TEMPERATURE RESPONSES OF LEAF DARK RESPIRATION IN THE UPPER TROPICAL FOREST CANOPY AND THEIR IMPLICATIONS FOR TROPICAL FOREST CARBON BALANCE

By

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To my parents, for valuing unsupervised play

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Global warming could decrease the net primary productivity (NPP) of tropical forests if warming increases plant respiration more than gross photosynthesis. To address the current lack of empirical data on foliar respiration in relation to temperature from tropical forest, I measured respiration rates of upper-canopy leaves of trees and lianas in a tropical forest in Panama, and analyzed the response of respiration to temperature in the short term (minutes to hours), and in the longer term (days).

Both *in situ* and laboratory measurements indicate that the short-term temperature response of respiration, expressed as Q_{10} (the proportional increase in respiration per 10°C warming), was higher than what is commonly assumed in global models. Respiration rates at 25°C (R_{25}) were also high compared to previously reported values for tropical forests. These results suggest that published global models may underestimate leaf respiratory carbon fluxes from tropical forests.

To assess whether mature leaves of tropical forest trees and lianas can acclimate to nighttime warming, I experimentally warmed upper-canopy leaves for 6–8 days. Respiration was down-regulated by on average 2.9% per degree of warming

above ambient temperature, while the Q_{10} remained unchanged. Acclimation was, however, not completely homeostatic. Accounting for acclimation when scaling respiration to the canopy under a 4°C warming scenario reduced the respiration flux compared to no-acclimation by 16% or 0.9 Mg carbon ha^{-1} yr^{-1}.

To identify general patterns in thermal acclimation of respiration I conducted a meta-analysis. This revealed that: 1) the greater the degree of warming, the less complete respiration acclimates, 2) the longer the duration of warming, the more complete respiration acclimates, 3) leaves that develop under the experimental temperature are better acclimated than leaves that are transferred from a lower temperature, and 4) growth forms and biomes do not differ in acclimation potential.

In conclusion, I found evidence that leaf respiration of tropical forest canopy trees and lianas exhibit higher sensitivity to short-term temperature changes than commonly assumed, but that long-term warming results in acclimatory down-regulation of respiration, in accordance with acclimation in temperate and boreal forest trees. This suggests that with gradual warming acclimation of respiration will minimize the potential decrease in NPP with global warming.

CHAPTER 1
INTRODUCTION

There is an urgent need to improve our ability to predict responses of tropical forests to climate change. Tropical forests account for more than 30% of global net primary production (NPP) (Malhi & Grace, 2000; Saugier *et al*., 2001; Huston & Wolverton, 2009), but they are thought to be close to their high temperature threshold (Doughty & Goulden, 2008). Warming by several degrees Celsius, as predicted for the coming decades (Diffenbaugh & Scherer, 2011) will push the majority of tropical forests into a climate envelope currently not occupied by closed-canopy forests (Wright *et al*., 2009). Mitochondrial respiration rates increase exponentially with warming. Global rise of temperature, especially during nighttime (Easterling *et al*., 1997) may thus have major impacts on the NPP of tropical forests if gross photosynthetic productivity does not increase with warming. However, despite the importance of tropical forests for global NPP insufficient empirical data are currently available to predict temperature responses of tropical ecosystems (Reed *et al*., 2012). Importantly, uncertainty about leaf respiration in tropical forests hinders accurate and precise modeling of carbon fluxes in tropical forests under current and future climates (Malhi *et al*., 2009).

How tropical forests will respond to climate warming cannot yet be answered, but in my dissertation research I have generated original empirical data on leaf respiration and its response to temperature over several timescales. These data are critically needed for predicting carbon balance of tropical forests under climate warming. Tropical forests characteristically maintain multiple canopy layers, as a result of which understory seedlings only receive 1–2% of above canopy solar radiation (Clark *et al*., 1996). In deep shade photosynthesis and respiration are very low (e.g., Kitajima, 1994),

so the largest proportion of the leaf respiratory flux in tropical forests comes from leaves in the sun-exposed upper canopy (Cavaleri *et al*., 2008). In my dissertation I therefore focus on dark respiration of sun-exposed upper-canopy leaves in a semi-deciduous tropical forest in Panama, where a construction crane enables canopy access. My dissertation consists of four chapters. Chapters 2–4 report on related studies that combine empirical data with simple models to explore the ecological implications of temperature responses of leaf dark respiration, while Chapter 5 synthesizes the current understanding of leaf respiratory acclimation to warming in a meta-analysis of published data from a range of growth forms and biomes.

In Chapter 2, I test the hypothesis that *in situ* rates of leaf respiration and the temperature response of respiration differ significantly among species and plant functional types, in association with differences in other leaf functional traits. To do so, I compare respiration data from 26 species of tree and liana collected with a new protocol for quantifying the short-term temperature sensitivity of leaf respiration under ambient temperature changes, using a series of pre-darkened leaves.

While the *in situ* measurements offer important insights into the temperature-response processes under ecologically relevant ambient temperature variation, greater degree of temperature control can be achieved in the laboratory, and individual leaves can be measured repeatedly. In Chapter 3, I report leaf-level temperature response curves of respiration measured in the laboratory for 123 upper-canopy leaves collected from 28 species of tree and liana. From these curves the temperature sensitivity of respiration, expressed as the Q_{10} (proportional increase in respiration with 10°C warming) could be determined at the leaf level. Leaf traits can vary considerable across

ecological scales (Messier *et al*., 2010), so to identify where most of the variance of leaf respiration traits occurs, the chapter explores how the variance in leaf respiration and Q_{10} can be decomposed into variance at the level of growth form (trees versus lianas), plant functional type based on tree successional status, species within growth form, and leaves within species.

Leaf respiration and Q_{10} values of canopy trees are challenging to measure, especially in tall and diverse tropical forests. Obtaining reliable estimates from more readily measured traits is therefore desirable. In Chapter 3, I use species-level trait averages to develop trait-based models of respiration and Q_{10}, which are subsequently used to estimate respiration and Q_{10} values for 24 tree and liana species. These leaf-level estimates are then scaled up to the canopy of the study site, making use of multi-year temperature data collected at the site and site-specific information on species relative abundance and leaf area index. For these calculations of nocturnal respiratory carbon fluxes, information on the instantaneous temperature response of dark respiration (e.g., the Q_{10}) is important, but short-term temperature responses cannot be translated into long-term responses of respiration to climate change.

Global warming has been more pronounced at night than during the day (Easterling *et al*., 1997; Alward *et al*., 1999) and it is likely that the nighttime temperatures will continue to rise asymmetrically (IPCC, 2007). Such asymmetric warming is expected to reduce NPP, as autotrophic respiration fluxes will rise with increasingly warmer nights, while daily net photosynthesis is unlikely to increase with daytime warming in the tropics (Cunningham & Read, 2002, 2003a; Doughty & Goulden, 2008; Doughty, 2011). Indeed, in several tropical forests, annual tree growth

is negatively correlated with annual means of daily minimum temperature, rather than with average temperature or atmospheric [CO_2] (Clark *et al*., 2003, 2010; Feely *et al*., 2007), suggesting a strong temperature sensitivity of nighttime respiration of these trees. However, acclimation of plant respiration to warming may reduce the potential decline in NPP (King *et al*., 2006; Smith & Dukes, 2013). Whether nighttime respiration in tropical tree and liana species can acclimate to elevated nighttime temperatures is currently unknown. The objective of Chapter 4 was to determine whether dark respiration of fully expanded leaves of tropical trees and lianas can acclimate to experimentally elevated nighttime temperatures. I report thermal acclimation responses of respiration in upper canopy leaves of three tree and two liana species after nighttime warming for 6–8 days. Acclimation is assessed by correlating average nighttime leaf temperature against the rate of leaf dark respiration at a set temperature for each species. These correlations are then used to calculate the canopy-level annual nighttime carbon flux associated with foliar respiration under several nighttime warming and acclimation scenarios.

Despite recent increased interest in thermal acclimation of respiration (e.g., Chen & Zhuang, 2013; Smith & Dukes, 2013; Wythers *et al*., 2013), general patterns in respiratory acclimation and how it impacts carbon fluxes from the terrestrial biosphere are still lacking. In Chapter 5, a meta-analysis of the published data from 30 sources attempts to identify general patterns of thermal acclimation of leaf dark respiration to warming of terrestrial plant species from across the globe. In total 237 temperature contrasts are included, representing 87 species of forbs, graminoids, shrubs, trees and lianas native to arctic and Antarctic, boreal, temperate and tropical ecosystems.

Global warming poses significant challenges on tropical forests through direct warming effects on high-temperature stress of photosynthesis (Doughty & Goulden, 2008; Doughty, 2011), effects of warming-induced changes in leaf-to-air vapor pressure deficit and potential drought-induced changes in NPP (Zhao & Running, 2010), and the increased burden of nighttime respiratory carbon loss (Clark *et al*., 2003,2010; Feeley *et al*., 2008). While the extent of these potential effects is still poorly understood, the field and laboratory measurements, the field experiments, data analyses, and model simulations I employ in this dissertation should contribute to a better understanding of how tropical forests may respond to climate warming.

CHAPTER 2
FOLIAR RESPIRATION AND ITS TEMPERATURE SENSITIVITY OF TREES AND LIANAS: *IN SITU* MEASUREMENTS IN THE UPPER CANOPY OF A TROPICAL FOREST

Background

Tropical forests account for more than one third of global terrestrial net primary productivity (NPP) (Malhi & Grace, 2000; Saugier *et al*., 2001), but lack of empirical data on leaf dark respiration (*R*) hinders efforts to reliably model carbon fluxes in tropical forests under current and future temperature regimes (Malhi *et al*., 2009). *R* increases with leaf temperature, and increases in *R* will reduce NPP if gross photosynthetic productivity remains constant or decreases. Global rise of temperature, especially during nighttime (Easterling *et al*., 1997), may thus have major impacts on the NPP of tropical forests (Galbraith *et al*., 2010). This mechanism is implicated in studies that report a negative correlation between tree diameter growth and annual means of daily minimum temperature (Clark *et al*., 2003, 2010). These results suggest that a temperature-driven increase in nighttime respiratory carbon loss reduced the net daily carbon gain available for tree growth.

To model plant respiratory carbon efflux from tropical forest trees in response to climate warming, both respiration at a set temperature (e.g., *R* at 25°C) and the temperature sensitivity of *R* need to be known. The latter is commonly described by the Q_{10}; the proportional increase in *R* with 10°C warming. Many coupled climate-vegetation models assume a constant value of 2.0 for Q_{10}, i.e., *R* doubles as temperature increases by 10°C (e.g., Cramer *et al*., 2001; Cox *et al*., 2004; Wang *et al*., 2011). However, Q_{10} is not constant over a wide range of temperatures. Rather, Q_{10} is lower when determined over higher temperature ranges (Tjoelker *et al*., 2001; Atkin *et al*.,

2005). Available data from tropical forests suggest that *R* and Q_{10} differ widely among tree species (Meir *et al*., 2001) and growth forms (Cavaleri *et al*., 2008). Unfortunately, reliable species-level measurements of *R* and Q_{10} are extremely scarce in tropical forests, hindering efforts to generalize patterns of *R* and its temperature sensitivity for tropical trees and lianas.

Species-level data on *R* and Q_{10} will be valuable both to parameterize carbon flux models, and to identify their relationships with other leaf functional traits. If *R* and Q_{10} correlate strongly with commonly measured traits, such as photosynthetic capacity, leaf mass per unit area (LMA), nitrogen or phosphorus content, and leaf lifespan, these relationships may be used to predict *R* and Q_{10} in species-rich tropical forests. Global analyses show that *R* per unit leaf mass at 25°C ($R_{25\ Mass}$) correlates positively with leaf nitrogen content and with photosynthetic capacity (Reich *et al*., 1998; S. J. Wright *et al*., 2004), but it remains unknown whether Q_{10} correlates with leaf functional traits and also whether *R* correlates with functional traits within the limited range of trait values observed among upper-canopy leaves in tropical forests. Identification of leaf traits that correlate with respiration characteristics would facilitate scaling up from leaf to ecosystem and biome-level processes, and may enable trait-based vegetation modeling in which traits rather than species identity are the starting point of the analysis (Van Bodegom *et al*., 2012).

Generalizable differences in *R* among plant functional types (PFTs) provide another means to scale up carbon flux estimates from individual leaves to the canopy in species-rich tropical forests. PFTs in tropical forests, such as lianas or tree species of different successional status, can be identified with remote sensing techniques (e.g.,

Lefsky *et al*., 2002; Kalacska *et al*., 2007; Alvarez-Añorve *et al*., 2012). If generalizable patterns of *R* and Q_{10} exist among PFTs, estimation of carbon fluxes of a given tropical forest could be facilitated when the relative abundance of different PFTs is known. Leaf traits of tree species differ among PFTs defined by their successional status, from the trait syndrome associated with high metabolic activity in fast-growing early-successional trees, to the syndrome associated with the conservative growth strategy of late successional trees (Reich *et al*., 1995; Kitajima & Poorter, 2008).

Lianas represent an important PFT in tropical forest canopies, and their abundance has increased in several tropical forests over recent decades (Phillips *et al*., 2002; I. J. Wright *et al*., 2004; Ingwell *et al*., 2010; Schnitzer & Bongers, 2011). Many lianas are fast growing, because they invest proportionally less in structural support and more in metabolism than trees. Indeed, in a Costa Rican rainforest, Cavaleri *et al*. (2008) found that lianas had on average higher *R* per unit leaf area at a given temperature than trees. Their data also showed that lianas had a marginally lower Q_{10} than trees, suggesting that lianas may have the competitive advantage of lower respiratory carbon loss at higher temperatures. However, these differences between trees and lianas are yet to be confirmed in another tropical forest.

The main objective of this study was to quantify *in situ* leaf respiration rates of trees and lianas in the upper canopy of a tropical forest, in relation to leaf temperature and leaf functional traits, and to determine whether plant functional types differ in respiration characteristics. More specifically, the following hypotheses were tested: 1) Leaf respiration rates and Q_{10} values vary among 26 species of tree and liana, showing significant differences among plant functional types. 2) Species differences in *R* and Q_{10}

are associated with differences in other leaf functional traits. We hypothesized that early-successional species would have higher *R* than later successional species, in concordance with general leaf trait syndromes associated with the species' successional status, and that lianas would have higher *R* than trees, in accordance with Cavaleri *et al*. (2008). We further hypothesized that *R* would correlate with leaf traits according to the predictions of the leaf economic spectrum (I. J. Wright *et al*., 2004). Species differences in Q_{10} may be caused by differences among species in factors controlling the rate of *R* at different temperatures (Atkin & Tjoelker, 2003), such that fast-growing species with high demand for respiratory products (energy and carbon skeletons) are likely to exhibit a greater increase in *R* with temperature than slow-growing species with lower respiratory demand. We thus expected Q_{10} to vary among PFTs and correlate with leaf traits associated with growth and metabolism, such as *R* and photosynthetic capacity.

We developed a new protocol to conduct *in situ* measurements of *R* of intact and pre-darkened leaves equilibrated with ambient temperature in the upper canopy. Leaf respiration of tropical canopy trees is frequently measured on cut-off branches taken to the laboratory (e.g., Cavaleri *et al*., 2008; Metcalfe *et al*., 2010; Van de Weg *et al*., 2012). Many such studies confirm the adequacy of laboratory measurements with cut branches with a pilot study, but the magnitudes of potential artifacts are rarely reported in the literature. Furthermore, many large tropical leaves cannot be enclosed entirely in a standard-sized gas exchange chamber, and warming only the portion of the leaf enclosed in the chamber may cause artifacts. The *in situ* measurement protocol used in

the study avoided these problems, and allowed sampling of a large number of leaves in ambient conditions of temperature and humidity.

Materials and Methods

Study Site and Species

The study was conducted in Parque Natural Metropolitano (PNM, 8°59'N, 79°33'W, 100 m a.s.l.), a seasonally dry tropical forest on the Pacific coast of the Republic of Panama, near Panama City. Annual rainfall at the site averages 1740 mm, most of which falls during the rainy season from May through December. The park is a 256-hectare natural reserve consisting of 80–150 year old secondary forest with tree heights up to 40 m. A 42-m tall construction crane with a 51-m long jib (Parker *et al*., 1992) allowing repeated non-destructive measurements of upper-canopy leaves (Kitajima *et al*., 2005). Tree and liana species were selected to represent a variety of functional types defined in terms of growth form (trees and lianas) and successional status (early-, mid- and late-successional tree species) (Pérez & Condit, 2012). Thirteen tree species and 13 liana species were selected from the upper forest canopy (Table 2-1). Additional leaf trait data had been collected previously at the same site for most of these species (S.J. Wright. Unpublished). Together these 26 species cover > 70% of the canopy area in reach of the crane (Avalos & Mulkey, 1999)

Field Measurements of Leaf Respiration Rates

All measurements were made in the wet season of 2010 (between late September and late November) when all study species had mature and non-senescing leaves. For each species two to five sun-exposed terminal shoots were selected on one to three individuals. Prior to sunset (6.00 p.m.) on the day before respiration measurements, 10 to 34 recently matured leaves were covered with thin aluminum foil,

so that they would not be exposed to any sunlight till the measurements. The abaxial side was not completely covered to allow free gas exchange during night. The following morning respiration was measured once on each leaf between 5.45 and 11.00 a.m. at ambient temperature. Before each measurement, we measured the leaf and ambient air temperature with a thermocouple. The air temperature in the upper canopy rose from 23–24°C pre-dawn to 28–31°C at 11.00 a.m., and the temperature of leaves that were kept covered with aluminum foil closely followed the ambient air temperature. Because the leaves were covered overnight our measurements avoided the effects of light-enhanced dark respiration (Atkin *et al*., 1998), and light-induced metabolites and respiratory gene expression (Florez-Sarasa *et al*., 2012) on respiration. Thus, our measurements attempted to estimate nighttime dark respiration in a manner to minimize the effects of respiration associated with carbohydrate exports and temporal variation in substrate availability during the night (Noguchi, 2005).

Leaf *R* was measured as CO_2 release rates with a portable infrared gas analyzer (LI-6400, Licor, Lincoln, NE, USA), at ambient humidity (70–90%) and a set CO_2 concentration of 400 ppm, maintained with the built-in CO_2 mixer. The block temperature of the LI-6400 was set to equal the ambient leaf temperature just before the measurement. Thus, during the respiration measurements, the leaf portion inside the gas exchange cuvette had the same temperature as the whole leaf, avoiding the potential measurement artifacts associated with warming a single leaf or leaf portion opposed to warming of the whole plant (Atkin *et al*., 2000) or stand (Griffin *et al*., 2002a). After the measurement at a single temperature, each leaf was harvested and brought back to the lab for additional trait measurements.

Functional Trait Data

Photosynthetic capacity (A_{max}) was measured on a separate set of 3–6 leaves per species, similar in sun exposure and apparent leaf age, between 9.00 and 10.00 a.m. at saturating irradiance of 1200 µmol photons m^{-2} s^{-1} and 400 ppm CO_2 at ambient temperature (range 26–29°C). After measurement of the leaf area with a LI-3000 leaf area meter (Licor), each leaf was dried ≥ 96 hours at 60°C for determination of dry mass and leaf mass per area (LMA). Five randomly selected leaves per species on which respiration was measured were ground for analysis of nitrogen (N) concentration with an elemental analyzer (Costech Analytical, Los Angeles, California, USA).

We report *R* on an area basis (R_{Area}) unless otherwise specified, but we also calculated respiration per unit leaf mass (R_{Mass}) by dividing *R* by LMA of each leaf. The median leaf lifespan and mean leaf phosphorus concentrations had been collected independently at the species-level from the same site (S.J. Wright. Unpublished). The species means for A_{max}, N and LMA in this independent data set match well with the species means determined in the current study (R^2 > 0.65 for each trait comparison).

Data Analysis

In our protocol, each leaf was measured only once at one specific temperature, and we estimated temperature dependence (Q_{10}) and respiration at a standardized temperature of 25°C at the species-level by pooling all measurements from each species. The temperature dependence of *R* was evaluated for each species from least square regression of natural-log transformed *R* on leaf temperature:

$$\ln(R) = a + bT_{leaf} \quad (2\text{-}1)$$

In equation. 2-1, a (the intercept) and b (slope) are species-specific constants, used to estimate species-specific Q_{10} as:

$$Q_{10} = e^{10b} \quad (2\text{-}2)$$

We report Q_{10} values calculated from the temperature response of R_{Area}, but also calculated Q_{10} of R_{Mass} (Table A-1). Confidence intervals for the Q_{10} estimates were calculated from the confidence intervals associated with the parameter b in equation 2-1. Subsequently, R_{25} of each leaf was calculated as:

$$R_{25} = \frac{R}{Q_{10}^{(T-25)/10}} \quad (2\text{-}3)$$

where *R* and T were the actual measurements for each leaf. The sample size varied from 10 to 34 leaves per species (Table 2-1), with larger sample size associated with species that could be sampled from multiple trees under the crane. For four out of 26 species the temperature range of *R* measurements did not include 25°C (with minimum measurement temperature between 25 and 27°C). Thus, we also calculated R_{27} in a similar manner as R_{25}, because 27°C fell within the temperature range for all species (Table A-1). However, we report only R_{25} values, because they are widely reported in the literature, and because trait correlations and comparisons among PFTs were similar for R_{27} and R_{25}.

Trait correlations were analyzed with Standardized Major Axis Regression using the SMATR package (Warton *et al*., 2012) in R (R Development Core Team, 2011). Slopes of trait correlations were compared between trees and lianas, but because no differences were found, all species were pooled. Not enough data were available for comparison of trait correlations among PFTs. Comparisons among species, PFTs, and growth forms were made using one-way ANOVAs and Tukey HSD *post hoc* tests, or Student's t-tests. Data were transformed to improve normality and homogeneity of

variance where necessary. All statistical analyses were performed in R version 2.14.1 (R Development Core Team, 2011).

Results

Respiration increased significantly with temperature for 22 of the 26 species (Fig. 2-1). The four species for which *R* did not increase significantly with temperature were included in the analyses to avoid biasing against low Q_{10} values. Both the elevation and the steepness of the slopes of the temperature response curves of ln*R* varied across species, resulting in considerable variation in R_{25} and Q_{10} (Table 2-1). The average R_{25} per unit leaf area ($R_{25\ Area}$) did not differ significantly among plant functional types (PFTs) (Fig. 2-2A). R_{25} expressed on a mass basis ($R_{25\ Mass}$) was higher in lianas than in trees (Table 2-2), but differences among early-, mid- and late-successional tree species were not significant (Fig. 2-2B).

The Q_{10} varied widely among species (range 1.24–3.66, mean 2.19; Table 2-1), exhibiting similar patterns across PFTs for Q_{10} derived from area- and mass-based *R*. Q_{10} did not differ significantly between trees and lianas (Table 2-2), but Q_{10} values were higher for early-successional trees than for each of the other PFTs (Fig. 2-2C). The mean Q_{10} for all 26 species was marginally greater than 2.0 ($Q_{10\ Area}$ = 2.18, P = 0.09; $Q_{10\ Mass}$ = 2.26, P = 0.03. t-test), although the 95% confidence intervals of the Q_{10} of individual species included 2.0 for 25 of 26 species.

$R_{25\ Area}$ correlated positively with photosynthetic capacity (A_{max}), LMA, and concentrations of N and P ($P < 0.01$ for all) (Fig. 2-3A-D; Table A-1). $R_{25\ Mass}$ showed positive correlations with A_{max}, N, P, and negative correlations with LMA and leaf lifespan (Fig. 2-3F-J; Table A-1). When analyzed separately the slopes of these correlations were the same for trees and lianas ($P > 0.1$ for all comparisons of slopes).

Whereas R_{25} showed consistent and strong correlations with other leaf functional traits, Q_{10} values did not correlate with *R* or any of the other leaf traits (Fig. A-1). Weighted regression analysis, in which species were weighted by the goodness of fit of the respiration temperature response curves improved the correlations, but did not produce significant results either (data not shown).

Discussion

In this study we determined *in situ* leaf dark respiration rates and Q_{10} values for tropical trees and lianas in the upper canopy of a seasonal tropical forest, using a new protocol to measure leaves darkened overnight. In our method, the whole leaf was equilibrated to the ambient temperature, and activation of photosynthetic metabolism was unlikely given continuous leaf darkening overnight till the measurement time.

Species-level estimates of R_{25} from our study are relatively high when compared with the available data of *R* measured on upper-canopy leaves of other tropical trees (Table 2-3). Variations in ecosystem characteristics and methods of respiration measurement may both have contributed to the differences among studies. *R* tends to be higher for trees on fertile soils than infertile soils when foliar N and P contents are accordingly high (Meir *et al*., 2001; Turnbull *et al*., 2005). The soil at PNM is less acidic and more fertile than many tropical rainforests (pH = 7.01; total exchangeable cations = 4.3 g kg^{-1}; available P = 5.81 mg kg^{-1}. B.L. Turner, pers. comm.[1]). However, mean N content per unit leaf mass was comparable to that at most other tropical sites, with the exception of nitrogen poor sites in Venezuela (Reich *et al*., 1998) and Australia (Pearcy, 1987). Consequently, R_{25} per unit leaf N was considerably higher at our site than at

[1] Email correspondence on 11 October 2012. Averages based on unpublished data.

most other tropical sites (Table 2-3), but comparable to the mean R_{25}/N from the eight tree species in Venezuela (Reich *et al*., 1998).

Another explanation for the relatively high rates of *R* we measured may be the large number of early- and mid-successional species that are fast growing and metabolically active. However, R_{25} of early-successional species was not systematically higher than in late-successional species on a unit area basis (Fig. 2-2A) and not significantly so on a per unit mass basis (Fig. 2-2B). It is also possible that measuring intact sun-exposed top canopy leaves contributed to the high rates of *R* in our study, contrasting with studies that used detached branches, or that measured leaves from a tower with which the most sun-exposed position of the upper canopy may not have been accessible. Studies that use detached branches generally report that tests have been made to assure that measurements of attached and detached leaves yield the same results (e.g., Cavaleri *et al*., 2008), but our measurements were also higher than *in situ* measured *R* (Table 2-3).

Significant Trait Correlations with Dark Respiration over a Small Trait Space

R_{25} only varied by a factor of 2.5 in our study, but nevertheless we found trait correlations similar to those of the mass-based leaf economics spectrum (I. J. Wright *et al*., 2004) in which *R* varied by more than an order of magnitude. Furthermore, these trait correlations also exist on a leaf area basis, with coefficients of correlation comparable to those found for leaf area based correlations reported in I. J. Wright *et al*. (2004). $R_{25\ Area}$ ranged from 0.7 to 1.8 μmol m^{-2} s^{-1} (Table 2-1) and this small interspecific variation correlated with other leaf traits that themselves cover a relatively small (2-4 fold) range of trait values (Table 2-1, Table A-2). Although the percent of interspecific variation in $R_{25\ Area}$ explained by other leaf traits was modest, the

relationship was robust despite the small range of R_{25} among species. This result thus offers potential for a trait-based approach to vegetation modeling (Van Bodegom *et al*., 2012).

Q_{10} did not correlate with any of the other functional traits we determined. Previous observations in which the temperature sensitivity of *R* correlated with leaf traits were made within species across depths in the canopy (Griffin *et al*., 2002b; Turnbull *et al*., 2003). In both cases Q_{10} correlated positively with leaf N content, but while Griffin *et al*. (2002b) found that lower canopy leaves of *Populus deltoides* had higher Q_{10} values and higher N (per unit leaf mass), Turnbull *et al*. (2003) found for several species that Q_{10} values were higher in top canopy leaves, as was N (per unit leaf area). There is no clear mechanistic explanation for why the relationship between leaf N and respiratory temperature response exists. Similar to our study, Bolstad *et al*. (1999) found no correlation between Q_{10} and leaf N for 18 deciduous temperate tree species, despite considerable variation in shade tolerance and associated photosynthetic properties of the species.

Interestingly, the average of the Q_{10} values across 26 species in our study was considerably higher than the 2.0 that is often used in coupled climate-vegetation models. Because the Q_{10} changes with the temperature interval over which it is measured Atkin *et al*. (2005) described a temperature dependent Q_{10}: $Q_{10} = 3.09-0.043*T$, where T is the measurement temperature. According to this relationship, a Q_{10} of 1.9 is predicted at 27°C, the average mid-point of the temperature intervals over which we determined the Q_{10}. Our Q_{10} data are comparable to Q_{10} measured on other tropical forest canopy leaves (Table 2-3). Both the fixed Q_{10} of 2.0 as used in many

coupled climate-vegetation models and the prediction from the temperature dependent Q_{10} of Atkin *et al*. might thus be underestimating the Q_{10} for tropical species.

Similarity in Respiration Characteristics among Plant Functional Types

We found limited patterns of Q_{10} across 26 species of tree and liana. Similarly Bolstad *et al*. (1999) found that interspecific variation in Q_{10} among 18 temperate tree species was apparently independent of PFT. We did, however, find that early-successional trees exhibited higher Q_{10} than later successional trees. There is no obvious mechanistic reason for this difference. Fast-growing, light-demanding species, such as the early-successional tree species in our study, have high demand for respiratory products, and they can achieve high *R* when respiratory substrate is available (Noguchi & Terashima, 1997). Conditions of high demand for respiratory products under non-limiting substrate supply can also result in high Q_{10} values (Slot *et al*., 2008). However, R_{25} was not significantly higher in early-successional species so it seems unlikely that the high Q_{10} we observed was solely driven by differences in metabolic demands among PFTs. More research will be needed to establish the generality of the pattern we observed. *R* measurements were made on a relatively large number of leaves per species, but because temperature dependence was not determined at the individual leaf level, wide 95% confidence intervals are associated with some of the Q_{10} estimates (Table 2-1). Future work should include leaf-level measurements of Q_{10} repeated within species so the intra-specific variation in Q_{10} as well as of *R* can be considered when analyzing patterns across large numbers of species and PFTs.

Differences between Trees and Lianas

$R_{25\ Mass}$ was higher, while Q_{10} values slightly lower in lianas than in trees, similar to the trend found by Cavaleri *et al*. (2008) in Costa Rica. The Q_{10} differences were, however, small and not statistically significant (Table 2-2). Given that the global temperature has risen just about 0.2°C per decade over the last 30 years (Hansen *et al*., 2006) the observed differences in respiration characteristics between trees and lianas were too small to suspect that temperature responses of respiration could have contributed to the increase in liana dominance in tropical forests over the last 30 years. Meanwhile, the similarity in area-normalized physiological traits of trees and lianas suggests that vegetation models could justifiably ignore growth form and consider all canopy leaves as equal, but use leaf traits to fine tune the model to account for underlying physiological differences among leaves of different species, plant functional types, and growth forms.

Concluding Remarks

Given the species richness of tropical forests, the lack of species-level respiration data from tropical canopy species has been striking; indeed, the paucity of respiration data is a major source of uncertainty in modeling carbon fluxes in tropical forests (Malhi *et al*., 2009). The R_{25} data generated from a total of 461 leaves measured *in situ* across 26 species of trees and lianas in the current study are a valuable addition. Furthermore, the results support that R_{25} can be estimated from easy-to-measure “soft” traits that are widely measured for tropical canopy trees, similar to the global trends (I. J. Wright *et al*., 2004). On the other hand, trees and lianas are similar in R_{25} and in R_{25}-trait correlations and large variation in *R* and leaf traits exists within each PFT. These results strongly support the notion that trait-based vegetation modeling is more promising than PFT-

based modeling of ecosystem-atmosphere fluxes (Van Bodegom *et al*., 2012). Especially in diverse tropical forests, bypassing species as a working unit and instead using trait data to model ecosystem processes would greatly simplify data collection. The mean Q_{10} across 26 species was greater than 2.0, the value often assumed in coupled climate-vegetation models. This significantly higher Q_{10} over the temperature range representative for current and near-future nighttime in the tropics should be considered for modeling carbon fluxes in tropical forests under climate warming scenarios.

Table 2-1. Species used, their plant functional type (PFT, ES: early-successional, MS: mid-successional, LS: late successional, L: liana) and their trait values. Respiration (R) at 25°C (R_{25}) and Q_{10} were determined from least square regression of *in situ* measurements of R of n leaves per species. Confidence intervals (CI) for Q_{10} were determined from the CI of the slope of the temperature response curves (Fig. 2-1). CIs of R_{25} were determined assuming normal distribution, as mean $R_{25} \pm 1.96$ *(standard error of R_{25}). Also reported are photosynthetic capacity (A_{max}), leaf mass per unit leaf area (LMA), % leaf nitrogen (N) determined for 5 leaves per species. Phosphorus (P) and leaf lifespan data were obtained from S.J. Wright (Unpublished). *nd*: no data available.

Species	PFT	n	$R_{25\ Area}$ (95% CI) µmol $m^{-2}s^{-1}$	$R_{25\ Mass}$ (95% CI) nmol $g^{-1}s^{-1}$	$Q_{10\ Area}$ (95% CI)	A_{max} µmol $m^{-2}s^{-1}$	LMA g m^{-2}	N %	P %	Lifespan days
Albizia guachapele (Kunth) Harms	ES	18	0.76 (0.7-0.8)	8.8 (8.0-9.4)	3.66 (1.8-7.6)	12.4	87	3.3	0.10	183
Annona spraguei Saff.	ES	11	0.95 (0.9-1.0)	10.7 (10.2-11.2)	2.79 (2.0-4.0)	13.5	89	2.8	0.17	216
Cecropia peltata L.	ES	13	1.25 (1.1-1.4)	12.5 (10.9-14.4)	3.14 (1.4-6.8)	19.8	100	2.8	0.17	114
Pittoniotis trichantha Griseb.	ES	12	1.19 (1.1-1.3)	17.5 (15.6-19.3)	2.58 (1.5-4.2)	13.5	68	2.3	0.14	180
Astronium graveolens Jacq.	MS	22	1.22 (1.2-1.3)	13.8 (13.1-14.9)	1.24 (0.8-1.8)	12.8	88	3.0	0.20	271
Castilla elastica var.costaricana, (Liebm) CC. Berg	MS	29	1.15 (1.1-1.2)	11.0 (10.5-11.5)	2.84 (2.2-3.6)	19.5	104	2.8	0.17	180
Ficus insipida Willd.	MS	13	1.45 (1.4-1.6)	9.9 (9.2-10.6)	1.70 (1.1-2.8)	23.4	147	2.3	0.17	92
Luehea seemannii Triana & Planch.	MS	34	1.14 (1.1-1.2)	10.9 (10.5-11.3)	2.12 (1.6-2.8)	19.8	105	2.4	0.15	186
Pseudobombax septenatum (Jacq.) Dugand	MS	22	1.17 (1.1-1.3)	11.5 (10.7-12.1)	1.51 (1.1-2.1)	16.3	102	2.3	*nd*	*nd*
Spondias mombin L.	MS	16	1.48 (1.4-1.6)	14.5 (13.8-15.0)	1.73 (1.3-2.3)	16.5	102	2.7	0.13	173
Zuelania guidonia (Sw.) Britt. & Millsp.	MS	13	1.36 (1.3-1.5)	11.0 (10.1-11.8)	2.42 (1.5-3.9)	17.3	124	2.0	*nd*	*nd*
Anacardium excelsum (Bertero & Balb ex Kunth) Skeels	LS	23	1.22 (1.2-1.3)	11.2 (10.8-11.5)	1.99 (1.7-2.3)	13.8	109	1.4	0.14	280
Chrysophyllum cainito L.	LS	25	0.81 (0.8-0.9)	7.0 (6.7-7.3)	1.94 (1.6-2.3)	14.1	116	1.8	0.10	153
Amphilophium paniculatum (L.) Kunth	L	23	0.84 (0.8-0.9)	12.8 (12.0-13.4)	1.75 (1.3-2.3)	10.9	66	2.8	0.17	122
Aristolochia tonduzii OC. Schmidt	L	20	1.02 (1.0-1.1)	12.6 (11.9-13.4)	2.19 (1.7-2.9)	12.1	81	3.1	0.15	173
Bonamia trichantha Hallier f.	L	18	0.80 (0.8-0.9)	11.7 (10.8-12.6)	1.82 (1.1-3.1)	10.4	69	3.2	0.18	179

Table 2-1. Continued.

Species	PFT	n	$R_{25\ Area}$ (95% CI) µmol $m^{-2}s^{-1}$	$R_{25\ Mass}$ (95% CI) nmol $g^{-1}s^{-1}$	$Q_{10\ Area}$ (95% CI)	A_{max} µmol $m^{-2}s^{-1}$	LMA g m^{-2}	N %	P %	Lifespan days
Cissus erosa Rich.	L	13	1.30 (1.2-1.4)	22.7 (20.4-24.8)	2.45 (1.6-3.8)	17.2	57	3.2	*nd*	89
Combretum fruticosum (Loefl.) Stuntz	L	13	1.09 (1.0-1.2)	14.5 (10.3-14.3)	2.19 (1.2-4.0)	17.7	75	2.3	0.16	154
Forsteronia myriantha Donn. Sm.	L	11	0.99 (0.9-1.1)	14.8 (13.4-16.2)	2.46 (1.2-5.1)	17.5	67	2.9	0.16	107
Gouania lupuloides (L.) Urb.	L	22	0.90 (0.8-1.0)	18.9 (16.7-21.7)	1.49 (1.0-2.3)	12.7	48	4.0	0.18	92
Mikania leiostachya Benth.	L	10	0.72 (0.6-0.8)	12.4 (10.8-13.9)	2.58 (1.1-6.1)	9.8	59	2.4	0.13	213
Phryganocydia corymbosa (Vent.) Bureau ex K. Schum	L	17	1.79 (1.6-2.0)	18.3 (16.7-19.5)	1.69 (1.1-2.6)	12.2	98	3.7	0.15	89
Serjania mexicana (L.) willd	L	11	1.01 (0.9-1.1)	10.9 (9.8-11.9)	2.52 (1.4-4.7)	12.9	93	2.4	0.15	*nd*
Stigmaphyllon lindenianum A. Juss.	L	12	1.05 (1.0-1.1)	13.2 (12.5-14.0)	1.62 (1.3-2.0)	16.3	80	2.7	0.14	150
Trichostigma octandrum (L.) H. Walt.	L	23	1.15 (1.1-1.2)	20.1 (18.6-21.5)	2.13 (1.3-3.6)	15.1	57	4.1	0.32	57
Vitis tillifolia Humb. & Bonpl. ex Roem. & Schult.	L	17	0.94 (0.9-1.0)	14.3 (12.9-15.7)	2.34 (1.0-5.5)	13.0	66	2.1	0.13	66

Table 2-2. Comparison of means (± 1 standard deviation) of physiological traits and leaf mass per unit area (LMA) of trees (13 species) and lianas (13 species). Respiration at 25°C (R_{25}), Q_{10}, and photosynthetic capacity (A_{max}) are shown on a per unit area (subscript "Area") and a per unit mass (subscript "Mass") basis. The ratio of respiration over photosynthetic capacity is also shown.

	Trees	Lianas
$R_{25\ Area}$ (µmol m^{-2} s^{-1})	1.17 ± 0.22	1.05 ± 0.27
$R_{25\ Mass}$ (nmol g^{-1} s^{-1})*	11.56 ± 2.63	15.17 ± 3.65
$Q_{10\ Area}$	2.28 ± 0.67	2.10 ± 0.37
$Q_{10\ Mass}$	2.36 ± 0.60	2.16 ± 0.55
$A_{max\ Area}$ (µmol m^{-2} s^{-1})*	16.37 ± 3.43	13.36 ± 2.64
$A_{max\ Mass}$ (nmol g^{-1} s^{-1})*	160.0 ± 26.3	197.0 ± 57.6
R_{25}/A_{max}	0.07 ± 0.01	0.08 ± 0.02
LMA (g m^{-2})§	103.2 ± 19.3	70.4 ± 14.6

* Trees and lianas different ($P < 0.05$), § $P < 0.01$

Table 2-3. Comparison of leaf dark respiration rates at 25°C (R_{25}), leaf nitrogen content (N), R_{25} per unit leaf nitrogen (R_{25}/N) and Q_{10} among studies of sun-exposed leaves from tropical forest trees and lianas (Means ± standard deviations of n species).

Location	Growth form	n	R_{25} (nmol $g^{-1}s^{-1}$)	N (mg g^{-1})	R_{25}/N (µmol (g N)$^{-1}$ s^{-1})	Q_{10}
Panama[1] (PNM - This study)	Trees	13	11. ± 2.6 6	24.5 ± 5.2	0.49	2.28
	Lianas	13	15.2 ± 3.7	29.9 ± 6.4	0.51	2.10
Costa Rica[2] (La Selva)	Trees	19	3.8	*nd*	0.32	2.34
	Lianas	6	6.8	*nd*		2.14
Venezuela[3] (San Carlos)	Trees	8	8.6 ± 4.4	16.2 ± 4.3	0.53	*nd*
Brazil[4] (Jaru)	Trees	6	2.8	25.0	0.11	2.30
Brazil[5] (Caxiuaña reserve)	Trees	8	4.6 ± 1.1	*nd*		
Brazil[6] (Flona-Tapajós)	Trees	6	7.4 ± 4.9	21.2 ± 6.4	0.35	*nd*
	Lianas	6	7.2 ± 2.9	20.8 ± 3.2	0.35	*nd*
Cameroon[4] (Mbalmayo)	Trees	6	4.7	25.7	0.18	2.00
Australia[7] (Curtain Fig NP)	Trees	1	4.4 ± 1.6	16.7 ± 4.1	0.26	*nd*
Indonesia[8] (Bogor)	Trees	3	*nd*	*nd*	*nd*	2.00

[1] current study; [2] Cavaleri *et al*. (2008), *R* measured on detached leaves; [3] Reich *et al.* (1998); [4] Meir *et al.* (2001); [5] Metcalfe *et al*. (2010); [6] Dominquez *et al.* (2007), *R* obtained from photosynthetic light response curves; [7] Pearcy (1987); [8] Stocker (1935). Where necessary, *R* values were converted into R_{25} using a Q_{10} of 2.0. *nd*: no data available.

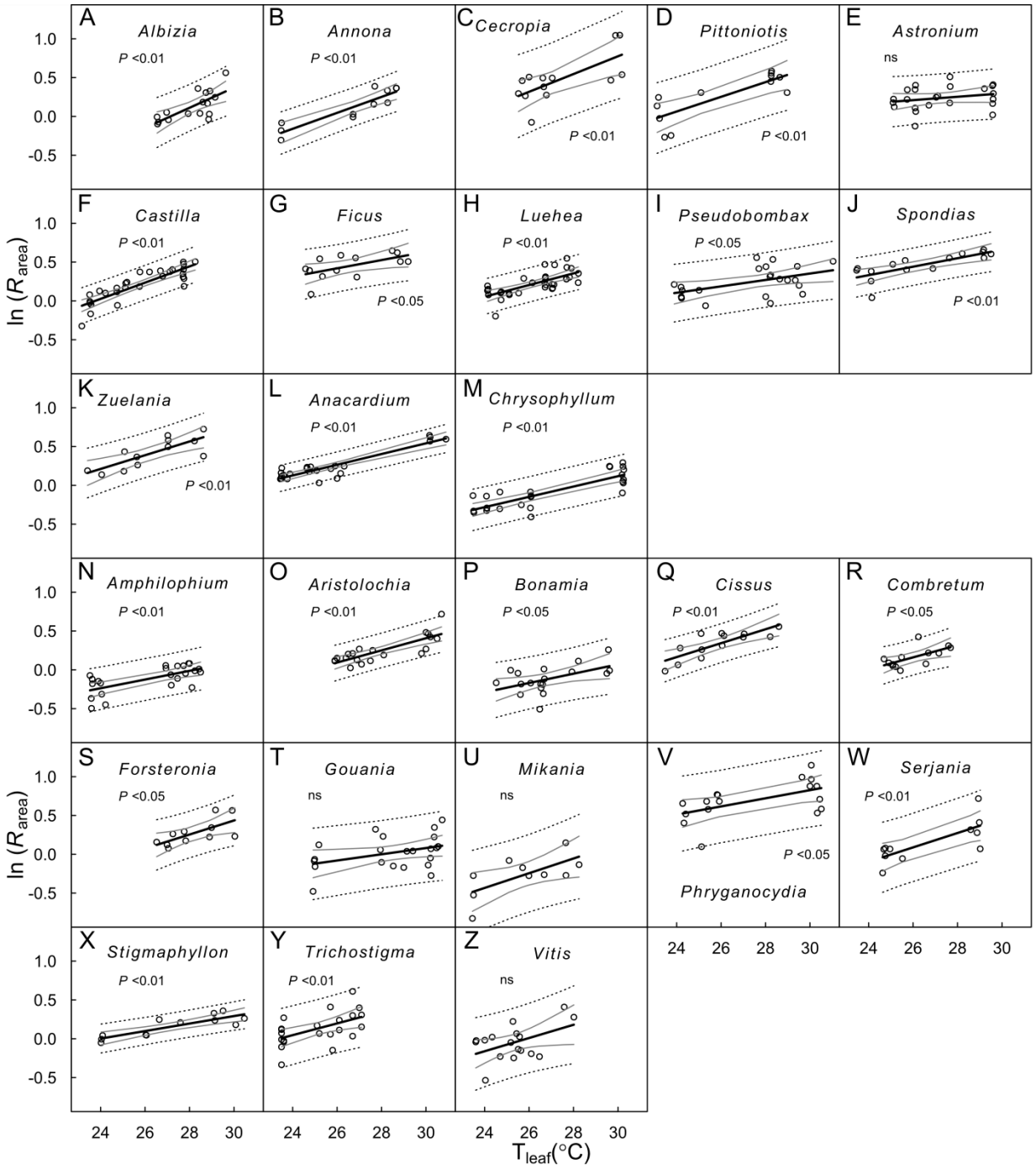


Figure 2-1. Natural-log transformed *in situ* leaf respiration rates (ln (R_{area})) plotted against leaf temperature (T_{leaf}) for 13 tree species (A-M) and 13 liana species (N-Z). Species are indicated by their genus name alone; see Table 1 for full species names and plant functional type the species belong to. Solid black lines represent the linear least square fits (significance of fit is indicated with *P* values); solid gray lines indicate the 95% confidence intervals of the fits; dashed black lines represent the 95% confidence intervals of the data.

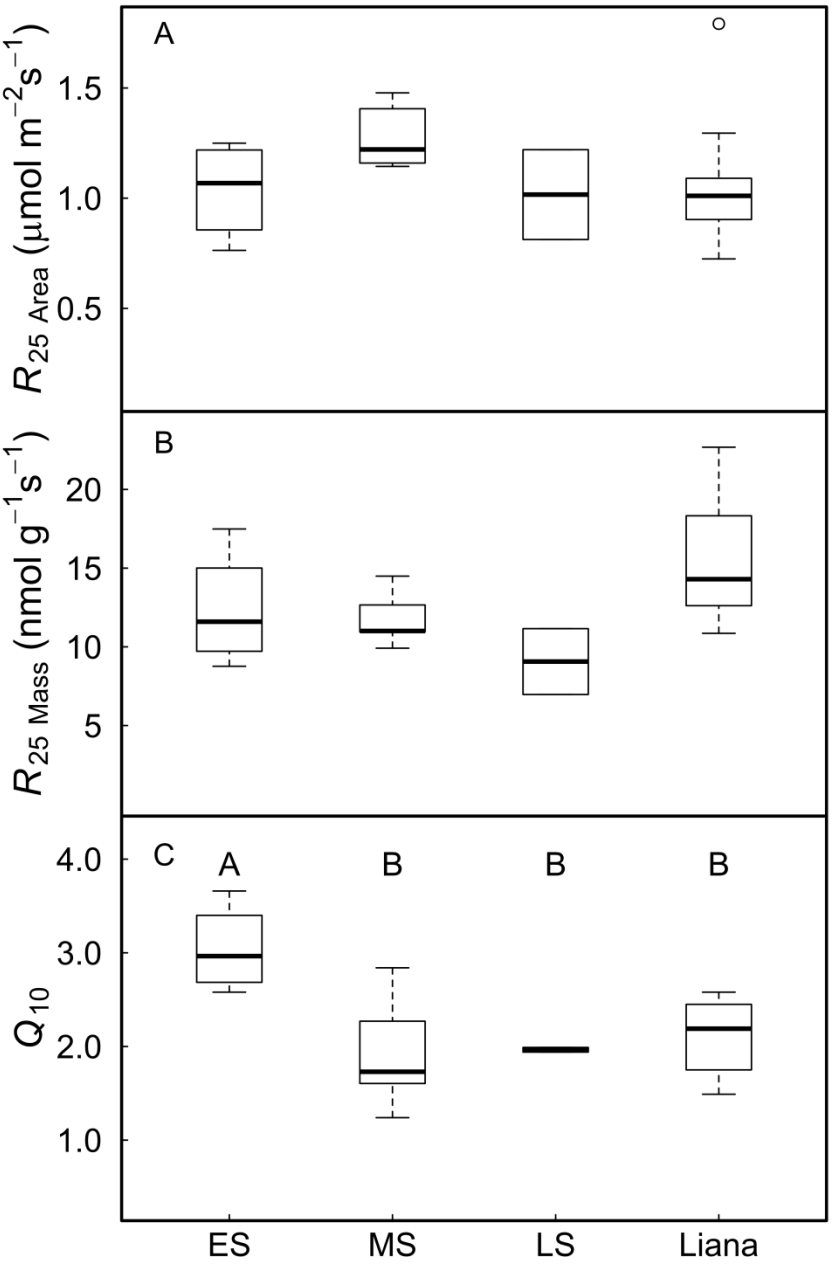


Figure 2-2. Variation of foliar respiration (*R*) characteristics within and among plant functional types (PFTs): early successional trees (ES, 4 species), mid-successional trees (MS, 7 species), late-successional trees (LS, 2 species), and lianas (13 species). A) *R* per unit leaf area at a temperature of 25°C (R_{25Area}). B) *R* per unit leaf mass at 25°C ($R_{25\ Mass}$). C) Q_{10} calculated from *R* per unit area. The box plots indicate the median, 25th and 75th percentile for each PFT. Whiskers extend to 1.5 times the interquartile range. Different letters indicate groups that are significantly different from one another ($P < 0.05$) (One-way ANOVA with Tukey *post hoc* testing).

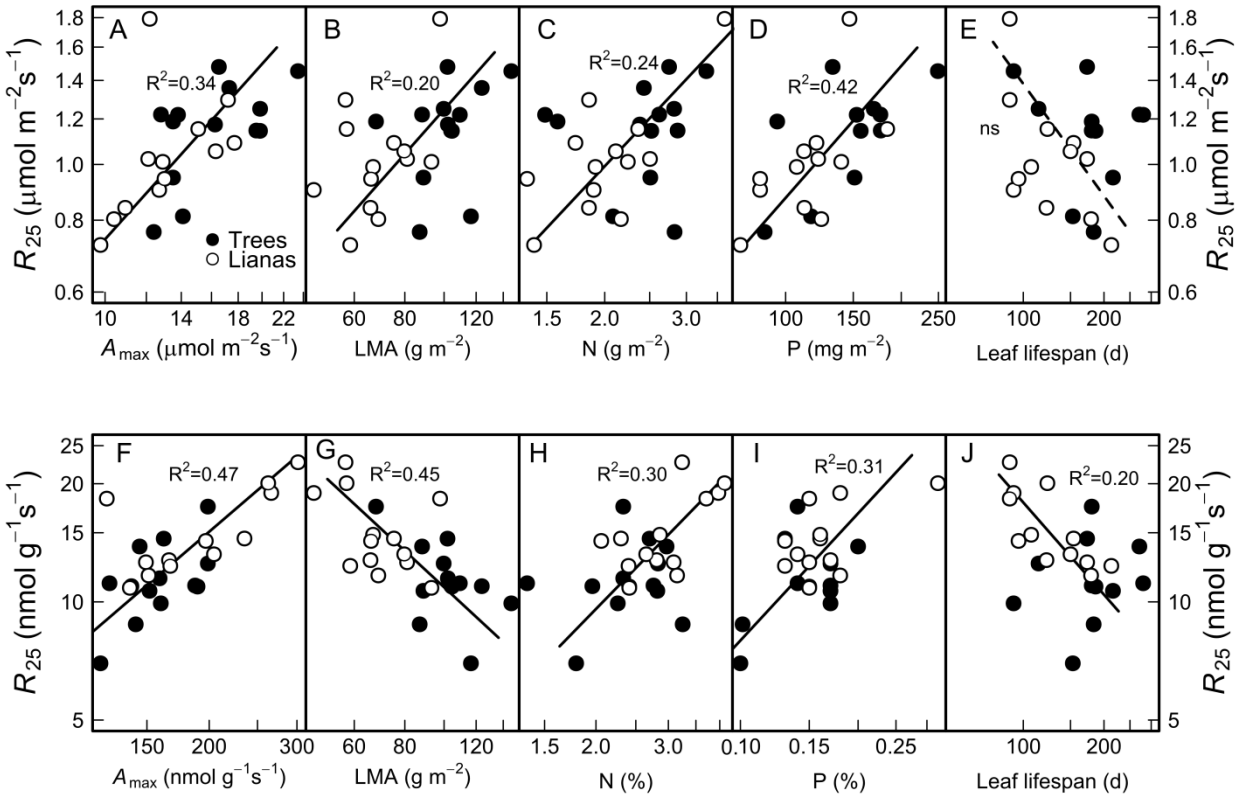


Figure 2-3. Correlations between respiration rates at 25°C (R_{25}) and other leaf traits. A-E) Traits per unit leaf area. F-J) Traits per unit leaf mass. All axes are on a natural-log scale. Each data point represents one species. A,F) R_{25} is correlated with photosynthetic capacity (A_{max}).B,G) R_{25} vs. leaf mass per unit area (LMA). C,H) R_{25} vs. leaf nitrogen content (N). D,I) R_{25} vs. leaf phosphorus content (P). E,J) R_{25} vs. leaf lifespan. Solid lines represent significant correlation of the traits (trees and lianas combined) ($P < 0.05$), as determined by standardized major axes regression. The dashed line in e indicated $P < 0.1$.

CHAPTER 3
TRAIT-BASED SCALING OF TEMPERATURE-DEPENDENT FOLIAR RESPIRATION IN A SPECIES-RICH TROPICAL FOREST CANOPY

Background

Tropical forests account for more than one third of global terrestrial gross primary productivity (GPP) (Beer *et al*., 2010), but 30% of this photosynthetically fixed carbon is released back into the atmosphere by leaf respiration (*R*) (Chambers *et al*., 2004; Malhi *et al*., 2011). Global rise of temperature, especially during nighttime (Kukla & Karl, 1993; Easterling *et al*., 1997) may have major impacts on the net primary productivity (NPP = GPP- autotrophic respiration), as autotrophic respiration increases with temperature. Tropical forests are likely to experience unprecedented warming within the next two decades (Diffenbaugh & Scherer, 2011), but as long as lack of empirical data on *R* and its temperature sensitivity hinders efforts to reliably model current carbon fluxes in tropical forests (Malhi *et al*., 2009), quantitative predictions of changes in these fluxes under future climates will remain ambiguous at best.

R of canopy trees is challenging to measure, especially in tall and diverse tropical forests. Eddy covariance techniques capture nocturnal respiration fluxes poorly (Goulden *et al*., 1996; Lavigne *et al*., 1997; Loescher *et al*., 2003) and do not allow for straight-forward partitioning of ecosystem respiration to its component sources. Thus, direct measurements of respiration are necessary for mechanistic prediction of forest canopy respiration. In tropical forests *R* is considered to be the largest component of autotrophic respiration (Chambers *et al*., 2004; Malhi *et al*., 2011; Malhi, 2012) and *R* is highest at the top of the canopy where leaves are exposed to full sun (Meir *et al*., 2001; Cavaleri *et al*., 2008). Therefore, to quantify the leaf respiratory carbon flux from tropical forests, fully-exposed upper-canopy leaves should be measured. Such data at the

species level are very scarce considering the diversity and importance of tropical forests, and the uncertainty associated with *R* flux estimates is consequently large (Malhi *et al*., 2009). Furthermore, to calculate the respiratory carbon flux from tropical forest canopies both *R* at a set temperature (e.g., *R* at 25°C; R_{25}) and the short-term temperature sensitivity of *R* need to be known. *R* roughly doubles with 10°C temperature increase; i.e., it has a Q_{10} of 2.0, although published values range from 1.1 to 4.2 (e.g., Azcón-Bieto & Osmond, 1983; Larigauderie & Körner, 1995; Tjoelker *et al*., 2001). Available data from tropical forests suggest that *R* and Q_{10} differ widely among species (Meir *et al*., 2001; Slot *et al*., 2013) and growth forms (Cavaleri *et al*., 2008), but not enough data are currently available to identify systematic patterns in Q_{10} among tropical forest trees and lianas.

The leaf economics spectrum (I. J. Wright *et al*., 2004) describes general correlations among photosynthesis (A_{max}) and *R*, and leaf structural and chemical properties, including leaf mass per unit leaf area (LMA), leaf longevity, and concentration of nitrogen (N) and phosphorus (P). Because these structural and chemical traits are easier to determine reliably than photosynthesis and respiration, these trait correlations are useful for estimating carbon (C) flux in species-rich forest canopies. Further, in many global vegetation models *R* is defined as a fixed fraction of maximum photosynthesis (e.g., HyLand , Levy *et al*., 2004; IBIS, Foley *et al*., 1996; LPJ, Sitch *et al*., 2003), or as a function of leaf N (e.g., HYBRID, Friend *et al*., 1997; NCAR LSM, Bonan *et al*., 2003). What is less certain is whether correlations with other leaf traits can be identified for the temperature sensitivity of *R*, in particular for the Q_{10}, which is widely used in C flux models.

Our ability to estimate *R* fluxes from tropical forests would greatly improve if the Q_{10} could be captured by a simple trait-based model in the way that *R* can be modeled from N or photosynthesis. Observational evidence suggests that Q_{10} values vary among species and with environmental conditions (Griffin *et al*., 2002b; Turnbull *et al*., 2003; Slot *et al*., 2008). It remains unknown whether this variation is predictable. The factor that restricts the rate of *R* changes with temperature (Atkin & Tjoelker, 2003). At low temperature the respiratory *capacity* is most limiting (because enzymatic reaction rates are low), while at high temperature *R* is more likely to be limited by the availability of respiratory substrate (primarily carbohydrates), or the demand for respiratory product (e.g., ATP: when there is low demand for ATP, the rate of respiratory electron transport becomes limited by the availability of ADP. Noguchi & Terashima, 1997). Two species with the same respiratory capacity but different substrate availability or demand for respiratory products are likely to have different temperature response curves. Furthermore, the factor that controls *R* may differ among species in relation to their plant functional type (PFT). For example, *R* in fast-growing, light-demanding species is more likely to be limited by substrate availability, while slow-growing, shade-tolerant species are more likely to be limited by the demand for respiratory products (Noguchi & Terashima, 1997). If species differ in the factor that controls *R* based on their respiratory substrate content and their PFT, it is likely that variation in Q_{10} values can be explained by traits associated with respiratory control and life history.

Here we set out to 1) quantify R_{25} and its temperature sensitivity for canopy leaves of a large number of common tropical forest trees and lianas, 2) statistically explain interspecific variation in R_{25} and Q_{10} in relation to leaf chemical and structural

traits, and 3) estimate the annual flux of *R* at the stand level in our study forest, using trait-based models identified under the second objective.

To achieve these goals we determined temperature response curves of respiration at the leaf-level for 123 leaves of 28 species of tree and liana from the upper canopy of a tropical forest in Panama, from which we calculated R_{25} and Q_{10} for each leaf. We chose to use R_{25} because it is widely used for comparison of respiration rates of plants from different biomes (e.g., Wright *et al*., 2006). We hypothesized that traits associated with the global leaf economics spectrum would also explain variation in respiration at the local scale.

Methods and Materials

Study Site

The study was conducted in Parque Natural Metropolitano (PNM, 8°59'N, 79°33'W, 100 m a.s.l.), a semi-deciduous moist tropical forest near Panama City on the Pacific coast of the Republic of Panama. Annual rainfall at the site averages 1740 mm, most of which falls during the rainy season (May through December). The park is a 256-hectare natural reserve consisting of 80–150 year old secondary forest with tree heights up to 40 m. A 42-m tall construction crane with a 51-m jib enables access to canopy leaves. Twenty eight species were selected from the upper forest canopy, representing a mix of plant functional types based on growth form and successional status; lianas (14 species), and trees classified as early-successional (5 species), mid-successional (7 species) and late-successional (2 species) (Table 3-1). Together these 28 species cover > 75% of the canopy area in reach of the crane (Avalos & Mulkey, 1999).

Respiration Measurements

For each species three to five sun-exposed terminal shoots were selected, where possible from multiple individuals. Twigs were collected pre-dawn at *ca*. 6 a.m., cut under water, or cut and immediately re-cut under water, and brought back to the laboratory in darkness and stored cool until measured. Dark respiration was measured on 3–8 whole, fully mature leaves per species at 5–7 temperatures between 20 and 32°C, in a Walz gas exchange cuvette with Peltier temperature control (GWK 3M, Walz, Mess- und Regeltechnik, Eiffeltrich, Germany), connected to a LI-6252 infrared gas analyzer (Licor, Lincoln, NE, USA). Petioles were cut under water and sealed with Parafilm in a 5-ml glass vial with water to prevent the leaves from drying out during the measurement. These 5–7 respiration measurements per took *ca.* 60–90 minutes to complete. All leaves were measured within 10 hours of collection. No trend in respiration rates with time since collection was detected within this period. All measurements were made in the wet season, between late August and early October, when all selected species had mature but non-senescing leaves.

Functional Trait Data

Leaf area was measured with a LI-3000 leaf area meter (Licor), and leaves were dried at 60°C to a constant mass. Leaf N was determined with an elemental analyzer (Costech Analytical, Los Angeles, California, USA). Concentrations of soluble carbohydrates (simple sugars and starch) were determined following Dubois (1956) with modifications. In short, simple sugars were extracted in 80% (v/v) ethanol by shaking at 27°C, followed by 2 two-hour incubations at 30°C. For each sample the supernatant from the three incubations was combined in a volumetric flask and brought to 10 ml. Glucose concentrations were determined colorimetrically at 487 nm via the phenol-

sulfuric acid method. Starch was hydrolyzed to glucose from the pellet in 1.1% hydrochloric acid at 100°C. Starch concentrations were determined as glucose equivalents. We measured photosynthetic capacity (A_{max}) on a separate set of 3–6 sun-exposed leaves per species at a saturating irradiance of 1200 µmol photons m^{-2} s^{-1}, 400 ppm CO_2 at ambient temperature (range 26–29 °C) with an LI-6400 (Licor) . All photosynthesis measurements were taken between 9.00 and 10.00 a.m. before mid-day stomatal closure was observed. Species means of foliar P were collected previously at the site, along with N, A_{max} and LMA (S.J. Wright. Unpublished). Species means for N, A_{max} and LMA in this independent data set correlated strongly with the species means determined in the current study ($R^2 > 0.65$ for each trait).

Quantification of *R* and Q_{10}

For each leaf, a linear regression line was fit to the $\log_{10}$-transformed leaf *R* versus leaf temperature (T_{Leaf}) according to:

$$\log_{10} R = a + bT_{Leaf} \quad (3\text{-}1)$$

where a and b, respectively the intercept and the slope of the response curve, are leaf-specific constants. Q_{10} values were calculated from these equations as:

$$Q_{10} = 10^{10b} \quad (3\text{-}2)$$

Subsequently, R_{25} of each leaf was calculated as:

$$R_{25} = \frac{R_{Tset}}{Q_{10}^{(T_{Set}-25)/10}} \quad (3\text{-}3)$$

where R_{Tset} is *R* measured at the set cuvette temperate (T_{set}). We averaged R_{25} over the 5–7 set cuvette temperatures to get a leaf-level R_{25} to use in our analyses.

Relationships of R_{25} and Q_{10} with other traits were examined with multiple regression models using species mean trait values. We developed regression models

for R_{25} and Q_{10} using leaf traits averaged at the species level rather than using the leaf level data. This is a conservative approach that avoids pseudo-replication within species (as multiple leaves within a species are not independent data points). To identify the best combination of predictor variables we used the subset method of variable selection in multiple regression (Miller, 2002) from the Leaps package version 2.9 in R. In this method the best combination of predictors for a subset of n predictors is identified (using R^2, $R^2_{adjusted}$ and Mallows' C_p (Mallows, 1973)) and this is repeated for all possible subset sizes.

Estimating Stand-level Respiration Fluxes

Our unpublished monitoring data at the study site show that leaf temperature (T_{Leaf}) is coupled closely to air temperature (T_{Air}), especially at night (T_{Leaf} = 0.999 * T_{Air}, R^2 = 0.91; T_{Leaf} of 5 tree species and 3 liana species monitored 5–8 days each). To estimate stand-level nighttime *R* flux we could therefore use a 17-year air temperature record from the site (data collected at 25m height on the crane at 60-minute (1995–2005) or 15-minute (2006–2011) intervals. http://biogeodb.stri.si.edu/physical_monitoring/research/metpark). We used four approaches to scale respiration of canopy leaves to the stand level, which differed in how estimates of R_A and Q_{10} were obtained and averaged across species. In model 1A, R_A and Q_{10} were estimated at the species level from their multiple regression relationships with other leaf traits. Model 2A, in contrast, used species averages of measured R_A and Q_{10}. To assess the functionality of a PFT-level flux prediction, both models were also run after substituting species-level estimates with R_A and Q_{10} values modeled from leaf traits averaged by PFT (1B), or with measured R_A and Q_{10} values averaged by PFT (2B). In all models, CO_2 efflux from

R was calculated by species for every 15- or 60-minute interval between 6 p.m. and 6 a.m. Where available, we used species-specific estimates of leaf area index (LAI; total leaf area per unit ground area, in $m^2\ m^{-2}$; five species in Kitajima *et al*., 2005) to calculate total CO_2 flux per unit ground area. For the remaining species we used mean LAI per growth form from Clark *et al*. (2008).

Dark respiration in shade leaves is reduced compared to that of fully exposed upper-canopy leaves, and we used site-specific data to estimate the degree of reduction in respiration of shade leaves in relation to the LAI of the trees. Transmittance of photosynthetically active radiation (PAR) decreases exponentially with LAI in the canopy of five of our focal tree species (Kitajima *et al*., 2005) such that

$$\%PAR \approx 100e^{-0.41 \times LAI\ above} \quad (3\text{-}4)$$

where "LAI above" refers to the number of leaf layers that shade the focal leaf layer. *R* decreases linearly with a decrease in % daily PAR such that

$$\%\ of\ R_{at\ full\ sun} \approx 22 + 0.78 \times \%PAR \quad (3\text{-}5)$$

(C. Rey-Sánchez & M. Slot. Unpublished). Combining equation 3-4 and equation 3-5 gives

$$\%\ of\ R_{at\ full\ sun} \approx 22 + 78e^{-0.41 \times LAI\ above} \quad (3\text{-}6)$$

Leaves in the second and third leaf layer of a tree canopy thus respire at 74% and 56% of leaves in the first (sun-exposed) layer respectively. For models 1A and 1B, however, the decrease in *R* with depth in the canopy was based on the change in the leaf traits that were predictors in the trait-based model of *R*.

To extrapolate to annual fluxes evergreen species were assumed to maintain LAI year-round, deciduous species had LAI = 0 in the 4-month dry season from January

through April, and semi-deciduous species maintained LAI for 10 months. For tropical forests both decreases and increases in dry season *R* have been reported (Domingues *et al*., 2005; Miranda *et al*., 2005; Stahl *et al*., 2013), so because dry season data were not available *R* was kept constant throughout the year. *R* was scaled to the stand level by determining the relative abundance of the canopy trees from their basal area in 2010 census data [Census data from PNM are collected by Smithsonian Tropical Research Institute Forest Dynamics project (Condit 1998; Hubbell *et al*., 2005; Condit *et al*., 2013)]. Because liana abundance was unknown at the species level, liana relative abundance was equally divided among species, and summed up to cover 30% of the crown area (Avalos & Mulkey, 1999). For each calendar year species-level *R* per hectare was summed up and multiplied by the molecular mass of C to get Mg C respired at night ha^{-1} yr^{-1}.

We also estimated *R* during the day (R_{Day}). Light inhibits *R*, so R_{Light} is lower than R_{Dark} at a given temperature (Sharp *et al*., 1984; Krömer 1995). We determined (R_{Light}) for saplings of four of our focal species using the Kok method (Sharp *et al*., 1984) and found an average reduction of respiration of 46% compared to R_{Dark} at a given temperature (n = 17 leaves). Light also reduces the Q_{10} but the extent of the reduction is variable (Atkin *et al*., 2000; Pons & Welschen, 2003). For calculation of R_{Day} we reduced the Q_{10} by 25% compared to the Q_{10} of R_{Dark}. The C flux from R_{Day} was calculated using day-time temperature data and a 46% reduction in *R* and 25% reduction in Q_{10} for all species.

Statistical Analyses

Comparisons among species, plant functional types, and growth forms were made using one-way ANOVAs and Tukey HSD *post hoc* tests, or Student's t-tests.

Where necessary, data were transformed to improve normality and homoscedasticity. The variance in respiration and Q_{10} data was broken down to variance at the species, PFT and growth form level using partial R^2 analysis. All statistical analyses were performed in R version 2.14.1 (R Development Core Team, 2011).

Results

Respiration at 25 °C

Respiration expressed on an area basis and standardized at 25°C (R_A) differed significantly among species (Fig. 3-1; Table 3-1), exhibiting 3-fold variation from the early-successional tree *Cecropia peltata* (1.99 ± 0.06 μmol m^{-2} s^{-1}; mean ± SD) to the liana *Cissus erosa* (0.60 ± 0.15 μmol m^{-2} s^{-1}). R_A of lianas (0.77 ± 0.13 μmol m^{-2} s^{-1}) was lower than R_A of trees (1.11 ± 0.41 μmol m^{-2} s^{-1}: t-test, P = 0.007). On average lianas had lower R_A than early-successional tree species, but other differences among PFTs were not significant. R_{25} expressed on a mass basis (R_M) also differed widely among species and among PFTs. Lianas and early-successional tree species had significantly higher R_M than late-successional species, with mid-successional species showing intermediate values that were not significantly different from the other PFTs (Fig. 3-1).

Q_{10} Values by Species, Plant Functional Type and Growth Form

Q_{10} values varied among species (mean 2.39, range 2.01 - 2.93), but did not differ systematically between trees (2.35 ± 0.18, mean ± SD) and lianas (2.45 ± 0.26) (Fig. 3-1). Different successional stages did not differ in mean Q_{10} values either, with early-successional (2.30 ± 0.19), mid-successional (2.36 ± 0.19) and-late successional species (2.26 ± 0.03) all falling within a narrow range. For all PFTs Q_{10} values were significantly higher than 2.0 (t-test, $P < 0.05$ for all PFTs). For 19 of the 28 species Q_{10} was also significantly greater than 2.0 at the species level.

Variance of Respiration and Q_{10}

Variation in R_A and R_M was considerable (see Fig. 3-1), with leaf-level values ranging more than 6-fold, and species means ranging 3-fold. Most of the variance existed among species (Fig. 3-2), but variation among leaves within species also explained 20–30% of the total variance. By comparison, Q_{10} values were less variable (leaf-level values ranged 2-fold and species means 1.5-fold). About 45% of the Q_{10} variance was explained by variation in Q_{10} values among leaves within species, with a similar percentage of variance explained by Q_{10} differences among species within PFT (Fig. 3-2). LMA, in contrast, differed significantly between trees and lianas (trees 106 ± 19, lianas 68 ± 15; $P < 0.001$. t-test) and growth form explained most of the variance in this trait.

Trait Correlations and Multiple Regression Models for Respiration and Q_{10}

Significant pair-wise correlations were found between R_A and area-based A_{max}, N, P, total non-structural carbohydrates (TNC_{Area}), and LMA (Table 3-2). To avoid overfitting of the model we chose to have maximally three predictors plus intercept in the model. The best three-parameter model for R_A contained the significant predictors P, A_{max} and LMA:

$R_A = 0.14 + (0.718 * P_{Area}) + (0.042 * A_{max}) - (0.009 * LMA)$

where R_A and A_{max} are in μmol m^{-2} s^{-1}, P_{Area} is in mg m^{-2} and LMA in g m^{-2}.

This model accounted for 64% of the variance in R_A in a subset of 24 species (12 tree, 12 liana species) for which data on all leaf traits were available (Table 3-3). The model's fit is illustrated by the partial residual plots in Fig. 3-3. The same set of parameters, when expressed on a mass basis (R_M and A_{max} in nmol g^{-1} s^{-1}, P in mg g^{-1}), constituted the best model for R_M:

R_M = 12.8 + (504 * P) + (0.005 * A_{max}) - (0.18 * LMA)

Q_{10} was best modeled from two independent predictors (Table 3-3):

Lianas: Q_{10} = 2.21 + (0. 021 * $TNC_{Area)}$

Trees: Q_{10} = 2.21 + (0. 021 * TNC_{Area}) - 0.281

where TNC_{Area} is in mg m^{-2}. Fig. 3-4 illustrates the combined effects of TNC_{Area} and growth form on Q_{10}. Pair-wise correlations were not significant for Q_{10} (Table 3-2) and models with more than two predictors included non-significant predictors. There was a marginally significant interaction between TNC_{Area} and growth form (P = 0.083, ANCOVA).

Stand-level Leaf Respiratory Carbon Flux

Multiple regression models of R_A explained more of the variance in respiration than models for R_M, so we scaled R to the stand level using R_A. Mean annual flux of nocturnal respiration between 1995 and 2011 was 4.5 ± 0.34 Mg C ha^{-1} (mean ± SD across years) when calculated using R_A and Q_{10} estimated from multiple regression models (model 1A), and 4.1 ± 0.33 Mg C ha^{-1} when using leaf traits averaged by PFT (model 1B) (Fig. 3-5; Table 3-4). The annual flux estimate based on species-specific R_A and Q_{10} measurements (model 2A) was 5.5 ± 0.35 Mg C ha^{-1}, and 5.3 ± 0.34 Mg C ha^{-1} when PFT-level means were used. In all estimates, circa 95% of the respiratory carbon flux came from trees and 5% from lianas. Of the C flux from trees 4–7% (range across the four models) came from early-successional species, 46–52% from mid-successional species and 42–50% from late-successional species.

R_{Day} was 2.9 ± 0.13 Mg C ha^{-1} yr^{-1} when R was estimated from leaf phosphorus content, A_{max} and LMA, and 2.7 ± 0.11 Mg C ha^{-1} yr^{-1} when using PFT averages of these leaf traits (Table 3-4). When species-level measurements of R_A and Q_{10} were

used R_{Day} was 2.6 ± 0.09 Mg C ha^{-1} yr^{-1}, while the R_{Day} flux estimated using PFT averages of measured R_A and Q_{10} was 3.4 ± 0.11 Mg C ha^{-1} yr^{-1} (Table 3-4). Estimates of the total carbon flux from foliar respiration ranged from 6.7 (model 1B) to 8.9 Mg C ha^{-1} yr^{-1} (model 2A).

Discussion

In this study we determined leaf-level dark respiration rates and Q_{10} values of upper-canopy leaves of tropical trees and lianas, and used these data to parameterize multiple regression models, enabling us to scale leaf respiration to annual stand level carbon efflux from other leaf functional traits. Annual carbon fluxes associated with *R* were comparable to those reported for lowland tropical forests in the Brazilian Amazon (Malhi *et al*., 2009).

Species and PFT Differences in Respiration Traits

R_A of lianas was lower than R_A of trees (Fig. 3-1), which is the opposite of what Domingues *et al*. (2007) and Cavaleri *et al*. (2008) found in other Neotropical forests. The LMA of lianas in the current study was, however, significantly lower than the LMA of trees, which was not the case in the above-mentioned studies. Liana leaves in the current study may thus simply have contained less metabolically active tissue per unit leaf area than trees. R_M of lianas was equal to that of early successional species, confirming that lianas have comparatively high metabolic activity.

In accordance with recent *in situ* measurements (Chapter 2) Q_{10} values were significantly higher than 2.0, the commonly assumed value that has been adopted in ecosystem process models (e.g., Thornton *et al*., 2002; Wang *et al*., 2009). Moreover, for tropical climates Q_{10} values even slightly lower than 2.0 have been predicted, based on the observation of declining Q_{10} with rising temperature interval over which *R* is

measured (Tjoelker *et al*., 2001; Atkin & Tjoelker, 2003). According to the linear function reported in Atkin & Tjoelker a Q_{10} of 1.97 is predicted at 26°C, the mid-point of the temperature interval over which we measured *R*. The temperature dependent Q_{10} has been incorporated in global vegetation models and ecosystem models as an improvement to the static Q_{10} (e.g., Wythers *et al*., 2005, 2013; Atkin *et al*., 2008; Ziehn *et al*., 2011; Chen & Zhuang, 2013). While it may be appropriate to expect the Q_{10} to decrease in relation to temperature within a plant, or of in plants within a climatic region (as originally suggested by Tjoelker *et al*., 2001), this observation cannot be extrapolated to predict Q_{10} values of tropical vegetation. The implication of doing so is that a low Q_{10} is predicted for the tropics, and that is clearly not supported by our data. Especially if the model uses a reference temperature of respiration that is lower than the mean temperature at the study site, underestimation of Q_{10} values can result in considerable underestimation of the calculated respiratory carbon efflux from the forest.

Multiple Regression of *R* and Q_{10}

We tried to identify trait-based models to predict R_A and Q_{10} that could be of use in modeling of carbon fluxes in tropical forests. Individual traits accounted for 21–47% of the explained variation in R_A and the best three-trait regression explained 64% of the variation. The best Q_{10} model uses total non-structural carbohydrate content per unit leaf area and growth form to explain 26% of the variation in Q_{10}, while single trait correlations were non-significant.

While N and A_{max} both correlate with *R* the fit is relatively poor across these co-occurring tropical species. Interestingly, P turned out to be a much better correlate of *R*. Meir *et al*. (2001) found that in a P-limited forest in Jarú, Brazil, P correlated more strongly with *R* than in a less P-limited system in Cameroon. Cavaleri *et al*. (2008)

working in a P-limited lowland rainforest in Costa Rica therefore hypothesized P to be a better correlate of *R* than N, which is less limiting at the site. While *R* did correlate positively with P, the correlation was not stronger than the correlation of *R* with N, suggesting that P did not limit *R* more than N did. Plant available P at PNM is on average 5.8 mg kg^{-1} (B. L. Turner, pers. comm.[1]), which is relatively high for a tropical forest soil. Indeed, the P_{Area} values in the current study are considerably higher than those in Meir *et al*. (2001) and Cavaleri *et al*. (2008). The greater strength of the correlation between *R* and P than between *R* and N is thus unlikely to reflect P limitation of this forest, but may instead reflect interspecific variation in non-metabolic N use in leaves, obscuring the correlation between *R* and N.

The best three-parameter model with P, A_{max} and LMA explained 64% of the variance in R_A. Meir *et al*. (2001) used stepwise regression to model R_A of leaves of tropical forest trees, and their best models also included P and LMA, but A_{max} was not considered in their analysis. With our data the full model of R_A as a function of P_{Area} and LMA alone would be significant and have an R^2 of 0.52, but LMA would not be a significant predictor in this model. N and LMA together explained 50–80% of the variance in *R* across sites and biomes, including tropical forest (Reich *et al*., 1998), but this study did not consider P and A_{max}. Although the observation that *R* correlates with P is not new, the fact that N was not included in our best models suggests that perhaps especially in tropical forests, P should be considered as a predictor of *R*, even if N is the better correlate across biomes (I. J. Wright *et al*., 2004).

[1] Email correspondence on 11 October 2012. Averages based on unpublished data.

A maximum of 26% of the observed variance in Q_{10} can be explained by TNC and growth form. When accounting for growth form, higher Q_{10} values are found in species with higher of TNC per leaf area. Thus, *R* of species with high TNC increases more with temperature than species with low TNC, suggesting that *R* in species with low TNC becomes substrate-limited at high temperature. Interestingly, however, substituting the concentration of simple sugars (the more immediate substrate for *R*) for TNC in the model causes the trend to be lost (i.e., no increase in Q_{10} with increase in the concentration of simple sugars). Furthermore, neither the concentration of simple sugars, nor TNC correlated significantly with Q_{10} in bivariate correlations. These factors may argue against the above explanation of progressive substrate limitation at high temperatures. While important for *R* and potentially for the Q_{10} of respiration, leaf sugar content is not a commonly measured trait in forest ecology. This calls into question the utility of a Q_{10} model that requires TNC and growth form to account for 26% of the interspecific variation in Q_{10}, when Q_{10} values of 85% of the species fall within the relatively narrow range between 2.15 and 2.70 and are not systematically different among PFTs.

Annual Leaf Respiratory Carbon Flux at the Stand-level

Estimates of annual nocturnal leaf respiratory carbon release were considerably lower for trait-based models that used multiple regression models to estimate R_A and Q_{10} than for models that used measured values of R_A and Q_{10}, despite the fact that the regression models were parameterized on the measured values. These differences are largely based on flux estimates for *Anacardium excelsum*, the species with the highest relative abundance at the site, and the largest single contributor to the stand-level C flux. *Anacardium* has average A_{max} and P concentration, but one of the highest LMA

values of all species (121 g m^{-2}; Table 3-1). Because LMA has a negative effect on R_A in the multiple regression model, R_A of *Anacardium*, and therefore of the study area, is underestimated. The use of PFT means, either of measured or modeled respiration traits resulted in a small reduction in flux estimates compared to the use of species-level data. In the following discussion we will use the flux calculations based on species-level measurement of R_A and Q_{10} to represent the best estimate of the respiratory carbon flux in the study forest.

Per year, nighttime *R* of canopy trees and lianas at PNM releases 5.5 Mg C ha^{-1}. This is almost identical to the nighttime *R* efflux of 5.6 Mg C ha^{-1} yr^{-1} that Malhi *et al*. (2009. With *R* data from Domingues *et al*., 2005), report for Tapajós in the Brazilian Amazon, but higher than the mean of 4.0 Mg C ha^{-1} reported for the Caxiuanã reserve in the eastern Amazon (Metcalfe *et al*., 2010). Although the dry season length at Tapajós is comparable to that at our site, the LAI estimates are higher (5.44 m^2 m^{-2}) than the values we used (mean for trees 3.66, with 30% cover of lianas with LAI of 0.73 m^2 m^{-2}). Furthermore, we conservatively assumed the dry season deciduous species (11 of 14 tree species) to be leafless for the full four months of the dry season, whereas the Tapajós site shows increased metabolic activity during dry season leaf flush (Huete *et al*., 2006). These calculations suggest that the nighttime *R* flux we calculated may be conservative, even though it is comparable to fluxes in other tropical forests. Leaf-level respiration rates at PNM are at the high end of the spectrum of values reported for tropical forest trees (Slot *et al*., 2013), which supports the notion that the nocturnal leaf respiratory C flux we report may be a slight underestimate of the true value for the study site.

R_{Day} added 3.5 Mg C ha^{-1} yr^{-1}, bringing the total R flux to 8.9 C Mg ha^{-1} yr^{-1}. The daytime flux was higher than reported in Malhi *et al*. (2009), who assumed R_{Light} to be 67% lower than R_{Dark} based on measurements on Snow Gum (*Eucalyptus pauciflora* Sieb. ex Spreng) (Atkin *et al*., 2000). Our estimate of 46% reduction of R in the light for four tropical tree species was almost identical to the 47% reduction Pons & Welschen (2003) reported for seedlings of *Eperua grandiflora* (Aubl.) Benth., another tropical tree species. Not enough data on R_{Light} are currently available to generalize the extent of reduction of R_{Light} relative to R_{Dark} in tropical versus temperate species. Total C flux was comparable to that at Tapajós (7.4 ± 4.0 Mg C ha^{-1} yr^{-1}). For Caxiuanã, the site where Melcalfe *et al*. (2010) estimated nighttime R to be 4.0 Mg C ha^{-1} yr^{-1}, Malhi *et al*. (2009) report a total leaf respiratory carbon flux of 8.9 ± 4.0 Mg C ha^{-1} yr^{-1}. This suggests either a very high daytime R flux, or, more likely, it illustrates how discrepancy in estimation methods results in grossly different flux estimates. Near Manaus, at a site with a shorter dry season than PNM the total annual flux is 10.1 ± 4.0 Mg C ha^{-1} yr^{-1} (Chambers *et al*., 2004; Malhi *et al*., 2009). Due to the large uncertainty in the estimates, it is currently not possible to conclusively state forest differences in total respiration fluxes.

Significance for Modeling

Our results suggest that within a tropical forest canopy, estimating R from either N or photosynthesis alone leaves a lot of variation unexplained. While across biomes these correlations may work well, within a single biome, or indeed a single forest, the value of these bivariate correlations is limited. We also looked at whether it was feasible to link Q_{10} to other leaf traits to facilitate future modeling efforts. The concentration of TNC and growth form together explained 26% of the variance in Q_{10}, but the physiological underpinning of this model is not clear and the explained variance is

modest. Rather than trying to model Q_{10} from other leaf traits, it will be important to improve our understanding of the dynamic nature of the Q_{10} in relationship to temperature, which currently appears to underestimate Q_{10} values of tropical trees. Our estimates of stand-level leaf respiratory carbon flux based on R_A and Q_{10} from trait-based models were comparable to fluxes reported for other tropical forests, suggesting that trait-based modeling indeed has potential. The parameterization of such trait-based models will, however, need to be done locally, or regionally to assure accurate predictions.

Table 3-1. Species codes, names, families, plant functional type (PFT. ES: early-successional, MS: mid-successional, LS: later-successional, L: lianas), and the number of leaves measured (n), dark respiration at 25°C per unit area (R_A) and mass (R_M), leaf mass per unit area (LMA), photosynthetic capacity (A_{max}), and concentrations of nitrogen (N), phosphorus (P), and total non-structural carbohydrates (TNC).

Code	Species	Family	PFT	n	R_A	R_M	Q_{10}	LMA	A_{max}	N	P	TNC
					µmol m^{-2} s^{-1}	nmol g^{-1} s^{-1}		g m^{-2}	µmol m^{-2} s^{-1}	%	%	mg g^{-1}
ALBG	*Albizia guachapele* (Kunth) Harms	Fabaceae	ES	4	0.76	8.2	2.50	94	12.4	3.8	0.10	159
ANNS	*Annona spraguei* Saff.	Annonaceae	ES	3	0.98	11.2	2.15	88	12.8	2.6	0.17	241
CECL	*Cecropia longipes* L.	Urticaceae	ES	3	1.82	19.5	2.20	94	20.6	2.5	0.20	155
CECP	*Cecropia peltata* L.	Urticaceae	ES	3	1.99	16.8	2.60	119	19.8	2.7	0.17	245
PITT	*Pittoniotis trichantha* Griseb.	Rubiaceae	ES	4	0.73	10.2	2.04	74	13.5	2.3	0.14	158
ASTG	*Astronium graveolens* Jacq.	Anacardiaceae	MS	3	1.27	12.9	2.15	98	12.6	2.4	0.20	160
CAS4	*Castilla elastica* (Liebm.) C.C.	Moraceae	MS	6	1.23	11.2	2.19	110	19.4	2.6	0.17	207
FIIS	*Ficus insipida* Willd.	Moraceae	MS	9	1.44	10.8	2.30	133	23.4	2.8	0.17	161
LUE	*Luehea seemannii* Triana & Planch.	Tiliaceae	MS	6	0.92	8.5	2.40	108	19.4	2.1	0.15	191
PSES	*Pseudobombax septenatum* (Jacq.) Dugand	Malvaceae	MS	5	0.90	7.0	2.70	127	16.2	2.0	*nd*	188
SPOM	*Spondias mombin* L.	Anacardiaceae	MS	4	0.93	12.1	2.48	78	16.5	2.6	0.13	109
ZUEL	*Zuelania guidonia* (Sw.) Britt. & Millsp.	Salicaceae	MS	3	0.91	8.1	2.30	114	17.3	2.0	*nd*	164
ANAE	*Anacardium excelsum* Bertero & Balb. ex Kunth Skeels	Anacardiaceae	LS	8	0.97	8.1	2.24	121	13.8	1.8	0.14	166
CHRC	*Chrysophyllum cainito* L.	Sapotaceae	LS	4	0.63	5.1	2.28	124	17.1	1.9	0.10	177
AMPP	*Amphilophium paniculatum* (L.)	Bignoniaceae	L	4	1.00	15.4	2.19	64	9.5	2.9	0.17	220
ARIC	*Aristolochia tonduzii* O.C. Schmidt	Aristolochiaceae	L	3	0.91	15.6	2.39	62	12.1	3.3	0.15	239
BONT	*Bonamia trichantha* Hallier f.	Convolvulaceae	L	7	0.73	10.0	2.93	74	10.4	2.3	0.18	266
CISE	*Cissus erosa* Rich.	Vitaceae	L	5	0.60	10.9	2.61	54	17.2	2.7	*nd*	210
COMF	*Combretum fruticosum* (Loefl.)	Combretaceae	L	5	0.80	12.0	2.75	69	17.7	2.8	0.16	196
GOUL	*Gouania lupuloides* (L.) Urb.	Rhamnaceae	L	4	0.68	14.8	2.30	47	12.7	4.2	0.18	233
MIKL	*Mikania leiostachya* Benth.	Asteraceae	L	3	0.66	10.7	2.01	63	9.8	2.1	0.13	102

Table 3-1. Continued.

Code	Species	Family	PFT	n	R_A µmol m^{-2} s^{-1}	R_M nmol g^{-1} s^{-1}	Q_{10}	LMA g m^{-2}	A_{max} µmol m^{-2} s^{-1}	N %	P %	TNC mg g^{-1}
ODOM	*Odontadenia macrantha* Willd. ex Roem & Schult	Apocynaceae	L	3	0.64	8.8	2.59	74	*nd*	2.1	0.09	128
PASV	*Passiflora vitifolia* Kunth	Passifloraceae	L	3	0.73	11.2	2.37	62	6.7	3.4	0.20	189
PHRC	*Phryganocydia corymbosa* (Vent.) Bureau ex K. *Schum.*	Bignoniaceae	L	3	0.76	6.9	2.53	110	12.2	3.2	0.15	94
PITC	*Pithecoctenium crucigerum* (L.) A.H. Gentry	Bignoniaceae	L	3	0.84	11.7	2.51	72	13.4	2.9	0.14	167
SERM	*Serjania mexicana* (L.) willd.	Malpighiaceae	L	4	0.64	9.0	2.56	71	12.9	2.9	0.15	177
STIH	*Stigmaphyllon hypargyreum* Triana & Planch.	Bignoniaceae	L	4	1.01	15.3	2.43	67	16.3	2.3	0.14	126
VITT	*Vitis tiliifolia* Humb. & Bonpl. ex Roem. & Schult.	Vitaceae	L	7	0.83	14.2	2.22	59	13.0	2.4	0.13	158

nd: no data available

Table 3-2. Results from tests of pairwise-correlation between respiration at 25°C per unit leaf area (R_A), per unit mass (R_M), and Q_{10}, and other traits: leaf phosphorus (P) and nitrogen (N) concentration, photosynthetic capacity (A_{max}), leaf mass per unit area (LMA), and total non-structural carbohydrate concentration (TNC). R_A and Q_{10} are correlated with area-based leaf traits; R_M with mass-based traits.

	R_A			R_M			Q_{10}		
	R^2	*P*	n	R^2	*P*	n	R^2	*P*	n
P	0.47	<0.001	24	0.23	0.018	24	0.0	ns	24
N	0.22	0.019	28	0.1	ns	28	0.0	ns	28
A_{max}	0.33	<0.01	27	0.04	ns	27	0.0	ns	27
LMA	0.21	0.013	28	0.17	0.028	28	0.0	ns	28
TNC	0.26	<0.01	28	0.06	ns	28	0.1	ns	28
Growth form	0.25	<0.01	28	0.03	ns	28	0.1	ns	28

Table 3-3. Parameter estimates of multiple regression analysis of R_A (µmol CO_2 m^{-2} s^{-1}), R_M (nmol CO_2 $g^{-1}s^{-1}$) and Q_{10}, against leaf phosphorus (P), photosynthetic capacity (A_{max}), leaf mass per area (LMA: g m^{-2}), TNC and growth form (Liana (L) vs. Tree (T)), where
Response = Intercept + *a*P + *b*A_{max} + *c*LMA + *d*TNC + *e*(Growth form).
R_A is regressed on area-based leaf traits (P, TNC: mg m^{-2}. A_{max}: µmol CO_2 m^{-2} s^{-1}); R_M on mass-based traits (P, TNC: mg g^{-1}. A_{max}: nmol $g^{-1}s^{-1}$). In the Q_{10} model TNC is expressed in g m^{-2}. Number of species (n), overall (multiple) R^2 and model significance or also shown.

Response	Intercept	P	A_{max}	LMA	TNC	Growth form	n	R^2	*P*
		a	*b*	*c*	*d*	*e*			
R_A	0.14	0.72**	0.042*	-0.009*			24	0.64	<0.001
R_M	12.8**	504*	0.005*	-0.182**			24	0.56	<0.001
Q_{10}	2.21***				0.021*	L: 0 T:-0.28**	28	0.26	<0.05

*, ** and *** indicate significance of the variable with $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Table 3-4. Distribution of stand-level foliar respiration carbon flux (Mg C $ha^{-1} yr^{-1}$) during night and day among plant functional types (Lianas, ES: early-successional, MS: mid-successional, LS: later-successional trees). Estimates are made using modeled and measured respiration (R_A) and Q_{10}, at the species level and the level of the plant functional type (PFT).

	Trait-based estimates of R_A and Q_{10}				Measured R_A and Q_{10}			
	Species means		PFT means		Species means		PFT means	
	Night	*Day*	*Night*	*Day*	*Night*	*Day*	*Night*	*Day*
Lianas	0.26	0.17	0.26	0.17	0.25	0.16	0.26	0.16
ES trees	0.17	0.11	0.20	0.13	0.18	0.11	0.28	0.18
MS trees	2.20	1.46	2.06	1.37	2.42	1.54	2.59	1.65
LS trees	1.83	1.20	1.53	1.01	2.61	1.64	2.16	1.36
Sum	4.46	2.94	4.05	2.68	5.46	3.45	5.29	3.35

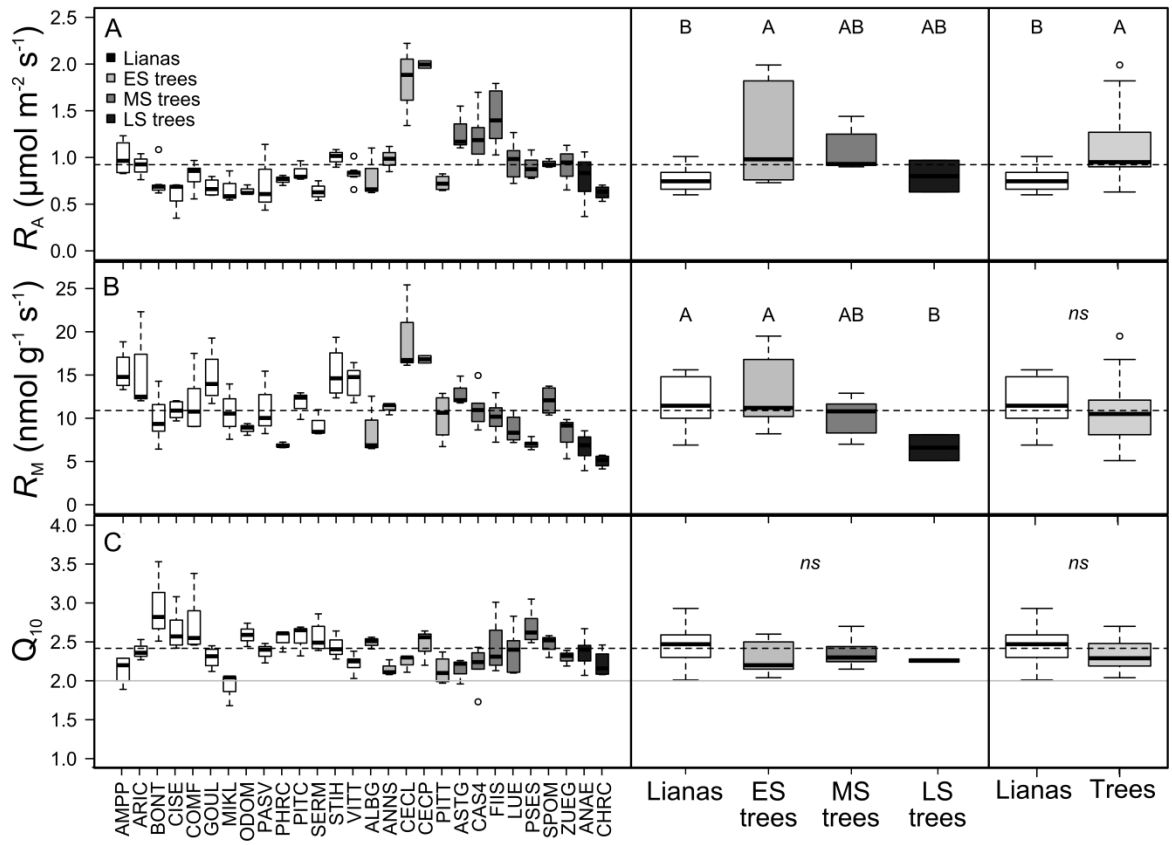


Figure 3-1. Variation of respiration traits within and among species (species codes as in Table 1); within and among plant functional types (PFT), and within and between growth forms. A) Respiration per unit leaf area (R_A). B) Respiration per unit leaf mass (R_M). C) Temperature sensitivity of respiration (Q_{10}) The overall means are indicated by the dashed lines. The gray line in C indicates Q_{10} = 2.0, the value widely used in C flux models. The tree species are early-successional (ES, 5 species), mid-successional (MS, 7 species), and late-successional (LS, 2 species). The box plots indicate the median, 25th and 75th percentile for each species, PFT and growth form. Whiskers extend to 1.5 times the interquartile range. Boxplots for PFT and growth form are calculated from species means. Different letters indicate groups that are significantly different from one another (P < 0.05) (One-way ANOVA with Tukey *post hoc* testing).

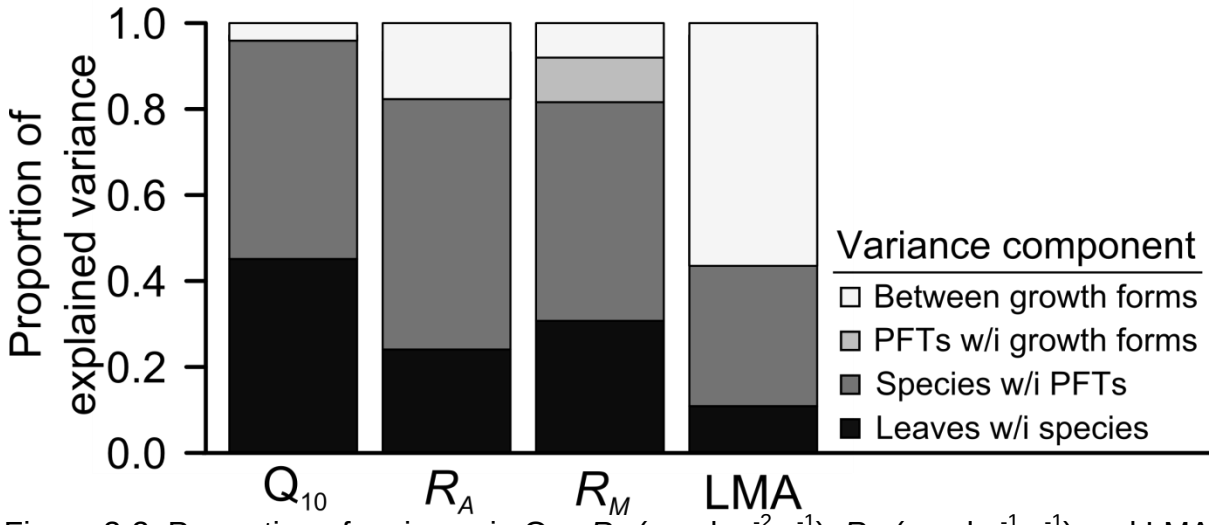


Figure 3-2. Proportion of variance in Q_{10}, R_A (µmol m^{-2} s^{-1}), R_M (nmol g^{-1} s^{-1}) and LMA (g m^{-2}) explained by variance within species, among species within plant functional type (PFT), among PFTs and between growth forms, as determined by partial R^2 analysis.

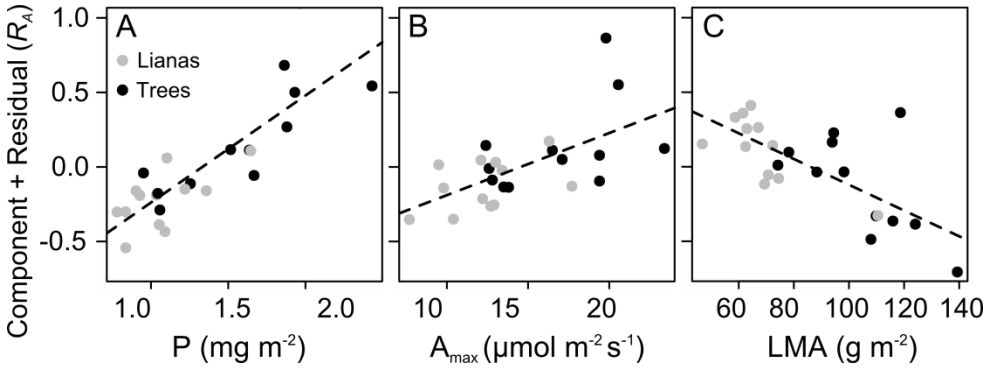


Figure 3-3. Partial residual plots for the best model of respiration per unit leaf area (R_A), in which R_A is regressed against phosphorus content per unit area (P_{Area}), photosynthetic capacity (A_{max}), and leaf mass per unit area (LMA). These plots show the predictor variable on the x axis, and on the y axis the residuals of the full model plus the partial regression coefficient of the predictor variable multiplied by the predictor variable. A) Phosphorus concentration per unit leaf area (P). B) Photosynthetic capacity (A_{max}). C) Leaf mass per unit area. Trees and lianas are combined in these analyses as growth form was not a significant predictor.

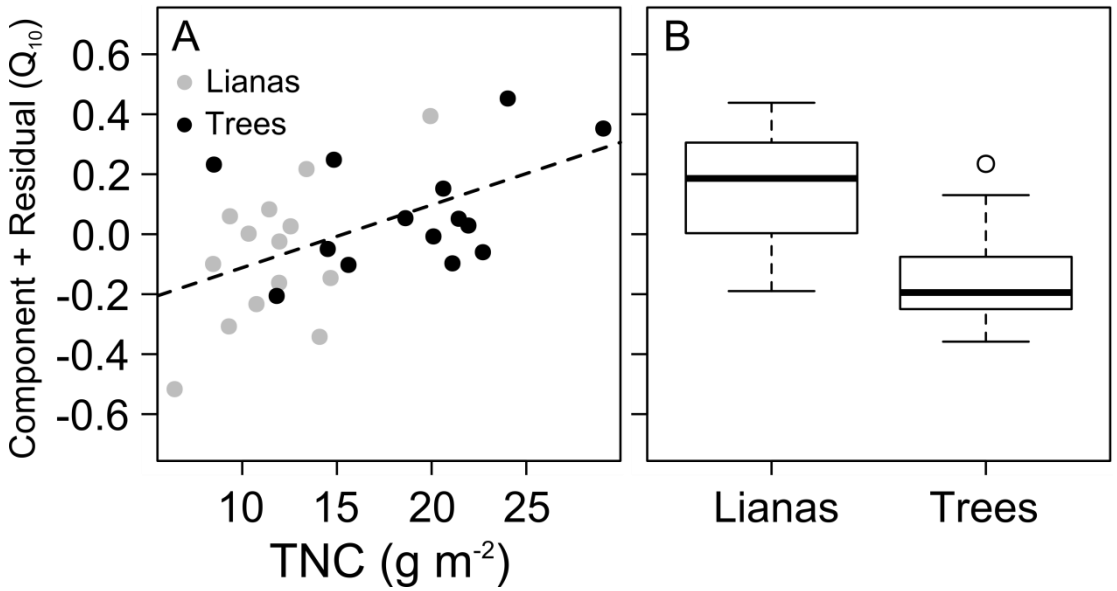


Figure 3-4. Partial residual plots for the best model of Q_{10}, in which Q_{10} is regressed against TNC_{Area} and growth form. A) The residuals of the full model + the regression coefficient of TNC_{Area} multiplied by TNC_{Area} are together plotted against TNC_{Area}. B) The sum of the full model residuals plus the regression coefficient of growth form multiplied by growth form is regressed against growth form on the x axis.

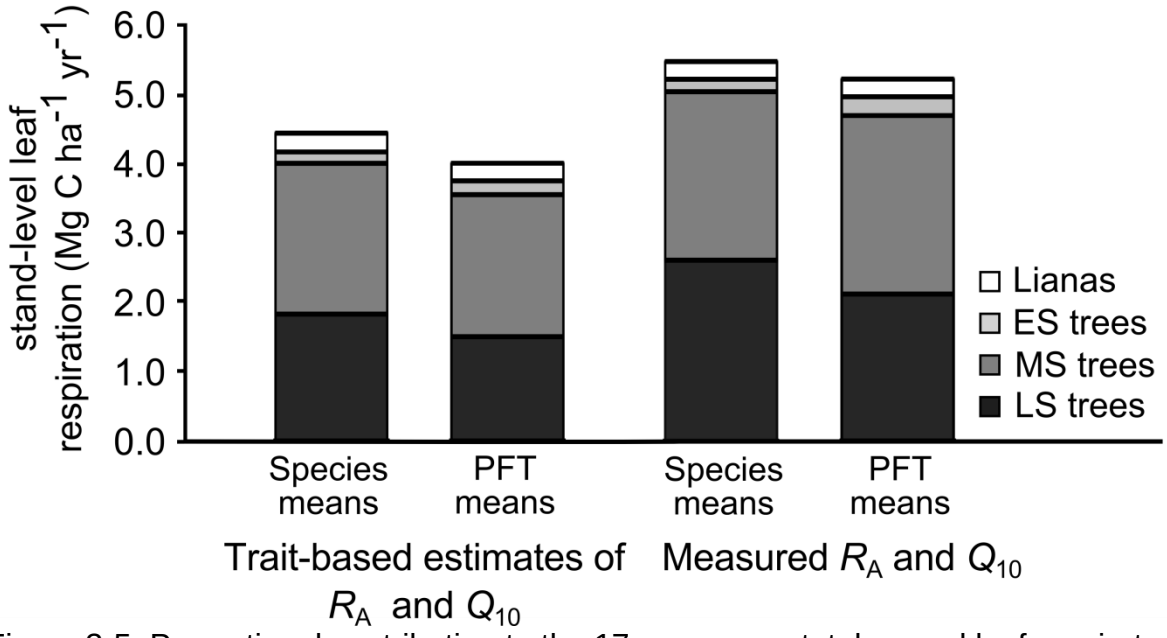


Figure 3-5. Proportional contribution to the 17-year mean total annual leaf respiratory carbon flux by lianas, early-successional (ES), mid-successional (MS) and late-successional (LS) tree species. R_A and Q_{10} were either modeled from leaf traits (models 1A,B. see main text) or measured values were used (models 2A,B).

CHAPTER 4
THERMAL ACCLIMATION OF LEAF DARK RESPIRATION TO EXPERIMENTAL NIGHTTIME WARMING IN TROPICAL CANOPY TREES AND LIANAS

Background

Contemporary tropical forests exist in a narrow temperature range (Janzen, 1967; Wright *et al*., 2009), close to what may be a high temperature threshold (Doughty & Goulden, 2008). Models predict unprecedented warming in the tropics over the current century (Diffenbaugh & Scherer, 2011), which will push the majority of tropical forests into a climate envelope currently not occupied by closed-canopy forests (Wright *et al*., 2009). The capacity of organisms to acclimate to a change in temperature is less likely to be under natural selection in thermally stable conditions such as tropical forests, than in temperate and boreal biomes (Janzen, 1967; Cunningham & Read, 2002; Ghalambor *et al*., 2006). Tropical forests are currently considered to be an important carbon sink (Phillips *et al*., 1998; Baker *et al*., 2004; Lewis *et al*., 2009), but how they will respond to climate warming is uncertain, as illustrated by the large discrepancy in predictions among dynamic global vegetation models (DGVMs) and earth system models (Ahlström *et al*., 2012; Cox *et al*., 2013). The physiological response of vegetation to temperature and CO_2 are the largest source of uncertainty in such models (Arneth *et al*., 2012; Booth *et al*., 2012; Huntingford *et al*., 2013), in part because thermal acclimation of photosynthesis and respiration of the vegetation is only addressed by some models, but not by others (Smith & Dukes, 2013). More importantly, the current lack of empirical data on thermal acclimation of tropical forest species hinders the improvement of the models and the likelihood of achieving predictions for tropical forests that are consistent across models.

Leaf dark respiration (non-photorespiratory mitochondrial respiration) is highly sensitive to changes in temperature, generally doubling with a 10°C rise in

temperature (i.e., Q_{10} = 2.0) (e.g., Amthor, 1984). Photosynthesis also increases with temperature, but peaks at a lower temperature than respiration (Hüve *et al*., 2011). Consequently, without perfect physiological acclimation, further temperature rise in tropical forests may reduce photosynthesis (Doughty, 2011) while increasing respiration, thus reducing NPP and the size of the potential carbon sink of tropical forests. Respiration is essential to growth and survival of plants, as it provides the energy and carbon skeletons for biosynthesis (Penning de Vries, 1975). However, the increase in respiration with temperature is primarily attributable to increased demand for cellular maintenance (Amthor, 1984; Ryan, 1991), and is not associated with increased growth. Respiration associated with growth is only indirectly affected by warming; it increases with warming only when growth itself is stimulated by rising temperature (Franz *et al*., 2004). In tropical species, however, warming generally results in a decrease in growth (see meta-analyses by Lin *et al*., 2010; Way & Oren, 2010; but see Cheesman & Winter, 2013). This suggests that unless respiration can sufficiently acclimate to warmer temperatures, carbon available for growth, and the potential of tropical forests to store carbon may diminish under future climate scenarios.

Acclimation of respiration to elevated temperature is characterized by a decreased rate at the new temperature compared to non-acclimated plants (Atkin & Tjoelker, 2003; Atkin *et al*., 2005. See Fig. 4-1). A reduction in respiration may also occur when warming leads to more rapid depletion of respiratory substrate (primarily simple sugars and starch), but substrate-limitation does not constitute acclimation. Net photosynthesis of tropical trees and lianas has been shown to decrease with warming a few degrees above current ambient temperatures (Doughty, 2011), suggesting limited capacity for physiological acclimation of tropical species. Whether

respiration in tropical tree and liana species can acclimate to elevated nighttime temperatures is currently unknown, but observations of reduced diameter increment of trees in a tropical lowland forest during years with above-average nighttime temperatures (Clark *et al*., 2003, 2010) suggest that acclimation may be incomplete at the very best.

The main objective of this study was to determine whether dark respiration of fully expanded leaves of tropical canopy trees and lianas can acclimate to elevated nighttime temperature. We further asked whether along with changes in respiration, warming results in changes in other leaf functional traits. Such parallel changes could provide indications of the mechanisms underlying the acclimation process. For example, several studies on temperate and boreal tree species have suggested an important role for leaf nitrogen and carbohydrates in thermal acclimation of respiration (Lee *et al*., 2005; Tjoelker *et al*., 2008, 2009). Furthermore, interspecific variation in changes of leaf traits with warming could potentially become valuable correlates or predictors of thermal acclimation of respiration. Finally, we aimed to calculate the effect of acclimation on whole-canopy leaf respiratory carbon release under elevated nighttime temperature regimes.

We addressed these objectives by experimentally warming branch segments of trees and lianas in the canopy of a semi-deciduous tropical forest in Panama. The result of the warming treatment varied from leaf to leaf, such that nighttime leaf temperatures ranged from ambient to *ca*. 8°C above ambient. We related average nighttime leaf temperature to the rate of leaf dark respiration at 25°C, and to the short-term temperature response of respiration. We chose 25°C as a set temperature because it is widely used for comparison of respiration rates of plants from different biomes (e.g., Wright *et al*., 2006), and it is close to the current mean nighttime

temperature at the study site. Studies with plants from arctic, boreal, temperate and alpine climates have shown that respiration of many species acclimates to changes in ambient temperature (Billings *et al*., 1971; Larigauderie & Körner, 1995; Collier, 1996; Arnone & Körner, 1997; Tjoelker *et al*., 1999a,b, 2008,2009; Atkin *et al*., 2000; Bolstad *et al*., 2003; Lee *et al*., 2005; Xu & Griffin, 2006; Xu *et al*., 2007; Bruhn *et al*., 2007; Ow *et al*., 2008a,b, 2010). Tropical forests experience minimal seasonal temperature changes (Wright *et al*., 2009), and because species have spent the past 2.6 million years of the Quaternary in conditions that were cooler than current tropical forests experience, it has been speculated that heat tolerance and the capacity to acclimate to elevated temperature may have been lost (Corlett, 2011). Our working hypothesis therefore is that respiration of these fully expanded mature leaves will not acclimation to nighttime warming and that warming consequently will lead to increased respiratory carbon release.

Materials and Methods

Study Site and Species selection

The study was conducted in Parque Natural Metropolitano, (PNM, 8°59'N, 79°33'W, 100 m a.s.l.) a seasonal tropical forest near the Pacific coast of the Republic of Panama, near Panama City. Annual rainfall at the site averages 1740 mm, most of which falls during the rainy season from May through December. Annual mean nighttime temperature at PNM between 1995 and 2012 was 24.5°C (range 23.3–26.1°C). This 256-hectare natural reserve consists of 80–150 year old secondary forest with tree heights up to 40 m. A 42-m tall construction crane with a 51-m long jib enables access to canopy leaves.

We selected three tree species (*Anacardium excelsum* (Bertero & Balb. ex Kunth) Skeels; *Luehea seemannii* Triana & Planch.; *Castilla elastica var.costaricana* (Liebm.) CC. Berg) and two liana species (*Bonamia trichantha* Hallier f;

Stigmaphyllon lindenianum A. Juss.) from the upper forest canopy. Henceforth the species will be referred to by their genus name only. Together these species contribute > 25% of the total canopy area (Avalos & Mulkey, 1999).

In Situ Warming Protocol

Nighttime warming of terminal shoots (or individual leaves on them) was achieved by infrared reflective frames fitted with flexible heat rope (Big Apple Herpetological, Inc., New York, USA), positioned 5–10 cm below the target leaves (Fig. 4-2). The design was adjusted to the architecture of the species; it was flat for most species with horizontal leaf display, but cone-shaped for *Anacardium* to account for this species' whorled leaf arrangement on vertically-oriented terminal branches. Identical frames without heat rope were fit on control shoots. The heat rope temperature was controlled by a thermostat, which triggered warming when air temperature dropped below 25°C. This method resulted in warming of leaf temperature only during night by an average of 2–4°C compared to the control leaves. In total 154 leaves were included in the experiment, of which 67 were successfully warmed (average warming > 1°C, and no warming > 10°C at any time) and 87 were used as controls. To assess the repeatability and consistency of the results we set up warming and control frames twice in *Anacardium* and *Luehea.* These repeated experiments were done on different branches and several weeks apart. Temperatures of warmed and control leaves were monitored with type T copper-constantan thermocouple wires attached to the abaxial side of the leaf, and recorded at 5-minute intervals with a Campbell 21X datalogger (Campbell Scientific, Logan, Utah, USA).

Dark Respiration Measurements

After 6–8 days of treatment, twigs were collected pre-dawn at *ca*. 6 a.m., immediately re-cut under water, and brought back to the laboratory in darkness for

measurements. Dark respiration was measured on whole leaves at 2–5 (mode = 3) temperature points between 20 and 32°C with a Walz gas exchange cuvette (GWK 3M, Walz Mess- und Regeltechnik, Eiffeltrich, Germany) connected to a LI-6252 infrared gas analyzer (Licor, Lincoln, Nebraska, USA). Petioles were cut under water and sealed in a 5 ml glass vial with Parafilm to protect the leaves against dehydration during measurement. For each leaf that was measured at 3 or more temperatures a least-square regression line was fit to the $\log_{10}$-transformed leaf respiration rate (R) versus leaf temperature (T_{Leaf}) data according to:

$$\log_{10}(R) = a + bT_{leaf} \quad (4\text{-}1)$$

where a and b, respectively the intercept and the slope of the response curve, are leaf-specific constants. Q_{10} values were calculated from these equations as:

$$Q_{10} = 10^{10b} \quad (4\text{-}2)$$

When R was measured at only two temperatures, Q_{10} was calculated as:

$$Q_{10} = \left(\frac{R_{T2}}{R_{T1}}\right)^{\left(\frac{10}{(T2-T1)}\right)} \quad (4\text{-}3)$$

where T1 and T2 are the lower and higher measurement temperatures respectively. Subsequently, respiration rate at 25 °C (R_{25}) of each leaf was calculated as:

$$R_{25} = \frac{R_{T_{Set}}}{Q_{10}^{(T_{Set}-25)/10}} \quad (4\text{-}4)$$

where R_{Tset} is R_{Leaf} measured at the set cuvette temperate (T_{Set}). We averaged R_{25} over the 2–5 set cuvette temperatures to get a leaf-level R_{25} to use in our analyses. All measurements were made in the wet season, between June and early October, when all selected species had mature but non-senescing leaves.

Functional Trait Data

Leaf area was measured with a LI-3000 leaf area meter (Licor) and leaf mass per unit area (LMA) was calculated as leaf mass (excluding petiole) after drying for >

96 hours at 60°C, divided by leaf area. Tissue N concentration was determined with an elemental analyzer (Costech Analytical, Los Angeles, California, USA). Concentrations of non-structural carbohydrates (soluble sugars and starch) were determined following Dubois (1956) with modifications. Briefly, simple sugars (monosaccharides) were extracted in 80% (v/v) ethanol from 10–15 mg ground sample by shaking followed by 2 two-hour incubations. The supernatant from each sample was collected in a volumetric flask and brought up to 10 ml. Starch was hydrolyzed to glucose from the pellet in 1.1% hydrochloric acid at 100°C. Glucose concentrations were determined colorimetrically with the phenol- sulfuric acid method.

We measured photosynthetic capacity (A_{max}) after 7 nights of warming in one warming experiment of *Anacardium* (16 leaves) and one *Luehea* experiment (12 leaves). A_{max} was measured at ambient temperature (range 29–31°C) and saturating irradiance of 2000 µmol photons m^{-2} s^{-1} with an LI-6400 (Licor). The CO_2 concentration during measurements was maintained at 400 ppm using the built-in CO_2 regulator, and relative humidity was kept between 65 and 85%. All photosynthesis measurements were taken before 9.00 a.m. to avoid mid-day stomatal closure, which can occur as early as 10 a.m. (Zotz *et al*., 1995; M. Slot, pers. obs.).

Data Analysis

To assess acclimation we calculated acclimation ratios using the set temperature method (Loveys *et al*., 2003) as:

$$Acclim_{\mathrm{SetTemp}} = \frac{R_{25\ \mathrm{Control}}}{R_{25\ \mathrm{Warmed}}} \qquad (4\text{-}5)$$

If acclimation has taken place $Acclim_{SetTemp}$ values are > 1.0. To determine to what extent acclimation approached complete homeostasis of respiration across temperatures we used the homeostasis method (Loveys *et al*., 2003):

$$Acclim_{Homeo} = \frac{R_{Control} \text{ at } T_{Control}}{R_{Warmed} \text{ at } T_{Warm}} \quad (4\text{-}6)$$

where T_{Warm} and $T_{Control}$ are the mean nighttime leaf temperatures experienced by warmed and control leaves respectively. When acclimation is completely homeostatic, $Acclim_{Homeo}$ is 1.0; values less than 1.0 indicate that acclimation of the warmed leaf is not completely homeostatic. Acclimation ratios are particularly useful in controlled experiments where temperature variation within a treatment is small. However, a simple dichotomous comparison of warmed leaves and controlled leaves would not account for the considerable variation in temperature within both warmed and control leaves in our experiments. We therefore also assessed acclimation by regressing R_{25} against the average nighttime leaf temperature over the week preceding the R_{25} measurement, where a steeper "acclimation slope" represents greater acclimation. Acclimation slopes and ratios were calculated by species, and by experiment within species for the two *Anacardium* and *Luehea* experiments. To calculate the average % decline in R_{25} per degree of nighttime leaf warming across species, R_{25} of every leaf was divided by R_{25} at the average $T_{Control}$ for each species.

Treatment and species effects were analyzed with analysis of variance; temperature effects on leaf traits were calculated as least square regression. All statistical analyses were performed in R version 2.14.1 (R Development Core Team, 2011).

Estimating Stand-level Respiration Fluxes

To assess the effect of acclimation of respiration to nighttime temperature on the annual nocturnal leaf respiratory carbon (C) flux at the stand level, we compared

different scenarios of warming and thermal acclimation with the leaf respiratory C flux under current climate. First we estimate the current flux. We have previously collected R_{25} and Q_{10} data for 28 species that constitute the canopy at our study site (Chapter 3). We combined this dataset with a 17-year temperature record from PNM (*http://biogeodb.stri.si.edu/physical_monitoring/research/metpark*) to calculate the leaf respiratory C flux under current temperature conditions. In short, we calculated respiration for each species based on 15- or 60-minute mean temperature and integrated that over 12-hour nights, assuming a 4 month dry season during which deciduous species are leafless and semi-deciduous species are leafless for 2 months. We let shade leaves respire at 50% the rate of sun leaves and assumed that species with a leaf area index (LAI: leaf area per unit ground area in $m^2\ m^{-2}$) > 1.0 had LAI minus 1 layers of shade leaves. Where available, we used species-specific estimates of LAI (Kitajima *et al*., 2005) to calculate total flux per unit ground area. For the remaining species we used mean LAI per growth form from Clark *et al*. (2008). Respiration was scaled to the stand level by determining the relative abundance of the canopy trees from their basal area in 2010 census data [Census data from PNM are collected by Smithsonian Tropical Research Institute Forest Dynamics project (Hubbell *et al*., 2005; Condit 1998; Condit *et al*., 2013)].

We re-ran the calculations for elevated nighttime temperature (current + 4°C) to estimate stand-level respiratory C efflux without acclimation. 4°C was chosen to represent end-of-century predictions for tropical South America (predicted rise of 2.5–4.7°C between 2000 and 2100; Cramer *et al*., 2004). Next, we accounted for acclimation by reducing R_{25} by 10% according to findings in the current study, and estimated the effect on C fluxes. Finally, we let R_{25} acclimate to the running average of nighttime temperature of the preceding week, and again total C efflux was

calculated. We let all canopy species acclimate equally, as our results (below) did not suggest acclimation differences among species and growth forms studied.

Results

Warming Effect on Respiration

The warming frames increased leaf temperature at night by an average of 2–4°C (Fig. 4-2). Average nighttime leaf temperature and species identity both had a significant effect on R_{25} (ANCOVA, $P < 0.01$), but their interaction was not significant. Leaves that had experienced warmer nights had lower respiration rates at 25°C (R_{25}). The qualitative pattern was consistent across species, and across repeated experiments within species (Fig. 4-3). The intra-specific variation in R_{25} that was not explained by leaf temperature was considerable, and when analyzing the effect of nighttime leaf temperature at the species level, it was only significant for the two experiments in *Anacardium*. Some of the leaf-to-leaf variation in R_{25} was attributable to differences in leaf nitrogen content. Consequently, the temperature dependence of R_{25} per unit leaf N (R_{25}/N) contained less intra-specific scatter (Fig. 4-3). All experiments showed a significant decline of R_{25}/N with leaf temperature ($P < 0.05$) except for the liana *Stigmaphyllon* ($P = 0.07$) and one of the *Luehea* experiments ($P = 0.09$). Across species R_{25} decreased by an average of 2.9% per degree of warming (Fig. 4-4), and R_{25}/N decreased by 4.2% per degree of warming.

$Acclim_{SetTemp}$ values for R_{25} were consistently > 1.0 and very similar among species, with values ranging from 1.04 to 1.14 (Table 4-1). $Acclim_{SetTemp}$ values for R_{25}/N were a little higher, with an overall mean of 1.17. Acclimation did not result in complete homeostasis of respiration; respiration of warmed leaves measured at their average nighttime temperature was higher than respiration of control leaves measured at the average nighttime temperature of control leaves ($Acclim_{Homeo}$ ratios between 0.73 and 0.94; Table 4-1). Even for *Castilla*, which exhibited the steepest

decline in R_{25} with nighttime temperature (Fig. 4-3), respiration rates at their average nighttime T_{Leaf} of 28.8°C were 10% higher than respiration rates of control leaves at 26.8°C (Fig. 4-1).

Q_{10} values ranged from 2.5 to 3.0, and did not differ systematically between trees and lianas (Table 4-1). Q_{10} was not affected by nighttime temperature.

Warming Effects on Other Leaf Traits

Nighttime leaf warming resulted in a significant increase in nitrogen per unit area (N_{Area}; +1.9% per °C relative to the mean of the control leaves; P = 0.002), sugar per unit area ($Sugar_{Area}$; +2.0% per °C; P = 0.019), and sugar to starch ratio (SS ratio; 2.9% per °C; P = 0.008). The increases in area-based N (N_{Area}) and simple sugars ($Sugar_{Area}$) were caused by marginally significant increases in both LMA and concentrations of N and Sugars per unit mass. The increase in N_{Area} with temperature contributed to the increased significance of the temperature response of R_{25} when expressed on a per unit N basis. Photosynthetic capacity was not affected by nighttime warming in either *Anacardium* or *Luehea* (t-test of control versus warming $P > 0.05$; no correlation with nighttime temperature at the leaf level).

Across-species Correlates of Acclimation

The effect of nighttime leaf temperature on R_{25} was consistent across species with little difference in the degree of acclimation among species. We explored whether these small differences among species correlated with leaf traits that are associated with plant metabolism, using trait means of control leaves to characterize the species. The acclimation slopes of the regression of R_{25} on the mean nighttime leaf temperature over the week preceding the measurements correlated strongly with species means of SS ratio (P = 0.050; R^2 = 0.77; Fig. 4-5). Small differences among species in the average leaf temperature during the week prior to respiration measurements also had a (marginally significant) effect on the extent of acclimation,

with *Castilla*, which had the highest mean nighttime temperature showing the strongest acclimation, and *Stigmaphyllon*, experiencing the coolest nights showing the smallest acclimation after *Bonamia* (Fig. 4-5). Acclimation slopes did not correlate significantly with leaf N content, LMA or species means of R_{25} (data not shown).

Consequence of Acclimation for Stand-level Respiration Fluxes

We estimated the leaf respiratory nighttime carbon efflux to be 4.1 Mg C ha^{-1} yr^{-1} for this site (Fig. 4-6). Increasing nighttime temperature by 4°C, not accounting for acclimation, increased the flux to 5.8 Mg C ha^{-1} yr^{-1}, an increase of 41%. Acclimation in which R_{25} was dependent on the average nighttime leaf temperature of the preceding week (based on data shown in Fig. 4-4) resulted in an annual nighttime C flux of 4.9 Mg C ha^{-1}. This represents a 16% reduction compared to 4°C warming without acclimation. The flux of warm-acclimated leaves under 4°C nighttime warming is still 19% higher than the current flux, highlighting the non-homeostatic nature of respiration in this system.

Discussion

Consistent Acclimation of Respiration to Elevated Nighttime Temperature

Respiration acclimated to higher nighttime temperature, as illustrated by the $Acclim_{SetTemp}$ values greater than 1.0 and by the consistent negative correlation between nighttime temperature and R_{25}. Respiration expressed per unit leaf nitrogen acclimated even more strongly. We warmed leaves in multiple common tree and liana species to assess the generality of the acclimation response and to obtain information useful for scaling carbon fluxes associated with leaf respiration up to the canopy level. The patterns we found were indeed similar among the experiments done in trees and lianas of different species. We can therefore interpret our observations as indicative of thermal acclimation occurring in trees and lianas of the common species in the upper forest canopy at PNM.

These results confirm the observations on arctic, boreal and temperate species that thermal acclimation of respiration of pre-existing leaves can be rapid (Billings *et al*., 1971; Atkin *et al*., 2000; Bolstad *et al*., 2003; Lee *et al*., 2005). Despite the thermal stability of the biome (Wright *et al*., 2009), tropical species have the capacity to thermally acclimate. In tropical tree species acclimation of photosynthesis to elevated daytime temperature has been found to be limited (Cunningham & Read, 2002, 2003a; Doughty, 2011). The fact that respiration does acclimate highlights the importance of maintaining optimal respiratory functioning under changing temperatures, while minimizing carbon loss from maintenance respiration. This is also illustrated by frequent observations in other biomes that respiration acclimates better to temperature than photosynthesis (e.g., Campbell *et al*., 2007; Ow *et al*., 2008a,b, 2010).

Correlates of Acclimation

Carbohydrate concentration was not lower in warmed leaves than in control leaves, nor did it decrease with average nighttime leaf temperature. Thus, our results are not an artifact of measuring respiration of warmed leaves that have become substrate limited. In fact, expressed per unit leaf area, the concentration simple sugars, the primary substrate of respiration, actually increased with temperature, as did the SS ratio. Nighttime warming can stimulate photosynthesis (Turnbull *et al*., 2002, 2004), which could in turn increase leaf sugar content, but photosynthetic capacity was not affected by warming. Warming can also restrict translocation of sugars when sieve plate pores get blocked by callose (McNairn & Currier, 1968). However, translocation blockage would also increase $Sugar_{Mass}$, but this was not significant.

The absence of correlation between R_{25} and Sugar suggest that R_{25} of these leaves is not substrate limited. Nor does it seem likely that there were treatment differences in demand for respiratory products through changes in sink strength away from the source leaves, as warmed and control leaves always came from the same plant. Most likely the decrease in R_{25} with temperature resulted from a change in respiratory capacity, associated with changes in concentrations or relative amounts of mitochondrial enzymes (Atkin *et al*., 2005). The change in N_{Area} with temperature may be associated with shifts in relative amounts of enzymes that differ in N content, but it is unclear what the functional significance of increased Sugar and SS ratio is with regards to thermal acclimation of respiration.

Leaf nitrogen and carbohydrates have repeatedly been associated with respiratory acclimation (Lee *et al*., 2005; Tjoelker *et al*., 2008, 2009). Interestingly, the species in which carbohydrates and nitrogen are associated with acclimation are mid- to high latitude tree species that routinely experience low temperatures. Acclimation to low temperature results in elevated respiration rates compared to non-acclimated plants (Atkin & Tjoelker, 2003). At the same time acclimation to low temperature tends to involve an increase in leaf sugar concentrations associated with cryoprotection (Kozlowski, 1992; Strand, 2003). It seems therefore unclear whether the correlation of carbohydrates with respiration across acclimation temperatures is related to respiratory acclimation per se or represents the composite of multiple acclimation processes including cold-hardening. Leaf nitrogen concentration is also higher in cold grown plants (Weih & Karlsson, 2001; Tjoelker *et al*., 1999b, 2008,2009; Lee *et al*., 2005), possibly in conjunction with elevated protein content to compensate for inhibition of photosynthesis at low temperature (Pyl *et al*., 2012). Interestingly, expressed per unit area, nitrogen content increased in warmed leaves

in our study. As a result, R_{25}/N decreased more strongly with temperature than R_{25} per unit leaf area did. The underlying mechanism and the functional importance of this are not clear. Acclimation to elevated temperature is likely to involve different processes than acclimation to low temperatures, and consequently the traits that correlate with the observed acclimation are likely to differ as well.

Acclimation in both Trees and Lianas

Acclimation was consistent among species, and there was no evidence that trees and lianas differed in their acclimatory capacity. Doughty (2011) found that A_{max} of canopy tree leaves was more negatively affected by *in situ* warming than A_{max} of liana leaves, suggesting lianas had a greater capacity for acclimation, but a mechanistic explanation for this observation was not identified. Boreal evergreen tree species acclimate more completely than broadleaved species (Tjoelker *et al*., 1999a,b), which fits the general assumption that species that experience large intra-annual temperature variations have greater acclimation capacity than species that do not. Studies using temperate species have not found systematic differences in acclimation capacity among different plant functional types (Campbell *et al*., 2007) or between inherently slow and fast growing species (Loveys *et al*., 2003). A more complete assessment of thermal acclimation of tropical trees and lianas will be necessary to verify the consistency of our results of comparable acclimation capacity in these growth forms.

Across species acclimation correlated positively with the sugar to starch ratio of leaves, but not with nitrogen or total non-structural carbohydrate (TNC) concentrations. Similarly, Loveys *et al*. (2003) found no relationship between concentrations of TNC and nitrogen and the capacity of species to thermally acclimate. The liana leaves in our experiment had significantly lower concentrations

of simple sugars and higher concentrations of starch, and consequently much lower sugar to starch ratios. The correlation between sugar to starch ratio and the acclimation slopes of leaves from different species was, however, not solely driven by the lianas (Fig. 4-5).

Consequences of Acclimation for Predicted Respiratory Carbon Fluxes from Tropical Forests

We let R_{25} of all canopy species acclimate to the same extent according to our observations from a series of 6–8 day warming experiments. The estimated effect of acclimation under 4°C nighttime warming was considerable at our study site; a reduction of nocturnal leaf respiratory carbon release of 0.9 Mg ha^{-1} yr^{-1} (16%) compared to a no acclimation scenario. Because acclimation did not result in maintenance of homeostatic respiration rates, the calculated flux was still 0.8 Mg C ha^{-1} yr^{-1} (19%) larger than the flux at current temperatures. However, acclimation of leaves developed at a new temperature tends to be greater than acclimation of pre-existing leaves (Loveys *et al*., 2003; Armstrong *et al*., 2006). As nights gradually warm over the coming decades, respiration is likely to acclimate to a greater degree than what we observed for pre-existing leaves, which would further mitigate the increase in carbon release from nighttime respiration.

Significance for Modeling

It has long been recognized that acclimation to temperature should be considered in global vegetation models, as it can reduce the magnitude of the positive feedback between climate and the carbon cycle in a warming world (King *et al*., 2006). Many DGVMs and ecosystem models still do not address acclimation, however, and many that do, use a temperature dependence of Q_{10} to represent acclimation (see for reviews of such models Wythers *et al*., 2005; Smith & Dukes, 2013). The use of a temperature-dependent Q_{10} is based on the observation that Q_{10}

values decrease with increasing temperature of measurement (Tjoelker *et al*., 2001; Atkin & Tjoelker, 2003). A temperature-dependent Q_{10} can contribute to success simulation of carbon fluxes in highly seasonal biomes (Chen & Zhuang, 2013; Wythers *et al*., 2013). However, implementing a temperature-dependent Q_{10} in a global model may result in low Q_{10} values being assigned to vegetation in tropical climates. In the current study Q_{10} values ranged from 2.5 to 3.0 for the temperature interval 22–32°C (midpoint 26°C) and Q_{10} did not decrease with warming. These values are much higher than what would be predicted for 26°C from the temperature-dependent Q_{10} model (Q_{10} = 3.09 - 0.043T (Atkin & Tjoelker, 2003). Q_{10} = 1.97 at T = 26°C), so for calculation of daily respiratory carbon fluxes the assumption of a declining Q_{10} would result in erroneous estimates. The use of a temperature-dependent Q_{10} in global models requires careful consideration of the timescale over which the Q_{10} responds to temperature. Considering acclimation of R_{25} to the temperature of the previous days or nights offers a more realistic approach, and is now supported by empirical data from tropical forests (this study) as well as from other biomes.

Concluding Remarks

Here we showed that mature leaves of tropical trees and lianas can acclimate to elevated nighttime temperatures in 6–8 days by down-regulating R_{25} independent of availability of respiratory substrate. Despite the short duration of the warming experiments the nature of the acclimation response suggested that the respiratory capacity of leaves was adjusted. Implementation of these acclimation responses into a simulation of stand-level nocturnal leaf respiration under 4°C warming reveals the potential to compensate simulated nighttime warming, a level of warming predicted for the end of the 21st century. Global models could be improved by accounting for

temperature acclimation of leaf respiration. Assuming respiration at a set temperature acclimates to the temperature of the previous 6–8 nights is preferable to assuming a temperature-dependent Q_{10}.

Table 4-1. The tree and liana species used in this study; their mean respiration rates at 25°C (R_{25}), R_{25} per unit nitrogen (R_{25}/N) and Q_{10} values in warmed and control leaves; and the 'set temperature' and 'homeostasis' acclimation ratios ($Acclim_{SetTemp}$, $Acclim_{Homeo}$), and the acclimation slopes for R_{25} and R_{25}/N, where the acclimation slope represents the change in R_{25} or R_{25}/N per °C (Δ flux °C^{-1}; more negative indicates stronger acclimation). For *Anacardium* and *Luehea* results from two experiments are shown. Different letters indicated significant differences between control and treatment at $P < 0.05$. Significant acclimation slopes ($P < 0.05$) are indicated with an *.

Species	Growth form		Treatment	R_{25}	R_{25}/N	Q_{10}	$Acclim_{SetTemp}$		$Acclim_{Homeo}$		Acclimation slope (Δ flux °C^{-1})	
				µmol m^{-2} s^{-1}	µmol (mg N)$^{-1}$ s^{-1}		R_{25}	R_{25}/N	R_{25}	R_{25}/N	R_{25}	R_{25}/N
Anacardium excelsum	Tree	1	Control	0.91	0.044	2.3	1.13	1.09	0.83	0.80	-0.047*	-0.0020
			Warmed	0.81	0.040	2.6						
		2	Control	0.75	0.040	2.6	1.12	1.08	0.84	0.82	-0.027*	-0.0011*
			Warmed	0.67	0.037	2.4						
Castilla elastica	Tree		Control	1.06^{a}	0.048^{a}	2.9	1.14	1.15	0.94	0.95	-0.062	-0.0032*
			Warmed	0.94^{b}	0.042^{b}	3.0						
Luehea seemannii	Tree	1	Control	0.94	0.050^{a}	2.7	1.04	1.14	0.84	0.92	-0.029*	-0.0021*
			Warmed	0.90	0.044^{b}	2.6						
		2	Control	0.99	0.045	2.5	1.14	1.24	0.82	0.89	-0.015	-0.0018*
			Warmed	0.88	0.036	2.7						
Bonamia trichantra	Liana		Control	0.73	0.059^{a}	3.0	1.05	1.21	0.73	0.84	-0.008	-0.0027*
			Warmed	0.69	0.048^{b}	2.9						
Stigmaphyllon lindenianum	Liana		Control	1.03^{a}	0.053^{a}	2.8	1.14	1.27	0.91	1.00	-0.021	-0.0026
			Warmed	0.91^{b}	0.042^{b}	3.0						

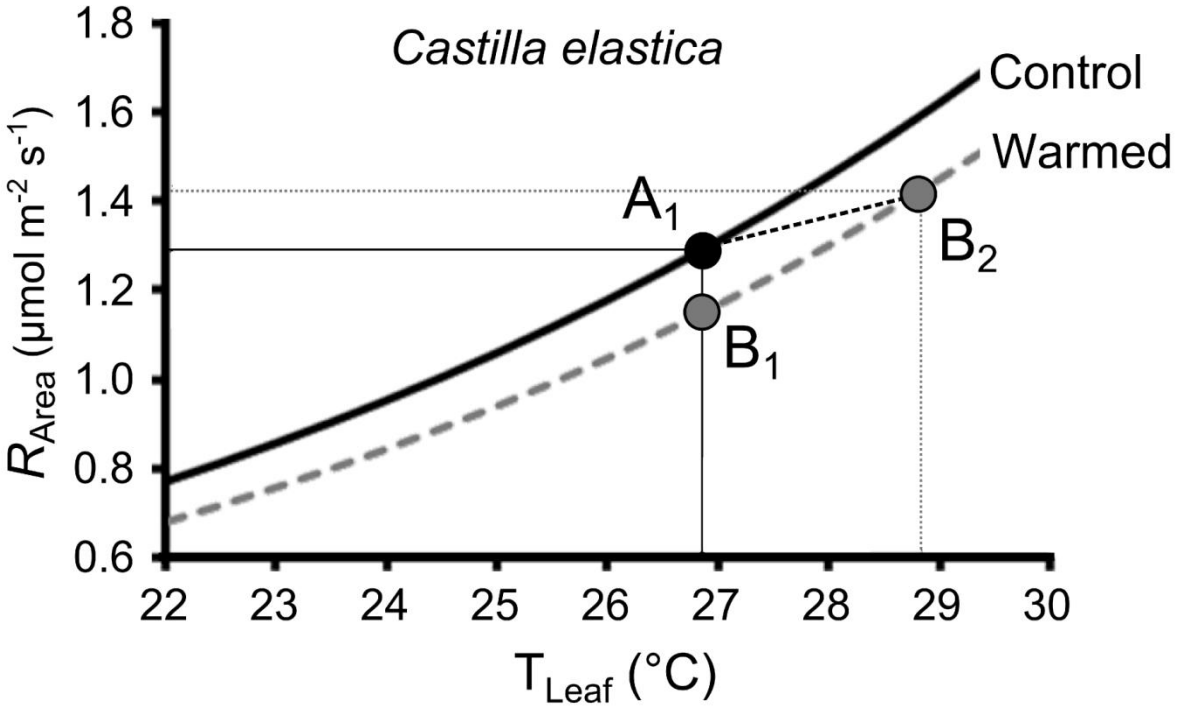


Figure 4-1. Example of acclimation, using the actual leaf temperature data and R_{25} and Q_{10} values for warmed and control leaves of *Castilla elastica*. Warmed leaves exhibit down-regulation of respiration at a set temperature ($A_1 > B_1$), but acclimated leaves respire more at their average nighttime temperature (28.8°C) than control leaves experiencing their average temperature (26.6°C) ($A_1 < B_2$), so acclimation is not completely homeostatic.

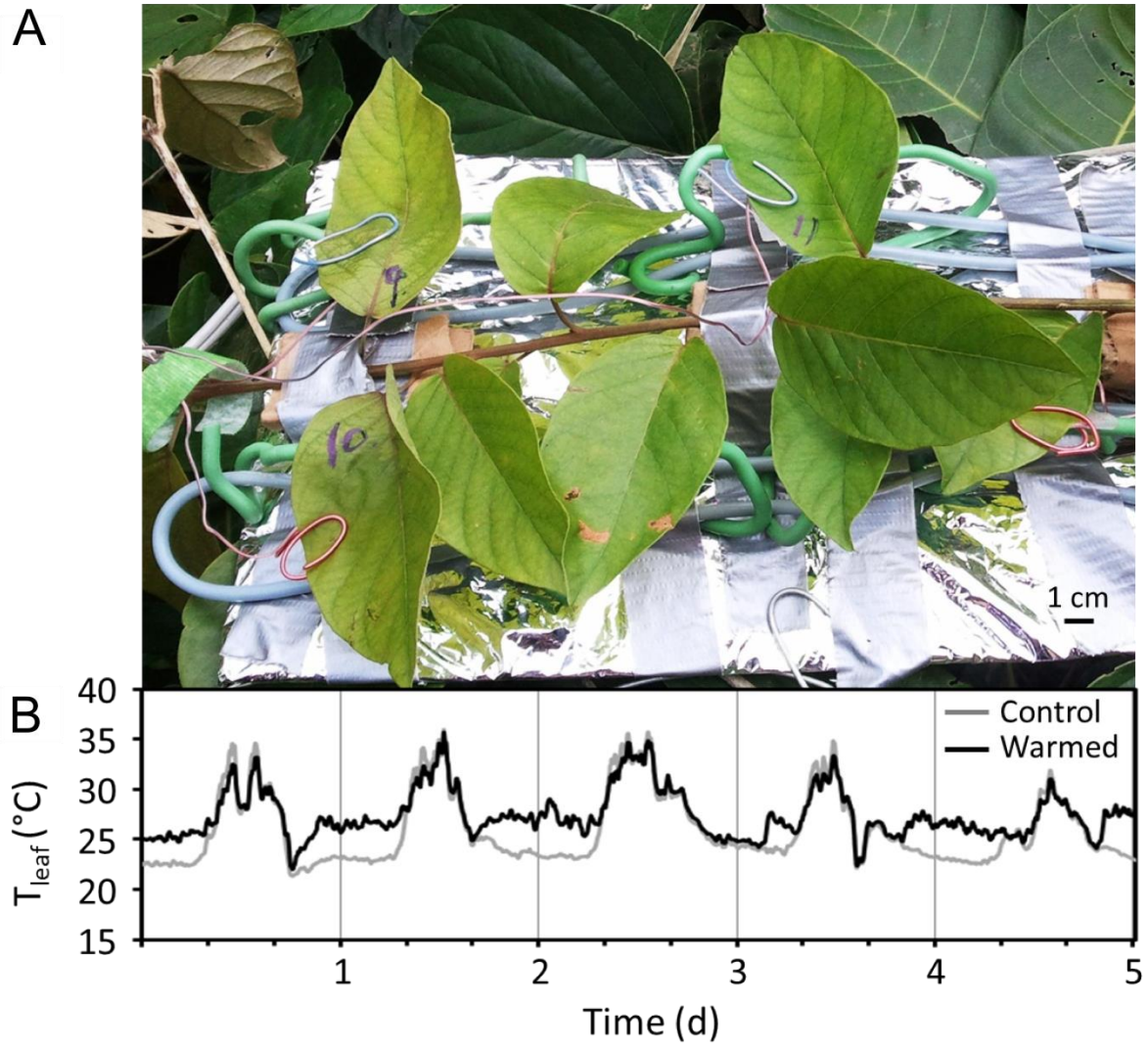


Figure 4-2. Example of experimental warming set up. A) Warming frame fit to the terminal shoot of the liana *Bonamia*. B) Average temperatures of control and warmed leaves of 5 days of the 6-day experiment with this species. Natural leaf angles were maintained as much as possible, but leaves were prevented from touching the heating rope by fitting rubber-coated twist-tie frames between the leaf and the heating rope.

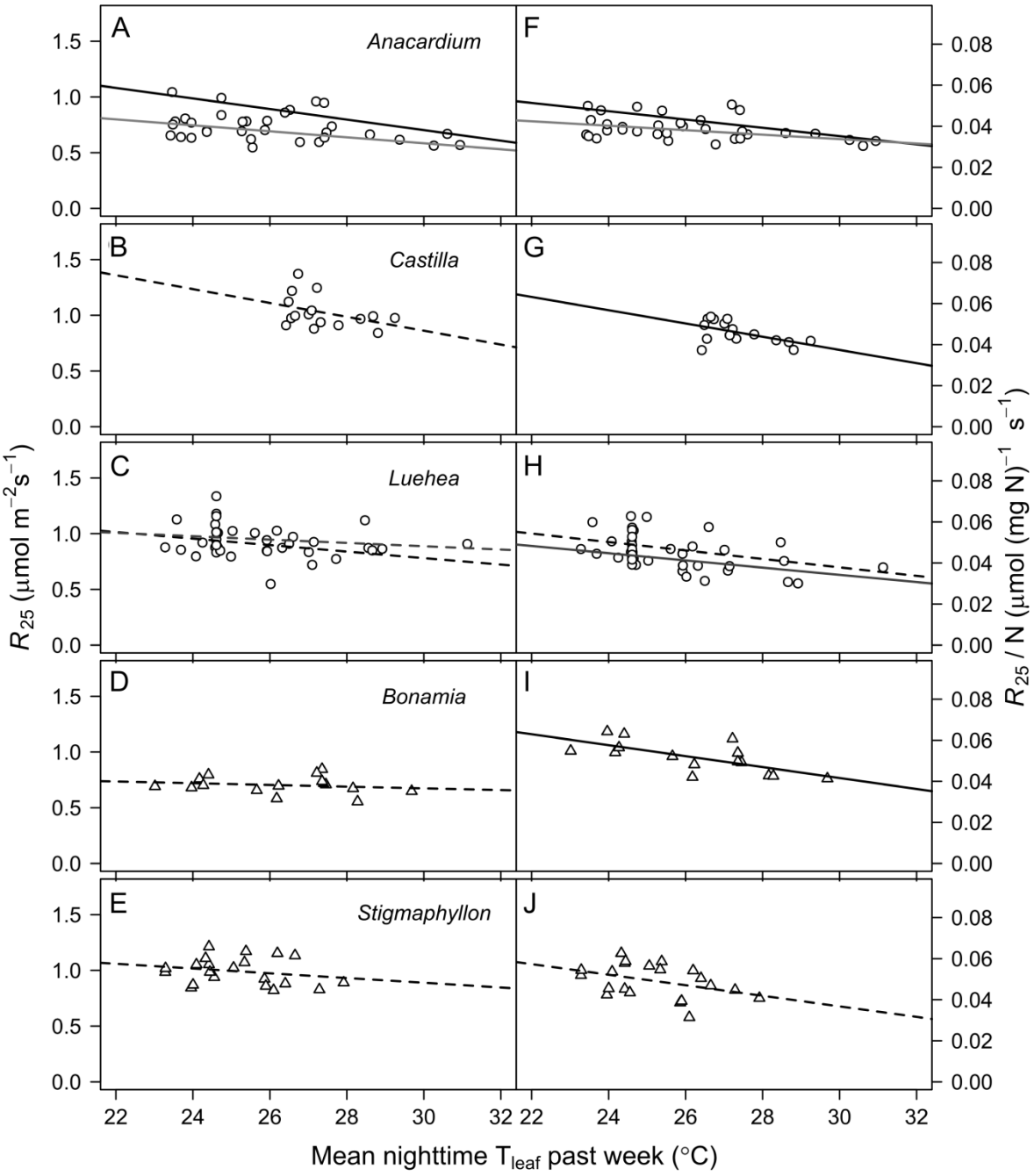


Figure 4-3. Leaf dark respiration at 25°C (R_{25}) in relation to the average nighttime temperature these leaves experienced in the preceding 6-8 days for the tree species *Anacardium*, *Castilla* and *Luehea* and the liana species *Bonamia* and *Stigmaphyllon*. A-E). R_{25} per unit leaf area. F-J) R_{25} per unit leaf nitrogen. For *Anacardium* and *Luehea* two experiments were performed. Trend lines for the different experiments are shown in different colors. Dashed lines are not significant at $P = 0.05$.

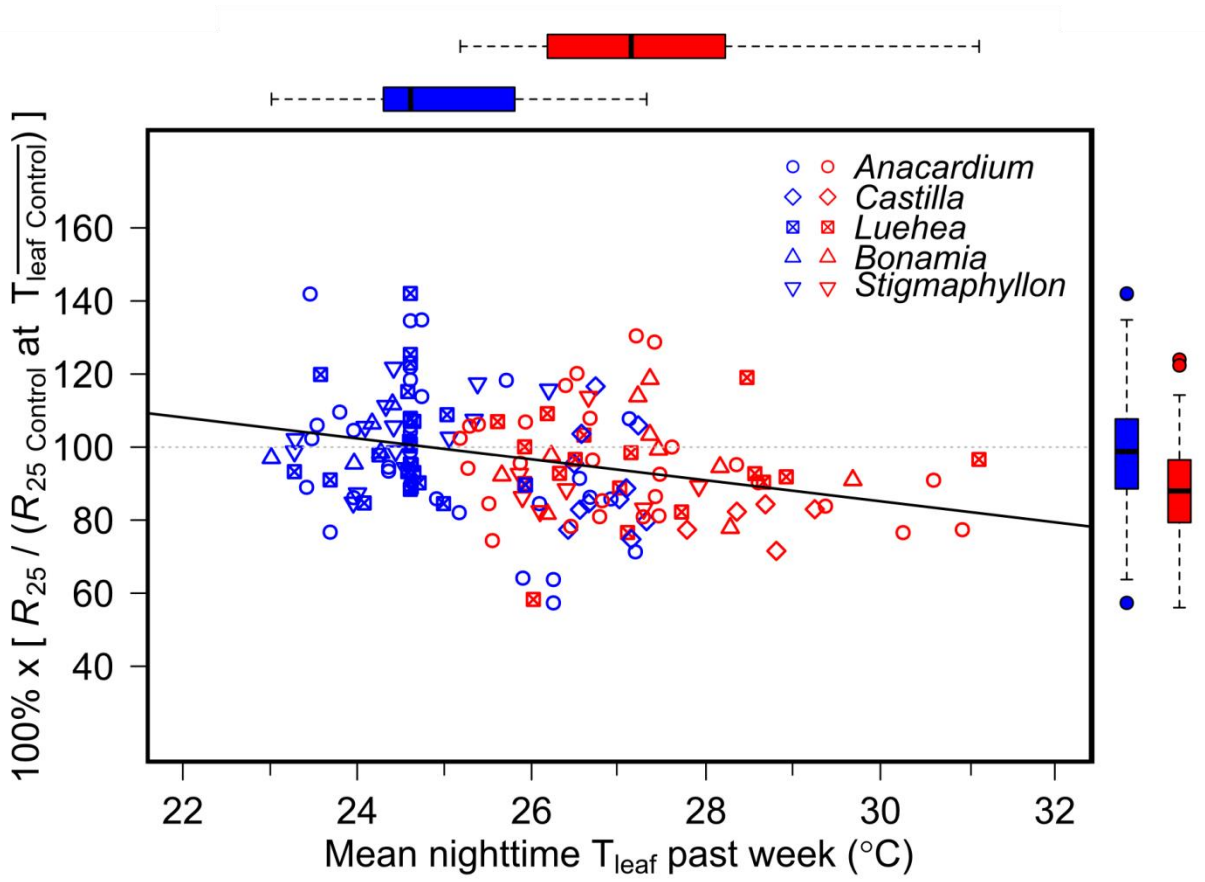


Figure 4-4. Respiration at 25°C (R_{25}) in relation to the average nighttime leaf temperature during the experiment, standardized by the species mean R_{25} of control leaves (blue) at their average nighttime temperature (= 100%). Horizontal box plots indicate the mean, median and spread of warmed (red) and control (blue) leaves across species. Vertical box plots show the mean, median and spread of standardized R_{25} values of warmed and control leaves. Notice that some control leaves are warmer than warmed leaves because of variation within species, among-branches, leaves and experiments; some experimental periods had overall higher temperatures (see also Fig. 4-3), control leaves in these experiments experienced were warmer nighttime temperature than warmed leaves in other experiments.

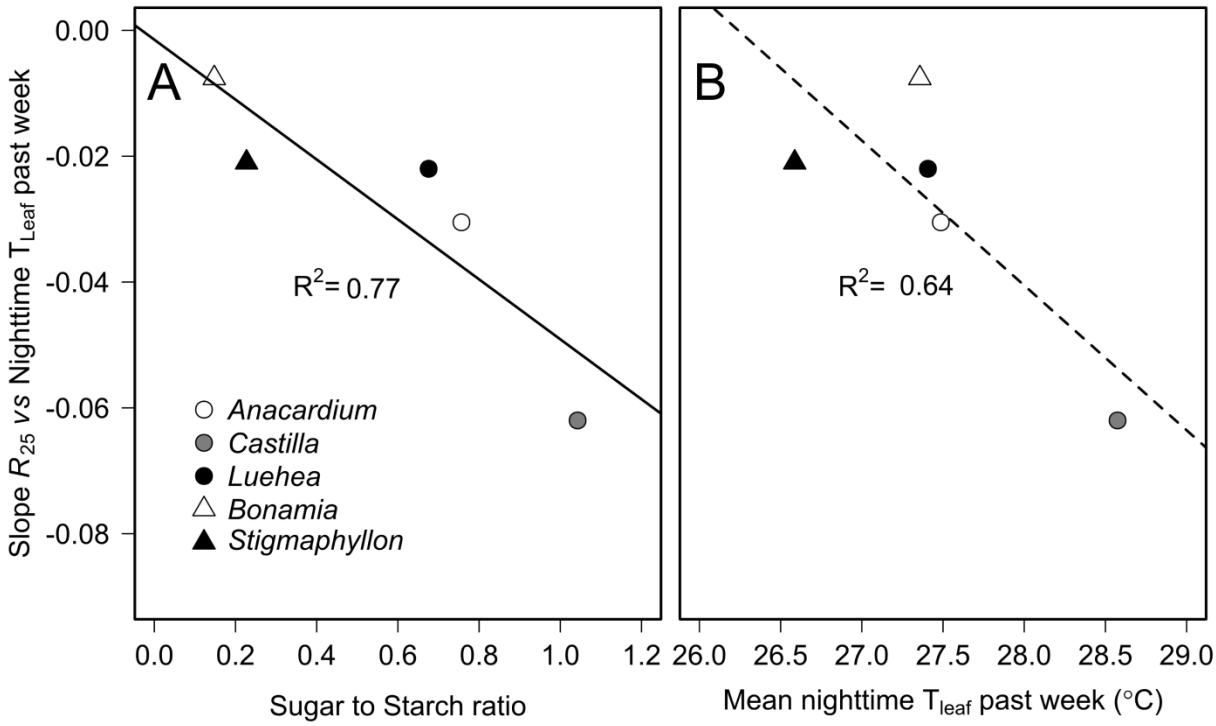


Figure 4-5. Correlations between species-level acclimation other leaf traits where acclimation is represented by the slope of R_{25} versus the mean nighttime leaf temperature (more negative indicates stronger acclimation). A) Acclimation vs. leaf sugar to starch ratio. B) Acclimation vs. average nighttime leaf temperature. The solid line in A indicates a significant correlation at $P < 0.05$; the dashed line is non-significant ($P = 0.1$).

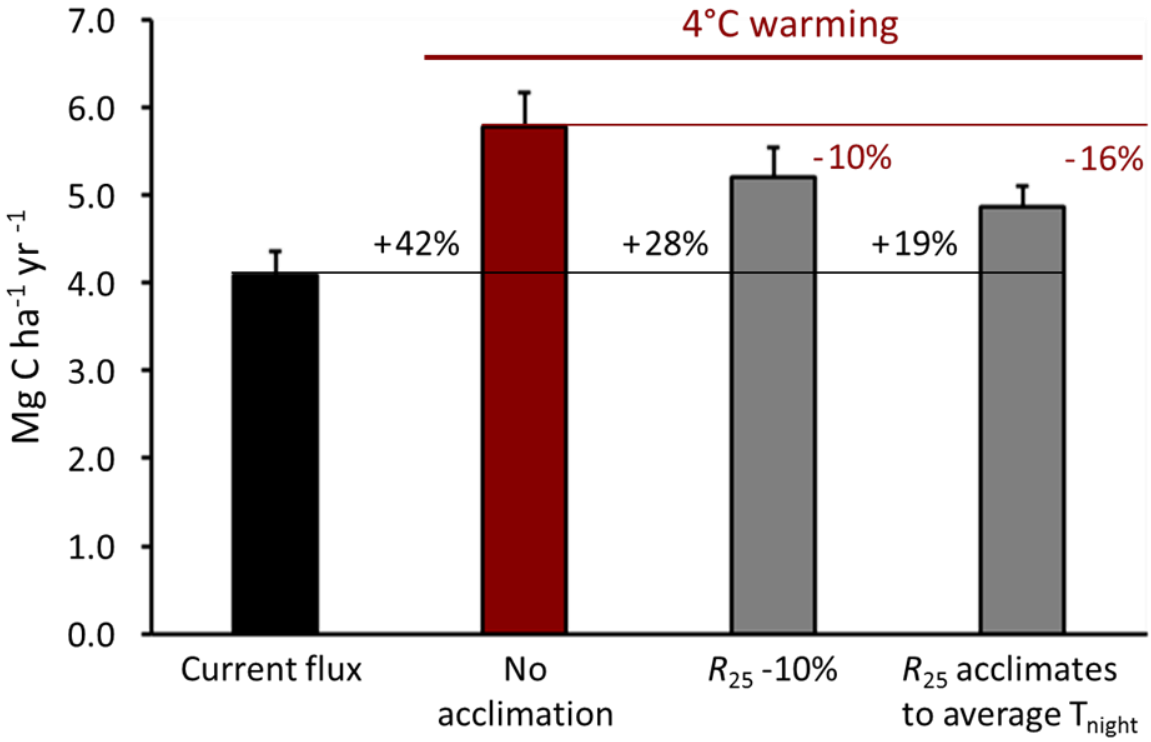


Figure 4-6. The estimated annual nighttime carbon efflux from leaf respiration at Parque Natural Metropolitano under 'current' temperature (1995–2011), and under 4°C warming with different acclimation scenarios; no acclimation, acclimation results in 10% reduction of R_{25}, or R_{25} acclimated to the average nighttime leaf temperature (T_{Leaf}) during the preceding week. In black numbers the percentage increase in fluxes relative to current are shown. Red numbers indicated the percentage decline in fluxes relative to the scenario of no acclimation. Error bars represent the standard deviation of flux estimates for the 17 years.

CHAPTER 5
GENERAL PATTERNS OF THERMAL ACCLIMATION OF LEAF DARK RESPIRATION ACROSS BIOMES AND PLANT TYPES

Background

Climate warming is predicted to increase the release of carbon dioxide from the terrestrial biosphere into the atmosphere, thus triggering a positive climate-terrestrial carbon feedback that accelerates warming (Cox *et al*., 2000; Luo, 2007). However, plant respiration (non-photorespiratory mitochondrial CO_2 release) may acclimate to warming and this acclimation may reduce the potential decline in net primary productivity (NPP) (King *et al*., 2006; Smith & Dukes, 2013). This chapter is an attempt to synthesize results from empirical studies on thermal acclimation of leaf dark respiration from across the globe. First, we briefly review the current understanding of thermal acclimation of respiration. We then discuss the aspects of tropical forests that lead to the supposition that tropical vegetation may respond differently to climate warming than cooler climate vegetation. Finally, we analyze published data on thermal acclimation of leaf dark respiration and discuss the results of the meta-analysis in the context of climate warming as anticipated for the current century.

Thermal Acclimation of Leaf Dark Respiration

Respiration increases exponentially with short-term temperature increment. This sensitivity of respiration to changes in temperature is primary driven by an increase in the demand for cellular maintenance, associated with increased protein turnover and membrane leakage at higher temperatures (Amthor, 1984; Ryan, 1991). Respiration is essential for growth and survival of plants as it provides energy and carbon skeletons for biosynthesis (Penning de Vries, 1975), but respiration associated with growth only increases with warming when growth itself is stimulated by rising temperature (Franz *et*

al., 2004). Thermal acclimation of respiration thus primarily involves changes in respiration associated with maintenance processes, rather than with growth.

Thermal acclimation of respiration is a physiological, structural, or biochemical adjustment by an individual plant in response to a change in temperature, that is manifested as an alteration in the short-term response to temperature (Smith & Dukes, 2013) (Fig. 5-1). Acclimation of respiration to a higher temperature regime results in a decreased rate at the new, elevated temperature compared to non-acclimated plants measured at that temperature (Atkin & Tjoelker, 2003; Atkin *et al*., 2005). Thermal acclimation of respiration functions to maintain optimal supply of ATP and carbon skeletons while minimizing carbon loss from respiration associated with maintenance processes. This may be achieved by changes in mitochondrial membrane composition to minimize ion leakage under warmer conditions (Raison *et al*., 1980), or by a reduction in the overall protein turnover rate, e.g., by a change in mitochondrial protein composition (Atkin *et al*., 2005). When respiration under warmed conditions equals the respiration rate exhibited by leaves under control conditions, homeostasis of respiration is maintained (Fig. 5-1).

Acclimation may occur within a few days of a temperature change (Rook, 1969; Billings *et al*., 1971; Atkin *et al*., 2000; Bolstad *et al*., 2003; Lee *et al*., 2005), but longer exposure to a new temperature may result in a greater degree of homeostasis (Smith & Hadley, 1974). As climate warming is a gradual process, the potential for acclimation needs to be assessed not just over a few days, but also following extended periods of warming. Furthermore, leaves developed under an experimental temperature are often more completely acclimated than fully formed leaves transferred to that temperature

(e.g., Campbell *et al*., 2007). Campbell *et al*. (2007) found no systematic differences in acclimation among different growth forms, but Tjoelker *et al*. (1999) found boreal evergreen tree species to acclimate better to experimentally imposed temperature differences than deciduous tree species. Given that most dynamic global vegetation models (DGVMs) use plant functional types to characterize vegetation, it would be valuable to identify systematic differences in acclimation potential among plant functional types if such differences were to exist.

Two types of acclimation of respiration have been identified (Atkin & Tjoelker, 2003) (Fig. 5-1). The type of acclimation indicates the mechanism underlying the acclimation process. Type I acclimation involves a decrease in the slope of the respiration-temperature response curve (i.e., lower short-term temperature sensitivity (Q_{10}) in warm-acclimated leaves), typically under influence of regulatory changes of existing respiratory enzymes (Atkin *et al*., 2005). Type II acclimation, a decrease in the elevation of the temperature response curve of respiration (i.e., lower respiration across the temperature range, without a change in Q_{10}), typically involves a change in overall respiratory capacity. The respiratory capacity may change under the influence of a change in the relative amounts of individual respiratory enzymes, or in the concentration of mitochondrial proteins (Atkin *et al*., 2005). Type II acclimation is expected to be more common for leaves developed at elevated temperature whereas Type I acclimation, associated with changes in existing enzymes, is thought to be more common in leaves that existed prior to the change in temperature (Atkin & Tjoelker, 2003; Atkin *et al*,. 2005). Ultimately, both types result in a reduction in respiration at warm conditions compared to non-acclimated leaves. It will nevertheless be valuable to identify patterns

in acclimation type to aid in predictions of changes in respiratory fluxes, as the short-term sensitivity (Q_{10}) changes in Type I but not in Type II.

Potential Differences in Warming Response of Tropical and Cool Climate Vegetation

Based primarily on mid- and high latitude acclimation studies, the current consensus is that most plant species can, in principle, acclimate to changes in temperature (Atkin & Tjoelker, 2003; Atkin *et al*., 2005), although acclimation responses are often species-specific (e.g., Larigauderie & Körner, 1995). There are, however, three important differences between lowland tropical forests and higher latitude ecosystems in their responses to climate warming.

First, tropical forests are close to their thermal optimum temperature (Doughty & Goulden, 2008) and further warming of tropical ecosystems will push the majority of tropical forests into a climate envelope currently not occupied by closed-canopy forest (Wright *et al*., 2009). Respiration at ambient temperature increases exponentially with mean annual temperature, and is thus already high in tropical forests (Wright *et al*., 2006). The absolute increase in respiration per degree of warming above ambient will be greater in the tropics than in high latitudes, as warming will occur along the steeper end of the exponential temperature response curve (Dillon *et al*., 2010). Compared to warming of cool-grown plants, a much greater down-regulation of respiration is required for acclimation to maintain homeostasis of respiration per degree of increase of temperature. This may constitute a considerably greater challenge than down-regulation of the relatively small change in absolute metabolic rates with warming on the cooler end of the temperature spectrum.

Second, tropical forests experience minimal seasonal temperature fluctuations (Wright *et al*., 2009), and the thermally stable environment of the tropics may not have favored evolution of the capacity to acclimate to temperature changes (Janzen, 1967; Cunningham & Read, 2003a; Ghalambor *et al*., 2006). Comparative studies of thermal acclimation of photosynthesis of temperate and tropical rainforest species indeed found that tropical species do not acclimate as completely as temperate species (Cunningham & Read, 2002, 2003b).

Third, for the past 2.6 million years of the Quaternary tropical regions have experienced conditions that were relatively cool compared to current and near-future temperatures, and natural selection would not have favored heat-protective genes and traits (Corlett, 2011, 2012). The proximity of tropical vegetation to experiencing supra-optimal leaf temperatures makes the issue of high temperature stress particularly pressing in the tropics. Heat stress can lead to protein denaturing (Vierling, 1991) and increased membrane fluidity (Quinn, 1989), factors that increase the respiratory demand for maintenance, and that are as such conflicting with an acclimatory decrease in respiration.

Very few studies on thermal acclimation have been done in the tropics and the predictions about respiratory acclimation in tropical forests are therefore difficult to make. By synthesizing data from around the globe in this meta-analysis we sought to determine the effects on thermal acclimation of respiration of 1) the biome of origin of the study species, 2) the duration of exposure to warming, 3) the developmental status of the leaf (pre-existing when temperature change was imposed, or newly-developed at the experimental temperature), 4) the growth form under investigation, and 5) the

degree of warming or the temperature difference across contrasting temperatures. General patterns in thermal acclimation across the globe may inform us about the likely response of tropical forests to climate warming.

Methods

Data Selection

We analyzed the results of studies where leaf dark respiration was measured for plants grown under different temperatures; measured repeatedly under changing ambient temperature conditions; were grown in common gardens at different ambient temperature regimes; or were exposed to experimental warming above ambient temperature. We searched Google Scholar and the Institute for Scientific Information (ISI) Web of Science for studies that 1) used non-cultivated plant species, 2) that exposed plants to at least two growth/acclimation temperatures, and 3) that measured respiration at the respective ambient temperatures, or at the same temperature for both groups, or both (e.g., by measuring full temperature response curves of respiration of control and warmed leaves). Most studies used growth cabinets to assess the effect of growth temperature or short-term temperature changes. Research on physiological cold-acclimation commonly uses the same set up, but these are not included in this study. In total 30 studies were included (Table B-1), reporting on 237 temperature contrasts of 87 species. While the motivation for this study was to synthesize information that could inform us about plant responses to climate warming, only a small number of the available studies compared respiration at ambient temperature with respiration of leaves warmed to above-ambient temperatures (18 out of a total of 237 species-by-temperature contrasts; 14 of 87 species). These studies report on species from alpine, arctic and Antarctic, boreal, temperate, and tropical climates and include

forbs, graminoids (sedges and grasses), and evergreen and deciduous shrubs, trees and lianas (Table 5-1). Studies that warmed plants or leaves during the night only were also included, as respiration does not necessarily acclimate to mean daily temperature (e.g., Atkin *et al*., 2000), and has been shown to acclimate to nighttime temperature instead (Bruhn *et al*., 2007). Data were extracted from tables, (enlarged) figures or from online supplementary information.

Data Analysis

To assess acclimation responses quantitatively we extracted information from these studies that enabled us to calculate 'acclimation ratios' based on the set temperature method and the homeostasis method (Fig. 5-2). For studies that used growth cabinets to expose plants to two or more different temperatures, the lower of the two was considered as the control temperature. Similarly, when temperature changes associated with seasons, changes in weather systems, or geographical range of common gardens were used, the lower temperature regime was considered to represent the control. To calculate $Acclim_{SetTemp}$ the control temperature was used as the set temperature whenever possible; sometimes a temperature intermediate to the control and warming temperature was used instead. For 56 temperature contrasts (41 species) data were available to calculate both acclimation ratios, but more commonly only $Acclim_{SetTemp}$ (139 contrasts, 39 species) or $Acclim_{Homeo}$ (43 contrasts, 36 species) could be calculated. Because $Acclim_{LTR10}$ (Fig. 5-2) requires information on both the initial Q_{10} and the long-term acclimation ratio LTR_{10}, this metric could not be calculated for most studies and was not included in the following analyses. It was not always possible to determine the uncertainty associated with the values used to calculate the acclimation ratios; variances were not always given; in some cases values were

extracted from fitted curves for which no confidence intervals were presented; or tables did not specify whether standard deviations of standard errors of the mean were presented. When sample size and standard errors were available, we tested whether respiration of control and warmed leaves were significantly different by assuming a normal distribution of the data and calculating 95% confidence intervals.

Acclimation Type

To properly assess which type of acclimation has occurred one would ideally have temperature response curves that go down to the low-temperature 'basal respiration'. In case of Type I acclimation the control and acclimation curve would 'hinge' at this point, whereas in case of Type II acclimation the curves would never intersect. Generally, however, respiration is not measured at low enough temperatures identify where the temperature response curve would intersect. It is more common to compare the slopes of log-transformed temperature response curves to test difference in slopes. When the slope of the warm-acclimated leaf is lower, Type I acclimation is supposed. If the slopes are not significantly different, but have lower intercept for warm-acclimated leaves then Type II acclimation is implicated. Accordingly, when data was available to compare slopes of control and acclimated leaves, results were classified as Type I or Type II, or no acclimation.

To explore whether certain environmental factors may influence either $Acclim_{SetTemp}$ or $Acclim_{Homeo}$ general linear models were tested for the following explanatory variables: biome of origin, growth form, temperature difference, duration of exposure, maximum nighttime temperature, method (warming above ambient, or not), and leaf developmental status (whether the measured leaves existed prior to exposure to the warmer temperature, or that they developed at the warm temperature). Leaf habit

(evergreen versus deciduous), was determined for woody species only, so in addition to the above models, we analyzed $Acclim_{SetTemp}$ and $Acclim_{Homeo}$ of the subset of woody species with models that included leaf habit. We also determined the most parsimonious significant linear regression model of $Acclim_{SetTemp}$ and $Acclim_{Homeo}$ that revealed minimal pattern in the residuals using the same candidate predictors as above. All statistical analyses were performed in R version 2.14.1 (R Development Core Team 2011).

Results

Temperature contrasts over which acclimation was determined ranged from 0.3°C in an *in situ* infrared heating experiment of eucalypt seedlings (Bruhn *et al*., 2007), to 21°C in a growth cabinet study on temperate tree, forb and graminoid species grown in hydroponics (Campbell *et al*., 2007). Respiratory response to change in temperature ranged from acclimation leading to complete homeostasis of respiration across the study temperatures, to no change in the instantaneous temperature response curve whatsoever. Acclimation to warmer temperatures resulted in a down-regulation of respiration at a set temperature in 179 out of 195 contrasts for which information on respiration at a set temperature was available (Fig. 5-3). This down-regulation was significant in 75% of the cases for which data was available to determine significance. Complete homeostasis ($Acclim_{Homeo} = 1.0$), or acclimation leading to over-compensation ($Acclim_{Homeo} > 1.0$) was, however, rare and the mean $Acclim_{Homeo}$ value (0.76 ± 0.29; mean ± SD) was significantly smaller than 1.0 (t-test, $t = -7.9$, $df = 97$, $P < 0.0001$). The tendency for down-regulation of respiration in warm-acclimated leaves was consistent across biomes and growth forms (Table 5-1).

Treatment and Species Effects on Acclimation of Respiration

Summary by biome appears to suggest lower $Acclim_{SetTemp}$ in the tropics than in cooler ecosystems (Fig. 5-4). However, this apparent pattern in $Acclim_{SetTemp}$ across biomes could be explained by the average degree of warming used in the experiments; in cooler ecosystems studies warmed plants by a greater degree than studies from the tropics (Table 5-1). When the effect of the degree of warming was accounted for, the biome of origin was no longer a significant predictor of either of the acclimation ratios. The mean maximum nighttime temperature that treatment plants were exposed to did not affect the acclimation potential of respiration either; studies at high temperature (warmest 33%: mean $T_{Night\ Max}$ = 25.6°C) did not result in systematically lower acclimation ratios than studies done at low temperatures (coldest 33%: mean $T_{Night\ Max}$ = 18.0°C) (Fig. 5-4 C,H).

The duration of warming had no effect on $Acclim_{SetTemp}$ (Fig. 5-4), and the mean $Acclim_{SetTemp}$ of studies exposing leaves to an experimental temperature for less than 25 days was not significantly different from the categories of warming 25–50 days and warming for more than 50 days. All three categories had mean $Acclim_{SetTemp}$ values that were significantly greater than 1.0 (t-test, $P < 0.001$ for all). Duration of warming had a positive effect on $Acclim_{Homeo}$ when only pre-existing leaves were considered; the longer pre-existing leaves were warmed, the more respiration approached homeostasis. $Acclim_{Homeo}$ of leaves developed at the experimental temperature was independent of duration of the experiment.

The developmental status of the leaves did not affect $Acclim_{SetTemp}$, but it did affect $Acclim_{Homeo}$; newly-developed leaves exhibited greater homeostatic acclimation of respiration than pre-existing leaves (Fig. 5-5). When other factors, such as duration and

the degree of warming were accounted for, leaf development status affected $Acclim_{Homeo}$ only in woody species (P = 0.04, Table 5-2).

There were no significant differences in $Acclim_{SetTemp}$ and $Acclim_{Homeo}$ among growth forms, with large variation existing within each (except for lianas, for which only two contrasts were included) (Fig. 5-4). In a simple one-factorial comparison evergreen and deciduous leaves did not differ significantly in either $Acclim_{SetTemp}$ or $Acclim_{Homeo}$. In the full model, however, leaf habit was significant at $P < 0.05$ for both acclimation ratios, with evergreen leaves exhibiting lower $Acclim_{SetTemp}$ values, but higher $Acclim_{Homeo}$ than deciduous leaves.

The degree of warming (temperature interval of the contrasts) had a significant effect on both $Acclim_{SetTemp}$ and $Acclim_{Homeo}$. However, whereas $Acclim_{SetTemp}$ increased with the degree of warming, $Acclim_{Homeo}$ decreased with the degree of warming (Fig. 5-4). There was a significant interaction between the duration and the degree of warming ($P < 0.05$; Table 5-2) that affected $Acclim_{SetTemp}$: $Acclim_{SetTemp}$ increased more strongly with the degree of warming in leaves that were warmed longer than in leaves warmed for a shorter duration (Fig. 5-6).

Leaf habit (evergreen versus deciduous) was significant for both $Acclim_{SetTemp}$ and $Acclim_{Homeo}$ in the subset of data for which information on leaf habit was available. Leaf developmental status (pre-existing versus newly-developed leaves) was also significant in the $Acclim_{Homeo}$ model of the woody taxa (Table 5-2). For both acclimation ratios the most parsimonious significant model only included the degree of warming.

***In Situ* Warming**

The eight studies that elevated temperature above ambient temperature in the field warmed leaves by an average of 2.0°C (median 2.2°C). This contrasts with a mean

temperature difference of 11.1°C (median 10.0°C) in the other studies. Median duration of the experimental warming was the same for the two groups at 30 days, whereas the mean duration was much longer for *in situ* studies because of two studies that warmed Scots pine trees in Finland for several years (Wang *et al*., 1995; Zha *et al*., 2002). Mean $Acclim_{SetTemp}$ with *in situ* warming was 1.05, which was significantly lower than the mean of the remaining studies (t-test, t = 9.8, $P < 0.0001$) (Fig. 5-5). $Acclim_{Homeo}$ of *in situ* warmed leaves was 0.96, marginally higher than in other studies (t-test, t = -3.4, $P = 0.056$). When the degree of warming was taken into consideration, results from the *in situ* warming studies no longer differed from the other studies.

Acclimation Type

The type of acclimation exhibited by warmed leaves could be determined for 54 temperature contrasts (11 on pre-existing leaves, 43 on leaves developed under the experimental temperature). Both pre-existing and newly-developed leaves exhibited Type II acclimation more often than Type I acclimation (Fig. 5-7A). Evergreens showed an even split between Type I and Type II acclimation, but deciduous species exhibited Type II acclimation in almost 75% of the cases. Interestingly, 4 out of 6 contrasts of pre-existing evergreen leaves showed Type I acclimation, whereas all 5 contrasts of pre-existing deciduous leaves exhibited Type II acclimation. Newly-developed leaves of both deciduous and evergreen species exhibited Type II acclimation in > 60% of the cases (Fig. 5-7C). Leaves that were warmed by less than 5°C exhibited Type I acclimation in the majority of the cases, whereas Type II acclimation was more common in leaves that were warmed by 5–10°C or by > 10°C (Fig. 5-7B). Type II acclimation was associated with a greater down-regulation of respiration (higher $Acclim_{SetTemp}$) and more homeostatic acclimation than Type I acclimation (Fig. 5-8).

Discussion

Several trends in respiratory acclimation could be identified from the current analysis of thermal acclimation data from multiple studies. There is an overwhelming tendency for reduction in respiration in warm-acclimated leaves when compared with control leaves at a set temperature, and there is no indication that different growth forms and plants from different biomes differ systematically in this respect. However, the two acclimation indices that we used gave conflicting results, which highlights a problem with quantitative assessment of acclimation.

Considerations for Quantifying Acclimation

When acclimation is simply defined as a change of the short-term temperature response curve of respiration, any significant deviation in elevation or slope indicates that acclimation has occurred. However, assessment of the degree of acclimatory changes requires careful consideration of the most relevant metric. Here we used two metrics of acclimation both of which give higher values when more acclimation has occurred. The higher the $Acclim_{SetTemp}$ value, the greater the down-regulation of respiration following warming, and thus the greater the degree of acclimation. Similarly, the higher $Acclim_{Homeo}$, the smaller the increase in respiration compared to leaves at control temperature, and the greater the degree of acclimation. However, the degree of warming, the strongest single predictor of both ratios, increases $Acclim_{SetTemp}$, while decreasing $Acclim_{Homeo}$. Figure 5-1 illustrates three acclimation scenarios for Type I and Type II acclimation of ‘warm’ and ‘hot’ acclimated leaves. The first scenario is partial acclimation, in which respiration of warm and hot grown leaves is down-regulated, but respiration is not homeostatic across temperatures. A greater degree of warming (i.e., “hot” > ”warm”) results in a greater $Acclim_{SetTemp}$ ($A_2/C_2 > A_2/B_2$), but in a smaller

$Acclim_{Homeo}$ value ($A_1/B_2 > A_1/C_3$). Partial acclimation occurred in 67 of the 99 cases for which $Acclim_{Homeo}$ could be calculated ($Acclim_{Homeo} \leq 0.95$; in 35 cases $R_{Control}$ at $T_{Control}$ was significantly higher than R_{Warm} at T_{Warmed}). Under the scenario of complete homeostasis, $Acclim_{SetTemp}$ increases with warming as before, while $Acclim_{Homeo}$ stays the same ($A_1/B_2 = A_1/C_3$). In 12 cases acclimation resulted in 'perfect' homeostasis ($Acclim_{Homeo}$ value between 0.95 and 1.05, and no significant difference between $R_{Control}$ at $T_{Control}$ and R_{Warm} at T_{Warmed}). Only in the scenario of over-compensation does $Acclim_{Homeo}$ increase with the degree of warming. This requires an enormous degree of down-regulation of respiration, corresponding with a very large decrease in Q_{10} (in case of Type I acclimation) or a considerable down-regulation of the respiratory capacity (in case of Type II acclimation). Indeed, over-compensation appears to be an uncommon phenomenon (10 cases in this meta-analysis with $Acclim_{Homeo} >1.05$, but only in one case was R_{Warm} at T_{Warmed} significantly smaller than $R_{Control}$ at $T_{Control}$). Clearly, complete homeostasis requires a considerable alteration of the short-term temperature response, and across wide temperature ranges, complete acclimation is often not achieved.

What, then, is the better indicator of the degree of acclimation? When Type I acclimation occurs $Acclim_{SetTemp}$ is dependent on the temperature at which it is determined ($A_1/C_1 > A_2/C_2$ in Fig. 5-1). The question when determining acclimation according to the set temperature method then is, what is the ecologically relevant temperature at which to determine respiration of plants that are grown at contrasting temperature regimes. The choice of reference temperature to determine respiration is often arbitrary and not necessarily of ecological relevance (Bruhn *et al*., 2007), and furthermore difficult to standardize across climate regions with contrasting temperature

regimes. In contrast, $Acclim_{Homeo}$ is not inherently dependent on the measurement temperature regardless of the type of acclimation, and it only considers environmentally relevant temperatures. $Acclim_{Homeo}$ thus appears to be the more useful indicator of acclimation. Estimates of $Acclim_{SetTemp}$ may, however, still contribute to improving global carbon flux estimates by implementing them in DGVMs. In such models respiration at a given temperature is generally calculated by adjusting a base rate of respiration at an (arbitrary) reference temperature to current temperature by multiplying this rate by a temperature sensitivity parameter, e.g., based on a Q_{10} value. With information on $Acclim_{SetTemp}$ the base rate of respiration itself can be made dependent on the acclimation temperature (e.g., nighttime temperature in the past week. See Chapter 4). While $Acclim_{Homeo}$ is biologically more relevant, $Acclim_{SetTemp}$ may thus still have its value in quantifying acclimation of respiration in dynamic models.

Biome-dependent Acclimation Potential?

Two recent meta-analyses found that warming of tropical plants decreases biomass accumulation whereas warming stimulates biomass accumulation in all other ecosystems and climate regions (Lin *et al*., 2010; Way & Oren, 2010). Furthermore, in a lowland tropical forest in Costa Rica tree diameter increment is reduced in years with above-average nighttime temperatures (Clark *et al*., 2003, 2010). These observations suggest that tropical species may be close to their thermal optimum and have limited capacity for thermal acclimation. After accounting for factors such as the degree of warming and the duration of the experiment, the biome of species origin had no effect on either $Acclim_{SetTemp}$ or $Acclim_{Homeo}$ in the current analysis. The relatively low values of $Acclim_{SetTemp}$ of the seven tropical species were explained by the small degree of warming the tropical species in this analysis were exposed to and do not preclude the

possibility for a greater degree of acclimation when warmed more. However, no studies to date have looked at the effect of warming tropical species by 5°C or more above their current ambient temperature under otherwise natural conditions to simulate predicted end-of-century temperatures. As an exception, in a growth cabinet study Cheesman & Winter (2013) exposed well-watered seedlings of the tropical pioneer tree species *Ficus insipida* Willd. and *Ochroma piramidale* (Cav. ex Lam.) Urb. to nighttime temperatures of up to 31°C while maintaining daytime temperature at 33°C. Krause *et al*. (2013) grew *F. insipida* at ever warmer conditions at 39°C, with nighttime temperature of 32°C. Mean $Acclim_{Homeo}$ values were 0.56 and 0.51 for *F. insipida* (R_{Warmed} at T_{Warm} > $R_{Control}$ at $T_{Control}$. $P < 0.05$) and 0.80 for *O. piramidale*. This suggests that warming to temperatures that are predicted for the end of the current century may have different consequences for the respiratory carbon flux of different species. Interestingly however, in both these studies seedlings accumulated significantly more biomass in the high nighttime temperature treatment, despite the fact that photosynthesis did not increase with warming. These results show that increase nighttime carbon loss does not necessarily impede tree growth in tropical forests. Species differences in thermal acclimation may have consequences for species composition, vegetation dynamics, and ecosystem functioning in a warmer world, so it will be important to verify the generality of this pattern with later successional tree species and to identify causes of interspecific variation in the capacity for thermal acclimation.

Near-homeostatic Acclimation to Development Temperature

Exposure to warmer temperature during leaf development leads to more homeostatic acclimation. Adaptive plasticity should optimize performance at new environmental conditions within the limits of the species' phenotypic plasticity. Leaves

transferred to warmer temperatures achieved a smaller degree of homeostasis, but the longer pre-existing leaves are exposed to the new temperature, the more they approach homeostasis.

Type I and Type II Acclimation in Pre-existing and Newly-developed Leaves

In contrast to previous reports (Atkin *et al*., 2005), pre-existing leaves exhibited Type II acclimation more frequently than Type I acclimation, similar to newly-developed leaves. In some of the species in which pre-existing leaves exhibited Type II acclimation nitrogen concentrations decreased with increasing temperature (Lee *et al*., 2005), which suggests down-regulation of the metabolic capacity. In other species, however, nitrogen concentration did not decrease with warming (see also Chapter 4). Clearly, pre-existing leaves can down-regulate the respiration capacity at higher temperatures, but the mechanism employed to do so is not well understood.

Acclimation and Climate Warming

As climate continues to warm, plants experience temperature changes from one year to the next that are relatively small compared to some of the temperature differences included in the current study. Small temperature differences are more likely to result in homeostatic rates of respiration than large temperature differences. Furthermore, newly-developed leaves maintain a greater degree of homeostasis than pre-existing leaves. Gradual warming is unlikely to expose leaves to dramatically higher mean annual or mean nighttime temperatures than those they experienced when they developed, especially in conditions where intra-annual temperature variations are small, such as in tropical forests. This suggests that most species are indeed likely to acclimate to a certain degree to warming. Warming is, however, not gradual, even if the rise in mean annual temperature change is. Heat waves will occur more frequently, and

with increasing intensity over the current century (Meehl & Tebaldi, 2004). Under heat-wave conditions pre-existing leaves will be exposed to temperatures considerably higher than their development temperature, and acclimation will at best result in limited homeostasis. We did not find evidence for biome differences in the capacity for acclimation, nor were leaves exposed to high maximum nighttime temperatures less likely to exhibit acclimation. However, whether the response of pre-existing leaves to extreme warming during a heat wave event differs by biome remains unknown.

Recent tropical seedling studies (Cheesman & Winter, 2013; Krause *et al*., 2013) show that even among pioneer species, large differences in thermal acclimation of respiration exist. Clearly more tropical data are needed to be able to make generalizations about the potential for thermal acclimation in the tropics. The current study included 13 temperature contrasts of seven tropical species. In recent global meta-analyses of temperature effects on biomass accumulation Way & Oren (2010) included ten data points for tropical and subtropical species out of 434 total data points, and in Lin *et al*. (2010) only nine out of 537 data points came from conditions of a mean annual temperature of 20°C or higher. Given the angiosperm diversity of tropical regions, the amount of empirical data from tropical forests is decidedly small and more data are needed to predict global patterns in the acclimation response of respiration, and to enable more reliable predictions of respiratory carbon fluxes by informing global circulation models (Malhi *et al*., 2009; Reed *et al*., 2012).

Table 5-1. Summary of studies of thermal acclimation of respiration in the lab (growth cabinets) and field. Mean $Acclim_{SetTemp}$ and $Acclim_{Homeo}$ (± SD) are shown by biome and growth form. Mean degree (range) of warming (ΔT), the number of temperature contrasts (n_{con}) and species (n_{sp}) are provided. See Appendix B for references.

Biome	Lab					Field				Total		Ref.
Growth form	n_{con}	n_{sp}	ΔT (°C)	$Acclim_{SetTemp}$	$Acclim_{Homeo}$	n_{con}	$n_{sp.}$	ΔT (°C)	$Acclim_{SetTem}$	$Acclim_{Homeo}$	n_{con}	n_{sp}	
Alpine											19	19	
Forbs	15	15	10.4 (10.0-11.0)	1.65 ± 0.32	0.62 ± 0.26						15	15	3,7,12
Graminoids	4	4	10.3 (10.0-11.0)	1.86	0.59 ± 0.19						4	4	7,12
Arctic & Antarctic											7	4	
Forbs	3	2	7.7 (5.0-10.0)	1.52 ± 0.40	0.94 ± 0.12						3	2	1,3,28
Graminoids	2	1	6.5 (5.0-8.0)	1.46 ± 0.22	1.04 ± 0.01						2	1	28
Shrubs													
Evergreen	2	1	15.0	1.42 ± 0.02	0.54 ± 0.07						2	1	20
Boreal											17	7	
Trees													
Deciduous	6	3	9.0 (6.0-12.0)	1.38 ± 0.35	0.68 ± 0.16	4	2	4.3 (3.4-5.3)	1.27 ± 0.49	*nd*	10	4	10,22
Evergreen	4	2	9.0 (6.0-12.0)	1.45 ± 0.24	0.78 ± 0.17	3	2	4.0 (1.5-8.0)	1.06 ± 0.19	*nd*	7	3	22-24,26,29
Temperate											189	46	
Forbs	68	17	12.1 (5.0-21.0)	1.56 ± 0.55	0.88 ± 0.42	3	3	1.1	0.94 ± 0.06	*nd*	71	20	1,12
Graminoids	30	7	13.0 (5.0-21.0)	1.76 ± 0.57	0.73 ± 0.35	2	2	1.6 (1.1-2.1)	1.17 ± 0.34	*nd*	32	9	6,9,12,14,30
Shrubs													
Evergreen	16	3	12.8 (6.0-21.0)	1.66 ± 0.60	0.71 ± 0.16						16	3	6,25
Trees													
Deciduous	17	5	11.8 (5.0-21.0)	1.93 ± 0.90	0.43 ± 0.18	10	5	5.3 (1.0-10)	1.33 ± 0.34	*nd*	27	7	4,6,10,13,15,16
Evergreen	36	7	12.8 (5.0-21.0)	1.55 ± 0.41	0.76 ± 0.18	7	3	1.8 (0.3-3.9)	1.06 ± 0.45	1.12 ± 0.30	43	7	2,5,6,14,16-18.21.27
Tropical											13	7	
Lianas													
Evergreen						2	2	2.9 (2.5-3.3)	1.10 ± 0.06	0.83 ± 0.05	2	2	19
Trees													
Deciduous	7	2	6.6 (3.0-10.0)	1.16	0.75 ± 0.17	3	3	2.8 (2.4-3.5)	1.08 ± 0.06	0.88 ± 0.04	10	4	8,11, 19
Evergreen						1	1	3.2	1.14	0.87	1	1	19

Table 5-2. *P*-values of models of the dependence of $Acclim_{SetTemp}$ and $Acclim_{Homeo}$ on species traits and experimental conditions. Biome, biome of species origin (Arctic/Antarctic, Alpine, Boreal, Temperate and Tropical); Growth Form, (Forbs, Graminoids, Shrubs, Trees, Lianas); Leaf habit, evergreen or deciduous (only a factor for a subset of 'woody' species); Pre-existing, leaves existing prior to experiencing the experimental temperature vs. leaves developed at the experimental temperature; Max T_{Night}, highest nighttime temperature in the experiment (e.g., the target nighttime temperature in a warming treatment); Method, the method used in the study (*in situ* warming above ambient, or all else); Duration, duration of exposure to experimental temperature; Degree of Warming, mean temperature difference between control and warmed leaves. The most parsimonious significant model only included Degree of Warming for both acclimation ratios.

	$Acclim_{SetTemp}$			$Acclim_{Homeo}$		
	Full model	Parsimonious model	'Woody'	Full model	Parsimonious model	'Woody'
Biome	0.44		0.20	0.54		0.23
Growth Form	0.62		0.82	0.75		0.09
Leaf habit			0.01			0.02
Pre-existing	0.75		0.28	0.19		0.04
Max T_{Night}	0.80		0.94	0.63		0.98
Method	0.52		0.75	0.28		0.09
Duration	0.97		0.88	0.57		0.09
Degree of Warming	<0.0001	<0.0001	<0.0001	<0.01	<0.0001	<0.01
Duration × Degree of Warming	0.04		0.05	0.64		0.18
Duration × Pre-existing	0.78		0.71	0.41		0.52
Model R^2	0.36	0.30	0.46	0.34	0.20	0.65
P	<0.0001	<0.0001	<0.0001	<0.01	<0.0001	<0.0001

Leaves acclimate to warming by down-regulating respiration (R). In the case of Type I acclimation, R is down-regulated at high temperature, but at low temperature R does not change (*i.e.*, the Q_{10} decreases). Under Type II acclimation R decreases at all temperatures (*i.e.*, no change in Q_{10}). The degree of acclimation can be expressed with the set temperature method $Acclim_{SetTemp} = \frac{R_{Control} \text{ at } T_{Set}}{R_{Warmed} \text{ at } T_{Set}}$ (e.g., A_2/B_2 or A_2/C_2 in the graphs below). The degree of homeostasis of R achieved by acclimation can be expressed by as: $Acclim_{Homeo} = \frac{R_{Control} \text{ at } T_{Control}}{R_{Warmed} \text{ at } T_{Warm}}$ (*e.g.*, A_1/B_2 or A_1/C_3 in the graphs below). Note that $Acclim_{SetTemp}$ increases with temperature under Type I, but not under Type II acclimation ($A_2/C_2 > A_1/C_1$).

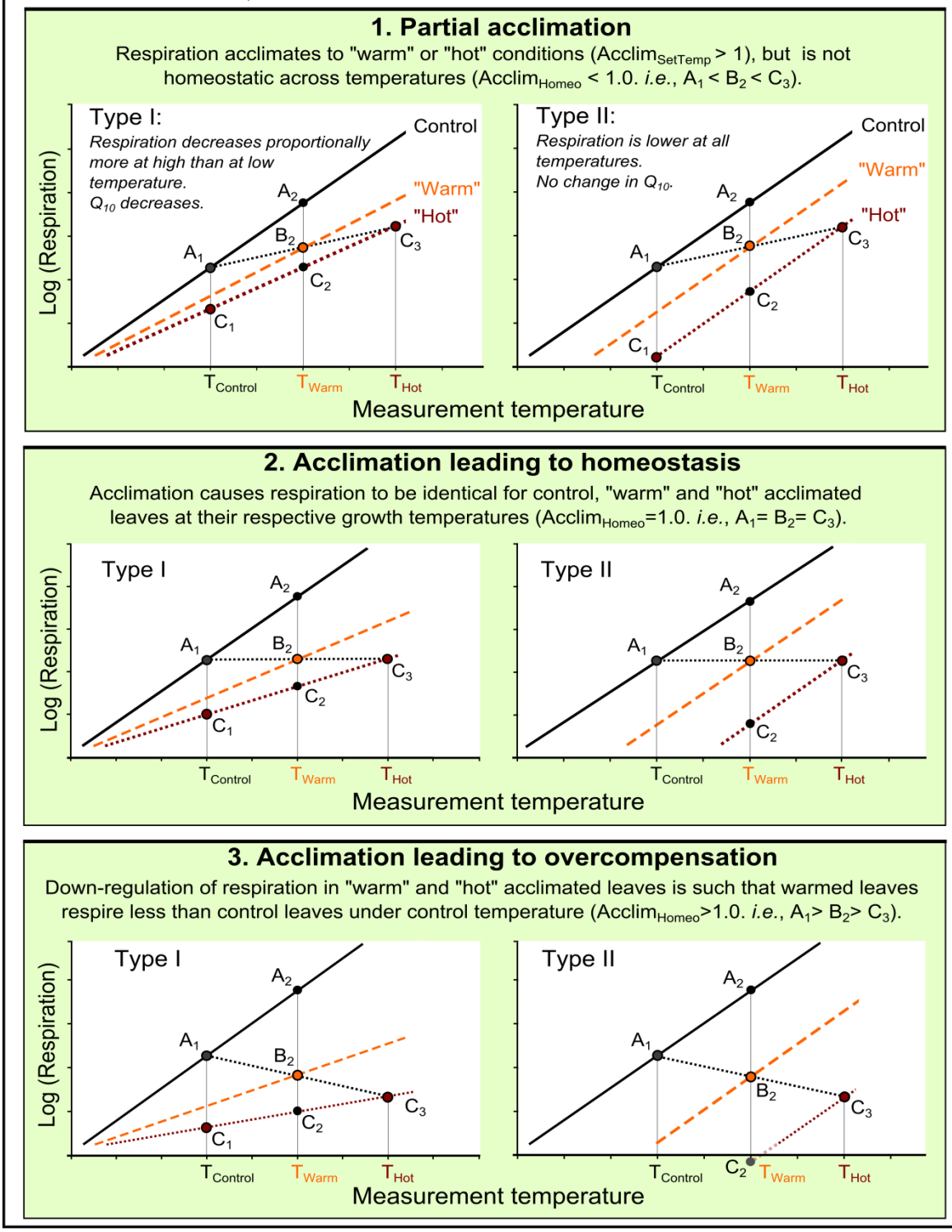


Figure 5-1. Acclimation of respiration illustrated.

Several methods have been proposed to quantify thermal acclimation of respiration. They are all based on quantifying the degree of down-regulation of respiration in warm-acclimated leaves, but they use different temperatures and time scales over which the change in respiration is assessed.

The **Set temperature method** ($Acclim_{SetTemp}$: Loveys *et al*. 2003) compares respiration of control and warmed leaves at a set temperature ($R_{Control}/R_{Warming}$). In principle, any temperature can be used, but when Type I acclimation (Box 1) has occurred, the ratio increases with the temperature used as T_{Set}. When respiration has acclimated $Acclim_{SetTemp} > 1.0$.

The **Long-Term acclimation Ratio** (LTR_{10}: Larigauderie & Körner 1995) compares respiration of warm-acclimated leaves at a high temperature with respiration of control leaves at a temperature 10°C lower (R_{Warmed} at $T_{Warm}/R_{Control}$ at T_{Warm}-10). In effect, the LTR_{10} represents the long-term Q_{10}. When R_{Warmed} at T_{Warm} is not significantly different from $R_{Control}$ at T_{Warm}-10, respiration has fully acclimated to warming.

$Acclim_{LTR_{10}}$ is a modification of LTR_{10} by Atkin *et al*. (2005) in which LTR_{10} is directly compared to the short-term Q_{10}. $Acclim_{LTR_{10}}$ is calculated as $1- [(LTR_{10}-1)/(Q_{10}-1)]$. The more the LTR_{10} has decreased following acclimation, the larger $Acclim_{LTR_{10}}$.

$Acclim_{Homeo}$ (Loveys *et al.* 2003) determines the degree of homeostasis of respiration in leaves acclimated to contrasting temperatures by comparing respiration of control leaves at control temperature to warmed leaves at warm temperature ($R_{Control}$ at $T_{Control}/R_{Warmed}$ at T_{Warm}). When $R_{Control}$ at $T_{Control}$ and R_{Warmed} at T_{Warm} are not significantly different, respiration has fully acclimated to warming. When the temperature difference between two sets of plants/leaves equals 10°C, $Acclim_{Homeo}$ is the inverse of LTR_{10}.

Under all three acclimation scenarios in Fig. 5-1 $Acclim_{SetTemp}$ will be > 1.0, so $Acclim_{SetTemp}$ cannot be used to determine whether acclimation results in homeostasis or not. LTR_{10}, $Acclim_{LTR_{10}}$ and $Acclim_{Homeo}$ are all 1.0 under complete homeostasis (or *e.g.*, 0.95–1.05, when allowing for some variation in the data). LTR_{10}> 1.0 under partial acclimation, and < 1.0 under over-compensation. $Acclim_{LTR_{10}}$ and $Acclim_{Homeo}$ are < 1.0 under partial acclimation, and > 1.0 under over-compensation.

Figure 5-2. Explanation of methods of quantification of acclimation.

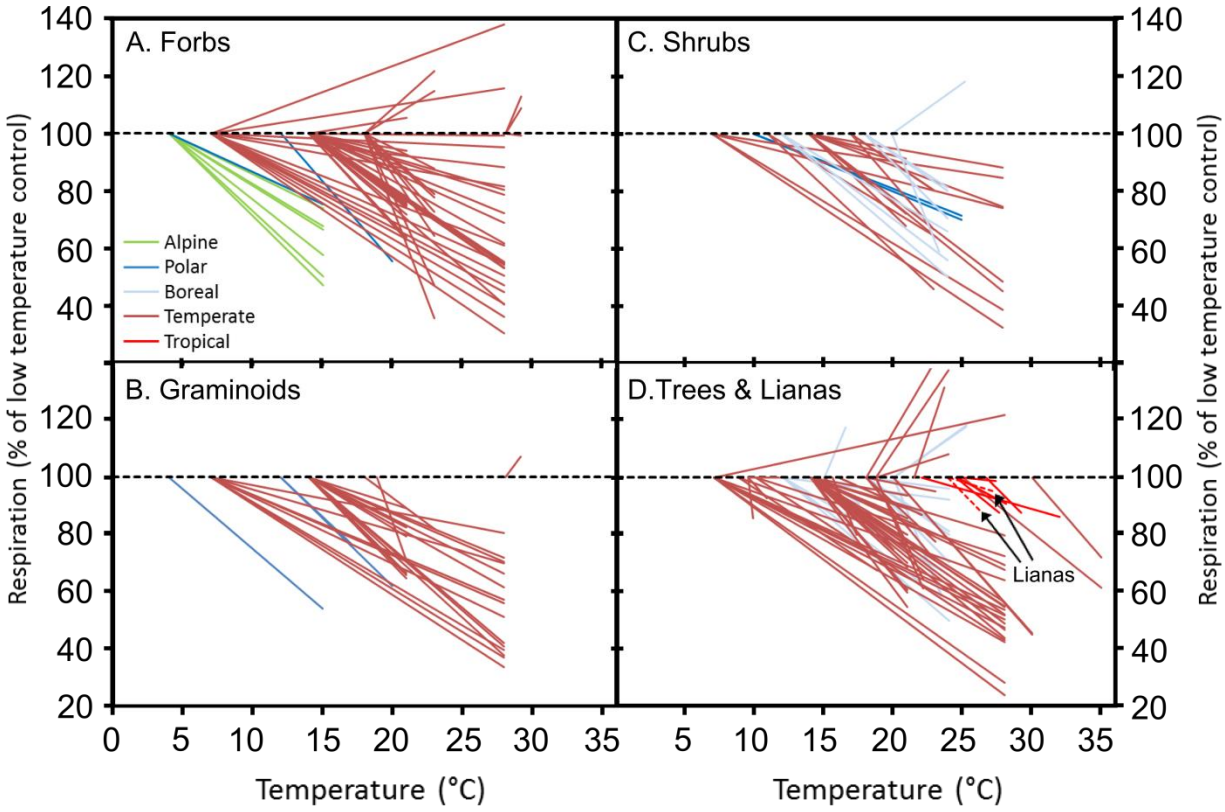
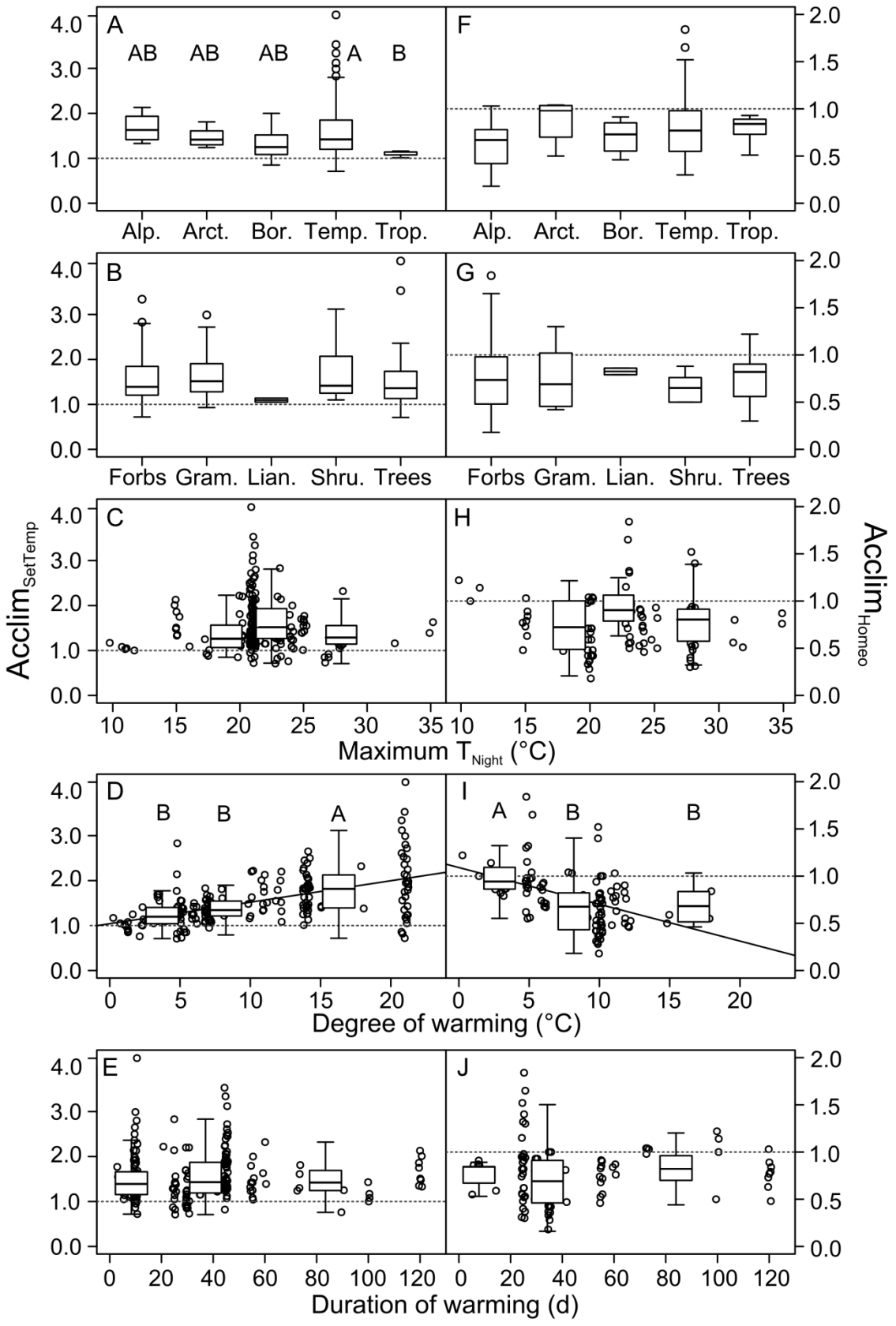


Figure 5-3. Relative change in respiration rate at a set temperature following acclimation of 64 plant species, based on 195 sets of respiration measurements at contrasting acclimation temperatures. A) Forbs. B) Graminoids. C) Shrubs. D) Trees and lianas. Respiration at the control temperature is set to 100% and respiration of warmed leaves was either taken directly from the published source, or calculated from the $Acclim_{SetTemp}$ value. All contrasts are shown in this graph, also if the change in respiration at a set temperature was not significant.

Figure 5-4. Acclimation parameters $Acclim_{SetTemp}$ (A-E) and $Acclim_{Homeo}$ (F-J) in relation to plant traits and experimental conditions. A,F) Effect of biome of origin (Alp., Alpine; Arct., Arctic/Antarctic; Bor., Boreal; Temp., Temperate; Trop., Tropical). B,G) Effect of growth form (Gram., Graminoids; Lian., Lianas; Shru., Shrubs). C,H) Effect of maximum nighttime temperature warmed plants were exposed to. D,I) Effect of degree of warming, or temperature difference across contrasts. E,J) Effect of duration of exposure to the experimental temperature. The continuous data in C-E and H-J were also binned in three groups of similar sample size and boxplots are shown at the median x-axis value of the bins. Two data points from multi-year warming experiments were omitted from the scatter plot in E, but were included in the box plot. Different letters indicate significant differences among groups at $P < 0.05$ (one-way ANOVA). The black lines in D and I represent significant linear regressions at $P < 0.05$.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Degree of warming (°C)
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J
Duration of warming (d)

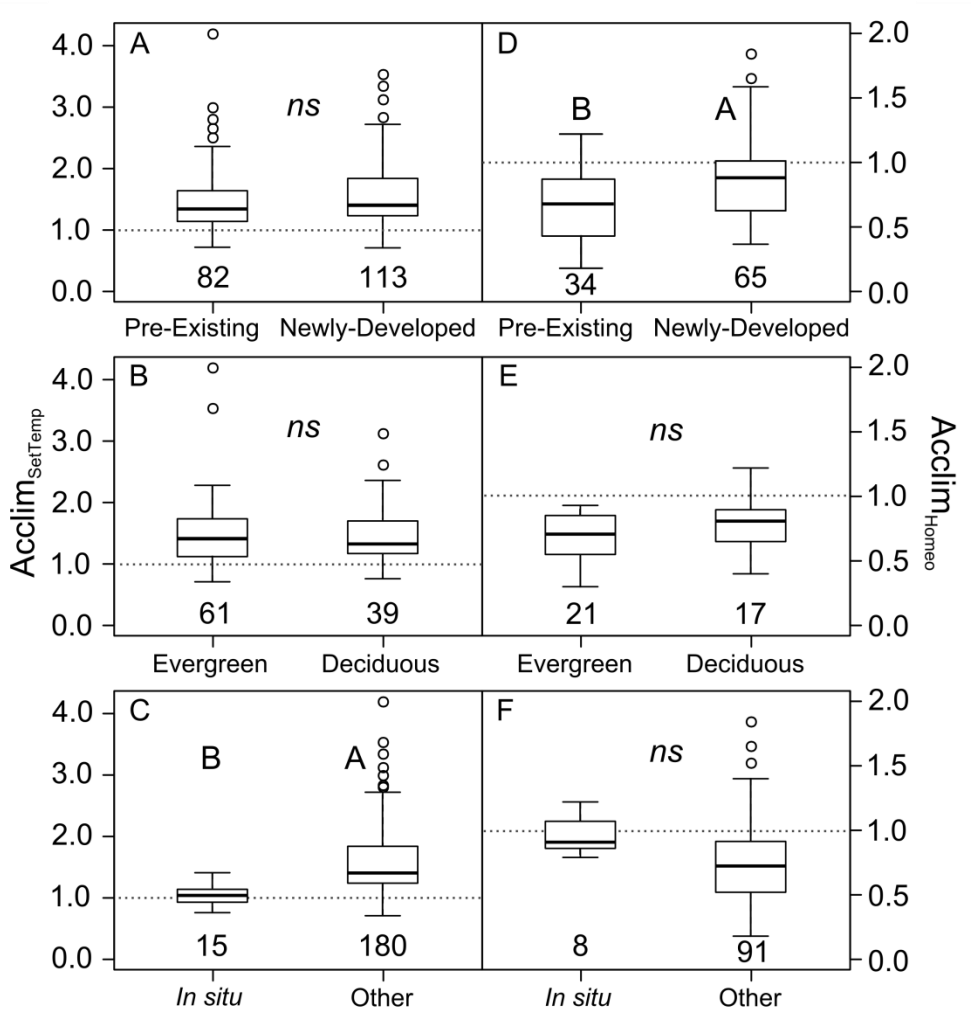

Figure 5-5. Acclimation parameters $Acclim_{SetTemp}$ (A-C) and $Acclim_{Homeo}$ (D-F) in relation to experimental conditions and leaf traits. A,D) Acclimation in relation to leaf developmental age (pre-existing at the time of warming vs. newly developed under warmed conditions). B,E) Acclimation in relation to leaf type (Evergreen *vs.* Deciduous). C,F) Effect of method of warming on acclimation (*in situ* warming above ambient, *vs.* other methods. Different letters indicate significant difference between groups at $P < 0.05$ (one-way ANOVA). The number of observations per category is shown below each boxplot.

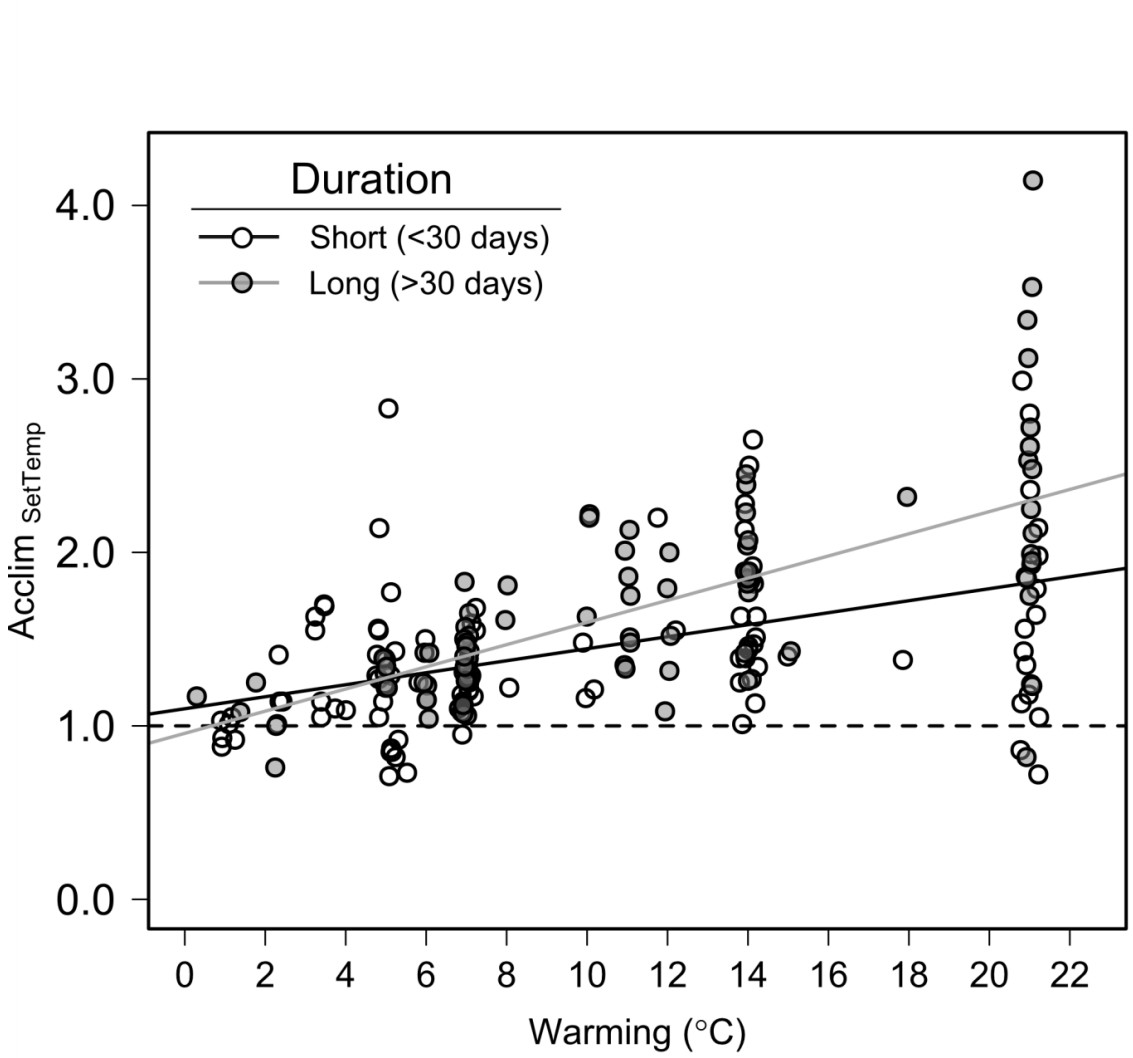

Figure 5-6. Interaction effect of duration of warming and degree of warming on $Acclim_{SetTemp}$, illustrated by the stronger temperature effect on leaves warmed for more than 30 days than for leaves warmed for up to 30 days. Data points are slightly jittered to reduce overlap and increase visibility.

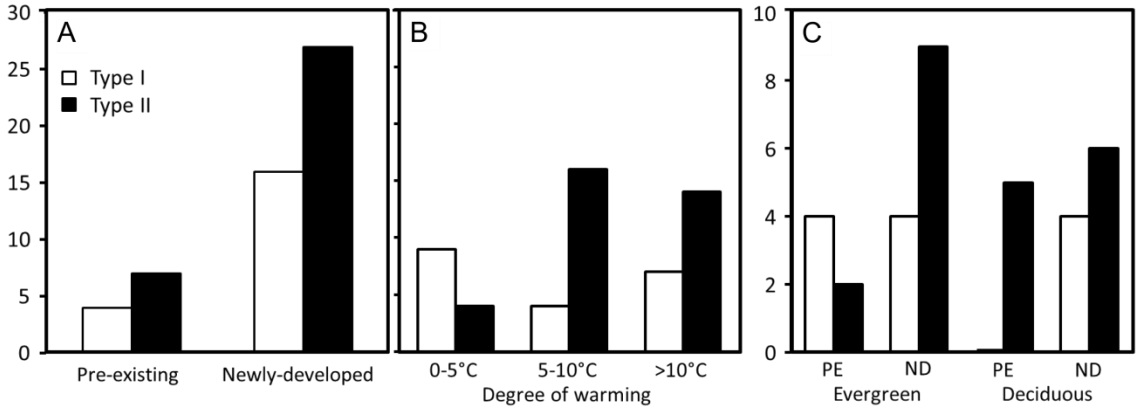

Figure 5-7. Frequency of Type I and Type II acclimation of respiration. A) Acclimation type for leaves present prior to warming (Pre-existing) and leaves developed under warmed conditions (Newly-developed). B) Acclimation type in relation to the degree of warming experienced. C) Acclimation type of Pre-existing (PE) and Newly-developed (ND) leaves of evergreen and deciduous woody species. In total 54 sets of observations on 41 species were included in A and B, and 34 sets of observations on 23 species in C.

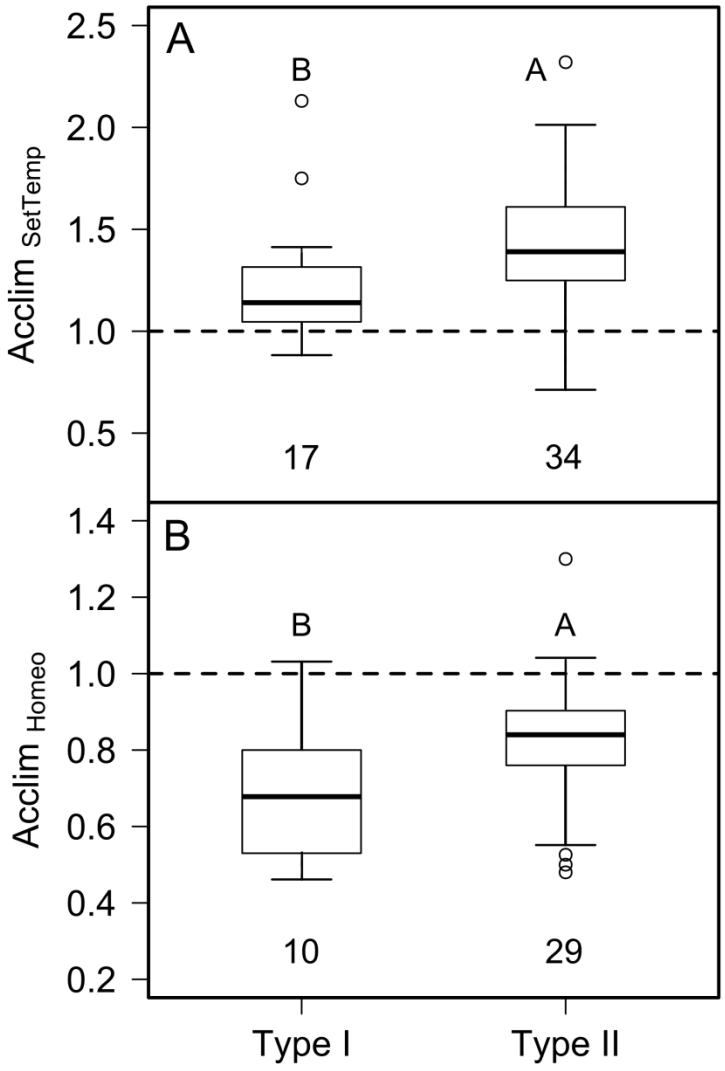

Figure 5-8. Acclimation parameters $Acclim_{SetTemp}$ and $Acclim_{Homeo}$ under Type I and Type II acclimation of respiration. A) $Acclim_{SetTemp}$. B) $Acclim_{Homeo}$. Different letters indicate significant difference between groups at $P < 0.05$ (one-way ANOVA). The number of observations per category is shown below each boxplot.

CHAPTER 6
CONCLUSIONS

There is uncertainty about the effects of climate change on tropical forests. While increased CO_2 in the atmosphere may, in theory, result in an increase in net primary productivity (NPP) by stimulating photosynthesis (Lloyd & Farquhar, 2008), rising temperatures may reduce NPP by increasing plant respiration more than gross photosynthetic rates. A decline in NPP can reduce the carbon sequestration service provided by tropical forests, possibly transforming them from net carbon sinks to carbon sources to the atmosphere, which would have important implications for the global carbon cycle. Yet, little is known about the temperature responses of leaf respiration in tropical forest. In my dissertation, I assessed the temperature response of leaf dark respiration of trees and lianas in the upper canopy of a tropical forest at different time scales. I determined the instantaneous physiological responses at the species level in the field (Chapter 2) and at the leaf level in the laboratory (Chapter 3), and I assessed thermal acclimation response to elevated nighttime temperatures in the longer term (6–8 days) (Chapter 4).

In Chapter 2, I report the results of *in situ* measurements of leaf dark respiration of 461 upper-canopy leaves of 26 species of tree and liana in a tropical forest in Panama using a new protocol that ensured thermal equilibrium of the measured leaves with the rest of the plant. Leaves were darkened with foil overnight until they were measured sequentially during ambient temperature rise in the morning. From these measurements temperature response curves of respiration were constructed for each of the 26 species, and values of Q_{10} (the proportional increase of respiration per 10°C warming) were calculated. These measurements revealed to respiration rates at 25°C

(R_{25}) were on the high end of what has previously been published for tropical forest trees. The high dark respiration rates may reflect the relatively high proportion of early- and mid-successional tree species in this secondary forest, or the relatively high fertility of the site. However, respiration in early- and mid-successional species was not significantly higher than in late-successional species within the site, and respiration rates were still high compared to published data when expressed per unit leaf nitrogen. Another possible reason for the comparatively high respiration rates was that I was better able to access fully sun-exposed leaves with the canopy crane than authors of previous studies, who used towers or sling shots to access canopy leaves. The Q_{10} values estimated from the temperature response curves of respiration were on average higher than what is commonly assumed in global models, which means that published global models may underestimate leaf respiratory carbon fluxes from tropical forests under warm conditions. Interspecific differences in respiration rates correlated positively with species differences in photosynthetic capacity, leaf nitrogen content, and leaf mass per unit leaf area, and negatively with median leaf lifespan. These trait correlations are in line with predictions from the leaf economic spectrum (I. J. Wright *et al*., 2004), which constrains leaf traits of plant species across the globe to a single axis of variation based on the return on investments. The results of the correlations among *in situ* respiration rates and leaf traits reported in Chapter 2 support the notion that even within the upper-canopy of a single tropical forest, species differences can be expressed as suites of traits associated with slow versus rapid metabolism.

The advantage of the ecological relevance of *in situ* measurements reported in Chapter 2 came at the cost of the inability to document temperature sensitivity for

individual leaves. Hence, the intra-specific variation in Q_{10} could not be quantified in Chapter 2. Chapter 3 addresses this concern by determining temperature sensitivity of leaf dark respiration on detached leaves in the laboratory, where leaf temperature could be controlled precisely and temperature response curves of respiration could be determined at the leaf level. I determined Q_{10} values for a total of 123 upper-canopy leaves from 28 species. Decomposing the variance in Q_{10} data revealed that > 40% of the total variance was explained by Q_{10} differences among leaves within species, with most of the remaining variance occurring at the level of species within plant functional types. The total variance in Q_{10} was, however, small compared to variance of R_{25}. In agreement with the results of *in situ* measurements reported in Chapter 2, the average Q_{10} was significantly greater than 2.0 (range of species means 2.0–2.9), and Q_{10} did not differ between growth forms or among plant functional types based on tree successional status. Q_{10} values were not related to leaf chemical and structural traits, and interspecific variation in Q_{10} thus appears to be independent of the leaf economic spectrum. Q_{10} could, however, be explained by a multiple regression model containing total non-structural carbohydrates and growth form (lianas versus trees), but only 26% of the variance could be explained. Among these 28 co-occurring species significant pair-wise correlations existed between R_{25} and other leaf traits similar to the results of the *in situ* measurements from Chapter 2. However, the best multivariate models of respiration did not include leaf nitrogen content, which is widely regarded as a key trait in the leaf economics spectrum, and is incorporated as a predictor of respiration in some coupled climate-vegetation models (e.g., Friend *et al*., 1997; Bonan *et al*., 2003). Instead, leaf phosphorus content was the strongest single predictor and the only

variable that was part of all significant multiple regressions of R_{25}. The best model contained leaf phosphorus content, photosynthetic capacity and leaf mass per unit area and explained 64% of the variance in R_{25}. Using these multiple regressions, I calculated the annual leaf respiratory carbon (C) flux from this forest to be 7.4 Mg C ha^{-1} yr^{-1}. This estimate is comparable to those from Amazonian tropical forests that maintain greater leaf area index and have a shorter dry season than my study site.

The instantaneous temperature sensitivity of respiration is valuable for calculation of respiration at different nighttime temperatures, but predictions of the long-term warming effects on respiration require information on temperature response of respiration at a different time scale. Acclimation of leaf respiration to elevated temperature regimes, i.e., down-regulation of respiration under warming, has been described in mid- to high-latitude plant species, and increasingly, attempts are made to incorporate algorithms for thermal acclimation of respiration into carbon flux models for regional (Chen & Zhuang, 2013; Wythers *et al*., 2013) and global scales (King *et al*., 2006; Atkin *et al*., 2008). In these models acclimation is uniformly applied to all growth forms and biomes, even though data are largely lacking from tropical forests. It has been suggested, however, that the temperature stability of tropical forests, both intra-annually and over geological time scale, might render the species less capable to acclimate to changing temperatures. Observations on high latitude tree species suggest that leaves from different plant functional types may differ in their capacity to acclimate to temperature (Tjoelker *et al*., 1999b), but no systematic differences in thermal acclimation were found among different growth forms (Campbell *et al*., 2007). While incorporation of acclimation algorithms into flux models has the potential to more

realistically predict future respiratory carbon fluxes (King *et al*., 2006), the current attempts to do so are based on relatively poorly tested assumptions. In an effort to reconcile the need to address thermal acclimation of respiration in carbon flux models with the lack of consensus on the underlying assumptions, I conducted two studies reported in Chapters 4 and 5.

First, using an *in situ* leaf warming experiment, I tested whether leaves of tropical forest trees and lianas can acclimate to nighttime warming by several degrees Celsius. Tropical forests experience little seasonal temperature fluctuations (Wright *et al*., 2009) and the capacity of organisms to respond to dynamic changes in temperature is expected to be limited in environments that commonly experience minimal temperature variation (Janzen, 1967; Cunningham & Read, 2002; Ghalambor *et al*., 2006). I therefore hypothesized that respiration of tropical leaves would not show active acclimation to nighttime warming. I examined changes in the temperature response of leaf respiration in terms of R_{25} and Q_{10} determined for individual leaves. While Q_{10} did not change with warming, a significant pattern of decline of R_{25} with nighttime leaf temperature during the previous week was found across leaves of three tree species and two liana species. Across species R_{25} decreased by an average of 2.9% per degree Celsius of leaf warming above the current ambient temperature. There was no evidence that this down-regulation of respiration was due to depletion of respiratory substrate, and thus the results of this experimental warming study suggest that these tropical canopy leaves actively acclimate to warmer nighttime temperatures. Despite the consistent pattern of down-regulation of respiration, acclimation did not result in complete homeostasis of respiration across temperatures; i.e., respiration rates at the

elevated experimental temperature were higher than the rates at the ambient (control) temperature. Nevertheless, the capacity of tropical trees and lianas to acclimate to warmer nights has the potential to reduce the magnitude of the positive feedback between climate and the carbon cycle in a warming world. This is the first report on thermal acclimation of respiration of tropical trees and lianas, and will be valuable for ecophysiologists and carbon flux modelers alike.

In Chapter 5, I report a meta-analysis of thermal acclimation of respiration based on data reported in published studies as well as my new data reported in Chapter 4. I compiled 237 temperature contrasts of 87 plant species, and tested for differences in acclimation among different biomes, growth forms, and experimental duration and degree of warming. Considerable variation existed among species, and within growth forms and biomes, but several patterns could be identified. 1) The larger the temperature difference plants were exposed to, the less complete did respiration acclimate (i.e., less complete homeostasis of respiration), 2) The longer leaves experienced a new temperature the greater the degree of homeostasis of respiration that was achieved, 3) When new leaves develop under warmed conditions, the degree of homeostasis is greater than for leaves developed under one cooler control conditions and transferred to warmer conditions. The available data do not suggest that plants of different growth forms and biomes differ significantly in acclimation of leaf dark respiration. This suggests that with gradual warming the capacity of respiration to acclimate will reduce the potential positive feedback of warming-stimulated respiratory CO_2 release across biomes.

In conclusion, I found evidence that leaf respiration of tropical forest canopy trees and lianas exhibits a higher sensitivity to short-term temperature changes (i.e., higher Q_{10}) than often assumed, but that longer term exposure to elevated nighttime temperature results in acclimatory down-regulation of respiration similar to what has been shown for plant species from thermally more variable regions than the tropics. Furthermore, the development of new leaves under elevated temperatures is likely to increase the degree of respiratory homeostasis that acclimation results in. The results presented in this dissertation thus suggest that long-term warming will not necessarily cause dramatic increases in leaf respiration loads in tropical forest trees, and that tropical forests may be more resilient to climate change that previously thought (Huntingford *et al*., 2013).

APPENDIX A.
SUPPLEMENTARY TABLES AND FIGURES TO CHAPTER 2

Table A-1. Results of standardized major axis regression analyses that are shown in Fig. 2-3. R_{25Area} (in μmol m^{-2} s^{-1}) is regressed against $A_{max\ Area}$ (in μmol m^{-2} s^{-1}), leaf mass per area (LMA; g m^{-2}), nitrogen content per unit area (N_{Area}; g m^{-2}), phosphorus per unit area (P_{Area}; mg m^{-2}) and leaf lifespan (in days). $R_{25\ Mass}$ (in nmol g^{-1} s^{-1}) is regressed against $A_{max\ Mass}$ (in nmol g^{-1} s^{-1}), LMA, N_{Mass} (mg g^{-1}), P_{Mass} (mg g^{-1}) and leaf lifespan.

Respiration Trait	Regression Trait	Elevation	Slope	R^2	P
$R_{25\ Area}$	$A_{max\ Area}$	-1.143	1.012	0.337	0.002
	LMA	-1.497	0.676	0.205	0.020
	N_{Area}	-0.261	0.859	0.237	0.012
	P_{Area}	-1.582	0.762	0.419	0.001
	Lifespan	1.434	-0.657	0.057	0.273
$R_{25\ Mass}$	$A_{max\ Mass}$	-1.330	1.090	0.474	<0.001
	LMA	2.949	-0.956	0.448	<0.001
	N_{Mass}	0.658	1.075	0.300	0.004
	P_{Mass}	1.978	1.076	0.315	0.005
	Lifespan	2.813	-0.780	0.195	0.035

Table A-2. Study species and families, plant functional types (PFT); early-successional (ES), mid-successional (MS), late-successional (LS) tree species; lianas (L). Q_{10} based on R_{Mass}-temperature response curves and its 95% confidence intervals; photosynthetic capacity on a leaf mass basis ($A_{max\ Mass}$), nitrogen (N) and phosphorus (P) content per unit leaf area, R per unit N, and the ratio of R at 25°C and photosynthetic capacity (R_{25}/A_{max}) are shown.

Species	Family	PFT	$Q_{10\ Mass}$ (95% CI)	$A_{max\ Mass}$ nmol $g^{-1}\ s^{-1}$	N_{Area} g m^{-2}	P_{Area} g m^{-2}	R_{25}/N µmol $(g\ N)^{-1} s^{-1}$	R_{25}/A_{max}
Albizia guachapele (Kunth) Harms	Fabaceae	ES	3.47 (1.27-7.45)	142	2.9	0.09	0.26	0.06
Annona spraguei Saff.	Annonaceae	ES	2.94 (2.28-3.83)	152	2.5	0.15	0.38	0.07
Cecropia peltata L.	Urticaceae	ES	3.16 (1.23-8.15)	199	2.8	0.17	0.45	0.06
Pittoniotis trichantha Griseb.	Rubiaceae	ES	2.60 (1.56-4.35)	199	1.6	0.10	0.76	0.09
Astronium graveolens Jacq.	Anacardiaceae	MS	1.87 (1.30-2.67)	145	2.6	0.18	0.46	0.10
Castilla elastica var.costaricana, (Liebm.) C.C. Berg	Moraceae	MS	2.89 (2.18-3.84)	187	2.9	0.18	0.39	0.06
Ficus insipida Willd.	Moraceae	MS	1.82 (1.11-2.98)	160	3.4	0.25	0.43	0.06
Luehea seemannii Triana & Planch.	Tiliaceae	MS	1.78 (1.36-2.37)	190	2.5	0.16	0.45	0.06
Pseudobombax septenatum (Jacq.) Dugand	Malvaceae	MS	1.70 (1.28-2.31)	159	2.3	*nd*	0.50	0.07
Spondias mombin L.	Anacardiaceae	MS	1.91 (1.51-2.23)	162	2.8	0.13	0.54	0.09
Zuelania guidonia (Sw.) Britt. & Millsp.	Flacourtiaceae	MS	2.57 (1.49-4.33)	140	2.5	*nd*	0.55	0.08
Anacardium excelsum (Bertero & Balb.ex Kunth) Skeels	Anacardiaceae	LS	1.93 (1.68-2.20)	126	1.5	0.15	0.80	0.09
Chrysophyllum cainito L.	Sapotaceae	LS	2.04 (1.72-2.42)	121	2.1	0.12	0.39	0.06
Amphilophium paniculatum (L.) kunth	Bignoniaceae	L	2.21 (1.67-2.93)	166	1.8	0.11	0.45	0.18
Aristolochia tonduzii O.C. Schmidt	Aristolochiaceae	L	2.45 (1.71-3.51)	164	2.5	0.12	0.41	0.08
Bonamia trichantha Hallier f	Convolvulaceae	L	1.88 (1.04-3.51)	151	2.2	0.12	0.36	0.08
Cissus erosa Rich.	Vitaceae	L	1.96 (0.81-3.40)	301	1.8	*nd*	0.71	0.08
Combretum fruticosum (Loefl.) Stuntz	Combretaceae	L	3.33 (1.99-5.59)	235	1.7	0.12	0.63	0.06
Forsteronia myriantha Donn. Sm.	Apocynaceae	L	1.65 (0.66-4.21)	220	1.9	0.11	0.51	0.07
Gouania lupuloides (L.) Urb.	Rhamnaceae	L	1.35 (0.75-2.42)	266	1.9	0.09	0.47	0.07
Mikania leiostachya Benth.	Asteraceae	L	2.55 (1.05-6.03)	167	1.4	0.08	0.51	0.07
Phryganocydia corymbosa (Vent.) Bureau ex K. Schum.	Bignoniaceae	L	1.89 (1.36-2.61)	125	3.6	0.15	0.49	0.15
Serjania mexicana (L.) willd.	Sapindaceae	L	2.25 (1.31-3.84)	139	2.2	0.14	0.45	0.08
Stigmaphyllon lindenianum A. Juss.	Malpighiaceae	L	1.60 (1.24-2.14)	204	2.2	0.11	0.49	0.06
Trichostigma octandrum (L.) H. Walt.	Bignoniaceae	L	1.99 (1.30-3.66)	263	2.3	0.18	0.49	0.08
Vitis tillifolia Humb. & Bonpl. ex Roem. & Schult.	Vitaceae	L	3.07 (1.26-7.47)	197	1.4	0.09	0.68	0.07

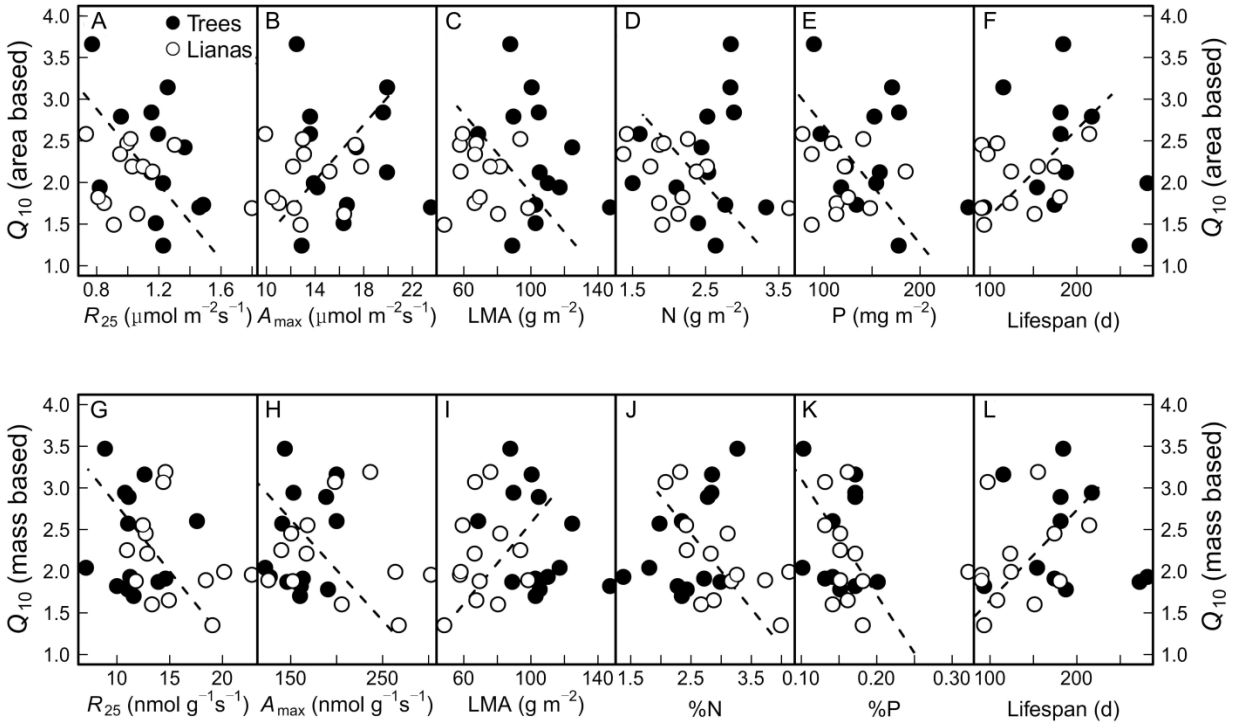

Figure A-1. Correlations between Q_{10} values, determined from species-level temperature response curves of area-normalized respiration data (A-F) and mass-normalized respiration data (G-L), and other leaf traits. A,G) Respiration at 25°C (R_{25}). B,H) Photosynthetic capacity (A_{max}). C,I) Leaf mass per unit area (LMA). D,J) Leaf nitrogen content (N). E,K) Leaf phosphorus content (P). F,L) Leaf lifespan. Dashed lines indicate non-significant correlations.

APPENDIX B
REFERENCES USED IN THE META-ANALYSIS IN CHAPTER 5

Studies used in the meta-analysis of studies of thermal acclimation of leaf dark respiration reported in Chapter 5. The numbers correspond to the reference numbers in Table 5-1.

1. **Arnone JA, Körner C. 1997.** Temperature adaptation and acclimation potential of leaf dark respiration in two species of *Ranunculus* from warm and cold habitats. *Arctic and Alpine Research* **29**: 122–125.

2. **Atkin OK, Holly C, Ball MC. 2000.** Acclimation of snow gum (*Eucalyptus pauciflora*) leaf respiration to season and diurnal variations in temperature: the importance of changes in the capacity and temperature sensitivity of respiration. *Plant, Cell & Environment* **23**: 15–26.

3. **Billings WD, Godfrey PJ, Chabot BF, Bourque DP. 1971.** Metabolic acclimation to temperature in arctic and alpine ecotypes of *Oxyria digyna*. *Arctic and Alpine Research* **3**: 277–289.

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7. **Chabot B, Billings W. 1972.** Origin and ecology of the Sierran alpine vegetation. *Ecological Monographs* **42**: 163–199.

8. **Cheesman AW, Winter K. 2013.** Elevated night-time temperatures increase growth in seedlings of two tropical pioneer tree species. *New Phytologist* **197**: 1185–1192.

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14. **Loveys BR, Atkinson LJ, Sherlock DJ, Roberts RL, Fitter AH, Atkin OK. 2003.** Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast- and slow-growing plant species. *Global Change Biology* **9**: 895–910.

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16. **Ow LF, Whitehead D, Walcroft AS, Turnbull MH. 2008b.** Thermal acclimation of respiration but not photosynthesis in *Pinus radiata*. *Functional Plant Biology* **35**: 448–461.

17. **Ow LF, Griffin KL, Whitehead D, Walcroft AS, Turnbull MH. 2010.** Seasonal variation in foliar carbon exchange in *Pinus radiata* and *Populus deltoides*: respiration acclimates fully to changes in temperature but photosynthesis does not. *Global Change Biology* **16**: 288–302.

18. **Rook DA. 1969.** The influence of growing temperature on photosynthesis and respiration of *Pinus radiata* seedlings. *New Zealand Journal of Botany* **7**: 43–55.

19. **Slot M, Rey-Sánchez AC, Winter K, Kitajima K**. Thermal acclimation of leaf respiration in tropical canopy trees and lianas. (Manuscript in preparation)

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BIOGRAPHICAL SKETCH

Martijn grew up in a small town in the north of The Netherlands, where he spent a comfortable youth not thinking much about trees other than as climbing objects and producers of pulp for the newspapers that he delivered every morning. Nonetheless, in 1997 he enrolled in Wageningen University to study forestry, specializing in forest ecology. When not studying, he read books, favoring the smoky comfort of Russian classics. Consequently, when the opportunity to go to Russia arose, he happily accepted, and in early 2002 he moved into a log cabin in central Siberia. There he got his first exposure to plant ecophysiology, working on cold-induced photoinhibition of Scots pine in the Siberian taiga as an intern at the Max Planck Institute for Biogeochemistry. After he earned his degree as a forestry engineer in Wageningen he worked in the tropical south of China for a few months, before starting a Master of Research in ecology at the University of York. While at York he continued to work on plant ecophysiology and tropical forest ecology.

After some years working as a field assistant, lab technician, intern, supermarket clerk, and garbage man, Martijn came to the University of Florida in 2007 to join the PhD program in the Department of Botany. Here, his interests in tropical forest ecology and plant ecophysiology were combined in his PhD project that focused on temperature response of dark respiration in tropical forest trees and lianas in Panama.

In summer 2013 Martijn will return to the tropics to start as a post-doctoral research associate at the Smithsonian Tropical Research Institute in Panama. He plans to read many more books and hopes to go back to climbing trees in old age.