STORED CARBON DECOUPLES SOIL CO_2 EFFLUX IN CHRONICALLY DISTURBED ECOSYSTEMS

by

DOUGLAS PATRICK AUBREY

(Under the Direction of ROBERT O. TESKEY)

ABSTRACT

The tight coupling of belowground autotrophic respiration with the availability of recently assimilated carbon, and its contribution towards soil CO_2 efflux, is an emerging paradigm in the ecophysiological literature. Here, we test the hypothesis that stored carbohydrates decouple the direct linkage between canopy photosynthesis and soil CO_2 efflux. Using longleaf pine (*Pinus palustris* L.), a species with an evolutionary history of frequent disturbance and large reserves of belowground stored carbon, we experimentally manipulated the transfer of recently assimilated carbon belowground. The first experiment temporarily disrupted the transfer of recently assimilated carbon belowground via canopy scorching, whereas the second experiment permanently terminated the transfer of recently assimilated carbon belowground via stem girdling and root trenching. Canopy photosynthesis was reduced to 20% of that in the control treatment immediately after the scorch treatment was applied; however, recovery occurred surprisingly rapidly, reaching 90% of that in the control treatment within one month. Despite the immediate reduction in canopy photosynthesis, soil CO_2 efflux was not impacted by the scorch treatment. Similarly, the scorch treatment did not impact fine root production, mortality, or standing crop. Fine root N concentrations were not diminished by scorching, but we observed some evidence that TNC concentrations in 3rd and 5th order roots decreased as TNC was presumably reallocated to maintain more metabolically active 1st order roots. Soil CO_2 efflux was not influenced by girdling or trenching treatments. Sugar and starch concentrations were impacted by girdling and trenching treatments, but depended on root type and temporal period. For example, sugar and starch concentrations of root orders 1-3 were not impacted by photosynthate exclusion treatments, but concentrations of root orders 4-6 and lateral roots decreased after treatment application suggesting that sugar and starch were mobilized from larger roots to maintain smaller, more metabolically active roots. Our results indicate that large belowground carbon reserves decouple the linkage between canopy photosynthesis and soil CO_2 efflux.

INDEX WORDS: Autotrophic respiration, chronic disturbance, evolutionary history, photosynthate assimilation, photosynthesis, *Pinus palustris*, soil CO_2 efflux, total non-structural carbohydrates, root nitrogen

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INTRODUCTION AND LITERATURE REVIEW

Two studies were conducted to test the hypothesis that stored carbohydrates decouple the direct linkage between current photosynthate assimilation and soil CO_2 efflux. These studies provide information to improve our basic understanding of the patterns and controls on soil CO_2 efflux—a major, yet poorly understood, component of the terrestrial carbon cycle. Both studies used longleaf pine (*Pinus palustris* L.)—a species with an evolutionary history of chronic disturbance and large reserves of belowground stored carbon—and experimentally manipulated the transfer of recently assimilated carbon belowground. The first experiment temporarily terminated the transfer of recently assimilated carbon belowground via canopy scorching and examined the impact on canopy photosynthesis, soil CO_2 efflux, fine root production, mortality, and nitrogen (N) and non-structural carbohydrate (sugar and starch) concentrations. The second experiment permanently terminated the transfer of recently assimilated carbon belowground via stem girdling and root trenching and examined the impact on soil CO_2 efflux and root N and non-structural carbohydrate concentrations.

Soil CO_2 Efflux

Forest ecosystems occupy approximately 42×10^{12} m^2 of earth’s land surface and account for the majority of terrestrial net primary productivity which makes them a major focus in global carbon budgets (Jobbágy & Jackson 2000; Geider *et al.* 2001). Ecosystem respiration (R_E) consumes approximately 77-85% of annual gross primary productivity in forest ecosystems across the globe (Law *et al.* 2002; Luyssaert *et al.* 2007; Baldocchi 2008), thus releasing the largest annual terrestrial CO_2 flux to the atmosphere—nearly 16 times that of annual fossil fuel combustion from 2000-2005 (Prentice 2001; IPCC 2007). Current estimates indicate soil CO_2 efflux contributes up to 88% of annual R_E (Davidson *et al.* 2006). A mechanistic understanding of R_E, and hence, soil CO_2 efflux, is critical to address questions related to interactions between the global carbon cycle and the climate system. However, soil CO_2 efflux is not a single respiratory process, but rather the sum of contributing respiratory processes utilizing different substrates.

Soil CO_2 efflux can be partitioned into autotrophic and heterotrophic sources based on the substrate utilized for metabolism. Substrate for all belowground respiratory processes originates primarily from autotrophic production in the form of aboveground plant litter, belowground root turnover, root exudates, recently assimilated carbon (recent photosynthate), and stored non-structural carbon (sugars and starch). The only substrate not entirely derived from autotrophic activity is soil organic matter which, in addition to autotrophic litter, consists of microbial and fungal biomass. Based on the substrate used for metabolism, we may consider the combined contributions of root and root-associated organisms as autotrophic respiration (R_A) and the contribution of non-root associated microorganisms as heterotrophic respiration (R_H). Thus, from a substrate perspective, R_A relies on carbohydrate production and storage whereas R_H relies mainly on detrital carbon pools, and to a lesser extent, root exudates. Whereas R_H is mainly influenced by abiotic factors, such as soil temperature and moisture, R_A appears more dependent on substrate availability. Thus, the tight coupling of root respiration with canopy photosynthesis has emerged as a contemporary hypothesis in the ecophysiological literature.

Coupling of Soil CO_2 Efflux with Canopy Photosynthesis

Isotopic labeling studies have provided evidence that soil CO_2 efflux and recent photosynthate are tightly coupled in many tree species. For example, a two day time lag between canopy assimilation of $^{14}CO_2$ and appearance of the label in soil CO_2 efflux has been observed in poplar (*Populus euramericana* cv. Eugeneii) (Horwath, Pregitzer & Paul 1994) and spruce (Carbone *et al.* 2007). Depletion of ^{13}C in soil CO_2 efflux and soil CO_2 was observed within one week following fumigation of loblolly pine (*Pinus taeda* L.) with $^{13}CO_2$ suggesting that the CO2 was derived from respiration of recent photosynthate (Andrews *et al.* 1999). Variations in natural abundance of carbon isotopes have suggested time lags of 1-4 days between canopy assimilation and soil CO_2 efflux (Ekblad & Högberg 2001; Ekblad *et al.* 2005). The carbon isotopic signature of R_E also suggests a tight coupling of canopy assimilation and soil CO_2 efflux (Hemming *et al.* 2005; Lai *et al.* 2005; Tang & Baldocchi 2005). Complimentary evidence has been derived from manipulative studies that terminate the transport of current photosynthate to root systems (e.g., girdling). Withholding current photosynthate from root systems should cause R_A to decline and the reduction should be proportional to the dependence of roots on current photosynthate. Many of these reports suggest soil CO_2 efflux is reduced within a week of girdling (Högberg *et al.* 2001; Frey, Hagedorn & Giudici 2006; Johnsen *et al.* 2007). Thus, time lags reported from both isotopic labeling and manipulative studies suggest that current photosynthate is used as substrate for belowground metabolism within one week.

Although evidence suggesting a tight coupling between canopy C assimilation and soil metabolic activity is growing, results from other manipulative studies challenge the universality of such a linkage across species and seasons. Species may respond differently to girdling even when girdling occurs during the same season and at the same site. For example, soil CO_2 efflux was reduced immediately after girdling European beech (*Fagus sylvatica* L.) in August but not until six weeks after girdling Norway spruce (*Picea abies* (L.) Karst.; Andersen *et al.* 2005). Interestingly, Olsson et al. (2005) reported soil CO_2 efflux of Norway spruce was reduced 27% within one week of June girdling. Soil CO_2 efflux was not influenced over two years following girdling of tulip poplar (*Liriodendron tulipifera* L.; Edwards & Ross-Todd 1979) or after defoliating hybrid poplar (*Populus* × *Canadensis* cv. Eugeneii; Kosola *et al.* 2001). Likewise, soil CO_2 efflux was not influenced one week after girdling a Norway spruce stand but was reduced 31% after one month and 57% after two months of girdling (Subke *et al.* 2004). Thus, girdling may not immediately (i.e., within the timeframe expected for recent canopy assimilates to cycle through the roots and diffuse through the soil into the atmosphere) result in decreased R_A. In the few studies where girdling has been applied during different seasons, different responses in soil CO_2 efflux have been observed. For example, soil CO_2 efflux was reduced 27% five days after June girdling of Scots pine (*Pinus sylvestris* L.) and 37% within five days after August girdling (Högberg *et al.* 2001). Soil CO_2 efflux was reduced 30% five days after autumn girdling of loblolly pine but no reductions were observed after spring girdling (Johnsen *et al.* 2007). Thus, the seasonal timing of girdling influences the observed lag time between canopy assimilation and soil CO_2 efflux and influences the magnitude of soil CO_2 efflux reduction. Thus, the linkage between canopy C assimilation and belowground utilization does not necessarily hold across species or within a species across seasons.

Importance of Stored Carbon for Root Respiration

The capacity of roots to maintain respiration rates following the termination of current photosynthate transport belowground highlights the importance of non-structural carbohydrates for root metabolism and provides insight into linkages between canopy photosynthesis and belowground utilization of carbohydrates. Non-structural carbohydrates are stored in the root system and represent available carbon capital that can be used for growth, repair, or maintenance (Chapin, Schulze & Mooney 1990; Le Roux *et al.* 2001). Relatively little is known about the use and turnover of non-structural carbohydrates (Körner 2003), but they likely buffer root systems against variable climate conditions (Trumbore 2006) or may perhaps be related to recovery from disturbance. Storage pools have commonly been considered a sink of low priority (Le Roux *et al.* 2001), but non-structural carbohydrates may accumulate at the expense of growth to maintain a given level of metabolic demand (Silpi *et al.* 2007). The non-structural carbohydrate content in root systems represents locally available carbohydrates for metabolism. From this perspective, it is possible that current photosynthate supply is a buffering mechanism to maintain basal non-structural carbohydrate content rather than considering non-structural carbohydrates as a buffering mechanism to maintain belowground metabolism during periods of low photosynthate supply.

There is likely a seasonal pattern which determines the substrate used for root metabolism. For example, periods of shoot and root growth should alternate throughout the growing season if a functional equilibrium is maintained between above- and belowground tree growth. Thus, we should also expect that seasonal demands for current photosynthate alternate between shoots and roots in accordance with growth periodicity. The majority of aboveground growth generally occurs during late spring and early summer. A concurrent decline in root growth during periods of shoot growth has been observed in various species (Reich *et al.* 1980; Willaume & Pagès 2006), including loblolly pine (Drew & Ledig 1980). During the period of rapid aboveground growth, most current photosynthate should be allocated to fuel aboveground growth and maintenance respiration with little or no current photosynthate transported belowground for root growth or maintenance. Therefore, we expect that root respiration should not rely on current photosynthate during periods of aboveground growth but will begin to rely on current photosynthate when aboveground growth slows in mid to late summer. This suspected seasonal pattern of R_A dependence on current photosynthate is supported by the different responses to girdling of Scots (Högberg *et al.* 2001) and loblolly pine (Johnsen *et al.* 2007) early and late in the growing season. This also suggests that R_A may be proportional to the amount of non-structural carbohydrates available at any given time.

Chapter Overview

Chapters 2 and 3 describe experiments which tested the hypothesis that stored carbohydrates decouple the direct linkage between photosynthate assimilation and soil CO_2 efflux in frequently disturbed ecosystems. The experiment described in chapter 2 temporarily disrupted the transfer of recently assimilated carbon belowground via canopy scorching and examined the impact on canopy photosynthesis, soil CO_2 efflux, fine root production and mortality, and nitrogen (N) and non-structural carbohydrate (sugar and starch) concentrations. The experiment described in chapter 3 permanently terminated the transfer of recently assimilated carbon belowground via stem girdling and trenching and examined the impact on soil CO_2 efflux and root N and non-structural carbohydrate concentrations. Chapter 4 synthesizes the major findings of the experiments described in chapters 2 and 3.

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CHAPTER 2

STORED CARBON DECOUPLES SOIL CO_2 EFFLUX FROM CANOPY PHOTOSYNTHESIS IN CHRONICALLY DISTURBED ECOSYSTEMS: IMPACT OF TEMPORARILY TERMINATING THE TRANSFER OF RECENTLY ASSIMILATED CARBON BELOWGROUND VIA CANOPY SCORCHING OF LONGLEAF PINE (*PINUS PALUSTRIS* L.)[1]

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Abstract

A tight linkage between belowground autotrophic respiration and recently assimilated carbon is an emerging paradigm in the ecophysiological literature. Here, we test the hypothesis that stored carbohydrates decouple the direct linkage between canopy photosynthesis and root respiration. Using longleaf pine (*Pinus palustris* L.), a species with an evolutionary history of frequent disturbance and large reserves of belowground stored carbon, we experimentally manipulated foliar leaf area and thus, canopy photosynthesis, via foliar scorching over two consecutive growing seasons following development of first-year needles. We monitored the impact of scorching on canopy photosynthesis, soil CO_2 efflux, fine root production, mortality, standing crop, nitrogen (N) concentration, and total nonstructural carbohydrate (TNC) concentration. Canopy photosynthesis was reduced to 20% of that in the control treatment immediately after the scorch treatment was applied; however, recovery occurred surprisingly rapidly, reaching 90% of that in the control treatment within one month. Despite the immediate reduction in canopy photosynthesis, soil CO_2 efflux was not impacted by the scorch treatment. Similarly, the scorch treatment did not impact fine root production, mortality, or standing crop. Fine root N concentrations were not diminished by scorching, but we observed some evidence that TNC concentrations in 3rd and 5th order roots decreased as TNC was presumably reallocated to maintain more metabolically active 1st order roots. Although the scorch treatment only temporarily terminated the belowground supply of photosynthate, the process of leaf area reconstruction represented an additional drain on stored carbon pools and some of this carbon may have been mobilized from belowground. However, lack of belowground response suggests that stored carbon of dominant plant species in frequently disturbed ecosystems may decouple the direct linkage between canopy photosynthesis and soil CO_2 efflux.

Introduction

Soil CO_2 efflux is the largest terrestrial source of carbon dioxide (CO_2) to the atmosphere (Subke, Inglima & Cotrufo 2006), yet the mechanistic controls of this important flux are currently under debate (Kuzyakov & Cheng 2001; Trumbore 2006; Vargas *et al.* 2010). A major difficulty in discerning these mechanisms is that soil CO_2 efflux is the product of two processes, belowground autotrophic and heterotrophic respiration, that are difficult to distinguish (Ryan & Law 2005; Baggs 2006; Kuzyakov 2006; Trumbore 2006), and are likely to be regulated differently (Carbone, Winston & Trumbore 2008). Multiple regulatory factors, such as temperature, water, nitrogen (N) availability, soil organic matter quality and quantity, and canopy photosynthesis have been correlated spatially and temporally with soil CO_2 efflux (Högberg *et al.* 2001; Seneviratne 2002; Ryan & Law 2005; Sulzman *et al.* 2005; Bahn *et al.* 2008; Högberg *et al.* 2008; Zhou, Talley & Luo 2009). Moreover, potential time lags between the influence of the regulatory drivers and detectable ecosystem responses increase the difficulty of discerning the mechanistic controls of soil CO_2 efflux (Baldocchi, Tang & Xu 2006; Stoy *et al.* 2007; Kuzyakov & Gavrichkova 2010; Mencuccini & Hölttä 2010).

The heterotrophic component of soil CO_2 efflux, which relies primarily upon soil organic matter as substrate, has received more attention than the autotrophic component, due in part to the importance of decomposition in nutrient mineralization (Aber *et al.* 1991; Högberg & Read 2006). However, a literature review (Hanson *et al.* 2000) and meta-analyses (Bond-Lamberty, Wang & Gower 2004; Subke, Inglima & Cotrufo 2006) suggest autotrophic respiration via roots and their associated mycorrhizal fungi account for, on average, *c.* 50% of soil CO_2 efflux in forest ecosystems. In addition, many studies have indicated that rates of belowground autotrophic respiration are directly related to—and dependent on—the availability of recently assimilated carbon (Ekblad & Högberg 2001; Högberg *et al.* 2001; Steinmann *et al.* 2004; Göttlicher *et al.* 2006; Keel, Siegwolf & Körner 2006; Carbone *et al.* 2007; Högberg *et al.* 2008; Plain *et al.* 2009; Bader & Körner 2010). Despite the growing body of literature that autotrophic respiration via roots and their associated mycorrhizal fungi is regulated by the availability of recent photosynthate (Högberg & Read 2006), data to the contrary can be found (Edwards & Ross-Todd 1979; Kosola *et al.* 2001; Guo, Mitchell & Hendricks 2004; Vargas, Trumbore & Allen 2009). Guo, Mitchell & Hendricks (2004) noted that much of the conflicting data were collected in ecosystems prone to disruptions in canopy photosynthesis via factors such as foliar scorching and insect defoliation. In turn, they hypothesized that the disturbance regime and corresponding selective advantage for carbon storage (i.e., total non-structural carbohydrate; hereafter referred to as TNC) among the dominant species of the ecosystem may impact the extent that soil CO_2 efflux is coupled with canopy photosynthesis.

Longleaf pine (*Pinus palustris* L.) ecosystems have an evolutionary history of chronic disturbance via burning with fires returning as frequently as every 1-3 years (Mitchell *et al.* 2006). Mature trees commonly withstand crown scorch consuming up to 100% of the foliage (Guo *et al.* 2008). Using longleaf pine (*Pinus palustris* L.) as a model for species with an evolutionary history of chronic disturbance, we experimentally manipulated foliar leaf area and thus, canopy photosynthesis, via foliar scorching over two consecutive growing seasons and monitored the impact of scorching on canopy photosynthesis, soil CO_2 efflux, and a variety of fine root indices. Our approach addresses the question: Will a reduction in current photosynthate assimilation produce a concomitant reduction in soil CO_2 efflux in a chronically disturbed ecosystem, or does stored carbon buffer the effect of disturbance and decouple the direct linkage between canopy photosynthesis and soil CO_2 efflux?

Materials and Methods

Study Site and Experimental Treatments

This study was conducted in a 50 ha longleaf pine (*Pinus palustris* L.) plantation located at the Joseph W. Jones Ecological Research Center in Newton, GA, USA (31º15 N latitude and 84º30 W longitude). The plantation was established in 1980 using an approximate 1.5 × 2 m planting grid, and longleaf pine was the exclusive overstory species (mean dbh = 16.9 cm and mean height = 14.3 m at the beginning of the experiment). The soil in the plantation is a Typic Quartzipsamment characterized by coarse sand that exceeds 2.5 m in depth, weak development of horizons due to mixing by fauna, low organic matter content, and lack of silt and clay (Goebel *et al.* 2001). The climate for this region has been characterized as humid subtropical with an average annual precipitation of 1310 mm distributed evenly throughout the year, and mean annual low and high temperatures of 9 and 28°C, respectively.

Foliar scorching (*c.* 80% needle scorch) was conducted in four 20 × 20 m plots. There were also four equivalent control plots. To reduce potential edge effects, sample collections were confined to the central 15 × 15 m subplot within each treatment plot, and plots were separated by at least a 20 m buffer zone. An 18 m hydraulic lift was used to access the canopy, and a hand-held torch connected to a propane tank was used to scorch the foliage for approximately 10 sec per needle flush. In the summers of 2006 and 2007, following the development of first year needle production, approximately 80% of the foliage (i.e., all needles except those on the apical branch of each tree) was killed on each tree in the scorch treatment plots. Following the scorch treatment, needles rapidly senesced, but most needles remained connected to the branches. Although we did not measure leaf elongation, needles of the post-scorch flush had nearly fully elongated within one month of applying the scorch treatment.

Xylem Sap Flux and Canopy Photosynthesis

Xylem sap flux was estimated with 30 mm Granier-type thermal dissipation probes (Granier 1985; Granier 1987) prior to and following recovery of the canopy from the first scorch treatment in 2006, but not the second scorch treatment in 2007. One thermal dissipation probe was installed at a height of 1.4 m on both the north and east side of two trees in three replicate plots of each treatment. Campbell CR10X and CR23X dataloggers measured the sensors every 30 sec and stored 15 min averages (Campbell Scientific Inc., Logan, Utah, USA). An on-site weather station recorded meteorological variables at 15 min intervals in synchrony with thermal dissipation measurements. Output from the two thermal dissipation probes was averaged per tree. Xylem sap flux density (J_s, g H_2O m^{-2} sapwood s^{-1}) was calculated after Granier, Huc & Colin (1992) and transpiration per unit leaf area (E_l, mmol H_2O m^{-2} leaf area s^{-1}) was calculated by multiplying J_s by the ratio of leaf area to sapwood area. Leaf area per tree was estimated by destructive harvesting (i.e., allometric relationships were used to predict leaf area as a function of dbh). Sapwood area was assumed to be the outer 30 mm band of xylem. This likely underestimated the conducting sapwood area somewhat, although the outer 20 mm has been shown to contain the area of maximum sap conductance (Cohen, Kelliher & Black 1985) which diminishes rapidly with depth in pine (Ford *et al.* 2004). Canopy photosynthetic rates were calculated from stomatal conductance (after Forrester, Collopy & Morris 2010) using the hybrid model of Katul, Ellsworth & Lai (2000). To determine the impact of scorching, photosynthetic rates of scorched trees were monitored through time and standardized by the calculated photosynthetic rates of control trees.

Soil CO_2 Efflux

Soil CO_2 efflux was measured monthly in 2006 and 2007 using a LI-6400-09 soil chamber and LI-6400XT portable photosynthesis system (LI-Cor Inc., Lincoln Nebraska, USA). Measurements were collected at 5 PVC soil collars (10.16 cm wide × 4.4 cm high inserted to a soil depth of 2.2 cm) randomly located in the center of each plot. Soil CO_2 efflux was measured between 11:00 and 14:00 h to standardize maximum daily rates. Soil temperature was measured concurrently at each sample location to a 5 cm depth using a LI-6000-09TC soil probe thermocouple (LI-Cor Inc., Lincoln, Nebraska, USA).

Fine Root Indices

Fine root (< 2 mm diameter) production and mortality estimates were measured using the minirhizotron approach described by Guo *et al.* (2008). Acrylic tubes were installed at a 45^o angle to a depth of 50 cm at three randomly located positions near the center of each plot. Each month, root images in numbered cells along each tube were recorded with digital photographs and analyzed with the software package RooTracker (Ver. 2.0, David C. Tremmel, Duke University Phytotron, Durham, NC). The longleaf plantation canopy had closed and the understory consisted of pine needles (i.e., only pine roots were captured in root images).

Fine root standing crop and N concentrations were assessed post-scorch (one month after treatment) in 2006 and 2007 using an intact soil monolith approach (Guo, Mitchell & Hendricks 2004). Three soil monoliths were collected in each plot by manually inserting a 30 cm l × 20 cm w × 30 cm d metal core. Extracted monoliths were placed on a two-layer sieve box to separate roots from soil. The top sieve had a 12.7 mm metal mesh and the bottom sieve had a 6.35 mm metal mesh. Once the soil had passed through the two-layer sieve, it was then passed through a 2 mm metal mesh sieve to remove fine roots that were not collected by the two-layer sieve box.

The collected roots were placed in plastic bags in a cooler for transport to the laboratory. In the laboratory, roots were sorted by branch order (Guo, Mitchell & Hendricks 2004) and oven-dried at 70°C to a constant mass. Root samples were then weighed, ground, and homogenized using a SPEX 8000-D mixer mill (SPEX, Edison, NJ). To determine N concentrations, sub-samples were analyzed on a FlashEA 1112 Series NC Soil Analyzer (Thermo Electron Corp., Waltham, MA).

Root samples for TNC assessments were collected and processed following the approach described by Guo, Mitchell & Hendricks (2004). Root tissues were analyzed for TNC in a two-step process to determine both sugar and starch concentrations (i.e., TNC concentrations were calculated as the sum of starch and sugar concentrations). Dried root samples were weighed then extracted overnight in 10 ml of 80% ethanol (v/v) followed by centrifuging at 2200 rpm for 15 min. The resulting supernatant was transferred to a 50 ml volumetric flask. The residual pellet was extracted again with 5 ml of 80% ethanol and centrifuged at 2200 rpm for 5 min. The supernatant was composited with that from the first extraction, diluted to 50 ml with 80% ethanol, and analyzed for sugars. The residue from the ethanol extractions was transferred to a glass tube, dried, and then boiled for three hours in 5 ml 3% HCl (w/w). The filtrate was then diluted to 50 ml with 3% HCl and analyzed for starch.

A modified phenol-sulfuric acid method was used for both sugar and starch analyses (Buysse & Merckx 1993). In a glass tube, 1 ml of solution was combined with 1 ml of 28% phenol in 400 ml of 80% ethanol and 5 ml of concentrated sulphuric acid. The tube was shaken for 1 min and allowed to stand for 15 min. Finally, absorption was measured at 490 nm on a Shimadzu UV-2101PC spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) and concentrations determined by standard curves.

Statistical Analyses

We analyzed the impact of the scorch treatment on canopy photosynthesis and recovery using a linear regression with time since scorching as the independent variable and scorch treatment photosynthetic rates expressed as a percent of control plot photosynthetic rates as the dependent variable. We analyzed soil CO_2 efflux, fine root standing crop, production, mortality, and N and TNC concentrations using a completely randomized multi-factorial repeated measures design. The experimental unit (plot) was treated as the random subject factor in all repeated measures analyses. Scorch treatment (n = 2) was treated as a fixed factor and year (n = 2) was treated as the fixed repeated factor when analyzing fine root standing crop, production, and mortality. A similar model was used for soil CO_2 efflux, but monthly sampling period (n = 19) was treated as the fixed repeated factor. Treatment effects on cumulative soil CO_2 efflux at the end of our observation period (i.e., December 2007) were analyzed using a one-way ANOVA with plot as a random factor. Root order (n = 3) was included as an additional fixed factor in the N and TNC concentration analyses. Fine root production, mortality, and N and TNC concentrations were proportional values and presented as such throughout the report; however, data were arcsine square root transformed to satisfy ANOVA assumptions (Zar 1996). Denominator degrees of freedom were estimated according to the Kenward-Roger method (Kenward & Roger 1997). Treatment means were compared using Fisher’s Least Significant Difference (LSD) test. When interactions occurred, we performed tests of simple main effects (Schabenberger, Gregoire & Kong 2000; Littell *et al.* 2006). All repeated measures analyses and the one-way ANOVA were performed using a mixed model procedure (PROC MIXED; Version 9.1.3, SAS Inc., Cary, NC, USA) with a type-I error rate of 0.05. Data presented in the text are means ± standard error.

Results

Canopy Photosynthesis and Soil CO_2 Efflux

Canopy photosynthesis was reduced to 20% of that in the control treatment immediately after the scorch treatment was applied; however, recovery occurred surprisingly rapidly reaching 90% of that in the control treatment within one month (Fig. 2.1). Prior to scorching, the average maximum canopy photosynthetic rate was 16.05 ± 4.33 μmol CO_2 m^{-2} sec^{-1} with an average reduction to 4.4 ± 1.43 μmol CO_2 m^{-2} sec^{-1} the day immediately following the scorch treatment application. Canopy photosynthesis recovered steadily in the scorch treatment, taking an average of 29.5 ± 4.5 days to reach 90% of control treatment assimilation rates.

Soil CO_2 efflux was not impacted by the scorch treatment ($P = 0.2254$) and seasonal dynamics ($P < 0.\ 0001$) were independent of treatment (i.e., no treatment × time interaction, Fig. 2.2). In 2006, soil CO_2 efflux rates increased from 2.0 μmol CO_2 m^{-2} sec^{-1} in May, to a peak of slightly more than 3.0 μmol CO_2 m^{-2} sec^{-1} in August, followed by a steady decline to less than 1.0 μmol CO_2 m^{-2} sec^{-1} in December. A similar pattern was observed in 2007 with maximum annual soil CO_2 efflux rates occurring in August followed by a steady decline through the remainder of the year. The scorch treatment was applied just after peak annual soil CO_2 efflux rates in 2006 and the subsequent flux rates in control and scorch treatments declined in a comparable manner. In 2007, the scorch treatment preceded the maximum annual soil CO_2 efflux rates, and the post-scorch efflux in both the control and scorch treatments increased to a peak in August (3.12 and 2.82 μmol CO_2 m^{-2} sec^{-1} for the control and scorch treatments, respectively) before falling consistently to the annual lows in December (0.84 and 0.72 μmol CO_2 m^{-2} sec^{-1} for control and scorch treatments, respectively). At the end of our observation period, the cumulative soil CO_2 efflux in the scorch treatment was statistically similar to the cumulative efflux in the control treatment ($P = 0.3838$, Fig. 2.3).

Fine Root Indices

The scorch treatment did not affect fine root production, mortality, or standing crop. Although both fine root production and mortality were higher in the scorch than the control treatment ($P = 0.0056$ and $P = 0.0414$, respectively), differences were evident prior to treatment application and seasonal dynamics (both $P < 0.0001$) were independent of treatment (i.e., no treatment × time interaction; Figs. 2.4a & 2.4b). Thus, the scorch treatment did not impact fine root production or mortality. Similarly, fine root standing crop was not influenced by the scorch treatment ($P = 0.3878$) and remained relatively stable between 2006 and 2007 ($P = 0.2019$). Averaged across all plots and both years, fine root standing crop was 1.66 ± 0.12 Mg ha^{-1}.

Fine root N concentrations varied significantly by root order in 2006, but not in 2007 (i.e., root order × year interaction, $P = 0.0043$), and were not influenced by the scorch treatment ($P = 0.7772$, data not presented). Regardless of treatment or year, fine root TNC concentrations varied significantly among root orders ($P < 0.0001$) as higher order roots exhibited higher TNC concentrations (Table 2.1). Fine root TNC concentrations were significantly influenced by the scorch treatment, but the effect was dependent upon root order and year (i.e., root order × scorch treatment × year interaction, $P = 0.0135$, Table 2.1). For example, TNC concentrations in the larger 3rd and 5th order roots were lower in the scorch treatment compared to the control treatment in 2007, but not in 2006. However, TNC concentrations of smaller and more metabolically active 1st order roots were similar in control and scorch treatments in 2007, but lower in the scorch treatment in 2006.

Estimates of Stored Carbon

To estimate the capacity for stored carbon to decouple the linkage between belowground autotrophic respiration and photosynthate assimilation, we assumed coarse root biomass to be 12% of the aboveground biomass (Nadelhoffer & Raich 1992). Aboveground biomass was estimated using site-specific allometric equations (unpublished data) that predicted stem and branch mass as a function of dbh. In addition, supplemental sampling showed that higher order lateral roots (8th order) were similar in TNC concentration to that of 5th order roots (i.e., 26% of dry weight; Table 2.1). Based on these assumptions, we estimated a belowground carbon storage pool of 125 g C m^{-2} at this plantation. If autotrophic respiration is assumed to account for 50% of total soil CO_2 efflux (Hanson *et al.* 2000), which was approximately 500 g C m^{-2} yr^{-1} in this plantation, then the belowground (both coarse and fine root) carbon storage pool represents roughly one-half of the annual belowground autotrophic respiration demand in this ecosystem. This estimate is likely conservative given the large tap and coarse root structure of longleaf pine (Hendricks *et al.* 2006).

Discussion

Recent studies have highlighted the direct linkage between canopy photosynthesis and belowground autotrophic respiration, which is a major constituent of soil CO_2 efflux in terrestrial ecosystems (Andrews *et al.* 1999; Steinmann *et al.* 2004; Körner *et al.* 2005; Keel, Siegwolf & Körner 2006; Carbone *et al.* 2007; Högberg *et al.* 2008; Plain *et al.* 2009; Bader & Körner 2010). Our results indicate that multiple canopy scorches, which initially reduced canopy photosynthesis by 80% (Fig. 2.1), had negligible and statistically non-significant impacts on soil CO_2 efflux (Figs. 2.2 and 2.3) in a system where the dominant species has an evolutionary history of chronic disturbance. Although we did not directly measure belowground autotrophic respiration in this study, previous work has shown that the autotrophic component of soil CO_2 efflux rivals that of the heterotrophic component at this site (Cheng *et al.* 2005). The sandy soil and low soil organic matter content further suggest that belowground autotrophic respiration represents a large portion of soil CO_2 efflux. Therefore, the autotrophic contribution to soil CO_2 efflux should have been more than adequate to detect a change in soil CO_2 efflux that resulted from only the autotrophic component following the termination of recent photosynthate belowground. During the first month after each scorch event, soil CO_2 efflux rates were comparable to those in the control treatment (Fig. 2.2). Similarly, the cumulative soil CO_2 flux after two scorch treatments remained comparable to that of the control treatment (Fig. 2.3). Furthermore, the scorch treatment did not influence fine root production and mortality (Fig. 2.4), or standing crop. Similarly, fine root N concentration, a surrogate for enzyme concentration and respiration rates (Ryan *et al.* 1996), was not impacted by scorch treatments. Associated assessments conducted in this and previous studies (Guo, Mitchell & Hendricks 2004; Sims *et al.* 2007; Guo *et al.* 2008) have indicated that key drivers of belowground autotrophic respiration were not significantly impacted by canopy scorching in this system. For example, in the same study plots, Sims *et al.* (2007) reported that production of mycorrhizal fungi, which are functional extensions of fine roots, were not impacted by scorch treatments.

A key dependent variable that was significantly affected by scorching was the TNC concentration of higher order roots (Table 2.1). Consistent with the findings of Guo, Mitchell & Hendricks (2004), following canopy scorching in 2007, the TNC concentration of higher order roots decreased while the TNC concentration of the lower order roots remained relatively stable. A proposed explanation for this pattern is that TNC in the higher order roots (i.e., larger diameter, secondary roots that presumably serve a carbon storage function) were transferred to the more distal lower order roots (i.e., smaller diameter, primary roots that function in concert with mycorrhizal fungi in resource uptake and assimilation) to help maintain the higher metabolic rates of feeder roots (Guo, Mitchell & Hendricks 2004; Varner *et al.* 2009; O'Brien 2010). Thus, TNC mobilized from higher order roots to maintain the relatively high metabolic activity and respiration rates of lower order roots and their mycorrhizal fungi may effectively decouple the direct linkage between canopy photosynthesis and belowground autotrophic respiration following canopy disturbance. However, the results from our study are not definitive as we did not observe a similar pattern in 2006. Rather, TNC concentrations of higher order roots were not affected by the scorch treatment, whereas TNC concentration of lower order roots decreased in response to the scorch treatment. It is not entirely clear why we did not observe the same response after each scorching event. However, it may be possible that TNC concentrations of 1st order roots were not reduced beyond a threshold in 2006 that would have required mobilization of TNC from higher root orders. Thus, even though TNC concentrations of 1st order roots were statistically lower in the scorch treatment in 2006, they may not have been low enough to influence metabolic activity (which is suggested by the lack of soil CO_2 efflux response) or require replenishment via mobilization from higher order roots.

The utilization of TNC to support belowground autotrophic growth and respiration is supported by results from other field studies. A ^{13}C tracer study conducted in a regenerating scrub oak ecosystem prone to frequent fires indicated that 33% of carbon in newly established roots originated from a source other than recent photosynthate (Langley, Drake & Hungate 2002). Similarly, a ^{14}C labeling experiment and associated studies (Czimczik *et al.* 2006; Schuur & Trumbore 2006; Carbone *et al.* 2007) in a black spruce (*Picea mariana*) forest, estimated the predominant fraction of TNC supporting root respiration was *c.* 3-5 years old. In addition, following complete denudation of leaf biomass by hurricane force winds in a tropical forest, new roots were produced using TNC reserves that had been stored 2 to 10 years prior to the disturbance (Vargas, Trumbore & Allen 2009). Moreover, Edwards and Ross-Todd (1979) indicated that girdling of tulip poplar (*Liriodendron tulipifera*) trees had no effect on soil CO_2 efflux over two years following the treatment. While these studies did not directly demonstrate mobilization of TNC from higher to lower order roots, the mechanism was clearly demonstrated by Guo, Mitchell & Hendricks (2004).

The findings in this and other studies indicating that stored carbon may buffer belowground autotrophic respiration from fluctuations in canopy photosynthesis contrast with results from a number of girdling studies. For example, Högberg *et al.* (2001) observed a 27% decline in soil CO_2 efflux within five days of girdling Scots pine (*P. sylvestris*) in the early summer and a 37% decline within five days of girdling in the late summer. In both instances, soil CO_2 efflux rates were approximately 50% lower than the corresponding control treatment by the end of the growing season. Similar results have been found when girdling European chestnut (*Castanea sativa* Mill.; Frey, Hagedorn & Giudici 2006), Norway spruce (*Picea abies* (L.) Karst.; Olsson *et al.* 2005), and European beech (*Fagus sylvatica* L.; Andersen *et al.* 2005). That girdling has resulted in relatively rapid and dramatic decreases in soil CO_2 efflux does indeed suggest a tight coupling of belowground autotrophic respiration with the availability of photosynthate; however, stored carbon appears to influence the strength of the coupling.

Although scorching and girdling influence the belowground availability of recent photosynthate in very different ways, we argue that life history traits related to ecosystem disturbance regimes and thus, allocation towards carbon storage, also influence the linkage between canopy photosynthesis and soil CO_2 efflux. Guo, Mitchell & Hendricks (2004) hypothesized that the disparate results regarding the linkage between canopy photosynthesis and soil CO_2 efflux may be reconciled, at least in part, by considering the evolutionary history of a species with respect to disturbance regimes. For example, in forests with infrequent but catastrophic disturbances, where dominant individuals are destroyed and ecosystem recovery occurs via regeneration of new individuals from seed, extensive carbon storage would not confer a selective advantage and soil CO_2 efflux would be more tightly coupled with canopy photosynthesis. In contrast, in forests with frequent but less intense disturbances, where dominant individuals generally survive and repair damaged tissues or regenerate from root sprouts, carbon storage confers a selective advantage and may decouple the linkage between canopy photosynthesis and soil CO_2 efflux (Kosola *et al.* 2001; Langley, Drake & Hungate 2002; Guo, Mitchell & Hendricks 2004; Sims *et al.* 2007; Vargas, Trumbore & Allen 2009; Varner *et al.* 2009; Clarke, Lawes & Midgley 2010; O'Brien 2010).

Just as the frequency and intensity of disturbances to photosynthate assimilation may vary widely among ecosystems, stored carbon and buffering responses to these disturbances may also vary widely among species. For example, girdling in a Norway spruce (Subke *et al.* 2004) stand had no effect on soil CO_2 efflux within one week (comparable to the results reported here for longleaf pine), but efflux rates were eventually reduced by 57% two months following girdling (comparable to the results reported by Högberg *et al.* 2001 for Scots pine). Also, the season of disturbance may influence the extent that soil CO_2 efflux is coupled with canopy photosynthesis. For example, girdling of loblolly pine (*P. taeda*) in the autumn resulted in a 30% decline in soil CO_2 efflux, whereas girdling in the spring had no effect (Johnsen *et al.* 2007). In addition, differences in carbon storage pool locations (i.e., above- versus belowground) and mobilization strategies may differ among species and systems. Furthermore, basic differences in growth habit between coniferous and deciduous species may influence the linkage between canopy photosynthesis and soil CO_2 efflux. For instance, the propensity of deciduous species to produce roots in the spring prior to leaf-out and to root sprout following disturbance to the aboveground stem likely influence carbon storage pools and patterns of carbon allocation to storage differently than conifers. Based on the available literature, the linkage between photosynthate assimilation and belowground utilization may vary widely among species, but also within species both spatially and temporally. Kuzyakov and Garvichkova (2010) identified four factors that, comprised of both biological and physical processes, affect the time lag between canopy photosynthesis and soil CO_2 efflux. These factors include: (i) plant height, which influences the transport time of photosynthate to the roots and the rhizosphere, (ii) vertical distribution of roots, soil temperature and moisture, which affect CO_2 diffusivity through the soil, (iii) plant physiological processes, such as assimilation rates, phloem transport rates, and carbon sink/source patterns, which will ultimately determine the supply of carbon to the soil, and (iv) environmental conditions, such as moisture stress, that affect physiological processes. We suggest that, in addition to these factors, ecosystem disturbance regimes and life history characteristics of the dominant species in an ecosystem will impact the time lag and coupling of soil CO_2 efflux and canopy photosynthesis.

Conclusions

Consistent with results from previous studies conducted in ecosystems with evolutionary histories of chronic disturbance, the results of this study suggest that stored carbon in fire-adapted longleaf pines may decouple the direct linkage between current photosynthate assimilation and soil CO_2 efflux. Consecutive years of canopy scorching did not significantly impact soil CO_2 efflux, suggesting that longleaf pines have adapted to maintain substantial carbon reserves to buffer disruption of canopy photosynthesis from frequent disturbance events.

While the method and degree of defoliation employed in this study has a direct ecological analogue in that longleaf pines occasionally experience and survive 100% needle scorch during natural and prescribed burning (Guo, Mitchell & Hendricks 2004; Guo *et al.* 2008), the disruption of current photosynthate allocation belowground was not as persistent as in girdling studies due to the relatively rapid recovery of leaf area. Nonetheless, the process of leaf area reconstruction itself represented an additional drain on stored carbon pools and some of this carbon may have been mobilized from the belowground carbon storage pool. Alternatively, aboveground stored carbon pools may have been mobilized to satisfy belowground metabolic requirements. Further work assessing the impact of girdling on soil CO_2 efflux across a range of evolutionary histories and carbon storage pools—from chronically disturbed ecosystems where the dominant species characteristically maintain large belowground carbon storage pools to catastrophically disturbed ecosystems where the dominant species characteristically maintain small belowground carbon storage pools—would be enlightening. There is currently a poor understanding of carbon storage pools and fluxes at ecosystem scales. Future investigations that address the patterns and mechanistic controls of TNC accretion and depletion may provide valuable insight into carbon cycling dynamics, which is critical to more accurately assess and predict the impact of natural and anthropogenic disturbances in terrestrial ecosystems (Williams *et al.* 1997).

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Table 2.1. Mean (SE) total non-structural carbohydrate (starch + sugar; TNC) concentrations expressed on a percent basis in 1st, 3rd, and 5th order fine roots (< 2 mm) for control and scorch treatments. Fine root TNC concentrations were significantly impacted by the scorch treatment, but the effect was dependent upon root order and year. Means sharing a letter are not significantly different (Fisher's LSD, α = 0.05).

	2006		2007	
Root Order	Control	Scorch	Control	Scorch
1st	11.91(1.81)f	8.46(0.70)g	11.23(0.45)f	11.34(1.86)fg
3rd	21.49(1.89)cde	19.79(0.86)de	25.91(0.49)ab	17.95(1.42)e
5th	22.04(0.84)cd	22.74(0.30)bcd	29.48(0.83)a	24.17(0.68)bc

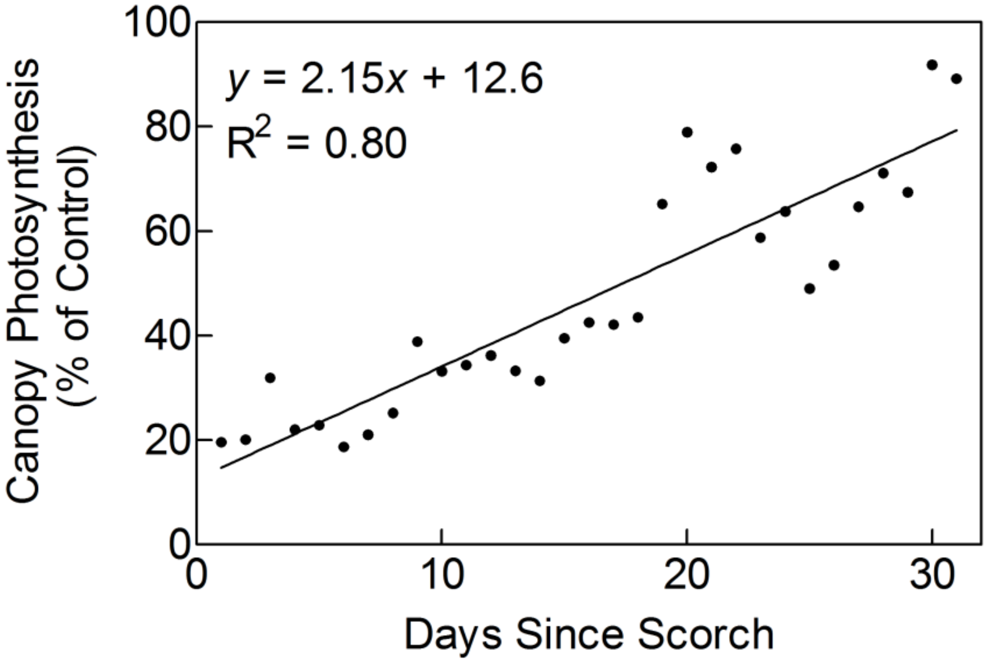

Figure 2.1. Recovery of canopy photosynthesis for scorch treatment plots expressed as a percent of control plot canopy photosynthetic rates. Photosynthetic rates in the scorch treatment were *c.* 20% of that in the control treatment immediately after treatment application, but recovered to *c.* 90% of that in the control treatment within one month. Each point on the figure represents a plot. Data are from the 2006 growing season.

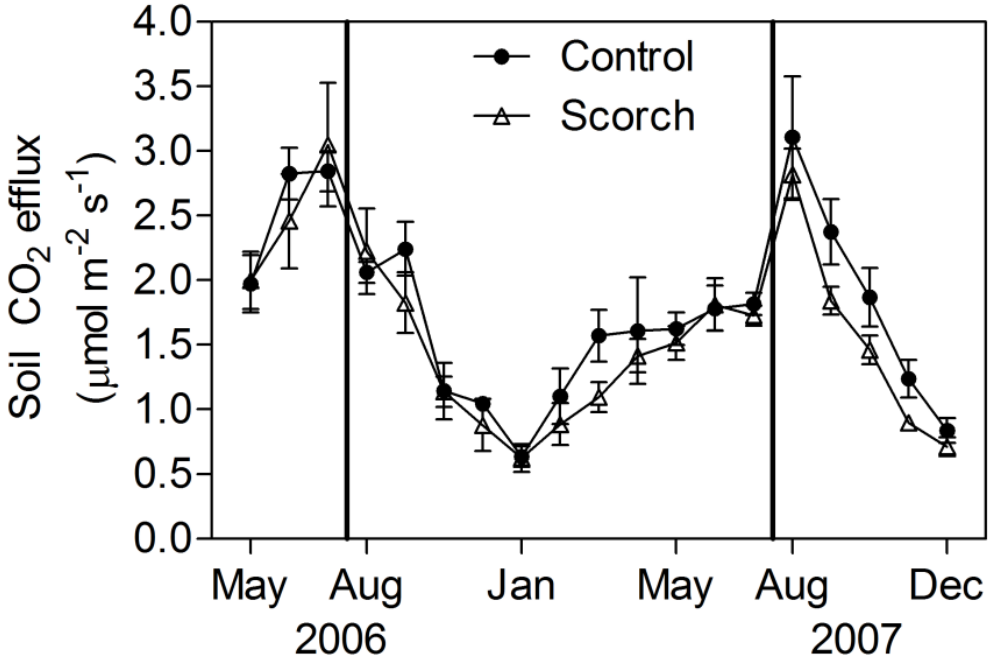

Figure 2.2. Soil CO$_2$ efflux (mean ± SE) for control and scorch treatments showed no treatment differences over two consecutive years and scorching applications. Vertical lines indicate when scorching occurred.

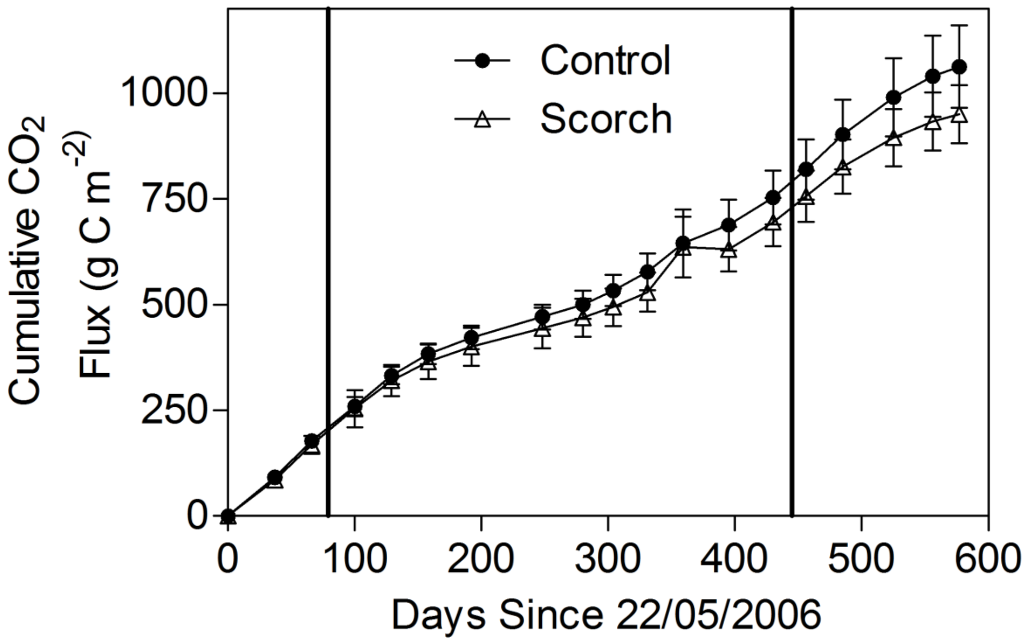

Figure 2.3. Cumulative soil CO_2 efflux for control and scorch treatments showed no treatment differences over two consecutive years and scorching applications. Vertical lines indicate when scorching occurred.

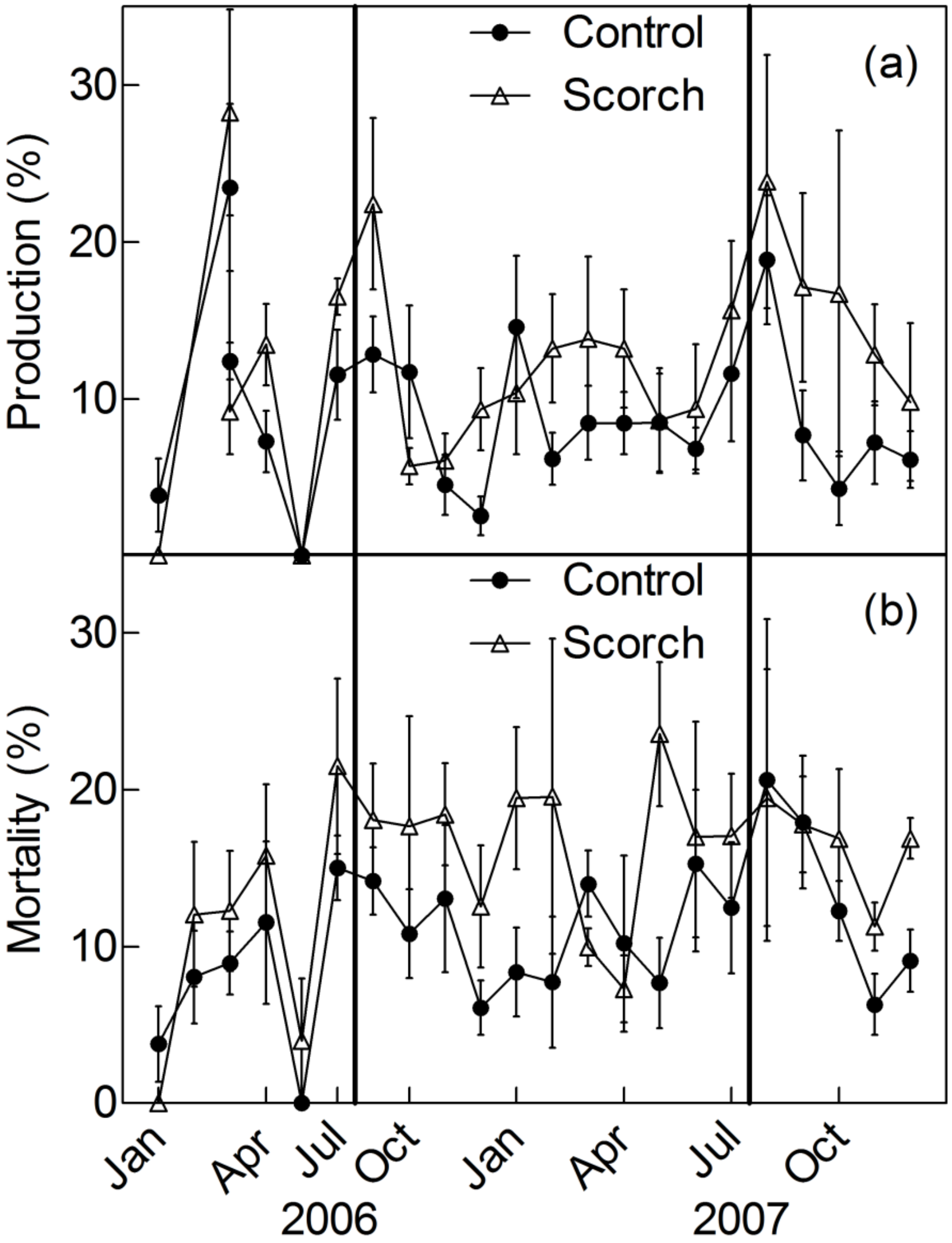

Figure 2.4. Mean ± SE (a) percent fine root (< 2 mm) production and (b) percent fine root mortality differed between treatments prior to treatment application and differences remained independent of treatment through time. Vertical lines indicate when scorching occurred.

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CHAPTER 3

STORED CARBON DECOUPLES SOIL CO_2 EFFLUX FROM CANOPY PHOTOSYNTHESIS IN CHRONICALLY DISTURBED ECOSYSTEMS: IMPACT OF PERMANENTLY TERMINATING THE TRANSFER OF RECENTLY ASSIMILATED CARBON BELOWGROUND VIA STEM GIRDLING AND ROOT TRENCHING OF LONGLEAF PINE (*PINUS PALUSTRIS* L.)[2]

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Abstract

The tight coupling of belowground autotrophic respiration with the availability of recently assimilated carbon, and its contribution towards soil CO_2 efflux, is an emerging paradigm in the ecophysiological literature. Here, we test the hypothesis that stored carbohydrates decouple the direct linkage between recently assimilated carbon and soil CO_2 efflux. Using longleaf pine (*Pinus palustris* L.), a species with an evolutionary history of chronic disturbance and large reserves of belowground stored carbon, we experimentally manipulated the transfer of recently assimilated carbon belowground using stem girdling and root trenching. Girdling treatments were applied in both May and August to examine potential seasonal dynamics in belowground dependence on recent photosynthate. Trenching was applied in May and differed from stem girdling in that it not only terminated the supply of photosynthate belowground, but also severed connections between the root branching network and the tap root, thus limiting the potential pool of carbohydrates available for mobilization. Soil CO_2 efflux was not influenced by photosynthate exclusion treatments and seasonal dynamics were independent of treatment. At the end of our observation period (243 days), the cumulative soil CO_2 efflux in the photosynthate exclusion treatments was statistically similar to that of the control treatment. Sugar and starch concentrations ranged from 2.5 to 25% and 8 to 35%, respectively, during our observation period and were impacted by photosynthate exclusion treatments, but treatment effects depended on root type and temporal period. For example, sugar and starch concentrations of root orders 1-3 were not impacted by photosynthate exclusion treatments, but concentrations of root orders 4-6 and lateral roots decreased after treatment application suggesting that sugar and starch were mobilized from larger order roots to maintain smaller order, more metabolically active roots. Our observations following girdling of a tree species with an evolutionary history of

chronic disturbance via fire are in contrast to those obtained in many girdling studies of tree species with evolutionary histories of infrequent, but catastrophic disturbance and suggest that the adaptation of large belowground carbon reserves decouples the linkage between canopy photosynthesis and soil CO_2 efflux.

Introduction

The combined contributions of belowground autotrophic and heterotrophic respiration, commonly measured and referred to as soil CO_2 efflux, account for the largest annual terrestrial CO_2 flux from the biosphere to the atmosphere. Although relationships between soil CO_2 efflux and its regulating factors (e.g., nutrient supply, moisture, and temperature) have been well documented, the mechanistic controls of this important flux remain unclear (Kuzyakov & Cheng 2001; Ryan & Law 2005; Davidson, Janssens & Luo 2006; Kuzyakov 2006; Trumbore 2006; Vargas *et al.* 2010). Despite its importance, soil CO_2 efflux is the most poorly understood component of the terrestrial carbon cycle (Trumbore 2006; Bond-Lamberty & Thomson 2010b; Bond-Lamberty & Thomson 2010a). Much of the uncertainty in determining the regulating factors of soil CO_2 efflux is due to its complexity—soil CO_2 efflux encompasses both autotrophic and heterotrophic belowground respiratory processes that are difficult to separate, involve different organisms, utilize different substrates, and may respond differently to environmental factors.

Understanding the mechanistic controls of belowground autotrophic and heterotrophic respiration is a prerequisite for understanding the mechanistic controls of soil CO_2 efflux. Until relatively recently, it was believed that soil CO_2 efflux was dominated by the heterotrophic component, which relies primarily on detrital carbon pools as substrate (see Hanson *et al.* 2000;

Högberg & Read 2006). Both isotopic and manipulative approaches have proven useful for separating the autotrophic and heterotrophic components of soil CO_2 efflux and have further demonstrated that the autotrophic component can rival—or even exceed—the heterotrophic component (Andrews *et al.* 1999; Ekblad & Högberg 2001). Isotopic approaches in particular have also demonstrated that carbon may be used as substrate for belowground autotrophic respiration (i.e., roots or mycorrhizal fungi) relatively quickly (*c.* 1-5 days) following assimilation (Horwath, Pregitzer & Paul 1994; Mikan *et al.* 2000; Johnson *et al.* 2002; Steinmann *et al.* 2004; Högberg *et al.* 2008). The tight coupling of root respiration, the primary component of belowground autotrophic respiration, with recently assimilated carbon is emerging as a contemporary paradigm in the ecophysiological literature.

Although results from isotopic studies indicate there can be rapid belowground utilization of recently assimilated carbon, they do not, however, provide information regarding the dependence of belowground autotrophic metabolism on the availability of recent photosynthate. For example, it is entirely possible—and rather appealing from an efficiency perspective—that recent photosynthate is utilized upon arrival belowground because it is already in a molecular form conducive to act as substrate for metabolism (i.e., sucrose or some other soluble sugar) as opposed to some other form that requires energy for conversion (i.e., starch). Manipulative studies that have disrupted the belowground transfer of recent photosynthate have demonstrated rapid and substantial declines in soil CO_2 efflux (Högberg *et al.* 2001; Subke *et al.* 2004; Andersen *et al.* 2005; Frey, Hagedorn & Giudici 2006) lending further support to the coupling paradigm while also providing evidence related to the belowground dependence on recent photosynthate. However, there are examples in the literature that conflict with the tight coupling paradigm (Edwards & Ross-Todd 1979; Kosola *et al.* 2001; Guo, Mitchell & Hendricks 2004; Vargas, Trumbore & Allen 2009).

Delayed responses of soil CO_2 efflux to the disruption of recently produced photosynthate may provide an indication of the functional importance of non-structural carbohydrates for root metabolism as well as insight into linkages between canopy photosynthesis and belowground utilization of carbohydrates. When stored in the root system, non-structural carbohydrates represent available carbon capital that can potentially be used for growth, repair, or maintenance (Chapin, Schulze & Mooney 1990; Le Roux *et al.* 2001). Relatively little is known about the use and turnover of non-structural carbohydrates (Körner 2003), but they likely buffer root systems against variable climate conditions (Trumbore 2006) or may perhaps be related to recovery from disturbance. Since carbon storage is beneficial for persisting through and recovering from disturbance events, a selective advantage for carbon storage should exist in species with an evolutionary history of chronic disturbance. Thus, root respiration and therefore, soil CO_2 efflux, may become decoupled from the availability of recent photosynthate in species that have adapted to chronic disturbance via increased carbon storage (Guo, Mitchell & Hendricks 2004).

Here, we test the hypothesis that stored carbohydrates decouple the direct linkage between current photosynthate assimilation and soil CO_2 efflux. Using longleaf pine (*Pinus palustris* L.) as a model for species with an evolutionary history of chronic disturbance and a high amount of stored root carbohydrates, we experimentally manipulated the transfer of recently assimilated photosynthate belowground using stem girdling and root trenching. We predicted that soil CO_2 efflux would not be impacted by the photosynthate exclusion treatments for an extended period (i.e., months) because root respiration would be maintained by mobilization of stored carbon from higher to lower order roots (Guo, Mitchell & Hendricks 2004).

Materials and Methods

Study Site and Experimental Design

This study was conducted in a longleaf pine plantation located at the Joseph W. Jones Ecological Research Center in Newton, GA, USA (31°15 W latitude and 84°30 N longitude). The plantation was established in 1987 using an approximate 1.25 × 2.5 m planting grid, and longleaf pine was the exclusive overstory species (mean diameter at breast height = 20.3 cm at the beginning of the experiment). A thinning of every third row was performed in 2006. The soil in the plantation is a loamy Arenic Utisol (Goebel *et al.* 2001). The climate for this region has been characterized as humid subtropical with an average annual precipitation of 131 cm distributed evenly throughout the year, and mean annual low and high temperatures of 9 and 28°C, respectively.

Twelve 30 × 30 m plots were established within the plantation in October 2009. Based on spatial proximity, we assigned groups of three plots to each of four blocks. Within each block, we randomly assigned one of three treatments to each plot. Belowground photosynthate exclusion treatments consisted of: control; early stem girdle, where all trees within a plot were stem girdled in early May; and late stem girdle, where all trees within a plot were stem girdled in early August. Girdling was performed by removing a 15-30 cm wide strip of bark and phloem around the circumference of the tree with a chainsaw at approximately 1.0 m above the ground. An additional trenched treatment plot was established within each block by trenching between neighboring tree rows in early May. Trenching to a depth of 1.0 m was performed mechanically between neighboring tree rows and the resulting trenched plots were devoid of trees and tap roots. Two layers of 6 mil black plastic sheeting were placed in the trench prior to back-filling with soil. The plastic sheeting extended beyond the top of the soil to ensure new root growth could not colonize the plots. Stem girdling terminated the supply of recent photosynthate to root systems, but maintained the interconnectedness of the root system, thus allowing for mobilization of carbon from storage pools to points of utilization. Trenching not only terminated the supply of recent photosynthate to root systems, but also severed connections between the root branching network and the tap root. Thus, we expected girdling and trenching to result in different rates of stored carbon depletion. Herbicide was applied to all plots in November 2009 and then as needed throughout the observation period to eliminate understory vegetation. The entire site was prescription burned in December 2009 to remove litter.

Soil CO_2 Efflux

Soil CO_2 efflux was measured at three PVC soil collars (10.16 cm wide × 4.4 cm high inserted to a soil depth of 2.2 cm) randomly located at the center of each plot using a LI-6400-09 soil chamber and LI-6400XT portable infrared gas analyzer (Li-Cor Inc., Lincoln, Nebraska, USA). Concurrent with soil CO_2 efflux measurements, soil temperature at 10 cm depth was measured using a LI-6000-09TC soil probe thermocouple (LI-Cor Inc., Lincoln, Nebraska, USA) and soil moisture integrated over 20 cm depth was measured using a C620 HydroSense soil water measurement system (Campbell Scientific, Logan, Utah, USA). To account for diel patterns in soil CO_2 efflux, which are mainly a function of temperature, we measured efflux of all plots within a single block consecutively and then measured efflux of all plots in the next block and so on. We systematically changed the initial block measured at each sampling effort so that the block measured initially during the first sampling effort became the final block sampled during the next effort and so on. Within each block, plots were measured in a random order. Although this approach should adequately account for diel patterns, we also normalized efflux values to a reference temperature of 25°C (i.e., E_{25}) following methods described by Lavigne, Foster & Goodine (2004).

Root Nitrogen and Nonstructural Carbohydrates

We collected root material from all treatment plots prior to treatment application (March), monthly during the growing season (May through November). Root categories were delineated according to order for fine roots and according to location (i.e., lateral and tap root categories). Specifically, we grouped 1st, 2nd, and 3rd order fine roots together (i.e., category 1-3) and 4th, 5th, and 6th order fine roots together (i.e., category 4-6). At each sampling effort, we randomly selected three individual trees within each plot and excavated a portion of the soil adjacent to the tree stem and collected fine root branching networks, lateral root sections connected to the tap root, and tap root samples. Fine root branching networks were completely removed from the soil and root orders were determined in the field. Lateral root sections were severed with a saw or hand clippers and removed from the soil. Tissue samples from tap roots were removed with a chisel at a depth of 10-20 cm. Individual trees were sampled only once during the experiment. Once collected from the soil, roots were placed in plastic bags according to category and put into a cooler for transport to the laboratory. Roots were washed of debris and oven-dried at 70°C to a constant mass then weighed, ground, and homogenized using a SPEX 8000-D mixer mill (SPEX, Edison, NJ).

Nitrogen (N), starch, sugar, and TNC (i.e., the sum of starch and sugar) concentrations were assessed for each root category. Sub-samples of dried root samples were weighed and analyzed on a FlashEA 1112 Series NC Soil Analyzer (Thermo Electron Corp., Waltham, MA) to determine N concentrations. A modified phenol-sulfuric acid method was used to determine both sugar and starch concentrations from sub-samples of dried roots (Buysse & Merckx 1993).

Statistical Analyses

We analyzed the impact of photosynthate exclusion treatments on soil CO_2 efflux and fine root N, sugar, starch, and TNC concentrations using a completely randomized block design with repeated measures. The experimental unit (plot) was considered as the random subject factor in all repeated measures analyses. Photosynthate exclusion treatment ($n = 4$) was considered as a fixed factor and sampling period ($n = 16$) was considered as the fixed repeated factor when analyzing soil CO_2 efflux, soil moisture, and soil temperature. A similar model was used to analyze root N, sugar, starch, and TNC concentrations, but the sampling period was less frequent ($n = 8$) and root category ($n = 3$) was included in the model as an additional fixed factor. The tap root category was not included in our statistical model because it did not occur in the trenched treatment. The trench treatment was applied to terminate connections between lateral and tap roots and, therefore, no tap roots were within trenched plots. Our main interest was to investigate treatment effects; therefore, we analyzed the full suite of treatments with the three root categories rather than all four root categories with only three treatments. We also analyzed a model that included only lateral and tap root categories in the three appropriate treatments (control, early girdled, and late girdled) and all sampling months. Treatment effects on cumulative soil CO_2 efflux at the end of our observation period (i.e., November 2011) were analyzed using a one-way ANOVA with plot as a random factor. Root N, sugar, starch, and TNC concentrations were proportional values and presented as such throughout the report; however, data were arcsine square root transformed to satisfy ANOVA assumptions (Zar 1996). Denominator degrees of freedom were estimated according to the Kenward-Roger method

(Kenward & Roger 1997). Treatment means were compared using Fisher's Least Significant Difference (LSD) test. When interactions occurred, we performed tests of simple main effects (Schabenberger, Gregoire & Kong 2000; Littell *et al.* 2006). All repeated measures analyses and the one-way ANOVA were performed using a mixed model procedure (PROC MIXED; Version 9.1.3, SAS Inc., Cary, NC, USA) with a type-I error rate of 0.05. Data presented in the text are means ± standard error.

Results

Soil CO_2 Efflux and Environmental Factors

Soil CO_2 efflux was not impacted by photosynthate exclusion treatments ($P = 0.3460$) and seasonal dynamics ($P < 0.\ 0001$) were independent of treatment (i.e., no sampling period × treatment interaction, Fig. 3.1a). Soil CO_2 efflux rates increased from 2.0 μmol m^{-2} sec^{-1} in March to a peak of 3.9 μmol m^{-2} sec^{-1} in August, followed by a steady decline to less than 1.1 μmol m^{-2} sec^{-1} in November. Similarly, E_{25} was not impacted by photosynthate exclusion treatments ($P = 0.2453$) and seasonal dynamics ($P < 0.\ 0001$) were independent of treatment (data not presented). At the end of our observation period, the cumulative soil CO_2 efflux in the photosynthate exclusion treatments was statistically similar to that of the control treatment ($P = 0.5242$, Fig. 3.1b).

Soil moisture, measured concurrently with soil CO_2 efflux, was not impacted by photosynthate exclusion treatments ($P = 0.5027$) and temporal dynamics ($P < 0.\ 0001$) were independent of treatment (i.e., no sampling period × treatment interaction, Fig. 3.1c). There was a non-significant trend of increased soil moisture in the trenched treatment relative to other treatments in late June, July, and early August, but this had no discernable effect on soil CO_2

efflux. Similarly, soil temperature, measured concurrently with soil CO_2 efflux, did not vary significantly among photosynthate exclusion treatments ($P = 0.8518$) and seasonal dynamics ($P < 0.0001$) were independent of treatment. Averaged across treatments, soil temperature increased from 16.4°C in March to a peak temperature of 31.9°C in September, followed by a steady decline to 15°C in November.

Root Nitrogen and Nonstructural Carbohydrates

Root [N] in the control treatment increased from minimum values of 0.27 ± 0.03% in March to maximum values of 0.39 ± 0.03% in September (Fig. 3.2b). Differences in the [N] among root categories were observed but depended on the temporal period (i.e., sampling period × root category interaction, $P < 0.0001$), and the impact of photosynthate exclusion treatments on [N] depended on both root category (i.e., root category × treatment interaction, $P < 0.0001$) and temporal period (i.e., sampling period × treatment interaction, $P = 0.0015$). Averaged across temporal period and photosynthate exclusion treatments, [N] was highest in root orders 1-3 (0.46 ± 0.01%), intermediate in root orders 4-6 (0.29 ± 0.01%), and lowest in lateral roots (0.21 ± 0.01%). The [N] of the three root categories was significantly different every month, with the exception of May and August when [N] of root orders 4-6 and laterals were similar (data not presented). None of the photosynthate exclusion treatments impacted [N] in root orders 1-3 or 4-6 relative to the control treatment, whereas [N] of lateral roots in both the early and late girdled treatments was lower than that of the control treatment (Fig. 3.2a). However, despite the differences in lateral root [N] elicited by the two girdling treatments, lateral root [N] in the trenched treatment remained similar to that of the control treatment (Fig. 3.2a). Treatments effects on [N] became evident in July and continued through November; however, treatment effects were not consistent. For example, [N] in the early girdled treatment was lower than that of the control treatment in July, October, and November, but similar in August and September (Fig. 3.2b). Lateral root [N] (0.20 ± 0.01%) was generally higher than that of tap roots (0.16 ± 0.01%), with the exception of May, July, and September when [N] was similar (i.e., sampling period × root category interaction, *P* = 0.0274); however, [N] of both root categories responded to photosynthate exclusion treatments similarly (i.e., no root category × treatment interaction, *P* = 0.2270).

Although there were some fluctuations during the observation period, [sugar] averaged across root categories in the control treatment remained relatively stable and did not exhibit a distinct seasonal pattern (Fig. 3.3) with the exception of root orders 1-3 where [sugar] decreased from a maximum of 9.0% in March to a minimum of 3.5% in August. Root [sugar] differed among root categories, was impacted by photosynthate exclusion treatments, and varied over time, but the individual effects were not independent (i.e., sampling period × root category × treatment interaction, *P* = 0.0309). Root [sugar] generally differed among root categories and was highest in root orders 4-6, intermediate in lateral roots, and lowest in root orders 1-3; however, in October and November, [sugar] of some root categories was similar within the early girdled and trenched treatments (data not presented). For example, [sugar] in the early girdled treatment in October was similar for root orders 1-3 and lateral roots and similar among all root categories in the trenched treatment. In November, [sugar] of root orders 1-3 was similar to that of root orders 4-6 and laterals in early girdled treatments, which remained different from each other, whereas [sugar] of root orders 1-3 and 4-6 was similar and [sugar] of root orders 4-6 and lateral roots were similar in the trenched treatment. The [sugar] of root orders 1-3 was not impacted by photosynthate exclusion treatment, regardless of temporal period (Fig. 3.3). However, [sugar] of root orders 4-6 and lateral roots was negatively impacted by photosynthate exclusion treatments, but the timing of impact differed. For example, [sugar] of root orders 4-6 in the early girdled and trenched treatments was lower than those of the control treatment in August and remained different through November, whereas the same treatment differences in [sugar] of lateral roots were not evident until September (Fig. 3.3). Although [sugar] of root orders 4-6 was similar in control and late girdled treatments throughout the observation period (Fig. 3.3), [sugar] of lateral roots in the late girdled treatments was lower than that of the control treatment in October and November (Fig. 3.3). Lateral root [sugar] (10.7 ± 0.4%) was higher ($P < 0.0001$) than that of tap roots (7.7 ± 0.3%); however, [sugar] of both root categories responded to photosynthate exclusion treatments similarly (i.e., no root category × treatment interaction, $P = 0.8961$).

Averaged across root categories and photosynthate exclusion treatments, [starch] decreased from a maximum of 25.4 ± 1.0% in March to a minimum of 11.9 ± 0.3% in October. Root [starch] generally—but not always—differed among root categories (i.e., sampling period × root category interaction, $P < 0.0001$), and varied over time ($P < 0.0001$), but was not impacted by photosynthate exclusion treatment ($P = 0.9001$). Averaged across photosynthate exclusion treatments and months, lateral roots exhibited the highest [starch] (19.1 ± 0.6%), followed by root orders 4-6 (17.8 ± 0.7%) and 1-3 (11.7 ± 0.3%). The only instances when [starch] was similar among root orders was in March, May, and September when [starch] of root orders 4-6 and lateral roots was similar (Fig. 3.4a). Although our statistical analysis indicated that [starch] was impacted by photosynthate exclusion treatments at certain points during the observation period (i.e., sampling period × treatment interaction, $P = 0.0012$), there actually was not a treatment effect as the statistical result was primarily due to pre-treatment differences. Specifically, the late girdled treatment exhibited lower root [starch] relative to the control treatment in March, but in June and July, root [starch] in the late girdled treatment was higher than that in the early girdled treatment (Fig. 3.4b). There was, however, a non-significant trend of lower root [starch] in the early girdled and trenched treatments relative to the control treatment in June, July, and August, but the trend diminished for the remainder of the observation period. Figure 3.5 shows [starch] of each root category as a function of photosynthate exclusion treatment for comparison with [sugar] (Fig. 3.4). Lateral root [starch] (19.2 ± 0.6%) was generally similar to that of tap roots (19.0 ± 0.4%), with the exception of March, May, and September when [starch] was higher in lateral roots (i.e., sampling period × root category interaction, *P* = 0.0003); however, [starch] of both root categories responded to photosynthate exclusion treatments similarly (i.e., no root category × treatment interaction, *P* = 0.8961).

Averaged across root categories in the control treatment, [TNC] decreased from a maximum of 38.9% in March to a minimum of 24.0 % in October. Root [TNC] differed among root categories, was impacted by photosynthate exclusion treatments, and varied over time, but the individual effects were not independent (i.e., temporal period × root category × photosynthate exclusion treatment interaction, *P* = 0.0309). In general, the patterns of root [TNC] related to root categories and photosynthate exclusion treatments followed those of [sugar], whereas temporal patterns of root [TNC] followed those of [starch]. Root [TNC] generally differed among root categories and was highest in root orders 4-6, intermediate in lateral roots, and lowest in root orders 1-3; however, in October and November, [TNC] of some root categories was similar within the early girdled and trenched treatments (data not presented). For example, in the early girdled treatment in October and November, and the trenched treatment in September and October, [TNC] was similar among all root categories. In November, [TNC] of root orders 1-3 and 4-6 was similar as was root orders 4-6 and lateral roots in the trenched treatment. The [TNC] of root orders 1-3 was not impacted by photosynthate exclusion treatment, regardless of temporal period (Fig. 3.6). However, [TNC] of root orders 4-6 and lateral roots was impacted by treatments, but not necessarily at the same time. For example, [TNC] of root orders 4-6 in the early girdled and trenched treatments was lower than that of the control treatment in August and remained different through November, whereas the same treatment differences in [TNC] of lateral roots were not evident until October (Fig 3.6). Although [TNC] of root orders 4-6 was similar in control and late girdled treatments throughout the observation period (Fig. 3.6), [TNC] of lateral roots in the late girdled treatments was lower than that of the control treatment in November (Fig. 3.6). The [TNC] of roots in control treatments varied through time. Lateral root [TNC] (30.0 ± 0.8%) was higher ($P < 0.0001$) than that of tap roots (26.7 ± 0.6%); however, [TNC] of both root categories responded to photosynthate exclusion treatments similarly (i.e., no root category × treatment interaction, $P = 0.9872$).

Discussion

The lack of response in soil CO_2 efflux following the termination of the supply of recent photosynthate belowground indicates that belowground autotrophic respiration of longleaf pine can be sustained for extended periods via stored carbon. That we did not observe declines in soil CO_2 efflux related to photosynthate exclusion treatments throughout the six months following treatment application is in contrast to results of many other girdling studies. For example, Högberg *et al.* (2001) observed a 27% decline in soil CO_2 efflux within five days of girdling Scots pine (*P. sylvestris*) in the early summer and a 37% decline within five days of girdling in the late summer. In both instances, soil CO_2 efflux rates were approximately 50% lower than the corresponding control treatment by the end of the growing season. Similar results were observed when girdling European chestnut (*Castanea sativa*; Frey, Hagedorn & Giudici 2006), Norway spruce (*Picea abies* (L.) Karst; Olsson *et al.* 2005), and European beech (*Fagus sylvatica* L.; Andersen *et al.* 2005). Girdling Eucalypts (*Eucalyptus grandis* × *urophylla*) elicited a much smaller impact on soil CO_2 efflux than did the studies described above (Binkley *et al.* 2006), whereas girdling tulip poplar (*Liriodendron tulipifera*) did not impact soil CO_2 efflux for at least two years (Edwards & Ross-Todd 1979). Interestingly, both the Eucalypt and tulip poplar share the life history characteristics of resprouting which indicates large belowground carbon storage (Bowen & Pate 1993; Clarke & Knox 2009; Schutz, Bond & Cramer 2009). Although longleaf pine does not resprout, the species has an evolutionary history of chronic disturbance via burning with fires returning as frequently as every 1-3 years (Mitchell *et al.* 2006) and mature trees commonly withstand fires that burn up to 100% of their foliage and refoliate their canopy using stored carbon (Guo *et al.* 2008). Thus, although the post-disturbance strategies of resprouting versus refoliating differ, they both rely on large pools of stored carbon. We believe that the degree of coupling between soil CO_2 efflux and canopy photosynthesis can be largely influenced by evolutionary histories that select for carbon storage.

Soil microclimate conditions remained similar among photosynthate exclusion treatments and should not have influenced soil CO_2 efflux. Similarities in soil temperature indicated similarities in canopy characteristics and subsequent light penetration to the forest floor. Similarities in soil moisture (Fig. 3.1c) indicated similarities in transpirational water use among girdled and control treatments. However, differences in soil moisture at depths below 20 cm could have potentially existed but were not measured. Although not statistically significant, the trend of increased soil moisture in the trenched treatment relative to the girdled and control treatments reflected the absence of transpirational water use (Fig. 3.1c). A potential concern with both girdling and trenching treatments is that root mortality introduces new organic matter into the soil that could be used as substrate and increase the heterotrophic component of soil CO_2 efflux (Kuzyakov 2006; Trumbore 2006). The impact of stimulating the heterotrophic component is primarily a concern when the goal is to separate the relative components form soil CO_2 efflux, but it could have potentially made it more difficult to detect a signal in soil CO_2 efflux that resulted only from the autotrophic component as was the case in our experiment. However, Scott-Denton, Rosenstiel and Monson (2006) showed that heterotrophic stimulation of soil CO_2 efflux from root mortality did not occur until the year following girdling in a subalpine forest dominated by lodgepole pine (*Pinus contorta*) and attributed this to the nonlinear rate of organic matter decay (Kirschbaum 1995; Trumbore 1997). The [sugar] of the most metabolically active roots was maintained at similar levels regardless of treatment and [starch] was not impacted by photosynthate exclusion treatments (Fig. 3.4). We would expect starch to be hydrolyzed to sugar if substrate for metabolism was insufficient. Furthermore, Binkley *et al.* (2006) found that girdling Eucalypts did not influence fine root biomass for at least five months. Finally, previous work in longleaf pine systems has shown that the autotrophic component of soil CO_2 efflux rivals that of the heterotrophic component (Cheng *et al.* 2005). Thus, we are relatively confident that our photosynthate exclusion treatments did not influence the heterotrophic component of soil CO_2 efflux and, therefore, we should have been able to detect an impact of the autotrophic component via changes in soil CO_2 efflux.

Both root [starch] and [sugar] were surprisingly high in longleaf pine. At times, [sugar] exceeded 20%, [starch] exceeded 30%, and resulting [TNC] exceeded 50% of dry weight. The seasonal pattern of root [TNC] in control trees was dominated by the seasonal pattern in [starch].

This was not surprising because root [starch] generally comprises a larger proportion of [TNC] than does [sugar] (Jordan & Habib 1996; Silpi *et al.* 2007; Regier *et al.* 2010). However, [sugar]—and not [starch]—was impacted by photosynthate exclusion treatments. This was surprising because the only other study that has disrupted the belowground availability of recent photosynthate and monitored both root [sugar] and [starch] observed treatment effects on [starch] while [sugar] remained stable (Jordan & Habib 1996). However, the [sugar] of the most metabolically active roots (root orders 1-3) were not influenced by photosynthate exclusion treatments (Fig. 3.3). The reductions in [sugar] of root orders 4-6 and lateral roots may have occurred in response to maintaining the [sugar] of root orders 1-3 via mobilization. That we did not observe concomitant reductions in soil CO_2 efflux suggests that there is an operating zone for [sugar] to maintain root metabolism at a given level and that our treatments had not yet depleted [sugar] below the threshold that would impact metabolism. That [starch] was both extremely high and not impacted by photosynthate exclusion treatment further suggests that [sugar] had not been reduced below the operating zone threshold in root orders 4-6 and lateral roots; otherwise, starch should have been hydrolyzed into sugars and both [sugar] and [starch] of a given root category would have been impacted. Alternatively, if [sugar] had been depleted below the operating level threshold in root orders 4-6 and lateral roots, then the contribution of these root categories towards soil CO_2 efflux must be insignificant relative to that of root orders 1-3.

Surprisingly, there were instances when root non-structural carbohydrates (primarily starch) increased between the October and November sampling periods despite the termination of recent photosynthate availability. For this to occur, either non-structural carbohydrates were mobilized from the stem (below the girdle), or portions of the root system were senescing and mobilizing non-structural carbohydrates to other parts of the root system. The lack of impact on soil CO_2 efflux and the fact that we did not observe large amounts of dead roots when collecting samples suggest that mobilization from the stem is the more likely scenario. However, longleaf pine root systems are extensive, so it is possible that the more distal portions of the root network were senescing. This would have gone unnoticed during our sampling as most of roots were collected within 1.0 m of a tree. This could be possible—even without detecting a signal in soil CO_2 efflux—because it was happening when efflux was at a seasonal minimum and would have therefore been more difficult to detect.

Similar patterns in longleaf pine root non-structural carbohydrate dynamics have been reported following canopy scorching (Guo, Mitchell & Hendricks 2004) and smoldering duff fires (Varner *et al.* 2009). Guo, Mitchell, and Hendricks (2004) proposed that non-structural carbohydrates in higher order roots (i.e., larger diameter, secondary roots that presumably serve a carbon storage function) are transferred to maintain the relatively high metabolic activity and respiration rates of lower order roots (i.e., smaller diameter, primary roots that function in concert with mycorrhizal fungi in resource uptake and assimilation). The utilization of non-structural carbohydrates to support belowground autotrophic growth and respiration is supported by results from other field studies. For example, a ^{13}C tracer study conducted in a regenerating scrub oak ecosystem prone to frequent fires indicated that 33% of carbon in newly established roots originated from a source other than recent photosynthate (Langley, Drake & Hungate 2002). Similarly, a ^{14}C labeling experiment and associated studies (Czimczik *et al.* 2006; Schuur & Trumbore 2006; Carbone *et al.* 2007) in a black spruce (*Picea mariana*) forest, estimated the predominant fraction of non-structural carbohydrates supporting root respiration was *c.* 3-5 years old. In addition, following complete denudation of leaf biomass by hurricane force winds in a tropical forest, new roots were produced using non-structural carbohydrates reserves that had been stored 2 to 10 years prior to the disturbance (Vargas, Trumbore & Allen 2009).

Ultimately, all substrate utilized for belowground autotrophic respiration is produced via photosynthesis, so the two processes are inextricably coupled and belowground autotrophic respiration is dependent on photosynthate production. Indeed, when available, recently assimilated C may be preferentially used as substrate for belowground autotrophic respiration even in a species with large belowground carbohydrate reserves. However, our results demonstrate that the processes can become temporally uncoupled when canopy photosynthesis is disrupted and belowground carbon storage is sufficiently large. In other words, stored carbon slows down the link between aboveground assimilation and belowground utilization of carbon. Our observations in longleaf pine suggest that stored carbon reserves are sufficiently large such that the linkage between above- and belowground processes is obscured to the point that it seemingly does not exist. Ecosystems that experience frequent disturbance and preferentially allocate carbon to storage should therefore be more resilient and capable of maintaining belowground respiration during periods of minimal or nonexistent carbon assimilation. A corollary of the large belowground storage pools may be that these systems are not carbon limited, but rather, nutrient limited and nutrient cycling might be very tight in such systems.

A direct link between canopy and soil processes has been described in boreal forests (Högberg *et al.* 2001; Olsson *et al.* 2005) and both evergreen and deciduous temperate forests (Irvine, Law & Kurpius 2005; Liu *et al.* 2006; Moyano, Kutsch & Rebmann 2008) as well as for grasslands (Johnson *et al.* 2002; Bahn *et al.* 2008) and crops (Kuzyakov & Cheng 2001; Moyano, Kutsch & Schulze 2007). However, there are a number of factors—both biological and physical—that may influence the degree to which canopy and soil processes are coupled. These factors include: (i) plant height, which influences the transport time of photosynthate to the roots and the rhizosphere (Mencuccini & Hölttä 2010), (ii) vertical distribution of roots (Jackson *et al.* 1996), soil temperature and moisture (Hirsch, Trumbore & Goulden 2004; Jassal *et al.* 2004; Jassal *et al.* 2005), which affect CO_2 diffusivity through the soil, (iii) plant physiological processes, such as assimilation rates, phloem transport rates (Kozlowski 1992; Mencuccini & Hölttä 2010), and carbon sink/source patterns and phenological changes (Cisneros-Dozal, Trumbore & Hanson 2006; Högberg *et al.* 2010; Wingate *et al.* 2010), which will ultimately determine the supply of carbon to the soil, and (iv) environmental conditions, such as moisture stress (Nobel 2009; Ruehr *et al.* 2009), that affect physiological processes. We argue that life history traits related to ecosystem disturbance regimes and thus, allocation towards carbon storage, also influence the linkage between canopy photosynthesis and soil CO_2 efflux. Guo, Mitchell, and Hendricks (2004) hypothesized that the disparate results regarding the linkage between canopy photosynthesis and soil CO_2 efflux may be reconciled, at least in part, by considering the evolutionary history of a species with respect to disturbance regimes. For example, in forests with infrequent but catastrophic disturbances, where dominant individuals are destroyed and ecosystem recovery occurs via regeneration of new individuals from seed, extensive carbon storage would not confer a selective advantage and soil CO_2 efflux would be more tightly coupled with canopy photosynthesis. In contrast, in forests with frequent but less intense disturbances, where dominant individuals generally survive and repair damaged tissues or regenerate from root sprouts, carbon storage confers a selective advantage and may decouple the linkage between canopy photosynthesis and soil CO_2 efflux (Kosola *et al.* 2001; Langley, Drake & Hungate 2002; Guo, Mitchell & Hendricks 2004; Sims *et al.* 2007; Vargas, Trumbore & Allen 2009; Varner *et al.* 2009; Clarke, Lawes & Midgley 2010).

Conclusions

Our observations following the girdling of longleaf pine, a tree species with an evolutionary history of chronic disturbance via fire and large reserves of belowground stored carbon, are in contrast to those obtained in many girdling studies of tree species with evolutionary histories of infrequent, but catastrophic disturbance and suggest life history traits may influence the degree of coupling between canopy photosynthesis and soil CO_2 efflux. Results from this study further suggest that the connection between aboveground assimilation and belowground utilization of carbon is not simple and that a variety of factors, including evolutionary history, need careful consideration when trying to relate canopy activity to belowground CO_2 production. Further work assessing the impact of girdling on soil CO_2 efflux across a range of evolutionary histories and carbon storage pools—from chronically disturbed ecosystems where the dominant species characteristically maintain large belowground carbon storage pools to catastrophically disturbed ecosystems where the dominant species characteristically maintain small belowground carbon storage pools—would be enlightening. The extent that adaptations related to evolutionary history reconcile the variation in reports regarding the linkage between canopy photosynthesis and soil CO_2 efflux is currently clouded by a poor understanding of carbon storage pools and fluxes at ecosystem scales. Future investigations that address the patterns and mechanistic controls of non-structural carbohydrate accretion and depletion may provide valuable insight into carbon cycling dynamics, which is critical to more accurately assess and predict the impact of natural and anthropogenic disturbances in terrestrial ecosystems (Williams *et al.* 1997).

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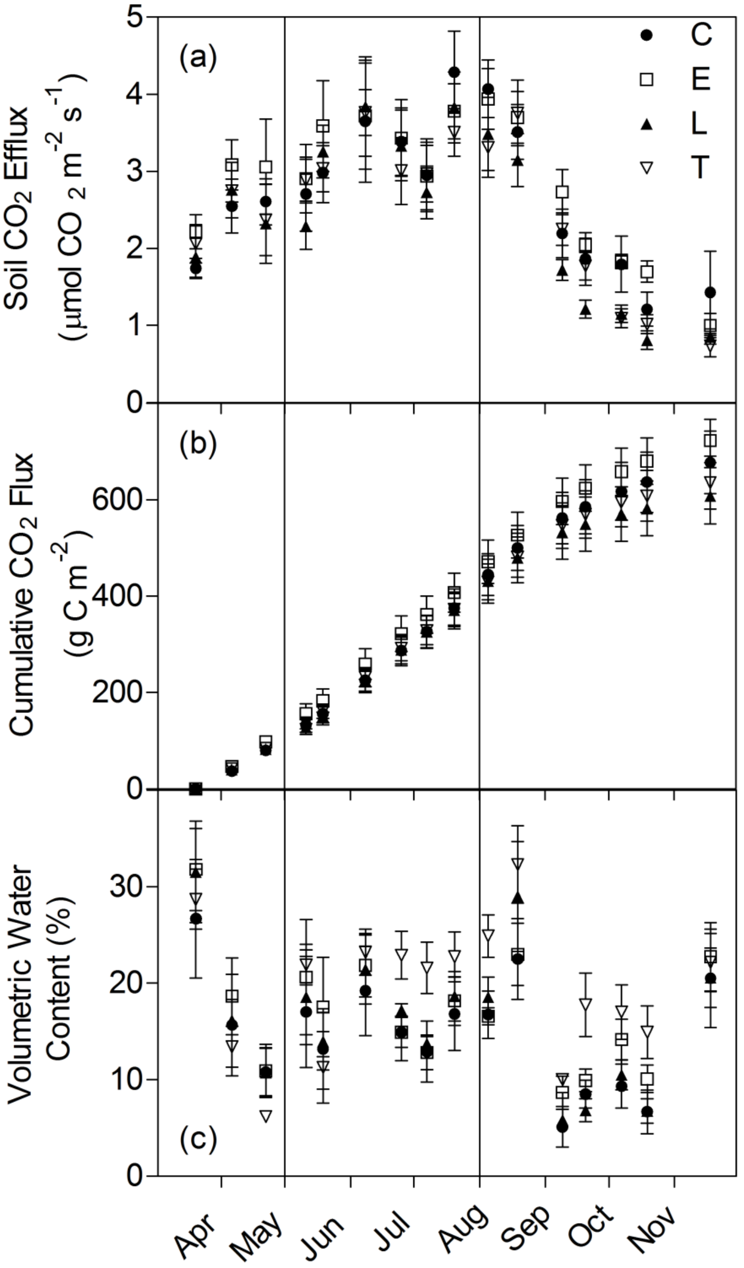

Figure 3.1. Mean ± SE (a) soil CO$_2$ efflux, (b) cumulative CO$_2$ flux, and (c) soil moisture for control (C), early girdled (E), late girdled (L), and trenched (T) treatments throughout the observation period. Vertical arrows indicate when girdling occurred. Trenching occurred at the same time as the first girdling treatment.

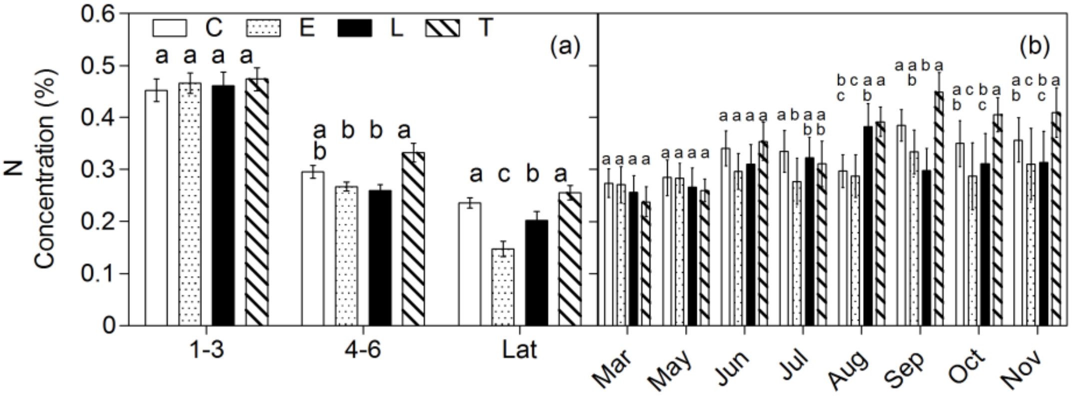

Figure 3.2. Treatment impacts on N concentration (mean ± SE) (a) of different root categories, and (b) throughout the observation period. Means sharing a letter within a given root category (a) or within a given month (b) are not significantly different (Fisher's LSD, $\alpha = 0.05$).

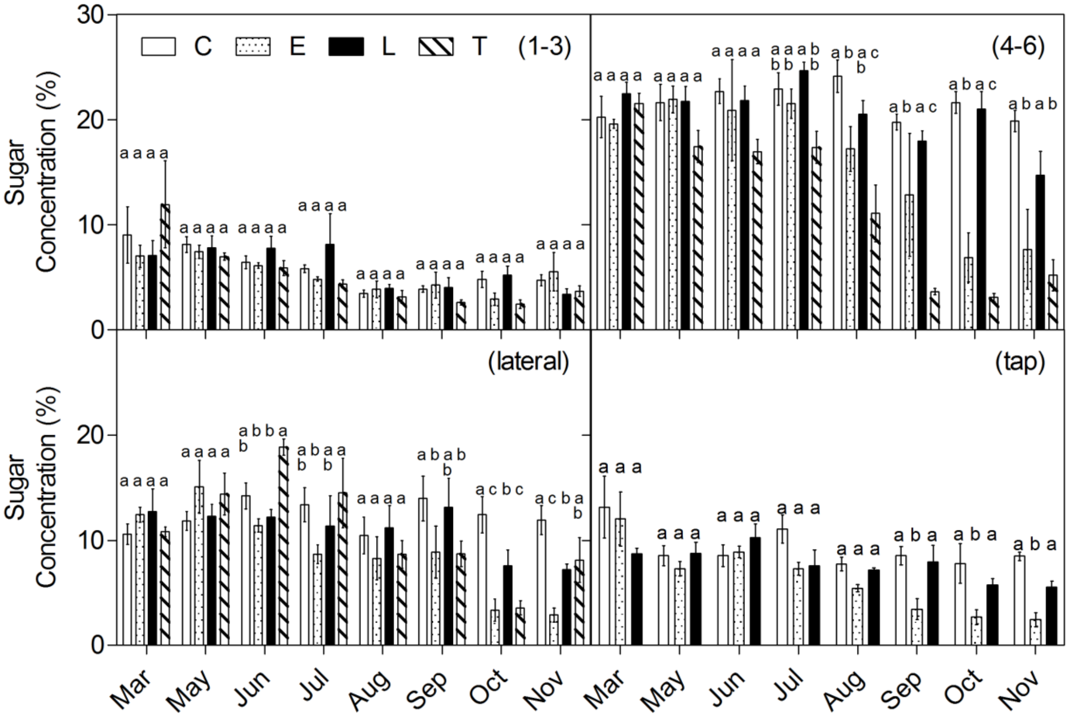

Figure 3.3. Treatment impacts on sugar concentration (mean ± SE) of root orders 1-3 (1-3), root orders 4-6 (4-6), lateral roots (lateral), and tap roots (tap) throughout the observation period. Means sharing a letter within a given a given month are not significantly different (Fisher's LSD, $\alpha = 0.05$).

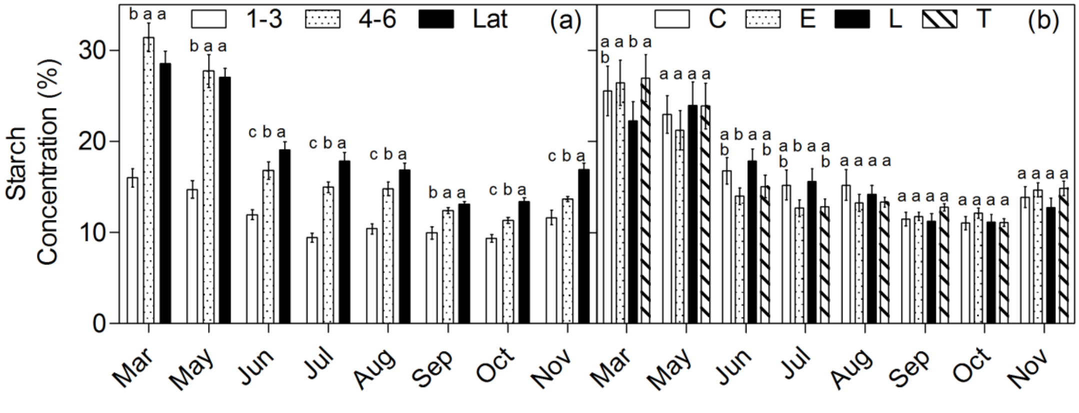

Figure 3.4. Starch concentration (mean ± SE) for (a) root categories and (b) treatments throughout the observation period. Means sharing a letter within a given a given month are not significantly different (Fisher's LSD, $\alpha = 0.05$).

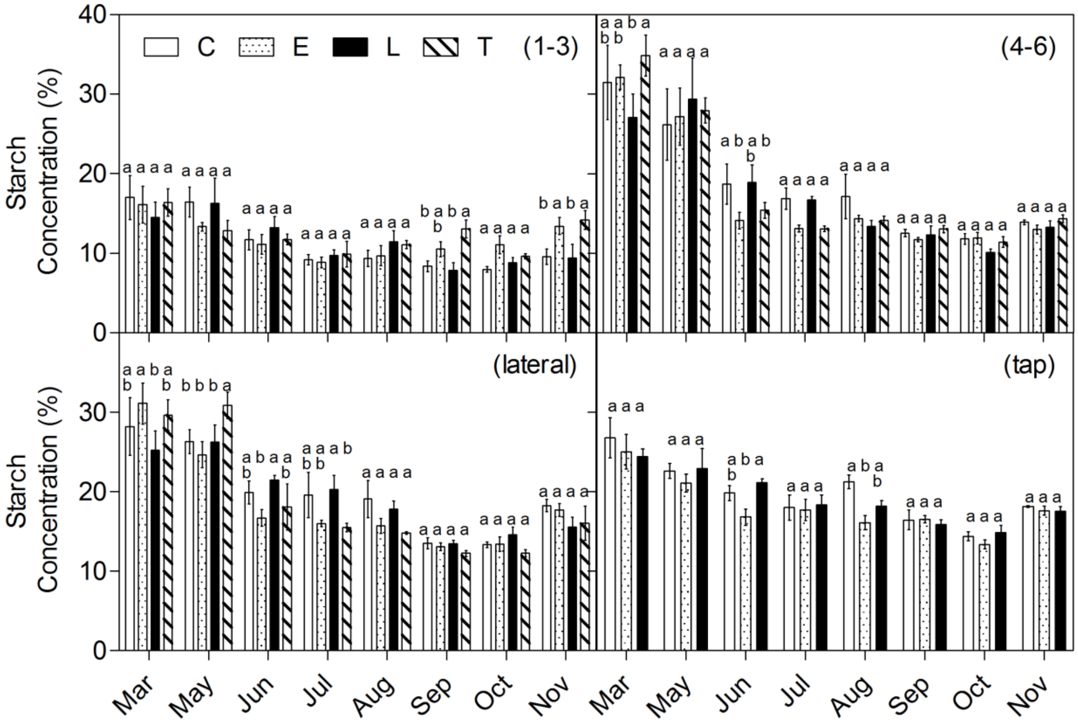

Figure 3.5. Treatment impacts on starch concentration (mean ± SE) of root orders 1-3 (1-3), root orders 4-6 (4-6), lateral roots (lateral), and tap roots (tap) throughout the observation period. Means sharing a letter within a given a given month are not significantly different (Fisher's LSD, $\alpha = 0.05$).

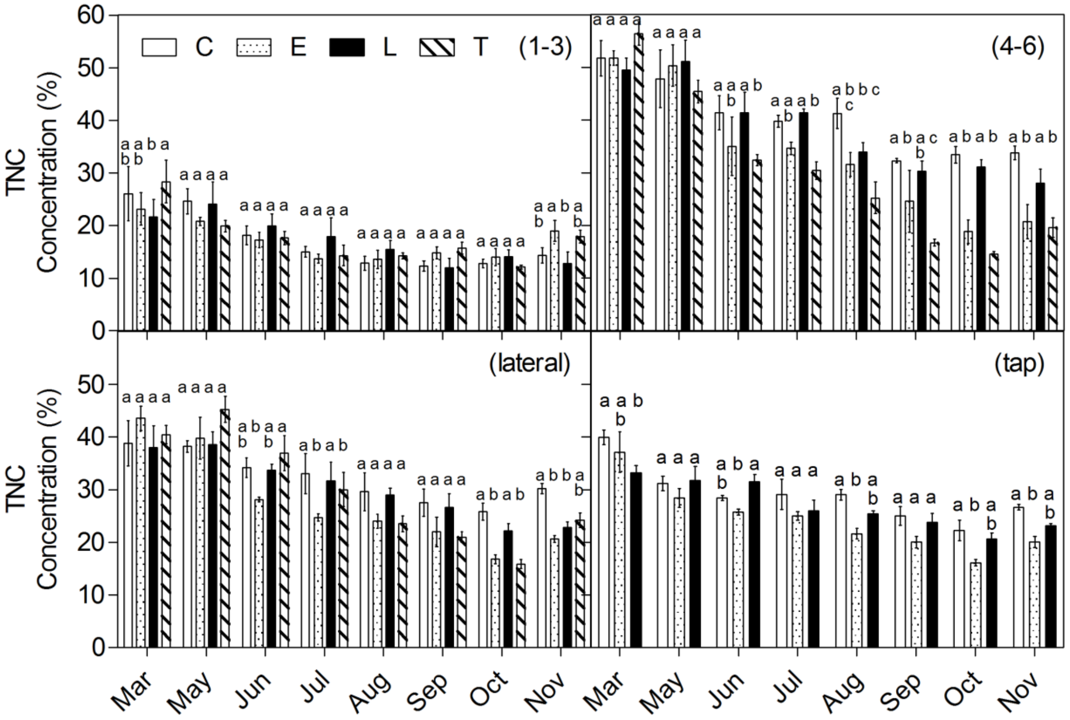

Figure 3.6. Treatment impacts on TNC (starch + sugar) concentration (mean ± SE) of root orders 1-3 (1-3), root orders 4-6 (4-6), lateral roots (lateral), and tap roots (tap) throughout the observation period. Means sharing a letter within a given a given month are not significantly different (Fisher's LSD, α = 0.05).

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CHAPTER 6

CONCLUSIONS

Summary of Previous Chapters

The previous chapters describe research that was conducted to improve our basic understanding of the mechanistic controls of soil CO_2 efflux—the largest annual terrestrial CO_2 flux from the biosphere to the atmosphere. Specifically, the research focused on understanding how the evolutionary history of a given species could influence the connection between canopy activity and root respiration, which is a major component of soil CO_2 efflux, in forest ecosystems. Both experiments used longleaf pine (*Pinus palustris* L.) as a model for species with an evolutionary history of chronic disturbance and experimentally manipulated the availability of recently assimilated carbon to the root system. The first experiment relied on canopy scorching to drastically—albeit temporarily—reduce the amount of leaf area and thus, canopy photosynthesis over two growing seasons. The second experiment relied on stem girdling and root trenching to completely and permanently terminate the belowground transport of photosynthate. Both experiments monitored treatment impacts on soil CO_2 efflux and a suite of root indices, such as nitrogen (N) and non-structural carbohydrate concentrations.

In the first experiment, scorching immediately reduced canopy photosynthesis to 20% of that in the control treatment; however, recovery occurred surprisingly rapidly as canopy photosynthesis in the scorch treatment reached 90% of that in the control treatment within one month. Soil CO_2 efflux was not impacted by the scorch treatment, nor was the cumulative flux of

CO_2 over the entire observation period. The scorch treatment did not impact fine root production, mortality, standing crop, or [N]. However, the scorch treatment did reduce total non-structural carbohydrate (TNC) concentrations of 3^{rd} and 5^{th} order roots after the 2007 treatment, but not after the 2006 treatment.

While the method and degree of defoliation employed in the first experiment has a direct ecological analogue in that longleaf pines occasionally experience and survive 100% needle scorch during natural and prescribed burning (Guo, Mitchell & Hendricks 2004; Guo *et al.* 2008), the disruption of current photosynthate allocation belowground was of a short duration due to the relatively rapid recovery of leaf area. Nonetheless, the process of leaf area reconstruction itself represented an additional drain on stored carbon pools and some of this carbon may have been mobilized from the belowground carbon storage pool. Alternatively, aboveground stored carbon pools may have been mobilized to satisfy belowground metabolic requirements.

In the second experiment, stem girdling and root trenching did not impact soil CO_2 efflux for at least six months after the perturbations. Similarly, the cumulative flux of CO_2 over the six month period was not impacted by either girdling or trenching. The [N], [sugar], and [starch] of the most metabolically active, lowest order roots were not impacted by girdling or trenching, but the [N] and [sugar] of higher order roots declined following perturbations.

The lack of response in soil CO_2 efflux following the disruption of canopy assimilation caused by scorching and the complete termination of belowground photosynthate availability caused by stem girdling and root trenching indicates that belowground autotrophic respiration can be sustained for extended periods in longleaf pine via stored carbon. In both experiments, we observed treatment-induced declines in the non-structural carbohydrate concentrations of higher order roots whereas the more metabolically active lower order roots generally maintained non-structural carbohydrate concentrations similar to that of the control treatment. Our results therefore suggest that stored carbon can buffer root respiration from disruptions in canopy photosynthesis by mobilizing stored carbon from higher order roots to lower order roots to maintain adequate substrate for metabolic demand.

Our observations following the disruption of canopy assimilation caused by scorching and the complete termination of belowground photosynthate availability caused by stem girdling and root trenching are in stark contrast to those obtained in girdling studies of tree species with evolutionary histories of infrequent, but catastrophic disturbance. Our results further suggest that life history traits may influence the degree of coupling between canopy photosynthesis and soil CO_2 efflux. Results from this study further suggest that the relationship between aboveground assimilation and belowground utilization of carbon is complicated and that a variety of factors, including evolutionary history, need careful consideration when trying to relate canopy activity to belowground CO_2 production. Further work assessing the impact of girdling on soil CO_2 efflux across a range of evolutionary histories and carbon storage pools—from chronically disturbed ecosystems where the dominant species characteristically maintain large belowground carbon storage pools to catastrophically disturbed ecosystems where the dominant species characteristically maintain small belowground carbon storage pools—would be enlightening. The extent that adaptations related to evolutionary history reconcile the variation in reports regarding the linkage between canopy photosynthesis and soil CO_2 efflux is currently clouded by a poor understanding of carbon storage pools and fluxes at ecosystem scales. Future investigations that address the patterns and mechanistic controls of non-structural carbohydrate accretion and depletion may provide valuable insight into carbon cycling dynamics, which is critical to more accurately assess and predict the impact of natural and anthropogenic disturbances in terrestrial ecosystems (Williams *et al.* 1997).

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