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INSTANTANEOUS PHOTOSYNTHETIC RESPONSE TO TEMPERATURE OF MATURE FOREST CANOPIES AND EXPERIMENTALLY WARMED SEEDLINGS

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INSTANTANEOUS PHOTOSYNTHETIC RESPONSE TO TEMPERATURE OF MATURE FOREST
CANOPIES AND EXPERIMENTALLY WARMED SEEDLINGS

By

Alida C. Mau

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

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In Applied Ecology

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This thesis has been approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE in Applied Ecology.

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Preface

The following chapters have been formatted for submission to peer-reviewed journals, and will be submitted for publication in the near future. None of the current chapters are under copyright, since this material has not been previously published. Chapters 2 and 3 were with the collaboration of Molly Cavaleri, who planned the experiments, and provided critical feedback and editing, while I performed the data collection, analysis, and writing. Chapter 2 will be published with additions of air temperature data, and have additional collaboration with Sasha Reed and Tana Wood. Chapter 3 will be included in a larger manuscript, when submitted for publication, likely with the contribution of Mickey Jarvi and Andrew Burton.

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Abstract

Tropical trees have been shown to be more susceptible to warming compared to temperate species, and have shown growth and photosynthetic declines at elevated temperatures as little as 3°C above ambient. However, regional and global vegetation models lack the data needed to accurately represent physiological response to increased temperatures in tropical forests. We compared the instantaneous photosynthetic responses to elevated temperatures of four mature tropical rainforest tree species in Puerto Rico and the temperate broadleaf species sugar maple (*Acer saccharum*) in Michigan. Contrary to expectations, leaves in the upper canopy of both temperate and tropical forests had temperature optima that are already exceeded by mean daily leaf temperatures. This indicates that tropical and temperate forests are already seeing photosynthesis decline at mid-day temperature. This decline may worsen as air temperatures rise with climate change if trees are unable to acclimate, increasing the likelihood that forests may shift from carbon sinks to sources.

A secondary study was conducted on experimentally warmed sugar maple seedlings to determine if photosynthesis had been able to acclimate to +5°C air temperature over four years. Species abundance models had predicted a decline of sugar maple within the Upper Peninsula of Michigan over the next 100 years, due to elevated temperature and altered precipitation. Instantaneous photosynthetic temperature response curves on both control and heated seedlings showed that the differences between treatments were not statistically significant, though there was a 16% increase in temperature optima and a 3% increase in maximum rates of photosynthesis in warmed

plots. Though evidence of acclimation was not significant, the seedlings did not fare poorly as the models suggest.

Chapter 1

Introduction

With the onset of climate changes, factors such as elevated carbon dioxide (CO₂) and elevated global air temperature can have variable effects on C₃ plant species (Alexander et al. 2013). Many species have adapted to specific temperature regimes, and may perform poorly if the environment is rapidly altered. The ability to acclimate photosynthesis or respiration to elevated temperatures may allow species to continue thriving in their current habitat, without major dieback and range shifts (Lee et al. 2005, Hikosaka et al. 2006, Yamori et al. 2014). However, species' ability to acclimate vary significantly by age and local climate factors. Seedlings, for example, have been hypothesized to have greater plasticity in terms of adapting to changing environmental conditions, while mature trees have more difficulty adjusting (Dreyer et al. 2001, Niinemets 2010).

Due to difficulty accessing forest canopies and the arduous nature of procuring canopy photosynthetic measurements, mature forests, especially in tropical ecosystems, have not been investigated as much as necessary (Bassow and Bazzaz 1997). Tropical forests alone contain approximately 2/3 of the world's terrestrial biomass (Brown and Lugo 1982). Thus the lack of knowledge of current temperature thresholds of these forests, and mature forest canopies in general can be detrimental in predicting changes to global carbon fluxes from climate change (Doughty and Goulden 2008, Cavaleri et al. 2015). If declines in photosynthesis in response to temperature are severe enough it is possible for forests of any type to shift from carbon sinks to carbon sources, further

exacerbating the effects of climate change, instead of mitigating CO₂ emissions (Booth et al. 2012).

Our study of canopy photosynthesis was done to serve as a baseline to evaluate how close different forest types were to temperature thresholds for photosynthetic capacity, prior to any acclimation. The first chapter of this thesis sought to compare instantaneous photosynthetic response to temperature in a deciduous, subtropical moist, and tropical wet forest, in addition to investigating within-canopy variability. It was hypothesized that tropical forests would be operating closer to the thermal threshold for photosynthesis than the temperate deciduous forest.

Though seedling warming experiments are relatively common, certainly more so than any canopy warming experiments, *in situ* seedling warming is still rare (Chung et al. 2013, Silva and Anand 2013). Many warming experiments are done in growth chambers or pots, with artificial light, precipitation, and unnatural soil conditions. For the second chapter, seedlings in the same temperate forest were experimentally warmed for three years to determine if they could shift rates of photosynthesis to acclimate to elevated temperature (Chung et al. 2013). It was hypothesized that the warmed seedlings would acclimate to warmer growth temperatures and have greater thermal optima and maximum rates of photosynthesis compared to those in control.

Both chapters relate to the overall goal of investigating photosynthetic thresholds, but vary in terms of the ages of the trees being sampled and species. Overall, it is important to investigate the effects of temperature on seedlings, for their potential range shifts, and mature trees for insight into shifts in carbon balance, to determine each group's

current limitations and potential ability to acclimate to climate change, in order to improve current knowledge and management strategies.

Chapter 2

Photosynthetic Temperature Responses within Temperate and Tropical Forest Canopies ¹

Introduction

Temperate and tropical forests make up much of the world's biomass, with tropical forests alone accounting for over 60% of terrestrial global carbon (Pan et al. 2013). Forests mitigate the effects of climate change, such as elevated temperature, through carbon dioxide (CO₂) uptake during photosynthesis. However, global surface-air temperature is expected to increase by 1 to 5°C by 2100, with an even greater increase expected in northern latitudes, along with an increase in short term heat waves with temperatures exceeding 31 to 33° C (Alexander et al. 2013, Kirtman et al. 2013). As temperatures increase, there is a theoretical thermal tipping point after which photosynthesis begins to decline while plant respiration may still be increasing. If declines in CO₂ uptake are severe enough that forest-wide respiration exceeds photosynthesis, forests could become net sources of carbon to the atmosphere (Doughty and Goulden 2008, Wood et al. 2012). Determining where this thermal threshold exists and whether different forest types are close to shifting from carbon sources to sinks is crucial to understanding global climate feedbacks. One method of doing so is to model what will happen to forest carbon balance with warming (Randerson et al. 2009). However, our ability to accurately parameterize these models is hampered by a lack of mechanistic data on physiological responses to warming of temperate and tropical trees (Medlyn et al. 2002). Many global vegetation models are based around that of Farquhar's

¹ This chapter is planned for submission for publication

model of photosynthesis (1980), but lack field data to parameterize the biochemical processes of photosynthesis, especially for tropical forests (Cavaleri et al. 2015, Vårhammar et al. 2015)

Understanding photosynthetic responses to warming across the whole canopy is needed to improve model representation and will greatly improve our understanding of how global carbon balance might alter with climate change. Due to the amount of C stored in tropical forests globally, any alteration in storage rates could have global consequences in terms of climate feedbacks and biogeochemical cycling (Cavaleri et al. 2015). Models analyzing the responses of tropical C storage to elevated temperature have produced inconsistent predictions on whether these forests will remain sinks or become sources (Clark 2004, Cavaleri et al. 2015). In part, this is because there is great variability among tropical forests in annual temperature, precipitation, soil types, geographic range, and species assemblage; likely showing a variety of responses to changing climate across different systems (Richards 1952, Holdridge 1967, Marín-Spiotta and Sharma 2013). Additionally, there are contrasting projected outcomes of the effect of increasing temperature on photosynthesis in the tropics due to the lack of field data as inputs (Cox et al. 2013). Large global shifts in photosynthesis could either mitigate or exacerbate the amount of CO₂ accumulating in the atmosphere, depending in part on the response of respiration to climate change (Atkin et al. 2008, Smith and Dukes 2013, Gonzalez-Meler et al. 2014, Weerasinghe et al. 2014). Since tropical forests in particular contain so much global carbon, any shifts in tropical photosynthesis can alter global carbon balances and feedbacks from climate change.

There is a dearth of ecophysiological data in tropical ecosystems from warming experiments, and mature tree warming experiments are rare in any latitude (Doughty and Goulden 2008, Cernusak et al. 2013, Gonzalez-Meler et al. 2014, Slot et al. 2014, Cavaleri et al. 2015). Though canopy acclimation studies will be crucial in determining whether different forest types can acclimate, instantaneous photosynthesis measurements are necessary to improve models and set a baseline to determine how close these forests are to the temperature optimums for photosynthesis. Even in temperate forests, there is a scarcity of data on the short term effects of elevated temperature on photosynthesis in mature forest canopies and for long-term temperature acclimation (Gunderson et al. 2010a, Filewod and Thomas 2014).

While additional data for model parameterization is needed, atmosphere-biosphere models are only as accurate as their species-specific plant physiological data, and different species show different thermal tolerances (Medlyn et al. 2002). While its physiological threshold to temperature is not well understood, *Acer saccharum*, currently one of the most prevalent species in the Great Lakes region and Northeastern United States, is not expected to do well under future warming scenarios (Allen et al. 2010, 2014). Additionally, currently climate models are lacking data on tropical forest responses to increasing temperature at the leaf and forest level, which can only be obtained through field studies (Huntingford et al. 2013).

Photosynthetic Response to Temperature

Light saturated photosynthetic response to temperature increases to a maximum (P_{max}) at an optimum temperature (T_{opt}), then decreases again, following a parabolic curve (Figure 1.1) (Berry and Bjorkman 1980, Farquhar et al. 1980, Hikosaka et al. 2006). As instantaneous leaf temperatures increase, photosynthesis typically increases as the rates of biochemical reactions increase from low-temperature limited enzymes (Berry and Bjorkman 1980, Yamori et al. 2014). In most temperate C_3 species photosynthetic decline occurs between 26 and 32°C, primarily due to stomatal closure (Farquhar et al. 1980). Elevated temperature can result in stomatal closure due to co-occurring increases in vapor pressure deficit in order to prevent water loss (Berry and Bjorkman 1980). The closure of stomata limits the ability for outside CO_2 to diffuse into leaves, thus reducing rates of net photosynthesis (Berry and Bjorkman 1980). There are many direct biochemical limitations to photosynthesis in response to temperature for C_3 species, though the two commonly used for model parameterization are the rate of RuBP (ribulose-1,5-bisphosphate) carboxylation (V_{cmax}) and the rate of RuBP regeneration from the electron transport chain (J_{max}) (Farquhar et al. 1980, Graham et al. 2003). At elevated leaf temperatures, limitations are most likely from rates of electron transport and lability of Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), though exact causes of decline are hard to generalize (Sage and Kubien 2007, Yamori et al. 2014). Irreversible leaf damage from high temperatures typically begins above 45°C as plasma membranes in the chloroplast become permeable and proteins begin to denature (Berry and Bjorkman 1980).

Temperate vs. tropical species photosynthetic response to temperature

Temperate species can be expected to have a lower overall T_{opt} , compared to tropical species, which have evolved under higher overall temperatures (Cunningham and Read 2002). Temperature optima are typically close to growing temperatures, with declines beginning above the temperature range which species are adapted to (Berry and Bjorkman 1980). Most temperate C_3 species have thermal optima between 20 and 35° C, peaking sharply at 30-35°C. Species adapted to warmer growing conditions, such as tropical trees, can exhibit T_{opt} 's above 35° C (Sage et al. 2008). However, the range of temperature from ambient to T_{opt} will likely be greater in temperate species, due to greater seasonal variability (Way and Oren 2010). Temperate species found at higher latitudes may also be temperature-limited, and therefore have a favorable response to climate change (Way and Oren 2010).

Tropical trees have been shown to be more susceptible to warming in comparison to temperate and boreal species, and have shown photosynthetic declines at temperatures of only 3°C above ambient air temperature (Bonan 2008, Way and Oren 2010). Tropical forests may already be operating close to their thermal optima, beyond which carbon uptake declines (Battaglia et al. 1996, Clark et al. 2003, Doughty and Goulden 2008). Temperate forests, on the other hand, have shown positive photosynthetic responses to increasing temperatures and a greater ability to acclimate to warmer temperatures (Gunderson et al. 2010a, Way and Oren 2010). Temperate species may have a greater range of tolerance due to greater seasonal and day to day temperature variation, and therefore may be better able to photosynthetically acclimate to higher temperatures

compared to tropical species (Cunningham and Read 2002, Kirschbaum 2004, Hikosaka et al. 2006, Gunderson et al. 2010b). Though some tropical species have demonstrated the ability for respiration to acclimate to increased night time temperatures, it is not as well known to what degree photosynthesis may do so (Doughty 2011, Slot et al. 2014). Tropical forests already operate within a narrow temperature range, and have shown strong species shifts in response to historic climate change, instead of an ability for individual trees to acclimate (Janzen 1967, Clark 2004, Slot et al. 2014, Cavaleri et al. 2015).

Leaf functional traits and photosynthetic capacity

Photosynthetic response to temperature may be predicted by using leaf functional traits and/or environmental gradients. For accurate model representation, it is also important to observe photosynthesis across the entire vertical transect of the canopy, as upper and lower branches have different traits and can respond differently to temperature (Sefcik et al. 2006). Canopy position, for example, can be a highly determinant factor in photosynthetic capacity. Upper canopy leaves of temperate deciduous species have been shown to have greater photosynthetic capacity, but they are also more susceptible to heat stress due to elevated temperatures and vapor pressure deficit (Catovsky et al. 2002). The upper 10% of sugar maple canopies have been shown to contribute 40% of total canopy carbon assimilation (Ellsworth and Reich 1993, Coble and Cavaleri 2014, Coble 2015). Upper canopy leaves of multiple tropical tree species have also been found to have higher and maximum rates of light saturated photosynthesis (A_{max}) (Kenzo et al. 2006). Light is generally limiting to photosynthesis across a vertical gradient within canopies due to

density of foliage limiting light access in the lower canopy (Niinemets 2007). Light can also be especially limiting in ecosystems with high annual cloud cover and mean annual precipitation (MAP), such as tropical rainforests (van de Weg et al. 2014). As light availability typically increases with canopy height, leaf temperature does as well. The direct radiation hitting upper canopy leaves warms sun leaves more than diffuse scattered light in lower shaded leaves (Dai et al. 2004). In many forests leaf temperatures can be 1-7 ° C above ambient air temperature, especially in the upper canopy (Koch et al. 1994). Therefore, upper canopy leaves with the greatest photosynthetic capacity are also likely to be the most vulnerable to temperature related declines and other environmental stresses (Catovsky et al. 2002).

Various leaf functional traits can also be strong predictors of photosynthetic capacity and, in turn, photosynthetic response to elevated temperature. Leaf nitrogen content (N) in canopies has been found to correlate with greater photosynthetic capacity in various forest types (Field 1986, Field and Mooney 1986, Evans 1989, Reich et al. 1998). In tropical forests in Puerto Rico, leaf mass and area based N showed a strong relationship with photosynthetic capacity across elevation gradients, which served as a proxy to temperature change (Harris et al. 2012). N is typically considered a limiting nutrient for photosynthetic capacity in temperate and tropical forests, though it has been found to have higher abundance in leaf tissue in cooler climates (Reich et al. 1994, Xiang et al. 2013). One study found that at any given leaf mass per area (LMA), temperate species exhibited higher concentrations of leaf N, indicating that N may be a better predictor of photosynthetic capacity in temperate systems than tropical ones (Xiang et al. 2013). Since temperate species are not expected to be high temperature limited and more

likely to acclimate, photosynthetic capacity may be a predictor of greater resilience to temperature related declines (Atkin et al. 2008). Northern *Acer saccharum* leaves, have been found not to be N limited, suggesting that N may not always be an accurate predictor of T_{opt} or maximum photosynthesis for this species (Gradowski and Thomas 2008).

LMA typically increases with height in forest canopies and has been found to have significant relationships with light saturated rates of photosynthesis (A_{max}), which have previously been used as a measure of photosynthetic capacity and could possibly be a strong predictor of maximum rates of photosynthesis (Xiang et al. 2013, Coble and Cavaleri 2014, Weerasinghe et al. 2014). LMA is used in many canopy photosynthesis models and can be used as a proxy for photosynthetic capacity since it is easier to measure than exact parameters of photosynthesis (Cornelissen et al., 2003). Like other traits strongly correlated with photosynthetic capacity, LMA is typically greatest in upper canopies (Coble, 2015).

Phosphorus (P) is needed for photosynthesis in creating and exporting triose-P in chloroplasts (Stitt 1997). Low levels of P can also limit RuBP reproduction for photosynthesis (Madhusudana Rao et al. 1986). Leaf-area based P is expected to be a strong predictor of photosynthesis since it has shown similar patterns across upper canopy gradients as light saturated rates of photosynthesis (Crous and Ellsworth 2004, Rozendaal et al. 2006). Thus, leaf P is likely to also be able to predict photosynthetic temperature response parameters (P_{max} and T_{opt}). P is one of the more limiting nutrients in tropical forests, due to high amounts of weathering, and should therefore be more

strongly correlated to photosynthetic parameters than in temperate forests, which are not as limited (Raaijmakers et al. 1995, Bloomfield et al. 2014).

This study sought to compare instantaneous photosynthesis response to temperature in mature canopies in three forest types: temperate deciduous, subtropical moist, and tropical wet. The measurements were done across vertical canopy gradients to determine how leaf temperature, T_{opt} , and P_{max} shifted with height. Due to the abundance of each forest type globally, and different predicted responses to elevated air temperature relating to climate change, it is crucial to compare the responses between the two forests and see how close each is to operating at its temperature optimum. Our specific hypotheses were:

A) Upper canopy leaves at all sites will have higher temperature optimums for photosynthesis (T_{opt}), higher maximum rates of photosynthesis (P_{max}), and both parameters will increase with increasing light availability.

B) T_{opt} and P_{max} will increase with increasing mean annual temperatures, and will be operating closer to photosynthetic thresholds in tropical forests.

C) T_{opt} and P_{max} will increase with increasing LMA at all sites but will be better predicted by leaf N in the temperate site, and leaf P at the tropical sites.

D) Maximum rates of electron transport (J_{max}) will begin to show temperature related declines at lower temperatures from declines in maximum rates of carboxylation (V_{cmax}).

Methods

Study Sites

Sampling took place at three sites, a temperate deciduous forest in Northern Michigan and a subtropical moist forest and tropical wet forest in Puerto Rico. Data were collected from four towers at the three locations (Table 2.1).

Temperate Deciduous Site

The temperate deciduous study site was located at the Michigan Tech Ford Center and Forest near L'Anse, MI (46.64 N, 88.48°W). Mean annual temperature (MAT) is 4.9°C and mean annual precipitation (MAP) is 879 mm, with 401 mm occurring during the growing season, typically May through September (Jarvi and Burton 2013). The soil at the site is an Allouez gravelly coarse sandy loam, extending to a depth of 40 cm (Coble and Cavaleri 2014). Species at the site included *Acer saccharum*, *Ulmus americana*, *Tilia americana*, *Betula alleghaniensis*, and *Ostrya virginiana*. *Acer saccharum* is the dominant species on the site, making up 97% of total tree density. The average height of the canopy was 23 m, while the average height to live crown was 13.8 (Coble et al. 2014). For additional information about site history see Campione et al. (2012).

Gas exchange and leaf trait data were acquired at the temperate site with 19 m tall mobile aluminum walk-up tower (Upright, Inc., Selma, CA, USA), with 10 tower sections, each 1.86 m tall (Table 2.1). It was constructed in the summer of 2012. Leaves were accessible from six heights (10.7, 12.6, 14.5, 16.4, 18.3, and 20.2 m), and the understory, (<1m). *Acer saccharum* (sugar maple), a broad leaved deciduous species, was the only species accessible from the tower.

Subtropical Moist Forest

The subtropical moist forest study site is in the municipality of Arecibo, Puerto Rico (18.40 °N, 66.73 °W), on a 114 ha privately owned forest reserve, El Tallonal. By the Holdridge life zone system, the site is a subtropical moist forest (Holdridge 1967). The wet season is from July to September, while the dry season lasts from January to March. The north central region of Puerto Rico where the subtropical moist forest is located is dominated by karst topography and has soils derived from limestone parent material (Monroe 1976). There were 57 tree species per hectare at the site, and the dominant tree species at the site was *Castilla elastica* (Lugo et al. 2008). The forest is estimated to be about 50 years old (Ewel and Whitmore 1973, Lugo et al. 2008). Historically, the area was used for grazing cattle and agriculture until 1950, when these lands were abandoned and naturally regenerated to forest (Ewel and Whitmore 1973). *Castilla elastica* was introduced to Puerto Rico from Africa and Asia near coffee plantations near the beginning of the 20th century (Costa et al. 2008). *Castilla elastica* is an early successional species and has been considered invasive in other forests (Kitajima et al. 2005). *Castilla elastica* is a fast growing, non-native, with very high LMA and light saturated photosynthesis (A_{max}) (Kitajima et al. 2005).

There were two identical towers at the site; each 25 m tall with thirteen 2 meter tall sections made of non-galvanized, painted steel tubing frames (BilJax, Archbold, OH). They were built in 2008 and are 12 meters apart. *Castilla elastica*, *Guarea guidonea*, and *Ocotea leucoxydon* were the only species measured from the towers. Leaves of these three species were sampled from ten heights across both towers. Other species were accessible at these and additional heights, however they were not sampled if they were not present at a minimum of two heights.

Tropical Wet Forest

The tropical wet forest was located in the Bisley Experimental Watershed of the Luquillo Experimental Forest within the El Yunque National Forest (18.31 °N, 65.74° W) (Holdridge 1967, Lugo and Batlle 1987, Scatena et al. 1993, Harris et al. 2012). Mean annual precipitation is 3936mm and mean annual temperature is 25°C, at an elevation of 361 meters above sea level (Scatena et al. 1993). The dry season is typically January through April, while the wet season ranges from May to November (Harris et al. 2012). The soils at the tropical wet forest are Cristal Tropohumults, clayey poorly drained soil with red mottles (Harris et al. 2012). The tropical wet forest as a whole averages 21 tree species per hectare as of 1998, with an average of 516 stems per ha (Weaver et al. 1986, Frangi and Lugo 1998). As of 2007, *Dacryodes excels* (common name: tabonuco), was the dominant tree species of the forest, with mature secondary Tabonuco as the main forest type (Heartsill-Scalley et al. 2007). *Dacryodes excelsa* is one of the dominant native trees in Puerto Rico, growing at elevations between 200 to 800 m (Lugo and Wadsworth 1990). *Dacryodes excelsa* is a long lived broad-leaved evergreen species, and it is estimated that mature trees live up to 400 years (Lugo and Wadsworth, 1990). Other species located near the tower include, *Prestoea montana*, *Casearia arborea*, *Inga laurina*, *Manilkara bidentata*, and *Sloanea berteriana* (Zimmerman et al. 1994). *Dacryodes excelsa* was the only species accessible across a canopy gradient in the forest. In the Luquillo Experimental Watershed, *Dacryodes excelsa* is the dominant species, with average heights of 30 to 35 meters. *Dacryodes excelsa* has historically shown much lower light saturated photosynthesis, with $2.7 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Ducrey 1994). The average height of the canopy was 20m as of 1996 (Reagan and Waide 1996). For

additional site history, see Birsdey and Weaver (1987), and Harris (2012). The site's tower is also a mobile aluminum walk-up tower (Upright, Inc., Selma, CA, USA) (Table 2.1). It was built in 1991 and has a footprint of 2.5 m². Leaves were accessible from 8 heights, though the only species sampled was *Dacryodes excelsa* at 6 tower heights.

Sampling Design

In the temperate forest, photosynthetic temperature response curves were conducted on two leaves of *Acer saccharum* at every accessible height of each tower, from 7 to 20 m, and five leaves from the understory at less than 1m. No A_{ci} curves were conducted in the temperate deciduous forest. Light response curves were conducted on two healthy mature leaves at the top (20.3 m), middle (10.7 m), and understory (<1 m). All sampling took place during the growing season, from July 7th to July 21st, 2014.

Only one species was sampled for temperature response curves at the tropical wet tower, *Dacryodes excelsa*. Two mature leaves were measured at every accessible height. Leaves were sampled at tower heights from 7 m to 25 m. A_{ci} curves were conducted on one leaf, each at 1.7 m, and 24.7 m, representing the middle, and upper canopy. Light response curves were taken on two healthy mature leaves at 24.7, 20.3, and 10.7 meters to extract the light saturation point. Sampling took place in two field campaigns: one from June 6th to June 9th, 2014 and another from August 6th to August 28th, 2014.

The subtropical moist site had 7 accessible species from the tower, but only three were sampled for temperature response curves: *Castilla elastica*, *Guarea guidonea*, and *Ocotea leucoxylon*. These species were chosen, because they had representatives accessible at a minimum of 2 heights. Two leaves of each species were sampled at each accessible height. *Castilla elastica* was sampled at 12, 14, 16, 20 and 22 m, *Guarea*

guidonea at 8 and 12 m, and *Ocotea leucoxydon* at 20, 22, and 25 m. A_{ci} curves were conducted only on *Castilla elastica* at 22 m. The light saturation point was used from light response curves of a previous study (Silva and Anand 2013). Sampling took place between 9 am and 5 pm in two campaigns: one from June 11th to June 13th, 2014 and another from August 13th to August 22nd, 2014.

Environmental Measurements

For all three sites, diffuse non-interceptance (DIFN) or fraction of sky that is not blocked by foliage (between 0 and 1), was measured at each study site as a measurement of light availability (Norman and Welles 1983). Two above canopy measurements were taken at the top of the tower, with two additional measurements taken at every lower tower sections thereafter, and two above canopy measurements were taken at the end of the transect. DIFN was only sampled in uniformly cloudy conditions at mid-day for all three sites. It was measured once during each field campaign in Puerto Rico, and during two days of the temperate field sampling. DIFN was measured with an LAI-2200 (Licor Biosciences, Lincoln, NE). A white diffuser cap was used to make any corrections to measurements taken in direct sunlight when uniformly cloudy conditions were not present. Leaf temperature was measured during uniform weather conditions, using a Fluke 572 infrared thermometer (Fluke Corporation, Everett, WA). Five leaves of all accessible species were measured at every tower height once an hour. Temperature was measured in June and August at the subtropical moist and tropical wet sites, and during July at the temperate deciduous site. This procedure was used at all three sites. Hourly changes in leaf temperature were then plotted across the upper canopy and mid canopy.

Photosynthesis measurements and parameter extractions

Photosynthesis measurements were taken with a Licor 6400XT with a 6400-02B Red/Blue light source (Licor Biosciences, Lincoln, NE). Light response curves were conducted with relative humidity between 50 and 60%, flow rate of 200 $\mu\text{mol s}^{-1}$ for temperate samples and 300 $\mu\text{mol s}^{-1}$ for tropical to maintain ambient relative humidity, CO_2 concentrations at ambient conditions of 400 ppm, and leaf temperature set at the ambient temperature of the leaf at the beginning of the curve. Photosynthetically active radiation (PAR) was decreased from 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the following intervals, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 100, and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The PAR value where A_{max} is reached was extracted by fitting light response curves to the Lobo model based on Michaelis-Menten equations (Lobo et al. 2013):

$$P_N = \left[\frac{f(I_o) \times I \times P_{gmax}}{f(I_o) \times I + P_{gmax}} \right] - R_D \quad \text{Equation 2.1}$$

Where P_N is the net photosynthetic rate, I for photosynthetic photon flux density, I_o is quantum yield at $I=0$, P_{gmax} is maximum gross photosynthetic rate, and R_D is the dark respiration rate (Baly 1935, Ye 2007, Lobo et al. 2013). The parameter extracted is I_{sat} or light saturation point (this value was used as the PAR input for temperature response and A_{ci} curves) it is extracted using the following:

$$I_{sat} = \sqrt{\frac{(\beta + \gamma)(1 + \gamma \times I_{comp})}{\beta - 1}} \quad \text{Equation 2.2}$$

Where β and γ are two dimensionless adjusting factors, and I_{comp} is the light compensation point (Baly 1935, Ye 2007, Lobo et al. 2013).

Temperature response curves were conducted using a water jacket (6400-88 expanded temperature kit, Licor Biosciences, Lincoln, NE) to increase and decrease leaf temperature. Leaf temperature increased by introducing heated water through a bev-a-line tube through the water jacket using gravity. After achieving the highest possible temperature (about 35°C), the temperature was incrementally decreased by adding ice water to the water jacket flow input and awaiting equilibrium at target temperatures. This was done to the lowest sampling temperature before dew point (about 22°C). The use of the water jacket allowed for a greater stabilization of temperatures and a greater temperature range than the Li-6400XT can typically achieve without the expanded temperature kit. Leaf temperature was measured at 22, 24, 27, 29, 30, 31, 32, 33, and 35 °C. Relative humidity was kept between 50 and 60%, and was regulated by flow (200 $\mu\text{mol}\cdot\text{s}^{-1}$ for temperate samples and 300 $\mu\text{mol}\cdot\text{s}^{-1}$ for tropical) and desiccant. CO_2 was kept at ambient concentrations of 400 ppm. PAR was kept at the light saturation point (I_{sat}) for each species, extracted from light response curves. T_{opt} was calculated by taking the second order polynomial function on the temperature response curves and solving for the highest point on the x-axis. P_{max} was extracted by solving for the photosynthetic value from the regression equation at T_{opt} (Figure 2.1) (Cavieres et al. 2000).

In order to determine the relationships between J_{max} , V_{cmax} , and temperature, photosynthetic CO_2 response (A_{ci}) curves were done at 25, 27, 30, 32, and 35 °C, starting with the first set of A_{ci} curves at 25°. The water jacket method for elevated temperatures

for temperature response curves was used to achieve the two highest temperatures. Relative humidity was kept between 50 and 60%, with flow set to 300 $\mu\text{mol s}^{-1}$. For each curve at each of the 5 temperature points, CO_2 was initially set at ambient, then decreased, and finally increased as follows: 400, 300, 200, 150, 100, 50, 400, 600, 800, 1000 ppm. PAR was once again set at the light saturation point (I_{sat}) for each species, extracted from light response curves. A_{ci} curves were fitted, and J_{max} and V_{cmax} extracted as in Sharkey et al. (2007). For extracting parameters on the temperature dependence of J_{max} and V_{cmax} , a modified version of the Arrhenius function was used (Johnson et al. 1942, Kattge and Knorr 2007).

Leaf Traits

Leaf area for all leaves measured for gas exchange at the temperate site was measured using a leaf area meter (LI-3100 Li-Cor Biosciences, Lincoln, NE). Leaf area of leaves measured for gas exchanged at the tropical and subtropical sites was measured by scanning pictures of leaves alongside a ruler (HP deskjet), then tracing the outline of the scanned image in ImageJ (Rasband, W.J 1997-2014, <http://imagej.nih.gov/ij>). Leaves were then dried for 24 hours at 65°C and weighed. Leaf mass per area (LMA gm^{-2}) was determined by taking the ratio of dry weight to leaf area. Samples were ground with a ball bearing grinder (8000M Mixer/Mill, Spex Sample Prep, Metuchen, NJ) for three minutes. Prior to elemental analysis, ground samples were dried for an additional 24 hours at 65°C, weighed with a Sartorius cubis microbalance (Data Weighing Systems, Elk Grove, IL) for 4-6mg of leaf material, and folded in to 5 by 9 mm tin capsules. Leaf nitrogen (N) analysis was done using an Elementar vario Microcube elemental analyzer

(Elementar Inc., Hanau, Germany). Leaf phosphorus (P) analysis was done with inductively coupled plasma optical emission spectrometry using a thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Optimal Emission Spectrometer (Precision Dynamics Corporation, San Fernando, Ca).

Data analysis

For comparing hourly leaf temperature at different heights, the canopy was divided into upper (>12 m) and lower (<12 m) canopy sections. The average hourly temperature in the upper and lower canopy for all species at one site was plotted against time, along with the standard error. Analysis of co-variance (ANCOVA) was used to compare all species and biome, along with height, against T_{opt} and P_{max} . An F-protected Least Significant Difference Test was performed to determine if means are significantly different among biomes. Comparison between maximum daily leaf temperature (T_{leaf}) and T_{opt} with height was done with simple linear regression. Simple linear regression was used to compare T_{opt} and P_{max} with N_{mass} , N_{area} , P_{mass} , P_{area} , and LMA. DIFN was transformed with a natural log function and also compared to T_{opt} and P_{max} using simple linear regression. Linear regression was separated by species, then all tropical species were separated by forest site, while *Acer saccharum* was the only species reported for the temperate forest biome. To compare T_{leaf} vs. T_{opt} , the maximum daily temperature at each height was used as T_{leaf} . The point where T_{leaf} and T_{opt} intersect was found by plotting T_{leaf} against T_{opt} from each temperature response curve. DIFN was transformed with a natural log so the relationship would become linear. All statistical analyses were performed in R (R Core Team, 2013).

Results

Within canopy variability of photosynthesis and temperature

Height as a predictor for T_{opt} was not significant for any species or biome, with the exception of *Guarea guidonia* (Table 2.2). As a predictor of T_{opt} the ln of DIFN was only significant for *Guarea guidonia* and the tropical biome (Table 2.2). For *Castilla elastica*, *Guarea Guidonia*, and the subtropical moist biome P_{max} increased with height (Table 2.2), though at the biome level it was not significant. For *Acer saccharum*, this relationship was also highly significant and therefore height and P_{max} for the temperate biome was too (Table 2.2). The relationship between height and P_{max} for all species and biomes was positive, while there was no relationship between height and T_{opt} (Figures 2.2 and 2.3). As a predictor of P_{max} , the ln of DIFN was significant for *Castilla elastica* and *Guarea guidonia* (Table 2.2). The relationship between P_{max} , and the ln of DIFN was positive for all species and biomes except *Ocotea leucoxyton* (Table 2.2).

Forest variability of photosynthesis and temperature

For all tropical species in both sites the mean of T_{opt} was greater than in the temperate forest (Table 2.3). T_{opt} showed a significant relationship with height, which also differed by species and forest type (Table 2.4). P_{max} also showed a significant relationship with height, by only in regard to species, not forest type (Table 2.4).

Ambient leaf temperature in the mid and upper temperature increased to a peak at mid-day, then declined (Figure 2.4). Maximum daily T_{leaf} response to height was found to be significant for all three sites, with a positive linear relationship (Figure 2.5). All

three sites had slightly different relationships between T_{opt} and T_{leaf} . At the temperate site, T_{opt} was greater than T_{leaf} in the understory and mid canopy, until they intersected at 11.4m, where T_{leaf} exceeded T_{opt} in the upper canopy (Figure 2.5). This intersection occurred at 27.6°C. At the wet tropical site (Figure 2.5), T_{opt} was slightly lower than T_{leaf} at all heights, and only began to intersect at the very top of the canopy (22.17 m) at 30.8°C. The subtropical moist site (Figure 2.5) showed a similar response as the temperate deciduous (a), where T_{opt} exceeded T_{leaf} in the lower canopy up to 10.6m and 29.4°C, where T_{leaf} then exceeded T_{opt} within the upper canopy.

Leaf functional traits

LMA as a predictor of T_{opt} was slightly significant for *Guarea guidonia*, and highly significant for *Acer saccharum* and the temperate biome (Table 2.2). As a predictor of P_{max} , the relationship with LMA was also significant for *Guarea guidonia*, *Acer saccharum*, and therefore the temperate biome (Table 2.2). LMA increased linearly with P_{max} and T_{opt} for these relationships (Figure 2.6).

For N_{area} , the relationship with T_{opt} was significant and linear for *Guarea guidonia* and *Acer saccharum* (Table 2.2). As a predictor of P_{max} , N_{area} had a significant, linear relationship for *Dacryodes excelsa*, *Guarea guidonia*, *Acer saccharum*, and for all biomes (Table 2.2 and Figure 2.7). The relationship between N_{mass} with T_{opt} and P_{max} was linear and only significant for *Acer saccharum* (and therefore the temperate biome) (Figure 2.7).

The relationship between T_{opt} and P_{area} was only significant for *Acer saccharum* and the temperate biome (Table 2.2). The relationship between P_{max} and P_{area} was also

only significant for *Acer saccharum* and the temperate biome (Table 2.2). The relationship between P_{mass} and T_{opt} was not significant for any species or biome, and for P_{mass} and P_{max} was only significant for *Guarea guidonia* (Table 2.2). For the species that had significant relationships with P_{area} or P_{mass} and T_{opt} and P_{max} x, P_{max} and T_{opt} increased linearly with P (Figure 2.8).

Mechanistic components of photosynthesis

For the data from August 2014, J_{max} response to temperature began to show declines around 30°C for upper canopy, and 35°C for mid canopy *Dacryodes excelsa* (Figure 2.9). For *Castilla elastica*, no declines were seen by 35°C, however J_{max} rates had begun to taper by this point. As for V_{cmax} , no declines were seen in response to temperature, and only began to taper at the maximum temperature of 32°C for upper canopy *Dacryodes excelsa* (Figure 2.9). The data from March 2015 showed earlier temperature related declines of J_{max} at 25°C in both the upper and lower canopy. V_{cmax} also appeared to show declines at 32°C (Figure 2.9).

Discussion

Within canopy variability of photosynthesis and temperature in relation to height and light

Upper canopy leaves only showed significantly higher T_{opt} in *Guarea guidonia*. The hypothesis that P_{max} would be greater in the upper canopy had more support, showing significance for *Acer saccharum*, *Castilla elastica*, and *Guarea guidonia*. The lack of significance could be due to a lack of complete canopy gradients from ground to the top

of crowns for individual species. However, there was a complete canopy gradient from the seedling level to the upper canopy for *Acer saccharum*, and that relationship between height and T_{opt} was still not significant (Table 2.2). Only *Guarea guidonia* had a significant relationship with height and T_{opt} , however, the sample size for this species was extremely small ($n=4$), and this species was only sampled at two heights, thus the regression for parameters of this species may not have a large enough sample size to draw accurate conclusions.

As for maximum rates of photosynthesis, It is likely that height was more significant predictor of P_{max} in temperate and not in tropical forests, because there were several temperature response curves done in the understory, whereas the lowest temperature response curve in either tropical forest was at 1.7 meters. With additional data from the understory of the Puerto Rican sites, height likely would have been a better predictor of P_{max} , as it has been correlated with light saturated rates of photosynthesis (A_{max}) in other studies (Kenzo et al. 2006, Weerasinghe et al. 2014).

Previous studies have found strong correlations between height and photosynthetic capacity and leaf temperature (Kenzo et al. 2006). It is much unexpected that T_{opt} and height would not be significant along with increases in canopy height. There is a scarcity of data from temperature response curves across canopy gradients due to ease of canopy access, thus it is difficult to determine if there is a significant relationship between T_{opt} and height in any forest type (Bassow and Bazzaz 1997, Cavieres et al. 2000, Vargas 2013). This implies that height is likely not a reliable predictor of T_{opt} .

Though light availability is considered one of the limiting factors for growth of tropical forest species, especially in rainforests, it was surprising to find that there was not a significant relationship with P_{max} (Xiang et al. 2013, Weerasinghe et al. 2014). The significant relationship between T_{opt} and the tropical biome was likely the result of availability of sunlight, as leaf temperature is typically greater for sun leaves than shaded ones (Ku et al. 1977, Smith and Nobel 1977). The lack of significance for the temperate biome could have been the result of sampling limitations, as the top of the tower in the temperate forest did not reach the very top of the canopy, and therefore samples were not possible at 100% DIFN.

Biome variability of photosynthesis and temperature

As predicted, both tropical forests had higher mean T_{opt} 's than temperate forests (Table 1.3). This was expected since T_{opt} typically corresponds with growth temperature, and MAT in the wet tropical and moist subtropical forests is over 20°C greater than the temperate deciduous forest (Berry and Bjorkman 1980, Cunningham and Read 2002). T_{opt} for tropical species has been seen to exceed 35°C, while most C₃ species have a range of 26 and 35°C. T_{opt} for all tropical species and the biome average (30.0°C) was greater than that of *Acer saccharum* (27.4°C) (Table 2.3). What was unexpected was the intersection of T_{opt} and T_{leaf} at the temperate site. It was hypothesized that the tropical sites would have leaf temperatures closer to T_{opt} than the temperate biome, but it was also not expected that T_{opt} would be exceeding T_{leaf} in the upper canopies of all three sites. The wet tropical forest had the least amount of canopy operating above T_{opt} , even less than the temperate deciduous forest (Figure 2.5). The intersection between current maximum leaf

temperature in upper canopies, and T_{opt} was lower at subtropical moist forest than the tropical wet forest. This was unexpected, since T_{opt} is typically around growing temperature, and mean annual temperature the subtropical forest is several degrees higher than the wet tropical forest (Table 2.1) (Hikosaka et al. 2006). That the canopy at the subtropical moist forest is operating above T_{opt} in a lower canopy position than the wet tropical forest was expected, since the mid canopy at the subtropical forest was much warmer than the tropical wet forest.

Leaf temperature is typically 1-7°C greater than corresponding air temperature, and also typically increases with height (Koch et al. 1994). For tropical tree species, it is unlikely that photosynthesis will be able to acclimate to elevated leaf temperature due to the limited range of annual temperature (Doughty and Goulden 2008). Thus T_{opt} will likely remain the same as T_{leaf} increases with the increase in air temperature from climate change.

Temperate and tropical forests already currently experience mid-day depression in photosynthesis due to elevated temperature (Koch et al. 1994, Peters et al. 2013). As maximum leaf temperatures typically occurred at the mid-day point, and rapidly declined (Figure 2.4), it is possible that the canopies are not operating at or above T_{opt} for a majority of the day. T_{leaf} measurements were also taken during the warmest times of annual growing season at all sites. In the case of *Dacryodes excelsa* at the wet tropical field site, P_{max} was generally lower than that of the subtropical moist species and *Acer Saccharum* in the temperate forest (Table 2.3). This could be in part, because of the life history traits of this species, as it typically occurs in older forests with high rainfall, and therefore low light availability. Even though photosynthetic capacity was much lower for

this tropical wet species than our study's northern hardwood forest species, due to the ability to photosynthesize year round, net canopy photosynthesis may actually be higher in tropical forests.

Previous studies have found that T_{opt} is associated with growth temperature, and that tropical forests generally have greater temperature optima for photosynthesis than temperate species (Cunningham and Read 2002, Silva and Anand 2013, Slot et al. 2014). Though our study found that the temperate site showed a similar canopy-wide relationship between T_{opt} and T_{leaf} as the subtropical moist, and may seem just as susceptible to declines in photosynthesis with rising air temperature, this may not be the case. Mature temperate trees are expected to be more successful at acclimating to warming than tropical forests, which have evolved under low diurnal, seasonal, and interannual temperature variation (Way and Oren 2010). However, species such as *Acer saccharum* are predicted to show decreases in abundance in Northern Michigan as the climate changes (Iverson and McKenzie 2013, Peters et al. 2013, 2014). The outcome of this would mean a decline of *Acer saccharum*, but not necessarily a decline in C assimilation of hardwood forests.

Unlike T_{opt} , however, tropical forests do not necessarily always have greater instantaneous rates of photosynthesis such as photosynthesis at temperature optima and light-saturated photosynthesis, as this study displayed (Reich et al. 1997, Wright et al. 2004, Xiang et al. 2013). Tropical forest canopies generally have greater rates of C assimilation than temperate forests, though at the leaf level, temperate species may exceed rates of those in the tropics. This is due to shorter growing seasons in temperate forests, and generally a greater abundance of photosynthetically active leaves within

tropical canopies (Reich et al. 1997). Thus, any potential declines in leaf level C assimilation of tropical leaves with increasing temperature could mean great reductions globally (Clark 2004, Doughty and Goulden 2008).

Leaf functional traits

Leaf phosphorus was not related to T_{opt} or P_{max} as expected for tropical forests, rejecting our hypothesis. Conversely, P_{area} 's relationship with T_{opt} and P_{max} was highly significant for *Acer saccharum* (Table 2.2). Mean values for mass and area-based phosphorus were also not significantly different between the temperate and tropical biomes, while leaf nitrogen concentrations were greater in the tropics than the temperate site (Table 2.3). This is highly unexpected, since phosphorus, an important substrate in photosynthesis, is not as limited in temperate forests as it is in tropical ecosystems due to high levels of rainfall and soil weathering (Raaimakers et al. 1995, Bloomfield et al. 2014).

Both nitrogen and phosphorus had greater overall significance on a leaf area basis than on the mass basis, which differs from other findings (Field and Mooney 1986, Reich et al. 1994). Previous studies in temperate forests have shown T_{opt} and P_{max} related to N_{area} and N_{mass} (Xiang et al. 2013). This could be indicative that nitrogen is one of the limiting components of photosynthesis in this ecosystem, more-so than in the tropical forests sampled. N_{area} was significant for the tropical wet and subtropical moist biomes in relationship to P_{max} , however the R^2 was lower than that of the temperate biome (Table 2.2). This is consistent with previous findings where nitrogen is a major limiting nutrient for photosynthesis in temperate forests (Cernusak et al. 2013, Hidaka and Kitayama

2013). Very young or very old tropical forests can also have nitrogen limitations for photosynthesis, which may explain why *Dacryodes excelsa*, which was found in the older of the two sampled tropical forests, also had a significant relationship with P_{max} and N_{area} , and a relatively high R^2 (Reich et al. 1994).

LMA was also a greater predictor of T_{opt} and P_{max} in temperate species than the tropical ones (Table 2.2). Average LMA was greatest in the tropical wet forest, followed by the subtropical moist forest, and lastly the temperate one (Table 2.3). This was expected, corresponding with the world-wide leaf economics spectrum (Wright et al. 2001). *Acer saccharum* has previously been found to have strong correlations between LMA and photosynthetic capacity within canopies, though the other species in this study have not been sampled for LMA across a vertical canopy gradient (Ellsworth and Reich 1992, 1993, Coble and Cavaleri 2014). The mean values of LMA differed significantly throughout the tropical biome and had greater ranges of standard error than any other leaf traits that were sampled (Table 2.3). In every study where LMA has been measured across a canopy, it has increased with height, regardless of the forest type (Cavaleri et al. 2010). Since maximum photosynthetic rates in this study did not increase significantly with height for most species, it is not a surprise that LMA also did not increase with P_{max} . This is likely that there was an insufficient portion of the canopy sampled to get a true gradient for LMA and photosynthetic capacity.

Other studies have found greater correlations between photosynthetic capacity and leaf functional traits (Reich et al. 1997, Wright et al. 2004, Xiang et al. 2013). Relationships between T_{opt} and these traits have rarely been measured. However, the

correlation between mass or area based nutrients and photosynthesis varies by study, with mass being a more successful predictor in some studies and area in others (Reich et al. 1998, Wright et al. 2004, Wright et al. 2005). In other studies, however, tropical forests have shown significant relationships between leaf phosphorus and photosynthesis, along with temperate forests with leaf nitrogen (Bassow and Bazzaz 1997, Cernusak et al. 2013, Hidaka and Kitayama 2013).

Mechanistic components of photosynthesis

As expected, J_{max} began to show temperature related declines prior to V_{cmax} (Figure 2.9). At the same canopy position, *Castilla elastica* had higher temperature optima for J_{max} and V_{cmax} than *Dacryodes excelsa*. This would be expected, since the subtropical moist forest at which *Castilla elastica* was sampled has a higher MAT (Table 2.4). Additionally, the *Dacryodes excelsa* curves which were sampled during the dry season, which is seasonally cooler showed lower optimum temperatures for J_{max} at the same heights as the August samples. This may be indicative of an ability for tropical species in the wet forest to acclimate to seasonal temperature changes, which also suggests greater acclimation potential to climate change. The series of A_{ci} curves at increasing temperature was pilot data, but the initial findings suggest that more of these types of data will be useful for the future. This enforces the ability to predict temperature optima for photosynthesis with mechanistic data, and also improve model parameterization (Doughty and Goulden 2008, Cernusak et al. 2013, Cavaleri et al. 2015).

These findings are similar to that of other studies, which have shown that J_{max} for individual species will show temperature-related declines prior to V_{cmax} (Hikosaka et al. 2006, Yamori et al. 2014). Additionally, tropical tree species that are adapted to higher growth temperature have also shown greater temperature optima for rates of electron transport (J_{max}) than those adapted to lower growth temperature (Vårhammar et al. 2015). The implications from that study, along with our own are that cooler sites in the tropics, such as the wet tropical site, may see temperature related declines in photosynthesis prior to those at warmer sites. This theory, however, conflicts with our results, where the subtropical moist forest, a warmer site, had a greater portion of its canopy currently operating above T_{opt} . J_{max} and V_{cmax} rates at elevated temperatures will help improve parameterization of coupled-carbon climate models where this data is currently limited, helping determine the temperature limitations for warmer tropical forests and whether they are caused by direct effects of temperature, impairing photosynthetic machinery, or indirect effects, such as stomatal closure (Medlyn et al. 2002). Mechanistic data of photosynthesis, especially from the tropics, is one of the greatest limitations in models currently in predicting the effects of climate change on changes in C exchange (Booth et al. 2012).

Conclusion

In the upper canopy of all forest types, T_{leaf} was found to exceed T_{opt} , indicating that leaves in the upper canopies of these forests are already showing declines as a result of high temperatures. The subtropical moist forest, which has the highest mean annual temperature of all sites had the greatest amount of canopy operating above T_{opt} . Contrary to expectations, T_{opt} exceeded T_{leaf} in the temperate biome, more so than the tropical wet

forest. Unless forest canopies can acclimate to increasing temperature and shift T_{opt} higher, a majority of the canopy of each site will be operating above T_{opt} by 2100, leading to potential global decreases in C storage.

As seen in previous studies, LMA and nitrogen (on and area and mass basis) were significant predictors of maximum rates of photosynthesis for the temperate forest, and showed a strong correlation with T_{opt} as well. The relationships in both tropical forests, however, were not as pronounced. Contrary to predictors, phosphorus was not a significant predictor of photosynthesis or T_{opt} in tropical forests as it was in temperate ones.

Tables

Table 2.1 Site information for each tower. Basal area (BA, m²ha⁻¹), trees per hectare (TPHA), species measured, elevation (meters above sea level), mean annual precipitation (MAP, mm), mean annual temperature (MAT, °C), tower height (m), tower footprint (m²).

Site	BA	TPHA	Species Measured	Elevation (m)	MAP (mm)	MAT (°C)	Tower height (m)	Tower footprint (m ²)
Temperate Deciduous	33	264	<i>Acer saccharum</i>	189	879	4.9	19	2.5
Subtropical Moist	42	1039	<i>Ocotea leucoxylo</i> , <i>Guarea guidonia</i> , <i>Castilla elastica</i>	122	1295	25.5	25	2.35
Tropical Wet	49	516	<i>Dacryodes excelsa</i>	361	3936	24.8	24.7	2.5

Table 2.2 R² from simple linear regression of height (m), LMA (g m⁻²), P_{area} (g m⁻²), P_{mass} (mg g⁻¹), N_{area} (g m⁻²), and N_{mass} (mg g⁻¹) in predicting T_{opt} (°C) and P_{max} (μmol CO₂ m⁻¹ s⁻²) by species and by biome. Levels of significance: * $p < .05$, ** $p < .01$, *** $p < .001$.

Species code/ Forest		n	Height	DIFN	LMA	P_{area}	P_{mass}	N_{area}	N_{mass}
<i>Dac</i>	T_{opt}	12	.01	.03	.05	.08	.05	.07	.02
	P_{max}	12	.10	.03	.10	.10	.10	.62**	.10
<i>Cas</i>	T_{opt}	14	.05	.15	.08	.04	.03	.08	.01
	P_{max}	14	.32*	.54**	.07	.09	.07	.06	.01
<i>Oco</i>	T_{opt}	6	.13	.32	.18	.11	.06	.01	.16
	P_{max}	6	.24	.34	.23	.25	.25	.22	.36
<i>Gua</i>	T_{opt}	4	.99**	.99**	.91*	.84	.83	.95*	.22
	P_{max}	4	.99**	.99**	.97**	.68	.95*	.94*	.45
<i>Acer</i>	T_{opt}	23	.06	.05	.57***	.39***	.06	.39***	.15*
	P_{max}	23	.68***	.07	.44***	.84***	.02	.38**	.22*
Tropical Wet	T_{opt}	12	.01	.03	.05	.08	.05	.07	.02
	P_{max}	12	.10	.03	.10	.10	.10	.62**	.10
Subtropical	T_{opt}	24	.11	.18	.04	.02	.03	.02	.09
Moist	P_{max}	24	.38**	.04	.08	.15	.01	.32**	.1
Temperate	T_{opt}	23	.06	.05	.57***	.39***	.06	.39***	.15*
	P_{max}	23	.68***	.07	.44***	.84***	.02	.38**	.22*

Table 2.3 Gas exchange and leaf trait variables. Mean values by species and forest. Species and biome, and corresponding species code. The number of samples (n) corresponds with the number of temperature response curves. Temperature optimum for photosynthesis (T_{opt} , °C), maximum rate of photosynthesis in response to temperature (P_{max} , $\mu\text{mol CO}_2 \text{ m}^{-1} \text{ s}^{-2}$), leaf mass per area (LMA, g m^{-2}), phosphorus on a leaf area basis (P_{area} , g m^{-2}), phosphorus on a leaf mass basis (P_{mass} , mg g^{-1}), nitrogen on a leaf area basis (N_{area} , g m^{-2}), and nitrogen on a leaf mass basis (N_{mass} , mg g^{-1}). Letter codes show levels of significant differences between means.

Species/ Forest	code	n	T_{opt}	P_{max}	LMA	P_{area}	P_{mass}	N_{area}	N_{mass}
<i>Dacryodes excelsa</i>	Dac	12	29.9b	4.9a	122.0c	.1a	.8a	1.7b	14.6a
<i>Castilla elastica</i>	Cas	14	30.0b	9.8c	87.7b	.1bc	1.4ab	2.2b	21.1a
<i>Ocotea leucoxyton</i>	Oco	6	30.3b	11.5c	114.7c	.1ab	.9a	2.9b	25.5a
<i>Guarea guidonia</i>	Gua	4	29.5b	7.0ab	77.7b	.1c	1.9b	2.5b	32.7b
<i>Acer saccharum</i>	Acer	23	27.4a	7.3b	44.5a	.1a	1.7b	.9a	21.2a
Tropical Wet		12	29.9A	4.9A	122.0A	.1A	.8A	1.7A	14.6A
Subtropical Moist		24	30.0A	9.76B	92.8B	.1A	1.0AB	2.1A	25.9A
Temperate		23	27.4B	7.3C	44.5C	.1A	1.7B	.9B	21.2A

Table 2.4 One way ANCOVA results for test of species, height, and species x height on temperature optimum (T_{opt}) and maximum rates of photosynthesis (P_{max}); and test of forest, height, and species x forest on temperature optimum (T_{opt}) and maximum rates of photosynthesis (P_{max}). Degrees of freedom (df), mean square, F-ratio, and levels of significance. Levels of significance: * $p < .05$, ** $p < .01$, *** $p < .001$.

	T_{opt}			P_{max}		
	df	Mean square	F-ratio	df	Mean square	F-ratio
Species	4	24.09	5.81***	4	61.61	8.05***
Height	1	68.51	15.5***	1	161.92	18.55***
Species x Height	4	12.99	3.45*	4	50.98	9.20***
error	53	3.77		53	5.54	
Forest	1	94.61	23.89***	1	9.58	.84
Height	1	68.51	15.5***	1	161.92	18.55***
Forest x Height	1	51.02	14.23***	1	2.38	0.37
error	56	3.59		56	8.83	

Figures

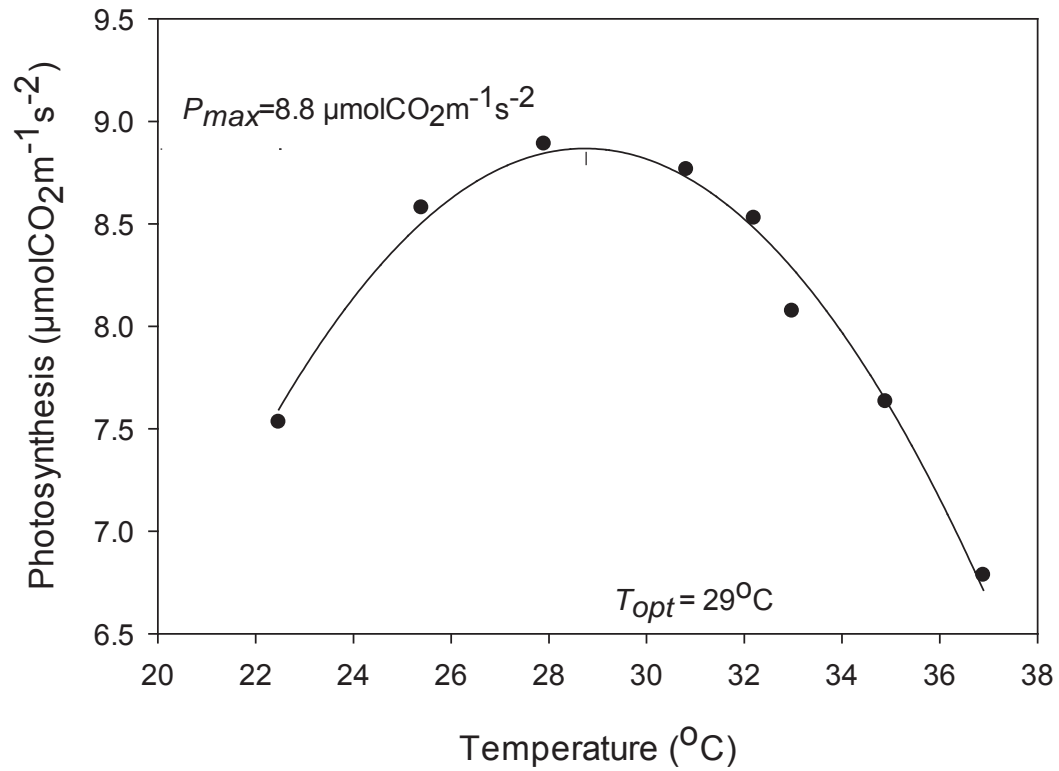


Figure 2.1 Photosynthesis response to temperature.

Example photosynthetic temperature response curve from *Dacryodes excelsa*.

Photosynthesis increases with temperature to a thermal optimum, then rapidly begins to decline, typically between 26 and 34°C (Farquhar et al. 1980). This thermal optimum is considered T_{opt} .

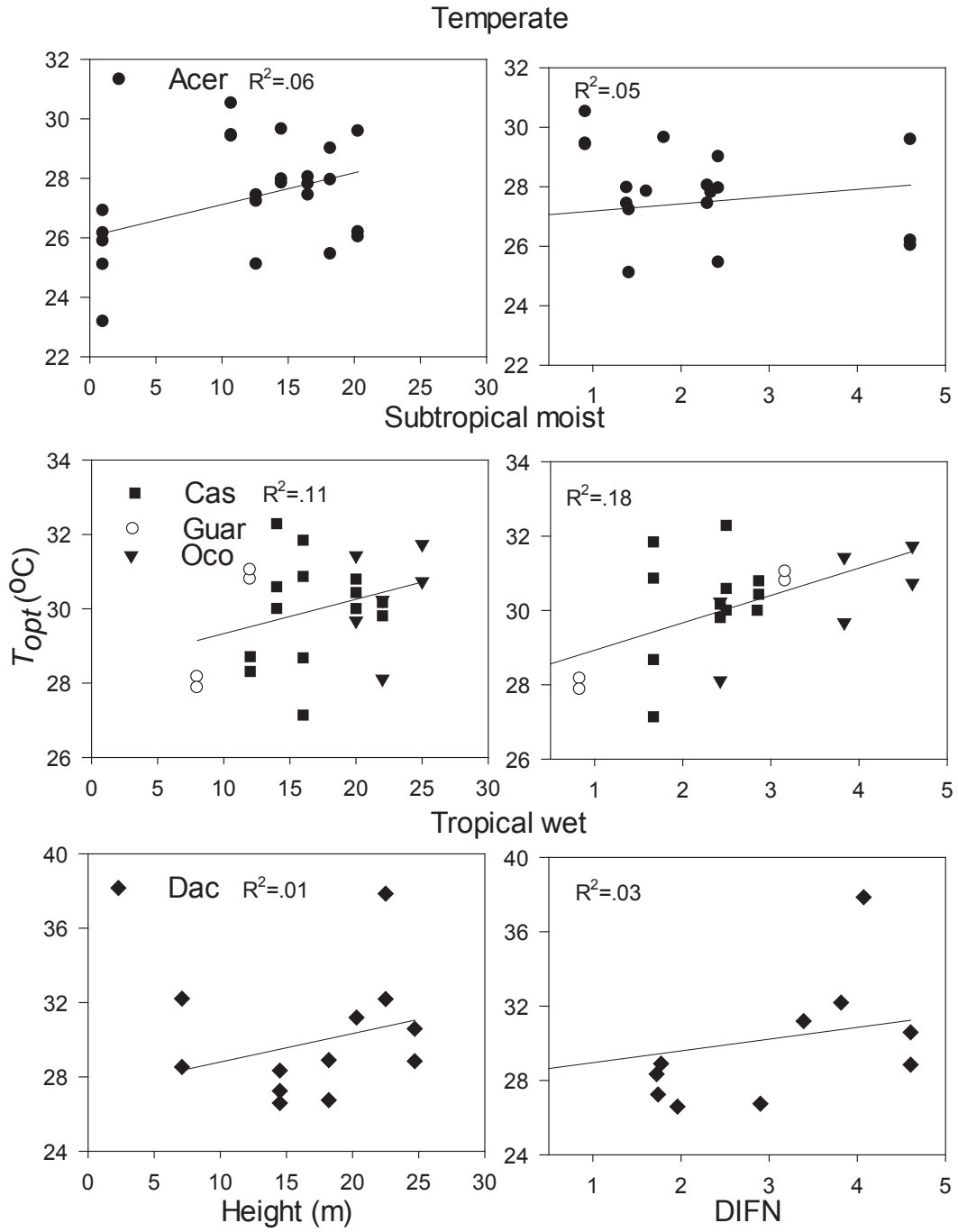


Figure 2.2 T_{opt} vs. Height and Light in tropical and temperate forests. Temperature optimum (T_{opt}) variation with height (m), T_{opt} (°C) variation with diffuse non-interceptance (DIFN). DIFN points are only from where temperature response curves were conducted.

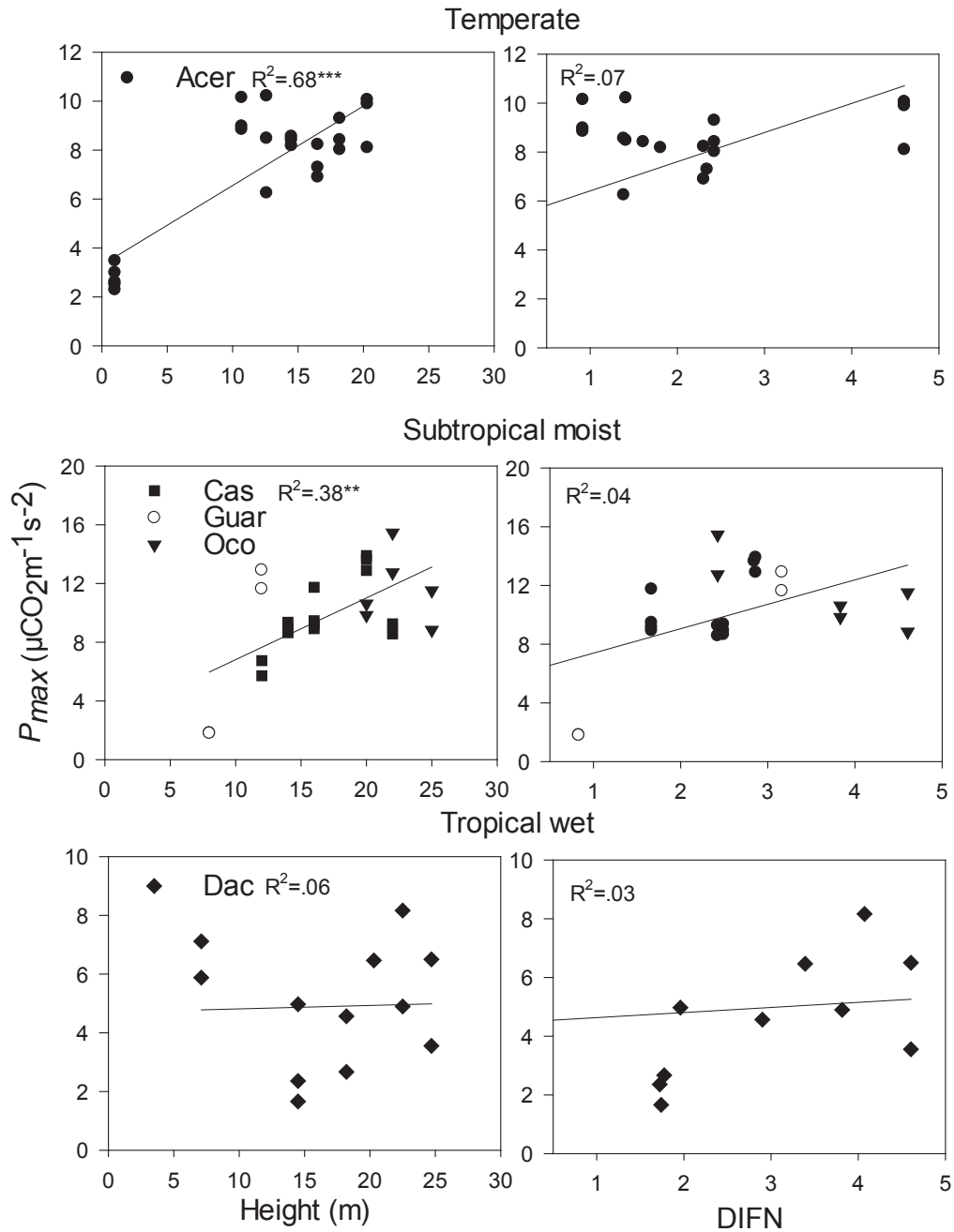


Figure 2.3 P_{max} vs. Height and Light in tropical and temperate forests. Maximum photosynthetic rates P_{max} ($\mu\text{mol CO}_2 \text{m}^{-1} \text{s}^{-2}$) and height, and P_{max} and DIFN. DIFN points are only from where temperature response curves were conducted. Pooled R^2 for all sites.

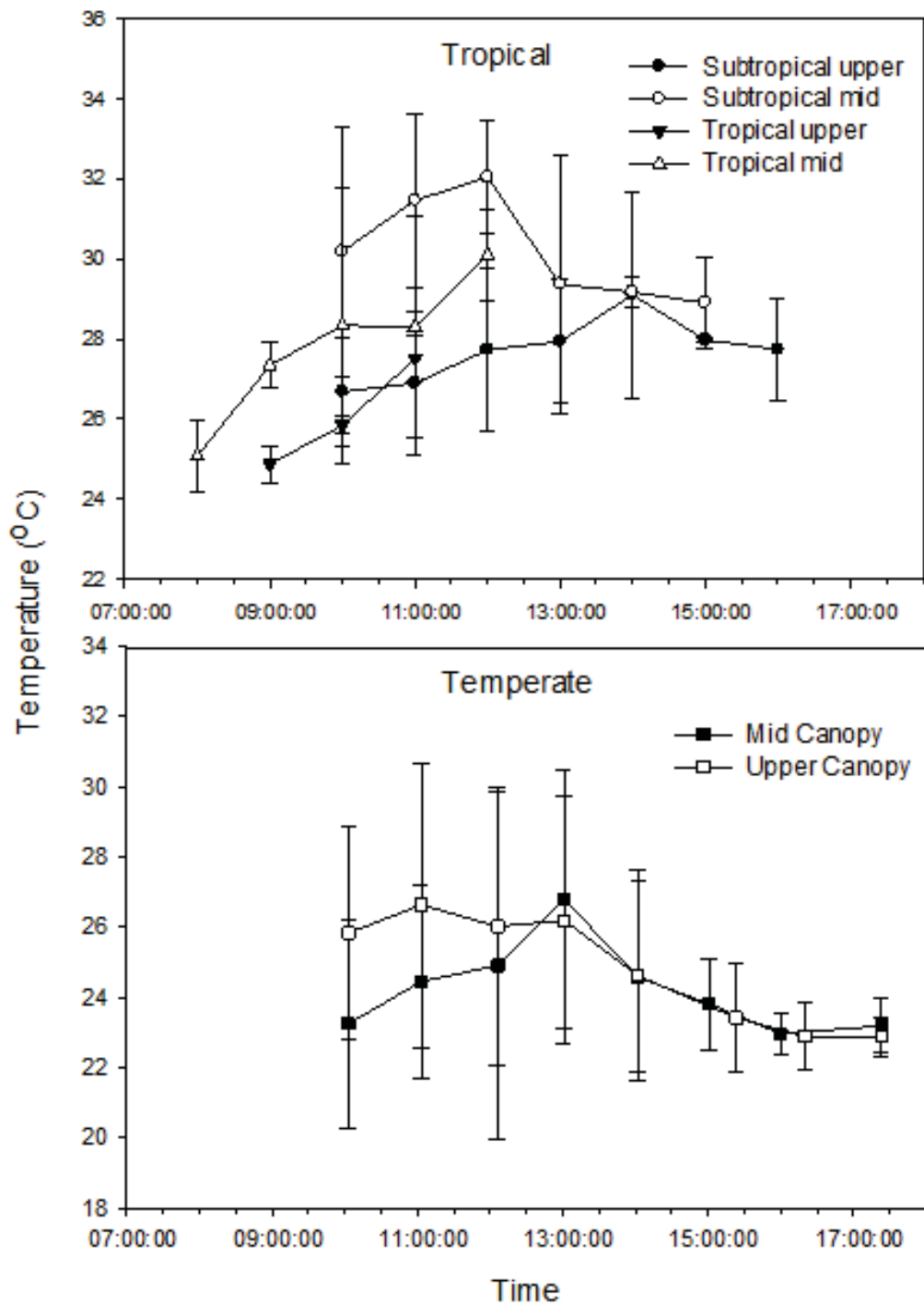


Figure 2.4 The mean of maximum hourly leaf temperature ($^{\circ}\text{C}$) of each sampling height, separated by upper and mid canopies in tropical forests and temperate forests. Upper canopy was defined as greater than 12m, while mid canopy was between 6 and 12 m.

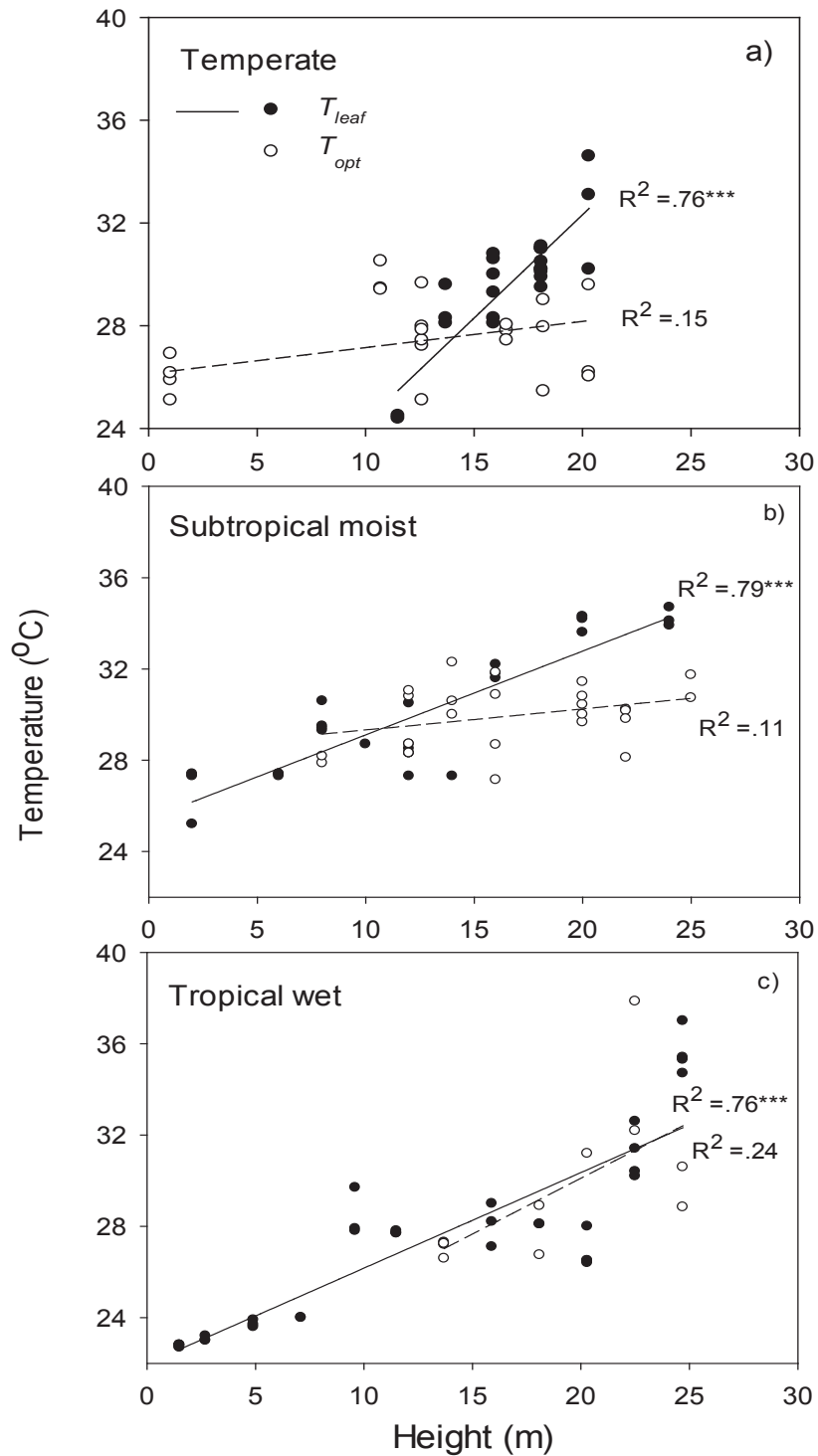


Figure 2.5 T_{opt} vs. $T_{leafmax}$ and height (m) comparisons. $T_{leafmax}$ is the maximum daily leaf temperature at each height, while T_{opt} was extracted from photosynthetic temperature response curves.

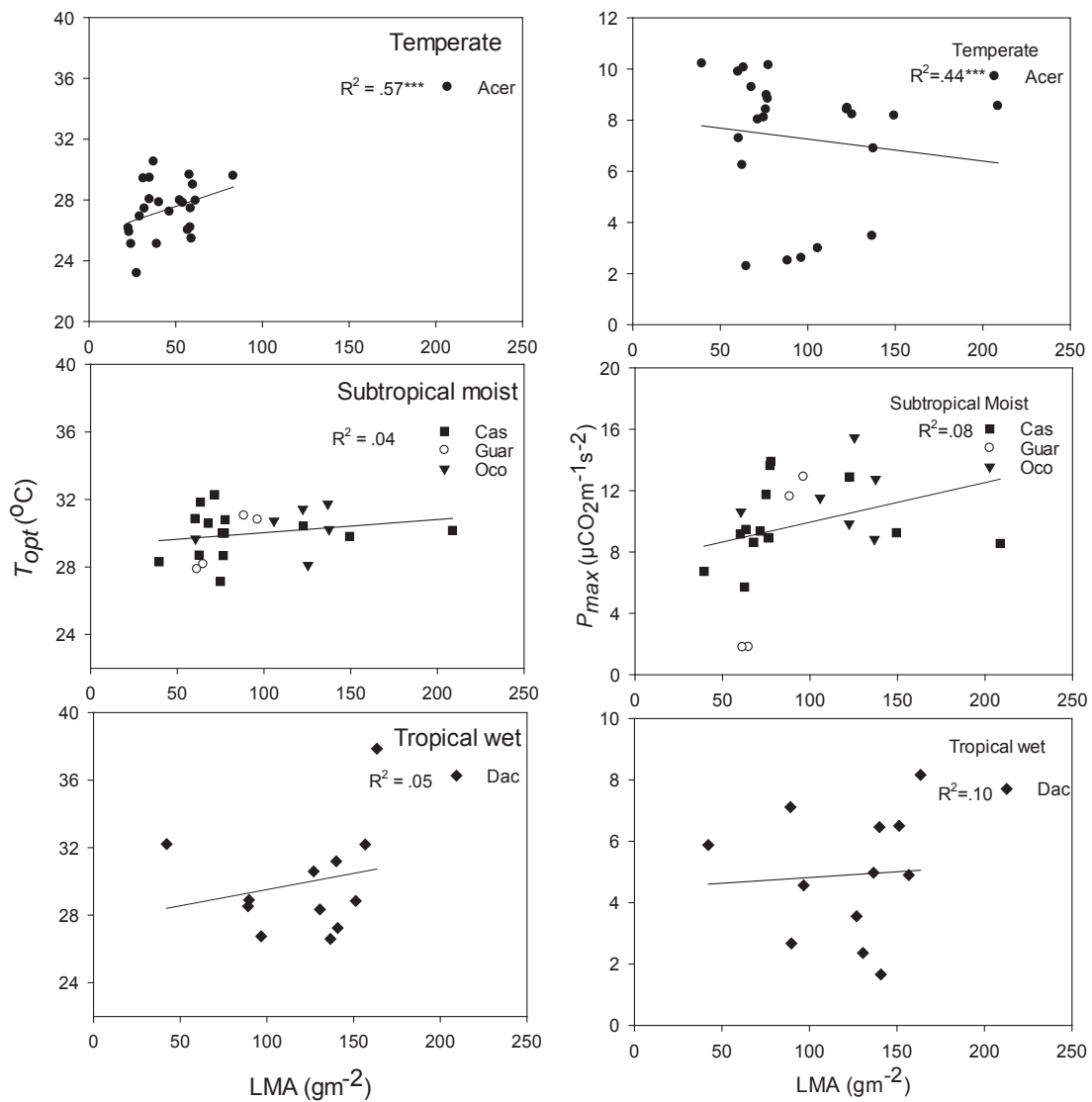


Figure 2.6 T_{opt} (°C) and P_{max} ($\mu\ mol\ CO_2\ m^{-1}\ s^{-2}$) vs. LMA ($g\ m^{-2}$) in tropical and temperate forests. T_{opt} variation with leaf mass per area (LMA) and P_{max} variation with LMA. Pooled R^2 for all sites.

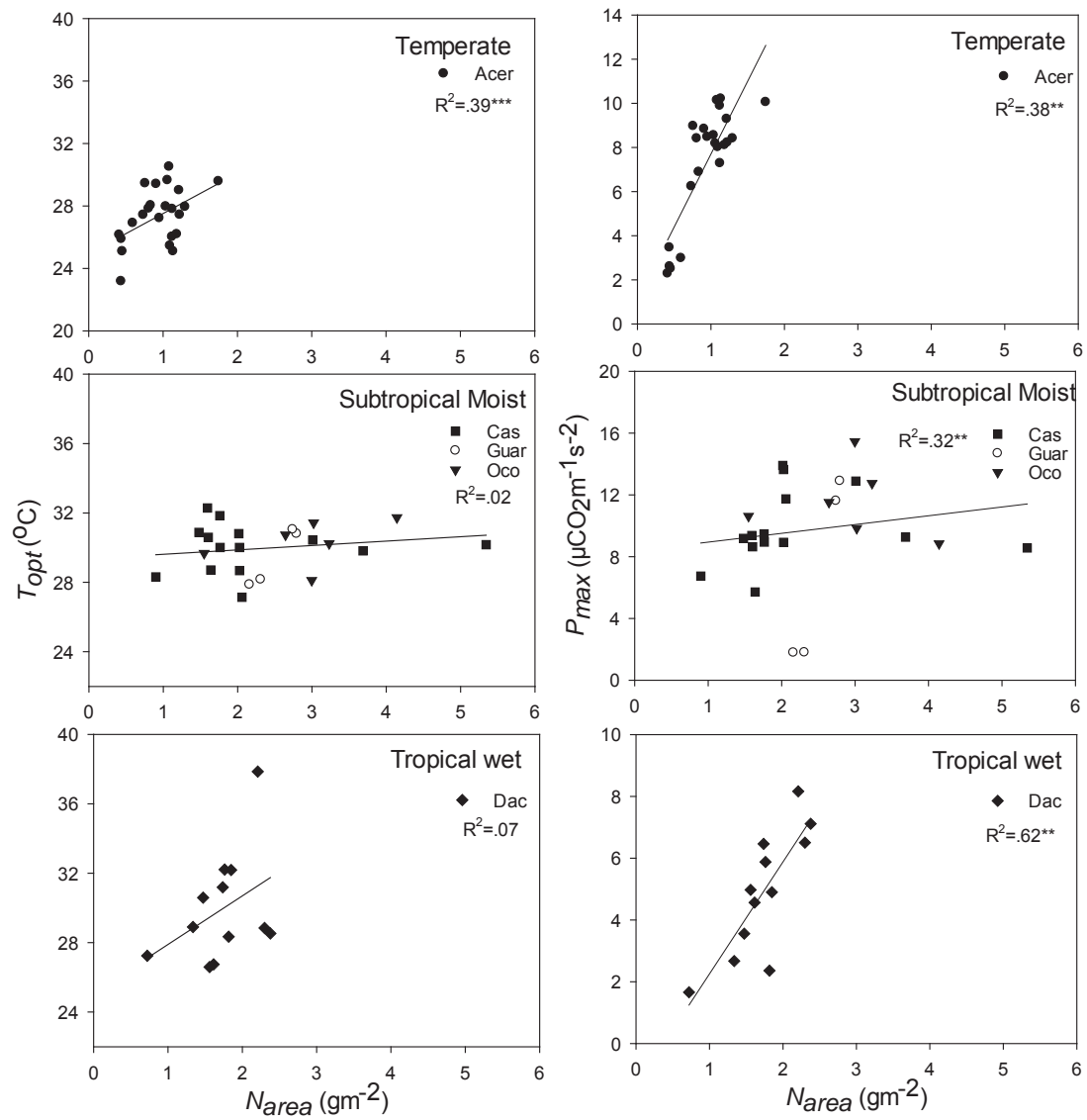


Figure 2.7 T_{opt} (°C) and P_{max} ($\mu\text{molCO}_2\text{m}^{-1}\text{s}^{-2}$) vs. N_{area} ($g\ m^{-2}$) in all forest types. Temperature optimum for photosynthesis (T_{opt}) variation with nitrogen on a leaf area basis (N_{area}). Maximum rates of photosynthesis (P_{max}) variation with N_{area} . Pooled R^2 for all sites.

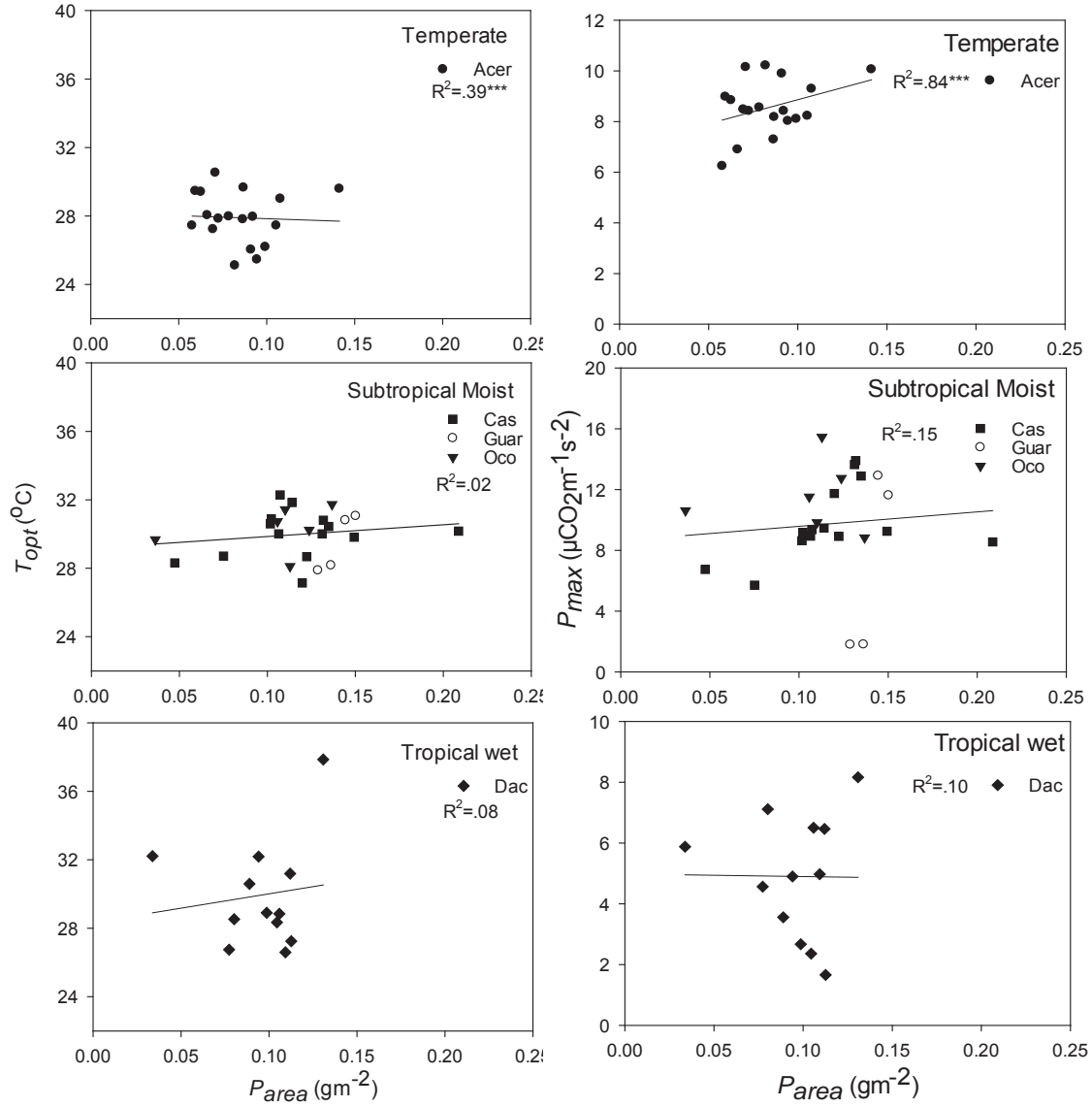


Figure 2.8 T_{opt} ($^{\circ}C$) and P_{max} ($\mu\text{mol}\ CO_2\ m^{-1}\ s^{-2}$) vs. P_{area} ($g\ m^{-2}$) in all forest types. Temperature optimum for photosynthesis (T_{opt}) variation with phosphorus on a leaf area basis (P_{area}). Maximum rates of photosynthesis (P_{max}) variation with P_{area} . Pooled R^2 for all sites.

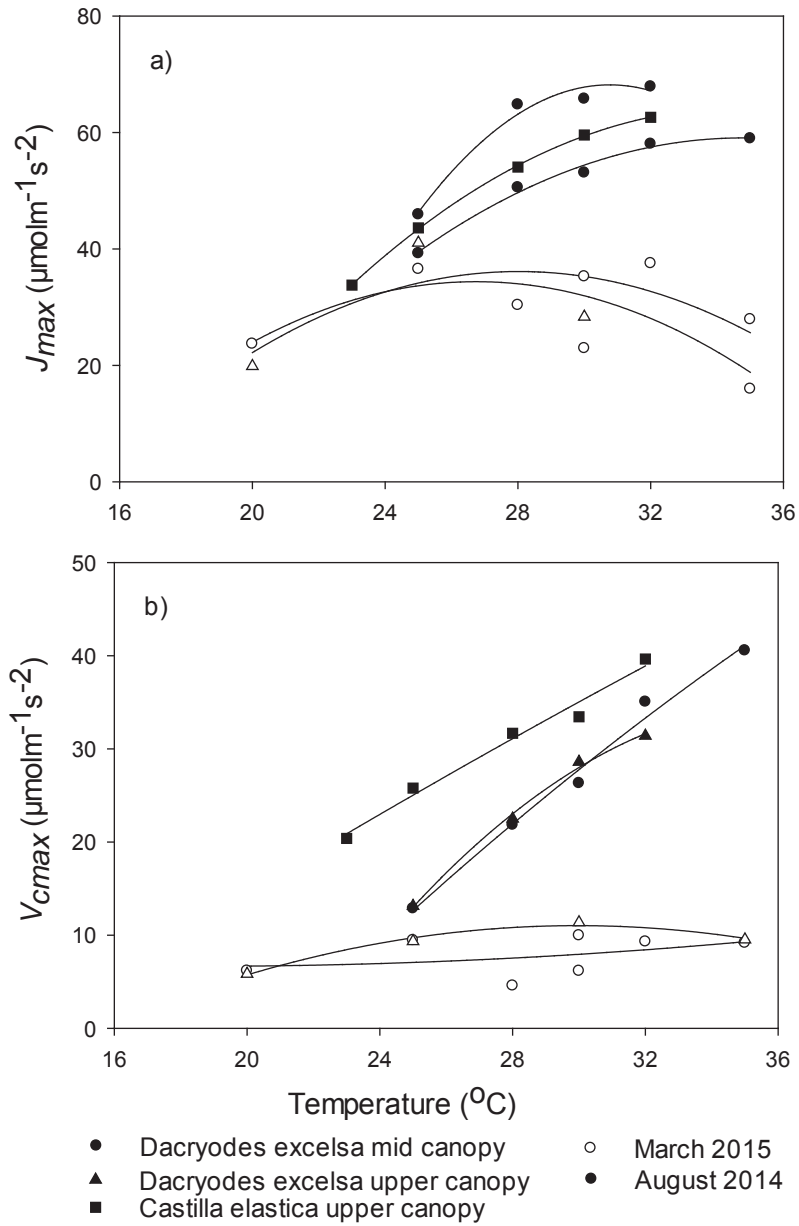


Figure 2.9 J_{max} ($\mu\text{molm}^{-1}\text{s}^{-2}$) and V_{cmax} ($\mu\text{molm}^{-1}\text{s}^{-2}$) vs. Temperature ($^{\circ}\text{C}$). Maximum rates of electron transport, and RuBP regeneration (J_{max}) and maximum rate of carboxylation (V_{cmax}) response to changing leaf temperature.

Chapter 3:

Photosynthetic acclimation of *Acer saccharum* seedlings subjected to elevated temperature *in situ*²

Introduction

With global warming, air temperatures are predicted to rise from 1 to 5° C by 2100 (Kirtman et al. 2013). Northern latitudes, such as the Upper Peninsula of Michigan are expected to have greater relative temperature increases than southern areas (Kirtman et al. 2013). Understanding the instantaneous and long term photosynthetic responses of seedlings to temperature, survival, and recruitment into the overstory is critical in predicting future forest carbon (C) balances and bioclimatic feedbacks.

Seedlings subjected to higher temperature regimes can have the ability to acclimate photosynthetic apparatus, thus avoiding declines in carbon assimilation due to heat stress. This leads to greater photosynthetic temperature optima (T_{opt}) above which photosynthesis begins to decline (Sage et al. 2008). Changes in growth temperature within species can increase T_{opt} , as plants grown at higher temperatures tend to have higher optimal temperatures for photosynthesis than individuals of the same species grown at lower temperatures (Berry and Bjorkman 1980, Hikosaka et al. 2006). As a result, trees grown at higher growth temperatures follow different developmental trajectories than non-warmed individuals, and therefore may have a greater photosynthetic capacity at higher measurement temperatures (Way and Oren 2010).

² This chapter contains material planned for submission for publication

Temperate forests have shown increases in net photosynthesis with increasing growth temperatures and an ability to acclimate to warmer temperatures (Way and Oren 2010). Temperate species, such as *Acer saccharum*, may have a large range of tolerance due to large seasonal variation in temperature, and therefore may be better able to photosynthetically acclimate to higher than average temperatures than those of other biomes (Cunningham and Read 2002). Many models predict forest dieback and shifts in species composition in response to elevated temperature from climate change. However acclimation is not always considered, and can alter how future forest populations react to increasing temperatures and mitigate the need to shift their range to survive (Gunderson et al. 2000, Kattge and Knorr 2007, Iverson and McKenzie 2013).

Sugar maple (*Acer saccharum*) is incredibly important in northern Michigan, both economically and ecologically. USDA Forest Inventory Analysis importance values of timber and ecological significance for this species range from 11 to 30, which are some of the highest in the United States (Iverson and Prasad 1998, Iverson and McKenzie 2013, 2014). However, with the onset of increasing temperatures, depending on the magnitude of increase, importance could either remain the same, or drop by over 50% (2014).

Leaf traits can be strong predictors of photosynthetic capacity, especially in regard to acclimation to elevated temperature. For example, nitrogen (N) is an important component of photosynthesis and growth, and leaf N concentrations can be strong predictors of photosynthetic capacity (Evans 1989, Reich et al. 1998, Han et al. 2004, Hikosaka et al. 2006). Leaf N has shown variations in leaves of the same species grown at high and low temperatures (Hikosaka et al. 2006). It therefore may not have a direct

correlation with photosynthetic capacity on the leaf mass or area basis. A change in leaf N between heated and non-heated seedlings could be a measureable indication that acclimation has occurred.

Differences in leaf mass per area (LMA) between ambient and warmed seedlings have varied across studies and species. Some studies have shown increases in LMA in various species grown above ambient temperature compared to control, while others showed a decrease (Hikosaka et al. 2006, Shen et al. 2009, Xiang et al. 2013, Weerasinghe et al. 2014). If leaves were not acclimating and also photosynthesis was declining with increased temperature, it is also likely that traits such as leaf N and LMA may also decline in heated plots.

Like leaf functional traits, causes of temperature related declines may differ between seedlings acclimated to different temperature regimes. Declines in C assimilation due to temperature could be from biochemical changes, or indirect effects. As with temperature, photosynthesis has been shown to increase with vapor pressure deficit (VPD) up to a point, and then decline (Guehl and Aussenac 1987, Dai et al. 1992). It has been hypothesized that increasing VPD, will be one of the major limiting mechanistic factors of photosynthesis as temperatures increase due to the concomitant decrease of stomatal conductance (g_s) with increasing temperatures and VPD (Ludlow and Jarvis 1971, Running 1976, Day 2000). Instantaneous water use efficiency (WUE), also known as the amount of carbon gained per unit of water lost, may also decline as temperatures rise, in part due to stomatal closure to avoid water loss, leading to an overall reduction in C assimilation (Eamus 1991, Keenan et al. 2013).

Instantaneous rates of photosynthesis can be modified by acclimation, and lead to the ability of plants to assimilate carbon at temperatures that would typically induce heat stress and stomatal closure (Smith and Dukes 2013). Instantaneous photosynthetic responses on small scales are currently being used to predict global plant acclimation of photosynthesis, since there is a dearth of knowledge on long-term responses and acclimation (Smith and Dukes 2013). This study of in situ warming and acclimation potential of an important northern hardwood species could help improve model parameters with the addition of long term effects of warming on photosynthetic apparatus.

This project sought to test the following hypotheses:

1. Heated seedlings will have higher T_{opt} and P_{max} than control, indicating photosynthetic acclimation to elevated temperature.
2. Leaf LMA and N will be greater in the heated plot than the control, indicating physical adaptation to elevated temperature.
3. Declines in photosynthesis beyond the temperature optima will be due to direct biochemical responses more so than indirect stomatal responses to elevated temperature.

Methods

Site

The study site was located at Michigan Technological University's Ford Center and Forest in Baraga County of the Upper Peninsula of Michigan (46.6°N, 88.5°W). Mean annual temperature (MAT) of this area is 4.9°C, while mean temperature during the growing season (May to September) is 15°C. The site receives 879 mm of precipitation annually, 401 mm of which come during the growing season (Burton et al. 2012). The soil within the plots is a Kallio cobbly silt loam extending to a depth of 40 cm.

Acer saccharum is the dominant tree in the forest, with 89.3% of basal area of all overstory trees (dbh > 5cm), though *Ulmus americana*, *Tilia americana*, *Betula alleghaniensis*, and *Ostrya virginiana* are also present. The stand has been managed for northern hardwood saw timber, using the selection harvest method with the last harvest in 1998 (Coble and Cavaleri 2014).

The plots used in this experiment were established in 2009 and were 10x10 m with 4 m buffers. Originally there were sixteen plots with four treatments: control, heated, irrigation (30% more precipitation above average), and irrigation and heated. The focus of the study was originally to determine acclimation of root respiration to increased precipitation and elevated temperature (Jarvi and Burton 2013). Infrared heat lamps model (MRM1215 heaters, Kalglo Electronics Co., Bethlehem, PA, USA) were placed in all heated plots to keep soil temperature at approximately 5°C above ambient. Lamps had been left on every growing season since 2011 and have been kept on 24 hours a day during that period. There were sixteen lamps per plot, located 1.5 m above the ground,

and temperature was manually adjusted to keep the +5°C above ambient uniform. Boardwalks were placed throughout the site to minimize soil compaction, and disturbance of the seedling layer during sampling.

Sampling Design

Photosynthetic measurements, including light response, temperature response, and assimilated CO₂ (A_{ci}) curves, were conducted during the 2014 growing season during two measurement campaigns in early June and September. There were three control plots, and three heated plots in the study. The infrared heat lamps with the most seedlings near it in the heated plots were turned on May 14th, 2014. Of the six plots used in this experiment, three were control and three were heated only. No water addition treatment plots were used. Plants were considered seedlings if they had two or more leaves, but with dbh below 5 cm and heights below 1.3 m.

Six total light response curves were conducted, one in each plot sampled on one leaf of *Acer saccharum*. The light saturation point (the point where photosynthesis reaches its maximum rate based on photosynthetically active radiation) was extracted from each curve, and the average for each plot type was used as the input for photosynthetically active radiation (PAR) in subsequent temperature response curves. Three temperature response curves were conducted in each plot, each on one leaf of *Acer saccharum* for a total of nine temperature response curves in each treatment. Seedling height ranged from 5cm to 1m, and age was impossible to determine. All seedlings had been within the plots for at least three years.

Photosynthesis Measurements

All photosynthesis measurements were taken with a Licor 6400XT with a 6400-02B Red/Blue light source (Li-Cor Biosciences, Lincoln, NE). Light response curves were conducted with relative humidity between 50 and 60%, a flow rate of 200 $\mu\text{mol s}^{-1}$, CO_2 concentrations at ambient conditions of 400 $\mu\text{mol mol}^{-1}$, and leaf temperature set at the ambient temperature of the leaf at the beginning of the curve. Leaves were allowed to equilibrate to maximum light conditions of 2000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ until photosynthesis and stomatal conductance were stable, prior to running the light response curve. Photosynthetically active radiation (PAR) was decreased from 2000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at the following intervals, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 100, and 0 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Light response curves were fitted with the following equation:

$$P_N = \left[\frac{f(I_0) \times I \times P_{gmax}}{f(I_0) \times I + P_{gmax}} \right] - R_D \quad \text{Equation 3.1}$$

Where P_N is the net photosynthetic rate, I is the photosynthetic photon flux density, I_0 is the quantum yield at $I=0$, P_{gmax} is the maximum gross photosynthetic rate, and R_D the dark respiration rate (Baly 1935, Ye 2007, Lobo et al. 2013). The light saturation point (I_{sat}) was extracted through fitting light response curves, and this point was used for the PAR setting of temperature response curves using the following equation:

$$I_{sat} = \sqrt{\frac{(\beta + \gamma)(1 + \gamma \times I_{comp})}{\beta - 1}} \quad \text{Equation 3.2}$$

Where β and γ are two dimensionless adjusting factors, and I_{comp} is the light compensation point (Baly 1935, Ye 2007, Lobo et al. 2013). The PAR value for the temperature response curves was extracted by fitting light response curves to the Lobo model based on Michaelis-Menten equations (Lobo et al. 2013).

Photosynthetic temperature response curves were conducted using a water jacket, as part of the 6400-88 expanded temperature kit, to increase and decrease leaf temperature (Li-Cor Biosciences, Lincoln, NE). Leaf temperature was initially set to ambient, then increased by allowing heated water to flow through a bev-a-line tube through the water jacket using gravity. After achieving the highest possible temperature (about 32°C) the temperature was incrementally adjusted to the lowest sampling temperature possible before dew point (about 16°C). The use of the water jacket allowed for a greater stabilization of temperatures and a greater temperature range than the Li-6400XT can typically achieve. For photosynthesis curves, leaf temperature was set to 16, 18, 20, 22, 24, 26, 28, 30, and 32°C. Relative humidity was maintained between 50 and 60%, and was regulated by flow (200 $\mu\text{mol s}^{-1}$) and desiccant. CO₂ was kept at ambient concentrations of 400 ppm. PAR was kept at the light saturation point for each species, extracted from light response curves. g_s , WUE, and VPD were automatically measured during each temperature response curve (Equations 3.1 and 3.2). T_{opt} was calculated by

taking the second order polynomial function of linear regression from the temperature response curves and solving for the highest point on the x-axis (Cavieres et al. 2000).

$$T_{opt} = -\frac{B}{(A^2 \times 2)} \quad \text{Equation 3.3}$$

Where A and B are as follows from the regression equation: $y = a^2 + b + c$. P_{max} was extracted by solving for the photosynthetic value from the regression equation at T_{opt} (Cavieres et al. 2000).

Leaf Traits

For all leaves measured for gas exchange, leaf area was measured using a leaf area meter (LI-3100 Li-Cor Biosciences, Lincoln, NE). Leaves were weighed, then dried for 24 hours at 65°C, ground for three minutes with a ball bearing grinder (8000M Mixer/Mill, Spex Sample Prep, Metuchen, NJ), and weighed again. Leaf mass per area (LMA) was determined by taking the ratio of dry weight to leaf area. Prior to elemental analysis, ground samples were dried for an additional 24 hours at 65°C, weighed with a Sartorius cubis microbalance (Data Weighing Systems, Elk Grove, IL), and for 4-6mg of leaf material was folded in to 5 by 9 mm tin capsules. N analysis was performed using an Elementar vario Microcube elemental analyzer (Elementar Inc., Hanau, Germany).

Data Analysis

T-tests were used to compare the means of photosynthesis measurements (T_{opt} , P_{max} , maximum rates of vapor pressure deficit (VPD_{max}), and photosynthesis at maximum

rate of vapor pressure deficit (A_{vpdmax}) and leaf traits (LMA, N_{mass} , and N_{area}) of the control and heated seedlings to determine if they were significantly different. One way ANCOVA tests were done to determine if treatment and temperature caused instantaneous WUE to differ. All statistical analyses were performed in R (R Core Team, 2013).

Results

Maximum rates of photosynthesis and optimum temperature with acclimation

There were no significant differences between the mean values of T_{opt} and P_{max} in the heated plots than the control plots, although the averages were 16% higher for T_{opt} and 3% higher for P_{max} in the heated plots and p-values were marginal (Table 3.1). The warmed seedlings had a sharper increase in photosynthesis from the low to maximum rate of photosynthesis, and had a T_{opt} that was more defined. The control seedlings, on the other hand, had less variation in rates of photosynthesis, and a more gradual peak at T_{opt} (Figure 3.2).

Variation in leaf functional traits in heated and control plots

There were no significant differences between the means of heated and control plots in regard to LMA, N_{mass} or N_{area} , although means were slightly higher for each in the heated plots for each trait (Table 3.1). For LMA, N_{mass} and N_{area} the respective differences were 11%, .28%, and 12%.

Direct and indirect effects of temperature on photosynthesis

Photosynthesis increased with VPD up to a maximum, then declined (Figure 3.2). Instantaneous water use efficiency increased linearly with temperature (Figure 3.3). Though was no significant difference between maximum g_s in the control plot or heated plots, and g_s did not follow a standard trend (Figure 3.3).

Discussion

Maximum rates of photosynthesis and optimum temperature with acclimation

The hypothesis that warmed seedlings would have higher T_{opt} and P_{max} was not supported on both counts. There was not a significant difference between the heated and control plots for either variable, despite a trend for slightly higher T_{opt} and P_{max} in the heated plots (Table 3.1). A previous study comparing photosynthetic rates between heated and unheated populations of *Acer saccharum* in open-top field chambers using resistance heaters found that heated seedlings had a statistically significant 12% increase in photosynthesis (Gunderson et al. 2010a). Though not significant, our study found that heated plots had a rate of photosynthesis that was 16% greater than that of control (Table 3.1 and Figure 3.3). The increase in T_{opt} , however, was relatively small, at only 3%, with little over a 1°C difference between treatments (Table 1). While it is difficult to determine if acclimation did truly occur, there appears to be a trend that may become apparent with increased sample size. Even at air temperatures 5°C above ambient, there were no major declines in photosynthesis, indicating that heated seedlings had not reached their thermal

optimum, and seedlings grown in ambient conditions could still show favorable responses to elevated temperatures. However, to avoid any eventual declines, seedlings would have to shift their thermal optima to higher temperature regimes. Across acclimation studies, leaves of seedlings have shown success acclimating to temperatures +2°C above ambient conditions and moderate success at +4°C above ambient leaf temperature, shifting their temperature optima and increasing carbon assimilation rates (Gunderson et al. 2010). In general, C₃ species have shown a greater ability to shift their photosynthetic response to temperature than other plant types, such as C₄ and CAM, in addition to species from northern portions of their range having greater acclimation potential than southern populations (Sage and Kubien 2007, Zhou et al. 2007, Yamori et al. 2014).

Since species survival begins with seedlings, this could be detrimental for *Acer saccharum* if it is truly unable to acclimate. Future forest ecology will be determined by what species of seedlings survive and are eventually recruited into the overstory. In the Upper Midwest and Great Lakes Region, forest type is not expected to change from temperate deciduous, however site conditions are predicted to favor species of oaks, forgoing acclimate of species that are currently present (Danz et al. 2011, Fisichelli et al. 2014). Thus acclimation will not only inform short term shifts of photosynthesis, but overall productivity (Smith and Dukes 2013). Changes in the carbon uptake of seedlings will unlikely to affect the carbon fluxes on the forest level, however, acclimation of mature trees could pose this threat. However, there are few data on mature tree acclimation of photosynthesis to altered climatic conditions.

Variation in leaf functional traits in heated and control plots

Averages of LMA, N_{area} , and N_{mass} were not significantly different between treatments, and the hypothesis that they would be greater in the warmed plot was rejected. LMA was slightly higher in the warmed plot than the control (Table 3.1). Since greater LMA has been found to correlate with greater photosynthetic capacity, it is possible that the warmed seedlings have acclimated slightly, but there was not enough statistical power to prove this (Poorter et al. 2009). However, since the warmed seedlings did not have significantly lower LMA, it is unlikely that they have responded negatively to the temperature. Other studies have found both increases and decreases in LMA with growth temperature, though changes were strongly influenced by light availability and minimum growth temperature, which our study did not explore (Poorter et al. 2009, Shen et al. 2009, Xiang et al. 2013).

Increases in N_{mass} of warmed seedlings were very small, while N_{area} increases were slightly greater (Table 3.1), though neither were significant. The degree to which leaf N mass or area can predict photosynthesis or temperature optima has varied by species and by study. N_{area} has been found to strongly correlate with light availability, more so than N_{mass} , and light is a major component of photosynthetic capacity of *Acer saccharum*, due to its shade tolerance (Frak et al. 2001, Le Roux et al. 2001, Meir et al. 2002, Coble and Cavaleri 2014). Significant changes between the heated and control leaf nitrogen concentrations may have indicated that leaf structure and photosynthesis had been altered in response to temperature, either in the form of acclimation if the heated plot had greater concentrations or declines in the quality of leaf structure and N if acclimation had not occurred. Both scenarios were possible, since seedlings of various species have shown an ability to acclimate to greater temperature by changing leaf

structure or nutrient allocations, but have also shown declines in leaf quality (Dreyer et al. 2001, Wright et al. 2004). A lack of acclimation would suggest that there could be declines C uptake, and eventual declines of the species itself, as was predicted for *Acer saccharum* (2014).

Direct and indirect effects of temperature on photosynthesis

Since neither stomatal conductance, VPD, nor WUE were shown to be significant, it was not possible to determine whether direct or indirect responses to temperature were responsible for declines in photosynthesis. However, T_{opt} of photosynthesis was several degrees greater than that of the temperature optimum of g_s , indicating that stomatal closure was likely not the main factor in photosynthetic decline. Generally g_s is much lower in seedlings than mature individuals (Reich et al. 1991, Thomas and Winner 2002). g_s also typically began to decline as soon as any environmental variables within the chamber were changed, thus any declines may not have been measurably caused by temperature and were simply the result of leaf stress from the experimental apparatus.

VPD did show similarities in limitations to photosynthesis to the temperature response curves (Figure 3.2). It is possible that, with increasing temperature, VPD is one of the indirect limitations to photosynthesis since the maximum rate of photosynthesis in response to temperature were very similar to maximum rates of photosynthesis in response to VPD (Table 3.1). There are currently few data showing the relationship between temperature acclimation and VPD.

Differences in instantaneous WUE were also not significant between heated and control treatments (Table 3.1). WUE also did not appear to be temperature limited, since it increased linearly without a decline. Like VPD, there are few data on the relationship between WUE increases and temperature, and more on the response to elevated CO₂ (Eamus 1991, Penuelas et al. 2008). Though this is another important component of climate change, elevated CO₂ is not expected to limit photosynthesis temperature, thus determining if VPD or g_s are limiting will be crucial (Bonan 2008, Alexander et al. 2013).

To determine more precise mechanistic shifts in photosynthesis and whether acclimation is occurring, it would be ideal to measure RuBP regeneration and Rubisco carboxylation. These two biochemical elements of photosynthesis, through the Farquhar C₃ photosynthesis model, can further explain acclimation of photosynthesis through shifts in temperature optima (1980). This can be done by conducting a series of CO₂ response (A_{ci}) curves at various temperatures to determine which is the limiting component of photosynthesis, and whether biochemical rates have changed between warmed and unwarmed plants (Sharkey et al. 2007). Acclimation studies on seedlings have utilized measurements of enzymatic processes to determine which leaf functions will adjust to elevated temperature, and whether acclimation is Type I or II (Smith and Dukes, 2013). The change in slope and intercept of instantaneous photosynthetic response curves can determine which form of acclimation may be occurring (Atkin et al. 2006).

Conclusion

Due to the small sample size, it was impossible to statistically detect if treatment differences in P_{max} , T_{opt} , and leaf traits were biologically significant. However, mean values for P_{max} , and T_{opt} were greater in the warmed plots than those in the control, indicating that some acclimation may have occurred. According to species range predictions, *Acer saccharum* is expected to show declines resulting from elevated temperature from climate change (Iverson and Prasad 1998). This would indicate an inability to acclimate, or at least not to the extent where it would maintain its current niche. If photosynthesis of *Acer saccharum* is not only acclimating to elevated temperatures, but also increasing with air temperature, there may not be the massive declines of sugar maple that were predicted in northern latitudes, and overall carbon assimilation of those forests may increase (2014). Few warming studies also look at the comprehensive indirect and direct effects of temperature on photosynthesis in *Acer saccharum*, which could further elucidate if and how acclimation is occurring. Studies where biochemical components of photosynthesis are sampled, in addition to WUE, LMA, and leaf nitrogen in warmed *in-situ* plots are rare. Significant evidence of acclimation in this study could not be shown for any variables, however this study did provide insight into other measurements that may improve understanding of acclimation of photosynthesis to temperature in future studies.

Table 3.1 Means and standard error for leaf traits in control and heated plots, and t-test p-values. Temperature optimum for photosynthesis, T_{opt} ($^{\circ}\text{C}$); maximum photosynthesis, P_{max} ($\mu\text{mol CO}_2 \text{ m}^{-1} \text{ s}^{-2}$); leaf mass per area, LMA (g m^{-2}); leaf nitrogen per unit area, N_{area} (g m^{-2}); leaf nitrogen on a mass basis, N_{mass} (mg g^{-1}); maximum vapor pressure deficit, VPD_{max} (kPa); rate of photosynthesis at VPD_{max} , P_{vpdmax} ($\mu\text{mol CO}_2 \text{ m}^{-1} \text{ s}^{-2}$); temperature optimum for maximum g_s , T_{gs} ($^{\circ}\text{C}$); maximum stomatal conductance, g_{smax} ($\text{mmol m}^{-2} \text{ s}^{-1}$); slope and of water use efficiency (WUE) response to temperature and the number of replicates (n).

	T_{opt}	P_{max}	LMA	N_{mass}	N_{area}	VPD_{max}	P_{vpdmax}	T_{gs}	g_{smax}	WUE	n
Control	26.50	3.30	25.63	18.80	.48	1.83	2.39	23.62	.06	2.05	9
	(.54)	(.24)	(1.4)	(.47)	(.03)	(.07)	(.26)	(.94)	(.01)	(.52)	
Heated	27.39	3.85	28.73	18.88	.54	1.81	3.81	22.52	.08	3.24	9
	(.50)	(.22)	(1.9)	(.96)	(.03)	(.16)	(.22)	(1.02)	(.02)	(.65)	
p-val	.24	.11	.20	.94	.28	.89	.14	.51	.37	.17	

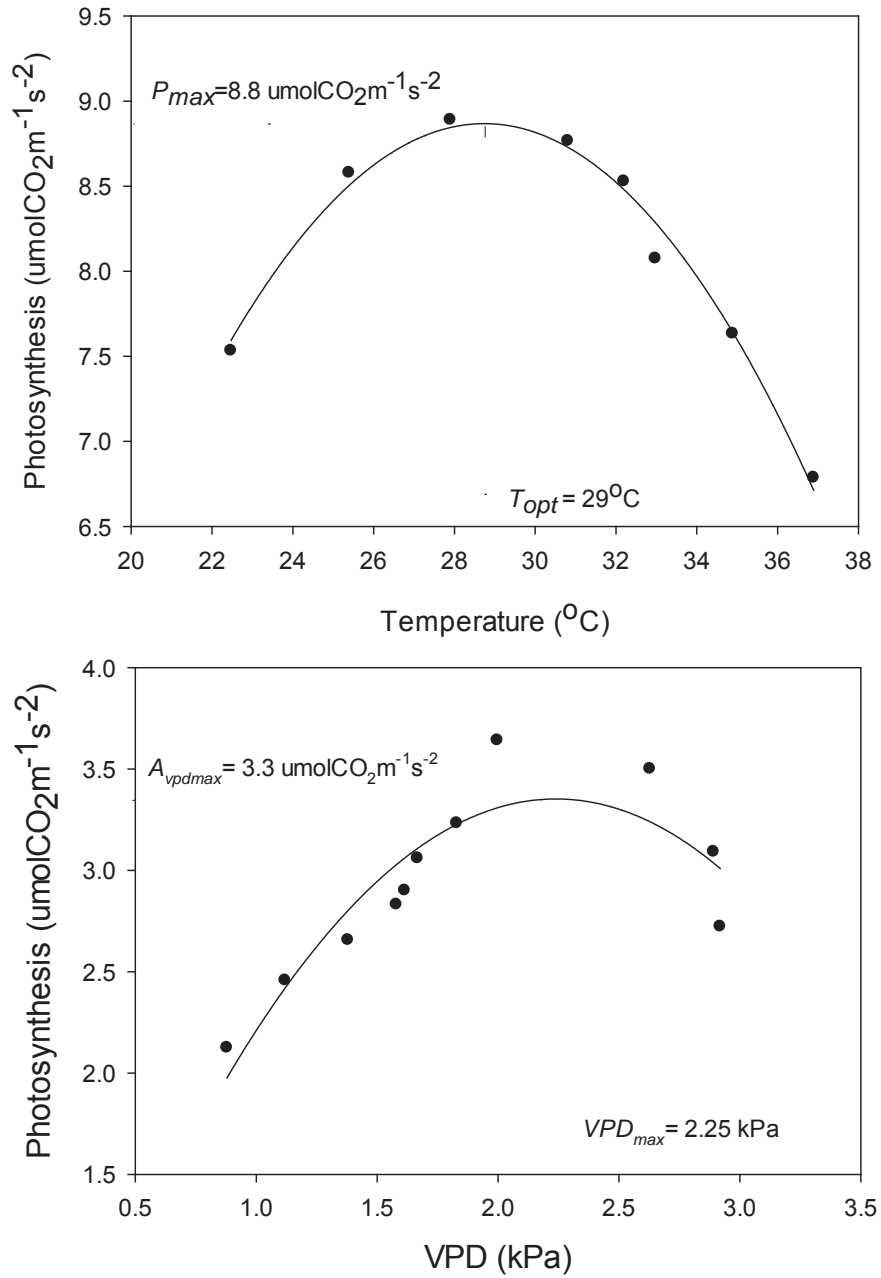


Figure 3.1 Example of a photosynthetic temperature response curve, where T_{opt} and P_{max} are noted and an example of VPD response to photosynthesis, where P_{vpdmax} and VPD_{max} are noted.

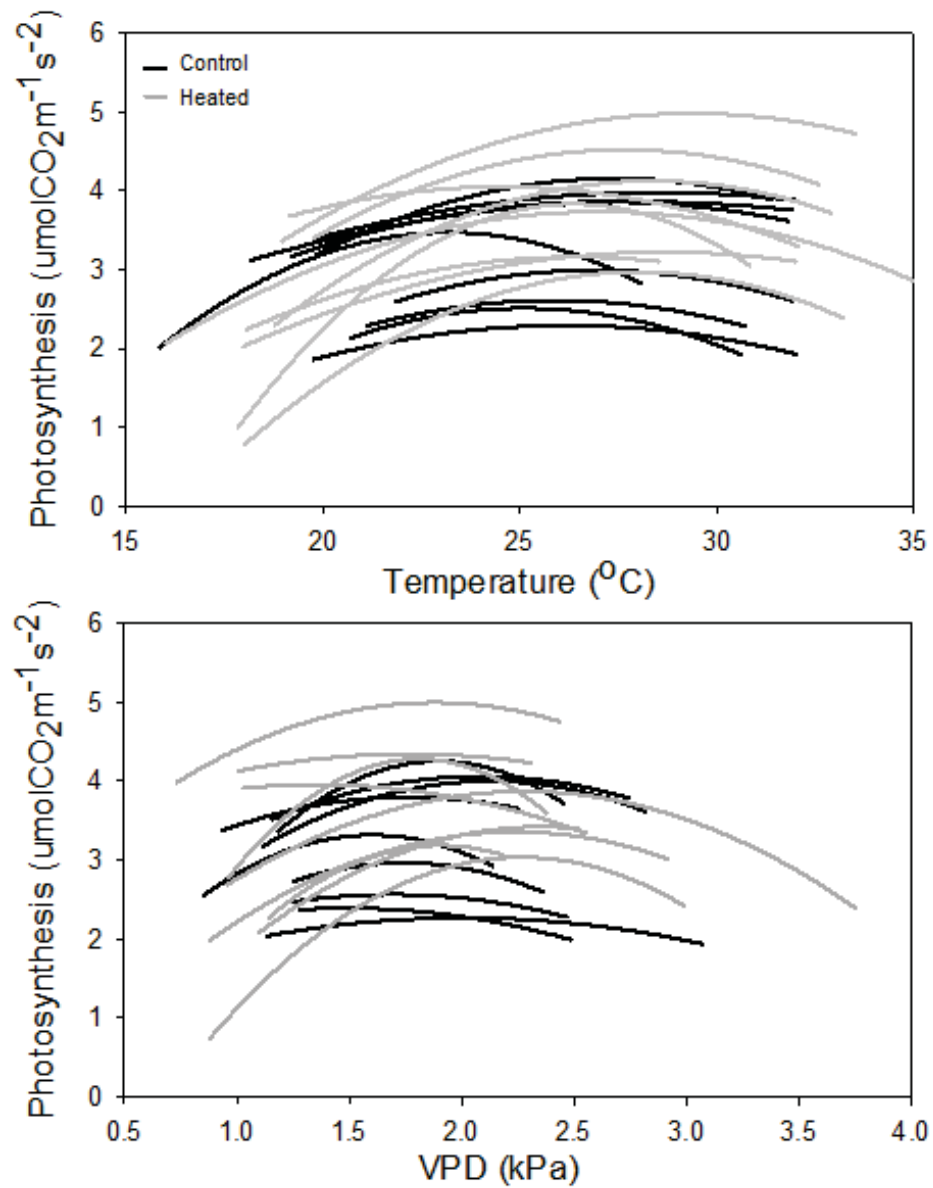


Figure 3.2. Control vs. heated responses to a) temperature ($^{\circ}\text{C}$) vs. photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-1} \text{ s}^{-2}$), vapor pressure deficit (VPD, kPa) vs. photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-1} \text{ s}^{-2}$). Photosynthesis's response to temperature and VPD were fitted with 2nd order polynomial functions.

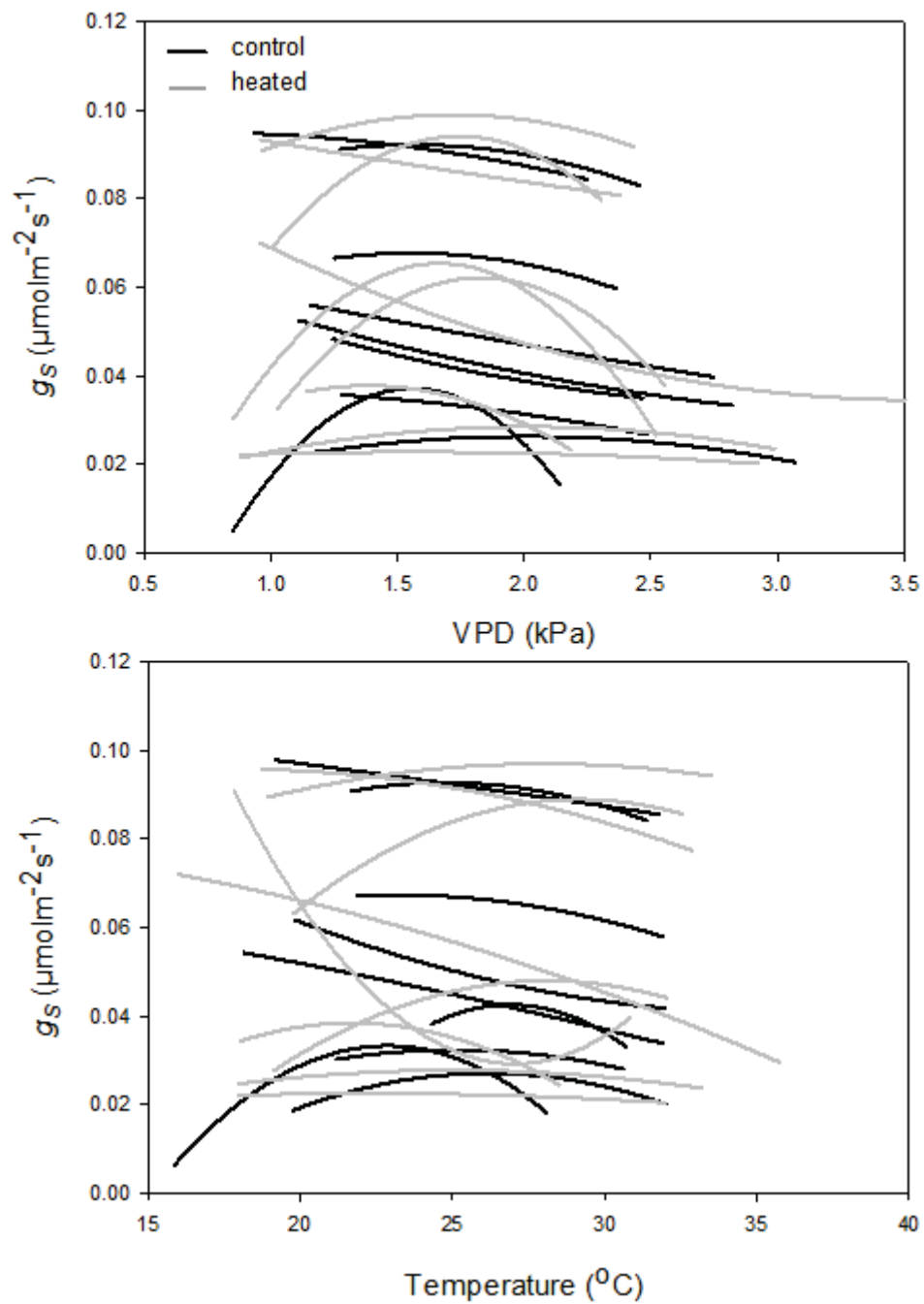


Figure 3.3 Stomatal conductance (g_s , $\mu\text{mol m}^{-2} \text{s}^{-1}$) response to vapor pressure deficit (VPD, kPa) and stomatal conductance (g_s , $\mu\text{mol m}^{-2} \text{s}^{-1}$) response to temperature ($^{\circ}\text{C}$).

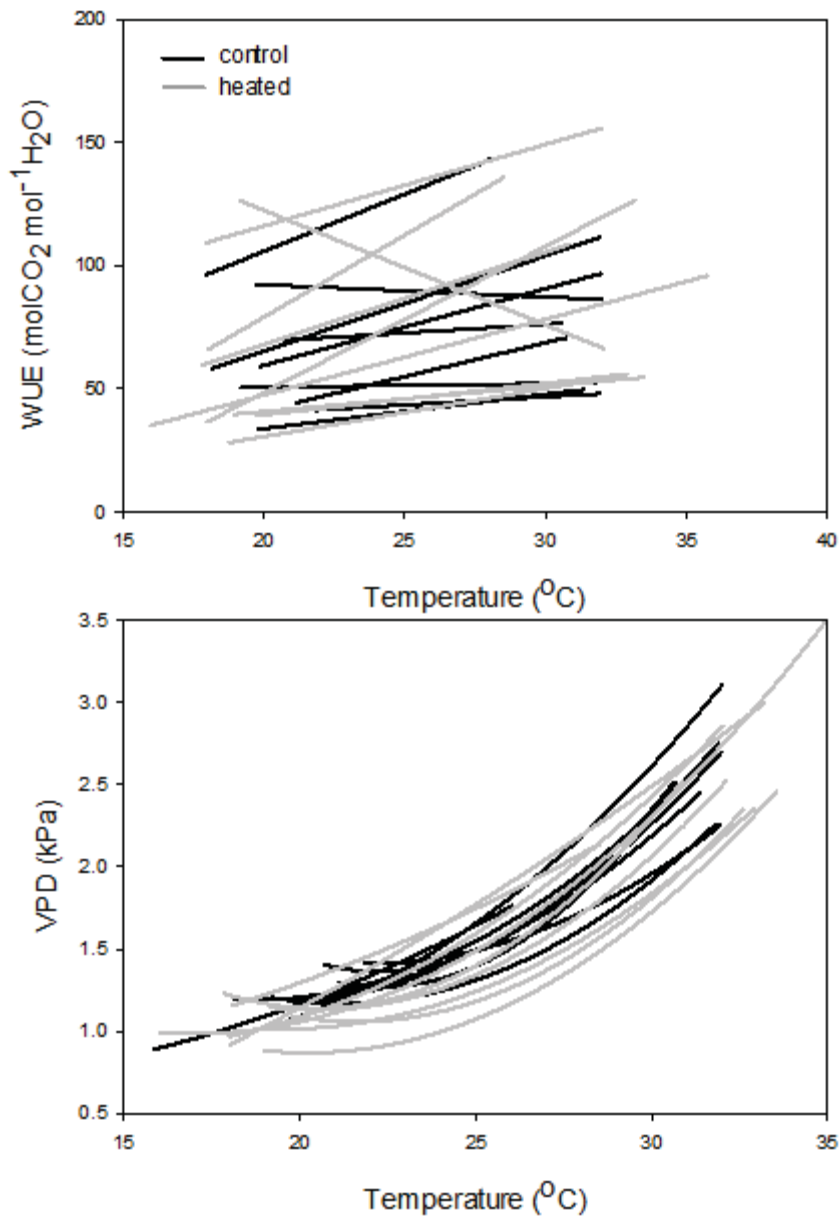


Figure 3.4 Instantaneous water use efficiency (WUE, molCO₂ mol⁻¹H₂O) in response to elevated temperature (°C) and vapor pressure deficit (VPD, kPa) response to temperature (°C). WUE fitted with a linear function and VPD with a second order polynomial.

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