1 **Title Page**

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

3 world

4 *Authors list:* Marielle N. Smith[1,2*], Tyeen C. Taylor[1,3], Joost van Haren[4], Rafael Rosolem[5,6],

5 Natalia Restrepo-Coupe[1,7], John Adams[4], Jin Wu[8], Raimundo C. de Oliveira[9], Rodrigo da

6 Silva[10], Alessandro C. de Araujo[11,12], Plinio B. de Camargo[13], Travis E. Huxman[14], Scott R.

7 Saleska[1*]

8 *Author affiliations:*

9 [1]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ

10 85721, USA; [2]Department of Forestry, Michigan State University, East Lansing, MI

11 48824, USA; [3]Department of Civil and Environmental Engineering, University of

12 Michigan, Ann Arbor, MI 48109, USA; [4]Biosphere 2, University of Arizona, 32540 S.

13 Biosphere Road, Oracle, AZ 85623, USA; [5]Department of Civil Engineering, University of

14 Bristol, Bristol, BS8 1TR, UK; [6]Cabot Institute, University of Bristol, Bristol, BS8 1UJ, UK;

15 [7]School of Life Sciences, University of Technology Sydney, Sydney, NSW, Australia;

16 [8]School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong SAR,

17 China; [9]Embrapa Amazônia Oriental, 68035-110 Santarém, Pará, Brazil; [10]Department

18 of Environmental Physics, University of Western Pará (UFOPA), Santarém, Pará, Brazil;

19 [11]Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil;

20 [12]Embrapa Amazônia Oriental, Belém, Pará, Brazil; [13]Laboratório de Ecologia Isotópica,

21 Centro de Energia Nuclear na Agricultura (CENA), Universidade de São Paulo, 13400-

22 970 Piracicaba, São Paulo, Brazil; and [14]Ecology and Evolutionary Biology & Center for

23 Environmental Biology, University of California, Irvine, CA 92629, USA

24   *Corresponding authors:*

25   *Marielle N. Smith, Department of Forestry, Michigan State University, East Lansing, MI

26   48824, USA. Tel: (520) 314-6346. Email: mariellenatashasmith@gmail.com

27   *Scott R. Saleska, Department of Ecology and Evolutionary Biology, University of

28   Arizona, 1041 E Lowell Street, Biological Sciences West Room 310, Tucson, AZ 85721,

29   USA. Tel: (520) 626-1500. Email: saleska@email.arizona.edu

30   *Keywords:* tropical forests, Biosphere 2, climate change, photosynthesis, thermal

31   tolerance

32

33   **Introductory paragraph**

34   Tropical forests may be vulnerable to climate change[1–3] if photosynthetic carbon uptake

35   currently operates near a high temperature limit[4–6]. 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 [CO_2], 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

49   showed consistent, negative sensitivity of GEP to VPD, but little direct response to

50   temperature. Importantly, in the mesocosm at low VPD, GEP persisted up to 38°C, a

51   temperature exceeding projections for tropical forests in 2100[10]. If elevated [CO_2]

52   mitigates VPD-induced stomatal limitation through enhanced water-use efficiency

53   (WUE) as hypothesised[9,11], tropical forest photosynthesis may have a margin of

54   resilience to future warming.

55

56   **Main text**

57   Tropical plants may be vulnerable to even small amounts of climate warming, having

58   evolved in climates with low thermal variability[12,13]. This vulnerability is highlighted by

59   observations suggesting that tropical forests are already functioning near their high-

60   temperature limit[4–6,14,15], together with projections that tropical regions will likely

61   experience unprecedented high temperatures that will soon push forests above such

62   limits[16].

63   A critical trait determining forest vulnerability to climate change is the thermal

64   sensitivity of photosynthesis. However, there is considerable debate over how different

65   component mechanisms of photosynthetic carbon uptake are influenced by climate as

66   temperatures increase above the apparent thermal optimum (T_{opt}) in tropical forests[4,9].

67   The temperature response curve of net ecosystem carbon uptake shows a decline at

68   high temperatures that may be caused by a decrease in photosynthesis (i.e. the balance

69   of gross photosynthetic carbon uptake and photorespiratory carbon emission) and/or

70   an increase in ecosystem respiration. We focus here on GEP (net ecosystem carbon

71   exchange minus ecosystem respiration, i.e. ecosystem photosynthesis), since the

72   relative impact of respiration is likely smaller due to low temperature sensitivity of

73   tropical ecosystem respiration over short timescales[17] and the small contribution of leaf

3

74   respiration to daytime CO_2 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 CO_2 (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 oxidation[8],

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 [CO_2] 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 spruce[23]—does so above the

95   T_{opt}. Analytical differentiation of direct and indirect effects from temperature-

96   response[31] and temperature×CO_2-response[18] 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 m[32]. 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 means[10]; 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

124   independent effects of temperature and VPD on GEP by performing separate

125   regressions on GEP-by-VPD and GEP-by-temperature, binning by temperature and VPD,

126   respectively (Methods).

127   Light-saturated GEP was maintained in the B2-TF to air temperatures at least

128   10°C higher than the threshold for natural tropical forests (Fig. 3a; Supplementary Figs

129   1 and 2). Whereas GEP distinctly declined above 27°C at the Amazon sites (K34, K67,

130   and K83) and 28°C at the seasonally dry tropical forest (Tesopaco), GEP showed little

131   response in the B2-TF until air temperatures exceeded 38°C. In contrast to the GEP-

132   temperature relationship, the response of GEP to VPD in the B2-TF was nearly identical

133   to the natural forest sites (Fig. 3b).

134   Using the B2-TF mesocosm, we were able to expose a tropical forest system to

135   lower VPD for a given temperature than is experienced in *in situ* sites (Fig. 2). This

136   experimental manipulation resulted in a reduced stomatal response, as evidenced by

137   the observed sustained GEP at high temperatures. In contrast, results from the *in situ*

138   forests suggest that the steeper relationship between temperature and VPD induced

139   more rapid stomatal closure with increasing temperatures. These results support the

140   hypothesis (H1) that VPD, rather than temperature *per se*, is the main driver of high-

141   temperature declines in photosynthesis.

142   We tested the consistency of support for H1 (indirect temperature effect) by

143   partially isolating the effect of each variable (VPD and temperature) on GEP at high

144   temperatures (≥ 28°C) with reciprocal binned regressions—regressing GEP on VPD

145   within bins of temperature (1°C bins) and vice versa (0.2 kPa bins) (Supplementary Fig.

146   3). At the B2-TF and all *in situ* sites, the mean response of GEP to increasing VPD across

147   temperature bins was negative (Fig. 4). Slopes were statistically distinguishable from

148   zero (two-tailed *t*-test, $p < 0.05$) for all datasets except K83. Across VPD bins, the mean

149   response of GEP to increasing temperature was either nonsignificant or positive

150   (Tesopaco and B2-TF, two-tailed t-test, $p < 0.05$). Taken together, these data from *in situ*

151   patterns of CO_2 flux suggest that, in common with the B2-TF, VPD is the major control

152   on GEP at high temperatures in tropical forests (H1).

153   Our observations of GEP responses to distinct VPD-temperature regimes at the

154   experimental mesocosm and *in situ* sites consistently indicate that the contribution of

155   H1 (stomatal sensitivity to VPD) to GEP reductions above T_{opt} is larger than H2 (direct

156   thermal restrictions on biochemistry), and that this trend persists for canopy air

157   temperatures well above those observed in the Amazon today, extending into the range

158   of future predictions for tropical forests[10] (Fig. 1). Although negative (direct) effects of

159   temperature on photosynthesis undoubtedly occur in concert with indirect (VPD)

160   effects at temperatures above T_{opt} (Fig. 4), alleviating VPD stress in the B2-TF enabled

161   GEP to continue up to air temperatures approaching lethal limits for photosynthesis

162   ($\sim 40°C$)[20]. Extending these findings, if the hypothesised increase in WUE under elevated

163   atmospheric $[CO_2]$ compensates for stomatal sensitivity to VPD, tropical trees may be

164   capable of maintaining high rates of photosynthesis at temperatures above those that

165   currently occur in this biome.

166   Given that upper canopy leaf temperatures can exceed air temperatures by a few

167   degrees[4,5], our results are consistent with leaf-level studies. Specifically, model studies

168   represent declines in tropical forest photosynthesis above leaf temperatures of 30°C as

169   predominantly due to indirect temperature effects through VPD[9], and empirical studies

170   show that direct, irreversible effects of temperature that damage the photosynthetic

171   machinery tend to occur at leaf temperatures of 40-50°C[20]; Supplementary Fig. 4;

172   Supplementary Note 1).

173   Our results go beyond previous ecosystem-scale studies[24,29,30] that have

174   examined this question, because we were able to experimentally investigate

175   temperatures in the B2-TF that are not expected to be experienced by *in situ* tropical

176   rainforests until late this century. Little work has been done at any scale that

177   experimentally decouples temperature and VPD while also assessing their impacts on

178   photosynthesis at temperatures above the apparent thermal optimum. Ecosystem-scale

179   studies[29,30] have used approaches similar to our binned regressions to differentiate the

180   effects of temperature and VPD on GEP. But it is not possible to manipulate VPD at this

181   scale except in an experimental mesocosm such as B2 (ref. 24; Methods). At the leaf-

182   level, studies at both high temperature and low VPD are reported to be rare due to the

183   methodological challenge of maintaining low VPD when temperatures in an enclosure

184   are high[27]. Combining natural observations with experimental manipulations is a

185   powerful and underutilised approach to understanding tropical forest responses to

186   future climates[33]. The B2-TF enables this approach in a uniquely large-scale, complex

187   tropical forest system.

188   The environmental conditions in the experimental mesocosm (B2-TF) differ

189   from the Amazonian sites in some key respects, in particular higher [CO_2] and lower soil

190   water content (Methods). However, our results are unlikely to be sensitive to these two

191   variables. Moderately elevated [CO_2] (25-38 ppm above the Amazonian sites) may have

192   enhanced photosynthetic capacity of the B2-TF, but studies have shown only small

193   effects of elevated [CO_2] on thermal tolerance[31,34]; see Methods), and lower soil

194   moisture would be expected to increase temperature sensitivity, not reduce it.

195   Thermal tolerance of tropical forests may also vary in time via thermal

196   acclimation and community assembly change. Photosynthetic acclimation to warming

197   can result in an increase in T_{opt}[11] or in the temperatures that are lethal for leaf

198   function[20] (Supplementary Fig. 4). However, evidence for acclimation in tropical species

199   is mixed[13,35]. Acclimation may involve a tradeoff that reduces maximum assimilation

200   rates[31] which, if not balanced by acclimation of respiration[11], provides a mechanism for

201   long-term reductions in carbon uptake that are not reflected by short-term temperature

202   response curves[14]. In the B2-TF, differential species mortality during two decades of

203   forest maturation led to an increase in the proportion of trees that emit isoprene[36], a

204   trait shown to differentiate the photosynthetic thermal tolerance of tropical plant

205   species[37]. Understanding future function of diverse tropical forests requires not only

206   understanding general physiological limitations, but also the extent of physiological

207   plasticity and variation among species.

208   The representation of photosynthetic sensitivities to VPD and temperature for

209   tropical trees varies among Earth system models[7], and accordingly, so does the relative

210   importance of indirect versus direct temperature effects[1,38]. Our results suggest that

211   models showing strong direct effects under current climate conditions should adjust

212   parameters that impose direct thermal restrictions on photosynthetic biochemistry at

213   high temperatures, and improve representation of stomatal conductance responses to

214   VPD, especially given potential interactions with changing atmospheric $[CO_2]$. Future

215   elevated $[CO_2]$ may increase WUE, though empirical support is mixed from eddy-

216   covariance data[22,39] and Free-Air Carbon dioxide Enrichment (FACE) experiments[11,21,40].

217   Higher WUE could reduce transpiration rates, resulting in further increases in leaf

218   temperatures, reduced atmospheric humidity, and consequently increased leaf-level

219   VPD. Understanding these integrated stomatal responses and feedbacks to climate is a

220   high research priority[7,11].

221   The analysis we present here is the first to examine the empirical response of

222   tropical forest photosynthesis to VPD and temperature at higher temperatures than are

223   currently found in Amazonian forests. We provide compelling evidence that stomatal

224   response to VPD is the primary mechanism for high-temperature photosynthetic

225   declines in tropical forests under current climate, and will likely continue to

226   predominate over direct biochemical responses to temperature until at least several

227   degrees of climate warming have been reached. This helps to resolve an outstanding

228   debate concerning the mechanism by which temperature limits photosynthesis, and

229   provides data to test and improve model predictions of tropical forest responses to

230   climate change. Although the actual response to future high temperatures will depend

231   critically on the degree to which VPD rises[7], and on leaf responses to VPD in the

232   presence of elevated atmospheric [CO_2], our findings suggest that tropical forest

233   photosynthesis does not currently operate close to a high temperature threshold, and

234   may be resilient to future warming.

235

236   **Methods**

237   **Study sites.** B2 is a large-scale Earth science facility near Tucson (Arizona, USA),

238   comprising five biomes, of which the B2 Tropical Forest Biome (B2-TF) is one. The B2-

239   TF has a complex vertical canopy structure including mature trees up to 13-17 m in

240   height[32], comprising a phylogenetically diverse assemblage of species typical of lowland

241   tropical rainforests in Southern and Central America[41]. The B2-TF provides a controlled

242   environment that can be sealed off from the outside world, allowing researchers to

243   measure forest responses to specific environmental variables[42–44]. Climate conditions

244   are maintained to be broadly similar to Amazonian forest sites[32], however, the B2-TF

245   receives less rainfall (1300 mm per year), mean temperature is higher (Fig. 1), there is a

246   stronger vertical temperature gradient (generated by heat trapped beneath the glass

247   enclosure and the shaded understory), and vapour pressure deficit (VPD) for a given

248   temperature is lower (Fig. 2). At the time of data collection, the facility was run as a

249   semi-closed system (closed in the daytime, open at night). In contrast to Amazonian

250   sites, there is no rainfall seasonality, but strong seasonality of temperature and VPD,

251   and extreme high temperatures are achieved during the five summer months (May-

252   September[32]). The dominant soil texture in the B2-TF is sandy clay loam[43], comprising

253   20-35% clay and >70% sand, which is similar to soil properties measured at K83 (18-

254   60% clay, 37-80% sand[45]), as are values of soil carbon and nitrogen (2% C and 0.1% N

255   in the B2-TF[41]; 2.1-2.8% C and 0.1-0.2% N at K83[45]. Soil volumetric water content (0.14-

256   0.25 cm^3 water cm^{-3} soil, ref. 44) tends to be moderately lower than values recorded at

257   K67 (0.20-0.44 cm^3 cm^{-3}, ref. 46) for the top 30 cm of the soil. Hence, increased plant

258   thermal tolerance in the B2-TF is unlikely attributable to reduced soil moisture stress.

259   Data from the Brazilian sites (K34, K67, and K83) are from Large-scale

260   Biosphere-Atmosphere Experiment in Amazonia (LBA) eddy covariance towers, part of

261   the Brazil flux network[47]. K67 and K83 are located within the Tapajós National Forest

262   (TNF), near Santarém, Pará. The TNF is a *terra firme* (upland) moist tropical forest,

263   receiving an average rainfall of 1993 mm per year and experiencing a 5-month dry

264   season between July and November[47]. The K34 site, located in the Cuieiras reserve, near

265   Manaus, Amazonas, is an old-growth *terra firme* tropical rainforest. This site receives

266   ~2400 mm rainfall per year and has a 3-month dry season from July until

267   September[48,49]. The tropical dry forest site (Tesopaco) in Sonora, Mexico experiences a

268   9-month dry season from October until June when the majority of the species lose their

269   leaves[50] (unlike the Brazilian sites, which are all evergreen forests); annual rainfall is

270   712 mm[51].

271   The mean atmospheric CO_2 concentration was moderately higher in the B2-TF

272   than in the natural forest sites (406 ppm, compared to 368 ppm at K34 and 381 ppm at

273   K83). The difference in [CO_2] is sufficient to moderately enhance photosynthetic

274   capacity in the B2-TF, but is unlikely to cause significant variation in the thermal

275   sensitivity of photosynthesis. For example, varying [CO_2] from 300 to 900 ppm

276   increased leaf-level T_{opt} of four tropical tree species by an average of only 2.2℃[31], and

277   varying [CO_2] from 360 to 500-1000 ppm for a variety of temperate zone plant types on

278   average led to a small increase in the lethal temperature (0.78℃[34]).

279

280   **Data selection and environmental drivers.** Overlapping net ecosystem exchange

281   (NEE), photosynthetically active radiation (PAR), temperature, and VPD data were

282   selected for the B2-TF from a non-gap-filled dataset compiled by ref. 52; this comprised

283   almost 4 months of data from 2000 and 2002. All complete years of overlapping NEE,

284   PAR, temperature, and VPD data were included for the three sites in the Brazilian

285   Amazon (K34, K67, and K83). According to this criteria, 3 years of data were included

286   for K34 (1999-2000 and 2003-2005), 7 years for K67 (2002-2006 and 2008-2011), and

287   3 years for K83 (2000-2003). We excluded periods when the tropical deciduous forest

288   site (Tesopaco) was dormant by using a leaf area index (LAI) threshold of >2.08 (mean

289   growing season LAI, with the growing season defined as periods when LAI ≥ 0.5). As a

290   result, we included data from 7 July to 20 September 2006 in the analyses presented.

291   Air temperature was measured at the height of the upper canopy (15 m) in the

292   B2-TF[44] and above the canopy for the natural tropical forest sites. Similar to natural

293   forests, the above-canopy and understory air temperatures are distinguished by the

294   shading effects of the canopy, however in the B2-TF the gradient is steeper[32]. In B2, the

295   high glass ceiling and the upper canopy surface bound a volume of air that is much

296   hotter than in natural forests, while air temperature in the shaded understory is more

297   similar to that of natural forests[32]. While measured canopy air temperature both in B2-

298   TF and the natural sites represents the hottest part of the canopy, this is also the region

12

299   that intercepts the most light and hence is likely the most important contributor to total

300   forest photosynthesis.

301   The sensitivity of VPD to temperature in our B2-TF dataset is both lower and

302   more variable (Fig. 2) than in the natural forests. The variable relationship between

303   VPD and temperature in the B2-TF depends on variation in the input of water vapour

304   (Supplementary Note 2; Supplementary Fig. 5). During normal operating conditions, soil

305   water was replenished twice weekly via nighttime rainfall events. During the daytime,

306   water vapour was added via misters, and the significant evapotranspiration from the

307   soil and multi-layer canopy was trapped in the glass enclosure, causing sustained high

308   humidity up to higher temperatures than is observed in natural systems open to

309   diffusion to the sky. The highest VPD in our B2-TF dataset comes from periods during

310   which rainfall was withheld for 4-6 weeks at a time. During these periods, the drying of

311   surface soil (less in magnitude than a TNF dry season, Supplementary Note 2) and

312   suspended use of misters contributed to reduced humidity, while tree water status was

313   maintained due to little change in deeper soil water (> 0.5 m, ref. 44). The ability to

314   significantly reduce VPD at high temperatures is a unique strength of large enclosures in

315   the B2 facility, as previously demonstrated with an experimental cottonwood stand[24].

316   We considered the impact of environmental characteristics unique to B2—

317   namely, low radiation levels (due to light interception of the space-frame) and wind

318   speeds—on the leaf to air temperature differential in comparison to natural forests. We

319   modelled leaf temperatures for the B2-TF and one Amazonian site (K34) at their site-

320   specific air temperature T_{opt} values (38 and 28 °C, respectively) using the R package

321   'tealeaves'[53] (Supplementary Note 3). Predicted leaf temperatures were higher than

322   measured air temperatures at both sites, but the mean leaf to air temperature

323   differential was lower in the B2-TF (0.51°C) than in the natural forest site (K34, 2.41°C;

324   Supplementary Fig. 6), predominantly due to reduced radiation in B2. As a result, the

325   predicted mean leaf T_{opt} increased (relative to the air temperature T_{opt} values) to

326   38.51°C for the B2-TF and 30.41°C for K34. Converting the T_{opt} values derived from air

327   temperatures to leaf temperatures reduces the difference between B2-TF and K34

328   optimum temperatures, but only by 1.9°C (from 10 to 8.1°C), giving us confidence in our

329   over overall conclusion that the B2 forest is considerably more temperature tolerant

330   than natural forest sites.

331

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

333   the biome:

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

334

335   where d[CO_2]a/dt is the rate of change in CO_2 concentration in the air inside the

336   mesocosm, M_a is the number of moles of air within the mesocosm per unit ground area

337   (m^2), F_{leak} is the CO_2 flux between the B2-TF and the neighbouring mesocosms due to air

338   leakage through the partition curtains, and F_{conc} is the rate of CO_2 uptake by the concrete

339   structure due to a carbonation reaction between CO_2 and calcium oxide[42].

340   NEE for natural forest sites was calculated from CO_2 fluxes according to methods

341   detailed in ref. 47 (for K34 and K83), ref. 54 (for K67), and ref. 50 (for Tesopaco). At all

342   natural sites, we filtered periods of low turbulent mixing known to produce erroneously

343   low NEE values using site-specific friction velocity (u^*) threshold values of 0.20, 0.22,

344   0.24, and 0.15 m s^{-1} for K34, K67, K83, and Tesopaco, respectively. We calculated gross

345   ecosystem exchange (GEE) from hourly (or for Tesopaco, half hourly) NEE

346   measurements, where GEE is NEE minus ecosystem respiration (R_{eco}). Here, we present

347   gross ecosystem productivity (GEP), calculated as negative GEE. R_{eco} was assumed to

348   equal night-time NEE values; as such, daily R_{eco} values for B2-TF, Tesopaco, and K67

349   were calculated as the mean of night-time NEE for each day; R_{eco} values for K67 were

350   further gap-filled by a linear interpolation of 50 night-time NEE measurements[54]. R_{eco}

351   for K34 and K83 was calculated as the mean of night-time NEE within a 5 to 15-day

352   window (30+ hourly values). GEP data for K67, K34, and K83 were gap-filled based on a

353   relationship with PAR[47].

354   While our data treatment accounts for seasonal variation in R_{eco}, we follow refs

355   47 and 17 and estimate daytime R_{eco} as the mean of nighttime NEE for each day or

356   window of several days. We do not fit nighttime NEE to a function of temperature, an

357   approach that is commonly used at higher latitude sites because little to no dependence

358   on temperature is observed at these tropical sites[17,47] (see below). Low temperature

359   variation in tropical sites leads to precipitation being the primary driver of variation in

360   soil respiration (the dominant component of R_{eco}[55]). We tested this assumption in our

361   datasets using linear regressions of nighttime NEE on temperature in monthly binned

362   data. All correlations were either non-significant or weak ($R^2 < 0.1$) with variable slope

363   signs, except for one month at K67 ($R^2 = 0.57$) showing decreasing R_{eco} with

364   temperature, and two months at K34 ($R^2 = 0.13$ and 0.29) and at the B2-TF ($R^2 = 0.21$

365   and 0.24) in each case showing decreasing and increasing R_{eco} with temperature,

366   respectively. These weak and variable relationships are consistent with studies at the

367   TNF sites during the dry season—the period of greatest diurnal temperature variation—

368   which recorded diurnal variation in soil respiration up to 1-3 µmol m^{-2} s^{-1} [56,57] and the

369   diurnal range in soil temperatures is similarly small in the B2-TF[43]. There is evidence

370   that leaf respiration and its temperature sensitivity are suppressed in the light[19] and is

371   regardless shown to have a small effect on light-saturated net photosynthesis in tropical

372   species[18]. We therefore expect any biases in GEP estimates resulting from

373   unaccountable daytime respiration to be small and unlikely to influence the relative

374   positions of site thermal optimums.

375   Light saturation curves were plotted between NEE and PAR for each site for all

376   available observations in order to estimate the light value at which GEP saturates. These

377   were as follows: 300 W m^{-2} (global incident radiation) for Tesopaco, 1000 μmol m^{-2} s^{-1}

378   (PAR) for K34, K67, and K83, and 200 W m^{-2} (downward shortwave radiation) for the

379   B2-TF. Mean values of light-saturated GEP values were calculated for 1°C temperature

380   bins and 0.2 kPa VPD bins. We scaled GEP to the maximum GEP value for each location

381   to compare the response of canopy-level photosynthesis in the B2-TF with natural

382   forest sites (Fig. 3), rather than the magnitude.

383   To simplify the figures in the main text, we combined the data for the three

384   Amazon forests (K34, K67, and K83) because the sites all experience broadly similar

385   environmental conditions, and exhibit similar responses of GEP to temperature and VPD

386   (Supplementary Fig. 2). Figs 1 and 2 present the raw data for all Amazon sites

387   combined, and Fig. 3 presents the mean GEP of Amazon sites (i.e. the mean of values for

388   K34, K67, and K83 shown in Supplementary Fig. 2), that have subsequently been scaled

389   to the maximum value.

390

391   **Data availability**

392   The datasets analysed in this study (eddy flux and environmental data) are available at

393   https://daac.ornl.gov/cgi-bin/dsviewer.pl?ds_id=1174 (for K34 and K83), and

394   https://ameriflux.lbl.gov/sites/siteinfo/BR-Sa1 (for K67). Datasets for Tesopaco and

395   the B2-TF are available at https://github.com/m-n-smith/B2-temp-paper-datasets.

396

397   **Code availability**

398   The R code used to conduct the analyses presented in this paper is available upon

399   request from the corresponding authors.

400

401   **References**

402   1.   Galbraith, D. *et al.* Multiple mechanisms of Amazonian forest biomass losses in three

403   dynamic global vegetation models under climate change. *New Phytol.* **187**, 647–665 (2010).

404   2.   Brienen, R. J. W. *et al.* Long-term decline of the Amazon carbon sink. *Nature* **519**, 344–348

405   (2015).

406   3.   Longo, M. *et al.* Ecosystem heterogeneity and diversity mitigate Amazon forest resilience to

407   frequent extreme droughts*. New Phytol.* **219**, 914–931 (2018).

408   4.   Doughty, C. E. & Goulden, M. L. Are tropical forests near a high temperature threshold?

409   *Journal of Geophysical Research: Biogeosciences* vol. 113 (2008).

410   5.   Mau, A., Reed, S., Wood, T. & Cavaleri, M. Temperate and tropical forest canopies are

411   already functioning beyond their thermal thresholds for photosynthesis. *Forests* vol. 9 47

412   (2018).

413   6.   Huang, M. *et al.* Air temperature optima of vegetation productivity across global biomes.

414   *Nat Ecol Evol* **3**, 772–779 (2019).

415   7.   Grossiord, C. *et al.* Plant responses to rising vapor pressure deficit. *New Phytol.* **226**, 1550–

416   1566 (2020).

417   8.   Sharkey, T. D. Effects of moderate heat stress on photosynthesis: importance of thylakoid

418   reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by

419   isoprene. *Plant Cell Environ.* **28**, 269–277 (2005).

420   9.   Lloyd, J. & Farquhar, G. D. Effects of rising temperatures and [CO2] on the physiology of

421   tropical forest trees. *Philosophical Transactions of the Royal Society B: Biological Sciences*

422   vol. 363 1811–1817 (2008).

423   10. IPCC. in *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to*

424   *the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. Stocker,

425   T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M.

426   Midgley) (Cambridge University Press, 2013).

427   11. Dusenge, M. E., Duarte, A. G. & Way, D. A. Plant carbon metabolism and climate change:

428   elevated CO2 and temperature impacts on photosynthesis, photorespiration and

429   respiration. *New Phytol.* **221**, 32–49 (2019).

430   12. Janzen, D. H. Why mountain passes are higher in the tropics. *Am. Nat.* **101**, 233–249 (1967).

431   13. Cunningham, S. C. & Read, J. Do temperate rainforest trees have a greater ability to

432   acclimate to changing temperatures than tropical rainforest trees? *New Phytol.* **157**, 55–64

433   (2003).

434   14. Sullivan, M. J. P. *et al.* Long-term thermal sensitivity of Earth's tropical forests. *Science* **368**,

435   869–874 (2020).

436   15. Lancaster, L. T. & Humphreys, A. M. Global variation in the thermal tolerances of plants.

437   *Proc. Natl. Acad. Sci. U. S. A.* **117**, 13580–13587 (2020).

438   16. Mora, C. *et al.* The projected timing of climate departure from recent variability. *Nature*

439   **502**, 183–187 (2013).

440   17. Hutyra, L. R. *et al.* Seasonal controls on the exchange of carbon and water in an Amazonian

441   rain forest. *Journal of Geophysical Research: Biogeosciences* vol. 112 (2007).

442   18. Slot, M. & Winter, K. In situ temperature relationships of biochemical and stomatal controls

443   of photosynthesis in four lowland tropical tree species. *Plant Cell Environ.* **40**, 3055–3068

444   (2017).

445   19. Tcherkez, G. *et al.* Leaf day respiration: low CO2 flux but high significance for metabolism

446   and carbon balance. *New Phytol.* **216**, 986–1001 (2017).

447   20. O'sullivan, O. S. *et al.* Thermal limits of leaf metabolism across biomes. *Glob. Chang. Biol.* **23**,

448   209–223 (2017).

449   21. Leakey, A. D. B. *et al.* Elevated CO2 effects on plant carbon, nitrogen, and water relations:

450   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-Yrízar, 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).

Acknowledgments

565

Author contributions

567　M.N.S., T.C.T., S.R.S., T.E.H., conceived the study, designed the analyses, and led the data

568　interpretation, with extensive help from J.v.H. and R.R. M.N.S. performed the data

569　analysis and drafted the manuscript, with substantial input from T.C.T., S.R.S., and T.E.H.

570　R.R. provided carbon exchange data for the B2-TF and advice on its analysis. N.R.C.,

571　R.C.d.O., R.d.S., A.C.d.A., P.B.d.C., and S.R.S. contributed to the installation, maintenance,

572　or analysis of eddy flux data from LBA tower sites. J.W. provided advice on binned

573　regression analysis. J.A. collected and analysed leaf-level chlorophyll fluorescence

574　measurements in the B2-TF. All authors contributed towards writing the final

575　manuscript.

576

Competing financial interests

578　The authors declare no competing financial interests.

579

580

581

582 **Figure Legends**

583

584 **Figure 1.** Air temperature distributions recorded at the B2-TF mesocosm (red), a

585 seasonally dry tropical forest (Tesopaco, gold), and Amazon forest sites (K34, K67, and

586 K83, blue). Dashed lines show the current mean temperatures at Amazon forest sites

587 (28°C) and the B2-TF (32°C); grey area shows the range of mean annual temperatures

588 projected for the Amazon region by 2100[10]. Only temperatures corresponding to light-

589 saturated gross ecosystem productivity (GEP) have been included.

590

591 **Figure 2.** Relationship between vapour pressure deficit (VPD) and air temperature for

592 the B2-TF mesocosm (red), a seasonally dry tropical forest (Tesopaco, gold), and

593 Amazon forest sites (K34, K67, and K83, blue). Boxplots represent median values

594 (horizontal lines at box midpoints), first and third quartiles (box bottom and top), while

595 vertical lines extending from the boxplots (whiskers) show the data that lies within 1.5

596 interquartile range of the lower and upper quartiles, and data points at the end of the

597 whiskers represent outliers. Vertical lines indicate the edges of five temperature bins,

598 evenly distributed across the full range of the dataset (binwidths = 4.63°C). Lines show

599 logistic growth equation fits for each site.

600

601 **Figure 3.** Light-saturated gross ecosystem productivity (GEP) versus (**a**) air

602 temperature and (**b**) vapour pressure deficit (VPD) for the B2-TF mesocosm (red), a

603 seasonally dry tropical forest (Tesopaco, gold), and Amazon forest sites (K34, K67, and

604 K83, blue). **a**, Points show the average GEP for each 1°C temperature bin, scaled to the

605 maximum GEP value for each forest site; **b**, points show the average light-saturated GEP

606 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 (μmol CO_2 m^{-2} s^{-1} / °C, red lines) and vapour pressure deficit (VPD, μmol

611 CO_2 m^{-2} s^{-1} / 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°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).

617

a

GEP scaled to max value vs Temperature (°C)

b

GEP scaled to max value vs VPD (kPa)

Legend:
- B2–TF
- Tesopaco
- K34, K67, K83