Physiological Mechanisms of Foliage Recovery after Spring or Fall Crown Scorch in Young Longleaf Pine (*Pinus palustris* Mill.)

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Abstract: We hypothesized that physiological and morphological responses to prescribed fire support the post-scorch foliage recovery and growth of young longleaf pine. Two studies conducted in central Louisiana identified three means of foliage regrowth after fire that included an increase in the gas exchange rate of surviving foliage for 3 to 4 months after fire. Saplings also exhibited crown developmental responses to repeated fire that reduced the risk of future crown scorch. Starch reserves were a source of carbon for post-scorch foliage regrowth when fire was applied in the early growing season. However, the annual dynamics of starch accumulation and mobilization restricted its effectiveness for foliage regrowth when fire was applied late in the growing season. As such, post-scorch foliage regrowth became increasingly dependent on photosynthesis as the growing season progressed. Additionally, the loss of foliage by fire late in the growing season interrupted annual starch dynamics and created a starch void between the time of late growing season fire and mid-summer of the next year. The occurrence of drought during both studies revealed barriers to foliage reestablishment and normal stem growth among large saplings. In study 1, spring water deficit at the time of May fire was associated with high crown scorch and poor foliage and stem growth among large saplings. We attribute this lag in stem growth to three factors: little surviving foliage mass, low fascicle gas exchange rates, and poor post-scorch foliage recovery. In study 2, May fire during a short window of favorable burning conditions in the tenth month of a 20-month drought also reduced stem growth among large saplings but this growth loss was not due to poor post-scorch foliage recovery. Application of this information to prescribed fire guidelines will benefit young longleaf pine responses to fire and advance efforts to restore longleaf pine ecosystems.

Keywords: crown architecture; drought; dry mass allocation; net photosynthesis; prescribed fire; starch dynamics; annual increment of stem growth; stomatal conductance; vapor pressure deficit

1. Introduction

Longleaf pine (*Pinus palustris* Mill.) was once a dominant tree species across the southeastern United States [1,2]. As industry advanced during the late nineteenth and early twentieth centuries, manufacturing and construction demands for lumber and naval stores were met, in part, by the steam-mechanized harvest of these old-growth longleaf pine forests [3]. Historically, the reoccurrence of low intensity fire was important to the sustained function of these fire climax ecosystems [4,5].

However, the natural occurrence of fire was suppressed and fire was excluded as a management tool in reforestation efforts [1,6]. In time, absence of fire across the landscape contributed to a change in *Pinus* dominance from longleaf pine to loblolly (*Pinus taeda* L.) and slash pines (*Pinus elliottii* Engelm.). Throughout the second half of the twentieth century, research to improve longleaf pine regeneration and restore longleaf pine ecosystems emerged [7–12]. These efforts, together with renewed use of prescribed fire and federal mandates to protect species intrinsic to longleaf pine ecosystems, set the stage for the America's Longleaf Restoration Initiative [13,14].

Longleaf pine seedlings and saplings may survive in a partially sunlit environment [15,16]. Under most conditions, however, longleaf pine requires a high level of sunlight to survive and become dominant in the forest canopy [6,17]. Competition control is necessary to achieve high sunlight because longleaf pine seedlings generally remain in the grass stage of development for 2 to 5 years [6,18]. Vertical stem growth is absent until the active growing stage begins when the stem reaches approximately 12 cm in height [19,20].

In young stands of longleaf pine, herbicides offer an effective option to control unwanted vegetation [21,22]. However, this method of vegetation management fails to perpetuate the diverse, natural ground-layer that benefits the flora and fauna of longleaf pine ecosystems [21,23–25]. In contrast, repeated fire keeps vegetative competition in check and promotes both timber and non-timber values in longleaf pine forests [26].

Wide-ranging stem growth responses to prescribed fire exist for longleaf pine. Growth losses after fire have been attributed to high fuel levels and fire intensities with the exclusion or delay of prescribed fire [27,28]. Regardless of fire return interval, high fire intensity risks death or injury of roots growing in duff or near the soil surface [29], and damage to xylem of the stem, branches, and shallow woody roots [30–32].

With repeated fire initiated at an early age and applied every 2 to 3 years, fuel accumulation is reduced, fire intensity is minimized, and growth loss by fire is absent or negligible. For example, when repeated fire in spring was initiated two years after planting, Haywood [21] found that longleaf pine growth was not negatively affected by five cycles of biennial fire. Similar results were observed elsewhere after three cycles of prescribed fire [33]. In a stem analysis of mature longleaf pines burned between 1976 and 1999, it was also reported that stand-level production over the rotation was not affected by fire despite the significant loss of annual stem increment in many years fire was applied [34]. In general, the annual stem increment of *Pinus* is sustained in the absence of foliage regrowth shortfalls after crown scorch or other means of defoliation [27,33–38].

Application of prescribed fire among vigorously growing longleaf pine seedlings and saplings may be necessary to insure high light availability and reduce establishment of woody competitors [39–41]. The stature of longleaf pine seedlings and saplings predisposes them to complete or nearly complete crown scorch. Longleaf pine has the potential to regrow scorched foliage by the end of the growing season after fire [42,43]. However, when stand environmental and climate conditions limit post-scorch foliage reestablishment, carbon endpoints such as growth and defense may suffer, and this risk is heightened during drought [32,44].

Knowledge of the environmental conditions and physiological processes that support post-scorch foliage recovery are needed to improve prescribed fire guidelines for young stands of longleaf pine and advance current efforts to restore longleaf pine ecosystems. Our present objective is a compilation of information from two studies that were established to investigate the physiological mechanisms of longleaf pine adaptation to fire. We hypothesized that there are physiological and morphological responses to repeated fire that sustain a carbon supply for foliage regrowth after a high level of crown scorch.

2. Materials and Methods

2.1. Study Sites

Study 1 was conducted at two sites in central Louisiana on the Calcasieu Ranger District of the Kisatchie National Forest, Rapides Parish, Louisiana, USA. At site 1 (31°6′ N, 92°36′ W), hereafter referred to as the upland site, a mixed pine-hardwood forest was harvested in 1996 and the area was roller-drum chopped and burned in 1997. At the time of study establishment, vegetation at the upland site was dominated by small hardwood trees, woody shrubs, and shade-tolerant herbaceous plants [45]. Soil at the upland site is a complex of Ruston fine sandy loam (fine-loamy, siliceous, semiactive, thermic Typic Paleudults), Malbis fine sandy loam (fine-loamy, siliceous, subactive, thermic, Plinthic Paleudults), and Gore very fine sandy loam (fine, mixed, active, thermic Vertic Paleudalfs). These soil series are moderately well, to well drained and very slowly, to moderately permeable with 0% to 12% slopes.

At site 2 (31°1′ N, 92°37′ W), hereafter referred to as the flatwoods site, a slash pine (*Pinus elliottii* Engelm.) plantation was harvested in the mid-1980s and the area was frequently prescribed burned to maintain a vegetation dominated by native bluestem grasses (*Schizachyrum* spp. and *Andropogon* spp.). In 1991, the area was sheared and windrowed, and then burned in 1993 and 1996 [46]. Soil at the flatwoods site is a complex of Malbis fine sandy loam and Beauregard silt loam (fine-silty, siliceous, superactive, thermic Plinthaquic Paleudults). The Beuaregard soil series is moderately well-drained, slowly permeable and has 0% to 5% slopes.

Study 2 was conducted in central Louisiana on the Winn Ranger District of the Kisatchie National Forest, Winn Parish, Louisiana, USA (32°3′ N, 92°51′ W). A loblolly pine plantation was harvested in early 2003 followed by chop and burn site preparation. The soil is Sacul fine sandy loam (fine, mixed, active, thermic Aquic Hapludults) which is moderately well-drained and slowly permeable with 2% to 25% slopes. Both studies are in the western Gulf coastal plain ecoregion with a ground layer potentially dominated by *Schizachyrium*, *Panicum*, and *Dichanthelium* grass species [47].

The climate at both study locations is humid subtropical. At study 1, regional 25-year (1980–2005) mean precipitation was 1479 mm and mean air temperatures in April through September and October through March were 24.1 °C and 12.8 °C, respectively [48]. Compared to the 25-year mean, annual precipitation during study 1 was 15% and 27% below average in 2003 and 2005, respectively, and 27% above average in 2004 (Figure 1a). Monthly mean air temperatures during study 1 were similar to the 25-year mean with the exception of January 2003 which was more that 20% cooler, and January 2005 which was more than 20% warmer than average conditions.

At study 2, regional 25-year (1988–2013) mean precipitation was 1421 mm and air temperatures in April through September and October through March were 24.7 °C and 12.0 °C, respectively [48]. The study was initiated during the eighth month of a prolonged drought across Winn Parish that began in May 2010 and continued through January 2012 [49]. A centrally located electronic weather station (HOBO Weather Logger, Onset Computer Corporation, Bourne, MA, USA) was used to record precipitation and air temperature at a 20 min interval in 2011 through 2013. Compared to the regional 25-year (1988–2013) mean, annual precipitation at study 2 was 53% lower in 2011, and 21% and 23% higher in 2012 and 2013, respectively (Figure 1b). Monthly mean air temperatures at study 2 were similar to the regional mean with the exception of January, March, and December 2012 which were more than 20% warmer than average conditions.
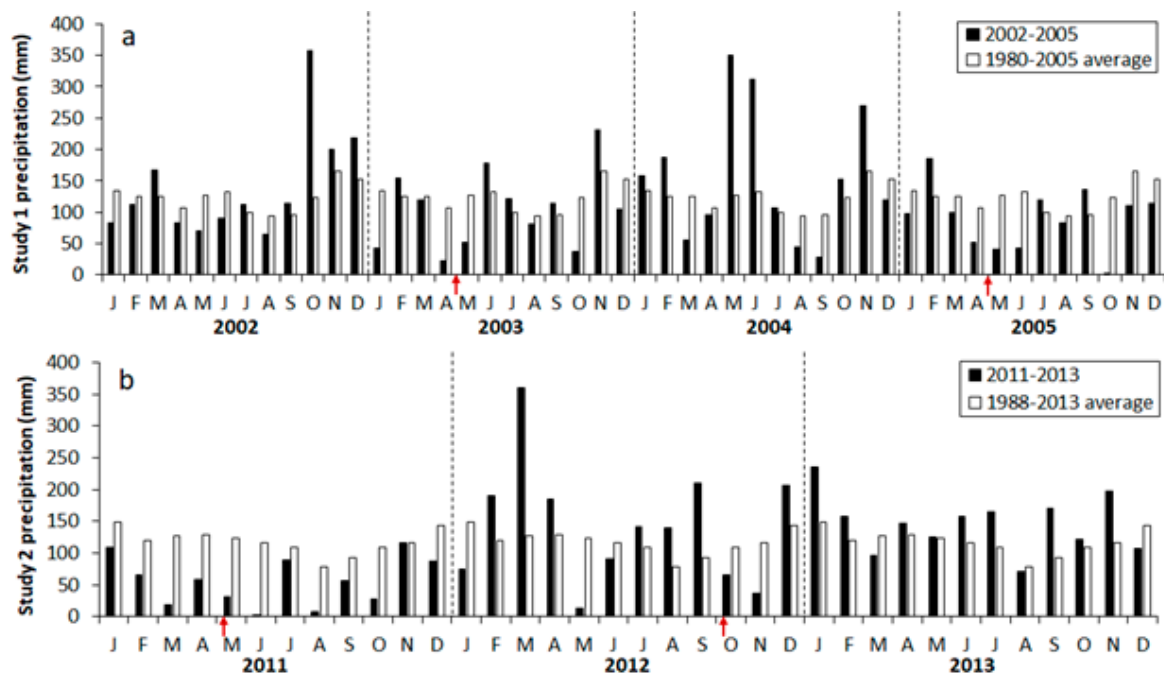
Figure 1. (**a**) Precipitation at study 1 in 2002 through 2005 and averaged between 1980 and 2005, and (**b**) at study 2 in 2011 through 2013 and averaged between 1988 and 2013. Arrows indicate prescribed fire application.

2.2. Study 1 Establishment

Study 1 used treatment plots among two prior studies established 5 years earlier on the upland and flatwoods sites in a randomized complete block experimental design with four blocks [21,46]. These previously established studies consisted of two blocks each at the upland and flatwoods sites. Container-grown longleaf pine seedlings from genetically improved Louisiana and Mississippi seed sources were grown by standard methods [7,50], and planted at the upland and flatwoods sites, respectively, at a spacing of 1.8 m × 1.8 m. Planting occurred in November 1997 at the upland site and March 1997 at the flatwoods site. Treatment plots (22 m × 22 m; 0.048 ha) contained 12 rows of 12 seedlings with the internal 8 rows of 8 seedlings as measurement plots.

In 2003, study 1 was established in a randomized complete block design with five blocks. Treatments were three of 10 treatments in the original two studies: (1) control (C)—no vegetation management activities after planting, (2) prescribed burn (SB)—plots were burned in spring every two or three years, and (3) herbicides (H)—herbicides were applied after planting to control herbaceous and woody vegetative competition. The SB plots were prescribed burned by a combination of backfires and strip headfires on 19 May, 1998 at the flatwoods site, and on 22 June, 2000, 21 May, 2003, and 26 May, 2005 at both sites. The H treatment at the upland site was banded applications of hexazinone in spring of 1998 and 1999 over unshielded seedlings and directed foliar spray of triclopyr to the foliage of arborescent shrubs in spring of 1998 and 1999 [21,46]. At the flatwoods site, the H plots were rotary tilled before planting in spring 1997 to reduce heavy grass cover. This was followed by banded applications of sethoxydim and hexazinone over unshielded seedlings in spring of 1997 and 1998 and directed foliar spray of triclopyr to foliage of arborescent shrubs in spring of 1998 and 1999 [45,46]. On the H plots of both sites, recovering brush was cut by hand one time in winter of 2001. Among the C, SB, and H treatment plots at each site in 2003, two blocks were identified at the upland site and three blocks were identified at the flatwoods site. Blocking was based on site location and the appearance of redoximorphic features indicative of drainage.

2.3. Study 2 Establishment

Container-grown longleaf pine from a Louisiana seed source were produced commercially (International Forest Co., Moultrie, GA, USA) and planted at a spacing of 1.8 m × 2.7 m in November 2004. The plantation was prescribed burned at age 4 years in April 2008. Two years later, study 2 was established by a randomized complete block design with three blocks. Blocks were delineated by topography and proximity to roads. Three treatment plots (1.4–4.3 ha) were identified in each block and subsequently partitioned by roads and plowed fire lanes. Treatments were (1) control (C)—no vegetation management activities after study establishment, (2) prescribed burned in spring (SB)—plots were burned by backfires on 16 May, 2011, and (3) prescribed burned in fall (FB)—plots were burned by backfires on 23 October, 2012. Three permanent fixed radius subplots (200 m^2) were randomly delineated in each of the nine treatment plots.

2.4. Fascicle Gas Exchange and Predawn Water Potential

2.4.1. Study 1

From dormant season total sapling heights in each treatment plot at the beginning of 2003, 2004, and 2005, three saplings within 10% of mean total height were randomly identified for fascicle gas exchange and predawn water potential measurements. Among these saplings, the midpoint of live crown length was determined and each half of the crown was identified as the upper or lower crown. Fascicle gas exchange was measured in the lower crown and distal one-third of the upper crown in the morning (09:00–12:00) and afternoon (12:30–15:30) during two or three measurement intervals in 2003, 2004, and 2005. In 2003 and 2005, one measurement interval occurred in May before SB and two measurement intervals occurred after SB. Measurement intervals consisted of three consecutive sunny days with two blocks at the upland and flatwoods sites measured on the first two days and the fifth block at the flatwoods site measured on the third day. To minimize time-of-day effects in the morning and afternoon, the gas exchange of one of the three measurement saplings per plot was recorded across the plots to be measured on that day, followed by two additional series of sapling measurements across the plots to be measured on that day. On measurement days, one mature fascicle from the upper crown of these three saplings was detached between 05:00 and 06:30 and enclosed in an ice chest. Fascicle predawn water potentials (PWP) were measured by a pressure chamber (PMS Instrument Co., Corvallis, OR, USA) within one hour of fascicle detachment.

Within 3 min of detaching two fascicles from the south side of saplings, light saturated net photosynthesis (A$_{sat}$; µmol CO$_2$ m^{-2} s^{-1}), stomatal conductance to water vapor (g$_w$; mol m^{-2} s^{-1}), and vapor pressure deficit (VPD, kPa) were quantified by a portable photosynthesis system (Model 6400, Li-Cor, Inc., Lincoln, NE, USA) and a standard 6 cm^2 needle cuvette equipped with a light-emitting diode (LED) light source (Model 6400-02B, Li-Cor, Inc., Lincoln, NE, USA). Tests before gas exchange measurements indicated that similar to loblolly pine [51], longleaf pine fascicles maintain normal gas exchange for at least three minutes after detachment. An average of 20 1-sec measurements was recorded after equilibration. Across eight measurement intervals, gas exchange was measured at ambient air temperature and relative humidity, a photosynthetically active radiation (PAR) of 1400 µmol m^{-2} s^{-1}, a CO$_2$ concentration of 400 ppm, and a flow rate of 200–300 µmol m^{-2} s^{-1}. Sampled fascicles were from the most recently mature flush and an effort was made to utilize foliage from lateral branches rather than the stem or terminal shoot. Fascicle surface areas in the cuvette were determined by the displaced needle volume method [52] and A$_{sat}$ and g$_w$ were expressed by fascicle surface area.

2.4.2. Study 2

Among saplings appearing to be of average stature in each fixed radius subplot, two were randomly chosen for measurements of fascicle gas exchange. Different saplings were chosen at each measurement interval. Morning (08:30–12:00) measurements were conducted 3, 5, and 12 months after SB and afternoon (12:30–16:00) measurements were conducted 12 months after SB. Morning

and afternoon measurements were conducted 8, 9, and 10 months after FB. Measurements in each interval were conducted by subplot and block using a portable photosynthesis system (Li-Cor, Inc. Model 6400) and a standard 6 cm^2 needle cuvette equipped with a LED light source (Li-Cor, Inc. Model 6400-02B). Two fascicles from the most recently mature flush of a lateral branch were detached, immediately placed in the needle cuvette, allowed to equilibrate, and A_{sat}, g_w, and VPD recorded at three 10-sec intervals. Total time from detachment to measurement was less than 3 min. Gas exchange was measured at ambient air temperature and relative humidity, PAR of 1800 μmol m^{-2} s^{-1}, a CO_2 concentration of 400 ppm, and a flow rate of 300 μmol m^{-2} s^{-1}. Fascicles were immediately removed from the cuvette and their diameter was measured to the nearest 0.01 mm. Fascicle surface area in the cuvette was estimated using fascicle diameter, number of needles per fascicle, and needle length in the cuvette [53]. Mean A_{sat} and g_w among the three measurements were expressed by fascicle surface area.

2.5. Woody Root and Terminal Shoot Starch

2.5.1. Study 1

At 10 sampling intervals between May 2003 before SB and April 2006, three to five small woody roots, 2–5 mm in diameter and 3 cm to 5 cm in length, were excavated with hand tools from the 0 cm to 10 cm soil depth approximately 0.5 m from the stem of the trees used for fascicle physiology measurements. Excavation of small woody roots allowed periodic starch assessment of saplings used for gas exchange measurements. Roots were washed with cold tap water, pooled by plot, transported to the laboratory in an ice chest containing dry ice, and frozen at −40 °C. Frozen root samples were lyophilized, ground through a 1-mm^2 mesh sieve, and analyzed (Dairyland Laboratories, Inc. Arcadia, WI, USA) for starch concentration using a modified Back Knudsen enzymatic assay [54]. Starch concentration was expressed as percentage of woody root dry mass.

2.5.2. Study 2

At 11 sampling intervals between February 2011 and June 2012, one longleaf pine sapling from each 200 m^2 C and SB subplot was felled at the groundline. Saplings were felled in a similar manner at 10 sampling intervals between January 2012 and October 2013 from each 200 m^2 C and FB subplot.

Harvested saplings appeared healthy and were of average stature by visual comparison among subplot saplings. The apical 15 cm of the terminal shoot was excised, and fascicles were removed and discarded. Terminal shoots were transported to the laboratory in an ice chest containing ice, frozen at −20 °C, lyophilized, and ground through a 1-mm^2 mesh sieve. Terminal shoot samples were analyzed (Dairyland Laboratories, Inc. Arcadia, WI, USA) for total starch concentration using the acid hydrolysis method [55] and starch concentration was expressed as percentage of terminal shoot dry mass. Prior to analyses of variance, data were transformed to square root values to establish normally distributed experimental errors.

2.6. Crown Architecture, Aboveground Biomass Allocation, and Growth

2.6.1. Study 1

From dormant season total sapling heights in each treatment plot at the beginning of 2003, 2004, and 2005, three one-third percentiles of total height per treatment plot were identified. One sapling was randomly chosen and harvested by one-third percentile from the two rows of saplings outside measurement plots. Among these saplings, groundline diameter was measured (d_g, cm) by handheld calipers. After saplings were felled at the groundline, branches were separated from the stem, foliage was separated from the branches, and all tissues were dried to equilibrium at 70 °C and weighed. All dry mass data except stem dry mass were transformed to square root values to establish normally distributed experimental errors prior to analyses of variance.

Among felled saplings in 2004 and 2005, total height (*h*, cm) from the groundline to the tip of the terminal bud, height from the groundline to the base of the live crown (HBLC), and live crown length were measured by a tape measure. The stem position representing the live crown midpoint was determined and numbers of live first-order lateral branches below (lower crown) and above (upper crown) the live crown midpoint were counted. Before dry mass determinations in 2004 and 2005, foliage and branches were partitioned by upper or lower crown. Live crown ratio (LCR) was calculated by the ratio of live crown length and total height, and ratios of upper and lower crown first-order lateral branch number (BNR) and dry mass (BMR) were calculated.

Among all living trees per treatment plot, d_g was measured by handheld calipers and *h* was measured by a height pole during the dormant season in early 2003, 2004, 2005, and 2006. Outside-bark stem volumes were estimated by the equation, $d_g^2 h$. Annual increments of stem growth per sapling (AI) in 2003, 2004, and 2005 were calculated by subtraction and expressed as dm^3.

2.6.2. Study 2

Stem, branch, and foliage biomass by total height class of C, SB, and FB saplings were quantified in September 2011, 2012, and 2013 [43]. At the beginning of study 2, mean total height among subplot saplings was 1.4 m with a coefficient of variation of 59%. These saplings were in the active growing stage of development characterized by minimal first-order lateral branch development and high variation in sapling stature [6]. Therefore, branch assessments were limited to total branch dry mass by sapling.

Similar to study 1, d_g and *h* were measured among subplot individuals that had emerged from the grass stage of development (i.e., at least 12 cm in height). Measurements were done during the dormant season in early 2011, 2012, and 2013 on the C, SB, and FB subplots. Outside-bark stem volumes were estimated by $d_g^2 h$ and AI per sapling were calculated by subtraction and expressed as dm^3.

2.7. Crown Scorch

In study 1, three saplings within 10% of mean total height by treatment plot were randomly identified and their percentage of scorched height was estimated one month after May fire in 2003 and 2005. In study 2, the percentage of scorched tree height among the two saplings per subplot chosen for gas exchange measurements was estimated 3 months after May 2011 fire and 8 months after October 2012 fire.

2.8. Statistical Analyses

2.8.1. Study 1

Fascicle physiology variables (A_{sat}, g_w, VPD, PWP) were expressed as the mean among three saplings per plot. By year and diurnal period, analyses of variance of mean A_{sat}, g_w, and VPD were conducted using a randomized complete block split-plot in time and space design. By year, analyses of variance of mean PWP were conducted by a randomized complete block split-plot in time design. The whole-plot effect was vegetation management treatment (C, no vegetation management; SB, prescribed fire in May 2003 and 2005; H, herbicide application during establishment). The time split-plot effect was measurement interval (two in 2004 and three each in 2003 and 2005). The space split-plot effect was crown level (lower or upper). Across 10 sampling intervals between May 2003 and April 2006, plot-level small woody root starch concentrations were evaluated by a randomized complete block split-plot in time design with vegetation management treatment as the whole-plot effect and sampling interval as the split-plot effect.

Using a randomized complete block design, analyses of covariance were applied to annual aboveground biomass and crown and branch variables using groundline diameter at the time of felling as the covariate. Similarly, analyses of covariance were applied to mean sapling annual increment of stem growth by sapling in 2003, 2004, and 2005 using 2003 mean plot dormant season d_g as the

covariate. All analyses of variance and covariance contained five blocks and three levels of vegetation management treatment.

Ordinary least squares regression was used to evaluate the rate of starch mobilization from small woody roots during four monthly sampling periods between May and August 2005 in response to three levels of vegetation management treatment. This method was also applied to d_g and foliage, branch, and stem dry masses among harvested saplings in 2005 to assess differences in regression parameters between the C and either the SB or H saplings. Mean square errors (s^2) between pairs of regression lines were evaluated for homogeneity [56], and when s^2 were homogenous at an α-level of 0.05, the regression parameters of pairs of lines were evaluated by the general linear test and *F*-statistic [57].

2.8.2. Study 2

Fascicle physiology variables (A_{sat}, g_w, VPD) were expressed as the mean of six measurements by plot (two per subplot) and terminal shoot starch concentration was expressed as the mean of three measurements by plot (one per subplot). Analyses of variance of mean A_{sat}, g_w, and VPD utilized a randomized complete block split-plot in time design. The whole-plot effect was the absence or presence of prescribed fire. The time split-plot effect was measurement interval. Mean terminal shoot starch concentration was evaluated by analyses of variance using a randomized complete block split-plot in time design among 11 sampling intervals in response to May 2011 fire and 10 sampling intervals in response to October 2012 fire. All analyses of variance and covariance contained three blocks and two levels of prescribed fire treatment: control (C) and either May 2011 fire (SB) or October 2012 fire (FB).

Groundline diameter and total height data were compromised by the loss of permanent tree tag data in 11 subplots in 2011 and one subplot in 2013. This prevented estimates of stem volume and AI on the compromised subplots and prohibited analyses of variance of AI. Alternatively, d_g and AI averaged among subplot saplings was calculated for the uncompromised subplots (2011: seven C subplots, five SB subplots, and four FB subplots; 2012: eight C subplots, nine SB subplots, and nine FB subplots). In 2011 and 2012, uncompromised subplots contained an average of 35 saplings (18 to 45 per subplot) and 33 saplings (14 to 44 per subplot), respectively. Using data from the uncompromised subplots, mean d_g in January 2011 and mean AI in 2011 were subjected to ordinary least squares regression to assess the effect of May 2011 fire on AI. Similarly, mean d_g in January 2012 and mean AI in 2012 were subjected to ordinary least squares regression to assess the effect of May 2011 fire or October 2012 fire on AI. Mean square errors (s^2) of pairs of regression lines were evaluated for homogeneity [56], and when s^2 were homogenous at an α-level of 0.05, the regression parameters of pairs of lines were evaluated by the general linear test and *F*-statistic [57].

Analyses of variance or covariance and ordinary least squares regression in both studies were conducted by the procedures of SAS statistical software (SAS Institute, Version 9.1, Cary, NC, USA). Among analyses of variance or covariance and ordinary least squares regression, the *F*-statistic was considered significant at an α-level of 0.05. Means were assessed by the Tukey–Kramer test and considered significantly different at an α-level of 0.05. Two co-occurring conditions defined marginal significance. These were an *F*-statistic or mean separation test that was significant at an α-level of 0.10 and a trend that was corroborated elsewhere in the data by significance at an α-level of 0.05.

3. Results

3.1. Fascicle Gas Exchange and Predawn Water Potential

3.1.1. Study 1

In the two years that May fire was applied, vegetation management treatment (VMT) and measurement interval had significant main and interaction effects on morning and afternoon A_{sat} and g_w (Table 1). Across the three measurement intervals in 2003, morning A_{sat} (Figure 2a) and g_w (Figure 2b) were significantly greater on the SB plots compared to the H plots by 12% and 25%,

respectively. Mean morning A_{sat} in 2003 was 16% less on day 128 compared to days 211 and 267 and this effect is attributed to a rainfall deficit in April and May.

**Table 1.** Probabilities of a greater *F*-value associated with diurnal values of upper and lower crown fascicle net photosynthesis (A_{sat}), stomatal conductance (g_w), vapor pressure deficit (VPD), and predawn fascicle water potential (PWP) in study 1 among longleaf pine saplings in 2003, 2004, and 2005 in response to three vegetation management treatments.

Source of Variation	Year	df [a]	Morning			Afternoon			PWP
			A_{sat}	g_w	VPD	A_{sat}	g_w	VPD	
Block		4	0.2619	0.1046	<0.0001	0.0224	0.3152	0.7769	0.7682
Treatment (T) [b]		2	0.0334	0.0144	0.0008	<0.0001	0.0007	0.0543	0.0446
Date (D) [c]		2	0.0004	0.0764	<0.0001	0.0022	0.0163	<0.0001	<0.0001
Crown (C) [d]	2003	1	<0.0001	<0.0001	0.0034	<0.0001	<0.0001	0.0001	—
D × T		4	0.5824	0.1659	0.2461	0.0222	0.0295	0.5265	0.9349
C × T		2	0.3026	0.3574	0.5679	0.2142	0.3391	0.5570	—
C × D		2	0.4768	0.5555	0.4667	0.0505	0.7081	0.6964	—
C × D × T		4	0.8174	0.8462	0.9475	0.0933	0.3655	0.9640	—
Block		4	0.0007	0.0008	0.2490	0.0888	0.0056	<0.0001	0.4212
T		2	0.1412	0.0805	0.0739	0.0598	0.0139	0.4548	0.1725
D		1	0.0834	0.0005	<0.0001	0.0007	0.0003	<0.0001	0.4709
C	2004	1	<0.0001	0.0045	0.0468	<0.0001	<0.0001	0.0448	—
D × T		2	0.5608	0.4691	0.4337	0.1240	0.011	0.7164	0.4025
C × T		2	0.4300	0.9294	0.9058	0.3231	0.3598	0.8199	—
C × D		1	0.2035	0.1480	0.7478	0.0275	0.0053	0.5197	—
C × D × T		2	0.9017	0.7219	0.9323	0.6726	0.9435	0.9924	—
Block		4	0.2028	0.4795	0.0001	0.0004	0.0029	<0.0001	0.2760
T		2	0.0020	0.0026	0.0209	<0.0001	<0.0001	<0.0001	0.0002
D		2	<0.0001	0.4142	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
C	2005	1	0.0003	0.005	0.2275	0.0697	0.1400	0.4631	—
D × T		4	<0.0001	<0.0001	0.0494	0.0007	<0.0001	0.0230	0.1091
C × T		2	0.3355	0.5853	0.6639	0.3167	0.2953	0.8039	—
C × D		2	0.0532	0.4613	0.7627	0.1478	0.5991	0.8438	—
C × D × T		4	0.4185	0.5085	0.9511	0.2122	0.2760	0.9456	—

[a] Degrees of freedom. [b] Treatments were no vegetation management activity after planting, prescribed fire on 22 June, 2000, 21 May, 2003, and 26 May, 2005, or herbicide control of herbaceous and woody vegetative competition. [c] Two measurement intervals occurred in 2004. Three measurement intervals occurred in 2003 and 2005 with one before and two after each May prescribed fire application. [d] Measurements of A_{sat} and g_w were conducted with fascicles collected below the midpoint of live crown length (lower crown) and in the upper one-third of the live crown above its midpoint (upper crown). For measurement of PWP, fascicles were collected between 05:00 and 06:30 from the portion of the upper crown accessible while standing next to the sapling.

Interaction between VMT and measurement interval significantly affected afternoon A_{sat} and g_w in 2003 and both morning and afternoon A_{sat} and g_w in 2005 (Table 1). Morning A_{sat} during the measurement interval after May 2005 prescribed fire was 94% greater on the SB plots compared to the C and H plots (Figure 2e). A similar afternoon effect was observed after May prescribed fires in 2003 (Figure 2c), and 2005 (Figure 2g) with 32% and 68% greater A_{sat}, respectively, on SB plots compared to C and H plots.

The g_w responses to interaction between VMT and measurement interval in 2003 and 2005 were similar to those of A_{sat}. On the measurement date after prescribed fire in 2003, afternoon g_w was 46% greater on the SB plots compared to the C and H plots, and this effect was also observed during the subsequent 2005 measurement interval on the SB and H plots (Figure 2d). In 2005, the magnitude of this effect appeared larger than in 2003 with 189% and 210% greater g_w in the morning (Figure 2f) and afternoon (Figure 2h), respectively, on the SB plots compared to the C and H plots.
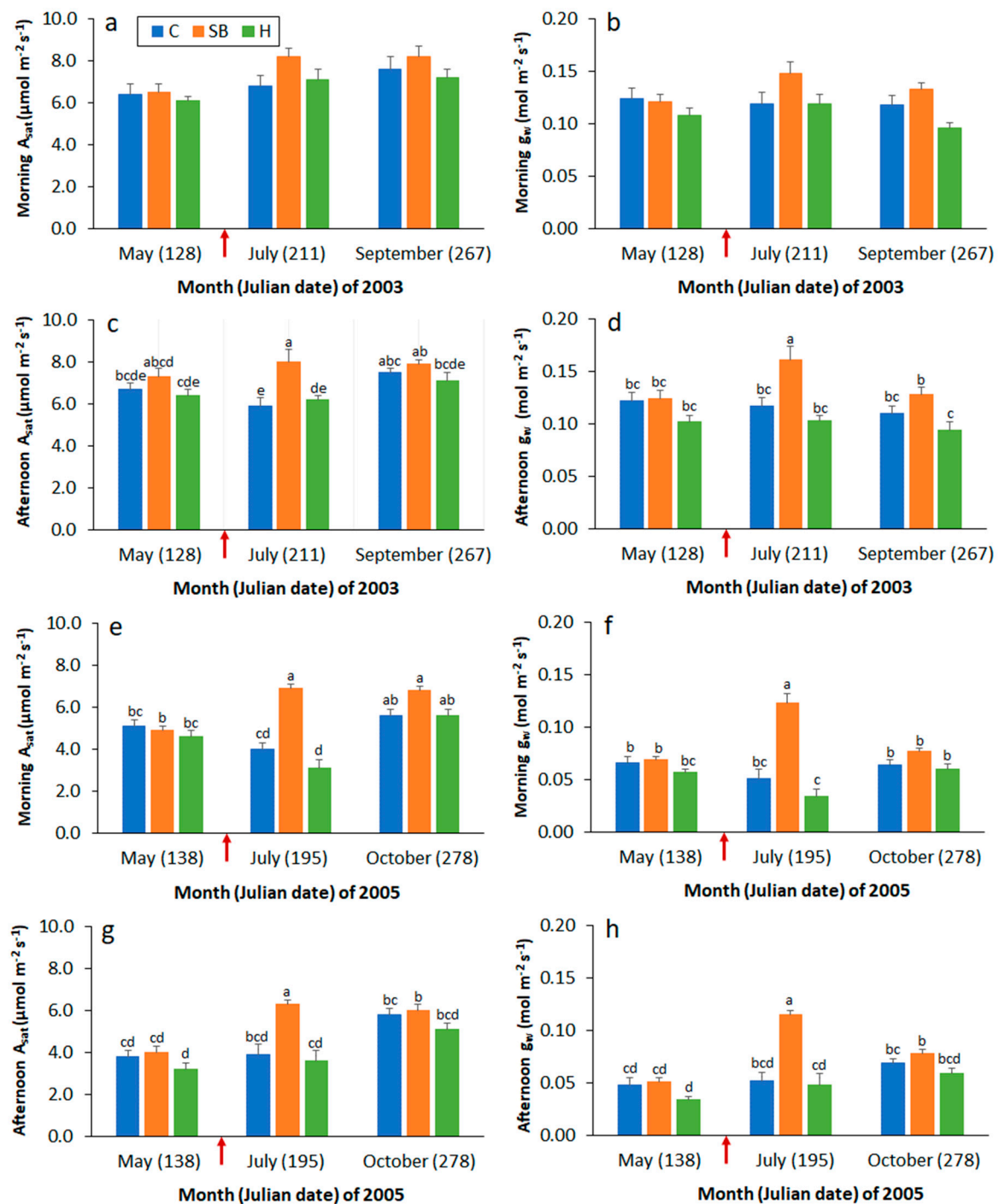
Figure 2. Mean fascicle-level net photosynthesis (A_{sat}) and stomatal conductance (g_w) among longleaf pine saplings in the (**a**,**b**) morning and (**c**,**d**) afternoon of 2003, and in the (**e**,**f**) morning and (**g**,**h**) afternoon of 2005 at study 1 in response to three vegetation management treatments (VMT) (C: no vegetation management activity after planting, SB: prescribed fire in June 2000, and May 2003 and 2005, H: herbicide control of herbaceous and woody vegetative competition). Arrows indicate prescribed fire application. Error bars represent the standard error of the mean. By year and diurnal period, A_{sat} and g_w means associated with a different lower-case letter are significantly different at the 0.05 level.

Fascicle gas exchange was not affected by VMT in the year without prescribed fire with one exception; afternoon g_w was significantly affected by interaction between VMT and measurement interval. On day 111 of 2004, afternoon g_w was 33% greater on the SB plots (0.125 mol m^{-2} s^{-1})

compared to the C and H plots (0.0.094 mol m^{-2} s^{-1}), but this effect was not observed on day 201 of 2004.

Morning A_{sat} and g_w in 2003, 2004, and 2005 and afternoon A_{sat} and g_w in 2003 were significantly affected by crown level as a main effect (Table 1). Across all three years, morning A_{sat} and g_w averaged 16% and 19% greater, respectively, in the upper crown compared to the lower crown. Afternoon A_{sat} and g_w in 2003 were 15% and 24% greater, respectively, in the upper crown compared to the lower crown. In 2004, interaction between crown level and measurement interval significantly affected afternoon A_{sat} and g_w. On day 111 of 2004, g_w was 42% greater in the upper crown (0.125 mol m^{-2} s^{-1}) compared to the lower crown (0.088 mol m^{-2} s^{-1}), but on day 201 of 2004, upper and lower crown g_w were equivalent (0.121 mol m^{-2} s^{-1}). A similar significant interaction effect on A_{sat} that was smaller in magnitude was observed in 2004. Afternoon A_{sat} and g_w in 2005 were not significantly affected by crown level as a main or interaction effect.

In 2003 and 2004, morning and afternoon VPD were significantly greater by 6% to 8% in the lower crown compared to the upper crown, but in 2005 VPD was not significantly affected by crown position (Table 1). In the year between May fires, VPD was not significantly affected by VMT, but in the two years that May fire was applied, VMT had a significant effect on morning VPD. Across the three measurement intervals in 2003, SB saplings experienced a significantly lower morning VPD (1.56 ± 0.04 kPa) compared to C (1.68 ± 0.05 kPa) and H saplings (1.70 ± 0.05 kPa). In 2005, significant interaction between VMT and measurement interval resulted in 19% lower morning VPD among SB saplings (1.48 ± 0.06 kPa) compared to C (1.77 ± 0.08 kPa) and H (1.89 ± 0.11 kPa) saplings on day 195. Before and after day 195 of 2005, morning VPD were similar among C, SB, and H saplings. Afternoon VPD was not affected by VMT in 2003. In 2005, significant interaction between VMT and measurement interval resulted in 16% lower afternoon VPD among SB saplings (2.69 ± 0.06 kPa) compared to C (3.22 ± 0.10 kPa) and H (3.20 ± 0.13 kPa) saplings on day 195, but similar afternoon VPD among C, SB, and H saplings on days 138 and 278.

Measurement interval had a significant effect on PWP in 2003 and 2005 (Table 1). In 2003, values of PWP on day 128 were significantly less than those on days 211 and 267, and in 2005, PWP on days 138 and 195 were significantly less than that on day 278. Low PWP on day 128 of 2003 is attributed to 79% and 60% less than normal precipitation in April and May, respectively, and low PWP on days 138 and 195 of 2005 is attributed to 52%, 68%, and 68% less than normal precipitation in April, May, and June, respectively. In the two years that May fire was applied, fascicle PWP was significantly affected by VMT. Across the three 2005 measurement intervals, PWP was significantly lower on the C and H plots (−0.63 MPa) compared to the SB plots (−0.45 MPa), and in 2003, PWP was lower on the H (−0.41 MPa) plots compared to the C and SB plots (−0.34 MPa) at the marginally significant α-level of 0.10.

3.1.2. Study 2

After prescribed fire in May 2011, morning A_{sat} and g_w were significantly affected by interaction between prescribed fire treatment and measurement interval (Table 2). Values of A_{sat} and g_w were significantly less on day 298 of 2011 than on day 150 of 2012 due to a rainfall deficit throughout most of 2011. By measurement interval, prescribed fire treatment in May 2011 did not affect morning A_{sat} (Figure 3a). However, on day 222 of 2011 and day 150 of 2012, morning A_{sat} was similar on the SB plots, but significantly different on the C plots. A similar morning g_w response to May 2011 fire was observed between day 222 of 2011 and day 150 of 2012 (Figure 3b).

Table 2. Probabilities of a greater *F*-value associated with mean diurnal observations of sapling longleaf pine fascicle net photosynthesis (A_{sat}), stomatal conductance (g_w), and vapor pressure deficit (VPD) in study 2 at three measurement intervals after prescribed fire in May 2011 or October 2012.

| Source of Variation	df [a]	Morning			Afternoon		
		A_{sat}	g_w	VPD	A_{sat}	g_w	VPD
May 2011 prescribed fire							
Block	2	0.2299	0.8771	0.0254	0.6009	0.2386	0.3983
Treatment (T) [b]	1	0.2019	0.3036	0.1118	0.4243	0.8215	0.9159
Date (D) [c]	2	<0.0001	<0.0001	0.0018	—	—	—
T × D	2	0.0437	0.0232	0.2143	—	—	—
October 2012 prescribed fire							
Block	2	0.7977	0.0014	0.0060	0.3610	0.0005	0.0422
T	1	0.9146	0.8036	0.0239	0.1455	0.0003	<0.0001
D	2	0.1283	0.0002	<0.0001	0.0684	<0.0001	0.0010
T × D	2	0.9167	0.7629	0.7436	0.6473	0.1471	0.2369

[a] Degrees of freedom. [b] Treatments were the absence or application of prescribed fire on 16 May, 2011 (Julian date 136) and the absence or application of prescribed fire on 23 October, 2012 (Julian date 296). [c] Morning measurements were conducted during three measurement intervals after 16 May, 2011 fire (Julian dates 222 and 298 of 2011, and 150 of 2012 which were 3, 5, and 12 months post-fire) and during three measurement intervals after 23 October, 2012 fire (Julian dates 150 of 2012, and 155 and 183 of 2013 which were 8, 9, and 10 months post-fire). Afternoon measurements were conducted on Julian date 150 of 2012 after May 2011 fire and on Julian dates 150 of 2012, and 155 and 183 of 2013 after October 2012 fire.
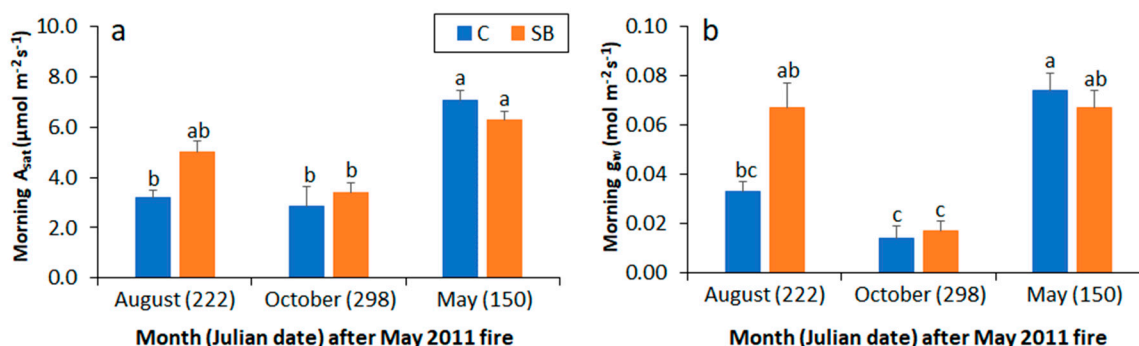
Figure 3. Mean fascicle-level (**a**) net photosynthesis (A_{sat}) and (**b**) stomatal conductance (g_w) in the morning at study 2 among longleaf pine saplings in response to no prescribed fire (C) or prescribed fire in May 2011 (SB). Error bars represent the standard error of the mean. Means associated with a different lower-case letter are significantly different at the 0.05 level.

Significant main effects of measurement interval on morning and afternoon g_w and VPD were observed after prescribed fire in October 2012 (Table 2). Morning and afternoon g_w were significantly greater on day 155 of 2013 (morning: 0.165 mol m^{-2} s^{-1}; afternoon: 0.144 mol m^{-2} s^{-1}) compared to days 183 and 215 of 2013 (morning: 0.121 mol m^{-2} s^{-1}; afternoon: 0.084 mol m^{-2} s^{-1}). Morning VPD was significantly less on days 155 and 183 of 2013 (2.20 kPa) compared to day 215 of 2013 (2.83 kPa).

Afternoon VPD differed significantly among all measurement intervals in 2013 with mean values of 2.72 kPa, 3.44 kPa, and 4.06 kPa on days 155, 183, and 215 of 2013, respectively. A significant main effect of October 2012 fire was observed among afternoon g_w and morning and afternoon VPD. Afternoon g_w was 26% higher (Figure 4b) and morning and afternoon VPD were 6% and 10% less (Figure 4c), respectively, among FB saplings compared to C saplings. Measurement interval and October 2012 fire did not significantly affect A_{sat} with morning and afternoon A_{sat} averages of 5.4 and 5.0 μmol m^{-2} s^{-1}, respectively, across the three measurement intervals (Figure 4a).
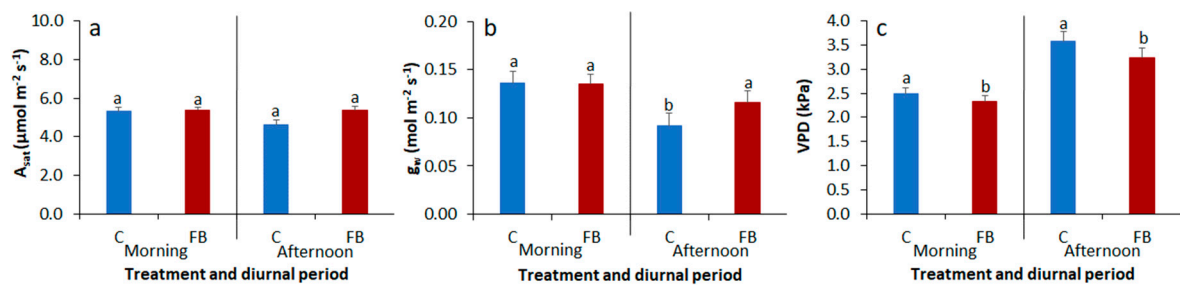
Figure 4. Mean longleaf pine sapling fascicle-level (**a**) net photosynthesis (A_{sat}), (**b**) stomatal conductance (g_w), and (**c**) vapor pressure deficit (VPD) in the morning and afternoon at study 2 averaged among three measurement intervals in response to no prescribed fire (C) or prescribed fire in October 2012 (FB). Error bars represent the standard error of the mean. Means associated with a different lower-case letter are significantly different at the 0.05 level.

3.2. Woody Root and Terminal Shoot Starch

3.2.1. Study 1

Small woody root starch concentration was significantly affected by sampling interval across 10 dates between May 2003 and April 2006 (Table 3). Interaction between VMT and sampling interval had a marginally significant effect on small woody root starch concentration ($p = 0.0763$). For the three VMT, linear regressions between sampling interval in 2005 and plot-level small woody root starch concentration were significant (C: $n = 20$, $p = 0.0001$, $r^2 = 0.5770$; SB: $n = 20$, $p < 0.0001$, $r^2 = 0.7581$; H: $n = 20$, $p < 0.0001$, $r^2 = 0.5926$). Mean square errors (s^2) between pairs of C, SB, and H regression lines were homogenous (C: $s^2 = 21.4\%$, SB: $s^2 = 25.6\%$, H: $s^2 = 28.1\%$). The y-intercepts between pairs of C, SB, and H regression lines were not significantly different. The slope of the SB regression line was significantly greater than that of the C regression line ($p = 0.0380$), and the slopes of the SB and C regression lines were similar to that of the H regression line (Figure 5).

Table 3. Probabilities of a greater *F*-value associated with mean starch concentrations (% of dry mass) of small woody roots at study 1 and the square root of mean terminal shoot starch concentrations at study 2 among longleaf pine saplings in response to vegetation management treatments that included prescribed fire.

Source of Variation	df [a]	Small Woody Roots	df	Terminal Shoot [d]	df	Terminal Shoot [d]
		Study 1 (C, SB, H)		Study 2 (C, SB)		Study 2 (C, FB)
Block	4	0.0217	2	0.4805	2	0.6016
Treatment (T) [b]	2	0.0726	1	0.1028	1	0.0864
Sampling date (D) [c]	9	<0.0001	10	<0.0001	9	<0.0001
T × D	18	0.0763	10	0.0359	9	<0.0001

[a] Degrees of freedom. [b] Study 1 treatments were no vegetation management activity after planting (C), prescribed fire on 22 June, 2000, 21 May, 2003, and 26 May, 2005 (SB), or herbicide control of herbaceous and woody vegetative competition (H). Study 2 treatments were the absence (C) or application of spring prescribed fire (SB) on 16 May, 2011 and the absence (C) or application of fall prescribed fire (FB) on 23 October, 2012. [c] In study 1, there were two sampling intervals in 2003, 2004, and 2006, and four sampling intervals in 2005 with one before fire application in 2003 and 2005, and one and three after fire application in 2003 and 2005, respectively. In study 2, there were 11 sampling intervals between February 2011 and June 2012 with two before May 2011 fire, and there were 10 sampling dates between July 2012 and October 2013 with two before October 2012 fire. [d] Before analyses of variance, terminal shoot starch concentrations in study 2 were transformed to square root values to establish normally distributed experimental errors.
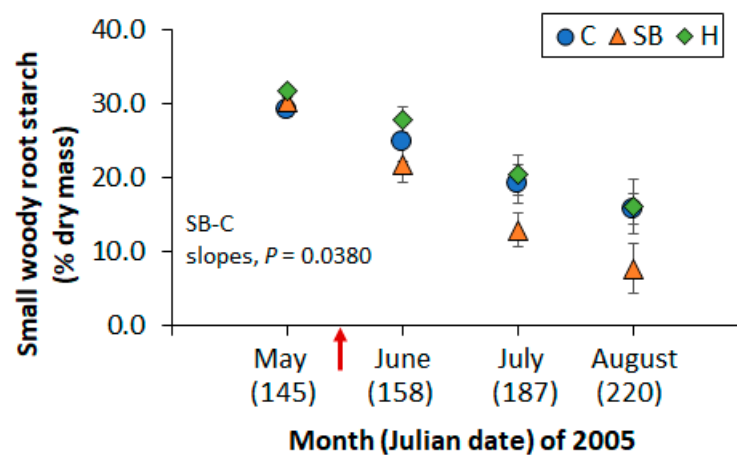
Figure 5. Mean starch concentration in small woody roots of sapling longleaf pines at study 1 in response to three vegetation management treatments (C: no vegetation management activity after planting, SB: prescribed fire in June 2000 and May 2003 and 2005, H: herbicide control of herbaceous and woody vegetative competition). Samples were collected in May 2005 (day 145) before prescribed fire, and in June (day 158), July (day 187), and August (day 220) after prescribed fire. Symbols are the mean of five plot values and the arrow indicates prescribed fire application in May. Bars represent the standard error of the mean.

3.2.2. Study 2

Square root-transformed terminal shoot starch concentrations were significantly affected by interaction between prescribed fire treatment and sampling interval during the 14- or 13-month monitoring periods after May 2011 or October 2012 fire, respectively (Table 3). On day 146 after May 2011 fire, square root-transformed terminal shoot starch concentration was significantly less among the SB saplings compared to the C saplings (Table 4). Beyond day 146 of 2011, square root-transformed terminal shoot starch concentration did not differ between the C and SB saplings. Between days 53 and 134 of 2013 after October 2012 fire, square root-transformed terminal shoot starch concentration was significantly less among the FB saplings compared to the C saplings (Figure 6). Beyond day 134 of 2013, square root-transformed terminal shoot starch concentrations were similar between the C and FB saplings. Unlike the C saplings, the FB saplings exhibited a significantly greater value of square root-transformed terminal shoot starch concentration on day 183 of 2013 compared to that on days 53, 85, and 134 of 2013.

Table 4. Non-transformed mean (standard error) starch concentration of terminal shoots among sapling longleaf pine at study 2 in response to no prescribed fire (C) or May 2011 prescribed fire (SB), or in response to no prescribed fire or October 2012 prescribed fire (FB).

Sampling Interval	Prescribed Fire Treatment [a]		
Month (Julian Date) and Year	C	SB	FB
May 2011 prescribed fire applied on Julian date 136			
February (49) 2011	3.6 (0.3) [fgb]	3.1 (0.3) [fgh]	—
March (84) 2011	8.6 (0.3) [abc]	9.9 (0.8) [ab]	—
May (146) 2011	5.5 (0.3) [cdef]	2.6 (0.1) [ghi]	—
June (167) 2011	5.6 (0.6) [bcdef]	3.9 (0.5) [fg]	—
July (194) 2011	5.3 (0.1) [cdefg]	5.0 (0.4) [defg]	—
September (262) 2011	4.1 (0.7) [efg]	2.8 (0.4) [fghi]	—
October (291) 2011	1.0 (0.4) [i]	1.4 (0.2) [hi]	—
February (48) 2012	4.8 (1.0) [defg]	3.3 (0.1) [fgh]	—
March (73) 2012	11.1 (1.0) [a]	11.1 (1.4) [a]	—
May (137) 2012	8.1 (0.7) [abcd]	6.8 (0.1) [bcde]	—
June (170) 2012	7.9 (1.1) [abcd]	7.7 (0.7) [abcd]	—
October 2012 prescribed fire applied on Julian date 296			
July (198) 2012	5.3 (0.2) [abcdfe]	—	7.0 (0.5) [abc]
September (270) 2012	2.1 (0.3) [ghij]	—	3.0 (0.4) [defghi]
October (302) 2012	0.9 (0.3) [ij]	—	1.0 (0.2) [ij]
February (53) 2013	4.7 (1.0) [bcdefgh]	—	0.8 (0.6) [j]
March (85) 2013	9.4 (0.3) [a]	—	2.4 (0.8) [fghij]
May (134) 2013	7.0 (0.5) [abc]	—	2.7 (0.8) [efghij]
June (155) 2013	6.6 (0.1) [abcd]	—	5.1 (0.9) [abcdefg]
July (183) 2013	5.7 (0.3) [abcde]	—	7.8 (0.5) [ab]
September (266) 2013	2.2 (0.4) [ghij]	—	3.6 (0.3) [cdefgh]
October (297) 2013	1.1 (0.1) [ij]	—	1.5 (0.3) [hij]

[a] Treatments were the absence (C) or application of prescribed fire on 16 May, 2011 (Julian date 136) (SB) and the absence or application of prescribed fire on 23 October, 2012 (Julian date 296) (FB). [b] Means by prescribed fire application date followed by different lower case letters are significantly different by the Tukey–Kramer test at the 0.05 level based on statistical analysis of square root-transformed values to establish normally distributed experimental errors.
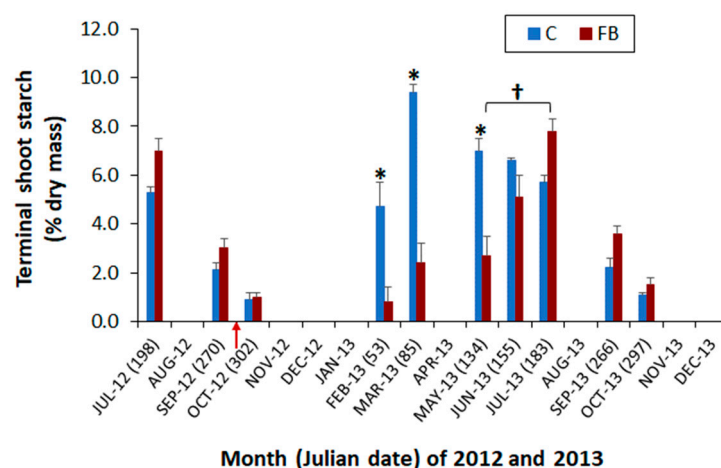
Figure 6. Mean terminal shoot starch concentration among sapling longleaf pine at study 2 in response to no prescribed fire (C) or prescribed fire in October 2012 (FB). The arrow indicates prescribed fire application. Error bars represent the standard error of the mean. Asterisks indicate significant differences between C and FB, and † indicates a significant difference between FB terminal shoot starch concentrations in May and July 2013 at the 0.05 level.

3.3. Aboveground Biomass and Crown Architectural Characteristics

Groundline diameter at the time of felling was a significant covariate in the analysis of variance of all aboveground biomass variables (Table 5). In 2003, VMT did not significantly affect square root-transformed values of total foliage or total branch dry mass of saplings but had a marginally significant effect on stem dry mass ($p = 0.0676$) with 29% less stem dry mass among SB saplings compared to H saplings (Figure 7c). One year later in 2004, VMT did not significantly affect square root-transformed values of total foliage dry mass but significantly affected square root-transformed values of total branch biomass with 26% less branch dry mass among SB saplings compared to H saplings (Figure 7e). Also in 2004, a marginally significant VMT effect on stem dry mass was observed ($p = 0.0504$) with less stem dry mass on SB plots compared to H plots at an α-level of 0.10 (Figure 7f).

Table 5. Probabilities of a greater *F*-value associated with analyses of covariance of aboveground biomass variables at study 1 in September 2003 and 2004, and October 2005 in response to three vegetation management treatments. Results are based on the destructive harvest of one sapling that was randomly chosen from among three one-third percentiles of total height per measurement plot.

Source of Variation	df [a]	Biomass Variable	2003	2004	2005
Covariate [b]	1		<0.0001	<0.0001	<0.0001
Block	4	Total foliage dry mass [d]	0.1255	0.0449	0.4066
Treatment (T) [c]	2		0.1196	0.1601	0.0038
Covariate	1		<0.0001	<0.0001	<0.0001
Block	4	Upper crown foliage dry mass [d]	0.0020	0.4509	0.4698
T	2		0.5598	0.7015	0.0504
Covariate	1		<0.0001	<0.0001	<0.0001
Block	4	Lower crown foliage dry mass [d]	0.4785	0.2564	0.2110
T	2		0.1349	0.4101	<0.0001
Covariate	1		<0.0001	<0.0001	<0.0001
Block	4	Total branch dry mass [d]	0.1076	0.0015	0.3597
T	2		0.2022	0.0306	0.0493
Covariate	1		-	<0.0001	<0.0001
Block	4	Upper crown branch dry mass [d]	-	0.1625	0.8209
T	2		-	0.3001	0.2491
Covariate	1		-	<0.0001	<0.0001
Block	4	Lower crown branch dry mass [d]	-	0.0730	0.2688
T	2		-	0.0798	0.0117
Covariate	1		<0.0001	<0.0001	<0.0001
Block	4	Stem dry mass	0.6413	0.2251	0.5976
T	2		0.0676	0.0504	0.0339

[a] Degrees of freedom. [b] The covariate was sapling groundline diameter at the time of felling. [c] Treatments were no vegetation management activity after planting, prescribed fire in June 2000 and May 2003 and 2005, or herbicide control of herbaceous and woody vegetative competition. [d] Before analyses of variance, data were transformed to square root values to establish normally distributed experimental errors.

In 2005, average values of square root-transformed total foliage and total branch dry mass, and stem dry mass among three saplings per plot were significantly affected by VMT (Table 5). The SB saplings had 33% less total foliage dry mass compared to C and H saplings (Figure 7g), and 30% and 32% less total branch and stem biomass compared to the H saplings (Figure 7h,i). Among individual saplings, linear regressions between foliage, branch, or stem dry mass and groundline diameter in 2005 were significant by vegetation management treatment (Foliage: C, $n = 13$, $p < 0.0001$, $r^2 = 0.7734$; SB, $n = 14$, $p = 0.0004$, $r^2 = 0.6591$; H, $n = 13$, $p < 0.0001$, $r^2 = 0.8538$; Branches: C, $n = 15$, $p < 0.0001$, $r^2 = 0.7104$; SB, $n = 14$, $p = 0.0001$, $r^2 = 0.7176$; H, $n = 15$, $p = 0.0001$, $r^2 = 0.6927$; Stem: C, $n = 15$, $p < 0.0001$, $r^2 = 0.7169$; SB, $n = 14$, $p < 0.0001$, $r^2 = 0.8571$; H, $n = 15$, $p < 0.0001$, $r^2 = 0.9016$). Mean square errors (s^2) between the SB and either the C or H regressions were homogenous (Foliage: C:

$s^2 = 0.30$ kg, SB: $s^2 = 0.09$ kg, H: $s^2 = 0.36$ kg; Branches: C: $s^2 = 0.15$ kg, SB: $s^2 = 0.11$ kg, H: $s^2 = 1.71$ kg; Stem: C: $s^2 = 5.38$ kg, SB: $s^2 = 1.15$ kg, H: $s^2 = 3.38$ kg).
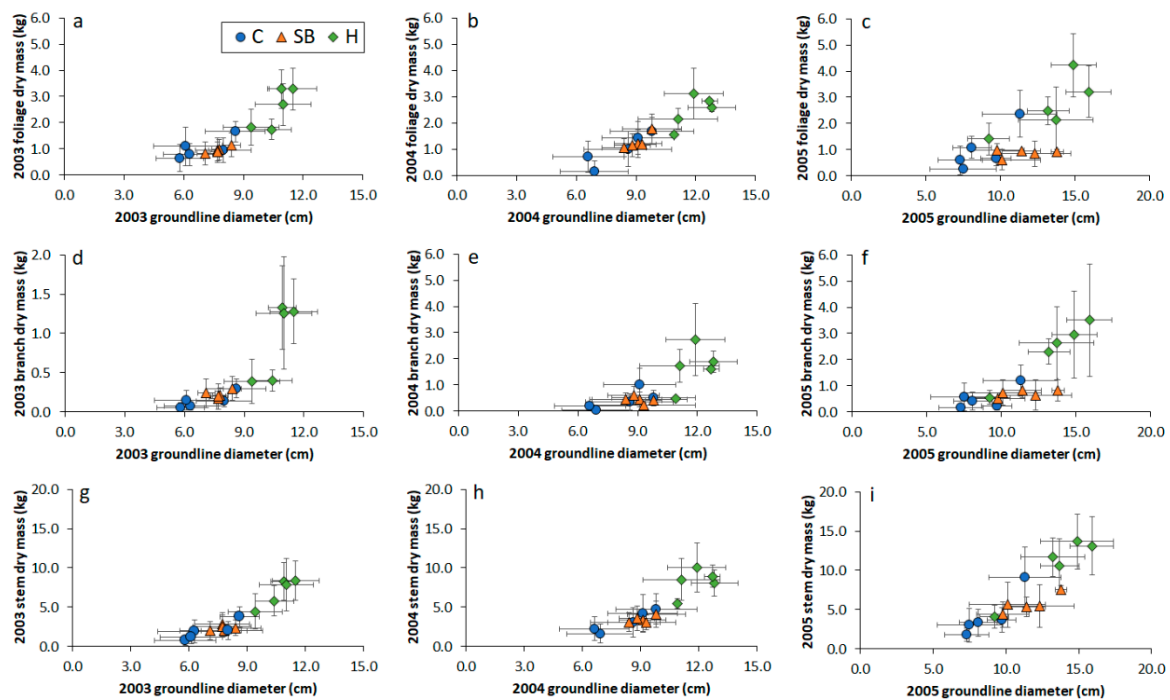
Figure 7. Relationships between longleaf pine (**a**,**d**,**g**) foliage, (**b**,**e**,**h**) branch, or (**c**,**f**,**i**) stem dry mass and sapling groundline diameter in 2003, 2004, and 2005 at study 1 in response to three vegetation management treatments (C: no vegetation management activity after planting, SB: prescribed fire in June 2000, and May 2003 and 2005, H: herbicide control of herbaceous and woody vegetative competition). Values are non-transformed means among three longleaf pine saplings per plot. Bars represent the standard error of the mean. Among all figures, the maximum *y*-axis range in 2003 and 2004 is 15.0 cm and that in 2005 is 20.0 cm. Among branch dry mass figures, the maximum *x*-axis range in 2003 is 2.0 kg and that in 2004 and 2005 is 6.0 kg.

The slope of the relationship between foliage dry mass and groundline diameter was significantly less for the SB saplings compared to the C saplings ($p = 0.0030$) (Figure 8a). No other regression parameters associated with C and SB sapling foliage, branch, or stem dry mass were significantly different. Between the C and H regressions, the y-intercept and slope of relationships between foliage (Figure 8b), branch (Figure 8d), or stem (Figure 8f) dry mass and groundline diameter were significantly different with consistently lower y-intercepts and slopes for the SB regressions compared to the H regressions (Foliage: slopes: $p < 0.0001$, y-intercepts: $p = 0.0102$; Branch: slopes: $p = 0.0022$, y-intercepts: $p = 0.0135$; Stem: slopes: $p = 0.0021$, y-intercepts: $p = 0.0350$).

When total foliage dry mass in 2005 was partitioned by crown level, square root-transformed values of lower crown foliage dry mass were significantly affected by VMT (Table 5), with a 54% reduction in lower crown foliage dry mass among SB saplings compared to C and H saplings. A marginally significant effect of VMT on square root-transformed values of upper crown foliage dry mass ($p = 0.0504$) was also observed in 2005 with 27% less upper crown foliage dry mass among SB saplings compared to H saplings. When total branch biomass in 2005 was partitioned by crown level, a 32% reduction in lower crown branch dry mass among the SB saplings was observed compared to the H saplings.
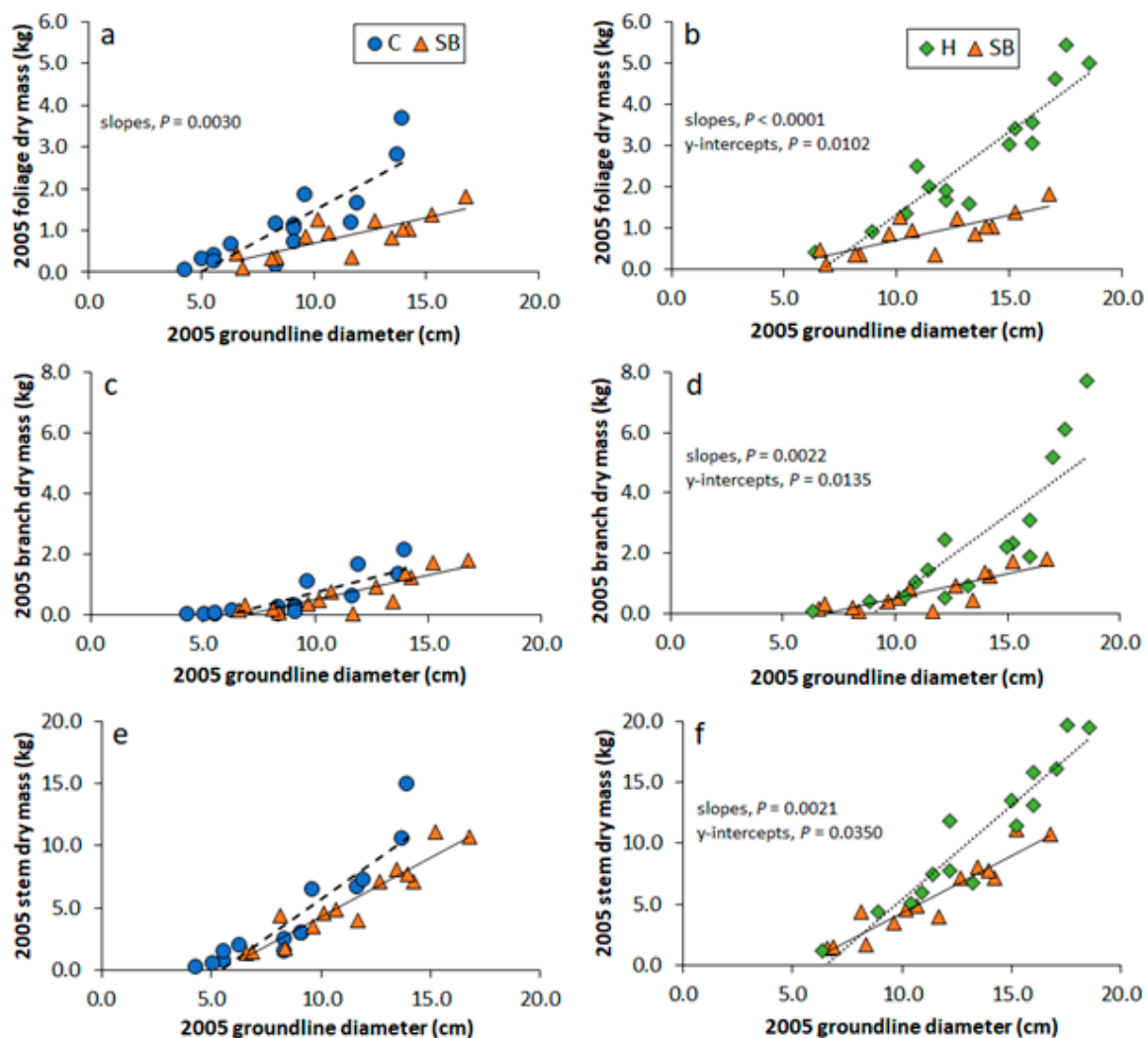
Figure 8. Linear regressions between non-transformed (**a**,**b**) foliage, (**c**,**d**) branch, or (**e**,**f**) stem dry mass and groundline diameter among saplings destructively harvested in October 2005. Foliage, branch, and stem linear regressions were significant by vegetation management treatment (C: no vegetation management activity after planting, SB: prescribed fire in June 2000, and May 2003 and 2005, H: herbicide control of herbaceous and woody vegetative competition). Significantly different slopes were observed between C and SB for foliage dry mass, and significantly difference slopes and y-intercepts were observed between H and SB for foliage, branch, and stem dry mass.

In study 1, VMT significantly affected crown architectural characteristics of harvested saplings in 2005 but not 2004 (Table 6). Live crown ratio was significantly less (21%) among SB saplings compared to C saplings in 2005. Also in 2005, the ratio of first-order lateral branch numbers in the upper and lower crown was significantly greater (37%) among SB saplings compared to C and H saplings. The effect of VMT on the ratio of first-order lateral branch dry mass in the upper and lower crown was marginally significantly ($p = 0.0802$) in 2005 with 21% greater values among SB saplings compared to C saplings. A marginally significant effect ($p = 0.0829$) of VMT on height to the base of the live crown was observed in 2005 with a 41% greater value among SB saplings compared to C saplings.

Table 6. Probabilities of a greater *F*-value associated with crown architectural characteristics among longleaf pine saplings at study 1 in September 2004 and October 2005 in response to three vegetation management treatments. Results are based on the destructive harvest of one sapling that was randomly chosen from among three one-third percentiles of total height per measurement plot.

Source of Variation	df [a]	Year	HBLC [d]	LCR [d]	FOBN [d]	BNR [d]	BMR [d]
Covariate [b]	1		0.0001	0.0885	<0.0001	0.0035	0.6762
Block	4	2004	0.2019	0.2319	0.1814	0.5464	0.8851
Treatment [c]	2		0.7210	0.8194	0.1233	0.4646	0.8285
Covariate	1		0.2056	0.0103	<0.0001	0.5294	0.4535
Block	4	2005	0.6796	0.1622	0.4984	0.3100	0.9064
Treatment	2		0.0829	0.0081	0.5528	0.0031	0.0802

[a] Degrees of freedom. [b] The covariate was sapling groundline diameter at the time of felling. [c] Treatments were no vegetation management activity after planting, prescribed fire in June 2000, and May 2003 and 2005, or herbicide control of herbaceous and woody vegetative competition. [d] HBLC: stem height to the base of the live crown; LCR: live crown ratio; FOBN: first-order lateral branch number; BNR: ratio of first-order lateral branch number in the upper and lower crown; BMR: ratio of first-order lateral branch dry mass in the upper and lower crown.
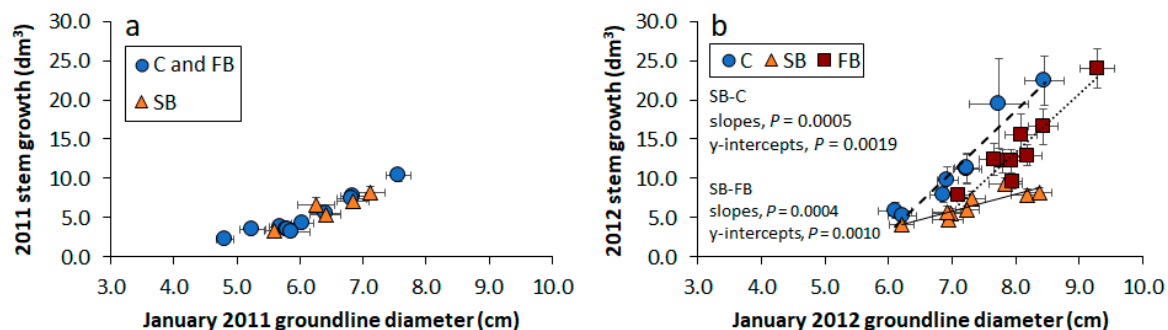
3.4. Stem Growth

In study 1, mean sapling groundline diameter in 2003 was a significant covariate in the analyses of variance of mean sapling annual increment of stem growth in 2003 ($p < 0.0001$), 2004 ($p < 0.0001$) and 2005 ($p < 0.0001$). Mean sapling annual increment of stem growth was not significantly affected by VMT in 2003 (Figure 9a) or 2005 (Figure 9c). While VMT had a significant effect on mean sapling annual increment of stem growth in 2004 ($p = 0.0079$) with greater values among C and SB saplings compared to H saplings, this effect does not appear to be biologically significant (Figure 9b).
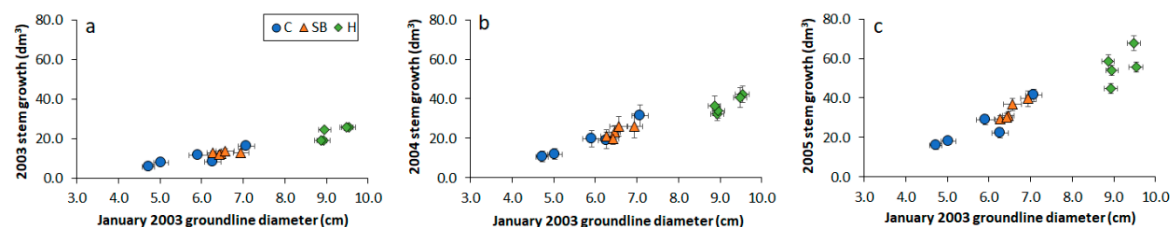
Figure 9. Relationships between longleaf pine mean sapling outside-bark annual increment of stem growth and mean sapling groundline diameter among plots at study 1 in (**a**) 2003, (**b**) 2004, and (**c**) 2005 in response to three vegetation management treatments (C: no vegetation management activity after planting, SB: prescribed fire in June 2000, and May 2003 and 2005, H: herbicide control of herbaceous and woody vegetative competition). The *x*-axis is truncated. Values are means among all live longleaf pine saplings per measurement plot (2003, 2004, and 2005: 71–138, 66–134, and 62–130 saplings per plot). Bars represent the standard error of the mean.

In study 2, linear regressions between mean sapling annual increment of stem growth in 2011 and mean sapling groundline diameter in January 2011 among subplots were significant for the unburned (C and FB) saplings ($n = 17$, $p = 0.0005$, $r^2 = 0.9085$) and the SB saplings (SB: $n = 5$, $p = 0.0167$, $r^2 = 0.8869$) (Figure 10a). These regression lines were characterized by homogenous s^2, and statistically similar slopes and y-intercepts. Linear regressions between mean sapling annual increment of stem growth in 2012 and mean sapling groundline diameter in January 2012 among subplots were significant for the C saplings ($n = 8$, $p = 0.0005$, $r^2 = 0.8818$), SB saplings ($n = 9$, $p = 0.0015$, $r^2 = 0.7858$), and FB saplings ($n = 9$, $p = 0.0003$, $r^2 = 0.8684$) (Figure 10b). Values of s^2 among pairs of C, SB, and FB regression lines in 2012 were homogenous at an α-level of 0.05 (C: $s^2 = 5.8$ dm^3; SB: $s^2 = 0.7$ dm^3; FB: $s^2 = 3.3$ dm^3). Slopes and y-intercepts of the C and FB regressions did not differ significantly by treatment, but slopes and y-intercepts of the SB and C regressions (slopes: $p = 0.0005$, y-intercepts: $p = 0.0019$) and SB and

FB regressions (slopes: $p = 0.0004$, y-intercepts: $p = 0.0010$) differed significantly by treatment. In both comparisons, the slopes and y-intercepts were significantly less for the SB regression compared to the C or FB regressions.

Figure 10. Relationships in (**a**) 2011 and (**b**) 2012 between longleaf pine mean sapling outside-bark annual increment of stem growth and mean groundline diameter among subplots in January at study 2 (16 subplots with 18–45 live saplings in 2011 and 26 subplots with 13–44 live saplings in 2012). Treatments were no prescribed fire (C) or prescribed fire in May 2011 (SB) or October 2012 (FB) with the C and FB subplots unburned in 2011. The *x*-axis is truncated. Bars represent the standard error of the mean. Significantly different slopes and y-intercepts were observed between SB and either C or H in 2012.

3.5. Crown Scorch

Among the three saplings by plot chosen for crown scorch assessment in study 1, an average of 4% to 73% of sapling total height was scorched by prescribed fire in May 2003, and an average of 65% to 100% of sapling total height was scorched by prescribed fire in May 2005. Means and coefficients of variation associated with plot values of scorched sapling height suggest that crown scorch was less severe and more variable in 2003 compared to 2005 (mean ± coefficient of variation, 2003: 37% ± 56%; 2005: 94% ± 11%). Among the six saplings by plot used for gas exchange measurements in study 2, an average of 52% to 65% of sapling total height was scorched by May 2011 fire (59% ± 4%), and an average of 53% to 61% of sapling total height was scorched by October 2012 fire (58% ± 7%). Means and coefficients of variation of sapling crown scorch were similar between the May 2011 and October 2012 fires.

4. Discussion

Recent decades of forest productivity research have documented a strong, positive relationship between foliage mass and stem production in intensively managed *Pinus* [58–61]. Past research has also reported that foliage regrowth after biotic or abiotic defoliation ensures *Pinus* survival and continued growth [37,62]. The vigor of longleaf pine is also tightly coupled with foliage mass [63,64], and in fire-adapted longleaf pine forests, the resilience of young trees is associated with reestablishment of consumed foliage after fire [42,43].

Foliage regrowth requires carbohydrate supplied to elongating shoots and developing buds and therefore, depends on physiological processes and environmental conditions that control carbon fixation and allocation. We observed three means of post-scorch foliage reestablishment in longleaf pine that included an immediate but temporary, positive response of fascicle gas exchange among surviving foliage in response to fire in the early growing season. At a smaller magnitude, fire in the late growing season also stimulated fascicle gas exchange. Second, availability of starch to regrow foliage depended on synchrony between *Pinus'* annual starch dynamics and the season of fire application. Finally, we observed developmental responses to biennial fire that minimized lower crown vulnerability to fire in the future.

4.1. Fascicle Gas Exchange Response to May Fire

In both studies, we found an increase in fascicle gas exchange for a limited period of time after May fire. In study 1, increases in A_{sat} and g_w were observed among the SB saplings in July, 2 months after May fire, but A_{sat} and g_w were similar between the C and SB saplings by the end of the growing season in 2003 (Figure 2c,d) and 2005 (Figure 2e–h). In study 2, severe drought that began 12 months before, and continued 8 months after May fire reduced gas exchange rates compared to those in study 1. Mean A_{sat} and g_w in August 2011 did not differ significantly between C and SB treatment. Water limitations to gas exchange in 2011 may have prevented observation of robust treatment effects on gas exchange at this time. However, comparisons of A_{sat} and g_w measured among the C and SB saplings in August 2011 and May 2012 suggest that gas exchange was augmented for 3 months after fire in May 2011 (Figure 3a,b).

Past research has attributed this pattern of gas exchange response after fire to increases in soil resource availability that persist until vegetation reestablishes. For example, when water supply was not limiting to gas exchange, foliar nitrogen concentration, driven by nitrogen uptake, was positively correlated with the gas exchange of *Pinus* foliage that survived fire [65,66]. Longleaf pine responds to water deficit by stomatal sensitivity and its performance is correlated with available water [67–70]. After fire, decreases in transpiring surface area and water use lead to increases in both available water [66,71] and fascicle-level gas exchange among surviving foliage [72–74].

When available water is low, stomatal function may be enhanced after partial defoliation by fire reduces whole-crown water use [44,73]. Alternatively, when available water is high, this effect may only be observed later in the day after high rates of transpiration deplete available water in the root-zone [65]. Between April and September, for example, precipitation was 20% greater in 2003 than 2005 (Figure 1a) and morning A_{sat} was similar between the C and SB saplings in July 2003 (Figure 2a). In contrast, morning A_{sat} in 2005 (Figure 2e) and afternoon A_{sat} in both years (Figure 2c,g) were higher among the SB saplings compared to the C saplings in July. This suggests that in 2003, these longleaf pine saplings exhibited sensitivity to a decrease in water supply that developed over the course of the day.

Despite treatment differences in gas exchange rates observed in July, by September 2003 in study 1 and October 2011 in study 2, A_{sat} and g_w were similar between the C and SB saplings. In early fall, foliage dry mass was comparable between the C and SB saplings in study 1 (Figure 7a) and study 2 [43]. In longleaf pine-bluestem woodlands similar to those in studies 1 and 2, the composition of herbaceous and woody vegetation changes in response to repeated fire with an increase in herbaceous plant cover and a decrease in shrub and tree numbers [27,46]. Regardless of these shifts in vegetation composition, pre- and post-fire water-use across a site may be similar once functional leaf area has recovered [66]. We attribute the short-lived nature of elevated A_{sat} and g_w after May fire in 2003 and 2011 to equilibration of functional leaf area and transpiration between the C and SB plots by September which was accomplished by the regrowth of both longleaf pine foliage and competing vegetation.

While measurements of whole-crown gas exchange were beyond the scope of these studies, observations made by other investigators provide insight regarding the potential role of enhanced post-fire gas exchange rates in prescribed burned longleaf pine. In a 20-year-old stand of longleaf pine under well-watered conditions, a 77% leaf area loss by scorch co-occurred with similar daily whole-crown transpiration values for two weeks between scorched and control trees having similar sapwood areas [73]. In another study, whole-tree sap flux among mature *Pinus rigida* L. was monitored for a 5-month period after fire [66]. It was reported that 5% to 10% crown scorch caused an immediate 27% reduction in whole-tree sap flux followed by up to a 25% increase in whole-tree sap flux for several months. In a different study, comparable whole-tree sap flux recovery in 26-year-old longleaf pines was observed after 80% crown scorch [75]. For *Pinus*, a positive correlation exists between water-use and whole-tree carbon fixation when water demands are met [76–78]. Under the soil moisture conditions in 2003, post-fire increases in afternoon A_{sat} and g_w among the SB saplings may have been accompanied by daily amounts of whole-crown carbon fixation comparable to those of C and H saplings of similar size.

4.2. Fascicle Gas Exchange Response to October Fire

After fire in October 2012, we observed a main effect of FB on g_w with greater afternoon g_w among FB saplings compared to C saplings through August 2013, 10 months after the October fire (Figure 4b). While six flushes are possible on an annual basis among longleaf pine saplings, the first three flushes produce the majority of new foliage and third flush fascicle elongation is nearly completed by late August [79]. As such, the positive response of afternoon g_w among FB saplings cannot be completely explained by the dynamics of transpiring foliage after fire.

Where young *Pinus* and woody competitors coexist, light limitations may reduce *Pinus* stomatal function and growth [80,81]. After October fire, A_{sat} and g_w in the morning and afternoon across the FB and C plots were comparable to the A_{sat} and g_w of young longleaf pine under favorable light and moisture conditions reported elsewhere [69,82,83]. Therefore, we do not attribute higher afternoon g_w on the FB plots to an increase in available light after October fire. Occurrence of this effect in the afternoon but not in the morning indicates that diurnal water supply may have been affected by October fire. It is possible that shifts in vegetative composition and vertical root distribution between the C and FB plots favored afternoon water availability on the FB plots.

Walter's two-layer niche-partitioning hypothesis [84] proposes that where water limitations to plant growth are common, herbaceous and woody plant roots are functionally separate [85,86]. Roots of herbaceous plants are prevalent in the upper portion of the soil profile, and while woody roots may coexist with herbaceous roots, they dominate the deep portion of the soil profile [87]. Competition for deep soil water between *Pinus* and other woody species has been observed with woody competition imparting at least as much or more soil resource stress on planted *Pinus* as herbaceous competition [88]. Over time, the repeated application of fire favors formation of a ground-layer and decreases the number of woody competitors in young stands of longleaf pine [9,21,27,45,89]. We speculate that a partial shift away from woody competition by October fire increased the availability of deep soil water to longleaf pine saplings leading to the observed 26% increase in afternoon g_w. Similar to observations of dissimilar morning and afternoon A_{sat} and g_w responses to May fire in 2003, favorable precipitation in 2013 may have isolated this response to the afternoon as root-zone water became depleted over the course of the day.

The resilience of longleaf pine on xeric sites and under prolonged drought advocates for its establishment where plant-available water is low [90,91]. In an assessment of fascicle gas exchange among mature longleaf, loblolly, and slash pines, similar net photosynthesis and stomatal conductance rates were observed among species, but longleaf pine exhibited consistently higher daytime fascicle water potentials than loblolly or slash pines [83]. At the other end of the spectrum, longleaf pine is also adapted to poorly drained flatwoods and savannas [47]. The moist growing conditions of 2013 revealed normal stomatal function among the C and FB saplings despite extreme afternoon VPD. Afternoon VPD during the June, July, and August measurement intervals ranged between 2.2 and 3.4, 2.7 and 4.1, and 3.1 and 5.2, respectively, and were under the control of high afternoon air temperatures (June: 35.8 ± 1.0 °C; July: 34.8 ± 1.1 °C; August: 38.9 ± 1.4 °C). Under similar conditions of ample available water and extreme air temperatures, normal stomatal function is not sustained in loblolly pine [92,93]. Compared to loblolly pine, therefore, longleaf pine may perform favorably on wet sites where extreme, high air temperatures are anticipated.

4.3. Fire and Starch Dynamics

The seasonal starch dynamics of plants characterizes their storage and mobilization of fixed carbon on an annual basis. In subtropical climates and under normal stand conditions, the majority of *Pinus* starch accumulates between the end of the active growing season (October–November) and the beginning of the next growing season (March–April) [94–97]. We also observed this pattern of starch accumulation in small woody roots and terminal shoots (Figures 5 and 6), as well as stem and taproot tissues [43]. Current photosynthate is the source of carbohydrate for starch in *Pinus* as demonstrated in loblolly pine seedlings three days after exposure to ^{14}C [98]. In this study, ^{14}C-labeled photosynthate

stored as ^{14}C-labeled root starch was 3-fold higher when ^{14}C exposure occurred in January compared to August.

As the growing season progresses, stored starch is mobilized and supplements current photosynthate allocated to *Pinus* foliage growth [99–103]. Because the stature of longleaf pine seedlings and saplings predisposes them to complete or severe crown scorch, the contribution of current photosynthate to their foliage regrowth after fire may be limited. After May fire, accelerated starch mobilization appeared to be an important source of carbohydrate for longleaf pine foliage regrowth. The role of starch reserves in foliage recovery after disturbance has also been observed among other species [62,104–106].

Unlike May fire, October fire disrupted the pattern of starch storage and mobilization in longleaf pine terminal shoots as well as stem and taproot tissues [43]. Starch failed to accumulate naturally among the FB saplings with 72% less terminal shoot starch in February through May 2013 compared to the C saplings (Figure 6). We propose that current photosynthate produced in unburned foliage of the FB saplings was allocated to new foliage growth creating a temporary void in starch reserves. By June 2013 which was 8 months after October fire, however, it appears that current photosynthate produced by both unburned foliage and recently mature, current-year first flush foliage restored terminal shoot starch.

4.4. Crown Architecture Response to Repeated Fire

We observed changes in longleaf pine crown architecture in response to repeated fire that reduced the risk of foliage consumption during future fires. In 2005, mean live crown ratio (LCR) was significantly less (21%) for the SB saplings compared to the C saplings. Also in 2005, a marginally significant effect of prescribed fire was observed on height to the base of the live crown (HBLC) with SB and C means of 2.2 m and 1.6 m, respectively.

In a review of fire resistance among the naturally occurring *Pinus* forests of Europe, the mortality of lower crown branches by fire was described as a fire avoidance mechanism [107]. The LCL and HBLC responses to repeated fire in our study represent this adaptation that reduces foliage consumption during future fires. After three fire intervals in study 1, this phenomenon was manifested as 37% and 21% higher ratios of primary lateral branch number (BNR) and dry mass (BMR), respectively, in the upper compared to the lower one-half of the live crown.

Light availability is generally higher in the upper crown compared to the lower crown [108,109], and *Pinus* gas exchange is positively correlated with the vertical distribution of available light in the canopy [53,110,111]. Our observations also detected higher gas exchange rates in the upper crown compared to the lower crown of longleaf pine saplings. Therefore, in addition to reducing the likelihood of foliage consumption during future prescribed fires, an upward shift of branch biomass in young, fire-maintained longleaf pine stands may indirectly benefit whole-crown carbon fixation by an increase in light acquisition.

4.5. Limitations to Foliage Re-Establishment after Fire

We observed several means of carbon support for foliage regrowth after scorch. However, barriers to foliage reestablishment associated with climate and fire intensity were also detected. For example, low precipitation in April through June (Figure 1a) contributed to an overall decrease in May and July gas exchange rates in 2005 compared to 2003 (Figure 2). At the same time, it is likely that spring water deficit in 2005 incited fire intensity by low fuel moisture content [112,113]. The result was more severe and less variable crown scorch in 2005 (94 ± 11%) compared to 2003 (37 ± 56%). Stored starch was a likely contributor to foliage regrowth among all individuals. As sapling size increased, however, retention of a threshold of surviving foliage may have been increasingly necessary to insure adequate carbon support for foliage re-establishment.

Relationships between foliage dry mass and d_g by harvested sapling in 2005 indicate that foliage re-establishment was influenced by sapling size (Figure 8a,b). Shortfalls in foliage dry mass between the SB saplings and either the C or H saplings increased proportionally with d_g. Although our saplings

were naturally variable in stature [114], little variation was observed in the high level of crown scorch they experienced in 2005. The respiratory demand for carbohydrate increases with tree size [115–117]. As such, in 2005 large saplings may not have had sufficient post-scorch starch reserves and surviving foliage to support basic metabolism and re-establishment of normal foliage mass (44). We propose that climate-driven reductions in A_{sat} regardless of sapling size and high crown scorch among large saplings challenged re-establishment of foliage mass after May 2005 fire in study 1.

In addition to water-limited gas exchange rates and high foliage loss by fire among large saplings, fire effects on small diameter lateral branches may have interfered with foliage regrowth in 2005. While first-order lateral branch number was not affected by SB, the resilience of higher order lateral branches may have been negatively affected by fire. This is because total branch dry mass among saplings of similar size was significantly less in 2005 and significantly less at a marginal level in 2004 among SB saplings compared to H saplings. Total branch dry mass among C saplings was intermediate between those of the SB and H saplings. It appears that repeated fire did not affect first-order lateral branch number, but led to less massive first-order lateral branches. Explanations for this include heat damage to the vascular cambium and xylem dysfunction by cell wall damage or cavitation in small diameter lateral branches of the lower crown [30,32,118]. Loss of these small lateral branches would reduce potential sites for vegetative bud development. At this point, however, we cannot conclude whether less massive first-order lateral branches evolved over the progression of fires in 2000, 2003, and 2005 or in response to high fire intensity incited by spring water deficit in 2005.

4.6. Limitations to Stem Growth after Fire

We conducted three evaluations of longleaf pine sapling stem growth in response to prescribed fire. In study 1, analyses of covariance were conducted among plot means of sapling annual increment of stem growth with plot mean groundline diameter in 2003 as a covariate. In this analysis, mean sapling annual increment of stem growth was similar between the C and SB plots but was smaller among the C and SB plots compared to the H plots in 2005 at age 8 years (Figure 9c). Equivalent, marginally significant trends were observed in 2003 and 2004 at ages 6 and 7 years, respectively (Figure 9a,b). In Study 1, similar treatment responses in sapling stem volume per hectare were reported through age 12 years [21]. Superior stem growth on the H plots validates the benefit of early and intensive vegetation management by herbicide application rather than repeated fire when maximizing juvenile growth takes precedence over restoration of non-timber ecosystem attributes.

Also in study 1, we defined d_g as an indicator of sapling stature in linear relationships between sapling stem dry mass and d_g in 2005. Comparison between the SB and H linear relationships of stem dry mass and d_g in 2005 revealed a smaller y-intercept and slope for the SB saplings compared to the H saplings (Figure 8f). A comparable but not significant trend was observed between the SB and C saplings in 2005 (Figure 8e). Slopes associated with foliage mass and d_g in 2005 showed that the magnitude of difference in foliage mass between both the SB and C saplings and the SB and H saplings increased significantly by sapling size. It is well established that foliage mass controls *Pinus* stemwood growth [59,60] and foliage regrowth after defoliation is needed to sustain *Pinus* stemwood growth [38,62]. Crown scorch risks a loss of both foliage and stem growth, but foliage regrowth by the end of the growing season after fire is possible and reduces this risk [43]. In 2005 of study 1, significantly smaller slopes for linear relationships between foliage or stem dry mass and d_g among the SB saplings compared to the C or H saplings suggests that under some conditions in young stands, the likelihood of foliage recovery after fire decreases and the risk of stem growth loss increases by sapling size.

In study 2, we also observed a negative effect of prescribed fire on longleaf pine sapling stem growth. However, this effect is not attributed to a lag in foliage regrowth after fire because scorched foliage mass was reestablished by September 2011, 4 months after May 2011 fire, and by September 2012, 11 months after October 2011 fire [43]. Specifically, linear regressions between subplot mean sapling annual increment of stem growth in 2012 and subplot mean d_g in January 2012 indicated that

the y-intercept and slope of the SB saplings were significantly less than those of the C or FB saplings. The pace of stem growth during the year after May 2011 fire decreased as d_g increased. Resolving the mechanism of this growth loss is not within the scope of our study. However, this response to May fire warrants further investigation to determine if it was caused by prescribed fire application during a short window of favorable burning conditions embedded in a prolonged drought. This line of research is supported by correlation between prolonged drought and tree mortality after wildfires in the western United States [32]. At this point, our observations of longleaf pine stem growth after May fire in 2005 and 2012 indicate that consequences of prescribed fire application during drought should be considered when maximum juvenile stem growth is desired. Guidelines for the application of prescribed fire in young stands of longleaf pine would benefit by further research to determine the relationship between water deficit, tree size, and post-scorch foliage dynamics.

5. Summary and Conclusions

Repeated fire sustains many ecological processes vital to longleaf pine ecosystem flora and fauna. For this reason, prescribed fire is an important tool in ongoing longleaf pine restoration efforts. The survival of prescribed burned longleaf pine seedlings is correlated with their size [39], and application of fire is not advocated until after the first growing season following planting [40]. Beyond this developmental stage, survival and vigorous growth among longleaf pine seedlings and saplings that experience severe crown scorch demonstrates the fire-tolerance of this species. We hypothesized that young longleaf pines withstand severe crown scorch by physiological and morphological attributes that promote foliage regrowth. We observed temporary increases in A_{sat} and g_w of non-scorched foliage after May fire which we attribute to an increase in available water caused by the temporary loss of functional leaf area across the site. Compared to g_w after May fire, a smaller and prolonged increase in g_w was observed after October fire which could not be attributed to aboveground vegetation dynamics after fire. Rather, we attribute elevated g_w after October fire to an anticipated increase in the ratio of herbaceous to woody vegetation that, in turn, reduced the diurnal uptake of deep soil water so that afternoon water availability was greater on the burned plots compared to plots that were not burned.

In response to prescribed fire in May, we also observed accelerated starch mobilization during the normal period of rapid foliage growth. This suggests that stored starch is a source of carbon to re-grow scorched foliage when fire coincides with high starch availability. The unimodal nature of starch dynamics in *Pinus* precludes starch deployment to foliage regrowth after late growing season fire because starch reserves are nearly depleted at this time. Thus, foliage regrowth after October fire depended primarily on current photosynthate produced by foliage that survived fire and recently mature, new foliage. Failure to accumulate a normal level of starch reserves during most of the growing season after October fire indicated that foliage regrowth was prioritized as a sink for current photosynthate. Among prescribed burned saplings, we also observed a shift toward the upper crown in number of first-order lateral branches and total branch biomass that reduced the likelihood of foliage damage by future fires.

Foliage mass was re-established by the end of the growing season after May 2003 fire in study 1, and by the end of the growing season after May or October fire in study 2 [43]. In contrast, poor re-growth of scorched foliage was observed in 2005 when May fire was applied during a 3-month precipitation deficit between April and June. In response to the difference in precipitation between 2003 and 2005 and its potential effect on fire intensity, crown scorch in May 2005 was more severe and less variable than in 2003. It is likely that the shortfall of new foliage growth among large SB saplings in 2005 was caused by sparse live foliage after May fire and water limitations to stomatal function that inhibited normal rates of carbon fixation through July. Linear relationships between foliage dry mass and groundline diameter 4 months after May 2005 fire indicate that re-establishment of scorched foliage was challenged among larger saplings. This, together with relationships between stem dry mass and groundline diameter between the SB and H saplings, indicate that shortfalls in foliage re-growth after

fire may have been manifested in low stem growth among large saplings. In 2012, linear relationships between annual stem growth and groundline diameter, averaged by subplot, also showed that stem growth among large saplings in the year after May fire was compromised.

Our results provide insight into optimal windows for prescribed fire in young stands of longleaf pine. When complete crown scorch is anticipated, seasonally available starch reserves are the primary supply of carbon to re-grow foliage. Thus, seedling and sapling vigor is maintained by application of fire when starch reserves are high. As sapling stature reaches a threshold that averts complete crown scorch, foliage is re-grown by starch reserves and current photosynthate. Carbon fixation by non-scorched foliage broadens the optimum window for fire application as a young longleaf pine stand develops. At the sapling stage of stand development, however, factors that affect fire intensity and carbon fixation such as prolonged periods of water deficit appear to play a role in post-fire foliage re-establishment and stem growth.

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