DISSERTATION

ECOSYSTEM RESPIRATION AND FOLIAR MORPHOLOGY OF A PRIMARY TROPICAL RAIN FOREST: THE EFFECTS OF CANOPY STRUCTURE AND ENVIRONMENTAL GRADIENTS

Submitted by
Molly A. Cavaleri
Graduate Degree Program in Ecology

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MOLLY A. CAVALERI ENTITLED "ECOSYSTEM RESPIRATION AND FOLIAR MORPHOLOGY OF A PRIMARY TROPICAL RAIN FOREST: THE EFFECTS OF CANOPY STRUCTURE AND ENVIRONMENTAL GRADIENTS" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work:

Adviser: Daniel Binkley

Co-Adviser: Michael Ryan

Alan Knapp

David Steingraeber

Department Head: William Lauenroth

ABSTRACT OF DISSERTATION

ECOSYSTEM RESPIRATION AND FOLIAR MORPHOLOGY OF A PRIMARY TROPICAL RAIN FOREST: THE EFFECTS OF CANOPY STRUCTURE AND ENVIRONMENTAL GRADIENTS

Wood and foliage are major components of ecosystem respiration, but estimates of large-scale rates for tropical rain forests are uncertain because of poor sampling in the upper canopy and across landscapes. Carbon balance models often rely on leaf mass per area (LMA) because it correlates with many plant physiological parameters. Researchers have long assumed variation in LMA to be a response to light (sun/shade leaf dichotomy), but LMA also reflects increases in leaf density that result from decreasing water potential with height. We used a portable scaffolding tower to measure plant respiration, LMA, and light from ground level to the canopy top across 55 sites in a primary tropical rain forest in Costa Rica. The first objective of this study was to extrapolate woody CO_2 efflux to the forest by characterizing its variation with canopy structure and landscape gradients. The second objective was to extrapolate foliar and total respiration to the forest by investigating the variation in foliar respiration with foliar parameters, canopy structure, and landscape gradients. The third objective was to determine whether LMA varied primarily because of light or water potential. Wood and foliage respiration rates increased with height and showed differences between plant functional groups. Wood respiration per unit ground area was 1.3 μmol CO_2 m^{-2} s^{-1} and foliar respiration was 3.5 μmol CO_2 m^{-2} s^{-1}, representing 14% and 37% of total ecosystem

respiration, respectively. Total ecosystem respiration (9.38 ± 1.43 μmol CO_2 m^{-2} s^{-1}) was 33% greater than eddy flux nighttime net ecosystem exchange for the same forest, suggesting that eddy flux studies reporting a large sink for tropical rain forests may be in error. We found LMA to be better related to height than light environment, supporting the hypothesis that the LMA gradient within forest canopies is primarily driven by a linear decrease in turgor pressure with height, caused by an increase in hydraulic resistance with gravity and longer path length. While light does affect LMA slightly, especially in the light-limited understory, the sun/shade leaf model taught in every plant physiology textbook is too simplistic to describe the large variation of LMA with vertical structure.

Molly Andrea Cavaleri
Graduate Degree Program in Ecology
Colorado State University
Fort Collins, CO 80523
Spring 2007

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CHAPTER 1:

INTRODUCTION

Tropical forests contribute up to one third of gross primary production globally (Melillo et al. 1993; Dixon et al. 1994), yet little is known about how the carbon balance of these systems will be altered with climate change. Recent studies disagree about whether tropical rain forests will act as carbon sources or sinks with increasing climatic temperatures. Several atmosphere-biosphere modeling studies suggest carbon source behavior over time as a result of increasing temperature (Kindermann et al. 1996; Braswell et al. 1997; Tian et al. 1998; Cox et al. 2000; Ito and Oikawa 2000; White et al. 2000; Cramer et al. 2001; Clark et al. 2003), while eddy covariance studies generally have concluded that tropical rain forests are acting as carbon sinks (Fan et al. 1990; Grace et al. 1995; Malhi et al. 1998; Loescher et al. 2003). A better understanding of ecosystem respiration at the landscape scale is a crucial first step in predicting how tropical forest ecosystem carbon balance may change with climate change.

Wood and foliage are major components of total ecosystem respiration, but estimates of ecosystem-scale rates are uncertain in tropical forests because of poor sampling in the upper canopy and across landscapes. A portable scaffolding tower was used to measure woody tissue and foliar CO_2 efflux from ground level to the canopy top across a range of sites of varying soil fertility in a primary tropical rain forest in Costa Rica. To examine the magnitude of carbon loss in this complex system, I extrapolated chamber CO_2 exchange measurements of foliage and woody material and combined this information with soil respiration (Schwendenmann et al. 2003) and coarse woody debris

respiration (Clark et al. 2002) to estimate ecosystem respiration. I then compared the results to three years of eddy flux nighttime net ecosystem exchange (NEE_{night}) data from the same location (Loescher et al. 2003). The eddy flux technique has several possible sources of error, including low turbulence at night and biased air movement, which can both result in a systematic underestimation of nighttime respiration (Baldocchi 2003). I present an independent estimate of ecosystem respiration for this tropical rain forest by summing extrapolated respiration measurements of component ecosystem parts.

Part of my overall research goal was to examine the effects of canopy structure and landscape gradients on foliage and wood respiration. I examined the variation in wood CO_2 efflux with season, plant functional group, height, soil fertility, and slope. I also characterized the variation in foliar respiration with temperature, plant functional group, foliar nutrients, leaf mass per area (LMA), soil nutrients, height, and photosynthetic capacity. Identifying these patterns was important for refining extrapolations and also for gaining a better understanding of the biology of the system. In the process of investigating variation in foliar respiration, I discovered an interesting pattern that contradicted most textbook ideas about LMA gradients within forest canopies. Further exploration produced a convincing story in which the vertical gradient of leaf water potential, rather than light environment, was primarily responsible for the increases in LMA within the canopy profile.

This dissertation contains the results of my research of the effects of tropical rain forest canopy structure and environmental gradients on aboveground respiration, and the effects of canopy gradients on foliar morphology. My research is summarized here in three chapters, each written as a manuscript for submission to peer-reviewed journals. As

such, each chapter contains its own abstract, introduction, methods, results, discussion and references.

Chapter I is the exploration of variation and extrapolation of wood CO_2 efflux in a primary tropical rain forest. Chapter II focuses on variation in foliar respiration and the extrapolation of component parts to estimate total ecosystem respiration. Chapter III explores the notion that the sun/shade leaf model may not be sufficient in describing canopy gradients in leaf morphology.

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CHAPTER 2:

WOOD CO_2 EFFLUX IN AN OLD-GROWTH TROPICAL RAIN FOREST

ABSTRACT

The balance between photosynthesis and plant respiration in tropical forests may substantially affect the global carbon cycle. Woody tissue CO_2 efflux is a major component of total plant respiration, but estimates of ecosystem-scale rates are uncertain because of poor sampling in the upper canopy and across landscapes. To overcome these problems, a portable scaffolding tower was used to measure woody tissue CO_2 efflux from ground level to the canopy top across a range of sites of varying slope and soil phosphorus content in a primary tropical rain forest in Costa Rica. The objectives of this study were to: (1) determine whether to use surface area, volume, or biomass for modeling and extrapolating wood CO_2 efflux, (2) determine if wood CO_2 efflux varied seasonally, (3) identify if wood CO_2 efflux varied by functional group, height in canopy, soil fertility, or slope, and (4) extrapolate wood CO_2 efflux to the forest. CO_2 efflux from small diameter woody tissue (<10 cm) was related to surface area, while CO_2 efflux from stems >10 cm was related to both surface area and volume. Wood CO_2 efflux showed no evidence of seasonality over two years. CO_2 efflux per unit wood surface area at 25° (F_A) was highest for the N-fixing dominant tree species *Pentaclethra macroloba*, followed by other tree species, lianas, then palms. Small diameter F_A increased steeply with increasing height, and large diameter F_A increased with diameter. Soil phosphorus and slope had slight, but complex effects on F_A. Wood CO_2 efflux per unit ground area was 1.34 ± 0.36 μmol m^{-2} s^{-1}, or 508 ± 135 g C m^{-2} yr^{-1}. Small diameter wood, only 15%

of total woody biomass, accounted for 70% of total woody tissue CO_2 efflux from the forest; while lianas, only 3% of total woody biomass, contributed one-fourth of the total wood CO_2 efflux.

INTRODUCTION

The balance between photosynthesis and plant respiration in tropical forest ecosystems has the potential to impact global carbon balance. Tropical forests account for more than one third of global plant carbon uptake (Saugier et al. 2001), and at least half of this carbon is released back into the atmosphere each year in plant respiration (Edwards et al. 1981; Chambers et al. 2004). The overall balance between carbon uptake and release remains unclear for tropical forests, because of inadequate knowledge of variation across landscapes and over time, and very limited measurements of respiration rates for tropical trees.

Woody tissue releases a proportion of total assimilated CO_2 back into the atmosphere, but estimates vary widely for tropical forests. The majority of recent estimates are in the range of 7-14% (Odum 1970; Ryan et al. 1994; Meir and Grace 2002; Chambers et al. 2004), while some earlier estimates are ~25% (Yoda 1967; Whitmore 1984), to as high as 50% (Müller and Nielson 1965). Early wood CO_2 efflux studies performed in tropical rain forests were based on detached samples (Müller and Nielson 1965; Yoda 1967; Yoda 1983; Whitmore 1984), which may have introduced errors associated with rapid diffusion of CO_2 upon excision (Teskey and McGuire 2005), or other methodological biases. More contemporary studies in tropical forests measured wood efflux *in situ*, but only from lower boles (Ryan et al. 1994; Nepstad et al. 2002;

Chambers et al. 2004). Several authors have agreed on the importance of measuring branch CO_2 efflux high in the canopy (Sprugel 1990; Ryan et al. 1996; Damesin et al. 2002; Vose and Ryan 2002), but until now this has not been attempted in a tropical forest.

Many recent studies in temperate systems have estimated wood CO_2 efflux in terms of maintenance vs. growth respiration (Ryan 1990; Ryan et al. 1995; Sprugel et al. 1995; Maier 2001; Damesin et al. 2002). Separating maintenance from growth respiration in tropical rain forests is difficult because they have no dormant season. Seasonality in wood CO_2 efflux has been found in tropical forests with distinct dry seasons (Nepstad et al. 2002; Chambers et al. 2004), but no seasonal study has yet been done in a tropical forest with a less pronounced dry season. I devised a novel approach to both measuring and extrapolating wood CO_2 efflux throughout the canopy, and also conducted a separate study to address the question of seasonality in woody tissue CO_2 for a tropical forest without a dormant season or a pronounced dry season.

Access has generally been the limiting factor for efforts to understand how woody tissue CO_2 efflux varies with canopy structure and across landscape gradients in tropical rain forests. This study presents results from an intensive two year field campaign where measured bole and branch CO_2 efflux using a portable scaffolding tower to access wood from forest floor to canopy top across gradients of soil fertility and slope in a primary tropical rain forest in Costa Rica. I was also able to estimate small diameter wood biomass and surface area distribution using the wood harvested from the tower transects, which greatly improved extrapolations of wood CO_2 efflux to the forest.

This study had four objectives. First, I sought to better understand the physiological sources of woody tissue CO_2 efflux by determining whether efflux

measurements were related to wood volume or surface area. These units are also important for modeling exercises and extrapolation, because preferred units (volume or area based) determine what stand-level information will be needed in order to estimate wood CO_2 efflux for the forest. For the second objective, I investigated seasonal variation in wood CO_2 efflux, and whether or not this variation tracked changes in rainfall, temperature, or light. The third objective was to characterize the sources variation in wood CO_2 efflux. For this, I constructed competing analysis of covariance (ANCOVA) models with both structural variables (height, diameter, plant functional group, branch or stem) and landscape variables (slope, soil phosphorus). The final objective was to estimate net woody tissue CO_2 exchange for the forest with estimates of wood biomass, surface area, and CO_2 efflux rates stratified by canopy height, diameter, and plant functional group.

MATERIALS AND METHODS

Study site

La Selva Biological Station is located in the Caribbean lowlands of northern Costa Rica (10°20' N, 83°50' W), at 37-150 m above sea level. La Selva is classified as premontane tropical wet forest in the Holdridge life-zone system (Hartshorn 1983). The mean annual rainfall is approximately 4 m, and the mean annual temperature is 26°C. Sampling occurred within La Selva's 515 ha of primary (old growth) forest. The average canopy height for the primary forest, including gaps, is approximately 20 m, and emergent trees range from 30-60 m (Clark et al. 1996; Clark et al. 2004). The basal area of the primary forest is approximately 24 m^2 ha^{-1}, with about 500 trees ha^{-1}, and a

quadratic mean diameter of 24 cm, based on an inventory of woody stems ≥10 cm in diameter (Clark and Clark 2000). Detailed information about La Selva soils and plant communities can be found in McDade et al. (1994).

Sample design: tower sites

This study was designed to test whether any landscape patterns in soil phosphorus or slope caused differences in woody CO_2 efflux. Phosphorus, rather than nitrogen, is likely limiting in this system (McDade et al. 1994), and previous studies of the primary forest at La Selva found fewer and larger trees in fertile flat sites, while a higher density of smaller trees were found on steep slopes (Clark and Clark 2000). I used a stratified random sample to locate sites across the landscape, with three slope x three soil phosphorus classes. A soil phosphorus map of the primary forest area of La Selva with cells of 10 by 10 meters was created by krigging data of phosphorus concentrations from the top 10 cm of soil sampled at a resolution of 50 m x 100 m. A digital elevation map provided slope for the same 10 x 10 m cells. The landscape was stratified into nine slope x phosphorus classes of equivalent area, using Arc's SLICE command (ArcGIS, Environmental Systems Research Institute, Redlands, CA, USA). Seventy-two possible tower sites were selected to the nearest meter using eight random coordinates from each of the nine classes, excluding swamps, permanent plots (to avoid disturbing long-term research), soils near streams, and sites close to trails. The 72 randomly selected sites were visited to assess the feasibility of tower construction, and 27 of these were discarded, approximately half because of wet or rocky terrain, and half because of large stems (>10 cm DBH) inside the tower footprint. Ultimately, 45 primary forest tower

sites were selected in this manner, five sites in each of the nine slope x phosphorus classes. Before each tower was constructed, slope was measured with a clinometer at the center of each tower site as the mean of two point measurements, taken 90° from one other. I used these slope data for all further analyses, rather than slope derived from the digital elevation map.

Because the original stratification did not capture any forest gaps, ten of the original 45 sites were selected as starting points for a procedure to locate ten additional "low canopy height" sites. At each of these ten sites, one low canopy height tower site was selected as the first location along a randomly oriented 50 m transect that had vegetation less than 16 m in height. At each new site, slope was measured and the krigged soil map was used to estimate soil phosphorus level.

At each original and low canopy tower site, an aluminum walk-up scaffolding tower (Upright, Inc, Dublin, Ireland) was constructed to the top of the canopy. These wood CO_2 efflux data represent sampling from 41 of the original 45 tower sites (including at least three towers in each of the nine slope x phosphorus classes), and 8 of the 10 low canopy height tower sites for a total of 49 towers. The wood biomass and wood surface area data used to estimate CO_2 efflux for the forest represent sampling from all 45 original tower sites, but no low canopy height sites, (because they did not represent the forest). While this paper focuses only on woody tissue net CO_2 exchange, the towers sampling design and construction were part of a larger project with the goal of characterizing canopy structure and function in a tropical rain forest.

Towers were constructed one section at a time, and all biomass within a section was harvested as each tower was built. Each section was 2.45 m x 1.86 m x 1.86 m

(LxWxH), with a footprint area of 4.56 m^2. The number of sections for each tower varied with canopy height, ranging from 1 section (1.86 m) to 24 sections (44.64 m). Harvested woody material was measured for length and diameter. After completing tower construction and harvesting, I measured CO_2 efflux on all intact woody species accessible from the side of the tower. Care was taken to avoid sampling near cut ends of stems or branches. The tower was then dismantled and moved to the nearest pre-selected random site. Each tower site was sampled only once, and tower construction and sampling occurred continuously from June 2003 to June 2005. These data represent 1226 wood CO_2 efflux measurements: two replicate measurements each of 613 individual branches or stems. Efflux samples represent over 110 species, 90 genera and 52 families. I separated woody species into four functional groups: trees, palms, lianas, and *Pentaclethra macroloba*, a leguminous tree species with 37% of the above-ground biomass (Clark and Clark 2000). Palm rachises measured in this study were woody and were included as branches.

The sampling scheme was not designed to take an unbiased sampling of wood biomass, surface area or CO_2 efflux for stems or branches >10 cm in diameter, because the tower could not be constructed within 1 m of large trees, or where large branches passed through the tower column. Therefore, large diameter stems and branches in the upper canopy were difficult to reach from the tower. To capture CO_2 efflux for large diameter wood, I measured efflux at approximately breast height on all woody stems surrounding the tower that had foliage represented anywhere in the tower footprint.

Sample design: seasonal measurements

To detect any seasonal changes in woody tissue net CO_2 exchange, an additional ten trees (five from each of two plots), were selected from long-term 0.5 ha plots in the primary forest landscape of La Selva (Clark and Clark 2000). I randomly chose canopy-level trees that represented five families, five genera, and six species, with above-buttress diameters ranging from 34 cm to 56 cm (Table 1). These ten trees were located in the same stand of primary forest, but were independent of the tower sampling sites. I sampled each tree once a month for 23 months from July 2003 to May 2005, with no measurements in December 2004 or January 2005 because of flooding. CO_2 efflux was measured in two locations on each tree, at heights ranging from 1.3 to 4.0 m, depending on buttress height.

CO_2 efflux measurements

Woody CO_2 efflux was measured using LCA-3 and LCA-4 open-system infrared gas analyzers (IRGA, Analytical Development Company, Hoddeson, UK). Woody tissue net CO_2 flux may be considered the sum of three terms: woody tissue respiration (+ flux), bark photosynthesis (- flux), and CO_2 dissolved in the xylem sap (+ if diffusing out, - if transported away) (Cernusak and Marshall 2000; McGuire and Teskey 2004; Bowman et al. 2005). I used unshielded clear polycarbonate custom-made chambers that allowed bark photosynthesis in an attempt to measure the sum of all three terms, throughout the vertical canopy transect. Chambers had neoprene gaskets and were clamped to stems or branches for measurements. Four chamber sizes were used, depending on the diameter of the woody material. Wood surface areas inside the four chambers were 7, 15, 16 and 22 cm^2, and sampled wood ranged from 0.7 cm to 90.0 cm in diameter. Chamber areas were

calculated as the area enclosed by the neoprene gasket + half the area of the gasket (assuming half the CO_2 from the area under the gasket would diffuse inside the chamber and half would diffuse outside the chamber). Small 9V battery-operated fans were installed to stir the air inside all but the smallest (7 cm^2) chambers. Air flow rates through the chambers ranged from 223-297 $\mu mol\ s^{-1}$ and chamber seals were checked with a flowmeter. Stable reference air CO_2 concentrations were maintained by drawing air through a 19 L mixing chamber. The difference in CO_2 concentration between the reference and the chamber was recorded after it had been stable for at least 2 minutes. I measured CO_2 efflux twice on each stem and branch, with measurements ~90° from one another, and replicates were averaged prior to analysis. Diameter was measured at the center point of chamber attachment.

At each CO_2 efflux measurement site, surface wood temperature was taken with a thermocouple thermometer. All fluxes were corrected to a reference temperature of 25 °C using a Q_{10} of 2.0. Ryan et al. (1994) found stem respiration Q_{10} values of 2.1 and 2.2 for two tree species at La Selva, and Meir et al. (2002) found mean stem respiration Q_{10} values of 1.6 and 1.8 for two tropical rain forests in Brazil and Cameroon.

I randomly selected subsamples of the harvested woody material for specific gravity measurements to calculate CO_2 efflux rates per unit biomass. Wood sample volume was measured by water displacement, and sample mass was measured after oven drying to constant mass at 60 °C. Stems measured for CO_2 efflux from the ground, where the wood was not representative of wood harvested in the tower footprint, were assigned specific gravity based on published values by species (Hidayat and Simpson 1994; Brown 1997; Segura and Kanninen 2005). When species were unknown or

specific gravity values were not found in the literature for a particular species, a La Selva stand-level mean specific gravity was used, 0.53 g cm^{-3} (Muller-Landau 2004).

Levy-Jarvis analyses: woody CO_2 efflux per surface area vs. volume

I used a graphical technique to discern the best units for expressing wood CO_2 efflux and for extrapolating to the forest (Levy and Jarvis 1998). If the CO_2 efflux rate is proportional to surface area, measured CO_2 efflux per unit volume will be positively and linearly correlated with the reciprocal of diameter. If the CO_2 efflux rate is proportional to volume (or biomass), measured CO_2 efflux on an area basis should be positively and linearly correlated with diameter. I examined the volume vs. surface area components for four canopy height classes: bottom 2 m (0-2 m), lower canopy (2-15 m), mid canopy (15-25 m), and upper canopy (25+ m).

Statistical analyses: seasonal changes in wood CO_2 efflux

The ten tree boles measured once a month for two years were analyzed with a repeated measures ANOVA in SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA) with individual tree as the "subject." I tested for differences in month, species, or their interaction on CO_2 efflux corrected to 25 °C. Monthly stem CO_2 efflux rates for individual species and averaged over all species were regressed against total monthly rainfall, mean monthly day and night temperature, and mean monthly photosynthetically active radiation from the La Selva Biological Station long term weather station data base (Organization for Tropical Studies; http://www.ots.duke.edu/).

Statistical analyses: sources of variation in CO_2 efflux across canopy and landscape gradients

I constructed an *a priori* set of candidate ANCOVA models with structural and environmental variables to describe both area-based wood CO_2 efflux corrected to 25 °C (F_A: μmol m^{-2} s^{-1}), and mass-based wood CO_2 efflux corrected to 25 °C (F_M: nmol kg^{-1} s^{-1}). Models were developed for two purposes: (1) to investigate the primary sources of variation in woody tissue CO_2 efflux and (2) to estimate efflux rates and errors for extrapolating rates to the forest. For each purpose, the most appropriate units were used as determined by the Levy-Jarvis analysis. The predictor variables considered were: natural log of diameter (lnD: continuous variable, cm); slope (S: continuous variable, degrees); plant functional group (G: liana, *P. macroloba*, tree or palm); soil phosphorus class (P: low P range: 0.65-0.86 mg g^{-1}; medium P range: 0.88-1.11 mg g^{-1}; high P range: 1.12-1.57 mg g^{-1}); canopy height class (H: bottom 2m = 0-2 m; lower canopy = 2-15 m; mid canopy = 15-25 m; upper canopy 25+ m); and wood type (WT: branch or stem). Diameter, F_A, and F_M were natural log-transformed to account for non-normal distributions and heteroscedasticity in the residuals.

I evaluated competing models using Akaike's Information Criterion (AIC), which penalizes a model based on its number of parameters. The best statistical model minimizes the value of AIC (Burnham and Anderson 1998). For each candidate model, maximum likelihood estimates of model parameters and AIC were calculated using PROC MIXED Method=ML in SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA). Normally distributed errors were verified by examining residuals after model fitting.

The best-fit models for both $\ln F_A$ and $\ln F_M$, as determined by the lowest AIC values, were used in all further analyses. Least-squares means for all interactions in the selected best-fit model were calculated for class variables and for specified values of continuous variables. Antilogs of least-squares means were plotted to make graphs easier to read, and only values within the range of diameters sampled for each class variable were plotted.

Estimating woody tissue CO_2 efflux for the forest

I estimated wood CO_2 efflux for wood <10 cm diameter using surface area, and for wood >10 cm using biomass, based on the results from the Levy-Jarvis analysis (Fig. 1). I used a two-part approach to assemble the biomass and surface area data needed for forest level estimates of wood CO_2 efflux, because the tower sampling was not designed to provide an unbiased estimate of wood >10 cm diameter (the tower could not be located over large stems). I used tower sample estimates of surface area for wood <10 cm diameter, and woody biomass from the eighteen 0.5 ha plots measured by Clark and Clark (2000) for wood >10 cm in diameter. Biomass from the tower samples for wood <10 cm diameter was subtracted from the total biomass calculated from the 0.5 ha plot data to estimate biomass for wood >10 cm in diameter. I used mean wood CO_2 efflux rates for different diameter classes, height classes, and functional groups paired with the appropriate biomass or surface area estimates to calculate total flux per unit ground area. Since wood CO_2 efflux rates differed little with slope and soil phosphorus, I did not use soil information in further estimates.

For wood <10 cm diameter, CO_2 efflux per surface area (F_A) and wood surface area per ground area estimates were stratified by functional group (trees, lianas, palms and *P. macroloba*), height class (bottom 2 m, lower canopy, mid canopy and upper canopy), and diameter class (0-2, 2-5, and 5-10 cm). Least squares means and 95% confidence limits were calculated for each group, height class, and at diameters 1, 3.5, and 7.5 cm (class mid-points) using the best-fit model for F_A. Means and confidence limits were then back-transformed and used with corresponding surface areas to estimate woody CO_2 for the forest for wood <10 cm diameter.

For wood >10 cm, CO_2 efflux per biomass (F_M) and biomass per ground area were estimated by diameter class (10-20, 20-30, 30-40, 40-60, 60-80, 80-100, and 100+ cm), and functional group (trees, lianas, palms and *P. macroloba*). Height was not used for these estimates because almost all of the large diameter efflux measurements were taken in the first two meters. Efflux rates for wood >10 cm were calculated for diameter class midpoints from the best fit model for F_M in a similar manner to that used for wood <10 cm. Woody biomass was estimated from eighteen 0.5 ha plots, established through stratified random sampling in the primary forest of La Selva. Above-buttress bole diameters were measured on all trees ≥10 cm in diameter in each 0.5 ha plot in 2004 (for more information on how plots were designed and sampled, see Clark and Clark 2000). I calculated total biomass for each tree, palm and *P. macroloba* in the eighteen plots using the following allometric equation from Chave et al. (2005):

$$TAGB = \rho * \exp(-1.239 + 1.980(\ln D) + 2.207(\ln D)^2 - 0.0281(\ln D)^3) \quad (1)$$

where $TAGB$ = total aboveground biomass (kg), ρ = wood specific gravity (g cm^{-3}), and D = above buttress diameter (cm). Specific gravity (ρ) used for trees = 0.53 g cm^{-3}

(Muller-Landau 2004), for *P. macroloba* = 0.60 g cm^{-3} (Segura and Kanninen 2005), and for palms = 0.31 g cm^{-3} (Baker et al. 2004). I used a different allometric equation for lianas from Gehring et al. (2004):

$$TAGB = \exp(-7.114 + 2.276(\ln D)) \quad (2)$$

Mean plot total biomass (kg m^{-2}) estimates by group were assumed to include small branches, but not small stems (only stems ≥10 cm were measured in the 0.5 ha plots). I subtracted the small branch biomass estimated from the tower footprint data from total biomass for each group to get the percent of total biomass by group that consisted of large diameter wood. Plot means and standard errors were calculated for large diameter wood biomass per ground area (kg m^{-2}) by plant functional group and diameter class.

RESULTS

Levy-Jarvis analysis: CO_2 efflux per surface area vs. volume

In the upper, mid, and lower canopy, the analysis showed that CO_2 efflux was related to surface area (Figs. 1A, 1B, 1C), and not to diameter (Figs. 1E, 1F, 1G). In the bottom 2 m, the analysis showed that CO_2 efflux was related to both volume and surface area (Fig. 1D, 1H).

Seasonal changes in wood CO_2 efflux

Stem F_A did not vary with month (P=0.90), species (P=0.18), or their interaction (P=0.34). Neither average F_A (Fig. 2A) nor F_A by species showed any trends with rainfall, temperature, or PAR (Figs. 2B-D).

Model selection results

Six predictor variables and all of their interactions could yield hundreds of possible models, so I limited the set of candidates prior to model selection. Using a limited set of *a priori* candidate models also reduces the effects of overfitting and erroneous correlations (Burnham and Anderson 1998). Preliminary full-model fits showed five of the six predictor variables to be highly significant, therefore all subsequent candidate models contained these five variables (lnD, G, P, H, and S); while wood type (WT) was found to be redundant and dropped from further analysis. Both lnF_A and lnF_M were modeled with linear combinations of the following predictor variables: lnD, G, P, H, and S; the 2-way interactions lnD*G, lnD*P, lnD*H, lnD*S, G*P, G*H, G*S, P*H, P*S, and H*S; and the 3-way interactions lnD*G*P, lnD*G*S, and lnD*P*S. The final *a priori* model set contained 143 models with 8 to 18 parameters. Surprisingly, the best-fit models with the lowest AIC values for predicting both lnF_A and lnF_M were exactly the same (Table 2). Model-derived least-squares means and 95% confidence limits of F_A and F_M are displayed for all height classes at five representative diameters (Table 3).

Sources of variation in wood CO_2 efflux

Because the Levy-Jarvis analysis showed that wood CO_2 efflux was related to surface area at all heights and diameters (Figs. 1A-D), I used efflux per unit surface area to investigate variation across canopy and landscape gradients. F_A increased with increasing slope, with greater effect in small diameter wood (Fig. 3A) and low soil P (Fig. 3D). The dominant tree species of this ecosystem, *P. macroloba*, had the highest F_A

at all diameters (Fig. 3B). Liana F_A sharply decreased with diameter, and the highest liana rates were comparable to high rates for *P. macroloba* at the smallest diameters (Fig. 3B). None of the lianas sampled were greater than 9 cm in diameter, and at this maximum diameter, lianas had lower F_A than all dicot tree species (Fig. 3B). Palms had the lowest overall F_A for all diameters, and showed little overall change in F_A with diameter (Fig. 3B). F_A of wood in the bottom 2 m increased with increasing diameter (Fig. 3C). F_A of small diameter wood in the canopy was much greater than F_A of small diameter wood in the bottom 2 meters, and F_A of wood less than 15 cm in diameter increased sharply with height, given the same diameter (Fig. 3C). CO_2 efflux rates for large diameter wood could be biased towards lower heights because 82% of the CO_2 efflux measurements from wood greater than 10 cm, and 92% of measurements from wood greater than 20 cm were taken less than two meters from the ground.

Small diameter wood biomass and surface area distribution

Biomass of all woody tissue <10 cm in diameter, including both branches and small stems, was 2.4 kg m^{-2}. A little over half of this total consisted of tree wood, while lianas, palms, and *P. macroloba* contributed about 15% each (Figs. 4A-D). Surface area of woody tissue <10 cm in diameter was 1.1 m^2 m^{-2}. Trees contributed a little less than half to the total surface area (Fig. 4F), lianas contributed almost one third to the total (Fig. 4H), and palms and *P. macroloba* contributed about 13% each (Figs. 4E and 4G). About 40% of tree small diameter biomass and surface area were in the lower canopy (2-15 m), with a fairly even distribution in the rest of the height classes (Figs. 4B and 4F). Over three-fourths of both *P. macroloba* and liana small diameter biomass and surface

area were found above 15 m, in the mid and upper canopy (Figs. 4A, 4E, 4D, and 4H), while palms accounted for virtually no biomass or surface area above 15 m (Figs. 4C and 4G). Total biomass for all wood <10 cm was distributed evenly among diameter classes, with about one-third per class (Figs. 4A-D). In contrast, the smallest diameter class (0-2 cm) accounted for 70% of the total <10 cm wood surface area (Figs. 4E-H). Both total small diameter biomass and total surface area were distributed similarly by canopy height, with approximately 10% in the bottom 2m, and about 30% in each of the lower, mid and upper canopy levels.

Large diameter wood biomass distribution

Small branches (<10 cm) were 11% of woody biomass for trees, 7% for *P. macroloba,* 17% for palms, and 49% for lianas, based on the small branches harvested from the tower transects and the 0.5 ha plot estimates of total woody biomass. Based on these percentages and the total biomass calculated from the 0.5 ha plot data, the total aboveground biomass of wood >10 cm diameter was 13.8 kg m^{-2}. The contribution of trees to this total was 59%, while *P. macroloba* contributed 35%, palms only 6%, and lianas less than 1% (Fig. 5A). The largest proportion (31%) of woody biomass >10 cm was in the 40-60 cm diameter class (Fig. 5A). The rest of the diameter classes were fairly evenly distributed with about 15% of the biomass each, except for 80-100 cm and 100+ cm, which accounted for only about 5% of the total large diameter biomass each (Fig. 5A).

Forest estimates of wood CO_2 efflux

Wood CO_2 efflux per unit ground area for woody tissue <10 cm in diameter was 0.95 ± 0.31 μmol m^{-2} s^{-1} (359 ± 118 g C m^{-2} yr^{-1}). Of this total, about 40% was from trees, 30% from lianas, 20% from *P. macroloba*, and 10% from palms (Figs. 4M-P). The upper, mid and lower canopy height classes each contributed about 30% each, while only about 10% of the small diameter wood CO_2 efflux came from the bottom 2 m (Figs. 4M-P). Over 70% of the CO_2 efflux for wood <10 cm came from the 0-2 cm diameter class, about 20% from the 2-5 cm class, and only about 10% from the 5-10 cm class (Figs. 4M-P). Because of their large surface area (Fig. 4H), lianas contributed a substantial portion of the total small diameter wood CO_2 efflux in the upper and mid canopy (Fig. 4P). The greatest proportion of tree biomass (Fig. 4B), surface area (Fig. 4F), and total CO_2 efflux (Fig. 4N) was in the lower canopy (2-15 m in height).

CO_2 efflux per unit ground area from wood >10 cm in diameter was 0.39 ± 0.05 μmol m^{-2} s^{-1} (149 ± 17 g C m^{-2} yr^{-1}). Of this total, about 60% was from trees, 30% from *P. macroloba*, 10% from palms, and <1% from lianas (Fig. 5C). Diameter classes (in cm) and their approximate percent contributions to total large diameter wood CO_2 efflux per unit ground area were as follows: 10-20 = 30%, 20-30 = 15%, 30-40 = 15%, 40-60 = 25%, 60-80 = 10%, and the largest two classes had less than 2% each (Fig. 5C). The largest proportion of *P. macroloba* CO_2 efflux was from diameters in the 40-60 range, while the largest proportion of tree CO_2 efflux was from diameters in the 10-20 cm range (Fig. 5C).

Total wood (all diameters) CO_2 efflux per unit ground area for this system was 1.34 ± 0.36 μmol m^{-2} s^{-1} (508 ± 135 g C m^{-2} yr^{-1}). Trees contributed 47% of the total, P. macroloba contributed 20%, lianas contributed 24%, and palms only contributed 9%

(Table 4). Wood that was <2 cm in diameter contributed half of the total efflux, and wood that was <10 cm accounted for 70% of total woody CO_2 efflux (Table 4). Less than 2% of the total woody efflux was from wood that was >80 cm (Table 4).

DISCUSSION

Levy-Jarvis analysis: CO_2 efflux per surface area vs. volume

The best units for expressing and extrapolating woody CO_2 efflux depend on whether the primary sources of CO_2 are surface area-based (Linder and Troeng 1980; Matyssek and Schulze 1988; Meir and Grace 2002; Chambers et al. 2004), or volume/biomass-based (Yoda 1983; Ryan 1990; Bowman et al. 2005). Some studies conclude that a mixture of several units and extrapolating procedures may be desirable (Lavigne et al. 1996; Damesin et al. 2002). One of the main issues for the use of one method over another is the attempt to partition maintenance vs. growth respiration sources. Generally, growth respiration is estimated using stem diameter growth data, while maintenance respiration may be estimated using sapwood volume and/or measurements taken when trees are dormant (Ryan 1990; Sprugel 1990; Ryan and Waring 1992; Ryan et al. 1994; Ryan et al. 1995; Sprugel et al. 1995). These well documented methods of measuring growth and maintenance respiration were not feasible in this study, because I had neither growth nor sapwood volume data.

According to Levy and Jarvis (1998), if the rate of CO_2 efflux is proportional to wood volume, efflux primarily reflects sapwood xylem parenchyma cell maintenance respiration; while a relationship with surface area reflects growth respiration of cambium and phloem cells. These divisions may not be useful for tropical forests, however,

because separating growth vs. maintenance respiration is difficult when there is no dormant season. Therefore, an increase of CO_2 efflux with volume could result from faster growth of larger diameter wood and/or a larger sapwood maintenance component. Whether CO_2 dissolved in xylem sap diffuses radially out of the bark, or is transported up the xylem stream could also be affected by both xylem volume and surface area for diffusion (Teskey and Mcguire 2002). As a result of these confounding factors, I will discuss volume and surface area CO_2 efflux sources, but not infer growth or maintenance respiration from their source.

In the upper, mid, and lower canopy, the relationship between the reciprocal of diameter and CO_2 efflux per unit volume (Fig. 1A, 1B, 1C) and the lack of relationship between diameter and efflux per unit area (Fig. 1E, 1F, 1G) indicate that wood CO_2 efflux for all wood above 2 m was primarily dependent on surface area, and not volume. These results contradict the findings of Yoda and others that respiration of small diameter wood was proportional to mass, while respiration of large diameter wood was proportional to surface area (Yoda et al. 1965; Yoda 1967). Unlike canopy rates, wood CO_2 efflux in the bottom 2 m was dependent on both volume and area (Fig. 1D, 1H). This is likely the result of the larger trees growing faster (Clark and Clark 2000) and more sapwood volume in larger trees. By simple geometry, the proportion of sapwood volume per unit surface area in large diameter wood is much greater than that of small diameter wood. These patterns in volume vs. area-based measurements are consistent with other studies of large diameter wood measured near the ground (Damesin et al. 2002; Meir and Grace 2002).

Seasonal changes in wood CO_2 efflux

Woody CO_2 efflux has been shown to vary seasonally in temperate forests (Sprugel 1990; Ryan et al. 1997; Damesin et al. 2002; Vose and Ryan 2002), where there are definite growing season and dormancy dynamics. Increased rates of stem CO_2 efflux (Nepstad et al. 2002; Chambers et al. 2004) and ecosystem respiration (Goulden et al. 2004) were found during the wet season in Brazilian rain forests, but the wet/dry season dynamics in this Costa Rican study site are not as pronounced. In the ecosystem of La Selva, there is documentation of seasonality in litterfall nutrient dynamics (Wood et al. 2005), soil respiration (Schwendenmann et al. 2003), and tree ring data (Fichtler et al. 2003). Trees in this system grow year-round, and while some tree species show seasonal patterns in growth (Hazlett 1987), the phenologies of all species are not synchronized the way they are in temperate forests or tropical forests with dry seasons. In this tropical rain forest, neither temperature, PAR, nor precipitation varied enough to affect woody CO_2 efflux rates of all species sampled. These results greatly simplify carbon balance modeling for this system.

Sources of variation in CO_2 efflux across canopy and landscape gradients

The best fit model for $\ln F_M$ accounted for 75% of the variation in CO_2 efflux (Table 2), but this is likely because F_M is autocorrelated with diameter (diameter is used to calculate F_M). The best fit model for $\ln F_A$ only accounted for 29% of the variation in CO_2 efflux (Table 2), likely because over 110 identified species and dozens more unidentified species were sampled. Nevertheless, general inferences based on the results

of the models will help us understand system processes, and how wood CO_2 efflux varies with canopy structure and landscape gradients.

EFFECTS OF SLOPE AND PHOSPHORUS

Initially I believed woody CO_2 efflux would increase with increasing soil P, based on evidence that P is likely limiting in this system (McDade et al. 1994) and the evidence that more nutrient-rich sites tend to have larger trees (Clark and Clark 2000). The situation is not this simple, however, as the effect of P seems to depend on slope, and the trend of higher F_A with higher slope (Figs. 3A and 5D) is most likely confounded with nutrient availability. The shallow slopes at La Selva tend to be inceptisols with higher available soil P, and the steeper slopes tend to be more acidic ultisols with less P available (McDade et al. 1994). At La Selva Biological Station, Schwendenmann et. al (2003) found higher rates of soil respiration where there were low levels of soil P, likely because more biomass of fine root mycorrhizae would be found in these sites, resulting in higher respiration rates from root/mycorrhizae complexes. Higher rates of tree root respiration where P is less available (steep, acidic ultisols) may have resulted in higher rates of measured aboveground woody CO_2 efflux. Teskey and McGuire recently found evidence that much of the CO_2 dissolved in stem xylem sap likely comes from root respiration and is transported upward in the xylem stream (pers comm). The idea that tree root respiration rates affect aboveground woody CO_2 efflux rates is supported by the {Slope*Phosphorus} interaction plot, in which the slope effect is only at low total P, where the effect of pH on P availability is likely to be more biologically important (Fig. 5D).

EFFECTS OF FUNCTIONAL GROUP

Whatever competitive advantage that allows *P. macroloba* to be dominant in this extremely diverse system also likely contributes to higher growth rates and thus higher woody respiration rates. CO_2 efflux rates of *P. macroloba* and all other tree species increase with increasing diameter on average (Fig. 5B), probably because growth (Clark and Clark 2000), and likely growth respiration also increase with diameter in this ecosystem.

The decrease in liana CO_2 efflux rates with increasing diameter (Fig 5B) could be the result of both higher growth rates and greater xylem CO_2 diffusion in liana branches, and lower maintenance respiration in liana stemwood. Lianas rely on the support of neighboring trees to reach the top of the canopy. Once there, they put proportionally more energy into producing leaf area than stem growth (Putz 1983). Thus, growth respiration rates of fine liana branches would likely be higher than liana stem growth respiration rates. Lianas have larger diameter xylem vessels than trees on average (Ewers and Fisher 1991; Fisher and Ewers 1995), and have been documented to transport more water than trees of similar diameters (Restom and Nepstad 2001). It would follow that lianas have the capacity to transport more dissolved CO_2 per unit surface area to the top of the canopy than the average tree, resulting in greater CO_2 diffusion out of small branches. Lianas also tend to have smaller stems than trees (Putz 1983), and thus a smaller volume contribution of stem maintenance respiration. The high rates of small diameter liana branches would not have been revealed if only lower stem measurements had been taken.

Palms in this ecosystem are generally located in the lower canopy; they rarely reach the upper canopy, where the highest overall efflux rates were found (Fig. 5C). Slower growth rates would also likely lead to lower woody growth respiration rates. The lower CO_2 efflux rates for small diameter palm parts may be explained by the fact that measured palm "branches" were actually palm frond rachises, which were usually green and thus likely re-fixing respired CO_2.

EFFECTS OF CANOPY POSITION

Wood type (branch vs. stem) did not explain significant variation when both diameter and height were included in the model, indicating branches and stems of the same size and in the same location had similar CO_2 efflux rates. In this tropical forest, where most trees have deliquescent morphology, the concept of stem vs. branch is more of a continuum, and often difficult to determine. Although Sprugel (1990) asserts that branch respiration may be qualitatively different from stem respiration, the subjective divisions of stem vs. branch categories were not as important to CO_2 efflux rates as the diameter and height of the woody tissue itself.

Wood CO_2 efflux in the bottom 2 m, which increased with increasing diameter (Fig. 3C), largely consisted of stems. Large diameter stems likely have both greater growth respiration *and* greater maintenance respiration rates per unit surface area than smaller diameter stems. Several studies of tropical trees also found an increase of stem CO_2 efflux on a surface area basis with increased stem diameter (Ryan et al. 1994; Meir and Grace 2002; Nepstad et al. 2002).

Wood in the upper canopy had much higher CO_2 efflux rates than wood of the same diameter lower in the canopy (Fig. 3C). Conversely, Yoda et al. (1965) found stems to have higher rates than branches, given the same diameter. Yoda and others measured CO_2 efflux on detached wood in enclosed chambers, where the diffusion effect of dissolved CO_2 in the xylem would be negligible, as the xylem CO_2 had likely already escaped prior to measurement. Indeed, the rapid increase in woody CO_2 efflux after excision is likely the result of rapid diffusion, as opposed to an increased respiration rate from wounding (Teskey and McGuire 2005).

Several possible driving mechanisms may be causing the trends of increasing CO_2 efflux with height for small diameter wood. First, within-tree woody respiration may increase closer to the leaves (higher in the canopy) because of the increased energy cost of both growing new cells, and loading and unloading carbohydrates into and out of the phloem from the xylem parenchyma cells (Sprugel 1990). This effect may also be amplified by the fact that leaves in full sun (higher in the canopy) have higher photosynthetic capacity and net photosynthesis than shade leaves (Ellsworth and Reich 1993; Dang et al. 1997; Carswell et al. 2000; Wilson et al. 2000; Hubbard et al. 2002). Second, wood respiratory potential, which is independent of xylem CO_2 diffusion, has been found to increase with increasing height in *Dacrydium cupressinum* (Bowman et al. 2005) and *Pseudotsuga menziesii* (Pruyn et al. 2002). A third possible explanation for higher CO_2 efflux rates of small branches high in the canopy is that they are growing faster. Small branches lower in the canopy may be older and have nearly stopped growing, while branches of the same size higher in the canopy may be younger and still growing rapidly. Finally, diffusion of CO_2 out of the xylem sap may also increase with

increasing height and decreasing diameter as a result of the upward movement of dissolved CO_2 during the day and thinner bark closer to the leaves. In several studies, diffusion of CO_2 dissolved in the xylem stream was found to be the primary source of measured CO_2 efflux (Teskey and Mcguire 2002; McGuire and Teskey 2004; Bowman et al. 2005). Further study is necessary to tease apart all of these possible reasons as to why woody tissue CO_2 efflux rates are so high at the top of the canopy.

Wood Surface area and biomass distribution

To my knowledge, no other dataset explores small diameter woody biomass and surface area distribution throughout the vertical canopy transect within a tropical rain forest. These data were crucial for extrapolating small diameter wood CO_2 fluxes, as surface area high in the canopy had a large effect on the estimate of total efflux (Figs. 4E-H and 4M-P).

The total woody biomass was 16.2 kg m^{-2}, with wood >10 cm contributing 13.8 kg m^{-2}, and wood <10 cm contributing 2.4 kg m^{-2}. This corresponds almost exactly with a previous estimate of total aboveground biomass for the primary forest of La Selva, 16.1 kg m^{-2} (Clark and Clark 2000). Perhaps this is not too surprising, considering the same eighteen 0.5 ha plot diameters were used in this study, however, Clark et al. used different allometric equations and did not take small vs. large diameter wood into account in their analysis.

This study also provided a novel way to estimate total liana biomass: 0.44 kg m^{-2}, or 3% of the estimated total woody biomass. When using plot-level above-buttress stem diameter data which only included stems ≥10 cm to estimate biomass, lianas were

essentially lost from the system (Fig. 5A). Using the same 0.5 ha plot data, Clark and Clark (2000) estimated liana biomass as 0.06 kg m^{-2}, or only 0.4% of the total biomass, underestimating liana biomass 7-fold. In a lowland tropical rain forest in the Amazon, Phillips et al. (2005) found liana biomass to be three times greater than the biomass in this Costa Rican rainforest. Amazonian forests possibly have a much greater proportion of lianas ≥10 cm, which accounted for 80% of the total liana biomass (Phillips et al. 2005).

Forest-level estimates of wood CO_2 efflux

Total woody tissue CO_2 efflux rate (508 ± 135 g C m^{-2} yr^{-1}), is approximately 40% of the estimated soil CO_2 efflux rate for the primary forest of La Selva (1027-1613 g C m-2 s-1, Schwendenmann et al. 2003), and approximately 20-30% of the estimated nighttime CO_2 net ecosystem exchange (1741-2668 g C m^{-2} s^{-1}), as estimated by the eddy covariance technique at La Selva from 1998-2000 (Loescher et al. 2003). Total woody tissue CO_2 efflux rate for this stand was almost twice the previous estimate of woody CO_2 efflux at La Selva (Ryan et al. 1994), which was only based two tree species. The estimate of this study is also about 20% higher than a recent estimate of woody tissue CO_2 efflux in an Amazonian tropical rain forest, which concluded that wood CO_2 efflux accounted for 20% of autotrophic respiration and about 14% of the total carbon assimilated by photosynthesis (Chambers et al. 2004). Both of these woody tissue efflux estimates were extrapolated solely based on stem measurements taken from the ground. In this study, a large portion of CO_2 flux would have been missed had small diameter wood had not been measured high in the canopy, especially lianas. Small diameter wood

(<10 cm) was only 15% of total woody biomass, but accounted for 70% of total woody CO_2 efflux (Table 4). Lianas were only 3% of the total woody biomass, but contributed one-fourth of the total woody CO_2 efflux (Table 4).

Uncertainties associated with these forest-scale woody CO_2 efflux estimates fall into two categories: uncertainties in the rates themselves and uncertainties in the data used to extrapolate these rates to the ecosystem. Three possible sources of error in the efflux rates per unit biomass or surface area are: (1) the lack of large diameter wood measurements high in the canopy, (2) the lack of a correction for seasonal temperature differences, and (3) the lack of nighttime measurements. The fact that I could not easily measure large branches or stems from the tower may not greatly bias estimates of large diameter wood CO_2 efflux because, while small diameter wood efflux has been found to increase with height, efflux rates of large diameter wood tend to remain unchanged with height (Sprugel 1990; Ryan et al. 1996; Damesin et al. 2002). The lack of a correction for seasonal temperature variation is also not likely to cause large errors, because the base temperature to which all CO_2 efflux rates were corrected (25 °C) was within a degree of the average annual temperature, and temperature shows only a small diurnal and seasonal amplitude (Agren and Axelsson 1980). If measurements had been taken only at the bases of stems, actual rates may have been underestimated by 30% or more by only measuring during the day, because much of the respired CO_2 is transported up the xylem stream with the sapflow (Teskey and Mcguire 2002; McGuire and Teskey 2004; Bowman et al. 2005). In this study, I have attempted to capture this "lost" respired CO_2 by measuring the full vertical transect of the canopy. During the day, when sapflow is at its peak, CO_2

in the xylem stream must eventually diffuse out of the tree, most likely high in the canopy where branch bark is thinnest.

Uncertainties in biomass and surface area estimates are likely to cause greater errors in ecosystem rates because of multiplicative effects and uncertainties in allometric equations. Even though surface area estimates for all woody tissue <10 cm in diameter were based on direct harvesting rather than allometry, they still could lead to substantial error if the towers sampling scheme did not adequately represent the forest with respect to small diameter wood distribution. Of the original randomly located set of possible tower sites, 37% were discarded because of rocky terrain or large stems, therefore it is reasonable to assume the tower sites are representative of at least 63% of the landscape. The allometric equations I used to estimate biomass for wood >10 cm in diameter could also be large sources of error, especially in diameter ranges >80 cm (Clark and Clark 2000). Less than 2% of the total woody CO_2 efflux came from wood that was >80 in diameter, however (Table 4), so this is likely not a huge source of error in these ecosystem estimates of wood CO_2 efflux.

MAIN CONCLUSIONS

- Stem CO_2 efflux showed no evidence of seasonality over a span of two years.
- Stem and branch CO_2 efflux rates per unit surface area at 25° (F_A) increased with woody tissue diameter for all dicot tree species, did not change with diameter for palms, and decreased with diameter for lianas.
- F_A was highest for the N-fixing dominant tree species *P. macroloba*, followed by other dicot tree species, lianas, and finally palms.

- Small diameter wood (<10 cm) F_A increased steeply with increasing canopy height.
- Total woody tissue net CO_2 exchange for this primary tropical rain forest was estimated as 1.34 ± 0.36 μmol m^{-2} s^{-1} (508 ± 135 g C m^{-2} yr^{-1}).
- Small diameter canopy wood is a substantial source of total woody CO_2 efflux, especially lianas.

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TABLES

Table 2.1. Species, family, and diameter of ten trees measured each month for the stem CO_2 efflux seasonal study. Each tree was measured once a month for 23 months.

Species	Family	Above buttress diameter (cm)	No. trees measured
Virola sebifera	Myristicaceae	44.1	1
Virola koschnyi	Myristicaceae	34.6	1
Apeiba membranaceae	Malvaceae	49.4	1
Cespedesia spathulata	Ochnaceae	46.7	1
Laetia procera	Flacourtiaceae	49.0	1
Pentaclethra macroloba	Fabaceae	36.7, 42.0, 49.4, 53.7, 55.6	5

Table 2.2. Predictor variables, their p-values, and model R^2 values for the best-fit model predicting both the natural log of area-based woody CO_2 efflux at 25°C (lnF_A), and the natural log of biomass-based woody CO_2 efflux at 25°C (lnF_M).

Predictor variable	Abbrev.	Coefficient p-values for model predicting lnF_A	Coefficient p-values for model predicting lnF_M	Factors or Units (if applicable)
ln(Diameter)	lnD	0.10	<0.0001	Continuous: cm
Plant functional group	G	0.01	0.03	*Pentaclethra. macroloba*, Tree, Liana, Palm
Soil phosphorus	P	<0.0001	<0.0001	Low P, Medium P, High P
Height class	H	<0.0001	<0.01	Bottom 2 m, Lower canopy, Mid canopy, Upper canopy
Slope	S	<0.0001	<0.0001	Continuous: degrees
ln(Diameter) x Group	(lnD*G)	0.02	0.04	NA
ln(Diameter) x Slope	(lnD*S)	0.02	0.05	NA
ln(Diameter) x Height	(lnD*H)	<0.0001	<0.0001	NA
Phosphorus x Slope	(P*S)	<0.01	<0.01	NA
R^2 of best fit model		0.29	0.75	

Table 2.3. Least-squares means, lower 95% confidence limits (LCL), and upper 95% confidence limits (UCL) for area-based (F_A, μmol m^{-2} s^{-1}) and mass- based (F_M, nmol kg^{-1} s^{-1}) CO_2 efflux rates corrected to 25°C in four canopy height classes at each of five representative diameters. Least-squares means and confidence limits were calculated as the antilogs of values from best-fit model results. Means were not extrapolated beyond the diameters actually sampled in each canopy class, resulting in empty cells.

	Bottom 2 m (0-2 m)	Lower canopy (2-15 m)	Mid canopy (15-25 m)	Upper canopy (25+ m)
Diameter cm	F_A (LCL, UCL) μmol m^{-2} s^{-1}	F_A (LCL, UCL) μmol m^{-2} s^{-1}	F_A (LCL, UCL) μmol m^{-2} s^{-1}	F_A (LCL, UCL) μmol m^{-2} s^{-1}
1	0.42 (0.33, 0.53)	0.93 (0.77, 1.12)	0.86 (0.68, 1.08)	0.87 (0.58, 1.29)
5	0.54 (0.48, 0.60)	0.64 (0.57, 0.72)	0.79 (0.67, 0.93)	1.37 (0.98, 1.92)
10	0.60 (0.53, 0.68)	0.54 (0.46, 0.64)	0.76 (0.58, 0.99)	1.67 (0.93, 3.01)
40	0.75 (0.59, 0.94)	-	-	-
80	0.83 (0.62, 1.12)	-	-	-
Diameter cm	F_M (LCL, UCL) nmol kg^{-1} s^{-1}	F_M (LCL, UCL) nmol kg^{-1} s^{-1}	F_M (LCL, UCL) nmol kg^{-1} s^{-1}	F_M (LCL, UCL) nmol kg^{-1} s^{-1}
1	447 (350, 570)	854 (701, 1041)	784 (585, 957)	669 (438, 1022)
5	106 (94, 119)	112 (99, 127)	140 (118, 166)	249 (175, 355)
10	57 (50, 64)	47 (39, 56)	68 (52, 90)	163 (88, 303)
40	16 (13, 21)	-	-	-
80	9 (6, 12)	-	-	-

Table 2.4. Total wood CO_2 efflux estimates (± standard errors) per unit ground area for the forest. Percent contributions to efflux by functional group and diameter class are displayed. Small diameter wood (<10 cm) was only 15% of total woody biomass, but accounted for 70% of total woody CO_2 efflux. Lianas were only 3% of the total woody biomass, but contributed one-fourth of the total woody CO_2 efflux.

Total Wood CO_2 Efflux		Percent of Total Wood CO_2 Efflux from Each Category					
Estimate ± SE	Units	Functional Group		Diameter Class			
1.34 ± 0.36	μmol CO_2 m^{-2} s^{-1}	Trees	47 %	0-2 cm	50 %	30-40 cm	4 %
508 ± 135	g C m^{-2} yr^{-1}	*P. macroloba*	20 %	2-5 cm	15 %	40-60 cm	7 %
		Lianas	24 %	5-10 cm	5 %	60-80 cm	3 %
		Palms	9 %	10-20 cm	9 %	80-100 cm	1 %
				20-30 cm	5 %	100+ cm	<1 %

FIGURES

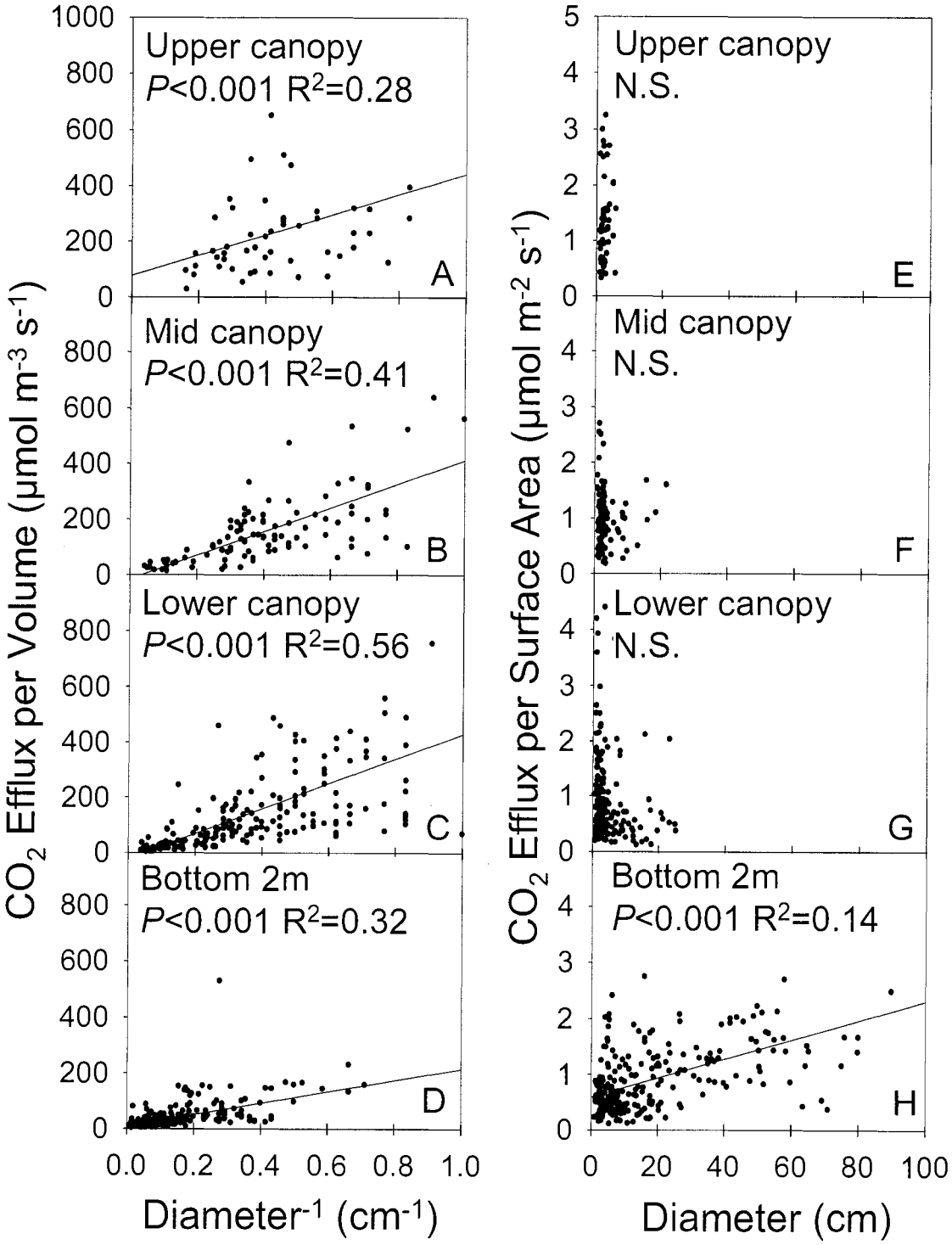
Upper canopy
P<0.001 R2=0.28
A
Mid canopy
P<0.001 R2=0.41
B
Lower canopy
P<0.001 R2=0.56
C
Bottom 2m
P<0.001 R2=0.32
D
Upper canopy
N.S.
E
Mid canopy
N.S.
F
Lower canopy
N.S.
G
Bottom 2m
P<0.001 R2=0.14
H
CO2 Efflux per Volume (µmol m-3 s-1)
CO2 Efflux per Surface Area (µmol m-2 s-1)
Diameter-1 (cm-1)
Diameter (cm)
0.0 0.2 0.4 0.6 0.8 1.0
0 20 40 60 80 100

Fig. 2.1. Wood CO_2 efflux measurements were corrected to 25°C and expressed on a volume basis vs. the reciprocal of diameter (Figs. 1A-1D), and on an area basis vs. diameter (Figs. 1E-1H), by canopy height class. In the upper, mid, and lower canopy, the relationship between the reciprocal of diameter and efflux per unit volume (Fig. 1A, 1B, 1C) and the lack of relationship between diameter and efflux per unit area (Fig. 1E, 1F, 1G) indicate that wood CO_2 efflux for all wood above 2 m was primarily dependent on surface area, and not volume. Unlike canopy rates, wood CO_2 efflux in the bottom 2 m was dependent on both volume and surface area (Fig. 1D, 1H). Data are also separated by canopy class: upper canopy, mid canopy, lower canopy and the bottom 2 m.

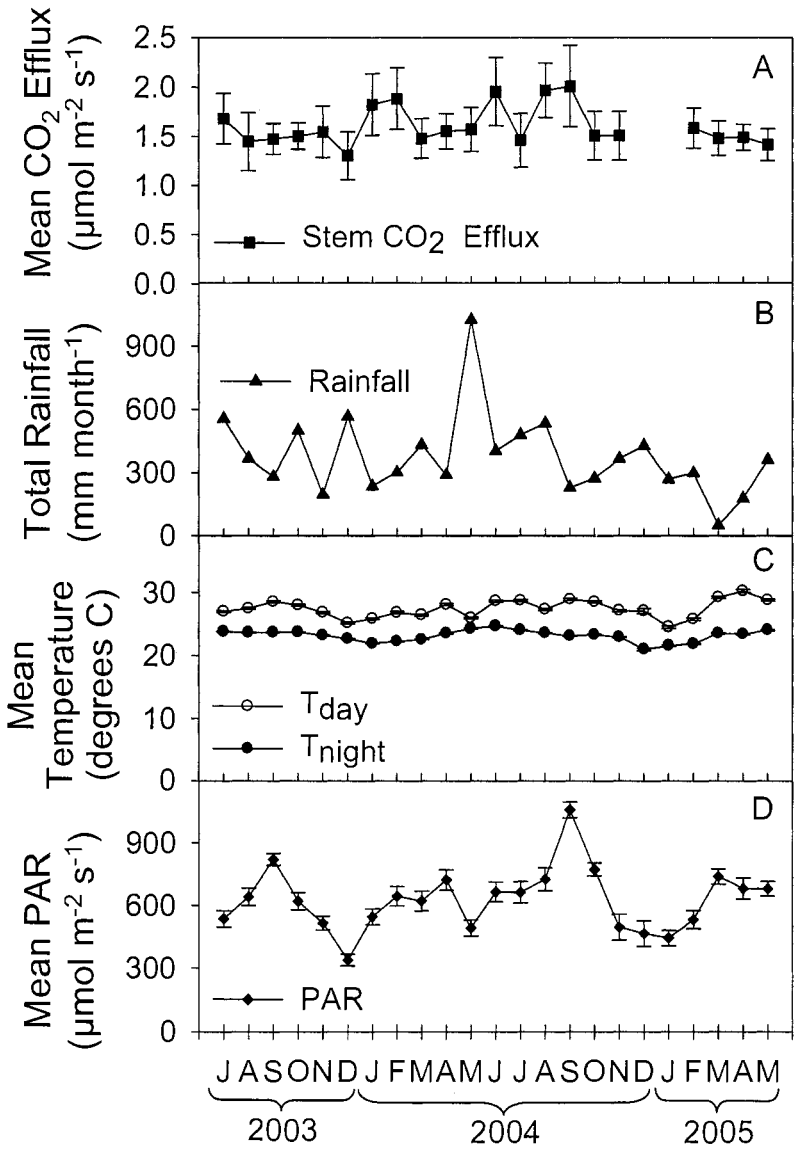

Fig. 2.2. Permanent plot mean monthly stem CO_2 efflux at 25°C (Fig. 2A), total monthly rainfall (Fig. 2B), mean monthly daytime and nighttime temperatures (Fig. 2C), and mean monthly PAR (Fig. 2D) are shown over 23 months. No clear seasonality in wood CO_2 efflux is evident in this system. The effect of "month" was not significant in a repeated measures ANOVA; and no correlations were found between monthly mean CO_2 efflux and rainfall, temperature or photosynthetically active radiation (PAR). Error bars represent standard error (some error bars may be hidden by symbols).

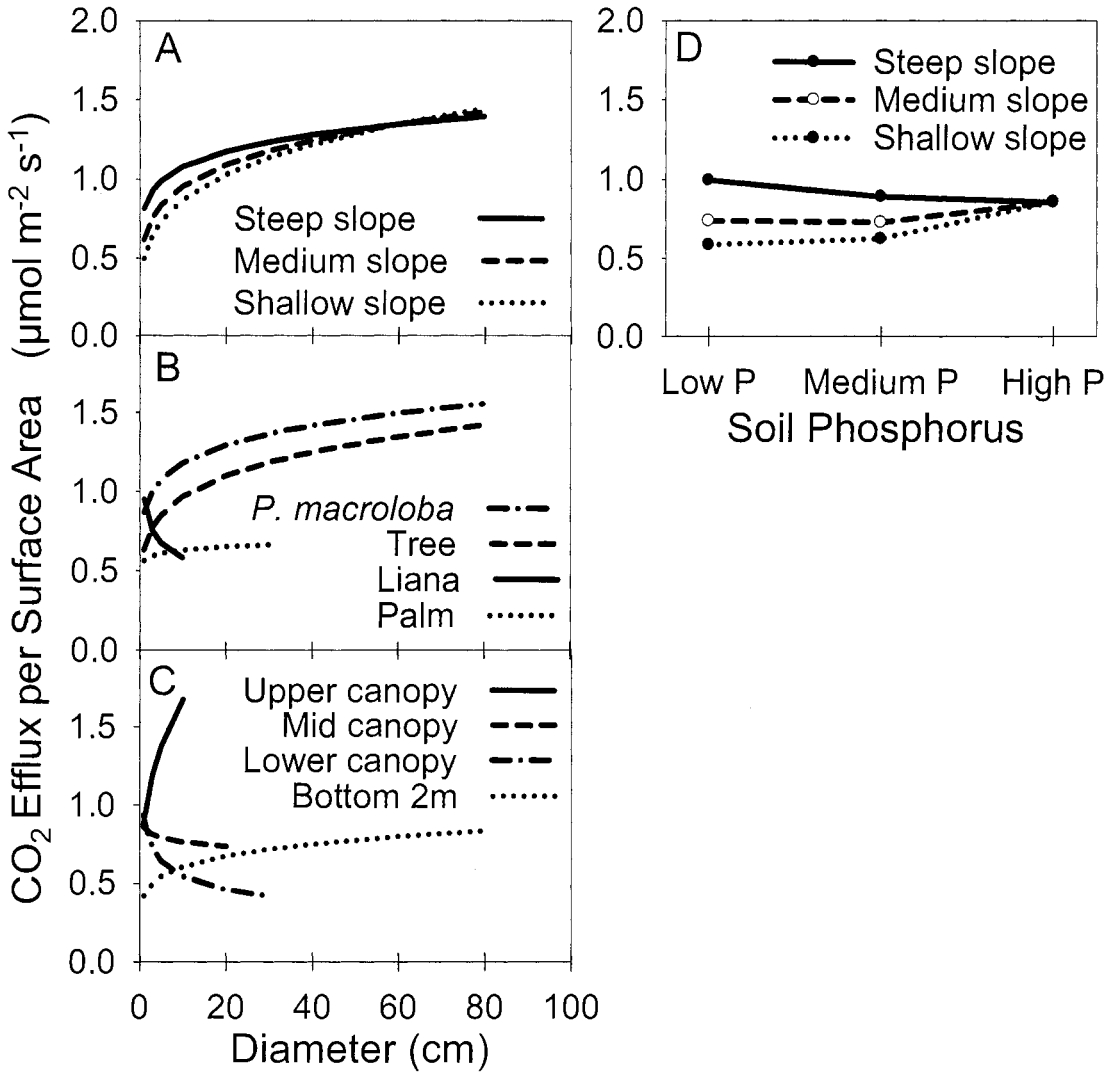

Fig. 2.3. Least-squares means for significant interactions in the best-fit ANCOVA model (see Table 2) predicting wood CO_2 efflux per unit surface area at 25°C in response to changes in diameter, plant functional group, soil phosphorus, slope, and canopy height. The effect of increasing CO_2 efflux with increasing slope was greatest in small diameter wood (Fig. 3A), and at low soil P (Fig. 3D). CO_2 efflux increased with diameter for trees and *P. macroloba* (Fig. 3B). For diameters less than 10 cm, CO_2 efflux increased steeply with height (Fig. 3C). Antilogs of both ln(CO_2 efflux) and ln(Diameter) are displayed. Least-squares means of interactions with slope (Figs. 3A and

3D) were calculated at the means of the upper, middle, and lower thirds of the data set (steep slope = 23.1°; medium slope = 12.4°; shallow slope = 4.2°). Least-squares means of interactions with the variable diameter (Figs. 3A-C) were calculated for the range of the diameters observed for each specific category over a possible range of 1 cm to 80 cm.

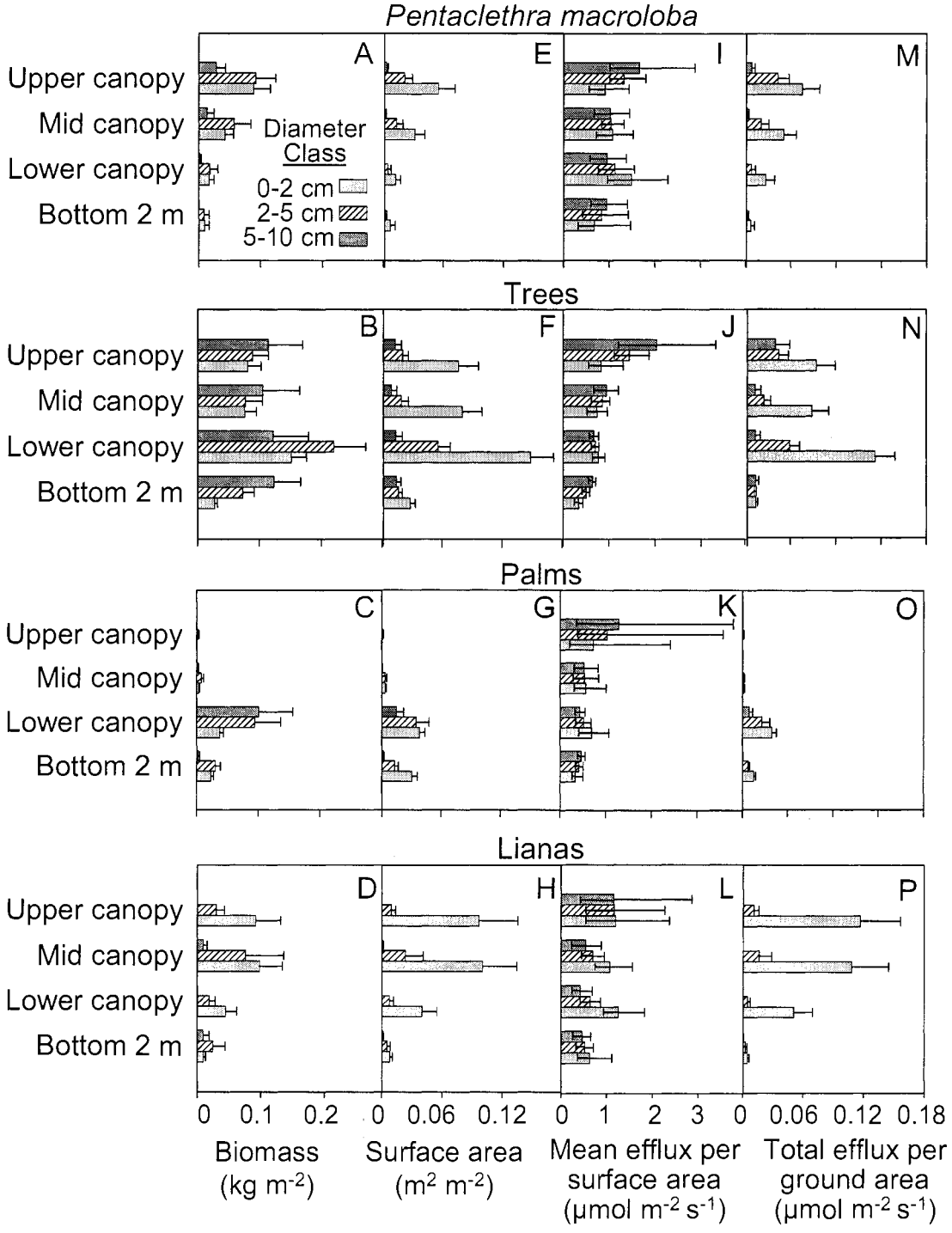

Fig. 2.4. Small diameter (<10 cm) wood biomass distribution (Figs. 4A-D), wood surface area distribution (Figs. 4E-H), mean CO_2 efflux rates at 25 °C per unit wood surface area (Figs. 4I-L), and total CO_2 efflux rates per unit ground area (Figs. 4M-P) by

functional group, canopy height class and diameter class. Lianas contributed a substantial portion of the total small diameter wood CO_2 efflux in the upper and mid canopy (Fig. 4P) because of their large surface area (Fig. 4H). The greatest proportion of tree biomass (Fig. 4B), surface area (Fig. 4F), and total CO_2 efflux (Fig. 4N) was in the lower canopy. Wood biomass and surface area per ground area were calculated as the mean tower small diameter wood biomass and surface area in each category. Error bars for Figs. 4A-H represent standard errors of the means among towers. CO_2 efflux per surface area values and errors were calculated as the antilogs of least-squares means and 95% confidence limits from the best-fit model, resulting in asymmetrical error bars for Figs. 4I-L. Mean tower wood surface area per ground area and standard errors were multiplied by CO_2 efflux per surface area model-derived means to yield CO_2 efflux per unit ground area values and errors (Figs. 4M-P).

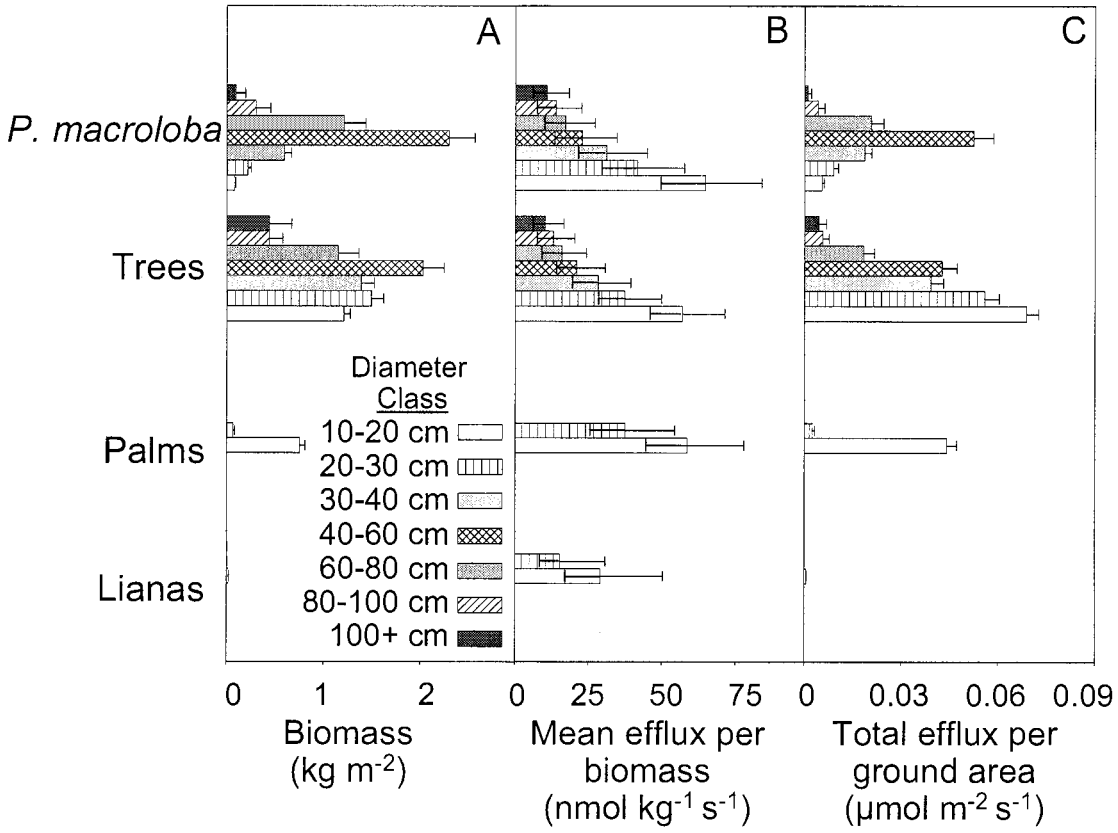

Fig. 2.5. Large diameter (>10 cm) wood biomass distribution (Fig. 5A), mean CO_2 efflux rates at 25 °C per unit biomass (Fig. 5B), and estimated CO_2 efflux rates per unit ground area (Fig. 5C) by functional group and diameter class. The largest proportion of *P. macroloba* CO_2 efflux was from diameters in the 40-60 range, while the largest proportion of tree CO_2 efflux was from diameters in the 10-20 cm range (Fig. 5C). In the large diameter range, lianas contributed essentially no CO_2 efflux (Fig. 5C), because very few lianas were greater than 10 cm in diameter (Fig. 5A). Large diameter biomass was calculated as mean plot biomass per category from eighteen 0.5 ha plots, minus small diameter branch biomass per category as calculated from towers transects (error bars in Fig. 5A represent standard errors of the mean plot data). CO_2 efflux per surface area

values and errors were calculated as the antilogs of least-squares means and 95% confidence limits from the best-fit model, resulting in asymmetrical error bars (Fig. 5B). Mean plot large diameter wood biomass area per ground area and standard errors were multiplied by CO_2 efflux per biomass model-derived means to yield CO_2 efflux per unit ground area values and errors (Fig. 5C). Means were not extrapolated beyond the diameters actually sampled for each functional group, resulting in empty cells for palms and lianas.

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CHAPTER 3:

FOLIAR AND ECOSYSTEM RESPIRATION IN AN OLD-GROWTH TROPICAL RAIN FOREST

ABSTRACT

The balance of photosynthesis and ecosystem respiration in tropical forests greatly influences the global carbon cycle. Foliar respiration is a major component of ecosystem respiration, yet extrapolations are uncertain in tropical rain forests because of access difficulties and poor estimates of leaf area index (LAI). A portable scaffolding tower was used to directly measure LAI and foliar respiration from 52 vertical transects throughout an old-growth tropical rain forest in Costa Rica. I characterized the variation in nighttime foliar respiration with structural, physiological, and environmental gradients. I extrapolated foliar respiration and combined this estimate with published values to estimate ecosystem respiration. The response of foliar respiration to temperature varied with plant functional group, but not with nutrients, leaf morphology, or height. Foliar respiration was linearly related to foliar nitrogen and phosphorus, with much stronger leaf area-based relationships than mass-based relationships. Respiration per unit leaf area, mass, nitrogen, and phosphorus increased with height, decreased with soil N, and varied with plant functional group. The relationship between respiration and photosynthetic capacity was non-linear, with photosynthetic capacity leveling off at about 10 μmol CO_2 m^{-2} s^{-1} as respiration increased from 1-2.5 μmol CO_2 m^{-2} s^{-1}. The ratio of photosynthetic capacity to respiration decreased with canopy height and increased with soil N. Using several methods of extrapolation, I estimated nighttime foliar respiration per unit ground area (R_{foliar}) as ~3.5-4.0 μmol CO_2 m^{-2}_{ground} s^{-1}. R_{foliar} estimated using nighttime

temperature data from a normal year (1999) was about 9% lower than when estimated with temperature from an El Niño Southern Oscillation year (1998). I estimated ecosystem respiration as 9.38 ± 1.43 μmol CO_2 m^{-2}_{ground} s^{-1} (soil=41%, foliage=37%, woody=14%, coarse woody debris=7%). This estimate was 33% greater than an eddy flux estimate of ecosystem respiration from the same forest, suggesting that studies reporting a large sink for tropical rain forests based on eddy flux measurements may be in error.

INTRODUCTION

Tropical forests account for more than one third of global plant productivity (Saugier et al. 2001), and at least half of this carbon is released back into the atmosphere as autotrophic respiration (Edwards et al. 1981; Chambers et al. 2004). A better understanding of autotrophic respiration at the landscape scale is a crucial first step in predicting how tropical rain forest ecosystem carbon balance may change with climate change. Foliage can account for 18-40% of total ecosystem respiration (Chambers et al. 2004; Curtis et al. 2005), yet extrapolations are uncertain in tropical rain forests because of access difficulties and the lack of unbiased leaf area index (LAI) estimates.

This study presents results from an intensive two-year field campaign where LAI and foliar respiration were measured across gradients of soil fertility in an old-growth tropical rain forest in Costa Rica. A portable scaffolding tower was used to access canopy foliage for respiration measurements, and to harvest foliage from forest floor to canopy top to estimate LAI. To my knowledge, this is the first foliar respiration estimate

for a tropical rain forest where the ecosystem extrapolation is based on detailed information of within-canopy variation in foliar respiration and LAI.

Foliar respiration standardized to a common temperature is influenced by many variables, including canopy height (Bolstad et al. 1999; Mitchell et al. 1999; Turnbull et al. 2003), foliar or soil nutrients (Ryan 1995; Reich et al. 1996; Mitchell et al. 1999; Meir et al. 2001; Turnbull et al. 2005), foliar morphology (Reich et al. 1998b; Mitchell et al. 1999; Meir et al. 2001) or species (Bolstad et al. 1999). To complicate matters further, the temperature response of foliar respiration can also change with any of the above variables (Atkin et al. 2005). Standardizing respiration measurements to a common temperature according to within-canopy and across-landscape variability in temperature response will greatly reduce uncertainty involved in extrapolating foliar respiration to the ecosystem.

Foliar dark respiration is a primary trait in the "leaf economics spectrum" (Wright et al. 2004). Dark respiration, leaf life span, photosynthetic capacity (A_{max}), leaf mass per area (LMA), foliar nitrogen (N), and foliar phosphorus (P) have been found to correlate with each other across plant functional groups and ecosystem types, revealing convergent evolution on a global-scale (Reich et al. 1997). Selection for high A_{max} in high resource habitats results in high foliar N, P, and respiration, and vice-versa, because photosynthesis requires large amounts of enzymes which have construction requirements (N and P) and maintenance costs (respiration) (Reich et al. 1998b). I expected foliar dark respiration to be linearly related to LMA, foliar N, P and A_{max}, in accordance with the leaf economics spectrum.

Some studies suggest that the more limiting the nutrient is, the tighter it will correlate with foliar respiration (Ryan 1995; Meir et al. 2001), and the ratio of respiration to foliar nitrogen (*R/N*) or foliar phosphorus (*R/P*) could also vary depending on which nutrients are overabundant or limiting. I expected soil P to have a greater effect on respiratory rates than soil N, because phosphorus, rather than nitrogen, is likely limiting in this rain forest (McDade et al. 1994). The ratio of photosynthetic capacity to respiration (A_{max}/R) may also vary in relation to nutrient limitation (Turnbull et al. 2005), and the ability of plants to maintain constant A_{max}/R may be related to thermal acclimation (Dewar et al. 1999). To characterize these sources of variation and compare them to variation in respiration per unit leaf area (R_A) and leaf mass (R_M), I analyzed the responses of R_A, R_M, *R/N*, *R/P*, and A_{max}/R to changes in soil N and P stocks, plant functional group, and canopy height.

Equipped with a full examination of the variability in foliar dark respiration within the canopy and across the landscape, I had numerous options for extrapolating foliar respiration to the ecosystem. I devised six different estimates of foliar dark respiration per unit ground area (R_{foliar}), including two complex and four simple methods. The more complex estimates used detailed information of within-canopy variability and temperature data, while the four simpler methods used overall means to see if I could provide realistic extrapolations of foliar respiration for this forest with less investment.

This study had five objectives. First, I sought to characterize the variation in foliar respiration with temperature. Second, I asked if respiration corrected to a common temperature of 25 °C varied with foliar nutrients, leaf mass per area (LMA), plant functional group, height, or soil nutrients. Third, I examined the relationship between

foliar respiration and photosynthetic capacity (A_{max}). Fourth, I used relationships identified in (1) and (2), LAI data from harvests, and temperature data to compare several methods of extrapolating foliar respiration to a ground-unit basis. Finally, I estimated ecosystem respiration by combining extrapolated foliar respiration per unit ground area with previously published values of woody, soil, and coarse woody debris (CWD) respiration, and compared the total to an estimate of eddy flux nighttime net ecosystem exchange (NEE_{night}) for the same location (Loescher et al. 2003). The eddy flux technique has several possible sources of error, including complex canopies, non-flat topography, still nighttime air, and biased air movement, which all can result in a systematic underestimation of nighttime respiration (Baldocchi 2003). Consequently, independent estimates of ecosystem respiration that help constrain estimates of nighttime effluxes should be extremely useful.

MATERIALS AND METHODS

Study site

La Selva Biological Station is located in the Caribbean lowlands of northern Costa Rica (elevation 37-150 m, 10°20' N, 83°50' W). La Selva, classified as premontane tropical wet forest in the Holdridge life-zone system (Hartshorn 1983), has a mean annual rainfall of ~4 m, and a mean annual temperature of 26 °C. This study includes sampling from within La Selva's 515 ha of old-growth forest. Further information about the soils and plant communities of La Selva can be found in McDade et al. (1994).

Tower construction and sampling scheme

The towers sampling design and construction were part of a larger project with the goal of characterizing canopy structure and function across environmental gradients in a tropical rain forest. Forty-five tower sites were located throughout the old-growth forest of La Selva using a stratified random sample with nine classes (three slope classes x three soil phosphorus classes), and five sites in each class (Cavaleri et al. 2006). Because the original stratification captured only one forest gap, ten of the original 45 sites were selected as starting points for a procedure to locate ten additional "low canopy height" sites. At each of these ten sites, one low canopy height tower site was selected as the first location along a randomly oriented 50 m transect that had vegetation less than 16 m in height.

At each randomly sampled site and each "low canopy height" site, an aluminum walk-up scaffolding tower (Upright, Inc, Dublin, Ireland) was constructed to the top of the canopy. Towers were constructed one 1.30 m x 1.86 m x 1.86 m (LxWxH) section at a time, harvesting all foliage within each section. A cantilever balcony installed on the top of the tower during harvesting nearly doubled the sample area to a total of 4.56 m^2. Tower heights varied from 1.86 m (1 section) to 44.64 m (24 sections). All harvested foliage was separated by height and plant functional group and measured with a leaf area meter (Li-3100, LI-COR Inc., Lincoln, NE) to estimate total LAI for each tower footprint, and proportion of LAI in each category. Plant functional groups for this study were: trees, palms, lianas (woody vines), and herbaceous plants (including herbs, epiphytes, vines, and ferns). After completing tower construction and harvesting, I measured photosynthesis on undamaged foliage accessible from the tower, then collected

adjacent foliage for nighttime respiration measurements. After all measurements were taken, the tower was dismantled and moved it to the nearest pre-selected random site.

Each tower site was sampled only once, and tower construction and sampling occurred continuously from June 2003 to June 2005. Photosynthesis and foliar respiration were sampled from 43 of the 45 randomly sampled tower sites, and 9 of the 10 "low canopy height" tower sites for a total of 52 sampled towers. I used LAI data from only the 45 randomly sampled tower sites to extrapolate foliar respiration to a ground-area basis.

Foliar gas exchange, structure and nutrients

Photosynthetic capacity (A_{max}), foliar respiration, foliar nitrogen (N), foliar phosphorus (P), and leaf mass per area (LMA) were measured for every species accessible from the tower, at every tower section in which the species was found. For each unique species at each unique tower section, A_{max} was measured *in situ*, and adjacent foliage segments were flagged for respiration sampling. Each flagged foliage segment (2-6 small leaves or one large leaf) was cut under water in the afternoon and placed in a water-filled floral tube so that cut surfaces were never exposed to air. Detached foliage samples were transported back to the lab for nighttime respiration measurements. Three replicates of A_{max}, and two replicates of respiration were measured for each unique species at each unique height, and replicates were averaged prior to statistical analyses. These data represent 990 foliar respiration measurements: two replicate measurements each of 495 plant samples, representing over 162 species and 53 families.

A_{max} was measured with an open-system portable infrared gas analyzer with an integrated blue-red light source inside the leaf chamber (Li-6400, LI-COR Inc., Lincoln, NE). Measurements were taken at a constant reference CO_2 concentration of 390 μmol mol^{-1} and an airflow of 500 μmol s^{-1}. The photosynthetic photon flux densities (PPFD) were determined as the saturating PPFD values from a photosynthesis/light curve on the same species at the same height. Saturating PPFD values ranged from 500-1500 μmol m^{-2} s^{-1} at heights <10 m, and from 1000-2000 μmol m^{-2} s^{-1} for heights >10 m.

Prior the construction of the first tower, I conducted a pilot study at La Selva Biological Station to ensure the validity of measuring foliar respiration on detached samples. I measured foliar respiration *in situ* on 42 attached samples at night, detached the same samples the next afternoon, and measured them again on the second night. Samples represented three functional groups and thirteen species: trees (seven species, n=25), herbaceous (one species of vine, n=4), and palms (five species, n=13). I measured palm and vine foliage from the ground, and I accessed canopy foliage of trees from a tall bridge. A repeated measures ANOVA with functional group as a factor and attached-detached as the within subjects factor showed no effect of detachment (d.f.=39, P=0.24). Several additional studies have also found no difference between respiration rates on attached vs. detached foliage (Bolstad et al. 1999; Mitchell et al. 1999; Turnbull et al. 2005).

I measured foliar dark respiration at night with LCA-3 and LCA-4 open-system infrared gas analyzers (Analytical Development Company, Hoddeson, UK). I clamped foliage into a clear polycarbonate custom-made chamber with a neoprene gasket (internal volume = 1750 mL, 12.5 cm x 28 cm x 5 cm), with only the stem or petiole protruding

during measurement. A small 9V battery-operated fan was installed to stir the air inside the chamber. Air flow rates through the chamber ranged from 330-340 μmol s^{-1} and chamber seals were checked with a flowmeter. Intake air was drawn through a 19 L mixing chamber to maintain stable reference CO_2 concentrations. I recorded the difference in CO_2 concentration between the reference and the chamber after it had been stable for at least 2 minutes. All foliage that was inside the chamber was measured with a leaf area meter (Li-3100, LI-COR Inc., Lincoln, NE) to determine respiration rates per unit leaf area. Foliage was dried to constant weight at 60 °C to calculate leaf mass per area (LMA g m^{-2}). Respiration measurements were taken in the dark between 7 pm and 5 am at ambient temperature. Foliage temperature was measured with a thermocouple thermometer.

For a subsample of 9 towers, I measured foliar respiration-temperature response curves on all accessible species x height combinations, excluding understory species. I did not measure plants in the first tower section (primarily understory herbs and ferns) because response curve measurements were time intensive, and ~ 90% of the leaf area in this forest consists of palms, trees and lianas (data not shown). Respiration-temperature response data included two replications each of: 31 tree samples (19 species), 13 liana samples (6 species), 8 palm samples (4 species), and one species from each of the herbaceous groups: fern, epiphyte, vine. A temperature-controlled cuvette with a peltier cell was attached to the LCA-3 infrared gas analyzer to measure response curves (Hubbard et al. 1995). A datalogger (Campbell 21X, Campbell Scientific, Logan, UT) controlled temperature and logged foliar respiration rates over the temperatures 15, 25, 30 and 35 °C. The intake air passed through a tube of $CaSO_4$ desiccant (Drierite, Xenia,

OH) to minimize condensation at the lower temperatures. As it became saturated, Drierite alone increased the CO_2 flow through the system. To correct for this, I took a CO_2 reading with no leaf in the chamber before and after each temperature curve and linearly interpolated between these two 'zero' points to calculate a zero for each measurement of the temperature curve.

Replicates of foliage samples measured for respiration and respiration-temperature response were bulked for nutrient analyses and ground in a Wiley mill with 20-mesh sieve. I analyzed foliar samples for N concentration with a LECO TruSpec CN Determinator, (LECO, Inc., St. Joseph, MI, USA). Foliar P concentrations were determined with nitric acid/ hydrogen peroxide digests and an Inductively Coupled Plasma Spectrometer (PerkinElmer 4300 Optima Dual View, Norwalk, CT, USA) by MDS Harris Laboratories, Lincoln, NE, USA.

Soil nutrient sampling

At each site, soil was sampled to a depth of 1 m with a 0.03 m diameter half-core auger before tower construction. Two subsamples were taken at a distance of 1 m from the tower base center and at a 180° angle from each other. Six to eight additional subsamples were taken at a distance of 2 m from the tower base center at regularly-spaced angles. Each subsample was separated into four layers by depth: 0-0.1 m, 0.1-0.3 m, 0.3-0.5 m, and 0.5-1 m. All subsamples for each tower were mixed by layer and organic material and stones removed. Samples were air-dried, sieved through a 2 mm screen, ground in a coffee mill and stored until nutrient analysis. Prior to analysis, samples were oven dried at 40 °C for 2-3 days and 20 g of each sample was finely ground

in an agate mill (Fritsch, Germany). Total N (mg g^{-1}) was analyzed by combustion with a C/N-Analyzer (CHN-O-RAPID, Elementar, Germany), and total P (mg g^{-1}) was analyzed with a HNO_3-pressure extraction and inductively coupled plasma spectrometry (ICP Spectro, Kleve, Germany). Stocks of N and P (Mg ha^{-1}) for each soil layer were calculated using the mean bulk density of each layer (0.67, 0.79, 0.85, 0.89 g cm^{-3} respectively at depths 0-0.1, 0.1-0.3, 0.3-0.5, and 0.5-1 m), measured from six permanent plots within the old-growth forest of La Selva Biological Station (DB Clark, unpublished data). N and P stocks for each layer were summed for cumulative soil N and P stocks by tower.

Data analysis

I used the following equation to model each respiration temperature response curve:

$$R_{Tleaf} = \beta_0 * \exp(T_{leaf} * \beta_1) \quad (1)$$

where β_0 and β_1 are model parameters, and R_{Tleaf} is respiration rate (μmol CO_2 m^{-2} s^{-1}) at the measured foliage temperature, T_{leaf} (°C). Q_{10}, the change in respiration rate with 10 °C change in temperature, is defined as $\exp(10 * \beta_1)$. I also modeled each respiration temperature response curve with a modified Arrhenius function described by Lloyd and Taylor (1994), shown below for a base temperature of 25 °C or 298 K:

$$R_{Tleaf} = R_A * [\exp((E_0/R_g)((1/298)-(1/T_{leaf})))] \quad (3)$$

where R_g is the gas constant (0.008314 kJ mol^{-1} K^{-1}), and E_0 (kJ mol^{-1} K^{-1}) is a parameter which describes the magnitude of temperature response, described as the energy of activation. I examined variation in Q_{10} and E_0 with simple linear regression (R_A, LMA,

foliar N, foliar P, soil N, soil P, and height), ANCOVA (functional group + height), and ANOVA (functional group) procedures. Based on the results of these analyses, I used functional group-specific Q_{10} values to standardize respiration rates to a base temperature of 25 °C. Fisher's LSD of the ANOVA determined functional group mean separation. For all further statistical analyses, I corrected respiration rates to 25 °C using:

$$R_A = R_{Tleaf}/(Q_{10}^{(Tleaf-25)/10}) \quad (4)$$

Mass-based respiration rates at 25 °C (R_M: nmol g^{-1} s^{-1}) were calculated with leaf mass per area (LMA: g m^{-2}) for each leaf. For both area and mass-based measurements, I used simple linear regressions to analyze variation in foliar respiration with LMA and foliar nutrients. In further analyses, I did not use slopes of these regressions to determine respiration per unit nitrogen (*R/N*: μmol g^{-1} N s^{-1}) or respiration per unit phosphorus (*R/P*: μmol g^{-1} P s^{-1}), because the area and mass-based slopes differed. Instead, I calculated *R/N* and *R/P* for each individual sample, which is the same value whether using mass or area-based measurements (LMA cancels out). The A_{max} vs. R_A relationship was modeled with a non-linear rectangular hyperbola.

I used ANCOVA procedures to model R_A, R_M, *R/N*, *R/P* and A_{max}/R_A with the following predictor variables: canopy height (m), soil N (Mg ha^{-1}), soil P (Mg ha^{-1}), and functional group (trees, lianas, palms, and herbaceous groups). Model predicted least-squares means and standard errors were displayed in figures. All statistical analyses were performed with SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA), with $\alpha = 0.05$.

Estimating foliar respiration per unit ground area and ecosystem respiration

I compared six estimates of foliar respiration per unit ground area (R_{foliar}), using two complex and four simpler methods of extrapolation (Table 2). I used half-hourly temperature data from 1999, a 'normal' year, and 1998, a strong El Niño Southern Oscillation (ENSO) year, to model estimates 1 and 2, respectively (Table 2). Using functional-group specific Q_{10} values, I rearranged equation 4 (estimating respiration at ambient air temperature, R_{Tair}, instead of R_{Tleaf}) and calculated mean deviations from respiration rates at 25 °C (R_{Tair}/R_A) for each functional group in each year. For both estimates 1 and 2, I multiplied these mean deviations by the model-predicted least-squares means for R_A, (Fig. 4E) to calculate R_{Tair} stratified by functional group and height. These values were multiplied by LAI stratified by the corresponding height and functional group (n=45 per height x group category, data not shown), and summed over categories to obtain a value per unit ground area (R_{foliar}, μmol CO_2 m^{-2}_{ground} s^{-1}). LAI was the largest source of variation, and standard errors were based on the variability in LAI from tower to tower. Half-hourly air temperatures were collected above the canopy from an eddy covariance tower located within the old-growth forest of La Selva (Loescher et al. 2003) using a 100 Ω platinum resistance thermistor (Omega Engineering, Stamford, CT, USA).

R_{foliar} estimates 3-6 were simpler because they were neither extrapolated using within-canopy variability of respiration, nor modeled with actual temperature data (all respiration measurements were corrected to 25 °C, Table 2). Estimate 3 was calculated by multiplying the overall tower mean and standard error of LAI (6.03 ± 0.32 m^2 m^{-2}_{ground}, n=45) by the overall sample mean R_A (0.59 μmol CO^2 m^{-2} s^{-1}, n=495). Estimates 4-6 were each calculated by multiplying the overall tower mean and standard error of total

N per unit ground area (N_{tot}=11.62 ± 0.65 g N m^{-2}_{ground}, n=45), by three different estimates of *R/N*. For estimate 4, I used the overall sample mean of *R/N* (0.32 μmol CO^2 $g^{-1}N$ s^{-1}, n=495). For estimate 5, I used the slope of the regression between R_A and N_A (0.34 μmol CO^2 $g^{-1}N$ s^{-1}, Fig. 2A), and for estimate 6, I used the slope of the regression between R_M and N_M (0.10 μmol CO^2 $g^{-1}N$ s^{-1}, Fig. 2D). For estimates 4-6, N_{tot} was calculated for each tower by summing: [total LAI (m^2 m^{-2}_{ground}) * mean LMA (g m^{-2}) * mean N_M (g g^{-1})] for each functional group in each tower section.

I estimated ecosystem respiration for the forest (R_{eco}) by adding the best estimate of R_{foliar} to published estimates of woody respiration (R_{woody}), soil respiration (R_{soil}), and coarse woody debris respiration (R_{CWD}) from the old-growth rain forest of La Selva Biological Station. Cavaleri et al.(2006) reported R_{woody} as 1.34 ± 0.36 μmol CO_2 m^{-2}_{ground} s^{-1}, based on extrapolated chamber measurements. To estimate R_{CWD}, I divided published values of downed CWD total carbon biomass (22.3 ± 2.7 Mg C ha^{-1}), by turnover time (9 yr) (Clark et al. 2002), and converted units to yield 0.66 ± 0.05 μmol CO_2 m^{-2}_{ground} s^{-1}. For R_{soil}, I used soil CO_2 efflux data from plots located in the same soil type as the eddy flux tower, Oxisols developed on old lava flows or 'residual' soils (2003). I calculated the mean ± 1 standard error of 6 soil chamber measurement plot-averages (3 plots x 2 years) and converted units for a value of 3.88 ± 0.22 μmol CO_2 m^{-2}_{ground} s^{-1} (2003).

I compared the summed value of ecosystem respiration to eddy flux nighttime net ecosystem exchange (NEE_{night}) for the same forest: 7.05 ± 0.69 μmol CO_2 m^{-2} s^{-1} (Loescher et al. 2003). This NEE_{night} estimate was based on data for turbulent nights only, when friction velocity (u^*) was greater than 0.4 m s^{-1} (Loescher et al. 2003).

RESULTS

Temperature response

Neither Q_{10} nor E_0 showed any relationships with soil nutrients, LMA, respiration at 25 °C, or foliar nutrients per unit leaf mass or area. Both Q_{10} and E_0 varied with height ($P<0.01$), but the differences were caused by the distribution of functional groups with height. Functional group explained 56% of the variability in both Q_{10} and E_0, and the addition of height to the models improved the r^2 by less than 1% in both cases. For all further analyses, respiration rates per unit area (R_A) and mass (R_M) were standardized to 25 °C using a different Q_{10} value for each plant functional group. Mean Q_{10} values were: herbaceous = 1.62, palm = 1.82, liana = 2.10, and tree = 2.18. Mean E_0 values for each group were: herbaceous = 35.5, palm = 44.4, liana = 55.6, and tree = 57.7 kJ mol^{-1} (Fig. 1).

Effects of nutrients, LMA, height, functional group on foliar respiration

R_A was linearly related to N_A, P_A, and LMA (Figs. 2A-C). R_M had weak relationships with both N_M and P_M, and the regression with LMA was not significant (Figs. 2D-F). Respiration rates at 25 °C per area, mass, N, and P varied with height and soil N, but not with soil P stocks (Table 3). Functional group was not significant in any model, but the height x group interaction was significant for both R_A and R/N (Table 3). Three-way interactions were pooled into error for all models. The ANCOVA predicting R_A had the highest r^2 (0.39, Table 3). Model-predicted least-squares means were plotted for each respiration variable for the height*soil N and height*group interactions (Fig. 3). R_A varied almost 6-fold, while R_M, R/N, and R/P were much less variable, at around 2 to

3-fold from the understory to the upper canopy. Respiration rates on any basis increased with height and decreased with soil N (Figs. A-D). The effects of soil N and more pronounced higher in the canopy, and respiration increased more steeply with height at the lowest soil N levels (Figs. 3A-D). Trees and lianas generally had higher respiration rates than palms and herbaceous groups, and liana rates increased more steeply with height than the other groups (Figs. 3E-H). The group difference was also more pronounced higher in the canopy (Figs. 3E-H).

Relationship between respiration and photosynthetic capacity

The relationship between area-based respiration at 25 °C (R_A) and photosynthetic capacity (A_{max}) was non-linear, with A_{max} leveling off at high R_A (Fig. 4A). The curve was described by a rectangular hyperbola ($P<0.0001$, $r^2=0.24$), where $A_{max} = (10.9*R_A)/(0.52+R_A)$. Photosynthetic capacity reached a maximum of about 10 μmol CO_2 m^{-2} s^{-1} as respiration increased from 1 to 2.5 μmol CO_2 m^{-2} s^{-1} (Fig. 4A). A_{max}/R_A varied with height and soil N, and all interactions were pooled into error (Table 3). Figure 4B shows the height effect at mean soil N (13.9 Mg ha^{-1}) and averaged over all functional groups, while Figure 4C shows the soil N effect at mean height (11.9 m) and averaged over all functional groups. The ratio A_{max}/R_A varied 2-fold from ~7 to14, decreased with height, and increased with soil N stocks (Fig. 4).

Foliar respiration per unit ground area and ecosystem respiration

Estimated R_{foliar} was ~9% higher for the ENSO year (estimate 2) compared with a normal year (estimate 1, Table 2). Estimate 6 of R_{foliar}, which used the slope of the R_M -

N_M regression to estimate R/M, was about 1/3 that of estimates 1-5 (Table 2). Three of the R_{foliar} estimates calculated using the simpler methods of extrapolation (estimates 3, 4, and 5) were similar to those estimated with the more complex methods (estimates 1 and 2, Table 2). Trees contributed the most to R_{foliar} (66%), with 15% from lianas, 12% from palms, and 7% from herbaceous groups.

R_{eco}, summed from R_{foliar}, R_{soil}, R_{woody}, and R_{CWD} was 9.38 ± 1.43 μmol CO_2 m^{-2}_{ground} s^{-1} (Fig. 5). I used R_{foliar} from estimate 1 (Table 2) because this extrapolation method was based upon the most information, and it was modeled with temperature data from a normal year. The contributions of each component part to R_{eco} were: soil=41%, foliage=37%, woody=14%, coarse woody debris=7%.

DISCUSSION

Temperature response

Q_{10} values (1.6-2.2) fall within the range of values for temperate deciduous forests of the U.S. (Q_{10} =1.4-3.2, Bolstad et al. 1999; Griffin et al. 2002; Turnbull et al. 2003; Turnbull et al. 2005). Values for both Q_{10} and E_0 are higher than values found in temperate rain forests of New Zealand (Q_{10} =1.3-2.0, E_0 =18-48, Turnbull et al. 2003; Turnbull et al. 2005), and slightly lower than values for tropical rain forests in Brazil and Cameroon (Q_{10} =2.0-2.3, Meir et al. 2001).

An increased respiration temperature response with height could be the result of increased substrate availability with increased light and net photosynthesis (Griffin et al. 2002). Variation in temperature response with soil or foliar nutrients has been attributed to changes in the maintenance of ion gradients, protein turnover, and cellular repair, all of

which require respiratory enzymes (Turnbull et al. 2003). Recent studies have shown a wide range of relationships between respiration temperature response, canopy position, and foliar nutrients. Foliar respiration temperature response in forests has increased (Turnbull et al. 2003), decreased (Griffin et al. 2002), and both decreased and increased with height (Bolstad et al. 1999). Temperature response has been found to increase (Turnbull et al. 2003; Turnbull et al. 2005) or remain constant (Bolstad et al. 1999) with foliar nutrients. I found no difference in Q_{10} or E_0 with either height or nutrients, indicating that the primary source of variation in temperature response was genetically controlled differences among species or functional groups, greatly simplifying subsequent modeling and extrapolation procedures. (Xu and Griffin 2006) also found that consistency in E_0 with height simplified further extrapolation.

Response to foliar nutrients, structure, height, functional group, and soil nutrients

As predicted by the leaf economics spectrum, respiration rates were correlated with foliar N, P, and LMA. Respiration is linked to photosynthesis, and 90% of plant N is in proteins, with Rubisco comprising up to half of all plant proteins (Lawlor 1993; Ryan et al. 1996). Phosphorus is necessary for protein synthesis, nucleic acids, plasma membranes, ADP phosphorylation and triose phosphate production (Amthor 1989; Stitt 1990).

The foliar properties in the leaf economics spectrum are all expressed on a leaf mass basis, and generally only 'sun' leaves are considered (Wright et al. 2004). When looking at the full vertical transect of forest canopies, however, foliar respiration tends to relate better to foliar N and P when expressed on a leaf area basis (Mitchell et al. 1999;

Meir et al. 2001; Xu and Griffin 2006). This pattern is likely a result of the co-variation of area-based leaf metrics with LMA, and the universal pattern of increasing LMA with height in forest canopies (Ford and Newbould 1971; Hutchison et al. 1986; Oberbauer and Strain 1986; Hollinger 1989; Niinemets and Kull 1995; Niinemets and Tenhunen 1997; Meir et al. 2001; Marshall and Monserud 2003; Koch et al. 2004). LMA also had a strong linear relationship with height (data not shown), and it is likely that the changes in LMA within the canopy profile underlie the strong covariance between area-based respiration and leaf nutrients.

In the leaf economics spectrum, a trade-off exists between short-lived leaves with high metabolic function vs. long-lived leaves with low metabolic function. The short-lived leaves have less protection against herbivory and mechanical damage, while long-lived leaves allocate more resources to structural rather than metabolic compounds (Reich et al. 1991; Reich et al. 1992; Reich et al. 1998a). Functional groups that generally have longer leaf life spans (herbaceous groups and palms) had the lowest overall values of respiration, and groups that generally have shorter leaf life spans (trees and lianas), had higher respiration rates. Lianas had the steepest increase in respiration with height of all the groups (Fig. 3). Once lianas reach the top of the canopy, they allocate more energy to leaf growth than stem growth (Putz 1983). Perhaps lianas are able to achieve higher rates of respiration and photosynthesis than trees because they rely on neighboring trees for support. Lianas are able to put increasing resources into metabolic compounds as light increases, whereas trees still need to allocate energy and nutrients to woody growth.

The overall sample mean for *R/N* was 0.32 μmol CO_2 g^{-1} N s^{-1} (Fig. 3), which was quite similar to *R/N* reported for *Pinus radiata* (0.31 μmol CO_2 g^{-1} N s^{-1} when standardized to 25 °C with the reported Q_{10} of 2.5(Ryan et al. 1996). A value reported for boreal and subalpine forests (0.53 μmol CO_2 g^{-1} N s^{-1} when standardized to 25 °C with the reported Q_{10} of 2.0, Ryan 1995) was 66% greater than the mean *R/N* of this study. Within forest canopies, respiration per unit nitrogen (*R/N*) is often less variable than R_M (Ryan 1995), and the variability of respiration per unit phosphorus (*R/P*) has not been well studied. Fertilization increased the variability in *R/N* of *Pinus radiata,* either because of an increased variability of the proportion of N in protein, or an increase in the variability in Rubisco activation (Ryan et al. 1996). *R/N, R/P* and R_M were all less variable than R_A, likely because of the influence of the LMA gradient with height on R_A. Without the influence of LMA, *R/N, R/P* and R_M all still increased with height, and were highest for trees and lianas (which dominate the upper canopy). Higher in the canopy where light is more abundant, more N and P may be allocated to respiratory and photosynthetic proteins, rather than other compounds such as those used in herbivory defense.

Both N_A and P_A explained a similar amount of variation in R_A (Fig. 2), therefore foliar phosphorus did not appear to constrain respiration more strongly than foliar nitrogen did, as Meir et al. (2001) reported in a tropical rain forest in Cameroon. Turnbull et al. (2005) found an increase in R_A with soil fertility along a soil chronosequence in New Zealand, but none of the respiratory variables in the present study varied with soil P, contrary to expectation. In fact, respiration decreased with increasing soil N stocks, which is difficult to interpret because respiration and foliar N

were positively correlated. In this forest it seems that soil N and foliar N are decoupled; soil N stocks are not related to N_{tot}, N_M or N_A (data not shown), supporting the assumption that nitrogen is not limiting in this system (McDade et al. 1994).

Respiration and photosynthetic capacity

Values of A_{max}/R_A by height and soil N varied from ~7 to14 (Fig. 4), with similar values found in temperate rainforests, deciduous, and coniferous forests (Turnbull et al. 2001; Vose and Ryan 2002; Turnbull et al. 2005). At high values of A_{max}, leaf metabolism appears to increase at a faster rate than the plant's ability to assimilate CO_2, indicated by the non-linear relationship between R_A and A_{max} (Fig. 4). Reich et al. found the relationship between R_A and A_{max} to be linear within biomes (indicating a constant ratio of A_{max}/R_A), but non-linear when several biomes and functional groups were plotted together (1998b). These data are from one biome, however, and the non-linearity is still present when only trees are plotted (data not shown). Turnbull et al. found A_{max}/R_A in a temperate rain forest to increase with increasing soil fertility (2005). A_{max}/R_A increased with increasing soil N content (Fig. 4C), primarily because of the decrease in R_A with increasing soil N; A_{max} did not change with soil N ($P = 0.59$, data not shown).

Although temperature response did not increase with height within groups, the groups with the highest Q_{10} tended towards the top of the canopy (trees and lianas). The high Q_{10} values in the upper canopy where temperatures are highest may or may not lead to exponential losses of carbon with increasing global temperatures, depending upon the ability of canopy foliage to acclimate. The availability of respiratory substrates such as non-structural carbohydrates can limit respiration temperature response, and lead to

thermal acclimation (Atkin and Tjoelker 2003; Xu and Griffin 2006). Dewar et al. (1999) suggested that the metabolic adjustment of non-structural carbohydrates that allows plants to acclimate to higher temperatures also can lead to constant A_{max}/R_A. Since A_{max}/R_A steadily decreased with canopy height (Fig. 4B), perhaps the plants in this canopy are not able to metabolically adjust to the higher temperatures in the upper canopy, possibly indicating an inability for these tropical plants to thermally acclimate.

Foliar respiration per unit ground area

R_{foliar} (3.5-4.0 μmol CO_2 m^{-2}_{ground} s^{-1}) was 35-50% higher than an estimate from the Amazon (Chambers et al. 2004). Estimates 1-3 of R_{foliar} were quite similar because the mean nighttime temperature in 1998 was 24.18 °C, and the mean temperature in 1999 was 23.14, which are both close to the standard temperature correction (25 °C) used in estimate 3. Three of the simpler estimates of R_{foliar} (estimates 3-5) were very similar to the more complex extrapolations (estimates 1 and 2), likely because the overall means for respiration, LAI and N_{tot} were based on good representations of the functional group and height distributions for the forest. Estimate 6, however, was quite low compared to the rest of the estimates because the correlation between R_M and N_M was poor, resulting in an underestimation of R/N (Fig. 2). I recommend not using the slope of R_M vs. N_M for ecosystem extrapolation within forest canopies. The percent contributions of each functional group to R_{foliar} were primarily driven by the percent contributions of LAI for each group.

While the difference between an ENSO and a normal year in R_{foliar} only represented an 9% increase in foliar respiration (0.3 μmol CO_2 m^{-2} s^{-1}), this could mean

the difference between carbon sink vs. source behavior for this forest. The balance between photosynthesis and respiration in tropical rain forests is very tight. In the ENSO year of 1998, the old-growth forest at La Selva was reported to range from a 0.01 μmol m^{-2} s^{-1} carbon source to a 0.35 μmol m^{-2} s^{-1} carbon sink (Loescher et al. 2003). Results vary widely about whether tropical forests presently act as carbon sources or sinks, or what will happen with future possible feedbacks due to climate change. Many atmosphere-biosphere modeling studies predict tropical forests will be an increased carbon source with global warming (Kindermann et al. 1996; Braswell et al. 1997; Tian et al. 1998; Cox et al. 2000; Ito and Oikawa 2000; White et al. 2000; Cramer et al. 2001; Clark et al. 2003). Several eddy flux studies, on the other hand, have concluded that tropical rain forests are presently acting as carbon sinks in non-ENSO years (Fan et al. 1990; Grace et al. 1995; Malhi et al. 1998; Loescher et al. 2003; but see Saleska et al. 2003).

Uncertainty in R_{foliar} could result from uncertainty in foliar respiration rates and/or LAI estimates. One source of error is the lack of a seasonality assessment in respiration rates. Foliar respiration and respiration temperature response have been found to change with season in temperate forests because of active growth early in the growing season or translocation later in the growing season (Vose and Ryan 2002; Atkin et al. 2005; Xu and Griffin 2006). In the old-growth forest of La Selva, studies have found seasonality in litterfall nutrients (Wood et al. 2005), soil respiration (Schwendenmann et al. 2003), and tree growth (Hazlett 1987). Woody respiration did not change seasonally, however, likely because species phenologies are not synchronized like they are in temperate forests (Cavaleri et al. 2006). In all extrapolations, I assumed the same was true for foliar

respiration because this forest has neither a distinct growing season nor a dormant season. I did take into account the effects of seasonal temperature changes on foliar respiration in R_{foliar} estimates 1 and 2 (Table 2), and only fully-expanded leaves were measured to minimize the effects of growth respiration.

Uncertainties in either the seasonality or the absolute value of LAI are more important than uncertainty in respiration rates because of the multiplicative effects when extrapolating. Studies in both temperate and tropical forests have found LAI of evergreen species to change seasonally (Curran et al. 1992; de Wasseige et al. 2003), and preliminary data (indirectly measured) indicate that LAI varies with season in the old-growth forest of La Selva (S.F. Oberbauer, unpublished data). Specific sites were not re-sampled over time, but the tower sampling was continuous for two years, so it is likely that much of the variability in seasonal LAI was captured. Another possible source of error in the estimate of LAI is if the sampling scheme did not adequately represent the forest. 37% of the original randomly located possible tower sites were discarded because of rocky terrain or large stems. It is therefore reasonable to assume the tower sites are representative of at least 63% of the landscape, which is a much greater percentage of the landscape than an eddy flux tower footprint. I am confident that these methods of extrapolating chamber respiration measurements represent the best available data for assessing ecosystem respiration of the old-growth forest of La Selva.

Ecosystem respiration

R_{eco} (9.38 ± 1.43 μmol CO_2 m^{-2}_{ground} s^{-1}) was 45% greater than an estimate for a tropical rain forest in Manaus, Brazil (Malhi et al. 1999), and about 20% greater than an

estimate for an Amazonian tropical rain forest (Chambers et al. 2004). Although total ecosystem respiration at La Selva was greater, the percentages of respiration from component ecosystem parts were quite similar at La Selva (foliage=37%, soil=41%, woody=14%, coarse woody debris=7%) and the Amazonian forest (foliage, including "understory" =38%, soil=41%, woody=14%, CWD=6%, Chambers et al. 2004).

R_{eco} from extrapolated measurements was 33% greater than the eddy flux NEE_{night} at La Selva (7.05 ± 0.69 μmol CO_2 m^{-2} s^{-1}, Loescher et al. 2003), even though NEE_{night} was based on turbulent nights only (Fig.5). Loescher et al. (2003) noted that the greatest uncertainty of their study was associated with NEE_{night}, and this uncertainty was an impetus for the present study. If these independent estimates of ecosystem respiration approximate the true value of NEE_{night}, the old growth forest at La Selva was likely a strong carbon source during the 1998 ENSO. Eddy covariance measurements are suspected to frequently underestimate NEE_{night} (Baldocchi 2003). The perception of tropical rain forests as strong sinks may need to be reconsidered if eddy covariance studies reporting a large sink for tropical rain forests (Fan et al. 1990; Grace et al. 1995; Malhi et al. 1998) have similarly underestimated NEE_{night}. These results emphasize the need for and value of independent estimates of NEE_{night} for constraining estimates of ecosystem carbon balance.

CONCLUSIONS

- Q_{10} and E_0 were constant across height, foliar and soil nutrients, LMA, and respiration at 25 °C, but functional groups dominating the upper canopy had higher Q_{10} and E_0 values than groups found lower in the canopy.

- As predicted by the leaf economics spectrum, foliar respiration, N, and P were correlated, and groups that generally have longer leaf-life spans (herbaceous groups and palms) had lower respiration rates than groups that generally have shorter leaf-life spans (trees and lianas).
- The influence of the LMA-height gradient resulted in both tighter correlations between area-based respiration vs. leaf nutrients, and greater variation in R_A than R_M, R/N or R/P.
- A_{max}/R_A decreased with height, possibly indicating an inability of plants to thermally acclimate to the higher temperatures of the upper canopy.
- Foliar respiration per unit ground area (R_{foliar}), estimated with ENSO year temperatures, was 9% greater when estimated with temperatures from a normal year, which could possibly be the difference between carbon sink vs. source behavior for this forest.
- Simple methods of extrapolation using overall mean rates of R_A, R/N, N_{tot}, and LAI produced realistic estimates of R_{foliar}, but I recommend not using the slope of the R_M vs. N_M regression to estimate R/N.
- I estimated total ecosystem respiration as 9.38 ± 1.43 μmol CO_2 m^{-2}_{ground} s^{-1}, which was 33% greater than eddy flux nighttime net ecosystem exchange for the same forest, suggesting that studies reporting a large sink for tropical rain forests based on eddy flux measurements may be in error.

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Table 3.1. Abbreviations used and their description.

Variable	Description	Units
A_{max}	Photosynthetic capacity	μmol CO_2 m^{-2} s^{-1}
A_{max}/R_A	The ratio of A_{max} to R_A	unitless
E_0	Energy of activation	kJ mol^{-1} K^{-1}
LAI	Leaf area index, leaf area per unit ground area	m^2 m^{-2}_{ground}
LMA	Leaf mass per unit leaf area	g m^{-2}
NEE_{night}	Nighttime net ecosystem exchange from eddy flux*	μmol CO_2 m^{-2}_{ground} s^{-1}
N_A	Foliar N per unit leaf area	g m^{-2}
N_M	Foliar N per unit leaf mass	mg g^{-1}
N_{tot}	Total mass of foliar N per unit ground area	g m^{-2}_{ground}
P_A	Foliar P per unit leaf area	g m^{-2}
P_M	Foliar P per unit leaf mass	mg g^{-1}
Q_{10}	Change in respiration with 10°C change in temp	unitless
R_{Tleaf}	Foliar respiration rate at T_{leaf}	μmol CO_2 m^{-2} s^{-1}
R_{Ta}	Foliar respiration rate at T_a	μmol CO_2 m^{-2} s^{-1}
R_A	Foliar respiration per unit leaf area at 25 °C	μmol CO_2 m^{-2} s^{-1}
R_M	Foliar respiration per unit leaf mass at 25 °C	nmol CO_2 g^{-1} s^{-1}
R/N	Foliar respiration at 25 °C per unit mass of foliar N	μmol CO_2 g^{-1} N s^{-1}
R/P	Foliar respiration at 25 °C per unit mass of foliar P	μmol CO_2 g^{-1} P s^{-1}
R_{eco}	Ecosystem respiration per unit ground area	μmol CO_2 m^{-2}_{ground} s^{-1}
R_{foliar}	Foliar respiration per unit ground area	μmol CO_2 m^{-2}_{ground} s^{-1}
R_{soil}	Soil respiration per unit ground area†	μmol CO_2 m^{-2}_{ground} s^{-1}
R_{woody}	Woody respiration per unit ground area‡	μmol CO_2 m^{-2}_{ground} s^{-1}
R_{CWD}	Coarse woody debris respiration per unit ground area§	μmol CO_2 m^{-2}_{ground} s^{-1}
T_a	Above-canopy temperature at night	°C or K
T_{leaf}	Leaf temperature at time of measurement	°C or K

* Data from Loescher et al. (2003).
† Data from Schwendenmann et al. (2003).
‡ Data from Cavaleri et al. (2006).
§ Data from Clark et al. (2002).

Table 3.2. Six estimates of foliar respiration extrapolated to the ecosystem (R_{foliar}, μmol CO_2 m^{-2}_{ground} s^{-1}), representing two complex (1-2) and four simpler (3-6) methods. See text for details about mean and error calculations.

Estimate Code	Temperatures used to model respiration, (mean ± 1 se)	Method of calculating estimate	Method of calculating error term	R_{foliar}
1) LAI-normal	1999, a normal year, half-hourly nighttime temps (23.14 ± 0.02°C)	Sum of [LAI mean * R_A mean] by group and height	Sum of [LAI se * R_A mean] by group and height	3.5 ± 0.8
2) LAI- Niño	1998, an ENSO year, half-hourly nighttime temps (24.18 ± 0.02°C)	Sum of [LAI mean * R_A mean] by group and height	Sum of [LAI se * R_A mean] by group and height	3.8 ± 0.9
3) LAI-mean	Standardized to 25 °C	[LAI overall mean * R_A overall mean]	[LAI overall se * R_A overall mean]	3.6 ± 0.2
4) *R/N*-mean	Standardized to 25 °C	[N_{tot} overall mean * *R/N* overall mean]	[N_{tot} overall se * *R/N* overall mean]	3.7 ± 0.2
5) R_A/N_A-slope	Standardized to 25 °C	[N_{tot} overall mean * slope of R_A/N_A]	[N_{tot} overall se * slope of R_A/N_A]	4.0 ± 0.2
6) R_M/N_M-slope	Standardized to 25 °C	[N_{tot} overall mean * slope of R_M/N_M]	[N_{tot} overall se * slope of R_M/N_M]	1.2 ± 0.1

Table 3.3. Predictor variable p-values for ANCOVA models of respiration per unit leaf area (R_A), mass (R_M), nitrogen (R/N), phosphorus (R/P), and the ratio of photosynthetic capacity to respiration (A_{max}/R_A). Soil P was not a significant predictor for any response variable, and was removed. Three-way interactions were pooled into error for all five response variables, and two-way interactions were pooled into error for A_{max}/R_A. See Figures 4 and 5 for model-predicted effects.

	Response Variables				
Predictor Variables	R_A	R_M	R/N	R/P	A_{max}/R_A
Height	<0.001	<0.01	<0.001	<0.01	0.001
Group	ns	ns	ns	ns	ns
Soil N	ns	ns	ns	ns	<0.001
Height*Group	0.01	ns	0.05	ns	-
Height*Soil N	0.05	<0.05	<0.05	<0.05	-
Group*Soil N	ns	ns	ns	ns	-
Height*Group*Soil N	-	-	-	-	-
Overall model	<0.001	<0.001	<0.001	<0.001	<0.001
Model r^2	0.39	0.18	0.20	0.16	0.06

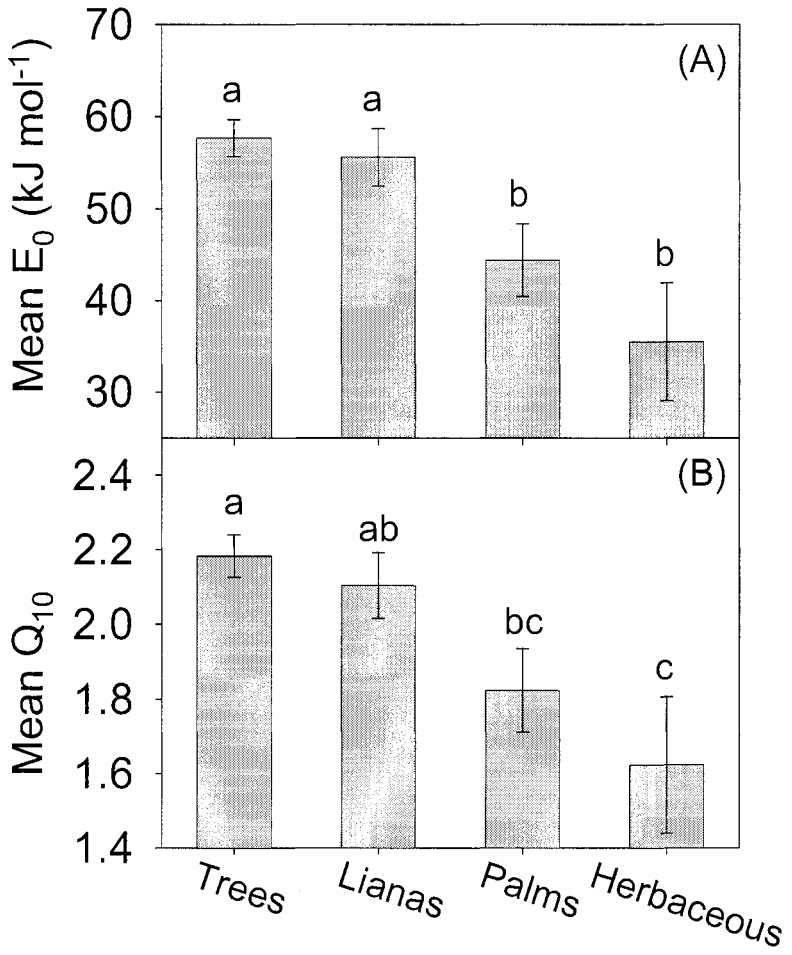

Figure 3.1. Foliar respiration least-squares mean Q_{10} and E_0 values by functional group. Both temperature response metrics were higher for trees and lianas, compared to palms and herbaceous groups. Means with the same letter are not significantly different, based on Fisher's LSD. Error bars are standard errors of the mean.

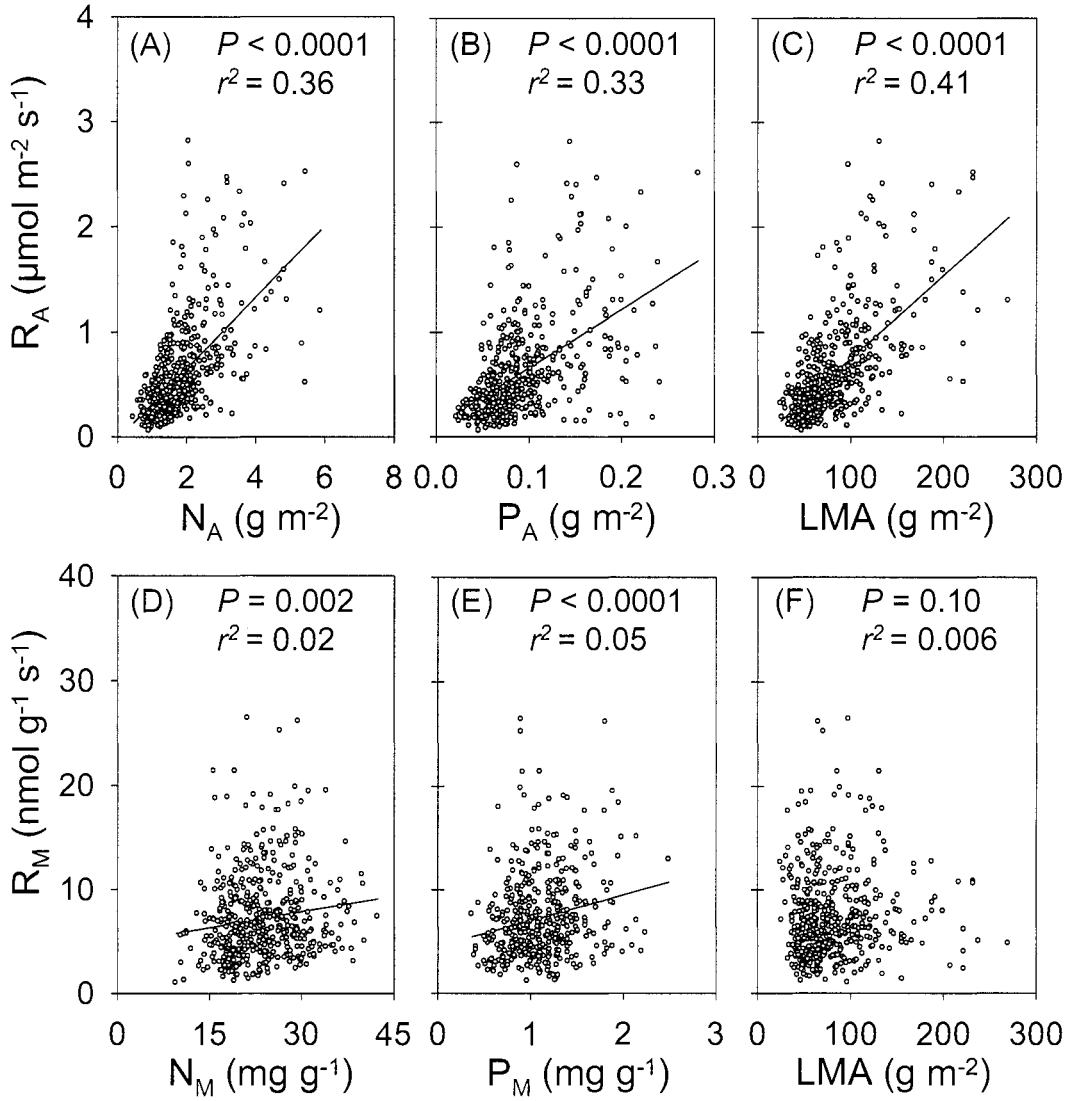

Figure 3.2. Regression plots between area- and mass- based foliar respiration, nitrogen, phosphorus, and leaf mass per area (LMA). Leaf area-based correlations between respiration and foliar nutrients (Plots A-B) were stronger than mass-based correlations (Plots D-E). The area-based relationships were likely driven by LMA. Equations for each significant (P<0.05) regression were: R_A=-0.03+0.34*N_A; R_A=0.04+6.5*P_A; R_A=-0.05+0.008*LMA; R_M=4.77+0.10*N_M; and R_M=4.3+2.5*P_M.

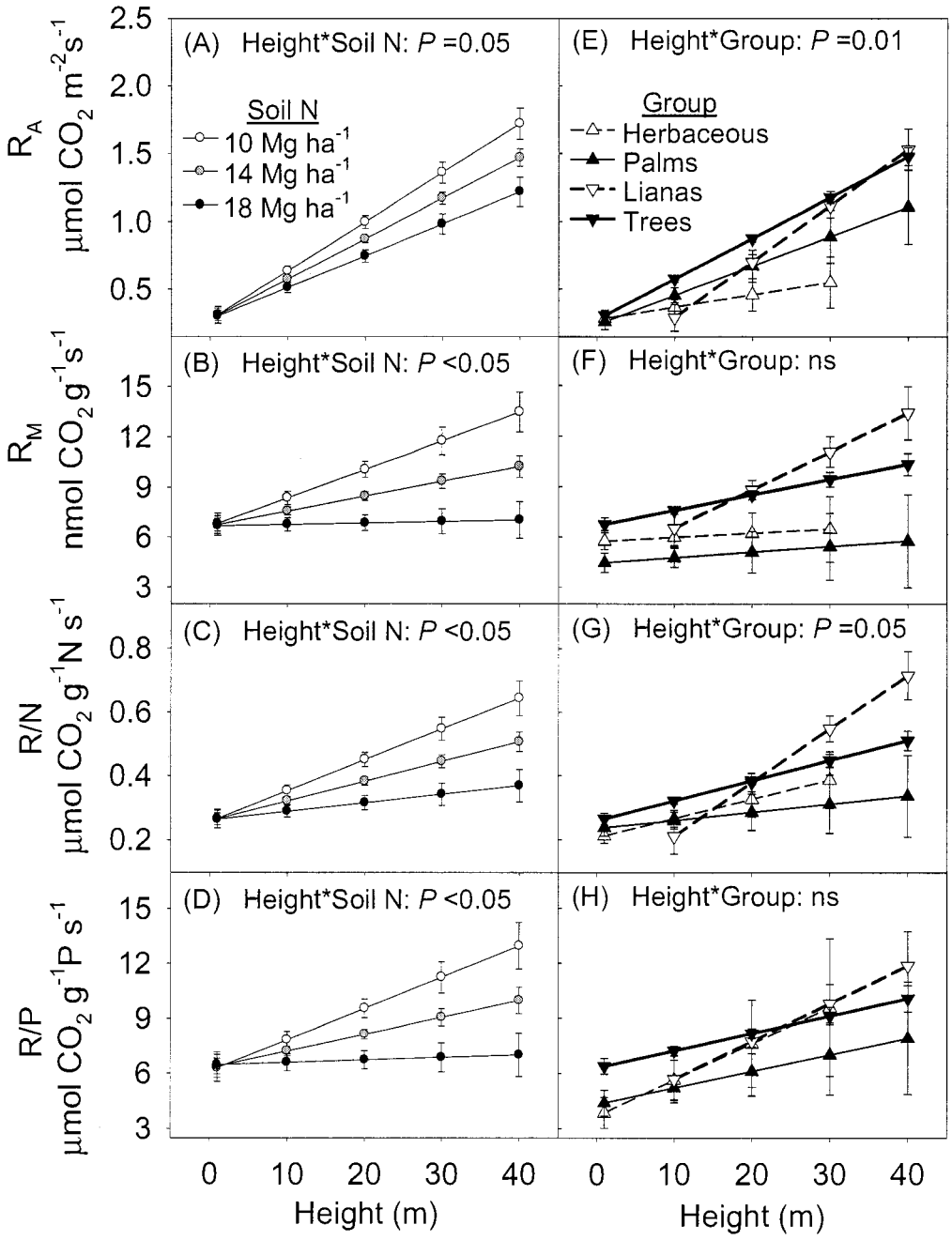

Figure 3.3. Model-predicted least-squares means and standard errors for height*soil N and height*group interactions from ANCOVAs predicting R_A, R_M, *R/N*, and *R/P* (Table 3). Respiration increased with height and decreased with soil N. Trees and lianas generally had higher respiration rates than palms and herbaceous groups. Effects of soil N and functional group were more pronounced higher in the canopy.

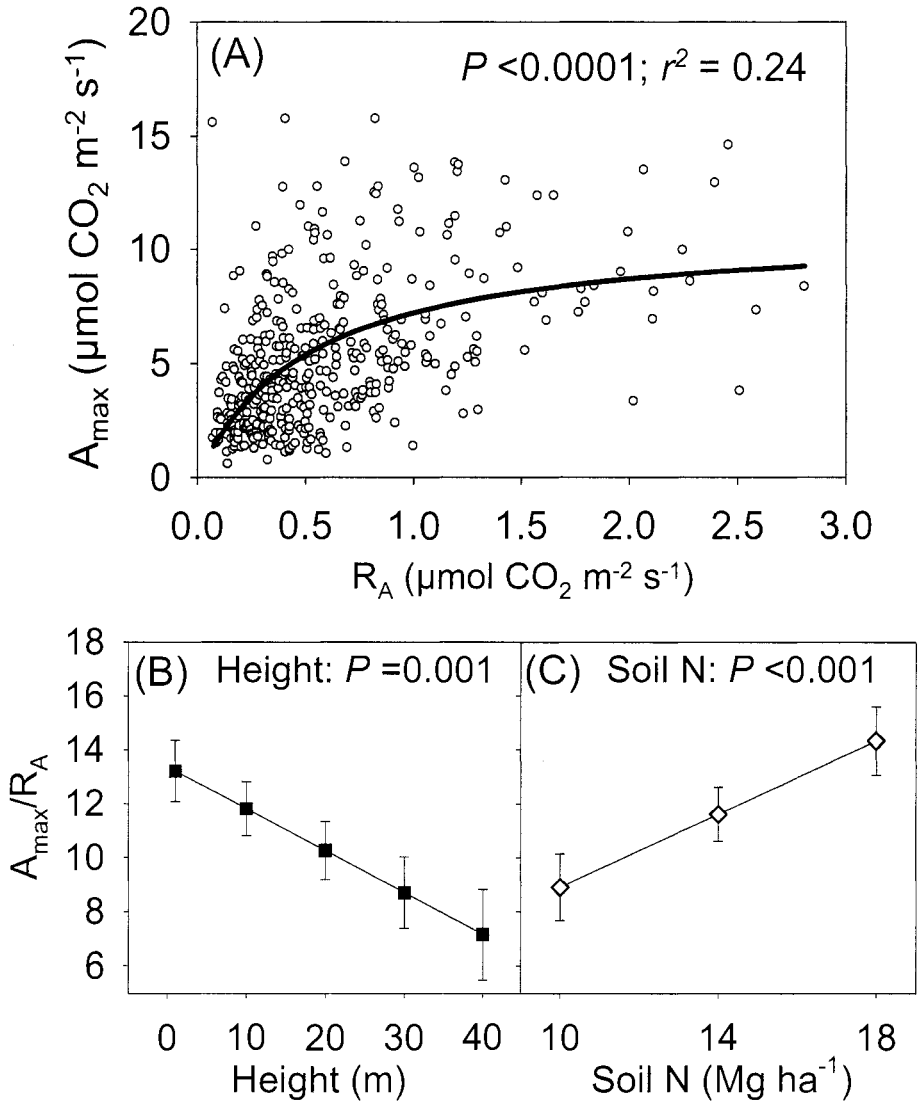

Figure 3.4. The relationship between area-based respiration at 25 °C (R_A) and photosynthetic capacity (A_{max}) (plot A), and the least-squares means and standard errors for height and soil N effects from the ANCOVA predicting A_{max}/R_A (plots B-C, Table 3). The curve in plot A was described by a rectangular hyperbola, where A_{max} = $(10.9*R_A)/(0.52+R_A)$. Plot B shows the height effect at mean soil N (13.9 Mg ha^{-1}) and averaged over all functional groups. Plot C shows the soil N effect at mean height (11.9 m) and averaged over all functional groups.

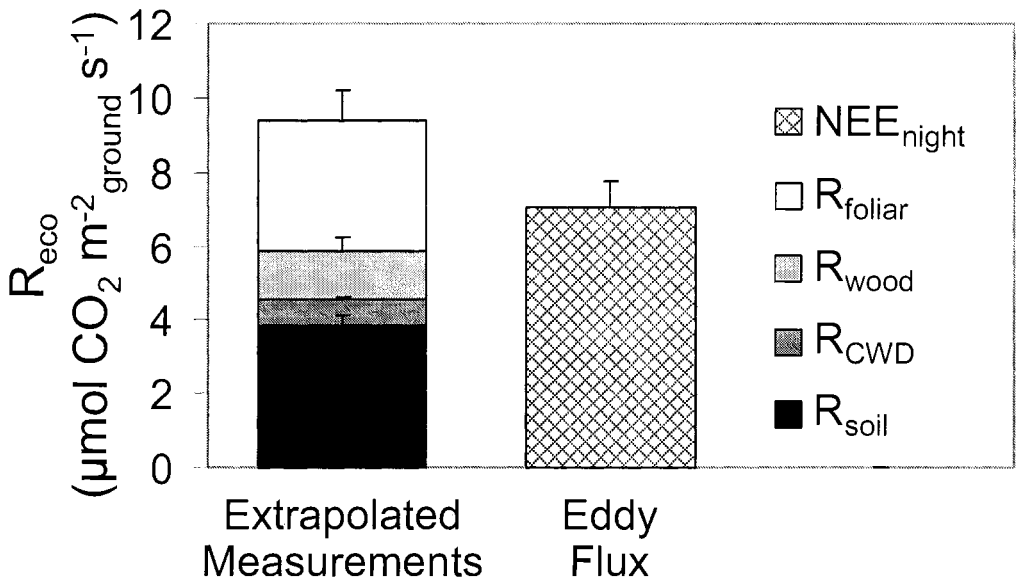

Figure 3.5. A comparison of ecosystem respiration (R_{eco}), as estimated by eddy flux nighttime net ecosystem exchange (NEE_{night}) vs. the summation of extrapolated measurements of component parts. R_{eco} from extrapolated measurements (9.38 ± 1.43 μmol CO_2 m^{-2} s^{-1}) was 33% higher than NEE_{night} (7.05 ± 0.69 μmol CO_2 m^{-2} s^{-1}, Loescher et al. 2003) for the same forest. NEE_{night} was based on data for turbulent nights only, when friction velocity (u^*) was greater than 0.4 m s^{-1} (Loescher et al. 2003). For the old-growth forest at La Selva, soil respiration was 3.88 ± 0.22 μmol CO_2 m^{-2} s^{-1} (Schwendenmann et al. 2003); woody respiration was 1.34 ± 0.36 μmol CO_2 m^{-2} s^{-1} (Cavaleri et al. 2006); coarse woody debris (CWD) respiration was estimated to be 0.66 ± 0.05 μmol CO_2 m^{-2} s^{-1}, from published total CWD carbon and turnover time (Clark et al. 2002); and foliage respiration was 3.5 ± 0.8 μmol CO_2 m^{-2} s^{-1} using estimate (1) of this study (Table 2).

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CHAPTER 4:

IS THE SUN/SHADE LEAF DICHOTOMY TOO SIMPLISTIC FOR FOREST CANOPIES?

ABSTRACT

The physiology and function of leaves commonly relates to leaf mass per area (LMA), the product of leaf thickness and leaf density. In all forest types, LMA increases from the understory to the canopy top. Classic experiments have shown that leaf thickness increases with increasing irradiance, leading many researchers to assume that the vertical gradient in LMA is primarily a response to the canopy light gradient. This "light centric" paradigm may not be the complete story, however. LMA also reflects increases in leaf density that result from decreasing water potential and turgor pressure with height. I tested two competing hypotheses: (1) LMA increases with height primarily because of a vertical light gradient, and (2) LMA increases with height primarily because of a vertical water potential gradient. This study presents results from a two year field campaign where light environment (% diffuse transmittance, TRANS), height and LMA were measured for all plant functional groups found along 55 vertical canopy transects in a primary tropical rain forest in Costa Rica. LMA showed a strong linear trend with height over all plant functional groups (200+ species), while the relationship between LMA and TRANS was weaker and non-linear. At low light levels, both light and water potential affect LMA, but multiple regression results and model predictions indicated that height had a greater overall influence on LMA than light. I conclude that the sun/shade leaf dichotomy is not the best explanation for the variation of LMA in forest canopies,

because the universal gradient of increasing LMA with canopy height depends more strongly on the vertical gradient in water potential.

INTRODUCTION

Classic papers by pioneer ecophysiologists showed leaf thickness to increase with increasing irradiance, and introduced the concept of "sun" vs. "shade" leaves found in most plant physiology textbooks. As a response to increased total irradiance, new leaves developed with longer, stacked palisade cells, and larger and more mesophyll cells, thus increasing leaf thickness and leaf mass per area (LMA g m^{-2}), the product of leaf thickness and leaf density (Nobel et al. 1975; Nobel 1977; Smith and Nobel 1978; Chabot et al. 1979; Gulmon and Chu 1981). These elegant early experiments were based primarily on herbaceous species and woody shrubs grown in chambers, with little effect of vertical gradients. Later studies found similar results for tree seedlings (Oquist et al. 1982; Ellsworth and Reich 1992) and vines (Hikosaka et al. 1994) in varying light environments, but these studies also lacked a major vertical gradient.

The subsequent discovery of vertical gradients of LMA within forest canopies fit nicely into this paradigm. A kg of leaves at the bottom of a tree canopy generally has 3-5 times the surface area of the same mass of leaves at the top of the canopy, in both conifer and broad-leaved species in temperate and tropical regions (Ford and Newbould 1971; Hutchison et al. 1986; Oberbauer and Strain 1986; Hollinger 1989; Niinemets and Kull 1995; Niinemets and Tenhunen 1997; Meir et al. 2001; Marshall and Monserud 2003; Koch et al. 2004). Models of forest canopy physiology require precise information on LMA because of its influence on vertical gradients of foliar nutrients, photosynthesis, and

respiration (Hollinger 1989; Ellsworth and Reich 1993; Niinemets and Tenhunen 1997; Mitchell et al. 1999; Meir et al. 2001). The overwhelming consensus for decades has been that LMA, and therefore leaf area- based gradients in photosynthesis, foliar nutrients, and respiration all increase with canopy height primarily because of foliar developmental responses to light (Ford and Newbould 1971; Dejong and Doyle 1985; Hutchison et al. 1986; Jurik 1986; Oren et al. 1986; Hollinger 1989; Brooks et al. 1991; Ackerly 1992; Ellsworth and Reich 1993; Evans 1993; Reich and Walters 1994; Niinemets and Tenhunen 1997; Kull and Niinemets 1998; Bond et al. 1999; Mitchell et al. 1999; Carswell et al. 2000; Griffin et al. 2002; Sack et al. 2006). I refer to this ecophysiology paradigm as "light centric" because light gradients are generally assumed to drive LMA gradients, and LMA gradients affect most other foliar physiological characteristics.

Several recent studies have suggested that light may not be the only (or the primary) driving force behind vertical gradients of LMA. In one of the first studies to remark upon this phenomenon, Niinemets et al. (1995) found the vertical LMA gradient within a conifer species to be related both to light and total tree height, suggesting the possible effects of gravity on LMA for taller trees. Marshall and Monserud (2003) found LMA to be similar at given sample heights, regardless of total tree height or light environment for western white pine, ponderosa pine and Douglas-fir. In another study of Douglas-fir, the increase in LMA related better to height than light environment (Woodruff et al. 2004). Finally, Koch et. al (2004) recently found xylem pressure to explain more variation in LMA of redwood canopies than the proportion of direct

radiation received by the foliage. Would the height profiles in LMA be explained better from a "light centric" paradigm or a new "water centric" paradigm?

The light gradient within forests is non-linear, while the water potential gradient is linear with respect to height. Canopy process models commonly use Beer's Law to describe the exponential decline of light with canopy depth caused by the interception and scattering of light by foliage (Wang 2003). The vertical gradient of water potential within xylem tissue is linearly related to height for two reasons: (1) hydraulic resistance is directly proportional to path length, or tree height (Tyree and Ewers 1991), and (2) even in the absence of transpiration, xylem tension increases by 0.01 MPa per meter of height, simply because of gravity (Scholander et al. 1965). Turgor pressure is expected to also decrease linearly with height, as found in Douglas-fir (Woodruff et al. 2004). Decreased turgor pressure, and therefore decreased cell expansion, is a passive response to decreased water potential (Hsiao 1973), and could result in denser, smaller foliage and higher LMA.

For this study, I set out to test the following two competing hypotheses: (1) LMA increases with height primarily because of a vertical light gradient, and (2) LMA increases with height primarily because of a vertical water potential gradient. A trend driven by water potential should show a linear relationship between LMA and height from the ground, whereas a light-driven pattern should show a linear relationship between LMA and light environment. Both factors are likely to exert some influence on foliage, so I used multiple regression analyses to determine the relative effects of light and height on LMA. I also examined how canopy gradients of LMA differed among tropical rain forest plant functional groups, in response to the recent emphasis on

modeling global-scale ecosystem function based on arrays of plant functional groups rather than biome types (Bonan et al. 2002).

This study presents results from a two year field campaign where light environment, height and LMA were measured for all plant functional groups found along 55 vertical canopy transects. A portable scaffolding tower was used to access foliage from forest floor to canopy top randomly across the landscape of a primary tropical rain forest in Costa Rica.

MATERIALS AND METHODS

Study site

Data were sampled in the primary forest of La Selva Biological Station, in the Caribbean lowlands of Costa Rica (elevation 37-150 m, 10°20' N, 83°50' W). La Selva is a premontane tropical wet forest (Hartshorn 1983), with mean annual rainfall of 4000 mm, and a mean annual temperature of 26 °C. The average canopy height for the primary forest (including gaps) is ~20 m, and individual emergent trees range from 30-60 m (Clark et al. 1996; Clark et al. 2004). Both woody and herbaceous functional groups reach great heights in the canopy, and some epiphytes and ferns grow high in the canopy without being rooted in the ground. Additional information about the soils and plants of La Selva can be found in McDade et al. (1994).

Sampling design and data collection

The towers sampling design and construction were part of a larger project with the goal of characterizing tropical rain forest canopy structure and function across

environmental gradients. Tower sites were located across the primary forest of La Selva using a stratified random sample (Cavaleri et al. 2006). At each tower site, an aluminum walk-up scaffolding tower (Upright, Inc, Dublin, Ireland) was constructed to the top of the canopy. Tower heights varied from 1.86 m (1 section) to 44.64 m (24 sections). Towers were constructed one section at a time, harvesting all foliage within each section. All harvested foliage was separated by height and the following plant functional groups: trees, palms, vines, lianas (woody vines), herbs and ferns, woody epiphytes, and non-woody epiphytes. Leaves of all species from each functional group within each tower section were bulked together in a bag and mixed thoroughly by hand. A subsample of several leaves was randomly selected from each bag to measure projected leaf area with an Li-3100 leaf area meter, (Li-Cor Inc., Lincoln, NE). Foliage subsamples were dried to constant mass at 60 °C to determine leaf mass per area (LMA g m^{-2}). These data represent 1262 samples from over 200 species: 62% tree species, 10% lianas, 9% ferns, 7% palms; herbs, epiphytes, and vines each represented 4% of the species sampled.

Light environment was measured at each tower section (every 1.86 m) with an LAI-2000 (Li-Cor Inc., Lincoln, NE). The LAI-2000 uses a hemispherical lens to measure transmittance of diffuse light (TRANS) from multiple angles (100% at canopy top). LAI-2000 measurements were taken in two-sensor mode, where one sensor was secured at the top tower section to automatically take readings, and the second sensor took measurements below the canopy at various heights. All TRANS measurements were taken at dawn or when sky was completely overcast, and a 180° view cap blocked the tower itself from the view field. A methodological comparison study (Gendron et al. 1998) showed %TRANS measured with an LAI-2000 to be one of the best methods of

estimating percent growing season photosynthetic photon flux density (%PPFD) in a deciduous forest. The relationship between %TRANS and %PPFD was linear and close to 1:1 with a slope of 0.9 and R^2 of 0.91 (Gendron et al. 1998). After all samples and measurements were taken, the tower was dismantled and moved to the next random site. Tower construction and sampling occurred continuously from June 2003 to June 2005, with each tower site sampled only once.

Statistical analyses

For all functional groups combined, I computed LMA means and standard errors for the following height classes: 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, and 30-40 m, and the following TRANS classes: 0-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, and 90-100%. I plotted LMA means at height class and TRANS class mid-points to display relationships and patterns in variability. For all data combined and for each separate functional group, I modeled LMA vs. height with simple linear regressions [LMA = $\beta_0 + \beta_1$ Height], and LMA vs. light with log-linear regressions [LMA = $\beta_0 + \beta_1$ ln(TRANS)]. I also modeled each data set with multiple regressions, combining both height and light terms [LMA = $\beta_0 + \beta_1$ Height + β_2 ln(TRANS)]. Interaction terms were not significant and were omitted from all models. I weighted all regressions with the inverse of the predictions for the unweighted full model to meet the assumptions of homoscedasticity. I evaluated models using both R^2 values and Akaike's Information Criterion (AIC), which penalizes a model based on the number of parameters. The best statistical model minimizes the value of AIC (Burnham and Anderson 1998). I used the multiple regression coefficients to plot weighted least

squares estimates of LMA at selected height and light levels for all functional groups combined. I computed an LMA plasticity index for each functional group as [(max LMA–min LMA)/max LMA] (Carpenter and Smith 1981). All statistical analyses were performed with SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

When all functional groups were combined, LMA increased linearly with height, and non-linearly with TRANS (Fig. 1). LMA increased steeply at TRANS levels between 0-25%, but there was little difference and large variability in LMA for light levels from 25-100% (Fig. 1). Standard errors increase higher in the canopy because of decreased sample size for these classes (Fig. 1). TRANS had a non-linear relationship with height, described by: Height= 4.3 + 4.67 ln(TRANS), R^2=0.50 (Fig. 2).

LMA increased linearly with height for all woody and herbaceous functional groups (Figs. 3 and 4), but height explained more of the variability in LMA for woody groups (40-58%) than herbaceous groups (18-29%, Table 1). The relationship between LMA and TRANS was log-linear for all functional groups (Figs. 3 and 4, Table 1). Plots of LMA vs. TRANS indicate that the light effect was primarily concentrated in light levels less than ~25% TRANS (Figs. 3 E-H and 4 D-F). The "height only" models explained more variation than the "light only" models in all groups except woody epiphytes (Table 1). When both height and TRANS terms were combined in multiple regressions, the coefficients for TRANS became non-significant in five of the seven functional groups, and became less significant in the remaining two groups and for all the data combined (Table 1). Multiple regressions that included both height and light

showed only marginal improvement over the height regressions, based on R^2 and AIC values (Table 1). Model predictions of LMA vs. height at four values of TRANS show that height had a much greater effect than light (Fig. 5).

Slopes and intercepts for the LMA x height linear regressions differed by functional group. Palms and epiphytes had the greatest overall values of LMA, while herbs and ferns had the lowest slope and the lowest overall values of LMA (Fig. 6). Trees, lianas, and vines had similar slopes for the linear regressions of LMA x height (Table 1, Fig 6). The plasticity index for herbaceous epiphytes was the greatest of all groups, while palms had the lowest plasticity index for LMA (Table 1).

DISCUSSION

Height is the primary driver of the LMA gradient, but light also has some effect

These results all strongly support hypothesis (2): the variation of LMA within a tropical rain forest canopy is primarily driven by height, likely because of the linear decrease in turgor pressure and capacity for cell expansion. Height explained the gradient in LMA much better than light environment for all functional groups, although light may have affected leaf morphology in the shaded understory. Chabot et al. (1979) hypothesized a plateau in LMA response when photon flux densities are saturating for photosynthesis. Ellsworth and Reich (1992) found all or most of the photosynthetic acclimation to high light in sugar maple to occur at 15% of full sunlight, and other studies have found little increased tree seedling growth above 20-30% sunlight (Logan 1965; Gottschalk 1985).

In contrast to this study, others have reported linear relationships between LMA and light, but these studies generally had few data points, especially in the high light range (Ellsworth and Reich 1993; Niinemets and Tenhunen 1997), weak linear relationships (Ackerly 1992), or were conducted on managed fruit trees where light gradients may be different than in closed-canopy forests (Dejong and Doyle 1985). Oberbauer et al. (1986) found LMA of a tropical tree species to increase both with canopy height *in situ* and with increasing light in growth chambers, but the maximum LMA achieved in the growth chambers was less than the maximum LMA in the canopy, suggesting something else may have been affecting LMA. Also in the Oberbauer et al. study (1986), the shape of the relationship between LMA and light within the canopy was not clear because a categorical height position variable was used. A recent study of open-grown arboretum deciduous trees came to the opposite conclusions of this study, and found variation in LMA to be more pronounced with differences in light (or "exposure") than height (Sack et al. 2006). This study also used categorical light and height variables, masking the shape of the LMA gradient, and these open-grown trees may have fundamentally different patterns of LMA than closed forest canopies.

Differences among functional groups

LMA is a primary trait in the "leaf economics spectrum" (Wright et al. 2004). LMA, along with leaf life span, photosynthesis, dark respiration, foliar N and P (all per unit leaf mass) correlate with each other across plant functional types and biomes, indicating remarkable global-scale convergent evolution (Reich et al. 1997). Leaf life span and LMA together describe the trade-off between long-lived leaves with greater

carbon allocation to structural rather than metabolic components (high LMA) vs. short-lived leaves with high metabolic activity and less physical protection (low LMA) (Reich et al. 1991; Reich et al. 1992; Reich et al. 1998). In the leaf economics spectrum, LMA and leaf life span are the central traits under evolutionary selection, and LMA alone has been dubbed "the most useful single indicator of leaf strategy" (Westoby et al. 2002). This slow/fast trade off at the leaf scale also predicts the slow/fast trade off at the plant level, as high LMA/long-lived species put more energy into plant survival than fast growth, while low LMA/short-lived species put more energy into growth than survival (Poorter and Bongers 2006; Sterck et al. 2006).

The leaf economics spectrum concept (Wright et al. 2004) was supported in this study because functional groups with longer leaf life spans (epiphytes and palms) had the highest overall values of LMA, and groups with the highest photosynthetic capacities (trees and lianas), had lower LMA values (Fig. 6). Low values of LMA have also been associated with shade tolerance (Jurik 1986; Bond et al. 1999), and the most shade tolerant functional groups in this study (vines, herbs and ferns) exhibited the lowest overall LMA values (Fig. 6).

The height gradient of LMA was greater than the differences between functional groups (Fig. 6). Sack et al. (2006) also found variation of LMA within species to be as great as variation between species, which could be of consequence when modeling ecosystem fluxes using only functional group differences in LMA. Leaf economics spectrum data (Reich et al. 1997) are already being used to link climate and ecosystem models (Bonan et al. 2002), and to model global-scale ecosystem structure and function (Moorcroft et al. 2001). Care must be taken when extrapolating with these data,

however, because they are generally composed of "sun leaf" measurements only, without regard to height gradients (Reich et al. 1991; Wright et al. 2004).

Palms had the lowest plasticity index and the lowest capacity to change morphologically with either height or light (Table 1). Herbaceous epiphytes exhibited both the highest overall and lowest overall values of LMA of all functional groups, and thus the highest plasticity index, indicating a greater capacity to morphologically adapt to changing environments (Table 1).

The height vs. LMA relationships were weak for herbaceous epiphytes and herbs and ferns (Table 1, Fig. 4B-C). No herbs were sampled above 4 m, and most ferns found above 4 m at La Selva are epiphytic (J.E. Watkins, pers. comm.), so the canopy samples in the 'herbs and ferns' group were likely all epiphytic ferns. The leaves of these herbaceous epiphytes and epiphytic ferns likely developed in canopy soil with no hydraulic connectivity to the ground. The effect of path length on hydraulic resistance and LMA would thus be circumvented, explaining the weaker height vs. LMA relationships. Koch et al. (2004) observed a similar phenomenon in redwoods, where an epiphytic redwood seedling had much lower LMA than adjacent foliage of the parent tree. LMA of woody epiphytes (Fig. 3D) had a tighter relationship with height than LMA of herbaceous epiphytes (Fig. 4C). Most of the woody epiphytes at La Selva are hemiepiphytes (C.L. Cardelus, pers. comm.), which retain hydraulic connection to the ground for a part of their life cycle. The long-lived leaves of these woody hemiepiphytes may have developed when this connection was still present, resulting in a tighter relationship between height and LMA.

Implications for physiological function

LMA relates to foliar physiology in several ways. Across species, LMA correlates with gas exchange rates and foliar nutrients per unit leaf *mass* because of the leaf economics spectrum and the universal correlations in life history strategies (Wright et al. 2004). Within forest canopies, LMA is predictive of gas exchange rates and foliar nutrients per unit leaf *area* because of the strong correlation of LMA with height, and the influence of LMA on rates per unit area (Hollinger 1989; Ellsworth and Reich 1993:Meir, 2001 #4; Niinemets and Tenhunen 1997; Mitchell et al. 1999). For example, photosynthetic capacity per unit area (A_{max}/area) is the product of LMA and A_{max}/mass. As a simplified illustration of the influence of LMA on canopy photosynthetic capacity, assume that a given plant has an A_{max}/mass value of 100 nmol g^{-1} s^{-1} throughout the canopy, but allow LMA to vary based on model-predicted estimates (Fig. 5). At 50% TRANS, a leaf 5 m in height would have an LMA of 66 g m^{-2}, while a leaf at 30 m would have an LMA value of 120 g m^{-2} (Fig. 5), which translates into A_{max}/area values of 6.6 and 12.0 μmol m^{-2} s^{-1}, respectively. Based solely on the changes in LMA due to height, A_{max}/area doubled from 5 to 30 m, even when light environment and A_{max}/mass were held constant.

Leaf thickness and leaf density

LMA is the product of two foliar characteristics: thickness and density (from Witkowski and Lamont 1991):

$$\frac{mass}{area} = \frac{mass}{(area * thickness)} * thickness = \frac{mass}{volume} * thickness = density * thickness \quad (1)$$

Witkowski et al. (1991) found foliar density and thickness to vary independently with soil moisture, nutrients, and light for several plant functional groups (vertical gradients were not investigated). I did not differentiate between foliar density and thickness, which likely varied independently within the vertical transect. A response to light is generally an increase in leaf thickness, because of lengthening and stacking of palisade cells (Nobel et al. 1975; Nobel 1977; Smith and Nobel 1978; Chabot et al. 1979). A response to decreased water potential is generally an increase in leaf density and a decrease in leaf area, because of tighter packing of smaller cells (Nobel 1977; Smith and Nobel 1978; Rascio et al. 1990). Niinemets and Kull (1995) found needle density to be related to total tree height, while needle thickness was related to canopy gap fraction (the study did not investigate sample height). In a study of the dominant tree species at La Selva Biological station, leaves from the top of the canopy were denser than leaves of seedlings grown at the same irradiance (Oberbauer and Strain 1986). Future studies could use this to great advantage in teasing apart the differing responses of LMA to vertical canopy gradients of light vs. water potential.

Conclusion

LMA is easily measurable and remarkably predictive of foliar physiological function, both within and across species. LMA was better related to height than light environment, supporting the hypothesis that the universal LMA gradient within forest canopies is primarily driven by a linear decrease in turgor pressure with height, caused by an increase in hydraulic resistance with gravity and longer path length. While light does affect LMA slightly, especially in the light-limited understory, the sun/shade leaf model

taught in every plant physiology textbook is too simplistic to describe the large variation of LMA with vertical structure. Perhaps our thinking needs to be shifted from a "light centric" to a "water centric" paradigm when investigating the vertical ecophysiological gradients in forest canopies.

Table 4.1. Regression coefficients, R^2 values, and Akaike's Information Criterion (AIC, lower is better) for the linear regressions between LMA and height, the log-linear regressions between LMA and TRANS, and the multiple regressions including both terms. 'Height only' models performed better than 'Light only' models in all cases except woody epiphytes, based on both R^2 and AIC values. Adding % transmittance (TRANS) to the Height model did not greatly improve model performance.

			Light only $LMA = \beta_0 + \beta_1 \ln(TRANS)$				Height only $LMA = \beta_0 + \beta_1 Ht$				Height and Light $LMA = \beta_0 + \beta_1 Ht + \beta_2 \ln(TRANS)$				
Functional Group	Plasticity Index	n	β_0	β_1	R^2	AIC	β_0	β_1	R^2	AIC	β_0	β_1	β_2	R^2	AIC
Trees	0.96	542	56**	13**	0.25	1421	45**	2.7**	0.43	1280	44**	2.4**	2.7*	0.43	1277
Lianas	0.91	164	55**	11**	0.27	372	41**	2.5**	0.55	292	40**	2.4**	N.S.	0.55	294
Palms	0.88	197	77**	14**	0.24	455	67**	3.6**	0.40	410	65**	3.0**	5.1**	0.42	404
Woody Epiphytes	0.93	19	N.S.	36**	0.59	50	30*	3.8**	0.58	50	N.S.	2.8*	N.S.	0.65	48
Vines	0.95	102	47**	6.4*	0.07	273	40**	2.1**	0.29	247	43**	2.5**	N.S.	0.30	247
Herbs and Ferns	0.90	132	54**	8.6**	0.15	310	56**	1.3**	0.18	304	54**	1.0**	N.S.	0.19	305
Herbaceous Epiphytes	0.98	106	43**	26**	0.17	429	47**	3.9**	0.18	427	40**	2.5*	N.S.	0.20	427
All Groups	0.98	1262	59**	12**	0.16	3680	52**	2.4**	0.27	3508	51**	2.2**	2.5*	0.27	3503

** P-value < 0.01; * P-value < 0.05; N.S. P-value > 0.05

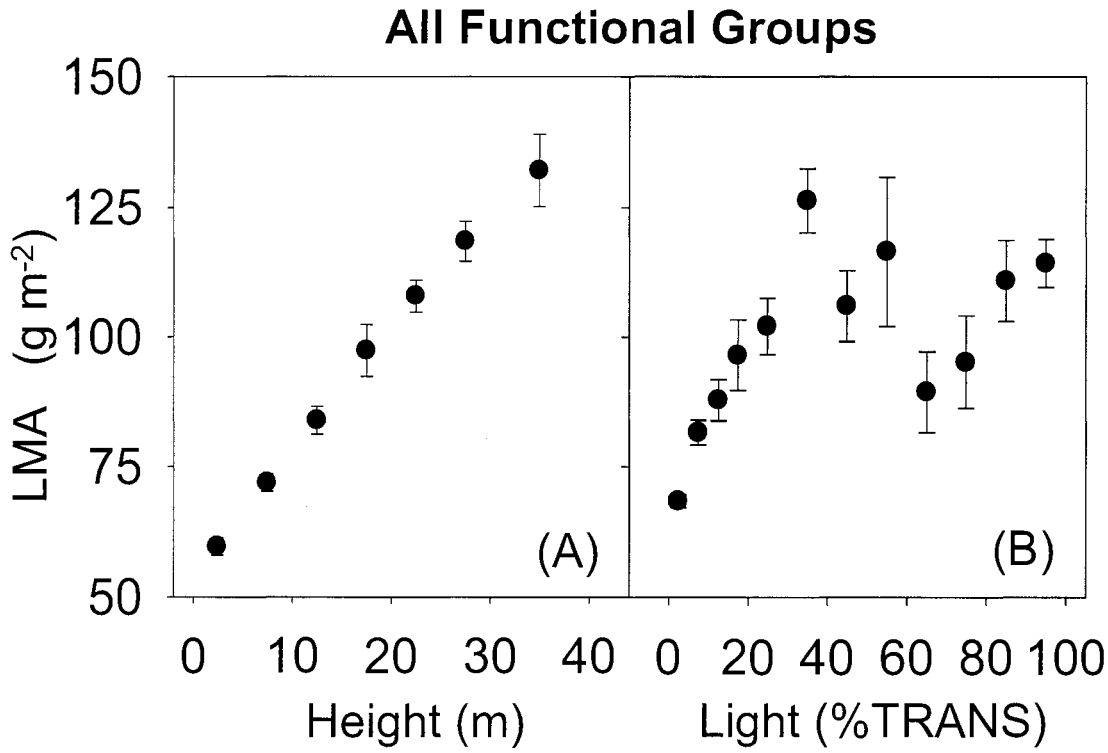

Figure 4.1. LMA means at height class and % transmittance (TRANS) class mid-points, bars represent standard errors. Leaf mass per area increased linearly with height, but non-linearly with % transmittance (%TRANS), indicating a greater influence of water potential than light environment on the increase of LMA across the entire canopy vertical gradient. LMA response to light likely plateaus beyond a photon flux density that is saturating for photosynthesis.

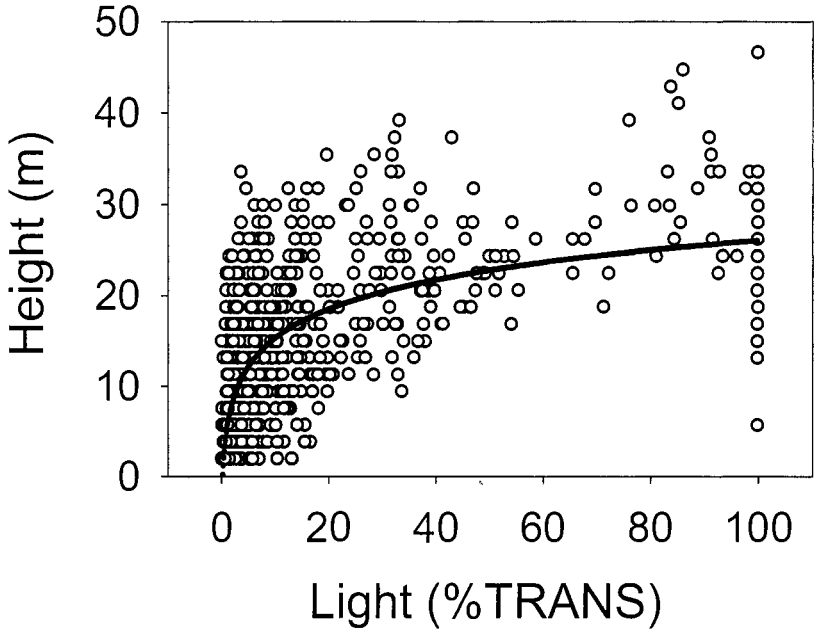

Figure 4.2. Height had a non-linear relationship with light environment, expressed as % transmittance (%TRANS). The relationship is described as follows: Height= 4.3 + 4.67 ln(TRANS), R^2=0.50.

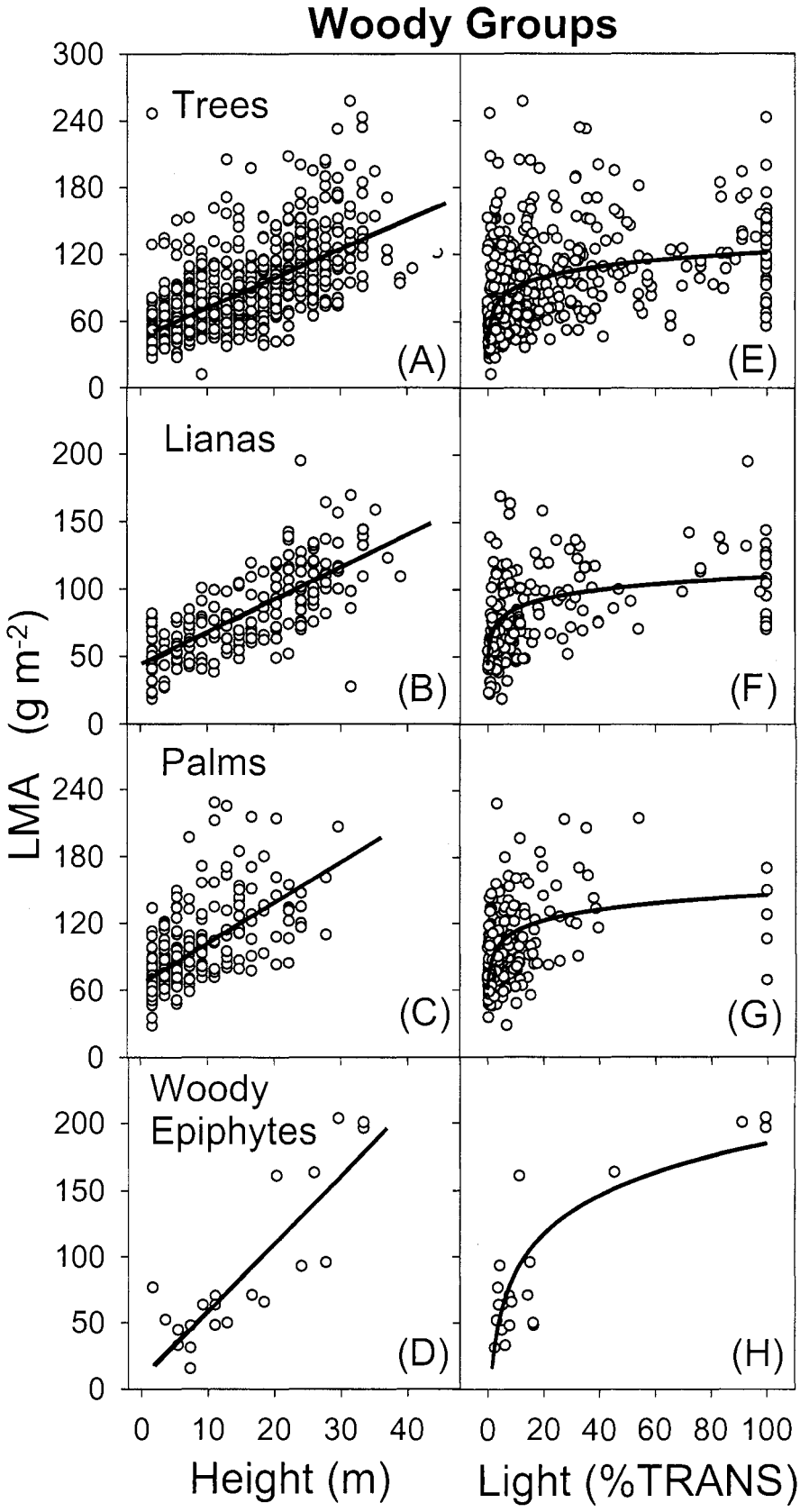
Woody Groups
Trees
Lianas
Palms
Woody Epiphytes
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
LMA (g m-2)
Height (m)
Light (%TRANS)
0
60
120
180
240
300
50
100
150
200
10
20
30
40
80

Figure 4.3. LMA vs. height and %TRANS by woody functional groups. LMA increased linearly with height for all woody functional groups (plots A-D). The non-linear relationship between LMA and %TRANS was described by a logarithmic regression for all woody functional groups (plots E-H). See Table 1 for equations and R^2 values.

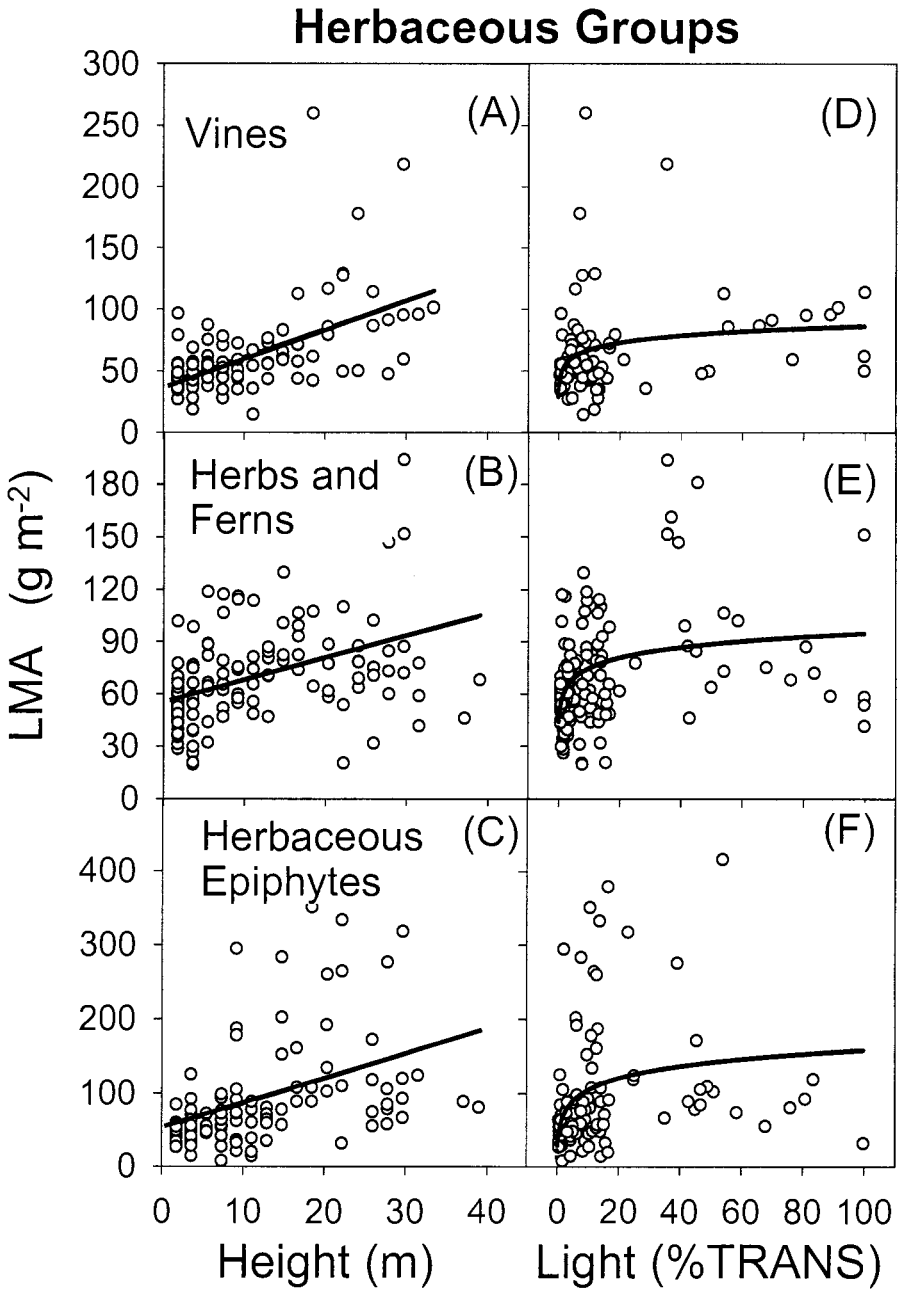

Figure 4.4. LMA vs. height and TRANS by herbaceous functional groups. LMA increased linearly with height for all herbaceous functional groups (plots A-C). Variation is greater for herbaceous groups than for woody groups. The non-linear relationship between LMA and %TRANS was described by a logarithmic regression for all woody functional groups (plots D-F). See Table 1 for equations and R^2 values.

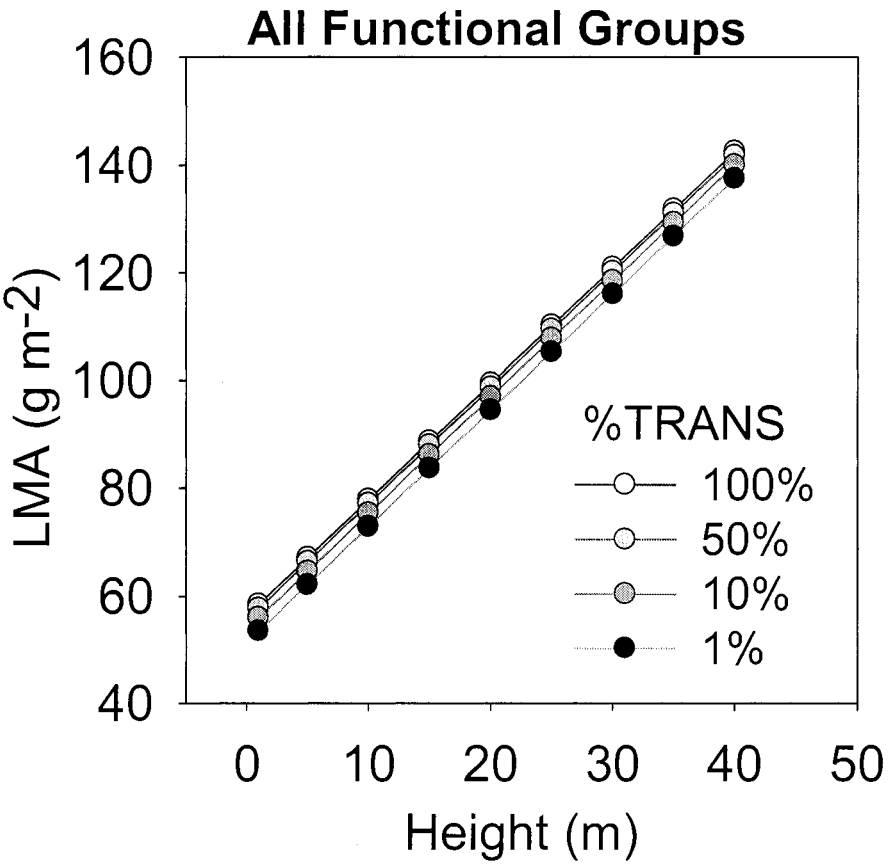

Figure 4.5. Model predictions of LMA vs. height at four values of %TRANS indicate that height had a much greater effect than light. LMA was modeled for all groups combined with a weighted least squares multiple regression: LMA =51 + 2.2 Ht + 2.5 ln(TRANS), R^2=0.27. See Table 1 for multiple regression model coefficients and R^2 values for each functional group individually.

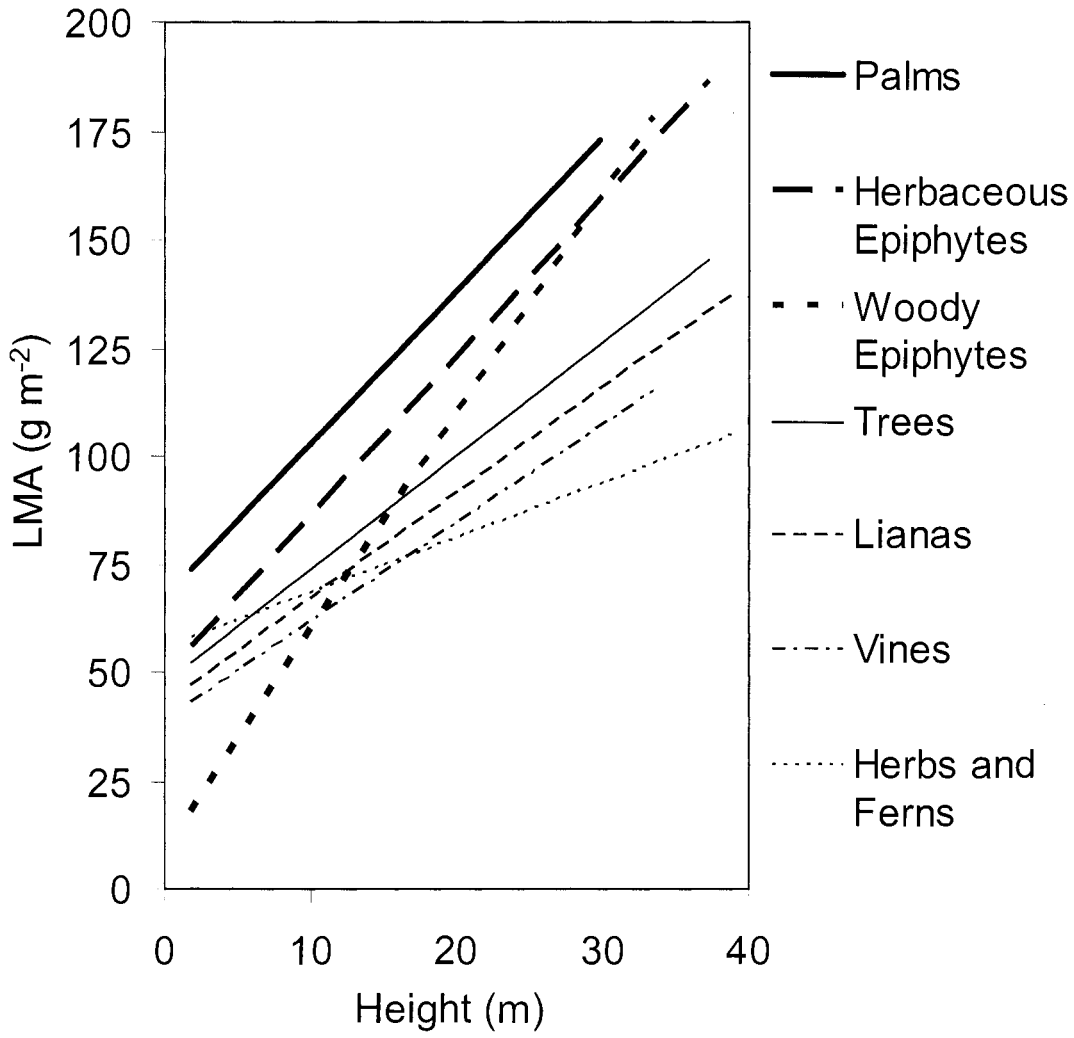

Figure 4.6. Slopes of LMA vs. height for each functional group. Although all functional groups showed significant linear relationships between LMA and height, slopes and intercepts differed because of different life histories, leaf economics, and hydraulic architecture. Palms and epiphytes had the greatest overall values of LMA, while herbs and ferns had the lowest slope and the lowest overall values of LMA. Woody epiphytes had the lowest intercept, and the greatest slope. Trees, lianas, and vines had similar slopes and intercepts. See Table 1 for intercept (β_0) and slope (β_1) values.

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CHAPTER 5:

DISSERTATION SYNTHESIS

The results of all three studies point to height a as key factor in the variation of aboveground plant respiration and foliar morphology for this system. Upper branches (especially lianas) contributed substantially to total plant respiration, as both wood and foliar respiration increased steeply with height. The height gradient in LMA dictated the strong relationships between area-based foliar respiration and nutrients. LMA was better related to height than light environment, supporting the hypothesis that the LMA gradient within forest canopies is primarily driven by a linear decrease in turgor pressure with height, caused by an increase in hydraulic resistance with gravity and longer path length. These results have great implications in the future interpretation and modeling of canopy physiological processes. Height is much simpler to measure and interpret than light environment. Physiological processes correlate better with height throughout the entire canopy profile, which could greatly simplify canopy process modeling over large scales. The novel aspects of the towers sampling design allowed an investigation of canopy physiology and structure in a manner that has never been attempted in a tropical rain forest.

This work has illuminated the tight interplay between CO_2 exchange and water relations, beyond stomatal control. Water potential and turgor pressure gradients may affect gas exchange more than previously thought, as turgor pressure likely drove the vertical gradients in LMA, which influenced the vertical variation in area-based foliar respiration. In another example, canopy branches (only ~15% of total woody biomass), accounted for 70% of total woody CO_2 efflux. This phenomenon is possibly the result of

dissolved CO_2 moving upwards in the xylem sap and diffusing to the atmosphere high in the canopy. More research is needed in areas where water relations and patterns of gas exchange intersect.

Through the course of this study, it became apparent that the 3-dimensional structures of both woody tissue and leaves were important factors to consider when extrapolating respiration rates to the ecosystem. For example, I extrapolated small diameter woody tissue (<10 cm) CO_2 efflux with wood surface area, while CO_2 efflux from stems >10 cm was extrapolated to the ecosystem with biomass. Similarly, the mass-based correlations between foliar respiration and foliar nitrogen were much weaker than the area-based correlations, indicating that mass-based R/N slopes were inappropriate to use in ecosystem extrapolation for this forest.

Total ecosystem respiration estimated by summing extrapolated components (3349 ± 803 g C m^{-2} yr^{-1}) was 26% greater than eddy flux nighttime net ecosystem exchange for the same forest, even when only turbulent nights were used in the eddy flux estimate. This discrepancy underscores the need for careful interpretation of eddy flux-derived respiration rates, and the difference could easily tip the balance between carbon sink vs. source behavior for the forest. I also found a substantial difference between foliar respiration extrapolated with niño vs. non- niño year nighttime temperatures, which could account for a 3% increase in total ecosystem respiration, again possibly being the difference between source and sink behavior. An important next step in the investigation of the carbon balance of this system is a refined estimate of gross primary production, which will be estimated using the canopy process model MAESTRA and detailed canopy physiology measurements.