BIOTROPICA ■■(■■): 1–8 2011 10.1111/j.1744-7429.2010.00746.x

An *In Situ* Leaf and Branch Warming Experiment in the Amazon

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ABSTRACT

Leaves and branches of mature trees, lianas, and gap species were warmed in an Amazonian forest for 4 mo to observe the effect of warming on photosynthesis, stomatal conductance, and transpiration. Electric resistance heaters increased air temperatures near the leaves by approximately 2°C. Sunlit leaf temperatures increased by 2–3°C on average, but during some periods leaf temperatures increased by > 5°C. Maximum photosynthesis (A_{max}) decreased significantly in the warmed leaves vs. the control leaves over the 13-wk study period with an average decrease in A_{max} of 1.4 μmol/m^{2}s (19% decrease from a mean A_{max} of 7.2 μmol/m^{2}s) when measured at 30°C and there were no signs of acclimation to higher temperatures within existing leaves. The decline in A_{max} was likely due to irreversible temperature damage caused by very high leaf temperatures and not due to C_i limitation of carboxylation. Warming had a larger negative impact on A_{max} in canopy level tree species than other tested functional groups such as lianas or gap species. Transpiration did not significantly increase in the warmed leaves compared with the control group. This study indicates that increased temperatures due to global warming could potentially decrease future tropical forest carbon uptake by a significant amount.

Abstract in Portuguese is available at http://www.blackwell-synergy.com/loi/btp.

Key words: Amazon; biosphere–atmosphere exchange; gas exchange; global warming; LBA-ECO; photosynthesis; warming experiment.

THERE IS DEBATE ON HOW FUTURE CLIMATE CHANGE WILL AFFECT TROPICAL FORESTS (Clark 2004, Lewis *et al.* 2004). Stomatal conductance will be affected by global warming either through hotter, drier conditions, or through increasing atmospheric CO_2 concentrations. Widespread stomatal closure over a large area in the Amazon could reduce rainfall because much rainfall is supplied by evaporation from the land surface (Salati & Vose 1984). Some modeling studies indicate that climate change may lead to a dieback of the Amazon forest as precipitation decreases (Cox *et al.* 2000, Betts *et al.* 2004). Approximately 20 percent of this decrease in precipitation is because increased atmospheric CO_2 concentrations lead to stomatal closure and decreased evapotranspiration (Betts *et al.* 2004). The simulated dieback of the Amazon contributed to an increase in global temperatures of about 1.5°C due to carbon cycle feedbacks in the model of Betts *et al.* (2004).

There is currently a lack of *in situ* experimental data on how stomatal conductance in tropical forest trees may respond to long-term increases in CO_2 or temperatures. There is experimental evidence that increased atmospheric CO_2 concentrations may decrease stomatal conductance (Field *et al.* 1995). High leaf temperatures may also lead to stomatal closure (Bazzaz 1998). High leaf temperatures increase leaf-to-air vapor pressure deficit (VPD), increasing evaporative demand and transpiration. This generally causes stomatal closure, which could decrease water vapor flux to the atmosphere. A pertinent question is whether increased VPD, and corresponding stomatal closure, will maintain, increase or decrease water vapor flux rates. Understanding how stomata respond to both short- and long-term warming are key to addressing this question.

Received 10 March 2010; revision accepted 23 October 2010.

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Extended warm temperatures may also affect CO_2 uptake and plant growth. Field observations have drawn attention to the possibility that tropical forest production is sensitive to temperature (Clark *et al.* 2003, Feeley *et al.* 2007). Leaf gas exchange studies using chambers have found that tropical leaf photosynthesis and stomatal conductance declines at higher temperatures (Koch *et al.* 1994, Ishida *et al.* 1999, Tribuzy 2005, Doughty & Goulden 2008). Field measurements of whole-ecosystem fluxes in tropical forests made using eddy covariance indicate that CO_2 uptake and canopy conductance decrease during warmer than average periods (Grace *et al.* 1995, Loescher *et al.* 2003, Goulden *et al.* 2004, Doughty & Goulden 2008). Currently, temperatures a few degrees outside the mean cause a steep decline in CO_2 uptake at both the leaf and canopy scale in tropical forests. For instance, Goulden *et al.* (2004) reported that temperatures higher than average tend to increase the reduction in net ecosystem exchange (NEE) possibly by affecting photosynthesis more than respiration and showed that NEE by an Amazonian forest decreased by 12 μmol/m^{2}s during periods that were only 3°C warmer than average. These observations support the idea that tropical forests currently operate near a high-temperature threshold, and that future increases in temperature may have a negative impact on primary production.

Tropical forests exist in a thermally stable environment and therefore the question of whether they will acclimate to higher temperatures is pertinent. Berry and Bjorkman (1980) define photosynthetic acclimation as 'environmentally induced changes in photosynthetic characteristics that result in an improved performance under the new growth regime' (Berry & Bjorkman 1980). Plants native to regions with greater climatic variability generally have greater acclimation potential than plants from regions with less climatic variability. There is evidence that tropical species are less likely than temperate species to acclimate to changing temperatures (Cunningham & Read 2003b). Tropical trees have a lower

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temperature optimum for carbon assimilation than for growth, which could cause increasing growth even as photosynthesis declines (Cunningham & Read 2003a, Raich *et al*. 2006). If tropical forests cannot acclimate to future higher temperatures caused by global warming, then a 2°C increase in air temperatures may cause a steep decline in CO_2 uptake.

Despite the importance of this issue, logistical difficulties have limited most tropical forest warming experiments. The goal of this paper is to test whether leaves and branches warmed by 2°C will decrease stomatal conductance and transpiration and substantially decrease CO_2 uptake. In a previous paper, I explored the effect of a short-term warming on tropical forest gas exchange (Doughty & Goulden 2008). I now explore whether the multi-month effect of warming on tropical forest gas exchange is the same as the short-term effect or whether leaves are able to acclimate to higher temperatures. I tested the following two hypotheses: (1) multi-month warming of tropical forest leaves will cause stomatal closure over extended periods that will decrease transpiration to the atmosphere; and (2) multi-month warming of tropical forest leaves will substantially decrease CO_2 uptake.

METHODS

SITE.—This study was conducted at the Floresta National (FLONA) do Tapajos as part of the Large-Scale Biosphere–Atmosphere Ecology Program (LBA-ECO). The FLONA Tapajos is located on the eastern side of the Tapajos River, extending from 50 to 150 km south of Santarem, Para, Brazil (3°01′15″ S, 54°58′20″ W). I worked at the km-83 (Goulden *et al*. 2004), km-67 (Saleska *et al*. 2003), and Seca Floresta (Nepstad *et al*. 2007) sites. The vegetation at the sites was closed tropical forest, with a canopy height of 30 m and emergent trees with heights up to 55 m. An analysis of precipitation showed the FLONA Tapajos was in the 27th driest percentile (± 3%) of Amazonian forests for both annual precipitation and length of the wet season (Saleska *et al*. 2003). At the site, air temperatures varied diurnally by 7°C (between 23°C and 30°C in October) and seasonally by 4°C (maximum daily temperature in October was 30°C and 26°C in February) (Goulden *et al*. 2004). The forest was on a broad, flat plateau. Soils were mainly yellow latosol clay (Haplic acrorthox) (Filho *et al*. 1993).

FIELD MEASUREMENTS.—The field campaign lasted from July 2005 to February 2006 and focused on six canopy top tree species and two canopy top liana species accessed with three canopy access towers and six gap specialist species in a gap at km-83 (Table 1). I studied two species accessible from a 30-m scaffold tower at LBA-ECO km-83 (Goulden *et al*. 2004), four species from a 45-m scaffold tower at km-67 (Saleska *et al*. 2003), and two species from a 30-m tower at the control site of the Seca Floresta experiment (Nepstad *et al*. 2007). Gap species ranged between 0.5 and 2 m tall and the leaves were exposed to the sky overhead.

I measured leaf-level gas exchange once a week at all sites from July 2005 to February 2006 on the same six canopy tree species, two canopy liana species, and six gap species using a portable gas exchange system (Li-6400, Li-Cor Biosciences, Lincoln, Nebraska, U.S.A.). Gas exchange rates were measured under a standardized set of conditions (leaf temperature 30°C and 37°C; PPFD 1000 μmol/m^2s; ambient CO_2). I used the Li-6400 Integrated Peltier coolers to control leaf temperatures. High leaf temperature of 37°C were chosen because in previous work significant stomatal closure at this temperature had been observed (Doughty & Goulden 2008), and the light level of 1000 μmol/m^2s as this was the forest's light-saturation point (Goulden *et al*. 2004). Two leaves were repeatedly measured on four branches on each of the eight canopy species (N = 64 each week), and the same leaf was returned to each week. On the rare occasion that a leaf abscised, another leaf was chosen

TABLE 1. *Species names, species abbreviations, functional group, average leaf lengths, and widths, number of leaves measured for* A_{max} *per species, and number of sap flow sensors per species for canopy top species at the km-83, -67, and Seca Floresta canopy towers and species in a gap (10 m radius) (Figueira* et al. *2008) near the km-83 eddy covariance tower.*

Species	Abbreviation	Functional group	Leaf length and width (cm)	# of leaves tested for A_{max}	# of sap flow sensors
Abuta sp.	Ab	Liana	13, 12	8	4
Caraipa sp.	Ca	Canopy tree	10, 5	8	4
Copaifera ducke	CD	Canopy tree	7, 3	8	4
Micropholis sp.	Mi	Canopy tree	14, 7	8	4
Protium punticulatum	PP	Canopy tree	15, 6	8	4
Sextonia rubra	SR	Canopy tree	12, 6	8	4
Tachigali mymercophyla	TM	Canopy tree	15, 6	8	4
Cariniana micrantha	CM	Gap	19, 7	4	1
Cecropia sciadophylla	CS	Gap	15, 9	4	1
Iryanthera sagotiana	IS	Gap	17, 5	4	1
Mabea subsessilis	MS	Gap	15, 6	4	1
Mabea sp.	Ma	Gap	9, 5	4	1
Protium sp.	Pr	Gap	11, 3	4	1

nearby. Two leaves were measured each week on each of the six gap species ($N = 12$). Each gas exchange measurement lasted 3–5 min, with a measurement taken every 20 sec. These 20 sec measurements were averaged together once the data had stabilized.

I measured A_{max} at 2 percent oxygen to estimate photorespiration in four tree species (*Caraipa* sp., *Copaifera ducke*, *Micropholis* sp., *Protium punticulatum*), two liana species (*Abuta* sp., unknown species) and three gap species (*Protium* sp., *Mabea subsessilis*, *Cariniana micrantha*) on warmed and nonwarmed leaves, at the start of the warming and toward its end (Bjorkman 1971). The composition of the air entering the gas exchange system was varied with an air flow valve, mixing 10 percent ambient air with 90 percent pure nitrogen from a tank, thereby reducing the oxygen concentration entering the system to around 2 percent. The gas mixture was then scrubbed of CO_2. I then used the Li-6400 CO_2 mixer to bring the CO_2 mixing ratio to 370 ppm. I determined the rates of photorespiration for leaves at 1000 μmol/m^{2}s and 30°C and 37°C by subtracting A_{max} at 2 percent oxygen from A_{max} at ambient oxygen. The gas exchange observations at 37°C were recorded before significant stomatal closure.

I added heat-balance sap flow sensors (Sakuratani & Abe 1985) on four branches on each of the six canopy top tree species and two liana species ($N = 32$), and one sap flow sensor on each gap species ($N = 6$). Every 2 min, sap flow and leaf temperature were measured with fine wire thermocouples (copper-constantan 0.005″ Omega, Stamford, Conneticut, U.S.A.) that were attached to the underside of leaves, and irradiance at the leaf surfaces with gallium-arsenide-phosphide sensors (Doughty & Goulden 2008). I calculated total leaf area for each branch by measuring the average area of a sub-sample of leaves and multiplied this by the total number of leaves. I then converted sap flow to leaf-area-based transpiration by dividing sap flow by total branch leaf area.

To determine the effect of warming on sap flow, I compared warmed with nonwarmed branches during midday (1000–1400 h local time), sunny (PPFD > 1200 μmol/m^{2}s) periods over 65 d (240–260 d before the warming and 261–305 d following the warming). Data were eliminated from several sap flow sensors whose rates of transpiration did not closely match transpiration rates calculated by leaf gas exchange due to sensor malfunction, patchy stomatal conductance, or cavatation. I only use sap flow data from tree species because many of the gap and liana sensors malfunctioned and I did not have an adequate sample size for statistical comparison.

WARMING METHODS.—I used electric resistance heaters powered by solar panels and a battery pack to continuously warm four individual leaves for each species for 90 d. Each heater had a 30 Ω constantan wire folded in aluminum foil into a 40 cm^2 rectangular shape. Six volts were run through the wire (1.2 W) and the heaters placed approximately 2 cm below the leaves by attaching them to the petiole with wire (Fig. 1). A nighttime comparison of top of canopy leaf temperatures between four heated leaves and a nonheated leaf from *Micropholis* sp. when the leaf thermocouple positions were visually verified, showed the electric resistance heaters warmed the leaves by an average of 1.96 ± 1.4°C (SD) at night (Fig. 2).

I used a passive method (black plastic sheets under the branches) to heat whole branches. The passive heaters were designed with light aluminum wire and black plastic so incident radiation not intercepted by the leaves was captured and used to heat the air near the branch during sunny periods. On each of my study species, on two of the four branches with sap flow sensors, the passive heaters were molded to be rectangular (10 × 20 cm), flat and approximately 10 cm below the lowest leaves on the branch. I compared air temperatures using thermocouples within the branches between three heated branches and a nonheated branch over a 3-day period to determine warming during sunny periods. The passive heaters warmed air temperatures an average of 1.95 ± 0.87°C (SD). There are potential sources of error associated with each method. The branch heaters only warmed the branches during sunny periods while the individual leaf heaters provided continuous and constant heat, but only warmed a small number of leaves.

FIGURE 1. A leaf warmed by a resistance heater in a heating experiment conducted at Floresta National do Tapajos, Brazil. Each heater added 1.2 W of energy to the leaf and was affixed several centimeters below the leaf. Heaters warmed the leaves continuously through the experiment.

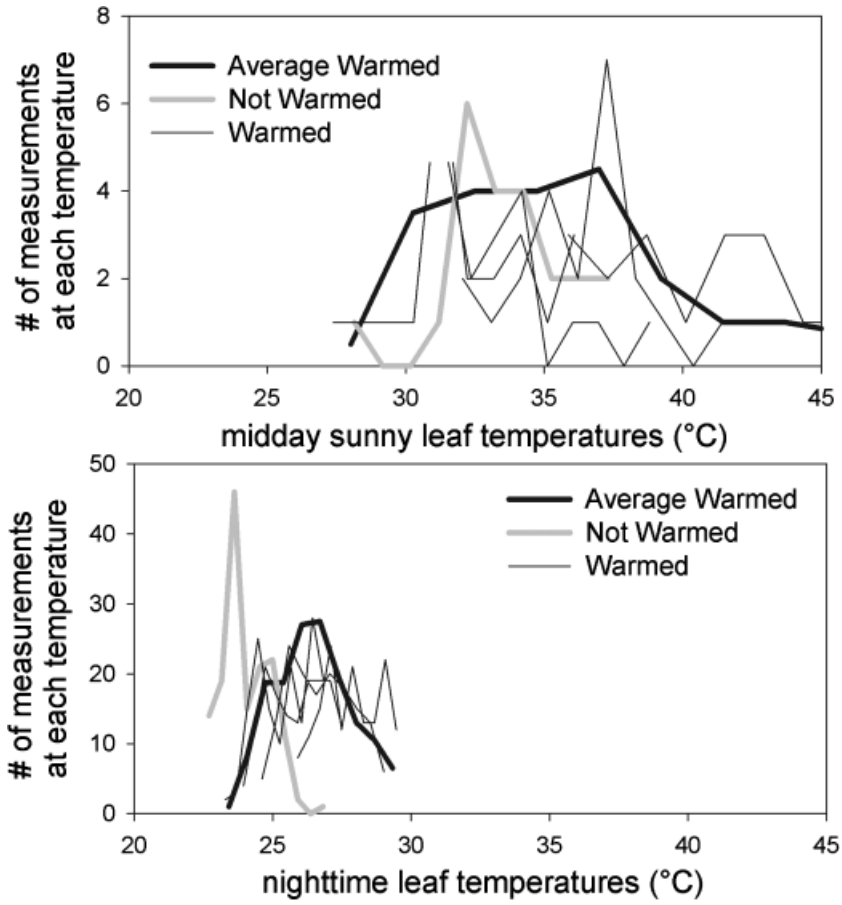

FIGURE 2. (Top) Sunny (PPFD > 1000 μmol/m^{2}s) midday (1000–1400 h local time) leaf temperatures for one normal (gray line), four heated (black line) leaves from *Micropholis* sp., and average heated leaf temperature (thick black line) warmed with electric resistance heaters on day 271 during a heating experiment conducted at Floresta National do Tapajos, Brazil. (bottom) Leaf temperatures for the same leaves between 2000 h and midnight local time on day 271. The *y*-axis represents the number of times each temperature was recorded between 1000–1400 h (top) and 2000–midnight local time. Leaf temperatures were measured using fine wire thermocouples threaded through the leaf and incident solar irradiance was measured using gallium-arsenide-phosphide sensors on nearby leaves.

DATA ANALYSIS.—To statistically analyze my gas exchange and sap flow data, I compared weekly measurements for all control and warmed leaves over a 13-wk period following warming using repeated measures two-way analysis of variance. I analyzed the effect of warming on each species separately and then as functional groups, grouping the six canopy tree species together, the two canopy top liana species together, and six gap species together.

RESULTS

EFFECTS OF WARMING ON A_{MAX}.—I compared leaf temperatures between a nonwarmed leaf and four warmed leaves of *Micropholis* sp. during noontime sunny conditions to quantify the combined effect of warming and any biophysical feedbacks, such as reduced latent heat evaporation on leaf temperature. Warming air temperature near the leaves by 2°C increased most leaf temperatures by 2–3°C, but increased leaf temperatures in some to up to 45°C for short periods (Fig. 2).

Warming leaves by 2°C significantly decreased A_{max} ($P < 0.05$ at 30°C and $P < 0.001$ at 37°C) compared with nonwarmed leaves when all six tree species were averaged together (Fig. 3A). There were no significant photosynthetic differences before the onset of warming. A_{max} in three of six individual tree species decreased significantly compared with the nonwarmed leaves at both 30°C and 37°C (Table 2). A_{max} decreased by an average of 1.4 μmol/m^{2}s over the 13 wk of warming when measured at 30°C and decreased by an average of 2.7 μmol/m^{2}s when measured at 37°C. The decrease was relatively constant over the period of study, except for an increased effect of temperature between weeks 4 and 6 when measured at 37°C. Some of the warmed leaves in this study showed visible signs of necrosis followed by abscission.

Stomatal conductance decreased significantly ($P < 0.05$) with warming compared with the nonwarmed control group when measured at 37°C and was close to significant ($P = 0.08$) when measured at 30°C for canopy top tree species (Fig. 3B). There were no significant differences in stomatal conductance before warming. The decrease in stomatal conductance was relatively constant over

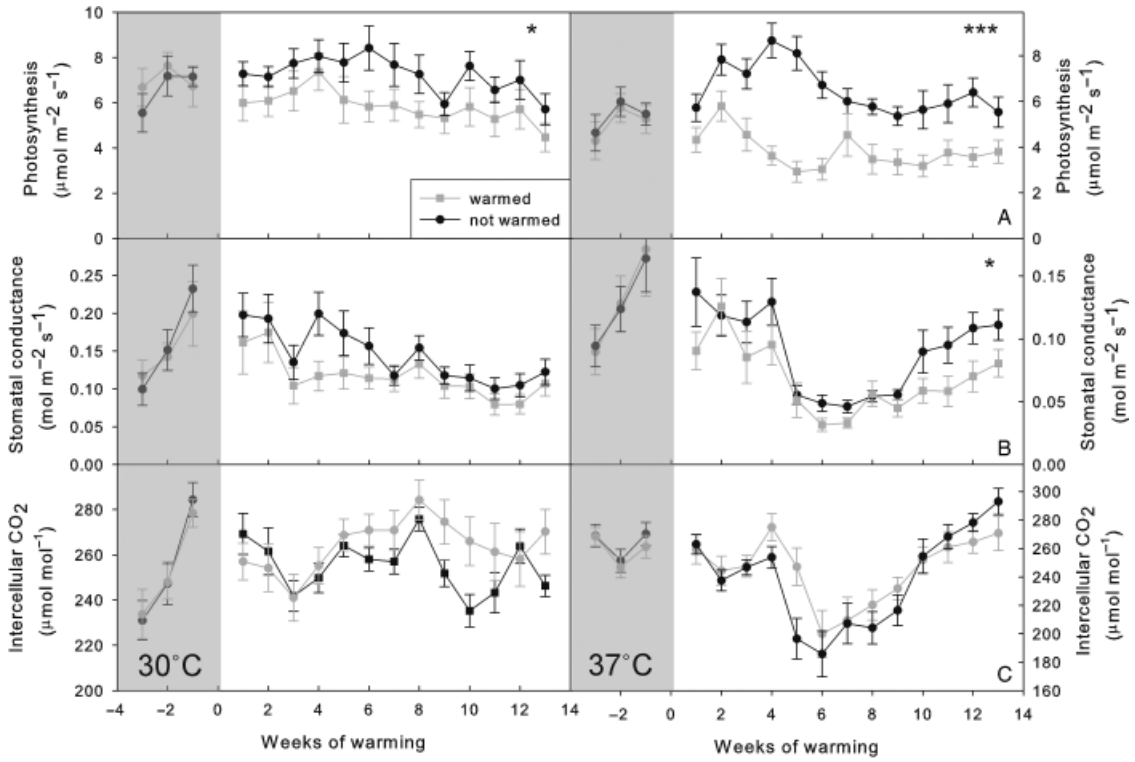

FIGURE 3. (A) A_{max} (μmol/m^{2}s); (B) stomatal conductance (mol/m^2/s); and (C) intercellular CO_2 concentrations (C_i) (umol mol^{-1}) for warmed leaves (gray squares) and nonwarmed leaves (black circles) (mean $\pm$ SE) in a heating experiment conducted at Floresta National do Tapajos, Brazil. Shaded region shows warmed and control leaves before the addition of electric resistance heaters. Gas exchange measurements (lasting 2–5 min) were made on sunlit leaves at the top of the canopy for six tree species using a portable gas exchange machine (Li-6400) at 30°C (left panel), and 37°C (right panel) (PPFD 1000 μmol/m^{2}s; ambient CO_2). Six to eight leaves (three to four warmed, three to four not warmed) from each of the six tree species ($N = 19$ warmed, $N = 22$ not warmed) were measured every week. Each week's data were compared using repeated measures two-way analysis of variance. Stars denote significant differences between the control and the warmed leaves $^*P < 0.05$, $^{***}P < 0.001$.

the entire 13-wk study. Intercellular CO_2 concentrations (C_i) in warmed leaves did not decrease relative to the control group and there were no significant differences between C_i concentrations (Fig. 3C).

Leaf size affects boundary layer conductance with larger leaves having larger boundary layers causing more warming of large leaves than smaller leaves. No significant correlation was found ($P > 0.05$), however, between average leaf size and the change in A_{max} with warming for each species (figure not shown).

To quantify the impact of high leaf temperatures on photorespiration, I measured A_{max} at 2 percent oxygen, which will eliminate most photorespiration. There were no significant differences ($P > 0.05$) in photorespiration between warmed and nonwarmed leaves (figure not shown). At 30°C, A_{max} at 2 percent oxygen (8.7 μmol/m^{2}s, $N = 73$) was significantly greater ($P < 0.001$) than A_{max} at ambient oxygen (4.7 μmol/m^{2}s, $N = 52$) (Fig. 4). A_{max} at 2 percent oxygen and 30°C (8.7 μmol/m^{2}s, $N = 73$) was significantly greater ($P < 0.05$) than A_{max} at 2 percent oxygen and 37°C (7.3 μmol/m^{2}s, $N = 67$).

EFFECTS OF WARMING ON TRANSPIRATION.—Of the 24 sap flow sensors initially installed on tree species, there was reliable data from nine sensors on warmed branches and four sensors on nonwarmed branches. These sensors all worked continuously for 20 d before the warming and 45 d following the warming. There was no significant increase ($P > 0.05$) in transpiration and a trend toward decreased transpiration in the warmed branches vs. the control branches (Fig. 5).

DISCUSSION

Previously at this study site, stand-level NEE was found to decline during warm periods, decreasing by 12 μmol/m^{2}s from maximum rates during periods that were only 3°C warmer than average (Doughty & Goulden 2008). A goal of this paper was to determine whether such reductions in maximum photosynthetic rates in tropical forests would continue in a warmer world or whether the trees would acclimate to warmer conditions.

When heated over a period of 13 wk, A_{max} decreased relative to the control by an average of 1.4 μmol/m^{2}s when measured at 30°C (Fig. 3A). The difference between the heated group and the control group did not change substantially over the 13-wk study, which suggests there is little acclimation to higher temperatures within existing leaves.

TABLE 2. *Significance between warmed leaves and nonwarmed leaves using repeated measures two-way analysis of variance for individual species and functional groups for A_{max}, transpiration, stomatal conductance, and C_i at 30°C and 37°C (PPFD 1000 μmol/m²s; ambient CO_2) for 3 wk before the warming and 13 wk after in a heating experiment conducted at FLONA Tapajos, Brazil. Columns are as follows:* D*, direction of the average photosynthetic response with heating (+ = more CO_2 fixed, − = less CO_2 fixed, 0 = no significant change);* ΔA_{max}*, light saturated photosynthesis;* ΔT*, transpiration;* Δg*, stomatal conductance;* ΔC_i*, intercellular CO_2 concentrations. Stars denote significant differences between the control and the warmed leaves* $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

	30°C					37°C				
Species name	*D*	ΔA_{max}	ΔT	Δg	ΔC_i	*D*	ΔA_{max}	ΔT	Δg	ΔC_i
TM	–	*	**	*	No	–	***	*	No	**
Mi	–	*	**	*	No	–	*	No	*	No
SR	–	***	*	***	No	–	***	*	*	No
CD	0	No	*	No	*	0	No	No	No	No
PP	0	No	No	No	*	0	No	No	No	No
Ca	0	No	No	No	No	0	No	**	No	No
6 tree species	–	*	*	No	No	–	***	**	*	No
Unidentified liana	0	No	No	No	No	0	No	No	No	No
Ab	0	No	*	*	*	0	No	*	No	No
2 liana species	0	No	No	No	*	0	No	No	No	No
6 gap species	0	No	No	No	–	0	No	No	No	No

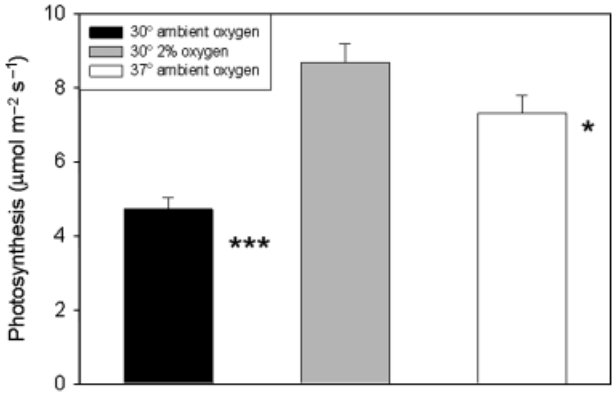

FIGURE 4. $A_{max} \pm$ SE for normal (black, ambient oxygen, $N = 52$) and low (gray, 2% oxygen, $N = 73$ at 30°, $N = 67$ at 37° white) O_2 concentration for four tree species, two liana species, and three gap species (PPFD 1000 μmol/m²s; ambient CO_2) in a heating experiment conducted at Floresta National do Tapajos, Brazil. At 37° A_{max} is recorded before significant stomatal closure. At ambient oxygen levels, the decline in A_{max} between 30 and 37° is due to photorespiration plus any other nonstomatal temperature effect. At 2 percent oxygen levels, the decline is due to nonstomatal, nonphotorespiration temperature effects. Stars denote significant differences between the center bar and the other bars: $^*P < 0.05$, $^{***}P < 0.001$.

High temperatures can affect tropical forest NEE in several ways. First, rising air temperatures will increase leaf temperatures and VPD, which may cause stomatal closure. Stomatal closure may limit photosynthesis by restricting the supply of CO_2 and limiting carboxylation. Second, the affinity of Rubisco for CO_2 decreases at high temperatures leading to increased photorespiration (Berry & Bjorkman 1980). Third, at very high temperatures, irreversible temperature damage to the photosynthetic machinery causes permanent declines in photosynthetic capacity (Berry & Bjorkman 1980).

There were no significant changes in C_i between the heated and not-heated groups. This suggests that the observed decrease in A_{max} was not due to a restricted supply of CO_2 and limited carboxylation. There were very high rates of photorespiration in all species tested, but no significant difference between rates of photorespiration in the warmed and nonwarmed groups. Photorespiration is likely driving much of the short-term decline in A_{max} between 30°C and 37°C (Fig. 3A), but it is unlikely to cause the multi-month decline in A_{max} in the warmed group. Pons and Welschen (2003) found similar high rates of photorespiration in a different tropical forest species (*Eperua grandiflora*).

Leaf respiration rates generally increase with temperature. Although dark respiration rates were not measured in this study, previous studies in temperate deciduous trees found that leaf respiration acclimated to rising temperatures (Lee *et al.* 2005). If Q_{10} rates do not change with warming, a Q_{10} rate of 2–2.3 (Meir *et al.* 2001), would cause an increase in respiration between 0.07 and 0.09 μmol/m²s if leaf temperatures increased by 2°C, assuming a baseline leaf respiration rate of 0.5 μmol/m²s. This indicates it is unlikely that increased leaf respiration would account for much of the 1.4 μmol/m²s decrease in A_{max} that was seen in the warmed leaves vs. the control.

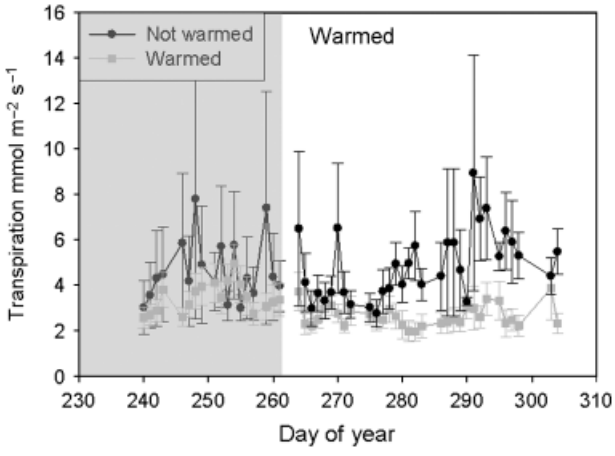

FIGURE 5. Transpiration rates measured by branch sap flow for sunny (irradiance > 1200 μmol/m²s) midday periods (1030–1400 h local time) for nine warmed branches and four nonwarmed branches on six tree species using passive black plastic heaters in a heating experiment conducted at Floresta National do Tapajos, Brazil. Five sunny periods were averaged each day for all branches and compared with nonwarmed branches using repeated measures two-way analysis of variance. The gray bar is the period before the heating.

Increasing air temperatures near leaves by 2°C, led to occasional very high leaf temperatures (> 45°C) (Fig. 2). The very highest measured temperatures appear anomalously warm compared with a large dataset on leaf temperatures from a prior study (Doughty & Goulden 2008). During certain periods, high temperatures likely led to temporary stomatal closure, reduced latent heat cooling and periods of extreme leaf temperatures hot enough to damage to the photosynthetic machinery.

The longer term decline in A_{max} in this study is likely due to irreversible temperature damage caused by short periods of very high leaf temperatures. Irreversible damage will often occur at 45–60°C (Berry & Bjorkman 1980, Cunningham & Read 2003b). I found that several of the larger, flatter warmed leaves reached temperatures that may cause such damage (Fig. 2). Therefore, it is likely that the 2°C increase in air temperature increased leaf temperature, and decreased evaporative cooling sufficiently to cause some of the leaves to reach leaf temperatures that damaged their photosynthetic machinery during sub-optimal conditions.

A second goal of this experiment was to determine how transpiration would react to a multi-month 2°C increase in air temperature. This is important to understand because temperature induced reductions in stomatal conductance and transpiration could decrease regional rainfall by reducing moisture cycling, and modifying atmospheric circulation due to greater warming of the land surface (Betts *et al*. 2004). Transpiration rates did not significantly increase with temperature and my data were not conclusive enough to determine whether transpiration decreased or remained the same with increased temperatures.

CONCLUSION

There were no obvious signs of photosynthetic acclimation within existing leaves and warming tropical forest leaves and branches by 2°C had an overall negative impact on canopy top tree species by decreasing A_{max}. The decline in A_{max} appeared to be related to high-temperature damage caused by short periods of increased maximum temperatures, possibly due to reduced latent heat cooling. This study had several limitations including a small number of species and leaves tested. In addition, acclimation could have occurred in the next generation of leaves without being detected by this study, because only a single cohort of leaves was heated. This study also did not address the important role of heating on increased canopy respiration. Nevertheless, this study is the first of its kind and future studies can better address these other issues.

ACKNOWLEDGMENTS

I thank Helber Freitas, Michela Figueira, Augusto Maia, and Albert de Sousa for field help; Mike Goulden, Scott Miller, Jim Randerson, Susan Trumbore, Diane Pataki, Deborah Clark, and anonymous reviewers for comments; UFPA in Santarem and Belem for help identifying species; and the LBA-ECO Santarem field office for support. This work was funded by NASA as a component of LBA-ECO and an Earth System Science Fellowship.

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