

ABSTRACT

HOLLINGSWORTH, STEPHANIE NICOLE. Physiological Mechanisms That Govern Size-Dependent Resprouting Success in Fire-Prone Savannas. (Under the direction of Dr. William Hoffmann.)

Plant success in any environment is dependent upon the ability to maintain a positive carbon balance, which becomes more difficult amid fire disturbance. Fire is a prevalent disturbance mechanism that plays an essential role in governing the vegetation structure of many ecosystems worldwide, such as grasslands, shrublands, and savannas. Fire can yield such profound changes in vegetation structure because it completely consumes aboveground biomass (topkill), but rarely destroys belowground organs, allowing fire-tolerant species to resprout from remaining belowground organs. Frequent fire prevents the opportunity for plants to recruit into larger fire-tolerant size classes, and thus it tends to favor the success of small growth forms at the expense of trees and large shrubs. Resprouting recovery commonly follows a curvilinear relationship, whereby small plants quickly exceed their pre-fire size but large plants may not fully recover their pre-fire size, a pattern exhibiting *negative allometry*. From the perspective of whole-plant carbon balance, it remains unclear why frequent fire favors the success of small plants over large plants. The goal of my dissertation research was to elucidate the physiological mechanisms that govern size-dependent resprouting success in fire-prone savannas to explain the community dynamics of savannas and other fire-dominated systems worldwide, and to improve our ability to predict species and community responses to fire. This dissertation aims to address the hypothesis that post-fire carbon balance is negatively correlated with plant stature across and within species, which can largely explain the patterns of post-fire resprouting, recovery, and replenishment of carbohydrate reserves in resprouting plants. To this end, I examined the recovery of aboveground biomass and changes in carbon assimilation in burned and control plants,

changes in root carbohydrates (CHO) and nitrogen (N), changes in allocation patterns after coppicing, and the effects of repeated burning on common understory plant species.

Compared to unburned sites, large resprouting plants recovered a smaller proportion of their pre-burn biomass and carbon relative to smaller sizes. Compared to large plants, small plants also showed the potential to assimilate more carbon relative to pre-burn root carbohydrate reserves. Within species, there was not a significant effect of fire on the concentrations of CHO or N, with respect to plant size. Trends in some species indicate that, in relative terms, plants with smaller CHO reserves may be able to produce more resprout biomass, than those with larger reserves. Contrary to our hypothesis, in plants that had all of their stems coppiced except the largest stem, there was no net benefit to the growth of the remaining stem. These coppicing experiments suggest that the relative contribution of one stem to the total plant biomass decreases with pre-burn size. The physiological patterns of biomass and carbon recovery described here not only exist within species, but also exist across species regardless of mechanisms specific to, and inherent in, functional type. Overall, my research suggests that: (1) the negative allometric pattern underlying resprouting that constrains the post-fire recovery of large plants, within and across species, arises very early in resprouting and has a self-reinforcing effect on whole-plant carbon balance; (2) these physiological mechanisms exists regardless of functional type and appear to be merely a function of plant size; (3) repeated annual burning strongly constrained the carbon that large plants could assimilate during resprouting, placing them at a relative disadvantage under frequent burning, compared to small plants.

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Physiological Mechanisms That Govern Size-Dependent Resprouting Success in Fire-Prone
Savannas

by
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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Plant Biology

Raleigh, North Carolina

2015

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DEDICATION

To my mom and dad, who have always provided support and encouragement.

BIOGRAPHY

Stephanie Nicole Hollingsworth was born in Toronto Canada in 1985, but grew up in Grand Rapids, Michigan. She graduated from high school in 2003 and went on to Grand Valley State University to study art. But after spending a semester in New Zealand learning about rainforest conservation and discovering a love of plants, she decided to pursue a career in science. She completed her Bachelors of Science in 2009 and began her doctorate in 2010 in the lab of Dr. Bill Hoffmann.

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CHAPTER 1

INTRODUCTION

Fire is a ubiquitous disturbance mechanism that plays a strong and direct role in governing the vegetation structure of many ecosystems worldwide (Bond et al. 2005), including North American tallgrass prairie (Dalglish et al. 2008; Adair et al. 2009; Koerner and Collins 2014), Mediterranean chaparral (DeSouza et al. 1986; Archibold 1995), Brazilian cerrado (Hoffmann et al. 2009), and Australian Eucalypt forests (Werner and Murphy 2001). Fire promotes a flammable ecosystem where climate would otherwise promote the dominance of forests (Bond et al. 2005). Specifically, many savannas require fire to maintain an open canopy of fire-resistant, shade-intolerant trees with an understory dominated by sun-loving C₄ grasses (Ratnam et al. 2011). Fire can yield such profound changes in vegetation structure largely because it kills aboveground biomass (topkill). However, fire rarely destroys belowground organs, allowing fire-tolerant species to resprout from remaining belowground organs, buds or other root material such as root crowns or lignotubers (Canadell and López-Soria 1998). Alternatively, fire suppression can allow trees to grow into larger size classes that are fire resistant (Harmon 1984). In the absence of fire, woody encroachment can occur relatively quickly (Bond et al. 2005; Scott et al. 2012). During these fire-free periods, woody plants are allowed sufficient time to invade open areas (Silva et al. 2001). Woody encroachment can result in the competitive exclusion of the grass layer and also reduce the flammability of the system through positive feedbacks (Beckage et al. 2009). Therefore, fire can have a dramatic effect on the vegetation structure and distribution of plant size-classes.

Frequent fire prevents the opportunity for smaller plants to recruit into larger fire-

tolerant size classes; juvenile woody plants may be maintained in a suppressed state below reproductive size amid frequent fire (Hoffmann 1999). These short fire cycles will tend to favor the success of small growth forms at the expense of trees and large shrubs (Hoffmann 1999; Hoffmann and Solbrig 2003). Resprouting recovery has been described as a curvilinear relationship (Figure 1.1), in which resprout biomass balances the biomass lost to fire (Grady and Hoffmann 2012). This curvilinearity is an important feature because above an equilibrial point, where the curve intersects a 1:1 line, larger sizes will not fully recover lost biomass. Within the same interval, resprouts of small plants may become larger than their pre-fire size, but large plants may not fully recover their pre-fire size. When log transformed, this curvilinear relationship appears as a straight line with a slope <1 (Figure 1.1b), a pattern exhibiting “negative allometry”, indicating that large plants experience a disproportionately negative resprouting response. Repeated topkill and resprouting will cause plant size to approach this equilibrial size over multiple fire cycles; however, as fire frequency increases, the resprout curve should shift downward, resulting in a smaller equilibrial size. It is unclear what physiological mechanisms are responsible for this pattern.

Resprouting plants regenerate from underground storage organs containing buds, storage carbohydrates and nutrients (Cruz and Moreno 2001b). This dissertation will address the physiological basis for negative allometry in resprouting plants.

Plant success in any environment is dependent upon the ability to maintain a positive carbon balance. Maintaining a positive carbon balance becomes more difficult in a cycle of repeated topkill and resprouting because fire has contrasting effects on carbon balance, by simultaneously imposing both positive and negative effects over short and long time scales.

For example, aboveground biomass and leaf area are destroyed, which imposes a constraint on whole-plant carbon balance by severely compromising photosynthesis when it is most critically needed. In contrast, as fire destroys aboveground biomass it also increases light availability to the understory vegetation that may have been previously shaded by taller species (Bond and Van Wilgen 1996; Platt et al. 2006). Repeated burning can have long lasting effects on carbon balance and patterns of biomass recovery. Plant responses to fire should therefore largely depend on the net effect of these positive and negative impacts.

Plants are able to mobilize stored carbon reserves to facilitate resprouting, which is an essential strategy to survive repeated burning (James 1984; Bond and Midgley 2003). Plants in fire-prone systems have evolved ways to balance photosynthetic investment with their respiratory demands amid frequent disruption to a positive carbon balance. Creating and storing belowground carbohydrates implies a tradeoff with the investment in aboveground biomass and photosynthetic tissue (Verdaguer and Ojeda 2002). Savanna species tend to allocate more resources to belowground organs than do forest congeners adapted to less frequent fire (Hoffmann et al. 2003; Hoffmann and Franco 2003). And the non-structural carbohydrates stored in these organs tend to be higher in resprouters compared to non-resprouting species (Pate et al. 1990; Bell et al. 1996; Knox and Clarke 2005), and are believed to be the main source of carbon for regenerating aboveground biomass after a disturbance (DeSouza et al. 1986; Miyanishi and Kellman 1986; Bowen and Pate 1993; Canadell and López-Soria 1998). Large belowground storage reserves may confer greater resprouting capacity, but require larger root systems that presumably impose greater respiratory demands. If repeated resprouting depletes root reserves, a carbon imbalance may

lead to plant mortality due to the respiratory burden of a large root system (Noble 2001). Carbohydrate storage buffers a plant against the consequences of future disturbances (Chapin et al. 1990; Kozlowski 1992). Changes in the size of these carbon reserves reflect the balance of carbon assimilation and consumption, and therefore serve as a measure of the carbohydrate status of a plant.

In addition to carbohydrate reserves, mineral nutrients such as nitrogen (N) commonly limit plant growth (Vitousek and Howarth 1991). Root N is correlated with root respiration rates and root N uptake (Reich et al. 1998), and can be an important source of the N for recovery of lost biomass. Therefore the size of root N storage should influence resprouting success. Fire may produce short-term flushes in soil N (Knoepp et al. 2009), but frequent fire may cause N limitation due to volatilization of organic matter (Vitousek and Howarth 1991, and references therein), or due to microbial immobilization of N (Dell, 1998). A meta-analysis of 185 data sets from 87 studies showed that fire significantly reduced the amount of litter N, increased soil NH_4^+ and NO_3^- in the first few months and returned to pre-fire levels within one year, however fire had no significant effect on litter N concentration, soil N total, or overall soil N concentration (Wan et al. 2001). Additionally, available soil N has not been shown to have significant effects on lignotuber size (Cruz and Moreno 2001a). We did not test for available soil N, but call into question how N concentrations and total root N affect resprouting success across plant sizes. It has been suggested that a minimum N pool may affect resprouting in some species (El Omari et al. 2003). Many studies have found that a single disturbance event would not significantly deplete nutrient concentrations, but that repeated burns could induce nutrient limitation (Miyanishi and Kellman 1986; Canadell

and López-Soria 1998).

Belowground organs not only are a main source of carbohydrate storage for resprouting plants, but are also the source of buds from which resprouting occurs (Wildy and Pate 2002). Resprouters may produce numerous resprout stems from a bank of dormant buds (Pate et al. 1990; Drewa et al. 2002). Multi-stemmed resprouters are abundant in open vegetation where being over shaded is less likely (Midgley 1996). Producing multiple stems increases the total leaf area and total crown volume, which improves the competitive success of a shorter statured plant (Kozovits et al. 2005). It has been suggested that competition for nutrients in large multi-stemmed plants may be greater than that of a single-stemmed plant of similar size (Midgley 1996). So would reducing the number of stems result in a net benefit to the growth of that individual? Maintaining a reserve of buds requires a large respiratory demand on the plant (Vesk and Westoby 2004), and thus has important implications for the physiological status of the plant during resprouting.

Elucidating the physiological mechanisms of size-dependent resprouting success would provide insights into plant persistence in fire-prone systems and explain the subsequent community dynamics of savannas and other fire-dominated systems worldwide. The longleaf pine-wiregrass (*Pinus palustris*–*Aristida stricta*) savanna at Fort Bragg in the Sandhills region of North Carolina provides a useful study system in which to examine the fire on plant physiological resprouting responses since prescribed burning has been in effect since 1991.

The goal of my dissertation research was to elucidate the physiological mechanisms that govern size-dependent resprouting success in fire-prone savannas to improve our ability to

predict species and community responses to fire. I will address this goal by examining the resprouting recovery of biomass and carbon, changes in carbon assimilation, changes in root carbohydrates and nitrogen, changes in allocation patterns after clipping, and the effects of repeated burning on common understory plant species. This dissertation aims to address the overarching hypothesis that post-fire carbon balance is negatively correlated with plant stature across and within species, and that this can largely explain patterns of post-fire resprouting, biomass and carbon recovery, and replenishment of carbohydrate reserves. In Chapter 2, I examined the allometry of biomass recovery and whole-plant carbon assimilation of nine understory species to understand its contribution to plant carbon balance and the size-dependency of post-burn resprouting. Chapter 3 examines the relationship between plant size and the concentration of root carbohydrates and root nitrogen, and how these concentrations change after fire. In Chapter 4, coppicing experiments were used to examine how the removal of supplementary shoots would affect the growth of the remaining stem in resprouting individuals. Chapter 5 addresses the patterns of negative allometry across 24 species and how plant responses to repeated fire could explain the success of small growth forms. Finally, the last chapter concludes with a summary chapter to describe the main findings of my dissertation, some limitations of the work, and recommendations for future research.

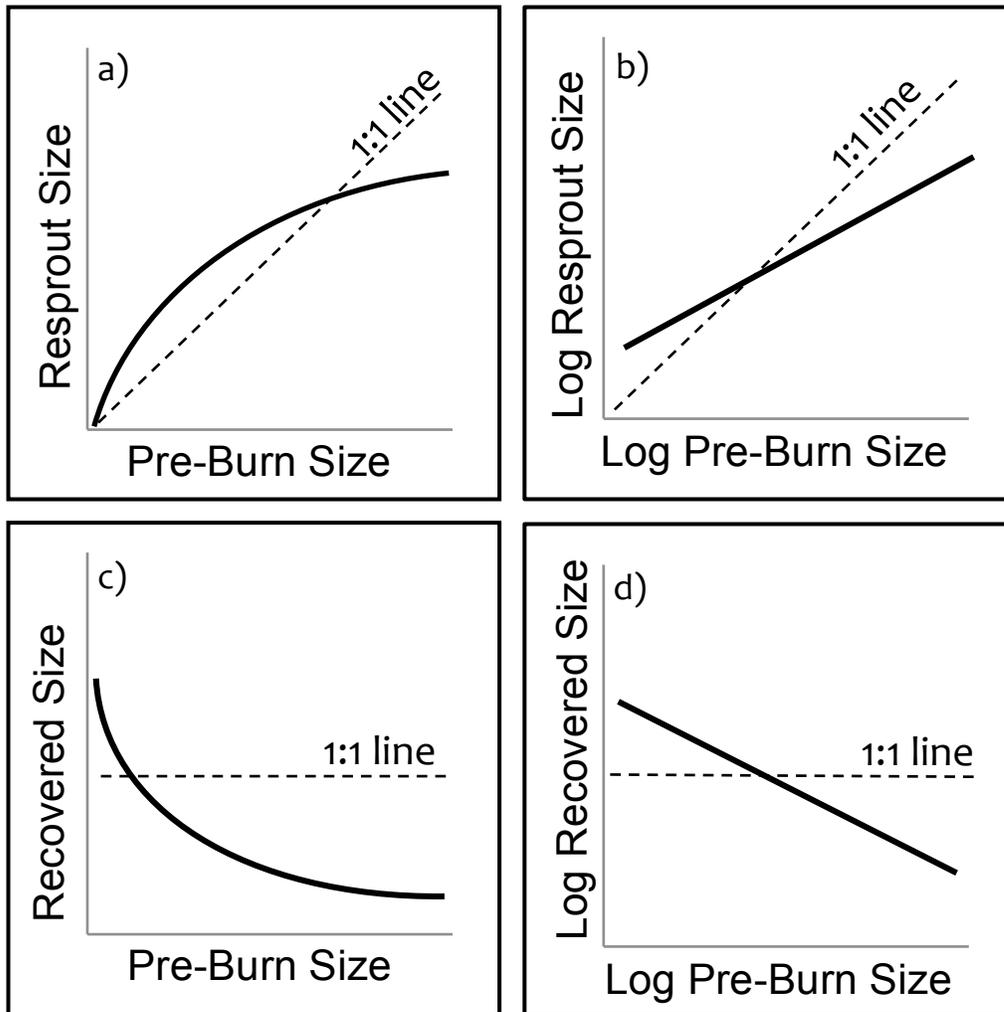


Figure 1.1 Hypothesized allometric patterns underlying the relationship between pre-burn and post-burn variables: (a) Allometric relationship between pre-burn and post-burn size, (b) log transformation of the relationship in a, (c) relationship between the recovered size (post-burn: pre-burn size) and the pre-burn size, and (d) log transformation of the relationship in c. The dashed line represents the 1:1 line, along which the post-fire resprout size equals pre-burn size, and the coefficient or slope (α) of the relationship is equal to 1. The solid line represents a “negative allometry” where $\alpha < 1$. Shallower slopes indicate a net benefit to smaller plants, consistent with a more negative allometry.

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CHAPTER 2

PLANT CARBON BALANCE EXPLAINS SIZE-DEPENDENT RESPONSES TO FIRE

ABSTRACT

The need to maintain a positive carbon balance is a universal constraint upon plants in any environment. The challenge of maintaining positive carbon balance is exacerbated when a plant is exposed to disturbances, such as fire, which destroy aboveground biomass (topkill) and therefore cause immediate carbon loss while compromising subsequent photosynthesis due to lost leaf area. When fire is frequent, it can limit the success of certain plant species while promoting others. To understand the physiological mechanisms underlying why small growth forms tend to be favored by frequent burning, we tested the hypothesis that post-fire carbon balance is negatively correlated with plant stature, and that this trend can largely explain patterns of post-fire resprouting, biomass and carbon recovery, and replenishment of carbohydrate reserves. We used *in situ* light measurements, photosynthetic light-response curves, and allometric estimates of leaf area to estimate potential whole-plant carbon assimilation over the first four months of resprouting in nine understory species. A colorimetric method was used to determine root carbohydrate concentrations. These metrics were used to determine whole-plant carbon status and quantify its contribution to recovery. Small plants recovered more biomass and carbon relative to pre-burn size. Compared to large plants, small plants also showed the potential to assimilate more carbon relative to pre-burn root carbohydrate reserves. We confirmed a pattern of resprouting that constrains the post-fire recovery of large plants, that we termed negative allometry, and reveal that it arises very early in resprouting and has a self-reinforcing effect on whole-plant carbon balance. We

argue that these physiological mechanisms can explain size-dependent resprouting success.

INTRODUCTION

The need to maintain a positive carbon balance is a universal constraint upon plants in any environment. Ultimately, growth, reproduction, and survival are dependent upon the ability of a plant to acquire more carbon through photosynthesis than it loses from respiration and tissue turnover (McCree 1986). This challenge is exacerbated when a plant is exposed to disturbances that destroy aboveground biomass (topkill), thereby causing immediate carbon losses while also compromising subsequent photosynthesis due to lost leaf area. Recovery of aboveground biomass relies on the mobilization of stored carbon reserves (Bowen and Pate 1993; Landhäusser and Lieffers 2002). By reducing woody growth, fire increases light availability to the understory vegetation (Bond and Van Wilgen 1996; Platt et al. 2006), which may aid resprouting plants in the quick recovery of aboveground biomass and renewal of positive carbon balance. Where disturbances are frequent, such as frequently burned savannas and grasslands, the loss of aboveground biomass is both complete and frequent. Although large trees may escape this fate, some juvenile trees, as well as shrubs and herbs will succumb to topkill. As a result, vegetation structure often shifts from a closed canopy forest dominated by hardwoods to an open canopy savanna with a diverse understory (Gilliam and Platt 1999; Bond et al. 2005). Frequent fire can therefore limit the success of certain plant species while promoting others, often favoring herbaceous and small-statured plants at the expense of trees and large shrubs (Hoffmann and Solbrig 2003).

Short fire return intervals have been shown to intensify the negative consequences for carbon balance in large plants. Grady and Hoffmann (2012) reported that a cycle of repeated

topkill and resprouting results in a stable size equilibrium resulting from a curvilinear relationship between pre-burn and resprout size. Without sufficient time between fires, the equilibrium size is reduced because plants are caught in a firetrap, precluding a full recovery of lost biomass (Grady and Hoffmann 2012). When log transformed, this curvilinear relationship appears as a straight line with a slope <1 , a pattern exhibiting “negative allometry”, indicating that large plants experience a disproportionately negative resprouting response (Figure 2.1); i.e. relative to small plants, large plants recover a smaller proportion of their pre-burn size. Over many years this can shift the overall vegetation structure in favor of small-statured plants. From the perspective of plant carbon balance it is unclear why frequent fire tends to favor small growth forms over large.

The objective was to develop a mechanistic framework to understand the physiological basis for these size-dependent responses to fire. I tested the hypothesis that post-fire carbon balance is negatively correlated with plant stature across species, and that this trend can largely explain patterns of post-fire resprouting, biomass recovery, and replenishment of carbohydrate reserves. To test this hypothesis, I investigated patterns of resprouting and estimates of potential whole-plant carbon assimilation in nine common understory plants under prescribed burning and control conditions in the longleaf pine-wiregrass savanna of the southeastern United States.

METHODS

Study sites and species

This research was conducted May–November 2011 at Fort Bragg Military Reservation in Hoke and Cumberland counties in North Carolina. Mean annual precipitation

is 1275 mm (1200 mm rain, 75 mm snow), and mean winter and summer temperatures are 6.9°C and 26°C, respectively (Sorrie et al. 2006). Fort Bragg is divided into a large number of burn units that have been managed independently with prescribed fire at intervals of approximately 3 years since 1991. Twelve study sites were selected at ecotones between the upland longleaf pine-wiregrass (*Pinus palustris*-*Aristida stricta*) savanna and stream-head wetlands, where plants have access to perennially wet soils, but are exposed to fire. Although all study sites had been regularly burned at 3-year intervals, they differed in the date of the most recent fires: four sites were last burned in the previous year (2010), four sites were last burned 2 years previously (2009), and four sites were burned 3 years previously (2008) and were suppressed from fire for the length of this project (unburned control sites). For the present study, all sites except the control sites were experimentally burned between June 10 and August 31, 2011. All burns were low-intensity surface fires similar to those implemented for routine maintenance of the sites.

I studied nine common understory species (Table 2.1), including 3 trees: *Acer rubrum* L. (red maple), *Oxydendrum arboreum* (L.) DC. (sourwood), *Persea palustris* (Raf.) Sarg. (swampbay); 4 woody shrubs: *Clethra alnifolia* L. (summer-sweet), *Gaylussacia frondosa* (L.) Torr. & A. Gray ex Torr. (dangleberry), *Ilex glabra* (L.) A. Gray (inkberry), *Lyonia lucida* (Lam.) K. Koch (pink fetterbush); and 2 herbaceous species: *Arundinaria tecta* (Walter) Muhl. (switchcane), *Eupatorium rotundifolia* L. (roundleaf thoroughwort). Hereafter, these species will be referred to by their genus. At least six of the nine species were found at each site.

Above and Belowground Biomass Recovery

In May 2011, at the beginning of the growing season, prior to burning, eight

individuals per species, when present, were tagged at each burn site. To reduce the effect of environmental heterogeneity, individuals were located within a single 5 x 5 meter sample area. Prior to burning, height and basal diameter were measured at 5 cm above the ground. These measurements were repeated at 1, 2, and 4 months after burning. By 4 months after the prescribed burns, growth of resprouts had ceased and leaves of deciduous species began to senesce. For suppression sites, measurements were taken at the beginning of June 2011 and measured 1, 2 and 4 months after initial measurement. For individuals with multiple stems, these measurements were performed for each stem. Species-specific allometric equations for pre-burn and resprouting plants (Appendix A) were used to non-destructively estimate leaf area and total aboveground biomass from the height of each tagged individual at each census. Pre-burn biomass refers to total aboveground biomass. For each species, a range of stem sizes was harvested, and the following measurements were collected: height, leaf area, stem weight, and leaf weight. These measurements were used to determine equations to predict total leaf area and total biomass based on measurements of stem height of tagged individuals in the study plots. A CI-202 portable laser leaf area scanner (CID Bio-Science, Inc., Camas, WA) was used to determine whole-plant leaf area. For each species, separate equations were developed for pre-burn and resprouting plants.

In 2012, root allometric equations were developed for each species to determine root mass based on total aboveground biomass. Plants of variable heights were harvested in sites three years since fire to account for variability in aboveground height. To develop these allometric relationships, stem heights of all individuals were measured before harvest. Stems, leaves, and roots were bagged separately, dried at 70 °C for 72-96 hours, and then weighed.

Allometric relationships within each species were significant ($P > 0.05$).

Photosynthesis Measurements

I performed leaf gas exchange measurements before and after fire to test for the effect of burning on maximum CO₂ assimilation rates (A_{\max}) used in the modeling synthesis described below. At the time of the initial pre-burn measurement, photosynthetic light-response curves were performed on plants to be burned and on unburned controls. Pre-burn light-response curves were performed on plants to be burned and on unburned controls. Pre-burn light-response curves were measured on 5–20 individuals per species per treatment, depending on abundance at each site. Measurements were performed using a portable infrared gas analyzer (LiCor 6400XT, Li-Cor Inc., Lincoln, NE), on fully expanded sunlit leaves between 10 am and 3 pm with the mean chamber temperature maintained at $25.0 \pm 0.5^\circ\text{C}$, and $400 \mu\text{mol mol}^{-1}$ CO₂ concentration, and a saturating light intensity of $1500 \mu\text{mol photon m}^{-2} \text{s}^{-1}$. Relative humidity within the chamber was controlled to match ambient conditions, but not to exceed 83%. Leaves were allowed to adjust to the light intensity for 2–5 minutes prior to measurement. An auto-logging program was set to record five measurements of gas exchange at each level of photosynthetically active radiation (PAR) ranging from 0–1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (1500, 1000, 500, 120, 60, 40, 20, 10, 0). Leaves were allowed to adjust for at least 5 minutes at each light intensity before gas exchange measurements were recorded. After fire, post-burn point measurements of A_{\max} were recorded at saturating light intensity ($1500 \mu\text{mol photon m}^{-2} \text{s}^{-1}$), matching the leaf and chamber conditions described above. An auto-logging program recorded five point measurements on 5–25 individuals per species per treatments, after an adjustment period of 2–5 minutes.

Measurements of Light Availability

To estimate light availability for the study plants, we combined overstory and understory measurements of light transmittance at each sampling site. Overstory transmittance was estimated using the Solar Pathfinder (Linden, TN), which provides hourly estimates of percent interception of direct sunlight by the canopy for each month of the year. Light interception was divided into classes of canopy closure (open, partial, and closed). To quantify light attenuation through the understory vegetation, the SS1 Sunscan Canopy Analysis System (Delta-T, Cambridge England) measured PAR at the corners of each sample site, at solar noon, and at three heights: 2 meters, 1 meter, and ground level. All measurements were made concurrently with measurements of incident PAR with a sensor placed in direct sunlight. Canopy-specific PAR (PAR for each canopy closure condition: closed, partial, open) was then calculated as the product of the baseline 1200 PAR and the average transmittance we obtained from the Sunscan. We estimated PAR that was specific to the height of each tagged individual as the product of the canopy-specific PAR and the fraction of the light transmitted from 2 meters to the height of each individual.

Modeling Carbon Assimilation

A simple model was used to estimate potential carbon (C) assimilation of resprouting plants, based on field measurements of leaf area, light availability, and photosynthesis. The objective was to estimate the relative capacity for post-fire carbon assimilation to contribute to recovery of aboveground biomass in plants differing in pre-burn size. Carbon assimilation was estimated for each individual per day as the product of the photosynthetic rate and leaf area, and integrated over the 4 months remaining in the growing season after the prescribed

fire.

The photosynthetic rate of each resprouting individual was estimated for each canopy closure class (each a fraction of the total incoming light), and time since fire (1, 2, 4 months post-fire) as

$$\phi \text{ PAR} + A_{\text{max}} - \sqrt{((\phi \text{ PAR} + A_{\text{max}})^2 - 4\theta \phi) - R_d} \text{ (Lambers et al. 2008)}$$

For each value of PAR at the height of each individual the parameters ϕ (quantum yield) A_{max} (light saturated rate of CO₂ assimilation), R_d (dark respiration), and θ (curvature parameter) were fitted to the data for the light-response curves described above using least squares nonlinear regression using R version 2.15.1 (The R Foundation for Statistical Computing, 2012).

Mean daylength was 13.75 h over the study period (35.1°N; <http://astro.unl.edu/classaction/animations/coordsmotion/daylighthouseexplorer.html>). Respiration of stems and roots was not directly measured, so we assumed 50% of the photosynthate was lost to respiration (Mooney 1972; Hopkins and Hüner 2009). This likely underestimates whole-plant respiration in the early stages of resprouting when leaf area is low relative to root mass. As we explain in the discussion, however, this underestimation during resprouting should be greatest in large individuals, resulting in conservative conclusions regarding the effect of plant size on post-fire carbon gain.

Root Carbohydrate Analysis

A colorimetric method was performed to determine starch and sugar concentration of roots (Chow and Landhäusser 2004). All root material was dried on the same day as collection at 70 °C for 72 hours (10–29 per species; 61–90 cm average height). Dried root

samples were ground using a Wiley mill with a 40-mesh screen. For each 50 mg sample, sugars were extracted three times using with 5 mL of 80% ethanol, by boiling samples in capped glass tubes in 95 °C water bath for 10 minutes each. After each extraction, tubes were centrifuged at 2500 rpm for five minutes and the supernatants of the three extractions were combined for sugar analysis. Then the extract was reacted with phenol-sulfuric acid, which allowed the sugars to be measured colorimetrically at an absorbance of 490 nm. To determine the starch concentration, the remaining starch in the residue was hydrolyzed to glucose by an enzyme mixture of α -amylase and amyloglucosidase, and then measured colorimetrically using a peroxidase-glucose oxidase-o-dianisidine solution. Starch was measured at an absorbance of 525 nm. Absorbances were converted to concentrations using standards to evaluate the efficiency of digestion and colorimetry.

There was no relationship ($P > 0.05$) between plant height and sugar, starch or total carbohydrate (CHO) concentration in roots. Therefore, the total CHO pool size for each tagged plant was calculated as the product of the species mean CHO and the allometrically determined root mass.

Analysis of Size-Dependent Responses to Fire

I used an allometric approach to test the hypothesis that, relative to small plants, large plants have lower whole-plant C assimilation and biomass recovery per unit of pre-burn biomass. Testing this relationship directly, as seen in Figure 2.1a, is subject to spurious correlations that can arise because the independent variable is used to calculate the dependent variable. This bias can be avoided by testing for an allometric relationship between whole-plant assimilation (or resprout biomass) and pre-burn biomass. Under the proposed

hypothesis, the relationship should exhibit *negative allometry* (i.e. allometric coefficient < 1). If negative allometry exists, the slope of the relationship is < 1 on a \log_{10} scale, and large plants experience a disproportionately negative response to fire; i.e., the resprouting variable is less than that of the pre-burn variable. Hereafter, this pattern will be referred to as negative allometry (Figure 2.1).

Statistical Analyses

Differences in resprout light saturated assimilation ($\mu\text{mol m}^{-2} \text{s}^{-1}$) were tested within each species between burned and control plants using a Student's T-test. Before burning, mean assimilation rates per species did not differ significantly between control sites and those that were scheduled to burn ($P > 0.5$). Within each species, I used a regression model with pre-burn biomass or pre-burn height as the independent variable and resprout variables as the dependent variable (both \log_{10} -transformed to fit normality): whole-plant leaf area, recovered biomass (resprout biomass: pre-burn biomass), carbon assimilated, recovered C (assimilated C: pre-burn biomass, and assimilated C: pre-burn CHO). We tested for homogeneity of slopes of C assimilated vs. pre-burn height (\log_{10} -transformed) among treatments using an analysis of covariance. All statistical analyses were performed using R version 2.15.1 (The R Foundation for Statistical Computing, 2012).

RESULTS

Light Availability & Photosynthetic Rates

Following fire, photosynthetic rates were higher due to greater light intensities combined with higher rates of light-saturated assimilation. Mean maximum light-saturated assimilation two months after fire was 36% higher in burned plants relative to controls across all species

($t = 13.6$, $df = 335$, $P < 0.001$), and the differences were significant within each species except for *Lyonia*, *Oxydendrum*, and *Persea* (Figure 2.2). Before burning, mean assimilation rates per species did not differ significantly between control sites and those that were scheduled to burn ($P > 0.5$). Before burning, the herbaceous species *Eupatorium* and *Arundinaria* had the highest photosynthetic rates (12.2 and $13.6 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). The increase in A_{max} after fire was reinforced by a 49% increase in understory light availability. Mean percent incident light remained high two months after fire, where available light was significantly higher (42.7%) than pre-burn light conditions ($t = -3.07$, $df = 93.8$, $P = 0.003$). But unburned control sites were not significantly changed over this time ($t = -0.45$, $df = 46.8$, $P = 0.65$).

Effect of Fire on Aboveground Biomass Recovery

All study plants were topkilled by fire, but 68% had resprouted within 1 month of burning. *Acer* and *Lyonia* had the lowest resprout rate of the study species (23.5% and 15%, respectively; Table 2.1). In contrast, the small woody shrub *Clethra* and the herbaceous species *Eupatorium* had a resprout rate of 100%. By the end of the growing season, an average of 91% of all burned plants resprouted, but *Lyonia*, continued to lag behind the others (75.0%).

Although fire dramatically reduced the total leaf area, the resprouting allometry (leaf area ~ stem height) was altered in favor of small plants, resulting in a more negative allometry in five species (Table 2.2). Resprouting leaf area allometry of *Eupatorium*, *Ilex*, *Lyonia*, *Oxydendrum*, and *Persea*, was significantly altered in favor of small plants, relative to unburned controls. In these species, the reduction in slope benefited small individuals

because they resprouted with a larger leaf area relative to their pre-burn size.

Biomass recovery within species displayed a consistent size-dependent trend, but the negative allometric relationship between resprout and pre-burn biomass was not significant in *Acer*, *Arundinaria*, and *Gaylussacia* (Table 2.3a). Net losses in plant biomass are shown as the fraction of pre-burn biomass recovered during 4 months of resprouting (Figure 2.3; Table 2.4a). Large plants experienced greater net reductions in plant mass relative to smaller plants, but this relationship was not significant for *Acer*, *Arundinaria*, and *Gaylussacia*. *Eupatorium* had many individuals that resprouted to a height twice that of their pre-burn size.

Effect of Fire on Carbon Assimilation and Recovery

All species in the burned plots exhibited negative allometry for the relationship between whole-plant C assimilation and pre-burn biomass (Table 2.3b), but in the unburned control sites this relationship was not significant for *Oxydendrum* and *Persea* (Table 2.3c). There was a significant positive relationship between whole-plant C assimilation and initial biomass within all species, both in resprouting individuals and those in the unburned controls (Figure 2.4; Table 2.4b). Burning significantly reduced the slope of the relationship between assimilated carbon and pre-burn biomass, relative to unburned controls in all species except *Acer* and *Clethra* (Table 2.5). This result reveals a greater negative effect on larger plants in burned sites relative to the unburned controls. Furthermore, the reduction in slope in burned treatments had a positive effect on the assimilation of small individuals, particularly for *Arundinaria*, *Eupatorium*, *Ilex*, and *Persea*, which is evident where the line of the burned treatment crosses above that of the unburned control (Figure 2.4).

Given the effect fire had on reducing the slope of the relationship between C assimilated

and initial biomass, we also present recovered C (post-fire C assimilated: pre-burn biomass) relative to pre-burn biomass (Figure 2.5; Table 2.4c). Large statured individuals of all species recovered significantly less C relative to their pre-burn size, when compared to smaller individuals. The significant negative relationship between recovered C and pre-burn biomass underscores the negative consequences experienced by large statured resprouting plants.

Effect of Fire on Root Carbohydrate Content

All species examined in this study had average root: shoot ratios between 1–2, except *Persea*, which had the greatest root: shoot ratio of 4.49. Root biomass was positively correlated with total aboveground biomass ($P < 0.05$). Across species, the total average root carbohydrate concentration per species ranged from 10.7% to 28.9% before burning. The ratio of resprout C assimilation to total pre-burn root CHO was significantly greater in small plants relative to large plants (Figure 2.6; Table 2.6). That is, compared to large plants, small plants assimilated a greater amount of C relative to their total pre-burn root CHO pool. The herbaceous species, *Eupatorium*, was able to assimilate 80–90 times more C relative to the available root CHO (Figure 2.6d), although most small statured plants were able to assimilate 10 times more C relative to their root CHO.

Table 2.1 Study species and characteristics are represented with the number of individuals, height range, and resprouting rate for nine common understory species. Nomenclature follows Flora of the Southern and Mid-Atlantic States by Alan S. Weakley (<http://www.herbarium.unc.edu/flora.htm>).

Growth Form	Species	Family	<i>N</i>	Height Range (cm)	1 month resprout rate (%)	4 month resprout rate (%)
Tree	<i>Acer rubrum</i> L.	Sapindaceae	62	5.5–260	23.5	82.4
	<i>Oxydendrum arboreum</i> (L.) DC.	Ericaceae	51	24–211	60.8	82.4
	<i>Persea palustris</i> (Raf.) Sarg.	Lauraceae	114	10–172	90.0	96.0
Shrub	<i>Clethra alnifolia</i> L.	Clethraceae	136	9-155	85.7	100
	<i>Gaylussacia frondosa</i> (L.) Torr. & A. Gray ex Torr.	Ericaceae	134	13.5–141	51.7	91.4
	<i>Ilex glabra</i> (L.) A. Gray	Aquifoliaceae	128	16–205	82.1	98.2
	<i>Lyonia lucida</i> (Lam.) K. Koch	Ericaceae	90	14–165	15.0	77.5
Herbaceous	<i>Arundinaria tecta</i> (Walter) Muhl	Poaceae	110	16–202	84.2	84.2
	<i>Eupatorium rotundifolium</i> L.	Asteraceae	55	10–134	100	100

Table 2.2 Allometric relationships between whole-plant leaf area and stem height for plants before and after fire. (a) ANCOVA results indicating a difference in slopes between unburned controls and resprouting plants 2 months after fire, (b) statistical significance for the relationship between whole-plant leaf area and stem height in resprouting plants, (c) statistical significance for the relationship between whole-plant leaf area and stem height in unburned control plants. See Appendix A for allometric equations.

(a) Leaf Area ~ Height (ANCOVA)				
Species	df	F	P	
<i>Acer rubrum</i>	22	0.73	0.40	
<i>Arundinaria tecta</i>	60	3.38	0.07	
<i>Clethra alnifolia</i>	114	2.16	0.15	
<i>Eupatorium rotundifolium</i>	32	32.7	<0.001	
<i>Gaylussacia frondosa</i>	94	2.75	0.100	
<i>Ilex glabra</i>	106	26.0	<0.001	
<i>Lyonia lucida</i>	52	51.2	<0.001	
<i>Oxydendrum arboreum</i>	66	13.2	<0.01	
<i>Persea palustris</i>	90	11.2	0.001	
(b) Leaf Area ~ Height (Resprout)				
Species	df	t	P	R²
<i>Acer rubrum</i>	11	43.7	<0.001	0.810
<i>Arundinaria tecta</i>	30	31.8	<0.001	0.885
<i>Clethra alnifolia</i>	57	22.2	<0.001	0.859
<i>Eupatorium rotundifolium</i>	16	10.8	<0.001	0.902
<i>Gaylussacia frondosa</i>	47	35.9	<0.001	0.910
<i>Ilex glabra</i>	53	21.0	<0.001	0.968
<i>Lyonia lucida</i>	26	17.1	<0.001	0.857
<i>Oxydendrum arboreum</i>	33	36.0	<0.001	0.879
<i>Persea palustris</i>	45	23.1	<0.001	0.806
(c) Leaf Area ~ Height (Pre-burn)				
Species	df	t	P	R²
<i>Acer rubrum</i>	11	37.7	<0.001	0.801
<i>Arundinaria tecta</i>	30	22.3	<0.001	0.895
<i>Clethra alnifolia</i>	57	39.5	<0.001	0.816
<i>Eupatorium rotundifolium</i>	16	60.2	<0.001	0.768
<i>Gaylussacia frondosa</i>	47	46.4	<0.001	0.895
<i>Ilex glabra</i>	53	26.0	<0.001	0.878
<i>Lyonia lucida</i>	26	42.6	<0.001	0.892
<i>Oxydendrum arboreum</i>	33	23.2	<0.001	0.821
<i>Persea palustris</i>	45	57.5	<0.001	0.950

Table 2.3 Statistical results of nonlinear regression analysis to determine negative allometry in the relationships between (a) pre-burn biomass and post-burn biomass, (b) pre-burn biomass and post-burn carbon assimilation, and (c) pre-burn biomass and carbon assimilation in unburned controls. Negative allometry ($P < 0.05$) exists if the slope of the relationship is significantly less than 1.

(a) Pre-Burn Biomass ~ Resprout Biomass					
Species	Equation	df	t	P	R²
<i>Acer rubrum</i>	$y=0.54x - 0.89$	11	1.54	0.07	0.23
<i>Arundinaria tecta</i>	$y=0.78x - 0.33$	30	1.15	0.13	0.35
<i>Clethra alnifolia</i>	$y=0.66x - 0.32$	57	4.27	<0.001	0.55
<i>Eupatorium rotundifolium</i>	$y=0.52x + 0.14$	16	5.53	<0.001	0.69
<i>Gaylussacia frondosa</i>	$y=0.70x - 0.71$	47	1.25	0.109	0.15
<i>Ilex glabra</i>	$y=0.37x - 0.08$	53	6.09	<0.001	0.19
<i>Lyonia lucida</i>	$y=0.30x - 0.46$	26	7.05	<0.001	0.26
<i>Oxydendrum arboreum</i>	$y=0.23x + 0.33$	33	2.69	0.005	0.02
<i>Persea palustris</i>	$y=0.66x - 0.28$	45	2.56	0.006	0.36
(b) Pre-Burn Biomass ~ Carbon Assimilated					
Species	Equation	df	t	P	R²
<i>Acer rubrum</i>	$y=0.68x + 0.19$	11	2.71	0.01	0.74
<i>Arundinaria tecta</i>	$y=0.68x + 0.39$	30	2.93	0.003	0.57
<i>Clethra alnifolia</i>	$y=0.72x + 0.57$	57	5.04	<0.001	0.75
<i>Eupatorium rotundifolium</i>	$y=0.43x + 0.59$	16	9.88	<0.001	0.76
<i>Gaylussacia frondosa</i>	$y=0.65x + 0.21$	47	5.24	<0.001	0.54
<i>Ilex glabra</i>	$y=0.48x + 0.51$	53	7.05	<0.001	0.43
<i>Lyonia lucida</i>	$y=0.69x + 0.19$	26	49.6	<0.001	0.94
<i>Oxydendrum arboreum</i>	$y=0.45x + 0.97$	33	4.43	<0.001	0.27
<i>Persea palustris</i>	$y=0.67x + 0.47$	45	4.33	<0.001	0.62
(c) Pre-Burn Biomass ~ Carbon Assimilated (Control)					
Species	Equation	df	t	P	R²
<i>Acer rubrum</i>	$y=0.82x + 0.48$	28	17.6	<0.001	0.99
<i>Arundinaria tecta</i>	$y=0.87x + 0.43$	30	10.1	<0.001	0.99
<i>Clethra alnifolia</i>	$y=0.80x + 0.62$	30	13.7	<0.001	0.99
<i>Eupatorium rotundifolium</i>	$y=0.88x + 0.58$	16	4.71	<0.001	0.98
<i>Gaylussacia frondosa</i>	$y=0.84x + 0.48$	30	8.67	<0.001	0.98
<i>Ilex glabra</i>	$y=0.82x + 0.51$	30	12.3	<0.001	0.99
<i>Lyonia lucida</i>	$y=0.79x + 0.35$	22	27.5	<0.001	0.99
<i>Oxydendrum arboreum</i>	$y=0.82x + 1.14$	6	1.89	0.054	0.92
<i>Persea palustris</i>	$y=0.98x + 0.39$	22	0.29	0.38	0.96

Table 2.4 Statistical results for the relationships between pre-burn biomass and the following recovery variables for resprouting plants 4 months after burning: (a) Recovered biomass (resprout biomass: pre-burn biomass), (c) carbon assimilated, and (d) recovered carbon (carbon assimilated: pre-burn biomass).

(a) Pre-Burn Biomass ~ Resprout Biomass					
Species	Equation	df	F	P	R²
<i>Acer rubrum</i>	$y = -0.46x - 0.88$	11	2.39	0.15	0.23
<i>Arundinaria tecta</i>	$y = -0.22x - 0.33$	30	1.30	0.26	0.35
<i>Clethra alnifolia</i>	$y = -0.33x - 0.32$	57	18.5	<0.001	0.55
<i>Eupatorium rotundifolium</i>	$y = -0.47x + 0.14$	16	30.7	<0.001	0.69
<i>Gaylussacia frondosa</i>	$y = -0.31x - 0.71$	47	1.56	0.21	0.15
<i>Ilex glabra</i>	$y = -0.63x - 0.08$	53	36.9	<0.001	0.19
<i>Lyonia lucida</i>	$y = -0.69x - 0.46$	26	49.9	<0.001	0.26
<i>Oxydendrum arboreum</i>	$y = -0.77x + 0.33$	33	7.29	0.01	0.02
<i>Persea palustris</i>	$y = -0.33x - 0.27$	45	6.50	0.01	0.36
(b) Pre-Burn Biomass ~ Carbon Assimilated					
Species	Equation	df	F	P	R²
<i>Acer rubrum</i>	$y=0.68x + 0.19$	11	34.7	<0.001	0.74
<i>Arundinaria tecta</i>	$y=0.68x + 0.39$	30	41.4	<0.001	0.57
<i>Clethra alnifolia</i>	$y=0.72 + 0.57$	57	175	<0.001	0.75
<i>Eupatorium rotundifolium</i>	$y=0.43x + 0.59$	16	54.2	<0.001	0.76
<i>Gaylussacia frondosa</i>	$y=0.65x + 0.21$	47	56.8	<0.001	0.54
<i>Ilex glabra</i>	$y=0.48x + 0.51$	53	41.3	<0.001	0.43
<i>Lyonia lucida</i>	$y=0.69x + 0.19$	26	407	<0.001	0.94
<i>Oxydendrum arboreum</i>	$y=0.45x + 0.97$	33	13.6	<0.001	0.27
<i>Persea palustris</i>	$y=0.67x + 0.47$	45	77.4	<0.001	0.62
(c) Pre-Burn Biomass ~ Carbon Assimilated: Pre-Burn Biomass					
Species	Equation	df	F	P	R²
<i>Acer rubrum</i>	$y= -0.31x + 0.19$	11	7.25	0.02	0.34
<i>Arundinaria tecta</i>	$y= -0.31x + 0.39$	30	8.69	0.006	0.20
<i>Clethra alnifolia</i>	$y= -0.27x + 0.56$	57	25.8	<0.001	0.30
<i>Eupatorium rotundifolium</i>	$y= -0.57x + 0.59$	16	97.6	<0.001	0.85
<i>Gaylussacia frondosa</i>	$y= -0.35x + 0.21$	47	16.6	<0.001	0.25
<i>Ilex glabra</i>	$y= -0.52x + 0.51$	53	48.9	<0.001	0.47
<i>Lyonia lucida</i>	$y= -0.31x + 0.18$	26	83.5	<0.001	0.75
<i>Oxydendrum arboreum</i>	$y= -0.54x + 0.97$	33	19.6	<0.001	0.35
<i>Persea palustris</i>	$y= -0.32x + 0.47$	45	18.5	<0.001	0.28

Table 2.5 ANCOVA results for the difference in slopes between unburned controls and resprouting plants 4 months after fire for the relationship between pre-burn biomass and carbon assimilated.

<i>4-Month Resprouts</i>	<i>df</i>	<i>F</i>	<i>P</i>
<i>Acer rubrum</i>	39	3.29	0.078
<i>Arundinaria tecta</i>	60	4.05	0.048
<i>Clethra alnifolia</i>	87	1.53	0.22
<i>Eupatorium rotundifolium</i>	32	56.3	<0.001
<i>Gaylussacia frondosa</i>	77	4.22	0.043
<i>Ilex glabra</i>	83	18.8	<0.001
<i>Lyonia lucida</i>	48	8.49	0.005
<i>Oxydendrum arboreum</i>	39	3.81	0.050
<i>Persea palustris</i>	67	7.41	0.008

Table 2.6 Statistical results for the relationships between pre-burn biomass and the recovery relationship for resprouting plants 4 months after burning: carbon assimilated: pre-burn carbohydrates.

Pre-Burn Biomass ~ Carbon Assimilated: Pre-burn Carbohydrate Pool					
Species	Equation	df	F	P	R²
<i>Acer rubrum</i>	$y = -0.32x + 1.53$	11	7.26	0.02	0.34
<i>Arundinaria tecta</i>	$y = -0.30x + 1.55$	29	6.36	0.01	0.15
<i>Clethra alnifolia</i>	$y = -0.28x + 1.25$	54	27.9	<0.001	0.33
<i>Eupatorium rotundifolium</i>	$y = -0.57x + 1.32$	12	87.1	<0.001	0.87
<i>Gaylussacia frondosa</i>	$y = -0.33x + 1.22$	45	15.2	<0.001	0.24
<i>Ilex glabra</i>	$y = -0.57x + 0.93$	50	62.5	<0.001	0.54
<i>Lyonia lucida</i>	$y = -0.31x + 0.59$	25	81.2	<0.001	0.76
<i>Oxydendrum arboreum</i>	$y = -0.62x + 1.49$	27	18.8	<0.001	0.39
<i>Persea palustris</i>	$y = -0.30x + 1.14$	44	15.4	<0.001	0.24

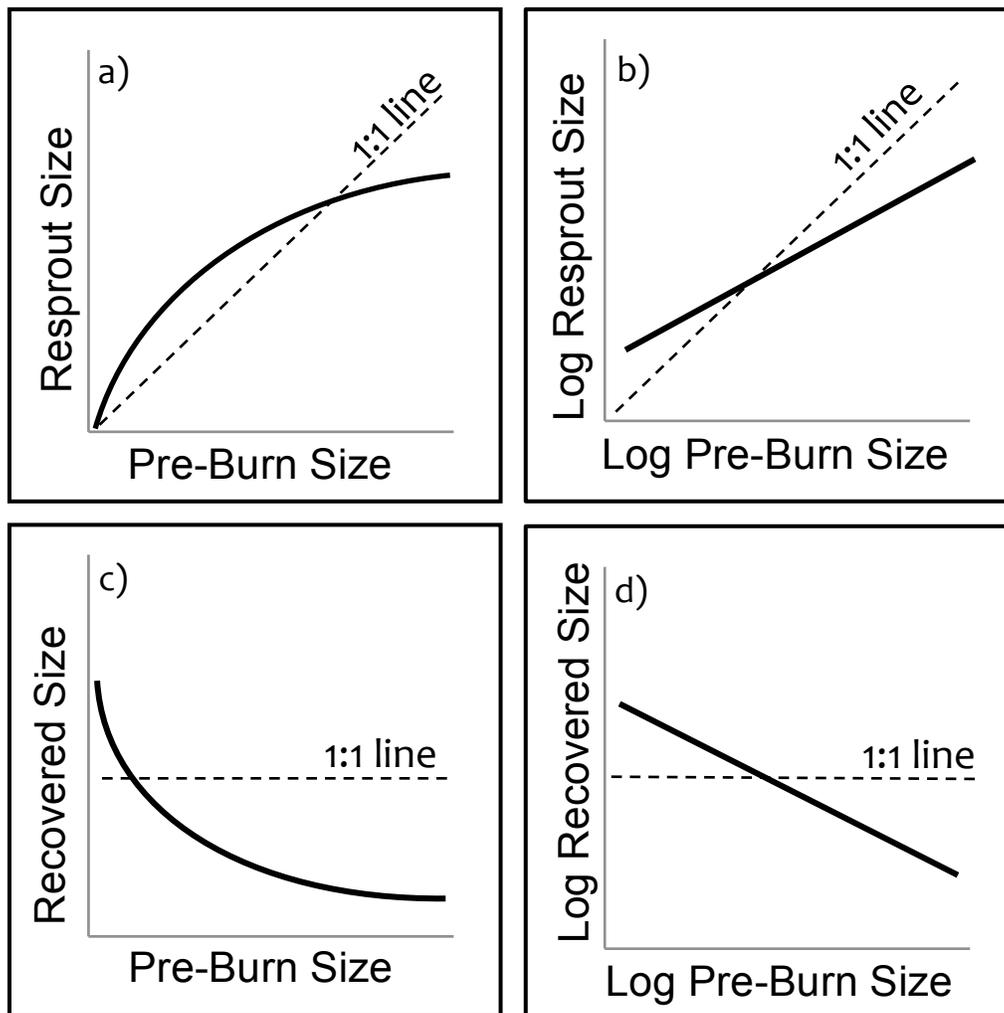


Figure 2.1 Hypothesized allometric patterns underlying the relationship between pre-burn and post-burn variables (modified from Grady & Hoffmann 2012): (a) Allometric relationship between pre-burn and post-burn size, (b) log transformation of the relationship in a, (c) relationship between the recovered size (post-burn: pre-burn size) and the pre-burn size, and (d) log transformation of the relationship in c. The dashed line represents the 1:1 line, along which the post-fire resprout size equals pre-burn size, and the coefficient or slope (α) of the relationship is equal to 1. The solid line represents a “negative allometry” where $\alpha < 1$. Shallower slopes indicate a net benefit to smaller plants, consistent with a more negative allometry.

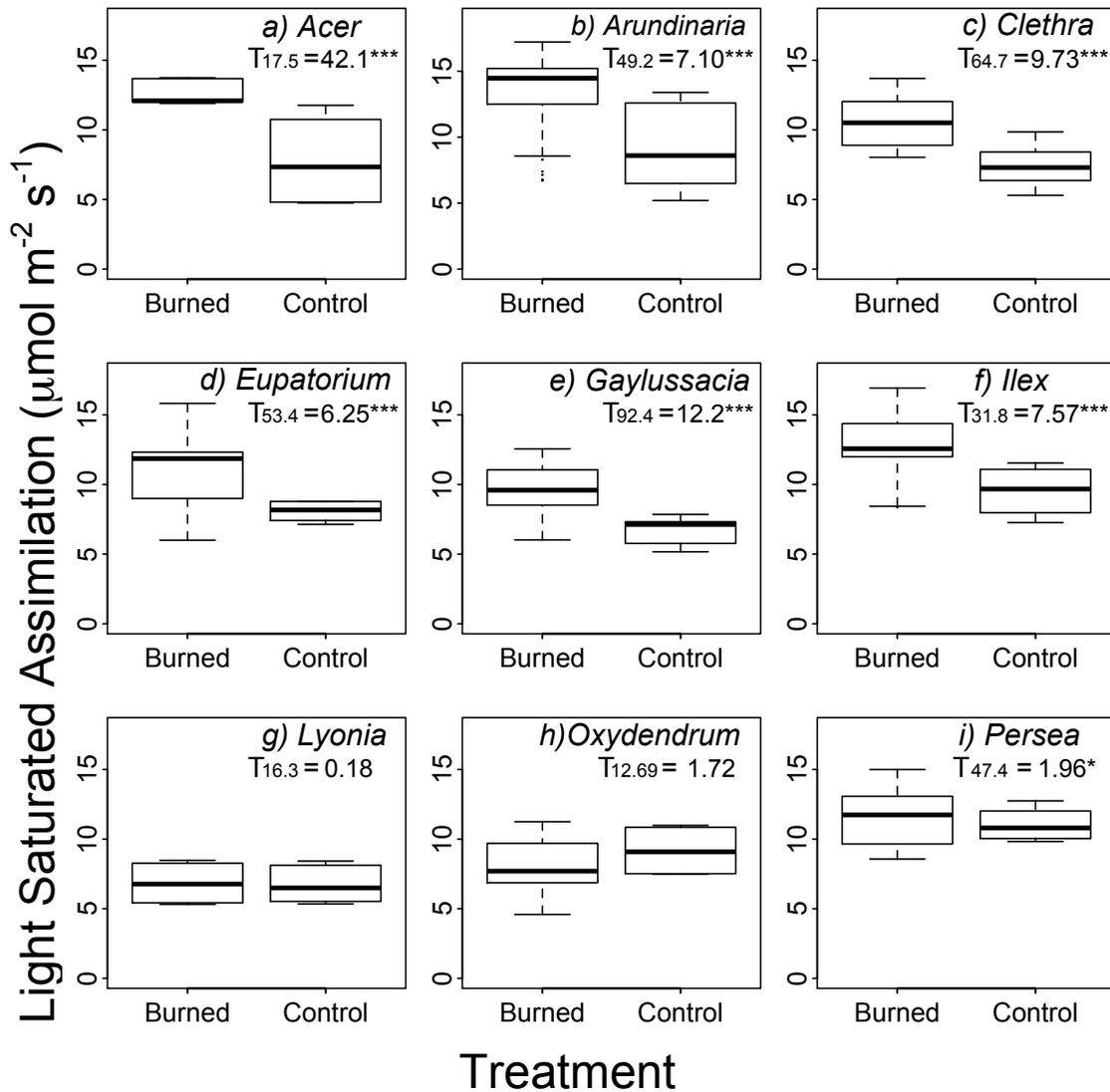


Figure 2.2 Mean light-saturated assimilation of sunlit leaves for nine common understory species in burned and unburned control sites two months after fire. Pre-burn gas exchange rates did not differ significantly between control sites and sites schedule to burn for (a) *Acer rubrum*, (b) *Arundinaria tecta*, (c) *Clethra alnifolia*, (d) *Eupatorium rotundifolium*, (e) *Gaylussacia frondosa*, (f) *Ilex glabra*, (g) *Lyonia lucida*, (h) *Oxydendrum arboreum*, (i) *Persea palustris*. $n = 101$ (burned), $n = 66$ (unburned). Asterisks indicate statistically significant effects of burn treatment: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

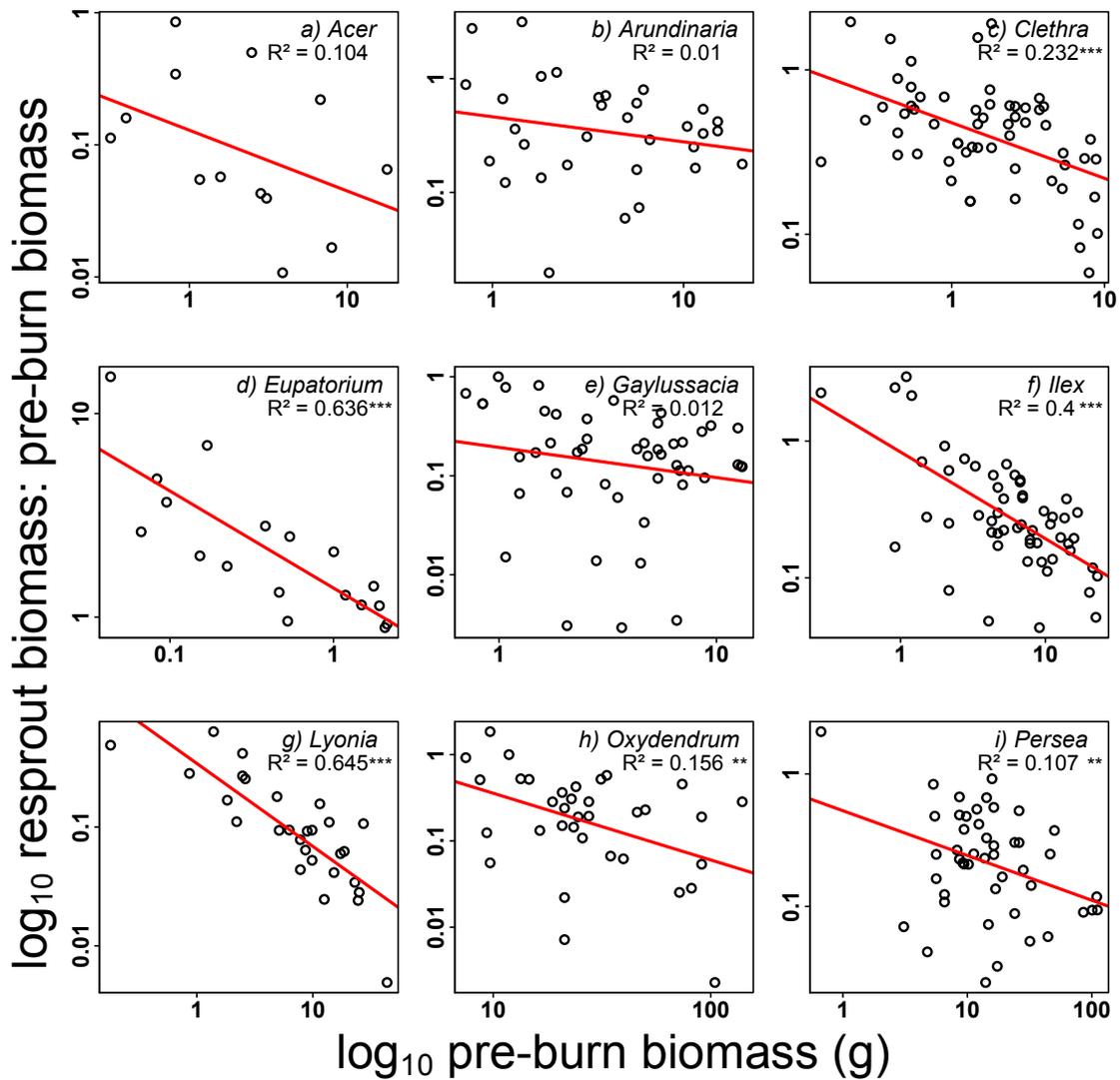


Figure 2.3 Relationship between pre-burn biomass and the fraction of pre-burn biomass that was recovered 4 months after burning for nine common understory species: (a) *Acer rubrum*, (b) *Arundinaria tecta*, (c) *Clethra alnifolia*, (d) *Eupatorium rotundifolium*, (e) *Gaylussacia frondosa*, (f) *Ilex glabra*, (g) *Lyonia lucida*, (h) *Oxydendrum arboreum*, (i) *Persea palustris*. Each point represents an individual of its respective species. a) n=13; b) n=32; c) n=59; d) n=18; e) n=49; f) n=55; g) n=28; h) n=35; i) n=47. Asterisks indicate statistically significant effects of pre-burn size: * P < 0.05; ** P < 0.01; *** P < 0.001.

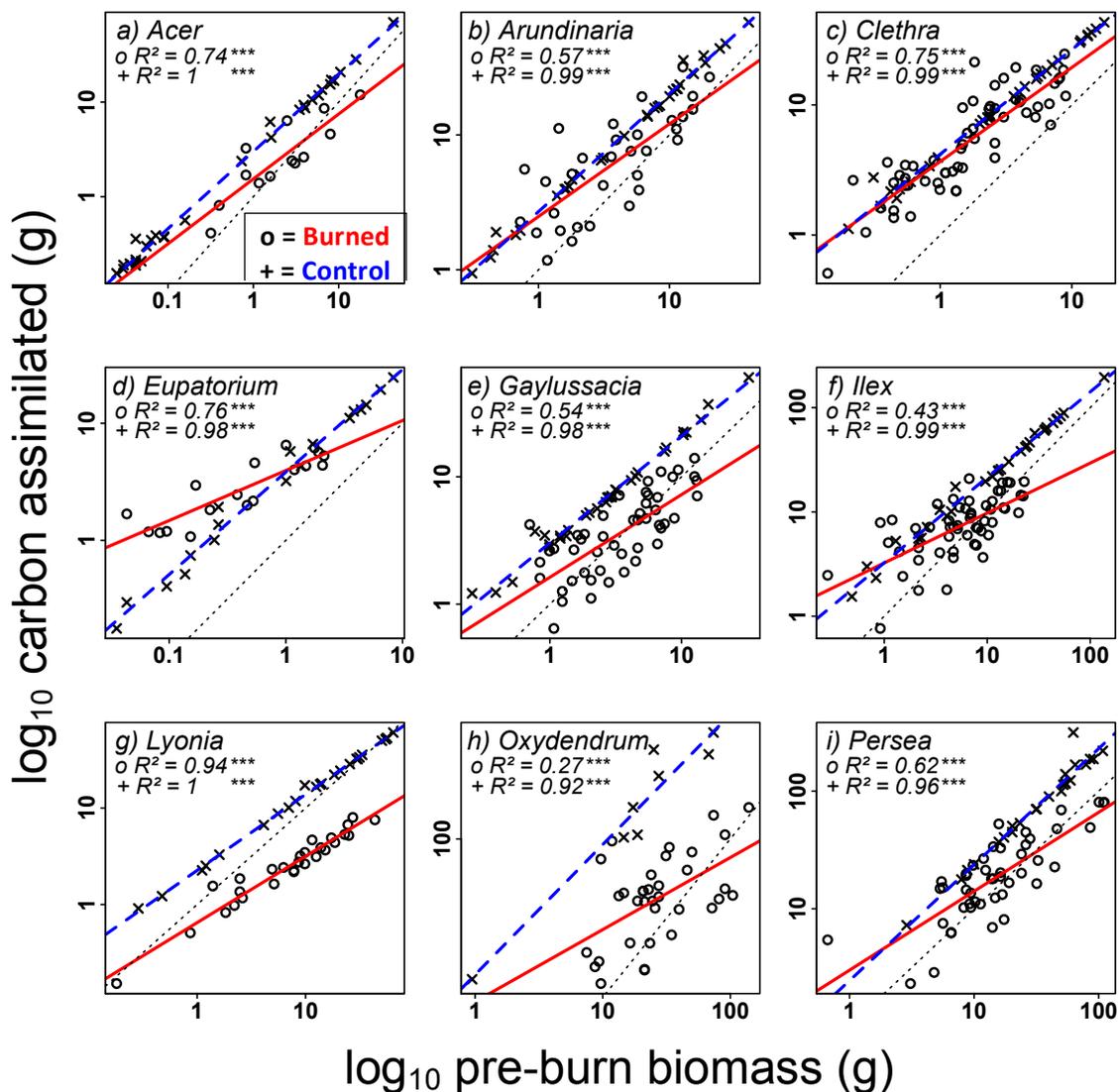


Figure 2.4 Relationship between pre-burn biomass and the total carbon assimilated after 4 months following a prescribed fire, for nine common understory species in burned (o) and unburned control sites (+). (a) *Acer rubrum*, (b) *Arundinaria tecta*, (c) *Clethra alnifolia*, (d) *Eupatorium rotundifolium*, (e) *Gaylussacia frondosa*, (f) *Ilex glabra*, (g) *Lyonia lucida*, (h) *Oxydendrum arboreum*, (i) *Persea palustris*. The dotted line represents the 1:1 line, below which plants assimilate a smaller fraction of carbon relative to pre-burn biomass. Shallower slopes in resprouting plants indicate a net benefit to smaller plants, consistent with a more negative allometry. Within each species, slopes between treatments were significantly different, except for *Acer* and *Clethra*. Each point represents an individual of the respective species in burned and unburned control sites, respectively: a) n=13, 30; b) n=32, 32; c) n=59, 32; d) n=18, 18; e) n=49, 32; f) n=55, 32; g) n=28, 24; h) n=35, 8; i) n=47, 24. Asterisks indicate statistically significant effects of pre-burn size: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

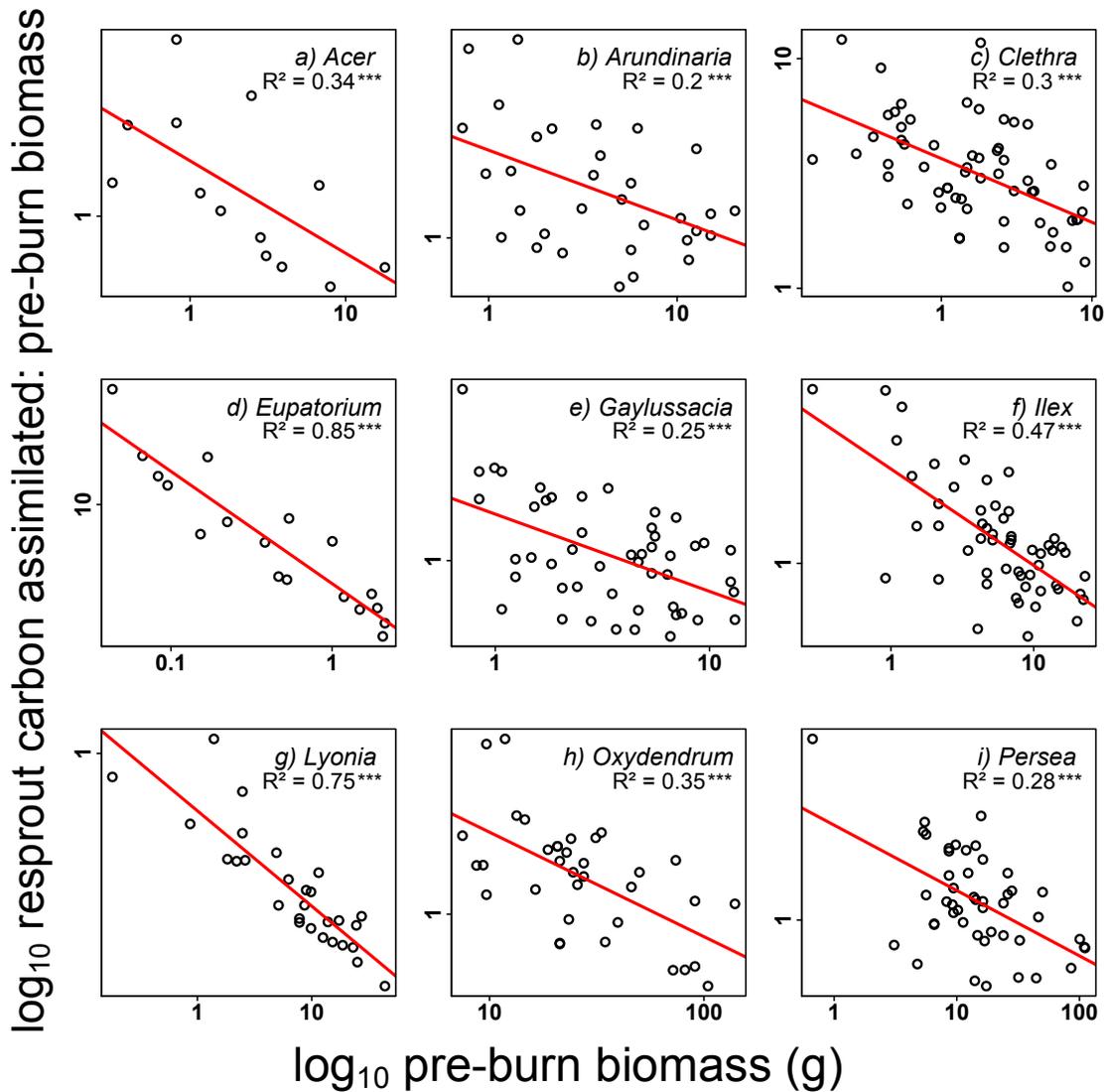


Figure 2.5 Relationship between pre-burn biomass and the fraction of pre-burn carbon that was recovered 4 months after burning for nine common understory species: (a) *Acer rubrum*, (b) *Arundinaria tecta*, (c) *Clethra alnifolia*, (d) *Eupatorium rotundifolium*, (e) *Gaylussacia frondosa*, (f) *Ilex glabra*, (g) *Lyonia lucida*, (h) *Oxydendrum arboreum*, (i) *Persea palustris*. Each point represents an individual of the respective species. a) n=13; b) n=32; c) n=59; d) n=18; e) n=49; f) n=55; g) n=28; h) n=35; i) n=47. Asterisks indicate statistically significant effects of pre-burn size: * P < 0.05; ** P < 0.01; *** P < 0.001.

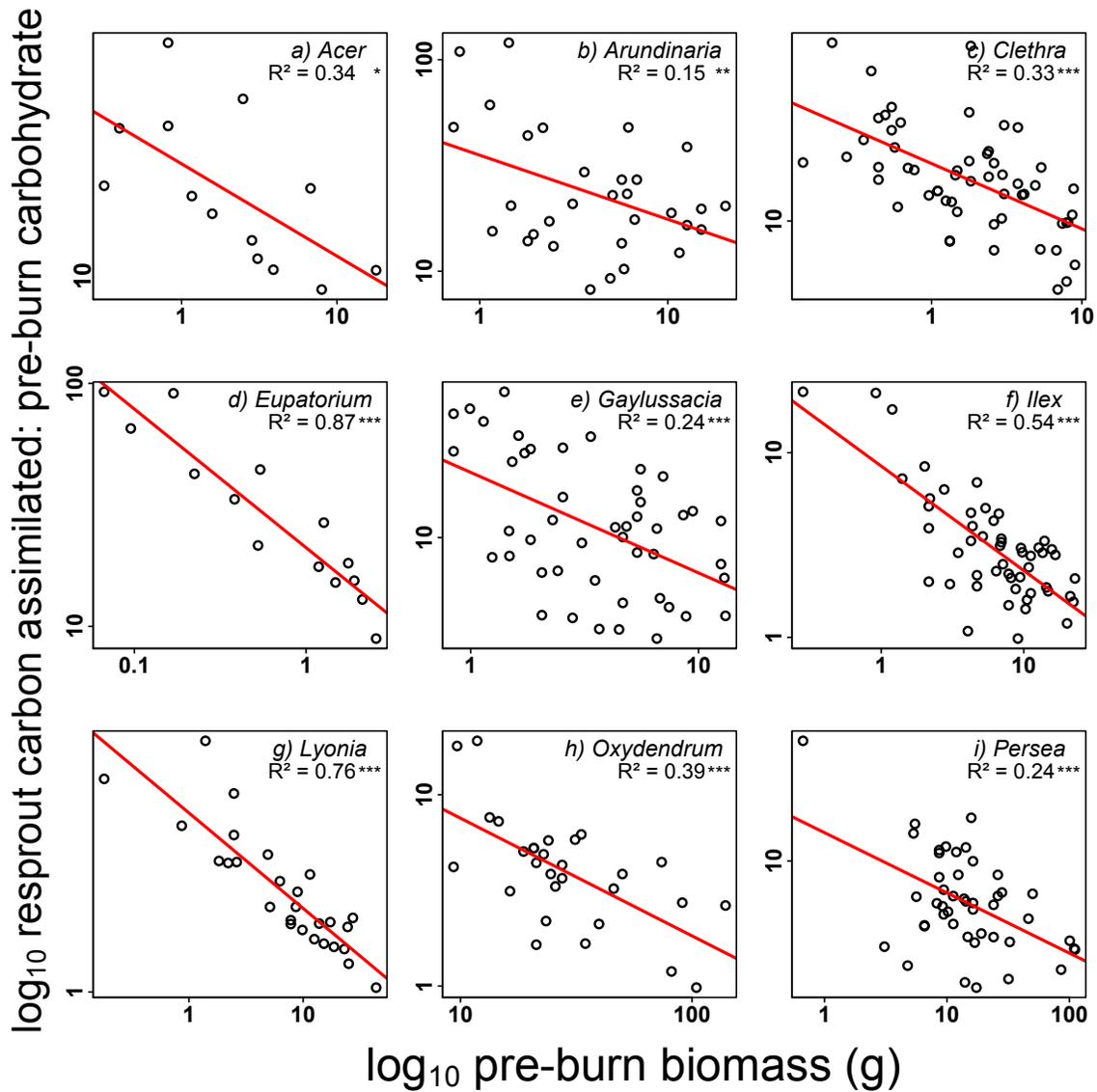


Figure 2.6 Relationship between pre-burn biomass and the ratio of resprout carbon assimilation to the pre-burn root carbohydrate pool (starch + sugar) for nine common understory species: (a) *Acer rubrum*, (b) *Arundinaria tecta*, (c) *Clethra alnifolia*, (d) *Eupatorium rotundifolium*, (e) *Gaylussacia frondosa*, (f) *Ilex glabra*, (g) *Lyonia lucida*, (h) *Oxydendrum arboreum*, (i) *Persea palustris*. Root carbohydrates were determined colorimetrically following Chow and Landhäuser (2004). Root mass was estimated allometrically from total aboveground biomass. Each point represents an individual of the respective species. a) n=13; b) n=31; c) n=56; d) n=14; e) n=49; f) n=52; g) n=27; h) n=34; i) n=46. Asterisks indicate statistically significant effects of pre-burn size: * P < 0.05; ** P < 0.01; *** P < 0.001.

DISCUSSION

It had been shown previously that resprouting plants exhibit a pattern of negative allometry in the post-fire recovery of biomass, which constrains the recovery of large plants (Grady and Hoffmann 2012). That is, fire causes a greater relative decline in aboveground biomass recovery in large plants than in small plants, thereby limiting the size that plants can attain when exposed to repeated topkill events. This research confirmed the negative allometry, and revealed that it arises very early during resprouting, and has a self-reinforcing effect on plant carbon balance. I demonstrated this self-reinforcing pattern by estimating whole-plant carbon assimilation of resprouting plants and quantifying its contribution to resprout biomass.

These data show that the physiological constraints underlying negative allometry result in a slower recovery for large plants and also allow small plants to regain positive carbon balance early in resprouting. In fact, in only four months of resprouting, small plants had the potential to assimilate sufficient carbon to replace the biomass lost to fire. Compared to unburned plants, there was a benefit to small sizes due to the change in the slope of the relationship between carbon assimilated and pre-burn size (Figure 2.4). In addition, small resprouting plants of a given pre-burn size were able to assimilate a larger proportion of C relative to their pre-burn CHO pool, compared to large plants (Figure 2.6). In the long term, this may promote small-statured species such as *Eupatorium*, and keep species such as *Ilex*, and *Gaylussacia* in smaller size classes.

I propose that small plants are able to use a smaller proportion of their CHO reserves during resprouting, and thus, are able to regain positive carbon balance earlier in resprouting than large plants. The initial rate of resprouting in small plants may allow them to

immediately recover a larger proportion of their leaf area. In relative terms, small plants may be able to assimilate more C than plants that are larger in size. There is likely a respiratory cost associated with a larger root system that would negatively affect recovery. The underestimation of resprout respiration in our model should be greatest in large individuals. Therefore, our conclusions regarding the effect of plant size on post-fire carbon gain would be conservative.

Smaller plants also benefited from the reduced competition for light, and the relative increase in leaf area immediately following fire. A higher photosynthetic rate in resprouts and enhanced assimilation may result from this increase in leaf area and perhaps an increase in the amount of chlorophyll per unit leaf mass (Fleck et al. 1998). An increase in the maximum photosynthetic rate after fire observed in this study and others (Fleck et al. 1998; Goorman et al. 2011), and an increase in hydraulic conductance (DeSouza et al. 1986; Goorman et al. 2011) or leaf nitrogen concentrations (Reich et al. 1990), would greatly contribute to the recovery of biomass in herbaceous species and small-statured individuals. There was no increase in the maximum photosynthetic rate of resprouting *Lyonia* and *Oxydendrum* and on average these species were also slower to resprout than the other seven species. These physiological mechanisms benefiting small plants seem to offset the limitations of having smaller absolute belowground reserves. Carbon assimilation in resprouting burned plants showed a more negative allometry compared to unburned controls (Figure 2.4). That is, due to the reduction in slope, small burned plants were able to assimilate more carbon relative to their pre-burn size, compared to small, unburned, control plants. This illustrates the capacity of small-statured plants to reestablish positive carbon balance and for rapid recovery. In addition, small plants received a net benefit from the

potential to assimilate more carbon relative to their pre-burn root carbohydrate status, which could indicate the dependence of herbaceous plants and small shrubs on current photosynthate immediately after leaf emergence, whereas large plants may be more limited by reserve mobilization throughout resprouting.

It remains unclear what proportion of resprouting is fueled by the influx of new photosynthate versus mobilization of root carbohydrates. Initial resprouting is dependent on stored reserves preceding leaf emergence after fire (Bowen and Pate 1993; Canadell and López-Soria 1998), but it is unclear how and when a shift occurs to utilize new assimilates. The size of belowground storage organs, such as lignotubers, appears to be negatively correlated with resprouting vigor (Cruz et al. 2003a; Wigley et al. 2009), but the concentration of non-structural carbohydrate reserves has not been shown to be strongly related to resprouting vigor (Richards and Caldwell 1985; Erdmann et al. 1993; Cruz et al. 2003b). Carbon isotope studies reveal that both current photosynthate and reserves may be used for resprouting, and that fire-prone trees may replenish root reserves using current photosynthates (Wigley et al. 2009). It has been suggested that there must be a balancing act (Schutz et al. 2009; Enright et al. 2011), but how communication and allocation between carbon pools is accomplished during resprouting remains elusive. Further studies should focus on this aspect of resprouting.

Persistence amid frequent fire requires the ability to accumulate carbon rapidly after fire for resprouting and replenishing carbohydrate reserves, which is critical for future resprouting events (Bond and Midgley 2001). Clipping and defoliation experiments reveal that if disturbance is both frequent and severe, starch reserves may be depleted beyond a point of recovery (Schutz et al. 2011). The inability of large plants to rapidly recover lost leaf

area and biomass becomes detrimental in frequent fire regimes, where they are unable to surpass a fire-vulnerable height (Wakeling et al. 2011). Rapid recovery is therefore particularly important for small plants early in resprouting at a time where fire offers equal access to available light across all size classes by eliminating competition with neighbors.

The physiological constraints on large plants we have described here may provide insights into the persistence equilibrium described by Grady and Hoffmann (2012). Plants exposed to repeated burning will tend to approach an equilibrial size in which post-burn size equals pre-burn size, where low resource sites or frequent disturbance reduce the curve, and thus the equilibrial size. This poses a problem for plants with an equilibrial point below their reproductive size. Without sufficient time to regain lost biomass (i.e. annual burning), the landscape may shift to being dominated by small statured plants. Therefore, recovery of aboveground biomass and replenishment of reserves may largely depend on the frequency and severity of the fire regime.

The physiological constraints underlying negative allometry have important implications for changes in understory diversity and community structure, but the outcomes are likely to be influenced by fire regime. Fire exclusion tends to increase woody plant dominance (Higgins et al. 2007), and increase tree density and canopy cover (VanderWeide and Hartnett 2011). The longleaf pine savannas of North Carolina support many rare and endemic plants that would be eliminated through competitive exclusion without frequent burning (Sorrie and Weakley 2001). In the Southeast USA, low-severity burns on short fire-return intervals of 1–3 years often increase the species richness (Dumas et al. 2007), and productivity of the herbaceous layer, with the greatest increases occurring under biennial burns (Brockway and Lewis 1997; Peterson and Reich 2008). A long fire-free interval would

be required for large statured plants to overcome the negative physiological consequences they experience during post-fire recovery. Ultimately, the physiological constraints underlying negative allometry, and size-dependent success, which have a self-reinforcing effect on whole-plant carbon balance very early in resprouting, have the potential for long-term effects on vegetation structure of savanna ecosystems. It is important to understand the dynamics of plant resprouting success in response to fire disturbance, not only to effectively use fire as a conservation and management tool, but also to explain the physiological mechanisms of plant persistence in fire prone habitats and their subsequent community dynamics.

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CHAPTER 3

SHORT-TERM EFFECTS OF FIRE ON ROOT CARBOHYDRATE AND NITROGEN RESERVES

ABSTRACT

Plants in fire-prone systems have evolved ways to balance photosynthetic investment with their respiratory demands amid frequent disruption to a positive carbon balance. Plants are able to mobilize stored carbon reserves to facilitate resprouting, which is an essential strategy to survive repeated burning. Carbohydrate storage buffers a plant against the consequences of future disturbances. Additionally, root nitrogen can be an important source of the N for recovery of lost biomass. Changes in the size of these reserves reflect the balance of carbon assimilation and consumption, and therefore serve as a measure of the carbohydrate status of a plant. I hypothesized that large-statured species undergo a greater depletion of reserves in response to burning. To address this, I harvested roots of nine common understory species before and after fire and quantified changes in concentrations and total pools of carbohydrate and nitrogen reserves. Within species, there was not a significant effect of fire on the nutrient concentrations with respect to plant size. Trends in some species indicate that, in relative terms, plants with smaller carbohydrate reserves may be able to regenerate more biomass per unit root CHO. This work provides some evidence that compared to larger individuals, smaller plants may be able to use CHO reserves more conservatively during resprouting, but N reserves appear not to be limiting after a single disturbance.

INTRODUCTION

Carbohydrate (CHO) reserves in belowground storage organs are commonly mobilized to renew lost leaf area and shoot biomass immediately following topkill (Miyanishi and Kellman 1986; Chapin et al. 1990; Bowen and Pate 1993). Plants capable of resprouting from underground storage organs typically maintain a larger reserve of stored carbohydrates than non-resprouting congeners (Pate et al. 1990; Bell et al. 1996; Knox and Clarke 2005), with a large store of starch contained in broader xylem rays (Bell et al. 1996). These reserves are critical for buffering the short-term, negative impacts of repeated fire, and allow for long-term persistence (Bell and Pate 1996; Cruz and Moreno 2001). Since resprouting consumes much of the stored CHO to recover leaf and shoot biomass, it is advantageous to be able to rapidly resume photosynthetic activity to replenish reserves between burns.

Plants may contain high levels of CHO and still fail to resprout (Wildy and Pate 2002). For example, repeatedly clipped plants that are kept in the dark generally fail to resprout even though CHO levels remain high (Richards and Caldwell 1985). Australian alpine grasses store high levels of root CHO, regardless of post-disturbance regeneration strategy (Tolsma et al. 2007). The fact that CHO reserves remain unused in repeatedly defoliated plants suggests that nutrients may be more limiting than carbon during severe or repeated disturbances (Chapin et al. 1990, and references therein).

Not only is CHO storage critical during plant growth, but mineral nutrients such as nitrogen (N) can often limit plant growth (Vitousek and Howarth 1991), and root N can be correlated to root respiration rates and root N uptake (Reich et al. 1998). Therefore, the size of root N storage should influence resprouting success. Fire can produce short-term flushes

of foliar N (DeSouza et al. 1986; Metzger et al. 2006), and soil N (Knoepp et al. 2009), but frequent fire may cause N limitation over time due to the volatilization of soil organic matter (Vitousek and Howarth 1991; Certini 2005). However, available soil N has not been shown to have significant effects on lignotuber size, which is related to resprouting vigor (Cruz and Moreno 2001). It has been suggested that a minimum N pool might be necessary for resprouting in some species (El Omari et al. 2003). Canadell and López-Soria (1998) found that a single disturbance event might not significantly deplete nutrient concentrations, but that repetitive fires would have a compound effect on nutrient limitation.

Although the importance of carbohydrate mobilization in resprouting is well documented, it is unclear how patterns of carbohydrate and nitrogen consumption and replenishment can explain plant recovery after fire. If repeated topkill and resprouting greatly depletes CHO or N, it may reduce resprouting vigor over multiple fire cycles, which can ultimately result in plant mortality (Noble 2001; Wildy and Pate 2002). Large plants have large root systems, and larger absolute carbohydrate reserves, yet it is unclear if reserves are utilized differently in large plants versus small plants. Over the course of resprouting, changes in the size of the reserves reflect the time-integrated balance of carbon assimilation and consumption, and therefore serve as a valuable measure of carbohydrate status of the plant.

The objective of this study was to determine if nutrient reserves are limiting across plant sizes during resprouting, and reveal the patterns of nutrient depletion and replenishment over the course of resprouting. We hypothesized that large-statured species undergo a greater depletion of reserves in response to burning due to higher respiratory demands. To address this hypothesis, we harvested roots of nine common understory species before and after fire

and quantified changes in concentrations and total pools of carbohydrate and nitrogen reserves in a mesic longleaf pine savanna. Within and across-species patterns were utilized to test this hypothesis.

METHODS

Study location and species

Belowground biomass was collected in June 2012 at Fort Bragg Military Reservation in Hoke and Cumberland counties in North Carolina. Mean annual precipitation is 1275 mm (1200 mm rain, 75 mm snow), and mean winter and summer temperatures are 6.9°C and 26°C, respectively (Sorrie et al. 2006). Fort Bragg is divided into >1000 burn units that have been managed independently with prescribed fire at intervals of approximately 3 years since 1991. Twelve collection sites were selected in ecotones between upland longleaf pine savanna and stream-head wetlands, where plants have access to perennially wet soils, but are exposed to fire.

I studied nine common understory species, including 3 trees: *Acer rubrum* L. (red maple), *Oxydendrum arboreum* (L.) DC. (sourwood), *Persea palustris* (Raf.) Sarg. (swampbay); 4 woody shrubs: *Clethra alnifolia* L. (summer-sweet), *Gaylussacia frondosa* (L.) Torr. & A. Gray ex Torr. (dangleberry), *Ilex glabra* (L.) A. Gray (inkberry), *Lyonia lucida* (Lam.) K. Koch (pink fetterbush); and 2 herbaceous species: *Arundinaria tecta* (Walter) Muhl. (switchcane), *Eupatorium rotundifolia* L. (roundleaf thoroughwort). Hereafter, these species will be referred to by their genus. *Above and Belowground Allometric Relationships*

Species-specific allometric equations for pre-burn and resprouting plants (Appendix A) were developed in 2011 to non-destructively estimate leaf area and total aboveground

biomass based on the height of each tagged individual. Pre-burn biomass refers to total aboveground biomass.

In 2012, root allometric equations were developed for each species by harvesting 10-20 plants of variable heights in sites three years post fire to predict root biomass from aboveground height. We measured height and diameter of all stems of each plant before harvest, and measured leaf area on the same day using a CI-202 portable laser area meter (CID Bio-Science Inc., Camas, WA USA). A minimum of 10 cm of coarse root material was harvested. Stems, leaves, and roots were bagged separately, dried at 70 °C for 72–96 hours, and then weighed.

Root Carbohydrate and Nutrient Analysis

A colorimetric method was used to determine starch and sugar concentration of roots (Chow and Landhäusser 2004). The root crown and primary coarse roots were collected before fire during the middle of the summer in June, and 1 and 2 months after fire. There was not a significant effect of time after fire on mean CHO ($P > 0.05$), so 2-month results are shown here. Roots were dried on the same day as collected at 70 °C for 72 hours (10–29 per species; 61–90 cm average height). Dried root samples were ground using a Wiley mill with a 40-mesh screen. For each 50 mg sample, sugars were extracted three times using with 5 mL of 80% ethanol, by boiling samples in glass tubes capped with marbles in a 95° C water bath for 10 minutes each. After each extraction, tubes were centrifuged at 2500 rpm for five minutes and the supernatants of the three extractions were combined for sugar analysis. Then the extract was reacted with phenol-sulfuric acid, which allowed the sugars to be measured colorimetrically at an absorbance of 490 nm. To determine the starch concentration, the remaining starch in the residue was hydrolyzed to glucose by an enzyme mixture of α -

amylase and amyloglucosidase, and then measured colorimetrically using a peroxidase-glucose oxidase-o-dianisidine solution. Starch was measured at an absorbance of 525 nm. Absorbances were converted to concentrations using standards to evaluate the efficiency of digestion and colorimetry.

The relationship between total root carbohydrate (CHO) concentration (sugars + starch) and height of the plant was not significant ($P > 0.05$). Therefore, the CHO pool size for each tagged plant in the study was calculated as the product of the mean CHO concentration per species and the allometrically estimated root mass.

The remainder of each root sample was sent to the North Carolina State University Environmental & Agricultural Testing Service in the Department of Soil Sciences to analyze N concentration.

Statistical Analyses

I tested for differences in mean CHO and N concentrations within each species between pre-burn and resprouting plants using a Student's T-test. Within each species, a regression model was used to test for the significance of plant height (cm) on the concentration of nutrients (CHO and N) before and after fire. Homogeneity of slopes was tested between times since fire using an analysis of covariance. All statistical analyses were performed using R version 2.15.1 (The R Foundation for Statistical Computing, 2012).

RESULTS

Carbohydrates

Across species, mean pre-burn CHO concentrations ranged from 8.4% to 28.7% (*Arundinaria* and *Clethra*, respectively), and 2 months after fire mean resprout CHO ranged

from 5.9% to 24.4% (*Arundinaria* and *Clethra*, respectively; Table 3.1). We observed a trend of reduced mean resprout CHO concentrations after fire, within species (Figure 3.1), but differences were only significant in *Eupatorium* ($t = 2.22$; $df = 20.9$; $P = 0.037$). There was not a significant effect of time after fire on mean CHO ($P > 0.05$). Before fire, there was a slight negative relationship between CHO concentration and pre-burn height, but within species, this relationship was only significant for *Clethra* ($t = 2.16$; $df = 17$; $P = 0.045$), *Ilex* ($t = -2.84$; $df = 21$; $P = 0.0098$), and *Persea* ($t = -3.28$; $df = 24$; $P = 0.0032$; Figure 3.2).

Across all observations, there was a negative relationship between resprout height and CHO concentration in the roots of resprouting plants ($F_{1,124} = 10.1$; $P = 0.002$; Figure 3.3), but this relationship was not significant for pre-burn measurements ($F_{1,190} = 2.49$; $P = 0.12$). There was a significant effect of fire ($F_{1,124} = 10.0$; $P = 0.002$), resulting in a more negative slope for resprouting plants.

There was a positive relationship between pre-burn root CHO and resprout biomass within species (Figure 3.4), but this relationship was not significant for *Acer* and *Oxydendrum* (Table 3.2a). In all species except *Acer*, *Eupatorium*, and *Lyonia*, the slope of the regression indicated a significant negative allometry (Table 3.2b), revealing that the resprout biomass of individuals with smaller initial CHO pools were proportionally larger than individuals with a larger CHO pool.

Nitrogen

Mean pre-burn N concentrations ranged from 0.17% to 0.83% (*Lyonia* and *Eupatorium*, respectively), mean resprout concentrations were between 0.18% and 0.79% (*Lyonia* and *Eupatorium*, respectively; Table 3.1), and significant differences were not observed within species as a result of fire (Figure 3.5). Nitrogen concentration showed a

negative trend with pre-burn plant height, but the relationship was only significant for *Gaylussacia* before fire ($t = 2.45$; $df = 27$; $P = 0.021$), *Oxydendrum* before fire ($t = -2.97$; $df = 7$; $P = 0.021$), and *Oxydendrum* after fire ($t = -2.70$; $df = 6$; $P = 0.036$; Figure 3.6).

Across all observations, there was a significant negative correlation between pre-burn plant height and root N concentration ($F_{1,193} = 4.62$; $P = 0.03$; Figure 3.7), but this relationship was not significant for resprouting plants ($F_{1,124} = 0.005$; $P = 0.95$). There was not a significant difference in the slopes due to burning ($P > 0.05$).

Root Carbohydrates & Nitrogen

Across all observations, there was a significant negative correlation between N and CHO concentration in the roots ($F_{1,136} = 59.7$; $P < 0.001$; Figure 3.8). That is, plants with larger concentrations of root CHO had smaller concentrations of root N. Burning had no significant effect on the slope of this relationship ($P > 0.05$). The relationship between N and CHO concentrations was not significant within species before or after burning ($P > 0.05$).

Table 3.1 Total number of plants harvested for nutrient analysis, concentration of resources in the roots of nine understory savanna species before prescribed fire and 2 months after fire. Values include concentration (mean + standard deviation in mg g⁻¹) of starch, soluble sugars, and nitrogen.

Species		N	Starch	Sugars	Total	Nitrogen
<i>Acer rubrum</i>	<i>pre-burn</i>	18	8.71 ± 5.39	5.10 ± 0.82	13.8 ± 5.39	0.253 ± 0.073
	<i>resprout</i>	6	13.33 ± 10.1	4.26 ± 0.96	17.6 ± 10.66	0.345 ± 0.131
<i>Arundinaria tecta</i>	<i>pre-burn</i>	23	3.90 ± 2.52	4.47 ± 1.80	8.37 ± 3.13	0.410 ± 0.105
	<i>resprout</i>	16	1.41 ± 1.60	4.54 ± 5.49	5.94 ± 6.50	0.456 ± 0.076
<i>Clethra alnifolia</i>	<i>pre-burn</i>	28	3.73 ± 2.70	24.9 ± 6.33	28.7 ± 6.52	0.282 ± 0.074
	<i>resprout</i>	18	2.19 ± 1.15	22.16 ± 7.82	24.4 ± 8.15	0.298 ± 0.052
<i>Eupatorium rotundifolium</i>	<i>pre-burn</i>	15	3.25 ± 3.61	9.09 ± 3.27	12.3 ± 5.27	0.825 ± 0.321
	<i>resprout</i>	17	1.41 ± 3.52	7.09 ± 3.33	8.50 ± 3.81	0.790 ± 0.346
<i>Gaylussacia frondosa</i>	<i>pre-burn</i>	22	5.95 ± 2.99	5.96 ± 1.76	11.9 ± 3.41	0.359 ± 0.098
	<i>resprout</i>	19	4.27 ± 3.58	6.50 ± 2.06	10.8 ± 3.50	0.398 ± 0.077
<i>Ilex glabra</i>	<i>pre-burn</i>	23	6.85 ± 4.09	6.53 ± 1.92	13.4 ± 4.93	0.323 ± 0.095
	<i>resprout</i>	18	3.58 ± 2.28	8.27 ± 4.36	11.8 ± 4.78	0.33 ± 0.144
<i>Lyonia lucida</i>	<i>pre-burn</i>	24	8.03 ± 2.79	6.71 ± 1.19	14.7 ± 3.20	0.175 ± 0.050
	<i>resprout</i>	14	6.98 ± 2.76	5.91 ± 1.02	12.9 ± 3.19	0.181 ± 0.025
<i>Oxydendrum arboreum</i>	<i>pre-burn</i>	9	11.19 ± 7.07	8.22 ± 3.1	19.4 ± 5.68	0.241 ± 0.057
	<i>resprout</i>	8	6.19 ± 5.62	8.71 ± 3.73	14.9 ± 8.43	0.22 ± 0.038
<i>Persea palustris</i>	<i>pre-burn</i>	26	12.15 ± 6.32	9.98 ± 5.23	22.1 ± 7.54	0.267 ± 0.095
	<i>resprout</i>	16	10.2 ± 4.61	10.12 ± 2.74	20.3 ± 4.60	0.277 ± 0.072

Table 3.2 Statistical results for the (a) relationships between total pre-burn root carbohydrates and the total biomass of resprouting plants 2 months after burning for nine common understory species and, (b) the significance of negative allometry for those relationships. Negative allometry, exists if the slope of the relationship is significantly less than 1 ($P < 0.05$), whereby larger plant sizes undergo larger negative consequences. Refer to Figure 3.3.

Pre-Burn Root Carbohydrate ~ Resprout Biomass						
	a) Significant Correlation				b) Negative Allometry	
Species	Equation	df	F	P	t	P
<i>Acer rubrum</i>	$y=0.47x - 0.70$	11	3.81	0.07	2.18	0.05
<i>Arundinaria tecta</i>	$y=0.36x - 0.64$	29	11.2	0.002	5.90	<0.001
<i>Clethra alnifolia</i>	$y=0.79x - 0.38$	54	85.6	<0.001	2.32	0.024
<i>Eupatorium rotundifolium</i>	$y=1.46x - 1.05$	12	43.4	<0.001	-2.06	0.061
<i>Gaylussacia frondosa</i>	$y=0.19x - 0.41$	45	6.01	0.018	10.5	<0.001
<i>Ilex glabra</i>	$y=0.55x + 0.24$	50	11.9	0.001	2.77	0.007
<i>Lyonia lucida</i>	$y=0.82x + 0.20$	25	9.16	0.006	0.65	0.52
<i>Oxydendrum arboreum</i>	$y=0.04x + 1.05$	27	0.19	0.66	9.10	<0.001
<i>Persea palustris</i>	$y=0.53x + 0.16$	44	26.6	<0.001	4.46	<0.001

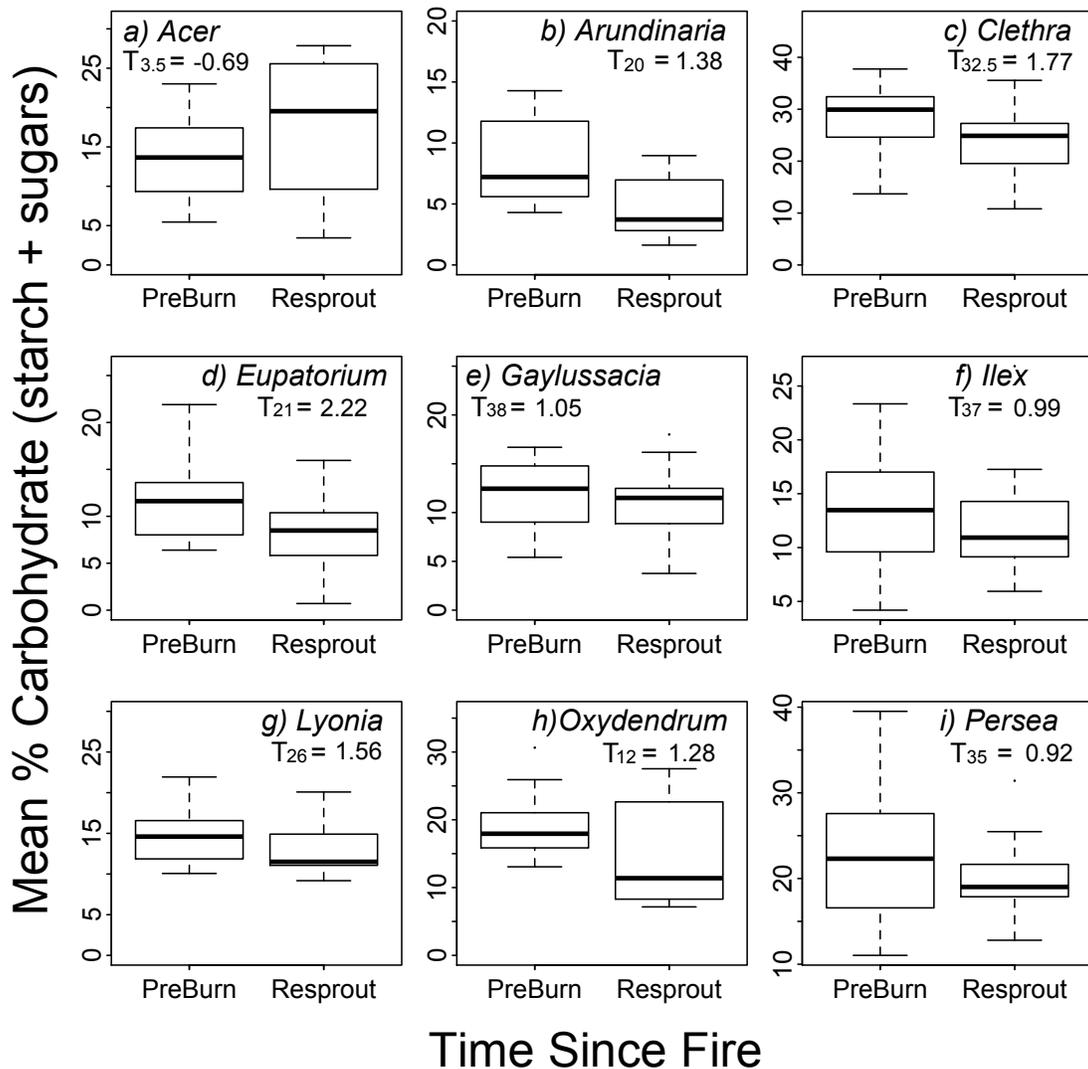


Figure 3.1 Mean root carbohydrate concentrations (sugar + starch) for nine common understory species before and two months after prescribed fire. (a) *Acer rubrum*, (b) *Arundinaria tecta*, (c) *Clethra alnifolia*, (d) *Eupatorium rotundifolium*, (e) *Gaylussacia frondosa*, (f) *Ilex glabra*, (g) *Lyonia lucida*, (h) *Oxydendrum arboreum*, (i) *Persea palustris*. n= 170 (pre-burn), n= 126 (resprout). The root crown and coarse roots were harvested and dried on the same day. A colorimetric method was performed on ground root samples to determine starch and sugar concentration. Pre-burn means did not differ significantly between control sites and sites schedule to burn.

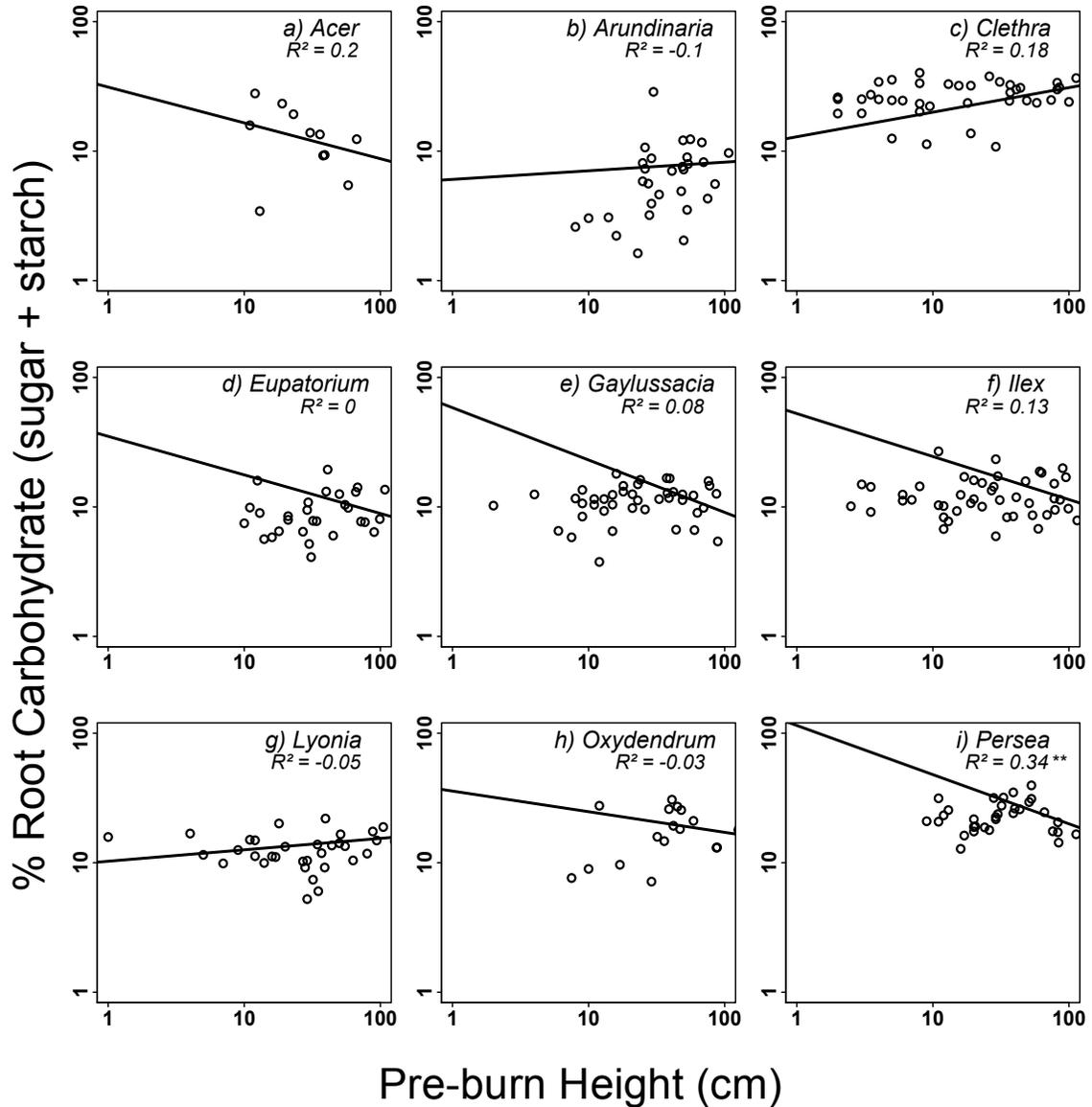


Figure 3.2 Allometric relationships between height and percent carbohydrate concentration (starch+sugars) in the roots of understory savanna species before fire (square; solid line) and two months after fire (circle; dashed line). The root crown and coarse roots were harvested and dried on the same day. A colorimetric method was performed on ground root samples to determine starch and sugar concentration. (a) *Acer rubrum*, (b) *Arundinaria tecta*, (c) *Clethra alnifolia*, (d) *Eupatorium rotundifolium*, (e) *Gaylussacia frondosa*, (f) *Ilex glabra*, (g) *Lyonia lucida*, (h) *Oxydendrum arboreum*, (i) *Persea palustris*. Asterisks indicate statistically significant effects of plant height: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$

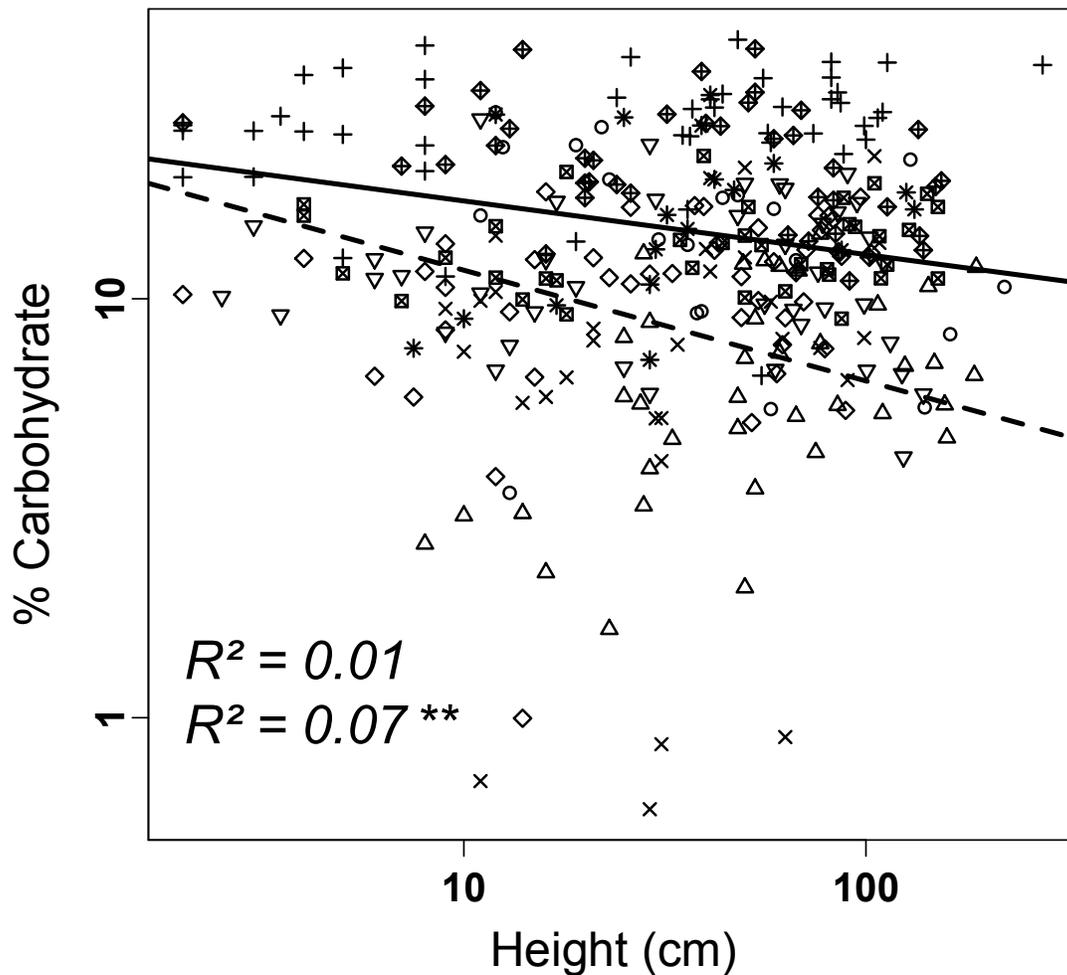


Figure 3.3 Relationship between height and root carbohydrate concentration for nine common understory savanna species before fire (solid line) and after fire (dashed line) across all observations. Slopes between lines were not significantly different. The root crown and coarse roots were harvested and dried on the same day. A colorimetric method was performed on ground root samples to determine starch and sugar concentration. (○) *Acer rubrum*, (Δ) *Arundinaria tecta*, (+) *Clethra alnifolia*, (×) *Eupatorium rotundifolium*, (◇) *Gaylussacia frondosa*, (▽) *Ilex glabra*, (⊠) *Lyonia lucida*, (*) *Oxydendrum arboreum*, (⊕) *Persea palustris*. Asterisks indicate statistically significant effects of total carbohydrate concentration: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$

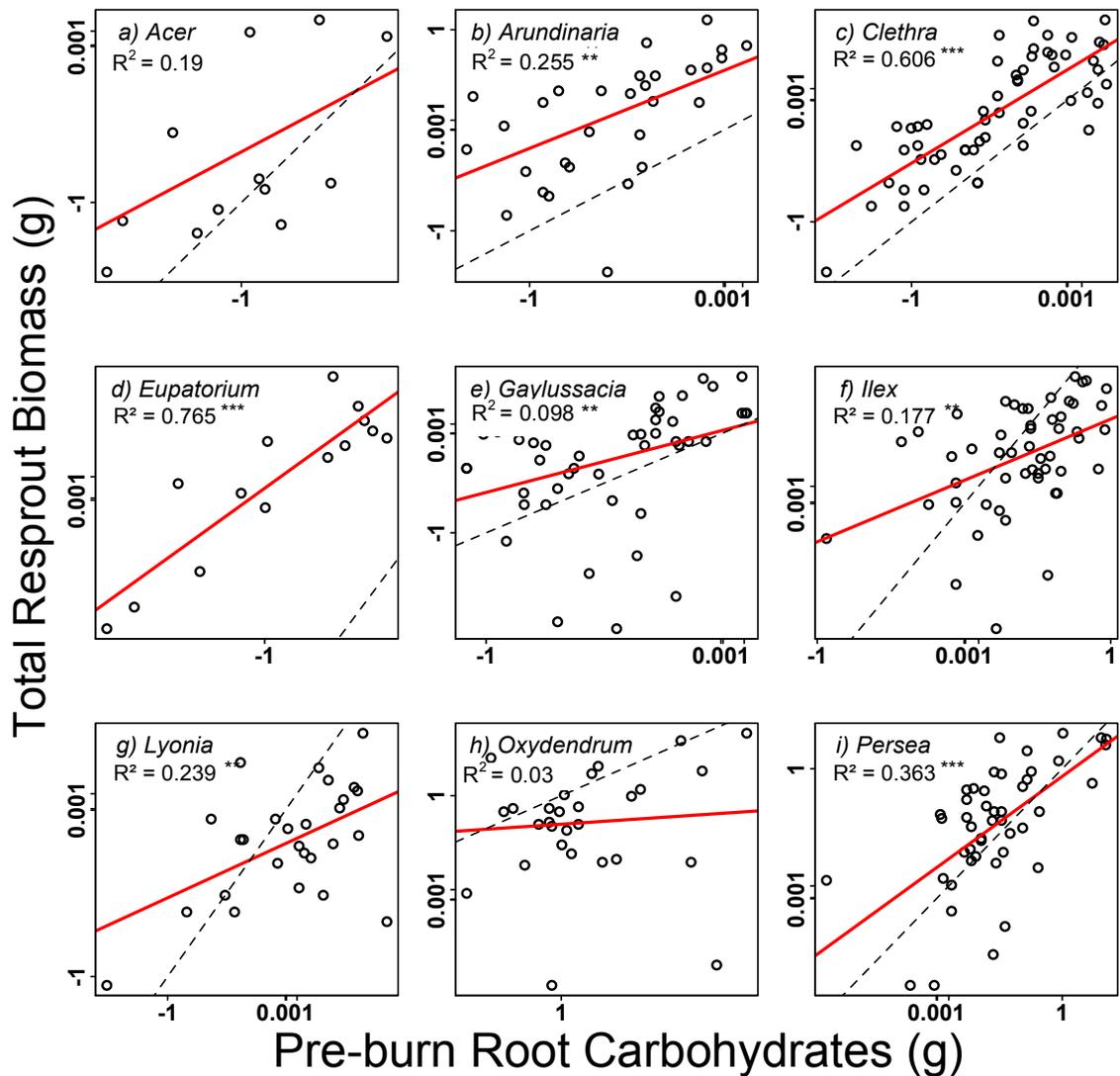


Figure 3.4 Relationship between total pre-burn root carbohydrates and total resprout biomass 4 months after fire for understory savanna species: (a) *Acer rubrum*, (b) *Arundinaria tecta*, (c) *Clethra alnifolia*, (d) *Eupatorium rotundifolium*, (e) *Gaylussacia frondosa*, (f) *Ilex glabra*, (g) *Lyonia lucida*, (h) *Oxydendrum arboreum*, (i) *Persea palustris*. The root crown and coarse roots were harvested and dried on the same day. A colorimetric method was performed on ground root samples to determine starch and sugar concentration. Root carbohydrate totals were calculated as the product of the average carbohydrate concentration and the root biomass (from allometry based on total plant biomass). The dotted line represents the 1:1 line, above which small plants resprouted more biomass per unit root carbohydrate compared to individuals with large carbohydrate totals (Table 3.1). Asterisks indicate statistically significant effects of pre-burn carbohydrate: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$

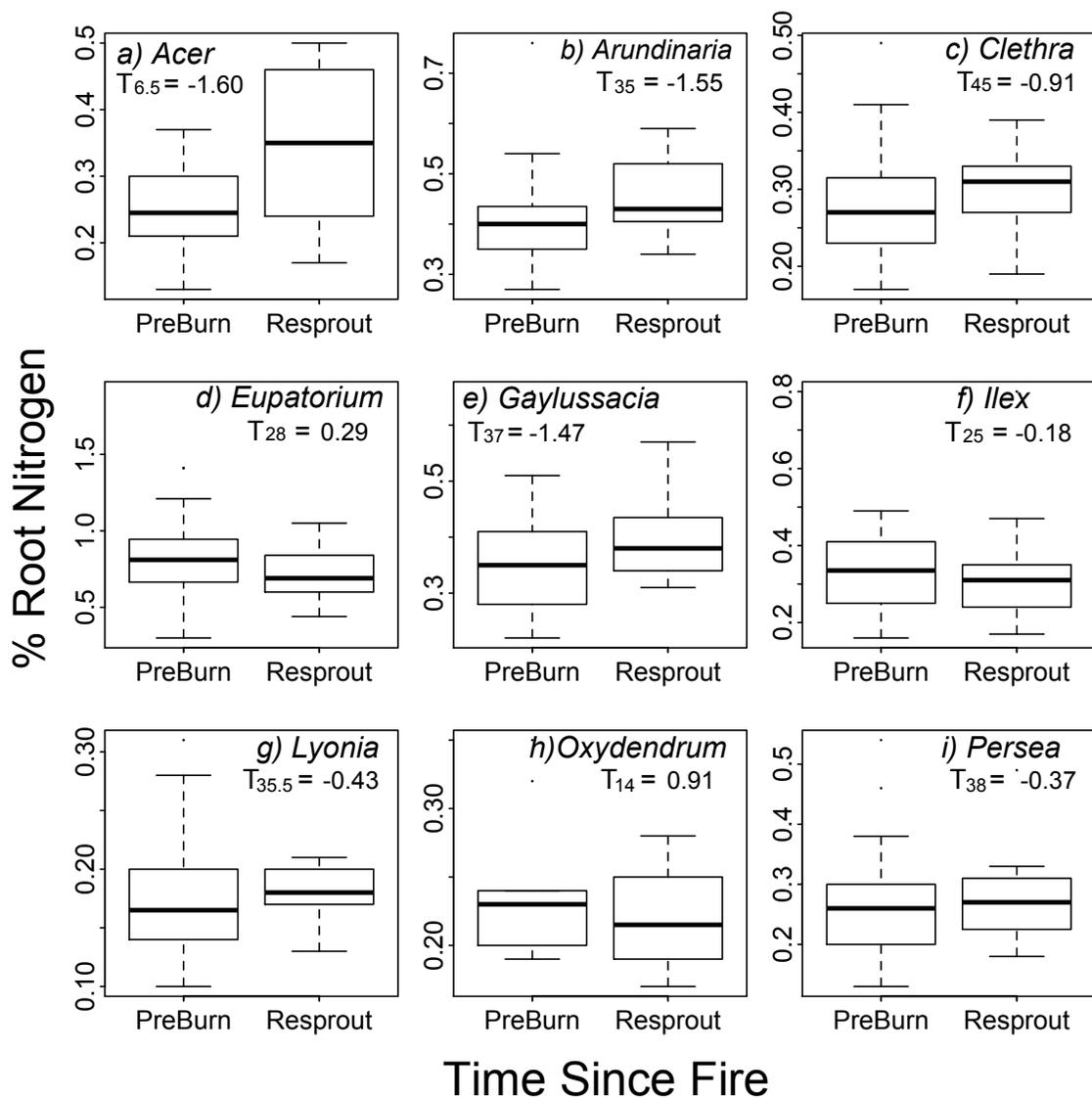


Figure 3.5 Mean root nitrogen concentrations of nine common understory species before fire and two months after prescribed fire. The root crown and coarse roots were harvested and dried on the same day. Means did not differ significantly between control sites and sites schedule to burn. (a) *Acer rubrum*, (b) *Arundinaria tecta*, (c) *Clethra alnifolia*, (d) *Eupatorium rotundifolium*, (e) *Gaylussacia frondosa*, (f) *Ilex glabra*, (g) *Lyonia lucida*, (h) *Oxydendrum arboreum*, (i) *Persea palustris*. n= 195 (Pre-burn), n= 126 (Resprout).

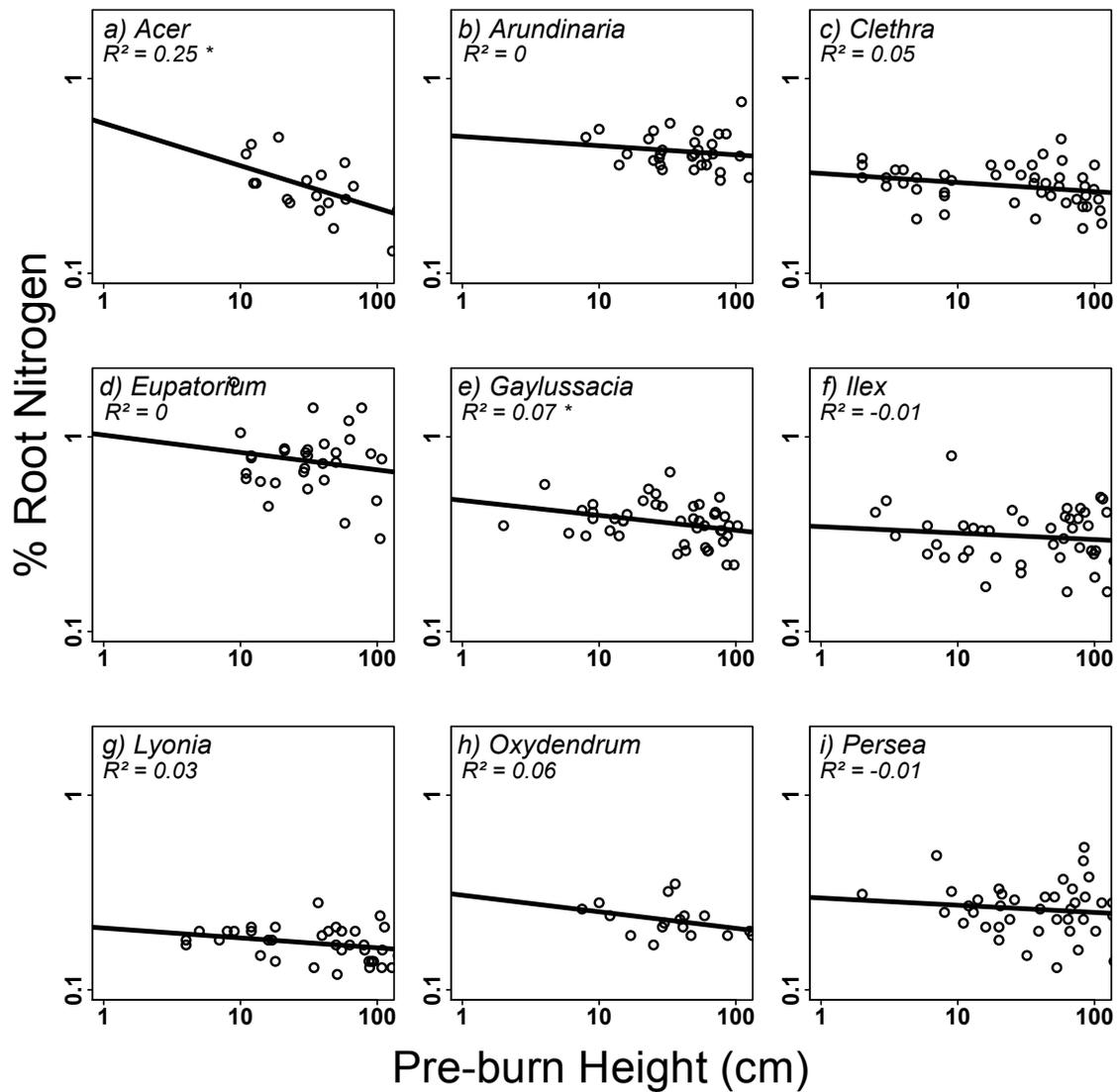


Figure 3.6 Relationships between height and percent nitrogen concentration in the roots of understory savanna species. Slopes of these relationships were not significantly different before and after fire. (a) *Acer rubrum*, (b) *Arundinaria tecta*, (c) *Clethra alnifolia*, (d) *Eupatorium rotundifolium*, (e) *Gaylussacia frondosa*, (f) *Ilex glabra*, (g) *Lyonia lucida*, (h) *Oxydendrum arboreum*, (i) *Persea palustris*. Asterisks indicate statistically significant effects of height: * $P < 0.05$

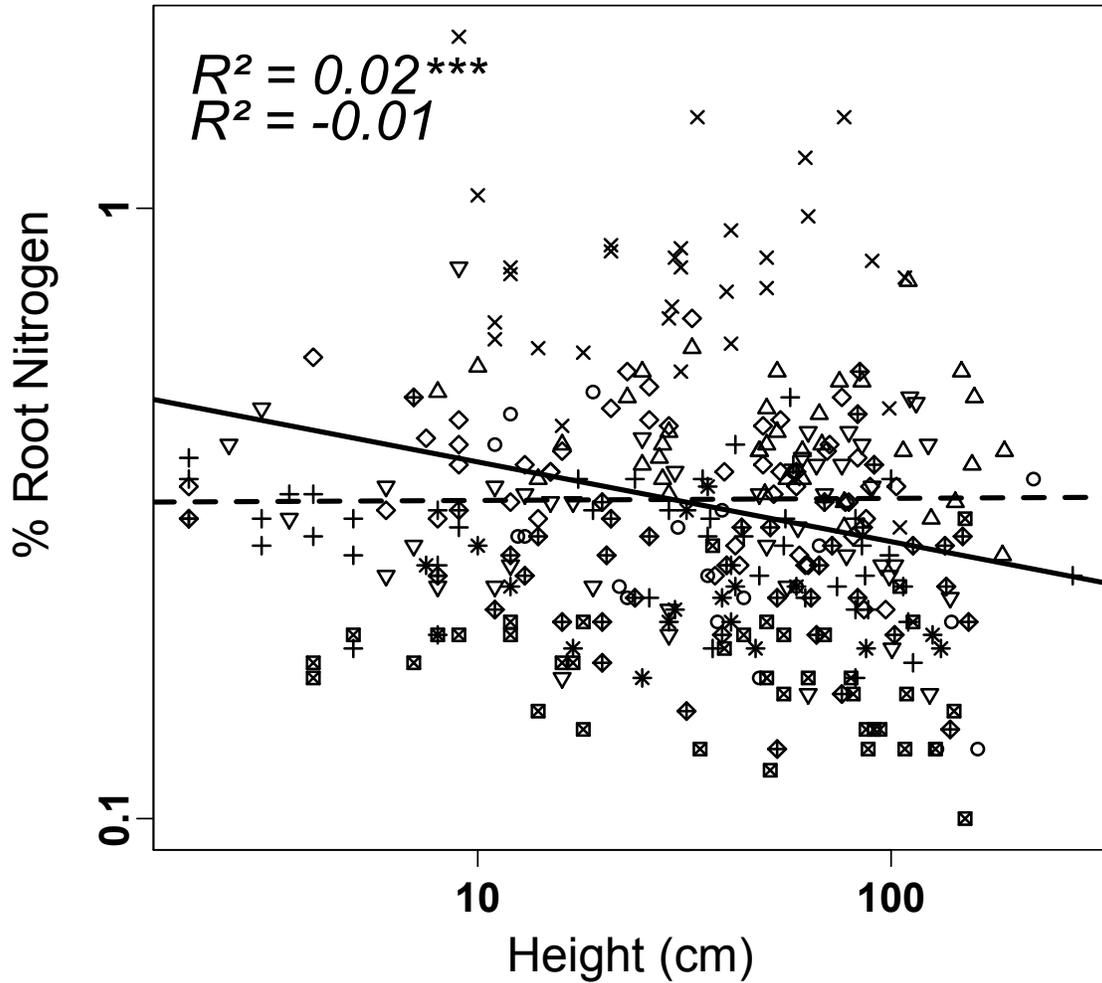


Figure 3.7 Relationship between height and root nitrogen concentration for nine common understory savanna species before fire (solid line) and after fire (dashed line) across all observations. Slopes were not significantly different before and after fire. The root crown and coarse roots were harvested and dried on the same day. A colorimetric method was performed on ground root samples to determine starch and sugar concentration. (○) *Acer rubrum*, (Δ) *Arundinaria tecta*, (+) *Clethra alnifolia*, (×) *Eupatorium rotundifolium*, (◇) *Gaylussacia frondosa*, (∇) *Ilex glabra*, (⊠) *Lyonia lucida*, (*) *Oxydendrum arboreum*, (⊕) *Persea palustris*. Asterisks indicate statistically significant effects of total carbohydrate concentration: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$

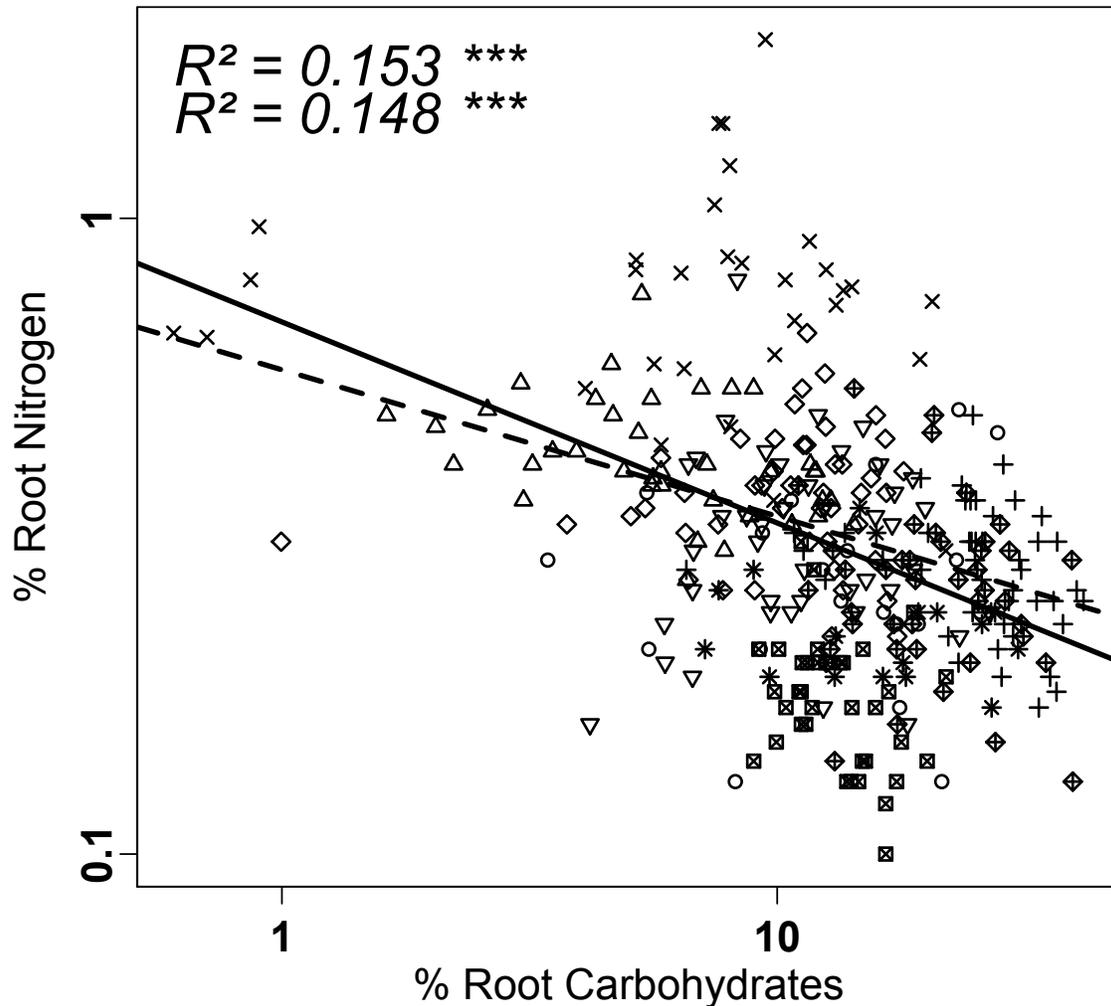


Figure 3.8 Relationship between total carbohydrate concentration and nitrogen concentration in the roots of nine common understory savanna species before fire (solid line) and after fire (dashed line). Slopes were not significantly different before and after fire. The root crown and coarse roots were harvested and dried on the same day. A colorimetric method was performed on ground root samples to determine starch and sugar concentration. (○) *Acer rubrum*, (Δ) *Arundinaria tecta*, (+) *Clethra alnifolia*, (×) *Eupatorium rotundifolium*, (◇) *Gaylussacia frondosa*, (∇) *Ilex glabra*, (⊠) *Lyonia lucida*, (*) *Oxydendrum arboreum*, (⊕) *Persea palustris*. Asterisks indicate statistically significant effects of total carbohydrate concentration: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$

DISCUSSION

The objective of this study was to determine the extent to which carbohydrate and nitrogen reserves explain resprouting success across plant size and to reveal the patterns of nutrient depletion and replenishment during resprouting in understory savanna plants.

Carbohydrate reserves are a critically limiting resource for resprouting (Landhäusser and Loeffers 2002), but within species we did not observe a drastic reduction in response to fire. Within species, patterns do not support our hypothesis that large-statured individuals would experience a greater depletion of either CHO or N reserves in response to burning (Figure 3.1). However, across all observations there was a larger reduction in carbohydrate concentration after fire in large plants compared to smaller plants (Figure 3.3). When we estimated the total carbohydrate pool for each individual in a larger set of individuals, within-species trends indicated that plants containing smaller pre-burn carbohydrate pools were able to produce a proportionally larger biomass, relative to those with larger pre-burn carbohydrate pools, illustrated by the negative allometry observed in some species (Figure 3.4). We suggest that either small-statured individuals use their carbohydrates more conservatively and rely heavily on concurrent photosynthate, or they are able to replenish these reserves within one growing season. In chapter 2, my results indicated that the pattern of carbon recovery arises early in resprouting and is self-reinforcing, which allows some small plants to replenish reserves in one growing season. Furthermore, these data suggest that smaller-statured individuals are able to regenerate more biomass per unit root CHO, and thus, are able to replenish CHO reserves quickly after a disturbance, relative to larger individuals.

Total CHO pool may be a stronger indicator of resprouting vigor than concentration itself (Moreira et al. 2012). Consistent with our observations of root CHO concentrations

(Figure 3.3), studies suggest that starch concentrations may be greater in smaller lignotubers (Cruz et al. 2003; Wigley et al. 2009), even though large storage organs may contain a greater absolute amount of CHO, as we observed. Carbohydrate concentration itself may not strongly predict resprouting vigor, but rather, individuals with smaller lignotubers may be more successful resprouters (Cruz et al. 2003).

It is possible that some species have adapted to store more root carbohydrates than they need for one disturbance event (Wildy and Pate 2002; Tolsma et al. 2007). The importance of the ability to replenish reserves with current photosynthate has been shown for plants undergoing experimental defoliation or shading, which experienced a delay in the recovery of initial CHO levels (Schutz et al. 2011), and consistently lower resprout biomass (Wildy and Pate 2002). Herbaceous plants may largely be able to bypass the use of stored CHO—after initial resprouting—by regaining leaf area rapidly and maintaining high photosynthetic rates (Richards and Caldwell 1985). This could explain the absence of significantly depleted CHO during resprouting, especially for small growth forms.

These results do not suggest that N reserves are depleted to a greater degree in large resprouting plants compared to small plants. However, plants with larger concentrations of root CHO may have a smaller capacity for storing root N. Burning also had no significant effect on root N concentration regardless of plant size. Nitrogen in the roots may not be limiting after a single fire event, as seen in other studies (Miyanishi and Kellman 1986). Canadell and López-Soria (1998) found that root N was only significantly depleted after multiple clipping events, but not after a single disturbance. Additionally, the effects of fire on root N are likely to be influenced by site and fire regime (Dress and Boerner 2003). Further studies on whole-plant N use are needed to determine if N is limiting amid frequent fire.

This work provides some evidence that compared to larger individuals, smaller plants undergo a smaller reduction in CHO reserves during resprouting, suggesting that small plants may rely more heavily on incoming photosynthates for resprouting or they are able to quickly replenish CHO reserves soon after resprouting. Further work should aim to reveal what fraction of incoming photosynthates are incorporated into root storage versus new growth, what is the turnover of root nutrients, and the long-term effects of fire frequency on nutrient consumption and replenishment during resprouting.

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CHAPTER 4

IS RESPROUTING IN LARGE PLANTS LIMITED BY MERISTEMS?

ABSTRACT

After fire, resprouting plants may produce numerous stems from a bank of dormant buds. Multi-stemmed resprouters are abundant in open vegetation where being over-shaded is less likely. Producing multiple stems increases the total leaf area and total crown volume, which increases the competitive success of shorter statured plants. It has been suggested that competition for nutrients in large multi-stemmed plants may be greater than that of a single-stemmed plant of similar size, due to the large respiratory demands of maintaining a large reserve of buds. To better understand the limitations and regulation of size-dependent resprouting success of multi-stemmed resprouters, I predicted that the growth of small plants would benefit from the reduction in stem number due to a reallocation of resources from multiple stems to a single remaining stem, whereas reducing the number of stems in a large plant would not benefit growth. To this end, I imposed coppicing treatments to half of the study individuals by removing all emerging shoots except the largest stem, throughout the growing season. Contrary to the predictions, there was no increase in the growth of the largest coppiced stem, relative to the uncoppiced treatment. The inherent allocation pattern in the uncoppiced plants suggests that the relative contribution of one stem to the total plant biomass decreases with increasing pre-burn size. These results highlight the advantages of being multi-stemmed in fire-prone systems.

INTRODUCTION

Woody plants exhibit a bimodal height distribution, resulting in a dichotomy between shrubs and trees (Scheffer et al. 2014). In savanna ecosystems, frequent fire tends to enhance this dichotomy by promoting the success of small-statured plants at the expense of larger plants. Resprouting shrubs are often multi-stemmed and tend to be shorter than non-resprouters, because resources are invested into multiple shoots at the expense of height (Midgley 1996). Additionally, the modular architecture of multi-stemmed shrubs allows them to maximize leaf area horizontally and increase the total crown size rather than merely increase in height (Küppers 1989; Kozovits et al. 2005), in order to take advantage of the improved light environment after fire to enhance carbon assimilation. Balancing the costs and benefits of this architecture is therefore essential to plant persistence amid fire. Understanding the way in which plants differentially optimize leaf area versus height may provide insights into what controls resprouting across plant sizes.

If multi-stemmed shrubs prioritize lateral leaf area over stem elongation following topkill, it is likely that resprouting recovery in large-statured plants may be bud-limited. Disturbance events that completely destroy aboveground biomass will favor the selection of large belowground storage reserves (Iwasa and Kubo 1997) that facilitate repeated resprouting (Bowen and Pate 1993; Paula and Ojeda 2009). Fire-prone savanna species tend to allocate a large proportion of resources to belowground organs (Hoffmann et al. 2003). However, it has been suggested that a minimum number of buds may also be required for persistence (Moreno and Oechel 1991). The number of resprouting shoots is positively correlated with the number of shoots before topkill in woody shrubs (Dacy and Fulbright 2009; Schafer and Just 2014), and this relationship has been shown to fit a curvilinear

function (Zammit 1988). In addition, the number of resprouting shoots is positively correlated with lignotuber size, also fitting a curvilinear relationship (Zammit 1988). This curvilinearity implies a negative allometric relationship (Figure 4.1). Carbon reserves and buds are both important for resprouting, but it remains unclear how these are limiting across plant sizes and how they can explain the resprouting success of small statured plants.

To better understand what limits and regulates size-dependent resprouting success in fire-prone ecosystems, I tested the hypothesis that post-topkill resprouting success (defined as total aboveground plant biomass) would be more limited by meristem number in large individuals relative to small individuals. I predicted that the suppression of supplementary shoots would cause a larger growth increase in smaller individuals compared to large individuals (Figure 4.2). That is, growth of small plants would benefit from the reduction in stem number due to a reallocation of resources from multiple stems to a single remaining stem, whereas reducing the number of stems in a large plant would not increase its size. To this end, I imposed coppicing treatments to half of the study individuals by removing all emerging shoots except the largest stem, throughout the growing season.

METHODS

Study sites and species

This study was conducted at Fort Bragg Military Reservation in Hoke and Cumberland counties in North Carolina in what is known as the Sandhills region. Mean annual precipitation is 1275 mm (1200 mm rain, 75 mm snow), and mean winter and summer temperatures are 6.9°C and 26°C, respectively (Sorrie et al. 2006). Fort Bragg covers over 64,000 hectares, mostly consisting of longleaf pine- wiregrass savanna (*Pinus palustris* Mill.; *Aristida stricta* Michx.). Since 1991, the Fort Bragg landscape has been divided into

management units that are burned approximately every three years with prescribed fire. Two quick-resprouting woody species were used in this study because of their relative abundance at Fort Bragg: *Oxydendrum arboreum* (L.) DC. (sourwood; deciduous; Ericaceae), *Persea palustris* (Raf.) Sarg. (swampbay; evergreen; Lauraceae). Hereafter, species will be referred to by genus. Within weeks of low intensity fires, both *Oxydendrum* and *Persea* are able to resprout from basal meristems and produce multiple stems from one root. They may persist in this multi-stemmed stage in a cycle of topkill and resprouting.

Plant Measurements

At the beginning of the growing season in late May and early June of 2013, individuals of *Oxydendrum* and *Persea* were tagged in 15 sites that burned 2-3 years prior. For each multi-stemmed individual, the height (cm) of each stem was measured. All plants were coppiced at about 1 cm above ground level in late June to simulate fire-induced topkill (Cruz et al. 2003a; Cruz et al. 2003b), and all plants within a 50 cm radius were removed to reduce competition that would occur with fire-induced topkill. During resprouting, treatments of “coppiced” (continual removal of all stems except the largest) or “uncoppiced” (all stems to remain intact) were assigned by ranking all heights within a species and evenly distributing treatments among pre-burn heights. Coppicing treatments began in early July, two weeks after initial removal of aboveground biomass, and continued every two weeks for 3 months. The heights of all stems in each treatment were measured at 6, 16, 20, 41 and 52 weeks after initial coppicing, but report data for 6, 20, and 52 weeks due to similar patterns seen in other times since topkill. After 3 months, new shoots ceased to emerge so coppicing treatments occurred monthly from November until the end of the growing season in December. Aboveground biomass was calculated using species-specific allometric equations

(Appendix A) for each individual stem, which were summed for individuals with more than one shoot.

Mortality was low despite repeated coppicing. One individual of *Oxydendrum* failed to resprout as a result of repeated coppicing in the first year, but it resprouted in the next growing season; two uncoppiced individuals failed to resprout after the initial topkill. There was no coppicing-induced mortality in the individuals of *Persea* by the end of the first growing season, but one uncoppiced individual failed to resprout after the initial topkill; however, in the next growing season, two individuals that were previously under 10 cm did undergo coppicing-induced mortality. However, there was a large reduction ($\frac{1}{2}$ of *Oxydendrum*; $\frac{1}{3}$ of *Persea*) in sample size due to wildfires in the summer of 2013.

Statistical Analyses

Within each species, I used a regression model with pre-topkill biomass or pre-topkill stem total as the independent variable and resprout variables as the dependent variable (both \log_{10} -transformed to fit normality): cumulative total of stems produced during resprouting, or resprout biomass. I tested for homogeneity of slopes among coppicing treatments using an analysis of covariance. Statistical analyses were performed in R version 3.0.2 (The R Foundation for Statistical Computing, 2012).

RESULTS

Repeated coppicing of resprouting plants resulted in the continual emergence of new shoots for approximately 4 months. In both the coppiced and uncoppiced treatments, there was a significant positive correlation between the total number of shoots before and after topkill (Figure 4.3a). Compared to the uncoppiced treatment, coppiced stems produced a greater number of shoots over the course of resprouting, relative to the pre-topkill stem total

(Table 4.1), but there was no significant difference in slope between the coppiced and uncoppiced treatments for either *Oxydendrum* or *Persea* at 6, 20, and 52 weeks of resprouting ($P > 0.05$). There was also a significant correlation between the total number of shoots produced and the pre-topkill biomass (Figure 4.3b; Table 4.2a), and a significant difference in slopes between treatments was observed in *Persea* after 20 and 52 weeks of resprouting (Table 4.2b).

Both *Oxydendrum* and *Persea* exhibited a significant positive correlation for the relationship between resprout biomass and pre-topkill biomass in both treatments during the growing season (Figure 4.4; Table 4.3a), but there was no significant difference in slope between the coppiced treatment and the largest stem of the uncoppiced treatment for either species during resprouting (Table 4.3b). There was, however, a difference in slope between the total biomass of the uncoppiced treatment and the largest stem of that treatment, for *Oxydendrum* at 6, 20 and 52 weeks, and for *Persea* at 20 and 52 weeks (Table 4.3c). This negative allometric relationship indicates that the relative contribution of one stem to the total biomass of the plant decreases with pre-burn size (Figure 4.4).

Within *Oxydendrum* and *Persea*, there was not a significant shift in the slope of the relationship of the largest biomass of the coppiced stem (Figure 4.5; $F_{3,101} = 3.1$, $P = 0.07$; Figure 4.6; $F_{3,137} = 3.0$, $P = 0.08$ respectively). There was however, a significant shift in the intercept of this same relationship (Figure 4.5; $F_{3,101} = 72.1$, $P < 0.001$; Figure 4.6; $F_{3,137} = 191$, $P < 0.001$ respectively).

Table 4.1 Statistical results for the relationships between resprout shoot number and pre-topkill shoot number for coppiced and uncoppiced resprouting plants of *Oxydendrum arboreum* and *Persea palustris* at 6, 20, and 52 weeks of resprouting. Refer to Figure 4.3a. There were no differences in slopes of different treatments ($P > 0.05$).

Time since topkill	Uncoppiced				Coppiced			
6 weeks	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>
<i>Oxydendrum arboreum</i>	76	1,36	57.5	<0.001	43	1,41	18.1	<0.001
<i>Persea palustris</i>	90	1,43	11.0	0.002	50	1,48	45.4	<0.001
20 weeks	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>
<i>Oxydendrum arboreum</i>	76	1,36	35.7	<0.001	42	1,40	15.9	<0.001
<i>Persea palustris</i>	90	1,43	24.3	<0.001	50	1,48	53.3	<0.001
52 weeks	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>
<i>Oxydendrum arboreum</i>	34	1,15	6.83	0.02	20	1,18	12.5	0.002
<i>Persea palustris</i>	72	1,34	12.4	0.001	41	1,39	17.2	<0.001

Table 4.2a Statistical results for the relationships between resprout shoot total and pre-topkill biomass for coppiced and uncoppiced resprouting plants of *Oxydendrum arboreum* and *Persea palustris* at 6, 20, and 52 weeks of resprouting. Refer to Figure 4.3b.

Time since topkill	Uncoppiced				Coppiced			
	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>
6 weeks								
<i>Oxydendrum arboreum</i>	76	1,36	44.5	<0.001	43	1,41	41.4	<0.001
<i>Persea palustris</i>	90	1,43	20.2	<0.001	50	1,48	41.9	<0.001
20 weeks								
<i>Oxydendrum arboreum</i>	76	1,36	41.0	<0.001	42	1,40	31.1	<0.001
<i>Persea palustris</i>	90	1,43	43.3	<0.001	50	1,48	22.6	<0.001
52 weeks								
<i>Oxydendrum arboreum</i>	34	1,15	6.47	0.02	20	1,18	11.7	0.003
<i>Persea palustris</i>	72	1,34	35.6	<0.001	41	1,39	8.09	0.007

Table 4.2b Statistical results for the difference in slopes between coppiced and uncoppiced resprouting plants 6, 20, and 52 weeks of resprouting for the relationship between resprout shoot total and pre-topkill biomass. Refer to Figure 4.3b.

Time since topkill	ANCOVA		
	<i>df</i>	<i>F</i>	<i>P</i>
6 weeks			
<i>Oxydendrum arboreum</i>	1,77	0.118	0.73
<i>Persea palustris</i>	1,91	0.007	0.9
20 weeks			
<i>Oxydendrum arboreum</i>	1,76	2.61	0.11
<i>Persea palustris</i>	1,91	10.6	0.001
52 weeks			
<i>Oxydendrum arboreum</i>	1,33	0.32	0.57
<i>Persea palustris</i>	1,73	13.5	<0.001

Table 4.3a Statistical results for the relationships between resprout biomass (g) and pre-topkill biomass (g) for coppiced and uncoppiced resprouting plants of *Oxydendrum arboreum* and *Persea palustris* at 6, 20, and 52 weeks of resprouting. Refer to Figure 4.4.

Time since topkill	Uncoppiced				Coppiced			
	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>
6 weeks								
<i>Oxydendrum arboreum</i>	76	1,36	9.48	0.004	43	1,41	11.6	0.001
<i>Persea palustris</i>	90	1,43	18.3	<0.001	50	1,48	45.1	<0.001
20 weeks								
<i>Oxydendrum arboreum</i>	76	1,36	62.9	<0.001	42	1,40	69.2	<0.001
<i>Persea palustris</i>	90	1,43	70.8	<0.001	50	1,48	121	<0.001
52 weeks								
<i>Oxydendrum arboreum</i>	34	1,15	37.8	<0.001	20	1,18	39.4	<0.001
<i>Persea palustris</i>	72	1,34	43.9	<0.001	41	1,39	121	<0.001

Table 4.3b Statistical results for the difference in slopes between the largest stems of the coppiced and uncoppiced resprouting plants 6, 20, and 52 weeks of resprouting for the relationship between resprout biomass (g) and pre-topkill biomass (g). Refer to Figure 4.4.

Time since topkill	ANCOVA		
	<i>df</i>	<i>F</i>	<i>P</i>
6 weeks			
<i>Oxydendrum arboreum</i>	1,77	0.2	0.65
<i>Persea palustris</i>	1,91	1.2	0.27
20 weeks			
<i>Oxydendrum arboreum</i>	1,76	0.0	0.99
<i>Persea palustris</i>	1,91	1.07	0.30
52 weeks			
<i>Oxydendrum arboreum</i>	1,33	0.09	0.76
<i>Persea palustris</i>	1,73	0.10	0.74

Table 4.3c Statistical results for the difference in slopes between the total biomass of the uncoppiced plants and the biomass of the largest stem of the uncoppiced plants after 6, 20, and 52 weeks of resprouting for the relationship between resprout biomass (g) and pre-topkill biomass (g). Refer to Figure 4.4.

Time since topkill	ANCOVA		
6 weeks	<i>df</i>	<i>F</i>	<i>P</i>
<i>Oxydendrum arboreum</i>	1,72	4.23	0.04
<i>Persea palustris</i>	1,86	0.03	0.96
20 weeks	<i>df</i>	<i>F</i>	<i>P</i>
<i>Oxydendrum arboreum</i>	1,72	25.6	<0.001
<i>Persea palustris</i>	1,86	6.53	0.01
52 weeks	<i>df</i>	<i>F</i>	<i>P</i>
<i>Oxydendrum arboreum</i>	1,30	8.32	0.007
<i>Persea palustris</i>	1,68	10.8	0.002

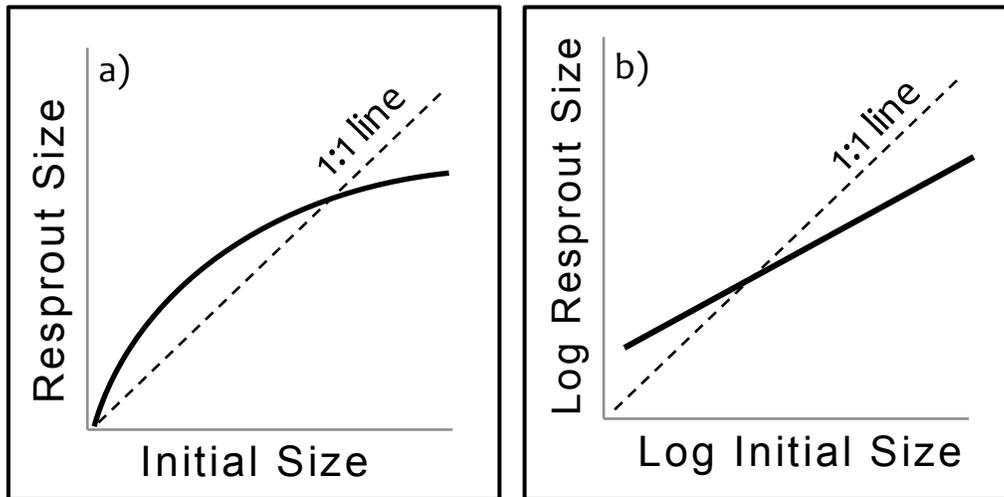


Figure 4.1 Hypothesized allometric patterns underlying the relationship between pre-burn and post-burn variables (modified from Grady & Hoffmann 2012): (a) Allometric relationship between pre-burn and post-burn size, (b) log transformation of the relationship in a. The dashed line represents the 1:1 line, along which the post-fire resprout size equals pre-burn size, and the coefficient or slope (α) of the relationship is equal to 1. The solid line represents a “negative allometry” where $\alpha < 1$. Shallower slopes indicate a net benefit to smaller plants, consistent with a more negative allometry.

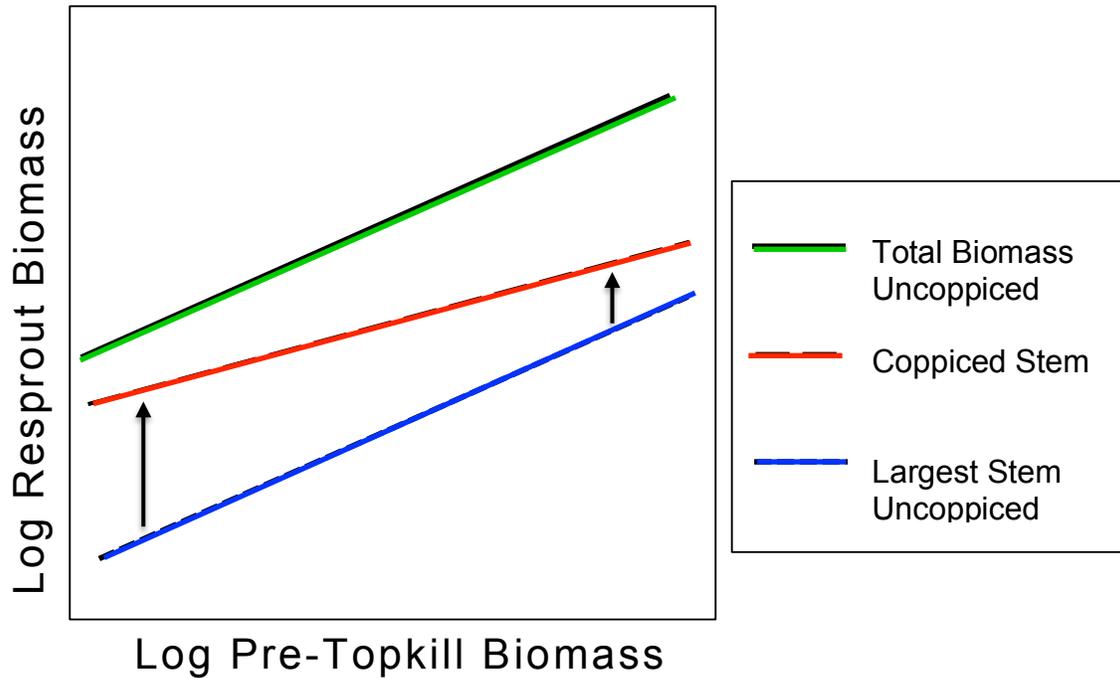


Figure 4.2 Expected results for the relationships between pre-topkill biomass and resprout biomass of coppiced plants (removal of all but the largest stem; red line), total biomass of uncoppiced plants (solid green line), and the largest stem of the uncoppiced plants (blue line). The total biomass of an uncoppiced individual includes the biomass of the uncoppiced largest stem of the same individual. We predicted that coppicing would cause a greater increase in the growth of the largest stem of the smaller sized plants (long arrow), than in larger plants (small arrow), and thus the slope of the coppiced treatment (red line) would be shallower than the slope of the largest stem of the uncoppiced plants (blue line).

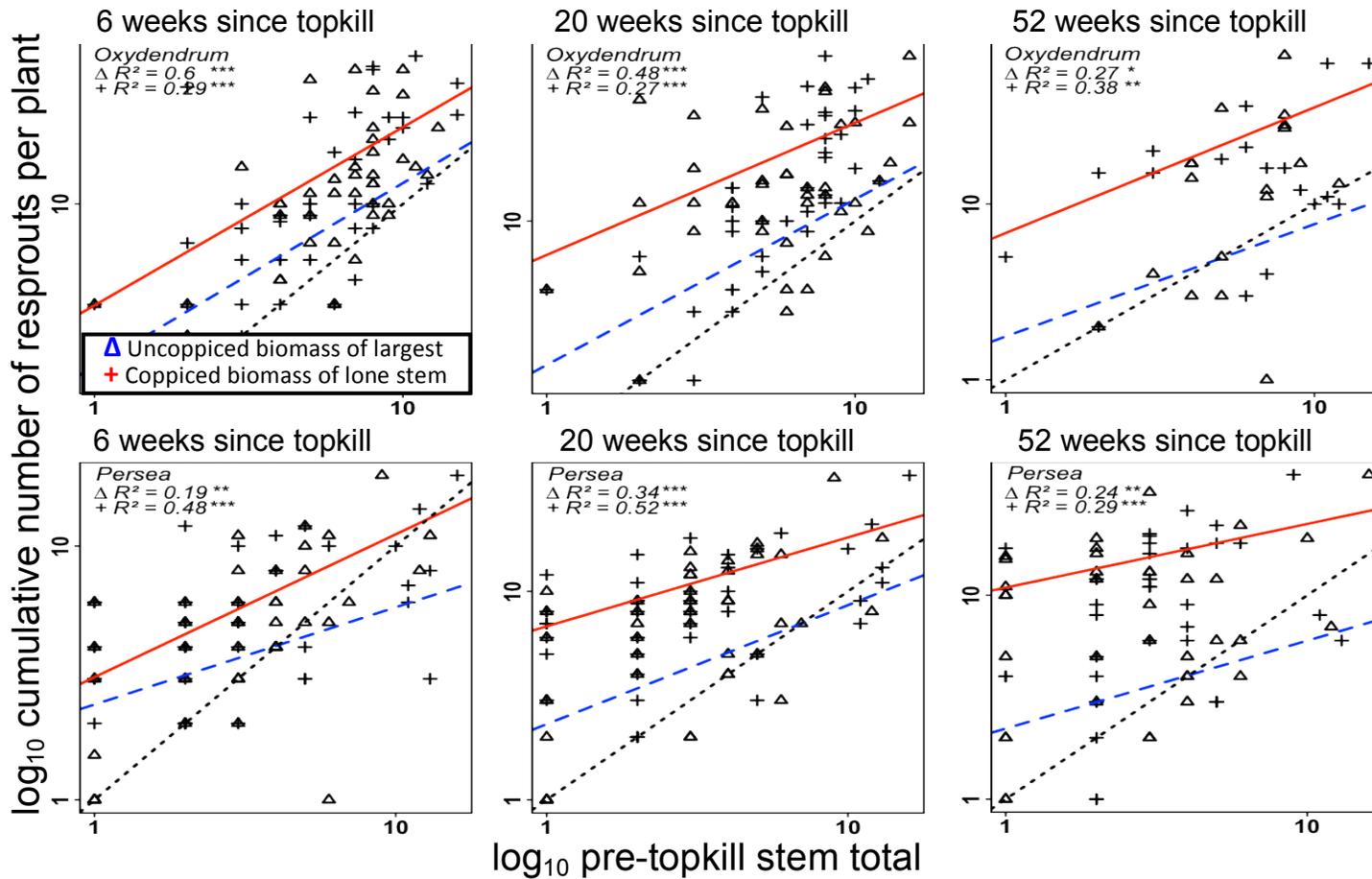


Figure 4.3a The total number of shoots that emerged during resprouting as a function of the total number of pre-topkill shoots for *Oxydendrum arboreum* and *Persea palustris* 6, 20, and 52 weeks after fire-simulated coppicing. Two treatments were imposed: continual coppicing of all stems except the largest (+ solid red line), and uncoppiced (Δ dashed blue line). Asterisks indicate statistically significant effects of pre-burn size: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

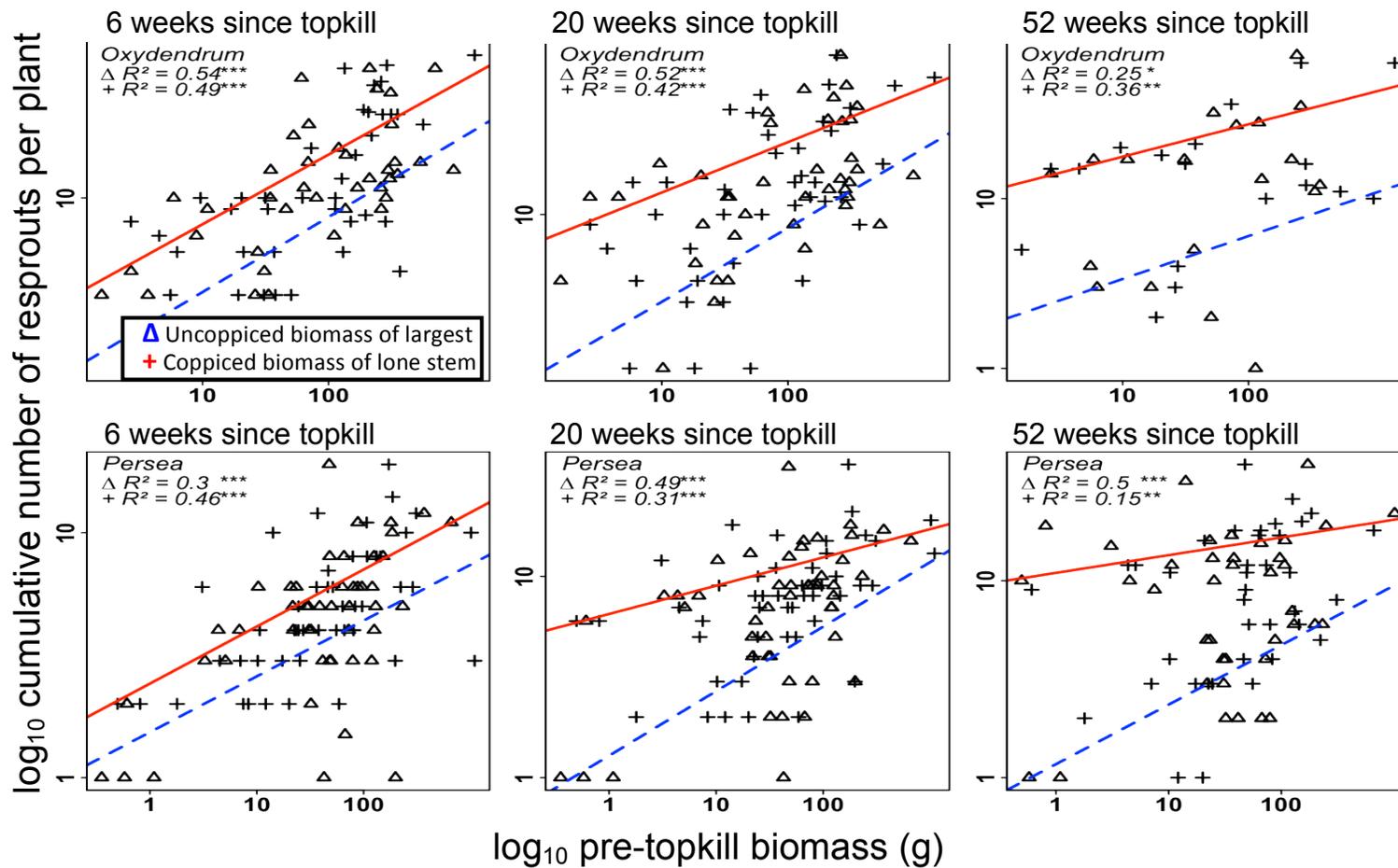


Figure 4.3b The total number of shoots that emerged during resprouting as a function of the pre-topkill biomass for *Oxydendrum arboreum* and *Persea palustris* 6, 20, and 52 weeks after fire-simulated coppicing. Two treatments were imposed: continual coppicing of all stems except the largest (+ solid line), and uncoppiced (Δ dashed line). Asterisks indicate statistically significant effects of pre-burn size: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

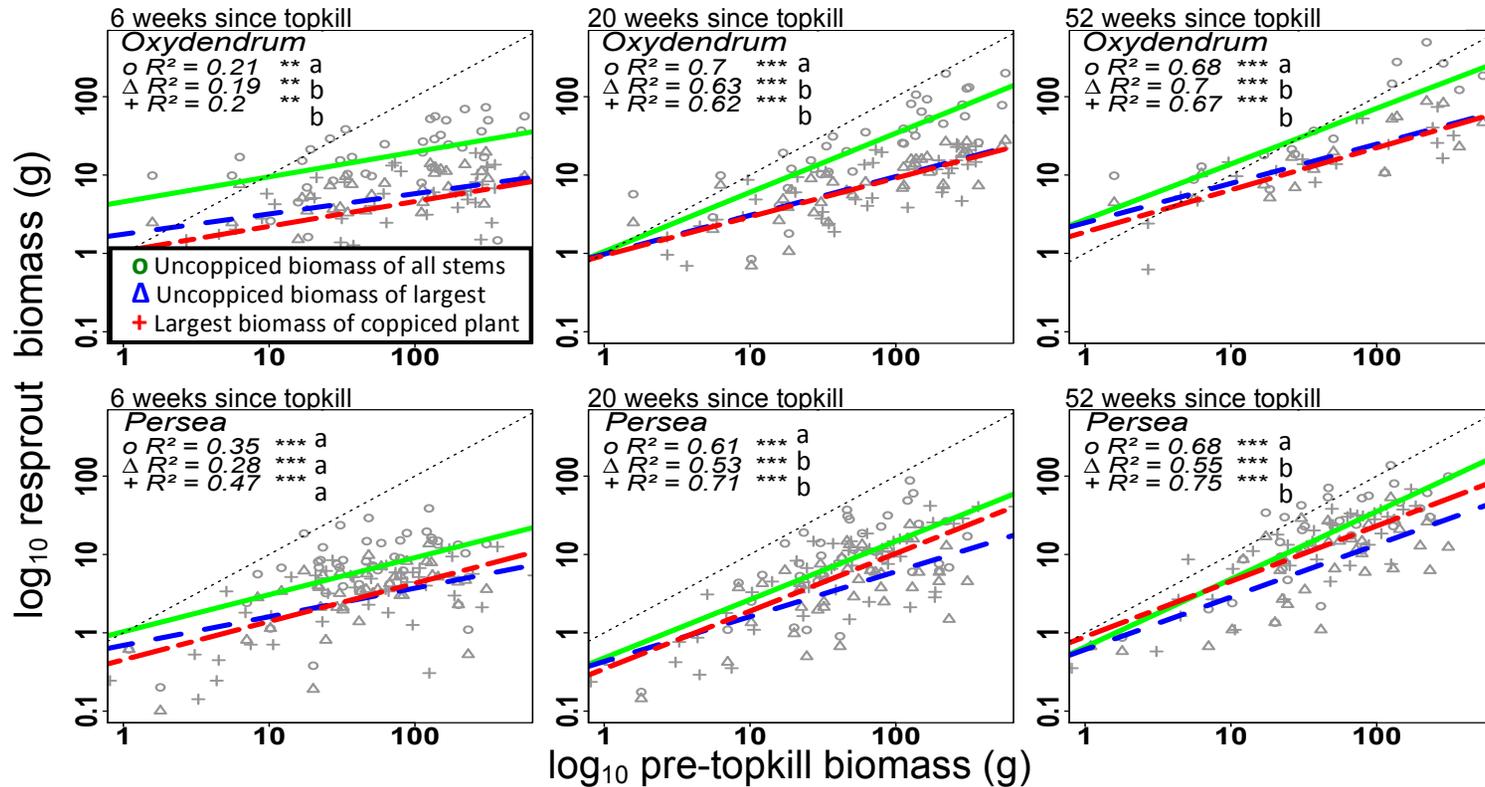


Figure 4.4. Resprout biomass (g) as a function of the pre-topkill biomass (g) for *Oxydendrum arboretum*, and *Persea palustris* at 6, 20 and 52 weeks after fire-simulated topkill. Two treatments were imposed: continual coppicing of all stems except the largest (+; red line), and uncoppiced. Uncoppiced treatments are shown separately in the relationships: biomass of the largest stem (Δ ; blue line), and total biomass of all stems of that individual, which also incorporates the biomass of the largest stem (O; green solid line). The dotted line represents the 1:1 line, below which plants experience greater negative effects to biomass recovery. Asterisks indicate statistically significant effects of pre-topkill size: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Different letters represent differences in slopes.

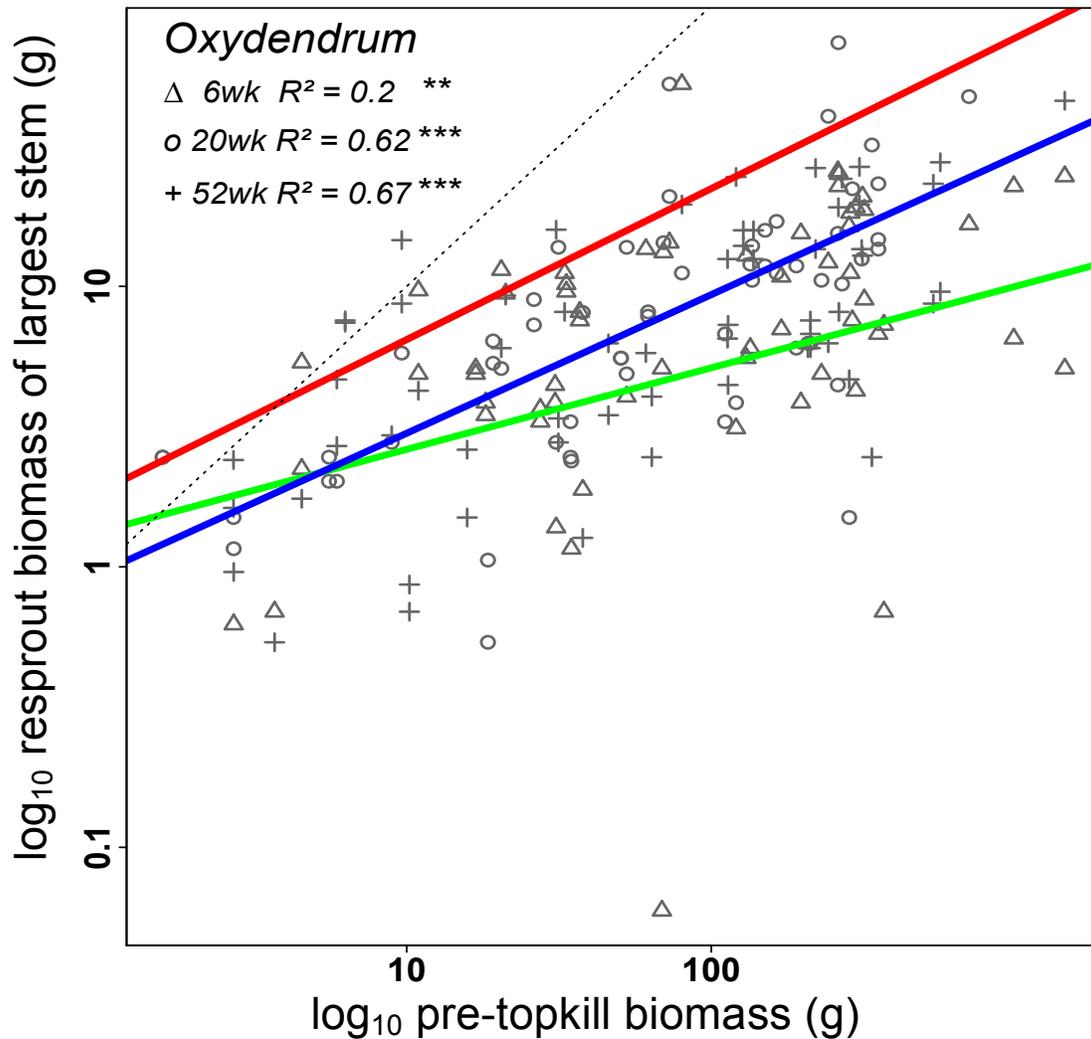


Figure 4.5 The resprout biomass of the largest stem in the coppicing treatment as a function of the pre-topkill biomass (g) for *Oxydendrum arboreum* 6 weeks (red line), 20 weeks (blue line), and 52 weeks (green line) after fire-simulated coppicing. The dotted line represents the 1:1 line, below which plants experience greater negative effects to biomass recovery. Asterisks indicate statistically significant effects of pre-topkill size: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

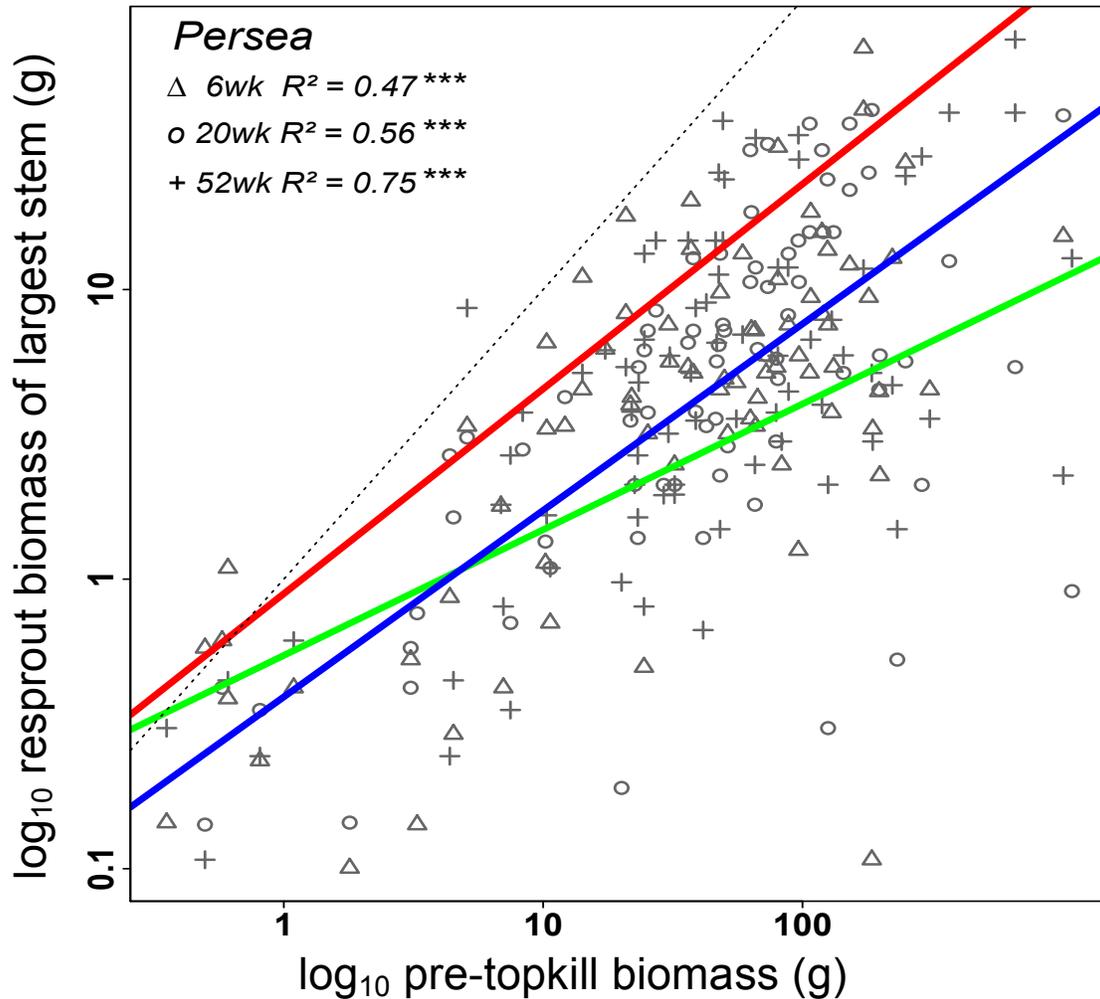


Figure 4.6 The resprout biomass of the largest stem in the coppicing treatment as a function of the pre-topkill biomass (g) for *Persea palustris* 6 weeks (red line), 20 weeks (blue line), and 52 weeks (green line) after fire-simulated coppicing. The dotted line represents the 1:1 line, below which plants experience greater negative effects to biomass recovery. Asterisks indicate statistically significant effects of pre-topkill size: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

Contrary to our hypothesis, the coppicing treatment did not alter biomass allocation patterns in resprouting plants in favor of small plants. The underlying patterns that were observed in the uncoppiced treatment were contrary to predictions. That is, compared to larger statured plants, a single stem of a small individual contributed a larger fraction of biomass to the total plant biomass. Small plants with fewer initial stems appeared to invest more in upward growth, initially allocating more reserves to fewer stems, whereas large plants may have a greater competitive allocation of resources among a larger number of buds. Since we did not observe a shift in allocation to the solo stem in small plants, smaller individuals appear to preferentially allocate more reserves to a fewer number of existing buds, and therefore cannot increase allocation. In large plants, I suggest that eliminating supplementary stems from a multi-stemmed individual would not benefit plant height or biomass growth because the largest stem is limited by growth rate (i.e. the largest stem is already growing as fast as possible and thus, the reallocation of resources cannot increase its growth). Therefore, the total biomass of a large individual is limited by the number of stems produced, even though each stem (or meristem initiation) is limited by growth rate.

This pattern highlights the advantages of being multi-stemmed in fire-prone systems; where light is rarely limiting, there is an advantage to maximizing leaf area production to cover as much ground as possible rather than invest in upward growth. Although, multi-stemmed plants tend to be shorter than single-stemmed trees (McMahon 1973; Midgley 1996), allocation ‘bet-hedging’ among multiple shoots ensures persistence amid frequent disturbances (Scheffer et al. 2014). It is likely that the coordination of buds and reserves after topkill demands a larger respiratory cost in large root systems (Vesk and Westoby 2004),

which would explain this trade-off. But for large resprouting plants, maximizing lateral photosynthetic leaf area appears to outweigh the disadvantages of self-shading among multiple branches and other maintenance requirements. In addition, Zammit (1988) found that small *Banksia* lignotubers retained a larger reserve of buds in relation to resprouted shoots, compared to large lignotubers, suggesting that they also invested in fewer shoots while they are small.

Removing supplementary shoots eliminated photosynthetic leaf area, and although carbohydrate reserves are essential for resprouting (Miyanishi and Kellman 1986; Hoffmann et al. 2004), we suggest that small individuals may rely more heavily on post-disturbance photosynthates than large plants. In South African fire-prone savannas, ¹⁴C-supplied resprouting shoots of *Acacia karoo* Hayne retained more carbon in their leaves and stems, and transported a smaller proportion to the roots than did intact plants, (Schutz et al. 2009). Coppicing experiments have shown variable effects on growth and starch reserves. Monthly defoliation of *Acacia* prevented stem thickening and reduced starch reserves, even though stem lengths were no different than in the controls (Schutz et al. 2011). Long-term coppicing (up to 66% stems per plant) of *Betula pubescens* Ehrh. reduced overall stem growth, but this constant browsing had no lasting effect on carbon limitation, even after seven years of simulated browsing (Palacio et al. 2008). These studies indicate that resprouting may not rely entirely on belowground carbon sources. Stem starch reserves may also play a large role in resprouting following partial coppicing (Erdmann et al. 1993). And grasses have been shown to support resprouting primarily from photosynthetic input (Richards and Caldwell 1985). Our low mortality rates suggest that intense repeated coppicing likely did not impose a carbon limitation on our study species during a single post-fire resprouting event.

Furthermore, flowering was observed in some *Oxydendrum* individuals after 52 weeks (data not shown).

It is possible that a continual investment in supplementary shoot growth prevented a shift in the allocation patterns during regrowth. The frequency with which our coppicing treatment took place demands a continual investment in multi-stemmed recovery, which could preclude reallocation of carbon to the remaining stem. Loescher (1990) suggested that the signal for starch hydrolysis might come from growing buds. It has also been suggested that a constraint on reserve mobilization may exist due to the large respiratory costs of maintaining a bud bank (Midgley 1996; Vesk and Westoby 2004). It is possible that in our coppicing experiment, the continual removal of coppiced stems may increase the respiratory demand on the plant, which may not accurately represent the true resprouting patterns after fire.

Resprouting success may be highly dependent on environmental conditions and disturbance regimes (Bellingham and Sparrow 2000). Resprouters may be more competitive in low-resource sites (Clarke et al. 2005), such as savannas, where conditions are unfavorable for fast growing species (Hoffmann et al. 2012). Many Australian alpine species store high levels of carbohydrates regardless of resprouting vigor, and resprout quickly even though fire has historically been infrequent there (Tolsma et al. 2007). Resprouting strategies will likely be influenced by the frequency and intensity of disturbance events. For example, under frequent-disturbance regimes bud banks may be more rapidly depleted than reserves (Bell and Pate 1996; Vesk and Westoby 2004). Additionally, fire intensity may reduce the number of resprouts per plant (Moreno and Oechel 1991). Multiple disturbance events, such as herbivory following a fire, can have drastic effects on resprouting capacity. An increase in

fire intensity may not only delay resprouting, but also increase susceptibility to herbivory (Moreno and Oechel 1991). Investment in herbivore defenses also requires a trade-off with starch storage that can have detrimental costs for growth (Paula and Ojeda 2011; Hean and Ward 2012). Overall, repeated disturbance events and herbivory could impose similar consequences as we observed, so these outcomes have important implications for long-term persistence.

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CHAPTER 5

EFFECTS OF MULTIPLE DISTURBANCES ON WHOLE-PLANT CARBON BALANCE: AN INTERSPECIES COMPARISON

ABSTRACT

An important characteristic of many plants inhabiting fire-prone environments is the ability to persist amid a cycle of repeated topkill and resprouting from belowground organs. Frequent fire often favors the success of small growth forms at the expense of trees and large shrubs. To address this, we investigated intra- and inter-species patterns of resprouting and estimates of potential whole-plant carbon assimilation in 24 common understory species in the longleaf pine savanna of the southeastern United States. Additionally, effects of repeated annual burning on carbon assimilation were examined. Repeated burning had a larger negative impact on the carbon assimilation of large plants compared to small plants. The physiological patterns of biomass and carbon recovery were shown not only exist within species, but also to exist across species regardless of mechanisms specific to or inherent in functional type. Across all species, annual burning caused a reduction in the slope of the relationship between carbon assimilated and pre-burn size after the second burn. Annual burning resulted in a downward shift in the equilibrium point of the amount of assimilated carbon per unit biomass. Because this pattern appears to be consistent across growth forms, it can explain patterns of species composition and vegetation structure across savannas with mild frequent fire, especially the success of herbaceous and small woody shrubs.

INTRODUCTION

Fire can fundamentally change the structure and composition of vegetation, often increasing the species richness and diversity of grasses and herbaceous plants (Brockway and Lewis 1997; Dumas et al. 2007; Mitchell et al. 2009). Savanna fires can occur as frequently as every 1–5 years (Frost & Robertson 1985; Glitzenstein et al. 1995), and historically fires occurred approximately every 2 years in the upland longleaf pine savannas of the southeastern USA (Stambaugh et al. 2011). Frequent burning can prevent competitive exclusion of grasses and forbs by larger shrubs and trees (Peterson and Reich 2008). As a result, flammable vegetation and an open savanna canopy will further prevent the invasion of dense stands of hardwoods (Gilliam and Platt 1999; Bond et al. 2005). Changes in fire frequency are therefore critical to the control of vegetation structure and species composition.

An important characteristic of many plants inhabiting fire-prone environments is the ability to persist amid a cycle of repeated topkill (destruction of aboveground biomass) and resprouting from belowground storage organs (Bond and Midgley 2003). However, resprouters do not dominate ecosystems worldwide due to the limitations of being shorter in stature than obligate seeding species (Midgley 1996). Frequent fire prevents the opportunity for plants to recruit into larger fire-tolerant size classes (Higgins et al. 2000), and thus it tends to favor the success of small growth forms at the expense of trees and large shrubs (Hoffmann and Solbrig 2003). Furthermore, if plants do not replenish carbon reserves between disturbances, it should follow that subsequent resprouting success may be reduced. Plant size will be maintained in a suppressed state if the plant does not reach a fire-resistant size (Gignoux et al. 1997), or does not surpass the pre-burn size (Grady and Hoffmann 2012). Thus, small plants will remain small, and large plants will be reduced to smaller size classes,

and remain at a reduced size if they cannot regain lost biomass (Figure 1). Log-transformed, this relationship exhibits a slope less than 1, a pattern we termed “negative allometry”. This “resprout curve,” described within species (Grady and Hoffmann 2012), can be explained by the recovery of positive carbon balance (Figure 2.4; Figure 2.5).

The need to maintain a positive carbon balance is a universal constraint upon plants in any environment. Ultimately, growth, reproduction, and survival are dependent upon the ability of a plant to balance photosynthesis and respiration (McCree 1986). This challenge is exacerbated when a plant is topkilled by fire, causing immediate carbon loss through destruction of aboveground biomass—including photosynthetic tissue—thereby compromising future carbon assimilation. Short fire return intervals often intensify the negative consequences to carbon balance in large plants.

In Chapter 2, I showed a consistent pattern of negative allometry in post-fire carbon assimilation within species, and that this negative allometry is self-reinforcing due to the influence of plant size on whole-plant carbon gain. Here, I hypothesize that these patterns not only characterize within-species responses to fire, but also the patterns across species. If confirmed, this would suggest the existence of universal constraints on plant responses to repeated fire that can largely explain the shifts in community composition that favor small growth forms. To address this, I investigated intra- and inter-species patterns of resprouting and estimates of potential whole-plant carbon assimilation in 24 common understory species in the longleaf pine savanna of the southeastern United States. Additionally, I wanted to understand how the patterns of carbon assimilation would be affected by repeated burning. I predicted that larger plants would incur larger negative consequences to resprout recovery and assimilation with repeated burning.

METHODS

Study sites and species

This research was at Fort Bragg Military Reservation in Hoke and Cumberland counties in North Carolina. Mean annual precipitation is 1275 mm (1200 mm rain, 75 mm snow), and mean winter and summer temperatures are 6.9 °C and 26 °C, respectively (Sorrie et al. 2006). Since 1991, Fort Bragg has conducted prescribed burned at intervals of approximately 3 years across independently managed burn units. Study sites were selected at ecotones between the upland longleaf pine-wiregrass (*Pinus palustris*–*Aristida stricta*) savanna and stream-head wetlands, where plants have access to perennially wet soils, but are exposed to fire. All study sites had been regularly burned at 3-year intervals until 2010, and burned annually thereafter. Sites were burned between June 10 and August 31. All burns were low-intensity ground fires similar to those implemented for routine maintenance of the sites.

I studied 23 common understory species, including three trees, four woody shrubs, and two herbs (Table 5.1): *Acer rubrum* L. (red maple), *Arundinaria tecta* (Walter) Muhl. (switchcane), *Carya tomentosa* (Lam.) Nutt. (mockernut hickory), *Clethra alnifolia* L. (summer-sweet), *Coreopsis major* Walter (tickseed), *Diospyros virginiana* L. (persimmon), *Eupatorium rotundifolium* L. (roundleaf thoroughwort), *Gaylussacia frondosa* (L.) Torr. & A. Gray ex Torr. (dangleberry), *Ilex glabra* (L.) A. Gray (inkberry), *Lyonia lucida* (Lam.) K. Koch (pink fetterbush), *Lyonia mariana* (L.) D. Don (piedmont staggerbush), *Magnolia virginiana* L. (sweetbay), *Morella caroliniensis* (Mill.) Small (southern bayberry), *Oxydendrum arboreum* (L.) DC. (sourwood), *Persea palustris* (Raf.) Sarg. (swampbay), *Quercus laevis* Walter (turkey oak), *Quercus marilandica* Münchh. (blackjack oak), *Rhexia*

alifanus Walter (savanna meadowbeauty), *Robinia nana* L. var. *nana* (Elliott) DC. (bristly locust), *Sassafras albidum* (Nutt.) Nees (sassafrass), *Tephrosia virginiana* (L.) Pers. (tephrosia), *Vaccinium formosum* Andrews (southern blueberry), *Vaccinium fuscatum* Aiton (highbush blueberry).

Above and Belowground Biomass Recovery

In May 2011, at the beginning of the growing season prior to burning, individuals of the first nine species were tagged at each burn site (Chapter 2). In May 2012, the 15 species were added to the study to improve interspecies comparisons and comparisons across growth form. Consequently, there is an additional year of resprouting data for the first set of species. To reduce the effect of environmental heterogeneity, individuals were identified and marked within a 5 x 5 meter sample area at each site. Prior to burning, we measured height and basal diameter at 5 cm above the ground. These measurements were repeated 4 months after burning at the end of the growing season. By 4 months after the prescribed burns, growth of resprouts had ceased and leaves of deciduous species began to senesce. Sites were burned each year, and so pre- and post-fire measurements were made from 2011–2013. For individuals with multiple stems, these measurements were performed for each stem. Species-specific allometric equations for pre-burn and resprouting plants (Appendix A) were used to non-destructively estimate leaf area and total aboveground biomass from the height of each tagged individual at each census. Pre-burn biomass refers to total aboveground biomass.

Photosynthesis Measurements

Leaf gas exchange measurements were performed before burning and on resprouting plants to test for the effect of burning on maximum CO₂ assimilation rates (A_{\max}) used in the modeling synthesis described below. Furthermore, for the first nine species, photosynthetic

light-response curves were performed on plants to be burned and on unburned controls. Pre-burn light-response curves were measured on 5–20 individuals per species per treatment, depending on abundance at each site. Measurements were performed using a portable infrared gas analyzer (LiCor 6400XT, Li-Cor Inc., Lincoln, NE), on fully expanded sunlit leaves between 10 am and 3 pm with the mean chamber temperature maintained at $25.0 \pm 0.5^\circ\text{C}$, and $400 \mu\text{mol mol}^{-1} \text{CO}_2$ concentration, and a saturating light intensity of $1500 \mu\text{mol photon m}^{-2} \text{s}^{-1}$. Relative humidity within the chamber was controlled to match ambient conditions, but not to exceed 83%. Leaves were allowed to adjust to the light intensity for 2–5 minutes prior to measurement. For the light-response curves, an auto-logging program was set to record five measurements of gas exchange at each level of photosynthetically active radiation (PAR) ranging from 0–1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (1500, 1000, 500, 120, 60, 40, 20, 10, 0). Leaves were allowed to adjust for at least 5 minutes at each light intensity before gas exchange measurements were recorded.

Post-burn point-measurements of A_{max} that were recorded on resprouting plants in 2011, and all additional species in 2012 were recorded at saturating light intensity ($1500 \mu\text{mol photon m}^{-2} \text{s}^{-1}$), matching the leaf and chamber conditions described above. An auto-logging program recorded five point measurements on 5–25 individuals per species per treatments, after an adjustment period of 2–5 minutes.

Modeling Carbon Assimilation

I used a simple model to estimate potential carbon (C) assimilation of resprouting plants, based on our field measurements of leaf area, and maximum light-saturated photosynthesis. The objective was to estimate the relative capacity for post-fire carbon assimilation to contribute to recovery of aboveground biomass in plants differing in pre-burn

size. In general, daily carbon assimilation was estimated for each individual as the product of the photosynthetic rate and leaf area, and these daily estimates were integrated over 4 months to obtain seasonal estimates of carbon assimilation. The average A_{\max} per species was used in these estimates. Respiration of stems and roots was not directly measured, so 50% of the photosynthate was assumed to be lost to respiration (Mooney 1972; Hopkins and Hüner 2009). This likely underestimates whole-plant respiration in the early stages of resprouting when leaf area is low relative to root mass. This underestimation during resprouting should be greatest in large individuals, resulting in conservative conclusions regarding the effect of plant size on post-fire carbon gain.

Analysis of Size-Dependent Responses to Fire

I used an allometric approach to test the hypothesis that, relative to small plants, large plants have lower whole-plant C assimilation and biomass recovery per unit of pre-burn biomass. Testing this relationship directly (Figure 5.1c, d) is subject to spurious correlations that can arise because the independent variable is used to calculate the dependent variable. This bias can be avoided by testing for an allometric relationship between whole-plant assimilation (or resprout biomass) and pre-burn biomass. Under the proposed hypothesis, the relationship should exhibit *negative allometry* (i.e. allometric coefficient < 1). If negative allometry exists, the slope of the relationship is < 1 on a \log_{10} scale, and large plants experience a disproportionately negative response to fire; i.e., the resprouting variable is less than that of the pre-burn variable.

Statistical Analyses

To test for trends across species in the first year of resprouting, I used a regression

model with mean pre-burn biomass as the independent variable and resprout variables as the dependent variable (both \log_{10} -transformed to fit normality): mean recovered biomass (resprout biomass: pre-burn biomass), mean C assimilated, and mean recovered C (assimilated C: pre-burn biomass). To test for homogeneity of slopes among growth forms I fit linear mixed models, using all observations over 3 years of resprouting, with burn occurrence (1st, 2nd, 3rd) and growth form as fixed effects, and species nested within growth form as a random effect to determine the effect of fire on resprout variables (as above). Models were fit using R version 2.15.1 (The R Foundation for Statistical Computing, 2012).

RESULTS

Effect of Fire on the Resprouting Rate

Annual burning had a negative effect on the rate of resprouting in many species (Table 5.1). Only 14 % of *Alnus* individuals successfully resprouted after fire in the first growing season, and failed to resprout in the second year. These individuals were therefore not included in the analysis. After the first burn, 90% of all individuals resprouted, and 75% resprouted after the second burn.

Effect of Fire on Aboveground Biomass Recovery

Across all species, there was a significant negative correlation between mean pre-burn biomass and recovered biomass in the first year of resprouting ($F_{1,21} = 4.9$; $P = 0.04$; Figure 5.2). On average, larger-statured species tended to recover a smaller proportion of their pre-burn biomass during the same post-fire growing season, relative to smaller plants; the slope of the relationship between mean pre-burn biomass and mean resprout biomass was less than 1, indicating negative allometry ($T_{21} = 2.03$; $P = 0.05$). Across all observations, after pre-burn biomass is taken into account, there was no difference among growth forms in post-

burn biomass ($F_{1,24} = 1.0$; $P = 0.40$; Table 5.2). That is, the observed differences in post-fire recovery among growth forms can be explain by their differences in pre-burn size.

Effect of Fire on Carbon Assimilation and Recovery

Across all species, there was a significant correlation between mean pre-burn biomass and carbon assimilated ($F_{1,21} = 16.4$; $P < 0.001$; Figure 5.3), and a significant negative correlation with recovered carbon ($F_{1,21} = 5.7$; $P = 0.03$; Figure 5.4). On average, larger statured species tended to assimilate less carbon relative to their pre-burn biomass, compared to smaller plants; negative allometry was significant for the relationship between mean pre-burn biomass and mean assimilated carbon ($T_{21} = 2.56$; $P = 0.018$). Across all observations, growth form was not a significant predictor of these patterns ($P > 0.05$; Table 5.2). The relationship for each burn exhibited significant negative allometry (Table 5.3).

Effect of Repeated Annual Burning

The relationship for each of the three burns showed a significant negative allometry (Table 5.3), indicating that larger sizes assimilated a smaller relative proportion of carbon, compared to smaller pre-burn sizes. Repeated burning had a significant effect on the slope of carbon assimilated ($F_{1,819} = 8.4$; $P < 0.001$; Figure 5.5), and also on the intercept ($F_{1,18} = 9.1$; $P = 0.007$). This caused a downward shift in the equilibrium point, which is the intersection of the resprout curve and the 1:1 line.

Table 5.1 Study species and characteristics are represented with the number of individuals for pre-burn allometry and tagged individuals and their rate of resprouting. Species without a resprout rate in the third year were part of the second cohort, whose inventory commenced in 2012. Nomenclature follows Flora of the Southern and Mid-Atlantic States by Alan S. Weakley (<http://www.herbarium.unc.edu/flora.htm>).

Growth Form	Species	Family	<i>N</i>	Burn 1 resprout rate (%)	Burn 2 resprout rate (%)	Burn 3 resprout rate (%)
Tree	○ <i>Acer rubrum</i> L.	Sapindaceae	67	82.4	66.7	46.2
	+ <i>Carya tomentosa</i> (Lam.) Nutt.	Juglandaceae	9	80.0	25.0	-
	∇ <i>Diospyros virginiana</i> L.	Ebenaceae	25	80.0	41.7	-
	⊞ <i>Magnolia virginiana</i> L.	Magnoliaceae	48	100	100	-
	▣ <i>Oxydendrum arboreum</i> (L.) DC.	Ericaceae	76	82.4	66.7	48.6
	■ <i>Persea palustris</i> (Raf.) Sarg.	Lauraceae	106	96.0	74.2	48.9
	● <i>Quercus laevis</i> Walter	Fagaceae	26	100	100	-
	▲ <i>Quercus marilandica</i> Münchh.	Fagaceae	55	96.4	100	-
	• <i>Sassafras albidum</i> (Nutt.) Nees	Lauraceae	8	100	100	-
	Shrub	× <i>Clethra alnifolia</i> L.	Clethraceae	137	100	86.3
* <i>Gaylussacia frondosa</i> (L.) Torr. & A. Gray ex Torr.		Ericaceae	107	91.4	71.4	36.7
◇ <i>Ilex glabra</i> (L.) A. Gray		Aquifoliaceae	143	98.2	93.8	67.3
⊕ <i>Lyonia lucida</i> (Lam.) K. Koch		Ericaceae	62	77.5	81.8	32.1
☆ <i>Lyonia mariana</i> (L.) D. Don		Ericaceae	27	84.2	58.3	-
⊗ <i>Morella caroliniensis</i> (Mill.) Small		Myricaceae	15	92.3	33.3	-
● <i>Robinia hispida</i> L. var. <i>nana</i> (Elliott) DC.		Fagaceae	7	100	100	-
■ <i>Vaccinium formosum</i> Andrews		Ericaceae	17	69.2	76.9	-
◇ <i>Vaccinium fuscatum</i> Aiton		Ericaceae	29	66.7	75.0	-

Table 5.1 Continued

Growth Form	Species	Family	N	Burn 1 resprout rate (%)	Burn 2 resprout rate (%)	Burn 3 resprout rate (%)
Herb	Δ <i>Arundinaria tecta</i> (Walter) Muhl	Poaceae	67	84.2	57.1	37.5
	⊠ <i>Eupatorium rotundifolium</i> L.	Asteraceae	48	100	96.9	93.8
	◇ <i>Coreopsis major</i> Walter	Asteraceae	15	100	87.5	-
	◆ <i>Rhexia alifanus</i> Walter	Melastomataceae	29	100	50.0	-
	● <i>Tephrosia virginiana</i> (L.) Pers.	Fabaceae	31	88.9	75.0	-

Table 5.2 Results of mixed models including all species with fire (i.e. burn occurrence) and growth form as fixed effects, and species nested within growth form as a random effect, with pre-burn biomass as the independent variable and resprout carbon assimilation as the dependent variable.

	<i>F</i>	<i>df</i>	<i>Df.res</i>	<i>P</i>
Carbon Assimilated				
Intercept	9.19	1	18.2	0.007
C Assimilated	105	1	2.76	0.0028
Growth Form	2.43	2	17.2	0.117
Burn	17.3	2	823	<0.001
C Assimilated*Burn	8.40	2	819	0.0002

Table 5.3 Relationships exhibiting negative allometry with pre-burn biomass as the independent variable and resprout carbon assimilation as the dependent variable, across all observations including 23 common understory plant species for three consecutive prescribed burns. Significant results indicate that the slope of the relationship is less than 1, indicating that larger sizes experience greater negative consequences to the response variable.

	<i>t</i>	<i>df</i>	<i>P</i>
Resprout Biomass			
Burn 1	8.84	486	<0.001
Burn 2	6.45	188	<0.001
Burn 3	3.51	159	0.0006
Carbon Assimilated			
Burn 1	10.6	486	<0.001
Burn 2	9.05	188	<0.001
Burn 3	5.92	159	<0.001

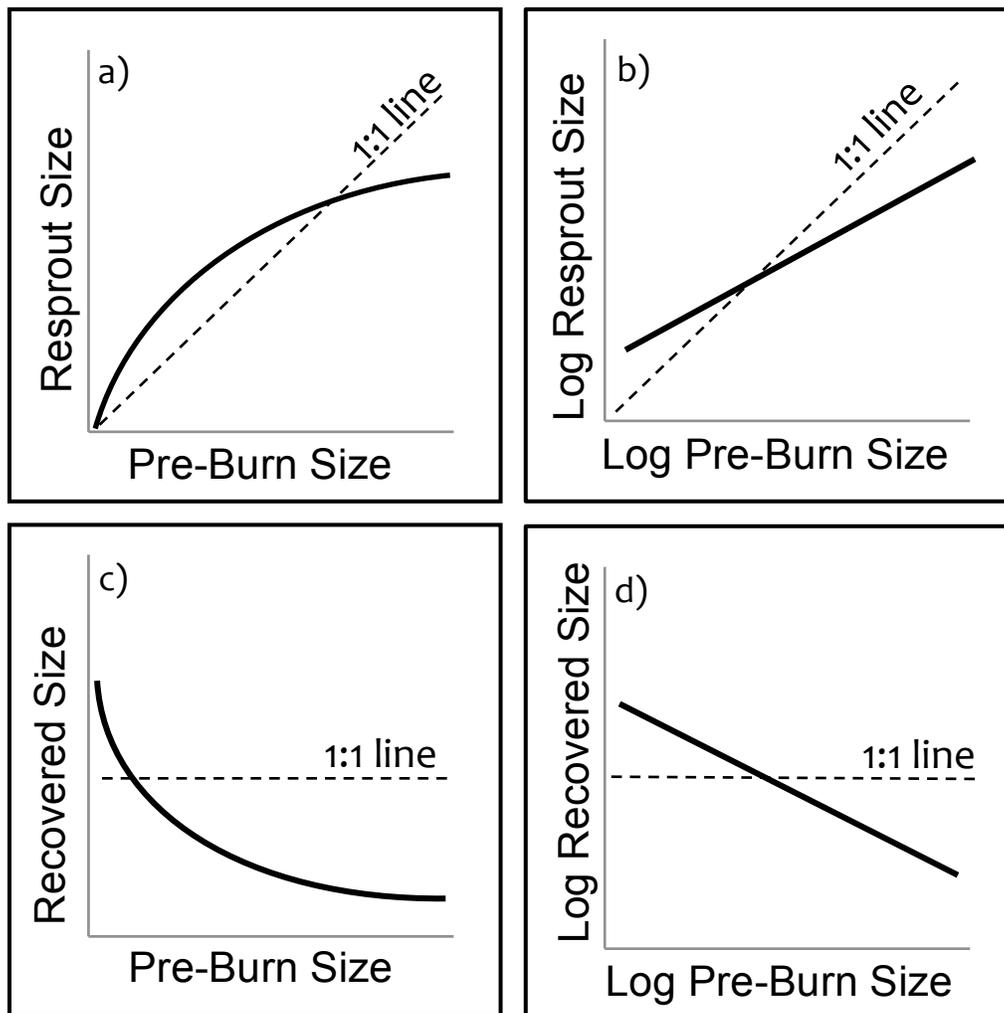


Figure 5.1 Hypothesized allometric patterns underlying the relationship between pre-burn and post-burn variables (modified from Grady & Hoffmann 2012): (a) Allometric relationship between pre-burn and post-burn size, (b) log transformation of the relationship in (a), (c) relationship between the recovered size (post-burn: pre-burn size) and the pre-burn size, and (d) log transformation of the relationship in (c). The dashed line represents the 1:1 line, along which the post-fire resprout size equals pre-burn size, and the coefficient or slope (α) of the relationship is equal to 1. The solid line represents a “negative allometry” where $\alpha < 1$. Shallower slopes indicate a net benefit to smaller plants, consistent with a more negative allometry.

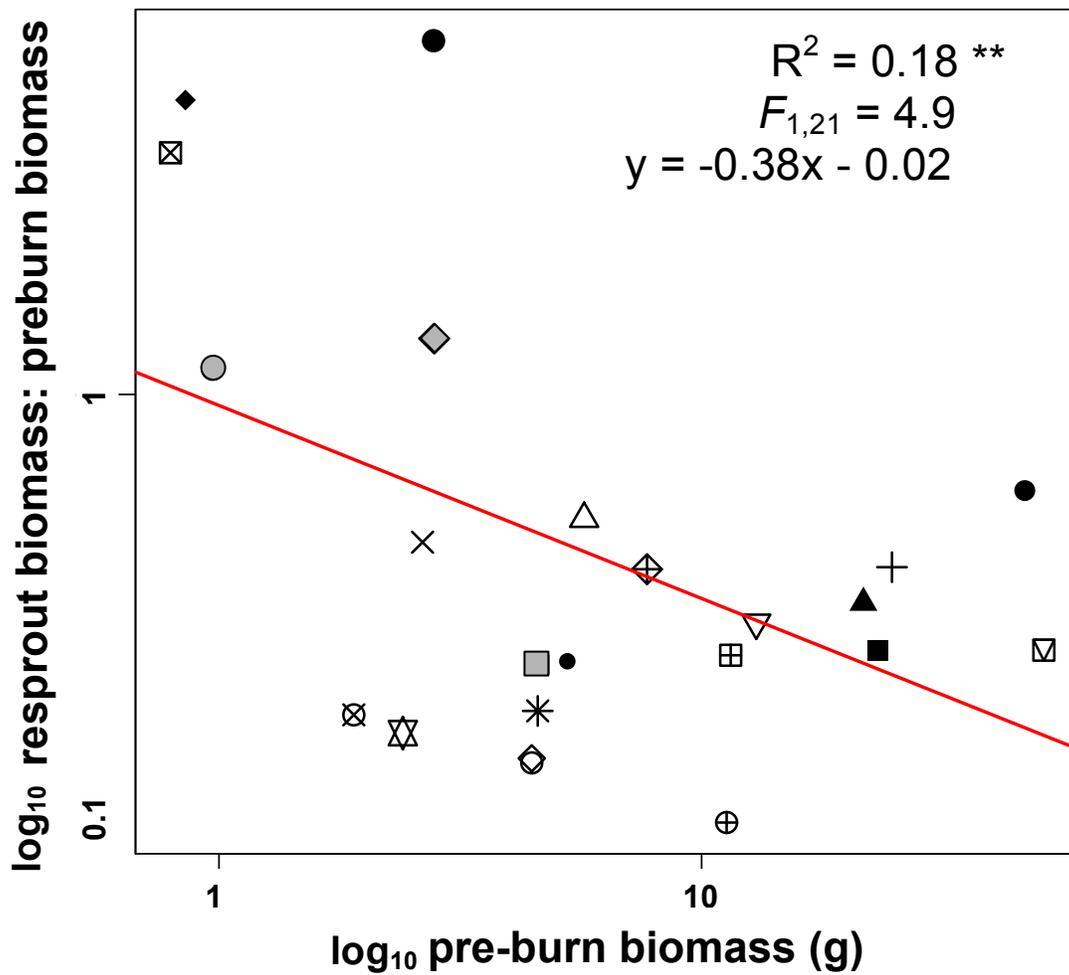


Figure 5.2 Across-species relationship between mean pre-burn biomass and mean recovered biomass (resprout biomass: pre-burn biomass) after four months of resprouting. Each point represents the mean value per species (Refer to Table 5.1). Asterisks indicate statistically significant effects of pre-burn size: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

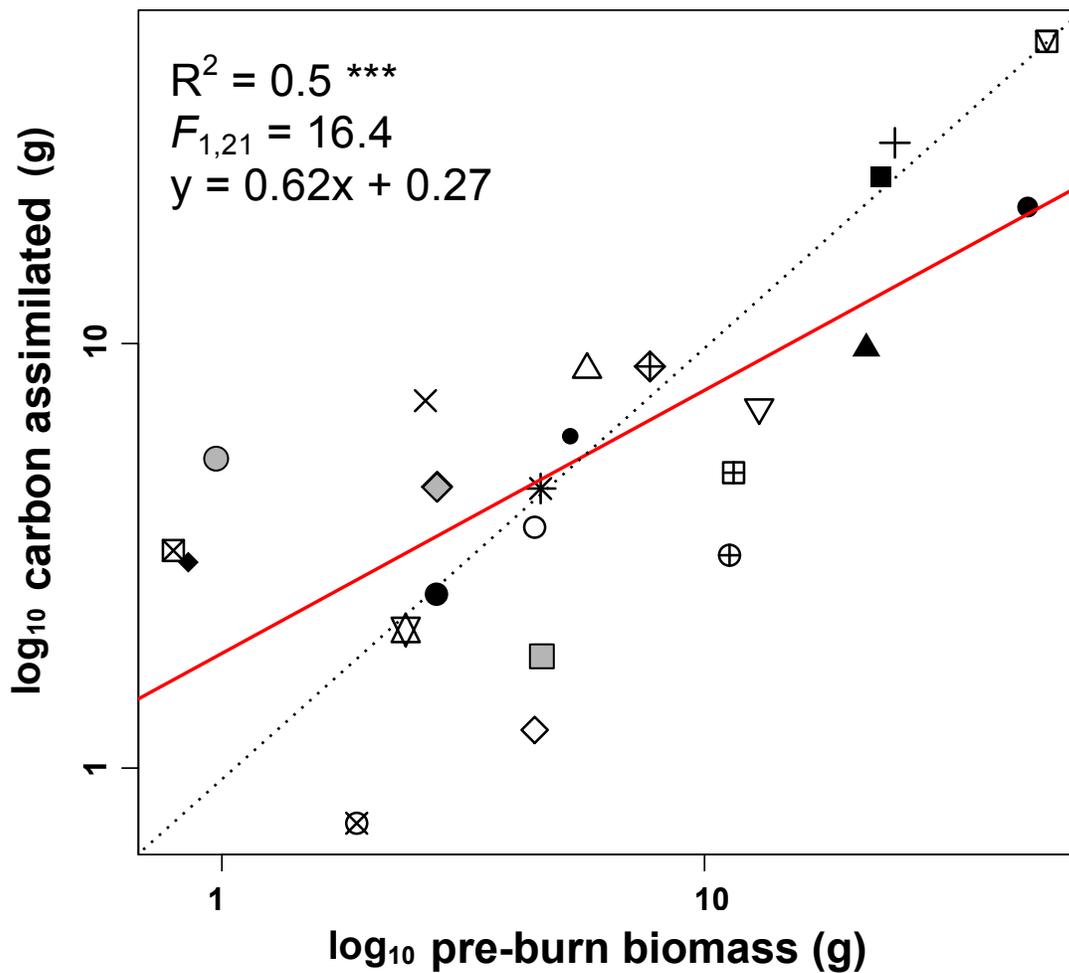


Figure 5.3 Across-species relationship between mean pre-burn biomass and mean potential carbon assimilated after four months of resprouting. Each point represents the mean value per species (Refer to Table 5.1). The dotted line represents the 1:1 line, above which plants assimilated a larger proportion of carbon relative to their pre-burn size. Asterisks indicate statistically significant effects of pre-burn size: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

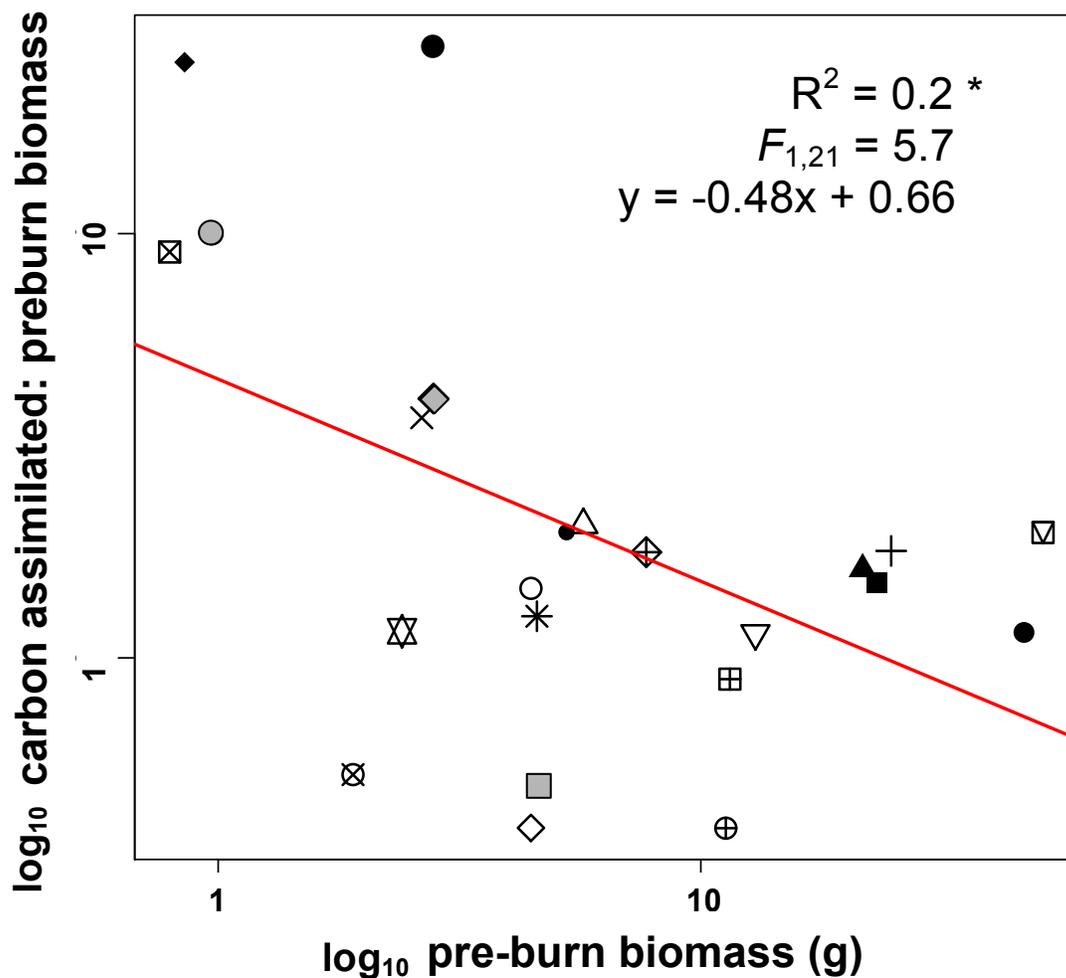


Figure 5.4 Across-species relationship between mean pre-burn biomass and mean recovered carbon (resprout carbon: pre-burn biomass) after four months of resprouting. Each point represents the mean value per species (Refer to Table 5.1). Asterisks indicate statistically significant effects of pre-burn size: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

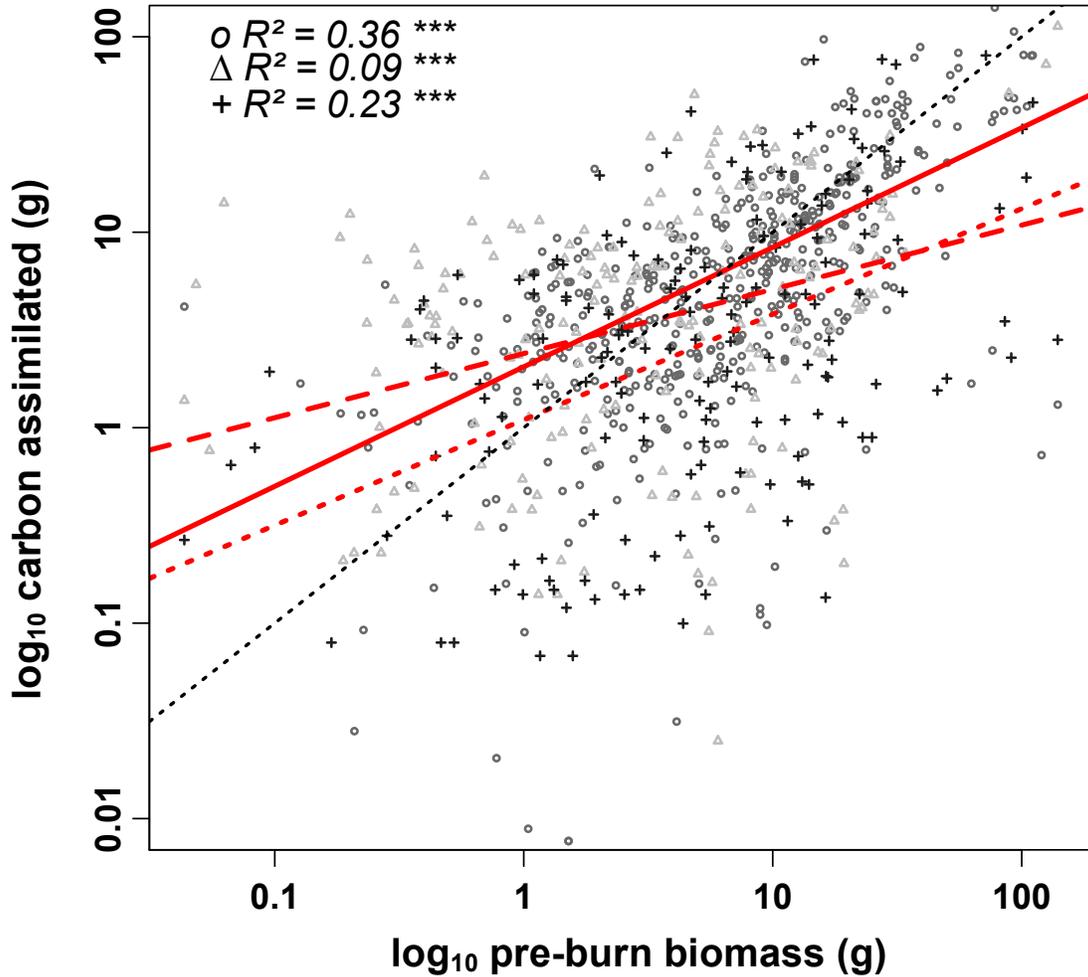


Figure 5.5 Relationship between pre-burn biomass and potential maximum carbon assimilation for three consecutive prescribed burns (solid line, 1st burn; long-dashed, 2nd burn; short-dashed, 3rd burn). Each point represents one individual across all observations of 23 common understory savanna species. The black dotted line represents the 1:1 line, below which plants assimilate a smaller fraction of carbon relative to pre-burn biomass. Shallower slopes in resprouting plants indicate a net benefit to smaller plants, consistent with a more negative allometry. Asterisks indicate statistically significant effects of pre-burn size: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

Frequent fire prevents the opportunity for plants to recruit into larger fire-tolerant size classes, and thus has a tendency to favor the success of smaller growth forms at the expense of trees and large shrubs. This study provides evidence to support the hypothesis that the physiological mechanisms underlying this size-dependent resprouting success exist regardless of functional type. Thus, the tendency for small plants to recover their pre-burn size quicker than large plants appears to be a function of plant size, and not related to constraints inherent in functional type itself.

I observed a similar inter-species patterns as described by Grady and Hoffmann (2012), in which larger individuals recovered a smaller fraction of their pre-burn size than smaller individuals, but results show that this pattern exists across species and growth forms. Negative allometry results in the intersection of the resprout curve and the 1:1 line, which corresponds to a point at which resprout size equals pre-burn size (Figure 5.1). If the relationship persists, plants should converge at this intersection, which is the equilibrium point.

Whether or not a plant reaches reproductive size in between fires will have important consequences for the composition of vegetation in fire-maintained systems. If reproductive size is below the equilibrium point, a plant will be suppressed at a smaller size class until a long enough fire-free interval. If plants in this study continued to be burned annually, plant size should eventually converge upon an equilibrium size. Because this pattern appears to be consistent across growth forms, it can explain patterns of species composition and vegetation structure across savannas with mild frequent fire, especially the success of herbaceous and small woody shrubs. The pattern I observed across species can explain the success of small

plants in frequently burned savannas, and in turn may shed light on the dynamics of size-class distributions in other fire-prone systems.

Repeated burning had a larger negative impact on the recovery of large plants compared to smaller plants. Annual burning caused a reduction in carbon assimilation relative to pre-burn size, causing a more negative allometry with successive fires (i.e. the shallower slope caused a downward shift in the equilibrium point with each consecutive burn). This is consistent with other patterns of resprout biomass repeated burning in the southeastern US (Harrington and Edwards 1999; Robertson and Hmielowski 2013).

This pattern is consistent across species in savannas with low-intensity fires, but these patterns may be altered, including the relative abundances of fire-tolerant versus fire-sensitive species, in systems prone to high severity fires. The vegetation composition of woody plants versus herbaceous plants has a large impact on the fuel characteristics that would influence the flammability at the community level (Morrison 2002).

High fire frequency is correlated to the prevalence of many rare and endangered plants in the southeast US (Sorrie and Weakley 2006), and thus the management of savannas depends on the fire frequency. Our results suggests that the longer periods of annual burning would have cumulative effects on the ability to assimilate carbon, and thus maximum resprout biomass, which could significantly alter savanna vegetation structure.

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CHAPTER 6

CONCLUSIONS

The goal of this research was to understand the physiological basis behind size-dependent plant resprouting success in fire-prone savannas to improve our ability to predict species and community responses to fire. I addressed this goal by examining resprouting recovery of biomass and carbon, changes in carbon assimilation, changes in root carbohydrates and nutrients, changes in allocation patterns after clipping, and effects of annual burning. The main hypothesis of this dissertation is that post-fire carbon balance is negatively correlated with plant stature across and within species, and that this can largely explain patterns of post-fire biomass recovery, and replenishment of carbohydrate reserves.

My dissertation research confirmed a pattern of resprouting that constrains the post-fire recovery of large plants, that we termed negative allometry, but reveal that it arises very early in resprouting and has a self-reinforcing effect on whole-plant carbon balance. We argue that these physiological mechanisms can explain size-dependent resprouting success. Specifically, this research found that (1) small plants tend to be reserve limited, indicating that they (a) undergo a rapid conversion of reserves into biomass, and (b) undergo a rapid recovery of positive carbon balance that further reinforces a rapid biomass recovery; (2) large plants are meristem limited, meaning that they are (a) slow to mobilize stored root reserves, (b) slow to recover positive carbon balance, which reinforces a slow recovery of lost biomass.

Within species, CHO and N concentrations did not change significantly after fire, nor were they significantly correlated with plant size. However, in relative terms, some plants exhibited the capacity to produce a proportionally larger resprout biomass relative to their CHO reserves, compared to plants with larger CHO reserves.

I tested whether coppicing supplementary stems in multi-stemmed plants would reduce competitive allocation from the root carbohydrates preferentially in small plants. Contrary to our hypothesis, there was no increase in the growth of the largest coppiced stem, relative to the uncoppiced treatment. The inherent allocation pattern in the uncoppiced plants suggests that the relative contribution of one stem to the total plant biomass decreases with pre-burn size.

These physiological patterns of biomass and carbon recovery were shown to not only exist within species, but also to exist across species regardless of mechanisms specific to or inherent in functional type. Additionally, annual burning can have negative consequences for resprout carbon assimilation, even when the timing of the burn allows for a full growing season.

An important characteristic of many plants inhabiting fire-prone environments is the ability to persist amid a cycle of repeated topkill and resprouting from belowground organs. Frequent fire prevents the opportunity for smaller plants to recruit into larger fire-tolerant size classes and therefore, short fire cycles will tend to favor the success of small growth forms at the expense of trees and large shrubs. Without sufficient time between fires, large statured plants will be prevented from both reaching a size that is fire-resistant and also under reproductive size. If large plants are able to surpass this threshold, the benefit of being large will be experienced, such as the ability to store more CHO. This dissertation confirms a curvilinear pattern that was previously described, which exhibits negative allometry, but provides physiological mechanisms to explain this pattern within and across species. These data suggest that size-dependent resprouting success can be explained by size rather than by functional type, and therefore has far-reaching implications for explaining patterns of species

distributions and vegetation composition. It is yet to be examined whether these patterns are widespread across disturbed systems.

This research was limited by unexpected logistical problems such as unfavorable burn conditions that prevented prescribed burning from taking place, and wildfires.

Additionally, fire intensity has an impact on the relative abundances of plant species that are fire-tolerant versus fire-sensitive (Morrison 2002). Prescribed burns at Fort Bragg were all ground fires, however, we did not control for fire intensity. Variability in fire intensity may have prevented resprouting in many species or individuals. Nevertheless, my results are likely to be representative of plant responses to fire in similarly disturbed communities.

Further resprouting studies should focus on what fraction of incoming photosynthates are incorporated into root storage versus new growth, what is the turnover of root nutrients, and the long-term effects of fire frequency on CHO and nutrient consumption (such as N) and replenishment during resprouting. Studies that could reveal the effects of long-term altered fire regimes on resprouting physiology, would not only improve our understanding of savanna dynamics, but the outcomes would benefit management and conservation efforts. This dissertation has demonstrated a pattern over a wide range of species; however, future research could focus on how widespread this pattern is for explaining resprouting success across disturbed ecosystems worldwide.

APPENDIX

APPENDIX A

ALLOMETRIC EQUATIONS

Allometric equations used to estimate leaf area (LA) from height (ht) measurements.

Species	Pre-burn Sites		Burned Sites	
	Equation	R ²	Equation	R ²
<i>Acer rubrum</i>	LA = 1.828 * (ht) – 1.789	0.943	LA = 1.693 * (ht) – 0.639	0.967
<i>Arundinaria tecta</i> ^a	LA = 1.691 * (ht) – 1.737	0.896	LA = 1.691 * (ht) – 1.737	0.896
<i>Clethra alnifolia</i>	LA = 1.483 * (ht) – 0.142	0.816	LA = 1.258 * (ht) + 1.346	0.859
<i>Eupatorium rotundifolium</i>	LA = 1.865 * (ht) – 2.718	0.768	LA = 1.426 * (ht) – 0.864	0.902
<i>Gaylussacia frondosa</i>	LA = 1.762 * (ht) – 1.089	0.895	LA = 1.570 * (ht) – 0.346	0.910
<i>Ilex glabra</i>	LA = 2.071 * (ht) – 2.764	0.878	LA = 1.490 * (ht) – 0.191	0.968
<i>Lyonia lucida</i>	LA = 1.834 * (ht) – 1.918	0.892	LA = 1.233 * (ht) + 0.122	0.857
<i>Oxydendrum arboreum</i>	LA = 2.029 * (ht) – 1.396	0.901	LA = 1.300 * (ht) + 1.671	0.948
<i>Persea palustris</i>	LA = 2.003 * (ht) – 1.755	0.950	LA = 1.714 * (ht) – 0.313	0.806

^aThe same equation was used to estimate leaf area of stems of *Arundinaria* in burned and unburned sites. For *Arundinaria*, the equation is based on individuals from pre-burn sites.

APPENDIX A Continued

Allometric equations used to estimate aboveground biomass (Bio) from height (ht) measurements.

Species	Pre-burn Sites		Burned Sites	
	Equation	R ²	Equation	R ²
<i>Acer rubrum</i> ^a	Bio = 2.14 *(ht) - 7.34	0.910	Bio = 2.14 *(ht) - 7.34	0.910
<i>Arundinaria tecta</i> ^a	Bio = 1.99 *(ht) - 6.82	0.955	Bio = 1.99 *(ht) - 6.82	0.955
<i>Clethra alnifolia</i>	Bio = 1.82 *(ht) - 5.96	0.886	Bio = 1.54 *(ht) - 4.33	0.796
<i>Eupatorium rotundifolium</i>	Bio = 2.10 *(ht) - 8.18	0.914	Bio = 1.82 *(ht) - 6.54	0.971
<i>Gaylussacia frondosa</i>	Bio = 2.06 *(ht) - 6.73	0.943	Bio = 1.85 *(ht) - 5.82	0.920
<i>Ilex glabra</i>	Bio = 2.43 *(ht) - 7.98	0.915	Bio = 1.54 *(ht) - 4.64	0.955
<i>Lyonia lucida</i>	Bio = 2.38 *(ht) - 7.99	0.907	Bio = 1.27 *(ht) - 4.33	0.844
<i>Oxydendrum arboreum</i>	Bio = 2.29 *(ht) - 7.35	0.916	Bio = 2.00 *(ht) - 6.05	0.895
<i>Persea palustris</i>	Bio = 2.21 *(ht) - 6.53	0.952	Bio = 1.89 *(ht) - 5.34	0.716

^a The same equation was used to estimate biomass of stems of *Acer* and *Arundinaria* in burned and unburned sites. The equation is based on individuals from pre-burn sites.

APPENDIX A Continued

Allometric equations used to estimate leaf area (LA) and aboveground biomass (Bio) from height (ht) measurements. Equations were used for both pre-burn and post-burn estimates.

Species	Leaf Area	R²	Aboveground Biomass	R²
<i>Carya tomentosa</i>	LA = 1.500*(ht) + 1.750	0.857	Bio = 1.932*(ht) - 4.082	0.923
<i>Coreopsis major</i>	LA = 1.738*(ht) - 2.555	0.941	Bio = 1.762*(ht) - 6.518	0.961
<i>Diospyros virginiana</i>	LA = 1.391*(ht) + 0.926	0.868	Bio = 1.765*(ht) - 4.698	0.887
<i>Lyonia mariana</i>	LA = 1.632*(ht) - 0.435	0.765	Bio = 1.863*(ht) - 5.743	0.819
<i>Magnolia virginiana</i>	LA = 1.398*(ht) + 0.222	0.643	Bio = 1.665*(ht) - 4.961	0.924
<i>Morella caroliniensis</i>	LA = 1.525*(ht) + 0.429	0.948	Bio = 1.651*(ht) - 4.369	0.954
<i>Quercus laevis</i>	LA = 1.745*(ht) - 0.0009	0.861	Bio = 1.925*(ht) - 4.695	0.908
<i>Quercus marilandica</i>	LA = 1.787*(ht) + 0.183	0.816	Bio = 1.997*(ht) - 4.775	0.917
<i>Rhexia alifanus</i>	LA = 1.841*(ht) - 3.348	0.915	Bio = 1.989*(ht) - 8.521	0.941
<i>Robinia hispida</i>	LA = 1.743*(ht) - 0.336	0.752	Bio = 1.419*(ht) - 3.667	0.675
<i>Sassafras albidum</i>	LA = 1.635*(ht) + 0.271	0.927	Bio = 1.984*(ht) - 5.422	0.941
<i>Tephrosia virginiana</i>	LA = 1.604*(ht) - 1.266	0.549	Bio = 1.729*(ht) - 6.213	0.636
<i>Vaccinium formosum</i>	LA = 1.197*(ht) + 0.816	0.755	Bio = 1.454*(ht) - 4.317	0.835
<i>Vaccinium fuscatum</i>	LA = 2.018*(ht) - 2.3026	0.867	Bio = 2.290*(ht) - 7.389	0.936