



Empirical evidence for resilience of tropical forest photosynthesis in a warmer world

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Tropical forests may be vulnerable to climate change^{1–3} if photosynthetic carbon uptake currently operates near a high temperature limit^{4–6}. Predicting tropical forest function requires understanding the relative contributions of two mechanisms of high-temperature photosynthetic declines: stomatal limitation (H1), an indirect response due to temperature-associated changes in atmospheric vapour pressure deficit (VPD)⁷, and biochemical restrictions (H2), a direct temperature response^{8,9}. Their relative control predicts different outcomes—H1 is expected to diminish with stomatal responses to future co-occurring elevated atmospheric [CO₂], whereas H2 portends declining photosynthesis with increasing temperatures. Distinguishing the two mechanisms at high temperatures is therefore critical, but difficult because VPD is highly correlated with temperature in natural settings. We used a forest mesocosm to quantify the sensitivity of tropical gross ecosystem productivity (GEP) to future temperature regimes while constraining VPD by controlling humidity. We then analytically decoupled temperature and VPD effects under current climate with flux-tower-derived GEP trends insitu from four tropical forest sites. Both approaches showed consistent, negative sensitivity of GEP to VPD but little direct response to temperature. Importantly, in the mesocosm at low VPD, GEP persisted up to 38 °C, a temperature exceeding projections for tropical forests in 2100 (ref. ¹⁰). If elevated [CO₂] mitigates VPD-induced stomatal limitation through enhanced water-use efficiency as hypothesized^{9,11}, tropical forest photosynthesis may have a margin of resilience to future warming.

Tropical plants may be vulnerable to even small amounts of climate warming, having evolved in climates with low thermal variability^{12,13}. This vulnerability is highlighted by observations suggesting that tropical forests are already functioning near their high-temperature limit^{4–6,14,15}, together with projections that tropical regions will likely experience unprecedented high temperatures that will soon push forests above such limits¹⁶.

A critical trait determining forest vulnerability to climate change is the thermal sensitivity of photosynthesis. However, there is considerable debate over how different component mechanisms of photosynthetic carbon uptake are influenced by climate as temperatures increase above the apparent thermal optimum (T_{opt}) in

tropical forests^{4,9}. The temperature response curve of net ecosystem carbon uptake shows a decline at high temperatures that may be caused by a decrease in photosynthesis (the balance of gross photosynthetic carbon uptake and photorespiratory carbon emission) and/or an increase in ecosystem respiration. We focus here on gross ecosystem productivity (GEP) (net ecosystem carbon exchange minus ecosystem respiration—that is, ecosystem photosynthesis), since the relative impact of respiration is probably smaller due to low temperature sensitivity of tropical ecosystem respiration over short timescales¹⁷ and the small contribution of leaf respiration to daytime CO₂ exchange in tropical species^{9,18,19}.

GEP may decline with warming as a result of stomatal closure, a mechanism for reducing water loss as the atmospheric demand for water vapour (vapour pressure deficit (VPD)) rises, which consequently reduces the uptake of CO₂ (H1, indirect temperature effect)⁷. High temperature can also disrupt the coordination of leaf biochemical components with different temperature optima, resulting in downregulation of the biochemistry underlying photosynthesis and accumulation of secondary stresses such as oxidation⁸, and very high temperatures degrade enzymes and reduce membrane stability^{9,20} (H2, direct temperature effects). While both hypotheses are presumed to contribute to observed plant responses to temperature over some range, a more precise understanding of their relative contributions at supra-optimal temperatures is critical for accurate prediction of forest function given future climate change. If direct effects are strong, temperature-induced changes to photosynthetic infrastructure pose a more immediate threat to forests, but if direct effects are weak, tropical forest photosynthetic processes may have a margin of resiliency to warming, especially if concurrent elevated atmospheric [CO₂] increases leaf water-use efficiency (WUE) and ameliorates the effect of higher VPD on leaf gas exchange^{9,11,21,22}.

A number of empirical studies at leaf^{23–28} and ecosystem scales^{24,29,30} suggest that declines in photosynthesis at high temperatures are associated with rising VPD, supporting H1. However, few of these studies experimentally decouple both temperature and VPD, and only one—a study of a boreal spruce²³—does so above T_{opt} . Analytical differentiation of direct and indirect effects from temperature-response³¹ and temperature × CO₂-response¹⁸ curves of photosynthesis in tropical plant leaves showed evidence for stomatal limitations above T_{opt} in some species (H1), and stronger

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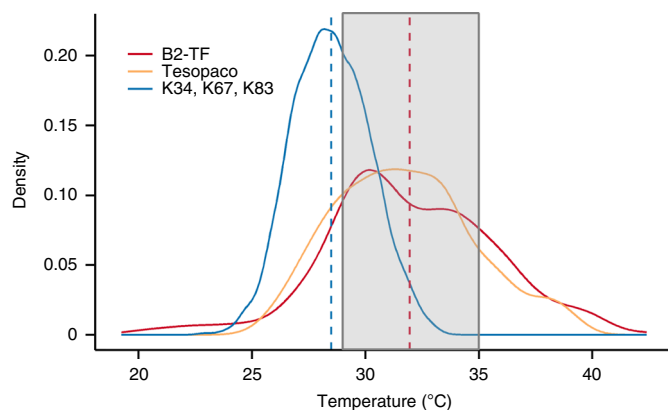


Fig. 1 | Air temperature distributions recorded at the B2-TF mesocosm, a seasonally dry tropical forest (Tesopaco) and Amazon forest sites (K34, K67 and K83). The dashed lines show the current mean temperatures at the Amazon forest sites (28 °C) and at the B2-TF and Tesopaco (32 °C); the grey area shows the range of mean annual temperatures projected for the Amazon region by 2100 (ref. ¹⁰). Only temperatures corresponding to light-saturated GEP have been included.

biochemical limitation (H2) or colimitation above T_{opt} in other species. Considering the narrow thermal niches to which tropical trees are expected to be adapted, a general paucity of data from tropical trees, and some empirical support for H2 at the leaf level, there is a clear need for ecosystem-scale experiments and observations that help us distinguish mechanisms of high-temperature photosynthetic declines in tropical forests.

To address this question, we used an experimental tropical forest with substantial climate control—the Biosphere 2 Tropical Forest biome (B2-TF; Arizona, USA). We compared the response of light-saturated GEP to air temperature and VPD in the B2-TF with those of three evergreen forest sites in the Brazilian Amazon (K34, K67 and K83) and of a tropical dry forest in Mexico (Tesopaco) (Methods). The B2-TF is a 0.2 ha enclosed mesocosm with a complex vertical canopy structure including mature trees up to 13–17 m (ref. ³²). The B2-TF allows assessment of the temperature sensitivity of tropical forest photosynthesis within the range of mean annual temperatures projected for Amazonia by 2100 (1–7 °C above present-day means¹⁰; Fig. 1) and up to 40 °C, approximately 6 °C higher than the maximum temperatures recorded at the Amazonian sites. Additionally, the sensitivity of VPD to temperature can be experimentally manipulated by controlling humidity, achieving a greater independence of the environmental factors that control photosynthesis than can be observed in natural forests (Methods).

To test whether declines in GEP above T_{opt} are due predominantly to indirect (H1) or direct (H2) temperature effects, we first quantified the response of light-saturated GEP to temperature and VPD in the experimental mesocosm, in which VPD and temperature were partially decoupled (B2-TF) (Fig. 2). Guided by the results from the experiment in the B2-TF, we analysed the in situ sites, in which temperature and VPD are highly correlated (K34, K67, K83 and Tesopaco). At all sites, we examined the independent effects of temperature and VPD on GEP by performing separate regressions on GEP-by-VPD and GEP-by-temperature, binning by temperature and VPD, respectively (Methods).

Light-saturated GEP was maintained in the B2-TF to air temperatures at least 10 °C higher than the threshold for natural tropical forests (Fig. 3a and Supplementary Figs. 1 and 2). Whereas GEP distinctly declined above 27 °C at the Amazon sites (K34, K67 and K83) and 28 °C at the seasonally dry tropical forest (Tesopaco), GEP showed little response in the B2-TF until air temperatures exceeded 38 °C. In contrast to the GEP–temperature relationship,

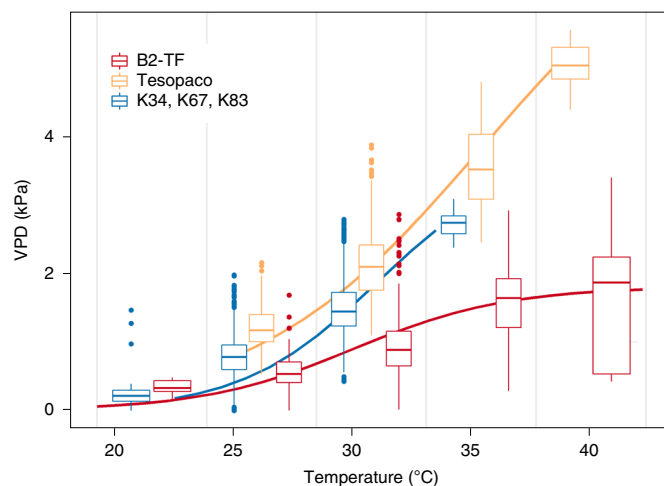


Fig. 2 | Relative to in situ tropical forests, VPD is reduced at high temperatures in the B2-TF by maintaining high humidity. Relationship between VPD and air temperature for the B2-TF mesocosm, a seasonally dry tropical forest (Tesopaco), and Amazon forest sites (K34, K67 and K83). The box plots represent median values (horizontal lines at box midpoints) and the first and third quartiles (upper and lower limits of box plots), while whiskers show the data that lies within the 1.5 interquartile range of the lower and upper quartiles, and data points at the ends of the whiskers represent outliers. The grey vertical lines indicate the edges of five temperature bins, evenly distributed across the full range of the dataset (bin widths, 4.63 °C). The lines show logistic growth equation fits for each site.

the response of GEP to VPD in the B2-TF was nearly identical to those in the natural forest sites (Fig. 3b).

Using the B2-TF mesocosm, we were able to expose a tropical forest system to a lower VPD for a given temperature than is experienced in in situ sites (Fig. 2). This experimental manipulation resulted in a reduced stomatal response, as evidenced by the observed sustained GEP at high temperatures. In contrast, the results from the in situ forests suggest that the steeper relationship between temperature and VPD induced more rapid stomatal closure with increasing temperatures. These results support the hypothesis (H1) that VPD, rather than temperature per se, is the main driver of high-temperature declines in photosynthesis.

We tested the consistency of support for H1 (indirect temperature effect) by partially isolating the effect of each variable (VPD and temperature) on GEP at high temperatures (≥ 28 °C) with reciprocal binned regressions—regressing GEP on VPD within bins of temperature (1 °C bins) and regressing GEP on temperature within bins of VPD (0.2 kPa bins) (Supplementary Fig. 3). At the B2-TF and all in situ sites, the mean response of GEP to increasing VPD across temperature bins was negative (Fig. 4). The slopes were statistically distinguishable from zero (two-tailed t -test, $P < 0.05$) for all datasets except K83. Across VPD bins, the mean response of GEP to increasing temperature was either nonsignificant or positive (Tesopaco and B2-TF, two-tailed t -test, $P < 0.05$). Taken together, these data from in situ patterns of CO_2 flux suggest that, as in the B2-TF, VPD is the major control on GEP at high temperatures in tropical forests (H1).

Our observations of GEP responses to distinct VPD–temperature regimes at the experimental mesocosm and in situ sites consistently indicate that the contribution of H1 (stomatal sensitivity to VPD) to GEP reductions above T_{opt} is larger than that of H2 (direct thermal restrictions on biochemistry), and that this trend persists for canopy air temperatures well above those observed in the Amazon today, extending into the range of future predictions for tropical forests¹⁰ (Fig. 1). Although negative (direct) effects of temperature on photosynthesis undoubtedly occur in concert with indirect (VPD) effects

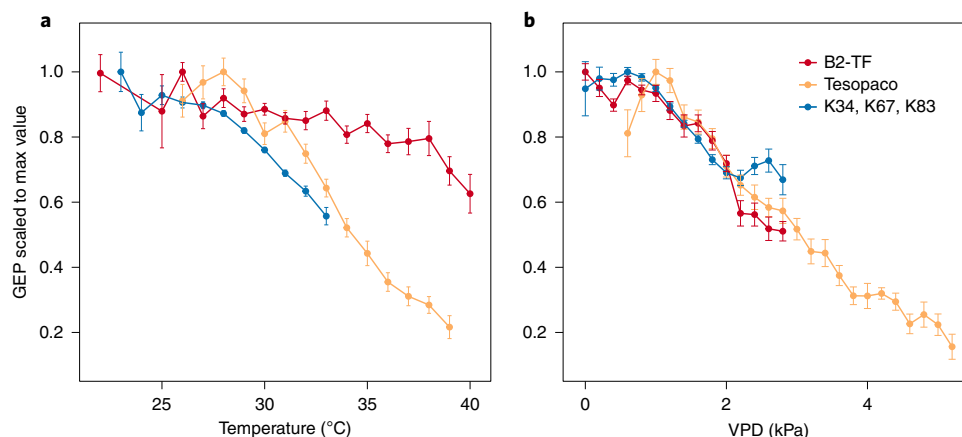


Fig. 3 | GEP is sustained to higher temperatures in the B2-TF than in situ tropical forests, whereas all forests respond similarly to VPD. a, b, The response of light-saturated GEP to air temperature (**a**) and VPD (**b**) for the B2-TF mesocosm, a seasonally dry tropical forest (Tesopaco) and Amazon forest sites (K34, K67 and K83). In **a**, the points show the average GEP for each 1°C temperature bin. In **b**, the points show the average GEP for each 0.2 kPa VPD bin. All points are scaled to the maximum GEP value for each site. The error bars indicate standard errors.

at temperatures above T_{opt} (Fig. 4), alleviating VPD stress in the B2-TF enabled GEP to continue up to air temperatures approaching lethal limits for photosynthesis ($\sim 40^{\circ}\text{C}$)²⁰. Extending these findings, if the hypothesized increase in WUE under elevated atmospheric $[\text{CO}_2]$ compensates for stomatal sensitivity to VPD, tropical trees may be capable of maintaining high rates of photosynthesis at temperatures above those that currently occur in this biome.

Given that upper-canopy leaf temperatures can exceed air temperatures by a few degrees^{4,5}, our results are consistent with leaf-level studies. Specifically, model studies represent declines in tropical forest photosynthesis above leaf temperatures of 30°C as predominantly due to indirect temperature effects through VPD⁹, and empirical studies show that direct, irreversible effects of temperature that damage the photosynthetic machinery tend to occur at leaf temperatures of $40\text{--}50^{\circ}\text{C}$ (ref. ²⁰) (Supplementary Fig. 4 and Supplementary Note 1).

Our results go beyond previous ecosystem-scale studies^{24,29,30} that have examined this question, because we were able to experimentally investigate temperatures in the B2-TF that are not expected to be experienced by in situ tropical rainforests until late this century. Little work has been done at any scale that experimentally decouples temperature and VPD while also assessing their impacts on photosynthesis at temperatures above the apparent thermal optimum. Ecosystem-scale studies^{29,30} have used approaches similar to our binned regressions to differentiate the effects of temperature and VPD on GEP. But it is not possible to manipulate VPD at this scale except in an experimental mesocosm such as Biosphere 2 (ref. ²⁴ and Methods). At the leaf level, studies at both high temperature and low VPD are reported to be rare due to the methodological challenge of maintaining low VPD when temperatures in an enclosure are high²⁷. Combining natural observations with experimental manipulations is a powerful and underused approach to understanding tropical forest responses to future climates³³. The B2-TF enables this approach in a uniquely large-scale, complex tropical forest system.

The environmental conditions in the experimental mesocosm (B2-TF) differ from the Amazonian sites in some key respects, in particular higher $[\text{CO}_2]$ and lower soil water content (Methods). However, our results are unlikely to be sensitive to these two variables. Moderately elevated $[\text{CO}_2]$ (25–38 ppm above the Amazonian sites) may have enhanced the photosynthetic capacity of the B2-TF, but studies have shown only small effects of elevated $[\text{CO}_2]$ on thermal tolerance^{31,34} (Methods), and lower soil moisture would be expected to increase temperature sensitivity, not reduce it.

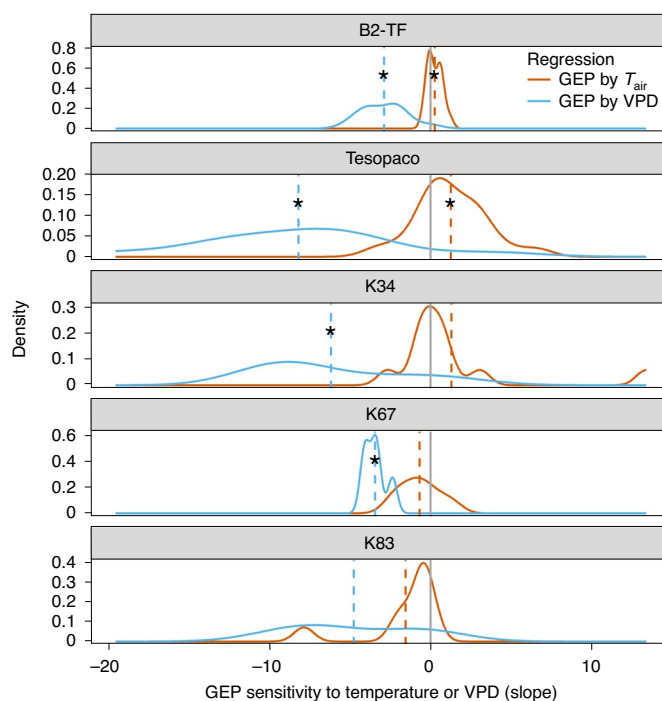


Fig. 4 | VPD has a negative effect on tropical forest GEP, while air temperature has a positive effect or no effect. Distributions of the sensitivity of GEP to air temperature ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ }^{\circ}\text{C}^{-1}$) and VPD ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ kPa}^{-1}$) derived from separate regressions between GEP and temperature, binning by VPD, and between GEP and VPD, binning by temperature. Data $\geq 28^{\circ}\text{C}$ have been selected for each site to examine the driving factor of high-temperature declines in GEP. The dashed lines show the mean slope value for each type of regression. The asterisks indicate mean slopes that are significantly different from zero ($P < 0.05$, two-tailed Student's t -tests).

The thermal tolerance of tropical forests may also vary in time via thermal acclimation and community assembly change. Photosynthetic acclimation to warming can result in an increase in T_{opt} (ref. ¹¹) or in the temperatures that are lethal for leaf function²⁰

(Supplementary Fig. 4). However, the evidence for acclimation in tropical species is mixed^{13,35}. Acclimation may involve a trade-off that reduces maximum assimilation rates³¹, which, if not balanced by acclimation of respiration¹¹, provides a mechanism for long-term reductions in carbon uptake that are not reflected by short-term temperature response curves¹⁴. In the B2-TF, differential species mortality during two decades of forest maturation led to an increase in the proportion of trees that emit isoprene³⁶, a trait shown to differentiate the photosynthetic thermal tolerances of tropical plant species³⁷. Understanding the future function of diverse tropical forests requires understanding not only general physiological limitations but also the extent of physiological plasticity and variation among species.

The representation of photosynthetic sensitivities to VPD and temperature for tropical trees varies among Earth system models⁷, and accordingly, so does the relative importance of indirect versus direct temperature effects^{1,38}. Our results suggest that models showing strong direct effects under current climate conditions should adjust parameters that impose direct thermal restrictions on photosynthetic biochemistry at high temperatures, and improve the representation of stomatal conductance responses to VPD, especially given potential interactions with changing atmospheric $[\text{CO}_2]$. Future elevated $[\text{CO}_2]$ may increase WUE, though empirical support for this possibility is mixed from eddy-covariance data^{22,39} and free-air carbon dioxide enrichment experiments^{11,21,40}. Higher WUE could reduce transpiration rates, resulting in further increases in leaf temperatures, reduced atmospheric humidity and consequently increased leaf-level VPD. Understanding these integrated stomatal responses and feedbacks to climate is a high research priority^{7,11}.

The analysis we present here examines the empirical response of tropical forest photosynthesis to VPD and temperature at higher temperatures than are currently found in Amazonian forests. We provide compelling evidence that stomatal response to VPD is the primary mechanism for high-temperature photosynthetic declines in tropical forests under current climate, and will likely continue to predominate over direct biochemical responses to temperature until at least several degrees of climate warming have been reached. This helps resolve an outstanding debate concerning the mechanism by which temperature limits photosynthesis, and provides data to test and improve model predictions of tropical forest responses to climate change. Although the actual response to future high temperatures will depend critically on the degree to which VPD rises⁷ and on leaf responses to VPD in the presence of elevated atmospheric $[\text{CO}_2]$, our findings suggest that tropical forest photosynthesis does not currently operate close to a high temperature threshold and may be resilient to future warming.

Methods

Study sites. Biosphere 2 is a large-scale Earth science facility near Tucson (Arizona, USA) comprising five biomes, of which the B2-TF is one. The B2-TF has a complex vertical canopy structure including mature trees up to 13–17 m in height⁴², comprising a phylogenetically diverse assemblage of species typical of lowland tropical rainforests in Southern and Central America⁴¹. The B2-TF provides a controlled environment that can be sealed off from the outside world, allowing researchers to measure forest responses to specific environmental variables^{42–44}. Climate conditions are maintained to be broadly similar to those at Amazonian forest sites³²; however, the B2-TF receives less rainfall (1,300 mm per year), the mean temperature is higher (Fig. 1), there is a stronger vertical temperature gradient (generated by heat trapped beneath the glass enclosure and the shaded understory) and the VPD for a given temperature is lower (Fig. 2). At the time of data collection, the facility was run as a semiclosed system (closed in the daytime, open at night). In contrast to Amazonian sites, there is no rainfall seasonality, but there is strong seasonality of temperature and VPD, and extreme high temperatures are achieved during the five summer months (May–September³²). The dominant soil texture in the B2-TF is sandy clay loam⁴³, comprising 20–35% clay and >70% sand, which is similar to the soil properties measured at K83 (18–60% clay, 37–80% sand⁴⁵), as are the values of soil carbon and nitrogen (2% C and 0.1% N in the B2-TF, ref. ⁴¹; 2.1–2.8% C and 0.1–0.2% N at K83, ref. ⁴⁵). The soil volumetric water content (0.14–0.25 cm^3 water per cm^3 soil, ref. ⁴⁴) tends to be moderately lower than the values recorded at K67 (0.20–0.44 $\text{cm}^3 \text{cm}^{-3}$, ref. ⁴⁶) for the top 30 cm of

the soil. Hence, the increased plant thermal tolerance in the B2-TF is probably not attributable to reduced soil moisture stress.

The data from the Brazilian sites (K34, K67 and K83) are from Large-scale Biosphere–Atmosphere Experiment in Amazonia (LBA) eddy covariance towers, part of the Brazil flux network⁴⁷. K67 and K83 are located in the Tapajós National Forest (TNF), near Santarém, Pará. The TNF is a *terra firme* (upland) moist tropical forest, receiving an average rainfall of 1,993 mm per year and experiencing a five-month dry season between July and November⁴⁷. The K34 site, located in the Cuieiras reserve, near Manaus, Amazonas, is an old-growth *terra firme* tropical rainforest. This site receives ~2,400 mm of rainfall per year and has a three-month dry season from July until September^{48,49}. The tropical dry forest site (Tesopaco) in Sonora, Mexico, experiences a nine-month dry season from October until June when the majority of the species lose their leaves⁵⁰ (unlike the Brazilian sites, which are all evergreen forests); the annual rainfall is 712 mm (ref. ⁵¹).

The mean atmospheric $[\text{CO}_2]$ was moderately higher in the B2-TF than in the natural forest sites (406 ppm, compared with 368 ppm at K34 and 381 ppm at K83). The difference in $[\text{CO}_2]$ is sufficient to moderately enhance photosynthetic capacity in the B2-TF but is unlikely to cause substantial variation in the thermal sensitivity of photosynthesis. For example, varying $[\text{CO}_2]$ from 300 to 900 ppm increased the leaf-level T_{opt} of four tropical tree species by an average of only 2.2 °C (ref. ³¹), and varying $[\text{CO}_2]$ from 360 to 500–1,000 ppm for a variety of temperate-zone plant types on average led to a small increase in the lethal temperature (0.78 °C, ref. ³⁴).

Data selection and environmental drivers. Overlapping net ecosystem exchange (NEE), photosynthetically active radiation (PAR), temperature and VPD data were selected for the B2-TF from a non-gap-filled dataset compiled by Rosolem et al.⁵²; this comprised almost four months of data from 2000 and 2002. All complete years of overlapping NEE, PAR, temperature and VPD data were included for the three sites in the Brazilian Amazon (K34, K67 and K83). According to these criteria, three years of data were included for K34 (1999–2000 and 2003–2005), seven years for K67 (2002–2006 and 2008–2011) and three years for K83 (2000–2003). We excluded periods when the tropical deciduous forest site (Tesopaco) was dormant by using a leaf area index (LAI) threshold of >2.08 (the mean growing season LAI, with the growing season defined as periods when $\text{LAI} \geq 0.5$). As a result, we included data from 7 July to 20 September 2006 in the analyses presented.

Air temperature was measured at the height of the upper canopy (15 m) in the B2-TF⁴⁴ and above the canopy for the natural tropical forest sites. Similar to natural forests, the above-canopy and understory air temperatures are distinguished by the shading effects of the canopy; however, in the B2-TF the gradient is steeper³². In the B2-TF, the high glass ceiling and the upper canopy surface bound a volume of air that is much hotter than in natural forests, whereas the air temperature in the shaded understory is more similar to that of natural forests³². While the measured canopy air temperature in both the B2-TF and the natural sites represents the hottest part of the canopy, this is also the region that intercepts the most light and hence is probably the most important contributor to total forest photosynthesis.

The sensitivity of VPD to temperature in our B2-TF dataset is both lower and more variable (Fig. 2) than in the natural forests. The variable relationship between VPD and temperature in the B2-TF depends on variation in the input of water vapour (Supplementary Note 2 and Supplementary Fig. 5). During normal operating conditions, soil water was replenished twice weekly via night-time rainfall events. During the daytime, water vapour was added via misters, and the substantial evapotranspiration from the soil and multilayer canopy was trapped in the glass enclosure, causing sustained high humidity up to higher temperatures than is observed in natural systems open to diffusion to the sky. The highest VPD in our B2-TF dataset comes from periods during which rainfall was withheld for 4–6 weeks at a time. During these periods, the drying of surface soil (less in magnitude than a TNF dry season, Supplementary Note 2) and suspended use of misters contributed to reduced humidity, while tree water status was maintained due to little change in deeper soil water (>0.5 m, ref. ⁴⁴). The ability to markedly reduce VPD at high temperatures is a unique strength of large enclosures in the B2 facility, as previously demonstrated with an experimental cottonwood stand²⁴.

We considered the impact of environmental characteristics unique to B2—namely, low radiation levels (due to light interception of the space-frame) and wind speeds—on the leaf-to-air temperature differential in comparison to natural forests. We modelled leaf temperatures for the B2-TF and one Amazonian site (K34) at their site-specific air temperature T_{opt} values (38 and 28 °C, respectively) using the R package *tealeaves*⁵³ (Supplementary Note 3). Predicted leaf temperatures were higher than measured air temperatures at both sites, but the mean leaf-to-air temperature differential was lower in the B2-TF (0.51 °C) than in the natural forest site (K34, 2.41 °C; Supplementary Fig. 6), predominantly due to reduced radiation in the B2-TF. As a result, the predicted mean leaf T_{opt} increased (relative to the air temperature T_{opt} values) to 38.51 °C for the B2-TF and 30.41 °C for K34. Converting the T_{opt} values derived from air temperatures to leaf temperatures reduces the difference between B2-TF and K34 optimum temperatures, but only by 1.9 °C (from 10 to 8.1 °C), giving us confidence in our overall conclusion that the B2-TF is considerably more temperature tolerant than natural forest sites.

Flux calculations. NEE in the B2-TF is calculated from the rate of change of CO_2 inside the biome:

$$NEE = \frac{d[CO_2]_a}{dt} M_a + F_{leak} + F_{conc} \quad (1)$$

where $\frac{d[CO_2]_a}{dt}$ is the rate of change in $[CO_2]$ in the air inside the mesocosm, M_a is the number of moles of air within the mesocosm per unit ground area (m^2), F_{leak} is the CO_2 flux between the B2-TF and the neighbouring mesocosms due to air leakage through the partition curtains, and F_{conc} is the rate of CO_2 uptake by the concrete structure due to a carbonation reaction between CO_2 and calcium oxide⁴².

NEE for natural forest sites was calculated from CO_2 fluxes according to methods detailed in ref. ⁴⁷ (for K34 and K83), ref. ⁵⁴ (for K67) and ref. ⁵⁰ (for Tesopaco). At all natural sites, we filtered periods of low turbulent mixing known to produce erroneously low NEE values using site-specific friction velocity threshold values of 0.20, 0.22, 0.24 and 0.15 m s^{-1} for K34, K67, K83 and Tesopaco, respectively. We calculated gross ecosystem exchange (GEE) from hourly (or for Tesopaco, half hourly) NEE measurements, where GEE is NEE minus ecosystem respiration (R_{eco}). Here, we present GEP, calculated as negative GEE. R_{eco} was assumed to equal night-time NEE values; as such, daily R_{eco} values for the B2-TF, Tesopaco and K67 were calculated as the mean of night-time NEE for each day. R_{eco} values for K67 were further gap-filled by a linear interpolation of 50 night-time NEE measurements⁵⁴. R_{eco} for K34 and K83 was calculated as the mean of night-time NEE within a 5- to 15-day window (30+ hourly values). The GEP data for K67, K34 and K83 were gap-filled on the basis of a relationship with PAR (ref. ⁴⁷).

While our data treatment accounts for seasonal variation in R_{eco} , we follow ref. ⁴⁷ and ref. ¹⁷ and estimate daytime R_{eco} as the mean of night-time NEE for each day or window of several days. We do not fit night-time NEE to a function of temperature, an approach that is commonly used at higher-latitude sites, because little to no dependence on temperature is observed at these tropical sites^{17,47} (see below). Low temperature variation in tropical sites leads to precipitation being the primary driver of variation in soil respiration (the dominant component of R_{eco} , ref. ⁵⁵). We tested this assumption in our datasets using linear regressions of night-time NEE on temperature in monthly binned data. All correlations were either non-significant or weak ($R^2 < 0.1$) with variable slope signs, except for one month at K67 ($R^2 = 0.57$) showing decreasing R_{eco} with temperature, and two months at K34 ($R^2 = 0.13$ and 0.29) and at the B2-TF ($R^2 = 0.21$ and 0.24) in each case showing decreasing and increasing R_{eco} with temperature, respectively. These weak and variable relationships are consistent with studies at the TNF sites during the dry season—the period of greatest diurnal temperature variation—which recorded diurnal variation in soil respiration up to $1\text{--}3\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ (refs. ^{56,57}). The diurnal range in soil temperatures is similarly small in the B2-TF⁴³. There is evidence that leaf respiration and its temperature sensitivity are suppressed in the light¹⁹, and they are regardless shown to have a small effect on light-saturated net photosynthesis in tropical species¹⁸. We therefore expect any biases in GEP estimates resulting from unaccountable daytime respiration to be small and unlikely to influence the relative positions of site thermal optima.

Light saturation curves were plotted between NEE and PAR for each site for all available observations to estimate the light value at which GEP saturates. These were as follows: 300 W m^{-2} (global incident radiation) for Tesopaco; $1,000\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ (PAR) for K34, K67 and K83; and 200 W m^{-2} (downward shortwave radiation) for the B2-TF. Mean values of light-saturated GEP were calculated for 1°C temperature bins and 0.2 kPa VPD bins. We scaled GEP to the maximum GEP value for each location to compare the response of canopy-level photosynthesis in the B2-TF with those of natural forest sites (Fig. 3), rather than the magnitude.

To simplify the figures in the main text, we combined the data for the three Amazon forests (K34, K67 and K83) because the sites all experience broadly similar environmental conditions and exhibit similar responses of GEP to temperature and VPD (Supplementary Fig. 2). Figures 1 and 2 present the raw data for all Amazon sites combined, and Fig. 3 presents the mean GEP of Amazon sites (that is, the mean of the values for K34, K67 and K83 shown separately in Supplementary Fig. 2) scaled to the maximum value.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The datasets analysed in this study (eddy flux and environmental data) are available at https://daac.ornl.gov/cgi-bin/dsviewer.pl?ds_id=1174 (for K34 and K83) and <https://ameriflux.lbl.gov/sites/siteinfo/BR-Sa1> (for K67). The datasets for Tesopaco and the B2-TF are available at <https://github.com/m-n-smith/B2-temp-paper-datasets>.

Code availability

The R code used to conduct the analyses presented in this paper is available upon request from the corresponding authors.

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Author contributions

M.N.S., T.C.T., S.R.S. and T.E.H. conceived the study, designed the analyses and led the data interpretation, with extensive help from J.v.H. and R.R. M.N.S. performed the data analysis and drafted the manuscript, with substantial input from T.C.T., S.R.S. and T.E.H. R.R. provided the carbon exchange data for the B2-TF and advice on its analysis. N.R.-C., R.C.d.O., R.d.S., A.C.d.A., P.B.d.C. and S.R.S. contributed to the installation, maintenance or analysis of the eddy flux data from the LBA tower sites. J.W. provided advice on the binned regression analysis. J.A. collected and analysed the leaf-level chlorophyll fluorescence measurements in the B2-TF. All authors contributed to writing the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41477-020-00780-2>.

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Study description	We test whether declines in gross ecosystem productivity (GEP) above the thermal optimum for photosynthesis (Topt) are predominantly due to indirect (H1) or direct (H2) temperature effects, using eddy flux data from four in situ tropical forest sites and a tropical forest mesocosm (Biosphere 2 Tropical Forest Biome).
Research sample	Flux measurements of CO ₂ from tropical forest eddy covariance sites in the Brazilian Amazon (K34, K67, and K83), Mexico (Tesopaco), and a tropical forest mesocosm (Biosphere 2 Tropical Forest Biome).
Sampling strategy	Net ecosystem exchange measurements were made hourly at K34, K67, K83, and B2, and half hourly at Tesopaco. This is a standard sampling procedure for eddy flux studies. All available flux data was included for each site, that provided overlapping net ecosystem exchange (NEE), photosynthetically active radiation (PAR), temperature, and vapor pressure deficit (VPD) data.
Data collection	NEE measurements in B2 were made according to Lin et al. 1998. NEE for natural forest sites was calculated from CO ₂ fluxes according to methods detailed in Restrepo-Coupe et al. 2013 (for K34 and K83), Hayek et al. 2018 (for K67), and Perez-Ruiz et al. 2010 (for Tesopaco). Further details are provided in the Methods section.
Timing and spatial scale	Overlapping NEE, PAR, temperature, and VPD data were selected for the B2-TF from a non-gap-filled dataset compiled by Rosolem et al. 2010; this comprised almost 4 months of data from 2000 and 2002. All complete years of overlapping NEE, PAR, temperature, and VPD data were included for the three sites in the Brazilian Amazon (K34, K67, and K83). According to this criteria, 3 years of data were included for K34 (1999-2000 and 2003-2005), 7 years for K67 (2002-2006 and 2008-2011), and 3 years for K83 (2000-2003). We excluded periods when the tropical deciduous forest site (Tesopaco) was dormant by using a leaf area index (LAI) threshold of >2.08 (mean growing season LAI, with the growing season defined as periods when LAI ≥ 0.5). As a result, we included data from 7 July to 20 September 2006 in the analyses presented. Measurements are at the ecosystem-scale, considered to be a ~1km radius around in situ flux tower sites, or for an area of 0.2 ha in the case of the B2-TF mesocosm.
Data exclusions	NEE data that did not coincide with measurements of PAR, temperature, and VPD were excluded, since we needed to have information on all these variables to test our hypotheses. In addition, NEE measurements collected during the dormant season at the tropical dry forest site (Tesopaco) were excluded using an LAI threshold of 2.08 (see response above and Methods for more details).
Reproducibility	We verify our findings on the high temperature controls on GEP from the B2 tropical forest mesocosm, where we experimentally manipulated the sensitivity of VPD to temperature against an analytical decoupling of temperature and VPD effects using in situ flux tower data. We find that the mechanisms driving high temperature declines in GEP in B2 are consistent with natural forests (predominantly controlled by indirect, or VPD effects).
Randomization	We do not have samples / individual organisms / participants, just in situ tropical forests and a tropical forest mesocosm. None of them were allocated into groups.
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