Changes in Water Use, Nitrogen Uptake, and Carbon Assimilation During Mortality of Loblolly Pine and Succession to Sweetgum

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Changes in water use, nitrogen uptake, and carbon assimilation during mortality of loblolly pine and succession to sweetgum

By

Nicole Hornslein

A Thesis
Submitted to the Faculty of Mississippi State University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Forestry in the Department of Forestry

Mississippi State, Mississippi
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Changes in water use, nitrogen uptake, and carbon assimilation during mortality of loblolly pine and succession to sweetgum

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As forests change, tree physiology responds to changes in resource demands. The impact of *Pinus taeda* (loblolly pine) mortality on physiology of successional hardwoods is unknown. *Liquidambar syraciflua* (sweetgum) and loblolly pine individuals were measured for resource-use during a simulated southern pine beetle mortality event where several pines underwent a girdling treatment. Sweetgum next to untreated pines had significantly higher sapflow every month, markedly throughout post-mortality months. Sapflow and photosynthetic capacity significantly declined in girdled pines before needle discoloration. Nitrogen concentration of senesced pine and sweetgum leaves significantly increased from pre-mortality to post-mortality. Pine mortality led to increases in sweetgum water use and leaf nitrogen content. A shift in species dominance from loblolly pine to sweetgum would reduce water lost by pine transpiration during sweetgum dormancy by approximately 154 mm. These data indicate significant responses to disturbance and seasonal resource demands in this forest type.
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CHAPTER I
INTRODUCTION

Historically, land in the southeastern United States has undergone extensive conversion to pine plantations for timber development (Schultz, 1997). Without silvicultural intervention, deciduous hardwood species will successfully establish in the understory of, and eventually replace, aging pine stands (Bormann, 1953; Wells et al., 1991; Schultz, 1997). Loblolly pine (*Pinus taeda* L.) forests have experienced localized mortality due to southern pine beetle (SPB; *Dendroctonus frontalis* Zimmermann) attack, and/or as forestry practices are abandoned and stands undergo succession to deciduous hardwoods (Coleman et al., 2008). In central Mississippi, USA, unmanaged forests have the potential to undergo succession from loblolly pine to hardwoods including sweetgum (*Liquidambar styraciflua* L.). As succession occurs, differences in physiological functioning between species will result in changes in resource use and availability. Additionally, these changes in species dominance will affect future availability of resources. This is particularly notable in forest type conversions from conifers to deciduous hardwoods (or vice-versa) due to known patterns of seasonal water use and carbon (C) uptake/assimilation, as well as differences in leaf habit of each forest type (Reich et al., 1995).

Water and nutrient acquisition through roots depend on a gradient of water potential that determine the rate of water transport throughout a tree’s sapwood, or
sapflow rate. Sapflow rate is measured as a method of estimating transpiration (Granier, 1987). Transpiration, a component of total evapotranspiration (ET), occurs when water vapor exits leaf stomata during the intake of carbon dioxide (CO₂) from the atmosphere. The rate at which these gases are exchanged through leaf stomata is referred to as stomatal conductance. The transpiration process is necessary in order for opened stomata to obtain enough atmospheric CO₂ for photosynthesis (i.e., carbon assimilation) to occur. Transpiration is influenced by climate variables including air temperature and relative humidity (which are used to calculate vapor pressure deficit; VPD), volumetric soil water content (soil moisture), and photosynthetically active radiation (PAR). These climate variables will be referred to as environmental parameters in this study.

The quantity of C and nitrogen (N) retained in leaf matter has impacts on forest nutrient cycling dynamics, which alter future nutrient availability and photosynthetic capacity (Reich et al., 1995). In terms of water use and availability, loblolly pine, as a conifer species, retains a portion of its needles year-round and transpires more water throughout the dormant season than deciduous hardwoods (Swank and Douglass, 1974; Stoy et al., 2006; Rao et al., 2011). Stand species composition, basal area, and patterns of seasonal rainfall are all large factors in determining species contribution to transpiration and water loss to the atmosphere (Pataki et al., 1998; Ewers et al., 2002). The impacts of these differences in seasonal water use dynamics can increase water yield in the dormant season, while decreasing water yield in the growing season. Thus, this forest type conversion from loblolly pine to sweetgum may alter future conditions of nutrient and hydrologic cycling in many southeastern forests.
This research quantified changes in forest functioning, including changes in tree-level water use, C uptake, and N uptake during overstory loblolly pine mortality. The objectives were to: 1) determine physiological outcomes of loblolly pine, throughout pine mortality 2) determine if mid-canopy sweetgum exhibit a physiological response to pine mortality, and 3) make stand-scale comparisons between loblolly pine and sweetgum water use relative to seasonal water stress and inundation. Physiological responses measured include sapflow rate, leaf N uptake, photosynthetic capacity, bole growth, bole respiration, leaf area and litterfall quantity.

It was hypothesized that under selective loblolly pine mortality: 1) control pines and sweetgum (particularly sweetgum next to girdled pines) would increase water use post-mortality, 2) increases in soil moisture deficit and VPD would negatively affect pine and sweetgum water use rates, 3) N concentration of leaf and needle litter would increase post-mortality, 4) N concentration in fresh leaves would be higher in sweetgum next to girdled pines than sweetgum next to control pines, leading to increases in photosynthetic capacity, 5) control pines and sweetgum would increase leaf area post-mortality, 6) control pines and sweetgum would increase bole growth in comparison to previous growing seasons, and 7) girdled pines would exhibit higher stem respiration. Due to known differences in conifer and deciduous hardwood physiology, a change in forest dominance from loblolly pine to sweetgum would alter availability of resources in the system, impacting hydrological, N, and C cycles.
CHAPTER II
LITERATURE REVIEW

2.1 Loblolly pine and sweetgum forests

2.1.1 Loblolly pine range

Loblolly pine is abundant in the southeastern United States, ranging from eastern Texas to southern New Jersey, inhabiting a total of 15 states (Baker and Langdon, 1990; Schultz, 1997). The range is fairly contiguous and varies from dry, upland locations to sharing space with hardwood species within the interior flatwoods ecosystem. Loblolly pine is successful from sea level to 900 meters in elevation (Schultz, 1997). Second to shortleaf pine \( (Pinus echinata \text{ Mill.}) \), loblolly is the most extensive pine of the southeastern United States (Schultz, 1997). It is highly adaptable to many habitats and shares its range with a multitude of grasses, forbs, shrubs, vines, hardwoods, and wildlife species (Baker and Langdon, 1990).

On well-drained sites, loblolly pine shares its range with longleaf pine \( (Pinus palustris \text{ Mill.}) \), shortleaf pine, and Virginia pine \( (Pinus virginiana \text{ Mill.}) \) (Baker and Langdon, 1990). Accompanying hardwoods on these sites include blackjack oak \( (Quercus marilandica \text{ Muenchh.}) \), post oak \( (Quercus stellata \text{ Wangenh.}) \), southern red oak \( (Quercus falcata \text{ Michx.}) \), white oak \( (Quercus alba \text{ L.}) \), hickories \( (Carya \text{ spp.}) \), sassafras \( (Sassafras albidum \text{ Nutt.}) \), and persimmon \( (Diospyros virginiana \text{ L.}) \). Along the eastern seaboard, it is found in association with American beech \( (Fagus grandifolia \text{ L.}) \).
Ehrh.), Carolina ash (Fraxinus caroliniana Mill.), and white ash (Fraxinus americana L.). Specifically to the Piedmont region, loblolly pine is found with southern red oak, northern red oak (Quercus rubra L.), post oak, white oak, scarlet oak (Quercus coccinea Muenchh.), shagbark hickory (Carya ovata Mill.), and mockernut hickory (Carya tomentosa L.) (Schultz, 1997).

On poorly-drained sites, pond pine (Pinus serotina Michx.) and spruce pine (Pinus glabra Walter) are common associates with loblolly pine (Baker and Langdon, 1990). Hardwoods on these sites include red maple (Acer rubrum L.), blackgum (Nyssa sylvatica Marshall), black willow (Salix nigra Marshall), and yellow-poplar (Liriodendron tulipifera L.) (Schultz, 1997). Interior flatwood sites that include loblolly pine, such as in central Mississippi, also commonly include sweetgum, cherrybark oak (Quercus pagoda Raf.), water oak (Quercus nigra L.), swamp chestnut oak (Quercus michauxii Nutt.), red maple, and yellow-poplar (Schultz, 1997).

Although loblolly pine can grow on a variety of soil types, it grows most productively on moderately acidic soils with somewhat poor drainage and very fine subsoil, such as clay (Baker and Langdon, 1990) and does not grow well on excessively drained soils (Schultz, 1997). Loblolly pine growth is most rapid in soils with pH from 4.5 to 6.0, but can still be found at sites with pH as low as 4.0 or as high as 6.7 (Jain et al., 1989). Over 75% of soils in its range are Ultisols (Schultz, 1997). Ultisols are most common in climates that are warm and humid and are common in forested landscapes in the southeastern United States (NRCS, 1999). Loblolly pines can withstand drought and flood conditions to a certain degree, but mature trees can tolerate seasonally flooded conditions while saplings cannot (Baker and Langdon, 1990). Many loblolly pine stands
with unmanaged mid-canopy hardwoods represent a transitional forest from pine dominance, to successional hardwoods (Quarterman and Keever, 1962).

2.1.2 Sweetgum range

Sweetgum is found in approximately 20 southern, eastern, and mid-western states as well as outside of the United States in Mexico, Guatemala, Belize, El Salvador, Honduras, and Nicaragua (Kormanik, 1990). Similarly to loblolly pine, sweetgum is a highly adaptable species; therefore, it is extremely abundant within its range (Kormanik, 1990), which extends farther north than loblolly pine (Wells et al., 1991). It occupies land with average annual rainfall from 1020 mm to 1520 mm and is most successful in moist and somewhat poorly-drained soils (Kormanik, 1990). In interior flatwood ecosystems, sweetgum is a mid-to-late-successional species in both poorly-drained and well-drained major bottoms, as well as mid-successional in well-drained minor bottoms (Hodges, 1997).

Although it is typically classified as moderately “shade intolerant,” sweetgum is successful at establishing a presence in pine understories and is common throughout its range due to its wide ecological amplitude (Bormann, 1953). Sweetgum is capable of reproducing by vigorous re-sprouting (Wells et al., 1991), but within most of its range, sweetgum is more successful at establishing new growth through seeds rather than sprouting mechanisms (Kormanik, 1990). Its seeds are moderately wind-dispersed (Wells et al., 1991), with the ability to travel approximately 200 meters from the main stem of the tree (Bormann, 1953). In poorly-drained sites, their roots tend to stay shallow and spread horizontally while roots of sweetgums in well-drained sites develop a deep taproot (Kormanik, 1990).
Sweetgum is found in both pure stands and in conjunction with other species in several forest types. Depending on topography and soil characteristics, sweetgum is associated with pin oak (*Quercus palustris* Münchh.), willow oak (*Quercus phellos* L.), American sycamore (*Platanus occidentalis* L.), American elm (*Ulmus americana* L.), or yellow-poplar (Kormanik, 1990). Additionally, it is a minor component of approximately 20 other forest types, including the loblolly pine-dominated system (Kormanik, 1990).


### 2.1.3 Exacerbated disturbance and forest succession

The pine-sweetgum forest type is undergoing changes due to environmental stressors as well as insect infestation. The SPB is one of the most destructive insects to southeastern pine forests (Ayres and Lombardero, 2000). Mortality from SPB is inflicted on trees by adult beetles boring into outer bark, eating phloem, and creating galleries in the cambium to lay eggs (Payne, 1980). Consequently, the tree is infected with blue stain fungi carried into the host by the beetles, which colonizes in parenchyma cells and tracheids in the xylem (Ballard et al., 1984; Kirisits and Offenthaler, 2002; Wullschleger et al., 2004). Growth of blue stain fungus within loblolly pines rapidly increases the rate of mortality by growing into the sapwood and releasing toxins into the xylem, clogging tracheids with resin (DeAngelis et al., 1986) and reducing the tree’s ability to transport water (Ballard et al., 1984; Långström et al., 1993; Wullschleger et al., 2004). This
process can be successfully simulated with girdling and fungal inoculation treatments (Hubbard et al., 2013).

Increased survival and dispersal of SPB (Williams and Liebhold, 2002) will have many consequences on forests and ecosystem services that they provide. Large, landscape-scale management of southern pines reduces forest heterogeneity, providing more hosts for SPB infestation (Raffa et al., 2008). However, genetic improvement of loblolly pine planting stock may have caused the recent decreases in SPB disturbance (Asaro et al., 2017). Conversely, Gan (2004) predicted that there will be 7.5 times higher SPB-caused damage in the southeastern United States due to climate change. Nonetheless, selective disturbance on loblolly will promote forest succession to mixed-hardwood stands (Williams and Liebhold, 2002; Coleman et al., 2008), in which the physiological outcomes are necessary to investigate.

As forest succession occurs, changes in phenological, morphological, and physiological characteristics of the species (Daley et al., 2007), as well as changes in microclimate and light attenuation (Matheny et al., 2014) have effects on C assimilation and water use. Without silvicultural intervention, loblolly pine stands will undergo succession to a hardwood-dominated system (Bormann, 1953; Wells et al., 1991; Schultz, 1997). Pine canopy closure creates an environment in which it is very difficult to perpetuate pine establishment in the understory. Although sweetgum is moderately intolerant of shade (Teskey and Shrestha, 1985), it will successfully establish in the understory of a pine canopy and become competitive because its qualities include the ability to grow into a very large tree, having wind-borne seed, and growing in a wide range of site conditions (Bormann, 1953). Loblolly pines are relatively poor competitors
with many hardwood species (Teskey and Shrestha, 1985) and sweetgums typically respond rapidly to removal of a pine overstory (Kormanik, 1990). In general, mid- and late-successional species respond positively to a disturbance on the dominant, early-successional species, particularly due to canopy gaps and newly increased light availability (Canham and Marks, 1985).

2.2 Physiological characteristics

2.2.1 Loblolly pine

Physiological success and productivity of loblolly pines are determined by water and nutrient availability, temperature, photosynthetically active radiation (PAR), disease, insects, allelopathy, and root growth patterns (Teskey et al., 1987). Productivity can be determined by the relationship between photosynthesis rate per unit leaf area and canopy leaf area index (LAI), the one-sided leaf area of the forest canopy per unit ground area (Teskey et al., 1987). Loblolly pines are shade intolerant, have very low photosynthesis rates in shaded environments (Teskey and Shrestha, 1985), and have high light-saturated photosynthesis in comparison to shade tolerant species (Bassow and Bazzaz, 1997). In aging loblolly pines, net primary productivity (NPP) is greatly reduced due to hydraulic limitations on photosynthesis rate (Drake et al., 2010). As with most tree species, the majority (75%) of their hydraulic resistance comes from roots and leaves; therefore leaf phenology and water stress both impact hydraulic conductivity and consequently, gas exchange rates (Domec et al., 2009).

Water stress, in particular, is likely to have the greatest effect on loblolly carbon assimilation rates, significantly reducing net photosynthesis (Teskey et al., 1987; Ellsworth, 2000). Trees must prioritize reducing the risk of embolism before balancing
carbon gain with water loss, especially during times of water stress (Buckley, 2005). Water stress, regulated by stomata (Buckley, 2005), is limiting to tree physiological functioning. Low humidity (or high VPD) creates atmospheric water stress which creates a more negative water potential at the leaf boundary layer, driving stomatal closure (Pallardy, 2008). Similarly, a drop in soil water potential due to low soil moisture force decreases in leaf water potential (Pallardy, 2008). High VPD and low soil moisture may occur as a single limitation, or in conjunction, with one stressor possibly becoming more limiting than the other.

Many allometric relationships exist between characteristics of loblolly pines. Crown characteristics and leaf area dynamics are often difficult to measure without destructive sampling; however crown components can easily be estimated by knowing diameter at breast height (DBH) (Baldwin, 1989) and sapwood area, which is linearly correlated to DBH (equation 2.1) (Blanche et al., 1985). Specifically, Blanche et al. (1984) determined that loblolly pine sapwood thickness is roughly estimated by:

\[
\text{Sapwood thickness} = 0.36 \times \text{DBH} + 0.224
\]  

Loblolly pines, and other fast-growing pine species, typically have thicker sapwood and higher water transport potential than slower growing species of the same age (Hillis, 1971; Blanche et al., 1984). Knowledge of sapwood area is important for estimating total tree water use because water is not transported at a uniform rate throughout the sapwood (Phillips et al., 1996; Ford et al., 2004). In mature loblolly pine, approximately 50% of total sapflow occurs in the outermost 4 cm of sapwood and sapflow rate decreases to less than 10% of total sapflow towards the heartwood in the innermost portion of sapwood (Ford et al., 2004). Similarly, Phillips et al. (1996) found a
59% reduction in sap flux density from the outermost to the innermost portions of sapwood in juvenile loblolly pines.

Many components of loblolly pine physiological functioning have correlations to environmental parameters due to stomatal sensitivities to light, VPD, and soil moisture. Unless trees are limited by water stress or low temperatures, PAR determines stomatal conductance and transpiration rate of loblolly pines (Schultz, 1997). When volumetric soil moisture content is less than 22% however, stomatal conductance and gas exchange rates are strongly correlated to upper soil layer moisture (Oren et al., 1998). In contrast, other studies found that VPD had the strongest relationship with loblolly pine sapflow, followed by PAR, with no correlation between sapflow and soil moisture (Ford et al., 2004) except during periods of severe soil moisture depletion (Phillips and Oren, 2001). In any case, soil water content partly determines ecosystem water balance through tree water use and transpiration rate. In a loblolly stand, Oren et al. (1998) found that up to 90% of the total evapotranspiration comes from the upper 0.35 m of soil while Gonzalez-Benecke et al. (2010) found that 73% of transpiration from the pine canopy comes from water in the upper 0.75 m of soil.

In addition to the effects of water stress on physiological functioning, nutrient availability also affects physiology particularly because leaf N content is related to maximum photosynthetic capacity (Bassow and Bazzaz, 1997; Drake et al., 2010; Ryan et al., 1997). Shade intolerant species such as loblolly pine typically have high leaf N content (Bassow and Bazzaz, 1997), facilitating higher photosynthesis rates. However, in aging stands, loblolly photosynthesis rates decrease due to limitations on stomatal conductance rather than N limitation (Drake et al., 2010). N fertilization increases
loblolly growth substantially in comparison to stand irrigation (Samuelson et al., 2008; Coyle et al., 2008) and stands that have been fertilized have higher average daily canopy transpiration in comparison to irrigated stands (Ewers et al., 1999). It is likely that mechanisms of transpiration and stomatal conductance are highly reliant on stand N availability (Samuelson et al., 2008). Inversely, litter with high C content and a high C:N ratio may immobilize soil N, creating a positive feedback loop between reduced N and photosynthetic capacity (Ryan et al., 1997).

Changes in photosynthetic capacity of loblolly pine have implications on C assimilation, and consequently, ecosystem productivity. Net ecosystem productivity (the difference of gross primary productivity and ecosystem respiration) is a good estimate of C assimilation rate, and is driven by increases in foliage and woody biomass (Maier et al., 2004). Past estimates of total C assimilated from photosynthesis and used during maintenance respiration in loblolly pine varies from 45-80% (Ryan et al., 1997; Maier et al., 1998), and pines exhibit positive linear relationships between respiration and site temperature (Ryan et al., 1994) and tissue N content (Maier et al., 1998). Specifically, stem respiration of loblolly pine contributes approximately 13% to whole-tree respiration, while all aboveground woody tissue contributes up to 15-25% to total ecosystem respiration (Maier et al., 2004). While Maier et al. (2004) found loblolly pine stands to be carbon neutral (neither a sink nor source), Noormets et al. (2010) estimated that a loblolly forest sequesters 361-835 g C m$^{-2}$ year$^{-1}$, which varies with yearly precipitation patterns.
2.2.2 Sweetgum

Sweetgum is a diffuse-porous species unlike many of its ring-porous hardwood competitors such as *Quercus*, *Carya* and *Ulmus* species. Consequently, its sapflow does not differ radially throughout the sapwood and water is transported throughout the xylem at a relatively constant rate (Phillips et al., 1996). Additionally, as a diffuse-porous species, sweetgum will typically have higher stomatal sensitivity to VPD than ring-porous species (Oren and Pataki, 2001). Most hardwood species, sweetgum included, have stomata on the lower epidermis of leaves (Pallardy, 2008) and stomata occur in a random arrangement in comparison to the rows of stomata present in pines. Sweetgum stomatal density is approximately 349 stomata per mm$^2$ leaf area in ambient CO$_2$ conditions (Herrick et al., 2004).

Broadleaf species typically exhibit feed-forward stomatal control (stomata are preferentially driven by assimilation of CO$_2$), as a response to VPD or evaporation rate (Jones, 1998; Pataki et al., 1998). High VPD increases stomatal conductance to approach maximum conductance, but sweetgum only approaches maximum conductance with significantly increased PAR (Pataki et al., 1998), and will increase efficiency of radiation use with significant increases in soil moisture (Allen et al., 2005). Previous studies suggest strong stomatal sensitivity to soil moisture deficit in sweetgum, compared to other hardwood species such as *Quercus* spp. and *Acer* spp. (Pataki et al., 1998; Bovard et al., 2005). Conversely, Oishi et al. (2010) found that when soil moisture is not a limiting factor, transpiration of sweetgum is similar to that of other deciduous hardwoods, but during drought conditions, sweetgum will out-compete other hardwoods for water resources.
Light availability may be the most important driver for sweetgum productivity; however, productivity is further enhanced by increases in water supply (Allen et al., 2005), and increases in soil N content (Coyle et al., 2008). When grown in shaded environments, net photosynthesis of sweetgum is relatively low in comparison to some of its hardwood competitors, such as yellow-poplar, red oak, and American beech (Teskey and Shrestha, 1985). Variation within sweetgum individuals between shade and sun leaves vary over twofold in terms of net photosynthesis rate from approximately 4.53 to 13.44 μmol m$^{-2}$ s$^{-1}$ at increasing levels of photosynthetic photon flux density (PPFD) (Harley et al., 1996). In many cases, leaf N content is linearly correlated with maximum photosynthesis rate (Bassow and Bazzaz, 1997), particularly so in hardwood species (Reich et al., 1995). Therefore, differences in photosynthetic capacity of sun and shade leaves may be influenced by higher N content of sun leaves (Harley et al., 1996). Nevertheless, these previous findings suggest that PAR, soil water, and N availability collaboratively and dynamically impact net primary productivity of sweetgum.

2.3 Physiological differences between pines and hardwoods

2.3.1 Water use

Quantifying the relationship between species composition and water use is complicated due to natural variation of the environment, but some relationships have been estimated (Bosch and Hewlett, 1982). Pine and hardwood species differ with respect to transpiration and stomatal conductance due to both physiological and structural differences (Ford et al., 2011). Pines often contribute to higher evaporative water loss on an annual basis due to greater foliar surface area that is retained throughout the “dormant” season (Swank and Douglass, 1974; Stoy et al., 2006; Rao et al., 2011).
Higher evapotranspiration in pine forests in comparison to deciduous hardwoods can also be attributed to the opaque nature of needles, allowing for increased absorption of solar radiation (Rao et al., 2011). Transpiration per unit leaf area of sweetgum is higher than loblolly pines, while pines have higher sapflow rates per unit stem area (Pataki et al., 1998). Daily peak sapflow in conifers usually occurs earlier in the day than hardwoods (Ewers et al., 2002), which is an advantage in areas where plants may experience high afternoon VPD, leading to water-stressed conditions. In some cases, sapwood area, LAI, and number of stems per unit ground area are better indicators of stand transpiration than species (Ewers et al., 2002). Tree sapwood area and LAI are important factors in determining transpiration rates across and within species.

Some exceptions exist to the trend of pines contributing to more evaporative water loss than deciduous hardwoods. In suburban forest conditions, Giraldo et al. (2015) found that sweetgum contributes to higher sapflow rates than loblolly pine throughout the summer growing season. Additionally, during periods of prolonged and extreme drought, pine plantations will transpire less than deciduous hardwoods due to pine’s higher sensitivity to climatic factors (Stoy et al., 2006), and higher water use efficiency (Hu et al., 2010). Oaks and hickories were found to transpire less per unit leaf area than eastern white pine (*Pinus strobus*); however, yellow-poplar transpired more (Ford et al., 2011). In regions where the majority of precipitation occurs throughout the summer growing season, such as Japan, Komatsu et al. (2008) found that annual transpiration of deciduous hardwood forests will be similar to that of conifer forests because neither deciduous hardwoods nor conifers are contributing to foliar interception in the dormant season due to the lack of precipitation.
2.3.2 Nutrient cycling

Accelerated forest succession from loblolly pine to sweetgum will alter nutrient cycling dynamics in terms of N availability. Leaf N content and resorption rate during senescence are important indicators of ecosystem N cycling. Leaf litter quality including litter N content may control leaf decomposition rate (Melillo et al., 1982), affecting N availability to competing species. Leaf N content of sweetgum in ambient conditions ranges from approximately 0.87 to 0.94% in eastern Tennessee, USA on silty clay loam soils (Iversen and Norby, 2008). Leaf N content of loblolly pine is approximately 0.76% in North Carolina, USA on sandy soils (Gough et al., 2004). N content in leaves is also an indicator of physiological functioning and is positively correlated to maximum photosynthetic rate (Bassow and Bazzaz, 1997; Ryan et al., 1997). Rubisco (an N-rich enzyme that performs carboxylation in the carbon reactions of photosynthesis) is often a limiting factor for photosynthesis; therefore the photosynthetic capacity parameter $V_{cmax}$, or maximum Rubisco-limited carboxylation rate, often depends on N availability (Wullschleger, 1993).

Conifers have relatively low photosynthetic N use efficiency relative to specific leaf area in comparison to hardwoods, which is a result of the different life spans of evergreen and deciduous leaves (Reich et al., 1995). N uptake and leaf N content of sweetgum is strongly correlated with net primary productivity (Norby and Iversen, 2006), which suggests that a release of N within the system due to mortality of loblolly pine may enhance sweetgum growth. However, leaf N content varies between individuals and seasons. Leaf N content increases with spring and summer plant growth, but pines and hardwoods both exhibit leaf N resorption to wood tissues during autumn and before leaf
abscission occurs (Pallardy, 2008). Resorption of N to woody tissues in hardwood species ranges from one-fourth to two-thirds of the leaf’s original N concentration (Pallardy, 2008).

2.3.3 Photosynthetic capacity

Factors that have an effect on photosynthesis rates include quantity of PAR, air temperature and VPD, wind speed, CO₂ concentrations, water and nutrient availability, stomatal conductance, and photosynthetic pathways (Baldocchi and Amthor, 2001). Photosynthesis rates are manipulated in the field and compared at a range of CO₂ levels to create CO₂ response curves (A/Cᵢ curves or assimilation (A) /internal CO₂ concentration (Cᵢ)). A/Cᵢ curves provide insight into intrinsic photosynthetic capacity by determining limitations of photosynthesis through calculations of maximum Rubisco activity (Vcₘₐₓ), maximum electron transport rate (Jₘₐₓ), and rate at which increasing CO₂ does not increase photosynthesis (Triose phosphate utilization-limited carboxylation rate - TPU).

Limitations on photosynthetic capacity differ between some pine and hardwood species (Reich et al., 1995). Net photosynthesis of loblolly pines will increase linearly as CO₂ increases until saturating CO₂ levels are reached; however, pines may have lower CO₂ uptake efficiency than some of their hardwood competitors (Teskey and Shrestha, 1985). Photosynthetic capacity of a given leaf within a forest is also likely to vary based on its position in the canopy (Ellsworth and Reich, 1993). Shade intolerant species typically have higher light-saturated photosynthesis rates than shade tolerant species (Bassow and Bazzaz, 1997). Under ambient CO₂ conditions, photosynthesis rates are approximately 0.038 µmol g⁻¹ s⁻¹ in loblolly pines and 0.0420 µmol g⁻¹ s⁻¹ in
sweetgum (Groninger et al., 1996). This would suggest higher forest CO₂ assimilation rates upon a transition to sweetgum dominance, if variability in site influences that impact productivity remains low.

2.4 Future stand conditions and physiological consequences

SPB-related disturbance accelerates forest succession to hardwoods (Coleman et al., 2008) and although there are some notable differences between hardwood and pine physiology, it is difficult to determine how these specific species in the interior flatwoods of central Mississippi will respond during successional changes. Hubbard et al. (2013) suggest increases in soil moisture will occur upon pine beetle-related mortality of a dominant species. When aging canopy trees undergo mortality, some surviving species increase transpiration while others decrease transpiration, which may be due to differences in hydraulic functioning between species, such as anisohydricity (Matheny et al., 2014). Photosynthetic capacity is related to the successional trajectory of a species (Ellsworth and Reich, 1993). Early-successional species have higher maximum net photosynthesis rates in comparison to late-successional species (Ellsworth and Reich, 1996).

Forest growth and productivity are strongly tied to environmental parameters and it is increasingly important to determine a forest’s relationship to climate as it is changing. The threats of anthropogenic climate change encourage more research on productivity of trees and their ability to assimilate C (Gan, 2004; Hu et al., 2010). Since CO₂ uptake by plants can be determined through calculation of transpiration losses and water use efficiencies, increasingly refined estimations of global gross primary productivity can be made with knowledge of tree physiological functioning and its
relationship with environmental drivers (Jasechko et al., 2013). Comparatively, some relationships exist between forests and the climate in which forest type is the catalyst for altered microclimate. Stoy et al. (2006) suggest that pine forests, in comparison to other land types, may alter convective precipitation events throughout the summer growing season due to higher latent heat flux or evapotranspiration of pines, resulting in heightened precipitation events.

Typically, high rates of C uptake occur following rain events, with low atmospheric VPD resulting in increased water use efficiency, soil moisture, and subsequently, leaf-level transpiration, which result in higher productivity (Hu et al., 2010). The southeastern United States is already heavily forested (Smith et al., 2004) and undergoing additional afforestation and reforestation, in which changes in climate and microclimate could result in a large change in fluxes due to the vastly contiguous forest cover. Drought is expected to increase across particular areas within the United States, including increases near the Gulf Coast (Dale et al., 2001). Prolonged drought may permanently reduce stomatal density and/or function, reducing the ability for leaves to obtain CO₂ even when water is present later in the growing season (Teskey et al., 1987). However, during short-term drought events, loblolly pine will usually maintain high sapflow (Pataki et al., 1998).

Change in climate may also change the distribution, extent, frequency, and magnitude of disturbance regimes and consequently, alter forest stand composition (Dale et al., 2001). The potential for severe drought in the future has the secondary effect of increasing forest susceptibility to pests and pathogens (Negron, 1998; Dale et al., 2001) due to trees being at a weakened and vulnerable state when drought stressed.
Furthermore, increases in temperature and changes in seasonality and quantity of precipitation can promote insect survival, reproduction, dispersal, and distribution (Dale et al., 2001).

In addition to increasing numbers of hosts and pests, changes in the climate can potentially predispose pines to SPB disturbance. Altered precipitation patterns that result in water stress will decrease a tree’s resistance to pathogens, making them more susceptible to bark beetle damage (Reeve et al., 1995; Ayres and Lombardero, 2000). Dobbertin et al. (2007) suggested that drought conditions created a level of drought stress within Scots pine (*Pinus sylvestris* L.) that reduced the trees’ resistance to pine bark beetle-driven mortality. Particularly, defenses are reduced when water stress results in decreased production of resin (Croisé and Lieutier, 1993).

It is important to determine mechanisms and timing of tree mortality in order to better understand and predict future forest dynamics and ecosystem processes (Hubbard et al., 2013), however it is difficult to determine when physiological functioning is altered during a tree mortality event such as after a pine beetle infestation or simulated SPB disturbance treatment. Needles may discolor and drop before sapflow velocity declines (Wullschleger et al., 2004), while physiological processes may decline without any indication of mortality by needle discoloration. In Norway spruce (*Picea abies* (L.) H. Karst) needle discoloration did not occur until the following spring after sapflow decreased to zero (Kirisits and Offenthaler, 2002). Hubbard et al. (2013) found that trees that were treated with phloem girdling continued to transport water until the following summer after the treatment was implemented, while beetle-attacked trees stopped transpiring months sooner.
Mortality and weakening of a dominant pine species by insect herbivory accelerates nutrient cycling by increasing the availability of nutrients in litter, and eventually, soil (Tchakerian and Coulson, 2011). Soil moisture and soil N content increases during the first few growing seasons after localized mortality of pines, improving site conditions for unaffected species (Hubbard et al., 2013). Soils directly beneath trees that have succumbed to beetle attack increase in available inorganic soil N, extractable ammonium (NH\textsubscript{4}\textsuperscript{+}) and nitrate (NO\textsubscript{3}\textsuperscript{−}), soil moisture (Clow et al., 2011), pH, and decrease in microbial biomass and calcium (Xiong et al., 2011). C input to the soil will increase with addition of coarse woody debris to the forest floor; however these inputs occur gradually in forests with SPB outbreaks (Coleman et al., 2008). In mountainous regions infested with mountain pine beetle (MPB), there were significant increases in soil N that occurred beneath the trees, while there were no significant effects on a larger watershed scale (Clow et al., 2011). The lack of an observed change in downstream water chemistry can be attributed to heterogeneity of MPB attack as well as the aridity of the landscape, leading to delayed leaching of soil nutrients to groundwater (Clow et al., 2011). Comparatively, other studies including Zimmerman et al. (2000) found significant changes in stream chemistry due to pine beetle attack, but the timing and magnitude of these changes are highly dependent upon specific hydrologic and geographic variables in each watershed (Mikkelson et al., 2013).

2.5 Literature review summary

Loblolly pine-dominated forests are common throughout the southeastern United States, and are often subjected to stressors of environmental changes and disturbances. Particularly, pine stands are becoming increasingly sensitive to pine beetle disturbance
(Dobbertin et al., 2007), which aids in expediting succession to hardwoods (Coleman et al., 2008). Forest transitions in this region of Mississippi will promote sweetgum dominance during mid-successional stages of the affected forests. Differences between loblolly pine and sweetgum water and nutrient use impact resource availability as well as stand hydrological and nutrient cycling. This study examined physiological changes pertaining to resource use and availability in this common forest type in order to predict future conditions based on environmental and forest stand changes. Specifically, the goal of this research was to estimate changes in pine and sweetgum water and nutrient use, and C uptake throughout the pine mortality process.
CHAPTER III
PHYSIOLOGICAL OUTCOMES OF LOBLOLLY PINE MORTALITY ON
LOBLOLLY PINE

3.1 Introduction

The physiological functioning of loblolly pine trees is likely to change when a mortality event occurs in a forest, particularly when the disturbance is selective such as SPB-induced mortality (Hubbard et al., 2013). Investigation of tree physiology under these conditions will aid in the understanding of how trees react to changes in resource demands for water, N, and C. This chapter documents the altered physiology of pines undergoing mortality as well as competing loblolly pines throughout the time that it takes for mortality to occur. Specifically, objectives include determining physiological response (in terms of changes in water use, N uptake, and components of C assimilation rates) to increasing availability of water and nutrients, and determining patterns of seasonal water use especially under conditions of inundation and drought stress.

Hypotheses are that there will be increases in water use, N uptake, and C assimilation in surviving pines, while stem respiration will increase in dying pines. These data will increase the understanding of changes in tree and forest functioning under conditions of forest disturbance and seasonal environmental stressors.
3.2 Methods

3.2.1 Site description

The research was conducted in the John W. Starr Memorial Forest (33°15'50.94"N, 88°53'6.47"W) in Winston County in east-central Mississippi. The forest is owned by Mississippi State University and its current primary use is for research within the College of Forest Resources. The approximately 900 m² forested study plot (Fig. 3.1) is located within an aging loblolly pine plantation containing mid-canopy hardwoods, at an elevation of approximately 102 m. It lies within the Noxubee River Watershed (HUC 03160108) (USGS, 2016) in the mixed coastal plain physiographic district. Mean annual temperatures, averaged between 1981-2010 range from 20.56°C – 32.50°C during the summer months (JJA) and from 0.47°C – 13.24°C during the winter months (DJF) (NOAA, 2010). Mean annual precipitation, averaged over the same 30-year range, was approximately 10.49 cm per month during the summer months and 13.79 cm per month during the winter months (NOAA, 2010). Total annual precipitation is, on average, 140.26 cm per year (NOAA, 2010).

The surface geology of this location is characterized as Porters Creek formation, which primarily consists of clay soil types (USGS, 2016). Specifically, the stand is located on clay soils classified as Urblo silt loam (NRCS, 2016), which are poorly drained and experience seasonal flooding. Over the study period, site inundation began in late December, 2015 and soils remained saturated throughout April, 2016. Aboveground, the vegetation is characterized by loblolly pine as the dominant overstory species. Sweetgum makes up the second-most abundant tree species, and the most abundant mid-canopy species. Additional hardwoods in the mid-canopy are water oak, willow oak, southern red
oak, and red maple. Understory herbaceous species including greenbriar (*Smilax* spp. L.) and poison ivy (*Toxicodendron radicans* L.) are also present. SPB activity was not present within the stand upon initial site preparation; however, signs of beetle disturbance on trees outside the boundary of the study plot became more abundant throughout the course of the study.

Figure 3.1  Plot boundary (hollow red polygon) in the John W. Starr Memorial Forest and Winston County, Mississippi, USA (inset map)
3.2.2 Treatments and experimental design

A loblolly pine stand undergoing succession to hardwoods was chosen in 2015 for data collection. Fifteen loblolly pines with no current bark beetle damage with DBH values ranging from 35.6 to 60.5 cm were selected for sampling. In July 2015, ten pines underwent a girdling treatment in which a 10 cm long ring of phloem tissue, approximately 30 cm above the forest floor, was removed from the entire circumference of the tree via methods of chainsaw and hatchet. Additionally, five of the girdled pines were inoculated with blue stain fungus in September, 2015 while the other five girdled pines were inoculated with an agar solution to serve as fungal controls. Trees were inoculated at four circumferential locations 5-10 cm above the girdle treatment. The remaining five loblolly pines serve as untreated controls to compare with the treated pines.

3.2.3 Environmental data

Environmental parameters of soil moisture, relative humidity, air temperature, and PAR were continuously collected throughout the sampling period to compare with tree physiological measurements. VPD was calculated from temperature and relative humidity using the Goff-Gratch equation (Goff and Gratch, 1946). Canopy air temperature and relative humidity were measured with a Rotronic HC2S3 sensor (Hauppauge, NY, USA) located approximately ten meters above the ground in a tree canopy. Soil moisture was measured with three time domain reflectometry (TDR) probes, model CS616 (Campbell Scientific Inc., Logan, UT, USA), located in the study plot approximately six meters from the central datalogger in three azimuthal directions. These sensors were connected to a CR1000 datalogger (Campbell Scientific Inc.) with data recorded at 30 minute intervals.
PAR was measured using a quantum sensor (LI 190, LI-COR Biosciences Inc., Lincoln, NE, USA) and LI-COR datalogger (LI-1400) located in a nearby canopy gap. Precipitation was measured with an Onset HOBO Data Logging Rain Gauge-RG3-M (TE525MM, Dallas, TX) that was located in a nearby forest clearing. For the six month period in which precipitation at the study location was missing, data were supplemented with local (33°26′9″ N, 88°50′46″ W) precipitation data (Weather Underground, The Weather Company, LLC; www.wunderground.com/history/airport/KSTF).

3.2.4 Water use

Water use rates were estimated by measuring sapflow using the Granier heat dissipation method (Granier, 1987). This method uses two probes containing thermocouples (sapflow probes) that were stacked vertically and inserted radially into the sapwood of each tree approximately one meter above ground level, with the upper of the two probes heated. The sapflow probes were protected from incoming solar radiation by strategically installing them on the northern side of each tree and by attaching an aluminum shield located above each set of probes. The deficit between the two sapflow probes’ temperatures is related to the velocity of sapflow \( u; \text{ g H}_2\text{O m}^{-2} \text{s}^{-1} \) through the following empirical equation 3.1:

\[
u = 119 \times ((\Delta TM - \Delta T)/\Delta T) - 1)^{1.23}
\]

where \( \Delta TM \) is the maximum temperature deficit between the two sapflow probes during nighttime (no/low flow) conditions and \( \Delta T \) is the deficit during positive sapflow (Granier, 1987). This follows the assumption that \( \Delta T \) will be higher when water is not moving and lower when water is moving past the sensors and dissipating heat produced by the upper
sensor upwards through the xylem. Total tree sapflow is calculated by the following equation 3.2:

\[ F = u * SA \]  

(3.2)

where \( F \) is total sapflow (g H\(_2\)O s\(^{-1}\)) and \( SA \) is the estimated sapwood area (m\(^2\)) (Granier, 1987). Sapwood area was estimated using the following equations (3.3-3.6) stemming from DBH measurements:

\[ SA = \pi * (HR + SD)^2 - HA \]  

(3.3)

where \( SD \) is equal to sapwood depth calculated by the equation developed by Blanche et al. (1984)

\[ SD = (0.36 * DBH) + 0.224 \]  

(3.4)

and \( HR \) is equal to heartwood radius estimated by the equation

\[ HR = \frac{DBH}{2} - SD - 2 \]  

(3.5)

where 2 cm is the estimated bark thickness and \( HA \) is equal to heartwood area calculated by the equation

\[ HA = \pi * HR^2 \]  

(3.6)

Radial decreases in flow rates through the sapwood were calculated using estimates developed from Ford et al. (2004) who found the outer-most portion of sapwood to have highest sapflow rates, with incremental decreases in rate until reaching the inner-most portion of the sapwood. Specifically, sapflow rates in this study were divided into four equal quadrants of sapwood area and estimated to be 100% of measured flow in the outermost quadrant, 73% of measured flow in the second quadrant, 57% of measured flow in the third quadrant, and 21% of measured flow in the innermost quadrant. These incremental reductions were used to account for the 150%
overestimation of sapflow that Ford et al. (2004) found from one point sapflow measurements over the entire sapwood area in loblolly pine.

Sapflow probes were connected to a CR1000 datalogger and AM 16/32B multiplexer (Campbell Scientific Inc.). Probes and dataloggers were powered using two deep cycle batteries and a solar panel located in a nearby canopy gap. Measurements were collected every 30 seconds and averaged over every 30 minutes. Data from the datalogger were collected on a weekly basis to be evaluated in the lab. Raw temperature deficits between the heated and reference probes were analyzed using the BaseLiner program developed by Duke University, converting the raw temperature differences into a sapflow rate via the empirical equation developed by Granier (1987) (Equation 3.1).

3.2.5 Photosynthetic capacity

Three girdled pines and three control pines were chosen to evaluate photosynthetic rates in August, 2016. $A/C_i$ curves were generated from measurements collected with a LI-COR 6400 photosynthesis system (LI-COR Biosciences Inc.) containing a red-blue light source and using an infrared gas analyzer chamber to estimate $CO_2$ and $H_2O$ leaf uptake and release, respectively. From each tree, a branch less than two cm in diameter was randomly removed from the canopy with a shotgun and immediately recut under water and measured in situ with the cut-end of branch submerged in a bottle of water. This step reduced the probability of embolism occurring, therefore reducing the risk of stomatal closure and lowered photosynthetic capacity (Hubbard et al., 1999). $A/C_i$ curves were made under saturating light conditions with PAR held constant at 1500 $\mu$mol m$^{-2}$ s$^{-1}$ while $CO_2$ concentrations were manipulated beginning at ambient levels (approximately 400 $\mu$mol mol$^{-1}$), reduced to 50 $\mu$mol mol$^{-1}$,
then raised sequentially to 800 µmol mol\(^{-1}\). Since pine needles do not take up the 6 cm\(^2\) chamber surface area, data were adjusted for the actual leaf area of sampled needles. Measured needles were clipped to the width of the chamber and preserved in a refrigerator until scanned with a known scaling factor onto the computer and measured for leaf area using ImageJ version 1.48 (National Institutes of health, USA, https://imagej.nih.gov/ij).

\(A/C_i\) curves were then fit by plotting CO\(_2\) assimilation rate (A) vs. CO\(_2\) concentration inside the leaf (C\(_i\)). Parameters of \(V_{cmax}\) (maximum Rubisco-limited carboxylation rate), \(J_{max}\) (maximum electron transport-limited carboxylation rate), and TPU (Triose phosphate utilization-limited carboxylate rate) were calculated using an \(A/C_i\) curve fitting model in a Microsoft Excel utility solver (Fig. 3.2) developed by Sharkey et al. (2007). Specifically, values (one, two, and three) were assigned to increasing inputs of C\(_i\) quantities to estimate if the points qualify as rubisco-limited (one), electron transport (RuBP-regeneration) limited (two), and TPU limited (three). Values were adjusted to best fit the curves and points that did not fit any curves were assigned a value of zero.
Figure 3.2  Example $A/C_i$ curve from the Microsoft Excel solver for one pine individual in which maximum photosynthetic carboxylation rates were determined.

Y axis represents carbon assimilation rate and X axis represents CO$_2$ concentration. Red, green, and yellow lines represent maximum carboxylation rates of photosynthesis that are Rubisco, electron transport, and Triose phosphate utilization limited, respectively. Blue points represent observed values of assimilation rate at varying CO$_2$ concentrations.

3.2.6  Leaf area dynamics and leaf litter content

Ten 0.75 m$^2$ litter traps were strategically placed within the stand to collect litter throughout the duration of the study. Senesced needles were collected to estimate litterfall quantity, C:N, and nutrient resorption rates. Leaf and needle litter were collected from the traps on a bi-weekly basis, sorted by species, oven-dried and weighed. Litterfall quantity was converted to LAI values with the following equation 3.7:

$$\text{LAI} = \frac{L \times \text{SLA}}{Z}$$

(3.7)

where $L$ is the oven-dried leaf litter weight (g), SLA is specific leaf area (cm$^2$ g$^{-1}$), and $Z$ is the litter trap area (cm$^2$). SLA was calculated with the following equation 3.8:
where ALA is the average leaf area (cm²) and ALW is the average leaf weight (g).

Approximately 40 needles were randomly selected from leaf litter, scanned with a LI 3100 (LI-COR Biosciences Inc.) leaf area scanner and weighed for determination of SLA. Loblolly pine needles were also used to determine senesced needle nutrient content. The needles were then ground into particles less than 0.25 mm in size and the samples were oven-dried at 60°C for additional water removal. The samples were analyzed for C and N content with an ECS 4010 Elemental Combustion System CHNS-O (Valencia, CA, USA).

In addition, foliage density was estimated as a measure of pine leaf area on an individual crown basis, to compare between girdled and control pine crowns. Pine foliage density was estimated using a LAI-2200 plant canopy analyzer (LI-COR Biosciences Inc.) during two periods of dormancy (March, 2016 and December, 2016) in order to avoid capturing mid-canopy hardwood crown foliage. Measurements were an average of four points at 90° azimuths beneath each tree during diffuse light conditions, and analyzed in the lab with FV2200 software (LI-COR Biosciences Inc.). To calculate crown volume, an oblong shape was created using x (distance from bole) and y (distance from forest floor) values with crown height data to represent each tree’s specific crown shape. Tree heights and height-to-base of live crown measurements were collected using a Tru Pulse 200 hypsometer (Laser Technology Inc, Centennial, CO, USA). Five crown radius measurements were collected using a standard 30 meter measuring tape and averaged to find crown width for each individual. Due to the shape of each tree’s crown and distance from the sensor (i.e. height to base of live crown), only data from 7° and 23° rings of the
LAI-2000 were used. Data from the remaining rings of the LAI-2000 were omitted, since they picked up foliage density of neighboring canopies rather than the intended individual. Several girdled pines were omitted from the December, 2016 measurements due to mortality and lack of needles present in individual crowns.

### 3.2.7 Bole growth

Fourteen of the fifteen pines in the study were cored at breast height after the second growing season (post-mortality; fall of 2016). The omitted pine was a control pine that died earlier in the season, its cause of mortality possibly due to previously unnoticed bark damage high on the bole of the individual. Cores were examined in the lab using a Lynx Dynascope stereo microscope (Vision Engineering, Surrey, England) and photographs were taken at approximately 5.0x magnification with a digital camera (AmScope FMA050, Irvine, CA, USA). Widths of growth rings were measured using ImageJ version 1.48 (National Institutes of health, USA, [https://imagej.nih.gov/ij](https://imagej.nih.gov/ij)) and differences in ring width were compared between girdled and control pines.

### 3.2.8 Stem respiration

Stem respiration of loblolly pines were measured in order to determine whether significantly different fluxes of CO$_2$ occur between girdled and control pines as well as between locations above vs. below the girdle in treated pines. Segments of 10 cm diameter PVC pipe were attached to the stem surface with gorilla glue® and sealed with silicone caulk. Two segments of PVC pipe were affixed to each pine: one below the girdle (or below where the hypothetical girdle would be on each control pine), 10-20 cm above the forest floor, and one above the girdle (or above where the girdle would be on each
control pine), approximately one meter above the forest floor. Depth of PVC pipe (bark surface to outer edge) was measured on each individual segment in three random locations inside the circumference of the pipe and averaged to determine an approximate depth of each pipe segment. Respiration was measured on a monthly (seasonally in winter) basis using a LI-COR 8100 Soil Gas Flux System and a 10 cm survey chamber (8100-102) (LI-COR Biosciences Inc.). Respiration data were adjusted based on the average measured depth of each PVC pipe and calculated using SoilFluxPro version 4.0 software (LI-COR Biosciences, Inc.).

3.2.9 Statistical analysis

Trees that were girdled but did not die or were a control tree but died were omitted from further analysis because they were statistically different from the remaining individuals in those treatment groups. Half-hourly sapflow data were summed to create daily totals and were averaged across treatment types. The daily averages of each treatment were then compared within each month (control vs. girdled), and between each year (month of 2015/pre-mortality vs. month of 2016/post-mortality) for each treatment category. These monthly values were compared using a linear mixed effect model (lme function) in R version 3.1.2 (R Core Team, 2015) with day of month as a random effect to account for the fact that these data are a repeated measure. Linear relationships between sapflow and VPD or soil moisture were determined using SigmaPlot version 13.0 (Systat Software, Inc. San Jose, CA, USA).

All leaf nutrient composition data were compared using analysis of variance (aov function) in R version 3.1.2. Specific analyses include between needles from control and girdled pines (green leaf samples), and between needles from pre-mortality and post-
mortality periods (senesced leaf samples). Photosynthetic parameters that were calculated ($V_{\text{cmax}}, J_{\text{max}},$ and TPU) from $A/C_i$ curves were compared between control and girdled trees using analysis of variance (aov function) in R version 3.1.2. Growth ring widths of control and girdled pines were compared between years using a paired t.test, and growth ring widths in each year were compared between control and girdled pines using analysis of variance (aov function) in R version 3.1.2. Average stem respiration across locations of each tree was compared between control and girdled pines for each month of sampling using analysis of variance (aov function) in R version 3.1.2.

3.3 Results

3.3.1 Environmental data

The environmental parameters of VPD, soil moisture, and PAR that were measured throughout the course of the study showed periods of water stress and resource availability within the site. Average daytime VPD and average daily soil moisture data were collected and calculated continuously throughout the experiment, however, periods of missing PAR data exist due to technical mishaps with the sensor and datalogger (Fig. 3.3; Table 3.1). Average daytime VPD was approximately 0.50 kPa in the winter (DJF) and 1.13 kPa in the summer (JJA). Soil moisture was approximately 53% in the winter (DJF) and 36% in the summer (JJA). Soil moisture was notably higher from December to May, and throughout this period, the study site was inundated. It should be noted, however, that soil moisture was notably lower towards the end of the second growing season (25%) than it was towards the end of the first growing season (34%). As of September 20, 2016, this region of Mississippi was experiencing a level 2 severe drought which escalated to a level 3 extreme drought and persisted until December 6, 2016.
Drought levels in the same months the prior year ranged from 0 to level 1 (abnormally dry) (National Drought Mitigation Center, 2015).

Figure 3.3 Graphed variation in environmental parameters including average daytime vapor pressure deficit (kPa), average daily soil moisture (cm$^3$ cm$^{-3}$), maximum daily photosynthetically active radiation (µmol m$^{-2}$ s$^{-1}$), and daily precipitation (mm).

Gaps in PAR data were due to technical mishaps with the datalogger.
Table 3.1 Average daytime vapor pressure deficit (VPD; kPa), daily soil moisture (cm$^3$ cm$^{-3}$), maximum daily photosynthetically active radiation (PAR; µmol m$^{-2}$ s$^{-1}$), and total precipitation (mm) and appropriate standard errors for each month of the study.

<table>
<thead>
<tr>
<th>Month</th>
<th>Average Daytime VPD (kPa) (SE)</th>
<th>Average Soil Moisture (%) (SE)</th>
<th>Average Daily Maximum PAR (µmol m$^{-2}$ s$^{-1}$) (SE)</th>
<th>Total Monthly Precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July, 2015</td>
<td>1.27 (0.11)</td>
<td>40 (0.63)</td>
<td>1740.55 (116.05)</td>
<td>75.95</td>
</tr>
<tr>
<td>Aug, 2015</td>
<td>1.14 (0.09)</td>
<td>36 (0.64)</td>
<td>1845.45 (0.85)</td>
<td>5.40</td>
</tr>
<tr>
<td>Sep, 2015</td>
<td>1.17 (0.08)</td>
<td>29 (0.42)</td>
<td></td>
<td>8.89</td>
</tr>
<tr>
<td>Oct, 2015</td>
<td>1.01 (0.12)</td>
<td>28 (0.95)</td>
<td></td>
<td>156.60</td>
</tr>
<tr>
<td>Nov, 2015</td>
<td>0.51 (0.06)</td>
<td>44 (0.70)</td>
<td></td>
<td>215.60</td>
</tr>
<tr>
<td>Dec, 2015</td>
<td>0.46 (0.04)</td>
<td>50 (0.86)</td>
<td>583.97 (50.55)</td>
<td>115.00</td>
</tr>
<tr>
<td>Jan, 2016</td>
<td>0.40 (0.05)</td>
<td>53 (0.36)</td>
<td>688.47 (59.57)</td>
<td>114.60</td>
</tr>
<tr>
<td>Feb, 2016</td>
<td>0.64 (0.06)</td>
<td>55 (0.26)</td>
<td>952.40 (78.31)</td>
<td>289.00</td>
</tr>
<tr>
<td>March, 2016</td>
<td>0.81 (0.10)</td>
<td>56 (0.10)</td>
<td>1136.38 (85.02)</td>
<td>180.60</td>
</tr>
<tr>
<td>April, 2016</td>
<td>0.97 (0.09)</td>
<td>54 (0.52)</td>
<td>1236.37 (107.75)</td>
<td>45.20</td>
</tr>
<tr>
<td>May, 2016</td>
<td>1.02 (0.08)</td>
<td>45 (0.95)</td>
<td>1510.57 (78.90)</td>
<td>21.40</td>
</tr>
<tr>
<td>June, 2016</td>
<td>1.15 (0.07)</td>
<td>39 (0.42)</td>
<td>1589.74 (37.42)</td>
<td>97.79</td>
</tr>
<tr>
<td>July, 2016</td>
<td>1.21 (0.08)</td>
<td>36 (0.52)</td>
<td>1634.65 (53.07)</td>
<td>22.86</td>
</tr>
<tr>
<td>Aug, 2016</td>
<td>1.03 (0.09)</td>
<td>34 (0.67)</td>
<td>1458.76 (55.06)</td>
<td>0.00</td>
</tr>
<tr>
<td>Sep, 2016</td>
<td>1.44 (0.10)</td>
<td>27 (0.29)</td>
<td>1431.65 (41.32)</td>
<td>5.33</td>
</tr>
<tr>
<td>Oct, 2016</td>
<td>1.50 (0.07)</td>
<td>24 (0.13)</td>
<td>1337.31 (29.67)</td>
<td>0.00</td>
</tr>
<tr>
<td>Nov, 2016</td>
<td>1.04 (0.07)</td>
<td>25 (0.72)</td>
<td>1192.90 (18.00)</td>
<td>86.61</td>
</tr>
<tr>
<td>Dec, 2016</td>
<td>0.44 (0.05)</td>
<td>39 (0.21)</td>
<td>629.85 (78.62)</td>
<td>110.20</td>
</tr>
</tbody>
</table>

Three months of PAR data are missing due to technical mishaps with the datalogger.

3.3.2 Water use

Inoculation treatments of the five girdled pines were not different from the remaining five girdled pines in the two months following the treatment ($p = 0.740$ and $p = 0.531$). The treatment was considered ineffective and all ten girdled pines were treated alike throughout the data analysis process. Evidence of pine mortality began late in the second growing season in August, 2016, with one girdled pine showing visual signs of death with needle discoloration and frass deposits on the forest floor. Sapflow ceased in several girdled individuals throughout October and November of 2016. By January, 2017,
seven of the ten girdled pines succumbed to the treatment, while three maintained green needles and continued to exhibit small quantities of sapflow. One of the control pines that underwent no treatment showed signs of mortality in November, 2016 with needle discoloration. Throughout the data collection process, it was noted that there was some external damage to this individual’s bark, which may have been related to its cause of mortality. The four trees (three girdled and alive, one untreated and dead) were omitted from statistical analysis.

During the first month of data collection, girdled pines contributed to higher average daily sapflow than control pines ($p < 0.001$; Fig. 3.4). Sapflow rates were not different throughout the following fall months; however, girdled pines began to significantly decrease sapflow rates in December, 2015 ($p < 0.001$; Fig. 3.4). Control pines continued to have at least 25% higher average daily sapflow for each month of the study with the exception of June, 2016, where sapflow did not differ between the two treatments ($p = 0.684$). Sapflow of girdled pines decreased at least twofold when comparing the same fall months in 2015 to 2016 (Table 3.2).
Figure 3.4  Monthly sums of average daily control and girdled pine sapflow rates (kg month$^{-1}$)

* denotes significant differences at $\alpha = 0.05$. Gray area represents period where mortality was visually observed due to needle discoloration.
Table 3.2  Average daily loblolly pine sapflow (kg day\(^{-1}\)) and standard error by month, year, and treatment for the duration of the study

<table>
<thead>
<tr>
<th>Average sapflow (kg H(_2)O day(^{-1}))</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Girdled</td>
</tr>
<tr>
<td>January</td>
<td>6.65 (0.71)</td>
<td>2.14 (0.85)</td>
</tr>
<tr>
<td>February</td>
<td>18.09 (2.00)</td>
<td>13.38 (1.33)</td>
</tr>
<tr>
<td>March</td>
<td>18.09 (2.00)</td>
<td>13.38 (1.33)</td>
</tr>
<tr>
<td>April</td>
<td>17.85 (1.64)</td>
<td>14.27 (1.26)</td>
</tr>
<tr>
<td>May</td>
<td>30.70 (1.65)</td>
<td>26.60 (1.61)</td>
</tr>
<tr>
<td>June</td>
<td>33.33 (1.88)</td>
<td>32.50 (2.02)</td>
</tr>
<tr>
<td>July</td>
<td>62.00 (4.15)*</td>
<td>92.45 (5.87)*</td>
</tr>
<tr>
<td>August</td>
<td>45.26 (1.99)</td>
<td>52.25 (1.09)*</td>
</tr>
<tr>
<td>September</td>
<td>23.67 (2.63)</td>
<td>22.39 (1.07)*</td>
</tr>
<tr>
<td>October</td>
<td>8.79 (1.54)</td>
<td>7.87 (0.89)*</td>
</tr>
<tr>
<td>November</td>
<td>13.30 (2.51)*</td>
<td>14.83 (1.43)*</td>
</tr>
<tr>
<td>December</td>
<td>13.74 (1.49)</td>
<td>7.92 (1.33)</td>
</tr>
</tbody>
</table>

* denotes significant difference between the same months of different years at \(\alpha = 0.05\). Bolded text denotes significant difference between treatments in the same month of the same year at \(\alpha = 0.05\).

In order to further investigate pine sapflow data, sapflow rate was analyzed as a response to increases in soil moisture and VPD. It was determined that sapflow was primarily limited by changes in soil moisture and a direct relationship between sapflow and VPD across all soil moistures was not detected. Therefore, the relationship between sapflow and VPD was analyzed by comparing sapflow sensitivity to VPD (slope of the relationship between sapflow and VPD) under distinct soil moisture ranges. The only combination of soil moisture level and pine treatment that had a significant effect on sapflow was between July and December of 2015 when soil moisture was above 40% \((p = 0.023)\). All other combinations of soil moisture level and pine treatment had no significant effect on sapflow and sapflow sensitivity to VPD.
Sapflow vs. soil moisture of girdled and control pines were compared for 2015 (pre-mortality) and 2016 (post-mortality) seasons (Fig. 3.5). Pre-mortality, a positive relationship existed between sapflow and soil moisture in both girdled pines ($y = 145.85x - 32.38; r^2 = 0.67; p < 0.001$) and control pines ($y = 119.77x - 21.38; r^2 = 0.52; p < 0.001$) when soil moisture was below 40%. In the same year, there was no relationship between sapflow and soil moisture in both girdled pines ($p = 0.272$) and control pines ($p = 0.142$) when soil moisture was above 40%. Post-mortality, when soil moisture was much more limiting, a positive relationship existed between sapflow and soil moisture in both girdled pines ($y = 71.05x - 8.14; r^2 = 0.26; p < 0.001$) and control pines ($y = 92.08x - 8.68; r^2 = 0.32; p < 0.001$) when soil moisture was below 40%. In the same year, there was a positive relationship between sapflow and soil moisture for girdled pines ($y = 113.14x - 29.72; r^2 = 0.78; p = 0.019$), and no relationship between sapflow and soil moisture for control pines ($p = 0.868$) when soil moisture was above 40%.
Figure 3.5  Relationships between sapflow (kg day\(^{-1}\)) of pines and soil moisture, in 2% increments of soil moisture using data from July-November of both years.

The slope of daily sapflow vs. daily VPD (sapflow sensitivity to VPD) was plotted against ranges of soil moisture content (Fig. 3.6). Pre-mortality, no relationships existed between sapflow sensitivity to VPD and soil moisture for girdled pines \((p = 0.099)\) and control pines \((p = 0.076)\) when soil moisture was below 40%. The same year, no relationships existed between sapflow sensitivity to VPD and soil moisture for girdled pines \((p = 0.143)\) and control pines \((p = 0.138)\) when soil moisture was above 40%. Post-mortality, no significant relationships existed between sapflow sensitivity to VPD and low soil moisture for girdled pines \((p = 0.737)\) and control pines \((p = 0.107)\). The
relationship between sapflow sensitivity to VPD and high soil moisture was unable to be detected when soil moisture was over 40% due to insufficient data as a result of low precipitation during the second year of the study.

Figure 3.6  Relationships between sapflow sensitivity to vapor pressure deficit (VPD; slope of sapflow rate vs. VPD; kg day\(^{-1}\) kPa\(^{-1}\)) and increases in soil moisture (%).

Slopes between sapflow and VPD were calculated for every 5% increase in soil moisture
Control and girdled pine did not exhibit strong responses to daily maximum PAR in any of the four seasons within a year of the study (Fig. 3.7). Girdled pines exhibited a positive response to PAR in spring (MAM) of 2016 ($y = 0.01x + 13.69; r^2 = 0.06; p = 0.027$). Girdled pines also exhibited a positive response to PAR in summer (JJA) of 2016 ($y = 0.02x + 2.83; r^2 = 0.10; p = 0.003$). No other relationships between sapflow and maximum daily PAR were significant at $\alpha = 0.05$.

![Figure 3.7](image_url)  
Figure 3.7  
Daily sapflow (kg day$^{-1}$) of girdled and control pines vs. maximum daily photosynthetically active radiation (µmol m$^{-2}$ s$^{-1}$)

Control pine sapflow between the same months of differing years was further investigated by sorting each month’s sapflow values into days with above and below average VPD (Table 3.3), and days with above and below average soil moisture (Table...
3.4). For each month, VPD values less than 1.25 kPa were assigned to the “below average VPD” category while values greater than 1.25 kPa were assigned as “above average VPD.” For July, August, and November, soil moisture values less than 40% were assigned to the “below average” category while values greater than 40% were assigned as “above average.” For September and October, the designation between below and above average soil moisture was 30%, due to seasonal water stress during these months. When trees were compared between years based on similar VPD, control pines significantly increased water use by 40% in October when VPD was above average and significantly decreased water use by 99% in November when VPD was below average (Table 3.3). Similarly, control pines increased water use by 48% in October when soil moisture was below average and decreased water use by 137% in November when soil moisture was below average (Table 3.4). Control pine sapflow also increased by 39% in September when soil moisture was below average, but the increase during this month was not detected when looking at sapflow at above or below average VPD values. There was no significant difference observed in control pine water use post-mortality when compared to the same months of the pre-mortality year.
Table 3.3  Control pine sapflow (and standard error; kg day$^{-1}$) compared between years during similar vapor pressure deficit (VPD) values (above 1.25 kPa and below 1.25 kPa) for each month

<table>
<thead>
<tr>
<th>Month</th>
<th>Below 1.25 kPa</th>
<th>Above 1.25 kPa</th>
<th>p-value</th>
<th>Below 1.25 kPa</th>
<th>Above 1.25 kPa</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>50.36 (3.57)</td>
<td>41.65 (6.10)</td>
<td>0.260</td>
<td>70.14 (2.32)</td>
<td>54.80 (9.09)</td>
<td>0.075</td>
</tr>
<tr>
<td>August</td>
<td>41.47 (2.36)</td>
<td>37.24 (2.01)</td>
<td>0.180</td>
<td>51.26 (1.13)</td>
<td>53.73 (2.07)</td>
<td>0.285</td>
</tr>
<tr>
<td>September</td>
<td>19.25 (3.71)</td>
<td>20.42 (3.52)</td>
<td>0.820</td>
<td>28.39 (2.65)</td>
<td>26.77 (1.78)</td>
<td>0.600</td>
</tr>
<tr>
<td>October</td>
<td>9.23 (1.87)</td>
<td>12.37 (3.23)</td>
<td>0.400</td>
<td>7.93 (1.13)</td>
<td>11.11 (0.45)</td>
<td>0.007</td>
</tr>
<tr>
<td>November</td>
<td><strong>13.30 (2.20)</strong></td>
<td><strong>6.67 (0.99)</strong></td>
<td>0.015</td>
<td>n/a</td>
<td>8.37 (0.64)</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Bolded values denote significance between years at $\alpha = 0.05$.

Table 3.4  Control pine sapflow (and standard error; kg day$^{-1}$) compared between years during similar soil moisture values (above and below 40% in July, August, September; above and below 30% in October, November) for each month

<table>
<thead>
<tr>
<th>Month</th>
<th>Below average soil moisture</th>
<th>Above average soil moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2015</td>
<td>2016</td>
</tr>
<tr>
<td>July</td>
<td>59.42 (2.63)</td>
<td>43.93 (5.51)</td>
</tr>
<tr>
<td>August</td>
<td>46.39 (1.82)</td>
<td>42.95 (2.32)</td>
</tr>
<tr>
<td>September</td>
<td>16.52 (2.42)</td>
<td><strong>23.00 (1.83)</strong></td>
</tr>
<tr>
<td>October</td>
<td>7.71 (1.14)</td>
<td><strong>11.39 (0.78)</strong></td>
</tr>
<tr>
<td>November</td>
<td><strong>16.71 (5.83)</strong></td>
<td><strong>7.06 (0.79)</strong></td>
</tr>
</tbody>
</table>

Bolded values denote significance between years at $\alpha = 0.05$.

3.3.3  Leaf area and leaf litter components

Pines dropped more needles in the post-mortality fall season (0.72 cm$^2$ cm$^{-2}$), than the pre-mortality fall season (0.60 cm$^2$ cm$^{-2}$) (Fig. 3.8; Table 3.5). Foliage density in the pre-mortality dormant season did not differ between control pines (0.43 m$^2$ leaf area m$^{-3}$ canopy volume) and girdled pines (0.40 m$^2$ leaf area m$^{-3}$ canopy volume) ($p = 0.629$).

Foliage density in the post-mortality dormant season was greater in control pines (0.25
m² leaf area m⁻³ canopy volume) than girdled pines (0.15 m² leaf area m⁻³ canopy volume) (p = 0.009). Additionally, foliage density significantly decreased from pre-mortality to post-mortality for both girdled pines (0.40 - 0.15 m² leaf area m⁻³ canopy volume; p <0.001) and control pines (0.43 – 0.25 m² leaf area m⁻³ canopy volume; p = 0.017).

Figure 3.8 Loblolly pine leaf litter quantity (cm² cm⁻²) throughout time

Table 3.5 Loblolly pine needle quantity and quality of senesced leaves collected from litter traps in the two seasons of the study

<table>
<thead>
<tr>
<th></th>
<th>2015/pre-mortality</th>
<th>2016/post-mortality</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litterfall quantity (cm² cm⁻²)</td>
<td>0.60</td>
<td>0.72</td>
<td>n/a</td>
</tr>
<tr>
<td>N concentration (%)</td>
<td>0.50 (0.01)</td>
<td>0.75 (0.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C concentration (%)</td>
<td>49.33 (1.13)</td>
<td>48.81 (0.62)</td>
<td>0.722</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>92.75 (2.58)</td>
<td>80.11 (3.48)</td>
<td>0.029</td>
</tr>
</tbody>
</table>
N concentration was higher in senesced leaves from the post-mortality collection season (0.75%) than senesced leaves from the pre-mortality collection season (0.50%) ($p < 0.001$; Table 3.5). C concentration did not differ between senesced needles collected in the post-mortality collection season (48.81%) and the pre-mortality collection season (49.33%) ($p = 0.722$; Table 3.5). C:N of senesced needles was lower in the post-mortality collection season (80.11) than the pre-mortality collection season (92.75) ($p = 0.029$; Table 3.5). Throughout the post-mortality collection season, pine resorption of leaf N steadily decreased with each collection of leaf litter ranging from 42.76% in the beginning of October to 18.33% in the end of November (Fig. 3.9).

![Figure 3.9](image_url)

**Figure 3.9** Loblolly pine nitrogen resorption (%) between senesced and fresh leaves in fall 2015 and 2016

N concentration of fresh needles that were collected in the 2016 summer growing season did not differ between leaves collected from girdled pines (0.88%) and control
pines (0.94%) \((p = 0.297; \text{Table 3.6})\). C concentration of fresh needles did not differ between leaves collected from girdled pines (45.95%) and control pines (43.66%) \((p = 0.514; \text{Table 3.6})\). C:N of fresh pine needles did not differ between girdled pines (53.23) and control pines (46.24) \((p = 0.365; \text{Table 3.6})\).

### Table 3.6

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control Pine</th>
<th>Girdled Pine</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>0.94 (0.04)</td>
<td>0.88 (0.06)</td>
<td>&lt;0.297</td>
</tr>
<tr>
<td>C (%)</td>
<td>43.66 (2.27)</td>
<td>45.95 (3.21)</td>
<td>0.514</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>46.24 (4.84)</td>
<td>53.23 (6.46)</td>
<td>0.365</td>
</tr>
</tbody>
</table>

#### 3.3.4 Photosynthetic capacity

All photosynthetic parameters measured in August, 2016 significantly differed between girdled and control pines (Table 3.7), and these were measured before signs of leaf mortality were observed. It should be noted, however, that two of the three girdled pines died later that season while one persisted throughout the winter. Average \( V_{cmax} \) of control pines was 63.50% higher than \( V_{cmax} \) of girdled pines. Average \( J_{max} \) of control pines was 67.10% higher than \( J_{max} \) of girdled pines. Average TPU of control pines was 44.28% higher than TPU of girdled pines.

### Table 3.7

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control pine ((\mu\text{mol m}^{-2} \text{s}^{-1}))</th>
<th>Girdled pine ((\mu\text{mol m}^{-2} \text{s}^{-1}))</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{cmax} )</td>
<td>182.00 (11.59)</td>
<td>111.33 (14.71)</td>
<td>0.020</td>
</tr>
<tr>
<td>( J_{max} )</td>
<td>186.00 (23.46)</td>
<td>111.33 (10.14)</td>
<td>0.042</td>
</tr>
<tr>
<td>TPU</td>
<td>14.37 (0.59)</td>
<td>9.96 (0.29)</td>
<td>0.003</td>
</tr>
</tbody>
</table>
3.3.5  **Bole growth**

Growth rings were measured at the end of the second growing season (post-mortality). Growth ring width of girdled pines, on average, was smaller in the second year (2016 growing season; 2.07 mm) than the first year (2015 growing season; 2.59 mm) ($p = 0.034$; Fig. 3.10). Growth ring width of control pines was not significantly greater in the second year (2.99 mm) than the first year (2.43 mm) ($p = 0.123$). Additionally, wider growth rings occurred in control pines (2.99 mm) than girdled pines (2.07 mm) in the second growing season ($p = 0.081$).

![Control and girdled loblolly pine growth ring width (mm) for the past five growing seasons. Girdling treatment occurred in 2015.](image)

Figure 3.10  Control and girdled loblolly pine growth ring width (mm) for the past five growing seasons. Girdling treatment occurred in 2015.
3.3.6 Stem respiration

Monthly respiration measurements “above” and “below” the girdle treatment on each pine individual were not determined to be significantly different from one another, therefore they were averaged. Values for each tree were averaged based on if the tree was a control pine or girdled pine. No monthly average CO$_2$ flux measurements were significantly different at $\alpha = 0.05$ level between control pines and girdled pines (Fig. 3.11). However, when stem respiration measurements were collected in August, CO$_2$ flux of girdled pines (3.90 $\mu$mol m$^{-2}$ s$^{-1}$) was somewhat higher than the flux of control pines (1.79 $\mu$mol m$^{-2}$ s$^{-1}$) ($p = 0.088$). Overall, although none of the measurements were significant, average monthly respirations was consistently higher in girdled pines than control pines.

Figure 3.11 Average (above and below girdle) monthly stem respiration ($\mu$mol m$^{-2}$ s$^{-1}$) of control and girdled loblolly pines in 2016

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3.4 Discussion

During the first two months of the study, girdled pines, on average, exhibited higher sapflow than control pines. There were no statistical differences between sapflow rates for the next three consecutive months, but by December of 2015, control pines began having significantly higher sapflow than girdled pines. Initial increases in girdled pine sapflow could be due to allocating more water resources to resin production, rather than transpiration. Ruel et al. (1998) found resin flow to double in loblolly pines after a bark-wounding treatment. Similarly, Guérard et al. (2007) found higher concentrations of C in damaged tissue than healthy tissue of Scots pine; however, they found that the majority of this C was stored as opposed to newly-acquired. The girdling treatment took approximately six months to significantly reduce sapflow. Despite the significant sapflow declines six months into this study, visual signs of mortality were not observed until approximately thirteen months after the girdling treatment. It was hypothesized that control pines would increase water use post-mortality, however, this was only detected in two post-mortality months when compared at similar soil moisture levels. Studies that had successful inoculation treatments (Yamaoka et al., 1990; Hubbard et al., 2013) found rapid declines in sapflow, up to ten days after pines were treated, which was much more rapid than the findings in the current study.

Relationships between sapflow, VPD, and soil moisture were very different between the two years of the study, likely due to the notably low precipitation that occurred in the second year. In 2015, when this region of Mississippi experienced typical quantities of precipitation, there was a positive relationship between sapflow (and sapflow sensitivity to VPD) and soil moisture when soil moisture was below 40%. These
data are consistent with findings from Oren et al. (1998) and Phillips and Oren (2001) who found correlations between loblolly pine sapflow and soil moisture during conditions of drought stress in forests containing clay and clay loam soils, similar to the forest soils in which this study took place. Comparatively, Ford et al. (2004) found sapflow to have highest correlations with VPD and PAR in a forest containing loamy sand soils, which are well drained. Pine sapflow did not have significant relationships to VPD or PAR, which suggests that sapflow in loblolly pine in this ecosystem, with poorly-drained soils, is primarily limited by soil moisture before limitations due to VPD and PAR were detected.

Negative relationships between sapflow (and sapflow sensitivity to VPD) and soil moisture when soil moisture was over 40% support that pines are limited by inundation. Baker and Langdon (1990) state that mature loblolly pines are moderately tolerant of inundated conditions, but are less tolerant if flooding over 0.30 meters is repeated in consecutive growing seasons. Lack of soil oxygen content is particularly limiting to water absorption by roots (Pallardy, 2008). Loblolly saplings especially cannot withstand flood conditions (Baker and Langdon, 1990), therefore stands like these will change dominance to flood-tolerant hardwoods (Coleman et al., 2008).

It was the intention of the senesced leaf nutrient component of this study to differentiate between needles from control pines and needles from girdled pines based on litter trap proximity to individual trees. After some analysis, however, it was determined that this differentiation was unable to be detected and leaf nutrient content of senesced needles was instead compared between pre-mortality and post-mortality fall seasons. Leaf N content of senesced needles significantly increased from pre-mortality to post-
mortality, causing a significant decrease in C:N. Leaf N content of live needles was 0.88% and 0.94% in girdled and control pines respectively, which is slightly higher than the Gough et al. (2004) estimate of 0.76%. Increases in senesced pine leaf N content is likely due to less N resorption of pines undergoing mortality. Therefore, upon mortality of the girdled loblolly pines, more N becomes available for uptake. Increases in soil N content and availability to plants may facilitate growth (Samuelson et al., 2008; Coyle et al., 2008) through increased gas exchange capabilities (Ewers et al., 1999). This is supported by the data from this study with significantly higher $V_{cmax}$ and growth ring width of control pines in comparison to girdled pines.

Many studies have shown the relationship between leaf N content and photosynthetic capacity (Bassow and Bazzaz, 1997; Drake et al., 2010; Ryan et al., 1997). Decreases in C:N suggest that N will not be immobilized in the soil (Berg and Ekbohm, 1983). Increased availability of N to surviving plants will increase photosynthetic capacity, and consequently, increase C assimilation. Eventually, however, it is likely that the woody debris will increase soil C content which would increase C:N. Woody litter from dead, downed trees immobilizes N throughout the wood decay process (Murty et al., 1996), potentially creating increasingly limited photosynthesis due to N limitation (Ryan et al., 1997).

As hypothesized, $V_{cmax}$, $J_{max}$, and TPU were all significantly lower in girdled pines than control pines. Similarly to the observed reductions in sapflow, the significant differences in these photosynthetic parameters occurred before visual signs of needle discoloration. Increased photosynthetic limitations in conjunction with reduced leaf area and growth rates suggest alteration of the C cycle in trees undergoing mortality. Girdled
trees will begin to act as a C source to the atmosphere as phloem transport of carbohydrates ceases to occur (Hubbard et al., 2013). Kurz et al. (2008) found that beetle outbreaks convert pine forests from a small C sink to a large C source, also by emitting C throughout the wood decaying process. Moreover, Ellsworth (2000) found that sunny day C assimilation was reduced by 45% during an extended drought. These findings suggest that a combination of water stress and pine mortality will impact forest C assimilation and source-sink relationships.

Foliage density significantly differed between girdled pines and control pines in the second dormant season in which measurements were collected, due largely to loss of crown foliage in several girdled pines. The decrease in foliage density of control pines between the two measurements may have been due to timing of needle replacement in pines. It was expected for the significant decrease to occur in girdled pines, but it was not expected that this would be evident in the control pines. The data perhaps would have been more interpretable if measurements were taken during the same month of dormancy over two seasons; December of 2015 and December of 2016, for instance. Due to time constraints of the duration of the study, the spring-fall measurements of control pine foliage density may not be representative of a reaction to the girdling treatment of adjacent trees. Nonetheless, the decrease in foliage density and increase in litterfall quantity suggest changes in N (Clow et al., 2011), C and hydrologic cycles (Hubbard et al., 2013), and ability to sequester C (Maier et al., 2004).

It was hypothesized that the girdling treatment would have an effect on stem respiration. C in the form of resin is transported to damaged tissue (Tisdale et al., 2003) and reduced phloem transport of carbohydrates should create an accumulation of
carbohydrate storage above the section of phloem that was girdled. Maier et al. (2010) found stem respiration to increase and decrease above and below a girdle, within 24 hours following the treatment. In comparison to Maier et al. (2010), who girdled each pine in two locations, each pine in the current study was girdled once and no significant trend in stem respiration above or below the girdling treatment was found. Additionally, the stem respiration component of this study was not implemented until six months following significant sapflow decline of girdled pines. Carbohydrate accumulation may not have been detected if trees already stopped producing carbohydrates before respiration measurements were collected. This is likely due to significant decreases in \( V_{cmax} \), \( J_{max} \), and sapflow of girdled pines relative to control pines before stem respiration was measured.

### 3.5 Conclusion

Mortality events in forests result in altered resource availability. SPB-simulated mortality of loblolly pine has measureable effects on many components of forest nutrient and hydrologic cycles, resulting in implications on future forest structure. Notably, measureable declines in photosynthetic capacity and sapflow rate significantly decreased one and seven months, respectively, before needle discoloration was observed. This component of timing during the mortality process is important, particularly during changes in climate. Loblolly pine contribution to carbon assimilation and ET are both significantly reduced before actions can be taken to combat pest and pathogen-related mortality. Estimations of N redistribution within the scope of this study suggest that N is more available to competing vegetation in the years following mortality, however future decomposition of woody debris will increase the C:N and potentially immobilize soil N,
resulting in a more N-limited system. A follow up to this study would be necessary in order to more accurately predict changes in nutrient and hydrologic cycling that occur in response to the SPB-simulated mortality event of loblolly pine.
CHAPTER IV
PHYSIOLOGICAL RESPONSE OF MID-CANOPY SWEETGUM TO SELECTIVE PINE MORTALITY

4.1 Introduction

Vegetation that exists in the mid-canopy is likely to increase physiological functioning by taking advantage of newly available resources upon disturbance of dominant canopy species (Canham and Marks, 1985). Particularly, after loblolly pine mortality or removal, succession is likely to be exacerbated due to gradual increases in availability of water, light, and nutrients (Coleman et al., 2008). However, it is unknown how physiological functioning of individuals will react to changes in resources and which resources will be most influential to physiological change, especially based on proximity to dying canopy trees. Investigation of mid-canopy sweetgum physiology under conditions of dominant loblolly pine mortality will aid in the understanding of how mid-successional species respond to changes in resource availability. The goal of this chapter is to document responses of mid-canopy sweetgum to localized mortality of loblolly pine across the time that it takes for pine individuals to die. Specifically, objectives are to determine physiological responses to increasing available water and nutrients, and determine patterns of seasonal water use under conditions of inundation and drought stress. The hypotheses for this chapter are that sweetgum next to dying pines will increase water use, nitrogen uptake, C uptake, and decrease photosynthetic limitations

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with newly available resources post-mortality. These data will increase the knowledge base of the successional dynamic between pines and hardwoods, especially under conditions of forest disturbance and seasonal environmental stressors.

4.2 Methods

4.2.1 Site description

The research was conducted in the John W. Starr Memorial Forest (33°15'50.94"N, 88°53'6.47"W) in Winston County in east-central Mississippi. See section 3.2.1 for a complete site description and map of the research location (Fig. 3.1).

4.2.2 Treatments and experimental design

Twelve sweetgums with DBH values ranging from 10.2 to 40.1 cm were selected for sampling to represent a range of DBH classes. The sweetgums were chosen due to proximity to girdled and control pines, which was approximately 1-1.5 meters in distance between pine-sweetgum pairs. Six sweetgum were located closer to girdled pines than they were to control pines while the remaining six sweetgum were located closer to control pines than to girdled. See section 3.2.2 for a complete explanation of the loblolly pine girdling treatment that occurred in July, 2015. By January, 2017, five girdled pines that successfully underwent mortality were located within close proximity of five of the six sweetgums next to girdled pines. The last sweetgum was located next to a girdled pine that remained alive throughout the study. Another sweetgum was located directly between pines of the two treatments. Therefore, statistical analysis was completed considering “sweetgum next to girdled pines” as only trees next to pines which
underwent mortality, and “sweetgum next to control pines” as only trees next to pines which remained alive.

4.2.3 Environmental data

Environmental parameters of soil moisture, relative humidity, air temperature, and PAR were continuously collected throughout the sampling period to compare with tree physiological measurements. See section 3.2.3 for a complete explanation of environmental data that was collected throughout the duration of this study.

4.2.4 Water use

Water use was estimated by measuring sapflow on the twelve sweetgum individuals throughout the 2015 and 2016 growing seasons. See section 3.2.4 for a complete description of the thermal dissipation method of measuring sapflow. Daily quantities of sapflow were determined for each individual by multiplying each measured sapflow rate by sapwood area for that individual. Sapwood area of each sweetgum was determined by using allometric equations developed by Oishi et al. (2008) and Ward et al. (2013) (equations 4.1 and 4.2 respectively).

\[
SA = 0.246 * DBH^{2.202} \tag{4.1}
\]

\[
SA = 0.82 * BA \tag{4.2}
\]

where SA is sapwood area in cm², DBH is the diameter at breast height measured in cm, and BA is basal area measured in cm². The Ward et al. (2013) method estimated sapflow approximately 40% higher than that of the Oishi et al. (2008) method. The average of the two estimates was used as the final estimate of sapwood area for each sweetgum individual.
4.2.5  Photosynthetic capacity

Photosynthesis measurements were collected on a subsample of six sweetgum individuals (three next to girdled pines, and three next to control pines). Branches less than two cm in diameter were removed from the canopy by clipping with a pole pruner and were then prepared for photosynthesis measurements in a similar fashion to the loblolly pines that were collected. See section 3.2.5 for a complete explanation of the photosynthesis measurements collected and $A/C_i$ curves that were calculated.

4.2.6  Leaf area dynamics, leaf litter content, and leaf anatomy

Sweetgum leaf litter was collected from litter traps and analyzed in the lab for LAI and leaf nutrient content. Leaves were collected bi-weekly throughout each fall season of the study. See section 3.2.6 for a complete explanation of leaf litter collection and sampling methodology for leaf nutrient content. Additionally, samples of fresh sweetgum leaves were collected to estimate stomatal length and density during both growing seasons and preserved by refrigeration in plastic bags. Patches of clear nail polish were painted in several random locations on the underside of each leaf and peeled off when dry to be placed onto a glass microscope slide. These were observed at 20x and 40x magnification with an Olympus Vanox compound light microscope (Waltham, MA, USA) and ten photos were taken at each magnification with a digital camera (AmScope FMA050, Irvine, CA, USA). Stomatal length and density were measured using ImageJ software version 1.48 (National Institutes of health, USA, [https://imagej.nih.gov/ij](https://imagej.nih.gov/ij)).
4.2.7 **Statistical analysis**

Due to unexpected mortality outcomes in four of the fifteen loblolly pines, two sweetgum individuals were omitted from statistical analysis. Therefore, the sample sizes for “trees next to girdled pines” and “trees next to control pines” were both equal to five sweetgums. Leaf anatomical characteristics including stomatal length and density were compared between years and between sweetgum next to control pines and sweetgum next to girdled pines using analysis of variance (aov function) in R version 3.1.2 (R Core Team, 2015). Sweetgum sapflow, leaf nutrient content, photosynthetic capacity, and growth were all organized and analyzed similarly to that of loblolly pine, which may be referenced in section 3.2.9.

4.3 **Results**

4.3.1 **Environmental data**

The environmental parameters VPD, soil moisture, and PAR that were measured throughout the course of the study show periods of water stress and resource availability within the site. These data are presented in section 3.3.1 (Fig. 3.3 and Table 3.1).

4.3.2 **Water use**

Throughout the entirety of the study, sweetgums adjacent to control pines contributed to higher average sapflow rates on a monthly basis, in comparison to sweetgums next to girdled pines (Fig. 4.1). The magnitude of difference between the two categories becomes greater during the second year of the study, as pine mortality becomes more apparent within the stand.
Sweetgum dormancy occurs December – April. Gray area represents when mortality was visually observed by needle discoloration. * denotes significance at $\alpha = 0.05$.

Sweetgums next to girdled pines increased sapflow from pre-mortality to post-mortality during October at below average VPD (309.15%; $p < 0.001$) and above average VPD (143.75%; $p < 0.001$) (Table 4.1), but decreased sapflow in August at below average VPD (30.45%; $p = 0.005$). Sweetgums next to control pines increased sapflow from pre-mortality to post-mortality at below average VPD during August (39.94%; $p < 0.001$) and October (155.78%; $p < 0.001$), and at above average VPD during September (19.13%; $p = 0.022$) and October (83.16%; $p < 0.001$) (Table 4.2).

Sweetgums next to girdled pines increased sapflow from pre-mortality to post-mortality
at below average soil moisture during October (208.39%; \( p < 0.001 \)) and November (434.78%; \( p < 0.001 \)), decreased sapflow at above average soil moisture during September (16.57%; \( p = 0.044 \)) (Table 4.3). Sweetgum next to control pines increased sapflow from pre-mortality to post-mortality at below average soil moisture during September (25.96%; \( p = 0.016 \)), October (107.29%; \( p < 0.001 \)), and November (72.27%; \( p = 0.040 \)), and at above average soil moisture during August (88.49%; \( p = 0.002 \)) and September (36.18%; \( p = 0.002 \)) (Table 4.4).

### Table 4.1
Sweetgum sapflow (and standard error; kg day\(^{-1}\)) next to girdled pine during each month on days with vapor pressure deficit below or above 1.25 kPa

<table>
<thead>
<tr>
<th>Month</th>
<th>Below average VPD</th>
<th>Above average VPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>14.19 (1.01)</td>
<td>16.50 (1.34)</td>
</tr>
<tr>
<td>September</td>
<td>10.84 (1.50)</td>
<td>14.98 (2.54)</td>
</tr>
<tr>
<td><strong>October</strong></td>
<td><strong>2.95 (0.76)</strong></td>
<td><strong>12.07 (1.26)</strong></td>
</tr>
<tr>
<td>November</td>
<td>2.61 (0.81)</td>
<td>4.71 (1.38)</td>
</tr>
</tbody>
</table>

Bolded values represent significance at \( \alpha = 0.05 \).

### Table 4.2
Sweetgum sapflow (and standard error; kg day\(^{-1}\)) next to control pine during each month on days with vapor pressure deficit below or above 1.25 kPa

<table>
<thead>
<tr>
<th>Month</th>
<th>Below average VPD</th>
<th>Above average VPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td><strong>16.30 (1.29)</strong></td>
<td><strong>22.81 (1.71)</strong></td>
</tr>
<tr>
<td>September</td>
<td>14.21 (1.43)</td>
<td>18.08 (2.42)</td>
</tr>
<tr>
<td><strong>October</strong></td>
<td><strong>6.83 (0.87)</strong></td>
<td><strong>17.47 (1.45)</strong></td>
</tr>
<tr>
<td>November</td>
<td>5.54 (0.91)</td>
<td>6.46 (1.56)</td>
</tr>
</tbody>
</table>

Bolded values represent significance at \( \alpha = 0.05 \).
In order to further investigate sweetgum sapflow data, sapflow rate was analyzed as a response to increases in soil moisture and VPD. It was determined that sapflow is primarily limited by changes in soil moisture and a direct relationship between sapflow and VPD across the entire soil moisture range was not detected. Therefore, the relationship between sapflow and VPD was analyzed by comparing sapflow sensitivity to VPD at varying soil moistures. No combinations of soil moisture level and sweetgum position relative to pine treatment were significant with respect to sweetgum sapflow or sapflow sensitivity to VPD.

Table 4.3  Sweetgum sapflow (and standard error; kg day$^{-1}$) next to girdled pine during each month on days with below average and above average soil moisture (40% in August, September; 30% in October, November)

<table>
<thead>
<tr>
<th>Month</th>
<th>Below average soil moisture</th>
<th>Above average soil moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2015</td>
<td>2016</td>
</tr>
<tr>
<td>August</td>
<td>17.22 (0.93)</td>
<td>16.23 (1.24)</td>
</tr>
<tr>
<td>September</td>
<td>10.31 (1.36)</td>
<td>13.78 (1.83)</td>
</tr>
<tr>
<td>October</td>
<td>3.22 (0.62)</td>
<td>9.93 (0.85)</td>
</tr>
<tr>
<td>November</td>
<td>0.92 (0.58)</td>
<td>4.92 (0.65)</td>
</tr>
</tbody>
</table>

Bolded values represent significance at $\alpha = 0.05$.

Table 4.4  Sweetgum sapflow (and standard error; kg day$^{-1}$) next to control pine during each month on days with below average and above average soil moisture (40% in August, September; 30% in October, November)

<table>
<thead>
<tr>
<th>Month</th>
<th>Below average soil moisture</th>
<th>Above average soil moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2015</td>
<td>2016</td>
</tr>
<tr>
<td>August</td>
<td>19.134 (1.12)</td>
<td>21.90 (1.49)</td>
</tr>
<tr>
<td>September</td>
<td>15.18 (1.16)</td>
<td>19.115 (1.561)</td>
</tr>
<tr>
<td>October</td>
<td>7.54 (0.70)</td>
<td>15.63 (0.97)</td>
</tr>
<tr>
<td>November</td>
<td>4.22 (1.28)</td>
<td>7.27 (1.43)</td>
</tr>
</tbody>
</table>

Bolded values represent significance at $\alpha = 0.05$. 65
Average daily sapflow was plotted against respective daily soil moisture percentage (Fig. 4.2). Pre-mortality, a positive relationship existed between sapflow and soil moisture for trees next to both girdled pines (\(y = 136.96x -29.67; r^2 = 0.66; p <0.001\)) and control pines (\(y = 110.10x -18.48; r^2 = 0.50; p <0.001\)) when soil moisture was below 40%. In the same year, there were no relationships between sapflow and soil moisture for trees next to girdled pines (\(p = 0.451\)), and control pines (\(p = 0.587\)) when soil moisture was above 40%. Post-mortality, positive relationships existed between sapflow and soil moisture in trees next to girdled pines (\(y = 58.65x -4.61; r^2 = 0.18; p<0.001\)) and control pines (\(y = 75.15x -4.31; r^2 = 0.22; p <0.001\)) when soil moisture was below 40%. No relationships existed between sapflow and soil moisture in trees next to girdled pines (\(p = 0.288\)) and control pines (\(p = 0.958\)) when soil moisture was above 40%.
Figure 4.2  Relationships between sapflow (kg day\(^{-1}\)) of sweetgum and soil moisture, in 2% increments of soil moisture using data from August-November of both years

Daily sapflow vs. daily VPD (sapflow sensitivity to VPD) was plotted against ranges of soil moisture content (Fig. 4.3). Pre-mortality, positive relationships existed between sapflow sensitivity to VPD and soil moisture for trees next to girdled pines (\(y = 4.33x -4.97; r^2 = 0.96; p = 0.020\)) and control pines (\(y = 3.94x -3.39; r^2 = 0.89; p = 0.050\)) when soil moisture was below 40%. No relationships existed between sapflow sensitivity to VPD and soil moisture for trees next to girdled pines (\(p = 0.241\)) and control pines (\(p = 0.085\)). Post-mortality, the relationships between sapflow sensitivity to VPD and soil moisture were not significant for trees next to girdled pines (\(p= 0.945\)) and trees next to
control pines. The relationship between sapflow sensitivity to VPD and soil moisture at all levels was negative for trees next to control pines (\( y = -1.64x + 3.91; p = 0.088 \)).

Figure 4.3  Relationships between sapflow sensitivity to vapor pressure deficit (VPD; slope of sapflow rate vs. VPD; kg day\(^{-1}\) kPa\(^{-1}\)) of sweetgum and soil moisture, in 5% increments of soil moisture using data from August-November of both years.

Sapflow of sweetgum next to girdled pine and sweetgum next to control pine did not exhibit a strong response to maximum daily PAR (Fig. 4.4). Sweetgum next to control pine responded positively to PAR in the fall (SON) of 2016 (\( y = 0.01x + 9.05; r^2 = \)).
0.07; \( p = 0.050 \)). No other relationships between sweetgum sapflow and maximum daily PAR were significant at \( \alpha = 0.05 \).

Figure 4.4  Daily sapflow (kg day\(^{-1}\)) of sweetgum next to girdled and control pines vs. maximum daily photosynthetically active radiation (\( \mu \text{mol m}^{-2} \text{s}^{-1} \))

4.3.3  Leaf area and leaf litter components

LAI calculated from litter trap leaf collection was higher in the post-mortality collection season (1.85 cm\(^2\) cm\(^{-2}\)) than the pre-mortality collection season (1.43 cm\(^2\) cm\(^{-2}\)) (Fig. 4.4) although no statistical analysis was run to determine if the increase was significant. N concentration of senesced leaves increased from 2015 (0.60%) to 2016 (0.67%) (\( p = 0.015 \); Table 4.5). C concentration of senesced leaves did not differ between leaves collected in pre-mortality (42.05%) and leaves collected post-mortality (42.25%) (\( p = 0.839 \); Table 4.5). Additionally, C:N of senesced leaves did not significantly differ
between leaves collected pre-mortality (68.24) and leaves collected post-mortality (64.24) \( (p = 0.237; \text{Table 4.5}) \). Lastly, resorption rate of leaf N content did not significantly differ between pre-mortality (56.03\%) and post-mortality (57.12\%) \( (p = 0.637; \text{Table 4.5}) \).

Figure 4.5  Sweetgum leaf area index of pre-mortality and post-mortality fall seasons estimated from litter trap leaf collections and specific leaf area of sweetgum leaves

Table 4.5  Senesced leaf litter quality and quantity from leaves collected from litter traps

<table>
<thead>
<tr>
<th></th>
<th>2015/pre-mortality</th>
<th>2016/post-mortality</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litterfall quantity (cm(^2) cm(^{-2}))</td>
<td>1.43</td>
<td>1.85</td>
<td>N/A</td>
</tr>
<tr>
<td>N concentration (%)</td>
<td>0.60 (0.02)</td>
<td>0.67 (0.02)</td>
<td>0.015</td>
</tr>
<tr>
<td>C concentration (%)</td>
<td>42.05 (0.87)</td>
<td>42.25 (0.33)</td>
<td>0.839</td>
</tr>
<tr>
<td>C:N</td>
<td>68.24 (1.80)</td>
<td>64.24 (1.36)</td>
<td>0.237</td>
</tr>
<tr>
<td>N resorption (%)</td>
<td>56.03</td>
<td>57.12</td>
<td>0.637</td>
</tr>
</tbody>
</table>
N concentration of fresh leaves collected pre-mortality did not differ between trees next to girdled pines (1.37%) and trees next to control pines (1.35%) \((p = 0.772)\). Likewise, N concentration of fresh leaves collection post-mortality also did not differ between trees next to girdled pines (1.65%) and trees next to control pines (1.50%) \((p = 0.157)\). Overall, however, N concentration of fresh leaves increased from the 2015 growing season (1.36%) to the 2016 growing season (1.56%) \((p = 0.002; \text{Table 4.6})\). C concentration of fresh leaves collected pre-mortality did not differ between trees next to girdled pines (37.10%) and trees next to control pines (37.93%) \((p = 0.655)\). C concentration of fresh leaves collected post-mortality also did not differ between trees next to girdled pines (40.28%) and trees next to control pines (41.11%) \((p = 0.463)\). Overall, however, C concentration of fresh leaves increased from the 2015 growing season (37.41%) to the 2016 growing season (40.75%) \((p = 0.005; \text{Table 4.6})\). C:N of fresh leaves collected in the 2015 growing season did not differ between trees next to girdled pines (27.13) and trees next to control pines (28.08) \((p = 0.587)\). C:N of fresh leaves collected in the 2016 growing season was not significantly higher in trees next to control pines (27.52) than trees next to girdled pines (24.46) \((p = 0.088)\). Overall, C:N did not differ between fresh leaves collected in 2015 (27.49) and leaves collected in 2016 (26.21) \((p = 0.295; \text{Table 4.6})\).

<table>
<thead>
<tr>
<th></th>
<th>2015</th>
<th>2016</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N concentration (%)</td>
<td>1.36 (0.04)</td>
<td>1.56 (0.05)</td>
<td>0.002</td>
</tr>
<tr>
<td>C concentration (%)</td>
<td>37.41 (0.68)</td>
<td>40.75 (0.99)</td>
<td>0.005</td>
</tr>
<tr>
<td>C:N</td>
<td>27.49 (0.80)</td>
<td>26.21 (1.17)</td>
<td>0.295</td>
</tr>
</tbody>
</table>

Table 4.6 Average sweetgum fresh leaf nutrient content in 2015 and 2016 summer growing seasons, from leaves clipped directly from the canopy.
4.3.4 Photosynthetic capacity and leaf anatomy

Measurements of photosynthetic parameters were collected once in August, 2016, approximately one month before needle discoloration was observed. Average $V_{\text{cmax}}$, $J_{\text{max}}$, and TPU in sweetgums next to girdled pine were higher than sweetgum next to control pine (Table 4.7), although the differences were not significant.

Table 4.7 Maximum photosynthetic carboxylation rates (µmol m$^{-2}$ s$^{-1}$) from measurements collected on one day during the summer growing season of 2016

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Next to control pine</th>
<th>Next to girdled pine</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{cmax}}$ (µmol m$^{-2}$ s$^{-1}$)</td>
<td>56.67 (22.88)</td>
<td>101.33 (22.24)</td>
<td>0.234</td>
</tr>
<tr>
<td>$J_{\text{max}}$ (µmol m$^{-2}$ s$^{-1}$)</td>
<td>61.67 (18.62)</td>
<td>82.67 (23.41)</td>
<td>0.521</td>
</tr>
<tr>
<td>TPU (µmol m$^{-2}$ s$^{-1}$)</td>
<td>4.63 (1.34)</td>
<td>5.93 (1.51)</td>
<td>0.554</td>
</tr>
</tbody>
</table>

Stomatal density did not differ between sweetgums next to control pines (275 stoma mm$^{-2}$) and sweetgums next to girdled pines (280 stoma mm$^{-2}$) in the 2015 growing season ($p = 0.484$; Table 4.8). Comparatively, stomatal density of sweetgums next to control pines (266 stoma mm$^{-2}$) was higher than stomatal density of sweetgums next to girdled pines (241 stoma mm$^{-2}$) in the 2016 growing season ($p = 0.017$; Table 4.8). This difference is due to a significant decrease in stomatal density of sweetgums next to girdled pines from 2015 to 2016 ($p < 0.001$) since sweetgum next to control pines did not change in terms of stomatal density between years ($p = 0.380$). Stomatal length show similar results to stomatal density. There was no difference between stomatal lengths of sweetgums next to control pines (13.9 µm) and sweetgums next to girdled pines (13.8 µm) in the 2015 growing season ($p = 0.633$; Table 4.8) but there was a significant difference between sweetgums next to control pines (13.8 µm) and sweetgums next to
girdled pines (13.4 µm) in the 2016 growing season ($p = 0.023$; Table 4.8). Similarly, this was due to a significant decrease in stomatal length of sweetgum next to girdled pines from 2015 to 2016 ($p = 0.035$) in comparison to no significant difference between years in sweetgum next to control pines ($p = 0.694$). Between all sweetgum, positive relationships exist between stomatal length and DBH ($y = 0.04x + 13.01$; $r^2 = 0.04$; $p < 0.001$) and between stomatal density and DBH ($y = 2.52x + 222.32$; $r^2 = 0.10$; $p < 0.001$).

Table 4.8  Stomatal density (mm$^{-2}$) and length (µm) averages for sweetgum next to control and girdled pines, for both summer growing seasons of the study

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Next to</td>
<td>Next to</td>
<td>Next to</td>
<td>Next to</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Girdled</td>
<td>Control</td>
<td>Girdled</td>
</tr>
<tr>
<td>Stomatal Density</td>
<td>275 (5)</td>
<td>280 (7)*</td>
<td>266 (7)</td>
<td>241 (10)*</td>
</tr>
<tr>
<td>(stoma mm$^2$ leaf area)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal Length</td>
<td>13.9 (0.2)</td>
<td>13.8 (0.2)*</td>
<td>13.8 (0.1)</td>
<td>13.4 (0.2)*</td>
</tr>
<tr>
<td>(µm)</td>
<td></td>
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</table>

Bolded values represent significance at $\alpha = 0.05$. 

73
4.3.5 **Bole growth**

Growth ring widths of sweetgum next to girdled pines (2.35 mm) and sweetgum next to control pines (2.12 mm) did not differ in the 2015 growing season ($p = 0.785$; Fig. 4.7). Similarly, growth ring widths of sweetgums next to girdled pines (1.95 mm) and sweetgums next to control pines (2.18 mm) did not differ in the 2016 growing season ($p = 0.758$; Fig. 4.7). Additionally, there was no difference in growth ring width between years sampled for sweetgum next to control pine ($p = 0.604$) and sweetgum next to girdled pine ($p = 0.539$; Fig. 4.7).
4.4 Discussion

This investigation of sweetgum physiology throughout the pine mortality process resulted in unexpected changes in sweetgum water use that differed from the aforementioned hypotheses. Throughout the entirety of the study, sweetgum individuals next to control pines had significantly higher sapflow than sweetgum individuals next to girdled pines. This is despite similar average DBH size of each sampling group (19.2 cm and 20.1 cm). Within most post-mortality months, at similar soil moisture and VPD conditions, sweetgums next to control pines had significantly higher sapflow rates than the same months of the pre-mortality year. These results suggest that loblolly pine mortality had an effect on sweetgum water use; live pines benefit neighboring sweetgum
more than a decrease in water use of dying pines benefits neighboring sweetgum. This detected response may be the result of hydraulic lift of loblolly pine, redistributing soil moisture from deep to shallow soil layers. Although this phenomenon has not been studied extensively, it has been shown to occur in longleaf pine with neighboring oak species (Espeleta et al., 2004). The results in the current study would suggest that decreasing water uptake from deep soil layers during mortality decreases hydraulic lift, which has a negative impact on sweetgum next to girdled pine.

Sweetgums next to control pines significantly increased sapflow from pre-mortality to post-mortality in most months at below and above average soil moisture, while sweetgum next to girdled pine only significantly increased sapflow in two months during below average soil moisture. There were some scenarios in which sweetgum next to girdled pine decreased sapflow between years, during above average VPD and soil moisture conditions (Tables 4.1 and 4.3) Reductions in sapflow and lack of sapflow response to soil moisture (Fig. 4.2) when soil moisture is above 40% are indicative of inundation limitations. Roots are physiologically limited by soil oxygen deficiency (Pallardy, 2008); therefore roots are incapable of water absorption during excessive flooding. Sweetgums next to girdled pines significantly decreased sapflow from pre-mortality to post-mortality at above average soil moisture during one month of the study, which would suggest that the trees were limited by excess soil moisture. During above average VPD conditions in the month of August, however, sweetgums next to girdled pines significantly decreased sapflow between 2015 and 2016. Sweetgum typically increase transpiration with increases in VPD (Oren and Pataki, 2001), but stronger relationships between sweetgums next to control pine and increases in PAR (Fig. 4.4)
correlate with findings from Pataki et al. (1998) that high VPD increases stomatal conductance, but more significantly so with increases in PAR.

The relationship between sapflow of trees next to control and girdled pine and soil moisture was positive in both years whenever soil moisture was below 40%. This relationship was not detected when soil moisture was above 40%; however, sweetgums next to girdled pines showed a negative, but negligible relationship in comparison to sweetgums next to control pines. Overall, the sweetgum in this study exhibited a higher response to soil moisture in the first year, when water was less limited. This corresponds with findings by Pataki et al. (1998) who found sweetgum to be sensitive to soil moisture in that small decreases in soil moisture will decrease sapflow in sweetgum, even when soils are relatively moist (Bates and Hall, 1981). Although sweetgum next to control pine were increasing sapflow post-mortality, they responded less positively to increases in soil moisture likely due to the drought stress occurring during the same time.

Although it is recognized for growing across a wide range of ecosystems and soil types (Kormani, 1990), some interpretations classify sweetgum as a flood-tolerant associate of minor bottoms (Hodges, 1997). Sapflow sensitivity to VPD decreased and increased when soil moisture was below and above 40%, respectively. Stomatal conductance has been found to increase with increases in VPD and PAR (Pataki et al., 1998) and with increases in soil moisture (Allen et al., 2005). In this study, sweetgum was primarily sensitive to changes in soil moisture, and secondarily sensitive to changes in VPD. A positive relationship was detected between increases in PAR and sweetgum sapflow next to control pines in the post-mortality season. It was hypothesized that this would occur in sweetgum next to girdled pines due to crown loss and increased access to
PAR. Instead, water stress had a larger effect on sweetgum next to girdled pine, which is likely what reduced those individuals’ ability to respond to increases in PAR.

Senesced leaf N content of sweetgum significantly increased from pre-mortality to post-mortality, which is consistent with findings of increased N use of understory vegetation after lodgepole pine (*Pinus contorta* Douglas) mortality (Hubbard et al., 2013). Increases in senesced leaf N content could be due to one of several possibilities. Firstly, as girdled pines became less productive, competition for nutrients in the system probably decreased, leaving more available for sweetgum to take up. Girdled pines also lost their entire crown foliage to the forest floor, redistributing large amounts of non-resorbed N to be decomposed in the soil organic layer. Additionally, sweetgum significantly increased water use rates in several post-mortality months in comparison to the same months pre-mortality. Since nutrients such as N (in the form of nitrate) enter a tree via water transport through the roots, increases in water use would suggest subsequent increases of N availability where N limitation is not a constraining factor.

Photosynthesis rates of sweetgum vary between sun and shade leaves (Harley et al., 1996), and the leaves collected in this study were all from shaded environments. When photosynthesis measurements were collected, length and density of stomata from trees next to girdled pines were significantly smaller than those next to control pines, which correspond with high water use of sweetgum next to control pines. Trees next to girdled pines had slightly higher $V_{cmax}$, $J_{max}$, and TPU, which is unexpected due to their low stomatal size and density. Lack of significance in photosynthetic parameters is consistent with leaf nutrient content data due to the positive correlation between leaf N content and photosynthetic capacity (Reich et al., 1995; Bassow and Bazzaz, 1997) and
Fresh leaf N content did not significantly differ between sweetgums next to control and girdled pines. Sweetgum stomata are notably sensitive to soil moisture deficits (Pataki et al., 1998; Bovard et al., 2005), so a decrease in stomatal size and density of sweetgum next to girdled pine may have been due to water stress. Herrick et al., (2004) found sweetgum stomatal density of sweetgum leaves to be 349 stoma per mm² leaf area, which is notably higher than estimates of the current study ranging from 241 – 280 stoma per mm² leaf area. This suggests that sweetgum at this site may be typically water stressed despite changes in site resource availability due to the girdling treatment.

Increases in growth and higher photosynthetic capabilities in sweetgums next to girdled pines were not detected within the scope of this study. Future increase in PAR availability to sweetgum should increase photosynthetic capacity until the trees are no longer light limited, since access to PAR is positively correlated to net primary productivity of sweetgum (Allen et al., 2004). Furthermore, large redistribution of N to the forest floor from pine leaf litter should facilitate increases in biomass accumulation, as long as the C:N ratio is conducive to N release and soil moisture conditions are not limiting. C assimilation, measured by growth ring width, was not affected within the time period of the study. Kneeshaw et al., (2002) suggested that after release from suppression, trees will initially allocate C to root growth in order to compensate for increased light availability and transpiration loss. Since root growth was not a measured component of this study, it is possible that changes in sweetgum C assimilation and storage occurred below ground.
4.5 Conclusion

Sweetgum physiological response to pine mortality is important to consider because although loblolly pine has many hardwood associates (oaks, ash, hickory, etc.) (Schultz, 1997), sweetgum, in the short-term, will outcompete these other hardwood competitors (Oishi et al., 2010), and respond rapidly to increases in light availability (Kormanik, 1990; Pataki et al., 1998; Allen et al., 2005). SPB-simulated mortality of loblolly pine had some measurable effects on physiological functioning of mid-canopy sweetgum. It was hypothesized that sweetgum next to girdled pines would take advantage of new resources and increase water use rate post-mortality, however this was more prominently exhibited in sweetgum next to control pines, particularly when soil moisture and VPD were below average. Overall, the detected responses in sweetgum physiology to the pine mortality event were increases in leaf N content and increased water use by sweetgum next to control pines, in which these suggest that N became more available for uptake and that loblolly pines may be contributing to some hydraulic lift of soil moisture. The resulting shift in forest dominance from loblolly pine to sweetgum would decrease annual water use, due to reduced sapflow of girdled pines as well as lower sapflow of sweetgum next to girdled pines. A follow up to this study would be able to better estimate changes that occur in the years following a mortality event, particularly to detect whether sweetgum increase photosynthetic capacity due to increased PAR access due to loss of pine crown foliage.
CHAPTER V
OVERALL CONCLUSIONS: COMPARISON OF PINE, SWEETGUM, AND STAND-SCALE ESTIMATIONS

5.1 Overview

It is to be expected that there will be differences between loblolly pine and sweetgum in terms of resource use due to known differences in conifer and hardwood physiological traits. It is unknown, however, to what magnitude resource use differs between these species under the circumstances of a SPB-simulated mortality event. Furthermore, this investigation occurred during extended drought conditions, which will become more frequent if future climate follows model predictions (Dale et al., 2001; Stocker, 2014). It is the goal of this chapter to document the comparisons between sweetgum and loblolly pine in terms of water use, leaf N content, photosynthetic capacity and bole growth. Additional investigation includes impacts on seasonal stand-level hydrological balance by estimating stand water use by species and focusing on when precipitation and foliar interception of precipitation typically occur in this region.

Sweetgum and loblolly pine water use, leaf nutrient content, and photosynthetic parameters were all determined from data collected following methodologies explained in Chapters 3 and 4. Additionally, site-level comparisons of water use were made using stand basal area data. These data are presented in Figures 5.2 and 5.3. Eleven plots were sampled with a 10-factor prism to determine an average basal area per hectare of each
species. Sapwood areas of these plots were estimated using equations 3.3, 4.1, and 4.2. Sapflow at the stand scale was calculated by multiplying average daily sapflow rates of girdled pines, control pines, and sweetgum by the appropriate sapwood area estimates for each species.

5.2 Water use

In general, measured sapflow was much higher in pines than sweetgum in the plot due to forest structure and mid-successional stage. Pines were older with larger sapwood area, while sweetgums were limited physiologically due to low access to PAR (Harley et al., 1996; Allen et al., 2005). High growing season (JJA) sapflow of sweetgum is consistent with other similar investigations of tree water use (Stoy et al., 2006; Giraldo et al., 2015). Additionally, loblolly pine will typically transpire less than hardwoods during extended drought due to higher sensitivity to climate (Stoy et al., 2006) and higher water use efficiency (Hu et al., 2010). This was detected in the second fall season in the study when sweetgum had high sapflow in comparison to loblolly pine. Pines contribute to higher evaporative loss of precipitation throughout winter dormant seasons (Swank and Douglass, 1974; Bryant et al., 2005; Stoy et al., 2006; Rao et al., 2011) which may result in additional dormant season water inputs with pine mortality, potentially prolonging the inundation period.

Measured sapflow rates that were scaled to the hectare level using stand basal area data were separated into two categories to represent a “control” forest and a “mortality” forest (Fig. 5.1 and 5.2). Data that were used to represent the control forest were from control pine and sweetgum next to control pine. Data that were used to represent the mortality forest were from girdled pine and sweetgum next to girdled pine.
Water use in the mortality forest was notably lower than the control forest for both loblolly pine and sweetgum, besides during the second fall season when water use was 268 mm and 256 mm for trees next to control pine and trees next to girdled pine, respectively (Fig. 5.1).

![Figure 5.1](image)

**Figure 5.1** Stand-scale estimation of forest water use (mm) by species in a typical pine-sweetgum forest (top) and in a pine-sweetgum forest undergoing a southern pine beetle-simulated mortality (bottom).

Sweetgum exhibit no sapflow during winter (DJF) due to seasonal dormancy.

In this forest, water use is approximately 1164.31 mm year\(^{-1}\) for control loblolly pine and 869.57 mm year\(^{-1}\) for mid-canopy sweetgum (average of next to control and next to girdled). These estimates are slightly larger than those made by Sun et al. (2010),
who found loblolly pine plantations to have 838 – 1087 mm year\(^{-1}\) of evapotranspiration. Control pines used approximately 154.33 mm of water during winter months (Table 5.1). Rainfall during these three months was 518.60 mm, which is slightly higher than the 30-year average at this location, 413.70 mm (NOAA, 2010). Yearly patterns in water availability were compared between the two forests (control forest and mortality forest) by subtracting monthly scaled water use from monthly precipitation from the 30 year climate normal for this location (Fig. 5.2). The estimated difference between precipitation and sapflow in the forest undergoing a pine mortality event remained positive throughout the year, suggesting water availability in the soil, and possibly, groundwater recharge. Alternatively, the difference between precipitation and sapflow in the control pine-sweetgum forest became negative in June and decreased throughout the summer growing season. This estimation suggests that the control pine and sweetgum are contributing to soil water depletion in the summer and fall months during this location.

Table 5.1 Seasonal water use (mm) of control pine, girdled pine, sweetgum next to control pine, and sweetgum next to girdled pine

<table>
<thead>
<tr>
<th>Months</th>
<th>Control pine</th>
<th>Girdled pine</th>
<th>Sweetgum next to control pine</th>
<th>Sweetgum next to girdled pine</th>
</tr>
</thead>
<tbody>
<tr>
<td>JA</td>
<td>383</td>
<td>342</td>
<td>126</td>
<td>101</td>
</tr>
<tr>
<td>SON</td>
<td>214</td>
<td>143</td>
<td>162</td>
<td>111</td>
</tr>
<tr>
<td>DJF</td>
<td>154</td>
<td>51</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>MAM</td>
<td>337</td>
<td>176</td>
<td>179</td>
<td>139</td>
</tr>
<tr>
<td>JJA</td>
<td>534</td>
<td>280</td>
<td>420</td>
<td>369</td>
</tr>
<tr>
<td>SON</td>
<td>170</td>
<td>60</td>
<td>268</td>
<td>256</td>
</tr>
</tbody>
</table>
5.3 **Leaf nutrient content**

Overall, senesced leaf and needle N concentration increased from pre-mortality to post-mortality while C concentration did not significantly differ between years (Fig. 5.3). Leaf N concentration of senesced sweetgum leaves was 20% higher than pine needles in the pre-mortality fall ($p<0.001$). Conversely, leaf N concentration of senesced sweetgum leaves was 12% lower than pine needles post-mortality ($p = 0.020$). Leaf C concentration of senesced pine needles was 17% higher than sweetgum leaves pre-mortality ($p <0.001$) and 16% higher post-mortality ($p <0.001$). C:N ratio of senesced loblolly pine needles was significantly higher than sweetgum leaves both pre-mortality ($p <0.001$) and post-
mortality ($p = 0.003$). Average tree resorption rate of N throughout the post-mortality season was 82% higher in sweetgum than loblolly pines ($p = 0.031$).

![Graph showing senesced leaf nutrient concentration of sweetgum and loblolly pine in the pre-mortality and post-mortality fall collection seasons. Letters denote significant difference at $\alpha=0.05$.]

Figure 5.3 Senesced leaf nutrient concentration of sweetgum and loblolly pine in the pre-mortality and post-mortality fall collection seasons

Letters denote significant difference at $\alpha=0.05$

When scaled to the hectare level, large increases in litterfall N occurred between pre-mortality and post-mortality (Fig. 5.4). Increased N uptake of sweetgum in addition to the decreased N resorption of loblolly pine resulted in measureable redistribution of N from the canopy to the forest floor. Lack of change observed in leaf C content in both loblolly pine and decreased C:N ratio suggests that N decomposition will not be immobilized.
Photosynthetic capacity and carbon storage

Photosynthetic capacity of sweetgum and control pines significantly differed in terms of $V_{\text{cmax}}$ and $J_{\text{max}}$. Control pine $V_{\text{cmax}}$ was 130% higher than sweetgum ($p = 0.006$). $J_{\text{max}}$ of control pine was 158% higher than $J_{\text{max}}$ of sweetgum ($p = 0.003$). Sweetgum and girdled pine did not significantly differ in terms of $V_{\text{cmax}}$ ($p = 0.274$) or $J_{\text{max}}$ ($p = 0.115$). Sweetgum had relatively low maximum saturating rates of photosynthesis in comparison to control pines, but this is typical of sweetgum when existing in the shaded understory (Teskey and Shrestha, 1985). Additionally, sweetgum leaves that were sampled for photosynthesis were shade leaves. Sun and shade leaves vary up to twofold in photosynthetic capacity and N concentration (Harley et al., 1996), suggesting that the
current study may have underestimated sweetgum photosynthesis rates. Reich et al. (1995) found that conifers have low photosynthetic N use efficiency in comparison to hardwoods, but our results of low leaf N content in pines corresponded to high maximum saturating rates of photosynthesis would suggest high photosynthetic N use efficiency in comparison to the sweetgums that were sampled (Figs. 5.3 and 5.5). Shade tolerance and canopy position may have had an effect on sweetgum photosynthetic parameters that were measured (Ellsworth and Reich, 1993; Bassow and Bazzaz, 1997).

![Average Rubisco limited carboxylation rate (V_{cmax}) and electron transport limited carboxylation rate (J_{max}) values of control pine, girdled pine, and sweetgum from one day of photosynthesis measurements](image)

**Figure 5.5**  Average Rubisco limited carboxylation rate ($V_{cmax}$) and electron transport limited carboxylation rate ($J_{max}$) values of control pine, girdled pine, and sweetgum from one day of photosynthesis measurements

Letters denote significant difference at $\alpha=0.05$

Sweetgum showed similar growth for the summer 2016 growing season as dying girdled pines (Fig. 5.6), but it is likely that new C inputs into sweetgum were allocated to belowground biomass (Kneeshaw et al., 2002). Additionally, although growth rings were
significantly wider in control pine than girdled pine in the 2016 growing season, the increase from 2015 to 2016 was not significant in control pine, which would also suggest that more C was allocated to root growth rather than stem growth. Soil respiration at this study did not differ between soils at the base of girdled pine and the base of control pine ($p = 0.154$), however this could be attributed to heterotrophic root respiration of girdled pine increasing similarly to autotrophic root respiration of control pine (Siegert et al., in press). On average, loblolly pines were decreasing growth ring width in the four pre-treatment growing seasons. Such reduction in productivity is typical in aging stands (Ryan et al., 1997). However, the increase (although nonsignificant) in growth ring width of control pines during the mortality year suggests they benefitted from the girdling treatment. Lacking pre-treatment data in terms of photosynthesis rates and leaf nutrient content make it difficult to detect in which way the pines increased physiological functioning to increase growth.
5.5 Overall conclusions

In summary, a SPB-related mortality event in addition to seasonal inundation and drought stress altered tree physiological functioning in this loblolly pine-sweetgum forest type. Such alterations have implications on site water, nutrient, and light availability that are likely to change future forest structure and functioning. In Mississippi, USA, the majority of precipitation occurs throughout winter months, when loblolly pines are contributing to water loss of the site by ET in two forms: foliar interception of precipitation and approximately 154 mm lost from transpiration. In many southern forests, clay soils and dormant season rainfall create inundated conditions with soil moisture approaching field capacity. Prolonged inundation periods can restrict growth and will promote more flood-tolerant hardwood species. Observed increases in sweetgum
water use during seasonal drought contributes to soil water depletion during summer and fall months. During drought conditions, sweetgum outcompete hardwood competitors (Oishi et al., 2010), so the combination of pine mortality and seasonal drought in this system is likely to promote sweetgum dominance. The initial increases in N redistribution to the forest floor and future accumulation of woody debris will favor species with low N-use efficiency in the initial years following mortality, to later favor species with high N-use efficiency if N inputs become immobilized. The data from this study indicate significant tree-level responses to disturbance and seasonal resource demands, which may alter structure and cycling dynamics in this pervasive forest type.
REFERENCES


