DISSERTATION

VARIATION IN CARBON CYCLING AMONG FOUR TREE SPECIES IN A TROPICAL RAIN FOREST

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ABSTRACT

VARIATION IN CARBON CYCLING AMONG FOUR TREE SPECIES IN A TROPICAL RAIN FOREST

Ecologists have long sought to explain how species affect ecosystem processes in tropical forests and more urgently now as tropical forests face climate and land-use change that shift species composition. Roughly 50 million hectares of primary forest diverse in species have been turned into tree plantations mostly in monodominant stands. To describe and predict the ecosystem processes of such plantations for regional and global assessments of forest carbon storage, we must understand how subtle differences in tree species traits affect forest carbon cycling. In a tropical rain forest of Costa Rica, twelve tree species were planted in monoculture plots, and four have survived to have almost two-fold difference in carbon stored in forest plant biomass. I examine the variation and its cause in this dissertation.

I began by examining the variation in foliar respiration and wood CO_2 efflux rates among species and canopy layers in Chapter 2. Understanding how species affect forest carbon cycling requires that autotrophic respiration be measured and placed in a complete carbon budget. However, extrapolating measurements of autotrophic respiration from chambers to ecosystem remains a challenge in tropical forests. High plant species diversity and complex canopy structure may cause respiration rates to vary and introduce bias in extrapolation. I examined if foliar respiration and wood CO_2 efflux rates vary among species and canopy layers and whether the variation was related to commonly used scalars, mass, N content, photosynthetic capacity, and wood size.

The variation in foliar respiration and wood CO_2 efflux rates showed that vertical sampling reduces bias more than temporal sampling. Foliar respiration rate increased three-fold with height and averaged ~0.74 μmol m^{-2} s^{-1} in overstory and ~0.25 μmol m^{-2} s^{-1} in the understory. Leaf mass per area, leaf nitrogen, and photosynthetic capacity explained some of the variation in foliar respiration rate, but canopy layer or height explained the most. Foliar respiration rate was similar among species. Chamber measurements of foliar respiration can be extrapolated to the canopy with rates and leaf area specific to each canopy layer or height class. If area-based rates are sampled throughout the canopy, foliar respiration may be extrapolated with total leaf mass by regressing the area-based rate against leaf mass per area to derive mean respiration rate per unit mass. Wood CO_2 efflux for overstory trees averaged 1.0 - 1.6 μmol m^{-2} s^{-1} for overstory trees and 0.6 - 0.9 μmol m^{-2} s^{-1} for understory species. The variation in wood CO_2 efflux rate was mostly related to wood size expressed as the ratio of wood surface area to mass. Wood CO_2 efflux rate was similar among species. Mean wood CO_2 efflux can be extrapolated to the stand using surface-area based rate, derived by regressing CO_2 efflux per unit mass against the ratio of surface area to mass, and total woody tissue surface area. The temperature response of foliar respiration was similar among species, and wood CO_2 efflux was similar between wet and dry seasons. Air temperature lacked strong seasonal trends.

I then constructed a complete and detailed annual carbon budget in Chapter 3, to examine if species difference in biomass carbon storage resulted from differences in gross primary production (GPP) or partitioning of GPP to components with lower turnover and respiration rates. Gross primary production and its partitioning control the behavior of ecosystem biogeochemistry models used for assessments in tropics and elsewhere, but they are difficult to measure at the detail necessary to parameterize the models and are often unknown at a site,

especially for tropical forests. As more primary forests are converted to monodominant plantations, determining how species affect carbon budgets is never more important.

I quantified the annual values of GPP, NPP, respiration, and biomass, and estimated fraction of GPP partitioned to components canopy, wood, and roots, fraction of component flux respired, and biomass turnover rate. Respiration, NPP, and biomass were measured for each component, and summed to estimate GPP and partitioning fractions. Biomass was estimated from annual inventories. Net primary production was estimated by summing biomass turnover and change in biomass carbon stored in components between annual inventories. Biomass turnover was measured using litter traps and fine root ingrowth cores. Respiration was scaled from chamber measurements made in Chapter 2.

I found that species differences in GPP, not partitioning, explained species differences in NPP and respiration for canopy, wood, and roots. Species took up 3070 – 4490 gC m^{-2} $year^{-1}$ as GPP, respired 2120 – 3200 gC m^{-2} $year^{-1}$, allotted 948 – 1280 gC m^{-2} $year^{-1}$ to NPP, and stored 7590 – 139000 gC m^{-2} as biomass. Species partitioned 33% of GPP to canopy, 26 – 37% to wood, and 35% to roots. Species respired more than half of fluxes partitioned to components, 59 – 72% in canopy, 63% in wood, and 81% in roots, and the high respiration rates of all components may have constrained the variation in partitioning fractions among species. Species with greater GPP had proportionally greater NPP, and species with greater wood NPP had larger biomass storage size and faster storage rate. Species difference in LAI explained species difference in GPP. Species difference in LAI was explained by both leaf mass and thickness, indicating that a morphological trait played a critical role in determining carbon cycling at the whole stand level. Accurate representation of leaf thickness in models may account for a majority of species differences in C fluxes. The current (2007 – 2010) data indicate that species

had accumulated LAI since a previous study (2003 – 2005) but declined in NPP, suggesting that increased LAI alleviated the decline in GPP or NPP and that the LAI – GPP relationship is empirical. Species will likely further decline in NPP or increase in turnover rate, and current partitioning fractions and turnover rates may not hold across time.

The decline in NPP can only be caused by a decline in GPP or an increase in respiration, and I examine the role of leaf carbohydrates in regulating photosynthesis and respiration to balance carbon budget at the whole tree level in Chapter 4. Trees are thought to balance their carbon budget with feedback from carbohydrate storage. Trees cannot increase storage size forever as NPP decline, and large storage size may decrease photosynthesis or increase respiration to remove excess carbohydrates. Some form of this feedback regulation is used in modeling plant growth and ecosystem biogeochemistry, to prevent carbohydrate storage from becoming too large. However, the storage pool serves as a source of carbohydrates during night and seasonal dormancy, and the pool size likely fluctuates before triggering any regulation. The changes in storage pool required to trigger the feedback regulation of photosynthesis or respiration remain unquantified, and thus their generality and importance in ecosystem processes are still unknown.

I tested the carbohydrate regulation of photosynthesis and respiration using girdling. Girdling severs phloem to stop carbohydrate export while leaving xylem intact for photosynthesis to continue, to accumulate carbohydrate in leaves simulating carbon imbalance. On the branches in the upper canopy, I varied girdling intensity by girdling in quarter increments and surrounded a target branch with fully girdled ones, to create a gradient in leaf carbohydrate content. Light saturated photosynthesis rate was tracked *in situ*, and foliar respiration rate and

leaf carbohydrate contents were measured after destructive harvest at the end of the treatment duration.

Girdling intensity had no effect on leaf carbohydrates, respiration, and reduced photosynthesis only under full girdling suggesting that leaf carbohydrate content is tightly regulated and thus decoupled from whole plant carbon balance. Girdling intensity did not vary leaf carbohydrate content and respiration in any species. Photosynthesis declined only under full girdling in three of four species, and one species did not respond at all. Because girdling also stops the export of hormones and reactive oxygen series, girdling may induce physiological changes unrelated to carbohydrate accumulation and may not be an effective method to study carbohydrate feedback in leaves. Leaf carbohydrate content may be regulated through phloem transport and sink activity (growth and storage elsewhere) in addiction to photosynthesis. Leaf carbohydrate content may be far removed or even decoupled from whole plant carbon balance, and may not play a role in regulating changes of GPP and respiration as trees age.

This dissertation shows that models of ecosystem biogeochemistry may represent carbon budgets of different species in monodominant stands reasonably well with LAI and without species-specific partitioning fractions. Despite morphological and physiological differences regulating carbon fluxes, species partitioned remarkably similar fraction of GPP to components and to respiration. Tree species in this environment may be constrained in partitioning of GPP. However, model predictions of carbon budgets based on LAI will not capture ontogenetic changes. Leaf carbohydrates may be decoupled from ontogenetic changes in whole tree carbon balance.

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Chapter 1

Introduction

In the rain forest, no niche lies unused. No emptiness goes unfilled. No gasp of sunlight goes untrapped. In a million vest pockets, a million life forms quietly tick. No other place on earth feels so lush. Sometimes we picture it as an echo of the original Garden of Eden – a realm ancient, serene, and fertile, where pythons slither and jaguars lope. But it is mainly a world of cunning and savage trees. Truant will not survive. The meek inherit nothing. Light is a thick yellow vitamin they would kill for, and they do. One of the first truths one learns in the rain forest is that there is nothing fainthearted or wimpy about plants.

– Diane Ackerman, The Rarest of the Rare: Vanishing Animals, Timeless Worlds

Twelve tree species were planted in monoculture plots in 1988 for an experiment in a tropical rain forest of Costa Rica (Fisher 1995), and four have survived and grown into distinctly different stands varying 6800 – 12000 gC m^{-1} in biomass (Russell et al. 2010). The four species produce biomass at the rate of 1100 – 1600 gC m^{-2} $year^{-1}$, and aboveground litter at 430 – 550 gC m^{-2} $year^{-1}$ (Russell et al 2010). These carbon cycling rates are twice as high as in average temperate forests (Luyssaert 2007). The magnitude of biomass production variation alone, ~500 gC m^{-2} $year^{-1}$, is greater than and the absolute vales of a typical boreal forests (Luyssaert 2007).

Ecologists have long sought to explain how species affect ecosystem processes, and increasingly with urgency as climate and land use change alter species composition and diversity (Chapin et al. 2000). In the tropics, ~50 million hectares of primary forests diverse in species have been converted into plantations mostly in monodominant stands (FAO 2011). Assessing and predicting how such changes influence ecosystem biogeochemistry requires us to understand

how different species affect forest carbon cycling. Species generally affect ecosystem processes strongly such that shifts in species composition or diversity significantly alter ecosystem functioning, but predicting the process, direction, and magnitude remains difficult (Chapin et al. 2012).

Forests in general and tropical forests in particular store great amounts of carbon as biomass, an ecosystem process that is increasingly important to assess and predict. Loss of biomass and soil carbon from deforestation is the second largest source of anthropogenic CO_2 emission (IPCC 2007), and programs such as United Nations Collaborative Programme on Reducing Emissions from Deforestation and Forest Degradation in Developing Countries (UN-REDD+) unfold to place financial value in forest carbon storage to prevent further deforestation. Such programs gain credibility and participation required to successfully conserve tropical forests if assessments and predictions of forest carbon storage are reliable.

Reliable assessment and prediction involves models of ecosystem biogeochemistry, but difficulty of measurement prevents model development, parameterization, and validation, especially for carbon fluxes that directly determine forest biomass. Measurements of carbon fluxes that regulate biomass in tropical forests are challenged by the forests' size, complexity, and heterogeneity. Measurements made on small scale on few individual trees must be scaled to the whole forest across time and space, and across variations in individuals, species, and soils. This challenge is severe for respiration that directly regulates forest biomass. Early studies suggested that the rate of net primary production (NPP) is not as high as expected from the rates of gross primary production (GPP) because much of GPP may be lost through high respiration (Kira 1978). However, high respiration and the role it plays in regulating tropical forest biomass remain questions (Malhi 2012), partly because respiration is difficult to measure at stand level

(Sprugel et al. 1995) but must be placed within a complete carbon budget to understand the underlying mechanisms that control forest carbon cycling (Ryan et al. 2004).

The objective of this dissertation is to construct a complete carbon budget to examine how different species affect carbon stored in forest plant biomass. I take advantage of the unique experimental stand in Costa Rica to ask three questions:

1. What scalars and sampling schemes best capture the variation in respiration rates among species and canopy layers within a stand?
2. What difference in species carbon cycling causes the difference in forest biomass carbon storage?
3. What is the role of carbohydrates in balancing carbon budget at the whole tree level?

I use leaf level measurements to examine the variation in foliar respiration and wood CO_2 efflux among species and canopy layers in Chapter 2. I scale the measurements to the stand level and place respiration within a complete and detailed carbon budget to examine how species affect forest biomass carbon storage in Chapter 3. I use experimental girdling to examine what role carbohydrates play in feedback regulation of photosynthesis and respiration to balance whole tree carbon budget in Chapter 4. I synthesize the results in Chapter 5.

Chapter 2

Variation in foliar respiration and wood CO_2 efflux rates among species and canopy layers in a wet tropical forest

Introduction

Autotrophic respiration consumes 30–70% of the carbon fixed in photosynthesis to supply energy for metabolism and growth (Charles-Edwards 1981, Ryan et al. 1997, Chambers et al. 2004, DeLucia et al. 2007, Litton et al. 2007, Luyssaert et al. 2007). It remains uncertain however whether autotrophic respiration will consume a greater fraction of photosynthesis at the expense of growth as forests respond to changes in temperature, precipitation, and species composition. Because of this uncertainty, predictions of future carbon balance remain difficult, particularly for tropical forests (Chambers et al. 2004, Malhi et al. 2011, Malhi 2012): will tropical forests remain a carbon sink (Fan et al. 1990, Grace et al. 1995, Cao and Woodward 1998, Phillips et al. 1998, Malhi et al. 1998, Loescher et al. 2003) or become a carbon source as temperatures increase (Kindermann et al. 1996, Braswell et al. 1997, Tian et al. 1998, White et al. 2000, Cox et al. 2000, Cramer et al. 2001, Clark et al. 2003)?

Crucial to prediction are fundamental questions about autotrophic respiration that can only be answered by measuring autotrophic respiration and placing it in a whole forest carbon budget. Is autotrophic respiration a constant fraction of photosynthesis (Waring et al. 1998, DeLucia et al. 2007, Litton et al. 2007)? Why then do black spruce (Picea mariana (Mill.) Britton, Sterns & Poggenburg (Ryan et al. 1997), and wet primary tropical forests (Chambers et al. 2004, Luyssaert et al. 2007, Malhi 2012) consume ~ 70% of photosynthesis compared to

assumed 50% (Waring et al. 1998, Litton et al. 2007)? Will the fraction of respiration to photosynthesis change as temperatures increase (Ryan 1991a, Atkin 2003, Atkin et al. 2005)? We will only answer these questions by placing autotrophic respiration in the context of a complete carbon balance (Ryan et al. 2004), by measuring the autotrophic respiration for studies where we have all of the other components of the carbon budget (Litton et al. 2007), and developing robust sampling and extrapolation protocols (Sprugel et al. 1995, Cavaleri et al. 2006, 2008).

Foliar respiration and wood CO_2 efflux rates can vary over 20x within a forest (Sprugel et al 1995), so schemes to sample, understand, and extrapolate respiration rates are critically important to producing estimates of aboveground foliar dark respiration and wood CO_2 efflux. Because respiration supports biochemical and physiological processes (Thornley and Cannell 2000, Amthor 2000), foliar respiration and wood CO_2 efflux rates per unit surface area generally vary with mass (Ryan 1990, Sprugel 1990, Wright et al. 2004), N content (Penning de Vries 1975, Field and Mooney 1986, Evans 1989, Ryan 1995, Reich et al. 2006), growth rate (Williams et al. 1987, 1989), and chemical composition of new tissue (Penning de Vries et al. 1974, Chapin 1989, Poorter and Bergkotte 1992).

Knowledge of these sources of variation does not yield simple schemes to extrapolate from the chamber to the ecosystem. The relationships between respiration and these predictor variables change within a stand, throughout the year, and with ontogeny and across time (Ryan 1990, Sprugel et al. 1995, Ryan et al. 2009). Other less known sources of variation, such as phloem transport, waste respiration, and translocation of CO_2 from elsewhere (Thornley and Cannell 2000, Amthor 2000, Teskey et al. 2008) also may alter the relationship between CO_2 efflux and scaling variables. The few extant studies in tropical forests showed that species

differences in wood CO_2 efflux were related to wood size (Yoda et al. 1965, Yoda 1967) but also to growth rate (Ryan et al. 1994, 2009, Robertson et al. 2010), and functional groups differed in foliar respiration and wood CO_2 efflux independently of mass or N content (Cavaleri et al. 2006, 2008).

In this study, we focus on determining how rates of foliar dark respiration and wood CO_2 efflux vary among species and canopy layers so we can understand where to best sample rates, what causes the variation in rates, and how to extrapolate those fluxes to produce unbiased estimates of aboveground autotrophic respiration. We hypothesized that foliar respiration and wood CO_2 efflux rates would vary among species and canopy layers because (1) for foliar respiration, leaves of different species and in different canopy layers have very different cellular activity related to differences in protein (N concentration), photosynthetic activity, and mass; (2) for foliar respiration, short-term temperature response will vary with respiration rate as it did for a primary forest (Cavaleri et al. 2008), (3) for wood CO_2 efflux, growth process dominate over maintenance of biomass and stems and branches of different species and canopy layers grow at different rates (Ryan et al. 1994). This variability with species and canopy layers thus (4) produce biased estimates of aboveground respiration flux if measurements are taken at a single point.

Materials and methods

Study site

We conducted this study at La Selva Biological Station, in the Atlantic lowlands of Costa Rica (10°26′N, 83°59′N). La Selva's climate is classified as Tropical Wet Forest in the Holdridge system (McDade 1994), with annual mean rainfall and temperature of 4000 mm and

26 °C. For 2009 and 2010, when measurements were taken, rainfall was 4500 mm and the temperature averaged 25°C. Soils at the site are acidic, highly leached, high in organic matter content, and classified as Mixed Haplic Haploperox (Kleber et al. 2007).

The site was cleared of primary forest in 1955, converted to pasture in 1956, and then continually grazed until 1987. In 1988, an experiment was established with eleven tree species and an abandoned pasture control, replicated over four blocks in a randomized complete block design (Fisher 1995). Plots were 50 x 50 m (0.25 ha), with a single-tree species planted in each plot except for the unplanted control. Understory plants were cleared for the first 3 years until the trees were established, but then allowed to regenerate naturally. By 2009, only four species had enough surviving trees for plot-level measurements, and these were the subjects of this study.

The four species studied were *Hieronyma alchorneoides* Allemao (HIAL), *Pentaclethra macroloba* (Willd.) Kunth. (PEMA), *Virola koschnyi* Warb. (VIKO), and *Vochysia guatemalensis* Donn. Sm. (VOGU). All are native to the surrounding primary forest, and *Pentaclethra* is the dominant species of canopy trees at La Selva, and the only N-fixing species of the four. The stands had aboveground biomass 5410 – 9870 gC m^{-2} comparable to 7200 gC m^{-2} of surrounding primary forest, and LAI of 5.2 – 6.5 similar to 6.0 in the surrounding forest. The planted trees dominated each species stand with them consisting on average 88% of aboveground biomass. Stand characteristics are in Table 2.1, and further details on the site and its history can be found in Fisher (1995) and Russell et al. (2010). We conducted this study as part of a larger project, ECOS (http://www.nrem.iastate.edu/ECOS/home), examining tree species effects on ecosystem processes (Raich et al. 2007, 2009, Russell et al. 2010, Russell and Raich 2012).

Sampling

Canopy at the site consisted of two distinct layers, overstory in the upper 15-35 m and understory in the lower 0-15 m. The overstory was occupied by foliage of the planted trees, but the understory included the planted trees, and other trees, forbs, grasses, and ferns from the surrounding forest. Species composition in the understory differed among the overstory species (Table 2.1).

Foliar respiration for the overstory trees was measured on ~20 branches (~16 near the top of the canopy and ~4 from lower in the canopy) from two to four individuals per plot using a 30 m scaffolding tower (Upright Inc., Dublin, Ireland) for access. Measurements were taken on one plot per species in 2009 during the wetter summer months and on a different plot in 2010 during the less wet winter and spring months. In the understory, 10–15 individuals were sampled per plot in all four blocks (two in summer 2009 and two in winter 2010).

Wood CO_2 efflux of the overstory trees was measured at 1.4 m height on 15 trees per plot for all four plots per species in summer 2009 and winter 2010. Wood CO_2 efflux was also measured from the scaffold tower on one to three stems at 1.8 m intervals above 1.4 m and on 10–15 branches in upper canopy in two of the four blocks (one in summer 2009 and one in winter 2010). Wood CO_2 efflux for woody understory plants were made on all four plots for the same sampling periods (0-6 trees per plot), but the sample was limited as only a few of the understory plants were large enough for measurement.

Ecophysiological measurements

Foliar respiration was measured on one to five leaves in a 1580 ml volume polycarbonate chamber on detached foliage at night. Branches were cut underwater in the afternoon, placed in

a floral tube with water without exposing the cut surface to air, and and CO_2 efflux measured at the lab in the dark from 20:00 and 02:00 (after > 2 hours of darkness). Attached and detached foliage had similar respiration rate in a previous study at La Selva (Cavaleri et al. 2008) and in several studies at other locations (Mitchell et al. 1999, Turnbull et al. 2005). Immediately after measurement, the foliage was measured for leaf area with a leaf area meter (LI-3100, LI-COR, Inc.). The foliage was then dried for 48 hours at 65 °C and measured for leaf dry mass, and ground to a powder with a Wiley mill and measured for leaf N with a C N analyzer (TruSpec CN, LECO, Inc., St. Joseph, Michigan, USA).

Wood CO_2 efflux was measured using clear polycarbonate chambers on intact stems or branches between 07:00 and 17:00. Because the chambers were clear, they allowed bark photosynthesis and our measurement was thus a sum of wood tissue respiration (+ flux), bark photosynthesis (– flux), and CO_2 dissolved in the xylem sap (Cernusak and Marshall 2000, McGuire and Teskey 2004, Bowman et al. 2005, Teskey et al. 2008). Wood surface area was estimated as the area inside the gasket creating the seal between the chamber and the wood. Wood volume sampled by the chamber was estimated by multiplying the volume of the underlying wood cylinder (height equal to chamber height) by the ratio of the surface area inside the chamber gasket to the surface area of the wood cylinder (generating a wedge-shaped slice). The volume was then converted to mass using species specific wood density.

All chambers had neoprene gaskets to form a seal and a small fan to mix the air inside but varied in size and shape. Chamber volume ranged from 16–47 mL for wood CO_2 efflux (enclosed wood surface area of 3–28 cm^2); different sized chambers were used to ensure fit on wood with different diameters. Chamber seals were checked with a flow meter, and wood and foliage surface temperature were measured with an infrared thermometer (OS423-LS, OMEGA

Engineering, Stamford, Connecticut, USA). CO_2 efflux was measured with an open-system LCA-3 (Analytical Development Company, Hoddeson, UK) infrared gas analyzer (IRGA) for 2009 measurements, or a lab-built closed-system instrument with Li-820 (Li-COR, Inc., Lincoln, Nebraska, USA) and CR10X data logger (Campbell Scientific, Logan, Utah, USA) for 2010 measurements. The open-system IRGA drew ambient air from a 19 L mixing container to maintain stable concentration of reference CO_2 during measurements, and the airflow rates through the chamber ranged between 200–340 μmol s^{-1} for measurements. Both instruments were regularly calibrated with a CO_2 standard.

Before detaching the branches to measure foliar respiration at night, the intact leaves were measured for photosynthesis using an open-system portable IRGA (LI-6400, LI-COR, Inc., Lincoln, Nebraska, USA). The measurements were taken on 5 fully expanded leaves, on the same branches sampled for respiration for foliage in the overstory but on different branches for foliage in the understory. Each leaf was measured once a day for 2–9 days for canopy foliage, and once for understory foliage. Photosynthesis was measured under a reference CO_2 of 390 μmol mol^{-1}; at an air flow rate of 500 μmol s^{-1}; and with a saturating level of photosynthetic photon flux density (2000 μmol m^{-2} s^{-1} for leaves at the canopy top and 1500 μmol m^{-2} s^{-1} for lower canopy and understory leaves) after the readings stabilized. Temperature and humidity were not controlled, and ranged 24.5–39°C and 0.5–2 kPa in vapor pressure deficit. Values reported are averages for each branch.

A subset of the foliage was also measured at night for the temperature response of foliar respiration by estimating Q_{10} from a temperature response curve. Of the foliage sampled for foliar respiration measurement, four branches from the overstory trees and three individuals of the understory plants from one block per species were measured for temperature response.

Temperature response was quantified with Q_{10}, the change in respiration rate with 10°C change in temperature, for foliar respiration measured 15, 20, 25, 30, and 35 °C in a temperature-controlled cuvette (Hubbard et al. 1995) and the closed-system IRGA described above. Foliar respiration was standardized to 25°C using Q_{10} specific to each of the four species and two canopy layers. Wood CO_2 efflux was also standardized to 25°C, using a Q_{10} of 2, because the wood of two tree species in the surrounding forest had Q_{10} of 2.1 and 2.2 (Ryan et al. 1994), and trees in tropical rainforests in Cameroon and Brazil had 1.8 and 1.6 (Meir and Grace 2002).

Data analysis

Foliar respiration and wood CO_2 efflux rates, standardized to 25ºC, and Q_{10} values were compared among species and between canopy layers using both a linear model ANOVA and a liner mixed effects model ANOVA at an experiment-wise $\alpha = 0.05$. A mixed effects model was used because the overstory trees and understory plants were sampled in different blocks in 2009 than in 2010 with unequal block replicates, and block nested within year was included as a random intercept. The model's independent variables were linear combinations of species and canopy layers, and the dependent variable was foliar respiration, the natural log of wood CO_2 efflux, or Q_{10}. Tukey-Kramer multiple comparison procedure was used to account for unbalanced sample sizes. The procedure yielded the same result for both fixed-effects only and mixed-effects models, and we present the results of the simpler fixed-effects only model.

Models of foliar respiration and wood CO_2 efflux and various predictor variables were constructed using both fixed- and mixed-effects models. Both model types produced comparable significance and R^2 values for the same candidate variable combinations. For simplicity, we report the result of fixed-effects model, but the reported R^2 values may slightly overestimate the

true value. Foliar respiration rate was modeled using three predictor variables: canopy layer (categorical; overstory or understory), species of planted trees (categorical; *Hieronyma*, *Pentaclethra*, *Virola*, or *Vochysia*), and a continuous variable of either LMA (g m^{-2}), leaf N content (g m^{-2}), or photosynthetic capacity (μmol m^{-2} s^{-1}). Because all understory samples were taken at ground level (at the same height), we examined the contribution of height of the foliage using height as the continuous variable replacing canopy layer. The predictor variables and their interactions were sequentially omitted from the full model (with all three variables and their interactions) and examined for their significance in predicting foliar respiration.

Wood CO_2 efflux was modeled with three predictor variables: canopy layer, species, and a continuous variable of wood surface area to mass ratio enclosed within the measurement chamber. The ratio was used to determine whether wood CO_2 efflux was related to surface area or mass (Levy and Jarvis 1998). The ratio was defined as mass per surface area for modeling area-based rate, and as surface area per mass for mass-based rate: if wood CO_2 efflux per unit surface area is related to mass per surface area, wood CO_2 efflux is related to mass, and if wood CO_2 efflux per unit mass is related to surface area per mass, wood CO_2 efflux is related to surface area (Levy and Jarvis 1998). We separately examined the contribution of wood height as the continuous variable. All analysis were done in R (R Core Team 2014), with lme4 (Bates et al. 2012), multcomp (Hothorn et al. 2008), and MASS (Venables and Ripley 2002) packages, and plotted with ggplot2 package (Wickham 2009).

Results

Foliar respiration rates varied more within a species than among species, and were higher for the overstory (Fig. 2.1a, $P < 0.01$). Average foliar respiration in the overstory was about

three times that in the understory: 0.78 vs. 0.27 μmol m^{-2} s^{-1} for *Hieronyma*, 0.70 vs. 0.19 μmol m^{-2} s^{-1} for *Pentaclethra*, 0.66 vs. 0.28 μmol m^{-2} s^{-1} for *Virola*, and 0.80 vs. 0.26 μmol m^{-2} s^{-1} for *Vochysia*. Pair-wise comparisons of foliar respiration rates within species and canopy layers (because of a significant interaction in the main effects, $P < 0.01$), showed higher rates for *Hieronyma* and *Vochysia* than for *Virola*, while *Pentaclethra* did not differ from others. Foliar respiration did not differ among species for the understory.

Wood CO_2 efflux was also as much as two times higher in the overstory than in the understory (Fig. 2.1b, $P < 0.01$), with means of 1.6 vs. 0.88 μmol m^{-2} s^{-1} for *Hieronyma*, 1.4 vs. 0.90 μmol m^{-2} s^{-1} for *Pentaclethra*, 0.97 vs. 0.87 μmol m^{-2} s^{-1} for *Virola*, and 1.0 vs. 0.60 μmol m^{-2} s^{-1} for *Vochysia*. Pair wise comparisons of CO_2 efflux rates within species and canopy layers (because of a significant interaction in the main effects, $P < 0.03$) showed that the overstory CO_2 efflux rate differed from the understory for all species except *Virola*. Overstory wood CO_2 efflux rates were higher in *Hieronyma* and *Pentaclethra* than in *Virola* and *Vochysia*, and rates for understory wood were similar among species.

Hypothesis 1: *The variation in foliar respiration among species and canopy layers is related to mass, N content, and photosynthetic capacity*

Foliar respiration rate varied with LMA, N content, and photosynthetic capacity strongly across canopy layers but only marginally within (Fig. 2.2). The variation in foliar respiration was explained well by the analysis of covariance models with only the single factor of LMA, N content, or photosynthetic capacity (Fig. 2.2, thin lines; Table 2.2). However, canopy layer explained more variability than those leaf traits, and with canopy layer in the model, model performance improved only slightly by adding LMA, N content, or photosynthetic capacity

(Table 2.2). The relationship between foliar respiration rate and LMA, N content, or photosynthetic capacity also had much lower slope within a canopy layer than across (Fig 2.2, thick lines).

Height related changes in LMA, N content, and photosynthetic capacity explained most of the variation in foliar respiration rate across canopy layers. We calculated foliar respiration rate per unit mass, per unit N, and per unit photosynthetic capacity to account for height related changes in LMA, N content, and photosynthetic capacity (Fig. 2.3). For every meter in height, the respiration rates increased only slightly with height for both mass based rate ($P < 0.01$, $R^2 = 0.07$, $y = 0.070x + 5.4$ nmol g^{-1} s^{-1}) and N based rate ($P < 0.01$, $R^2 = 0.11$, $y = 0.0038x + 0.25$ μmol g^{-1} N s^{-1}), while photosynthetic capacity based rate did not change ($P = 0.73$, $y = 0.066$). Height alone explained area based respiration rate well (Fig. 2.3, $P < 0.01$, $R^2 = 0.68$, $y = 0.021x + 0.23$ μmol m^{-2} s^{-1}), and model R^2 slightly but always increased when canopy layer was replaced with height for area based respiration rate (Table 2.2).

Photosynthetic capacity best explained the variation among species in foliar respiration (Table 2.2). In the model analysis, adding species failed to improve the model fit if the models already contained canopy layer and photosynthetic capacity ($P = 0.08$) but improved fit if the models contained canopy layer and LMA or N content (Table 2.2). Surprisingly, N content explained the least amount of the variation among species, primarily because *Pentaclethra*, the N-fixing species, had much lower foliar respiration rate per g N ($P < 0.01$). Its foliage on average contained 42 % more N than other leaves of overstory species, but had similar foliar respiration rate per unit area (Fig. 2.1).

Hypothesis 2: *Short-term temperature response of foliar respiration will vary with respiration rate*

Instantaneous temperature response (Q_{10}) of foliar respiration did not vary with respiration rate ($P = 0.80$). The values of Q_{10} were similar for all species and canopy layers, except for *Pentaclethra* foliage in the overstory. The mean values of Q_{10} were, *Hieronyma* = 1.6, *Pentaclethra* = 2.6, *Virola* = 1.6, *Vochysia* = 1.8 for overstory; and 1.9, 1.7, 1.5, and 1.4 respectively in the understory.

Hypothesis 3: *The variation in wood CO_2 efflux is related to growth process (surface area) not maintenance (biomass)*

Surface area better explained the variability in wood CO_2 efflux than did mass (Fig. 2.4), but both were significant ($P < 0.01$; Fig. 2.4). The greater R^2 for the relationship indicating surface area (0.49 vs. 0.31) suggests that growth processes contribute more to efflux than does the maintenance of woody tissues (Levy and Jarvis 1998). We used rates based on surface area for further analysis to account for the relationship between growth processes and wood CO_2 efflux, and the variation in the efflux rate per surface area was only marginally related to canopy layer ($R^2 = 0.07$, $P < 0.01$) species ($R^2 = 0.05$, $P < 0.01$), or the two combined ($R^2 = 0.14$, $P < 0.01$). Unlike foliar respiration, wood CO_2 efflux per unit surface area slightly decreased with height ($P < 0.01$, $R^2 = 0.01$, slope = 0.99).

Hypothesis 4: *Variability in foliage respiration and wood CO_2 efflux with species and canopy position will bias ecosystem estimates if measurements are taken at a single point*

Because foliar respiration rate at the leaf level was higher in the overstory than in the understory, measurements taken only in either one would produce biased estimates of foliar respiration for the ecosystem (Fig. 2.5). As an example, consider a forest with an LAI of six, four in the overstory and two in the understory, with the mean foliar respiration rates of this study, 0.25 μmol m^{-2} s^{-1} in the understory and 0.74 μmol m^{-2} s^{-1} in the overstory. This would yield an ecosystem estimate of 3.5 μmol m^{-2} ground s^{-1}, compared with 4.4 μmol m^{-2} s^{-1} if just the overstory was sampled, or 1.5 μmol m^{-2} s^{-1} if just the understory were sampled. A sampling scheme focused on fewer samples would also likely bias the ecosystem estimate, given the large within-species variability.

Unlike foliar respiration, variation in wood CO_2 efflux was not well explained by the canopy layer when differences in the ratio of wood surface area to mass were accounted for, and extrapolations based on surface area are unlikely to produce bias estimates of wood CO_2 efflux. Wood surface area is rarely measured however, and most often wood mass is used to extrapolate chamber measurements to the stand. Mass based measurements of wood CO_2 efflux increases for wood as diameter decreases, and because smaller diameter branches and stems have higher efflux, extrapolation using rates per mass and wood biomass will underestimate stand wood CO_2 efflux (Fig. 2.6). With a wood mass of 20,000 g m^{-2}, and 1/3 of that with diameter < 10 cm, wood CO_2 efflux for the stand would be 1.2 μmol m^{-2} s^{-1} (using the mean efflux for wood with diameter > 10 cm in this study of 0.06 nmol CO_2 g^{-1} s^{-1}). Accounting for higher mean wood CO_2 efflux rate for small wood (0.22 nmol CO_2 g^{-1} s^{-1}) yielded a stand-level estimate of 2.3 μmol m^{-2} s^{-1}. The larger the fraction of large wood in the forest, the lower the bias would be.

The temperature response of foliar respiration differed among species, but the difference produced only a minor bias compared with a single temperature response because temperature varies very little in this forest (Fig. 2.7). Agren and Axelsson (1980)(Agren and Axelsson 1980) derived a formula to calculate the effect on respiration sums from variation in daily and annual temperature relative to constant temperature, and we calculated how this effect changes with Q_{10}. The difference in the lowest to highest Q_{10} we observed (1.4 to 2.6) increased the annual CO_2 efflux estimated from mean temperature by 1.04 to 1.16 with an daily and annual amplitude of 2°C and 8 °C, within a range of historic values (McDade 1994). Annual fluxes could be estimated with low bias from mean annual temperature and common Q_{10}.

Discussion

Foliar respiration was only weakly related to mass, N, and photosynthetic rate within the overstory and understory

Leaf N content, photosynthetic capacity, or LMA were of minor importance in explaining the variability of foliar respiration within a canopy layer (Fig. 2.2). These weak relationships suggest that predictions of the worldwide leaf economic spectrum (Wright et al. 2004, Shipley et al. 2006) may not be appropriate for explaining differences within a canopy. This is not to say that the maintenance of dry mass and proteins, especially those associated with photosynthesis, is unrelated to foliar respiration. Across canopy layers, the variability of foliar respiration did follow the predictions of the spectrum as we hypothesized (Fig. 2.2), consistent with existing data in the neotropics (Oberbauer and Strain 1986, Meir et al. 2001, Domingues et al. 2005, Cavaleri et al. 2008, Ryan et al. 2009, Metcalfe et al. 2010). However, the weak relationships within a canopy layer suggests that foliar respiration in these species includes components

unrelated to maintenance respiration, such as overflow respiration to decrease excess carbohydrates, respiration to fuel phloem loading, and respiration for ion gradient maintenance (Penning de Vries 1975, Bouma et al. 1995, Cannell and Thornley 2000, Amthor 2000). Presence of these components is supported by existing data as well. In the surrounding primary forest, foliar respiration increased with height even for mass based and N based rates accounting for height related changes in LMA and N content (~0.08 nmol g^{-1} s^{-1} and ~0.004 μmol g^{-1} N s^{-1} for every meter; Cavaleri et al 2008). Mass based foliar respiration rate increased 43% under imposed drought from rainfall exclusion (Metcalfe et al. 2010), and area based foliar respiration rate increased 60 – 250% during the dry season in the Amazon (Miranda et al. 2005). Hourly rates of foliar respiration varied diurnally between 0.34 – 0.74 μmol m^{-2} s^{-1} without a clear pattern (Chambers et al. 2004). Determining how foliar respiration reflects components unrelated to maintenance respiration will not only improve the accuracy of stand level estimates and account for special and temporal variations, but also of prediction of foliar respiration response under climate change.

Wood CO_2 efflux rates and patterns between plantation secondary forests and primary forests

Wood CO_2 efflux rates and their variability were generally similar to the studies in the primary forests. The rates we observed are consistent with those measured at the ground level on two different species of the primary forest in an earlier study (~1.0 μmol m^{-2} s^{-1}, Ryan et al 1994), on wood > 10 cm in diameter in lowland Amazon forest (~1.1 μmol m^{-2} s^{-1}, Robertson et al 2010) and in *Eucalyptus* plantations in Hawaii and Brazil (0.06 nmol g^{-1} s^{-1} this study; ~0.06 nmol g^{-1} s^{-1}, Ryan et al 2009). We found that wood CO_2 vary considerably from 0.09 to 3.9 μmol m^{-2} s^{-1}, and the variation was mostly unrelated to species and canopy layer. The variability

was also large in primary forests (in μmol m^{-2} s^{-1}: 0.1 – 5.2, Meir & Grace 2002, 0.03 – 3.6, Chambers et al 2004, ~0 – 4.5, Cavaleri et al 2006, ~0 – 4.5, Robertson et al 2010), but smaller for two species of trees in the surrounding primary forest (0.3 – 2.1, Ryan et al. 1994) perhaps due to smaller sample size. The variability was related to both growth and maintenance processes, and the relationships were fairly similar among species and canopy layers, also consistent with other studies (Ryan et al. 1994, Meir & Grace 2002, Robertson et al 2010). Respiratory cost of growth and maintenance may be well conserved within a functional group in tropical forests.

An exception was the higher wood CO_2 efflux from large diameter wood and the lack of increase in wood CO_2 efflux with height compared to the observations in the primary forest (Cavaleri et al. 2006). Wood CO_2 efflux for larger diameter wood averaged 1.2 μmol m^{-2} s^{-1} at ground level and slightly decreased with height in the secondary forest, compared to ~0.8 at the ground level increasing to ~1.7 μmol m^{-2} s^{-1} in the upper canopy of the primary forest (Cavaleri et al. 2006). The difference may be related to some combination of greater proportion of large size classes, higher growth rate for branches in upper canopy (Ryan et al. 1994, 2009), or composition of species or functional groups (Cavaleri et al. 2006). These interpretations are complicated by limits to radial diffusion of CO_2 in wood. The interpretations assume that local processes alone cause the variation in chamber measurements, but wood tissue, especially cambium, limits radial diffusion of CO_2 and causes CO_2 from elsewhere in the stem or roots to dissolve in xylem and phloem streams and be transported to the site of measurement (McGuire and Teskey 2004, Spicer and Holbrook 2005, Teskey et al. 2008, Aubrey and Teskey 2009, Trumbore et al. 2012). The diffusion barrier itself may explain why wood CO_2 efflux was proportional to surface in this study.

Sampling to estimate annual aboveground autotrophic respiration in tropical forests

Our results suggest a vertical transect to reduce bias in estimates of annual aboveground autotrophic respiration for a wet tropical forest. Sampling within a canopy layer or at any position within the canopy fails to measure the substantial variation in foliar respiration within the upper and lower canopy and with height (Fig. 2.2), primarily driven by the differences in respiration rates among sampling positions and not the distribution of LAI (Fig. 2.5). Cavaleri et al. (2008) showed that when full vertical transect is taken, overall mean respiration rate and LAI produces similar estimates compared to more complex models with height structure. Taken together, they suggest that simple extrapolation models with mean respiration rate and stand LAI produces unbiased estimates of ecosystem foliar respiration as long as the vertical transect is made to capture the variability in respiration rate along height. Similarly, unbiased estimates of ecosystem wood CO_2 efflux may require a vertical transect, although our results suggests that stand level estimates may be made with little bias if enough small diameter wood can be sampled at the ground level, as wood size was the primary cause of the variation in wood CO_2 efflux. Our finding contradicted the observations in the primary forest, and thus vertical transect should be sampled, if only to test whether wood CO_2 efflux changes with height.

Though ecosystem respiration may be uniquely aseasonal in wet tropical forests, the variation in foliar respiration and wood CO_2 efflux may be common in all forests. The forest in this study has some seasonality in air temperature and rainfall, with slightly less wet season in the spring months (McDade 1994). We measured wood CO_2 efflux during the wet season in 2009 and again during the less wet season in 2010 on the same individual, and all species had similar wood CO_2 efflux at 1.4 m height ($P = 0.26$), except *Hieronyma*. The difference in wood CO_2 efflux for *Hieronyma* was small, 1.9 μmol m^{-2} s^{-1} in 2009 and decreased to 1.6 μmol m^{-2} s^{-1}

in 2010 ($P < 0.01$). Wood CO_2 efflux at breast height showed no clear seasonality in a more detailed measurement in the primary forest (Cavaleri et al. 2006). The smaller temperature fluctuation also reduces the effect of Q_{10} on annual estimates of respiratory flux (Fig. 2.7). This evidence combined support the idea that plant respiration can be estimated and studied from measurements made once or twice a year (Yoda et al. 1965, Ryan et al. 1994, Chambers et al. 2004, Cavaleri et al. 2006). However, tropical forests may not be unique in the variation in foliar respiration and wood CO_2 efflux within and among canopy layers. Foliar respiration varies within a canopy in other forests likely as a function of light and height (Brooks et al. 1991, Bolstad et al. 1999, Griffin et al. 2001, Law et al. 2001, Rayment et al. 2002, Turnbull et al. 2003). Wood CO_2 efflux varies within canopy also, as a function of size and height (Lavigne 1988, Sprugel 1990, Edwards and Hanson 1996, Damesin et al. 2002, Ceschia et al. 2002).

Conclusions

Foliar respiration varied a little among species and more substantially between canopy layers. The variation was related to LMA, leaf N, and photosynthetic capacity across canopy layers, but only marginally within, perhaps because foliar respiration includes a substantial contribution from components unrelated to maintenance. Wood CO_2 efflux varied slightly among species and canopy layers and much more within, and the variation was related to the ratio of wood mass to surface area. Wood CO_2 efflux may depend on wood growth, but other factors such as diffusion and CO_2 dissolved in xylem stream may need to be accounted for. Temperature response was similar for all but *Pentaclethra*, and relatively constant temperature reduced the effect of different Q_{10} in producing a bias in annual estimates. Our results suggest that chamber measurements of foliar respiration can be extrapolated to the canopy with rates and

leaf area specific to each canopy layer or height class. Alternatively, if area-based rates are sampled throughout the canopy, mean respiration rate per unit mass derived by regressing the area-based rate against leaf mass per area can be extrapolated to the stand using total leaf mass. Mean wood CO_2 efflux rate per unit surface area, derived by regressing CO_2 efflux per unit mass against the ratio of surface area to mass, can be extrapolated to the stand using total woody tissue surface area. For these species and this forest, vertical sampling may yield more accurate estimates than would temporal sampling.

Tables

Table 2.1. Mean and maximum values of density, diameter at breast height and height, and leaf area index (LAI) for overstory (planted) trees and understory plants of each species' plot.

Planted species	Stem Density (trees ha^{-1})		Diameter (cm)		Height (m)		LAI	
	Mean	Max	Mean	Max	Mean	Max	Over-story	Under-story
Hieronyma alchorneoides (HIAL)	165	176	25	33	23	47	3.7	1.6
Pentaclethra macroloba (PEMA)	294	380	21	27	14	52	5.0	1.5
Virola koschnyi (VIKO)	226	284	24	30	20	43	3.7	2.6
Vochysia guatemalensis (VOGU)	255	280	31	40	24	62	3.1	3.1

Data taken in 2009 survey as part of a larger study (ECOS, http://www.nrem.iastate.edu/ECOS/home), and LAI taken from Russell et al (2010).

Table 2.2. Values of R^2 for models predicting foliar respiration rate (μmol CO_2 m^{-2} s^{-1}). All models had $P < 0.01$ except for the species only model (NS). Continuous variables were LMA (g m^{-2}), N content (g m^{-2}), photosynthetic capacity (μmol CO_2 m^{-2} s^{-1}), and height (of foliage sample taken, m), and categorical variables were canopy layer (overstory or understory) and species (*Hieronyma*, *Pentaclethra*, *Virola*, or *Vochysia*).

Predictor variables	R^2
Species	NS
Canopy layer	0.65
Species × Canopy layer	0.67
LMA	0.50
LMA × Species	0.58
LMA x Canopy layer	0.66
LMA × Canopy layer × Species	0.70
LMA x Height x Species	0.73
N content	0.45
N content × Species	0.63
N content × Canopy layer	0.66
N content × Canopy layer × Species	0.72
N content × Height × Species	0.75
Photosynthetic capacity	0.61
Photosynthetic capacity × Species	0.63
Photosynthetic capacity × Canopy layer	0.71

Photosynthetic capacity × Canopy layer × Species	0.72
Photosynthetic capacity × Height × Species	0.75

Figures

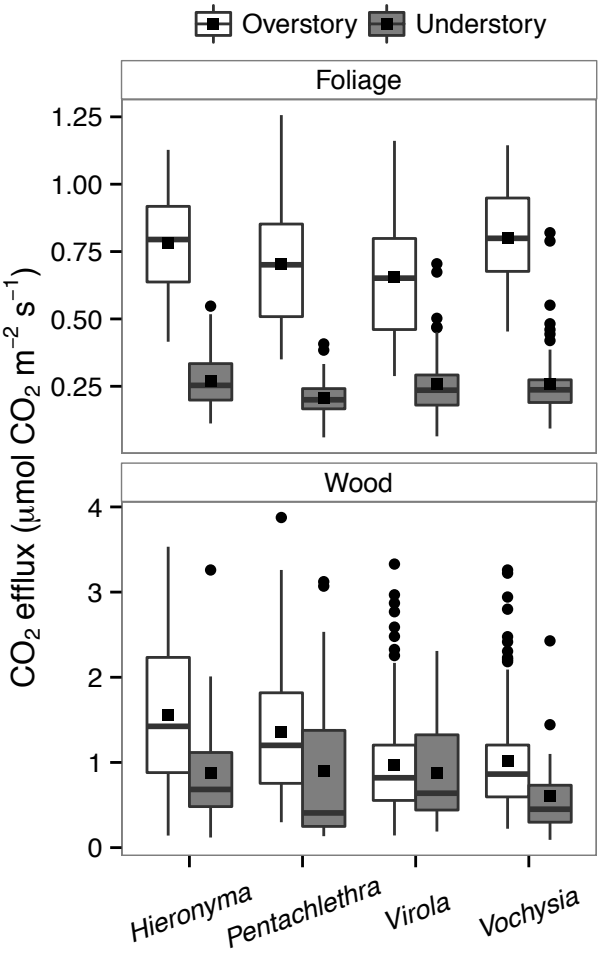

Figure 2.1. Box plots of per unit area foliar respiration and wood CO_2 efflux rates show that the rates vary more within than between species and are generally higher in the overstory. Open boxes represent overstory, and grey boxes represent understory. Solid squares show means. The data were normally distributed for foliage, but were not for wood with skew toward zero and long tail of larger flux values.

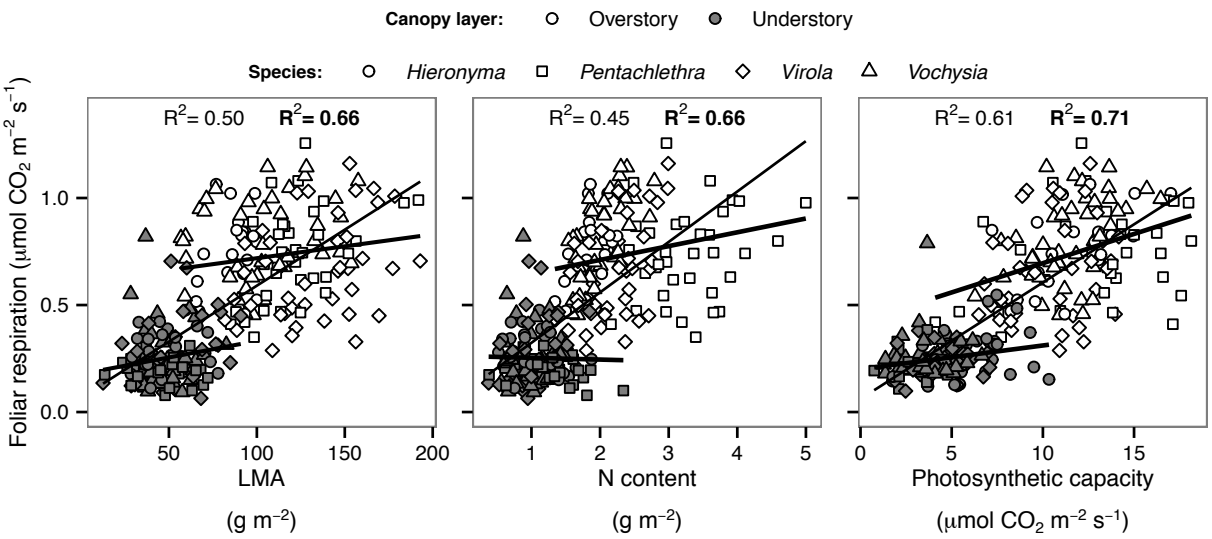

Figure 2.2. The variation in foliar respiration was related to LMA, N content, and photosynthetic capacity across canopy layers but only marginally within. Filled points represent overstory, and open points represent understory. Circles represent *Hieronyma*, squares *Pentaclethra*, diamonds *Virola*, and triangles *Vochysia*. Thin lines were drawn across canopy layers, using the models from Table 2.1, with only LMA (intercept = 0.067, slope = 0.0052), N content (0.088, 0.24), or photosynthetic capacity (0.061, 0.054). Thick lines were drawn using the models with canopy layer and LMA (intercept = 0.61, slope = 0.011 for overstory; 0.18, 0.011 for understory), N content (0.58, 0.064; 0.26, -0.01), or photosynthetic capacity (0.42, 0.027; 0.20, 0.011).

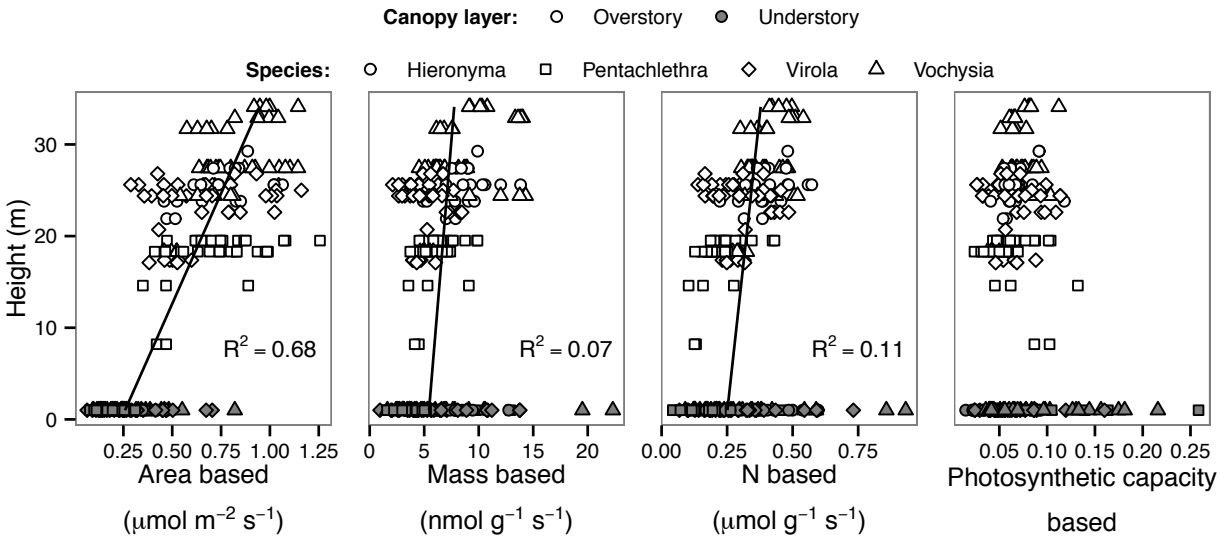

Figure 2.3. Relationship between height and foliar respiration calculated as leaf area, mass, N, and photosynthetic capacity based rates show that the increase in foliar respiration with height is mostly explained by incrases in LMA, N content, and photosynthetic capacity. Filled points represent overstory, and open points represent understory. Circles represent *Hieronyma*, squares *Pentaclethra*, diamonds *Virola*, and triangles *Vochysia*. See text for regression line equations.

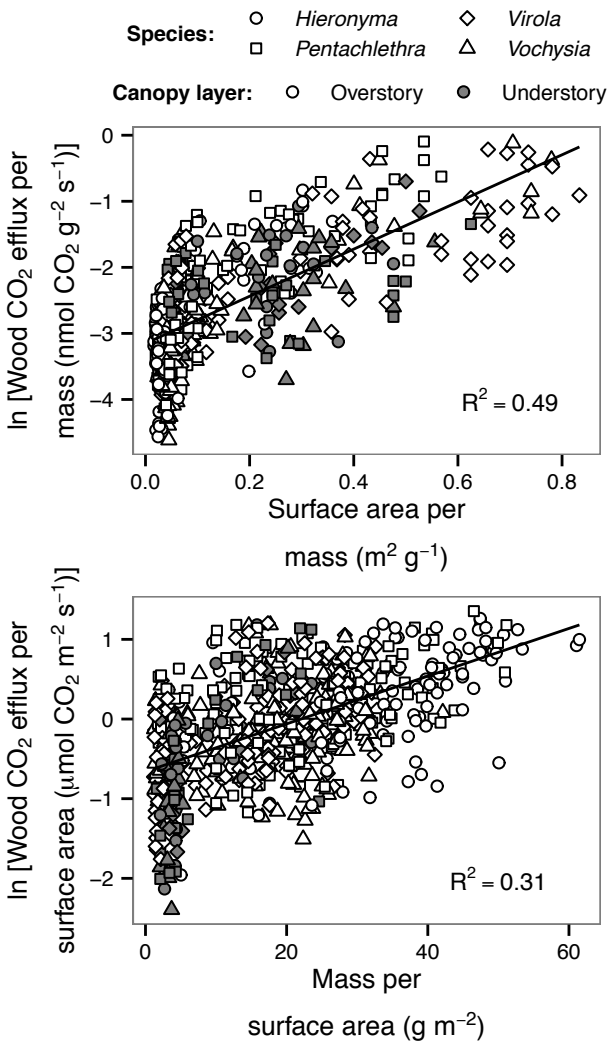

Figure 2.4. The variation in ln-transformed rate of wood CO_2 efflux per mass was related to surface area per mass (top) more than log-transformed rate of wood CO_2 efflux per surface area was related to mass per surface area (bottom). Regression lines were drawn with intercept = -3.2 and slope = 3.6 for top plot, and -0.66 and 0.030 for bottom plot.

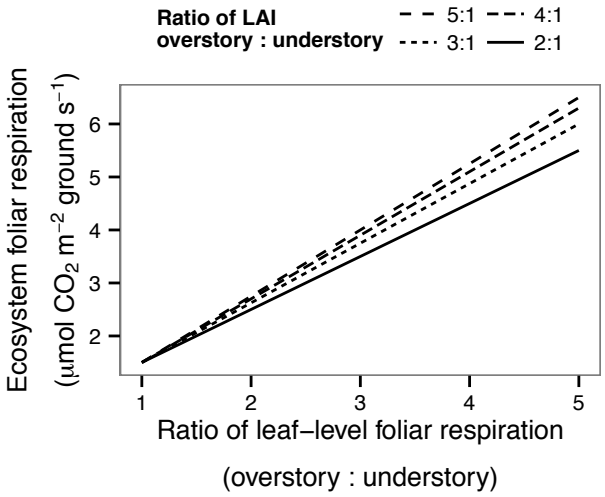

Figure 2.5. Change in total foliar respiration estimated from leaf-level rates, assuming total LAI of 6 and understory respiration rate of 0.25 μmol m^{-2} s^{-1}. Total foliar respiration was underestimated if the overstory rate was unaccounted for, and the bias increased with the ratio of overstory to understory rates. The bias also increased slightly with the ratio of overstory to understory LAI.

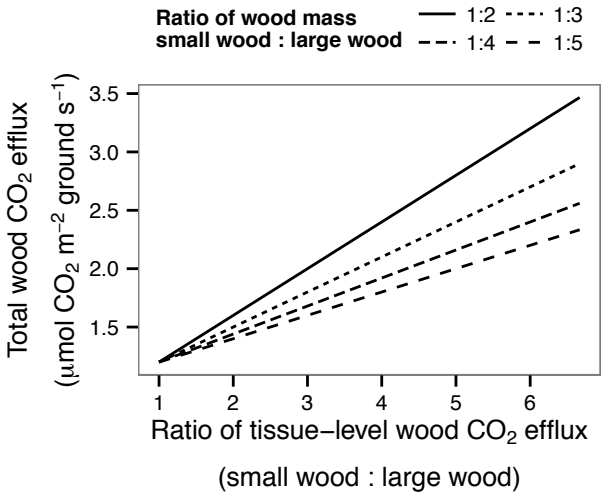

Figure 2.6. Change in total wood CO_2 efflux estimated from tissue-level rates, assuming total wood mass of 20,000 g m^{-2} and large wood CO_2 efflux rate of 0.06 nmol g^{-1} s^{-1}. Total wood CO_2 efflux was underestimated if wood CO_2 efflux rate for small wood was unaccounted for, and the bias increased with the ratio of small to large wood rates. The bias decreased with the ratio of small to large wood mass.

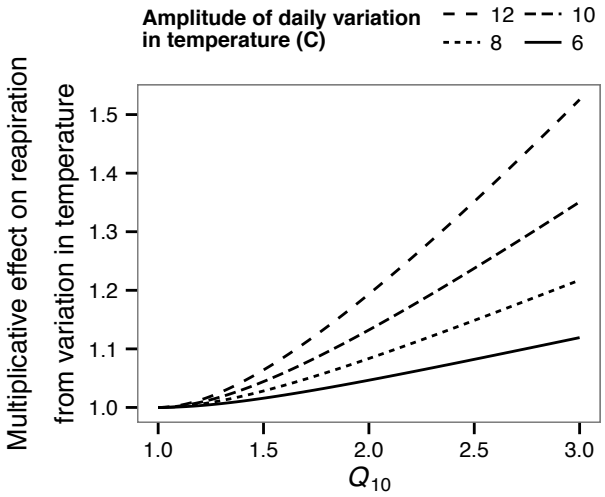

Figure 2.7. Variation in Q_{10} increased the multiplicative effect of temperature variation on annual respiration estimated using constant temperature. The variation in temperature was assumed to follow sinusoidal cycle daily and annually, and the amplitude of the annual cycle is $1/4^{th}$ of the amplitude of the daily cycle.

Chapter 3

Gross primary production and its partitioning among four tree species of a tropical rain forest

Introduction

Tree species significantly affect forest carbon storage and storage rate through subtle differences in gross primary production (GPP), partitioning of GPP, and turnover biomass pools, but it remains unclear how these key functions vary among species in the same environment. In a tropical rain forest of Costa Rica, four tree species simultaneously planted in monodominant stands have nearly a two-fold difference in biomass after 17 years (6510 – 11900 gC m^{-2}, Russell et al. 2010). They also differ substantially in belowground NPP and litter production (Russell et al. 2010). These differences have two possible causes. One is a difference in GPP, and another is a difference in partitioning of GPP to forest components with slower turnover and lower partitioning to respiration, such as wood.

Gross primary production and its partitioning to components of differing respiration and turnover rates control the behavior of ecosystem biogeochemistry models used for assessments in tropics and elsewhere (Dufresne et al. 2002, Ise et al. 2010, Weng and Luo 2011, Friend et al. 2014), but they are difficult to measure at the detail necessary to parameterize the models and are often unknown at a site, especially for tropical forests. Most measurements are on biomass (Tilman 1988, Poorter et al. 2012a), and partitioning of GPP is inferred even though biomass patterns reflect both flux and turnover and may not predict partitioning (Litton et al. 2007). Measuring flux and partitioning improves the description of controlling processes, and may

constrain the variety of modeling approaches to partitioning of GPP (Franklin et al. 2012). Because the approaches vary, the models disagree in assessing the current conditions and predicting how terrestrial C storage responds to climate and land use change (Huntingford et al. 2013, De Kauwe et al. 2013). Tropical forests have enormous fluxes of GPP, NPP, respiration, and turnover (Luyssaert et al. 2007), and small differences in the fluxes determine biomass C storage and storage rate. More than 60 million hectares of tropical forests are planted, most in monocultures (FAO 2011). Model performance at such sites improves with better description and understanding of how species affect fluxes and partitioning.

A simple approach to species effect is to assume that tree species at a site have similar fraction of GPP partitioned to components. Partitioning of GPP is described as

$$Q_i = \eta_i Q, \quad (1)$$

where Q_i is component flux (often in gC m^{-2} ground per unit of time), Q is GPP (gC m^{-2} ground per unit of time), and η_i is the fraction of GPP partitioned to the component i (sums to 1 across components, unitless). The components are typically foliage, woody tissue, and fine roots for simplicity, to minimize the complication that arises from one component changing into another, say a branch growing past its size class to become stem. If species have similar fraction of GPP partitioned to components, Eqn. 1 yields a hypothesis that the fraction is independent of species difference in GPP (η_i does not vary with Q across species), and thus species difference in GPP causes and is directly proportional to the difference in component flux (as in $Q_i = \eta_i Q$).

Component flux is next partitioned to respiration and NPP, and a simple approach is to assume that species have similar fraction of component flux partitioned. Partitioning of component flux is described as

$$Q_i = \gamma_i Q_i + (1 - \gamma_i) Q_i, \quad (2)$$

where γ_i is the fraction of component flux respired (unitless). The term $\gamma_i Q_i$ describes component respiration and $(1-\gamma_i)Q_i$ describes component NPP, and components are foliage, wood, and fine roots. If species have similar fraction of component flux respired, Eqn. 2 yields a hypothesis that the fraction is independent of species differences in component flux (γ_i does not vary with Q_i across species), and thus species difference in component flux causes and is directly proportional to the differences in respiration and NPP of foliage, wood, and roots (as in $Q_i = \gamma_i Q_i + (1-\gamma_i)Q_i$).

Net primary production and turnover then describe the rate of C storage in plant biomass, and a simple approach is again to assume that species have similar turnover rate. Biomass turnover is described as

$$\frac{dW_i}{dt} = NPP_i - \lambda_i W_i, \quad (3)$$

where W_i is plant biomass (gC m^{-2} ground), and λ_i is turnover rate of biomass (unit $time^{-1}$) of components foliage, wood, and fine roots. The term dW_i/dt is biomass storage rate of a component, and $\lambda_i W_i$ is their litter production, if other losses such as herbivory and leaching are minor. The definitions of equation symbols are listed in Table 3.1. If species have similar component turnover rates, Equation 3 yields a hypothesis that species difference in biomass storage rate is related to species difference in NPP and biomass. If litter production ($\lambda_i W_i$) is low in a component however, such as in wood, NPP dominates biomass storage rate ($dW_i/dt \approx NPP_i$). Species difference in biomass storage rate is then proportional to the difference in NPP.

Partitioning fractions (η_i, γ_i) represent critical points at which species influence ecosystem C storage and storage rate, but species may be constrained by physical and physiological requirements of biomass and thus have partitioning fractions related to biomass fractions. For example, respiration increases with biomass ($R_i \propto W_i$), N, and NPP at component or whole plant level because respiration fuels metabolism for biomass maintenance and growth

(Thornley 1970, Hesketh et al. 1971, Ryan 1991b). Metabolism of a whole stand, whether respiration, NPP, or GPP, increases with mass (GPP $\propto W$) because xylem transport constraints plant size and function (Niklas and Enquist 2001, West et al. 2009). Thus partitioning fractions may be proportional to biomass fractions (R_i/GPP $\propto W_i/W$). However, partitioning appears not to reflect biomass fractions across sites (Litton 2007), as other factors such as morphology and tissue density affect biomass sizes and differ among species (Reich 2002). Furthermore, the hypotheses from Eqn. 1 and 2 appear to be approximately true across sites (Litton et al. 2007), but not likely true within a site if resources or stand age vary (Ryan et al. 2004, Stape et al. 2008, Ryan et al. 2010, Stape et al. 2010). These together suggest that partitioning fractions are functions of physiology, resource availability, and ontogeny and may not be proportional to biomass fractions.

We examined how C fluxes, partitioning, and turnover differ among tree species in the experimental forest of Costa Rica. Our objectives were to quantify the annual values of GPP (Q), component flux (Q_i), NPP, respiration, and biomass (W_i), and estimate fraction of GPP partitioned to component (η_i), fraction of component flux respired (γ_i), and turnover rate (λ_i). We tested the following hypotheses.

1) Fraction of GPP partitioned to components foliage, wood, and roots does not vary with GPP across species. Therefore, species differences in GPP are directly proportional to the differences in component flux.
2) For foliage, wood, and roots, fraction of component flux respired does not vary with component flux across species. Therefore, species differences in component flux are directly proportional to the differences in NPP and respiration.

3) Species are similar in component turnover rate. Therefore, species differences in biomass storage rate of components are related to the differences in NPP and biomass.
4) Species differences in GPP are proportional to total biomass.
5) Partitioning fractions are proportional to biomass fractions.

Methods

Study site

The experimental site is in a broad-leaved evergreen rainforest at La Selva Biological Station, in the Atlantic lowlands of Costa Rica (10° 26′ N, 83° 59′ N). The climate at La Selva is classified as Tropical Wet Forest in the Holdridge system (McDade 1994), with mean annual rainfall of 4000 mm and mean annual temperature of 26 °C. Soils at the site are classified as Mixed Haplic Haploperox (Kleber et al. 2007) and are acidic, highly leached, low in base saturation, and high in organic matter content.

The site was cleared of primary forest in 1955 by logging, with the non-harvested material burned, converted to pasture with C4 grasses in 1956, and grazed until 1987. In 1988, the experimental plantation was established with 11 tree species in monodominant stands and an abandoned pasture control, each in 0.25-ha (50 x 50 m) plots, replicated over four blocks covering 12-ha in a randomized complete block design (Fisher 1995). Understory plants were manually cleared for the first three years for the planted trees to establish but were then allowed to regenerate naturally. By 2007, four tree species had continuous canopies necessary for plot level measurements, and these species were the subjects of this study.

The four tree species were *Hieronyma alchorneoides* Allemao, *Pentaclethra macroloba* (Willd.) Kunth., *Virola koschnyi* Warb., and *Vochysia guatemalensis* Donn. Sm., all native

species of the surrounding primary forest. *Pentaclethra* is the only leguminous N-fixing species, and the dominant species of canopy trees (~15% basal area, McDade et al 1994) at La Selva. In 2005, the planted trees contained 80 – 89% of the aboveground biomass (Russell et al. 2010), with the remainder in the understory. Further details on the site and its history can be found in Fisher (1995) and Russell et al (2010).

Each species plot was categorized into three plant components, canopy, wood, and coarse + fine roots. Canopy included leaves and reproductive tissues, wood included any woody tissue aboveground and stump ≥ 10 cm in diameter and stump roots ≥ 1.0 cm in diameter within 1 m from the center of the stem and 1 m depth, and coarse + fine roots included any roots between the planted trees and within 1 m depth.

Mass and Carbon budget

Component biomass (W_i) was measured in 2007 – 2010 with methods described in Russell et al (2010). Briefly, all trees with DBH ≥ 2.5 cm were measured for diameter and height in annual inventories, and canopy and wood mass calculated using allometric regressions specific to each species and summed within a plot. Data for canopy and wood mass of plants with DBH < 2.5 cm were taken from Russell et al. (2010), and were measured during 2004 – 2005. Fine roots (0 – 2 mm) were measured by coring to 30 cm depth. Coarse roots between planted trees were measured in a soil pit to 1 m depth in 2005, and data were taken from Russell et al. (2010).

Component fluxes (Q_i) and GPP (Q) were estimated as sum of component NPP and respiration (Möller et al. 1954, Ryan et al. 2004, Litton et al. 2007, Ryan 1991b), such that respiration and NPP summed to component flux ($Q_i = NPP_i + R_i$), and component fluxes

summed to GPP (Q = sum of Q_i). Partitioning fractions were estimated as defined in Eqns. 1 and 2, and turnover rate was estimated as litterfall / biomass ($\lambda_i W_i / W_i$). Our carbon budget excludes foliar dark respiration in light, volatile organic compound emissions, organic matter leaching from biomass, and herbivory. The method thus underestimates GPP by unknown quantities. Carbon fluxes through volatile organic compound emissions and herbivory are relatively small in tropical forests (Clark et al. 2001), and the other unmeasured fluxes are likely minor in magnitude compared to what was measured. We have no evidence that the unmeasured fluxes vary among species.

We estimated component NPP, litter production and biomass storage rate using methods described in Russell et al. (2010), with data from annual inventories during 2007 – 2010. Canopy NPP was estimated as a sum of litterfall and biomass storage rate. Canopy litterfall was measured in four 1.3 x 0.4 m traps per plot twice a month, and biomass storage rate was estimated allometrically using DBH and height data from annual inventories during 2007 – 2010. Canopy NPP included herbaceous ground layer, and the values for the ground layer were assumed to equal biomass measured in 2005. Wood NPP was estimated as sum of branch (wood ≥ 10 cm in diameter) litterfall and biomass storage rate. Biomass storage rate was estimated allometrically from the annual inventories, and branchfall was measured in 3.0 x 3.0 m quadrats every three month during 2008 and 2009. There was no significant stem mortality. Fine root NPP was assumed to equal fine root ingrowth, as fine root biomass did not change in previous measurements (Valverde-Barrantes et al. 2007). Fine root ingrowth was measured in cores to 15 cm depth. Coarse root NPP was estimated in 2005, as biomass divided by age of the trees. The measurements of root NPP are problematic and may be an underestimate since the root-ingrowth

method misses the production of very fine roots (Fahey 1994). See Russell et al. (2010) for more details.

Canopy and wood respiration was estimated by scaling chamber measurement data taken 2009 – 2010 from Chapter 1 to the stand, and coarse + fine root respiration estimated by using total belowground carbon flux. For canopy, chamber measurements were taken on ~20 samples in the upper canopy, and 10 – 15 individuals in the ground layer. The samples were cut under water, taken to the lab, and measured at night. Wood CO_2 efflux was measured on 10 – 15 branches and stems at in the upper canopy, and ~15 stems at the ground level. The measurements were made using clear polycarbonate chambers connected to an open or closed system IRGA. For canopy respiration, leaf-level foliar respiration rates were averaged for the upper canopy and the ground layer in each species stand, scaled to the stand with respective mean dry mass. The rates were extrapolated to annual values with mean annual nighttime temperatures of 2007 – 2010 and temperature response specific to species and canopy layers taken from Chapter 1. The annual rates were then averaged. For wood respiration, chamber measurements were scaled with dry mass similarly to canopy respiration and extrapolated using mean annual temperature. The chamber measurements were averaged separately for wood $\geq$ 10 cm and < 10 cm in diameter for each species and multiplied by their respective dry mass. Stumps were included as wood $\geq$ 10 cm and stump roots as < 10 cm. The rates were then extrapolated using mean annual temperatures and averaged. Because our measurements were taken on intact wood, they include CO_2 dissolved in xylem stream, and may include some from coarse + fine roots and soil (McGuire and Teskey 2004, Teskey et al. 2008). Coarse + fine roots respiration was estimated from total belowground carbon flux. Total belowground carbon flux was calculated as soil CO_2 efflux – litter C input + changes in soil organic matter C (Raich and

Nadelhoffer 1989, Litton et al. 2007). Coarse + fine roots NPP, stump and stump roots NPP and respiration were further subtracted to estimate coarse + fine roots respiration. This method includes as respiration production and turnover of very fine roots and respiratory activity of roots and their associated micbrobiota.

Statistical analysis

Species difference in fluxes and partitioning fractions were tested with a linear model ANOVA, followed by pair-wise contrasts with Tukey-Kramer multiple comparison adjustment at experiment-wise $\alpha = 0.05$. Errors were quantified for species level means of plot level values and only reflect variance among plots, not uncertainties in each component estimates at plot level. The hypotheses were tested by regressing the plot level values. Gross primary production, component flux, respiration, and NPP were either calculated from biomass or fluxes summed, and direct comparison involves autocorrelation. We used ratios instead (i.e. partitioning fractions and turnover rates) to minimize the autocorrelation involving mass. However, component flux (Q_i) is part of GPP (Q), respiration and NPP part of component flux (Q_i) and litterfall ($\lambda_i W_i$) and biomass storage rate (dW_i/dt) part of component NPP as Eqns. 1, 2, and 3 show. Regressing a ratio fluxes against a flux would still involve some autocorrelation, for example Q_i / Q against Q. This autocorrelation causes unknown portion of positive relationships tested. All analysis were done in R (R Core Team 2014), with multcomp (Hothorn et al. 2008), and MASS (Venables and Ripley 2002) packages, and plotted with ggplot2 package (Wickham 2009).

Results

Species differed in biomass, GPP, component flux, NPP, and respiration at whole stand level (Table 3.2), and the differences in NPP, respiration, and biomass reflected GPP (Fig. 3.1). Species averaged 9810 gC m^{-2} in mass varying 77% (*Virola*) to 142% (*Vochysia*), and averaged 3650 gC m^{-2} $year^{-1}$ in GPP varying 84% (*Virola*) to 123% (*Vochysia*) of the mean. Respiration averaged 2560 gC m^{-2} $year^{-1}$, ranging 83% (*Virola*) to 125% (*Vochysia*), and NPP averaged 1090 gC m^{-2} $year^{-1}$, ranging 87% (*Virola*) to 117% (*Vochysia*). Species differed in component mass for wood, in component respiration for canopy and wood, and in component NPP for wood and coarse + fine roots (Table 3.2).

Hypothesis 1: Fraction of GPP partitioned to foliage, wood, and roots does not vary with GPP across species.

Fraction of GPP partitioned was independent of species differences in GPP (Fig. 3.2, right column), and thus species differences in GPP explained the differences in component fluxes. Therefore, component flux for canopy, and coarse + fine roots increased directly proportional to GPP across species (Fig. 3.2, left column). However, component flux for wood may be multiplicatively proportion to GPP to some degree, since *Vochysia* had significantly higher mean value of fraction of GPP partitioned to wood (Table 3.3). Species differed only in fraction of GPP partitioned to wood, ranging 0.26 in *Hieronyma* to 0.37 in *Vochysia* (Table 3.3). Site averages of fraction of GPP partitioned to a component were 0.33 for canopy, 0.32 for wood, and 0.35 for coarse + fine roots (Table 3.3).

Hypothesis 2: Fraction of component flux respired does not vary with component flux across species.

Except for canopy, fraction of component flux respired was independent of species difference in component flux to wood and coarse roots (Fig. 3.3 left column). Therefore, species difference in component flux explained the differences in respiration and NPP for wood and coarse + fine roots. In those components, NPP increased with component flux ($P < 0.01$, $R^2 > 0.39$). Respiration increased with NPP for wood and marginally for coarse + fine roots (Fig. 3.3 right column). The exception was canopy, where only respiration, not NPP, reflected the species difference in component flux. Fraction of component flux respired increased with component flux (Fig. 3.3 top left), and thus respiration increased multiplicatively with component flux. Component flux was unrelated to NPP for canopy ($P = 0.27$), and respiration was independent of NPP in that component (Fig. 3.3 top right).

Fraction of GPP respired from the whole stand was similar among species and averaged 0.71 ($P = 0.82$, Table 3.3). At component level, species differed only in fraction of component flux respired for canopy (Table 3.3). Site averages of the fraction were 0.63 and 0.64 for canopy and wood, and 0.81 for coarse + fine roots.

Hypothesis 3: Species are similar in turnover rate of components, and species difference in biomass storage rate is related to the difference in NPP and biomass.

Species differed in turnover rate ($P < 0.01$ for both canopy and branch). Mean turnover rate for canopy was higher in *Hieronyma* at 1.8 $year^{-1}$, compared to 1.2 in *Pentaclethra*, 1.3 in *Virola*, and 1.2 $year^{-1}$ in *Vohchysia* (at $P < 0.05$ in pair-wise contrasts). The mean rate for branch was higher in Vochysia at 0.095 $year^{-1}$, compared to 0.039 in *Hieronyma*, 0.043 in *Pentaclethra*, and 0.052 $year^{-1}$ in *Virola* ($P < 0.05$). The analysis focused on canopy and branches (wood > 10

cm in diameter), and excluded larger wood and coarse roots for lack significant mortality, and fine roots because their biomass did not change in a previous study (see methods, (Valverde-Barrantes et al. 2007)).

Species were similar in litter production for canopy ($P = 0.26$) but differed for branch only ($P < 0.01$), and biomass storage rate was similar among species for both components ($P = 0.57$ for canopy, $P = 0.38$ for branch), but increased with NPP for branch (Fig. 3.4 right column). For canopy, litter proudction averaged 472 gC m^{-2} $year^{-1}$ in *Hiernoyma*, 421 in *Pentaclethra*, 415 in *Virola*, and 385 in *Vochysia*, and biomass storage rate averaged 6.4, 8.1, 8.1, and 8.5 gC m^{-2} $year^{-1}$ respectively. For branch, litter production averaged 89 gC m^{-2} $year^{-1}$ in *Hiernoyma*, 97 in *Pentaclethra*, 88 in *Virola*, and 200 in *Vochysia*, and biomass storage rate averaged rate averaged 40, 49, 46, and 57 gC m^{-2} $year^{-1}$.

Hypothesis 4: Species difference in GPP is proportional to total biomass.

Species difference in GPP was proportional to biomass (Fig. 3.5, right). Our estimate of GPP involves biomass, and the two variables are autocorrelated. We compared GPP and LAI to reduce the autocorrelation. Leaf area index increased with biomass ($P < 0.01$, $R^2 = 0.72$), and was estimated with leaf mass and specific leaf area and involved another variable independent from biomass. Gross primary production increased with LAI (Fig. 3.5, left), providing additional evidence that GPP increased with biomass.

Hypothesis 5: Partitioning fractions are proportional to biomass fractions.

Biomass fraction (W_i/W) was related to fraction of GPP partitioned to component for wood and coarse + fine roots but not for canopy (Fig. 3.6). Species with greater wood fraction had greater fraction of C uptake partitioned there and less to canopy and coarse + fine roots (Fig. 3.6). Component flux increased with biomass for wood in a tight relationship ($P < 0.01$, $R^2 = 0.82$), but marginally for coarse + fine roots ($P = 0.05$, $R^2 = 0.19$) and for canopy ($P = 0.09$, $R^2 = 0.13$).

Increase in respiration with biomass explained this relationship. Fraction of GPP respired in component (R_i / Q) increased with biomass fraction (Fig. 3.7). Respiration increased with biomass in a tight relationship for wood ($P < 0.01$, $R^2 = 0.80$), but only marginally for canopy ($P = 0.07$, $R^2 = 0.16$) and coarse + fine roots ($P = 0.09$, $R^2 = 0.14$). Species with greater biomass fraction in wood respired a greater fraction of GPP in wood and less in canopy and coarse + fine roots (Fig. 3.7)

Discussion

Our results indicate that species difference in LAI caused most of the difference in biomass C storage size and storage rate. Gross primary production increased with LAI. Species difference in GPP in turn explained the difference in component flux, and component flux explained the difference in NPP for wood with low turnover and low respiration. Species difference in wood NPP then explained stand level difference in biomass storage size and storage rate because wood had low turnover rate and was by far the biggest component. This may be related to respiration dominating partitioning of GPP. All species respired more than half of component flux, and high respiration caused partitioning fractions to be proportional to biomass

fractions. Below we discuss some mechanism behind the patterns in respiration, LAI control of GPP, how partitioning fractions may change as stands age, and modeling implications.

Canopy and wood respiration, not temperature or nutrient availability, explained high whole stand respiration

Compared to previous studies, the ratio of respiration to NPP was high for wood but not for canopy, suggesting that the temperature effects of maintenance respiration is not responsible for the high whole stand respiration. Litton et al. (2007) reported global values of ~2:1 (or 66% of component flux respired) for foliage, but ~1:2 (35% of component flux respired) for aboveground wood. Our values were ~2:1 (64%) for both canopy and wood, and the ratio should have been higher than the global average for both canopy and wood if temperature effect on respiration is responsible. If wood respiration consumed only 35% of component flux instead of observed 64%, whole stand respiration would be 60% of GPP, comparable to 57% reported in Litton et al. (2007). As wood respiration increased with wood NPP (Fig. 3.3), processes associated with growth may be responsible for the high wood respiration.

Tests of hypothesis 1 and 2 indicate that canopy respiration increased multiplicatively with GPP, and implies that whole stand respiration also increased multiplicatively with GPP. Component respiration was proportional to GPP in other components. We examined the effect of canopy respiration on whole stand respiration using Eqns. 1 and 2 and the estimates for the partitioning fractions. We predicted whole stand respiration from observed GPP and stand mean partitioning fractions, but increased the canopy respiration multiplicatively with GPP using the equation in Fig. 3.3 ($\gamma_i = 0.38 + 2.1\times10^{-4}Q_i$). Predicted ratio of respiration to observed GPP at whole stand level increased with GPP ($R / Q = 0.60 + 2.5\times10^{-5}\ Q$). We then fitted this equation

to the observations (Fig. 3.8). Though the fit was marginal, the correlation coefficients were identical to those derived from observation ($\gamma = 0.61 + 2.4\times10^{-5}\ Q$, $P = 0.07$, $R^2 = 0.16$). Clearly, this approach is flawed in that relationship derived from observation is fitted to the same observation. However, multiplicative increase in canopy respiration must also increase whole stand respiration multiplicatively with GPP, if all other partitioning fractions remain fixed. We thus postulate that the species difference in canopy respiration explains the difference in stand level respiration. The increase in canopy respiration suggests that additional GPP has greater respiratory cost. We discuss this further in section on LAI control on GPP.

Our definition of coarse + fine root respiration may also have contributed to the high whole stand respiration. Whole stand respiration was 70% of GPP, compared to global average of 57% and 60 – 70% range reported for tropical forests (Litton et al. 2007, Malhi 2012). We estimated coarse + fine roots respiration by subtracting root NPP from total belowground carbon flux. Coarse + fine roots respiration thus includes root exudates, rhizospere respiration, and production and turnover of very fine roots. Without coarse + fine roots, fraction of GPP respired at whole stand level was 0.64.

High whole stand respiration is unlikely to be related to nutrient availability. Increasing nutrient availability tends to decrease biomass fraction of fine roots, increase the ratio of LAI to fine roots, increase partitioning of GPP to aboveground components, and increase the ratio of NPP to GPP (Tilman 1988, Litton et al. 2007, Vicca et al. 2012, Fernández-Martínez et al. 2014). These trends support the concept that in nutrient rich sites, individual trees partition greater fraction of GPP to leaf and wood to increase carbon gain, whereas they partition more to fine roots for nutrient gain in nutrient poor sites. We found that GPP increased with LAI, supporting increased carbon gain, but increased GPP increased partitioning to wood to only a marginally if

at all, and did not change partitioning to canopy and coarse + fine roots. Coarse + fine root respiration was proportional to GPP, even though coarse + fine root respiration included root exudates and respiration from root associated microbes. Root exudates and microbial respiration would have increased if greater fraction of GPP was partitioned there to increase nutrient uptake (Kuzyakov 2002, Drake et al. 2011, Vicca et al. 2012). The ratio of canopy to coarse + fine root component fluxes did not change with GPP either ($P = 0.87$).

Increased LAI alleviated ontological decline in GPP

Gross primary production increased with LAI across species, and this may suggest that LAI increases GPP across time. Leaf area index increased since 2003 – 2005, from 5.2 – 6.5 (*Hieronyma* and *Pentaclethra*) to 6.7 in *Hieronyma*, 7.8 in *Pentaclethra*, 7.0 in *Virola*, and 10 in *Vochysia*, reaching values similar to those in typical plantations (8.7, (Asner et al. 2003)), and surpassing ~6 in the surrounding primary forest (Russell et al. 2010). Both the planted overstory trees and the understory plants contributed to the increase since 2003 – 2005. The overstory trees continued to accounted for similar proportion to the total LAI, except in *Vochysia* where overstory LAI increased from 51% to 64%. As canopy biomass was relatively similar among species (Table 3.2), species difference in leaf thickness explains the difference in LAI. With the equation for regressing LAI against GPP (Fig. 3.5), 2003 – 2005 LAI equals 2700 – 3100 gC m^{-2} $year^{-1}$ in GPP.

However, GPP likely declined since 2003 – 2005 despite the increased LAI, because stands declined in NPP. On average, NPP declined to 72% of 2003 – 2005, when NPP was 1100 gC m^{-2} $year^{-1}$ in *Virola* to 1600 gC m^{-2} $year^{-1}$ in *Hieronyma* (Russell et al. 2010). These values would equal 48 – 60% of the GPP estimated with 2003 – 2005 LAI, slightly higher in the global

range of 30 – 70% (DeLucia et al. 2007, Litton et al. 2007), higher than 27 – 46% in primary forests in tropics (Malhi 2012), and much higher than ~30% for 2007 – 2010. It is thus likely that GPP was higher during 2003 – 2005 and has since declined. This indicates that GPP is not predictable from 2007 – 2010 relationship between LAI and GPP. The LAI – GPP relationship has changed over time, and likely had higher intercept, shallower slope, or both in 2003 – 2005.

Respiration also suggests that GPP has declined. Respiration has likely declined similarly to NPP, or stayed the same since 2003 – 2005. Lower NPP requires lower growth respiration, and respiration generally decreases with NPP (Litton et al. 2007), and respiration was related to NPP in wood and perhaps in coarse + fine roots in 2007 – 2010. Since 2003 – 2005, root respiration estimated from total belowground carbon flux has remained similar except in *Hieronyma* (Russell et al. 2010). The stand level respiration from leaf and wood (~760 for both gC m^{-2} $year^{-1}$) for 2007 – 2010 were similar to those in surrounding forest at ~870 and 570 gC m^{-2} $year^{-1}$ respectively. This combined with decreased NPP suggests that GPP has declined since 2003 – 2005.

Component flux to canopy suggests that the increase in LAI since 2003 – 2005 may have alleviated the ontological decline in GPP. Canopy had high respiration rate, and the respiration rate increased multiplicatively with GPP, indicating that LAI is both a cause and a consequence of GPP. Maintaining LAI (leaf mass) requires respiration and leaf production to replace turnover, and additional leaf area beyond certain LAI absorbs less light per area, and light absorption saturates at LAI of 5 – 6 (Asner et al. 2003). If leaves are thought of as an investment to gain GPP, return on investment should decrease with LAI, if LAI becomes a consequence of GPP. We calculated return on investment as (GPP – component flux for canopy) / component flux for canopy. Return on investment for LAI in 2007 – 2010 averaged 2.1 and did not change

with LAI across species even at LAI > 10 ($P = 0.15$), suggesting that greater LAI since 2003 – 2005 is a cause of and has yet to become predominantly a consequence of greater GPP. Thus greater LAI may have alleviated the ontological decline in GPP.

Current partitioning fractions and turnover rates may not hold as stands age

Despite the decline in NPP, species had accumulated biomass since 2003 – 2005 (Russell et al. 2010), suggesting that NPP will further decline for the stands to reach steady state (Ryan et al. 2004). Biomass was already comparable to the surrounding primary forest (Russell et al. 2010), and since then has increased to 118% in *Hieronyma*, 130% in *Pentaclethra*, and 117% in *Virola* and *Vochysia*. Mass of both canopy and wood components increased, 106% (*Virola*) to 134% (*Vochysia*) for canopy mass and 119% (*Virola*) to 132% (*Pentaclethra*) for wood mass. For biomass to reach steady state, NPP must equal litter production (including mortality). Stand level turnover rate must increase to ~0.11 $year^{-1}$ if the stands reach steady state at current stand mean biomass of 9810 gC m^{-2} and NPP of 1090 gC m^{-2} $year^{-1}$. This turnover rate is twice as much as the turnover rate of branches, suggesting that NPP will likely decline further.

The decline in NPP may induce changes in partitioning fractions since wood NPP declined more than canopy NPP. In 2007 – 2010, canopy NPP was 90% (*Hieronyma*), 87% (*Pentaclethra*), 105% (*Virola*), and 95% (*Vochysia*) of 2003 – 2005 values, but wood NPP had declined to 54, 48, 65, and 71% respectively. Fraction of GPP partitioned to canopy thus likely has increased since 2003 – 2005. Fraction of component flux respired may have increased for canopy, if the stands followed ontological change observed in *Eucalyptus* plantations (Ryan et al. 2004).

Turnover rate will likely increase as well, though not from turnover of canopy or branches but from individual tree mortality. Turnover rate for canopy and branch was proportional to component NPP (Fig 3.4), perhaps from self-shading as the trees grow taller, and may decrease as NPP and height growth decline. The decrease may be small as turnover rate for canopy and branch in 2007- 2010 remained similar since 2003 – 2005 (500 – 580 gC m^{-1} $year^{-1}$ from 370 - 550 gC m^{-1} $year^{-1}$, Russell et al. 2010) and to those in the surrounding forest (~440 gC m^{-1} $year^{-1}$, (Parker 1994)). This suggests that canopy and branch turnover rates are near steady state, and any increase in stand level turnover would come from individual tree mortality. Site-mean basal area was 27 m^{-2} ha^{-1}, slightly higher than 24 m^{-2} ha^{-1} in the surrounding primary forest (Clark and Clark 2000). Considering that the stands are still accumulating biomass, increase in mortality is likely.

Implications for modeling

Our results suggest that models may use similar partitioning fractions and turnover rates for different species at a site. Partitioning fractions may be estimated from biomass fractions. The relative lack of difference in partitioning fractions among species suggests that species within a vegetation type at a site responds similarly in C fluxes to their environment at annual time scale.

Critical parameters to represent species difference may be morphological. Much of the differences in component flux, NPP, and respiration were explained by GPP, and GPP in turn increased with LAI. Though LAI varied from 6.0 – 12, leaf mass was relatively similar at 270 – 360 gC m^{-1}, indicating that bulk canopy leaf thickness critically controls GPP, component flux, NPP, and respiration. Species generally vary more in morphology than in biomass fractions

(Poorter et al. 2012b), and morphology may better represent species difference in models than biomass fractions, partitioning fractions, or turnover rates.

Our results also suggests that ontological shift in partitioning fractions and turnover rates are more critical to represent species difference in modeling than species differences at one point in time. The trends across time in NPP and LAI suggests that partitioning fractions and turnover rates change over time, and these ontological changes may depend on species. Species did not differ in NPP during 2003 – 2005, but by 2007 – 2010, significant differences appeared for NPP, respiration, and GPP.

Conclusion

Species difference in LAI explained the difference in carbon storage in plant biomass. Gross primary production increased with LAI, and species differences in GPP, not partitioning, explained the differences in component flux. Component flux in turn explained species difference in NPP and respiration at component level, except for canopy respiration. Species difference in wood NPP explained biomass storage size and storage rate. Canopy respiration increased multiplicatively with component flux. Wood respiration consumed 20% of GPP, much higher fraction than reported global average. At whole stand level, respiration consumed 70% of GPP. Species had accumulated LAI since 2003 – 2005, but declined in NPP, suggesting that increased LAI alleviated the decline in GPP and or NPP. Models may assume constant partitioning fractions across species at a site, but likely not across time. Species will likely further decline in NPP or increase in turnover rate, and current partitioning fractions and turnover rates may not hold across time. Morphology, especially leaf thickness, may be more critical to representing species differences in C fluxes.

Tables

Table 3.1. Definition of symbols for equations 1, 2, and 3.

Symbol	Definition	Units
Q	GPP; total photosynthesis minus foliar respiration in light	gC m^{-2} unit $time^{-1}$
Q_i	Component flux; C flux to plant biomass component i	gC m^{-2} unit $time^{-1}$
W_i	Component mass; mass of plant component i	gC m^{-2}
η_i	Fraction of Q partitioned to component i	unitless
γ_i	Fraction of Q_i respired	unitless
λ_i	Turnover rate for component; fraction of W_i that becomes litter	unit $time^{-1}$

Table 3.2. Dry mass (gC m^{-2}) and C fluxes (gC m^{-2} $year^{-1}$) of each species. Different letters indicate significant differences among species in pairwise contrasts at $\alpha = 0.05$.

		Canopy	Wood	Coarse + fine roots	Whole stand
R	*Hieronyma*	$777^{ab} \pm 63$	$600^{a} \pm 36$	1132 ± 176	$2510^{ab} \pm 190$
	Pentaclethra	$659^{a} \pm 83$	$801^{ab} \pm 129$	933 ± 92	$2390^{ab} \pm 263$
	Virola	$610^{a} \pm 35$	$577^{a} \pm 81$	937 ± 51	$2120^{a} \pm 70$
	Vochysia	$1000^{b} \pm 82$	$1060^{b} \pm 81$	1137 ± 182	$3200^{b} \pm 337$
	Site mean	761	760	1030	2560
NPP	*Hieronyma*	479 ± 24	$357^{a} \pm 23$	$305^{a} \pm 26$	$1140^{ab} \pm 34$
	Pentaclethra	429 ± 43	$379^{a} \pm 63$	$180^{b} \pm 20$	$988^{a} \pm 111$
	Virola	423 ± 25	$332^{a} \pm 37$	$193^{b} \pm 29$	$948^{a} \pm 55$
	Vochysia	393 ± 21	$594^{b} \pm 43$	$296^{a} \pm 29^{a}$	$1280^{b} \pm 38$
	Site mean	431	416	244	1090
Component flux (or GPP)	*Hieronyma*	$1260^{ab} \pm 74$	$956^{a} \pm 74$	1440 ± 188	$3650^{ab} \pm 182$
	Pentaclethra	$1090^{a} \pm 107$	$1180^{ab} \pm 175$	1110 ± 93	$3380^{ab} \pm 311$
	Virola	$1030^{a} \pm 35$	$909^{a} \pm 106$	1130 ± 72	$3070^{a} \pm 100$
	Vochysia	$1400^{b} \pm 94$	$1660^{b} \pm 108$	1430 ± 195	$4490^{b} \pm 372$
	Site mean	1190	1180	1280	3650
Mass	*Hieronyma*	270 ± 23	$9260^{ab} \pm 680$	438 ± 33	$9970^{ab} \pm 693$
	Pentaclethra	360 ± 45	$8090^{a} \pm 1480$	378 ± 37	$8830^{a} \pm 1510$
	Virola	335 ± 24	$6800^{a} \pm 1060$	457 ± 80	$7590^{a} \pm 1120$

Vochysia	341 ± 30	13100[b] ± 990	475 ± 80	13900[b] ± 1050
Site mean	326	9306	473	9810

Table 3.3. Fraction of GPP partitioned to component (η_i), and fraction of component flux partitioned to respiration (γ_i) for each species. Different letters indicate significant differences among species in pairwise contrasts at $\alpha = 0.05$.

		Canopy	Wood	Coarse + fine roots	Whole stand
Fraction of GPP partitioned to component (η_i)	*Hieronyma*	0.35 ± 0.02	0.26^{a} ± 0.04	0.39 ± 0.04	
	Pentaclethra	0.32 ± 0.01	0.34^{ab} ± 0.02	0.33 ± 0.03	
	Virola	0.34 ± 0.01	0.29^{ab} ± 0.03	0.37 ± 0.03	
	Vochysia	0.31 ± 0.01	0.37^{b} ± 0.01	0.32 ± 0.02	
	Site mean	0.33	0.32	0.35	
Fraction of component flux respired (γ_i)	*Hieronyma*	0.62^{ab} ± 0.02	0.63 ± 0.01	0.78 ± 0.03	0.69 ± 0.03
	Pentaclethra	0.60^{a} ± 0.03	0.68 ± 0.03	0.84 ± 0.02	0.71 ± 0.03
	Virola	0.59^{a} ± 0.02	0.63 ± 0.03	0.83 ± 0.02	0.71 ± 0.04
	Vochysia	0.72^{b} ± 0.02	0.64 ± 0.02	0.78 ± 0.03	0.72 ± 0.03
	Site mean	0.63	0.64	0.81	0.71

Figures

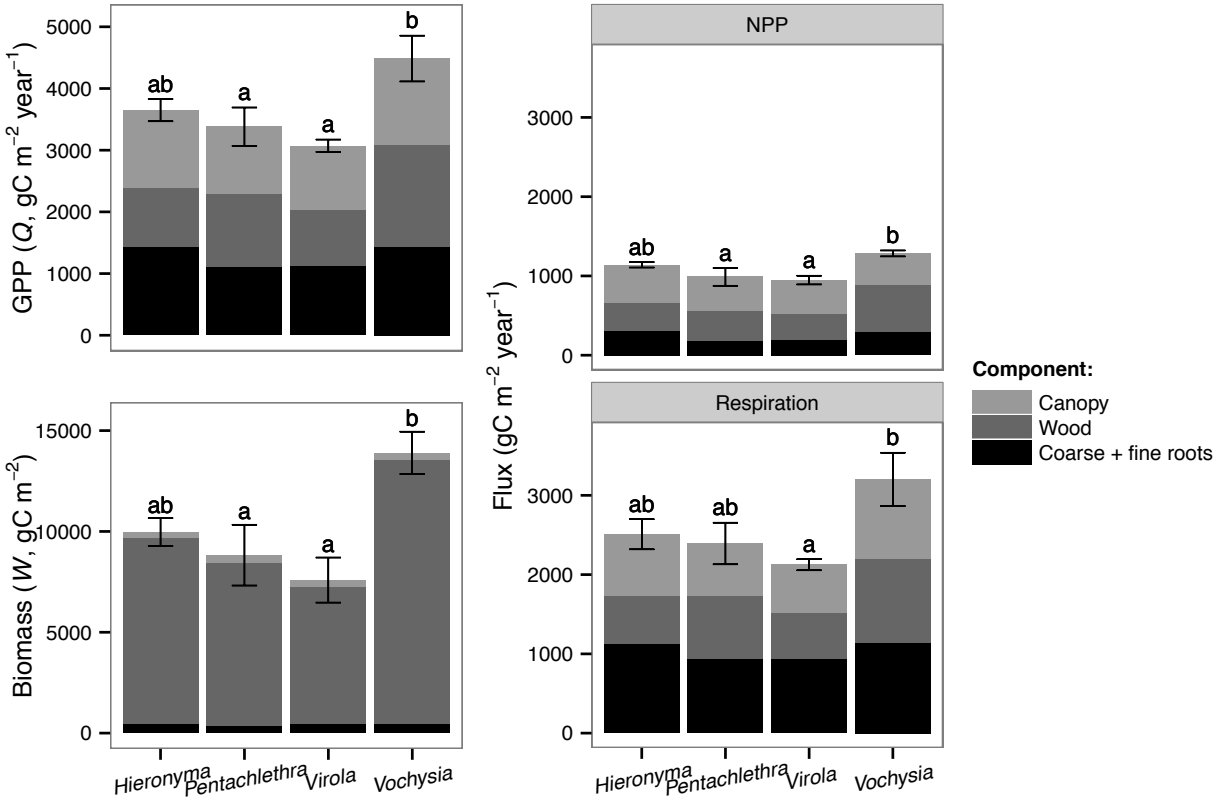

Figure 3.1. Species differed in GPP (*Q*), biomass (*W*), NPP, and respiration (R). Error bars show standard error of the mean among blocks ($n = 4$ for each species), and different letters indicate significant differences in sum of components in pair-wise contrast ($P < 0.05$). Shades of grey indicate components: light grey is canopy, dark grey is wood, and black is coarse + fine roots. Wood includes stumps and stump roots.

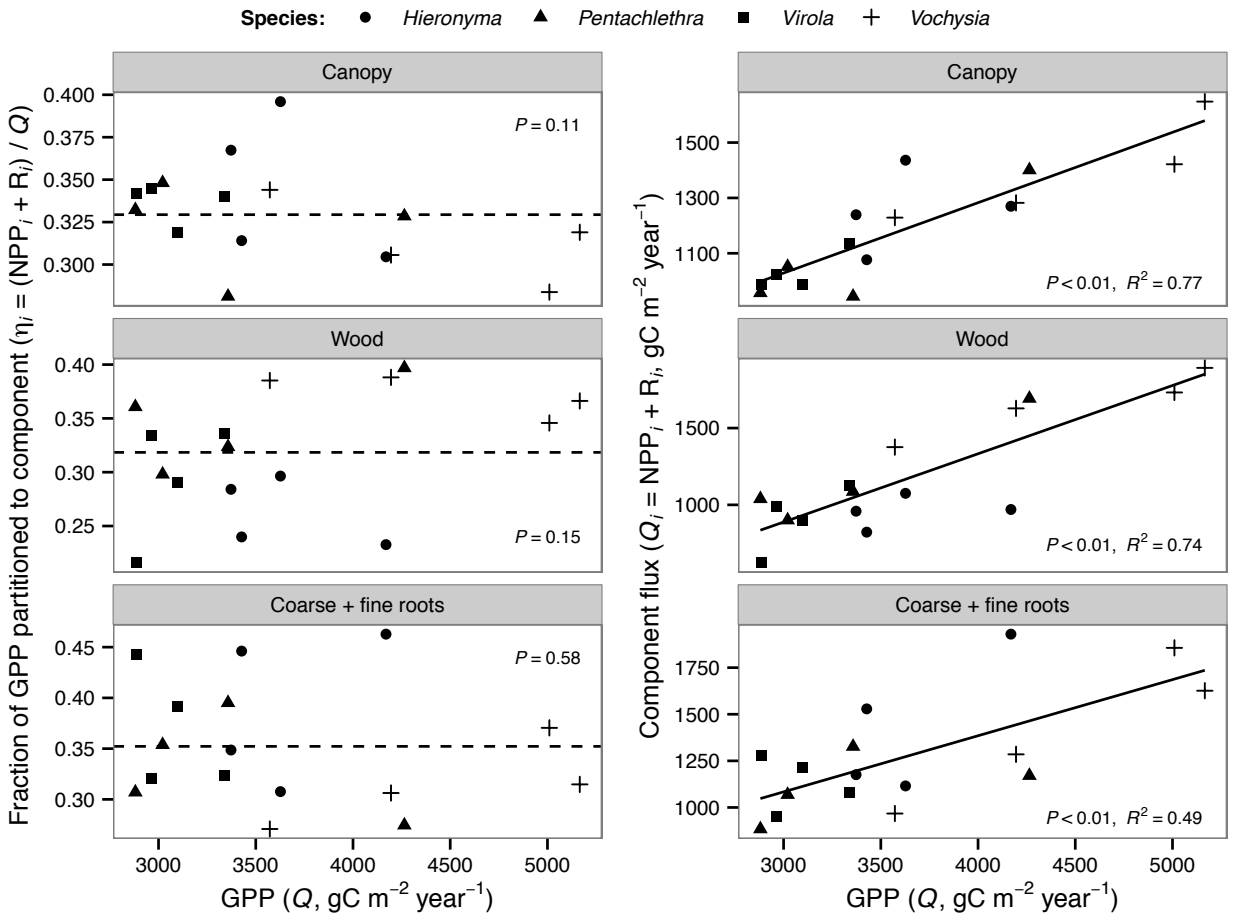

Figure 3.2. Fraction of GPP partitioned to component did not vary with GPP (left column), and thus component flux to canopy, wood, and coarse + fine roots increased proportionally with GPP across species (right column). Dotted lines indicate mean across species of fraction of GPP partitioned to canopy, wood, and coarse + fine roots (see Table 3.2 for values). The regression lines were drawn with $y = 0.32x$ for canopy, $y = 0.33x$ for wood, and $y = 0.35x$ for coarse + fine roots.

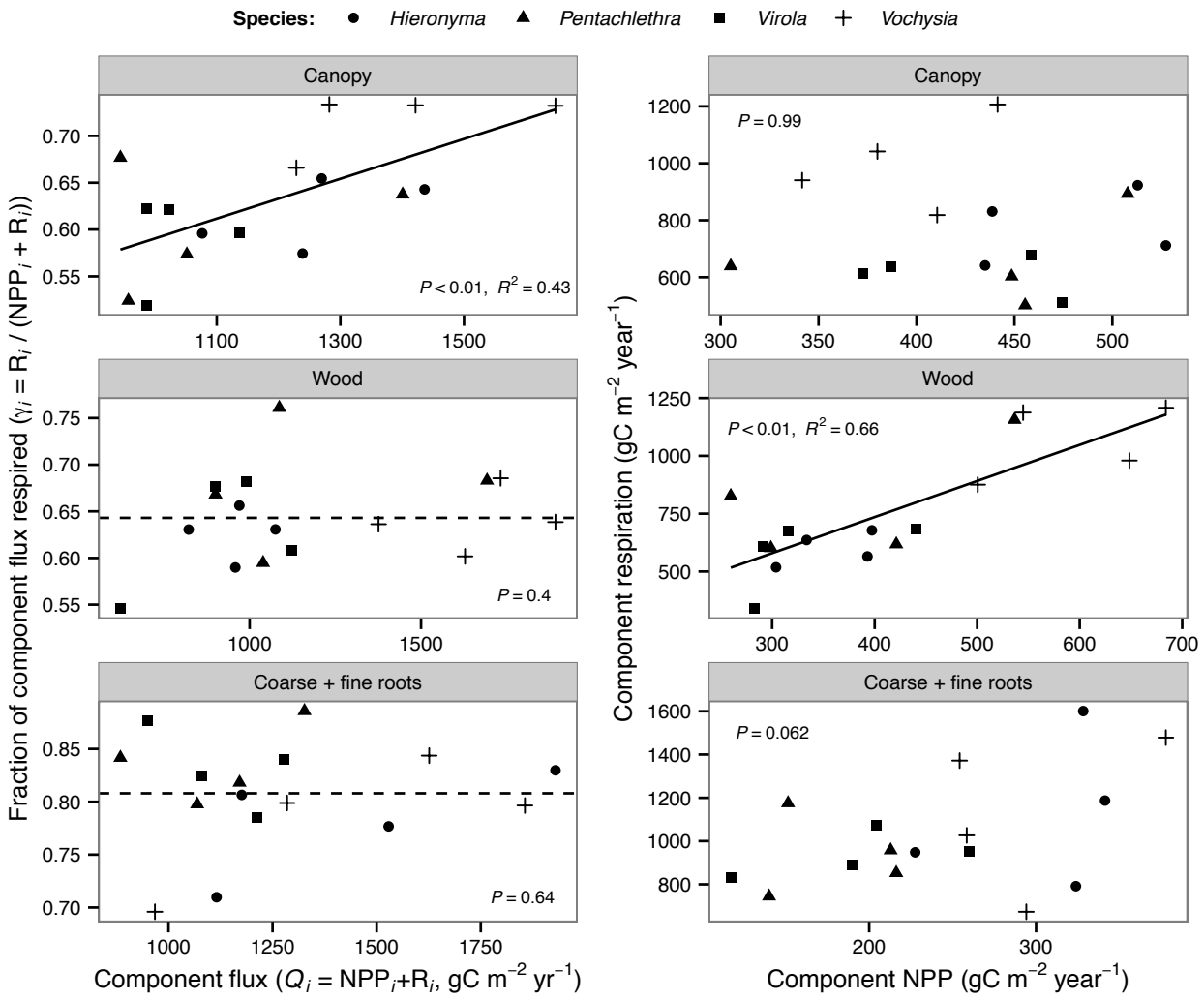

Figure 3.3. Fraction of component flux respired did not change with component flux for wood and coarse + fine roots (left column), and thus NPP and respiration increased with component flux for those components. Respiration increased with NPP in wood, and marginally in coarse + fine roots (right column). For canopy, fraction of component flux respired increased with component flux (top left), and respiration did not increase with NPP (top right). Dotted lines indicate mean values of fraction of component flux respired across species for each component (see Table 3.2 for values). Regression lines were $y = 0.38 + 2.1\times10^{-4}x$ for canopy and $y = 1.6x$ for wood.

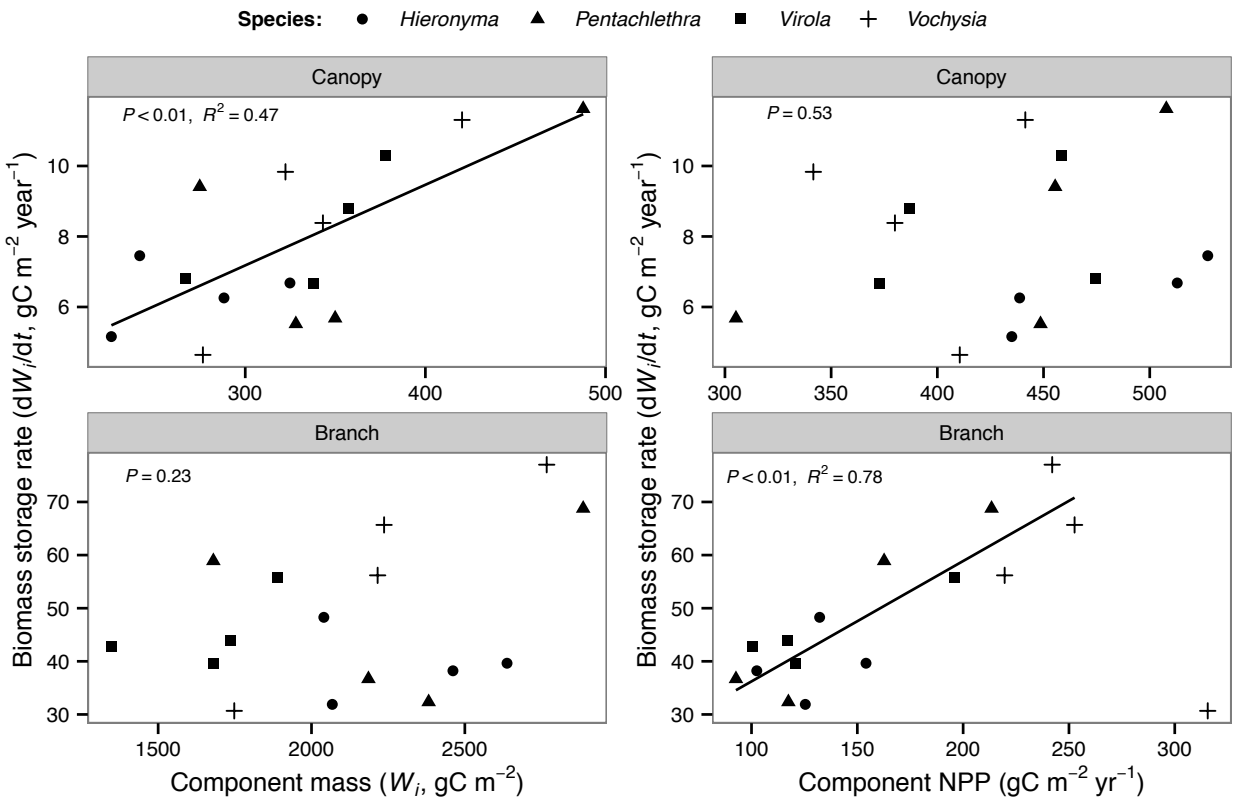

Figure 3.4. Species difference in biomass storage was related to the difference in mass for canopy (left column), and related to NPP for branch (right column). Regression line for branch in left column was $y = 11 + 0.24x$, excluding one extreme value for *Vochysia*. Regression lines for right column were $y = 3.8\times10^{-3}x$ for canopy and $y = 4.3\times10^{-4}x$ for branch.

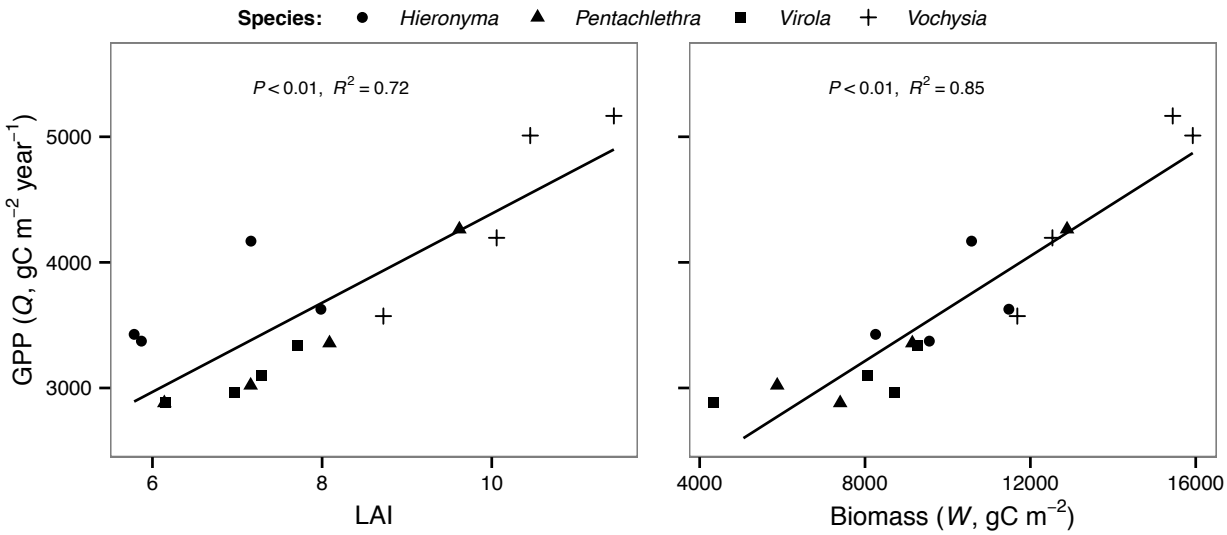

Figure 3.5 GPP increased with LAI (left) and total plant biomass (right). Regression lines were $y = 840 + 350x$ for LAI and $y = 1.5\times10^3 - 0.21x$ for biomass.

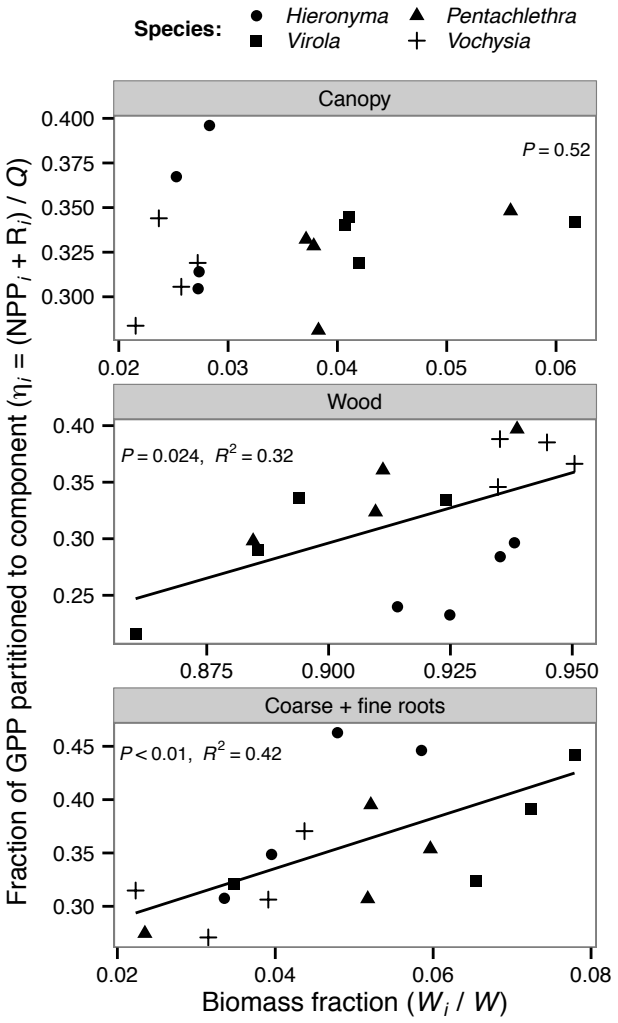

Figure 3.6. Biomass fraction was related to fraction of GPP partitioned to component for roots and wood, but not for canopy. Regression lines were $y = -0.82 + 1.2x$ for wood, and $y = 0.24 + 2.4x$ for coarse + fine roots.

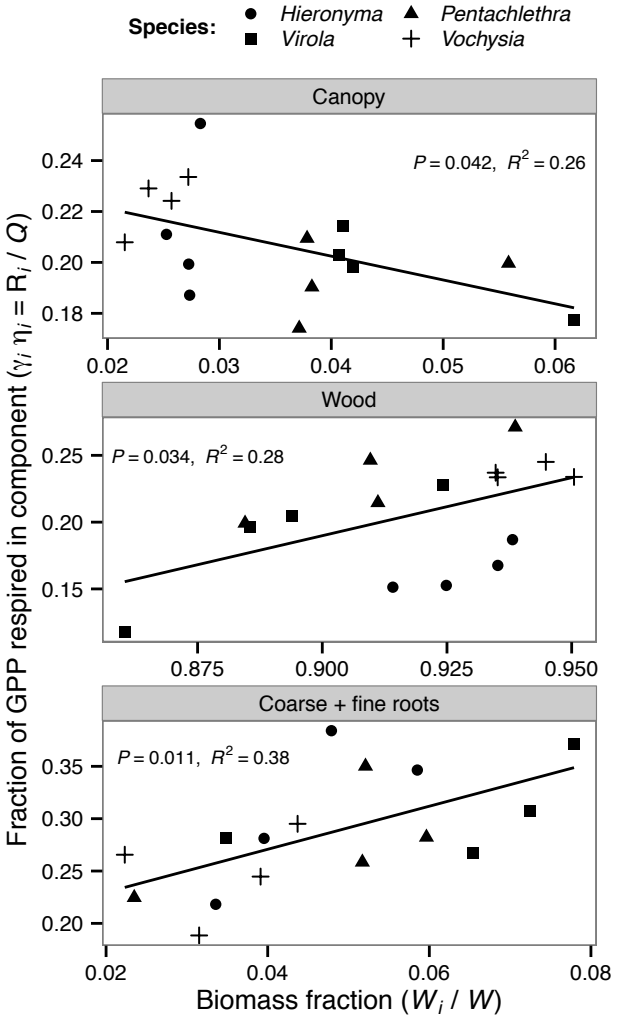

Figure 3.7. Biomass fraction was related to respiratory flux for all components. Regression lines were $y = 0.24 – 0.94x$ for canopy, $y = -0.59 + 0.87x$ for wood, and $y = 0.19 + 2.1x$ for coarse + fine roots.

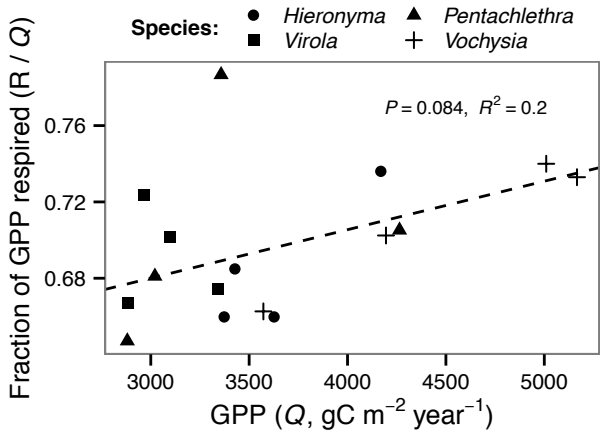

Figure 3.8. Relationship between GPP and fraction of GPP respired for the whole stand, fitted to a predicted relationship using Eqn. 1 and 2, and observed increase in fraction of component flux respired for canopy (from Fig. 3.3, dotted line). Whole stand fraction of GPP respired may have

Chapter 4

Carbohydrate regulation of photosynthesis and respiration from girdling in four trees species of a tropical rain forest

Introduction

Plants are thought to balance their carbon budget with feedback from carbohydrate storage (Paul and Foyer 2001, Fatichi et al. 2014), but the controls and mechanisms at whole plant scale remain unclear. The simplest view is that photosynthesis (source) supplies carbohydrates for metabolism and growth (sink), and the difference between source and sink fluxes determines the size of the storage pool (Chapin et al. 1990, Körner 2003). Clearly the storage pool cannot increase or decrease infinitely, supporting the idea that the pool size must play a role in the feedback regulation of source and sink fluxes.

In the feedback regulation, the storage pool size may increase or decrease photosynthesis (Boussingault 1868, Ewart 1896, Paul and Foyer 2001), and respiration may remove excess carbohydrates when the storage pool becomes too large (Lambers 1982, Cannell and Thornley 2000, Amthor 2000). This feedback regulation has gained attention as a way to increase crop yields (Cui et al. 2003, Reynolds et al. 2005, Ainsworth and Bush 2011), a mechanism that dampens plant response to higher CO_2 in the atmosphere (Körner 2003), and a different perspective for modeling plant growth and ecosystem biogeochemistry (Génard et al. 2008, Yin and Struik 2010, Nikinmaa et al. 2013, Fatichi et al. 2014). However, the storage pool serves as a source of carbohydrates during night and seasonal dormancy, and the pool size likely fluctuates before triggering any regulation. The changes in storage pool required to trigger the feedback

regulation of photosynthesis or respiration remain unquantified, and thus their generality and importance in ecosystem processes are still unknown.

The mechanism of carbohydrate regulation of photosynthesis is well documented at the cellular level, with pathways identified that both increase and decrease photosynthesis through changes in biochemistry and stomatal behavior. When experimental manipulations increase sucrose and starch concentrations in leaves, Rubisco and other Calvin-cycle enzymes decrease, and the rates of RuBP regeneration, carboxylation and electron transport decline (Stitt et al. 1991, Goldschmidt and Huber 1992, Krapp and Stitt 1995). Carbohydrate levels also control the expression of photosynthetic and phloem transport genes (Sheen 1990, Krapp et al. 1993, Koch 1996, Chiou and Bush 1998). Stomata may be involved, where accumulating carbohydrates causes stomatal closure perhaps to optimize carbon gain and water use (Mäkelä 1996, Nikinmaa et al. 2013). However, carbohydrate regulation of photosynthesis at cellular level has been tested mostly *in vitro* with high levels of carbohydrate concentration (Paul and Foyer 2001). It has been field tested in some crop, but only on a few fruit trees and even less on non-agricultural trees (Sweet and Wareing 1966, Herold 1980, Harrell and Williams 1987, Myers et al. 1999, Urban and Alphonsout 2007, Domec and Pruyn 2008, Nebauer et al. 2011).

The removal of excess carbohydrate in respiration is thought to occur through alternative electron pathway, but the role of alternative pathway and whether such waste respiration occurs remain speculative becasue alternative pathway activity is difficult to measure (Lambers et al 2008). Ubiquitous in plant mitochondria, the alternative pathway diverges from the cytochrome pathway—the primary energy-producing path—at the electron transfer from ubiquinone to O_2, and so bypasses proton pumping for ATP production (Rasmusson et al. 2004). The alternative pathway produces a third of ATP that the cytocrome pathway does (Lambers et al. 2008). The

alternative pathway is often theorized as a mechanism to remove excess carbohydrates as waste respiration (Lambers 1982, Millar et al. 1998, Cannell and Thornley 2000, Amthor 2000). Alternative path activity is difficult to measure directly *in situ* however, and the ecophysiological significance of the alternative pathway and whether waste respiration occurs in nature remain ambiguous (Lambers et al. 2008).

A common method of testing carbohydrate regulation of photosynthesis and respiration uses girdling to sever phloem and accumulate carbohydrates in leaves. Girdling stops the export of carbohydrates out of the leaf while leaving xylem intact for water and nutrient transport and for photosynthesis to continue (Noel 1970). Carbohydrates should then accumulate in the leaf, and simulate a carbon imbalance where source activity exceeds sink activity. Physiological changes are then tracked, often through measurements of leaf gas exchange, and carbohydrate accumulation is quantified by measuring the concentration of simple sugars and starch (non structural carbohydrates, NSC) in the leaves harvested at the end of the experiment. Studies have used girdling for studies of carbohydrate feedback (Myers et al. 1999, Iglesias et al. 2002, Urban and Alphonsout 2007, Cheng et al. 2008, Domec and Pruyn 2008, De Schepper et al. 2010, Fan et al. 2010, Nebauer et al. 2011).

We tested carbohydrate regulation of photosynthesis and respiration using branch girdling on four tree species in a tropical rainforest of Costa Rica. For branches in the upper canopy, we girdled fully to stop carbohydrate export, incompletely in quarter fractions to reduce export by degree, and surrounded an intact branch with girdled ones to increase export. We hypothesized that increased girdling intensity would 1) increase leaf carbohydrate content, 2) decrease photosynthesis rate inversely proportional to carbohydrate content, and 3) increase respiration rate proportional to carbohydrate content. Fig. 4.1 shows our hypothesis.

Methods

Study site

We conducted this study at La Selva Biological Station, in the Atlantic lowlands of Costa Rica (10°26′N, 83°59′N), with climate categorized in the Holdridge system as Tropical Wet Forest (McDade 1994) and mean annual rainfall of 4000 mm and temperature of 26 °C, with precipitation averaging > 100 mm each month. The measurements were taken from June to Septemeber in 2009 and from January to June in 2010. Rainfall was ~4500 mm/yr and the temperature 25°C for both 2009 and 2010. The soil is an acidic, highly leached, organic matter rich oxisol classified as Mixed Haplic Haploperox (Kleber et al. 2007). The native vegetation is broad-leaved evergreen tropical rainforest.

The site is part of a larger study examining tree species effects on ecosystem processes (ECOS, http://www.nrem.iastate.edu/ECOS/home, (Russell et al. 2010, Russell and Raich 2012). The site was cleared of primary forest and converted to pasture in 1956 then grazed until 1987. In 1988, experimental plantations were established with eleven tree species and unplanted control, replicated over four blocks in a randomized complete block design (Fisher 1995). Understory plants were cleared during plantation establishment and for three years afterwards, but then allowed to regenerate naturally. Plots were 50 x 50 m (0.25 ha), with a single-tree species planted in each plot except for the unplanted control. By 2008, only four tree species had survival adequate for whole-plot measurements, and these species were the subjects of this study.

The four species were *Hieronyma alchorneoides* Allemao (HIAL), *Pentaclethra macroloba* (Willd.) Kunth. (PEMA), *Virola koschnyi* Warb. (VIKO), and *Vochysia guatemalensis* Donn. Sm. (VOGU). All are native to the surrounding primary forest, and *Pentaclethra* is the dominant species of canopy trees at La Selva and the only N-fixing species of

the four. By 2008, each species had formed a stand with aboveground biomass similar to the surrounding forest (~8500 gC m^{-2} mean total aboveground biomass, (Russell et al. 2010). The stands were growing fast at aboveground net primary productivity of ~1200 gC m^{-2} $year^{-1}$ (Russell et al. 2010). Further details on site history and it's carbon and nitrogen cycle characteristics can be found in Fisher (1995), Russell et al (2010), and Russell and Raich (2012).

The girdling treatments were made on the branches of upper canopy, in one block per species in 2009 and in another block per species in 2010. The branches were 2-4 cm in diameter supporting 5-10 fully expanded sun leaves in the upper canopy, and accessed from a 30 m portable scaffolding tower (Upright Inc., Dublin, Ireland).

Treatments

We varied girdling intensity generate a gradient of phloem export rate. We girdled in quarter increments (0, ¼, ½, ¾, and full) by stripping bark and phloem in a band 1.5 cm wide near the proximal end of the branch. We also surrounded an intact branch with completely girdled ones, to increase demand for phloem export for the target branch. Branches not immediately connected to the girdled ones served as controls. For 2009 measurements, complete girdle and control treatments were established on four branches per treatment for each species; in 2010, all treatments were made on three to four branches per treatment for each species.

Photosynthesis and stomatal conductance measurements were made periodically for six to 20 days after treatment for all replicates on fully expanded leaves. Branches were then cut under water, transported with cut ends in water to the lab, measured for respiration and leaf area, and dried for quantification of leaf mass per area and NSC. A few branches did not survive

through the end of the photosynthesis measurements because of wind damage or herbivory by leaf cutter ants.

Measurements of response variables

Photosynthesis and stomatal conductance were measured as a light saturated rate, and photosynthetic capacity assessed as the response to CO_2 concentration (*A*-*C*i curve). Measurements were made on five fully expanded leaves per branch with an open-system portable IRGA (LI-6400, LI-COR, Inc., Lincoln, Nebraska, USA). Light saturated photosynthesis rate and stomatal conductance were measured under a reference CO_2 of 390 μmol mol^{-1}, at an air flow rate of 500 μmol s^{-1}, and with a saturating level of photosynthetic photon flux density of 2000 μmol m^{-2} s^{-1} after an acclimation time when the readings stabilized. Because the site was fairly remote and rain was frequent, we could not control for measurement time of day, temperature, and humidity. Measurements were made between 07:00 and 16:00, under temperature ranging from 24.5–39°C. Measurements were excluded from the analysis if vapor pressure deficit at the leaf surface exceeded 3 kPa. Response of photosynthesis rate to CO_2 concentration (A-C_i curve) was taken to estimate maximum rates of caboxylation (V_{cmax}) and electron transport (J_{max}), on a subset of foliage by varying the reference CO_2 concentration (400, 300, 200, 100, 50, 400, 600, 800 μmol mol^{-1}). A subset of *Hieronyma* leaves were measured for mid day leaf water potential using a pressure bomb.

Response of foliar respiration was measured using a chamber made from clear polycarbonate connected to an IRGA, on detached foliage at night. The branches were cut underwater in the afternoon, placed in a floral tube with water without exposing the cut surface to air, and the cut surface kept in water until after respiration measurement. The foliage was

taken back to the lab and measured at night between 20:00 and 02:00 in the dark and under ambient temperature. Detached and attached foliage had similar respiration rate in a previous study at La Selva (Cavaleri et al. 2008) and in several other studies (Mitchell et al. 1999, Turnbull et al. 2005). The foliage was placed inside the chamber and sealed with neoprene gaskets, and the seal checked with a flow meter. The chamber was 1580 mL in volume, and the air inside was mixed with a small fan. The chamber was connected to an infrared gas analyzer (IRGA): open-system LCA-3 (Analytical Development Company, Hoddeson, UK) for 2009 measurements, or a lab-built closed-system with Li-820 (Li-COR, Inc., Lincoln, Nebraska, USA) and CR10X data logger (Campbell Scientific, Logan, Utah, USA) for 2010 measurements. The open-system IRGA drew ambient air from a 19 L mixing container to maintain stable concentration of reference CO_2 during measurements. The airflow rates through the chamber ranged between 270–340 μmol s^{-1}. Both instruments were regularly calibrated with a CO_2 standard. Foliar respiration rates measured at ambient temperature were standardized to 25°C using estimated Q_{10} specific to each species from a previous study (*Hieronyma* = 1.6, *Pentaclethra* = 2.6, *Virola* = 1.6, *Vochysia* = 1.8; Chapter 1).

Carbohydrate response was quantified by measuring NSC content, leaf mass per area (LMA), and C and N contents of foliage dried immediately after respiration measurements. The foliage was measured for leaf area with a leaf area meter (LI-3100, LI-COR, Inc), dried for 48 hours at 65 °C and measured for leaf dry mass, ground to a fine powder, and measured for leaf N and C with a C-N analyzer (TruSpec CN, LECO, Inc., St. Joseph, Michigan, USA). The foliage was measured for NSC content using an enzymatic assay (Wong 1990, Hoch et al. 2002). Briefly, ~2 mg of powdered leaf was extracted with 0.75 mL distilled water at 120 °C in closed centrifuge tubes fitted with silicone O-rings for three hours for starch, and separate ~4 mg

powder was extracted with 1.5 mL distilled water at 100 °C for an hour for glucose and fructose. Glucose and fructose were enzymatically converted to gluconat-6-phosphate on a 96-well plate and measured photometrically. Sucrose was hydrolyzed to glucose and fructose using invertase at 40 °C for an hour, and starch to glucose using amyloglucosidase at 40 °C overnight. Resulting monosaccharides were measured as described above, and concentration calculated by subtracting free glucose and sucrose concentrations. All values of NSC content are expressed as g glucose equivalent per unit leaf area, and C and N content values are expressed as g per unit leaf area.

We used a leaf area basis to express the values instead of the more common dry mass basis because leaf area is constant in fully expanded leaves (barring herbivory), while leaf mass will change if carbohydrates accumulate or are depleted. Concentrations, mg NSC g^{-1} dry mass for example, will underestimate the accumulation of NSC because both the numerator and the denominator will increase during accumulation. In addition, both photosynthesis and respiration are expressed on leaf area basis, and a common denominator for x and y simplifies the interpretation of regressions. For example, NSC content in g m^{-2} (x/a) regressed against photosynthesis in μmol CO_2 m^{-2} s^{-1} (y/a) will yield a slope of photosynthesis per NSC in μmol CO_2 g^{-1} s^{-1} (y/x), but NSC concentration in mg g^{-1} (x/b) regressed against photosynthesis μmol CO_2 m^{-2} s^{-1} (y/a) will yield a slope of μmol CO_2 g dry mass g^{-1} NSC s^{-1} (yb/xa). Thus the slope yb/xa is directly proportional to y/x only if b/a remains constant over the range of y and x, and in cases of leaf area (a) and mass (b), b/a is expected to change as carbohydrates accumulate or deplete.

Statistical analysis

The response variables were compared among girdling intensity and species in a linear model ANOVA, followed by pair-wise contrasts between treatments and control with Tukey-Kramer multiple comparison adjustment at experiment-wise $\alpha = 0.05$. We also used a linear model ANCOVA with girdling intensity as covariate to assess trends in the response variables. Analyzing the 2009 and 2010 data separately and combined yielded similar significance, and we present the result of the combined analysis. Photosynthesis rate, stomatal conductance, and foliar respiration rate were further analyzed with a linear model ANCOVA with three predictor variables, treatment, species, and either LMA, C, N, or NSC contents as a covariate. All analysis were done in R (R Core Team 2014), with multcomp (Hothorn et al. 2008), and MASS (Venables and Ripley 2002) packages, and plotted with ggplot2 package (Wickham 2009).

Results

Hypothesis 1: Increased girdling intensity will increase leaf carbohydrate content

Girdling intensity created no clear or consistent trends in leaf carbohydrate and N content (Table 4.1, Fig. 4.2). Though girdling intensity was significant ($P = 0.03$) in the ANOVA model for glucose and fructose content, no pair-wise comparison of treatment to control showed significant differences in LMA, C, N, glucose and fructose, starch, or NSC contents whether compared among or within species. Sucrose content was near zero for all samples and not shown. Species showed diverging trends in treatment means (Fig. 4.2). Girdling intensity slightly increased LMA and C content in *Virola* (ANCOVA with girdling intensity as covariate, $P = 0.03$ and 0.02 respectively, Fig. 4.2), and marginally decreased N content in *Hieronyma* and increased in *Virola* (ANCOVA, $P = 0.1$ and 0.02, Fig. 4.2). Measures of carbohydrate contents

were well related to each other, but less for starch (C with glucose and fructose, $P < 0.01$, $R^2 = 0.84$; C with LMA, $P < 0.01$, $R^2 = 0.98$; C with starch, $P < 0.01$, $R^2 = 0.41$; glucose and fructose with starch, $P < 0.01$, $R^2 = 0.47$).

Hypothesis 2: Increased girdling intensity will decrease photosynthesis inversely proportional to carbohydrate content

Increased girdling intensity decreased photosynthesis only under full girdling ($P < 0.01$), while *Vochysia* did not respond at all (Fig. 4.3). By the end of treatment duration, full girdling reduced light saturated photosynthesis rate to 31 – 41% of control in *Hieronyma* (4.1 under full girdle vs 10 μmol CO_2 m^{-2} s^{-1} for control), *Pentaclethra* (1.8 vs 13 μmol CO_2 m^{-2} s^{-1}), and *Virola* (1.8 vs 13 μmol CO_2 m^{-2} s^{-1}), but remained similar in *Vochysia* (10 under full girdle vs 11 μmol CO_2 m^{-2} s^{-1} for control; all at $P < 0.05$ in pair-wise contrast with control, Fig. 4.3). Photosynthesis rate started to decline on second day under full girdling, continued to decline to fourth day, and remained low thereafter. Other girdling intensities created no trend in photosynthesis rate (ANCOVA without full girdle, $P = 0.90$; no significance in pair-wise contrasts), except for surround girdling in *Vochysia* (at $P < 0.05$ in pair-wise contrast with control, Fig. 4.3). The decline in photosynthesis rate was accompanied by a decline in photosynthetic capacity, with V_{cmax} and J_{max} declining only under full girdling and in three species, similarly to photosynthesis. By the end of the treatment duration, V_{cmax} and J_{max} declined to 8.2 – 24 % of control in *Hieronyma* (12 vs 50 μmol CO_2 m^{-2} s^{-1} for V_{cmax}; 27 vs 135 μmol CO_2 m^{-2} s^{-1} for J_{max}), *Pentaclethra* (3.1 vs 38 μmol CO_2 m^{-2} s^{-1}; 7.4 vs 129 μmol CO_2 m^{-2} s^{-1}), and *Virola* (6.6 vs 42 μmol CO_2 m^{-2} s^{-1}; 15 vs 114 μmol CO_2 m^{-2} s^{-1}), but remained similar in *Vochysia* (29 vs 38 μmol CO_2 m^{-2} s^{-1}; 58 vs 86 μmol CO_2 m^{-2} s^{-1}; all at $P < 0.05$ in pair-wise

contrasts). Girdling at less than full had no effect on intercellular concentration of CO_2 (P = 0.21).

Stomatal conductance declined in concert with the decline in light saturated photosynthesis rate for full girdle treatments. The decline in stomatal conductance for the full-girdle treatments started within the first five days and remained low until the end of the treatment ($P < 0.01$). The decline was 17 – 26% of control in *Hieronyma* (0.074 under full girdle vs 0.31 mol H_2O m^{-2} s^{-1} for control), ~17% of control for *Pentaclethra* (0.033 vs 0.19 mol H_2O m^{-2} s^{-1}) and 25% of control for *Virola* (0.11 vs 0.42 mol H_2O m^{-2} s^{-1}); *Vochysia* showed no decline (0.21 vs 0.28 mol H_2O m^{-2} s^{-1}; all at $P < 0.05$ in pair-wise contrasts). The decline in stomatal conductance was unlikely to have been an artifact of girdling decreasing leaf water potential, because for the one species measured (*Hieronyma*), leaf water potential was unaffected by full girdling at the end of leaf measurements. Stomatal conductance remained unchanged under partial girdling in all species (ANOVA without full girdle, P = 0.77).

Patterns in photosynthesis varied with leaf C, starch and N content (Fig. 4.4), but the patterns differed for the full girdling versus all other treatments. For all treatments except for full girdling, photosynthesis increased with C, starch, and N content per unit leaf area ($P < 0.01$). For the full-girdling treatment, photosynthesis decreased as C, starch, and N content increased ($P < 0.01$). Photosynthesis was unrelated to glucose + fructose content (P = 0.24).

Hypothesis 3: Increased girdling intensity will increase respiration proportional to carbohydrate content

Respiration rate decreased with girdling intensity for *Pentaclethra*, but was unaffected by any treatment for the rest of the species (P = 0.31, Fig. 4.5). Respiration rate differed among

species and averaged 0.77, 0.66, 0.76, and 0.88 μmol CO_2 m^{-2} s^{-1} for *Hieronyma*, *Pentaclethra*, *Virola*, and *Vochysia* ($P < 0.01$). Respiration rate increased with N in three species ($P < 0.01$) and marginally in *Vochysia* ($P = 0.06$). Respiration rate increased with glucose + fructose and C content in *Hieronyma* and *Virola*, but decreased with C content in *Pentaclethra* and *Vochysia* ($P < 0.01$). Foliar respiration increased as starch content increased in *Hieronyma* ($P < 0.01$), but was unrelated to respiration in the three other species ($P > 0.18$).

Discussion

The results did not support our hypotheses (Fig. 4.6). None of the predicted responses (carbohydrate concentrations, photosynthesis and photosynthetic capacity, respiration) varied with girdling intensity. Only full girdling prompted lower photosynthesis and photosynthetic capacity in three of four species, but this response was unaccompanied by increased carbohydrate content or respiration. For possible causes of these patterns, we examine our hypothesis and results, and outline implications for further study on carbohydrate storage feedbacks in regulating tree carbon balance.

Phloem transport and sink activity tightly regulate leaf carbohydrate content

The results did not support our hypotheses that girdling intensity would trigger carbohydrate accumulation in leaves (Fig. 4.1). Full girdling severs phloem and dramatically decreases carbohydrate export (Noel 1970) as confirmed by studies that examine the connectivity of photosynthesis and soil CO_2 efflux (Hogberg et al. 2001, Andersen et al. 2005, Olsson et al. 2005, Binkley et al. 2006, Frey et al. 2006, Levy-Varon et al. 2012). However, leaf carbohydrates remained unchanged even with $3/4^{th}$ of phloem removed, and were

unaccompanied by any adjustment of C input (photosynthesis) or C loss (respiration). Even with full girdling, one species, *Vochysia*, showed no response in any of the measured variables. These results suggest that leaf carbohydrate content is tightly regulated, not only by photosynthesis, but also by some combination of sink activity and phloem transport.

The partial girdling results suggest that the path of phloem transport can change to bypass blockage and maintain phloem export and carbohydrate content in the leaf. In terminal branches, each functioning phloem sieve tubes likely connect to minor veins of leaves. The phloem sieve tubes directly connected to a leaf may conduct most of the phloem export out of the leaf, where carbohydrates flow in a path restricted to the vertical section extended down from the petiole. This sectorial phloem transport has been confirmed in herbaceous plants (Watson and Casper 1984, Marshall 1996, Vuorisalo and Hutchings 1996, Fetene et al. 1997, Preston 1998) and in an oak (De Schepper et al. 2013a). Each leaf thus exports carbohydrates through a specific subset of sieve tubes surrounding a branch, and the leaf loses the primary transport conduit when those specific sieve tubes no longer functions. Carbohydrates may then accumulate in the leaf, or get re-routed to another set of sieve tubes that serve a different leaf. In latter case, carbohydrate levels likely remain unchanged along with photosynthesis and foliar respiration, and our results support this interpretation. De Schepper et al (2013) also observed that partial girdling rerouted the sectorial phloem flow in Oak, and speculated that the mechanism for this re-routing may be the leakage and retrieval system along a phloem path. Along a phloem transport path to a terminal sink, carbohydrates continually flow out from phloem to feed lateral sinks, and a portion is reloaded back on to phloem (Thorpe and Lang 1983, van Bel 2003, De Schepper et al. 2013b). This leakage and retrieval system may allow carbohydrates to flow laterally among phloem tubes, allowing for re-routing of phloem transport in case of obstruction. The retrieval occurs

mainly through active loading pathways (Patrick et al. 2001), and should lead to higher respiration rate of branches above the partial girdle.

As phloem transport seemed to have rerouted through 1/4th of original quantity of sieve tubes, our results also suggest that each phloem sieve tubes have high conductivity, capable of supporting transport load much grater than the leaf directly connected can generate. De Schepper et al (2013) observed reduced phloem flow rate under partial girdling, but we detected no evidence of reduced transport rate. The exception may be *Virola*, in which leaf carbohydrate content slightly increased with girdling intensity. In contrast, leaf carbohydrate content remained unchanged even after 20 days under 3/4th girdling in *Hieronyma*, suggesting that species may differ in phloem conductivity.

The results from the partial girdling treatments further suggest that the species tested regulate phloem loading to maintain phloem transport rate. Though debated for trees (Thompson 2006, Mencuccini and Hölttä 2009, Knoblauch and Peters 2010, Jensen et al. 2011), phloem transport is generally accepted to be driven by hydrostatic pressure: carbohydrates are loaded on to phloem sieve tubes for water to follow by osmosis, generating turgor pressure that drives transport downstream (Münch 1930, van Bel 2003, Jensen et al. 2011). Thus transport rate depends on phloem carbohydrate concentration gradient, generated by phloem loading. That leaf carbohydrates did not accumulate even with 3/4th of phloem severed in this study indicates that phloem transport rate was maintained – through considerably fewer phloem sieve tubes – by generating much greater turgor pressure. Thus phloem loading likely increased with girdling intensity to maintain phloem transport rate and leaf carbohydrate content.

It is unclear which phloem loading mechanisms are responsible however. The strategies for phloem loading vary among species (Rennie and Turgeon 2009), but are either passive or

active. In phloem loading, carbohydrates flow from mesophyll to companion cells surrounding phloem sieve tubes and then to phloem sieve tubes. Carbohydrates diffuse from mesophyll to companion cells in passive loading, whereas in active loading, membrane sugar transporters or sugar concentrating steps use energy and force carbohydrates from mesophyll into companion cells. Woody species tend to be passive loading (Gamalei 1991, Rennie and Turgeon 2009, Fu et al. 2011), while *Pentaclethra* belongs to Fabaceae that includes some species with active loading strategies (Fu et al. 2011). Active loading species tend to have lower soluble sugar (transport molecules) and higher starch (a storage molecule) concentrations than passive loading species (Rennie and Turgeon 2009, Fu et al. 2011). Glucose + fructose concentration was indeed lower in *Pentaclethra* than in other species (16 compared to 47 mg g^{-1}, $P < 0.01$), but starch was also low in *Pentaclethra* (26 compared to 75 mg g^{-1} in *Hieronyma*, 42 in *Virola*, and 26 in *Vochysia*). Sucrose, a primary transport molecule, is typically high for passive loading species (Fu et al. 2011), but was near zero in all of the species of this study. If the species were active loading, the metabolic activity should have been high at the loading site between leaf mesophyll and minor veins, but foliar respiration rate remained similar under all girdling intensities. It is thus difficult to parse which species use what loading strategies.

The lack of response in *Vochysia* to full girdling suggests that the sink activity elsewhere may also regulate phloem transport rate and leaf carbohydrate content. Full girdling in *Vochysia*, and perhaps partial girdling in all species, may have increased sink activities in the branch section distal to the girdle, as some combination of enhanced growth (De Schepper et al. 2010), respiration (Wang et al. 2006, Johnsen et al. 2007, Domec and Pruyn 2008), or carbohydrate storage (Daudet et al. 2005, Cheng et al. 2008, De Schepper et al. 2010). Apical growth may have increased as well, though branches supporting sun leaves are typically a net exporter of

carbon. These responses require the conversion of transport sugars into some other form at the site of phloem unloading. Phloem unloading appears to be a passive process (Patrick and Offler 1996, Patrick et al. 2001), and thus the concentration of transport sugars must decrease in tissues surrounding phloem sieve tubes to increase phloem unloading. This is done by converting transport sugars to some other form, such as glucose or starch, for metabolism or storage. Such conversion may be strongly regulated, and this view is supported by the observations that gene expression for sugar metabolism increased in girdled fruit trees (Nebauer et al. 2011), and phloem sucrose concentration and osmotic pressure remained unchanged above a cold girdle that reduced phloem flow (Gould et al. 2004). It is thus possible that the branches above the girdle increased in sink activity and may have turned into a storage organ, especially in *Vochysia*. Photosynthesis rate under girdling can remain high if significant sink organs are present (Legros et al. 2009, Nebauer et al. 2011).

The active regulation of leaf carbohydrate content likely does not involve waste respiration in the leaf, but any response in respiration is difficult to interpret because metabolic activity changes with phloem transport, storage, or growth. Carbohydrate concentration affects foliar respiration rate only sometimes, in soybean and amaranth under high temperature (Bunce 2007), but not in Mango (Urban and Alphonsout 2007, Urban et al. 2010) and in the four species of this study. In species with active loading strategies, higher foliar respiration rate may reflect the metabolic requirement for phloem loading only and not necessarily wastage respiration. Higher branch respiration may also reflect increased active reloading along phloem path or enhanced conversion of transport sugars.

Full girdling may affect photosynthesis through changes other than carbohydrate accumulation

The complex and interrelated responses to girdling raise the possibility that unintended factors caused the decline in photosynthesis. Because girdling severs phloem, it stops the export of not just carbohydrates but of all materials including auxin, abscisic acid, and reactive oxygen series (Mahouachi et al. 2009, Turgeon and Wolf 2009, Turnbull and Lopez-Cobollo 2013). They may have accumulated simultaneously yet independently of carbohydrates, directly causing stomatal closure, which in turn reduced measured photosynthesis rate (Setter et al. 1980, Harrell and Williams 1987, Roper and Williams 1989). In some girdling studies, photosynthesis declined before carbohydrates accumulated in citrus (Nebauer et al. 2011), only under high temperature and independently of carbohydrate accumulation in young apple (Fan et al. 2010), and not at all in oil palm (Legros et al. 2009).

The reduction in stomatal conductance we observed is unlikely to have come from decreased xylem hydraulic conductivity. The reduction was not immediate, occurred only under full girdling, and not at all in *Vochysia*. Internal concentration of CO_2 did not change. Though only measured on *Hieronyma*, midday leaf water potential increased under full girdling, reflecting the stomatal closure (-0.74 MPa under full girdling and -1.1 MPa under control; P = 0.02). Previous girdling studies observed similar reduction in stomatal, leaf conductance, or transpiration rate (Harrell and Williams 1987, Proietti 2003, Franck et al. 2006, Urban and Alphonsout 2007, Wu et al. 2008, Domec and Pruyn 2008, Fan et al. 2010, Urban et al. 2010, Nebauer et al. 2011). They attributed the cause to assimilate accumulation because girdling had no effect on predawn and midday water potential or leaf water content (Proietti 2003, Franck et al. 2006, Domec and Pruyn 2008), or because girdling had no effect on internal concentration of CO_2 (Harrell and Williams 1987, Proietti 2003, Urban 2004, Urban and Alphonsout 2007, Wu et

al. 2008, Fan et al. 2010). These studies suggest that stomata respond to decreased photosynthesis from assimilate concentration, to optimize carbon gain and water use (Mäkelä 1996, Nikinmaa et al. 2013). However, it remains unclear which factors, hormones, reactive oxygen series or carbohydrates, caused the decline in stomatal conductance and photosynthesis, and girdling alone cannot provide clear evidence.

Does leaf carbohydrate content indicate plant C balance?

Leaf carbohydrate content remained unchanged and well above zero at all girdling intensities, suggesting that leaf carbohydrate concentration maybe tightly regulated independent of demands elsewhere in the tree. Studies have used as evidence the presence or lack of change in NSC pool to infer carbon balance, for both negative (Lacointe et al. 2004, Palacio et al. 2012), and positive (Hoch et al. 2003, Chew and Bonser 2009, Sanz-Perez et al. 2009). Our results show that neither interpretation warrants confidence. It is unclear what the accumulation of leaf carbohydrates indicates when fully girdled leaves showed no change in carbohydrate content yet decreased photosynthesis, and when phloem transport proceeded seemingly unaltered with only 1/4 of sieve tubes intact.

Sink regulation of photosynthesis assumes a passive buildup to carbohydrates when sink activity declines, but this assumption weakens as more precise understanding emerges on the regulations of leaf carbohydrate content and phloem transport. *Arabidopsis* actively regulates leaf starch content diurnally to match day-time gain and night time use (Gibon et al. 2004, Scialdone et al. 2013), and accumulate carbohydrates when starved of C (Smith and Stitt 2007). Our results suggest that some tree species also tightly regulate carbohydrate content through phloem transport and sink activity. Sink regulation of photosynthesis may still occur, perhaps

through signaling pathways other than directly by carbohydrate accumulation, and understanding of such pathways would improve our ability to model and predict plant carbon balance.

Conclusion

Girdling intensities did not vary leaf carbohydrate content and respiration, and photosynthesis declined only under full girdling in three of four species. The results suggest that leaf carbohydrate content is highly regulated through phloem transport and sink activity elsewhere in addition to photosynthesis. The capacity for phloem transport may be high. Because it may induce physiological changes unrelated to carbohydrate accumulation, girdling may not be an effective method to study the link between carbohydrate storage, photosynthesis, and respiration, at least in some species. Leaf carbohydrate content may be decoupled from whole plant carbon balance and may not serve as a signal for sink limitation of photosynthesis.

Tables

Table 4.1. Predictor variable *P* values for ANOVA models of girdling intensity and species explaining either C, N, LMA, glucose and fructose, starch, or NSC (glucose, fructose, and starch). Values < 0.05 are in bold.

	Predictor variables		
Response variables	Girdling intensity	Species	Girdling × Species
C content (g C m^{-2})	0.13	**< 0.01**	0.75
N content (g N m^{-2})	0.76	**< 0.01**	0.58
LMA (g m^{-2})	0.12	**< 0.01**	0.78
Glucose+fructose (g m^{-2})	**0.03**	**< 0.01**	0.10
Starch (g m^{-2})	0.42	**< 0.01**	0.99
NSC (g m^{-2})	0.27	**< 0.01**	0.95

Figures

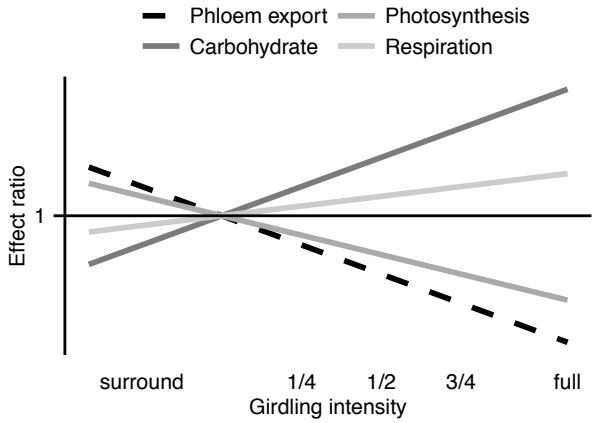

Figure 4.1. Graphical representation of hypothesis, showing the effect ratios of response variable to varying girdling intensity. Effect ratio is defined as treatment over control. We predict that increased girdling intensity decreases phloem export (dashed line), leaf carbohydrate content will increase (dark grey line), photosynthesis will decrease (grey line), and respiration will increase (light grey line). We measured the latter three (solid lines).

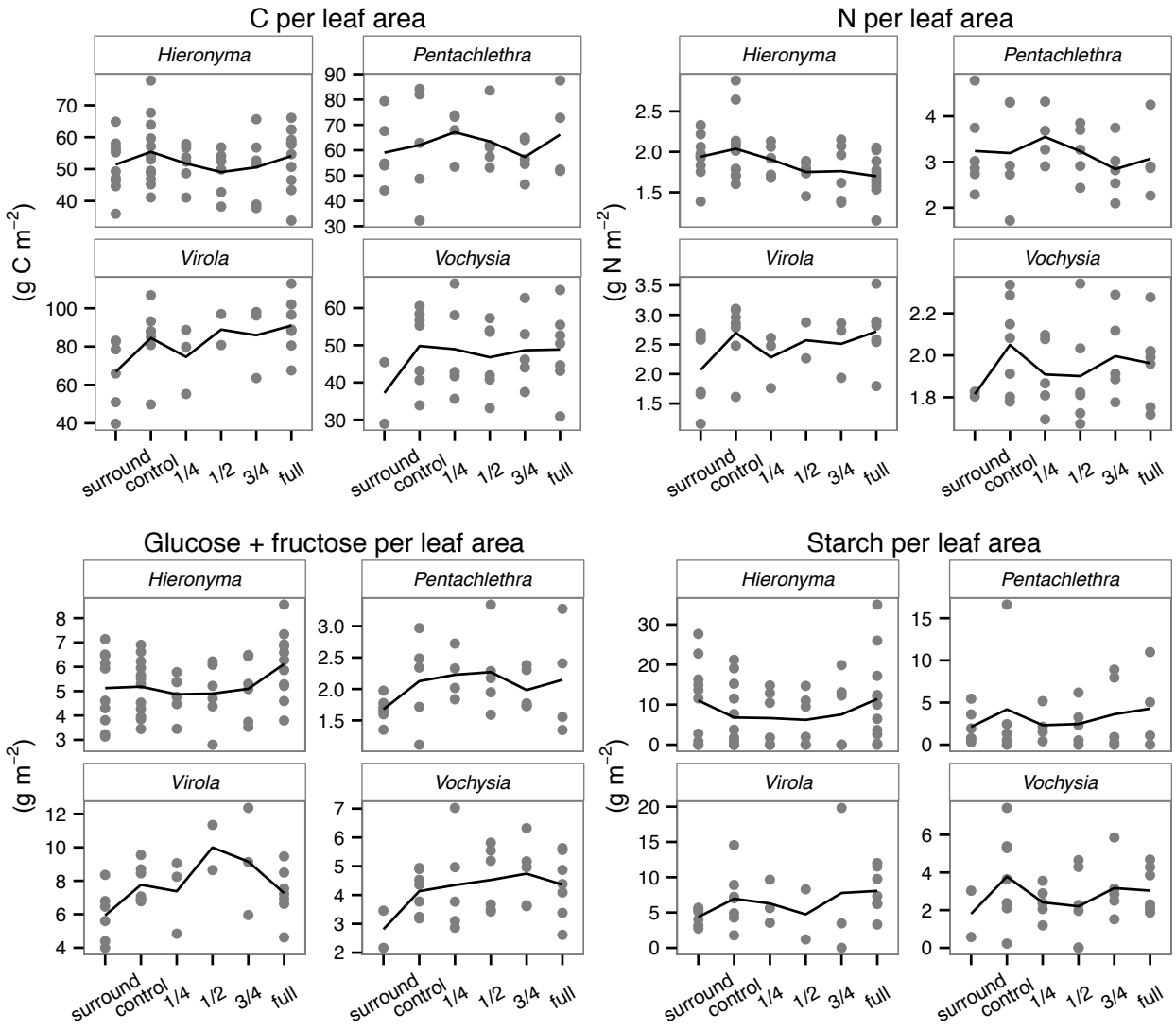

Figure 4.2. Effects of girdling treatments on C (g C m^{-2}), N (g N m^{-2}), glucose and fructose (g m^{-2}), and starch (g m^{-2}) contents on leaf area basis, showing that the increased girdling intensity created no clear trends in any species, except for N content per leaf area in *Hieronyma*, and C and glucose and fructose contents in *Virola*. Lines connect treatment means. Response of LMA was virtually identical to C content, with tight relationships between LMA and C content for each species ($P < 0.01$, $R^2 = 0.98$), and not shown here.

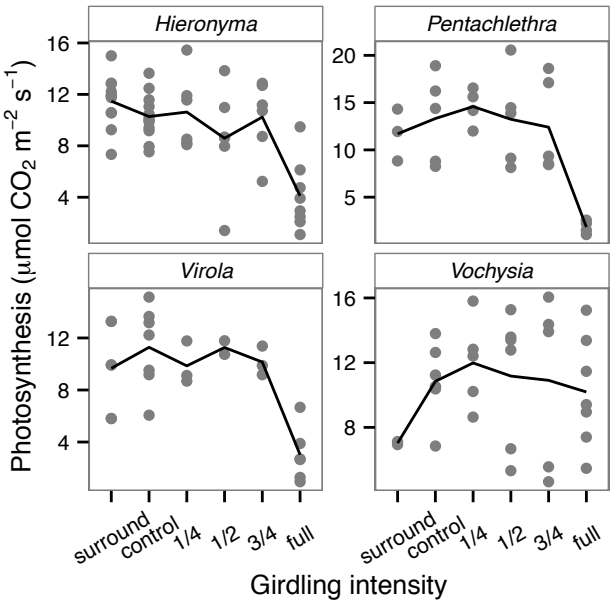

Figure 4.3. Response of light saturated photosynthesis rate to girdling intensity at the end of treatment duration. Girdling intensity decreased photosynthesis rate only under full girdling, in *Hieronyma*, *Pentaclethra*, and *Virola*, but not in *Vochysia*. Lines connect treatment means.

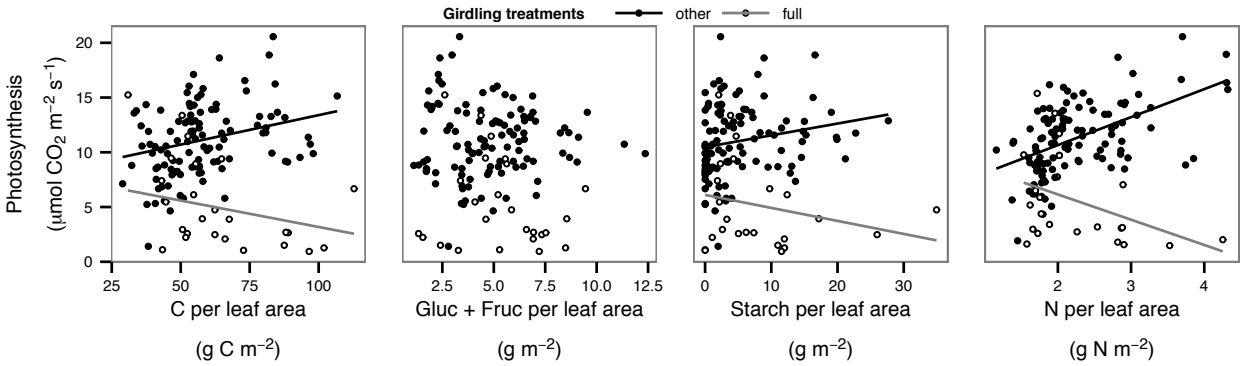

Figure 4.4. Correlations between light saturated photosynthesis rate and per leaf area contents of C, glucose + fructose, starch, or N show that full girdling reduced photosynthesis by mechanisms unrelated to leaf carbohydrate or N contents. Open circles and grey lines represent values and trend lines for full girdling intensity, and filled circles and black lines represent values and trend lines for other girdling intensities and control. Photosynthesis was unrelated to glucose and fructose content ($P = 0.24$). Photosynthesis decreased under full girdling but increased under other treatments with C (top right, $P < 0.01$, $R^2 = 0.39$; lines drawn with $y = -0.048x + 8.0$ for full girdling, and $y = 0.054x + 8.0$ for other treatments), starch ($P < 0.01$, $R^2 = 0.40$; $y = -0.12x + 6.1$ for full girdling, and $y = 0.11x + 10.5$ for other treatments), and N ($P < 0.01$, $R^2 = 0.47$; $y = -2.4x + 10.5$ for full girdling, and $y = 2.6x + 5.1$ for other treatments).

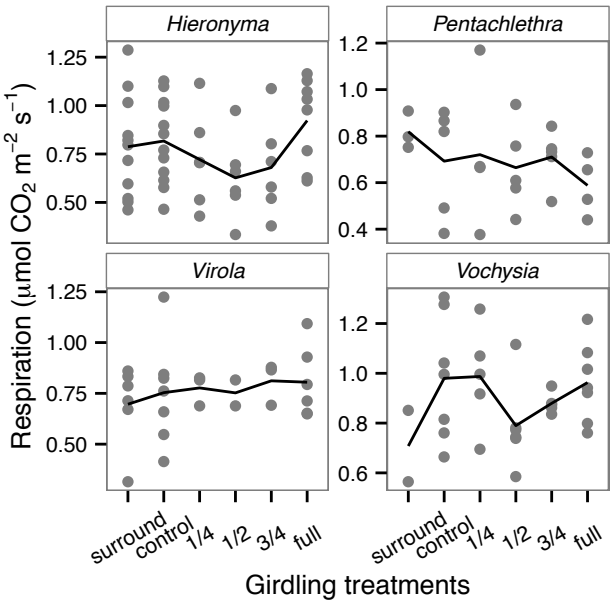

Figure 4.5. Foliar respiration shows no response to girdling treatments. Lines connect treatment means.

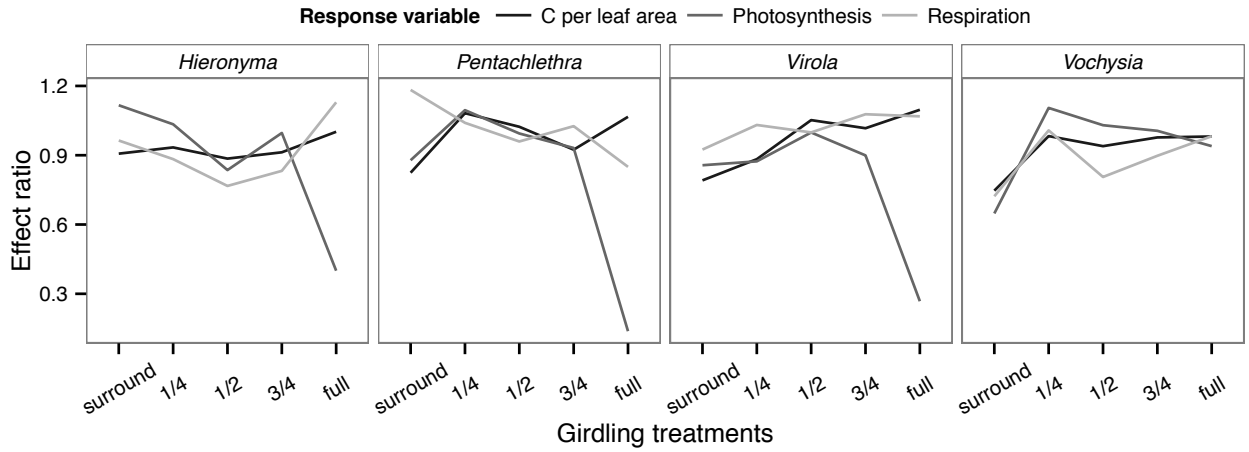

Figure 4.6. Summary of the effect ratios treatment to control for each response variables under girdling treatments shows that photosynthesis declined under full girdling disproportionately compared to other response variables under rest of the treatments, and

References

Chapter 5

Conclusions

This dissertation sought to construct a complete carbon budget to examine how species affect carbon stored in forest plant biomass in a tropical rainforest of Costa Rica. Planted in monoculture plots, four tree species have nearly two-fold difference in biomass after 17 years. In Chapter 2, I quantified the variation in leaf level measurements of foliar respiration and wood CO_2 efflux for scaling the measurements to the stand level estimates without bias. In Chapter 3, I estimated complete annual carbon budget to examine how species affect forest biomass carbon storage. In Chapter 4, I experimentally tested how carbohydrates regulate photosynthesis and respiration to balance whole tree carbon budget. This dissertation represents a step forward in understanding tree species effects on carbon cycling in tropical forests.

Constructing a complete carbon budget in detail requires scaling leaf-level measurements of respiration rates across space and time. This is a challenge ecologists face in tropical forests and elsewhere, and forms the basis of ecosystem biogeochemistry. Foliar respiration and wood CO_2 efflux rate can vary 20x in a stand (Sprugel et al. 1995), and unbiased estimates require characterization of the variation in rates within a stand. Diverse species and complex canopy structure further complicate scaling in tropical forests. In Chapter 2, I found that respiration rates vary vertically among canopy layers, and this variation was caused by a change in leaf thickness. Respiration rate varies very little among species, and since temperature fluctuates only slightly from season to season, respiration rate largely remained similar between seasons. The results show that the vertical sampling may be critical in tropical forests.

In Chapter 3, I constructed a complete and detailed carbon budget to examine how species affect biomass carbon storage at the forest scale. I found that species differences in GPP caused the differences in NPP, respiration, biomass storage, and storage rate. Gross primary production in turn increased with LAI. This suggests that ecosystem biogeochemistry models may use partitioning fractions estimated in this chapter to parameterize for simulations for monoculture stands in similar environments.

Of greater impact may be species difference in morphology and ontology. Species difference leaf thickness mostly explained the difference in LAI. Species had accumulated high LAI but declined in NPP since 2003 – 2005, suggesting that either GPP had declined or respiration had consumed increasingly greater proportion of GPP over time. Morphology is relatively easily to measure for parameterizing models, but assessing ontological change in carbon fluxes requires repeated and accurate measurements under logistically difficult conditions. A different approach may be required.

Whole stand respiration consumed 70% of GPP, higher than most forests measured (DeLucia et al. 2007, Litton et al. 2007). All species respired more than half of carbon flux to components, 63% in foliage, 63% in wood, and 81% in roots. Foliar respiration and wood CO_2 efflux rates from Chapter 2 were similar to those in the surrounding primary forest (Cavaleri et al. 2006, 2008), suggesting that the surrounding forest may also respire large proportion of GPP. If the respiration rate reflects the cost of maintaining biomass in high temperature (Reich et al. 2006), partitioning to respiration should be high elsewhere in the tropics and would increase as temperature raises. However, partitioning to respiration varies within the tropics (Malhi 2012), indicating that some other mechanism such as carbohydrate supply may contribute to the high

respiration rate. Rising temperature may cause respiration to consume a greater proportion of GPP at the expense of NPP, and the causes of high respiration deserves further investigation.

In Chapter 4, I examined the role of leaf carbohydrates in regulating photosynthesis and respiration to balance whole tree carbon budget. I used girdling in varying intensity to alter leaf carbohydrate content. I found that girdling did not change leaf carbohydrate at all, and photosynthesis decreased only under most extreme girdling intensity in three out of four tree species. Respiration did not change either. These results suggest that leaf carbohydrate content is tightly regulated by phloem transport and thus decoupled from whole plant carbon balance. The results also suggest that tree species may differ greatly in phloem transport.

Photosynthesis was relatively more sensitive than respiration to girdling treatments, suggesting that GPP may further decline as the stands age despite large LAI. The decline in NPP may increase whole tree carbohydrate storage, but likely not in foliage. As a result, respiration may increase in wood or roots, but again not in foliage. Leaf carbohydrates likely do not directly regulate GPP but perhaps indirectly through hormonal signaling, and ontogenetic control of GPP and respiration remains unclear.

Models may represent carbon budget differences among species in monodominant stands reasonably well with species differences in LAI and similar partitioning fractions, but projections requires better understanding of ontogenetic control of GPP and respiration. It is rather remarkable that LAI alone explained most of species differences in GPP, respiration, NPP, biomass storage, and storage rate. Species difference in leaf thickness caused much of the differences in carbon cycling but apparently not by species difference in phloem transport. Tree species in this environment may be constrained in partitioning of carbon fluxes.

Literature Cited

Agren, G. I., and B. Axelsson. 1980. Population respiration: A theoretical approach. Ecological Modelling 11:39–54.

Ainsworth, E. A., and D. R. Bush. 2011. Carbohydrate export from the leaf: A highly regulated process and target to enhance photosynthesis and productivity. Plant Physiology 155:64–69.

Amthor, J. S. 2000. The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. Annals of Botany 86:1–20.

Andersen, C., I. Nikolov, P. Nikolova, R. Matyssek, and K.-H. Häberle. 2005. Estimating "autotrophic" belowground respiration in spruce and beech forests: decreases following girdling. European Journal of Forest Research 124:155–163.

Asner, G. P., J. M. O. Scurlock, and J. A Hicke. 2003. Global synthesis of leaf area index observations: implications for ecological and remote sensing studies. Global Ecology and Biogeography 12:191–205.

Atkin, O. 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. Trends in Plant Science 8:343–351.

Atkin, O. K., D. Bruhn, V. M. Hurry, and M. G. Tjoelker. 2005. The hot and the cold: unravelling the variable response of plant respiration to temperature. Functional Plant Biology 32:87–105.

Aubrey, D. P., and R. O. Teskey. 2009. Root-derived CO_2 efflux via xylem stream rivals soil CO_2 efflux. New Phytologist 184:35–40.

Bates, D., M. Maechler, and B. Bolker. 2012. lme4: Linear mixed-effects model using S4 classes. http://CRAN.R-project.org/package=lme4.

Binkley, D., J. Stape, E. Takahashi, and M. Ryan. 2006. Tree-girdling to separate root and heterotrophic respiration in two Eucalyptus stands in Brazil. Oecologia 148:447–454.

Bolstad, P. V., K. Mitchell, and J. M. Vose. 1999. Foliar temperature-respiration response functions for broad-leaved tree species in the southern Appalachians. Tree Physiology 19:871–878.

Bouma, T. J., R. De Visser, P. H. Van Leeuwen, M. J. De Kock, and H. Lambers. 1995. The respiratory energy requirements involved in nocturnal carbohydrate export from starch-storing mature source leaves and their contribution to leaf dark respiration. Journal of Experimental Botany 46:1185–1194.

Boussingault, J. B. 1868. Agronomie, chimie agricole et physiologie. Second edition. Mallet Bachelier, Paris.

Bowman, W. P., M. M. Barbour, M. H. Turnbull, D. T. Tissue, D. Whitehead, and K. L. Griffin. 2005. Sap flow rates and sapwood density are critical factors in within- and between-tree variation in CO_2 efflux from stems of mature *Dacrydium cupressinum* trees. New Phytologist 167:815–828.

Braswell, B. H., D. S. Schimel, E. Linder, and B. Moore. 1997. The response of global terrestrial ecosystems to interannual temperature variability. Science 278:870–873.

Brooks, J. R., T. M. Hinckley, E. D. Ford, and D. G. Sprugel. 1991. Foliage dark respiration in *Abies amabilis* (Dougl.) Forbes: variation within the canopy. Tree Physiology 9:325–338.

Bunce, J. A. 2007. Direct and acclimatory responses of dark respiration and translocation to temperature. Annals of Botany 100:67–73.

Cannell, M. G. R., and J. H. M. Thornley. 2000. Modelling the components of plant respiration: Some guiding principles. Annals of Botany 85:45–54.

Cao, M., and F. I. Woodward. 1998. Dynamic responses of terrestrial ecosystem carbon cycling to global climate change. Nature 393:249–252.

Cavaleri, M. A., S. F. Oberbauer, and M. G. Ryan. 2006. Wood CO_2 efflux in a primary tropical rain forest. Global Change Biology 12:2442–2458.

Cavaleri, M. A., S. F. Oberbauer, and M. G. Ryan. 2008. Foliar and ecosystem respiration in an old-growth tropical rain forest. Plant Cell and Environment 31:473–483.

Cernusak, L. A., and J. D. Marshall. 2000. Photosynthetic refixation in branches of Western White Pine. Functional Ecology 14:300–311.

Ceschia, E., C. Damesin, S. Lebaube, J.-Y. Pontailler, and E. Dufrêne. 2002. Spatial and seasonal variations in stem respiration of beech trees (*Fagus sylvatica*). Annals of Forest Science 59:801–812.

Chambers, J. Q., E. S. Tribuzy, L. C. Toledo, B. F. Crispim, N. Higuchi, J. dos Santos, A. C. Araujo, B. Kruijt, A. D. Nobre, and S. E. Trumbore. 2004. Respiration from a tropical forest ecosystem: Partitioning of sources and low carbon use efficiency. Ecological Applications 14:S72–S88.

Chapin, F. S. 1989. The cost of tundra plant structures - Evaluation of concepts and currencies. American Naturalist 133:1–19.

Chapin, F. S., E. D. Schulze, and H. A. Mooney. 1990. The Ecology and Economics of Storage in Plants. Annual Review of Ecology and Systematics 21:423–447.

Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. Nature 405:234–242.

Chapin, F. S., III, P. Matson, and P. Vitousek. 2012. Species Effects on Ecosystem Processes. Pages 321–336–336 *in* Principles of Terrestrial Ecosystem Ecology. Springer New York, New York, NY.

Charles-Edwards, D. A. 1981. The mathematics of photosynthesis and productivity. Academic Press.

Cheng, Y. H., O. Arakawa, M. Kasai, and S. Sawada. 2008. Analysis of reduced photosynthesis in the apple leaf under sink-limited conditions due to girdling. Journal of the Japanese Society for Horticultural Science 77:115–121.

Chew, S., and S. Bonser. 2009. The evolution of growth rate, resource allocation and competitive ability in seeder and resprouter tree seedlings. Evolutionary Ecology 23:723–735.

Chiou, T.-J., and D. R. Bush. 1998. Sucrose is a signal molecule in assimilate partitioning. Proceedings of the National Academy of Sciences 95:4784–4788.

Clark, D. A., S. Brown, D. W. Kicklighter, J. Q. Chambers, J. R. Thomlinson, J. Ni, and E. A. Holland. 2001. Net primary production in tropical forests: An evaluation and synthesis of existing field data. Ecological Applications 11:371–384.

Clark, D. A., S. C. Piper, C. D. Keeling, and D. B. Clark. 2003. Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984–2000. Proceedings of the National Academy of Sciences 100:5852–5857.

Clark, D. B., and D. A. Clark. 2000. Landscape-scale variation in forest structure and biomass in a tropical rain forest. Forest Ecology and Management 137:185–198.

Cox, P. M., R. A. Betts, C. D. Jones, S. A. Spall, and I. J. Totterdell. 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408:184–187.

Cramer, W., A. Bondeau, F. I. Woodward, I. C. Prentice, R. A. Betts, V. Brovkin, P. M. Cox, V. Fisher, J. A. Foley, A. D. Friend, C. Kucharik, M. R. Lomas, N. Ramankutty, S. Sitch, B. Smith, A. White, and C. Young-Molling. 2001. Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. Global Change Biology 7:357–373.

Cui, K., S. Peng, Y. Xing, S. Yu, C. Xu, and Q. Zhang. 2003. Molecular dissection of the genetic relationships of source, sink and transport tissue with yield traits in rice. Theoretical and Applied Genetics 106:649–658.

Damesin, C., E. Ceschia, N. Le Goff, J. M. Ottorini, and E. Dufrêne. 2002. Stem and branch respiration of beech: from tree measurements to estimations at the stand level. New Phytologist 153:159–172.

Daudet, F. A., T. Ameglio, H. Cochard, O. Archilla, and A. Lacointe. 2005. Experimental analysis of the role of water and carbon in tree stem diameter variations. Journal of Experimental Botany 56:135–144.

De Kauwe, M. G., B. E. Medlyn, S. Zaehle, A. P. Walker, M. C. Dietze, T. Hickler, A. K. Jain, Y. Luo, W. J. Parton, I. C. Prentice, B. Smith, P. E. Thornton, S. Wang, Y.-P. Wang, D. Wårlind, E. Weng, K. Y. Crous, D. S. Ellsworth, P. J. Hanson, H. Seok Kim, J. M. Warren, R. Oren, and R. J. Norby. 2013. Forest water use and water use efficiency at elevated CO 2: a model-data intercomparison at two contrasting temperate forest FACE sites. Global Change Biology 19:1759–1779.

De Schepper, V., J. Bühler, M. Thorpe, G. Roeb, G. Huber, D. van Dusschoten, S. Jahnke, and K. Steppe. 2013a. ^{11}C-PET imaging reveals transport dynamics and sectorial plasticity of oak phloem after girdling. Plant Physiology 4:400.

De Schepper, V., K. Steppe, M.-C. Van Labeke, and R. Lemeur. 2010. Detailed analysis of double girdling effects on stem diameter variations and sap flow in young oak trees. Environmental and Experimental Botany 68:149–156.

De Schepper, V., T. De Swaef, I. Bauweraerts, and K. Steppe. 2013b. Phloem transport: a review of mechanisms and controls. Journal of Experimental Botany 64:4839–4850.

DeLucia, E. H., J. E. Drake, R. B. Thomas, and M. Gonzalez-Meler. 2007. Forest carbon use efficiency: is respiration a constant fraction of gross primary production? Global Change Biology 13:1157–1167.

Domec, J. C., and M. L. Pruyn. 2008. Bole girdling affects metabolic properties and root, trunk and branch hydraulics of young ponderosa pine trees. Tree Physiology 28:1493–1504.

Domingues, T. F., J. A. Berry, L. A. Martinelli, J. Ometto, and J. R. Ehleringer. 2005. Parameterization of canopy structure and leaf-level gas exchange for an eastern Amazonian tropical rain forest (Tapajos National Forest, Para, Brazil). Earth Interactions 9.

Drake, J. E., A. Gallet-Budynek, K. S. Hofmockel, E. S. Bernhardt, S. A. Billings, R. B. Jackson, K. S. Johnsen, J. Lichter, H. R. McCarthy, M. L. McCormack, D. J. P. Moore, R. Oren, S. Palmroth, R. P. Phillips, J. S. Pippen, S. G. Pritchard, K. K. Treseder, W. H. Schlesinger, E. H. DeLucia, and A. C. Finzi. 2011. Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO2. Ecology Letters 14:349–357.

Dufresne, J. L., L. Fairhead, H. Le Treut, M. Berthelot, L. Bopp, P. Ciais, P. Friedlingstein, and P. Monfray. 2002. On the magnitude of positive feedback between future climate change and the carbon cycle. Geophysical Research Letters 29:43–1–43–4.

Edwards, N. T., and P. J. Hanson. 1996. Stem respiration in a closed-canopy upland oak forest. Tree Physiology 16:433–439.

Evans, J. R. 1989. Photosynthesis and nitrogen relationship in leaves of C-3 plants. Oecologia 78:9–19.

Ewart, A. J. 1896. On assimilatory inhibition in plants. Journal of the Linnean Society of London, Botany 31:364–461.

Fan, P. G., L. S. Li, W. Duan, W. D. Li, and S. H. Li. 2010. Photosynthesis of young apple trees in response to low sink demand under different air temperatures. Tree Physiology 30:313–325.

Fan, S.-M., S. C. Wofsy, P. S. Bakwin, D. J. Jacob, and D. R. Fitzjarrald. 1990. Atmosphere-biosphere exchange of CO_2 and O_3 in the central Amazon Forest. Journal of Geophysical Research 95:16851.

FAO. 2011. State of the World's Forests. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fatichi, S., S. Leuzinger, and C. Körner. 2014. Moving beyond photosynthesis: from carbon source to sink-driven vegetation modeling. New Phytologist 201:1086–1095.

Fernández-Martínez, M., S. Vicca, I. A. Janssens, J. Sardans, S. Luyssaert, M. Campioli, F. S. Chapin III, P. Ciais, Y. Malhi, M. Obersteiner, D. Papale, S. L. Piao, M. Reichstein, F. Rodà, and J. Peñuelas. 2014. Nutrient availability as the key regulator of global forest carbon balance. Nature Climate Change 4:471–476.

Fetene, M., C. Benker, and E. Beck. 1997. The pathway of assimilate flow from source to sink in *Urtica dioica* L., studied with ^{14}C under ambient atmospheric conditions. Annals of Botany 79:585–591.

Field, C. B., and H. A. Mooney. 1986. The photosynthesis-nitrogen relationship in wild plants. Pages 22–55 *in* T. J. Givnish, editor. On the economy of plant form and function. Cambridge University Press, Cambridge.

Fisher, R. F. 1995. Amelioration of degraded rain forest soils by plantations of native trees. Soil Sci. Soc. Am. J. 59:544–549.

Franck, N., P. Vaast, M. Genard, and J. Dauzat. 2006. Soluble sugars mediate sink feedback down-regulation of leaf photosynthesis in field-grown *Coffea arabica*. Tree Physiology 26:517–525.

Franklin, O., J. Johansson, R. C. Dewar, U. Dieckmann, R. E. McMurtrie, Å. Brännström, and R. Dybzinski. 2012. Modeling carbon allocation in trees: a search for principles. Tree Physiology 32:648–666.

Frey, B., F. Hagedorn, and F. Giudici. 2006. Effect of girdling on soil respiration and root composition in a sweet chestnut forest. Forest Ecology and Management 225:271–277.

Friend, A. D., W. Lucht, T. T. Rademacher, R. Keribin, R. Betts, P. Cadule, P. Ciais, D. B. Clark, R. Dankers, P. D. Falloon, A. Ito, R. Kahana, A. Kleidon, M. R. Lomas, K. Nishina, S. Ostberg, R. Pavlick, P. Peylin, S. Schaphoff, N. Vuichard, L. Warszawski, A. Wiltshire, and F. I. Woodward. 2014. Carbon residence time dominates uncertainty in terrestrial vegetation responses to future climate and atmospheric CO2. Proceedings of the National Academy of Sciences 111:3280–3285.

Fu, Q., L. Cheng, Y. Guo, and R. Turgeon. 2011. Phloem loading strategies and water relations in trees and herbaceous plants. Plant Physiology 157:1518–1527.

Gamalei, Y. 1991. Phloem loading and its development related to plant evolution from trees to herbs. Trees-Structure and Function 5:50–64.

Génard, M., J. Dauzat, N. Franck, F. Lescourret, N. Moitrier, P. Vaast, and G. Vercambre. 2008. Carbon allocation in fruit trees: from theory to modelling. Trees-Structure and Function 22:269–282.

Gibon, Y., O. E. Bläsing, N. Palacios-Rojas, D. Pankovic, J. H. M. Hendriks, J. Fisahn, M. Höhne, M. Günther, and M. Stitt. 2004. Adjustment of diurnal starch turnover to short days: depletion of sugar during the night leads to a temporary inhibition of carbohydrate utilization, accumulation of sugars and post-translational activation of ADP-glucose pyrophosphorylase in the following light period. The Plant Journal 39:847–862.

Goldschmidt, E. E., and S. C. Huber. 1992. Regulation of Photosynthesis by End-Product Accumulation in Leaves of Plants Storing Starch, Sucrose, and Hexose Sugars. Plant Physiology 99:1443–1448.

Gould, N., P. E. H. Minchin, and M. R. Thorpe. 2004. Direct measurements of sieve element hydrostatic pressure reveal strong regulation after pathway blockage. Functional Plant Biology 31:987–993.

Grace, J., J. Lloyd, J. McIntyre, A. C. Miranda, P. Meir, H. S. Miranda, C. Nobre, J. Moncrieff, J. Massheder, Y. Malhi, I. Wright, and J. Gash. 1995. Carbon dioxide uptake by an undisturbed tropical rain forest in southwest Amazonia, 1992 to 1993. Science 270:778–780.

Griffin, K. L., D. T. Tissue, M. H. Turnbull, W. Schuster, and D. Whitehead. 2001. Leaf dark respiration as a function of canopy position in *Nothofagus fusca* trees grown at ambient and elevated CO_2 partial pressures for 5 years. Functional Ecology 15:497–505.

Harrell, D. C., and L. E. Williams. 1987. Net CO_2 assimilation rate of grapevine leaves in response to trunk girdling and gibberellic acid application. Plant Physiology 83:457–459.

Herold, A. 1980. Regulation of photosynthesis by sink activity - the missing link. New Phytologist 86:131–144.

Hesketh, J. D., D. N. Baker, and W. G. Duncan. 1971. Simulation of Growth and Yield in Cotton: Respiration and the Carbon Balance1. Crop Science 11:394.

Hoch, G., A. Richter, and C. Körner. 2003. Non-structural carbon compounds in temperate forest trees. Plant Cell and Environment 26:1067–1081.

Hoch, G., M. Popp, and C. Körner. 2002. Altitudinal increase of mobile carbon pools in Pinus cembra suggests sink limitation of growth at the Swiss treeline. Oikos 98:361–374.

Hogberg, P., A. Nordgren, N. Buchmann, A. F. S. Taylor, A. Ekblad, M. N. Hogberg, G. Nyberg, M. Ottosson-Lofvenius, and D. J. Read. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346–363.

Hubbard, R. M., M. G. Ryan, and D. L. Lukens. 1995. A simple, battery-operated, temperature-controlled cuvette for respiration measurements. Tree Physiology 15:175–179.

Huntingford, C., P. Zelazowski, D. Galbraith, L. M. Mercado, S. Sitch, R. Fisher, M. Lomas, A. P. Walker, C. D. Jones, B. B. B. Booth, Y. Malhi, D. Hemming, G. Kay, P. Good, S. L. Lewis, O. L. Phillips, O. K. Atkin, J. Lloyd, E. Gloor, J. Zaragoza-Castells, P. Meir, R. Betts, P. P. Harris, C. Nobre, J. Marengo, and P. M. Cox. 2013. Simulated resilience of tropical rainforests to CO2-induced climate change. Nature Geosci 6:268–273.

Iglesias, D. J., I. Lliso, F. R. Tadeo, and M. Talon. 2002. Regulation of photosynthesis through source: sink imbalance in citrus is mediated by carbohydrate content in leaves. Physiologia Plantarum 116:563–572.

IPCC. 2007. Climae change 2007: synthesis report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

Ise, T., C. M. Litton, C. P. Giardina, and A. Ito. 2010. Comparison of modeling approaches for carbon partitioning: Impact on estimates of global net primary production and equilibrium biomass of woody vegetation from MODIS GPP. Journal of Geophysical Research 115:G04025.

Jensen, K. H., J. Lee, T. Bohr, H. Bruus, N. M. Holbrook, and M. A. Zwieniecki. 2011. Optimality of the Münch mechanism for translocation of sugars in plants. Journal of The Royal Society Interface 8:1155–1165.

Johnsen, K., C. Maier, F. Sanchez, P. Anderson, J. Butnor, R. Waring, and S. Linder. 2007. Physiological girdling of pine trees via phloem chilling: proof of concept. Plant Cell and Environment 30:128–134.

Kindermann, J., G. Wurth, G. H. Kohlmaier, and F. W. Badeck. 1996. Interannual variation of carbon exchange fluxes in terrestrial ecosystems. Global Biogeochemical Cycles 10:737–755.

Kira, T. 1978. Community architecture and organic matter dynamics in tropical lowland rain forests of Southeast Asia with special reference to Pasoh Forest, west Malaysia. Pages 561–590 *in* T. B. Tomlinson and M. H. Zimmermann, editors. Tropical Trees as Living Systems. Cambridge University Press, New York.

Kleber, M., L. Schwendenmann, E. Veldkamp, J. Rˆflner, and R. Jahn. 2007. Halloysite versus gibbsite: Silicon cycling as a pedogenetic process in two lowland neotropical rain forest soils of La Selva, Costa Rica. Geoderma 138:1–11.

Knoblauch, M., and W. S. Peters. 2010. Münch, morphology, microfluidics - our structural problem with the phloem. Plant Cell and Environment 33:1439–1452.

Koch, K. E. 1996. Carbohydrate-modulated gene expression in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47:509–540.

Körner, C. 2003. Carbon limitation in trees. Journal of Ecology 91:4–17.

Krapp, A., and M. Stitt. 1995. An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: Changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. Planta 195:313–323.

Krapp, A., B. Hofmann, C. Schafer, and M. Stitt. 1993. Regulation of the expression of *rbc*S and other photosynthetic genes by carbohydrates - a mechanism for the sink regulation of photosynthesis? Plant Journal 3:817–828.

Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 165:382–396.

Lacointe, A., E. Deleens, T. Ameglio, B. Saint-Joanis, C. Lelarge, M. Vandame, G. C. Song, and F. A. Daudet. 2004. Testing the branch autonomy theory: a $^{13}C/^{14}C$ double-labelling experiment on differentially shaded branches. Plant Cell and Environment 27:1159–1168.

Lambers, H. 1982. Cyanide-resistant respiration: A non-phosphorylating electron transport pathway acting as an energy overflow. Physiologia Plantarum 55:478–485.

Lambers, H., F. S. Chapin III, and T. L. Pons. 2008. Plant Physiological Ecology. Second edition. Springer, New York.

Lavigne, M. B. 1988. Stem growth and respiration of young balsam fir trees in thinned and unthinned stands. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 18:483–489.

Law, B. E., F. M. Kelliher, D. D. Baldocchi, P. M. Anthoni, J. Irvine, D. Moore, and S. Van Tuyl. 2001. Spatial and temporal variation in respiration in a young ponderosa pine forest during a summer drought. Agricultural and Forest Meteorology 110:27–43.

Legros, S., I. Mialet-Serra, A. Clement-Vidal, J. P. Caliman, F. A. Siregar, D. Fabre, and M. Dingkuhn. 2009. Role of transitory carbon reserves during adjustment to climate variability and source-sink imbalances in oil palm (*Elaeis guineensis*). Tree Physiology 29:1199–1211.

Levy, P. E., and P. G. Jarvis. 1998. Stem CO_2 fluxes in two Sahelian shrub species (*Guiera senegalensis* and *Combretum micranthum*). Functional Ecology 12:107–116.

Levy-Varon, J., W. Schuster, and K. Griffin. 2012. The autotrophic contribution to soil respiration in a northern temperate deciduous forest and its response to stand disturbance. Oecologia 169:211–220.

Litton, C. M., J. W. Raich, and M. G. Ryan. 2007. Carbon allocation in forest ecosystems. Global Change Biology 13:2089–2109.

Loescher, H. W., S. F. Oberbauer, H. L. Gholz, and D. B. Clark. 2003. Environmental controls on net ecosystem-level carbon exchange and productivity in a Central American tropical wet forest. Global Change Biology 9:396–412.

Luyssaert, S., I. Inglima, M. Jung, A. D. Richardson, M. Reichsteins, D. Papale, S. L. Piao, E. D. Schulzes, L. Wingate, G. Matteucci, L. Aragao, M. Aubinet, C. Beers, C. Bernhoffer, K. G. Black, D. Bonal, J. M. Bonnefond, J. Chambers, P. Ciais, B. Cook, K. J. Davis, A. J. Dolman, B. Gielen, M. Goulden, J. Grace, A. Granier, A. Grelle, T. Griffis, T. Grunwald, G. Guidolotti, P. J. Hanson, R. Harding, D. Y. Hollinger, L. R. Hutyra, P. Kolar, B. Kruijt, W. Kutsch, F. Lagergren, T. Laurila, B. E. Law, G. Le Maire, A. Lindroth, D. Loustau, Y. Malhi, J. Mateus, M. Migliavacca, L. Misson, L. Montagnani, J. Moncrieff, E. Moors, J. W. Munger, E. Nikinmaa, S. V. Ollinger, G. Pita, C. Rebmann, O. Roupsard, N. Saigusa, M. J. Sanz, G. Seufert, C. Sierra, M. L. Smith, J. Tang, R. Valentini, T. Vesala, and I. A. Janssens. 2007. CO_2 balance of boreal, temperate, and tropical forests derived from a global database. Global Change Biology 13:2509–2537.

Mahouachi, J., D. Iglesias, M. Agust, and M. Talon. 2009. Delay of early fruitlet abscission by branch girdling in citrus coincides with previous increases in carbohydrate and gibberellin concentrations. Plant Growth Regulation 58:15–23.

Malhi, Y. 2012. The productivity, metabolism and carbon cycle of tropical forest vegetation. Journal of Ecology 100:65–75.

Malhi, Y., A. D. NOBRE, J. Grace, B. Kruijt, M. G. P. Pereira, A. Culf, and S. Scott. 1998. Carbon dioxide transfer over a Central Amazonian rain forest. Journal of Geophysical Research 103:31593.

Malhi, Y., C. Doughty, and D. Galbraith. 2011. The allocation of ecosystem net primary productivity in tropical forests. Philosophical Transactions of the Royal Society B-Biological Sciences 366:3225–3245.

Marshall, C. 1996. Sectoriality and physiological organisation in herbaceous plants: an overview. Vegetatio 127:9–16.

Mäkelä, A. 1996. Optimal control of gas exchange during drought: theoretical analysis. Annals of Botany 77:461–468.

McDade, L. A. 1994. La Selva: Ecology and Natural History of a Neotropical Rain Forest. University of Chicago Press, Chicago.

McGuire, M. A., and R. O. Teskey. 2004. Estimating stem respiration in trees by a mass balance approach that accounts for internal and external fluxes of CO_2. Tree Physiology 24:571–578.

Meir, P., and J. Grace. 2002. Scaling relationships for woody tissue respiration in two tropical rain forests. Plant Cell and Environment 25:963–973.

Meir, P., J. Grace, and A. C. Miranda. 2001. Leaf respiration in two tropical rainforests: constraints on physiology by phosphorus, nitrogen and temperature. Functional Ecology 15:378–387.

Mencuccini, M., and T. Hölttä. 2009. The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. New Phytologist 185:189–203.

Metcalfe, D. B., R. Lobo-do-Vale, M. M. Chaves, J. P. Maroco, L. Aragao, Y. Malhi, A. L. Da Costa, A. P. Braga, P. L. Goncalves, J. De Athaydes, M. Da Costa, S. S. Almeida, C. Campbell, V. Hurry, M. Williams, and P. Meir. 2010. Impacts of experimentally imposed drought on leaf respiration and morphology in an Amazon rain forest. Functional Ecology 24:524–533.

Millar, A. H., O. K. Atkin, R. Ian Menz, B. Henry, G. Farquhar, and D. A. Day. 1998. Analysis of respiratory chain regulation in roots of soybean seedlings. Plant Physiology 117:1083–1093.

Miranda, E. J., G. L. Vourlitis, N. P. Filho, P. C. Priante, J. H. Campelo, G. S. Suli, C. L. Fritzen, F. de Almeida Lobo, and S. Shiraiwa. 2005. Seasonal variation in the leaf gas exchange of tropical forest trees in the rain forest–savanna transition of the southern Amazon Basin. Journal of Tropical Ecology 21:451–460.

Mitchell, K. A., P. V. Bolstad, and J. M. Vose. 1999. Interspecific and environmentally induced variation in foliar dark respiration among eighteen southeastern deciduous tree species. Tree Physiology 19:861–870.

Möller, C. M., D. Möller, and J. Nielsen. 1954. Graphic presentation of dry matter production of European beech. Det Forstlige Forsogsvaesen i Danmark 21:327–335.

Münch, E. 1930. Die Stoffbewegungen in der Pflanze. Gustav Fischer, Jena.

Myers, D. A., R. B. Thomas, and E. H. DeLucia. 1999. Photosynthetic responses of loblolly pine (Pinus taeda) needles to experimental reduction in sink demand. Tree Physiology 19:235–242.

Nebauer, S. G., B. Renau-Morata, J. L. Guardiola, and R. V. Molina. 2011. Photosynthesis down-regulation precedes carbohydrate accumulation under sink limitation in Citrus. Tree Physiology 31:169–177.

Nikinmaa, E., T. HöLttä, P. Hari, P. Kolari, A. Mäkelä, S. Sevanto, and T. Vesala. 2013. Assimilate transport in phloem sets conditions for leaf gas exchange. Plant Cell and Environment 36:655–669.

Niklas, K. J., and B. J. Enquist. 2001. Invariant scaling relationships for interspecific plant biomass production rates and body size. Proceedings of the National Academy of Sciences 98:2922–2927.

Noel, A. R. A. 1970. The girdled tree. Botanical Review 36:162–195.

Oberbauer, S. F., and B. R. Strain. 1986. Effects of canopy position and irradiance on the leaf physiology and morphology of *Pentaclethra macroloba* (Mimosaceae). American Journal of Botany 73:409–416.

Olsson, P., S. Linder, R. Giesler, and P. Högberg. 2005. Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Global Change Biology 11:1745–1753.

Palacio, S., R. Hernández, M. Maestro-Martínez, and J. J. Camarero. 2012. Fast replenishment of initial carbon stores after defoliation by the pine processionary moth and its relationship to the re-growth ability of trees. Trees-Structure and Function 26:1627–1640–1640.

Parker, G. G. 1994. Soil fertility, nutrient acquisition and nutrient cycling. Pages 54–63 *in* L. A. McDade, K. S. Bawa, H. A. Hespenheide, and A. S. Hartshorn, editors. La Selva Book: Ecology and Natural History o a Neotropical Rain Forest. University of Chicago Press, Chicago, Illinois.

Patrick, J. W., and C. E. Offler. 1996. Post-sieve element transport of photoassimilates in sink regions. Journal of Experimental Botany 47:1165–1177.

Patrick, J. W., W. Zhang, S. D. Tyerman, C. E. Offler, and N. A. Walker. 2001. Role of membrane transport in phloem translocation of assimilates and water. Australian Journal of Plant Physiology 28:697–709.

Paul, M. J., and C. H. Foyer. 2001. Sink regulation of photosynthesis. Journal of Experimental Botany 52:1383–1400.

Penning de Vries, F. W. T. 1975. The cost of maintenance processes in plant cells. Annals of Botany 39:77–92.

Penning de Vries, F. W. T., A. H. M. Brunsting, and H. H. van Laar. 1974. Products, requirements and efficiency of biosynhtesis: A quantitative approach. Journal of Theoretical Biology 45:339–377.

Phillips, O. L., Y. Malhi, N. Higuchi, W. F. Laurance, P. V. Nunez, R. M. Vasquez, S. G. Laurance, L. V. Ferreira, M. Stern, S. Brown, and J. Grace. 1998. Changes in the carbon balance of tropical forests: Evidence from long-term plots. Science 282:439–442.

Poorter, H., and M. Bergkotte. 1992. Chemical composition of 24 wild species differing in relative growth rate. Plant Cell and Environment 15:221–229.

Poorter, H., K. J. Niklas, P. B. Reich, J. Oleksyn, P. Poot, and L. Mommer. 2012a. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist 193:30–50.

Poorter, L., E. Lianes, M. Moreno-de las Heras, and M. Zavala. 2012b. Architecture of Iberian canopy tree species in relation to wood density, shade tolerance and climate. Plant Ecology 213:707–722.

Preston, K. A. 1998. The effects of developmental stage and source leaf position on integration and sectorial patterns of carbohydrate movement in an annual plant, *Perilla frutescens* (Lamiaceae). American Journal of Botany 85:1695–1703.

Proietti, P. 2003. Changes in photosynthesis and fruit characteristics in olive in response to assimilate availability. Photosynthetica 41:559–564.

R Core Team. 2014, May 22. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raich, J. W., A. E. Russell, and O. Valverde-Barrantes. 2009. Fine root decay rates vary widely among lowland tropical tree species. Oecologia 161:325–330.

Raich, J. W., A. E. Russell, and R. Bedoya-Arrieta. 2007. Lignin and enhanced litter turnover in tree plantations of lowland Costa Rica. Forest Ecology and Management 239:128–135.

Raich, J. W., and K. J. Nadelhoffer. 1989. Belowground carbon allocation in forest ecosystems - global trends. Ecology 70:1346–1354.

Rasmusson, A. G., K. L. Soole, and T. E. Elthon. 2004. Alternative NAD(P)H dehydrogenases of plant mitochondria. Annual Review of Plant Biology 55:23–39.

Rayment, M. B., D. Loustau, and P. J. Jarvis. 2002. Photosynthesis and respiration of black spruce at three organizational scales: shoot, branch and canopy. Tree Physiology 22:219–229.

Reich, P. B. 2002. Root-shoot relations: Optimality in acclimation and adaptation or the "Emperor's New Clothes"? Pages 205–220 *in* Y. Waisel, A. Eshel, T. Beeckman, and U. Kafkafi, editors. Plant Roots: The Hidden Half, Third Edition. Marcel Dekker, Inc New York.

Reich, P. B., M. G. Tjoelker, J. L. Machado, and J. Oleksyn. 2006. Universal scaling of respiratory metabolism, size and nitrogen in plants. Nature 439:457–461.

Rennie, E. A., and R. Turgeon. 2009. A comprehensive picture of phloem loading strategies. Proceedings of the National Academy of Sciences 106:14162–14167.

Reynolds, M. P., A. Pellegrineschi, and B. Skovmand. 2005. Sink-limitation to yield and biomass: a summary of some investigations in spring wheat. Annals of Applied Biology 146:39–49.

Robertson, A. L., Y. Malhi, F. Farfan-Amezquita, L. E. O. C. Aragao, J. E. Silva Espejo, and M. A. Robertson. 2010. Stem respiration in tropical forests along an elevation gradient in the Amazon and Andes. Global Change Biology 16:3193–3204.

Roper, T. R., and L. E. Williams. 1989. Net CO_2 assimilation and carbohydrate partitioning of grapevine leaves in response to trunk girdling and gibberellic acid application. Plant Physiology 89:1136–1140.

Russell, A. E., and J. W. Raich. 2012. Rapidly growing tropical trees mobilize remarkable amounts of nitrogen, in ways that differ surprisingly among species. Proceedings of the National Academy of Sciences 109:10398–10402.

Russell, A. E., J. W. Raich, R. B. Arrieta, O. Valverde-Barrantes, and E. González. 2010. Impacts of individual tree species on carbon dynamics in a moist tropical forest environment. Ecological Applications 20:1087–1100.

Ryan, M. G. 1990. Growth and maintenance respiration in stems of *Pinus contorta* and *Picea engelmannii*. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 20:48–57.

Ryan, M. G. 1991a. Effects of climate change on plant respiration. Ecological Applications 1:157–167.

Ryan, M. G. 1995. Foliar maintenance respiration of subalpine and boreal trees and shrubs in relation to nitrogen content. Plant Cell and Environment 18:765–772.

Ryan, M. G. 1991b. A simple method for estimating gross carbon budgets for vegetation in forest ecosystems. Tree Physiology 9:255–266.

Ryan, M. G., D. Binkley, J. H. Fownes, C. P. Giardina, and R. S. Senock. 2004. An experimental test of the causes of forest growth decline with stand age. Ecological Monographs 74:393–414.

Ryan, M. G., J. L. Stape, D. Binkley, S. Fonseca, R. A. Loos, E. N. Takahashi, C. R. Silva, S. R. Silva, R. E. Hakamada, J. M. Ferreira, A. M. N. Lima, J. L. Gava, F. P. Leite, H. B. Andrade, J. M. Alves, and G. G. C. Silva. 2010. Factors controlling Eucalyptus productivity: How water availability and stand structure alter production and carbon allocation. Forest Ecology and Management 259:1695–1703.

Ryan, M. G., M. A. Cavaleri, A. C. Almeida, R. Penchel, R. S. Senock, and J. L. Stape. 2009. Wood CO_2 efflux and foliar respiration for *Eucalyptus* in Hawaii and Brazil. Tree Physiology 29:1213–1222.

Ryan, M. G., M. B. Lavigne, and S. T. Gower. 1997. Annual carbon cost of autotrophic respiration in boreal forest ecosystems in relation to species and climate. Journal of Geophysical Research-Atmospheres 102:28871–28883.

Ryan, M. G., R. M. Hubbard, D. A. Clark, and R. L. Sanford. 1994. Woody-tissue respiration for *Simarouba amara* and *Minquartia guianensis*, two tropical wet forest trees with different growth habits. Oecologia 100:213–220.

Sanz-Perez, V., P. Castro-Diez, and R. Joffre. 2009. Seasonal carbon storage and growth in Mediterranean tree seedlings under different water conditions. Tree Physiology 29:1105–1116.

Scialdone, A., S. T. Mugford, D. Feike, A. Skeffington, P. Borrill, A. Graf, A. M. Smith, M. Howard, and D. Weigel. 2013. Arabidopsis plants perform arithmetic division to prevent starvation at night. eLife 2.

Setter, T. L., W. A. Brun, and M. L. Brenner. 1980. Effect of obstructed translocation on leaf abscisic acid, and associated stomatal closure and photosynthesis decline. Plant Physiology 65:1111.

Sheen, J. 1990. Metabolic repression of transcription in higher plants. The Plant Cell Online 2:1027–1038.

Shipley, B., M. J. Lechowicz, I. Wright, and P. B. Reich. 2006. Fundamental trade-offs generating the worldwide leaf economics spectrum. Ecology 87:535–541.

Smith, A. M., and M. Stitt. 2007. Coordination of carbon supply and plant growth. Plant Cell and Environment 30:1126–1149.

Spicer, R., and N. M. Holbrook. 2005. Within-stem oxygen concentration and sap flow in four temperate tree species: does long-lived xylem parenchyma experience hypoxia? Plant Cell and Environment 28:192–201.

Sprugel, D. 1990. Components of woody-tissue respiration in young *Abies amabilis* (Dougl.) Forbes trees. Trees 4:88–98.

Sprugel, D. G., M. G. Ryan, R. J. Brooks, K. A. Vogt, and T. A. Martin. 1995. Respiration from the organ level to the stand. Pages 255–299 *in* W. K. Smith and T. M. Hinckley, editors. Resource Physiology of Conifers. Academic Press, Inc., San Diego.

Stape, J. L., D. Binkley, and M. G. Ryan. 2008. Production and carbon allocation in a clonal *Eucalyptus* plantation with water and nutrient manipulations. Forest Ecology and Management 255:920–930.

Stape, J. L., D. Binkley, M. G. Ryan, S. Fonseca, R. A. Loos, E. N. Takahashi, C. R. Silva, S. R. Silva, R. E. Hakamada, J. M. D. Ferreira, A. M. N. Lima, J. L. Gava, F. P. Leite, H. B. Andrade, J. M. Alves, G. G. C. Silva, and M. R. Azevedo. 2010. The Brazil Eucalyptus Potential Productivity Project: Influence of water, nutrients and stand uniformity on wood production. Forest Ecology and Management 259:1684–1694.

Stitt, M., A. Schaewen, and L. Willmitzer. 1991. “Sink” regulation of photosynthetic metabolism in transgenic tobacco plants expressing yeast invertase in their cell wall involves a decrease of the Calvin-cycle enzymes and an increase of glycolytic enzymes. Planta 183:40–50.

Sweet, G. B., and P. F. Wareing. 1966. Role of plant growth in regulating photosynthesis. Nature 210:77–79.

Teskey, R. O., A. Saveyn, K. Steppe, and M. A. McGuire. 2008. Origin, fate and significance of CO_2 in tree stems. New Phytologist 177:17–32.

Thompson, M. V. 2006. Phloem: the long and the short of it. Trends in Plant Science 11:26–32.

Thornley, J. H. 1970. Respiration, growth and maintenance in plants. Nature 227:304–&.

Thornley, J. H. M., and M. G. R. Cannell. 2000. Modelling the components of plant respiration: Representation and realism. Annals of Botany 85:55–67.

Thorpe, M. R., and A. Lang. 1983. Control of import and export of photosynthate in leaves. Journal of Experimental Botany 34:231–239.

Tian, H., J. M. Melillo, D. W. Kicklighter, A. D. McGuire, J. V. K. Helfrich, B. Moore, and C. J. Vörösmarty. 1998. Effect of interannual climate variability on carbon storage in Amazonian ecosystems : Abstract : Nature. Nature 396:664–667.

Tilman, D. 1988. Plant Strategies and the Dynamics and Structure of Plant Communities. Princeton University Press, Princeton, NJ.

Trumbore, S. E., A. Angert, N. Kunert, J. Muhr, and J. Q. Chambers. 2012. What's the flux? Unraveling how CO_2 fluxes from trees reflect underlying physiological processes. New Phytologist 197:353–355.

Turgeon, R., and S. Wolf. 2009. Phloem transport: Cellular pathways and molecular trafficking. Annual Review of Plant Biology 60:207–221.

Turnbull, C. G. N., and R. M. Lopez-Cobollo. 2013. Heavy traffic in the fast lane: long-distance signalling by macromolecules. New Phytologist 198:33–51.

Turnbull, M. H., D. T. Tissue, K. L. Griffin, S. J. Richardson, D. A. Peltzer, and D. Whitehead. 2005. Respiration characteristics in temperate rainforest tree species differ along a long-term soil-development chronosequence. Oecologia 143:271–279.

Turnbull, M. H., D. Whitehead, D. T. Tissue, W. S. F. Schuster, K. J. Brown, and K. L. Griffin. 2003. Scaling foliar respiration in two contrasting forest canopies. Functional Ecology 17:101–114.

Urban, L. 2004. Effect of fruit load and girdling on leaf photosynthesis in *Mangifera indica* L. Journal of Experimental Botany 55:2075–2085.

Urban, L., and L. Alphonsout. 2007. Girdling decreases photosynthetic electron fluxes and induces sustained photoprotection in mango leaves. Tree Physiology 27:345–352.

Urban, M. A., D. M. Nelson, G. Jimenez-Moreno, J. J. Chateauneuf, A. Pearson, and F. S. Hu. 2010. Isotopic evidence of C-4 grasses in southwestern Europe during the Early Oligocene-Middle Miocene. Geology 38:1091–1094.

Valverde-Barrantes, O. J., J. W. Raich, and A. E. Russell. 2007. Fine-root mass, growth and nitrogen content for six tropical tree species. Plant and Soil 290:357–370.

van Bel, A. J. E. 2003. The phloem, a miracle of ingenuity. Plant Cell and Environment 26:125–149.

Venables, W. N., and B. D. Ripley. 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York.

Vicca, S., S. Luyssaert, J. Peñuelas, M. Campioli, F. S. Chapin, P. Ciais, A. Heinemeyer, P. Hogberg, W. L. Kutsch, B. E. Law, Y. Malhi, D. Papale, S. L. Piao, M. Reichstein, E. D. Schulze, and I. A. Janssens. 2012. Fertile forests produce biomass more efficiently. Ecology Letters 15:520–526.

Vuorisalo, T., and M. Hutchings. 1996. On plant sectoriality, or how to combine the benefits of autonomy and integration. Vegetatio 127:3–8–8.

Wang, W. J., Y. G. Zu, H. M. Wang, X. Y. Li, T. Hirano, and T. Koike. 2006. Newly-formed photosynthates and the respiration rate of girdled stems of Korean pine (*Pinus koraiensis* Sieb. *et* Zucc.). Photosynthetica 44:147–150.

Waring, R. H., J. J. Landsberg, and M. Williams. 1998. Net primary production of forests: a constant fraction of gross primary production? Tree Physiology 18:129–134.

Watson, M. A., and B. B. Casper. 1984. Morphogenetic constraints on patterns of carbon distribution in plants. Annual Review of Ecology and Systematics 15:233–258.

Weng, E., and Y. Luo. 2011. Relative information contributions of model vs. data to short- and long-term forecasts of forest carbon dynamics. Ecological Applications 21:1490–1505.

West, G. B., B. J. Enquist, and J. H. Brown. 2009. A general quantitative theory of forest structure and dynamics. Proceedings of the National Academy of Sciences 106:7040–7045.

White, A., M. G. R. Cannell, and A. D. Friend. 2000. CO_2 stabilization, climate change and the terrestrial carbon sink. Global Change Biology 6:817–833.

Wickham, H. 2009. ggplot2: Elegant Graphics for Data Analysis. Springer.

Williams, K., C. B. Field, and H. A. Mooney. 1989. Relationships among leaf construction cost, leaf longevity, and light environment in rain-forest plants of the gegus *Piper*. American Naturalist 133:198–211.

Williams, K., F. Percival, J. Merino, and H. A. Mooney. 1987. Estimation of tissue construction cost from heat of combustion and organic nitrogen content. Plant Cell and Environment 10:725–734.

Wong, S. C. 1990. Elevated atmospheric partial pressure of CO_2 and plant growth. Photosynthesis Research 23:171–180.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M.-L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428:821–827.

Wu, B. H., H. Q. Huang, P. G. Fan, S. H. Li, and G. J. Liu. 2008. Photosynthetic responses to sink-source manipulation in five peach cultivars varying in maturity date. Journal of the American Society for Horticultural Science 133:278–283.

Yin, X., and P. C. Struik. 2010. Modelling the crop: from system dynamics to systems biology. Journal of Experimental Botany 61:2171–2183.

Yoda, K. 1967. Comparative ecological studies on three main types of forest vegetation in Thailand III. Community respiration. Nature & Life in SE Asia 5:65.

Yoda, K., K. Shinozaki, H. Ogawa, K. Hozumi, and T. Kira. 1965. Estimation of the total amount of respiration in woody organs of trees and forest communities. Journal of Biology, Osaka City University 16:15–26.