temperature is challenging because leaf temperature and VPD are highly correlated under typical measurement conditions (Pearson correlation coefficient  $\rho = 0.93$  in the current dataset; Supporting Information Fig. S1), which induces problems of multicollinearity for standard regression techniques. Further, both relationships are intrinsically nonlinear. Because of these complications, we used multiple statistical approaches to analyze the leaf temperature responses with and without statistically accounting for the VPD effect. We further evaluated the efficacy of these approaches by attempting to recover simulated temperature and VPD responses. We complement these analyses of temperature and VPD effects on photosynthesis with analyses of stomatal sensitivity to VPD, and estimation of the stomatal slope parameter from the Unified Stomatal Optimization model (Medlyn *et al.*, 2011), which is related to water use efficiency. All data were analyzed in R v.4.3.1 (R Development Core Team, 2023).

**Nonlinear parabolic temperature optima** The overall response of light-saturated net photosynthesis ( $A$ ) as a function of leaf temperature ( $T_{\text{leaf}}$ ) was fitted for each site using the pooled data across species with a standard parabolic function following Gunderson *et al.* (2010), but including random effects as:

$$A = A_{\text{opt}} - b \times (T_{\text{leaf}} - T_{\text{opt}})^2 + u_i + \epsilon \quad \text{Eqn 1}$$

where  $A_{\text{opt}}$  is the photosynthesis rate at the optimum temperature ( $T_{\text{opt}}$ ),  $b$  is a constant that is proportional to the width of the curve,  $u_i$  represents a species-level random intercept term, and  $\epsilon$  is a normally distributed error term. Next, we modified this function to incorporate an additional dependency on VPD as:

$$A = A_{\text{opt}} - b \times (T_{\text{leaf}} - T_{\text{opt}})^2 + b_2 \times \sqrt{\text{VPD}} + u_i + \epsilon \quad \text{Eqn 2}$$

where  $b_2$  estimates the sensitivity of  $A$  to the square root of VPD. In model comparison, the square root dependency on VPD performed better than a log or linear VPD term (data not shown).

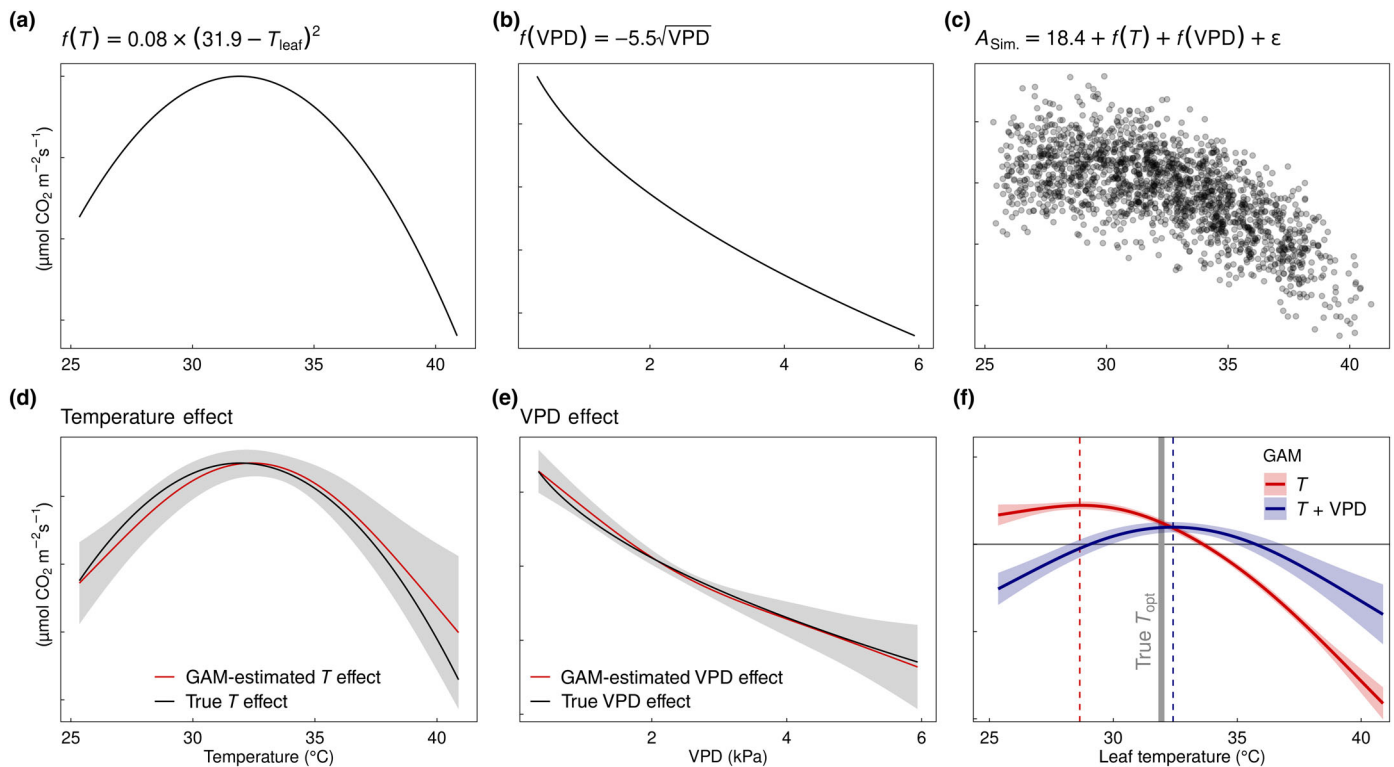
These curves were fitted with the *nlme* function in the NLME package (Pinheiro *et al.*, 2022).

**Generalized additive models** The responses of photosynthesis and stomatal conductance ( $g_s$ ) to temperature and VPD were then analyzed with Generalized Additive Models (GAMs) using the MGCV package (Wood, 2017). The general GAM form used was:

$$y = s(T_{\text{leaf}}) + s(\text{VPD}) + u_i + \epsilon \quad \text{Eqn 3}$$

where  $y$  represents either  $A$  or  $g_s$ ,  $s$  represents smoothing functions,  $u_i$  is a species-level random intercept term, and  $\epsilon$  is a normally distributed error term. The  $s(\text{VPD})$  term was dropped for the temperature-only GAMs. We also fit a set of GAMs where the smoothing functions for  $T_{\text{leaf}}$  and VPD were allowed to vary depending on whether the species were trees or lianas. We fit and compared different model main effects using Maximum Likelihood and used Restricted Maximum Likelihood for the final estimation. GAM smoothing functions are highly flexible, where the user can specify a level of penalization to reduce ‘wiggliness’ in the smoother and improve model goodness of fit (Wood, 2017). The smoothing functions were specified as the thin-plate-spline shrinkage smoother and set to approximate five knots to further reduce overfitting from measurement extremes and outliers (Marra & Wood, 2011). We specified the select option in model fitting to allow smoothing terms to be penalized to zero if they do not contribute to model fit. This level of penalization and constraint on the maximum ‘wiggliness’ reduces the influence of outliers on the smoothing function. We also tested interaction effects between leaf temperature and VPD using tensor product smooths; however, interactions were weak and only nominally improved goodness of fit (not shown); therefore, we focused on the GAM fits as per Eqn 3 and did not explore the interaction further.  $T_{\text{opt}}$  estimates and corresponding uncertainty was extracted from the GAMs by calculating the 95% simultaneous confidence interval (CI) range of the effect of the temperature smooth at its peak. The full range of the smooth was then subset by the lower and upper values of the CI, and the minimum and maximum temperatures were used as estimates of the 95% CI range of  $T_{\text{opt}}$ . When including a random effect for species, the  $T_{\text{leaf}}$  smooth did not have a hump shaped peak in some model fits when VPD is accounted for; therefore,  $T_{\text{opt}}$  was not identifiable in those models.

**Model validation through simulation** We simulated data and examined the ability of each statistical approach to recover the underlying data generating process (Fig. 1). We used the photosynthesis dataset for trees from San Lorenzo to specify the temperature and VPD range. We simulated rates of photosynthesis as the additive contribution of a parabolic temperature response (Fig. 1a) and a negative square root VPD response (Fig. 1b) with normally distributed error. Specifically, the temperature response including  $T_{\text{opt}}$ , the VPD response, and variance were prescribed by using estimated parameters from fitting the San Lorenzo tree data to Eqn 2 (Fig. 1c). The GAM form of Eqn 3 was then used



**Fig. 1** Illustration of the process of simulating temperature and vapor pressure deficit (VPD) dependent photosynthesis ( $A_{\text{sim}}$ ) and demonstration of the recovery of the functional forms of the prescribed temperature and VPD responses.  $A_{\text{sim}}$  is simulated from the addition of nonlinear temperature and VPD effects, with normally distributed error. Panels (a) and (b) show the functional forms of a hypothetical parabolic temperature effect and a negative square root VPD effect, respectively. Parameters and variance for producing the simulating functions were estimated by fitting Eqn 2 to the tree data from San Lorenzo. (c)  $A_{\text{sim}}$  plotted against temperature along with the data generating function used to simulate  $A_{\text{sim}}$ . (d) The true temperature effect as in (a), and the GAM-estimated temperature effect. (e) The true VPD effect as in (b), and the GAM-estimated VPD effect. (f) A temperature-only GAM is compared with the GAM that has both temperature and VPD effects. The estimated  $T_{\text{opt}}$  from each model is compared to the true  $T_{\text{opt}}$  value from the data generating function. Offsets have been added to overlay the simulated and GAM-estimated curves, and numbers have been removed from the y-axis to avoid confusion. The shaded regions indicate a 99% confidence interval of the GAM fits, including for the true  $T_{\text{opt}}$  in (f).

to estimate the underlying functional forms of the temperature and VPD response of the simulated data following the same approach used in the previous section (Fig. 1d,e). For the photosynthesis GAM fits,  $T_{\text{opt}}$  was extracted by isolating the fit smoothing function to the corresponding temperature of the smoothing function's peak value. The same procedure was followed for the parabolic function using Eqns 1, 2. The R script used to produce the simulation and figures is available in Notes S1.

**Binned linear leaf temperature and VPD responses** To facilitate comparison with previous attempts to disentangle temperature and VPD effects on measured  $\text{CO}_2$  exchange of tropical forests (e.g. Tan *et al.*, 2017; Wu *et al.*, 2017), we also used a binning approach, in which photosynthesis is linearly regressed against temperature within narrow bins of VPD, and against VPD within narrow bins of temperature. To be consistent with the use of a random species effect in our other analyses, we fit the models with a species-level random intercept, using the linear mixed model *lmer* function from the LME4 package (Bates *et al.*, 2015). This binning approach does not completely resolve the covariance between temperature and VPD; it can be sensitive to the specification of bin

widths; does not fully account for differing model goodness of fit between bins; and results in a loss of variance in the binned data. We therefore focus our interpretation on the GAM analyses and use the binning approach for comparative purposes. For each site, the pooled data was split into 12 narrow VPD bins of variable range but with a similar number of observations. We regressed  $A$  against  $T_{\text{leaf}}$ , while allowing the  $T_{\text{leaf}}$  slope to vary across VPD bins. Likewise, data were split into 12  $T_{\text{leaf}}$  bins of varying range but with a similar number of observations. Here, we regressed  $A$  as a function of VPD, while allowing the VPD slope to vary across  $T_{\text{leaf}}$  bins. Sample sizes and ranges for each bin are shown in Fig. S2. The same analyses were performed for stomatal conductance in relation to  $T_{\text{leaf}}$  and VPD.

**Stomatal parameters** We calculated the stomatal sensitivity to VPD as the  $m$  parameter, following Oren *et al.* (1999), from:

$$g_s = g_{s,\text{ref}} - m \times \log_e(\text{VPD}) \quad \text{Eqn 4}$$

where  $g_s$  is stomatal conductance and  $g_{s,\text{ref}}$  is the reference stomatal conductance, for which we use the 90<sup>th</sup> percentile of  $g_s$  at  $\text{VPD} < 1.4$  kPa.

The stomatal slope parameter  $g_1$  scales inversely with water use efficiency, and as such this might relate to the stomatal response to high temperature or high VPD. Optimal stomatal theory predicts that drought adapted species have a lower marginal water cost of carbon gain and therefore lower  $g_1$  values than species from wet climates (Heroult *et al.*, 2013). The parameter was calculated following Lin *et al.* (2015):

$$g_s = 1.6 \times (1 + g_1/\sqrt{\text{VPD}}) \times (A/c_a) \quad \text{Eqn 5}$$

where  $c_a$  is the  $\text{CO}_2$  concentration of the atmosphere surrounding the leaf. In this calculation, the  $g_0$  parameter is removed from the original model of Medlyn *et al.* (2011). Data associated with temperatures  $< 30^\circ\text{C}$  were excluded for these calculations as these data points deviated from the tight linear relationship necessary to estimate these parameters accurately.

The  $m$  and  $g_1$  parameters were calculated from the data pooled across species by study site, by growth form (i.e. trees vs lianas), as well as at the species level.

## Results

### Patterns of VPD and temperature response

We estimated  $T_{\text{opt}}$  across the populations of upper canopy leaves by site from both the parabolic temperature model (Eqn 1), and the GAM (Eqn 3), and refit the models with an additional VPD term (Eqns 2, 3). The GAM models had improved goodness of fit metrics over the parabolic model (Table 1). All model approaches indicated higher  $T_{\text{opt}}$  at the seasonally dry forest (Parque Metropolitan) than at the wet forest (San Lorenzo).

$T_{\text{opt}}$  estimated by the temperature-only GAM was  $29.8^\circ\text{C}$  at San Lorenzo and  $31.6^\circ\text{C}$  at Metropolitan (Fig. 2; Table 1). When VPD was included in the GAMs,  $T_{\text{opt}}$  was  $33.1^\circ\text{C}$  at San Lorenzo, which was  $3.3^\circ\text{C}$  higher than when VPD was ignored (Fig. 2; Table 1). At Parque Metropolitan, however, accounting for VPD yielded a temperature response that did not reach an optimum within the measured temperature range (Fig. 2; Table 1). When fitting the temperature response as a parabolic function with Eqn 1, photosynthesis peaked at  $28.8^\circ\text{C}$  at San Lorenzo and at  $30.3^\circ\text{C}$  at Parque Metropolitan (Table 1). At their respective  $T_{\text{opt}}$ ,  $A$  equaled  $11.2 \mu\text{mol m}^{-2} \text{s}^{-1}$  at San Lorenzo and  $12.6 \mu\text{mol m}^{-2} \text{s}^{-1}$  at Parque Metropolitan. When accounting for VPD (Eqn 2),  $T_{\text{opt}}$  was  $3.4^\circ\text{C}$  and  $8.3^\circ\text{C}$  higher than without VPD in the model at San Lorenzo ( $T_{\text{opt}} = 32.2^\circ\text{C}$ ) and Parque Metropolitan ( $T_{\text{opt}} = 38.6^\circ\text{C}$ ), respectively (Table 1).

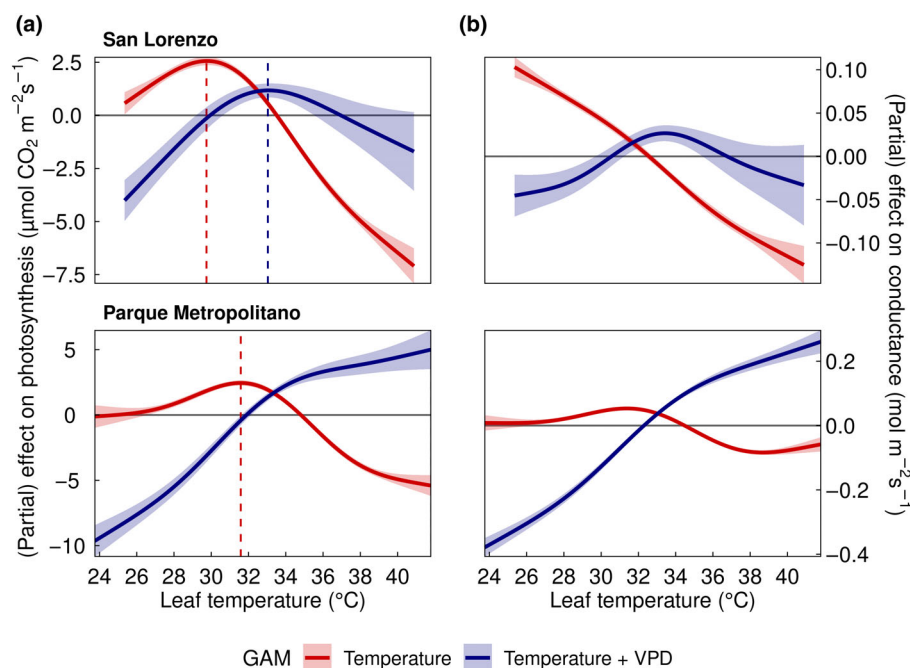
$T_{\text{opt}}$  increased from  $30.0^\circ\text{C}$  to  $34.9^\circ\text{C}$  at San Lorenzo and from  $31.6^\circ\text{C}$  to  $36.3^\circ\text{C}$  at Parque Metropolitan when adding VPD to GAMs without a random species effect (Fig. S3). The fact that the temperature + VPD GAM did not peak at Parque Metropolitan in Fig. 2 appears to be related to the behavior of lianas, of which there were many more at Parque Metropolitan (nine species, and 47% of all measurements) than at San Lorenzo (five species, 17% of all measurements): When running these models with an interaction term on growth form, a clear peak can be identified for trees at both sites, whereas the lianas do not peak at either side when VPD is included in the models (Table 1; Fig. S4). The two species with the highest average and maximum measurement temperature and the highest measurement VPD at Parque Metropolitan were both lianas, but average and maximum measurement temperature and VPD and their ranges did not differ systematically between trees and lianas (Fig. S5).

**Table 1** Optimum temperature ( $T_{\text{opt}}$ ) estimates for net photosynthesis and goodness of fit metrics for different statistical models.

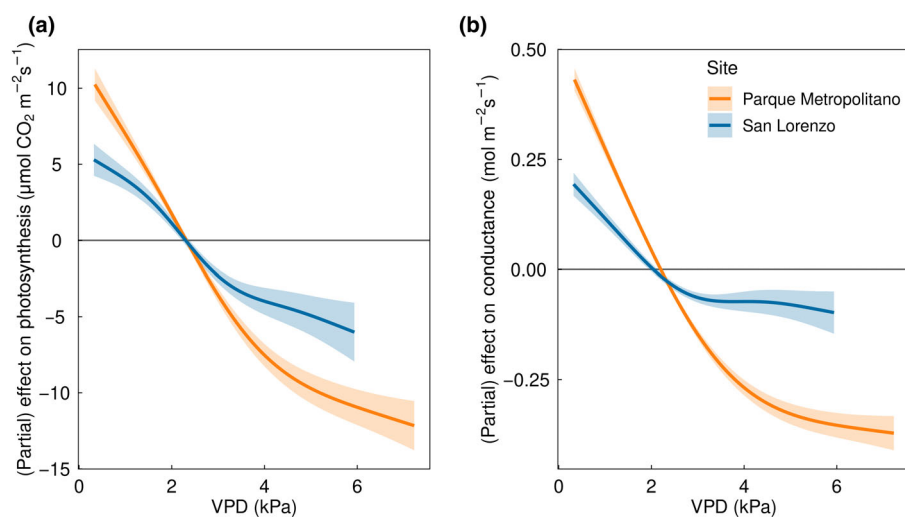
Model		Growth form	$T_{\text{opt}}$ ( $^\circ\text{C}$ )	(95% CI)	$R^2$	RMSE	AIC
San Lorenzo							
GAM	$T_{\text{leaf}}$	All	29.8	28.6–30.9	0.71	2.24	9366
	$T_{\text{leaf}} + \text{VPD}$	All	33.1	31.2–35.2	0.72	2.17	9244
	$T_{\text{leaf}}$ , by growth form	Trees	29.6	28.4–30.8	0.71	2.23	9358
		Lianas	30.4	28.5–31.9			
$T_{\text{leaf}} + \text{VPD}$ , by growth form	Trees	32.7	30.6–34.9	0.73	2.16	9235	
	Lianas	40.9*	34.3–40.9				
Parabola	$T_{\text{leaf}}$	All	28.8	28.4–29.1	0.69	2.29	9546
	$T_{\text{leaf}} + \sqrt{\text{VPD}}$	All	32.2	31.4–32.9	0.71	2.24	9455
Parque Metropolitan							
GAM	$T_{\text{leaf}}$	All	31.6	30.5–32.6	0.77	2.37	8164
	$T_{\text{leaf}} + \text{VPD}$	All	41.8*	35.1–41.8	0.82	2.10	7729
	$T_{\text{leaf}}$ , by growth form	Trees	31.4	30.0–32.7	0.79	2.28	8030
		Lianas	31.5	30.1–32.7			
	$T_{\text{leaf}} + \text{VPD}$ , by growth form	Trees	35.9	33.5–38.4	0.83	2.03	7639
Lianas		41.8*	39.5–41.8				
Parabola	$T_{\text{leaf}}$	All	30.3	30.1–30.6	0.75	2.48	8401
	$T_{\text{leaf}} + \sqrt{\text{VPD}}$	All	38.6	37.3–39.9	0.80	2.22	8024

All models included a species-level random intercept term. Generalized Additive Models (GAMs, Eqn 3) and parabolic functions (Eqns 1, 2) were run with and without explicitly accounting for vapor pressure deficit (VPD). Shown are  $T_{\text{opt}}$  and its 95% confidence interval, as well as the variance explained by the model ( $R^2$ ), the root means squared error (RMSE), and the model comparison Akaike Information Criterion (AIC).

\* $T_{\text{opt}}$  could not be determined within the measured temperature range.



**Fig. 2** Partial effects of temperature on photosynthesis (a) and stomatal conductance (b) at San Lorenzo and Parque Metropolitan, based on Generalized Additive Models (GAM) with a species-level random intercept. Shaded areas indicate the 95% confidence intervals of the GAM fits. Vertical dashed lines in (a) indicate the temperature optima for GAM fits with and without the vapor pressure deficit (VPD) term.



**Fig. 3** Partial effects of vapor pressure deficit (VPD) on photosynthesis (a) and on stomatal conductance (b) at San Lorenzo and Parque Metropolitan based on Generalized Additive Models (GAM) with a species-level random intercept. Shaded areas indicate the 95% confidence intervals of the GAM fits.

When regressing  $A$  against temperature within VPD bins, temperature had a positive, or non-negative effect on  $A$  until VPD exceeded 2.5–3.5 kPa, which occurred when temperatures exceeded 33–35 $^{\circ}\text{C}$  (Fig. S2). The  $A$ - $T_{\text{leaf}}$  slopes within VPD bins (the linear regressions in Fig. S2a,c), changed sign at 34.4 $^{\circ}\text{C}$  at San Lorenzo, and at 37.2 $^{\circ}\text{C}$  at Parque Metropolitan, yielding VPD-corrected  $T_{\text{opt}}$  estimates that were, respectively, 5.8 $^{\circ}\text{C}$  and 7.1 $^{\circ}\text{C}$  higher than when  $T_{\text{opt}}$  was calculated from Eqn 1.

The partial effects of VPD on  $A$  when accounting for temperature in the GAMs were consistently negative at both study sites (Fig. 3a). The effect of VPD on photosynthesis was stronger when temperature was included in the model (Fig. S6). The negative effect of VPD on  $A$  also emerges from the binning exercise illustrated in Fig. S2: regardless of temperature,

$A$  consistently decreases with increasing VPD within narrow  $T_{\text{leaf}}$  bins.

Stomatal conductance ( $g_s$ ) showed similar relationships to temperature and VPD as  $A$  (Fig. 2b). The strong negative effect of VPD on  $g_s$  when accounting for temperature, followed a near identical pattern to the VPD effect on  $A$  (Fig. 3b), illustrating that  $A$  was strongly (log-linearly) correlated with  $g_s$  ( $P < 0.001$ ,  $R^2 = 0.61$  and  $0.69$  at San Lorenzo and Parque Metropolitan, respectively) (see also Fig. S7).

Like the  $A$ - $T_{\text{leaf}}$  slopes, the  $g_s$ - $T_{\text{leaf}}$  slopes within VPD bins decreased with increasing VPD, especially at San Lorenzo (Fig. S8). The  $g_s$ -VPD slopes increased moderately with  $T_{\text{leaf}}$ ; that is, at higher temperatures, VPD had a less negative effect on  $g_s$  than at low temperature. This is also reflected by the flattening out of the additive effect of VPD on  $g_s$  at high VPD (Fig. 3b).

## Simulation analyses

The simulation analyses we performed achieved two things. First, it showed that when VPD exerts a negative effect on photosynthesis, the apparent  $T_{\text{opt}}$  of photosynthesis is inevitably lower than the true  $T_{\text{opt}}$  (Fig. 1c,f). Second, it showed that the GAMs were able to recover the true shape of the temperature and VPD responses from the simulated data, despite temperature and VPD being highly correlated and the GAMs not having any predetermined functional form (Fig. 1). The parabolic model (i.e. Eqn 2) could also recover the simulated  $T_{\text{opt}}$  when given the correct predetermined functional forms (not shown), but when the true temperature response deviates from a parabolic form, the GAM would perform far better.

## Stomatal model parameters

The  $g_1$  parameter was moderately lower at San Lorenzo ( $3.32 \mu\text{mol m}^{-2} \text{s}^{-1} \text{kPa}^{0.5}$ , 95% CI (3.25–3.39)), than at Parque Metropolitan ( $3.55 \mu\text{mol m}^{-2} \text{s}^{-1} \text{kPa}^{0.5}$ , 95% CI (3.49–3.62)) when it was calculated from the pooled data (Table S1). Likewise, averaged across species-level estimates  $g_1$  was slightly, but not significantly ( $t$  test,  $P=0.34$ ) lower at San Lorenzo ( $3.09 \pm 0.23 \mu\text{mol m}^{-2} \text{s}^{-1} \text{kPa}^{0.5}$ , Mean  $\pm$  SEM) than at Parque Metropolitan ( $3.39 \pm 0.20 \text{kPa}^{0.5}$ ). Species-level values ranged from 1.41 in the liana species *Vantanea depleta*, to 5.42 in the tree species *Anacardium excelsum* (Table S2).  $g_1$  values did not differ systematically between trees ( $3.46 \mu\text{mol m}^{-2} \text{s}^{-1} \text{kPa}^{0.5}$ , 95% CI (3.4–3.51)) and lianas ( $3.49 \mu\text{mol m}^{-2} \text{s}^{-1} \text{kPa}^{0.5}$ , 95% CI (3.4–3.59)).  $g_1$  values were similar for evergreen ( $3.18 \pm 0.17 \mu\text{mol m}^{-2} \text{s}^{-1} \text{kPa}^{0.5}$ ) and deciduous species ( $3.17 \pm 0.30 \mu\text{mol m}^{-2} \text{s}^{-1} \text{kPa}^{0.5}$ ).

The  $m$  value, indicating stomatal sensitivity to VPD, was 46% higher at San Lorenzo ( $184 \text{mmol m}^{-2} \text{s}^{-1} \log_e (\text{kPa})^{-1}$ , 95% CI (178–189)) than at Parque Metropolitan ( $126 \text{mmol m}^{-2} \text{s}^{-1} \log_e (\text{kPa})^{-1}$ , 95% CI (120–132)) when calculated from the data pooled by site (Table S1). When calculated at the species level,  $m$  averaged  $185 \pm 21 \text{mmol m}^{-2} \text{s}^{-1} \log_e (\text{kPa})^{-1}$  ( $\pm$  SEM) at San Lorenzo and  $129 \pm 21 \text{mmol m}^{-2} \text{s}^{-1} \log_e (\text{kPa})^{-1}$  at Parque Metropolitan. This difference in mean species-level  $m$  values between the sites was not statistically significant ( $t$  test,  $P=0.06$ ). At Parque Metropolitan,  $m$  was 15% lower in lianas than in trees when it was calculated from the pooled data, whereas at San Lorenzo  $m$  of lianas was 20% higher than that of trees.  $m$  averaged  $144 \pm 17 \text{mmol m}^{-2} \text{s}^{-1} \log_e (\text{kPa})^{-1}$  in evergreen species and  $189 \pm 34 \text{mmol m}^{-2} \text{s}^{-1} \log_e (\text{kPa})^{-1}$  in deciduous species ( $t$  test,  $P=0.16$ ) Across species,  $m$  increased strongly ( $R^2=0.77$ ) with maximum  $g_s$  ( $g_{s,\text{ref}}$  in Eqn 4), indicating that species with high maximum  $g_s$  exhibiting greater stomatal sensitivity to VPD than species with low maximum  $g_s$  (Fig. S9).

## Discussion

We have provided evidence that stomatal closure in response to elevated vapor pressure deficit is the main driver of the short-term temperature response of photosynthesis of canopy

trees in two tropical forests in Panama. When statistically accounting for VPD, temperature does not have a negative effect on photosynthesis until  $c. 33\text{--}36^\circ\text{C}$  is reached, yielding a VPD-corrected true temperature optimum that is  $3.5\text{--}4^\circ\text{C}$  higher than when estimating  $T_{\text{opt}}$  without considering VPD. This VPD-corrected  $T_{\text{opt}}$  roughly corresponds to the  $T_{\text{opt}}$  of  $V_{\text{cmax}}$  and  $J_{\text{max}}$  of tropical tree species (Slot & Winter, 2017b; Dusenge *et al.*, 2021; Choury *et al.*, 2022). For lianas the effect of VPD on  $T_{\text{opt}}$  was even greater, as no optimum in the temperature response was reached when accounting for VPD, and as a result, when pooling data from trees and lianas by site, no VPD-corrected  $T_{\text{opt}}$  could be identified at Parque Metropolitan. The lower  $T_{\text{opt}}$  estimate when ignoring VPD is an inevitable outcome of the combined direct temperature effect on photosynthesis, and the negative indirect effect, through changes in VPD and stomatal conductance. The seasonally dry forest at Parque Metropolitan exhibited slightly greater stomatal sensitivity to VPD than the wet forest at San Lorenzo but had higher apparent and VPD-corrected thermal optima. We show that Generalized Additive Models provide a powerful tool for disentangling temperature and VPD effects on photosynthesis and for determining both the apparent  $T_{\text{opt}}$ , and the VPD-corrected  $T_{\text{opt}}$ .

## Indirect warming effects drive photosynthesis response to temperature

The stomatal response to VPD can account for the decline in photosynthesis between the apparent  $T_{\text{opt}}$  of  $c. 30\text{--}32^\circ\text{C}$  and the VPD-corrected  $T_{\text{opt}}$  of  $c. 33\text{--}36^\circ\text{C}$ . The temperature response of photosynthesis of canopy trees over this temperature range is thus not primarily driven by *direct* warming effects on metabolic or biochemical processes, but by the *indirect* effect of warming through changes in VPD, supporting our first two hypotheses. While other studies have reported an important role for direct temperature effects, most notably Scafaro *et al.* (2023), our results are consistent with model simulations based on environmental conditions in the Amazon (Lloyd & Farquhar, 2008; Rowland *et al.*, 2014), and with Eddy Covariance studies for tropical forests in SE Asia (Tan *et al.*, 2017) and the Amazon (Wu *et al.*, 2017) in which VPD and temperature effects were disentangled using a binning approach similar to the one presented in Fig. S2. Our results also align with Smith *et al.* (2020), who compared the temperature response of gross ecosystem productivity (GEP) between forests in the Brazilian Amazon and Mexico (evergreen, and mostly dry season deciduous, respectively), and a tropical forest mesocosm in the Biosphere 2 facility in Arizona, USA. In the experimental Biosphere 2 setting, VPD was low even when temperature was high ( $< 2.0 \text{kPa}$  at  $40^\circ\text{C}$ ), whereas VPD at the field sites rose in a near-exponential fashion with temperature, as is typical in natural settings (reaching  $c. 5.0 \text{kPa}$  at  $40^\circ\text{C}$  in Mexico). While GEP of the forests in Brazil and Mexico decreased quite steeply above  $30^\circ\text{C}$  – roughly corresponding to the apparent  $T_{\text{opt}}$  of photosynthesis in the current study – GEP at Biosphere 2 decreased moderately with temperature, and only decreased more steeply above  $38^\circ\text{C}$ , a temperature comparable to the VPD-corrected  $T_{\text{opt}}$  in the current study. Eddy Covariance

and other stand-level analyses rely on assumptions about ecosystem respiration to isolate carbon uptake and its temperature sensitivity, but our analyses of almost 4000 leaf-level measurements convincingly show that VPD is driving a significant part of the temperature response of net photosynthesis in both wet and seasonally dry tropical forests.

The  $T_{\text{opt}}$  estimate without accounting for VPD (what we have called the 'apparent  $T_{\text{opt}}$ ') might be relevant in some studies, for example when comparing species growing at the same (micro) climate. However, when mechanistic understanding of direct temperature effects is the objective, it will be critical to correct  $T_{\text{opt}}$  for VPD. While VPD is strongly affected by temperature, it is also a function of atmospheric moisture content, so the VPD effect should not be treated as a direct temperature effect (Mills *et al.*, 2024). Failing to account for VPD might misattribute the cause of decreasing photosynthesis between the apparent  $T_{\text{opt}}$  and the true  $T_{\text{opt}}$  to direct temperature effects. Nonetheless, the VPD-corrected  $T_{\text{opt}}$  does not represent the optimum of a single process but reflects the outcome for light-saturated net photosynthesis of a composite of VPD-independent direct temperature effects on processes affecting photosynthesis of  $C_3$  plants, including  $V_{\text{cmax}}$ ,  $J_{\text{max}}$ , photorespiration, and Rubisco deactivation.

Because temperature and VPD strongly covary under natural conditions, our dataset did not contain measurements at high temperature and low VPD, or at low temperature and high VPD, and it is possible that statistical inference of the independent temperature and VPD effects would be slightly different in a dataset that contains measurements across the full range of possible VPD values at each temperature. Collecting such data requires careful leaf-level manipulations which can be aided by the extended humidity control of new commercially available photosynthesis systems such as the LI-6800 (Li-Cor). However, deviating significantly from realistic combinations of temperature and VPD is unlikely to improve our understanding of their effects under field conditions.

### Independent temperature and VPD effects

Many studies have shown that stomatal conductance decreases with increasing VPD (reviewed in Grossiord *et al.*, 2020), but few studies have investigated how stomata respond to temperature alone and the mechanisms are not well understood (Buckley, 2019; Mills *et al.*, 2024). The GAMs shown in Fig. 2 indicate that the independent effect of temperature on stomatal conductance is positive at Parque Metropolitan, consistent with most experimental results (Fredeen & Sage, 1999; Mott & Peak, 2010; Urban *et al.*, 2017; Mills *et al.*, 2024), but has a peaked temperature response at San Lorenzo.

### Contrasting rainfall regimes differ minimally in the role of VPD in short-term temperature responses, but lianas differ from trees

We hypothesized that the wet forest and the seasonally dry forest would show different responses to VPD, because stomatal closure with increasing VPD would be advantageous when at risk of

hydraulic failure. Stomatal conductance indeed declined more steeply with increasing VPD at the seasonally dry forest at Parque Metropolitan than at the wetter San Lorenzo site (Fig. 3b). The stomatal sensitivity parameter  $m$  was, however, moderately higher at San Lorenzo than at Parque Metropolitan (Tables S1, S2). This discrepancy might be explained by the fact that unlike the GAM-derived VPD response,  $m$  estimates are disproportionately affected by measurements at low VPD due to the  $\log(\text{VPD})$  term in Eqn 4. Slot & Winter (2017a) demonstrated that the decrease in photosynthesis above  $T_{\text{opt}}$  was associated with a small, but statistically significant decrease in the  $c_i/c_a$  ratio at Parque Metropolitan, but not at San Lorenzo, further suggesting moderately stronger stomatal limitation at Parque Metropolitan. A steeper decline in stomatal conductance could reduce the apparent  $T_{\text{opt}}$  estimate, but  $T_{\text{opt}}$  estimates were higher for Parque Metropolitan than for San Lorenzo. The difference in both apparent  $T_{\text{opt}}$  and VPD-corrected  $T_{\text{opt}}$  might be the result of thermal acclimation leading to an increase in  $T_{\text{opt}}$  at the warmer forest at Parque Metropolitan, or it may reflect species filtering or adaptation, as there is virtually no species overlap between the sites.

Stomatal slope parameter  $g_1$  did not differ systematically between San Lorenzo and Parque Metropolitan. Wu *et al.* (2020), who investigated a subset of the species we studied, did not find differences between these sites either in either  $g_1$  or parameters of alternative stomatal conductance models. Likewise, several other recent studies of leaf hydraulic traits found no significant differences between the two sites (Pivovarov *et al.*, 2021; Smith-Martin *et al.*, 2022). What does, however, differ significantly, is the percent of deciduous species, which is much greater at Parque Metropolitan (Condit *et al.*, 2000). Deciduousness is a critical drought avoidance strategy (e.g. Wolfe *et al.*, 2016) that may obviate the need for higher leaf-level drought tolerance at the seasonally dry forest. Indeed, deciduous and evergreen species did not have systematically different  $g_1$  or  $m$  values.

Interestingly, when analyzing lianas only, the VPD-corrected temperature effect on photosynthesis was consistently positive at both sites. This may explain why no VPD-corrected  $T_{\text{opt}}$  could be determined with the GAM approach when pooling trees and lianas at Parque Metropolitan, where lianas made up > 40% of the measured species. The greater contrast between the temperature-only and the temperature + VPD models suggests that lianas experience a stronger VPD effect on photosynthesis. This is not reflected by the  $m$  and  $g_1$  parameters – which, unlike the GAMs used here, are determined with prescribed functional forms – nor by previous analyses of stomatal limitation of photosynthesis in trees vs lianas (Slot & Winter, 2017a). Further study of the physiology of lianas will thus be required to explain the observed patterns.

### Long-term effects of rising temperature and VPD

We focused on the effect of VPD on the short-term temperature response of photosynthesis, but in the long term, acclimation can change short-term temperature responses (e.g. Berry & Björkman, 1980; Hikosaka *et al.*, 2006; Slot & Winter, 2017c; Kumarathunge *et al.*, 2019; Slot *et al.*, 2021; Crous *et al.*, 2022).



Acclimation of photosynthesis of three tropical tree species to elevated temperatures increased the apparent  $T_{opt}$  and decreased the  $J_{max}/V_{cmax}$  ratio, without changes in stomatal sensitivity to temperature and VPD (Slot & Winter, 2017c). By contrast, acclimation to understory warming at Duke Forest, USA, resulted in increased stomatal sensitivity to VPD for two of the three species studied (Marchin *et al.*, 2016). Likewise, Schönbeck *et al.* (2022) found increased stomatal sensitivity to VPD in warm-grown saplings of two of the three temperate tree species they grew in a factorial temperature by VPD experiment in Switzerland. To better understand the mechanisms underlying tropical plant responses to long-term changes in temperature, similar experiments with tropical forest species are needed in which temperature and VPD are independently manipulated.

### Concluding remarks

Rising temperatures and associated increases in VPD have significant impact on tropical forest photosynthesis (Tan *et al.*, 2017; Smith *et al.*, 2020), growth (Rifai *et al.*, 2018; Hubau *et al.*, 2020), and mortality (McDowell *et al.*, 2018; Bauman *et al.*, 2022; Doughty *et al.*, 2023), with strong consequences for the role tropical forests can play in mitigating anthropogenic climate change (Nölte *et al.*, 2023). Predictions of these changes would benefit from improved mechanistic understanding of the independent roles of temperature and VPD in affecting the photosynthetic physiology of tropical trees. We have shown that the temperature effect on photosynthesis can be misinterpreted when examined independent of co-occurring VPD effects. The VPD-independent direct effect of temperature on photosynthesis peaks at *c.* 33–36°C in trees in these forests, and the decrease in carbon uptake between the apparent temperature optimum of 30–31°C and the true optimum temperature reflects stomatal closure in response to rising VPD. Upper canopy leaves rarely exceed 35°C for extended periods of time (Rey-Sánchez *et al.*, 2016), so under current temperature regimes VPD dominates the apparent temperature response across ecologically relevant temperature ranges.

VPD has been increasing in tropical South America and Africa (Barkhordarian *et al.*, 2019; Rifai *et al.*, 2019), and rising VPD reduces global vegetation growth (Yuan *et al.*, 2019). Reductions in rainfall in many tropical areas (e.g. Duffy *et al.*, 2015) will exacerbate the impact of rising VPD on photosynthesis in the tropics. While the negative VPD effect might be compensated by increasing atmospheric CO<sub>2</sub> concentrations (Lloyd & Farquhar, 2008; Rifai *et al.*, 2022), ongoing warming and associated increases in VPD could potentially negate the CO<sub>2</sub> fertilization effect (Li *et al.*, 2023). Experimental data on a wide range of tree species are urgently needed to establish the long-term effects of elevated VPD and temperature on photosynthesis of tropical trees.

### Acknowledgements

This research was supported by the Smithsonian Tropical Research Institute. CEE was the recipient of a Smithsonian

Institution postdoctoral fellowship. SWR received support from the Australian Research Council Discovery Grant (DP240101938). The upper canopy photosynthesis data could not have been collected without the expert assistance of crane operators Edwin Andrades and Ocard Saldaña. Four anonymous reviewers provided helpful comments and suggestions on earlier versions of this paper.

### Competing interests

None declared.

### Author contributions

MS, KW and SWR designed the study. MS collected the original data. SWR analyzed the data, conducted the simulations, and made the figures. All authors interpreted the results. MS wrote the manuscript, with significant contributions from SWR, KW and CEE.

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### Data availability

Data from Slot & Winter (2017a) are available at: <https://repository.si.edu/handle/10088/30581>. R scripts used to produce the analyses and figures are attached in Notes S1.

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Vapor pressure deficit and its correlation with leaf temperature.

**Fig. S2** Photosynthesis plotted against leaf temperature and VPD, and predicted photosynthesis as a linear function of leaf temperature with variation in VPD, and VPD with variation in temperature for San Lorenzo Parque Metropolitan.

**Fig. S3** Generalized Additive Model derived partial effects of temperature on photosynthesis and stomatal conductance in the absence of species-specific intercepts.

**Fig. S4** Generalized Additive Model derived partial effects of temperature on photosynthesis and stomatal conductance for trees and lianas at San Lorenzo and Parque Metropolitan.

**Fig. S5** Mean and range of measurement temperature and VPD for each species measured at San Lorenzo and Parque Metropolitan.

**Fig. S6** Generalized Additive Model derived partial effects of VPD on photosynthesis for San Lorenzo and Parque Metropolitan.

**Fig. S7** Photosynthesis in relation to stomatal conductance at different VPD and different temperatures in San Lorenzo and Parque Metropolitan.

**Fig. S8** Stomatal conductance plotted against leaf temperature and VPD, and predicted photosynthesis as a linear function of leaf temperature with variation in VPD, and VPD with variation in temperature for San Lorenzo Parque Metropolitan.

**Fig. S9** Stomatal sensitivity to VPD plotted against the maximum stomatal conductance at low VPD.

**Notes S1** Zip file containing R scripts used to produce the analyses and figures.

**Table S1** Stomatal slope parameter  $g_1$ , and stomatal sensitivity to VPD parameter  $m$  estimated at the level of the site (San Lorenzo vs Parque Metropolitano), at the level of growth form (trees vs lianas), and contrasting growth forms by site.

**Table S2** Stomatal slope parameter  $g_1$ , and stomatal sensitivity to VPD parameter  $m$  estimated at the species level at San Lorenzo and Parque Metropolitano.

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