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# TROPICAL PLANT PHYSIOLOGICAL RESPONSES TO EXPERIMENTAL WARMING AND HURRICANE DISTURBANCE

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# TROPICAL PLANT PHYSIOLOGICAL RESPONSES TO EXPERIMENTAL WARMING AND HURRICANE DISTURBANCE

By

Rob Tunison

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

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This dissertation has been approved in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY in Forest Science.

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# Author Contribution Statement

The work presented in this dissertation was performed collaboratively between researchers at Michigan Technological University (MTU), the United States Geological Survey (USGS) Southwest Biological Science Center, and the United States Department of Agriculture - Forest Service – International Institute of Tropical Forestry (USDA-FS-IITF). The Tropical Responses to Altered Climate Experiment (TRACE), where the research was conducted, was conceptualized and created by Dr. Molly A. Cavaleri (MTU), Dr. Sasha C. Reed (USGS), and Dr. Tana E. Wood (USDA-FS-IITF). The data in all chapter were collected and analyzed by Rob Tunison. Chapters 1, 3, 4, & 5 were written solely by Rob Tunison. Chapter 2 is currently under review and was primarily written by Rob Tunison with substantial edits from Dr. Molly A. Cavaleri, Dr. Sasha C. Reed, and Dr. Tana E. Wood.

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# Abstract

Tropical forests exchange more carbon with the atmosphere than any other biome, but their responses to climate change are largely understudied. The work presented in this dissertation uses a tropical understory warming experiment that was hit by a hurricane disturbance to investigate how plant physiology responds to multiple types of climate change pressures. We measured the potential for root respiration, leaf photosynthesis & respiration, and leaf thermotolerance acclimation to experimental warming while recovering from hurricane disturbance. We found there was thermal acclimation via decreased ecosystem root respiration, increased photosynthetic thermal niche breadth, and decreased quantum efficiency for some species at higher temperature. We also saw that photosynthesis was highest in early successional stages, when the canopy was more open, but photosynthetic rates were lower in experimentally warmed plots. Together those results point towards an overall reduction of carbon assimilation to tropical forest ecosystems. We found that plants at our study site were adapted for early successional stages and will likely perform better than other successional stage plants in a future climate scenario with higher atmospheric temperatures and more frequent hurricane disturbances. As the plant communities stand now, the forests at our site will likely capture less carbon from the atmosphere than they do now; however, with changing communities, they could maintain the high rates of carbon assimilation as they have done historically.

# 1 Introduction

Tropical forests exchange more carbon with the atmosphere than any other terrestrial biome (Bond-Lamberty et al. 2004), but little is known about how tropical ecosystem carbon balance will respond to a warming climate (Cavaleri et al. 2015). Many studies measure plant physiological functions in tropical ecosystems, but few address how tropical plant photosynthesis and respiration will respond under experimental warming conditions (Slot et al. 2014, Slot and Winter 2017, Carter et al. 2020, Noh et al. 2020). These warming experiment responses are important because they describe how plants will acclimate to warmer temperatures. Plants exhibiting a temperature acclimation response will have different physiological processes after warming treatment than before resulting in different physiological parameters that would better inform global carbon cycle models estimating carbon cycling under future climate scenarios with higher temperatures. Furthermore, since many of the studies showing thermal acclimation have been performed in temperate ecosystems, they may not represent tropical forest functions which may already be operating at a thermal maximum (Doughty and Goulden 2008, Mau et al. 2018). Acclimation is important for long-lived sessile organisms, like shrubs and trees, because it gives them a flexible range to change their physiological functions to be better suited to changing conditions. Acclimation differs from adaptation because it encompasses the bounds of what a single organisms genetics can do, while adaptation is a population level genetic change that occurs across generations.

Plant roots can exhibit thermal acclimation of carbon processes by downregulating respiration to conserve carbon at higher temperatures. Root respiratory acclimation can be driven by decreased production of enzymes that are more efficient at higher temperatures, adenylate control, and/or substrate limitation (Atkin and Tjoelker 2003, Jarvi and Burton 2018, O'Leary et al. 2019). The ability of plants to acclimate respiratory response to higher temperatures has been observed in temperate ecosystems which have larger seasonal temperature variation (Loveys et al. 2003, Atkinson et al. 2007, Jarvi and Burton 2018), but plants in tropical ecosystems may not have the capacity to acclimate due to the narrow range of temperatures they experience (Cunningham and Read 2003, Slot and Kitajima 2015).

Similar to the respiratory responses of roots, foliar tissues can acclimate through downregulating respiratory response at higher temperatures (Atkin and Tjoelker 2003), but they can also acclimate photosynthetic systems to operate more efficiently at higher temperatures (Berry and Bjorkman 1980). This acclimation response allows plants to be competitive by fixing more carbon at higher temperatures than plants that do not acclimate. More carbon being sequestered into plants via photosynthesis will reduce atmospheric $CO_2$, resulting in a negative feedback loop that dampens atmospheric warming caused by $CO_2$ greenhouse gas effect. However, if tropical plants are already at or beyond their thermal threshold for photosynthesis (Cunningham and Read 2003, Mau et al. 2018) and not able to acclimate, a positive feedback could occur where warming causes higher $CO_2$ release through respiration and lower photosynthetic $CO_2$ fixation resulting in higher atmospheric $CO_2$ concentration that causes more warming.

With increasing frequency and intensity of heatwaves (IPCC 2018), tropical plants could be temporarily pushed past the thermal limits of photosynthetic machinery. The high temperature thermotolerance of plants is the point where the light reactive systems of photosynthesis break down. If heat-shock does not cause mortality in plants, it can diminish the photosynthetic capacity of leaves (Krause et al. 2015, Wang et al. 2016, Marias et al. 2017, Drake et al. 2018). While photosynthetic capacity can rebound following heat-shock in tropical leaves (Marias et al. 2017), leaves may operate at sub-optimal photosynthetic capacity for a period of time. However, if plants acclimate their thermotolerance response to heat-shock after long-term warming, they may able to rebound from heat-shock more quickly.

Global atmospheric warming is expected to increase intensity and frequency of tropical cyclones which can cause major disturbance events in forests (Lugo 2008, Knutson et al. 2019). Tropical cyclones, or hurricanes in the Atlantic Ocean, can alter forest canopies by defoliating trees, breaking stems, and causing tree mortality (Uriarte et al. 2019). Defoliation temporarily decreases forest carbon uptake due to loss of photosynthetic tissues, but forest productivity increases in the long-term as new growth goes into woody biomass while fallen timber is sequestered into soil (Uriarte and Papaik 2007). However, under more frequent disturbance events, defoliation can decrease productivity without sequestering more woody biomass (Ito 2010). Hurricane disturbances can cause soil nutrient pulses through fine litter deposition and changes in canopy throughfall nutrient fluxes that affect soil biogeochemical cycling (Lodge et al. 1991, Heartsill-Scalley et al. 2007, Liu et al. 2018). Canopy defoliation temporarily changes soil moisture and streamflow through diminished evapotranspiration (Scatena and Larsen 1991, Heartsill-Scalley et al. 2007). Many ecological processes disrupted by hurricane disturbance are temperature dependent and little is known of how disturbance recovery will be affected by higher atmospheric temperatures.

The work presented in this dissertation addresses the thermal and hurricane acclimation potential of several physiological parameters related to photosynthesis and respiration in plants. All of the work presented here was performed at the Tropical Responses to Altered Climate Experiment (TRACE), a tropical forest understory warming experiment in eastern Puerto Rico that warms forest understory plots +4 °C above ambient temperature. In September 2017, Puerto Rico was hit by two major hurricanes that changed the canopy dynamics at the TRACE site, giving the unique opportunity to measure the physiological responses to hurricane recovery under higher temperatures. The second chapter of the dissertation investigates the thermal acclimation potential and hurricane responses of fine-root respiration using pre- and post-hurricane root respiration measurements under warming and ambient conditions. The third chapter looks at foliar photosynthetic and respiratory responses to post-hurricane recovery under experimental warming conditions for two understory shrubs. The fourth chapter assesses the potential for tropical plants to acclimate high-heat thermotolerance to experimental warming and changing canopy during post-hurricane recovery. The fifth chapter is a synthesis of the conclusion in this dissertation and a summary of what these results mean for carbon cycling in the tropics under climatic pressures from atmospheric warming and more frequent hurricane disturbances.

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# 2 Respiratory acclimation of tropical forest roots in response to *in situ* experimental warming and hurricane disturbance

## 2.1 Abstract

Climate projections predict higher temperatures and more frequent hurricanes in the tropics. Tropical plants subjected to these stresses may respond by acclimating their physiology. We investigated tropical forest root respiratory acclimation in response to hurricane disturbance and *in situ* experimental warming in eastern Puerto Rico. We measured root specific respiration, root biomass, and root traits at the Tropical Responses to Altered Climate Experiment (TRACE), where understory vegetation is warmed +4 °C above ambient temperature. Our measurements span 5 years, including before and after two major hurricanes, to quantify root contributions to ecosystem carbon fluxes in a tropical forest. Experimental warming did not affect root specific respiration rates, but did result in decreased fine-root biomass, thereby decreasing ecosystem-level root respiration in warmed plots. Root specific respiration became more variable after the hurricane disturbance. Root respiration in this tropical forest did not acclimate to higher temperatures, but decreased root biomass indicates root contribution to soil carbon dioxide efflux may be lower than expected with warming. Lower root biomass may also limit nutrient and water uptake, having potential negative effects on carbon assimilation. Our results show that warming and hurricane disturbance have strong potential to affect tropical forest roots, as well as ecosystem carbon fluxes.

## 2.2 Introduction

Tropical forests exchange more carbon with the atmosphere than any other type of ecosystem, and tropical soils represent about 30% of soil organic carbon, globally (Jobbagy and Jackson 2000, Tarnocai et al. 2009). Both ongoing and predicted climate change have the potential to dramatically alter tropical forest carbon stocks and fluxes. For example, with higher projected global atmospheric temperatures (IPCC 2018) and increased frequency and intensity of tropical cyclone disturbances (Knutson et al. 2019), tropical plants will face environmental stresses that could alter how they use carbon for energy. This would have implications not only for carbon fluxes aboveground, but also for belowground carbon cycling, including in roots. Root respiration is estimated to account for 30%-50% of total soil carbon dioxide ($CO_2$) efflux (Bond-Lamberty et al. 2004), but is rarely explicitly measured in carbon flux studies; thus anthropogenic effects on roots may represent a large and poorly quantified feedback to global change.

Plant respiration is a temperature-sensitive process that increases exponentially with increasing measurement temperatures as enzymatic metabolism increases. In response to short-term or long-term exposure to warmer growth temperatures, plant respiration can acclimate by down-regulating either respiratory basal rates or temperature sensitivity to use carbon stores more efficiently at higher temperatures (Atkin and Tjoelker 2003). As such, with increased temperatures root respiration acclimation responses could mitigate total contributions to global soil $CO_2$ efflux, thereby decreasing the strength of the reinforcing feedback between respiratory $CO_2$ emissions and global warming (Tjoelker 2018). While the signal accuracy of carbon flux responses to warming in Earth System Models (ESMs) is improving, the role of tropical ecosystems in terrestrial carbon exchange is highly variable in those models (Cavaleri et al. 2015). Understanding the broad spectrum of root structural and functional responses, including respiration, to increased temperature will inform ESMs to better estimate future biogeochemical cycling (Warren et al. 2015).

Soil $CO_2$ efflux is primarily the respiratory byproduct of invertebrates, bacteria, fungi, and roots. Soil organisms may have different magnitudes of respiration response to higher temperatures, and parameterizing how heterotrophs and autotrophs differ in response to warming will both improve representation of soil $CO_2$ efflux in ESMs, and also enhance our understanding of ecosystem function (Subke and Bahn 2010, Hopkins et al. 2013). Root respiration can be parameterized within ESMs using temperature-dependent values of basal metabolism (e.g., $R_{25}$; respiration rate at 25 °C) or temperature sensitivity (e.g., $Q_{10}$; rate of respiration change over a 10 °C change in temperature). $R_{25}$ and $Q_{10}$ are modeled parameters derived from measuring respiration rates at different temperatures, and thermal acclimation can be characterized by a decrease in either parameter under higher growth temperatures (Atkin and Tjoelker 2003).

Ecosystems subjected to frequent disturbances can generate a “resilience debt”, which is when aspects of ecosystem function are no longer sustained due to increasing frequency of disturbance (Johnstone et al. 2016). Our study site, the Tropical Responses to Altered Climate Experiment (TRACE), a tropical wet forest understory and soil warming experiment, was hit by two major hurricanes in 2017 that knocked down much of the canopy causing a "pulse" disturbance on top of the "press" disturbance from our experimental warming. Tropical cyclones (also called hurricanes and typhoons depending on location) can reduce root biomass in the long-term even though root growth rates can increase within months following hurricane disturbance and then significantly decrease below pre-hurricane levels after 6 months (Silver and Vogt 1993). For example, 10 months after a hurricane disturbance at the TRACE site, Yaffar et al. (2021) found root biomass increased 2.8-fold in the control (unwarmed) plots, but increased only 1.6-fold in roots exposed to a year of experimental warming before the disturbance. This reduction in biomass production in previously warmed plots relative to control plots persisted for nearly a full year, despite no active warming during this time-frame. These results suggest a legacy effect of warming may have contributed to the "resilience debt" accrued through both pulse (e.g., hurricanes) and press (e.g., chronic warming) disturbances. Slower root regrowth in experimentally warmed plots after hurricanes also suggests there is the potential for a smaller root contribution to total soil $CO_2$ efflux following hurricanes in a warmer climate, if root specific respiration rates remain unchanged or heterotrophic soil respiration rates increase with warming (e.g., Wood et al. In Revision).

Very few studies have evaluated temperature effects on root respiration of tropical species. A greenhouse study of Australian tropical tree seedlings found evidence of root specific respiration acclimation in response to experimental warming for only one of eight species studied (Noh et al. 2020). An *in situ* experimental soil warming study in a Panamanian tropical forest which used root exclusion methods and soil $CO_2$ efflux partitioning found no evidence of root thermal acclimation (Nottingham et al. 2020).

However, to our knowledge, there are no studies directly measuring root specific respiration response to warming *in situ*, nor any studies showing the interacting effects of long-term warming and hurricane disturbance on tropical root respiration.

Similar to the leaf economic spectrum, where acquisitive leaf traits are associated with short-lived leaves and conservative traits are associated with long-lived leaves (e.g. Wright et al. 2004), roots exhibit a similar economic spectrum, where acquisitive roots have higher root specific respiration rates (RSR, nmol $CO_2$ $g^{-1}$ $s^{-1}$), nitrogen concentrations ($N_{root}$, %), specific root length (SRL, cm $g^{-1}$), and lower root tissue density (RTD, g $cm^{-3}$) than conservative roots (Roumet et al. 2016). Root morphological traits have been found to change in tropical seedlings when experimentally warmed by increasing both SRL and $N_{root}$, and decreasing RTD (Noh et al. 2020). These responses to warming are characteristic of roots with nutrient acquisitive strategies, and they usually result in higher respiration rates (Roumet et al. 2016). Trait responses to warming can be species-specific, but the amount of variation in morphological traits that affect root respiration can be nearly as high within a plant as among plants within the ecosystem (Paradiso et al. 2019). Overall, roots in the TRACE site became longer per unit mass (i.e., greater SRL) after the hurricanes in Puerto Rico (Yaffar et al. 2021), suggesting some plants may have switched to a more acquisitive strategy after the disturbance, or the signal may be indicative of a shift in community composition towards more acquisitive species (Kennard et al. 2020).

Here we sought to take advantage of a unique opportunity to assess the effects of warming and hurricane disturbance on root biomass, growth, respiration, acclimation, and traits. We hypothesized that (H1) long-term *in situ* experimental warming would not induce acclimation (i.e., down-regulation) of root specific respiration, and that post-hurricane root specific respiration would increase in warmed plots to compensate for lower root mass and/or pulses of available soil nutrients. We also hypothesized that (H2) ecosystem root respiration at 25 °C ($R_{25}$; scaled up using root biomass) before the hurricanes would be lower in warmed plots than control plots because of reduced root biomass under warming, and that this trend would continue after the hurricanes even when plots were not warmed due to legacy effects of the warming treatment. Finally, we hypothesized that (H3) roots would move towards a more acquisitive strategy for nutrient uptake in warmed plots and after the hurricanes through increased $N_{root}$ and SRL, and decreased root tissue density, traits which are associated with higher rates of root specific respiration.

## 2.3 Materials and Methods

### 2.3.1 Site description

This study was conducted from 2017-2020 at the Tropical Responses to Altered Climate Experiment (TRACE), a subtropical wet forest understory and soil warming experiment located in the Luquillo Experimental Forest in Puerto Rico (18.32465° N, 65.73058° W). Mean annual temperature at the site is 24 °C (Kimball et al. 2018a) and mean annual precipitation is ~3500 mm (Murphy et al. 2017). Acidic, red clay soils at the TRACE site are classified as Ultisols (Scatena 1989). The TRACE project consists of three ambient temperature (i.e., control) plots and three experimentally warmed plots, each hexagonally shaped with 4 m diameter. Experimental plots were warmed 4 °C above the average surface temperature of the control plot temperatures using infrared (IR) heaters 1-2 m above the vegetation (Kimball et al. 2018a). Ambient temperature control plots had metal plates pointing towards the plot vegetation to mimic the potential infrastructure effects of the infrared heaters. Initially after the hurricanes, the heaters were at about 2.5 m above the ground and were moved to their max height of 4 m in July 2020 as the understory vegetation height increased. Each plot had similar composition of understory plant species and were mainly composed of *Psychotria brachiata*, *Piper glabrescens*, *Guarea guidonia*, *Miconia racemosa*, *Inga vera,* and *Prestoea acuminata*.

In September 2017, two major hurricanes made landfall on Puerto Rico, causing heavy disturbance of the forest canopy and severely damaging the electrical infrastructure of the island. The TRACE warming infrastructure was repaired by July 2018, and experimental warming was restarted on September 28, 2018 after one year of post-hurricane baseline data collection without warming under new canopy conditions. There was a successional shift in plant species diversity within a year after the hurricanes, during which there was a large population of grasses in the understory (Kennard et al. 2020). Grasses and herbaceous plants were shaded out by the early successional tree *Cecropia schreberiana*, which had not been in the plots previously, within two years after the hurricanes (unpublished data). Over the course of the experiment, warming was started in September 2016, paused for a year after the hurricanes in September 2017, and restarted in September 2018. Warming has been continuous to date, except for periodic maintenance. Prior to the hurricanes, surface soils (0-10 cm; where most of the roots are located) were warmed +3.6 °C over control soils, and an average of ~+3 °C warming effect was observed at soil depths up to 50 cm (Kimball et al. 2018). After the hurricanes, however, higher understory foliage density caused the warming effect in the upper 10 cm of soil to be reduced to ~+1 °C (Fig. 2.1), and negligible warming was detected in the deeper soils.

### 2.3.2 Experimental design

For each root specific respiration campaign, we took 3 plot replicate cores for each of the 6 plots, totaling 18 cores per campaign and 9 cores per treatment. Each sampling day, from 8:00 to 17:00 AST, one core from each plot was removed and sampled in a randomized order. Cores were weighed, roots were separated from soil, and soil-free roots were measured for respiration. Two different types of root cores were removed from the plots: fresh bulk soil cores and root ingrowth cores. Fresh cores were taken by driving a 5.08 cm diameter PVC pipe into the first 10 cm of undisturbed soil. Root ingrowth cores were 10 cm deep, 5.08 cm diameter plastic tubes with 1.5 mm mesh that were placed in the hole left when each fresh core was taken. Root ingrowth cores were filled with 275 g homogenized root-free soil taken from a 10 cm -deep soil pit located just outside of the study area, which is based on mean bulk density of soils for the site (Yaffar et al. 2021). Distinction between these cores is important because roots in a root ingrowth core represent newer growth that may have higher respiration rates due to higher enzymatic activity associated with potentially higher metabolic demand required for growth, and due to differences in soil structure between the two methods. Fresh cores are more representative of the activity in the soil, while ingrowth cores allowed us to record more samples without removing too much material from the experimental plots. Fresh cores were collected in March 2017, March 2018, September 2018, and March 2019 and root ingrowth cores were collected in July 2017, March 2018, July 2018, September 2018, March 2019, November 2019, March 2020, and October 2020.

### 2.3.3 Environmental data

Hourly measurements of soil temperature and moisture in the first 10 cm was averaged among the three surface soil temperature/moisture probes (CS655, Campbell Scientific, Logan, UT) located at the edge, midway to the center, and center of each plot and then averaged over the 10 days before cores were extracted. Deeper soil temperature and moisture (20-30 cm and 40-50 cm) data were not used in this study because the focal roots were only 0-10 cm deep. Hourly vegetation temperature measured using IR thermometers (SI-121, Apogee Instruments, Logan, UT) was averaged over the 10 days before cores were extracted.

### 2.3.4 Respiration measurements

Root specific respiration measurements (nmol $CO_2$ $g^{-1}$ root $s^{-1}$) were taken on soil-free fine roots (<2 mm diameter) picked from the cores for 20 total person-minutes (two people x 10 minutes of picking). This timing was selected to minimize a decay in respiration rates before the roots were measured. After the first 10 minutes, the remaining roots were separated from the soil to measure total fine root biomass per core. Respiration was measured by placing soil-free roots in a 0.116 L aluminum cuvette equipped with a type-T temperature probe in a closed loop system in-line with a CA-10 infrared gas analyzer (IRGA; Sable System, North Las Vegas, NV, USA) and a Sable System SS-4 subsampling pump totaling 0.186 L of system volume. Data were collected from the system using a Sable System UI-3 connected to a computer with Sable Systems Expedata software. Flow rate for the system was set to 400 ml min$^{-1}$ with the Sable Systems SS-4. Data collection was initiated when the system was at stable temperature, and %$CO_2$ and air temperature within the cuvette were measured for 5 minutes using Expedata software.  Respiration rates were measured at three different temperatures (23 °C, 25 °C, and 30 °C) to generate a temperature response curve from which temperature sensitivity parameters were extracted. Root respiration was initially measured at 25 °C for each core and then measured at either 23 °C or 30 °C before finally being measured at the remaining temperature of 23 °C or 30 °C. Root temperature was controlled by placing root cuvettes in insulated lunchbox coolers filled with water near the target temperatures. Air temperature within the cuvette was controlled with addition of hot water or ice to the water bath to heat or cool the cuvette air, respectively. Following each measurement, roots were removed from the cuvette and placed in the next cuvette at the following temperature in the sequence. All respiration measurements were taken within an hour of the soil cores being removed from the plots.

Respiration rate was converted from $\Delta\%CO_2$ s$^{-1}$ to nmol $CO_2$ s$^{-1}$ by multiplying $\Delta\%CO_2$ s$^{-1}$ by the nmol of air in the system, which was calculated as follows:

$$\text{nmol CO}_2\ \text{s}^{-1} = \Delta\%\text{CO}_2\ \text{s}^{-1} * \left(\frac{PV}{RT}\right) * 10^{-9}\text{nmol mol}^{-1} \qquad \text{Eq. 1}$$

where $P$ is pressure (atm), $V$ is system volume (L), $T$ is measurement temperature (K), and $R$ is the ideal gas constant (0.0821 atm L $mol^{-1}$ $K^{-1}$). Root specific respiration (nmol $CO_2$ $g^{-1}$ $s^{-1}$) was calculated as the respiration rate divided by the dry mass of roots used in the measurement. Within the hour when roots are being measured, root respiration rate decays, therefore a correction factor was applied to respiration response. Corrected respiration rate (*CRR*; nmol $CO_2$ $g^{-1}$ $s^{-1}$) was calculated as follows:

$$CRR = RR + t * c \qquad \text{Eq. 2}$$

where *RR* is measured respiration rate (nmol $CO_2$ $g^{-1}$ $s^{-1}$), *t* is seconds since roots were extracted from ground (s), and *c* is a coefficient of 0.6107 (nmol $CO_2$ $g^{-1}$). The correction coefficient was calculated in a pilot study by repeating measurements on several sets of roots at the same temperature over two hours during which respiration rate decayed (data not shown).

Root specific respiration temperature response parameters were calculated by fitting *CRR* values from each core to a curve using a non-linear least squares regression with the ‘nls’ function in R statistical language (R Core Team 2020) with the equation below:

$$CRR = \beta_0 * \ e^{\beta_1 T} \qquad \text{Eq. 3}$$

where $T$ is measurement temperature and $\beta_0$ and $\beta_1$ are modeled parameters used for extracting $R_{25}$ and $Q_{10}$. For each temperature curve, $Q_{10}$ (unitless) was calculated using the equation:

$$Q_{10} = e^{\beta_1 * 10} \quad \text{Eq. 4}$$

and $R_{25}$ (nmol $CO_2$ $g^{-1}$ $s^{-1}$) was calculated using the equation:

$$R_{25} = \beta_0 * e^{\beta_1 * 25} \quad \text{Eq. 5}$$

Area-based estimations of root specific respiration (ecosystem root respiration, g $m^{-2}$ $d^{-1}$), were scaled up from root biomass per unit ground surface area of 10 cm deep cores. Ecosystem root respiration at 25 °C was calculated as:

$$Ecosystem\ root\ respiration = \frac{biomass}{A} * R_{25} \quad \text{Eq. 6}$$

where *biomass* is the core total fine root biomass (g), and *A* is the area of the upper surface of each core ($2.0268 \times 10^{-3}$ $m^2$).

### 2.3.5 Root trait measurements and soil nutrients

After respiration measurements, living roots were sorted from dead roots, imaged with an EPSON Perfection V800 Scanner containing an acrylic tray filled with DI water, and analyzed for root length, diameter, surface area, and volume using WinRHIZO software (Regent Instruments Inc., Quebec, Canada) to calculate SRL (cm $g^{-1}$), mean diameter (mm), and RTD (g $cm^{-3}$). Living and dead roots were dried separately at 65 °C for 48 hours and weighed to determine total dry biomass.

Roots were measured for percent carbon and nitrogen, and soil from the collection cores was subsampled for soil ammonium ($NH_4^+$, µg g dry soil$^{-1}$), nitrate ($NO_3^-$, µg g dry soil$^{-1}$), and extractable phosphate ($PO_4^{3-}$, µg g dry soil$^{-1}$). Dried roots used for respiration and morphological trait measurements were ground in a ball-mill (SPEX 8000D, SPEX Sample Prep, Metuchen, NJ, USA) and analyzed for percent carbon and nitrogen on an elemental analyzer (Elementar Vario Micro Cube, Elementar Inc., Langenelsbold, Germany). Core soil extractable $NH_4^+$ and $NO_3^-$ were assessed on fresh soils the same day as collection by extracting soil with 2 N KCl with shaking for 1 hour, allowing extracts to sit overnight, filtering through Whatman #1 filter paper (GE Healthcare, Chicago, IL), freezing immediately, and storing frozen until analysis (based on Robertson et al. 1999 as described in Reed et al. 2008). Extracts were measured using an indophenol blue and cadmium reduction colorimetric assay on a discrete analyzer (Unity Scientific Smartchem 200 Discrete autoanalyzer, Milford, MA). Soil Bray-extractable $PO_4^{3-}$ was extracted by shaking fresh soil with Bray solution (dilute HCl and $NH_4F$) for 1 minute (Bray and Kurtz 1945). Bray extractions were filtered through Whatman #1 filter paper, frozen, and stored frozen until analysis. Extracts were measured with an ascorbic acid molybdate colorimetric analysis on a SmartChem 200 Discrete Analyzer. All extracts were shipped frozen from Puerto Rico to an analytical chemistry laboratory in Moab, UT (US Geological Survey Southwest Biological Science Center).

### 2.3.6 Statistical analyses

Root specific respiration temperature curve parameters ($R_{25}$, $Q_{10}$), root biomass per unit ground area, ecosystem root respiration, and specific root length relationships with warming treatment effect and hurricane effects were analyzed separately for root ingrowth and fresh cores using two-way ANCOVAs, where sampling campaign was used as a time proxy for hurricane effect. Post-hoc Tukey HSD tests were used to determine individual relationships when there was statistical significance in ANCOVAs. $R_{25}$ relationships with root trait measurements (specific root length, root tissue density, $N_{root}$, root mean diameter) and soil nutrients ($NH_4^+$, $NO_3^-$, $PO_4^{3-}$) for root ingrowth versus fresh core measurements were analyzed with linear models that had the independent variable and core type as interacting variables. All statistical analyses were performed using base R 4.1.2 (R Core Team 2022).

## 2.4 Results

### 2.4.1 Root specific respiration

Root specific respiration did not acclimate in response to experimental warming. We did, however, find evidence that root specific respiration in ingrowth cores increased immediately following hurricane disturbance effect, then returned to pre-hurricane rates. There were no significant differences in $R_{25}$ or $Q_{10}$ between control and warmed plots, indicating there was no treatment effect before the hurricanes with warming turned on, no legacy effect from warming during regrowth with warming turned off, and no treatment effect during regrowth with warming turned on following hurricane disturbance (Fig. 2.2 Table 2.1). $Q_{10}$ was no different across campaigns for either core type (Fig. 2.2b); however, there were differences in $R_{25}$ between sampling campaigns following the hurricanes for roots in root ingrowth cores, which had higher $R_{25}$ in March 2018 than in

September 2018 (Fig. 2.2a; Table 2.1). $R_{25}$ did not correlate with plot vegetation temperature, soil surface temperature, or soil surface volumetric water content (Table 2.2), suggesting the root specific respiration at our site was independent from seasonality of temperature and precipitation. Furthermore, a lack of soil temperature effect on $R_{25}$ is support that there is no thermal acclimation of $R_{25}$ at our site.

### 2.4.2 Fine root biomass and ecosystem root respiration

While root specific respiration showed no treatment effect, both fine root biomass in the upper 10 cm and ecosystem root respiration at a standard temperature of 25 °C (i.e., fine root respiration per unit ground area in the upper 10 cm), showed statistically significant responses to the experimental warming treatment in the fresh cores. Ingrowth cores showed no effect of either treatment or campaign on root biomass or ecosystem root respiration (Fig. 2.3, Table 2.1). In the fresh cores, however, root biomass was lower in warmed plots than control plots, even post-hurricane when warming was not active, as there were no significant interactions of treatment and sampling campaign (Fig 2.3a., Table 2.1). Ecosystem root respiration of fresh cores showed a marginally significant interaction between treatment and campaign, where rates were lower in warmed plots compared to control in the pre-hurricane campaign only (Fig. 2.3b, Table 2.1).

### 2.4.3 Relationships between root specific respiration, root traits, and soil nutrients

Root specific respiration correlated with some root traits but not with soil nutrient variables. $R_{25}$ increased with increasing fine root $N_{root}$ in root ingrowth cores but not in fresh cores (Fig. 2.4a, Table 2.2). $R_{25}$ was not significantly correlated with specific root length (Fig. 2.4b) or root mean diameter (Fig. 2.4c) but was negative correlated with root tissue density for both types of cores (Fig. 2.4d, Table 2.2). $R_{25}$ did not correlate with soil $PO_4^{3-}$, $NO_3^-$ , $NH_4^+$, total soil N, total soil C, or soil C:N for either core type (Fig. 2.5, Table 2.2). SRL increased dramatically for approximately 9 months following the hurricanes and then returned to pre-hurricanes levels within 15 months for both fresh and root ingrowth cores (Fig. 2.6a, Table 2.3).

## 2.5 Discussion

### 2.5.1 Root respiration response to warming

Pre-hurricane root specific respiration did not show a down-regulation under the warming treatment through mechanistic shifts in $R_{25}$ or $Q_{10}$, partially supporting H1 that long-term warming would not induce acclimation of root specific respiration. However, we also predicted that post-hurricane root specific respiration would increase in warmed plots, which did not occur. We expected lower root biomass and higher nutrient pulses in warmed plots to cause higher root specific respiration rates post-hurricane (H1). While we did observe lower root biomass in the warmed plots for fresh cores, and post-hurricane soil nutrient pulses were found to be greater in warmed plots (Reed et al. 2020), these trends did not lead to higher metabolic demand for root respiration per unit root mass. Respiratory thermal acclimation is well studied in leaves (Atkin and Tjoelker 2003, Loveys et al. 2003, Slot and Kitajima 2015, Carter et al. 2020) but is represented by only a few studies with conflicting results on thermal acclimation potential for roots. In temperate hardwood forest warming experiments, ecosystem fine-root respiration has been observed to acclimate to warming through down-regulation of root biomass (Melillo et al. 2011) and through down-regulation of root specific respiration at a standard measurement temperature of 18 °C (Jarvi and Burton 2013). Loveys et al., (2003) did not observe root respiratory acclimation for greenhouse-grown temperate herbaceous plants, though they did see foliar thermal respiratory acclimation; while Atkinson et al. (2007) found no change in root $Q_{10}$ for greenhouse-grown temperate herbaceous plants. Thermal root respiratory acclimation was observed in a greenhouse study of sub-tropical citrus plants (Bryla et al. 1997) and in one of four tropical species in a greenhouse study (Noh et al. 2020). There was no evidence of thermal acclimation of root respiration in an *in situ* soil warming experiment in a tropical ecosystem in Panama (Nottingham et al. 2020).

Prior to the 2017 hurricanes, warmed plots maintained much lower root biomass for fresh cores, showing only half as much root biomass as the unwarmed controls (Fig. 2.3a). This lower root biomass translated into much lower ecosystem root respiration rates (i.e., per unit ground area; Fig. 2.3b). This warming effect on root biomass and thus root ecosystem respiration was not seen in the root ingrowth cores, suggesting it was not warming’s effect on root growth capacity that caused the response. We expected warming to decrease ecosystem root respiration rates compared to control plots due to lower fine root biomass under warming (H2), and this hypothesis was partially supported by our data for pre-hurricane fresh cores. However, H2 was not supported post-hurricane because ecosystem root respiration was not affected by previous warming for root ingrowth or fresh cores when warming was not active, suggesting there was no legacy effect from warming, or that the treatment effect on root biomass was not strong enough to be detected in our ecosystem root respiration response. While there was no difference in ecosystem root respiration for post-hurricane fresh cores when warming was active, there appears to be an emerging trend where control plots had a higher ecosystem root respiration rate. As succession proceeds, we predict to see this trend towards the values we observed for pre-hurricane warmed cores. While not statistically different, estimated means of ecosystem root respiration were about 50% lower in the warmed plots than ambient temperature plots after the hurricanes when warming was turned back on because the warming treatment caused root biomass to decrease, but $R_{25}$ had higher values and more variation for both control and warmed plots.

Overall, we found that ecosystem root respiration was lower in experimentally warmed plots, which is contrary to results from another *in situ* tropical warming experiment that did not see a difference in root respiration contributions to total soil respiration for warmed plots (Nottingham et al. 2020). The difference between our observed reduction in ecosystem root respiration and lack of difference observed by Nottingham et al. (2020) was likely attributed to our direct measurement of root respiration and biomass, while the Nottingham et al. (2020) study used indirect measurements of root respiration contributions to soil respiration via the use of root exclusion methods. If the root biomass changes in the Nottingham et al. (2020) study are consistent with our observations, it is likely that the heterotrophic contributions to overall soil respiration were higher than observed in our study. Higher heterotrophic respiration could be indicative of higher nutrient turnover by microbial processes that could be leveraged by plants producing fewer roots that can uptake more nutrients with less biomass. Microbial biomass for C and N is higher in warmed plots when warming is active, but there is no effect on microbial C and N after the hurricanes when warming was not active (Reed et al. 2020).

If this is not an acclimation response, but rather a stress response, there could be indirect, detrimental effects on other plant processes like photosynthesis, water uptake, and nutrient acquisition. The TRACE warming experiment includes understory vegetation warming which can indirectly influence belowground biomass allocation and belowground $CO_2$ efflux. A limitation on photosynthetic uptake due to warming, which occurred for our understory shrubs (Carter et al. 2020), may result in belowground root respiratory substrate limitation because of the linkages between above and belowground fluxes in tropical systems (Gutiérrez del Arroyo and Wood 2020, 2021). However, it was probably more likely that our patterns were driven by a limitation in root growth rather than respiratory substrate limitation, as we found no treatment effects in root specific respiration, but we did find a down-regulation of ecosystem root respiration which was largely driven by root biomass. Decreased fine root biomass could also limit water uptake which would decrease chances of survival during periods of soil water deficit. In addition, lower root biomass could decrease nutrient uptake, limiting the potential for aboveground plant structures to assimilate $CO_2$.

Experimentally warmed temperate species have been found to allocate more carbon to belowground biomass than ambient temperature plants, and the effect sizes of increased fine root biomass in warmed plants decrease with decreasing latitude, meaning warming affects root biomass production less the closer you get to tropics (Wang et al. 2021). Experimental warming in temperate ecosystems can make temperatures closer to the photosynthetic temperature optimum, thereby increasing potential carbon fixation. In contrast, tropical plants are already at or near their photosynthetic temperature optima (Doughty and Goulden 2008, Mau et al. 2018). This is important for contextualizing our root results, which show warming-induced decreases in biomass are contrary to the trend for non-tropical plants. Taken together, these temperate and tropical patterns suggest belowground carbon allocation may be mediated by total photosynthate production even in a warming world.

### 2.5.2 Hurricane disturbance response

Increased root specific respiration for root ingrowth cores immediately after hurricanes followed by a decrease several months later is likely caused by a quickly changing plant community composition after the hurricanes. The root ingrowth cores contained only new root growth, so they were likely dominated by the most recently grown plants. Our field site was about 70% bare ground, 20% herbaceous cover, and 10% woody cover before the hurricanes and shifted to 6% bare ground, 70% herbaceous cover, and 14% woody cover in September 2018, about a year after the hurricanes (Kennard et al. 2020). Furthermore, plant community composition shifts causing respiration shifts is supported by changes in other root dimensional traits like root tissue density and specific root length because herbaceous plants are faster living and are likely associated with more acquisitive root traits that typically have higher root specific respiration.

Independent of warming or hurricane effects, $R_{25}$ was about 1.7 times higher in root ingrowth cores than fresh cores (Fig. 2a), suggesting that new root growth had higher metabolic demand than established roots had for maintenance and nutrient acquisition. Additionally, root ingrowth core $R_{25}$ was more variable after the hurricanes, possibly due to species-specific differences in $R_{25}$ and the dynamic changes in plant communities following disturbances. During the September 2018 campaign, low $R_{25}$ values could be due to the plots being dominated by grasses while all other campaigns had lower grass density (Kennard et al. 2020). Root ingrowth cores had higher variability following hurricanes, while fresh cores remained constant following hurricanes indicating that newly developed fine root $R_{25}$ was more variable than established fine root $R_{25}$. Higher $R_{25}$ variability in new fine roots, but not established roots, could indicate shifts in community composition. Though both root ingrowth and fresh cores were subjected to the same successional changes, root ingrowth cores were composed of strictly new growth, while fresh cores represented a mix of established roots and new roots. Additionally, root ingrowth cores contained the same homogenized soil that contained similar microbial communities and aggregates were broken up with no large rocks or other obstructions while fresh cores were intact undisturbed soils that had been "incubating" in place for years.

Higher root specific respiration in root ingrowth cores than fresh cores suggest root ingrowth cores have higher respiratory demand associated with new root growth or nutrient uptake while fresh cores are more associated with maintenance respiration. Post-hurricane variation of root specific respiration for root ingrowth cores but not fresh cores suggest the variation in root specific respiration is mainly influenced by new growth, and maintenance respiration is relatively unchanged. Our data suggest the "resilience debt" (i.e., higher disturbance frequency leading to ecosystem dysfunction) for roots is more strongly affected by warming than hurricanes because the biomass in the fresh cores from the control plots recovered to pre-hurricane levels, while the biomass in the warmed plots was unchanged after the hurricanes, and was consistently lower than biomass in the control plots.

### 2.5.3 Relationships Between Root Respiration, Root Traits, and Soil Nutrients

We expected a nutrient pulse after the hurricanes to cause roots to switch to more acquisitive strategies for nutrient uptake (H3). Root specific respiration, however, was apparently not affected by extractable soil $NO_3^-$, $NH_4^+$, or $PO_4^{3-}$. Root biomass did not increase after the hurricanes, but our temporal resolution may not have been fine enough to capture the sharp peak in root biomass immediately after hurricane disturbance observed in other studies (Silver and Vogt 1993, Yaffar et al. 2021). H3 was partially supported in that $R_{25}$ showed a positive relationship with $N_{root}$, and a negative relationship with root tissue density, which are both indicators that the higher respiration roots were exhibiting more acquisitive root traits and lower respiration roots were more conservative (Roumet et al. 2016). While some of the higher respiration from root ingrowth cores could be a product of a more acquisitive strategy, much of it is likely from new root growth, which also has high metabolic demand and is associated with higher N concentration for growth. Specific root length increased following the hurricanes, while root specific respiration remained constant, indicating root morphology changed to make more efficient use of energy by increasing root length, while still using the same amount of energy per unit mass.

### 2.5.4 Conclusions

Overall, we did not observe acclimation of tropical forest root respiration to experimental warming through direct controls over respiratory mechanisms, but we did see ecosystem root respiratory acclimation through decreased fine-root biomass in the upper 10 cm of soil. Decreased root biomass was likely driven by experimental warming, though it is unclear if the warming effect was caused by above or belowground effects of warming. While the hurricanes increased the variability we observed in many of our responses, most variables appeared to return to pre-hurricane levels within two years. Hurricanes make it challenging to study the effects of warming alone, but offer valuable insight into root responses to disturbance. Lower ecosystem root respiration in a warmer world means that tropical roots may have a smaller contribution to atmospheric $CO_2$ than they do now, but decreased root biomass could have downstream implications for aboveground processes caused by potential changes in nutrient and water uptake. We speculate these changes could result in decreased $CO_2$ assimilation through photosynthesis, which could be confirmed through modeling net ecosystem productivity.

## 2.6 Tables and Figures

Table 2.1: ANOVA table for fresh and root ingrowth cores with Treatment, Campaign, and their interaction as independent variables. Statistically significant p values are denoted with bold text. $R_{25}$ is root specific respiration (nmol g root$^{-1}$ s$^{-1}$), $Q_{10}$ is temperature sensitivity (unitless), root biomass (g), and ecosystem root respiration is estimated root respiration based on $R_{25}$ and root biomass (g C g root$^{-1}$ d$^{-1}$).

| Core Type | Factor | $R_{25}$ | | $Q_{10}$ | | Root Biomass | | Ecosystem Root Resp | |
|---|---|---|---|---|---|---|---|---|---|
| | | df | P | df | P | df | P | df | P |
| Root Ingrowth | Treatment | 1 | 0.82 | 1 | 0.98 | 1 | 0.25 | 1 | 0.67 |
| | Campaign | 7 | **0.013** | 7 | 0.30 | 7 | 0.21 | 7 | 0.070 |
| | Treatment:Campaign | 7 | 0.12 | 7 | 0.83 | 7 | 0.39 | 7 | 0.23 |
| Fresh Cores | Treatment | 1 | 0.29 | 1 | 0.14 | 1 | **<0.001** | 1 | **<0.001** |
| | Campaign | 3 | 0.29 | 3 | 0.33 | 3 | 0.17 | 3 | 0.10 |
| | Treatment:Campaign | 3 | 0.35 | 3 | 0.99 | 3 | 0.13 | 3 | 0.051 |

Table 2.2: Linear models of $R_{25}$ response to root traits, soil chemistry, and temperature/soil moisture. Each value is the p value for the $R_{25}$ response to the independent variable in the first column. Core type indicates a difference between root ingrowth and fresh cores and the interaction indicates $R_{25}$ having a differential response to the independent variable between core types.

| | | Independent Var | Core Type | Var:Core Type |
|---|---|---|---|---|
| Root Traits | $N_{root}$ % | **<0.001** | 0.052 | **<0.001** |
| | Specific root length (cm $g^{-1}$) | 0.93 | **0.005** | 0.92 |
| | Root tissue density (g $cm^{-3}$) | **0.002** | 0.09 | 0.32 |
| | Root mean diameter (mm) | 0.25 | 0.37 | 0.63 |
| Soil Chemistry | Soil $PO_4^{-3}$ (µg $g^{-1}$) | 0.71 | **0.005** | 0.78 |
| | Soil $NO_3^-$ (µg $g^{-1}$) | 0.12 | **<0.001** | 0.25 |
| | Soil $NH_4^+$ (µg $g^{-1}$) | 0.95 | **<0.001** | 0.45 |
| | Soil Carbon % | 0.62 | 0.49 | 0.83 |
| | Soil Nitrogen % | 0.76 | 0.77 | 0.95 |
| | Soil C:N | 0.45 | 0.35 | 0.45 |
| Environ-mental | Vegetation Temperature (˚C) | 0.18 | 0.75 | 0.87 |
| | Soil Temperature (˚C) | 0.33 | 0.68 | 0.44 |
| | Soil Moisture ($cm^3$ $cm^{-3}$) | 0.77 | 0.73 | 0.54 |

Table 2.3: ANOVA table for fresh and root ingrowth cores with Treatment, Campaign, and their interaction as independent variables. Statistically significant p values are denoted with bold text. SRL is specific root length (cm $g^{-1}$), RTD is root tissues density (g $cm^{-1}$), and $N_{root}$ is nitrogen content (%).

| | | SRL | | RTD | | $N_{root}$ | |
|---|---|---|---|---|---|---|---|
| Core Type | Factor | df | P | df | P | df | P |
| Root Ingrowth | Treatment | 1 | 0.06 | 1 | 0.80 | 1 | 0.48 |
| | Campaign | 7 | **<0.001** | 7 | **0.046** | 7 | **0.004** |
| | Treatment:Campaign | 7 | **0.047** | 7 | 0.52 | 7 | **<0.001** |
| Fresh Cores | Treatment | 1 | 0.35 | 1 | 0.83 | 1 | **0.044** |
| | Campaign | 3 | **<0.001** | 3 | 0.17 | 3 | **<0.001** |
| | Treatment:Campaign | 3 | 0.27 | 3 | 0.81 | 3 | 0.29 |

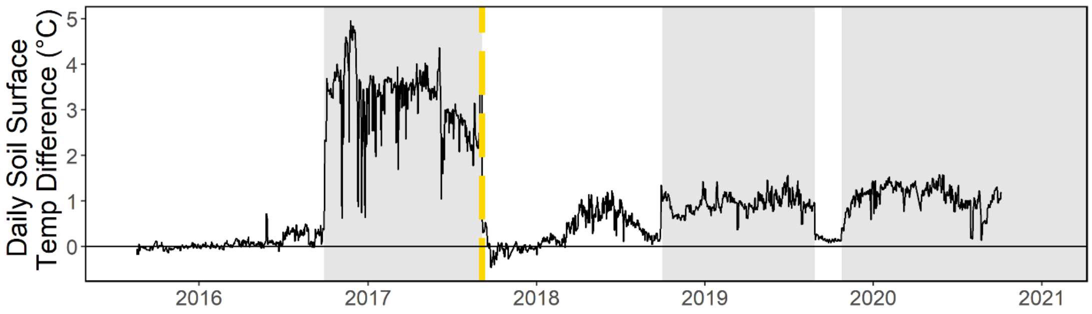

Figure 2.1: Daily difference between the means of soil temperature in the upper 10 cm of soil for warmed and control plots. Shaded blocks show where warming was turned on and white blocks show where warming was not turned on. Hurricanes occurred at dashed yellow lines.

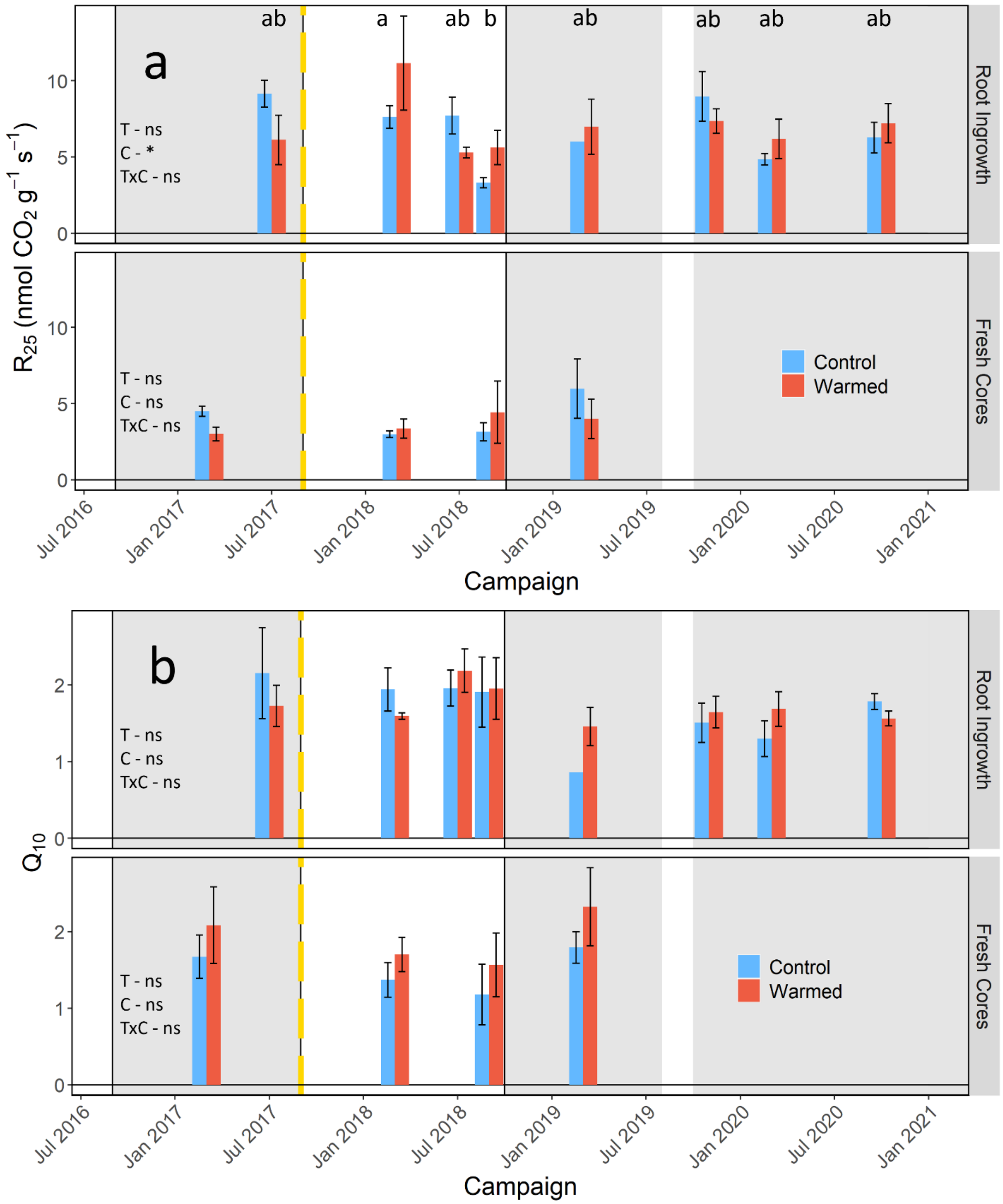

Figure 2.2: $R_{25}$ (2a) and $Q_{10}$ (2b) for control and warmed plots during each sampling campaign. Root ingrowth campaigns are in the top panel and fresh core campaigns are in the bottom panel for each plot. Shaded blocks show where warming was turned on and

white blocks show where warming was not turned on. Hurricanes occurred at dashed yellow lines. The legend in the bottom left shows where there is statistical significance for treatment (T), campaign (C), and treatment x campaign interaction (TxC) in an ANCOVA. Characters indicate statistical significance between campaigns.

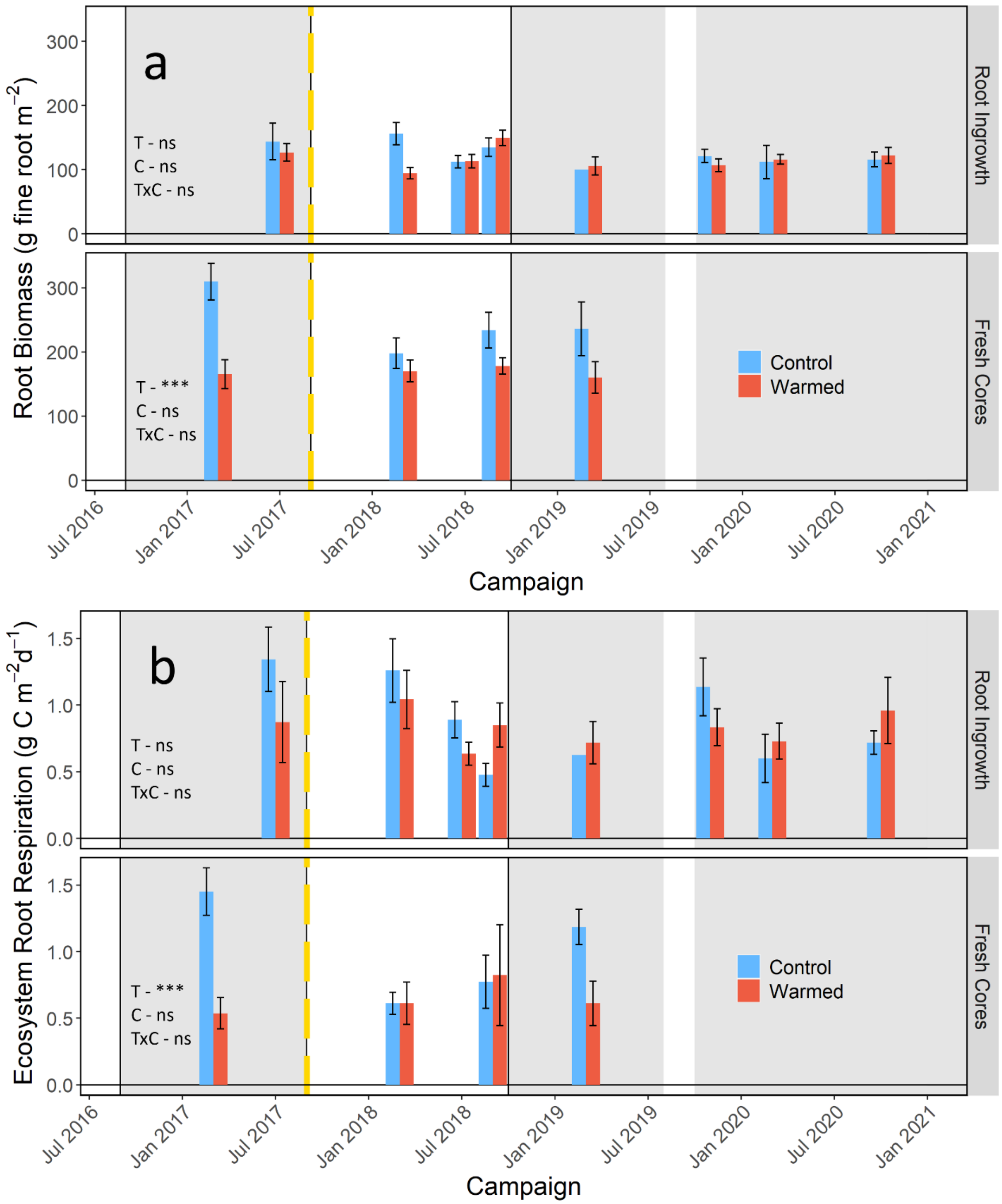

Figure 2.3: Root biomass per unit ground area in the upper 10 cm of soil (3a) and estimated fine ecosystem root respiration at 25 °C per unit ground area for control and warmed plots during each sampling campaign (3b). Root ingrowth campaigns are in the

top panel and fresh core campaigns are in the bottom panel for each plot. Shaded blocks show where warming was turned on, and white blocks show where warming was not turned on. The legend in the bottom left shows where there is statistical significance for treatment (T), campaign (C), and treatment x campaign interaction (TxC) in an ANCOVA.

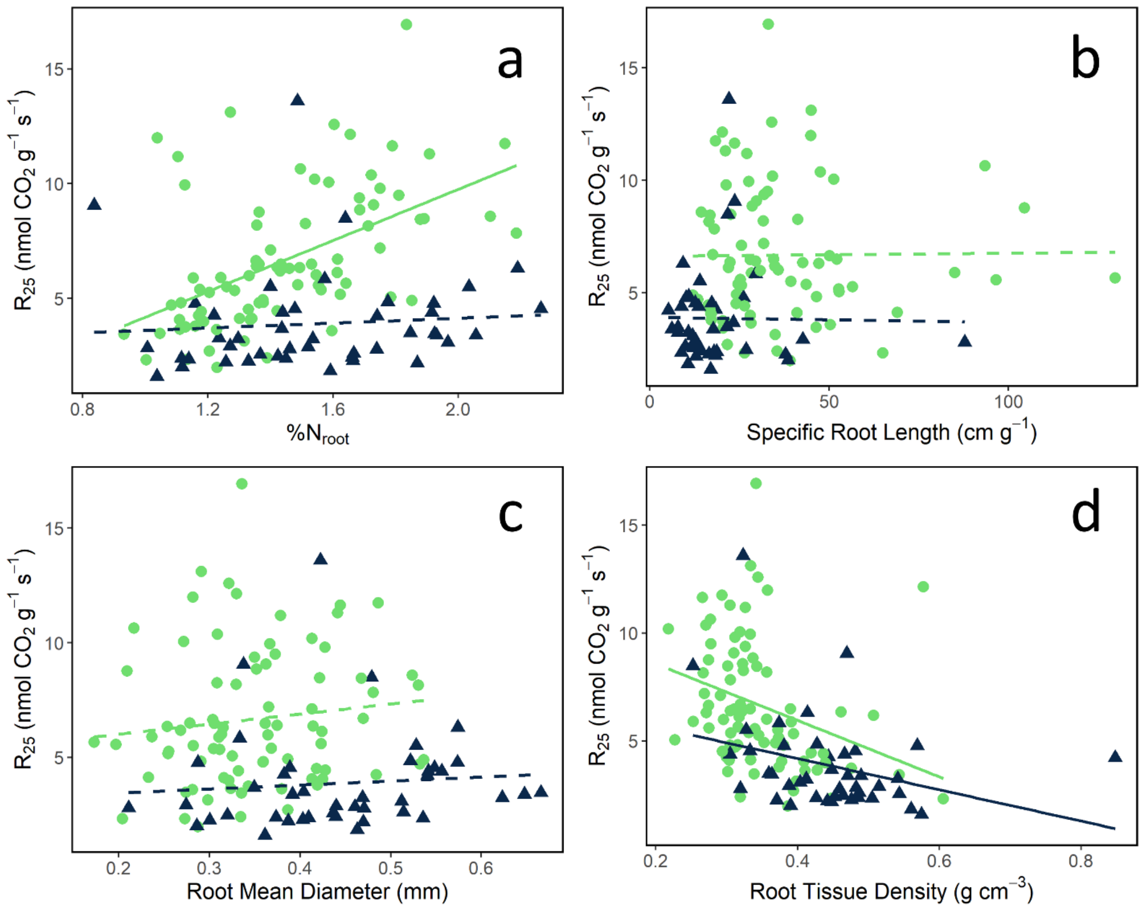

Figure 2.4: $R_{25}$ relationships with root nitrogen content (4a), specific root length (4b), root mean diameter (4c) and root tissue density (4d). Root ingrowth cores are denoted by green circles and fresh cores are denoted by blue triangles. Solid lined indicate a significant correlation and dashed lines are not significant. $R_{25}$ increased with higher root nitrogen content and decreased with higher root tissue density. $R_{25}$ was not affected by root length or diameter.

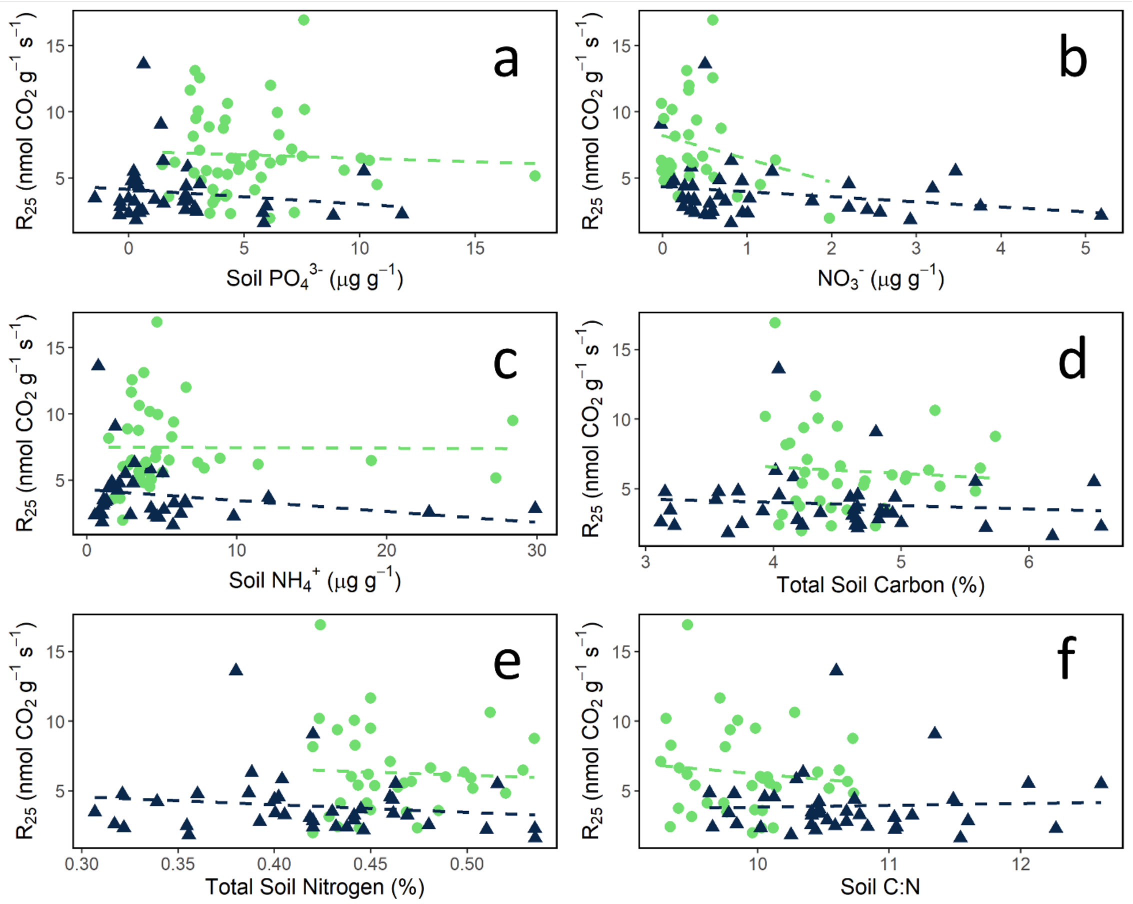

Figure 2.5: $R_{25}$ relationships with soil phosphate (5a), soil nitrate (5b), soil ammonium (5c), total soil carbon (5d) total soil nitrogen (5e) and soil C:N (5f). Green circles are root ingrowth cores and blue triangles are fresh cores. Solid lined indicate a significant correlation and dashed lines are not significant. The were no significant relationships between $R_{25}$ and soil nutrients.

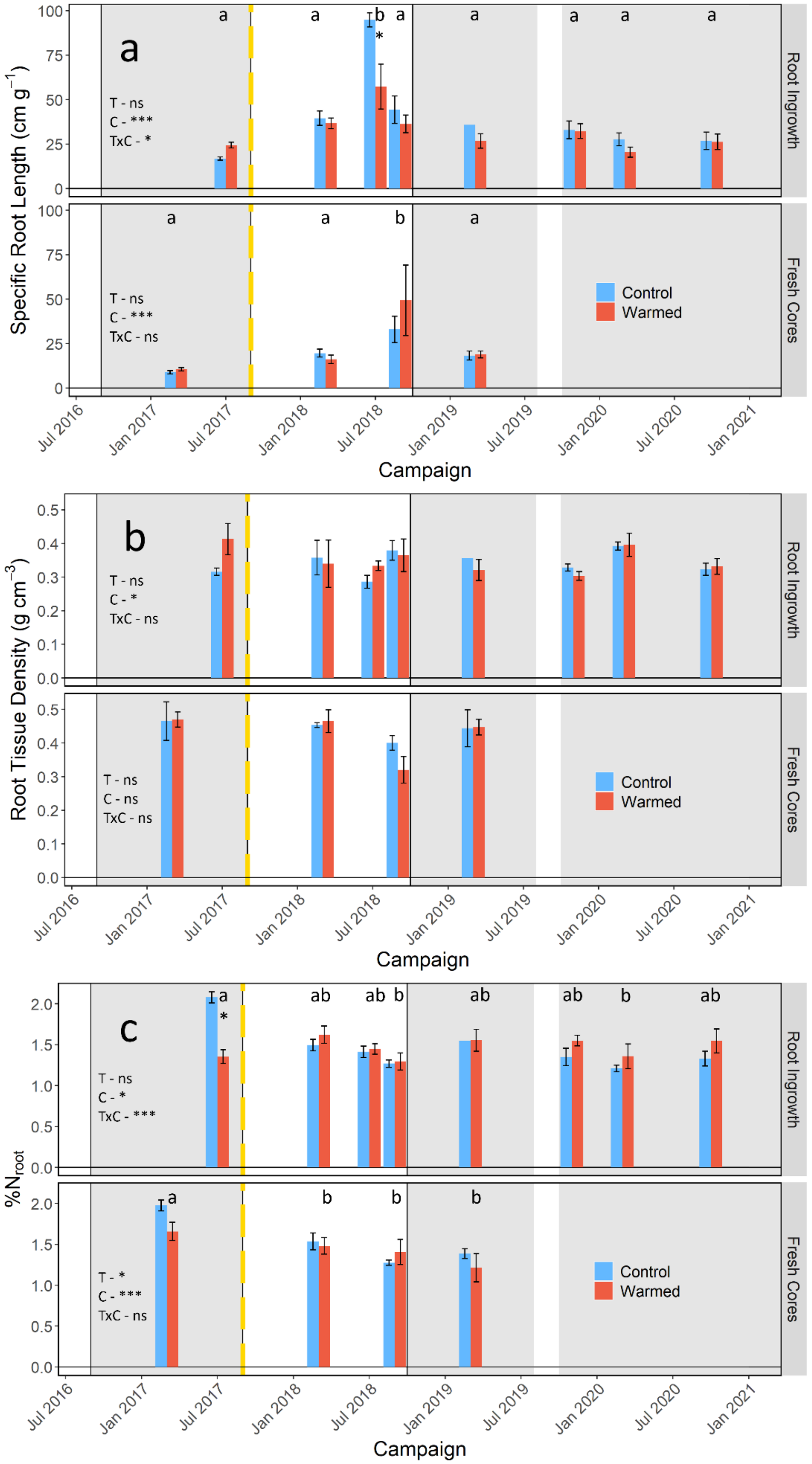
a
Specific Root Length (cm $g^{-1}$)
100
75
50
25
0
T - ns
C - ***
TxC - *
a
a
b
*
a
a
a
a
a
Root Ingrowth
T - ns
C - ***
TxC - ns
a
a
b
a
Fresh Cores
Control
Warmed
Jul 2016
Jan 2017
Jul 2017
Jan 2018
Jul 2018
Jan 2019
Jul 2019
Jan 2020
Jul 2020
Jan 2021
Campaign
b
Root Tissue Density (g $cm^{-3}$)
0.5
0.4
0.3
0.2
0.1
0.0
T - ns
C - *
TxC - ns
Root Ingrowth
T - ns
C - ns
TxC - ns
Fresh Cores
Control
Warmed
Jul 2016
Jan 2017
Jul 2017
Jan 2018
Jul 2018
Jan 2019
Jul 2019
Jan 2020
Jul 2020
Jan 2021
Campaign
c
%$N_{root}$
2.0
1.5
1.0
0.5
0.0
T - ns
C - *
TxC - ***
a
*
ab
ab
b
ab
ab
b
ab
Root Ingrowth
T - *
C - ***
TxC - ns
a
b
b
b
Fresh Cores
Control
Warmed
Jul 2016
Jan 2017
Jul 2017
Jan 2018
Jul 2018
Jan 2019
Jul 2019
Jan 2020
Jul 2020
Jan 2021
Campaign

Figure 2.6: SRL (6a), RTD (6b), and $N_{root}$ (6c) for control and heated plots during each sampling campaign. Root ingrowth campaigns are in the top panel and fresh core campaigns are in the bottom panel for each plot. Shaded blocks show where warming was turned on and white blocks show where warming was not turned on. Hurricanes occurred at dashed yellow lines. The legend in the bottom left shows where there is statistical significance for treatment (T), campaign (C), and treatment x campaign interaction (TxC) in an ANCOVA. Characters indicate statistical significance between campaigns.

## 2.7 References

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# 3 Tropical understory photosynthetic and respiratory responses to long-term experimental warming and hurricane disturbance recovery in a Puerto Rico Forest

## 3.1 Abstract

As global atmospheric temperature rises, it exerts temperature pressures on plants that alter their physiological functions. While temperate species, which experience higher variability in seasonal temperature than tropical plants, can acclimate photosynthetic and respiratory functions to higher temperatures, less is known about the potential for tropical plants to thermally acclimate. Furthermore, climate change is predicted to increase the intensity and frequency of hurricanes, which, in combination with higher temperatures, could further alter plant physiological processes. Plants that can acclimate to both of these stressors are more likely to persist during rapid climatic changes. The degree to which understory plants can acclimate to temperature dependent climate changes drives how understory plants contribute to understory carbon processes at higher atmospheric temperatures.We assessed the potential for tropical plants to acclimate physiological processes to experimental warming and hurricane disturbance response using an *in situ* tropical understory warming experiment in eastern Puerto Rico called the Tropical Responses to Altered Climate Experiment (TRACE), which was subjected to two hurricanes in 2017. We measured photosynthetic and respiratory responses of two

tropical understory shrubs in warmed and ambient temperature plots over 4 campaigns spanning 2 years. We found there are some indicators that plants at our site can thermally acclimate photosynthetic and respiratory responses to experimental warming. We also observed that both of our study species were able to acclimate to hurricane disturbance. Though there were signs of thermal acclimation, the overall photosynthetic rate was lower in the warmed plots showing an overall net reduction in carbon assimilation potential. One species, *Piper glabrescens*, underwent a mortality event after the hurricanes in only the warmed plots, suggesting that the pressures of both warming and hurricane disturbance may put some species at risk of mortatlity. Together, these show that the carbon assimilation potential of tropical forests may be diminished in a changing climate and the acclimation we see is likely a short-term solution for a long-term problem.

## 3.2 Introduction

The contribution of tropical ecosystem carbon balance to global carbon cycling is largely understudied (Cavaleri et al. 2015) even though tropical forests represent 15% of terrestrial land area (Pan et al. 2013). Given that climate change models predict an increase in atmospheric temperatures and more frequent and intense hurricanes within the next century (IPCC 2018), elucidating the responses of these ecosystems to warming and disturbance is becoming increasingly important. Ecosystems assimilate atmospheric carbon dioxide ($CO_2$) via autotrophic photosynthesis and release $CO_2$ to the atmosphere through autotrophic and heterotrophic respiration. The balance of photosynthesis and respiration is what defines the net productivity of an ecosystem. Biological carbon exchange with the atmosphere involves many temperature-dependent processes, therefore ecosystem carbon balance is dependent on the metabolic constraints of the organisms in them. Individual organisms that can change their metabolic function to operate more efficiently at different temperature are able to acclimate, whereas populations of organisms that change function to operate more efficiently at different temperatures are adapting though population-level genetic changes. Trees and shrubs can be long-living organisms; we are interested in their acclimation potential, which is more relevant than population level changes in the context of global climate change in the next 80 years. Acclimation potential is especially important for long-living tropical plant species because they have historically lived in a narrower range of temperatures than temperate species and may not have evolved the capacity to acclimate to large temperature changes (Cunningham and Read 2003).

Photosynthesis is a temperature dependent metabolic process that increases in rate until it reaches an optimal temperature, then decreases at higher temperatures (Berry and Bjorkman 1980). Thermal acclimation of photosynthesis would allow plants to assimilate more $CO_2$ at higher temperatures via 1) increases in the temperature optimum for photosynthesis ($T_{opt}$, °C), 2) increases in photosynthesis at the temperature optimum ($A_{opt}$, µmol $m^2$ $s^{-1}$) (Berry and Bjorkman 1980, Way and Yamori 2014), or 3) increases in the thermal photosynthetic niche breadth (maintaining high photosynthesis at a broad range of temperatures) (Ω, °C) (Slot and Winter 2017). At our tropical understory warming experiment site in Puerto Rico, photosynthetic thermal acclimation has only been observed through increases in Ω in *Psychotria brachiata* after a year of closed-canopy understory warming (Carter et al. 2020). Acclimation can also be driven through changes in carbon fixation limited by activity of ribulose-1,5-bisphosphate carboxylase-oxygenase (rubisco), the enzyme that converts $CO_2$ to organic compounds, via changes in maximum rubisco carboxylation rate ($V_{cmax}$) or electron transport rate ($J_{max}$) (Farquhar et al. 1980). Globally, there is evidence that plants can acclimate photosynthesis to seasonal variation in temperature through rubisco limitation (Kumarathunge et al. 2019), but there is little evidence of this in the tropics.

Photosynthetic rates are also limited by soil moisture; which controls the rate that water is conducted from the roots to the leaves via stomatal conductance. Lower soil moisture leads to less stomatal conductance, and therefore decreasing the ability for carbon assimilation because closed stomata means that no $CO_2$ can enter the leaf for photosynthesis (Stocker et al. 2018). Soil moisture availability can be limited by several factors, and has been shown to be reduced by warming (Xu et al. 2013). Tropical forests under long-term experimental soil moisture deficit can maintain photosynthesis, but at the cost of higher respiration (Rowland et al. 2015). While many plants employ strategies like decreasing leaf area or limiting stomatal conductance to reduce water loss during periods of water deficit, we are studying broadleaf evergreen tropical rainforest understory species not known to regulate water loss through changes in leaf morphology. Stomatal conductance increases at higher temperatures (Urban et al. 2017), but the plants previously studied at our site have limited stomatal conductance acclimation response to warming at our site (Carter et al. 2020).

Plant respiration is a temperature dependent process that increases in rate until it reaches an optimal temperature and then decreases (Atkin et al. 2000), but the temperature at which dysfunction is reached is usually far in excess of the temperature optimum for photosynthesis. Acclimation of respiration to higher temperatures would allow plants to conserve more carbon at higher temperatures through down-regulation of basal metabolic rate at a standardized temperature, which for the tropics might be 25 °C ($R_{25}$, µmol $m^2$ $s^{-1}$), or the amount of change over 10 °C ($Q_{10}$, unitless) (Atkin and Tjoelker 2003). Without thermal acclimation of either respiration or photosynthesis, the net amount of carbon assimilated will be lower at higher temperatures.

While photosynthetic and respiratory rates are affected along a temperature gradient, they are also dependent on changes in other environmental variables like light availability. Under climate scenarios with more frequent and intense hurricane disturbances, understory light availability will be highly dynamic with post-disturbance light being more available in the early-successional understory than later-successional periods. During disturbance events like the 2017 hurricane María, which had double the tree mortality of similar size hurricanes in Puerto Rico, canopy conditions were drastically changed (Uriarte et al. 2019). Some species are able to exploit changes in canopy dynamics by increasing photosynthetic $CO_2$ uptake by acclimating to high-light environments through increases in leaf mass per area (LMA, $m^2$ $g^{-1}$) and nitrogen concentrations (%N) (Wen et al. 2008), but usually at the cost of higher respiration from higher metabolic demand (Wen et al. 2008). Hurricanes can also have large impacts on changes in community composition (Kennard et al. 2020, Alonso-Rodríguez et al. 2022) which could affect ecosystem-level carbon assimilation due to species variation in photosynthetic capabilities, but little is known about how plant physiology changes in response to hurricane recovery under warming conditions.

Our overall objectives were to investigate the effects of both experimental warming and post-hurricane canopy closure on the photosynthetic and respiratory acclimation potential of woody shrubs, *Psychotria brachiata* and *Piper glabrescens.* Specifically, we hypothesized that (H1) *P. brachiata* and *P. glabrescens* would photosynthetically acclimate to experimental warming, but that post-hurricane canopy closure would cause a decline in carbon uptake rates. Based on prior work at TRACE, we did not expect (H2) foliar respiration to acclimate to experimental warming or change with post-hurricane canopy closure dynamics.

## 3.3 Methods

### 3.3.1 Study Site

We performed our study at the Tropical Responses to Altered Climate Experiment (TRACE), an understory warming experiment located in Luquillo, Puerto Rico (18° 19' 28” N, 65° 43' 50" W). Mean annual temperature (MAT) at TRACE is ~24 °C and mean annual precipitation (MAP) is ~3500mm (García-Martino et al. 1996). Soils at the site are acidic, clay soils classified as ultisols (Scatena 1989). In September 2017, two major hurricanes, Irma and María, defoliated the canopy and knocked down many large stem trees at the TRACE site, with hurricane María causing double the tree mortality of previous hurricanes of similar size (Uriarte et al. 2019). Prior to the hurricanes, the canopy was closed and dominant canopy trees as the site were *Syzygium jambos*, *Ocotea leucoxylon*, *Casearia arborea*, and *Prestoea montana* (Alonso-Rodríguez et al. 2022).

TRACE has three ambient temperature control plots and three warmed plots that are hexagonally shaped with a 4 m diameter. Warmed plots are experimentally warmed using infrared heaters to maintain vegetation temperature 4 °C above the mean of ambient temperature control plots (Kimball et al. 2018b). Control plots are equipped with dummy heaters to control for the presence of heater infrastructure in the warmed plots.

### 3.3.2 Environmental measurements

Plot level vegetation temperature ($T_{veg}$) and plot level soil volumetric water content (VWC) were continuously measured and averaged each hour at the TRACE site. $T_{veg}$ is collected using an infrared radiometer (SI-121, Apogee Instruments, Logan, UT) aimed at the vegetation in the center of the plot. Soil surface VWC was measured using 3 soil temperature and moisture probes (CS655, Campbell Scientific, Logan UT) that were installed at a depth of 0-10 cm in the soil. The sensors were located at the edge of the plot, the center of the plot, and midway between those points. For $T_{veg}$ and VWC values used in our analyses, we averaged the hourly measurement for the 10 days preceding each physiological measurement to describe the growing conditions the plant was experiencing leading up to measurement.

As the canopy recovered from the 2017 hurricanes, we tracked successional changes in canopy structure by measuring leaf area index (LAI; $m^2$ $m^{-2}$) at each of the plots. We measured LAI using an LI-2200c plant canopy analyzer (LI-COR, Biosciences, Lincoln, NE, USA) with a quarter cover lens cap. Six replicate measurements were taken at each plot at each time point with the lens facing inwards towards the plot from each of the 6 warming infrastructure support poles at each plot. These measurements were taken 31 times from January 2018 through September 2022.

We modeled canopy recovery after the hurricanes by fitting sigmoidal curves to the LAI data with respects to days since hurricane disturbance. LAI disturbance response curves were fit to the data at each plot using the equation

$$LAI = \frac{a}{e^{-b*(D_H*c)}}, \qquad \text{Eqn 1}$$

where LAI is leaf area index, $D_H$ is days since hurricanes, a is the maximum LAI value, b is the steepness of LAI recovery, and c is the inflection point of the sigmoidal curve. LAI for each plot levels out between 700 and 1400 days after the hurricanes, with some plots approaching their pre-hurricane LAI values of about 6 (Fig 3.1).

### 3.3.3 Study Design

The two plant species used in this study were *Psychotria brachiata*, which was common among all plots, and *Piper glabrescens*, which was common among 5 of 6 plots, the exception of which is an ambient temperature plot. *P. brachiata* is an early-successional shrub that flourishes in open canopy and persists in shaded understory (Valladares et al. 2000). *P. glabrescens* is described as an early- to mid-successional shrub adapted to closed canopy conditions (Myster and Walker 1997, Alonso-Rodríguez et al. 2022). Three individuals per species per plot were sampled for temperature response curves. The top-most fully expanded leaf on a plant was selected to control for leaf age and ensure the leaf had developed under experimental treatment conditions. Photosynthesis-temperature responses curves were measured on each sampled leaf in each campaign and then respiration-temperature responses curves were measured on the same leaves after the photosynthesis campaign had ended (about 1-2 months). Each campaign was planned to included both photosynthetic and respiratory measurements. Data collection campaigns were performed March-May 2019, February 2020, May-June 2020, and January-April 2021. The February 2020 campaign was a partial campaign that was cut short due to COVID-19 restrictions, with only some leaves measured for photosynthesis and none measured for respiration. Warmed *P. glabrescens* were visually stressed and had been dropping leaves and by 2021, there were no *P. glabrescens* in the warmed plots due to mortality; only *P. brachiata* were measured during the final 2021 campaign.

### 3.3.4 Photosynthetic measurements

Photosynthesis temperature response curves were taken using a LI-COR LI-6800 IRGA (LI-COR Biosciences, Omaha, NE, USA) equipped with a 6 $cm^2$ round aperture. Chamber controls were set at chamber pressure ($\Delta P$) of 0.2 kPa, flow rate of 400 µmol $s^{-1}$, mixing fan at 10,000 rpm, reference $CO_2$ at 400 µmol $mol^{-1}$, and VPD at 2 kPa. Light levels were selected by finding a point where a subsample of plants was beyond irradiance saturation using light response curve before the campaign began. Light levels ranged from 500 µmol $m^{-2}$ $s^{-1}$ to 800 µmol $m^{-2}$ $s^{-1}$. Set points for photosynthesis temperature response curves were 22 °C, 25 °C, 27 °C, 30 °C, 33 °C, 35 °C, 37 °C, and 40 °C. During periods where ambient temperature was too high for the instrument to reach 22 °C, the lowest possible temperature was used instead (normally around 23 °C). Photosynthesis temperature response curves were fit using the method from June et al. (2004) with the formula:

$$A_{net} = A_{opt} * e^{-\left(\frac{T_{leaf} - T_{opt}}{\Omega}\right)^2}, \qquad \text{Eqn 2}$$

where $A_{net}$ is net photosynthesis, $T_{leaf}$ is leaf temperature, $T_{opt}$ is the optimum temperature for photosynthesis, $A_{opt}$ is photosynthetic rate at $T_{opt}$ and Ω describes the how flat the curve is at the peak (i.e., photosynthetic thermal niche breadth). Curves were fit using the ‘nls’ function in base R (R Core Team 2022).

Water use efficiency at 25 °C ($WUE_{25}$, µmol $CO_2$ $mmol^{-1}$ $H_2O$) was measured using the 25 °C measurements from the photosynthesis-temperature response curves and was calculated as net photosynthetic assimilation at 25 °C ($A_{net25}$, µmol $CO_2$ $m^{-2}$ $s^{-1}$) over stomatal conductance at 25 °C ($g_{sw25}$, mmol $H_2O$ $m^{-2}$ $s^{-1}$).

We extracted parameters from photosynthesis-$CO_2$ response (A-$C_i$) curves to address the mechanisms behind photosynthetic temperature acclimation using maximum rubisco carboxylation rate ($V_{cmax}$) and maximum electron transport rate ($J_{max}$). $V_{cmax}$ and $J_{max}$ were modeled from A-$C_i$ curves which were measured at 25 °C, 27 °C, 30 °C, 33 °C, 35 °C, 37 °C, and 40 °C to obtain the temperature optima of parameter rates. A-$C_i$ were measured with an LI-6800 equipped with a 6800-01A head and 6 $cm^2$ aperture set at chamber pressure (ΔP) of 0.2 kPa, flow rate of 400 µmol $s^{-1}$, mixing fan at 10,000 rpm, variable [$CO_2$] , PAR at 800 µmol $m^{-2}$ $s^{-1}$, and VPD at 1 kPa. Each A-$C_i$ curve was sampled in a sequence of chamber $CO_2$ concentration ($c_a$) at 400 ppm, 300 ppm, 200 ppm, 100 ppm, 50 ppm, 5 ppm, 400 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm, and 1200 ppm with measurements taken within 2 minutes after the previous measurement. $V_{cmax}$ and $J_{max}$ were extracted from each A-$C_i$ curve at each temperature using the R package 'plantecophys' (Duursma 2015).

$V_{cmax}$ and $J_{max}$ -temperature response curves were used to model the optimum temperatures for $V_{cmax}$ and $J_{max}$ ($T_{optV}$ and $T_{optJ}$) and the maximum rates at temperature optima ($V_{opt}$ and $J_{opt}$). $V_{cmax}$ and $J_{max}$ -temperature response curves were fit using the Medlyn et al. (2002) formula

$$f(T_k) = k_{opt} * \left( \frac{H_d * e^{\frac{H_a*(T_k - T_{opt})}{T_k*R*T_{opt}}}}{H_d - H_a * \left( 1 - e^{\frac{H_d*\left(T_k - T_{opt}\right)}{T_k*R*T_{opt}}} \right)} \right), \qquad \text{Eqn 3}$$

where $T_k$ is $T_{leaf}$ in Kelvin, $k_{opt}$ is $V_{cmax}$ or $J_{max}$ at the temperature optimum for either (µmol $m^{-2}$ $s^{-1}$), $H_a$ is modeled activation energy (kJ $mol^{-1}$), $T_{opt}$ is the modeled temperature optimum for the parameter (K), R is the universal gas constant (8.314 J $K^{-1}$ $mol^{-1}$), and $H_d$ is the difference between of the parameter at $T_{opt}$ and measured $V_{cmax}$ or $J_{opt}$, which was held constant at 200 kJ $mol^{-1}$ (Medlyn et al. 2002).

## 3.3.5 Foliar Respiration Measurements

We measured respiration temperature response curves using the same leaves used for photosynthesis response curves. A LI-COR LI-6800 fitted with a 6800-13 6x6 large leaf chamber was used for respiration measurements to maximize leaf surface area measured. Respiration was measured after dusk when photosynthesis was least active, ranging from 22:00-03:00. Chamber controls were set at chamber pressure (ΔP) of 0.1 kPa, flow rate of 1,000 µmol $s^{-1}$, mixing fan at 10,000 rpm, reference $CO_2$ at 400 µmol $mol^{-1}$, PAR at 0 µmol $m^{-2}$ $s^{-1}$, and VPD at 2 kPa. Set points for respiration temperature response curves were 25 °C, 30 °C, 33 °C, 35 °C, 37 °C, and 40 °C. During times when the instrument would not reach 40 °C, a lower max temperature was used (normally around 38 °C).

Respiration temperature response curves were fit using a non-linear least squares regression to a curve with the equation

$$R_d = \beta_0 * e^{\beta_1 T_{leaf}}, \qquad \text{Eqn 4}$$

where $R_d$ is dark respiration, $T_{leaf}$ is leaf temperature (°C), and $\beta_0$ & $\beta_1$ are modeled parameters. $Q_{10}$ was extracted from the curve using the equation

$$Q_{10} = e^{\beta_1 * 10} \qquad \text{Eqn 5}$$

and $R_{25}$ was extracted from the curve using the equation

$$R_{25} = \beta_0 * e^{\beta_1 * 25}. \qquad \text{Eqn 6}$$

### 3.3.6 Leaf Traits

After leaves were measured for photosynthesis and respiration, we calculated leaf mass per area (LMA, g $m^{-2}$) by dividing dry leaf mass by leaf area. We calculated LMA by scanning leaves with an Epson Stylus NX420 flatbed scanner and analyzed for area using ImageJ v.1.50 software. Leaves were then dried at 65 °C for 48 hours, weighed for dry-weight, and ground using a ball-mill (SPEX 8000D, SPEX Sample Prep, Metuchen, NJ, USA). Nitrogen content was measured using an elemental analyzer (Elementar Vario Micro Cube, Elementar Inc., Langenelsbold, Germany).

### 3.3.7 Statistical Analyses

We evaluated $A_{opt}$, $T_{opt}$, Ω, $R_{25}$, $Q_{10}$, and LMA responses to experimental warming treatment, LAI, $T_{veg}$, and the interactions of warming treatment with LAI and $T_{veg}$ using linear models. We evaluated $A_{net25}$, $g_{sw25}$, and $WUE_{25}$ responses to experimental warming and VWC using linear models. We evaluated $V_{opt}$, $J_{opt}$, $T_{optV}$ and $T_{optJ}$ responses to experimental warming using unpaired t-tests. All statistical analyses were performed in base R (R Core Team 2022).

## 3.4 Results

### 3.4.1 Photosynthetic thermal acclimation

We found that both *P. glabrescens* and *P. brachiata* had lower $A_{opt}$ in warmed plots than control plots (control plots were higher by ~1.4 and ~1.8 µmol $m^{-2}$ $s^{-1}$, respectively) and that $A_{opt}$ decreased for both species as LAI increased post hurricane

(Table 3.1, Fig 3.2). $T_{opt}$, however, was unaffected by experimental warming or LAI in both species (Table 3.1, Fig 3.2). Thermal niche breadth (Ω) was higher in warmed than control plots for *P. brachiata*, but was not different between warmed and control plots for *P. glabrescens* (Table 3.1, Fig 3.2). As LAI increased, Ω increased for *P. brachiata* and decreased for *P. glabrescens* (Table 1, Fig 2).

*P. brachiata* $A_{opt}$ was unaffected by plot level vegetation temperature ($T_{veg}$), while *P. glabrescens* $A_{opt}$ decreases as $T_{veg}$ increased. For both species, $T_{opt}$ was unaffected by $T_{veg}$ (Table 3.2, Fig 3.2). Ω was unaffected by $T_{veg}$ in *P. brachiata*, but Ω decreased as $T_{veg}$ increased for *P. glabrescens* (Table 3.2, Fig 3. 2).

### 3.4.2 Stomatal conductance and water use efficiency responses to soil water content

Stomatal conductance at 25 °C ($g_{sw25}$) was unaffected by experimental warming and soil surface volumetric water content (VWC) for both species (Table 3.3, Fig 3.4). Net photosynthesis at 25 °C ($A_{net25}$) was lower in warmed than control *P. brachiata*; however, control *P. brachiata* $A_{net25}$ decreased as VWC increased. *P. glabrescens* $A_{net25}$ was unaffected by experimental warming, but increased as VWC increased in both control and warmed plots, though the increase was more pronounced in the control plots (Table 3.3, Fig 3.4). Water use efficiency at 25 °C ($WUE_{25}$; $A_{net25}/g_{sw25}$) was higher in warmed than control *P. brachiata*, and $WUE_{25}$ decreased in control plots as VWC increased, but remained constant in warmed plots (Table 3.3, Fig 3.4). *P. glabrescens* $WUE_{25}$ was unaffected by experimental warming and VWC. $WUE_{25}$ for *P. brachiata* decreased as LAI increased for unwarmed control plants, but remained constant for warmed plants (Table 3.3, Fig 3.4). *P. glabrescens* $WUE_{25}$ decreased as LAI increased, independent of experimental warming effect (Table 3.1, Fig 3.4).

### 3.4.3 $V_{cmax}$ and $J_{max}$ Acclimation

$V_{opt}$ was higher in warmed *P. brachiata*, and lower in warmed *P. glabrescens* than control plants (Table 3.4, Fig 3.5). $J_{opt}$ was unaffected by warming for *P. brachiata*, but was lower in warmed *P. glabrescens* (Table 3.4, Fig 3.5). $T_{optV}$ was unaffected by warming for both species and was higher than $T_{opt}$ by ~14 °C and ~9 °C for *P. brachiata* and *P. glabrescens*, respectively. $T_{optJ}$ was ~2 °C higher in warmed than controlled *P. brachiata* and ~1.5 °C lower in warmed than controlled *P. glabrescens* (Table 3.4, Fig 3.5).

### 3.4.4 Respiratory thermal acclimation

*P. brachiata* $R_{25}$ was not affected by experimental warming, but did decrease as LAI increased (Table 3.1, Fig 3.3). *P. glabrescens* $R_{25}$ was lower in warmed plots but was unaffected by changing LAI (Table 1, Fig 3). *P. brachiata* $Q_{10}$ increased as LAI increased in warmed plots, but was unaffected by LAI in control plots (Table 3.1, Fig 3.3). *P. glabrescens* $Q_{10}$ was higher in warmed plot than control plots and decreased as LAI increased, while *P. glabrescens* $Q_{10}$ increased in control plots as LAI increased (Table 3.1, Fig 3.3). $R_{25}$ and $Q_{10}$ were unaffected by $T_{veg}$ for both species (Table 3.2, Fig 3.3).

# 3.5 Discussion

## 3.5.1 Experimental warming response

We found photosynthetic and respiratory-temperature response variables were affected by experimental warming, post-hurricane canopy dynamics, and soil surface water content, indicating there is some thermal acclimation to long-term warming even while recovering from hurricane disturbance. We saw notable differences in the magnitude and direction of responses to experimental warming and hurricane recovery in the two species we measured. *P. brachiata* acclimated to warming via upregulation of Ω, but *P. glabrescens* did not show any signs of thermal acclimation to experimental warming.

While we did not see photosynthetic thermal acclimation through upregulation of $A_{opt}$ and $T_{opt}$ seen in other tropical warming experiments (Cheesman and Winter 2013, Slot and Winter 2017), we did observe upregulation of Ω, or thermal niche breadth, in *P. brachiata*. To our knowledge, upregulation of Ω of tropical plants in response to warming has only been documented at our study site in *P. brachiata* during pre-hurricane experimental warming (Carter et al. 2020). However, $A_{opt}$ was lower for *P. brachiata* in warmed plots which means that while the thermal niche the leaves can occupy is higher, the amount of carbon leaves can potentially assimilate is diminished.

Curiously, for *P. brachiata*, $V_{opt}$ was higher in the warmed plots, indicating that rubisco carboxylation rates are higher in warmed plot, which could confer higher photosynthetic rates, which we do not observe. This indicates that rubisco carboxylation rate is not the rate limiting factor for *P. brachiata* at these temperatures. $J_{opt}$ did not vary between treatments for *P. brachiata*, so electron transport rate is also not likely a rate limiting factor for assimilation. While $T_{optJ}$ was higher for warmed *P. brachiata*, the temperatures are ~9 °C higher than $T_{opt}$, further indicating that electron transport rate is not limiting photosynthesis at $T_{opt}$. Warmed *P. brachiata* could be limited by foliar nitrogen concentration, which could limit the other high nitrogen demanding processes. In *P. glabrescens*, both $V_{opt}$ and $J_{opt}$ were much lower in warmed plants and are likely an important mechanism describing the decrease in photosynthetic rate for warmed *P. glabrescens*. *P. glabrescens* also had a lower $T_{optJ}$, but it was still higher thaNn $T_{opt}$ and likely not the reason for shifts in overall photosynthetic rate. Unlike non-tropical species that can acclimate rubisco activity to seasonal temperature variability (Kumarathunge et al. 2019), the species we studied at our site, which have a narrow range of temperatures, did not show a potential for thermal acclimation of rubisco activity.

Similar to *P. brachiata*, lower $A_{opt}$ for warmed *P. glabrescens* shows that *P. glabrescens* will have overall lower photosynthesis, but we did not observe any changes that would suggest it is acclimating to experimental warming. Decreases in *P. glabrescens* $A_{opt}$ are largely driven by increasing $T_{veg}$, indicating that instead of acclimating to higher temperatures, higher temperatures were detrimental to the plant and could have contributed to their mortality. $J_{opt}$ and $V_{opt}$ rates were both nearly 50% lower in warmed than control *P. glabrescens* and are likely limiting $A_{opt}$ as temperature increases.

*P. brachiata* stomatal conductance was not affected by variation in soil surface VWC, while $A_{net25}$ decreased as soil surface VWC increased in control plots. This resulted in $WUE_{25}$ decreasing as soil surface VWC increased in control plots. Warmed *P. brachiata* $WUE_{25}$ remains unchanged across a soil surface VWC gradient, but control $WUE_{25}$ decreases at higher soil surface VWC. This could be indicative of an acclimation effect where warmed *P. brachiata* are able to perform photosynthesis consistently at the cost of overall lower photosynthesis, but control plants have lower photosynthetic rates at high soil moisture with overall higher photosynthetic rates. *P. glabrescens* are more abundant during periods of drought, but do not respond well to experimental warming (Alonso-Rodríguez et al. 2022), suggesting that the warming itself is the cause of mortality in *P. glabrescens*, and not the soil water deficit.

*P. brachiata* respiration did not acclimate to warming, and in fact the warmed *P. brachiata* became more sensitive to high-temperatures as the canopy closed which is the opposite direction we expected. *P. glabrescens* respiration was lower in the warmed plots indicating that they were acclimating respiration to the warmed plots; however, since warmed *P. glabrescens* all died, that conservation of carbon at high temperatures via low respiration rate may have cost the plant important metabolic resources for survival.

### 3.5.2 Hurricane recovery response

As we expected, *P. brachiata*, an early-successional shrub, performed well when there was higher light post-hurricane and the photosynthetic rates decreased as the canopy closed, allowing it to persist when there is less light availability. Though leaf-area based photosynthetic rates were lower, the number of *P. brachiata* were higher in the warmed plots (Alonso-Rodríguez et al. 2022), and they may be fixing more carbon at a plot level due to higher numbers of plants. *P. glabrescens* also benefited from the high-light conditions post-hurricane by upregulating photosynthetic rates when the canopy was more open. *P. glabrescens* acclimation of photosynthesis to high-light aligns with it being an early successional plant, but higher atmospheric temperatures might push *P. glabrescens* to being strictly a mid-successional plant.

Maximum quantum efficiency, measurement of stress where high values indicate low stress, for warmed *P. brachiata* at the TRACE site was lower in leaves from control plants (Tunison et al. In Prep), suggesting that light processes could be limiting photosynthesis for warmed *P. brachiata* at TRACE. While high-light induced photoinhibition of photosystem I has been observed in the shade-established congener, *Psychotria rubra* (Huang et al. 2015), our lower photosynthetic rates were associated with low-light and warming, rather than high-light photoinhibition.

*P. brachiata* respiration was highest after the hurricanes when the canopy was more open and can be attributed to the higher metabolic demand of the higher post-hurricane photosynthetic rates; however, *P. glabrescens* respiration was not affected by changing light conditions which means the plants are fixing more carbon at high-light, without higher metabolic contributions to photosynthesis.

Hurricane recovery cannot be disentangled from duration of warming, which could show the emergence of acclimation over long periods of time. However, since the responses for photosynthesis and respiration parameters trend in the same direction as LAI increases for both control and warmed plants, this is a good indicator that hurricane recovery is affecting photosynthesis and respiration through changes in canopy, rather than duration of warming treatment. Warmed plots have a different range of LAI values than control plots, which is not necessarily related to warming treatment, and is more likely related to number of large-stemmed trees knocked down during the hurricane.

### 3.5.3 Conclusions

Overall, we see that warming decreases leaf area-based photosynthetic rates and there are partial signs of acclimation to warming though changes in a larger thermal niche breadth and maintenance of high water use efficiency during periods of lower water availability. Both species in our study were able to acclimate physiological processes to changing light conditions after the hurricanes, but having pressures of both warming and disturbance could negatively affect some species. With overall lower photosynthetic rates under warmer conditions, independent of canopy cover, there will likely be lower photosynthetic carbon assimilation in tropical understory plants at higher atmospheric temperatures. While there is some evidence that respiration is thermally acclimating, it may not be enough to offset the lowered carbon assimilation. These lower photosynthetic rates confer lower $CO_2$ uptake from the atmosphere on a global scale, lessening the sink strength of tropical forests and resulting in more atmospheric $CO_2$ which in turn increases global temperatures. Our future goals are to use biomass estimations from the plots to model how these physiological processes will affect overall carbon cycling in the plots under future climatic changes in temperature and disturbance frequencies.

## 3.6 Tables and Figures

Table 3.1: ANCOVA stats for physiological responses to experimental warming and LAI

| | | n | Treatment | LAI | Treatment:LAI |
|---|---|---|---|---|---|
| *P. glabrescens* | $A_{opt}$ | 40 | **0.0062** | **0.0065** | 0.17 |
| | $T_{opt}$ | 40 | 0.13 | 0.96 | 0.54 |
| | $\Omega$ | 40 | 0.16 | **0.029** | 0.90 |
| | $R_{25}$ | 33 | **0.029** | 0.49 | 0.41 |
| | $Q_{10}$ | 33 | **0.027** | 0.39 | **0.024** |
| | LMA | 31 | 0.37 | 0.065 | 0.057 |
| | $WUE_{25}$ | 46 | 0.98 | **0.011** | 0.89 |
| *P. brachiata* | $A_{opt}$ | 74 | **0.045** | **< 0.001** | 0.89 |
| | $T_{opt}$ | 74 | 0.72 | 0.45 | 0.82 |
| | $\Omega$ | 74 | **< 0.001** | **0.0042** | 0.11 |
| | $R_{25}$ | 64 | 0.39 | **< 0.001** | 0.80 |
| | $Q_{10}$ | 64 | 0.73 | **0.0043** | **0.04** |
| | LMA | 71 | 0.10 | **< 0.001** | 0.43 |
| | $WUE_{25}$ | 85 | 0.07 | **0.0084** | **0.039** |

Table 3.2: ANCOVA stats for physiological responses to experimental warming and vegetation temperature

| | | n | Treatment | Tveg | Treatment:Tveg |
|---|---|---|---|---|---|
| *P. glabrescens* | $A_{opt}$ | 40 | **0.0036** | **0.00019** | 0.76 |
| | $T_{opt}$ | 40 | 0.12 | 0.56 | 0.26 |
| | $\Omega$ | 40 | 0.14 | **0.012** | 0.095 |
| | $R_{25}$ | 33 | **0.017** | **0.015** | 0.36 |
| | $Q_{10}$ | 33 | **0.033** | 0.34 | 0.12 |
| | LMA | 31 | 0.43 | 0.63 | 0.88 |
| | $WUE_{25}$ | 46 | 0.057 | 0.22 | 0.092 |
| *P. brachiata* | $A_{opt}$ | 50 | 0.087 | 0.42 | 0.13 |
| | $T_{opt}$ | 50 | 0.93 | 0.83 | 0.062 |
| | $\Omega$ | 50 | **0.000824** | 0.80 | 0.21 |
| | $R_{25}$ | 42 | 0.75 | 0.27 | 0.46 |
| | $Q_{10}$ | 42 | 0.19 | 0.33 | 0.24 |
| | LMA | 48 | 0.16 | 0.13 | **0.0089** |
| | $WUE_{25}$ | 57 | 0.17 | 0.18 | 0.17 |

Table 3.3: ANCOVA stats for physiological responses to experimental warming and soil volumetric water content (VWC)

| | | n | treatment | VWC | treatment:VWC |
|---|---|---|---|---|---|
| *P. glabrescens* | $WUE_{25}$ | 36 | 0.57 | 0.43 | 0.77 |
| | $A_{net25}$ | 36 | 0.10 | **0.031** | 0.070 |
| | $g_{sw25}$ | 36 | 0.19 | 0.10 | 0.11 |
| *P. brachiata* | $WUE_{25}$ | 57 | **0.034** | **0.0097** | **0.024** |
| | $A_{net25}$ | 57 | **0.00087** | **0.0057** | **0.0024** |
| | $g_{sw25}$ | 57 | 0.14 | 0.60 | 0.22 |

Table 3.4: Unpaired t-test stats for warming treatment effect on $V_{opt}$, $J_{opt}$, $T_{optV}$ and $T_{optJ}$

| | | n | treatment |
|---|---|---|---|
| *P. glabrescens* | $V_{opt}$ | 10 | **0.0031** |
| | $J_{opt}$ | 11 | **0.0065** |
| | $T_{optV}$ | 10 | 0.34 |
| | $T_{optJ}$ | 11 | **0.048** |
| *P. brachiata* | $V_{opt}$ | 18 | **0.043** |
| | $J_{opt}$ | 18 | 0.33 |
| | $T_{optV}$ | 18 | 0.15 |
| | $T_{optJ}$ | 18 | **0.013** |

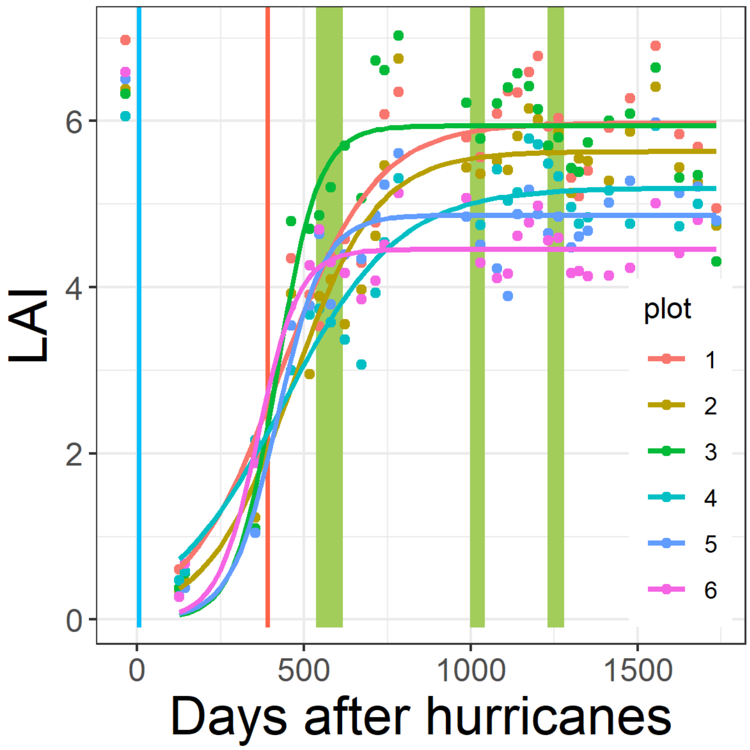

Figure 3.1: Leaf area index over time for each TRACE plot, following hurricane disturbance on day 0. The blue vertical line is the day when Hurricane Irma hit Puerto Rico, and the red line is when warming at the TRACE site was restarted. The vertical green bars are periods during which photosynthesis and respiration were measured.

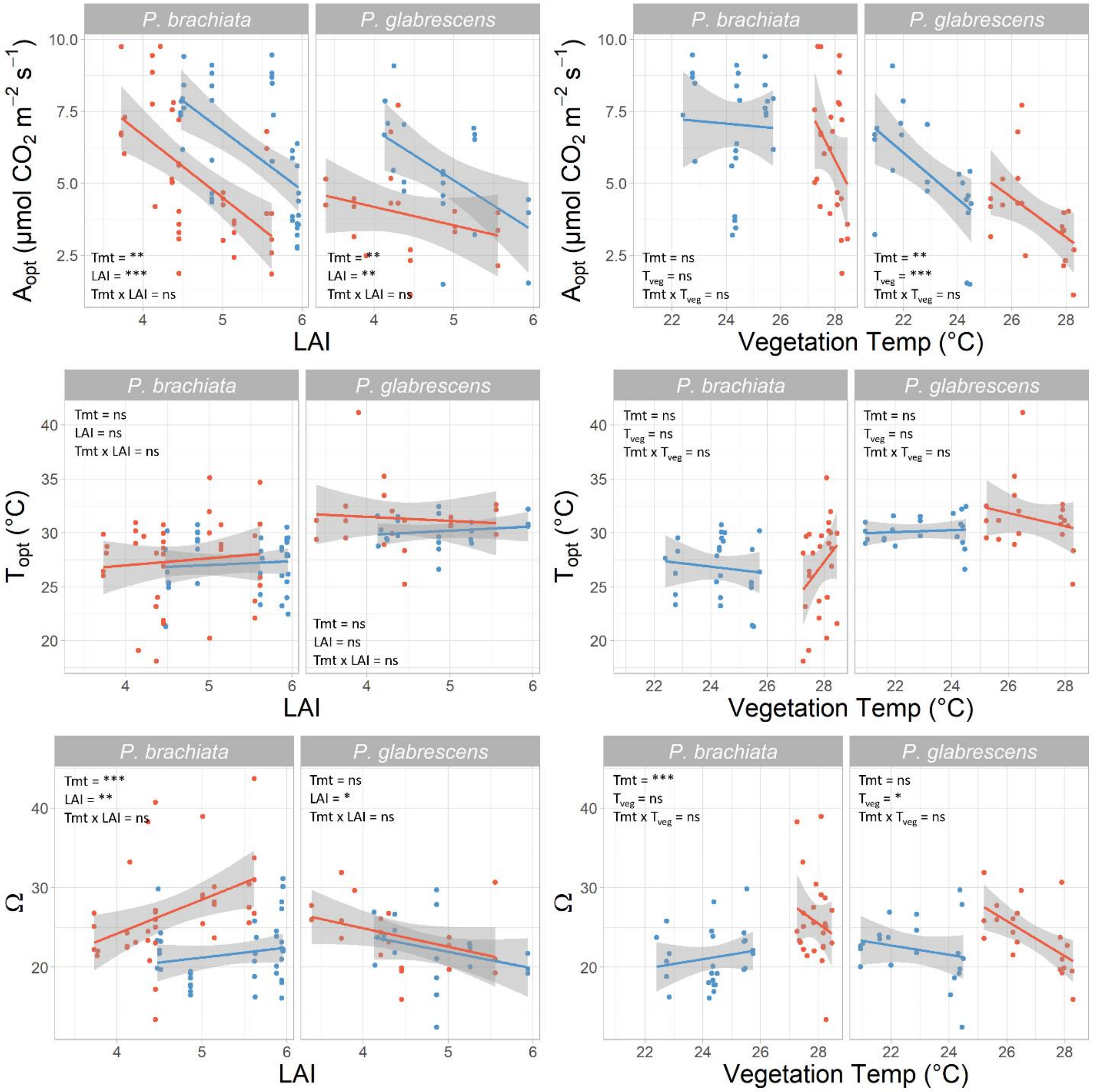

Figure 3.2: Photosynthetic temperature response curve parameter responses to experimental warming, LAI, and vegetation temperature. Red dots and lines are warmed plants, and blue dots and lines are control plants. Statistical significance is shown in each panel with Tmt as treatment effect, LAI or vegetation temperature as the continuous

variable, and the interaction of the Tmt with the continuous variable, where * is $p <0.05$, ** is $p < 0.01$, and *** is $p < 0.001$.

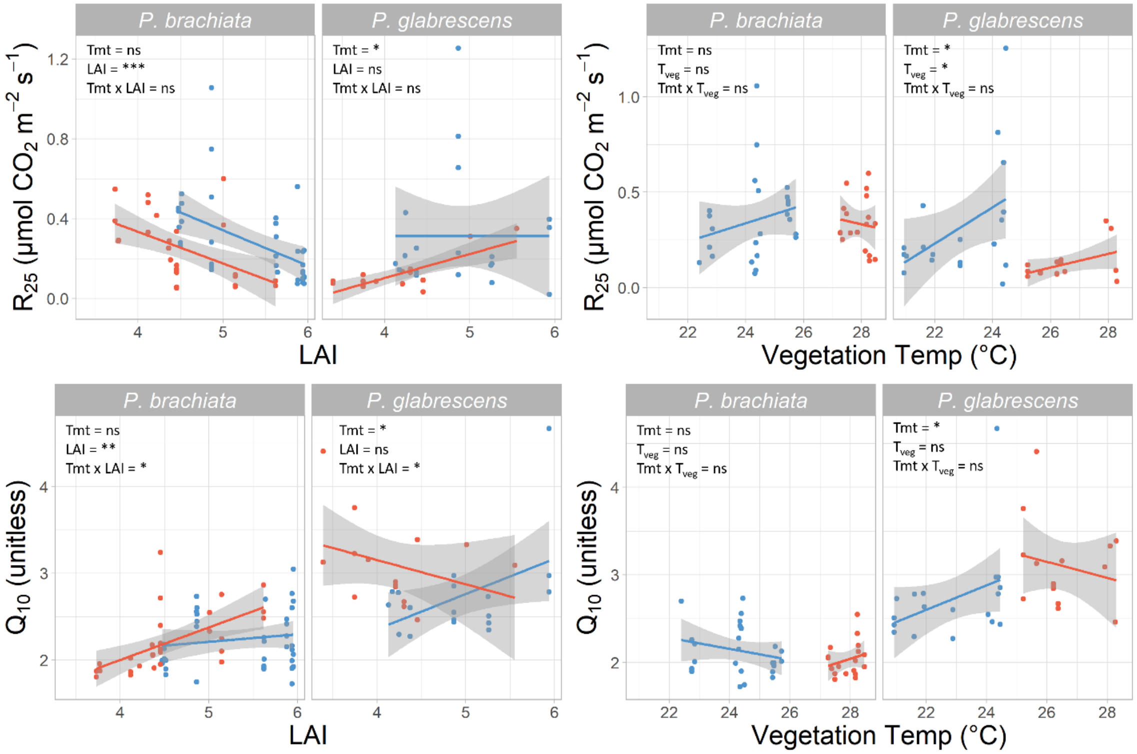

Figure 3.3: Respiration temperature response curve parameter responses to experimental warming, LAI, and vegetation temperature. Red dots and lines are warmed plants and blue dots and lines are control plants. Statistical significance is shown in each panel with Tmt as treatment effect, LAI or vegetation temperature as the continuous variable, and the interaction of the two with * is $p < 0.05$, ** is $p < 0.01$, and *** is $p < 0.001$.

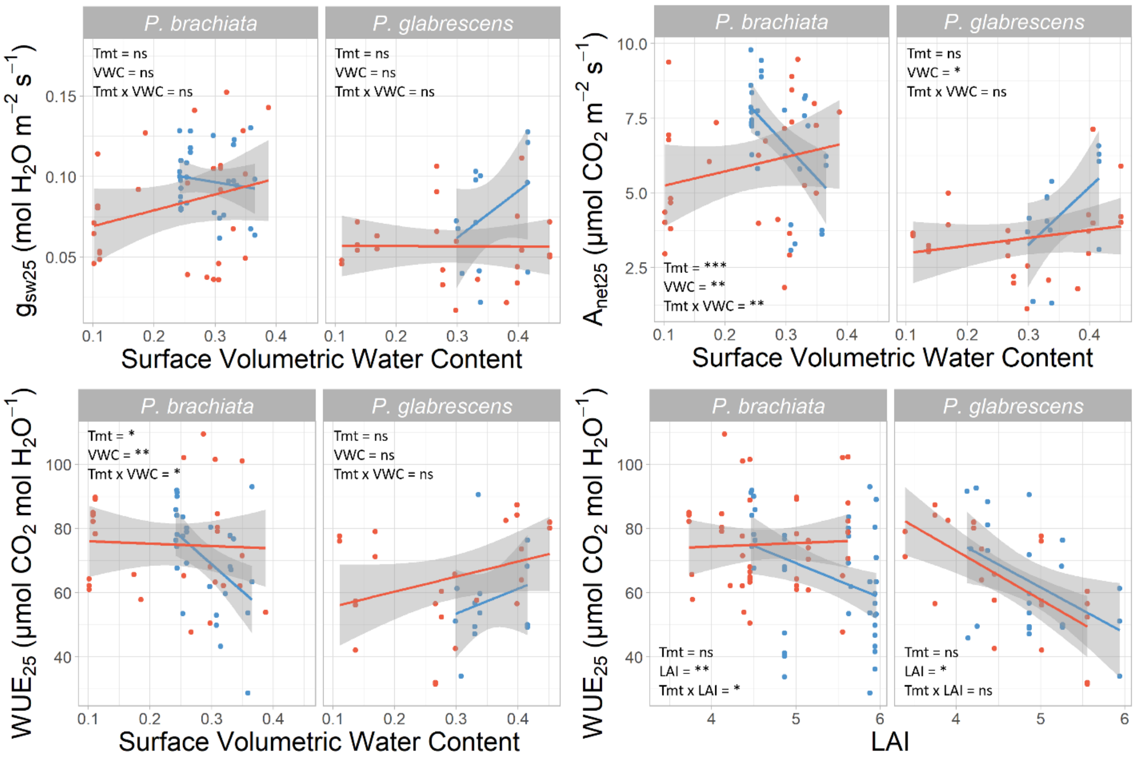

Figure 3.4: Stomatal conductance, photosynthesis, and WUE at 25 °C responses to experimental warming and soil volumetric water content and WUE$_{25}$ responses to experimental warming and LAI. Red dots and lines are warmed plants and blue dots and lines are control plants. Statistical significance is shown in each panel with Tmt as treatment effect, surface volumetric water content or LAI as the continuous variable, and the interaction of the two with * is $p < 0.05$, ** is $p < 0.01$, and *** is $p < 0.001$.

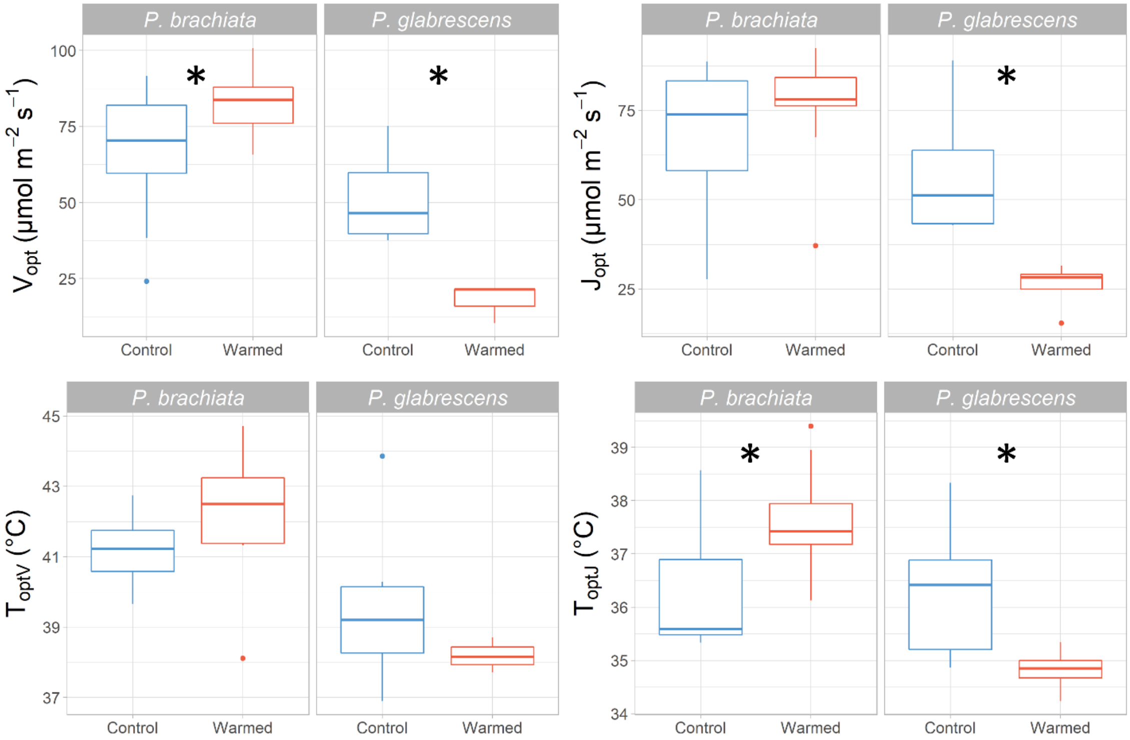

Figure 3.5: $V_{opt}$, $J_{opt}$, $T_{optV}$, and $T_{optJ}$ responses to experimental warming. Red boxes are warmed plants and blue boxes are control plants. Statistical significance is shown in each panel with a star indicating differences in treatment.

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# 4 Foliar thermotolerance is more affected by post-hurricane canopy dynamics than experimental warming in Puerto Rican tropical understory shrubs

## 4.1 Abstract

With increasing global atmospheric temperature and more frequent high-temperature events expected in the next 100 years due to climate change, plants could be subject to higher thermal stress and could be pushed to their high-temperature thermal limits. Additionally, hurricane disturbances, which are projected to become more intense and frequent, change understory canopy dynamics and can increase solar radiation and temperature in the understory. These changing conditions will exert pressure on photosynthetic systems which could respond by changing their physiology to survive at higher temperature extremes, described as thermotolerance. The ability of plants to acclimate their thermotolerance to higher temperatures could mitigate damage during high-temperature events and give plants that acclimate an advantage over plants that do not. We investigated the potential of three tropical understory species to acclimate thermotolerance to long-term experimental warming and hurricane disturbance recovery by measuring thermotolerance using fluorometric methods at the Tropical Responses to Altered Climate Experiment (TRACE). TRACE is a long-term, understory warming experiment located in eastern Puerto Rico that warms forest understory plots +4 °C above ambient temperatures. We observed thermotolerance acclimation to warming in one species, *Cecropia schreberiana*, which showed an increase in thermotolerance with

increasing plot temperature for only warmed plants. *Piper glabrescens* showed a higher thermotolerance at higher plot temperature, but experimental warming did not affect the response. All three species responded to hurricane disturbance by increasing thermotolerance when the canopy was more closed. Only *C. schreberiana* showed response to changing soil moisture and had higher thermotolerance when soil moisture was low. Overall, this shows that different tropical understory species will have variable thermotolerance responses to warming and disturbance, but ultimately long-term of +4 °C warming alone may not be enough to induce thermotolerance acclimation at out site.

## 4.2 Introduction

Frequency and intensity of extreme temperature events are increasing (IPCC 2018). Extreme temperature is a strong selective force guiding evolution (Gutschick and BassiriRad 2003) with unknown consequences for tropical plants experiencing a narrower range of temperatures in their evolutionary history than temperate species. Though the air temperature around tropical leaves may have a narrow annual range, leaf temperature can fluctuate drastically when leaves are exposed to high solar radiation (Leakey et al. 2003). High leaf temperature can be regulated through evaporative loss of water at the leaf surface, which is regulated through stomatal conductance (Urban et al. 2017). Despite these thermoregulatory mechanisms, leaf temperature can still exceed air temperature when temperature and solar radiation are high (Doughty and Goulden 2008, Fauset et al. 2018). Periods of high solar radiation when stomatal conductance is low can increase leaf temperatures that are near the temperatures at which loss of function can occur for photosystem II in tropical plants (Krause et al. 2010). Under the RCP8.5 scenario with a potential +4 °C increase in global temperatures by 2100 (IPCC 2018), plants could be at their thermotolerance limits without acclimation.

Thermotolerance is the limit at which the light reactions of photosynthesis are limited by temperature. Thermotolerance can be measured using fluorometric methods to measure the temperature at which light reactions for photosynthesis lose function. These light reactions do not operate at full capacity and define a relative baseline of stress for the plant. Stress in plants, including heat stress, can be measured as maximum potential quantum efficiency (QE) of photosystem II ($F_v/F_m$) using a chlorophyll fluorometer on a dark-adapted leaf (Maxwell and Johnson 2000). Variable fluorescence ($F_v$) is the difference between maximum fluorescence ($F_m$) and minimum fluorescence ($F_o$). As temperature increases, $F_v/F_m$ decreases following a sigmoidal shaped curve until photosystem II is non-functioning (Valladares and Pearcy 1997). The temperature where photosystem II has a 50% loss of function ($T_{50}$) is often considered the limit of thermotolerance; however, the temperature where photosystem II has 15% loss of function ($T_{crit}$) describes the threshold after which small changes in temperature drastically reduce function in photosystem II (Knight and Ackerly 2003, Curtis et al. 2014). In addition to temperature stress, drought stress in plants can lower QE in plants (Shahenshah and Isoda 2010). Releasing warmed plants of any water stress during measurement could show the signal of thermotolerance response to temperature without drought response.

In a recent review of on thermotolerance that included high temperature thermotolerance on non-agricultural tropical species (Geange et al. 2021), few studies quantified thermotolerance using chlorophyll fluorescence techniques in tropical rainforests (Krause et al. 2010, 2015, Sastry and Barua 2017, Sastry et al. 2018, Slot et al. 2019, 2021, Feeley et al. 2020). A survey of tropical plant thermotolerance showed that thermotolerance was positively associated with mean annual temperature, a trend present in and among many species (Slot et al. 2021). Some tropical dry-forest plants exhibit thermotolerance acclimation by having higher thermotolerance in the hot-dry versus the cool-wet season (Sastry and Barua 2017). This will be the first study measuring thermotolerance on tropical plants exposed to long-term, *in situ* experimental warming.

Hurricanes can cause large changes to understory canopy dynamics when strong winds knock down large-stem trees and defoliate the upper canopy, causing more light to reach the understory during early successional stages. Understory tropical plant species that are frequently exposed to disturbance, like those in Puerto Rico, could be subject to higher thermotolerance stress than systems not disturbed by hurricanes because they are more frequently exposed to sustained high solar radiation that occurs after wind disturbance events open the forest canopy. Hurricanes change understory dynamics that can decrease survivability of tropical seedlings either directly though changes in light and temperature conditions, or indirectly through density-dependent conspecific competition (Comita et al. 2009, Bachelot et al. 2020). Some tropical forest species have lower thermotolerance for shaded leaves than for sun-exposed leaves (Slot et al. 2019). It is possible that earlier in succession, when understory solar radiation is higher, thermotolerance will be higher and QE will be lower. Additionally, hurricanes affect biogeochemical cycling by sending a pulse of nutrients into the leaf litter and soil at rates of 1-2 times the turnover during a single event relative to a typical year (Lodge et al. 1991). This pulse of nutrient could result in higher nitrogen availability for plants, but little is known about the role of leaf nitrogen effects on thermotolerance.

While tropical plant thermotolerance temperatures exceed the high temperature $CO_2$ compensation point where photosynthetic carbon assimilation is zero and photosynthetic machinery is no longer photosynthesizing via carbon reactions (Slot et al. 2019), the thermotolerance trait is more attributed to survival of photosynthetic machinery during heatwaves (Drake et al. 2018). Under experimental heatwave conditions following a year of *in situ* experimental warming of *Eucalyptus parramattensis,* photosynthesis was decoupled from stomatal conductance, causing experimentally warmed plants to have a sustained evaporative cooling effect without photosynthetic carbon fixation while increasing thermotolerance by +2 °C (Drake et al. 2018). Thus, during periods of high temperature stress that is beyond the maximum temperature of photosynthetic carbon fixation, leaf water availability may be playing a role in thermotolerance acclimation.

We hypothesize that (H1) plants exposed to long-term experimental warming will acclimate to higher temperatures by increasing the temperature at which there is functional loss of the light reaction of photosynthesis. This response will be evident through experimental warming and seasonal variability in plot-level maximum temperature. (H2) Thermotolerance will be highest after the hurricanes when solar radiation is highest in the understory and thermotolerance will be highest in plants limited by soil moisture. (H3) Thermotolerance will be positively dependent on leaf nitrogen concentration.

## 4.3 Methods

### 4.3.1 Study Site

Our work was done at the Tropical Responses to Altered Climate Experiment (TRACE) a tropical forest understory warming experiment in the Luquillo Experiment Forest in Eastern Puerto Rico. TRACE has three 4 m diameter hexagonal shaped experimentally warmed plots that heat the understory vegetation +4 °C above ambient temperature using infrared heaters 2-4 m above the ground (maintained 1-2 m above the foliage) and three ambient temperature plots with the same "dummy" infrastructure, but unwarmed (Kimball et al. 2018b). The mean annual temperature at the site is ~25 °C and the mean annual precipitation is ~3500 mm of rainfall (Scatena 1989). Two major hurricanes, Irma and Maria, made landfall in Puerto Rico in September 2017, disturbing the forest canopy by knocking down most of the large-stemmed trees and completely defoliating the few remaining large stems. All thermotolerance measurements were taken after the hurricanes, when succession was causing changes in canopy structure, microclimate, and plant community diversity.

The site has a large diversity of plants, and only few species are common amongst most of the plots – *Psychotria brachiata* was common among all the plots, *Piper glabrescens* was common among 5 of the plots, and *Cecropia schreberiana* was common among all the plots from after the hurricanes to until around October 2019, after which the tree crowns grew above the height of the heaters and were no longer subjected to experimental warming.

### 4.3.2 Environmental variables

Leaf area index (LAI; $m^2_{leaf}\ m^{-2}_{ground}$) was used as a proxy for light environment. LAI was measured at each plot using LI-COR LI-2200 (LI-COR Biosciences, Lincoln, NE, USA) equipped with a quarter cover lens cap. LAI measurements were taken around 7-9 am when light was diffuse at the site. LAI measurements in plot replicates of 6 were repeated 31 different days starting in January 2018 and continuing through September 2022. Each of the six replicates was measured at each of the six support poles for the warming infrastructure and measured towards the interior of the plot. Concurrent "open canopy" measurements taken from an open area at the Sabana Field Research Station about 100 m from the study site was used as a comparison for on plot level LAI. LAI at each plot was fitted to a sigmoidal shaped curve so we could estimate the LAI at each plot during the time when thermotolerance measurements were being recorded.

$$LAI = \frac{a}{e^{-b*(D_H*c)}}, \qquad \text{Eqn 1}$$

where LAI is leaf area index, $D_H$ is days since hurricanes, a is the maximum LAI value, b is the steepness of LAI recovery, and c is the inflection point of the sigmoidal curve.

Plot level temperature was measured using Apogee infrared thermometers (SI-121, Apogee Instruments, Logan, UT, USA) aimed at the center of the plot and data were collected and averaged at each hour using a CR-1000 data logger (Campbell Scientific, Logan , UT, USA). We used the max hourly temperature during the 10 days preceding the thermotolerance measurement to assess thermotolerance responses to plot vegetation temperature. Surface soil moisture was measured using a CS-655 soil temperature/moisture probe (Campbell Scientific, Logan , UT, USA) embedded 0-10 cm in the soil and averaged hourly using a CR-1000 datalogger. We used mean soil moisture during the 10 days preceding the thermotolerance measurement to assess thermotolerance responses to soil moisture.

### 4.3.3 Thermotolerance measurements

Three individuals per species per plot (3 plants x 3 species x 6 plots) were measured during each campaign. The top-most fully expanded leaf on each stem was measured for thermotolerance. When a species was absent from a plot, three extra individuals of that species were randomly selected from the plots with the same treatment. We measured thermotolerance in two ways, immediately following collection (“immediate”) and after a time of water saturation (“rehydrated”). Immediate leaves were collected pre-dawn using red light headlamps and placed in plastic bags in a dark cooler to prevent field station security lights from biasing measurements. Leaf samples were brought back to a darkened lab for measurement. In June 2019 and January 2020, rehydrated leaves were collected at 17:00 the day before measurement by cutting the base of the petiole underwater to prevent cavitation. Excised leaves were placed in deionized water-filled floral tubes and placed in a dark cabinet over night to saturate with water and become dark-adapted. Eight 1.27 cm diameter discs were punch from each leaf, wrapped in moistened cheesecloth, and placed in 2x3 cm watertight plastic bags. Leaf discs for immediate and rehydrated leaves were incubated in water baths using a sous vide (ANOVA A2.2-120V0US, Anova Culinary, San Francisco, CA, USA) at 25 °C, 35 °C, 40 °C, 45 °C, 47 °C, 50 °C, 52 °C, and 60 °C, for 30 minutes (method adapted from (Knight and Ackerly 2003)). Leaf discs were removed from the water baths and set out at room temperature for 30 minutes to recover. A FluorPen FP-100 (Photon Systems Instruments, Drásov, Czech Republic) handheld fluorometer was used to measure $F_v/F_m$ using the OJIP protocol. Fluorometric data were fit to a sigmoidal curve using the equation:

$$F_v/F_m = \frac{a}{1+e^{-b*(t-c)}}, \tag{Eqn 2}$$

where $t$ is incubation temperature (°C), $a$ is the highest value for $F_v/F_m$ (maximum quantum efficiency, QE), $b$ is the steepness of the curve, and $c$ is the point where $F_v/F_m$ is at 50% loss of function ($T_{50}$, °C). From this curve, $T_{crit}$ (°C) is calculated as the value of t where $F_v/F_m$ is 85% of the fitted QE parameter (Fig. 4.1). Thermotolerance curves were fit using the ‘nls’ function in base R (R Core Team 2022).

### 4.3.4 Leaf Trait Measurements

After fluorescence measurement, the leaf discs were pooled by individual and dried for 48 hours in a 65 °C drying oven and massed to get dry leaf mass for leaf mass per area (LMA; g cm$^{-2}$) measurements. The dried leaves were ground using a ball-mill (SPEX 8000D, SPEX Sample Prep, Metuchen, NJ, USA) and analyzed for carbon and nitrogen on an elemental analyzer (Elementar Vario Micro Cube, Elementar Inc., Langenelsbold, Germany).

### 4.3.5 Data analysis

Thermotolerance parameters $T_{50}$, $T_{crit}$, and QE were used to determine if thermotolerance responds to experimental warming treatment using a three-way ANOVA with species, experimental warming treatment, and LAI as explanatory variables. Thermotolerance measures were also analyzed with ANCOVAs with warming treatment, LAI, max temperature, soil volumetric water content, and the interaction of warming treatment with each of the independent variables as explanatory variables. The hydration experiment was tested for *P. brachiata* and *P. glabrescens* using an ANCOVA with each thermotolerance variable in response to treatment, hydration, and the interaction of treatment and hydration during the June 2019 and January 2020 measurement campaigns. All statistical analyses were performed using R (R Core Team 2022).

## 4.4 Results

LAI increased in a sigmoidal shaped curve as the canopy closed after the hurricanes. LAI was lowest immediately after the hurricanes and increased during the first two years after the hurricanes until leveling out (Fig 4.2). Pre-hurricane LAI values were around 6 and leveled out between 4-6 when the canopy closed for each plot. The variability in these values is likely dependent on the amount of large stem plants near the plot measured were knocked down during the hurricanes and the surviving large-stem plants that leafed out after the hurricanes.

There were species level differences in thermotolerance independent of experimental warming, but associated with changes in LAI (Table 4.2, Fig 4.3). $T_{50}$ was highest in *C. schreberiana* and *P. glabrescens* and increased for all species as the LAI increased. $T_{crit}$ was highest for *C. schreberiana* and lowest in *P. glabrescens* (Table 4.2, Fig. 4.3). QE was for highest for *C. schreberiana* and lowest in *P. glabrescens* (Table 4.2, Fig. 4.3).

We did not observe any acclimation of thermotolerance to experimental warming through changes in $T_{50}$ or $T_{crit}$ for the species we measured (Table 4.2, Fig. 4.3). However, for *P. brachiata*, there was an interacting effect of warming treatment and LAI where $T_{crit}$ increased as LAI increased, but only in the warmed plots while $T_{crit}$ remained unchanged in the control plots as LAI increased (Table 4.2, Fig 4.3). We did observe an increase in QE for warmed *P. glabrescens* and a decrease for in QE for warmed *P. brachiata*, and no experimental warming effect on QE for *C. schreberiana* (Table 4.2, Fig 4.3).

We did obseve changes in thermotolerance associated with max temperatuere in the 10-days preceeding thermotolerance measurement. *C. schreberiana* $T_{50}$ increased as temp increased and the temperature difference was more pronounced in warmed plots than in control plaots (Table 4.3, Fig 4.4). *P. glabrescens* $T_{50}$ increased as max temp increased, but was not affected by warming treatment. *C. schreberiana* $T_{crit}$ followed the same trend as $T_{50}$, but *P. glabrescens* and *P. brachiata* $T_{crit}$ were not affected by max temperature or warming treatment (Table 4.3, Fig 4.4). *P. glabrescens* QE was lower in control plots than warmed plots and QE was negatively dependent on max temperature for both control and warming treatments.

Changes in closing canopy cover, measured as increasing LAI, caused increases in $T_{50}$ for all three species. As the canopy closed, *C. schreberiana* $T_{50}$ increased ~2 °C, *P. glabrescens* $T_{crit}$ increased ~2 °C, and *P. brachiata* $T_{crit}$ increased ~2 °C. As the canopy closed, *C. schreberiana* $T_{crit}$ increased ~2 °C, *P. glabrescens* $T_{crit}$ increased ~4 °C, and *P. brachiata* $T_{crit}$ increased ~2 °C, but only in warmed plots (Table 4.2, Fig. 4.3). QE decreased in *P. glabrescens* as the canopy closed, QE increase for *P. brachiata* as the canopy closed, and QE was unaffected by canopy changes for *C. schreberiana* (Table 4.2, Fig. 4.3). Thermotolerance for warmed *C. schreberiana* leaves increased as soil moisture decreased suggesting that warmed *C. schreberiana* plants were acclimating to lower soil moisture (Table 4.4, Fig. 4.5).

Leaf hydration caused an increase in $T_{50}$ for *P. brachiata* in June 2019, but *P. brachiata* $T_{50}$ was unaffected by hydration in January 2020 (Table 4.5, Fig 4.6). *P. glabrescens* $T_{50}$ and $T_{crit}$ were unaffected by hydration in June 2019 and then *P. glabrescens* $T_{50}$ and $T_{crit}$ both decreased for hydrated leaves in January 2020 (Table 4.5, Fig 4.6). QE was lower in warmed *P. brachiata* leaves independent of hydration in both June 2019 and January 2020.

Leaf nitrogen % did not have any effects on QE or thermotolerance through changes in $T_{50}$ or $T_{crit}$ for *P. brachiata* or *P. glabrescens*, the only two species on which we measured N% (Table 4.6).

## 4.5 Discussion

### 4.5.1 Warming effects on thermotolerance and QE

We observed little thermotolerance acclimation to experimental warming as we hypothesized in H1, but we did observe that QE responded to experimental warming in both *P. brachiata* and *P. glabrescens*. While there was not an experimental warming effect on thermotolerance, thermotolerance does increase as temperature increases for *C. schreberiana* and *P. glabrescens*. The trend of thermotolerance response to temperature is similar in magnitude of other studies that looked at tropical plant thermotolerance responses to mean annual temperature in Colombia and Panama (Feeley et al. 2020, Slot et al. 2021); however, because our plots had effectively the same mean annual temperature as each other and we think the $T_{max}$ of the ten days prior to measurement captures this same effect. Additionally, *C. schreberiana* $T_{50}$ and $T_{crit}$ only increased with temperature in warmed plot, suggesting it is acclimating to increased temperature for plant exposed to experimental warming. A lack of thermotolerance acclimation in *P. brachiata* indicates that either the plants we were measuring do not exhibit this response to long-term warming, or that our experimental treatment was not high enough to induce a response. Ecologically, this means that under long-term warming at the expected temperatures of +4 °C by the year 2100, some tropical plants may not have the mechanisms to acclimate their upper threshold of temperature tolerance during periods of extreme heat. However, if there are repeated and frequent periods of extreme heat, some plants, like *Eucalyptus parramattensis* measured by Drake et al. (2018), may be able to mitigate high-heat damage through thermotolerance acclimation. We think this response is likely due to extreme heat response as opposed to long-term warming like the treatment at our experiment.

Our expectations of lower QE in warmed plots were predicated on thermotolerance being higher in warmed plot and energy that would be directed towards maintenance and repair of photosystems would be redirected to increasing thermotolerance. However, since we do not see thermotolerance acclimation to warming, it is likely that the changes in QE are caused by other stress responses. *P. brachiata* exhibited an expected response by having a lower QE in warmed plots, indicating that warmed leaves are more stressed. This stress could be a direct response from the heat or it could be an indirect response from potentially higher pathogens in the warmed plots (Delgado-Baquerizo et al. 2020). *P. glabrescens* exhibited an unexpected response because QE was higher in warmed plots indicating that the photosystems were less stressed than in control plots; however, warmed *P. glabrescens* were undergoing mortality while control *P. glabrescens* were not. Control *P. glabrescens* were apparently performing well, while warmed *P. glabrescens* were possibly redirecting their resources to repairing and maintaining photosystems while other systems apparently failed.

### 4.5.2 Effects of post-hurricane recovery on thermotolerance

*P. brachiata* exhibited a lower QE in warmed plots than control plots, but *P. glabrescens* had a higher QE in warmed plots than control plots, which is the opposite direction than we hypothesized. Contrary to our expectations, *C. schreberiana, P. glabrescens*, and *P. brachiata* all showed an increase in $T_{50}$ with increased LAI while *C. schreberiana* and *P. glabrescens* had the same response for $T_{crit}$ and only warmed *P. brachiata* had an increase in $T_{crit}$ while control *P. brachiata* $T_{crit}$ remained constant while LAI increase.

We expected thermotolerance to be higher when LAI was lower and the canopy was open because there would be more incoming solar radiation that would cause the plants to redirect resources to dissipate and tolerate higher heat; we observed the opposite direction response with increases in thermotolerance of ~2-4 °C as the canopy closed. It could be that overall, higher solar radiation had negative impacts on light and heat responses that caused lower thermotolerance. All the plants we measured were early successional plants with *P. glabrescens* and *P. brachiata* as understory plants and *C. schreberiana* as a canopy plant (Wen et al. 2008, Alonso-Rodríguez et al. 2022), however, they were all measured below the upper canopy and as the upper canopy closed and increased plot level LAI, the plants we measured were all subjected to similar amounts of solar radiation exposure in each plot. The increase in thermotolerance as a response to closing canopy could be a ubiquitous response among the early successional plants at our site. It is odd that control *P. brachiata* plants did not show an increase in $T_{crit}$ while the canopy closed, but did show an increase in $T_{50}$. This trend indicates that as the canopy closed, the steepness of the sigmoidal curve for functional decay over increased measurement temperature decreased for control *P. brachiata*, while the whole curve shifted right in warmed *P. brachiata*.

Curiously, each species showed a different response to QE as the canopy closed. *C. schreberiana*, a very fast growing early successional upper-canopy tree, did not have a QE response to closing canopy. It is possible that *C. schreberiana*, a high-light adapted plant, has mechanisms to repair and protect photosystems that keep photosystems independent of canopy cover. *C. schreberiana* do not typically grow in the understory under a closed canopy so it is possible their photosystems are less sensitive to changing light conditions. *P. glabrescens* had a decrease in QE as the canopy closed suggesting they were becoming more stressed, which is counterintuitive because high-light can cause photoinhibition through higher D1-turnover resulting in lower QE (Tao et al. 2021). Lower QE is also a product of protection from short periods of overexcitation during high periods of light (Külheim and Jansson 2005). The closed canopy *P. brachiata* at our site may be showing a lower QE due to these photoprotective mechanisms. Higher stress *on P. glabrescens*, which is slow-growing relative to other plants in the plots (anecdotal), could be caused by inter-specific competition because other plants were able to take advantage of high-light conditions earlier in succession while the canopy was more open. *P. brachiata* had an expected response where QE increased as the canopy closed and lower solar radiation caused a release from stress.

### 4.5.3 Soil moisture limitations

As we hypothesized, thermotolerance was higher when soil moisture was more limiting, but only in *C. schreberiana*. This trend was only observed in warmed *C. schreberiana* suggesting that warming acclimation is related to thermotolerance and water limitation which aligns with another study at our site showing that drought tolerance is higher for *C. schreberiana* (Alonso-Rodríguez et al. 2022). However, *P. brachiata* and *P. glabrescens* thermotolerance not acclimating to limited water could be because maintaining high-heat thermotolerance without evaporative cooling is energetically expensive (Wahid et al. 2007). *P. brachiata* seedlings at out study site increased under warming conditions while not performing well during drought (Alonso-Rodríguez et al. 2022). *P. brachiata* seedlings had a higher abundance during drought, but lower seedling and sapling abundance under warming (Alonso-Rodríguez et al. 2022). These drought benefits to *P. glabrescens* are apparently limited to unwarmed plants since the warmed plants did not survive and thermotolerance was not related to soil moisture.

### 4.5.4 Water saturation

In June 2019, we observed an increase in P. brachiata $T_{50}$, supporting our hypothesis that hydrated leaves will have higher thermotolerance, but that response did not persist to January 2020. However, *P. glabrescens* were unaffected by hydration in June 2020 and then have a decrease in $T_{50}$ and $T_{crit}$ for hydrated leaves. In June 2019, when ambient temperatures were about +4 °C higher than in January 2020, there were little effects of hydration on thermotolerance. Hydrated *P. brachiata* leaves had an increase in $T_{50}$, while all other parameters remained constant. However, in January 2020, non-hydrated *P. glabrescens* leaves had a higher $T_{50}$ and $T_{crit}$, suggesting that non-hydrated *P. glabrescens* leaves had a higher thermotolerance when seasonal temperatures were cooler. A lack of interaction effects between hydration and warming treatment on thermotolerance indicates that hydration effects on thermotolerance are independent of warming treatment effects.

### 4.5.5 Effects of leaf traits and species on thermotolerance

*P. brachiata* and *P. glabrescens* did not show any response to variable N%. A lack of effects of foliar N% on thermotolerance is interesting because foliar N% is associated with overall photosynthetic potential. Because foliar N% is not causing a change in thermotolerance, we think the budget of nitrogen in the leaf is going to overall photosynthesis and thermotolerance may not be one of the most demanding systems for nitrogen. Since thermotolerance causes changes in membrane fluidity (Tovuu et al. 2013), we speculate that the nitrogen using metabolic demand that causes changes in membrane fluidity is so much smaller than the proportion that goes to more nitrogen intensive processes like carboxylation that the signal, if there is one, is in discriminant from the carbon fixation signal. Soil nitrogen is not associated with higher QE (Lin et al. 2013) while N% is correlated with LMA (Luo et al. 2021), we speculate that these leaves have a budget for how much nitrogen is directed to photosystem repair and maintenance, while the rest of the available nitrogen is budgeted to carbon reactions.

### 4.5.6 Summary

Overall, a lack of thermotolerance acclimation to increased temperatures, and a lower thermotolerance earlier in succession suggests understory plants at our site are at more risk of temperature induced mortality under future climate scenarios with higher temperatures and more frequent and intense hurricane disturbances. Each of the plants we measured showed different stress responses to temperature and canopy cover showcasing the variability in responses that plants have to environmental stimuli that reflect their evolutionary history. While some high-light loving, early successional plants, like *C. schreberiana*, might be resistant to warming and hurricane disturbance, other understory low-light tolerant plants, like *P. glabrescens*, may not have the mechanisms to survive both warming and disturbance.

## 4.6 Tables and Figures

Table 4.1: ANCOVA table for thermotolerance and QE response to species, experimental warming treatment, LAI, and the interaction of each. Values in table are p values and bold values are statistically significant at $p < 0.05$.

| | n | Species | Treatment | LAI | S x T | S x L | T x L | S x T x L |
|---|---|---|---|---|---|---|---|---|
| $T_{50}$ | 198 | **<0.001** | 0.17 | **<0.0001** | 0.62 | 0.13 | 0.34 | 0.43 |
| $T_{crit}$ | 198 | **<0.001** | 0.39 | **0.0021** | 0.53 | **0.028** | 0.07 | 0.83 |
| QE | 198 | **<0.001** | 0.89 | **<0.001** | **0.011** | **0.0012** | 0.13 | 0.56 |

Table 4.2: ANCOVA table for thermotolerance and QE response to experimental warming treatment and LAI. Values in table are p values and bold values are statistically significant at $p < 0.05$.

| | | n | Treatment | LAI estimate | Treatment x LAI estimate |
|---|---|---|---|---|---|
| *P. glabrescens* | $T_{50}$ | 51 | 0.31 | **0.00041** | 0.45 |
| | $T_{crit}$ | 51 | 0.29 | **0.013** | 0.79 |
| | QE | 51 | **0.011** | **0.025** | 0.92 |
| *P. brachiata* | $T_{50}$ | 78 | 0.92 | **0.000013** | 0.26 |
| | $T_{crit}$ | 78 | 0.65 | 0.24 | **0.031** |
| | QE | 78 | **0.032** | **0.000013** | 0.22 |
| *C. schreberiana* | $T_{50}$ | 51 | 0.086 | **0.000039** | 0.36 |
| | $T_{crit}$ | 51 | 0.82 | **0.018** | 0.32 |
| | QE | 51 | 0.83 | 0.15 | 0.37 |

Table 4.3: ANCOVA table for thermotolerance and QE response to experimental warming treatment and 10-day max temperature. Values in table are p values and bold values are statistically significant at $p < 0.05$.

| | | n | Treatment | Max Temp | Treatment x Max Temp |
|---|---|---|---|---|---|
| *P. glabrescens* | $T_{50}$ | 51 | 0.33 | **0.0053** | 0.32 |
| | $T_{crit}$ | 51 | 0.31 | 0.10 | 0.86 |
| | QE | 51 | **0.0090** | **0.0045** | 0.92 |
| *P. brachiata* | $T_{50}$ | 57 | 0.73 | 0.61 | 0.89 |
| | $T_{crit}$ | 57 | 0.73 | 0.13 | 0.54 |
| | QE | 57 | 0.19 | 0.84 | 0.18 |
| *C. schreberiana* | $T_{50}$ | 51 | 0.12 | **0.036** | **0.049** |
| | $T_{crit}$ | 51 | 0.81 | **0.044** | **0.018** |
| | QE | 51 | 0.84 | 0.62 | 0.52 |

Table 4.4: ANCOVA table for thermotolerance and QE response to experimental warming treatment and soil surface volumetric water content. Values in table are p values and bold values are statistically significant at $p < 0.05$.

| | | n | Treatment | Soil VWC | Treatment x Soil VWC |
|---|---|---|---|---|---|
| *P. glabrescens* | $T_{50}$ | 45 | 0.16 | 0.68 | 0.23 |
| | $T_{crit}$ | 45 | 0.98 | 0.42 | 0.38 |
| | QE | 45 | **0.0082** | 0.76 | 0.43 |
| *P. brachiata* | $T_{50}$ | 58 | 0.97 | 0.62 | **0.024** |
| | $T_{crit}$ | 58 | 0.98 | 0.38 | **0.013** |
| | QE | 58 | 0.25 | 0.38 | 0.37 |
| *C. schreberiana* | $T_{50}$ | 51 | 0.13 | **0.049** | 0.10 |
| | $T_{crit}$ | 51 | 0.82 | 0.10 | **0.071** |
| | QE | 51 | 0.84 | 0.66 | **0.043** |

Table 4.5: ANCOVA table for thermotolerance and QE response to experimental warming treatment and hydration experiment in June 2019 and January 2020. Values in table are p values and bold values are statistically significant at $p < 0.05$.

| | | | n | Treatment | Hydration | Treatment x Hydration |
|---|---|---|---|---|---|---|
| 2019 June | *P. glabrescens* | $T_{50}$ | 35 | 0.45 | 0.59 | 0.62 |
| | | $T_{crit}$ | 35 | 0.82 | 0.69 | 0.87 |
| | | QE | 35 | 0.39 | 0.86 | 0.18 |
| | *P. brachiata* | $T_{50}$ | 27 | 0.088 | **0.041** | 0.15 |
| | | $T_{crit}$ | 27 | 0.49 | 0.26 | 0.78 |
| | | QE | 27 | **0.029** | 0.81 | 0.97 |
| 2020 January | *P. glabrescens* | $T_{50}$ | 29 | 0.52 | **0.027** | 0.97 |
| | | $T_{crit}$ | 29 | **0.0035** | **0.00055** | 0.61 |
| | | QE | 29 | **0.017** | **0.016** | 0.56 |
| | *P. brachiata* | $T_{50}$ | 33 | 0.074 | 0.68 | 0.85 |
| | | $T_{crit}$ | 33 | 0.72 | 0.20 | 0.59 |
| | | QE | 33 | **0.037** | 0.60 | 0.70 |

Table 4.6: ANCOVA table for thermotolerance and QE response to experimental warming treatment and foliar nitrogen (N%). Values in table are p values and bold values are statistically significant at $p < 0.05$.

| | | n | Treatment | N% | Treatment x N% |
|---|---|---|---|---|---|
| *P. glabrescens* | $T_{50}$ | 32 | 0.68 | 0.72 | 0.73 |
| | $T_{crit}$ | 32 | 0.38 | 0.46 | 0.31 |
| | QE | 32 | 0.93 | 0.099 | 0.77 |
| *P. brachiata* | $T_{50}$ | 45 | 0.88 | 0.52 | 0.89 |
| | $T_{crit}$ | 45 | 0.35 | 0.72 | 0.32 |
| | QE | 45 | 0.87 | 0.57 | 0.69 |

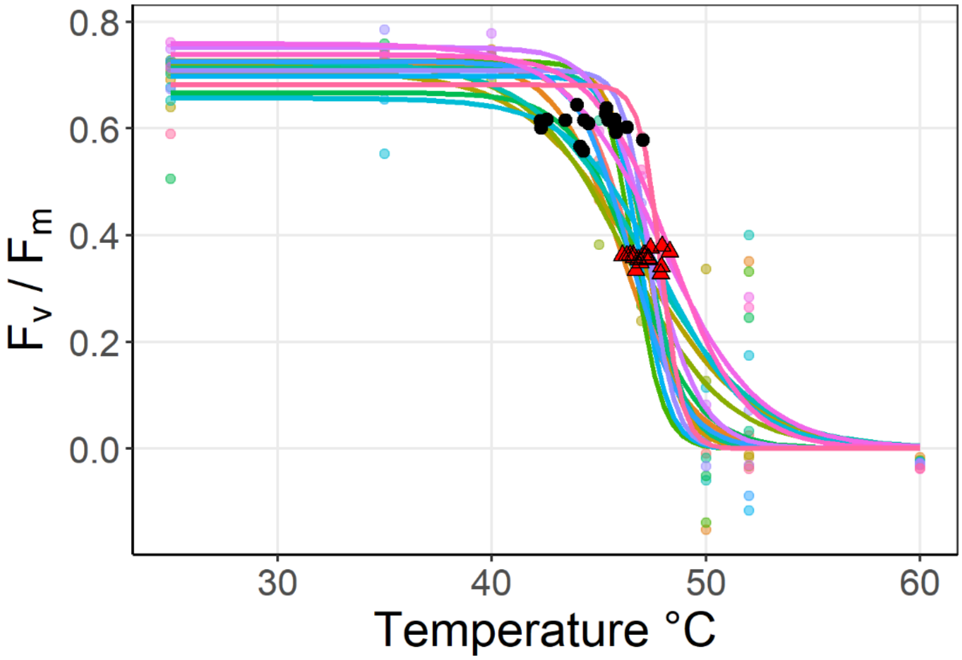

Figure 4.1: Thermotolerance curves showing the change in $F_v/F_m$ as temperature increases. These data are the December 2018 *P. brachiata* curves showing $T_{crit}$ as black dots, $T_{50}$ as red triangles, and QE as the $F_v/F_m$ values of the curve where it is flat at lower temperatures.

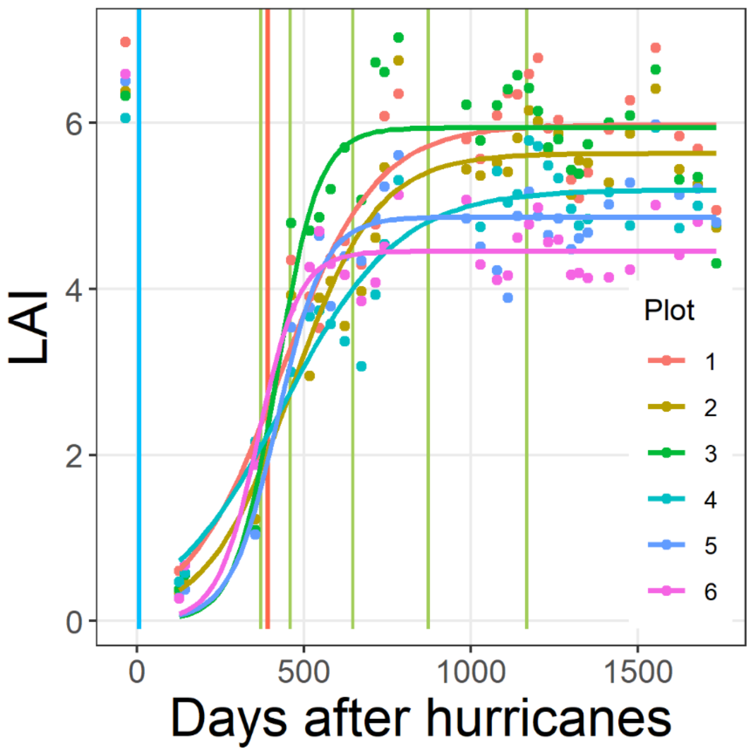

Figure 4.2: LAI response to hurricane recovery at each of the TRACE plots. The vertical blue line a days = 0 is when hurricane Irma hit and the red line is when experimental warmign was turned back on. The green lines indicate times when thermotolerance data were collected. Pre-hurricane LAI was around 6 and decreased to nearly zero before following a sigmoidal shaped response for increasing canopy cover.

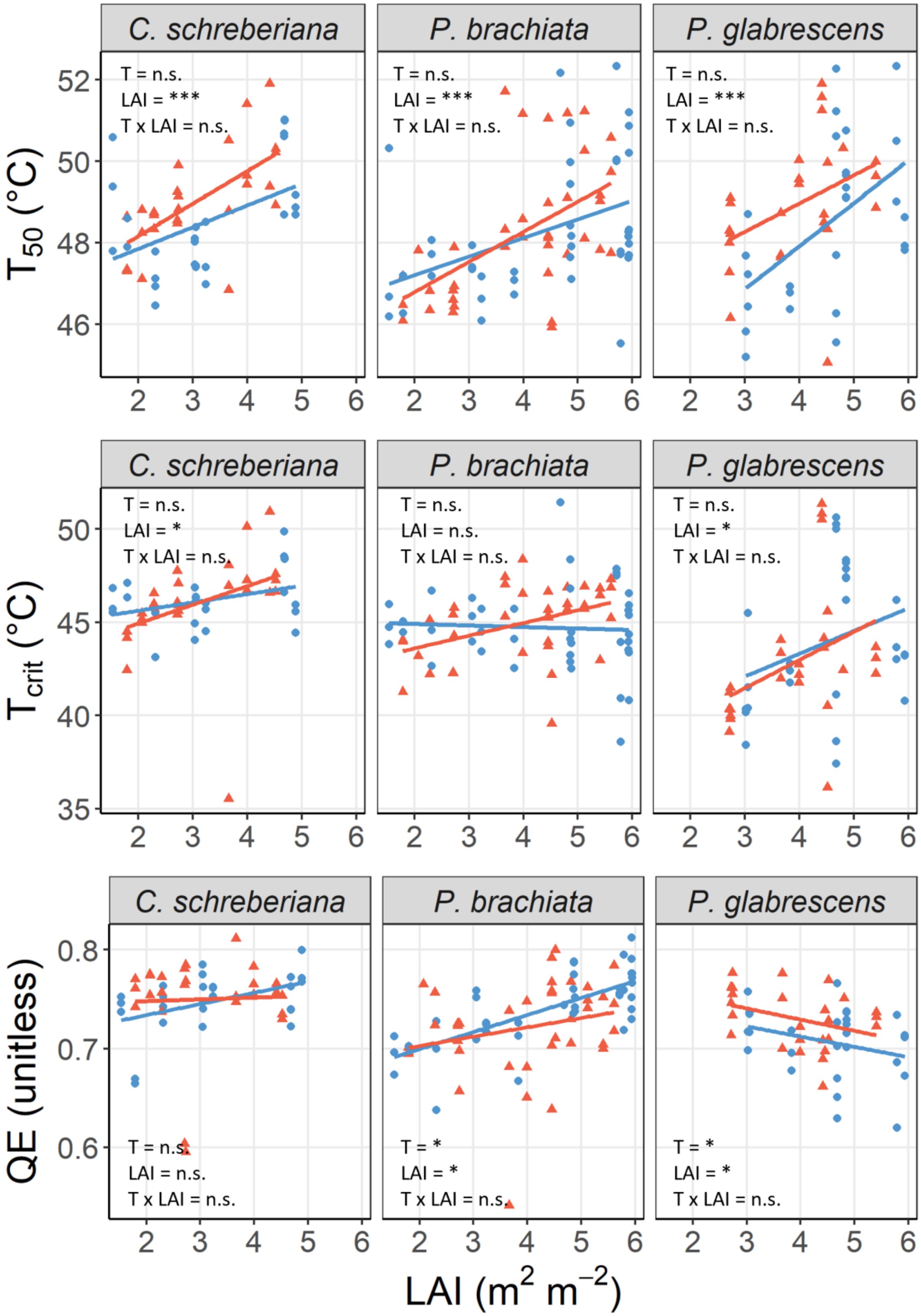
C. schreberiana
P. brachiata
P. glabrescens
T = n.s.
LAI = ***
T x LAI = n.s.
T = n.s.
LAI = ***
T x LAI = n.s.
T = n.s.
LAI = ***
T x LAI = n.s.
$T_{50}$ (°C)
46
48
50
52
C. schreberiana
P. brachiata
P. glabrescens
T = n.s.
LAI = *
T x LAI = n.s.
T = n.s.
LAI = n.s.
T x LAI = n.s.
T = n.s.
LAI = *
T x LAI = n.s.
$T_{crit}$ (°C)
35
40
45
50
C. schreberiana
P. brachiata
P. glabrescens
T = n.s.
LAI = n.s.
T x LAI = n.s.
T = *
LAI = *
T x LAI = n.s.
T = *
LAI = *
T x LAI = n.s.
QE (unitless)
0.6
0.7
0.8
2
3
4
5
6
LAI ($m^2$ $m^{-2}$)

Figure 4.3: $T_{50}$, $T_{crit}$, and QE response to LAI and experimental warming treatment for *C. schreberiana*, *P. brachiata*, and *P. glabrescens*. Red triangles and blue dots indicate measurements in warmed plots and control plots, respectively. Statistical significance is indicated in the corner with experimental warming treatment (T), LAI, and the interaction of treatment and LAI (T x LAI) ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***, no significance = n.s.)

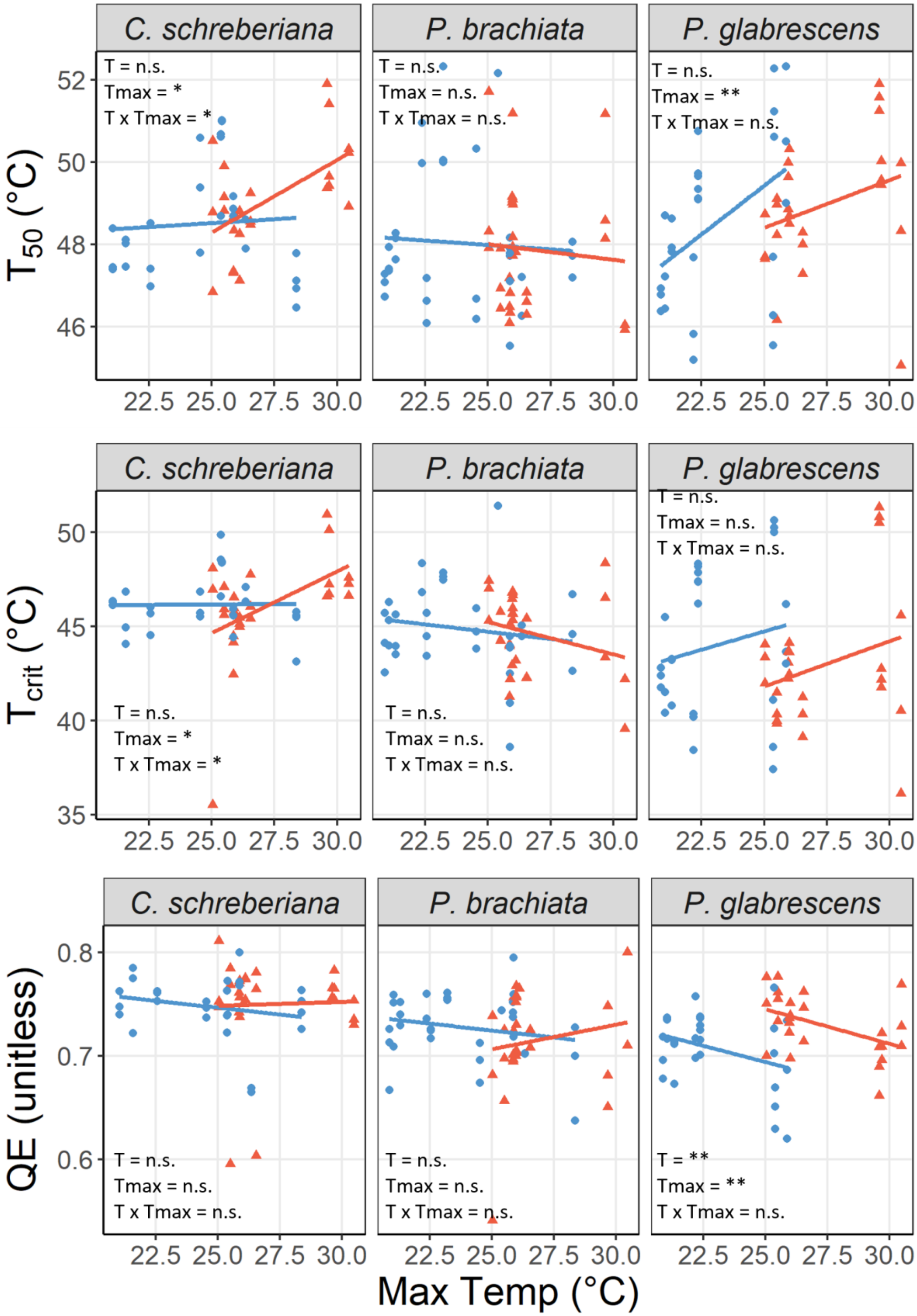
C. schreberiana
P. brachiata
P. glabrescens
T = n.s.
Tmax = *
T x Tmax = *
T = n.s.
Tmax = n.s.
T x Tmax = n.s.
T = n.s.
Tmax = **
T x Tmax = n.s.
$T_{50}$ (°C)
52
50
48
46
22.5 25.0 27.5 30.0
C. schreberiana
P. brachiata
P. glabrescens
T = n.s.
Tmax = *
T x Tmax = *
T = n.s.
Tmax = n.s.
T x Tmax = n.s.
T = n.s.
Tmax = n.s.
T x Tmax = n.s.
$T_{crit}$ (°C)
50
45
40
35
22.5 25.0 27.5 30.0
C. schreberiana
P. brachiata
P. glabrescens
T = n.s.
Tmax = n.s.
T x Tmax = n.s.
T = n.s.
Tmax = n.s.
T x Tmax = n.s.
T = **
Tmax = **
T x Tmax = n.s.
QE (unitless)
0.8
0.7
0.6
22.5 25.0 27.5 30.0
Max Temp (°C)

Figure 4.4: $T_{50}$, $T_{crit}$, and QE response to max vegetation temperature in the 10 days preceding the thermotolerance measurement and experimental warming treatment for *C. schreberiana*, *P. brachiata*, and *P. glabrescens*. Red triangles and blue dots indicate measurements in warmed plots and control plots, respectively. Statistical significance is indicated in the corner with experimental warming treatment (T), $T_{max}$, and the interaction of treatment and $T_{max}$ (T x $T_{max}$) ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***, no significance = n.s.)

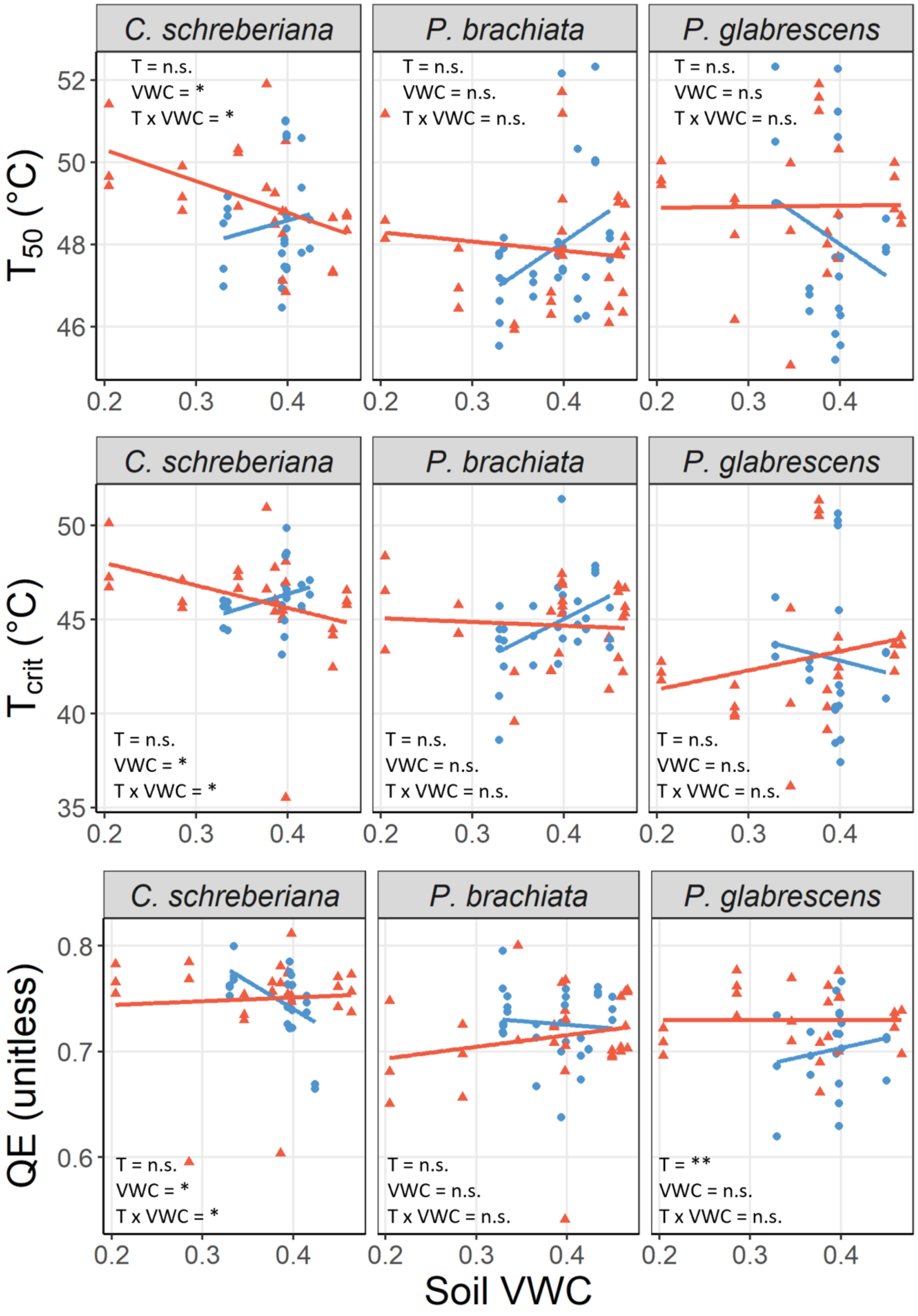
C. schreberiana
P. brachiata
P. glabrescens
T = n.s.
VWC = *
T x VWC = *
T = n.s.
VWC = n.s.
T x VWC = n.s.
T = n.s.
VWC = n.s
T x VWC = n.s.
$T_{50}$ (°C)
52
50
48
46
0.2
0.3
0.4
C. schreberiana
P. brachiata
P. glabrescens
$T_{crit}$ (°C)
50
45
40
35
T = n.s.
VWC = *
T x VWC = *
T = n.s.
VWC = n.s.
T x VWC = n.s.
T = n.s.
VWC = n.s.
T x VWC = n.s.
0.2
0.3
0.4
C. schreberiana
P. brachiata
P. glabrescens
QE (unitless)
0.8
0.7
0.6
T = n.s.
VWC = *
T x VWC = *
T = n.s.
VWC = n.s.
T x VWC = n.s.
T = **
VWC = n.s.
T x VWC = n.s.
0.2
0.3
0.4
Soil VWC

Figure 4.5: $T_{50}$, $T_{crit}$, and QE response to mean surface soil volumetric water content in the 10 days preceding the thermotolerance measurement and experimental warming treatment for *C. schreberiana*, *P. brachiata*, and *P. glabrescens*. Red triangles and blue dots indicate measurements in warmed plots and control plots, respectively. Statistical significance is indicated in the corner with experimental warming treatment (T), VWC, and the interaction of treatment and VWC (T x VWC) ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***, no significance = n.s.)

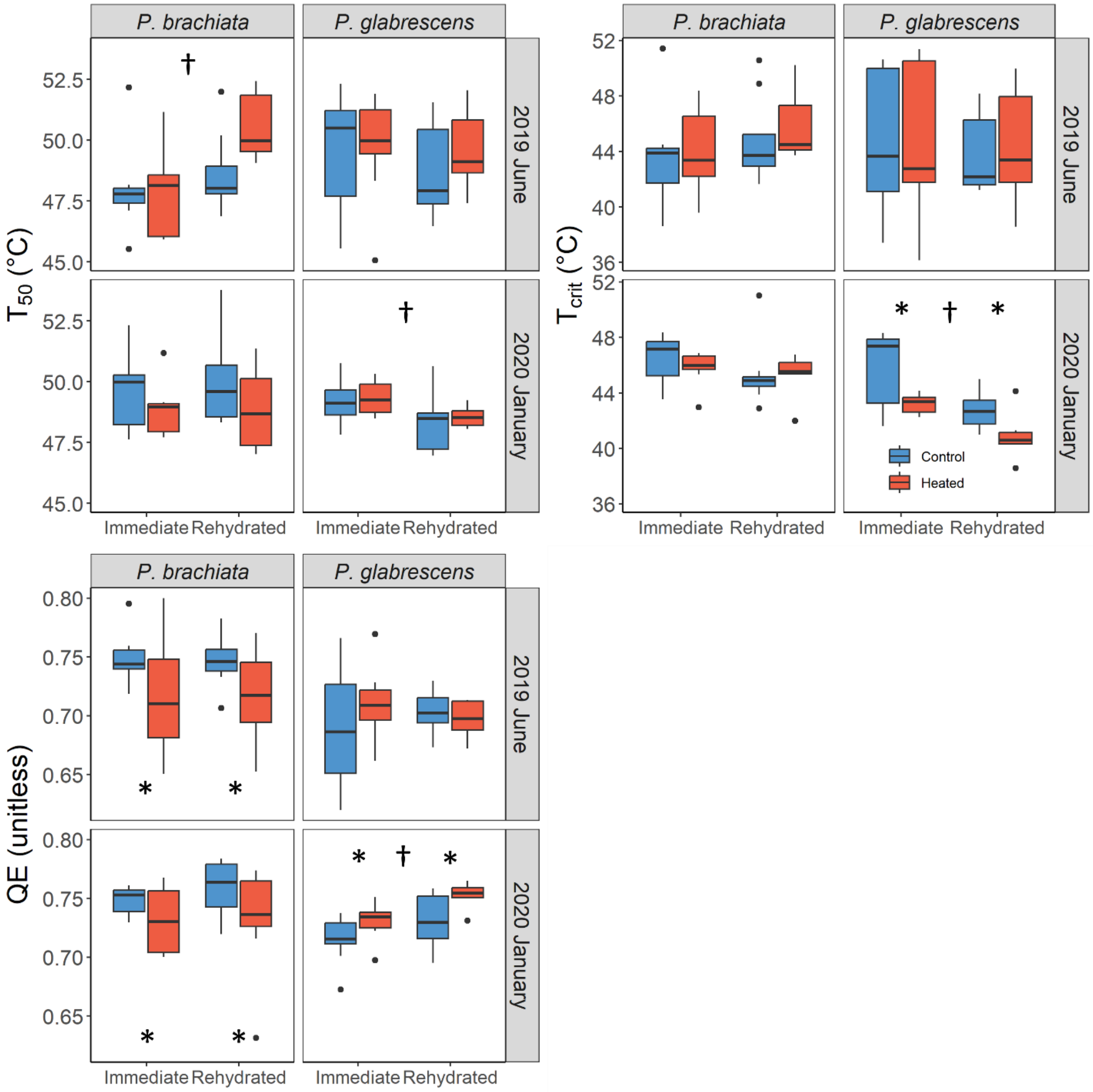

Figure 4.6: $T_{50}$, $T_{crit}$, and QE response to hydration experiment for warmed and control leaves during June 2019 and January 2020. The asterisk (*) indicates differences in warming treatment values and the dagger (†) indicates differences between immediate and rehydrated leaves.

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# 5 Dissertation Conclusion

In summary, we found that several photosynthetic and respiratory systems could acclimate to hurricane disturbance and experimental warming, but there were likely costs to each of the thermal acclimation parameters. Because the plants we studied are at a location that is subject to frequent hurricane disturbances, they showed short-term acclimation responses that showed they were able to take advantage of the rapidly changing canopy conditions and resulting microclimate changes.

Root specific respiration did not thermally acclimate to experimental warming through changes in physiological processes, but overall ecosystem root respiration did downregulate because there was lower root biomass. Root specific respiration became more variable in the short period after the hurricanes, but returned to pre-hurricane levels after about a year. The cause for short-term post-hurricane changes in root specific respiration is likely due to changes in plant communities, which was highly dynamic in early succession.

Foliar photosynthesis and respiration showed a small potential for thermal acclimation to warming, but the overall response was lower photosynthesis and respiration. There was a boost to photosynthetic and respiratory rates early in succession when the canopy was more open. The lower photosynthetic rates due to warming were consistent across changes in canopy conditions, showing that the warming and hurricane effects were independent of each other. Additionally, there was some response for photosynthetic mechanisms to changing soil moisture in warmed plots, showing that some warmed plants can maintain high water use efficiency during periods of soil water deficit.

Thermotolerance did not acclimate to warming and was lower when post-hurricane canopy cover was lower, possibly putting the plants at our site at more risk for loss of function at high temperatures. There was some acclimation effect of thermotolerance to some warmed plant species that had low soil moisture conditions where thermotolerance was likely protecting plants from high temperatures when they did not have the capacity to thermoregulate via evaporative cooling (Cavaleri 2020). Thermotolerance did increase with increasing plot level maximum temperatures for some species, and at a rate that is consistent with other species in the region (Slot et al. 2021). These thermotolerance results show that there are several potential causes for variation in thermotolerance and they do not necessarily rely strictly on direct temperature effects.

I speculate that the lower root biomass in warmed plots is caused by lower overall photosynthate production and less is transported below ground to the roots. Also, there was a big boost to post hurricane fine-root biomass in control plots and to a lesser degree in warmed plots (Yaffar et al. 2021) that could be supported by the initial increase in photosynthate production in the early stages of succession when the canopy was open and photosynthetic rates were higher. We do not have a complete story for how the root biomass changes using minirhizotron data presented by Yaffar et al. (2021), but data have been continuously collected since that publication and are concurrent with the data presented here and we could use it to see if photosynthetic rates are the cause for total amount of fine-root biomass.

Thermotolerance and photosynthesis have opposite directions of response to increasing canopy cover where thermotolerance is low when photosynthesis is high. This could be the a tradeoff that the plant is making to use more energy to photosynthesize more by using less energy to put into heat-shock response. While this tradeoff may be risky for plant in more arid ecosystems, I doubt that it is currently risky for the plants in this ecosystem because they usually have high soil moisture availability and can use that resource to thermoregulate without the risk for thermal damage. While the trait does not seem risky for the plants now, it could become risky if the plant experience more frequent and longer periods of drought, which is projected to increase in the region (Allen et al. 2010). While some species at the site respond better to drought and others respond better to warming (Alonso-Rodríguez et al. 2022), the combination of both stressors may put some species at risk of mortality due to soil moisture deficit limiting thermoregulation while warming puts them nearer to their thermotolerance temperature. Though higher plant community diversity can protect moist tropical forests species from mortality due to multiple abiotic and biotic stressors (McDowell et al. 2018), hurricane disturbances could limit the species in these communities to early succession species if the systems accumulate enough ‘resilience debt’ that it cannot rebound to original community compositions (Johnstone et al. 2016). A change in community composition could limit the diversity of traits that could protect the system from multiple types of disturbances (drought, warming, hurricane, biotic, etc...) and put it at more risk from single modal disturbance events. The benefit of the community being composed of strictly early-successional species is that it will have communities that are resilient to hurricane disturbances and will likely be able to fix more carbon at higher rates in those conditions. I think this is a relatively Panglossian view of carbon fixation and is relevant through the lens of carbon fixation as an ecosystem service. It is something that could be beneficial to humans through mitigating atmospheric greenhouse gases, but at the potential loss of community diversity, which is intrinsically valuable. The work we are doing is not a conservation project, but rather a project looking to quantify the carbon processes so we can better estimate future carbon cycling processes which has huge conservation implications.

The future work I plan to do is use the carbon exchange parameters from these studies to model carbon cycling at this site under future projected climate scenarios that have higher temperature and more frequent disturbances. We will be using the Energy Exascale Earth System Model – Functionally Assembled Terrestrial Ecosystem Simulator (E3SM-FATES) to model amount of carbon exchanged with this ecosystem and the atmosphere. If the plant communities remain as they are now, I predict that the overall carbon assimilation will be lower and it will be reflected by a lower carbon release from the system through respiration. Overall I think this system will become less of a sink under warming and more frequent disturbances than it has been historically.

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