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## Nonstructural Carbohydrate Concentrations of Pine Trees as a Function of Evolutionary History

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NONSTRUCTURAL CARBOHYDRATE CONCENTRATIONS OF PINE TREES AS A FUNCTION OF  
EVOLUTIONARY HISTORY

by

JOSHUA MIMS

(Under the Direction of Doug Aubrey)

ABSTRACT

Nonstructural carbohydrates (NSC) can supply substrate during periods when current photosynthate is unavailable or inadequate to meet metabolic demands. I hypothesized that natural selection has favored higher nonstructural carbohydrate concentrations ([NSC]) in species that have an evolutionary history of frequent disturbance and tested this using three southern pine species that have evolved under a continuum of disturbance frequencies (evolutionary history of fire ~ longleaf > slash > loblolly). Stem and root samples were collected from 12 similar-sized individual trees of each species during time periods that reflect the annual minimum and maximum [NSC]. A modified colorimetric method was performed on the samples to determine [NSC]. Both [sugar] and [starch] differed among species, between organs, and exhibited seasonal patterns; however, these individual effects were not always independent of each other. Longleaf roots exhibited higher [sugar] than loblolly and slash; however, [sugar] in the stems were similar among species. Both [sugar] and [starch] of roots were higher than stems in March. Roots exhibited seasonal changes in [sugar] and [starch] (higher in October for sugar and higher in March for starch). The results suggest that evolutionary history of disturbance partly explains patterns of [NSC] observed in southern pines as longleaf—the species with an evolutionary history of frequent fire—exhibited the highest [NSC] in roots; however, similarities between slash and loblolly indicate that other factors must also be considered. The results are likely explained by the

different life histories exhibited in the juvenile stages of these pines; specifically, patterns of allocation to above- and belowground tissues. I suggest that the increased belowground storage observed in adult longleaf pine may simply be a relic of selective pressures imposed at the juvenile stage.

INDEX WORDS: nonstructural carbohydrates, stored carbon, longleaf, slash, loblolly, disturbance history, evolutionary history, fire, insect

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JOSHUA MIMS

B.S., Georgia Southern University, 2012

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MASTERS OF SCIENCE

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## DEDICATION

I would like to dedicate this to my grandparents T.M. and Marie Mims and Frankie Cothorn. It's through their example of hard work and all their support that helped me through this master's program. I also dedicate this to my parents Richard and Cathy Mims, whom, I am grateful for all their support. Finally, I dedicate this to my major advisor Dr. Doug Aubrey. I couldn't have asked for a better advisor and I thank him for being an excellent role model.

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## CHAPTER 1

### INTRODUCTION

Stored carbon is important in supplying substrate when current photosynthate (i.e., sources) is unavailable or inadequate to meet the substrate demands (i.e., sinks) of physiological processes, such as growth, defense, and maintenance. On a seasonal basis, nonstructural carbohydrate (NSC) concentrations are largely controlled by different patterns in source/sink relationships that result from changes in physiological activity and carbon allocation. The seasonality exhibited by deciduous trees clearly demonstrates both the perennial requirement for NSC reserves as well as the seasonal pattern in concentrations. Specifically, the refoliation of deciduous trees each spring obviously requires NSCs since photosynthate production is very small or negligible when the primary photosynthetic organs are lacking. Likewise, deciduous trees rely on NSC pools to maintain respiratory processes during the dormant season. In general, carbon demand is increased during the growing season and NSC compounds are mobilized until photosynthate production meets metabolic demands (Hoch *et al.*, 2003) and NSC concentrations are lowest just after the growing season where photosynthate is low or completely unavailable, but metabolic substrate demand continues to draw upon reserves (Richardson *et al.*, 2013).

Disturbance events exert stress on plants that can affect carbon assimilation and use to different degrees depending on frequency and intensity. Post-disturbance recovery from catastrophic disturbances usually depends on the recruitment of new individuals from seed. On the other hand, post-disturbance recovery from chronic disturbances usually depends on the repair and regeneration of damaged tissues. Hence, there is a selective pressure for carbon storage for individuals experiencing chronic disturbances. Chronic disturbances such as fire can scorch foliage and decrease the production of photosynthate to levels below what is required to meet respiratory demands (Aubrey *et al.*, 2012). In

such a case, NSCs are mobilized to compensate for the insufficient supply of current photosynthate required to maintain the metabolism of existing cells as well as to refoliate the canopy, thus decreasing the NSC pool. This strategy of storing carbon may be providing an advantage for individuals who live in environments with frequent disturbances; however, the extent to which disturbance frequencies and NSC pool size are correlated have not been thoroughly researched.

Subtle differences in the evolutionary history of closely related species should result in differences in stored carbon. Longleaf pine (*Pinus palustris*), slash pine (*Pinus elliottii*), and loblolly pine (*Pinus taeda*) are commonly occurring tree species in temperate forests of the southeastern US that can be placed on a continuum with respect to fire frequency. Among these species, longleaf pine experience fire with the highest frequency. Natural fires can occur every year or up to every 5 to 10 years for longleaf pine (Platt, 1991). On the other end of the continuum is loblolly pine—a species that experiences fire with the lowest frequency. Further, loblolly are considered the least fire-resistant of these Southern three pine species (Hare, 1965). Slash pine experience fire at an intermediate frequency. In a century, slash pines can experience a range of 3 to 15 natural fires (Landers, 1991).

These Southern pine species can also be placed on a continuum based on their resistance to insect attacks. Boring bark beetles are a biotic disturbance that may have also played a role in the evolution of *Pinus* species (Guerard *et al.*, 2007; Keeling & Bohlmann, 2006; Warren *et al.*, 1999). These species differ in their resistance to boring insects and can be placed along a continuum similar to fire frequency. Longleaf and slash pines have oleoresin that has moderate to high viscosity, flow, and yield along with a mixture of monoterpenes and resin acids that make them more resistant to bark beetle attack than loblolly (Friedenberg *et al.*, 2007; Hodges *et al.*, 1979). Loblolly is the least resistant to bark beetle attack and loblolly stands have been shown to experience greater number of infestations than longleaf (Friedenberg *et al.*, 2007).

The aim of this study is to determine if there is a relationship between NSC concentrations and disturbance frequency among the three dominant southern pine species. Specifically, this study focuses on determining if natural selection has favored higher NSC concentrations in a species that has evolved under greater disturbance frequencies relative to other closely related species. Here we investigate the relationship of stored carbon and disturbance history across these co-occurring pine species. It is hypothesized that disturbance history influences NSC concentrations of woody perennial species such that species with an evolutionary history of frequent disturbance will exhibit higher concentrations of stored carbon relative to species that have evolved under less frequent disturbance regimes. Using the gradient of fire frequency and insect resistance that the dominant southern pines have evolved under, we expect NSC concentrations will follow along disturbance continuums. In that, we predict that longleaf pine trees will exhibit the highest NSC concentrations and loblolly will exhibit the lowest NSC concentrations.

## CHAPTER 2

### MATERIALS AND METHODS

#### *Study Site Description*

The study was conducted on a 5.26 ha mixed pine forest on privately owned land in Hazlehurst, Georgia, USA (31°41'N, 82°34'W) at approximately 59m above sea level. This area receives a mean annual precipitation of 123.67cm with a mean annual high temperature of 25.61°C and a mean annual low temperature of 12.22°C. The study site consists of planted loblolly and slash pine trees with naturally regenerated longleaf pine and various oak species scattered throughout. The study site is comprised of 57.7% Kershaw sand and 42.3% Troup sand ("Web Soil Survey," 2015).

#### *Experimental Design*

Twelve trees from each species between 20 to 25cm dbh were randomly selected and inspected to ensure there were no diseases or obvious visible injuries that could have affected the amount of carbon stored. Stem cores and coarse root samples were collected from the same individual trees on October 13, 2013 and March 9, 2014. Stem core samples were taken from each tree at breast height using an increment borer. The stem cores were cut to represent a section from the center of the tree to the phloem. Coarse roots were traced from the base of each tree and a representative section was removed with clippers. All coarse root sections were approximately 1.5cm in diameter. Tissue samples were sealed in plastic bags and placed on ice in a cooler to slow enzymatic activity before being transported to the laboratory. Samples were placed in an oven at 60°C until they were completely dry. Dried samples were ground to a fine powder using an 8000D Mixer/Mill ball grinder (SPEX Sample Prep, Metuchen, NJ, USA) and transferred into plastic scintillation vials.

### *NSC Analysis*

Stem and root tissue samples were analyzed for nonstructural carbohydrates in a two-step process to determine sugar and starch concentrations. Sugar and starch concentrations were analyzed following a modified phenol-sulfuric acid method (Buysse & Merckx, 1993). First, 60.0mg of plant powder from each sample was weighed and placed into plastic centrifuge tubes. To extract the soluble sugars, 10mL of 80% ethanol was added to each centrifuge tube and then the samples were stored in a refrigerator overnight. The next day, the samples were allowed to reach room temperature before being placed in a centrifuge for 20 minutes at 6,000 rpm. The resulting supernatant was then poured through filter paper into 50mL volumetric flasks being careful not to pour out any plant powder. Five mL of 80% ethanol was added to each centrifuge tube containing the samples and placed in the centrifuge for an additional 15 minutes. The supernatant was extracted into the corresponding 50mL volumetric flask. This process was repeated for a total of three supernatant extractions. Each volumetric flask was brought to volume with 80% ethanol and poured into 20mL glass scintillation vials to be stored in the refrigerator until spectrophotometer analysis.

After the soluble sugars were extracted from the samples, the resulting plant powder pellet was analyzed for starch concentrations. The centrifuge tubes were placed on a heating block at 90°C until each of the pellets was dry. Five mL of 3% HCl were added to each centrifuge tube which was then placed on the heating block set at 100°C to allow the samples to digest for three hours. The samples were allowed to cool and then poured into a funnel with filter paper to let the extracted starch filter into 50mL volumetric flasks. Each flask was brought to volume with 3% HCl and poured into 20mL glass scintillation vials which were stored in the refrigerator until spectrophotometer analysis. Replicates of each sample were also processed.

To determine the unknown concentrations of our samples, a set of sugar and starch standard solutions were produced. The absorbance values for each set were determined using the GENESYS 20 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Using the linear equations from the absorbance values of these standards, the concentrations of our samples could be determined using their absorbance values and Beer's Law. To determine the absorbance values of our samples, 1mL of sample reacted with 1mL of 28% phenol and 5 mL of concentrated sulfuric acid. Each of these reactants were added to a 20mL glass scintillation vial, capped, and vortexed. The resulting solution sat for 15 minutes before it was transferred into plastic cuvettes to be analyzed in the spectrophotometer for absorbance. Both sugar and starch samples underwent this reaction. Total nonstructural carbohydrate (TNC) concentration was determined by adding sugar and starch concentrations for each sample.

Species-specific allometric equations were used to estimate NSC content above and below ground for each tree. The allometric equations used to estimate biomass for our study species came from an article by Gibson *et al* (1985). These equations were chosen because they were derived from stands that had all three of our study species with similar dbh measurements and site characteristics and provided both above and belowground biomass equations for each of the species. Aboveground equations estimated total stem wood while belowground equations estimated stump and taproot wood. We applied dbh and height measurements from our sample trees to estimate biomass and then multiplied these biomass estimates by our NSC concentrations to estimate NSC content.

### *Statistical Analysis*

We compared NSC concentrations and content as a function of species, organ, and month using repeated measures ANOVA. Our experimental unit was the individual trees (n=12). Time (n=2) was treated as a repeated factor, while species (n=3) and organ (n=2) were treated as fixed factors. Treatment means were compared using Tukey's Honestly Significant Difference (HSD) test. Significant

interactions were decomposed using tests of simple main effects. All analyses were performed using JMP Pro 10 software with  $\alpha=0.05$ .

## CHAPTER 3

### RESULTS

#### *Sugar Concentrations*

Sugar concentrations differed among species, between organs, and changed through time; however, their individual effects were not always independent of each other. Longleaf roots exhibited higher sugar concentrations than loblolly and slash, which exhibited similar sugar concentrations (Fig. 3.1); however, sugar concentrations of stems were similar among all species (species  $\times$  organ interaction;  $P < 0.0001$ ). Roots exhibited 536% higher sugar concentrations than stems ( $P < 0.0001$ ; Fig. 3.2) and this effect was independent of time or species. Sugar concentrations were 17% higher in October than March for roots, but remained similar through time in stems (time  $\times$  organ interaction;  $P < 0.0138$ ; Fig. 3.3).

#### *Starch Concentrations*

Starch concentration differed among species, between organs, and changed through time; however, these individual effects were not independent of each other (i.e., species  $\times$  organ  $\times$  time interaction). Longleaf roots exhibited the highest starch concentrations in March when loblolly and slash were similar; however, loblolly roots exhibited a higher starch concentration than slash in October, whereas longleaf root starch concentration was similar to both (Fig. 3.4). Stems of all species exhibited similar starch concentrations and these remained similar through time (Fig. 3.4). Regardless of the differences in root starch concentrations among species, root starch concentrations were higher in March than October (Fig. 3.4). Regardless of species, root starch concentrations were higher than stem starch concentrations in March; however, stem and root concentrations were similar in October for longleaf and loblolly, whereas the stem concentrations in slash was higher than the root starch concentration (Fig. 3.4).

### *TNC Concentrations*

TNC concentrations differed among species, between organs, and changed through time; however, their individual effects were not always independent of each other. Longleaf roots exhibited higher TNC concentrations than loblolly and slash, which exhibited similar TNC concentrations (Fig. 3.5); however, TNC concentrations of stems were similar among all species (species x organ interaction;  $P < 0.0001$ ). Roots exhibited 64% higher TNC concentrations than stems ( $P < 0.0001$ ; Fig. 3.6) and this effect was independent of time or species. Roots exhibited 33% higher TNC concentrations in March than October for roots, but remained similar through time in stems (time x organ interaction;  $P < 0.0001$ ; Fig. 3.7).

### *Stand Characteristics*

DBH height and biomass were measured or calculated for all the individuals of the mixed stand of pine species. There were no significant differences in dbh among the three study species. The average dbh measurements for my pine species ranged from 21.6cm to 22.1cm ( $P = 0.5467$ ; Fig. 3.8). Height differed among all three of my species. Slash pine was the tallest species with an average height of 18.3m. Next, longleaf exhibited an average height of 14.4m. The shortest species was loblolly with an average height of 12.7m ( $P < 0.0001$ ; Fig. 3.9). Similarly to height, the biomass of the stems differed among all three pine species. Slash had the greatest stem biomass with an average biomass of 136.9kg. Next, longleaf exhibited an average stem biomass of 115.3kg. With an average biomass of 88.3kg, loblolly had the lowest stem biomass ( $P < 0.0001$ ; Fig. 3.10). Unlike the stems, the root biomass did not differ among my three study species. The average root biomass for longleaf, slash, and loblolly ranged from 17.6kg to 26.2kg.

### *Sugar Content*

Sugar content differed among species, between organs, and changed through time; however, their individual effects were not always independent of each other. Longleaf roots exhibited higher sugar

content than slash and loblolly, which exhibited similar sugar content (Fig. 3.11); however, sugar content in the stems were similar among all species (species x organ interaction;  $P < 0.0001$ ). Roots exhibited 60% higher sugar content than stems in October; however, stem and root sugar content were similar in March (Fig. 3.12). Sugar content in the roots and stems remained similar through time (time x organ interaction;  $P < 0.001$ ; Fig. 3.13).

#### *Starch Content*

Starch content differed among species, between organs, and changed through time; however, their individual effects were not always independent of each other. Slash stems exhibited higher starch content than loblolly and longleaf, which exhibited similar starch content (Fig. 3.14); however, starch content of roots were similar among all species (species x organ interaction;  $P < 0.0001$ ). Stems exhibited 312% higher starch content than roots ( $P < 0.0001$ ; Fig. 3.15) and this effect was independent of time or species. Roots exhibited 84% higher starch content in March than October, but starch content remained similar through time in stems (time x organ interaction;  $P < 0.0008$ ; Fig. 3.16).

#### *TNC Content*

TNC content differed among species, between organs, and changed through time; however, their individual effects were not always independent of each other. Slash stems exhibited higher TNC content than loblolly and longleaf, which were similar, while longleaf roots exhibited higher TNC content than loblolly, whereas slash exhibited an intermediate TNC content that was similar to both longleaf and loblolly (Fig. 3.17); (species x organ interaction;  $P < 0.0001$ ). Stems exhibited 190% higher TNC content than roots ( $P < 0.0001$ ; Fig. 3.18) and this effect was independent of time or species. Roots exhibited 38% higher TNC content in March than October for roots, but remained similar through time in stems (time x organ interaction;  $P < 0.0146$ ; Fig. 3.19).

## CHAPTER 4

## DISCUSSION

*Species Differences*

Longleaf exhibited the highest NSC concentrations of the three southern pine species investigated; however, the concentrations exhibited by the other species did not follow the continuum of disturbance frequency as I had predicted. Instead, slash and loblolly exhibited similar NSC concentrations. Therefore, the evolutionary history of disturbance frequency and the resulting resilience to disturbance only partly explains the patterns of carbon storage exhibited by these three pine species. Deviations from my predictions likely indicate that multiple evolutionary pressures have selected for stored carbon in these pine species. Below I consider other disturbance agents as well as different growth strategies exhibited by these species at key points in their life history that may have influenced selection for stored carbon.

Evolutionary history of insect attacks could potentially explain the differences in NSC observed among the southern pine species. NSC plays an important role in the production of resin and other secondary compounds that help defend against beetle attack (Guerard *et al.*, 2007). Also, NSC may help activate defensive mechanisms that are induced by a beetle attack (E. Christiansen & Ericsson, 1986; Erik Christiansen *et al.*, 1987; Guerard *et al.*, 2007; Lahr & Sala, 2014; Lombardero *et al.*, 2000). The beetles feed on the phloem, cambium, and outer sapwood which can lead to the girdling of the tree (A. A. Berryman, 1972; Friedenber *et al.*, 2007). Also, these beetles have associated fungi that can contribute to host mortality through mycelial penetration of tissues, the release of toxins, or interactions with host defenses (Paine *et al.*, 1997). The success of defending off insects and associated pathogens depends on the quality and quantity of these defensive compounds (A. A. Berryman, 1972; Erik Christiansen *et al.*, 1987; Hodges *et al.*, 1979; Keeling & Bohlmann, 2006; Warren *et al.*, 1999). Trees with greater NSC concentrations may be better suited to survive an attack by beetles because there is more substrate

available for synthesis of defense compounds (E. Christiansen & Ericsson, 1986; Warren *et al.*, 1999). This means there may be a selective advantage for pine tree species with greater NSC concentrations to better defend against insect attacks. The greater NSC concentrations found in longleaf may be an evolutionary adaptation to beetle attacks, which have led them to be less susceptible to beetle attack in present day.

I hypothesized that fire would be the major selective pressure for carbon storage for these southern pine species which all exhibit some fire-adapted traits (Fonda, 2001; Keeley, 2012; Pausas, 2015). While all three species are considered to be fire resistant, they can however, be placed along a continuum based on their relative degree of fire resistance (De Ronde, 1982; McCune, 1988). On one end of the continuum, longleaf is considered to be the most fire resistant species while loblolly is regarded as the least fire resistant. Slash is considered to be more fire resistant than loblolly, but less resistant to fire than longleaf (De Ronde, 1982; Hare, 1965; McCune, 1988). These three pine species share similar fire-adapted traits that contribute to their fire resistance. For example, all three species exhibit some degree of self-pruning which helps prevent fires from climbing branches to the crown (He *et al.*, 2012; Keeley, 2012; Keeley *et al.*, 2011; O'Brien *et al.*, 2008; Kenneth W Outcalt, 2000; Pausas, 2015). All three species exhibit relatively thick tree bark that insulates the cambium from extreme temperatures associated with fire (He *et al.*, 2012; Keeley, 2012; Keeley *et al.*, 2011; O'Brien *et al.*, 2008; Kenneth W Outcalt, 2000; Pausas, 2015; Pausas & Keeley, 2009; Van Lear & Harlow, 2000), though the bark of longleaf and slash exhibits greater insulating efficiency than that of loblolly (Hare, 1965). One major difference in fire-adapted traits among these three species is the early growth strategy exhibited by seedlings.

Whereas loblolly and slash seedlings exhibit a strategy of fast growth to escape low to moderate surface fires, longleaf exhibits a different strategy in which the seedling remains in a fire-resistant grass stage. If a fire occurs before a loblolly or slash seedling can extend their canopy high enough to escape

damage, then they are susceptible to mortality from crown scorching (Burns, 1983; Keeley, 2012; O'Brien *et al.*, 2008). However, longleaf seedlings tolerate low-intensity fires before the canopy grows tall enough to escape surface fires by remaining within the grass stage for five to ten years (Keeley, 2012). While in the grass stage, the apical meristem is protected from low intensity surface fires by moisture rich sheath of needles (O'Brien *et al.*, 2008; Van Lear & Harlow, 2000) and an extensive root system develops (Heyward, 1933; O'Brien *et al.*, 2008; Kenneth W Outcalt, 2000) that stores reserves to help refoliate scorched needles and fuel rapid stem growth (Fonda, 2001; He *et al.*, 2012; Keeley, 2012; O'Brien *et al.*, 2008; Van Lear & Harlow, 2000). Indeed, once the stem begins growing vertically, it rapidly extends the canopy high enough aboveground to avoid low intensity surface fires (Fonda, 2001; He *et al.*, 2012; Keeley, 2012; O'Brien *et al.*, 2008; Van Lear & Harlow, 2000).

Pine seedlings must endure various disturbances to survive and grow to a sexually mature stage to pass on genes to their offspring. The growth strategy exhibited by slash and loblolly seedlings requires longer return intervals of fire disturbances to assure that their seedlings are tall enough to survive wildfires (Kenneth W Outcalt, 2000). However, the rapid stem growth of longleaf seedlings, allows for this species to thrive in ecosystems with shorter return intervals of fire (Keeley, 2012; O'Brien *et al.*, 2008; Van Lear & Harlow, 2000). From this, fire seems to be the greatest bottleneck for pine seedlings to survive to sexually mature adults. The increased carbon storage which fuels rapid stem growth provides an advantage that increases the fitness of longleaf seedlings allowing them to survive frequent fire regimes to become sexually mature adults (Fonda, 2001; He *et al.*, 2012; Keeley, 2012; O'Brien *et al.*, 2008; Van Lear & Harlow, 2000). Thus, there would have been a selective advantage for increased belowground carbon storage during the evolutionary history of longleaf. Under this framework, the higher NSC concentrations observed in mature longleaf may simply be a relic of the selective pressure imposed at the seedling stage. This relic of the juvenile stage provides benefits to adult longleaf such as,

providing carbon to help refoliate and maintain belowground respiration after a fire disturbance (Aubrey *et al.*, 2012).

Disturbance is considered to be a strong selective pressure in the evolution of life histories (Sousa, 1984; White, 1979). Disturbance regimes include frequency, predictability, and severity. Generally, selection strength increases as these factors increase (Lytle, 2001; White, 1979). With this in mind, we assess the selection strength of insect and fire disturbances on carbon storage at different points of longleaf life history. Wood-boring insects feed on phloem for nutrition (A. A. Berryman, 1972; Friedenber *et al.*, 2007) and phloem that is rich in carbohydrates is more nutritious and preferred by wood-boring insects (Raffa *et al.*, 1993). Longleaf seedlings in the grass stage have very little phloem making them less desirable to wood-boring insects as opposed to more mature trees with larger volume of more nutritious phloem (A. A. Berryman, 1972; Alan A Berryman, 1976; Erik Christiansen *et al.*, 1987). Based on this, we would not expect there to be any selective pressure for carbon storage on juvenile pines imposed by insects. Despite the extreme severity of insect infestations, (see Billings 1994 and Raffa *et al.* 2008), it is probably not as strong of a selective force for carbon storage compared to fire disturbance, certainly not at the juvenile stage.

My results suggest that the selective pressure of fire on stored carbon is more influential at the juvenile stage than at the adult stage. As stated before, fire seems to be the greatest bottleneck preventing seedlings from reaching sexual maturity. The increased carbon storage in longleaf seedlings provides an advantage that was selected to help longleaf survive frequent fire regimes (Fonda, 2001; He *et al.*, 2012; Keeley, 2012; O'Brien *et al.*, 2008; Van Lear & Harlow, 2000). Fire is relatively predictable due to flammable fuel loads and seasonal storms that cause lightning ignited wildfires (K. W. Outcalt, 2008). The thin, long needles of pines result in fuel loads with low compaction which help attribute to the low severity of fire (Pausas, 2015). The highly flammable needles assure that fuel loads are

frequently burned which prevent the buildup of fuel that would result in high severity fire (Fonda, 2001; Pausas, 2015). Damage from fire may not be as severe as damage from insects, but its predictability and high frequency make fire the most likely selective driver for the carbon storage observed in longleaf.

### *Spatial and Temporal Dynamics*

The dynamics of NSC are considered to reflect the source-sink relationships within trees (Chapin *et al.*, 1990; Dietze *et al.*, 2014; Hoch *et al.*, 2003; Lacoïnte, 2000; Richardson *et al.*, 2013). In my study, seasonality was exhibited in the roots, but not the stems. As expected, roots in October exhibited the lowest NSC concentrations because carbon demand during the growing season is greater than the supply of photosynthate, so these reserves are drawn upon to help satiate carbon demand decreasing the size of the NSC pools. On the other hand, roots in March exhibited the highest NSC concentrations because carbon demand during the dormant season is less than the supply of photosynthate, so the NSC pools accumulate up to the point of spring growth (Chapin *et al.*, 1990; Korner, 2003; Sala *et al.*, 2012; Wiley & Helliker, 2012). Roots of larch (*Larix gmelinii*) and ash (*Fraxinus mandshurica*) trees were reported to have decreased NSC during the beginning of the growing season in response to the increased carbon demand (Mei *et al.*, 2015).

Unlike the roots, the NSC concentrations of stems in my study did not exhibit any seasonal dynamics. This conforms to results from Hoch *et al.* (2003) where ten temperate forest species did not exhibit significant seasonal differences in the NSC concentrations of stem. Likewise, *Pinus sylvestris* did not exhibit seasonal differences in the stem in a study by Terziev (1997). However, NSC concentrations in the stems and roots of *Larix gmelinii* and *Fraxinus mandshurica* trees displayed similar seasonal dynamics (Mei *et al.*, 2015). Although coarse roots and stems are usually the main storage organs for NSC (Dietze *et al.*, 2014; Hoch *et al.*, 2003; Wurth *et al.*, 2005), leaves and branches can also function as storage organs (Chapin *et al.*, 1990; Korner, 2003; Richardson *et al.*, 2013; Wurth *et al.*, 2005). The

mobilization of NSC from every storage organ plays a role in the dynamics of NSC within a tree (Richardson *et al.*, 2013). The seasonal dynamics exhibited by roots in my study may be attributed to the mobilization of reserve materials from the roots to the stems. For example, the stems could be maintaining near-constant concentrations of NSC due to mobilization of NSC from the roots and/or other storage organs.

Opposing seasonal trends of sugar and starch concentrations are relatively common and are likely due to the hydrolysis of starch—the longer term storage component of NSC—to sugar—the shorter term component of NSC that represents available substrate (Richardson *et al.*, 2013; Terziev *et al.*, 1997; Wong *et al.*, 2003). The roots in my study exhibited an opposing trend as well. Sugar concentrations were significantly higher in October than March, while starch concentrations were higher in March than October. Richardson *et al.* (2013) attributed the opposing trends to the interconversion between sugar and starch components. This would help explain the trends because during the growing season starch concentrations decrease because they are being hydrolyzed into sugar to be used to meet high carbon demand. During the dormant season, excess sugars are converted into starch for storage because the carbon demand is lower (Chapin *et al.*, 1990; Korner, 2003; Sala *et al.*, 2012; Wiley & Helliker, 2012). However, with only two sampling dates, my data cannot provide a complete picture of what may actually be happening in the months between these two dates.

Although roots exhibited higher concentrations of NSC than stems, stems represented the largest pool of NSC due to the larger biomass of the stem in comparison to the root biomass. A similar pattern was observed by Barbaroux *et al.* (2003), where beech and oak trees exhibited the greatest concentrations of NSC in the roots and branches, but the largest pool of NSC was located in the stem. Likewise, *Larix gmelinii* and *Fraxinus mandshurica* trees have exhibited the largest pools of NSC in the stem (Mei *et al.*, 2015).

Increased NSC storage in the roots is more common in species that experience aboveground disturbances (Iwasa & Kubo, 1997). Root reserves in the fire-adapted longleaf pine have been shown to maintain belowground respiration for long periods following severe foliar scorching events (Aubrey *et al.*, 2012). Perhaps resprouters are the best example of increased root storage of NSC for post-disturbance recovery. Resprouters like *Stiralinia latifolia* have been known to allocate most of its NSC reserves in the roots to aid in recovery from severe fire disturbances (Bowen & Pate, 1993). One reason for this increased storage of NSC reserves to belowground organs is that NSC is better protected belowground from aboveground disturbances (Clarke *et al.*, 2013; Iwasa & Kubo, 1997). This may help explain why all of the species in our study exhibited higher concentrations in the root than in the stems.

Starch was the major NSC component for each organ, especially in the stems. Averaging across species and month, starch accounted for 63.2% and 90.5% of the total NSC pool in the roots and stems, respectively. In many studies, starch has been shown to be the major component of NSC. For example, tropical trees studied by Würth *et al.* (2005) exhibited a sugar to starch ratio of 1:2.5 in the stem and 1:2 in coarse roots. Also, the majority of temperate forest tree species studied by Hoch *et al.* (2003) exhibited significantly higher starch concentrations compared to sugar concentrations in the stem.

## CHAPTER 5

### CONCLUSION

I found that patterns of stored carbon in mature southern pine trees could not be predicted simply by their evolutionary history of disturbance as I had predicted. Indeed, longleaf had higher NSC concentrations than slash and loblolly. However, slash and loblolly exhibited similar concentrations of NSC instead of following along the continuum of disturbance frequency. To help explain these deviations from my predicted results, I investigated differences in early growth strategies of seedlings. The growth strategies of these southern pines are adapted to help seedlings survive in ecosystems with frequent fire. Especially for longleaf, that stores reserves in roots while in the seedling grass stage to help fuel rapid stem growth to extend canopy above the threat of surface fires. In this sense, fire seems to be the greatest bottleneck that seedlings must endure to survive to sexually mature adults. Factors of disturbance regimes were used to assess the selection strength of insect and fire disturbances for stored carbon. Despite the low severity of surface fires, the high frequency and its predictability make fire the most likely selective driver for stored carbon. These results suggest that the patterns of stored carbon observed in mature trees may be a relic of selective pressures that affected carbon storage in juvenile trees. Future studies should investigate seedlings and saplings of these species to determine if these patterns do, in fact, emerge at the juvenile stage.

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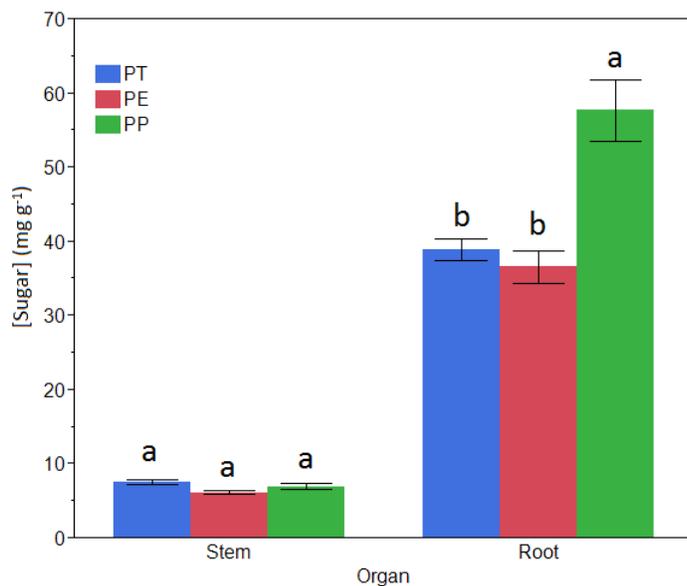
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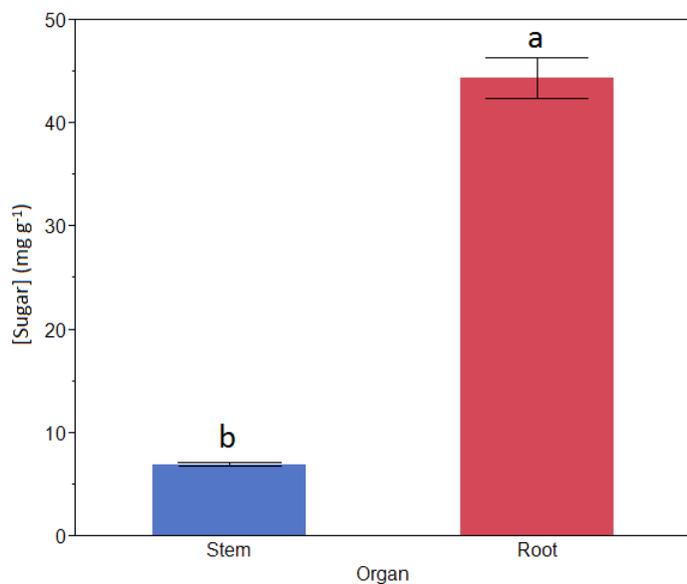
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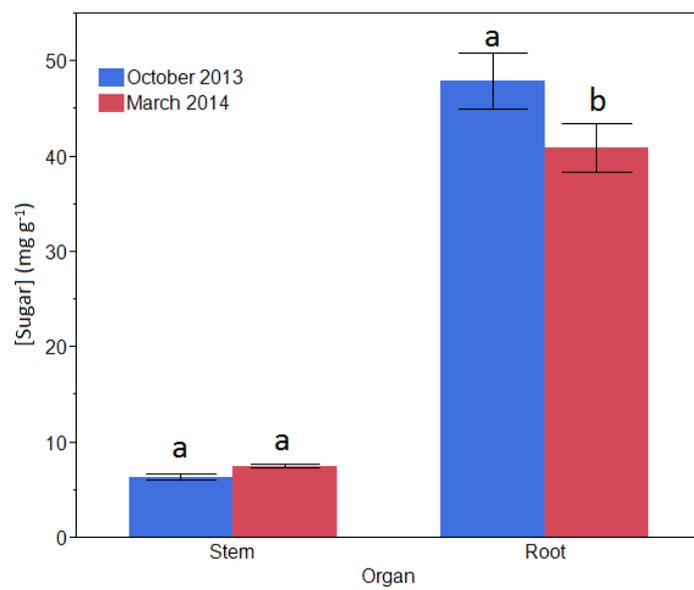
## APPENDIX A



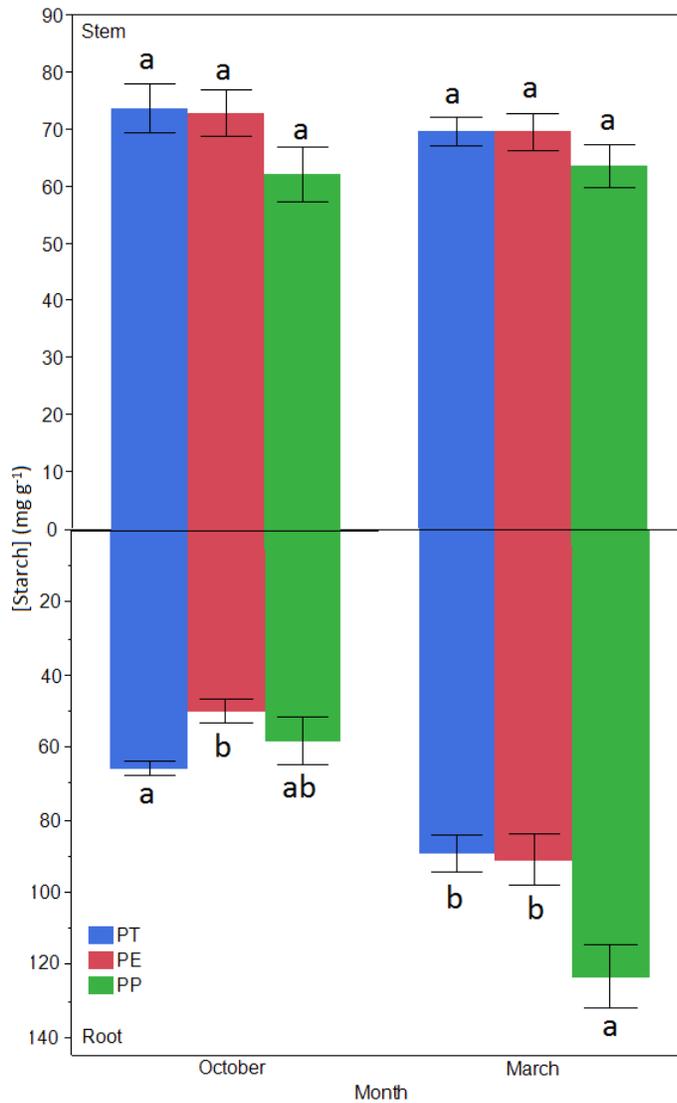
**Figure 3.1:** Mean ( $\pm$ SE) sugar concentrations ( $\text{mg g}^{-1}$ ) for species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) by organ (stem and root) averaged across months (October 2013 and March 2014). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



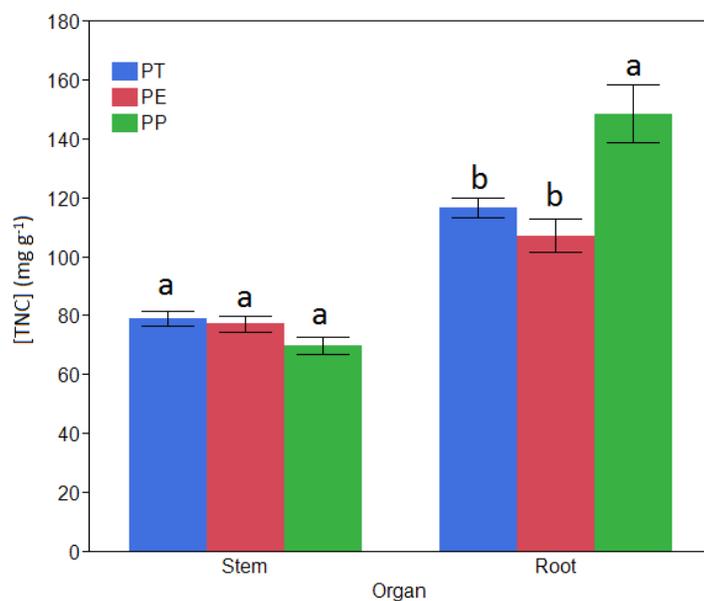
**Figure 3.2:** Mean ( $\pm$ SE) sugar concentrations ( $\text{mg g}^{-1}$ ) between organ (stem and root) averaged across species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) and months (October 2013 and March 2015). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



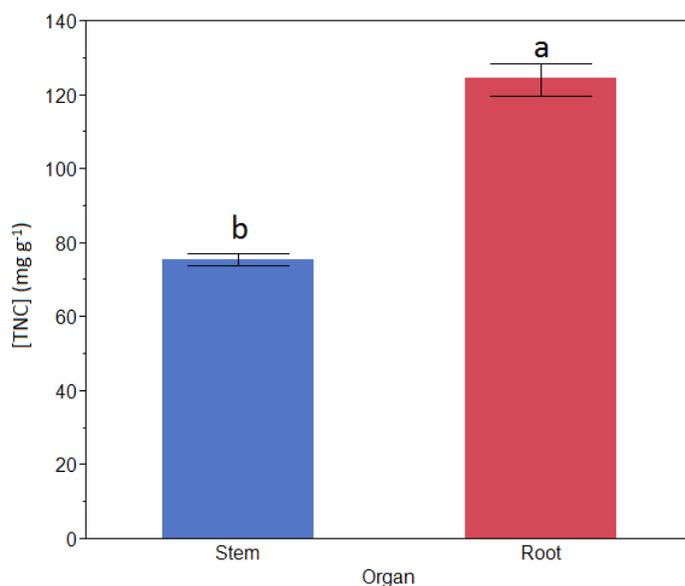
**Figure 3.3:** Mean ( $\pm$ SE) sugar concentrations ( $\text{mg g}^{-1}$ ) for organ (stem and root) by month (October 2013 and March 2014) averaged across species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



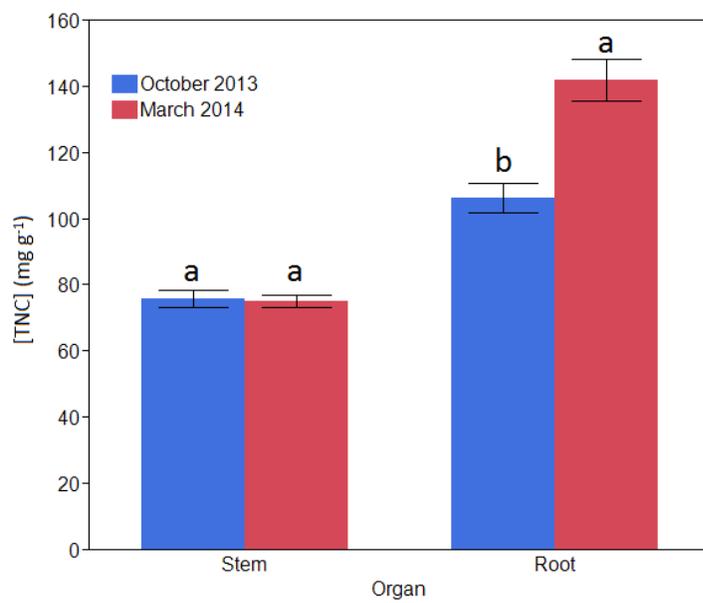
**Figure 3.4:** Mean ( $\pm$ SE) starch concentrations ( $\text{mg g}^{-1}$ ) for species (PT= *Pinus tadea*, PE= *Pinus eliottii*, and PP= *Pinus palustris*) by month (October 2103 and March 2014) by organ (stem and root). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



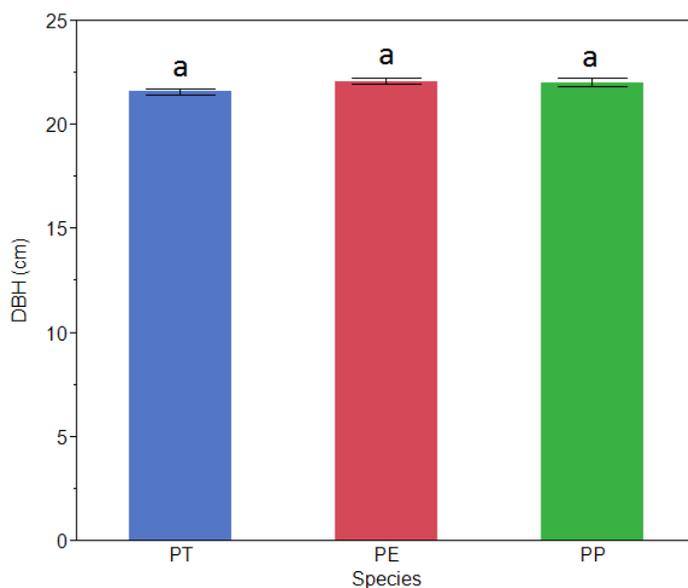
**Figure 3.5:** Mean ( $\pm$ SE) TNC concentrations ( $\text{mg g}^{-1}$ ) for species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) by organ (stem and root) averaged across months (October 2013 and March 2014). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



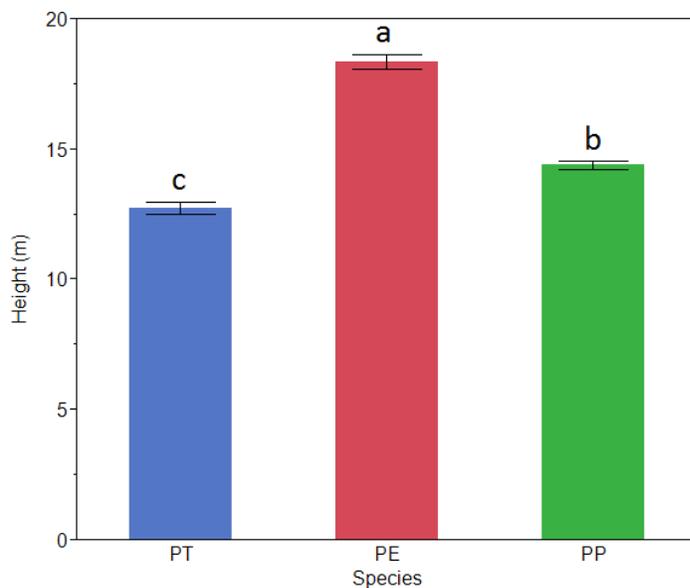
**Figure 3.6:** Mean ( $\pm$ SE) TNC concentrations ( $\text{mg g}^{-1}$ ) between organ (stem and root) averaged across species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) and months (October 2013 and March 2015). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



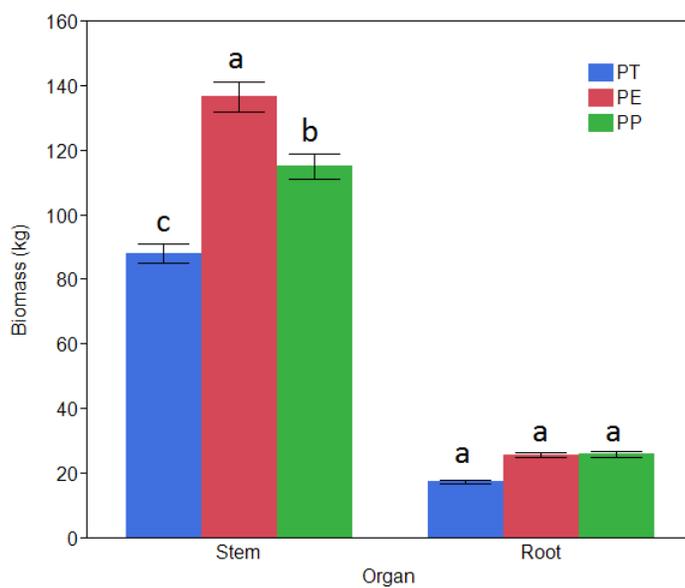
**Figure 3.7:** Mean ( $\pm$ SE) TNC concentrations ( $\text{mg g}^{-1}$ ) for organ (stem and root) by month (October 2013 and March 2014) averaged across species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



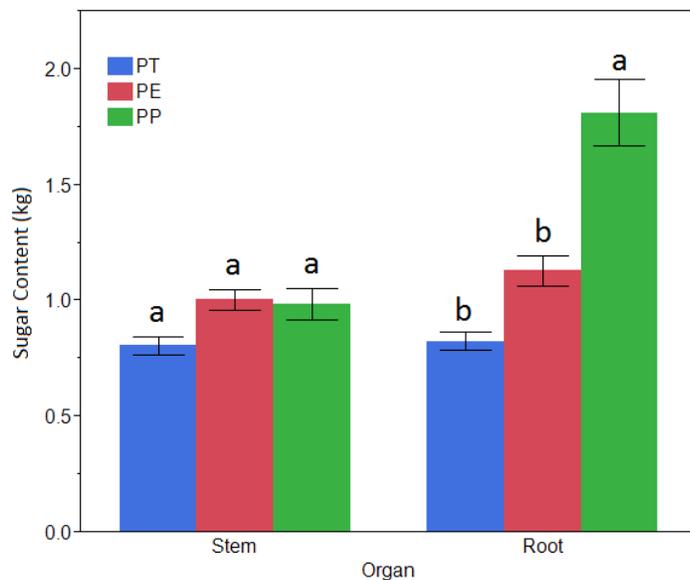
**Figure 3.8:** Mean ( $\pm$ SE) DBH (cm) for species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*). Bars with different letters indicate significant differences ( $\alpha=0.05$ ).



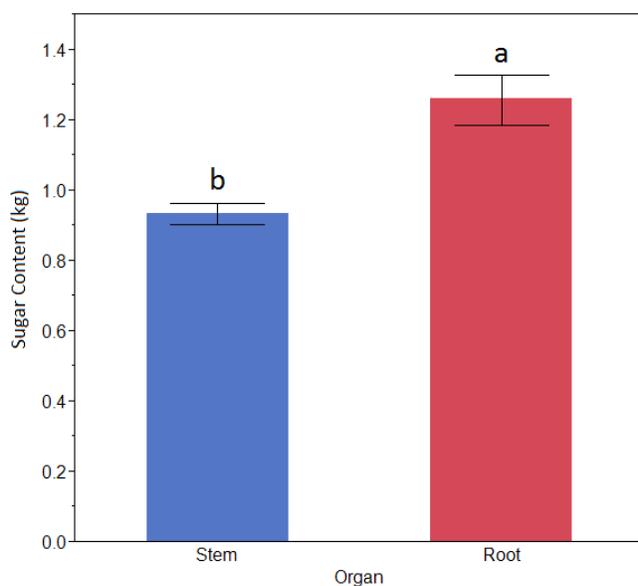
**Figure 3.9:** Mean ( $\pm$ SE) heights (m) for species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*). Bars with different letters indicate significant differences ( $\alpha=0.05$ ).



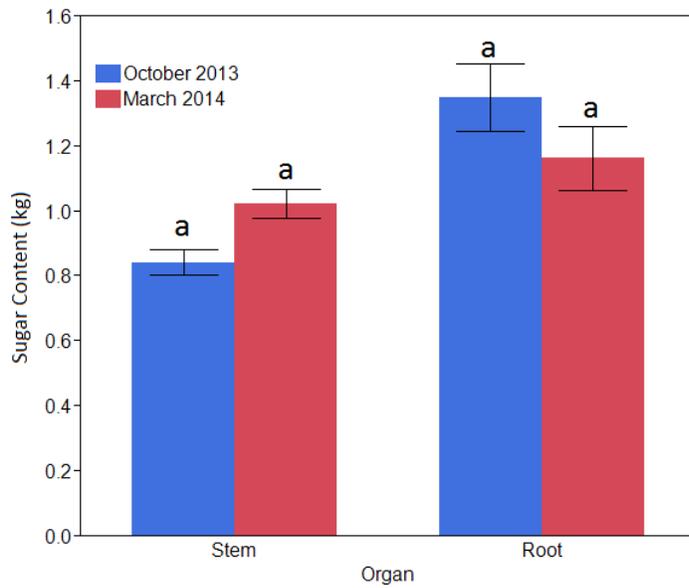
**Figure 3.10:** Mean ( $\pm$ SE) biomass (kg) for species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) by organ (stem and root). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



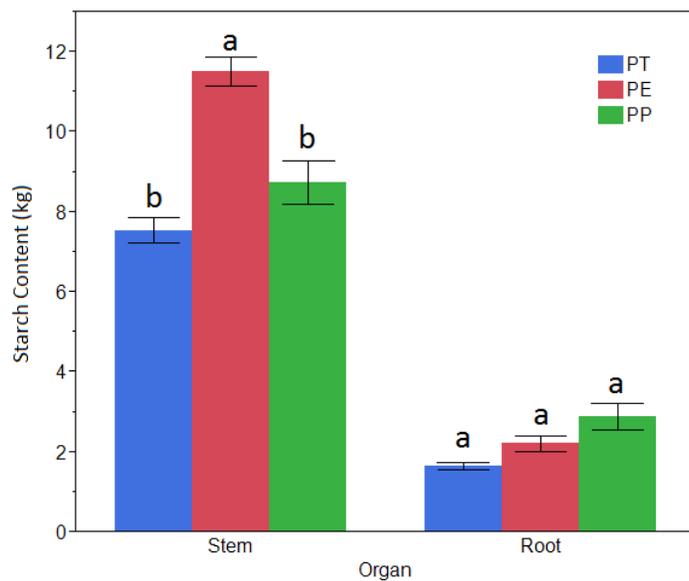
**Figure 3.11:** Mean ( $\pm$ SE) sugar content (kg) for species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) by organ (stem and root) averaged across months (October 2013 and March 2014). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



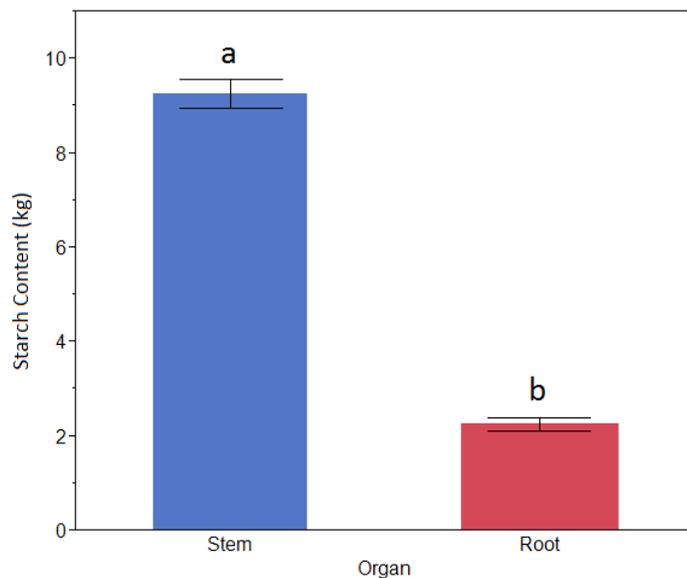
**Figure 3.12:** Mean ( $\pm$ SE) sugar content (kg) between organ (stem and root) averaged across species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) and months (October 2013 and March 2015). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



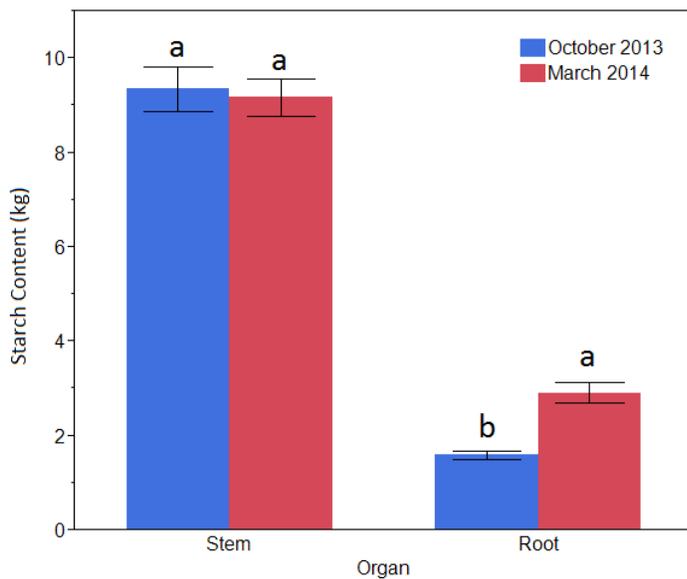
**Figure 3.13:** Mean ( $\pm$ SE) sugar content (kg) for organ (stem and root) by month (October 2013 and March 2014) averaged across species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



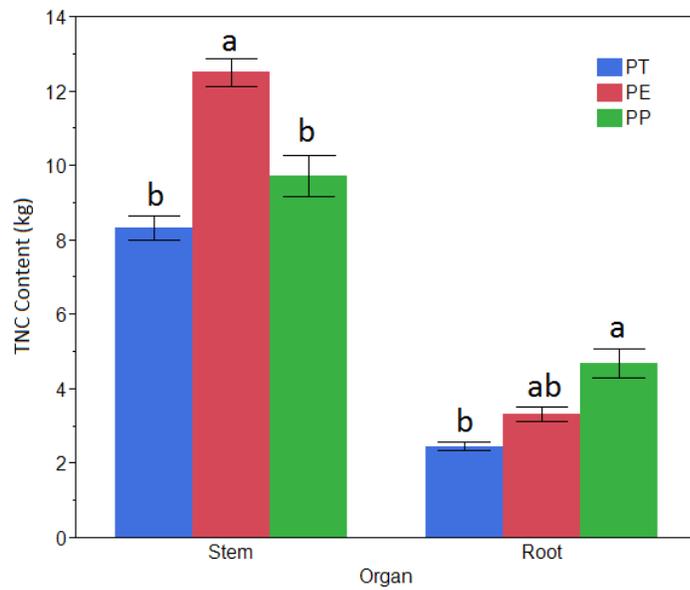
**Figure 3.14:** Mean ( $\pm$ SE) starch content (kg) for species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) by organ (stem and root) averaged across months (October 2013 and March 2014). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



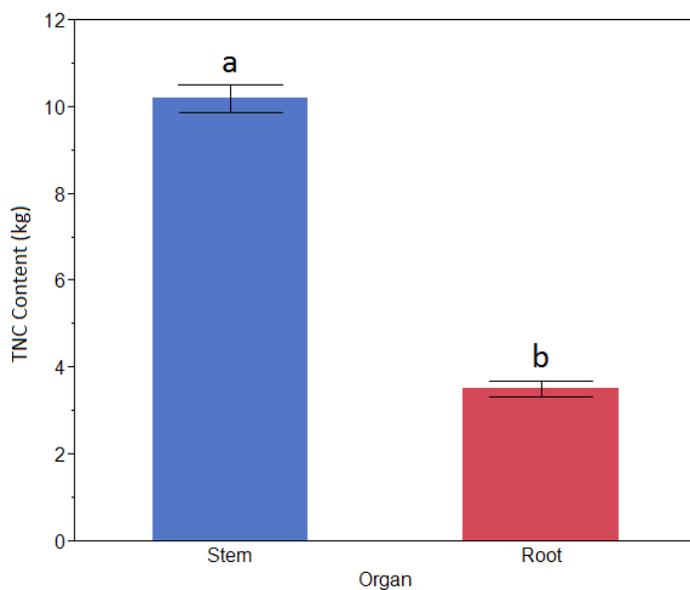
**Figure 3.15:** Mean ( $\pm$ SE) starch content (kg) between organ (stem and root) averaged across species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) and months (October 2013 and March 2015). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



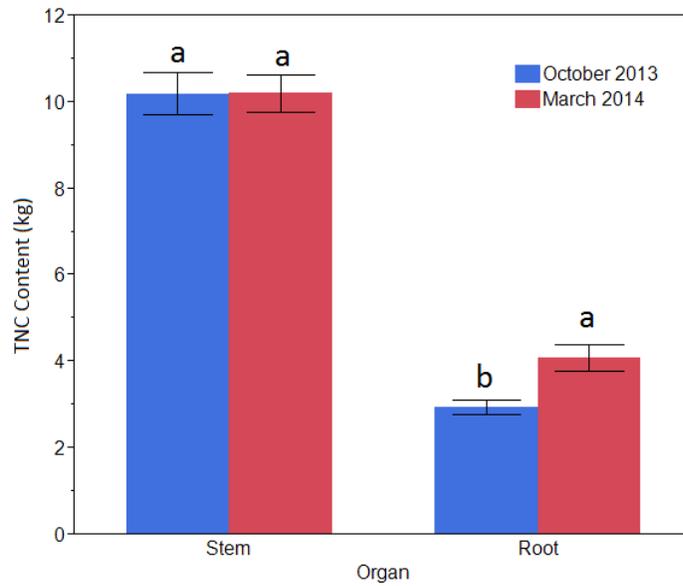
**Figure 3.16:** Mean ( $\pm$ SE) starch content (kg) for organ (stem and root) by month (October 2013 and March 2014) averaged across species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



**Figure 3.17:** Mean ( $\pm$ SE) TNC content (kg) for species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) by organ (stem and root) averaged across months (October 2013 and March 2014). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



**Figure 3.18:** Mean ( $\pm$ SE) TNC content (kg) between organ (stem and root) averaged across species (PT= *Pinus tadea*, PE= *Pinus elliottii*, and PP= *Pinus palustris*) and months (October 2013 and March 2015). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).



**Figure 3.19:** Mean ( $\pm$ SE) TNC content (kg) for organ (stem and root) by month (October 2013 and March 2014) averaged across species (PT= *Pinus tadea*, PE= *Pinus elliotii*, and PP= *Pinus palustris*). Bars with different letters within an organ indicate significant difference ( $\alpha=0.05$ ).