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1 **Title Page**

2 *Title:* Empirical evidence for resilience of tropical forest photosynthesis in a warmer
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32

33 **Introductory paragraph**

34 Tropical forests may be vulnerable to climate change¹⁻³ if photosynthetic carbon uptake
35 currently operates near a high temperature limit⁴⁻⁶. Predicting tropical forest function
36 requires understanding the relative contributions of two mechanisms of high-
37 temperature photosynthetic declines: stomatal limitation (H1), an indirect response due
38 to temperature-associated changes in atmospheric vapour pressure deficit (VPD)⁷, and
39 biochemical restrictions (H2), a direct temperature response^{8,9}. Their relative control
40 predicts different outcomes—H1 is expected to diminish with stomatal responses to
41 future co-occurring elevated atmospheric [CO₂], whereas H2 portends declining
42 photosynthesis with increasing temperatures. Distinguishing the two mechanisms at
43 high temperatures is therefore critical, but difficult because VPD is highly correlated
44 with temperature in natural settings. We used a forest mesocosm to quantify for the
45 first time the sensitivity of tropical gross ecosystem productivity (GEP) to future
46 temperature regimes while constraining VPD by controlling humidity. We then
47 analytically decoupled temperature and VPD effects under current climate with flux
48 tower-derived GEP trends *in situ* 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 2100¹⁰. If elevated [CO₂] mitigates VPD-induced stomatal limitation through enhanced water-use efficiency (WUE) as hypothesised^{9,11}, tropical forest photosynthesis may have a margin of resilience to future warming.

Main text

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 (i.e. the balance of gross photosynthetic carbon uptake and photorespiratory carbon emission) and/or an increase in ecosystem respiration. We focus here on GEP (net ecosystem carbon exchange minus ecosystem respiration, i.e. ecosystem photosynthesis), since the relative impact of respiration is likely smaller due to low temperature sensitivity of tropical ecosystem respiration over short timescales¹⁷ and the small contribution of leaf

74 respiration to daytime CO₂ exchange in tropical species^{9,18,19}.

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

91 A number of empirical studies at the leaf^{23–28} and ecosystem-scale^{24,29,30} suggest
92 that declines in photosynthesis at high temperatures are associated with rising VPD,
93 supporting H1. However, few of these studies experimentally decouple both
94 temperature and VPD, and only one—a study of a boreal spruce²³—does so above the
95 T_{opt} . Analytical differentiation of direct and indirect effects from temperature-
96 response³¹ and temperature×CO₂-response¹⁸ curves of photosynthesis in tropical plant
97 leaves showed evidence for stomatal limitations above T_{opt} in some species (H1), and
98 stronger biochemical limitation (H2) or co-limitation above T_{opt} in other species.

99 Considering the narrow thermal niches to which tropical trees are expected to be
100 adapted, a general paucity of data from tropical trees, and some empirical support for
101 H2 at the leaf level, there is a clear need for ecosystem scale experiments and
102 observations that help us distinguish mechanisms of high temperature photosynthetic
103 declines in tropical forests.

104 To address this question, we used an experimental tropical forest with
105 significant climate control—the Biosphere 2 Tropical Forest Biome (B2-TF; Arizona,
106 USA). We compared the response of light-saturated GEP to air temperature and VPD in
107 the B2-TF to that of three evergreen forest sites in the Brazilian Amazon (K34, K67, and
108 K83) and of a tropical dry forest in Mexico (Tesopaco) (Methods). The B2-TF is a 0.2 ha
109 enclosed mesocosm with a complex vertical canopy structure including mature trees up
110 to 13-17 m³². The B2-TF allows assessment of the temperature sensitivity of tropical
111 forest photosynthesis within the range of mean annual temperatures projected for
112 Amazonia by 2100 (1-7°C above present-day means¹⁰; Fig. 1) and up to 40°C,
113 approximately 6°C higher than maximum temperatures recorded at the Amazonian
114 sites. Additionally, the sensitivity of VPD to temperature can be experimentally
115 manipulated by controlling humidity, achieving a greater independence of the
116 environmental factors that control photosynthesis than can be observed in natural
117 forests (see Methods).

118 To test whether declines in GEP above T_{opt} are predominantly due to indirect
119 (H1) or direct (H2) temperature effects, we first quantified the response of light-
120 saturated GEP to temperature and VPD in the experimental mesocosm in which VPD
121 and temperature were partially decoupled (B2-TF) (Fig. 2). Guided by the results from
122 the experiment in B2, we analysed the *in situ* sites in which temperature and VPD are
123 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; 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, the response of GEP to VPD in the B2-TF was nearly identical to the natural forest sites (Fig. 3b).

Using the B2-TF mesocosm, we were able to expose a tropical forest system to 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, 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 ($\geq 28^\circ\text{C}$) with reciprocal binned regressions—regressing GEP on VPD within bins of temperature (1°C bins) and vice versa (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). 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 CO₂ flux suggest that, in common with 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 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 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 (~40°C)²⁰. Extending these findings, if the hypothesised increase in WUE under elevated atmospheric [CO₂] 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 40-50°C²⁰; Supplementary Fig. 4; 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 B2 (ref. 24; 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 underutilised 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 [CO₂] and lower soil water content (Methods). However, our results are unlikely to be sensitive to these two variables. Moderately elevated [CO₂] (25-38 ppm above the Amazonian sites) may have enhanced photosynthetic capacity of the B2-TF, but studies have shown only small effects of elevated [CO₂] on thermal tolerance^{31,34}; see Methods), and lower soil moisture would be expected to increase temperature sensitivity, not reduce it.

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} ¹¹ or in the temperatures that are lethal for leaf function²⁰ (Supplementary Fig. 4). However, evidence for acclimation in tropical species

is mixed^{13,35}. Acclimation may involve a tradeoff 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 tolerance of tropical plant species³⁷. Understanding future function of diverse tropical forests requires not only understanding 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 representation of stomatal conductance responses to VPD, especially given potential interactions with changing atmospheric [CO₂]. Future elevated [CO₂] may increase WUE, though empirical support is mixed from eddy-covariance data^{22,39} and Free-Air Carbon dioxide Enrichment (FACE) 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 is the first to examine 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 to 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 [CO₂], 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. B2 is a large-scale Earth science facility near Tucson (Arizona, USA), comprising five biomes, of which the B2 Tropical Forest Biome (B2-TF) is one. The B2-TF has a complex vertical canopy structure including mature trees up to 13-17 m in height³², 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 variables⁴²⁻⁴⁴. Climate conditions are maintained to be broadly similar to Amazonian forest sites³², however, the B2-TF receives less rainfall (1300 mm per year), 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 vapour pressure deficit (VPD) for a given temperature is lower (Fig. 2). At the time of data collection, the facility was run as a

semi-closed system (closed in the daytime, open at night). In contrast to Amazonian sites, there is no rainfall seasonality, but 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 soil properties measured at K83 (18-60% clay, 37-80% sand⁴⁵), as are values of soil carbon and nitrogen (2% C and 0.1% N in the B2-TF⁴¹; 2.1-2.8% C and 0.1-0.2% N at K83⁴⁵. Soil volumetric water content (0.14-0.25 cm³ water cm⁻³ soil, ref. 44) tends to be moderately lower than values recorded at K67 (0.20-0.44 cm³ cm⁻³, ref. 46) for the top 30 cm of the soil. Hence, increased plant thermal tolerance in the B2-TF is unlikely attributable to reduced soil moisture stress.

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 within the Tapajós National Forest (TNF), near Santarém, Pará. The TNF is a *terra firme* (upland) moist tropical forest, receiving an average rainfall of 1993 mm per year and experiencing a 5-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 ~2400 mm rainfall per year and has a 3-month dry season from July until September^{48,49}. The tropical dry forest site (Tesopaco) in Sonora, Mexico experiences a 9-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); annual rainfall is 712 mm⁵¹.

The mean atmospheric CO₂ concentration was moderately higher in the B2-TF than in the natural forest sites (406 ppm, compared to 368 ppm at K34 and 381 ppm at K83). The difference in [CO₂] is sufficient to moderately enhance photosynthetic

capacity in the B2-TF, but is unlikely to cause significant variation in the thermal sensitivity of photosynthesis. For example, varying $[CO_2]$ from 300 to 900 ppm increased leaf-level T_{opt} of four tropical tree species by an average of only $2.2^{\circ}C^{31}$, and varying $[CO_2]$ from 360 to 500-1000 ppm for a variety of temperate zone plant types on average led to a small increase in the lethal temperature ($0.78^{\circ}C^{34}$).

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 ref. 52; 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 $LAI \geq 0.5$). 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 B2, the high glass ceiling and the upper canopy surface bound a volume of air that is much hotter than in natural forests, while air temperature in the shaded understory is more similar to that of natural forests³². While measured canopy air temperature both in 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 likely 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; Supplementary Fig. 5). During normal operating conditions, soil water was replenished twice weekly via nighttime rainfall events. During the daytime, water vapour was added via misters, and the significant evapotranspiration from the soil and multi-layer 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 significantly 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 B2. 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 forest is considerably more temperature tolerant than natural forest sites.

Flux calculations. NEE in the B2-TF is calculated from the rate of change of CO₂ inside the biome:

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

where $d[CO_2]_a/dt$ is the rate of change in CO₂ concentration in the air inside the mesocosm, M_a is the number of moles of air within the mesocosm per unit ground area (m²), F_{leak} is the CO₂ flux between the B2-TF and the neighbouring mesocosms due to air leakage through the partition curtains, and F_{conc} is the rate of CO₂ uptake by the concrete structure due to a carbonation reaction between CO₂ and calcium oxide⁴².

NEE for natural forest sites was calculated from CO₂ 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 (u^*) 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 gross ecosystem productivity (GEP), calculated as negative GEE. R_{eco} was assumed to

equal night-time NEE values; as such, daily R_{eco} values for 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). GEP data for K67, K34, and K83 were gap-filled based on a relationship with PAR⁴⁷.

While our data treatment accounts for seasonal variation in R_{eco} , we follow refs 47 and 17 and estimate daytime R_{eco} as the mean of nighttime NEE for each day or window of several days. We do not fit nighttime 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} ⁵⁵). We tested this assumption in our datasets using linear regressions of nighttime 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 $1\text{--}3\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ ^{56,57} and 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 is 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 optimums.

Light saturation curves were plotted between NEE and PAR for each site for all available observations in order to estimate the light value at which GEP saturates. These were as follows: 300 W m⁻² (global incident radiation) for Tesopaco, 1000 μmol m⁻² 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 values 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 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). Figs 1 and 2 present the raw data for all Amazon sites combined, and Fig. 3 presents the mean GEP of Amazon sites (i.e. the mean of values for K34, K67, and K83 shown in Supplementary Fig. 2), that have subsequently been scaled to the maximum value.

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). 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.

References

1. Galbraith, D. *et al.* Multiple mechanisms of Amazonian forest biomass losses in three dynamic global vegetation models under climate change. *New Phytol.* **187**, 647–665 (2010).
2. Brien, R. J. W. *et al.* Long-term decline of the Amazon carbon sink. *Nature* **519**, 344–348 (2015).
3. Longo, M. *et al.* Ecosystem heterogeneity and diversity mitigate Amazon forest resilience to frequent extreme droughts. *New Phytol.* **219**, 914–931 (2018).
4. Doughty, C. E. & Goulden, M. L. Are tropical forests near a high temperature threshold? *Journal of Geophysical Research: Biogeosciences* vol. 113 (2008).
5. Mau, A., Reed, S., Wood, T. & Cavaleri, M. Temperate and tropical forest canopies are already functioning beyond their thermal thresholds for photosynthesis. *Forests* vol. 9 47 (2018).
6. Huang, M. *et al.* Air temperature optima of vegetation productivity across global biomes. *Nat Ecol Evol* **3**, 772–779 (2019).
7. Grossiord, C. *et al.* Plant responses to rising vapor pressure deficit. *New Phytol.* **226**, 1550–1566 (2020).
8. Sharkey, T. D. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant Cell Environ.* **28**, 269–277 (2005).
9. Lloyd, J. & Farquhar, G. D. Effects of rising temperatures and [CO₂] on the physiology of tropical forest trees. *Philosophical Transactions of the Royal Society B: Biological Sciences* vol. 363 1811–1817 (2008).
10. IPCC. in *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to*

- the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (ed. Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley) (Cambridge University Press, 2013).
11. Dusenke, M. E., Duarte, A. G. & Way, D. A. Plant carbon metabolism and climate change: elevated CO₂ and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytol.* **221**, 32–49 (2019).
 12. Janzen, D. H. Why mountain passes are higher in the tropics. *Am. Nat.* **101**, 233–249 (1967).
 13. Cunningham, S. C. & Read, J. Do temperate rainforest trees have a greater ability to acclimate to changing temperatures than tropical rainforest trees? *New Phytol.* **157**, 55–64 (2003).
 14. Sullivan, M. J. P. *et al.* Long-term thermal sensitivity of Earth's tropical forests. *Science* **368**, 869–874 (2020).
 15. Lancaster, L. T. & Humphreys, A. M. Global variation in the thermal tolerances of plants. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 13580–13587 (2020).
 16. Mora, C. *et al.* The projected timing of climate departure from recent variability. *Nature* **502**, 183–187 (2013).
 17. Hutrya, L. R. *et al.* Seasonal controls on the exchange of carbon and water in an Amazonian rain forest. *Journal of Geophysical Research: Biogeosciences* vol. 112 (2007).
 18. Slot, M. & Winter, K. In situ temperature relationships of biochemical and stomatal controls of photosynthesis in four lowland tropical tree species. *Plant Cell Environ.* **40**, 3055–3068 (2017).
 19. Tcherkez, G. *et al.* Leaf day respiration: low CO₂ flux but high significance for metabolism and carbon balance. *New Phytol.* **216**, 986–1001 (2017).
 20. O'sullivan, O. S. *et al.* Thermal limits of leaf metabolism across biomes. *Glob. Chang. Biol.* **23**, 209–223 (2017).
 21. Leakey, A. D. B. *et al.* Elevated CO₂ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J. Exp. Bot.* **60**, 2859–2876 (2009).

- 451 22. Keenan, T. F. *et al.* Increase in forest water-use efficiency as atmospheric carbon dioxide
452 concentrations rise. *Nature* **499**, 324–327 (2013).
- 453 23. Fredeen, A. L. & Sage, R. F. Temperature and humidity effects on branchlet gas-exchange in
454 white spruce: an explanation for the increase in transpiration with branchlet temperature.
455 *Trees* **14**, 161–168 (1999).
- 456 24. Barron-Gafford, G. A., Grieve, K. A. & Murthy, R. Leaf- and stand-level responses of a
457 forested mesocosm to independent manipulations of temperature and vapor pressure
458 deficit. *New Phytol.* **174**, 614–625 (2007).
- 459 25. Vargas G, G. & Cordero S, R. A. Photosynthetic responses to temperature of two tropical
460 rainforest tree species from Costa Rica. *Trees* **27**, 1261–1270 (2013).
- 461 26. Slot, M., Garcia, M. N. & Winter, K. Temperature response of CO₂ exchange in three tropical
462 tree species. *Funct. Plant Biol.* **43**, 468–478 (2016).
- 463 27. Slot, M. & Winter, K. The effects of rising temperature on the ecophysiology of tropical
464 forest trees. in *Tropical Tree Physiology: Adaptations and Responses in a Changing*
465 *Environment* (eds. Goldstein, G. & Santiago, L. S.) 385–412 (Springer International
466 Publishing, 2016).
- 467 28. Santos, V. A. H. F. dos *et al.* Causes of reduced leaf-level photosynthesis during strong El
468 Niño drought in a Central Amazon forest. *Glob. Chang. Biol.* **24**, 4266–4279 (2018).
- 469 29. Wu, J. *et al.* Partitioning controls on Amazon forest photosynthesis between environmental
470 and biotic factors at hourly to interannual timescales. *Glob. Chang. Biol.* **23**, 1240–1257
471 (2017).
- 472 30. Tan, Z.-H. *et al.* Optimum air temperature for tropical forest photosynthesis: mechanisms
473 involved and implications for climate warming. *Environmental Research Letters* **12**, 054022
474 (2017).
- 475 31. Slot, M. & Winter, K. In situ temperature response of photosynthesis of 42 tree and liana
476 species in the canopy of two Panamanian lowland tropical forests with contrasting rainfall
477 regimes. *New Phytol.* **214**, 1103–1117 (2017).

- 478 32. Arain, M. A., Shuttleworth, W. J., Farnsworth, B., Adams, J. & Sen, O. L. Comparing
479 micrometeorology of rain forests in Biosphere-2 and Amazon basin. *Agric. For. Meteorol.*
480 **100**, 273–289 (2000).
- 481 33. Cavaleri, M. A., Reed, S. C., Smith, W. K. & Wood, T. E. Urgent need for warming experiments
482 in tropical forests. *Glob. Chang. Biol.* **21**, 2111–2121 (2015).
- 483 34. Taub, D. R., Seemann, J. R. & Coleman, J. S. Growth in elevated CO₂ protects photosynthesis
484 against high-temperature damage. *Plant Cell Environ.* **23**, 649–656 (2000).
- 485 35. Doughty, C. E. An in situ leaf and branch warming experiment in the Amazon. *Biotropica* **43**,
486 658–665 (2011).
- 487 36. Taylor, T. C. *et al.* Isoprene emission structures tropical tree biogeography and community
488 assembly responses to climate. *New Phytol.* **220**, 435–446 (2018).
- 489 37. Taylor, T. C., Smith, M. N., Slot, M. & Feeley, K. J. The capacity to emit isoprene differentiates
490 the photosynthetic temperature responses of tropical plant species. *Plant, Cell Environ.* **42**,
491 2448–2457 (2019).
- 492 38. Rowland, L. *et al.* Modelling climate change responses in tropical forests: similar
493 productivity estimates across five models, but different mechanisms and responses.
494 *Geoscientific Model Development* **8**, 1097–1110 (2015).
- 495 39. Tan, Z.-H. *et al.* Interannual and seasonal variability of water use efficiency in a tropical
496 rainforest: Results from a 9 year eddy flux time series. *J. Geophys. Res. D: Atmos.* **120**, 464–
497 479 (2015).
- 498 40. Gray, S. B. *et al.* Intensifying drought eliminates the expected benefits of elevated carbon
499 dioxide for soybean. *Nature Plants* **2**, 1–8 (2016).
- 500 41. Leigh, L. S., Burgess, T., Marino, B. D. V. & Wei, Y. D. Tropical rainforest biome of Biosphere
501 2: Structure, composition and results of the first 2 years of operation. *Ecol. Eng.* **13**, 65–93
502 (1999).
- 503 42. Lin, G. *et al.* An experimental and modeling study of responses in ecosystems carbon
504 exchanges to increasing CO₂ concentrations using a tropical rainforest mesocosm. *Funct.*

- 505 *Plant Biol.* **25**, 547–556 (1998).
- 506 43. Lin, G. *et al.* Ecosystem carbon exchange in two terrestrial ecosystem mesocosms under
507 changing atmospheric CO₂ concentrations. *Oecologia* **119**, 97–108 (1999).
- 508 44. Rascher, U. *et al.* Functional diversity of photosynthesis during drought in a model tropical
509 rainforest - the contributions of leaf area, photosynthetic electron transport and stomatal
510 conductance to reduction in net ecosystem carbon exchange. *Plant, Cell Environ.* **27**, 1239–
511 1256 (2004).
- 512 45. Silver, W. L. *et al.* Effects of soil texture on belowground carbon and nutrient storage in a
513 lowland Amazonian forest ecosystem. *Ecosystems* **3**, 193–209 (2000).
- 514 46. Davidson, E. A., Nepstad, D. C., Ishida, F. Y. & Brando, P. M. Effects of an experimental
515 drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and
516 nitric oxide in a moist tropical forest. *Glob. Chang. Biol.* **10**, 1 (2008).
- 517 47. Restrepo-Coupe, N. *et al.* What drives the seasonality of photosynthesis across the Amazon
518 basin? A cross-site analysis of eddy flux tower measurements from the Brasil flux network.
519 *Agric. For. Meteorol.* **182–183**, 128–144 (2013).
- 520 48. Araújo, A. C. *et al.* Comparative measurements of carbon dioxide fluxes from two nearby
521 towers in a central Amazonian rainforest: The Manaus LBA site. *J. Geophys. Res.* **107**,
522 (2002).
- 523 49. Gonçalves, L. G. de *et al.* Overview of the Large-Scale Biosphere–Atmosphere Experiment in
524 Amazonia Data Model Intercomparison Project (LBA-DMIP). *Agric. For. Meteorol.* **182–183**,
525 111–127 (2013).
- 526 50. Perez-Ruiz, E. R. *et al.* Carbon dioxide and water vapour exchange in a tropical dry forest as
527 influenced by the North American Monsoon System (NAMS). *Journal of Arid Environments*
528 **74**, 556–563 (2010).
- 529 51. Álvarez-Yépiz, J. C., Martínez-Yrizar, A., Búrquez, A. & Lindquist, C. Variation in vegetation
530 structure and soil properties related to land use history of old-growth and secondary
531 tropical dry forests in northwestern Mexico. *Forest Ecology and Management* **256**, 355–366

(2008).

52. Rosolem, R., James Shuttleworth, W., Zeng, X., Saleska, S. R. & Huxman, T. E. Land surface modeling inside the Biosphere 2 tropical rain forest biome. *J. Geophys. Res.* **115**, G4 (2010).
53. Muir, C. D. tealeaves: an R package for modelling leaf temperature using energy budgets. *AoB Plants* **11**, plz054 (2019).
54. Hayek, M. N. *et al.* A novel correction for biases in forest eddy covariance carbon balance. *Agric. For. Meteorol.* **250**, 90–101 (2018).
55. Chambers, J. Q. *et al.* Respiration from a tropical forest ecosystem: partitioning of sources and low carbon use efficiency. *Ecological Applications* **14**, 72–88 (2004).
56. Saleska, S. R. *et al.* Carbon in Amazon forests: unexpected seasonal fluxes and disturbance-induced losses. *Science* **302**, 1554–1557 (2003).
57. Goulden, M. L. *et al.* Diel and seasonal patterns of tropical forest CO₂ exchange. *Ecological Applications* **14**, 42–54 (2004).

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Author contributions

M.N.S., T.C.T., S.R.S., 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 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 eddy flux data from LBA tower sites. J.W. provided advice on binned regression analysis. J.A. collected and analysed leaf-level chlorophyll fluorescence measurements in the B2-TF. All authors contributed towards writing the final manuscript.

Competing financial interests

The authors declare no competing financial interests.

Figure Legends

Figure 1. Air temperature distributions recorded at the B2-TF mesocosm (red), a seasonally dry tropical forest (Tesopaco, gold), and Amazon forest sites (K34, K67, and K83, blue). Dashed lines show the current mean temperatures at Amazon forest sites (28°C) and the B2-TF (32°C); grey area shows the range of mean annual temperatures projected for the Amazon region by 2100¹⁰. Only temperatures corresponding to light-saturated gross ecosystem productivity (GEP) have been included.

Figure 2. Relationship between vapour pressure deficit (VPD) and air temperature for the B2-TF mesocosm (red), a seasonally dry tropical forest (Tesopaco, gold), and Amazon forest sites (K34, K67, and K83, blue). Boxplots represent median values (horizontal lines at box midpoints), first and third quartiles (box bottom and top), while vertical lines extending from the boxplots (whiskers) show the data that lies within 1.5 interquartile range of the lower and upper quartiles, and data points at the end of the whiskers represent outliers. Vertical lines indicate the edges of five temperature bins, evenly distributed across the full range of the dataset (binwidths = 4.63°C). Lines show logistic growth equation fits for each site.

Figure 3. Light-saturated gross ecosystem productivity (GEP) versus (a) air temperature and (b) vapour pressure deficit (VPD) for the B2-TF mesocosm (red), a seasonally dry tropical forest (Tesopaco, gold), and Amazon forest sites (K34, K67, and K83, blue). a, Points show the average GEP for each 1°C temperature bin, scaled to the maximum GEP value for each forest site; b, points show the average light-saturated GEP for each 0.2 kPa VPD bin, scaled to the maximum GEP value for each site. Error bars are

607 standard errors.

608

609 **Figure 4.** Distributions of the sensitivity of gross ecosystem productivity (GEP) to air
610 temperature ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / ^\circ\text{C}$, red lines) and vapour pressure deficit (VPD, μmol
611 $\text{CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{kPa}$, blue lines) derived from separate regressions between GEP and
612 temperature, binning by VPD, and between GEP and VPD, binning by temperature. Data
613 $\geq 28^\circ\text{C}$ have been selected for each site to examine the driving factor of high
614 temperature declines in GEP. Dashed lines show the mean slope value for each type of
615 regression. Stars indicate mean slopes that are significantly different from zero ($p <$
616 0.05 , two-tailed Student's t -tests).

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