Impact of Tree Inoculation by *Leptographium terebrantis* on Soil Microbial Communities in Commercial Loblolly Pine Stand

by

Shrijana Duwadi

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 5, 2019

Keywords: *Leptographium terebrantis, Pinus taeda*, soil microbial biomass, new roots, ectomycorrhizae

Approved by

Lori G. Eckhardt, Chair, Professor of Forestry and Wildlife Sciences
Ryan Nadel, Assistant Research Professor
Emily A. Carter, Research Soil Scientist
Mary A. Sword Sayer, Research Plant Physiologist
Yucheng Feng, Professor of Soil Microbiology

Abstract

A variety of abiotic and biotic stressors, including root-feeding bark beetles and pathogenic ophiostomatoid fungi, are associated with root disease of *Pinus* spp. Our research goal was to analyze if a tree inoculation by ophiostomatoid fungus, *Leptographium terebrantis* affects soil microbial biomass, new root growth, and ectomycorrhizal colonization of fine roots in a commercial loblolly pine stand in Eufaula, AL. The study design included three replicates of five treatment levels. We also examined soil physiochemical composition and foliar nutrients prior to the inoculation treatment.

The treatment effect on microbial biomass and ectomycorrhizal colonization of fine roots were insignificant. Seasonal variation in microbial biomass and soil C/N ratio was evident, both before and after the inoculation treatment. Microbial biomass responds positively to soil moisture and soil organic matter. The treatment effect on new root growth was insignificant until December 2018. A difference in new root growth among treatments was observed in February 2019. In 2017 and 2018, new root growth was rapid in the spring and summer, while it declined in the fall. Maximum new roots during the two-year study period were observed at 28.3 and 35.4 cm depths. New root growth was not significant for the treatment and control pairs within the treatment plots. Ectomycorrhizal colonization varied by depth and was highest at the 20-30 cm soil depth. More acidic soil favored ectomycorrhizal colonization in our commercial loblolly pine stand.

Total soil N, total S, available Mg, and pH were significantly different among treatments before inoculation. Excluding available Cu and Al, soil chemical properties were significantly different among depths. Except for foliar Mn, pre-inoculation foliar nutrients were not significantly different among treatments.

Our study has allowed us to understand the response of soil biological properties to loblolly pine infection with *L. terebrantis* as well as the importance of soil moisture, soil organic matters and balanced soil pH for overall stand health.

Acknowledgments

I am deeply grateful to my advisor, Dr. Lori G. Eckhardt, for without her mentoring and motivation completing this work would have been impossible. I would also like to express my gratitude to my committee members, Dr. Ryan Nadel, Dr. Emily A. Carter, and Dr. Mary A. Sword Sayer for their guidance and constructive insight throughout the project and completion of this thesis. Dr. Yuchung Feng's teaching was invaluable in clearing my preconceived notions regarding soil microbes.

I would like to thank Forest Health Cooperative and the School of Forestry and Wildlife Sciences for providing funding for research. I am thankful to USDA Forest Service at Auburn, Alabama and Pineville, Louisiana for the technical support. Special thanks are due to Dr. Zhaofei Fan and Dr. B. G. Lockaby for statistical assistance and suggestions.

I am much obliged to Robin Governo, Luis Mendez, John Mensah, Jessica Ahl, Dalton Smith, Andrea Wahl, Sarah Paedan, Preston E. Steele, Jr., James W. Dowdell, Charles Essien, Ashton Newman, Travis Waller, Hamed Majidzadeh, Sawyer Manson, Marcus Willford, Jaylil Collins, Micah Walker, Terra Vick, Olivia Wilkes, Alec Wellham, and Jace McCauley for lending their skills and knowledge that was much required for the completion of this project.

Finally, to my family and Mr. H for keeping up with my tantrums, I would like to say thanks a lot!

Table of Contents

List of Tables

List of Figures

Chapter One

Introduction and literature review

1.1. Loblolly pine

Loblolly pine (*Pinus taeda* L.) is a widely distributed tree in the southeastern United States from southern New Jersey to central Florida and throughout the Gulf States to eastern Texas. It spreads over the flat Atlantic Coastal Plain, Piedmont Plateau, and the southern extent of the Cumberland Plateau to mountainous Appalachian Highlands with the elevation of 150-365 m (Baker & Langdon, 1990). Since the early 1960s, loblolly pine has been intensively planted in commercial forestry plantations across the southeastern United States due to its rapid growth and ease of establishment. Currently, this species is grown on more than 11 million ha across the southeastern United States (Hugget et al., 2013) and its acreage is projected to increase in the future (Wear & Gries, 2012).

Low temperature and inadequate rainfall limit the northern and western range of loblolly pine in the United States (Wahlenberg, 1960). Loblolly pine is the principal commercial tree species in the southeastern United States and contributes to almost 50% of the pine growing stock (Wear & Gries, 2012), with billions of dollar worth of trees harvested each year for lumber and pulpwood production (Moore et al., 2013).

Besides its commercial value, loblolly pine is important from an ecological perspective. Its seeds are an important food source for many birds and small mammals (Wahlenberg, 1960). Loblolly pine forests provide habitat for wildlife including white-tailed deer, bobwhite quail, wild turkey, mourning doves (Smeltzer et al., 1977) and some endangered species (Baker & Langdon, 1990; Moore et al., 2013) such as the red-cockaded woodpecker (Halls, 1977).

Loblolly pine plays an important role in improving water quality (Moore et al., 2013) and stabilizing soil because of its fast growth and good litter production (Baker & Langdon, 1990). In addition, this species is used for shade and as a wind and noise barrier. Mature loblolly pine has thick bark and a tall crown which make it resistant to damage by fire (Brown & Davis, 1973; Wade, 1985).

1.2. Forest decline

1.2.1. General concepts of forest decline

During the second half of the 20th century, classical concepts of forest pathology such as Koch's postulates, the disease triangle, and the one pathogen-one disease theory couldn't adequately explain frequent forest disease outbreaks (Olivia et al., 2013). Several new theories were introduced to explain forest decline, such as forest decline being the outcome of repeated modifications to stress in trees, subsequently making them susceptible to secondary pathogens (Houston, 1992). Similarly, forest decline has also been attributed to global climate change and environmental pollution (Auclair et al., 1992). Of all the forest decline theories, the spiral of decline theory proposed by Paul Manion is presently the most accepted theory. Manion (1991) indicated that a decline is defined as "an interaction of interchangeable, specifically ordered abiotic and biotic factors to produce a gradual general deterioration, often ending in the death of trees." According to this theory, pre-disposing factors, inciting factors, and contributing factors are involved in tree vigor decline. Pre-disposing factors include permanent stress caused by factors such as climate, topography, or genotype. Inciting factors can trigger rapid decline in a short period of time and examples of these factors include frost, drought, or thinning (Eckhardt & Menard, 2009; Eckhardt et al., 2016). Contributing factors include biotic components that cause further tree decline and premature mortality (Manion, 1991). Contributing factors may be

bark beetles and ophiostomatoid fungi in the southern pine forests (Ekchardt et al., 2007; Eckhardt & Menard, 2009).

1.2.2. World declines

The decline of forest trees throughout the world has been studied and documented for decades. Hardwood tree species declines include oak (*Quercus spp*.) decline that is mostly incited by drought events in the eastern United States (Kessler, 1992) and Ozark Highlands (Jenkins & Pallardy, 1995). Other hardwoods in decline include paper birch (*Betula papyrifera* Marsh.) in northern Michigan (Jones et al., 1993) and wandoo (*Eucalyptus wandoo* Blak.) throughout western Australia (Hooper & Sivasithamparam, 2005). Bruce & Allen (1991) reported that sugar maple (*Acer saccharum* Marsh.) decline had a significant correlation with climatic extremes such as summer drought and low soil water recharge during summer.

Also, declines have been studied in silver fir (*Abies alba* Mill.) (Skelly & Innes, 1994; Camarero et al., 2002) and red spruce (*Picea rubens* Sarg.) forests (Siccama et al., 1982; Johnson et al., 1985; Battles & Fahey, 1996). Decline symptoms in many other conifer species have been reported including rapid whitebark pine (*Pinus albicaulis* Engelm.) decline caused by white pine blister rust (*Cronartium ribicola* Fisch.) (Keane & Stephen, 1993), chlorotic decline of ponderosa pine (*Pinus ponderosa* Laws.) (Parmeter et al., 1962), lodgepole pine (*Pinus contorta* Doug.) decline (Mather et al., 2010), red pine (*Pinus resinosa* Sol.) decline (Klepzig et al., 1995), longleaf pine (*Pinus palustris* Mill.) decline associated with root-infecting fungi (Otrosina et al., 1999), littleleaf disease of shortleaf pine (*Pinus enchinata* Mill.) (Campbell & Copeland, 1954), and loblolly pine decline (*Pinus taeda* L.) (Eckhardt et al., 2007).

1.2.3. Southern pine decline

The major species of pines that are grown extensively in the southern states include shortleaf pine, loblolly pine, longleaf pine, and slash pine (*Pinus elliottii* Engelm.) (Wear & Gries, 2012). Among these, loblolly pine is most susceptible to southern pine decline, shortleaf pine is frequently affected, while longleaf and slash pines are rarely susceptible to this problem (Alabama Forestry Commission, 2008). Southern pine decline occurs when stressed trees allow root-feeding bark beetles to vector ophiostomatoid fungi (Ophistomatales: Ophistomataceae) such as *Leptographium terebrantis* Barras & Perry, *Grosmannia huntii* (R.C. Rob. Jeffr.) Zipfel, Z.W. de Beer and M.J. Wingf., *L. huntii* M. J. Wingf., *L. procerum* (W.B. Kendr.) M. J. Wingf., and *L. serpens* (Goid.) Siemaszko into the root system (Matusick et al., 2013).

The first case of decline in loblolly pine was reported in the southeastern United States in the Oakmulgee and Tuscaloosa Ranger Districts of the Talladega National Forest in 1959 (Brown & McDowell, 1968). Due to similar symptomatologies, loblolly pine decline was first diagnosed as little leaf disease (Campbell & Copeland, 1954) and the symptoms included short chlorotic needles, sparse crowns, reduced radial growth, and finally mortality (Lorio, 1966; Hess et al., 1999).

The decline of shortleaf pine in the southeastern United States is promoted by little leaf disease (Campbell & Copeland, 1954). The interaction of susceptible host trees with specific site characteristics and the presence of the oomycete *Phytophthora cinnamomi* Rands. leads to the development of little leaf disease symptoms in shortleaf pine (Campbell & Copeland, 1954; Oak & Tainter, 1988). The symptoms include stunted, yellow or chlorotic needles, small cones, and sprouting at the base of the stem (Campbell & Copeland, 1954). Finally, tree death occurs within 6 years of early symptom appearance (Lawson, 1990).

The decline of longleaf pine in the southeastern Unites States and its association with root-infecting fungi was reported by Otrosina et al. (1999). From a study done to determine factors involved in the decline of longleaf pine after prescribed fire near New Ellenton, South Carolina, it was found that root pathogen damage, soil factors, and physiological dysfunction were associated with decline symptoms where stands were prescribed burned (Otrosina et al., 2002).

1.2.3.1. Loblolly pine decline

Two observations indicate that the loblolly pine forests have a greater vulnerability to decline compared to the other major southern pine species in the southeastern United States. First, due to its high timber quality, loblolly pine is the most widely planted species among the four major southern pines (Alabama Forestry Commission, 2008). As a result, the pine decline disease complex is more commonly observed in loblolly pine plantations compared to forests dominated by shortleaf, longleaf, or slash pines (Alabama Forestry Commission, 2008). Second, on a global scale, forest declines and mortality over the past 20 years have been increasingly reported (Allen et al., 2010; Anderegg et al., 2012). Regionally, over the past 20 years, observations of the pine decline disease complex in loblolly pine have also increased (Eckhardt et al., 2007). Among landowners that experience this problem, there is concern about how loblolly pine growth and site productivity are affected (Eckhardt, 2003).

1.2.3.2 Root: crown equilibrium and mechanisms to cope with decline

Balance between the root system and crown can be described as the relationship where by water and mineral nutrients absorbed by the root system are utilized by above-ground portions of plants and carbohydrate fixed by foliage sustains the vigor and growth of roots (Harley & Smith, 1983; Watson, 1991). Hence, change in this equilibrium, especially due to loss of root system

function can cause a detrimental effect on tree growth, health, and vigor. Above-ground plant parts contribute to this equilibrium, in part by supplying a significant amount of carbon (C) produced by photosynthesis for the production and maintenance of roots and soil microbial communities (McMurtrie & Wolf, 1983). This source of carbohydrate is approximately 50-70% of the net C fixed during photosynthesis (Nisbet & Mullins, 1986; Santantonio & Grace, 1987).

Growth and maintenance of the root system and crown are largely dependent on their supply of, and demand for carbohydrates and nitrogen (N) (Brouwer, 1962). Some theoretical models (Thornley, 1972a, b; Wareing & Patrick, 1975) predict that the crown-root ratio on nutrient deficient sites (Tetreault et al., 1978; Keyes & Grier, 1981; Waring, 1983) favors a greater portion of net photosynthetic C allocation to the root system (Nadelhoffer et al., 1985). Also, it is anticipated that on sites with low fertility, fine root production will be greater than on nutrient sufficient sites (Comeau & Kimmins, 1989; Keys & Grier, 1981; Nisbet & Mullins, 1986; Vogt et al., 1987). Thus, a higher root-shoot ratio may maintain tree vigor on poor sites.

With the progression of decline in southern pines, fine root deterioration occurs (Brown & McDowell, 1968) and primary root infection with *Leptographium* and tree mortality increase (Eckhardt, 2003). Additionally, *L. terebrantis* can cause resin-soaked lesions and blue staining of primary roots as well as cause significant damage to fine roots or reduce their number beyond the lesions (Alabama Forestry Commission, 2008). In response to infection, root xylem cells form tyloses to compartmentalize against the spread of the pathogen (Yadeta & Thoma, 2013). This can further interrupt water and nutrient translocation (Joseph et al., 1998) to and from the root system and significantly affect root-shoot equilibrium.

Alternatively, limited soil nutrient availability might directly contribute to the decline process (Coyle et al., 2015). Though, loblolly pine is nutrient demanding (Jokela et al., 2010), it

is often grown in nutrient deficient and severely eroded soils (Coyle et al., 2015) and outside its natural range (Mead, 2013; Moya et al., 2013; Urban et al., 2013). This can aggravate the occurrence of root mortality associated with the pine decline disease complex.

Tree roots contribute to the formation of soil organic matter which plays a major role in maintaining soil physical properties (Ilorkar & Totey, 2001; Kumar et al., 2004), and in shaping the plant and microbial communities that affect ecosystem function (Wardle, 1992; van der Heijden et al., 2008; Berg & Smalla, 2009). Therefore, knowledge regarding the role of fungal root infection in the pine decline disease complex and its effect on interaction between roots and soil properties can aid the formulation of preventative and remedial responses to this problem.

1.2.3.3. Loblolly pine root system infection vectored by insects

In the southeastern United States, the ophiostomatoid fungi vectored by root feeding bark beetles and other insects are capable of deteriorating *Pinus* woody roots near the soil surface, affecting whole plant physiology, and ultimately contributing to decline. *Leptographium terebrantis* is one of the many ophiostomatoid fungi which cause lesions in the phloem and resin-soaking in the xylem of naturally occurring and artificially inoculated woody roots of mature trees (Harrington & Cobb, 1983; Klepzig et al., 1996). The pathogen thrives inside xylem ducts and disturbs whole-plant water transport (Oliva et al., 2014). When the storage and transport systems of water and carbohydrates are compromised in roots, foliage health and retention in the crown suffers followed by a reduction in radial growth, and possible mortality (McDowell et al., 2008). Water deficits worsen the loss of xylem hydraulic conductivity (McDowell et al., 2008; Choat et al., 2012). In addition, when low xylem water potentials occur due to pathogen effects on hydraulic conductivity, phloem transport is also reduced, and this limits the allocation of carbohydrates for metabolic function (Sevanto et al., 2014). Damaged

root systems have a negative impact on water and nutrient uptake that eventually leads to growth losses and tree mortality (Lucash et al., 2005).

1.2.4. Factors associated with southern pine decline

1.2.4.1. Predisposing factors

Loblolly pine tolerates poorly drained soils with a clay subsoil and performs well in moderately acidic, moderately well to poorly drained soil (Baker & Langdon, 1990; Londo & Ezell, 2011). Very wet, water-logged, eroded, and shallow soil doesn't favor loblolly pine growth (Baker & Langdon, 1990). The least productive soils for loblolly pine are eroded soils with very plastic subsoils (Fowells, 1965; Ralston, 1978). Sandy loam, loam, or sandy clay loam soils that are moderately well or well-drained have been found at pine decline sites (Eckhardt & Menard, 2009). However, pine decline is not always associated with a definite soil type. Rather than soil type, tree age, topography, and soil organic matter content are associated with pine decline (Alabama Forestry Commission, 2008).

Outside of commercial timberland, most forest soils in the southeastern United States are only moderately fertile and soil fertility is likely a contributing factor in pine decline (Coyle et al., 2015). Some studies have suggested that limited soil N availabilities can contribute to stand dieback (Eckhardt & Menard, 2009), as N is usually considered limiting to the forests in the southeastern United States (Pritchett & Smith, 1975). Birk & Matson (1986) reported that N deficiency in loblolly pine adversely affected starch metabolism, which in turn affected new needle growth in the spring. Therefore, it is possible that nutrient deficient conditions, especially N deficiency, pre-disposes trees to decline.

In established loblolly pine stands, soil bulk density (BD) ranges from 1.2-1.5 g/cm^3 near the surface of the soil to more than 2.0 g/cm^3 in the subsoil or on compacted or degraded sites

(Schultz, 1997). Soil BD affects soil porosity and water holding capacity and it therefore, affects soil-tree-water relationships and potential tree growth. Assessment of loblolly pine decline on the Oakmulgee Ranger District of the Talladega National Forest in Alabama by Eckhardt et al. (2003) found that the declining plots had comparatively higher BD values and lower total porosity values compared to the healthy control plots. Also, as soil depth increased, the ratio of exchangeable Ca and Al was relatively constant in the control plots but decreased with increasing depth due to higher subsoil Al levels in the declining plots.

In a study done to assess the topographical features associated with loblolly pine decline in Central Alabama, Eckhardt & Menard (2008) found a significant role of aspect in decline. Trees in plots with southeast/south/southwest aspects and steep slopes were more likely to suffer decline than trees in plots with northeast/north aspects and mild to no slopes. An increase in decline incidence when south/southwest aspects occurred with a steep slope may have been due to the negative effects of extreme aspect and slope on available water (Eckhardt & Menard, 2008).

1.2.4.2. Inciting factors

Short term stress factors such as insect defoliation, frost, drought, and root injury, which are otherwise not capable of killing healthy trees under normal circumstances, act as inciting factors in southern pine decline (Eckhardt et al., 2016). Similarly, silvicultural practices like heavy thinning and excessive fire regimes, or periodic climatic events like high wind and moisture stress can incite decline where predisposing and contributing factors have created physiological stress and a loss of tree vigor (Eckhardt & Menard, 2009). Recently, wild pigs (*Sus scrofa* L.) have been proposed as a possible inciting factor because they disturb the ecosystem (Wickland, 2014), particularly by feeding activities (Bratton, 1975; Lacki & Lancia 1986).

Removal of tree bark during their feeding process can stress trees and make them susceptible to attack by bark beetles (Eckhardt et al., 2016).

1.2.4.3. Contributing factors

Bark beetles and ophiostomatoid fungi are usually associated with each other (Paine et al., 1997). Southern pine decline in loblolly pine is associated with the root-feeding bark beetle species, *Hylastes salebrosus* Eichhoff and *Hylastes tenuis* Eichhoff, and the regeneration weevil species *Hylobius pales* Herbst and *Pachylobius picivorus* Germar (Eckhardt et al., 2007). *Hylastes* spp. make galleries and oviposit in woody roots (Matusick et al., 2013) of loblolly pine trees (Wood, 1982; Klepzig et al., 1995; Jacobs & Wingfield, 2001). They often introduce *L. terebrantis, L. serpens* and *G. huntii* into the tree root system (Eckhardt & Menard, 2009). *Pachylobius picivorus* and *H. pales* cause the mortality of pine seedlings (Edmonds et al., 2000) and they vector *L. terebrantis* and *L. procerum*. Both root feeding bark beetles and regeneration weevils introduce the vectored fungi into woody roots while feeding and constructing galleries (Eckhardt & Menard, 2009).

1.3. *Leptographium terebrantis*

1.3.1. Background

Leptographium terebrantis is the fungus commonly found in the woody roots of declining conifers throughout the United States and Canada (Harrington, 1988). In the southeastern United States, *L. terebrantis* is reported to infect longleaf pine (Otrosina et al., 2002), slash pine, and loblolly pine (Eckhardt et al., 2007). Apart from that, it has been isolated from eastern white pine (*Pinus strobus* L.) (Harrington, 1988), Scots pine (*Pinus sylvestris* L.) in Massachusetts (Highley & Tattar, 1985), and red pine in Wisconsin (Klepzig et al., 1991). It was also found in the woody roots of lodgepole pine in British Columbia (Morrison & Hunt, 1988),

and Douglas-fir (*Pseudotsuga menziesii* Mirb.) and pinyon pine (*Pinus edulis* Engelm.) in the western United States and Canada (Harrington, 1988).

1.3.2. Morphology

Jacobs and Wingfield (2001) described the morphology of *L. terebrantis* as the "typical *Leptographium.*" *Leptographium terebrantis* is usually identified based on its mycelial or hyphal branching. Differentiation in the hyphal branching patterns among *L. terebrantis* and closely related species is minimal. *Leptographium terebrantis* can be differentiated from *L. procerum* by the absence of rhizoid like structures joining conidiophores. Conidiophores of *L. terebrantis* occur singly or in groups, and usually are at the end of aerial mycelia (Jacobs & Wingfield, 2001).

1.3.3. Impact

Barras & Perry (1971) described the first association of *Leptographium terebrnatis* with loblolly pine. *Leptographium terebrantis* along with three other *Leptographium* anamorphs; *L. procerum* (Kendrick) M.J. Wingfield, G. alacris T. A. Duong, Z. W. de Beer & M. J. Wingf*ield* [formerly *L. serpens* (Goidanich) Siemaszko], and *L. lundbergii* Lagerberg & Melin, were isolated from loblolly pine decline site in central Alabama (Eckhardt, 2003). Otrosina et al. (1997) reported the isolation of *L. terebrantis* from woody pine roots in southern pine stands attacked by southern pine beetle. *Leptographium terebrantis* was found to be associated with red pine (*Pinus resinosa* Sol. ex Aiton.) decline complex in Wisconsin (Klepzig et al., 1991). Raffa & Smalley (1988) found that inoculating red and jack pines (*P. banksiana* Lamb.) with *L. terebrantis* decreased host resistance to root weevils and black turpentine beetle (*Dendroctonus terebrans* Olivier.).

Apart from various pine species, *L. terebrantis* has been isolated from grand fir [*Abies grandis* (Dougl. ex D. Don) Lindl.], mountain hemlock [*Tsuga mertensiana* (Bong.) Carriere], subalpine fir [*Abies lasiocarpa* (Hooker) Nuttall], western hemlock [*Tsuga heterophylla* (Raf.) Sarg.], western larch (*Larix occidentalis* Nuttall), western red cedar (*Thuja plicata* Donn ex D. Don), and white spruce [*Pica glace* (Moench) Voss], (Morrison & Hunt, 1988). Among western North American conifers, *L. terebrantis* was identified as a secondary invader that was introduced into the root system by bark beetles (Harrington & Cobb, 1983).

1.3.4. Decline management

Some management techniques may reduce decline progression in commercial forest lands including protection of soil and roots from damage by compaction, soil fertilization to replenish nutrient deficiencies, subsoiling during site preparation, reducing stand rotation age, or clear-cut harvesting if a stand age is greater than 40 years old (Eckhardt & Menard, 2009). Frequent thinning is one of the many silvicultural practices that increases tree vigor and it has been used to manage forest stands for decades (Keen, 1958; Graham & Knight, 1965).

1.4. Impact of forest disturbances on soil components

1.4.1. Impact on soil microbial biomass

1.4.1.1. Microbial biomass concept

Soil microbial biomass (MB) constitutes living and dead microorganisms, especially bacteria and fungi, and makes about 5% of the total organic C and N in the soil. After the death of microbes, the nutrients held by these organisms are released and available for plant uptake (Hoyle et al., 2018). Microbial biomass is also considered an indicator of soil quality and changes in total soil organic C (Hoyle et al., 2018). Microbial biomass is affected by soil clay content, soil pH, and soil organic C (Hoyle et al., 2018). Water and C content in soil change as

the result of climate, soil type, and management practices and this in turn affects MB (Hoyle et al., 2018). Also, rainfall is considered a limiting factor to MB abundance (Hoyle et al., 2018).

1.4.1.2. Response to disturbances

Forest ecosystems play a major role in the global C cycle. The CO_2 produced by soil microbial respiration contributes significantly to the flux of C from forest soil to the atmosphere (Holden & Treseder, 2013). There are significant relationships between changes in MB following disturbances and soil microbial respiration (Holden & Treseder, 2013). Changes in various soil properties following disturbances (both biotic and abiotic) can change soil MB and respiration, thus affecting C balance in forest ecosystems (Holden & Treseder, 2013).

In recent years, biotic disturbances like insect infestations and disease outbreaks have been pronounced throughout North America, and these events have affected the soil C cycle (Hicke et al., 2012). For example, biotic disturbances that cause tree defoliation increase the influx of dead plant litter into soils (Hicke et al., 2012), and biotic disturbances that increase insect biomass and frass deposition enhance the availability of mineral nutrients in the soil (Lovett et al., 2002). Soil MB and respiration may increase when biotic disturbances such as defoliation result in increases in soil fertility or labile C content (Holden & Treseder, 2013).

1.4.2. Impact on root system

Forest trees usually have a large root system. To maintain the root system, forest trees require a high amount of carbohydrates which is approximately 20-47% of photosynthetically fixed C (Smucker, 1993) and this value may increase under stressful environmental conditions (Smucker, 1984; Nobel, 1991). Pathologists view roots as a potential host for pathogenic and parasitic fungi (Graham, 2002). If abiotic stresses are present during the growing season, negative effects of root infection on plants are pronounced (Allmaras et al., 1988).

The spread of vascular wilt pathogens such as *Leptographium* spp., *Ophiostoma* spp., and *Ceratocystis* spp. is slowed by compartmentalization due to the *in situ* production of C compounds that block vascular conduits (Shigo & Tippett, 1981; Bonsen et al., 1985; Yadeta & Thomma, 2013). As part of this response, vascular wilt pathogens might induce premature tree mortality through hydraulic failure of xylem or, on the other hand, C starvation by a decrease in the downstream supply of C in phloem (Oliva et al., 2014). This can ultimately lead to the deficiency of water and C in belowground plant parts and cause failure of the root system.

1.4.3. Impact on mycorrhiza

A mycorrhiza is a symbiotic association between a fungus and the roots of a vascular host plant (Kirk et al., 2001). In forest trees, the symbiotic fungus forms a mantle and Hartig Net in host tree roots without penetrating root cells or the endodermis to form the ectomycorrhizal (EM) association (Marks & Foster, 1973).Various factors like host age, soil N, pH, and heavy metal contamination, auxin production by the EM fungus, and cation supply influence the formation and composition of EM fungi in root systems of higher plants (Mason et al., 1986; Chai et al., 2013). Newly produced photosynthate plays a major beneficial role in EM fungal growth and maintenance (Söderström & Read, 1987; Högberg et al., 2001; Johnson et al., 2002; Steinman et al., 2004; Högberg et al., 2010).

In this relationship, plant hosts provide C to the fungal partner of this symbiosis (Högberg & Högberg, 2002). In return, EM fungi protect fine roots of trees from pathogenic infection. This is accomplished by (i) utilizing sugars and therefore decreasing the food supply to pathogens, (ii) providing a barrier against disease, (iii) producing antibiotics, and (iv)supporting the root microbial community (Zak, 1964). Pathogenic infection in the xylem may trigger

hydraulic failure, and as a result, decrease the belowground C supply (Oliva et al., 2014) and subsequently, EM abundance and the vigor and growth of forest trees.

1.4.4. Impact on Carbon (C) cycle

Of the Earth's total land surface, 65% is covered by forests which contain 80% of the soil C among terrestrial ecosystems (Landesberg & Gower, 1997). The C flux of forest ecosystems is dominated by the production and turnover of fine roots and as such, roots are recognized as a major component of terrestrial C (King et al., 2002). Carbon allocated as current photosynthate to leaves, storage, metabolism, and root exudates also has an important effect on soil C storage, but these effects vary depending on plant type and age, its microbial root symbionts, and environmental variables such as nutrient availability (Litton et al., 2007).

The nutrient cycle is affected by biotic and abiotic disturbances like plant disease, fire, harvesting, and hurricanes (Foster & Bhatti, 2012). In the forests of North America, insect pests and pathogen disturbances are widespread and thus, affect the global forest C cycle (Hicke et al. 2012). Although most disease and insect pest outbreaks are localized, some have the potential to affect a wide range of the forest (Dale et al., 2001). Thus, tree growth reduction and mortality following pathogen spread is responsible for significant effects on the C cycle in North American forests. Bark beetles like mountain pine beetle and southern pine beetle that vector pathogenic fungi feed within tree phloem (Hicke et al., 2012) and result in a substantial modification of the C cycle (Elkinton & Liebhold, 1990; Candau et al., 1998) by killing trees.

Forest pathogens mostly affect the C cycle by causing tree growth reductions (Tkacz & Hansen, 1982; Hansen et al., 2000; Woods et al., 2005) or by killing less vigorous trees (Hicke et al., 2012). For example, blue stain fungi primarily kill the host tree by blocking water and nutrient conducting tissues with fungal hyphae (Six & Wingfield, 2011). These fungi are usually

vectored by beetles, as in loblolly pine decline, where *Leptographium* spp. is vectored by root-feeding bark beetles.

The sudden outbreak of insect pests and pathogens can be consequential to forest C cycling over the long-term (Hicke et al., 2012). However, if the stand recovers after being subjected to an infestation, there may be an increase in stand productivity (Romme et al., 1986; Brown et al., 2010). Also, the impact of disturbance on the C cycle is determined by the type of disturbance. Short term defoliation can decrease net primary productivity (NPP) and net ecosystem productivity (NEP) for a while, but NEP will recover when plants produce new leaves and photosynthesis rates are restored. On the other hand, widespread tree mortality due to pathogen infestation for many years or due to bark beetle outbreak can affect the forest C cycle over the long-term (Dymond et al., 2010; Pfeifer et al., 2011).

1.5. Objectives

The major goal of this research project was to study the interactions between soil biology and the artificial stem inoculation of loblolly pine with *L. terebrantis*, with a focus on soil microbial biomass, new root growth, and ectomycorrhizal colonization in a commercial *P. taeda* stand. The specific objectives are mentioned as follows:

1. To assess soil and foliar nutrients prior to *L. terebrantis* inoculation of loblolly pine trees;
2. To analyze the effect of *L. terebrantis* inoculation of loblolly pine trees on soil microbial biomass;
3. To analyze the effect of *L. terebrantis* inoculation of loblolly pine trees on new root growth; and
4. To analyze the effect of *L. terebrantis* inoculation of loblolly pine trees on the ectomycorrhizal colonization of fine roots.

Chapter 2

Soil physiochemical properties and foliar nutrient analysis prior to inoculating loblolly pine stand with *Leptographium terebrantis*

2.1. Abstract

Soil moisture, bulk density, and soil nutrients are important factors determining the proper growth and natural distribution of loblolly pine trees. Moisture stress and nutrient deficiency can affect the health of trees and make them susceptible to decline. Soil and foliar samples from a commercial loblolly pine stand in Eufaula, Alabama were collected in 2017 to determine the differences in soil properties and foliar nutrients among treatments prior to inoculating loblolly pine trees with *Leptographium terebrantis*. Significant differences in total N, total S, available Mg, and pH were found among treatments and across soil depths. Excluding available Cu and Al, soil chemical properties were significantly different among soil profiles. The foliar nutrients were not significantly different among treatments except for Mn. The project is ongoing, and we suspect that inoculation treatment will bring change in tree physiology, thus indirectly affecting soil physical and chemical properties.

2.2. Introduction

For the past 50 years, the decline of loblolly pine (*Pinus taeda* L.) health throughout the southeastern United States has been frequently reported (Campbell & Copeland, 1954; Lorio, 1966; Brown & McDowell, 1968; Oak & Tainter, 1988; Hess et al., 1999). The decline phenomenon is complex and associated with root-feeding bark beetles and ophiostomatioid fungi (Eckhardt & Menard, 2009). A soil biological property such as a decrease in the number of fine roots has been associated with the decline (Hess et al., 2002). However, it is still unknown which physical and chemical properties of a commercial loblolly pine stand influences tree health following stem inoculation with *Leptographium terebrantis* Barras & Perry.

Soil is part of the natural habitat for plants (Blume et al., 2015). Plants use soil nutrients for growth and in due course release the nutrients back to the soil through litter accumulation and decay (Omoro et al., 2011). This phenomenon has been described as a "nutrient pump" (Evans, 1992; van Noordwijk & Purnomosidhi, 1995). Plants have the potential to influence soil physiochemical characteristics by affecting soil pH, texture, water holding capacity, and nutrient availabilities (Johnston, 1986). They also contribute to soil formation and development processes which include organic matter (OM) accumulation, profile mixing, and nutrient cycling (Nkongolo & Plassmeyer, 2010). The infestation of a tree by a pathogen can cause repeated growth reductions and premature tree mortality, thus changing residue decomposition in soil (Hicke et al., 2012). Due to the indivisible relationship between plants and soil, tree decline is expected to affect soil properties. However, this hypothesis of ours still needs validation.

Researchers have made an attempt to evaluate forest declines by comparing the nutritional status of healthy and declining stands (Kaupenjohann et al., 1989). But decline cannot be explained by changes in soil nutritional status alone (Saxe, 1993) because several factors can

contribute to the appearance of similar symptoms (Linder, 1987). Analysis of foliar nutrients can be helpful for understanding both soil supply and nutrient uptake and subsequently formulate site-specific management practices to maintain the optimal nutrient status in trees (Linder, 1987; Richardson et al., 1999; Sypert, 2006), especially during the period of forest decline.

A field study was carried out in Eufaula, Alabama to investigate soil properties and foliar nutrient concentrations before an on-site stem inoculation of loblolly pine trees with *L. terebrantis*. It is expected that pathogen action will trigger an inadequate carbon (C) supply to the root and mycorrhizal network. This effect on the growth and maintenance of roots and ectomycorrhizae will be followed by the loss of root system function and a reduction of OM deposition in the soil. The hypothesis of this research is that before the inoculation treatment, soil and foliar nutrient concentrations will be similar among treatments. The aim of this study at present is to assess the pre-inoculation (i) soil physiochemical properties among treatments at different soil depths and (ii) foliar nutrient concentrations among treatments. The project is ongoing, and the post-inoculation soil and foliar samples are yet to be collected.

2.3. Materials and methods

2.3.1. Site description

The research site was located near Eufaula, Alabama, in Barbour County in a loblolly pine plantation managed by Rayonier Inc. (32°1'13.10"N, 85°12'31.76"W). The plantation is located within the east Gulf coastal plain physiographic region of Alabama. The study site was dominated by fine sandy loam soil. Loblolly pine seedlings were planted in January 2003 to establish the plantation which was intensively managed until the time of study establishment (Alan Wilson, personal communication).

Fifteen treatment plots were established in 2015 (Figure 2.1). The average area of each plot was 76.38 m^2. At the time of study establishment, the trees were 14 years old and when the inoculation treatments were established, the trees were 16 years old. In 2014, prior to the study establishment, thinning was done so each plot retained one pair of trees in parallel rows, approximately 1 m apart within the planting row and 3.048 m apart between planting rows (Figure 2.2). A metal tag was attached to each tree denoting the tree number and a diameter band was attached to the tree at breast height. A weather station (WatchDog 2000, Spectrum Technologies Inc.) was installed to record air temperature, solar radiation, relative humidity, precipitation, and wind speed of the study area.

The experimental design consisted of 15 plots arranged in a completely randomized design (CRD) with 5 treatments and 3 replicates (Figure 2.1). The treatments for the study were: control (no inoculum or wound), wound (no inoculum), low inoculation, medium inoculation, and high inoculation which were randomly assigned to five trees in one plantation row of each 15 pair-lined plots (Figure 2.1, Figure 2.2).

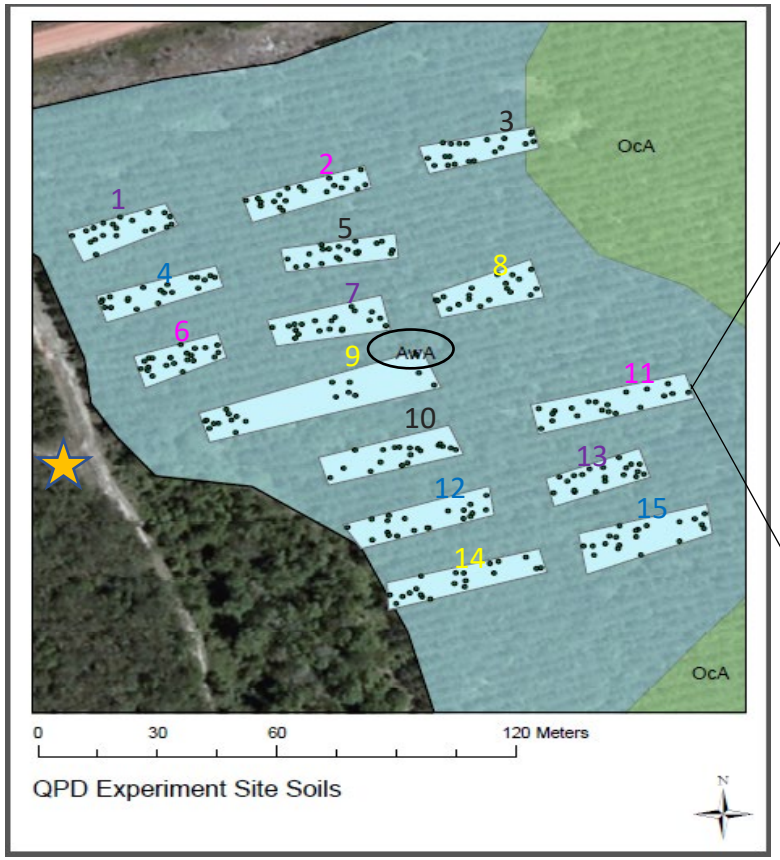


Figure 2.1. Map of the study site near Eufaula, Alabama. Treatment plots are represented by rectangles and soil series are represented by background color (AwA: Annemaine-Wahee complex). Small circles represent pre-thinning tree locations. Filled yellow star indicates the location of weather station.

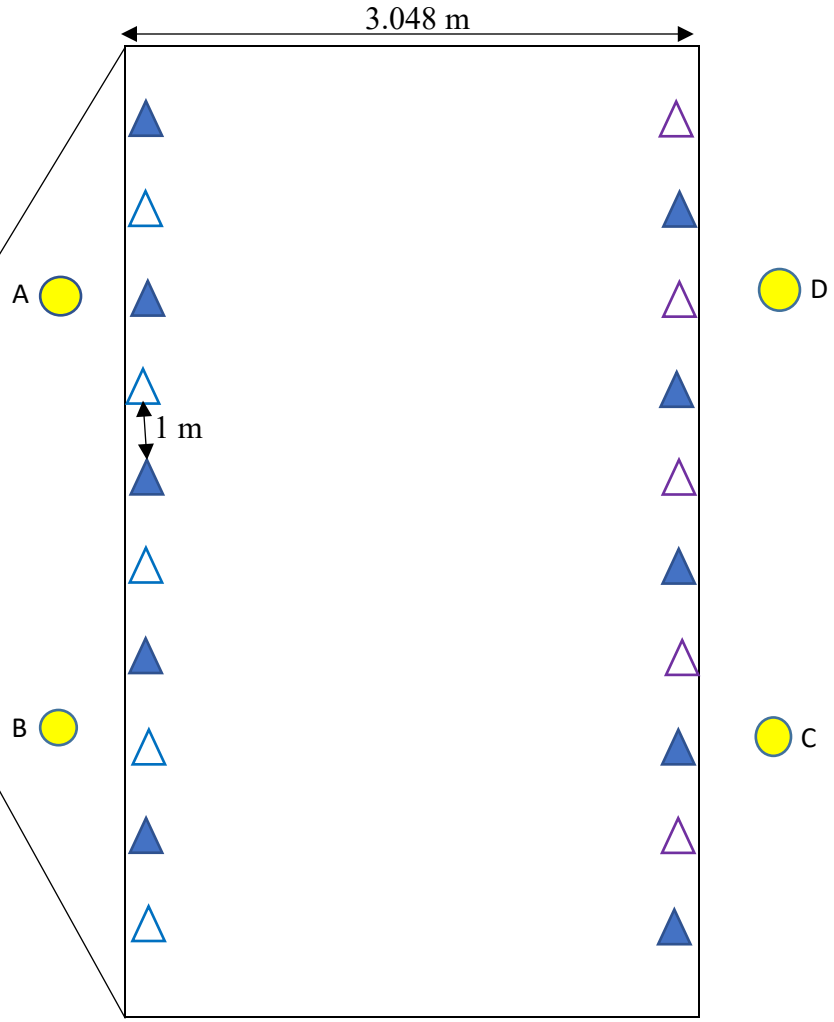


Figure 2.2. Post-thinning field layout of tree arrangement and distance between them in each treatment plot. Unfilled purple triangles indicate inoculated trees, unfilled blue traingles indicate control trees, and solid blue traingles indicate other trees. Letters (A-D) indicate the points from where soil cores were removed in each plot.

2.3.2 Experiment design and inoculation treatment

Two preliminary small-scale experiments were conducted before inoculating the trees near Eufaula, Alabama primarily, to (i) select an appropriate virulent fungal isolate of *L. terebrantis* and (ii) to determine the levels of low, medium, and high inoculation treatment. To select the most virulent *L. terebrantis* isolate from 42 isolates, an extensive seedling inoculation experiment was done according to Devkota & Eckhardt (2018). From the study, ATCC accession no. MYA-3316 was found as the most virulent *L. terebrantis* isolate to loblolly pine in comparison to 41 other isolates.

To identify the levels of inoculation treatment, a preliminary field inoculation experiment was carried out at the Solon Dixon Forestry Education Center, Andalusia, Alabama. Following the sterilization of toothpicks at 121°C and 0.103 MPa for 60 minutes, *L. terebrantis* isolate (ATCC accession no. MYA-3316) was grown in toothpicks imbibed with malt extract agar for approximately 24 days at 23°C in the dark (Devkota et al., 2018).

Figure 2.3. Toothpicks with *Leptographium terebrantis* inserted at 16 radial points of four rows in loblolly pine saplings at the Solon Dixon Center, Andalusia, Alabama. Toothpicks were clipped to facilitate covering the inoculation zone with a duct tape.

The inoculation treatment of trees near Eufaula, Alabama was carried out on March 13 and 14, 2017. The treatments were applied in accordance with Devkota et al. (2018), and based on the response of loblolly pine to different densities of virulent *L. terebrantis* from the Andalusia, Alabama study (Devkota et al., 2018).

Figure 2.4. Toothpicks with *Leptographium terebrantis* inserted in loblolly pine tree at the study site near Eufaula, Alabama.

The treatments for the study were: control (no inoculum or wound), wound (no inoculum), low inoculation, medium inoculation, and high inoculation. Five randomly chosen trees in one plantation row of each pair-lined plot received one of the five inoculation treatments. Control trees were left untouched. To apply rest of the treatments, selected trees were wrapped in plastic transparencies before drilling the holes. Plastic transparencies were marked with pre-determined inoculation points for different inoculation treatments with a permanent marker. A 5 mm deep hole was drilled at each predetermined inoculation point. The holes were drilled perpendicular to the surface of the stem using a sterilized 1.5 mm drill bit. One toothpick was inserted per hole. The toothpick insertion method simulated fungal transfer from maturation feeding activities of root-feeding bark beetles. Toothpicks were left inserted, clipped down to the bark, and covered with duct tape. Wounded trees had one fungus-free toothpick inserted per 1.2 cm ground-line diameter. Trees that received low, medium, or high inoculation treatments had one toothpick infected with *L. terebrantis* inserted per 10.0 cm, 2.4 cm, and 1.2 cm of ground-line diameter, respectively. The inoculation points were radially equidistant from each other. Each inoculation point was replicated 4 times vertically and equally spaced.

2.3.3. Soil sampling, processing and analysis

Soil samples for physiochemical property analysis were collected on March 2, 8 and 9, 2017; one week prior to inoculating loblolly pine trees with *L. terebrantis*. Four soil cores of approximately 6 × 50.8 cm were removed from each plot (Figure 2.5). Samples were collected from outside the periphery of the longer side of each plot (Figure 2.2). Soil cores were capped, placed in a cooler, and transported to the USDA Forest Service lab in Auburn, Alabama within 3 hours of collection where they were kept at 4°C until being processed.

Figure 2.5. Pneumatic soil core sampler with a plastic tube inside it used to collect soil cores.

During processing, each core was cut in 10.16 cm increments (Figure 2.6) and further divided vertically into halves. Coarse materials including stones, root pieces, pine needles and other plant parts were manually removed. One half of each 10.16 cm sample was placed in an oven at 105°C until the dry weight stabilized. The other half was stored in the cooler for nutrient analysis. Prior to the nutrient analysis, the remaining half of soil samples were weighed and allowed to air dry

Figure 2.6. Each soil core was divided into 10.16 cm increments. Brownish soil is from the top and the reddish soil is from the bottom of the core.

until the weight stabilized. The total amount of moisture in each 10.16 cm soil segment was calculated by adding the amount of moisture lost by drying (oven-drying and air-drying). The gravimetric soil moisture (%GMC) and bulk density (BD) of soil were calculated as:

%GMC = [weight of water at collection (g)/dry soil weight (g)] ×100

BD = dry soil weight (g)/ soil volume (cm^3)

The air-dried soil samples were composited by plot and depth and sieved using a soil sieve with 2 mm openings (No.10). Soil samples were then sent to the Soil Health Assessment Center, University of Missouri, Columbia, Missouri where pH and nutrients were analyzed. Soil pH was determined in both water (pH_{H2O}) and 0.01 M $CaCl_2$ solution (pH_{salt}) (Kalra & Maynard, 1991). Percentage (%) total C (Ctot), % organic C (Corg), % total nitrogen (Ntot), and % total sulfur (Stot) were analyzed via combustion analyzer (Kowalenko, 2006). An extraction with 1M KCl was used to determine soil aluminum (Al) and manganese (Mn) (Kachurina et. al, 2008). The quantity of available phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), zinc (Zn), copper (Cu), and iron (Fe) were determined according to the Mehlich-3 procedure (Mehlich, 1984) and expressed in mg kg^{-1}. The effective cation exchange capacity [ECEC in cmol(+)/kg] was computed from the summation of exchangeable Ca, Mg, K, Na, and Al, while the effective exchangeable base content [EB in cmol(+)/kg] was calculated as the sum of exchangeable Ca, Mg, K, and Na.

2.3.4. Foliage sampling, processing, and nutrient analysis

Pre-inoculation foliage samples were collected between February 13-17, 2017. In each plot, an upper crown shoot of four randomly chosen trees was obtained by shooting its woody branch using a 0.22 caliber rifle. The first flush foliage of 2016 was taken from the shot branch. About 25 fascicles from the branch tissue of each tree were placed in a paper bag. Foliage

samples were transported to the Forest Health Dynamics Laboratory (FHDL) at Auburn University, Auburn, Alabama on dry ice to ensure moisture retention.

During processing, foliage samples were force air-dried at 70°C for 72 hours and ground in a Wiley mill or ball mill grinder to pass a 0.5 mm mesh screen. The ground samples were sent to Waypoint Analytical, Memphis, Tennessee for nutrient analyses. The nutrients analyzed were N, P, K, Ca, Mg, S, and Na that were expressed in %, and B, Zn, Mn, Fe, Cu, and Al that were expressed in mg kg^{-1}.

2.3.5. Statistical Analysis

The statistical analyses of the pre-inoculation soil and foliar nutrients data were completed using SAS version 9.4 (SAS Institute Inc. 2010, Cary, NC). The BD and %GMC of twenty-10.16 cm soil segments (4 cores × five-10.16 cm increments) were averaged to calculate the BD and %GMC of each plot and were analyzed by a one-way ANOVA (PROC GLM) with treatment as the main effect. Soil samples that were composited within plot by depth were analyzed for soil pH and nutrients by depth (0 -10.16 cm, 10.16 -20.32 cm, 20.32- 30.48 cm, 30.48- 40.64 cm, and 40.64- 50.8 cm). Soil chemical properties were assessed with by a two-way ANOVA (PROC GLM) in a CRD with treatment and soil depth as main effects and treatment × depth as an interaction effect. Distribution curves were created for soil chemical properties that were significantly different among depths (Jobbagy & Jackson, 2001). Foliar nutrient concentrations were analyzed by a one-way ANOVA (PROC GLM) with treatment as the main effect. The main and interaction effects were considered significant at $P \leq 0.05$ unless otherwise noted. Tukey's Multiple Range test (PROC GLM) was used to compare the means among treatments and depths.

2.4. Results

2.4.1. Pre-inoculation soil physiochemical properties evaluation

Before the inoculation treatment, a significant differences in BD and %GMC were not found among treatments (Table 2.1). The average BD and %GMC at the 0-50.8 cm depth in the study area were 1.4 g cm^{-3} and 12%, respectively. Significant differences in Ntot and Stot, available Mg, and pH were found among treatments and across depths. Soil chemical properties were significantly different across depths except for Al and Cu. The interaction between treatment and depth was insignificant for all soil chemical properties (Table 2.2).

The Ntot and Stot in the low inoculation treatment were significantly higher than in the medium inoculation treatment (P= 0.0224 and 0.0445, respectively). Available Mg was significantly higher in the high inoculation treatment than in the control (P=0.0474) and low inoculation (P=0.0137) treatments. The $pH_{(H2O, salt)}$ in the medium inoculation treatment was significantly higher than in the control (P=0.0055, 0.0102), wound (P=0.0001, 0.0004), and low inoculation (P<0.0001, 0.0009) treatments, while the pH_{H2O} in the high inoculation treatment was significantly higher than in the low inoculation treatment (P=0.0039) (Figure 2.7).

Along with Ctot, Ntot, and Stot, the concentration of available P, K, Ca, Mg, Zn, Mn, and Fe, as well as EB and ECEC were significantly higher in top 10 cm of the soil profile compared to all other depths and tended to decrease with depth. Available Na did not follow this trend. At the 50.8 cm depth, a non-significant increase in available K, Mg, Zn, and Stot, was noticed compared to similar values at the 40.6 cm depth. This trend was significant for ECEC. Soil pH_{H2O} was significantly greater at the 20.3-30.5 cm depth than the 0-10.2 (P=0.0150) and 40.6-50.8 cm (P=0.0086) depths. Soil pH_{salt} was significantly greater at the 10.2-20.3 (P=0.0320) and 20.3-30.5 cm (P=0.0080) depths than the 40.6-50.8 cm depth (Figure 2.8, Figure 2.9).

Table 2.1. Probabilities of a greater *F*-value from a one-way ANOVA for bulk density and gravimetric soil moisture of a mature loblolly pine stand near Eufaula, Alabama before an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

	df	*F*-value	*P>F*
Bulk density	4	0.51	0.7289
% gravimetric soil moisture	4	1.53	0.2672
Error	10		

Table 2.2. Probabilities of greater *F*-value from a two-way ANOVA for soil chemical properties of a mature loblolly pine stand near Eufaula, Alabama before an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Soil properties	Treatment (T)			Depth (D)			T × D		
	df	*F*-value	*P>F*	df	*F*-value	*P>F*	df	*F*-value	*P>F*
Ctot	4	2.48	0.0555	4	32.07	<0.0001	16	0.66	0.8217
Ntot	4	2.86	0.0329	4	97.51	<0.0001	16	0.70	0.7759
P	4	2.50	0.0545	4	23.89	<0.0001	16	0.28	0.9965
K	4	0.97	0.4323	4	35.84	<0.0001	16	0.83	0.6477
Ca	4	1.08	0.3786	4	34.05	<0.0001	16	0.27	0.9970
Mg	4	3.31	0.0175	4	19.26	<0.0001	16	0.70	0.7772
S	4	2.58	0.0481	4	35.29	<0.0001	16	1.34	0.2130
Na	4	1.07	0.3792	4	3.74	0.0097	16	0.67	0.8061
Zn	4	2.30	0.0714	4	16.47	<0.0001	16	1.00	0.4730
Al	4	2.16	0.0872	4	2.07	0.0990	16	1.02	0.4554
Mn	4	1.25	0.3015	4	24.33	<0.0001	16	0.22	0.9992
Fe	4	0.60	0.6649	4	22.61	<0.0001	16	0.22	0.9992
Cu	4	0.96	0.4399	4	1.89	0.1271	16	0.56	0.8983
EB	4	1.61	0.1871	4	36.36	<0.0001	16	0.35	0.9872
ECEC	4	1.57	0.1961	4	9.21	<0.0001	16	1.17	0.3259
pH_{H2O}	4	10.58	<0.0001	4	4.30	0.0045	16	0.50	0.9380
pH_{Na}	4	6.55	0.0003	4	3.85	0.0084	16	0.69	0.7877
Error	50								

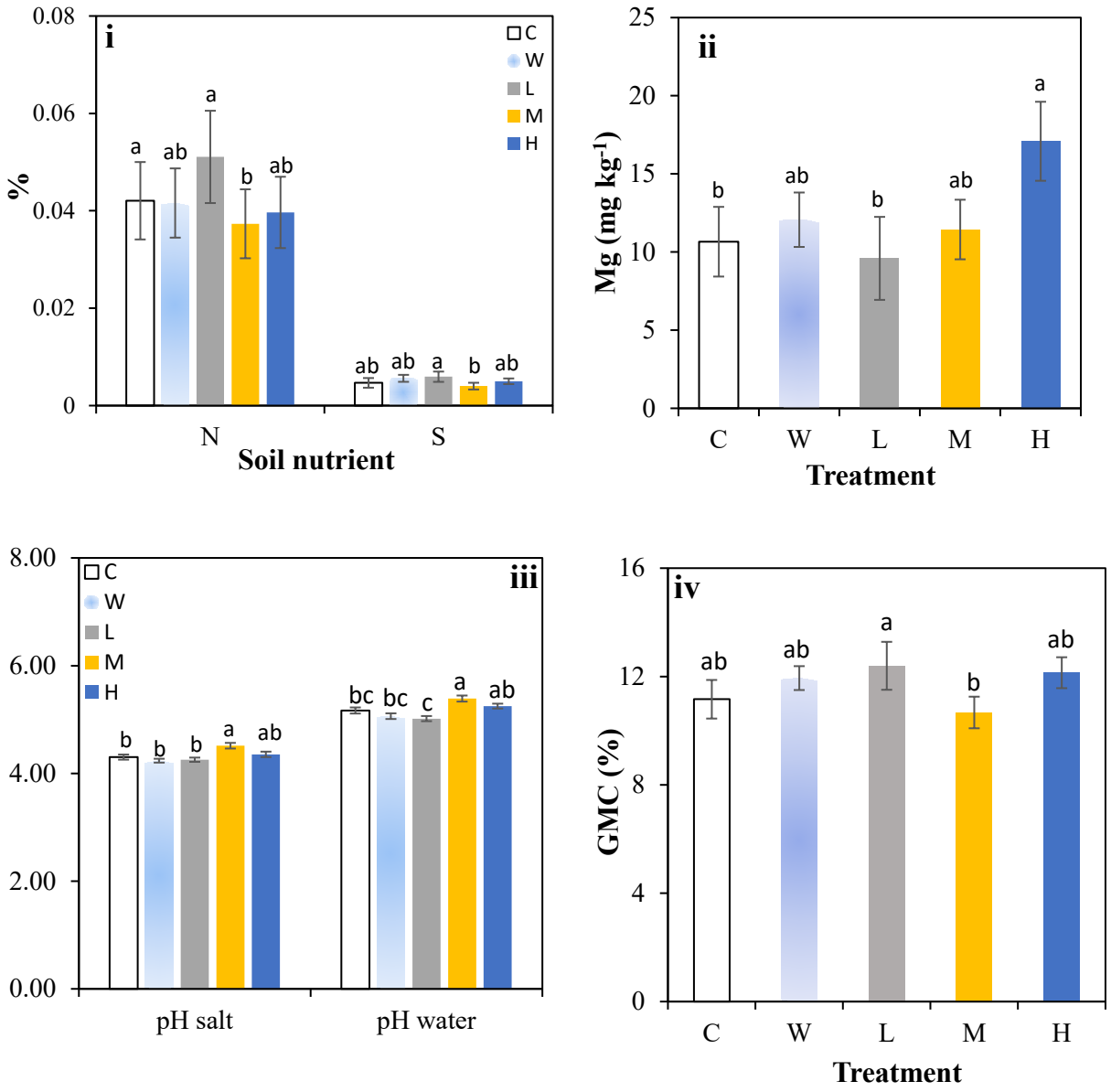


Figure 2.7. Average **(i)** total N and total S, **(ii)** exchangeable Mg, **(iii)** pH, and **(iv)** gravimetric soil moisture (%GMC) among treatments before the application of inoculation treatment on loblolly pine trees. Treatments were control (C), wound (L), low (L), medium (M), and high (H) inoculation. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

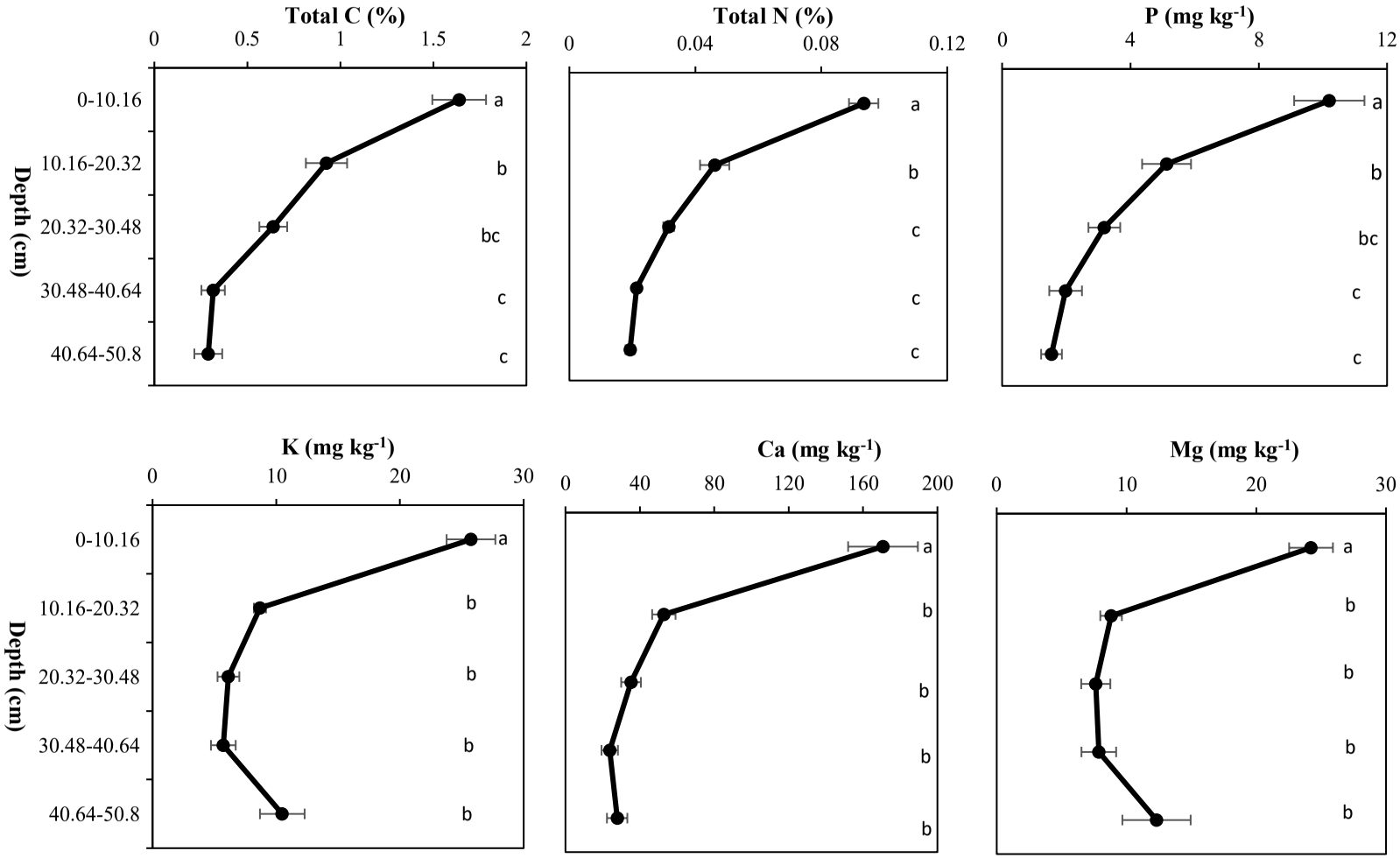


Figure 2.8. Soil nutrients comparison across top 50.8 cm depth in loblolly pine stand. The analyzed nutrients include total C, total N, available P, available K, available Ca, and available Mg. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

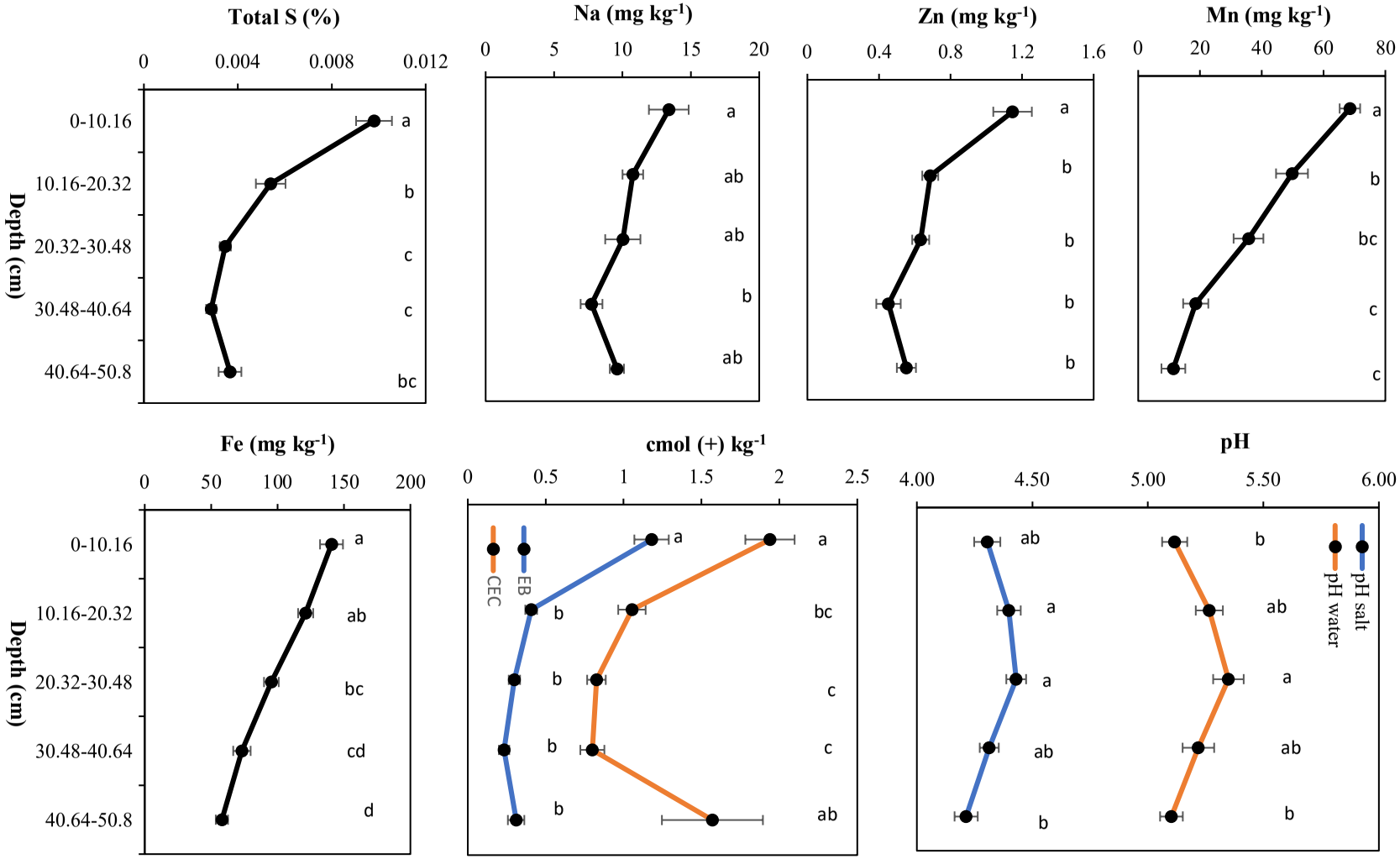


Figure 2.9. Soil properties comparison across top 50.8 depth in loblolly pine stand .The analyzed properties include total S, available Na, available Zn, extractable Mn, available Fe, exchangeable base, effective cation exchange capacity, pH_{salt}, and pH_{water}. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

2.4.2. Pre-inoculation foliar nutrients evaluation

Except for Mn, foliar nutrient concentrations among treatments were not significantly different before inoculation treatment (Table 2.3). The foliar Mn concentration of trees assigned the low inoculation treatment was significantly higher than those of trees assigned the control (P=0.0003), medium (P=0.0396), or high inoculation treatment (P=0.0011) (Figure 2.10).

Foliar N and P concentrations were near the threshold levels of sufficiency of 1.2% and 0.12%, respectively, for loblolly pine. Foliar S, K, Mg, Ca, Zn, and Cu were slightly above the threshold levels of sufficiency of 0.10%, 0.30%, 0.08%, 0.15%, 10-20 ppm, and 2-3 ppm, respectively, for loblolly pine, while foliar B, Mn, and Fe concentrations exceeded the threshold levels of sufficiency at 4-8 ppm, 20-40 ppm, and 20-40 ppm, respectively, for loblolly pine (Wells et al., 1973; Pritchett & Comerford, 1983; Allen, 1987; Jokela, 2004) (Figure 2.11).

Table 2.3. Probabilities of greater F-value from a one-way ANOVA for foliar nutrient concentrations of a mature loblolly pine stand near Eufaula, Alabama before an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Nutrients	df	F-value	$P>F$
N	4	1.46	0.2266
P	4	1.80	0.1431
S	4	1.09	0.3717
K	4	0.46	0.7671
Ca	4	0.85	0.4989
Mg	4	0.72	0.5832
Na	4	1.58	0.1932
B	4	2.53	0.0509
Fe	4	0.65	0.6302
Cu	4	1.10	0.3666
Zn	4	0.42	0.7954
Mn	4	6.30	0.0003
Al	4	1.63	0.1799
Error	55		

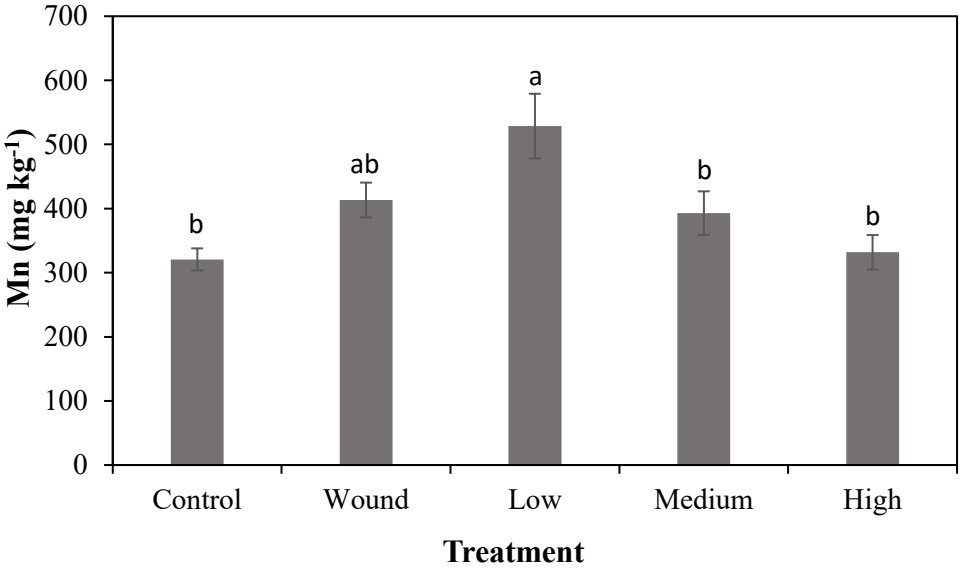


Figure 2.10. Average foliar manganese (Mn) concentration among trees assigned for control, wound, low, medium, and high inoculation treatments with *Leptographium terebrantis*. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

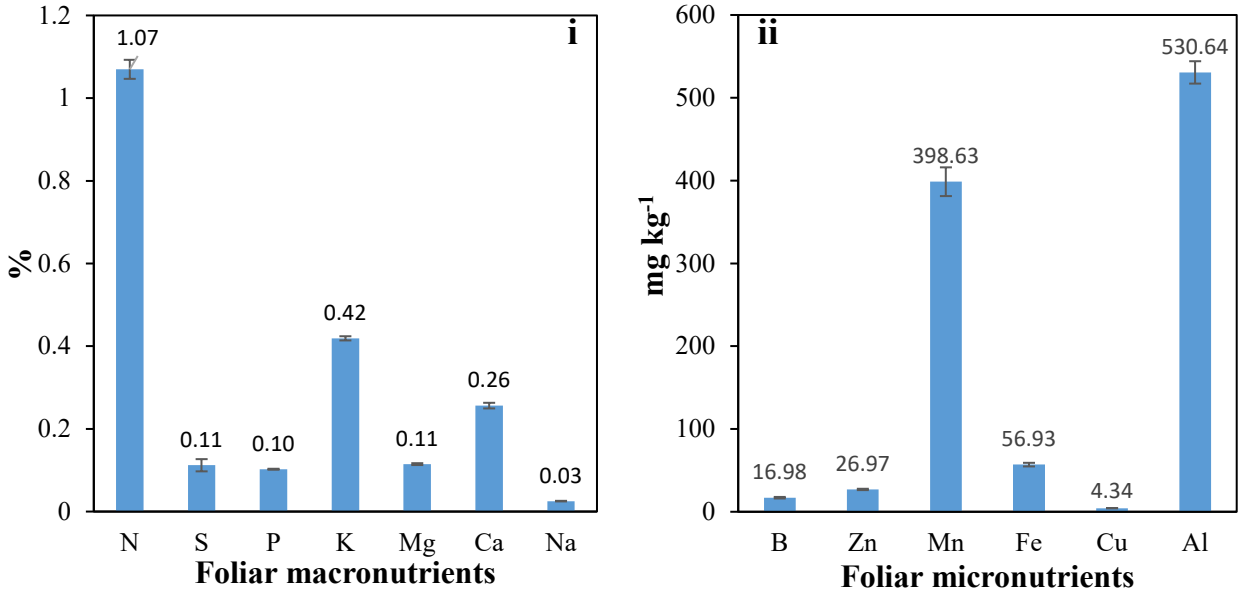


Figure 2.11. Average concentrations of foliar **(i)** nitrogen (N), sulphur (S), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), **(ii)** boron (B), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), and aluminum (Al) in the study site before the application of treatments to loblolly pine trees. Error bars represent the standard error of the mean.

4.5. Discussion

Prior to inoculating loblolly pine trees with *L. terebrantis*, differences in soil chemical properties were noted among sites assigned for treatments. Therefore, the variation in Ntot, Stot, available Mg, and pH suggest that other plot level factors are causing the differences. The observation of unusually high or low values for analyzed properties in one or more plots may have caused the differences among treatments. The average Ntot of the study site was 0.4 %. A significantly higher Ntot in plots assigned the low inoculation treatment compared to that in plots assigned the medium inoculation treatment may be attributed to the presence of 50% more Ntot in plot 12 (0.6%) compared to that in plot 13 (0.3%). Significantly higher Stot in plots assigned the low inoculation treatment compared to the plots assigned the medium inoculation treatment may be attributed to the fact that plots assigned the low inoculation treatment had approximately 37% more Stot (0.006%) than plots 1 and 13 (0.0038%) which were assigned the medium inoculation treatment.

Although average available soil Mg in the top 50 cm at the study site was 12%, it was exceptionally high in plot 6 (23 mg kg^{-1}) compared to that in plots 14 (8 mg kg^{-1}) and 15 (4 mg kg^{-1}). Plots 6, 14, and 15 were assigned the high, control, and low inoculation treatments, respectively. This may explain the observation of significantly lower available soil Mg in the plots assigned the control and low inoculation treatments compared to that in plots assigned the high inoculation treatment. Similarly, lower than average soil pH in plots 9, 10, and 15 and higher than the average soil pH in plot 13 may have contributed to the observation of significantly lower pH in plots assigned the control, wound, and low inoculation treatments compared to plot assigned the medium inoculation treatment.

Values of Ntot, Stot, and available P, K, Ca, Mg, Zn, Mn, and Fe were highest in the top 10 cm soil profile which suggests that these nutrients may be limiting factors for the growth of loblolly pine and thus have shallow vertical distribution (Jobbagy & Jackson, 2001). This observation was important since N and P play a significant role in determining forest productivity (Ballard, 1984; Binkley, 1986; Tamm, 1991; Vitousek, 2004), while N, P, and K are considered as the indicators of nutrient supply in forest soils (Schoenholtz et al., 2000). However, available Na did not show similar vertical distribution as other nutrients did and was consistent with Bowen (1979) and Thompson et al. (1997) who suggested that such observation may be because of the low demand of Na by trees as the content of Na in plant tissues is low.

The soil pH increased steadily between the 0 cm and 30.5 cm depths and decreased below the 30.5 cm depth. The average soil pH of the surface soil was within the range (4.5-6.0) that is reported to favor rapid growth of loblolly pines. The average BD of the 50 cm deep soil at our study site was 1.4 g cm^{-3} which fell within the range of 1.2-1.5 g cm^{-3} that is usually reported in established loblolly pine stands (Schultz, 1997). A large amount of loblolly pine needles deposition in our study site, followed by their incorporation with the mineral soil and decomposition may have contributed to this lower BD. Our explanation is supported by the fact that 70-90% of the forest floor in loblolly pine stand composed of needles in various stages of decomposition (van Lear & Goebel, 1976; Jorgensen et al., 1980).

The significantly higher foliar Mn concentration in plots assigned the low inoculation treatment compared to plots assigned the control, medium, and high inoculation treatments may be attributed to the observation of a considerable difference in foliar Mn on plot 15 (718 mg kg^{-1}) when compared to plots 8 (282 mg kg^{-1}), 7 (332 mg kg^{-1}), and 2 (277 mg kg^{-1}) which were assigned the control, medium, and high inoculation treatments, respectively. Foliar nutrients

approaching deficient levels were total N and P. Our result was confirmed by Martin & Jokela (2004) who reported that in spite of annual fertilization, foliar N in loblolly pine plantation remained below the critical value of 1.2%. Consistent with the study of Jokela (2004), who reported that the foliar Mn concentration in southern pines usually exceeds the threshold of 20-40 mg kg^{-1}, our study showed a considerably high value of foliar Mn concentration which averaged 400 mg kg^{-1} among plots. High concentrations of Fe and B in foliage may be due to an increase in the absorption of these nutrients by ectomycorrhizae (Lapeyrie, 1990; Mitchell et al., 1990).

The pre-inoculation analysis showed that the significant difference in soil chemical properties existed among plots before the inoculation treatments were applied. Post-inoculation soil and foliar sample collections are scheduled for July 2019. It is anticipated that inoculating trees with *L. terebrantis* will indirectly lead to soil resource limitations due to the onset of decline and its effect on foliage, fine roots, ectomycorrhizae, and their role in the nutrient pump at the study site.

Chapter 3

Fate of soil microbial biomass in the *Leptographium terebrantis* inoculated loblolly pine stand

3.1. Abstract

As the decline associated with *Leptographium terebrantis* has been reported to affect fine roots of loblolly pine trees throughout the southeastern United States, it is unknown if this fungus can affect soil microbial communities. A field study in Eufaula, Alabama was conducted in 2016 and 2017 to investigate microbial biomass responses to an on-site stem inoculation of loblolly pine trees with *L. terebrantis*. The pre- and post-inoculation microbial biomass and soil organic C/N ratio were analyzed to account for seasonal variations and variations due to the inoculation treatment of trees. The microbial biomass was determined by chloroform fumigation-extraction method and quantified by assessing the amount of carbon and nitrogen available in the living microorganisms present in the top 10 cm of soil. Though the treatment effect was insignificant, in 2 years of the study period, seasonal variation in analyzed soil parameters was evident, both before and after the inoculation treatment. Based on our study, it appears that microbial biomass responds positively to soil moisture and soil organic matters.

3.2. Introduction

Loblolly pine (*Pinus taeda* L.) is a commercially important tree species in the southeastern United States (Rauscher, 2004). However, a recent study suggests that pine stands in this region risk experiencing southern pine decline (Meyerpeter, 2012). This decline phenomenon is complex and is associated with root-feeding bark beetles and ophiostomatioid fungi (Hess et al., 1999; Hess et al., 2002). It has some unmistakable symptoms described in the literature that include sparsely crowned trees with short chlorotic needles and reduced radial growth followed by tree death (Hess et al., 1999, Hess et al., 2002). Below the soil surface, only a few symptoms have been recognized in association with decline. A decrease in the number of fine roots and their mortality has been observed in affected loblolly pines (Brown & McDowell 1968; Hess et al., 2002). Also, biotic factors such as pathogenic fungi and bark beetles are reported to inhabit the stressed trees (Manion & Lachance, 1992; Capretti & Battisti, 2007). It is unknown if *Leptographium terebrantis* Barras & Perry is a threat to soil microbes associated with declining loblolly pines.

Forest decline is a global problem triggered by various disturbances such as climate change, habitat fragmentation, and pathogen infestation. (Sapsford et al., 2017). *Leptographium terebrantis* is a pathogenic fungus which can disrupt water transportation in the xylem (Joseph et al., 1998) and lead to the decline and death of *Pinus* spp. (Paine et al., 1997). Pathogenic infection in the xylem may trigger a hydraulic failure, and as a result, decrease the allocation of carbon (C) belowground (Oliva et al., 2014). Since microbes are affected by changes in soil C (Hoyle et al., 2018), we suspect that without a regular supply of C to the root and mycorrhizal network, soil microbial function will be hindered.

In forest ecosystems, soil microbes play a major role in the decomposition of soil organic matter (SOM) (Swift et al., 1979; Holden & Treseder, 2013), and the release of essential nutrients for plant uptake (Hoyle et al., 2018). Likewise, above-ground vegetation is responsible for releasing approximately 20% of photosynthate into the soil (Bais et al., 2006). Microbes in the rhizosphere get C from root exudates and SOM (Haichar et al., 2008). Due to this microbe-plant interaction, a decline in tree health is expected to affect soil microbial communities. Factors affecting tree health include storms, insect outbreaks, and pathogen infestation (Goetz et al., 2012). Though soil microbes are sensitive to various environmental factors such as temperature (Allison & Treseder, 2008; Frey et al., 2008; Rustad et al., 2001), and moisture (Hawkes et al., 2011; Salamanca et al., 2003), an effect on them following the pathogenic infestation of forests is poorly understood (Holden & Treseder, 2013). Additionally, it is unknown if tree infection by the pathogenic fungus, *L. terebrantis* hinders soil microbial function.

A field study was conducted in Eufaula, Alabama to investigate soil microbial biomass (MB) before and after an on-site stem inoculation of loblolly pine trees with *L. terebrantis*. Over the course of 5 years, it is expected that pathogen action reduces water translocation to the tree crown. Reduction in photosynthesis and loss of leaf area will result followed by a decreased C allocation to roots and exudates from roots. The hypothesis of the present research is two-fold. First, it is hypothesized that before the inoculation treatment, MB will be similar among treatments. Second, it is hypothesized that after inoculation treatment and the appearance of significant treatment effects on tree and fine root growth, MB will be affected. In this study we specifically examined (i) if the MB and soil organic C/N ratio among treatments differed before and after the inoculation treatment and (ii) the seasonal dynamics of the soil organic C/N ratio.

3.3. Materials and methods

3.3.1. Site description

The research site was located near Eufaula, Alabama, in Barbour County in a loblolly pine plantation managed by Rayonier Inc. (32°1'13.10"N, 85°12'31.76"W). The plantation is located within the east Gulf coastal plain physiographic region of Alabama. The study site was dominated by fine sandy loam soil. Loblolly pine seedlings were planted in January 2003 to establish the plantation which was intensively managed until the time of study establishment (Alan Wilson, personal communication).

Fifteen treatment plots were established in 2015 (Figure 3.1). The average area of each plot was 76.38 m^2. At the time of study establishment, the trees were 14 years old and when the inoculation treatments were established, the trees were 16 years old. In 2014, prior to the study establishment, thinning was done so each plot retained one pair of trees in parallel rows, approximately 1 m apart within the planting row and 3.048 m apart between planting rows (Figure 3.2). A metal tag was attached to each tree denoting the tree number and a diameter band was attached to the tree at breast height. A weather station (WatchDog 2000, Spectrum Technologies Inc.) was installed to record air temperature, solar radiation, relative humidity, precipitation, and wind speed of the study area.

The experimental design consisted of 15 plots arranged in a completely randomized design (CRD) with 5 treatments and 3 replicates (Figure 3.1). The treatments for the study were: control (no inoculation or wound), wound (no inoculation), low inoculation, medium inoculation, and high inoculation which were randomly assigned to five trees in one plantation row of each 15 pair-lined plots (Figure 3.1, Figure 3.2).

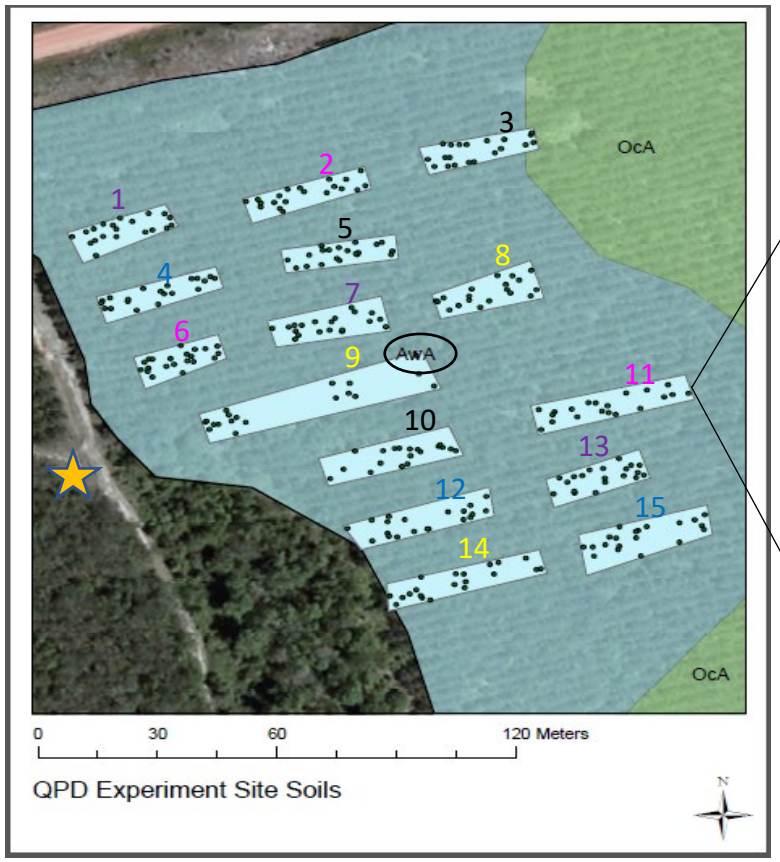


Figure 3.1. Map of the study site near Eufaula, Alabama. Treatment plots are represented by rectangles and soil series are represented by background color (AwA: Annemaine-Wahee complex). Small circles represent pre-thinning tree locations. Filled yellow star indicates the location of weather station.

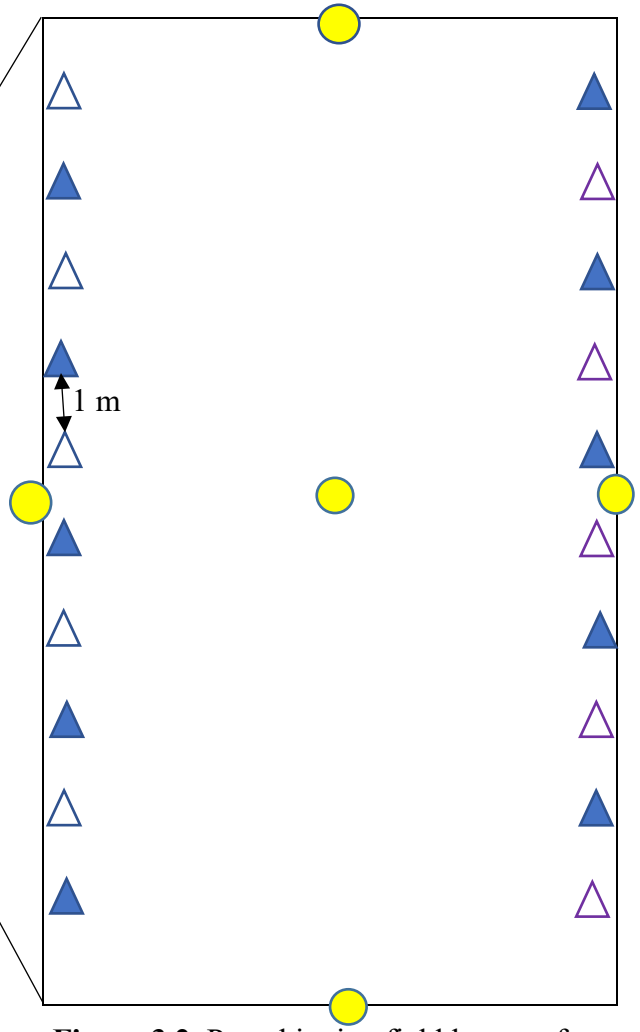


Figure 3.2. Post thinning field layout of tree arrangement and distance between them in each treatment plot. Unfilled purple triangles indicate inoculated trees, unfilled blue triangles indicate control trees, and solid blue triangles indicate other trees. Yellow circles represent microbial biomass collection point in each plot.

3.3.2 Experiment design and inoculation treatment

Two preliminary small-scale experiments were conducted before inoculating the trees near Eufaula, Alabama primarily, to (i) select an appropriate virulent fungal isolate of *L. terebrantis* and (ii) to determine the levels of low, medium, and high inoculation treatment. To select the most virulent *L. terebrantis* isolate from 42 isolates, an extensive seedling inoculation experiment was done according to Devkota & Eckhardt (2018). From the study, ATCC accession no. MYA-3316 was found as the most virulent *L. terebrantis* isolate to loblolly pine in comparison to 41 other isolates.

To identify the levels of inoculation treatment, a preliminary field inoculation experiment was carried out at the Solon Dixon Forestry Education Center, Andalusia, Alabama. Following the sterilization of toothpicks at 121°C and 0.103 MPa for 60 minutes, *L. terebrantis* isolate (ATCC accession no. MYA-3316) was grown in toothpicks imbibed with malt extract agar for approximately 24 days at 23°C in the dark (Devkota et al., 2018).

Figure 3.3. Toothpicks with *Leptographium terebrantis* inserted at 16 radial points of four rows in loblolly pine saplings at the Solon Dixon Center, Andalusia, Alabama. Toothpicks were clipped to facilitate covering the inoculation zone with a duct tape.

The inoculation treatment of trees near Eufaula, Alabama was carried out on March 13 and 14, 2017. The treatments were applied in accordance with Devkota et al. (2018), and based on the response of loblolly pine to different densities of virulent *L. terebrantis* from the Andalusia, Alabama study (Devkota et al., 2018).

Figure 3.4. Toothpicks with *Leptographium terebrantis* inserted in loblolly pine tree at the study site near Eufaula, Alabama.

The treatments for the study were: control (no inoculum or wound), wound (no inoculum), low inoculation, medium inoculation, and high inoculation. Five randomly chosen trees in one plantation row of each pair-lined plot received one of the five inoculation treatments. Control trees were left untouched. To apply rest of the treatments, selected trees were wrapped in plastic transparencies before drilling the holes. Plastic transparencies were marked with pre-determined inoculation points for different inoculation treatments with a permanent marker. A 5 mm deep hole was drilled at each predetermined inoculation point. The holes were drilled perpendicular to the surface of the stem using a sterilized 1.5 mm drill bit. One toothpick was inserted per hole. The toothpick insertion method simulated fungal transfer from maturation feeding activities of root-feeding bark beetles. Toothpicks were left inserted, clipped down to the bark, and covered with duct tape. Wounded trees had one fungus-free toothpick inserted per 1.2 cm ground-line diameter. Trees that received low, medium, or high inoculation treatments had one toothpick infected with *L. terebrantis* inserted per 10.0 cm, 2.4 cm, and 1.2 cm of ground-line diameter, respectively. The inoculation points were radially equidistant from each other. Each inoculation point was replicated 4 times vertically and equally spaced.

3.3.3. Soil microbial biomass (MB) sampling and analysis

Soil samples for MB were collected in January, April, July, and October 2016 and January, April, July, and October 2017. Five mineral soil (0-10 cm) samples were collected from each plot. The forest floor was removed before collecting samples. One sample was collected from plot center and one each from the plot edge in the four cardinal directions from the plot center (Figure 3.2). Samples from each plot were pooled in a plastic bag, placed in a cooler and transported to the processing lab at Auburn University, Auburn, Alabama within three hours of collection.

Samples were prepared for MB analysis within one week after collection. A soil sieve with 2 mm openings (No.10) was used to sieve soil samples to remove roots and coarse material from the soil. After sieving, soil from each plot was divided into two 18.5 g samples and poured into 125 ml Erlenmeyer flasks. Using the Chloroform Fumigation Extraction (CFE) technique, one sample was fumigated with alcohol-free chloroform to kill soil microbes, while another sample was left undisturbed (Vance et al., 1987). Fumigated samples were left airtight in a chloroform vapor-saturated atmosphere in the dark for 24 hours to completely kill soil microbes. After that, 125 ml of 0.5M K_2SO_4 was poured in flasks containing the fumigated or un-fumigated samples, flasks were subjected to shaking on an electric shaker for 30 minutes, and soil solutions were vacuum filtered through No. 5 Whatman filter paper to obtain a clear filtrate. The filtrate was stored in the freezer at -20°C until analyses could take place. Total organic C (TOC) and total organic N (TON) were analyzed using a TOC-V and N combustion analyzer (Shimadzu Scientific Instruments, Columbia, MD). The microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were reported as µg/g of dry soil and calculated in accordance with the formula in Paul et al. (1999).

(i) $MBC = (C_{fumigated} - C_{unfumigated})/k_{EC}$

(ii) $MBN = (N_{fumigated} - N_{unfumigated})/k_{EN}$

Where $C_{fumigated}$ = amount of organic C present in fumigated sample,

$C_{unfumigated}$ = amount of organic C present in un-fumigated sample,

$N_{fumigated}$ = amount of organic N present in fumigated sample, and

$N_{unfumigated}$ = amount of organic N present in un-fumigated sample

Since, dissolved organic C and N have less than 100% extraction efficiencies, MBC was calculated using a C extraction coefficient (k_{EC}) of 0.45 (Wu et al., 1996) and MBN was calculated using a N extraction coefficient (k_{EN}) of 0.54 (Brookes et al., 1985).

3.3.4. Soil moisture analysis

Soil samples collected at the 0-10 cm depth and processed for MB analysis were also used for assessing soil moisture. A 10 g soil sample from each pooled soil sample per plot was weighed in an aluminum tin and placed in the oven at 105°C for 72 hours. Gravimetric soil moisture (GMC) was reported as g/g of dry soil and calculated as:

GMC= weight of water lost after oven drying (g)/dry weight of the soil (g)

3.3.5. Statistical Analysis

Statistical analyses were completed using SAS version 9.4 (SAS Institute Inc. 2010, Cary, NC). Data collected before the inoculation treatment were analyzed by a two-way ANOVA (PROC GLM) with treatment and time as main effects and treatment × time as an interaction effect. Soil moisture concentration was found to be significantly different among treatments, and so were MBC, MBN, and soil C/N ratio. We suspected that plot level variation in GMC, MBC, MBN, and soil organic C/N ratio were due to relationships among these variables. Therefore, data collected after the inoculation treatment were analyzed by Pearson's correlation

(PROC CORR) for relationships among GMC, MBC, MBN, and soil C/N ratio to identify covariates responsible for influencing ANOVA results for post-inoculation MBC and MBN.

Initially, a two-way ANOVA (PROC GLM) was used to test if the post-inoculation MBC, MBN, and soil organic C/N ratio varied significantly among treatments and time of collection. Pearson's correlation results indicated that soil properties may have impacted the response variables. The post-inoculation MBC was significantly correlated ($P<0.05$) with soil organic C/N ratio and MBN was significantly correlated with GMC. Using an appropriate covariate, ANCOVA (PROC GLM) was used to test the effects of covariate, inoculation treatment, time, and their interactions on MBC and MBN.

The main and interaction effects were considered significant at $P\leq0.05$ unless otherwise noted. Tukey's Multiple Range test (PROC GLM) was used to compare the means among treatments and depths.

3.4 Results

3.4.1. Stand environment and soil moisture

Compared to the 25-year average between 1994 and 2018 (NOAA, 2019) of Barbour County, Alabama, precipitation during the MB study period from January 2016 through October 2017 was reduced by 14.8%. During the significant decline in MB in October 2016, precipitation was 0 mm. Precipitation was negligible in September 2016 which was one month before soil sample collection in October 2016 (Figure 3.5).

Values of GMC were significantly different among treatments and sampling time both before and after the inoculation (Table 3.1). The pre-inoculation GMC was significantly higher in the low treatment than in the high inoculation treatment ($P=0.0311$). The post-inoculation GMC was significantly higher in the low treatment than in the wound ($P=0.0066$) and medium

inoculation treatments (P=0.0059) (Figure 3.6). Differences across seasons are shown in Figure 3.7 and presented in Table 3.2.

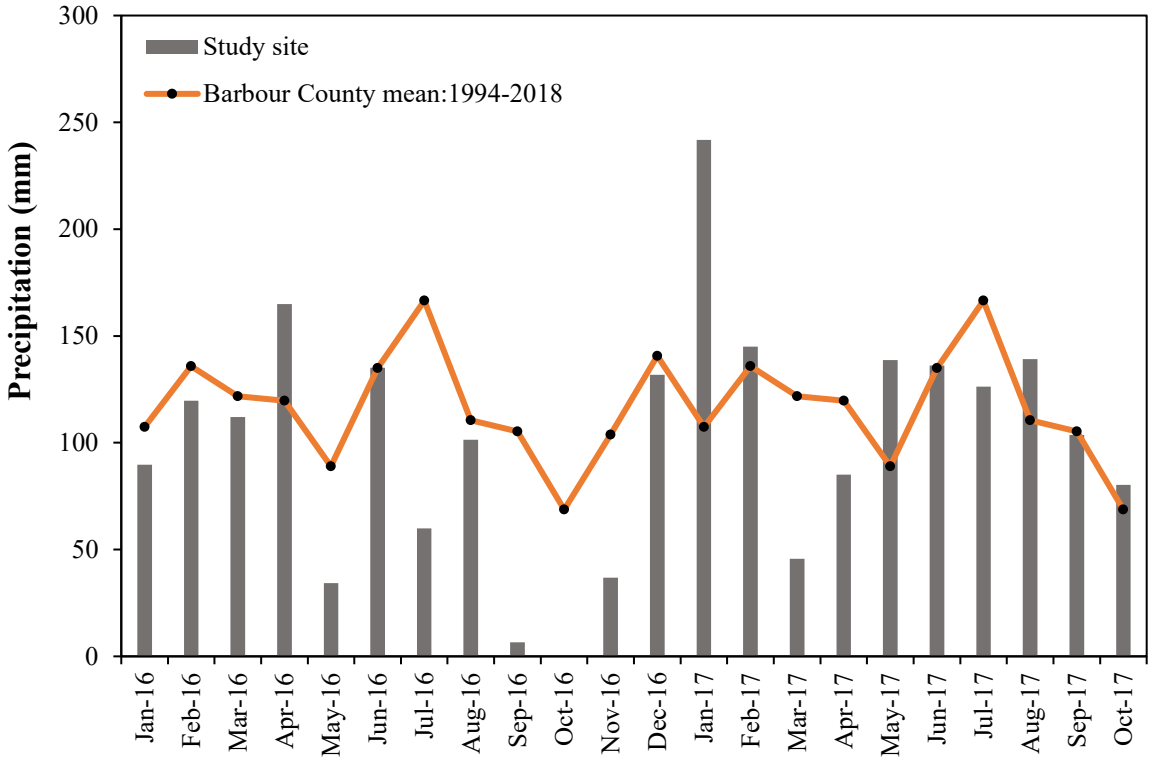


Figure 3.5. Mean monthly precipitation (mm) between 1994 and 2018 in Barbour County, Alabama and total monthly precipitation between January 2016 and October 2017 in a mature loblolly pine stand near Eufaula, Barbour County, Alabama.

Table 3.1. Probabilities of a greater *F*-value from a two-way ANOVA for gravimetric soil moisture of a mature loblolly pine stand near Eufaula, Alabama before and after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Pre-inoculation				Post-inoculation			
Source	df	*F*-value	*P>F*	Source	df	*F*-value	*P>F*
Treatment (Trt)	4	2.71	0.0404	Trt	4	4.77	0.0042
Time (T)	4	121.66	<0.0001	T	2	7.08	0.0030
Trt× T	16	0.71	0.7744	Trt× T	8	0.81	0.9828
Error	50			Error	44		

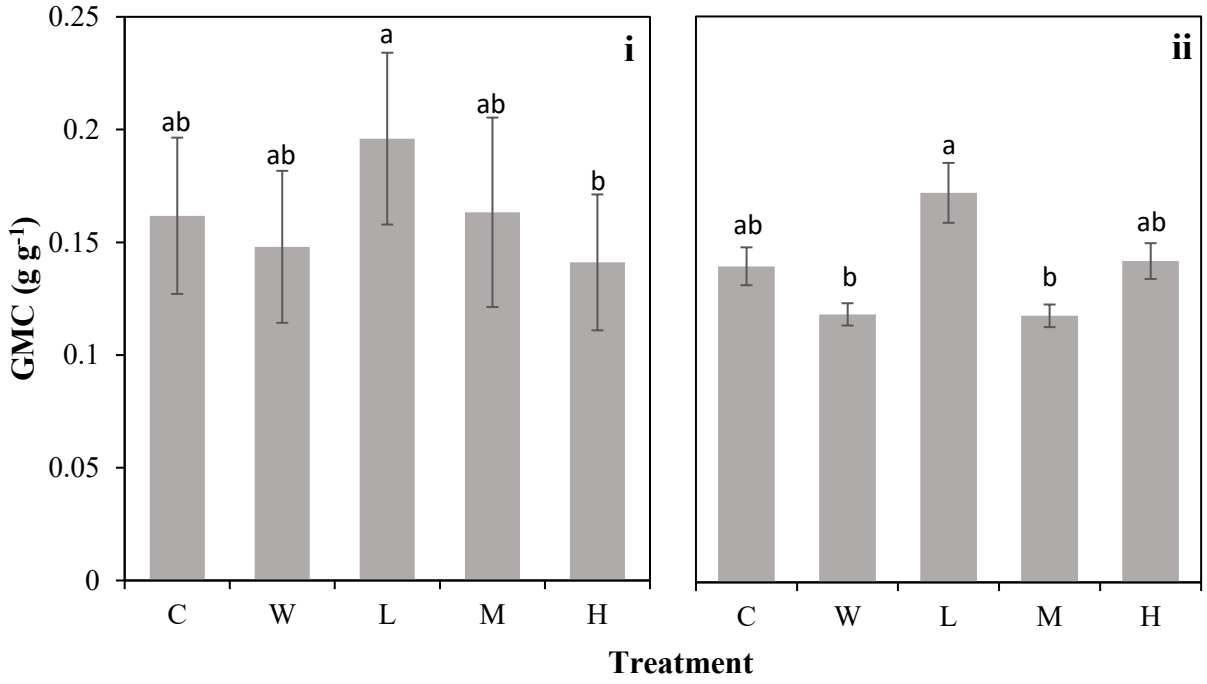


Figure 3.6. Average gravimetric soil moisture (GMC) among treatments **(i)** before and **(ii)** after the application of inoculation treatments on loblolly pine trees. Treatments were control (C), wound (L), low (L), medium (M), and high (H) inoculation. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

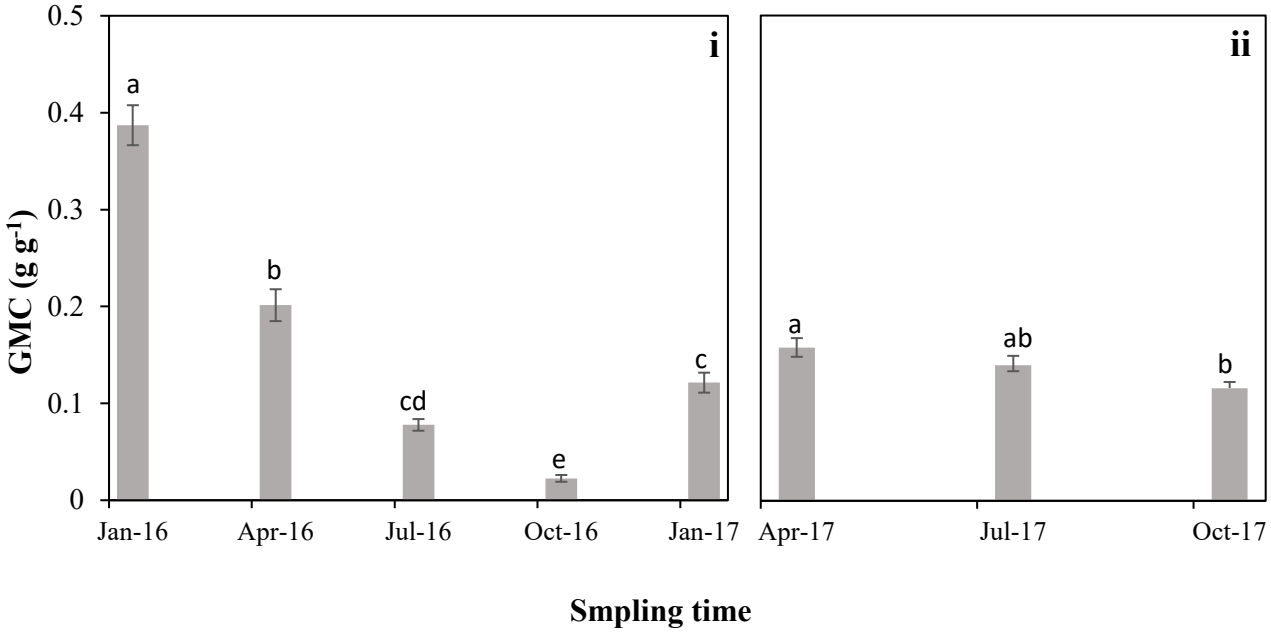


Figure 3.7. Average gravimetric soil moisture (GMC) among sampling time **(i)** before and **(ii)** after the application of inoculation treatments on loblolly pine trees. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

Table 3.2. *P*-value for significantly different gravimetric soil moisture among sampling times before and after the inoculation treatments were applied on loblolly pine trees.

Pre-inoculation sampling time pairing			*P*-value	Post-inoculation sampling time pairing	*P*-value
January 2016	vs.	April 2016	<0.0001		
January 2016	vs.	July 2016	<0.0001		
January 2016	vs.	October 2016	<0.0001		
January 2016	vs.	January 2017	<0.0001		
April 2016	vs.	July 2016	<0.0001		
April 2016	vs.	October 2016	<0.0001	April 2017 vs. October 2017	0.0021
April 2016	vs.	January 2017	0.0005		
July 2016	vs.	October 2016	0.0298		

3.4.2. Microbial biomass carbon (MBC) and nitrogen (MBN)

Before the inoculation treatment, MBC and MBN were correlated with both GMC and soil organic C/N ratio. However, after the treatment MBC and MBN were only correlated with soil organic C/N ratio and GMC, respectively (Table 3.3).

Values of MBC were significantly different among treatments and sampling time both before and after treatments were applied (Table 3.4, Table 3.5). The pre-inoculation MBC was significantly higher on the low treatment than on the control (P=0.0313) and high inoculation treatments (P=0.0311). The post-inoculation MBC was significantly higher on the low inoculation treatment than on the control treatment (P=0.0481) (Figure 3.8). The ANCOVA results indicated that the post-inoculation difference in MBC among treatments was not due to inoculation treatment but was due to covariance between MBC and soil organic C/N ratio (Table 3.7). Differences among sampling times are shown in Figure 3.8 and presented in Table 3.6. The interaction effect of soil organic C/N ratio and sampling time on post-inoculation MBC was found (Table 3.7, Figure 3.10i).

The pre-inoculation MBN was significantly different among treatments and sampling time, while the post-inoculation MBN was significantly different among treatments (Table 3.4, Table 3.5). The pre-inoculation MBN was significantly higher in the low treatment than in wound (P=0.0140) and high inoculation treatments (P=0.0070). The post-inoculation MBN was significantly higher in the low inoculation treatment than in wound (P=0.0185) and medium inoculation treatments (P=0.0811) (Figure 3.9). The ANCOVA results indicated that the post-inoculation difference in MBN among treatments was not due to inoculation treatment but was due to covariance between MBN and GMC (Table 3.7) and the relation is shown in Figure 3.10. Differences among sampling times are shown in Figure 3.9 and presented in Table 3.6. The post

inoculation MBN was significantly lower in April 2017 compared to July 2017 (P<0.0001) and October 2017 (P=0.0001) (Figure 3.10ii).

Table 3.3. Pre and post-inoculation Pearson's correlation coefficient between microbial biomass and gravimetric soil moisture (GMC) or soil organic C/N ratio along with their respective *P*-values.

	Microbial biomass carbon				Microbial biomass nitrogen			
	Pre-inoculation		Post-inoculation		Pre-inoculation		Post-inoculation	
Soil properties	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value
GMC	0.6934	<0.0001	0.1073	0.4836	0.4688	<0.0001	0.7262	<0.0001
Organic C/N	0.4711	<0.0001	0.3322	0.0258	0.2489	0.0313	0.0784	0.6086

Pre-inoculation: n=75 (5 seasons×5 treatments×3 replicates)
Post-inoculation: n=45 (3 seasons×5 treatments×3 replicates)

Table 3.4. Probabilities of a greater *F*-value from a two-way ANOVA for microbial biomass carbon and nitrogen in a mature loblolly pine stand near Eufaula, Alabama before an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Source	df	Microbial biomass carbon		Microbial biomass nitrogen	
		F-value	*P>F*	*F*-value	*P>F*
Treatment (Trt)	4	2.93	0.0298	4.39	0.0040
Time (T)	4	16.11	<0.0001	13.45	<0.0001
Trt× T	16	0.81	0.6725	0.81	0.6728
Error	50				

Table 3.5. Probabilities of a greater *F*-value from a two-way ANOVA for microbial biomass carbon and nitrogen in a mature loblolly pine stand near Eufaula, Alabama after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Source	df	Microbial biomass carbon		Microbial biomass nitrogen	
		F-value	*P>F*	*F*-value	*P>F*
Treatment (Trt)	4	2.39	0.0728*	3.19	0.0270**
Time (T)	2	4.10	0.0267**	1.53	0.2327
Trt× T	8	0.32	0.9514	0.05	0.9999
Error	44				

* significant at *P*<0.10 level, ** significant at *P*<0.05 level

Table 3.6. *P*-value for significantly different microbial biomass carbon (MBN) and nitrogen (MBN) among sampling times before and after the inoculation treatments were applied on loblolly pine trees.

Pre-inoculation sampling time pairing			*P*-value		Post-inoculation sampling time pairing	*P*-value
			MBC	MBN		MBC
January 2016	vs.	July 2016	<0.0001			
January 2016	vs.	October 2016	<0.0001	<0.0001		
January 2016	vs.	January 2017	0.0016			
April 2016	vs.	July 2016	0.0012			
April 2016	vs.	October 2016	<0.0001	0.0007		
July 2016	vs.	October 2016		<0.0001	October 2017 vs. July 2017	0.0229
January 2017	vs.	October 2016		0.0002		

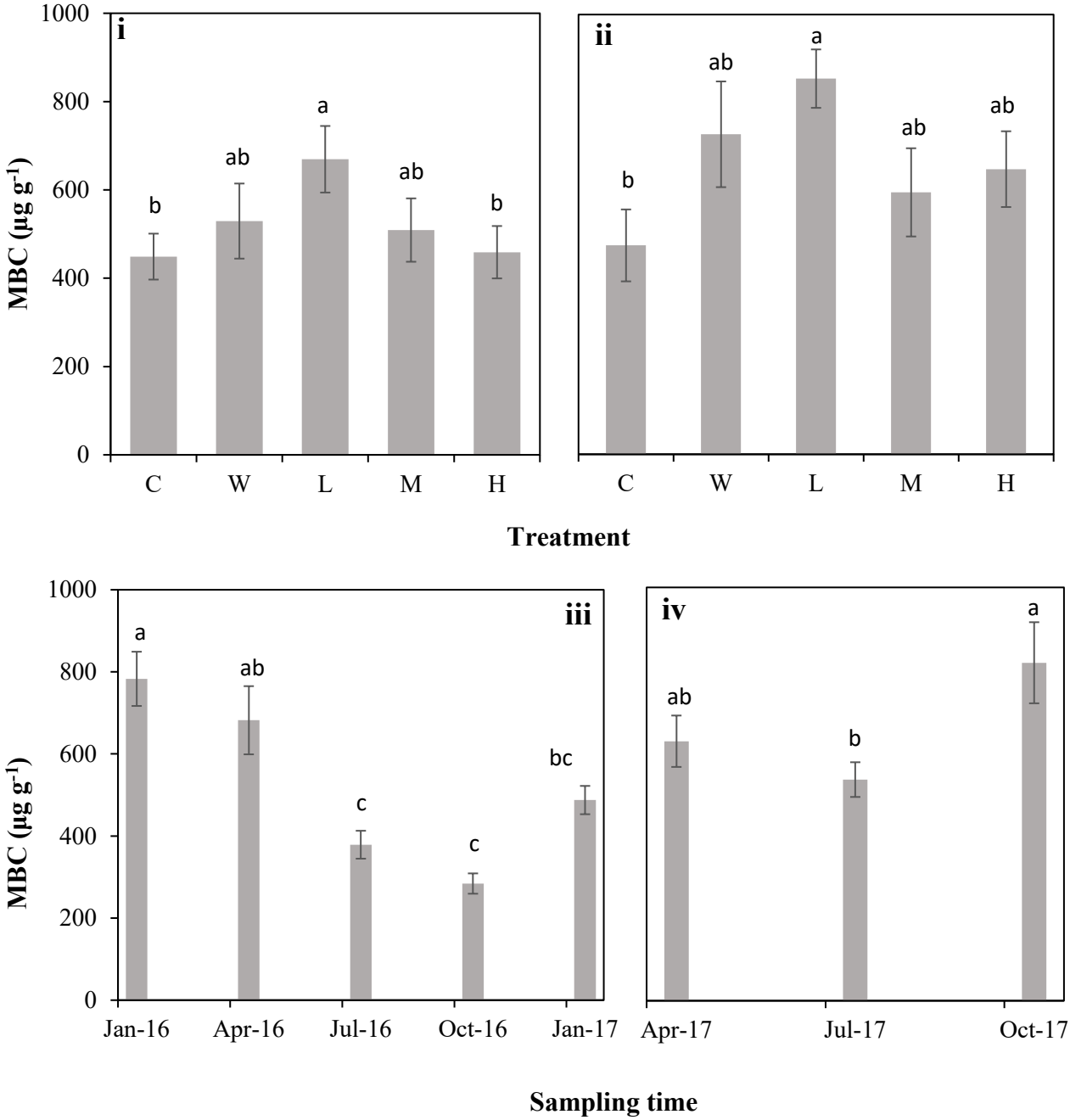


Figure 3.8. Average microbial biomass carbon (MBC) among treatments **(i)** before and **(ii)** after the application of inoculation treatments and among sampling time **(iii)** before and **(iv)** after the application of inoculation treatments on loblolly pine trees. Treatments were control (C), wound (L), low (L), medium (M), and high (H) inoculation. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

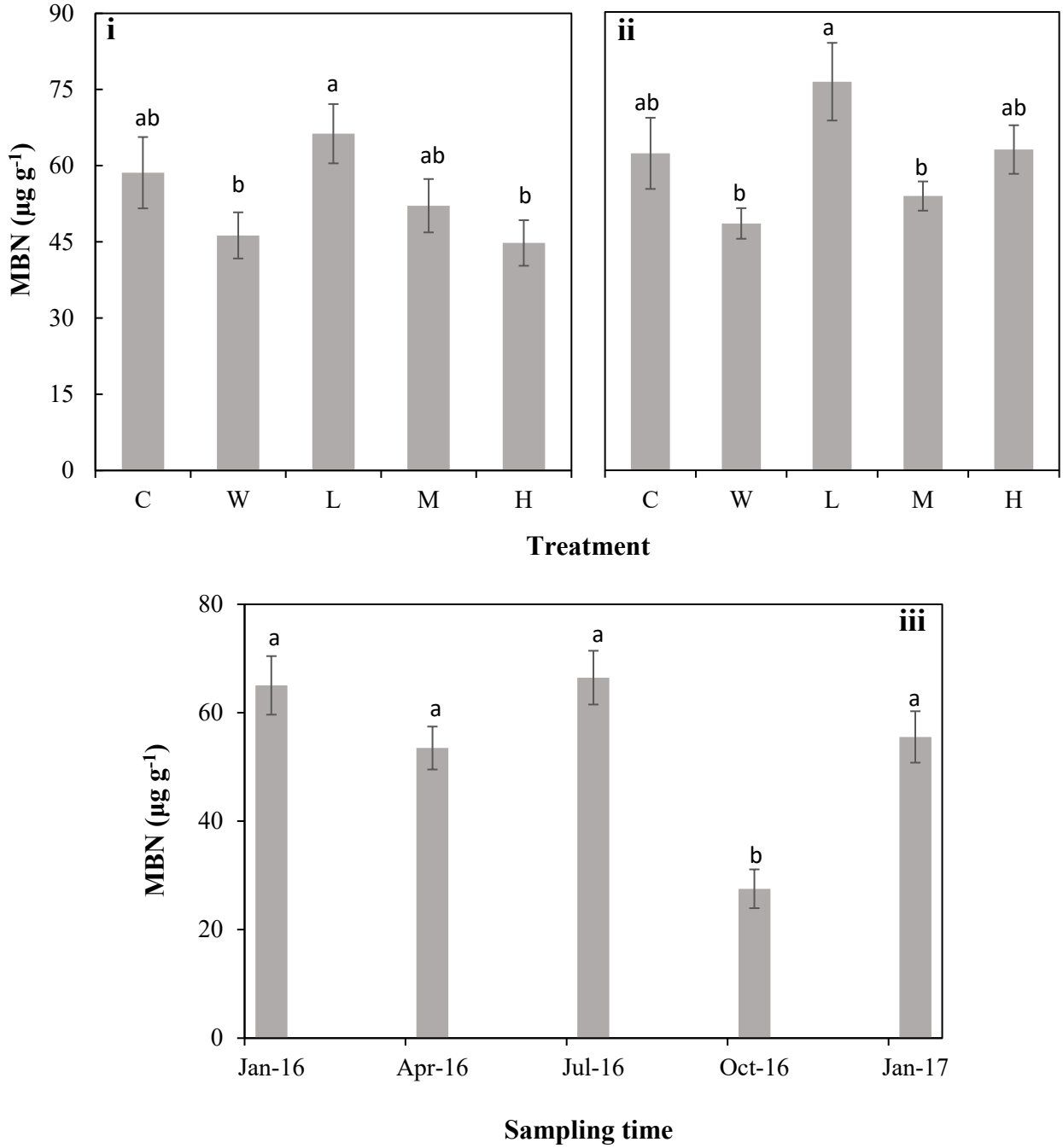


Figure 3.9. Average microbial biomass nitrogen (MBN) among treatments **(i)** before and **(ii)** after application of inoculation treatments and among sampling time **(iii**) before the application of inoculation treatments on loblolly pine trees. Treatments were control (C), wound (L), low (L), medium (M), and high (H) inoculation. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

Table 3.7. Probabilities of a greater *F*-value from ANCOVA for microbial biomass carbon and microbial biomass nitrogen in a mature loblolly pine stand near Eufaula, Alabama after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.The covariates were soil organic C/N ratio for microbial biomass carbon analysis and soil moisture content for microbial biomass nitrogen analysis.

Microbial biomass carbon				Microbial biomass nitrogen			
Source	df	*F*-value	*P>F*	Source	df	*F*-value	*P>F*
Treatment (Trt)	4	0.35	0.8383	Trt	4	0.39	0.8292
Time (T)	2	4.46	0.0302	T	2	1.16	<0.0001
Organic C/N ratio	1	0.02	0.8881	SMC	1	6.09	<0.0001
Trt× T	8	0.98	0.4868	Trt× T	8	0.93	0.9700
Trt× C/N	4	0.26	0.9015	Error	29		
T× C/N	2	4.10	0.0381				
Trt× T× C/N	8	0.93	0.5174				
Error	15						

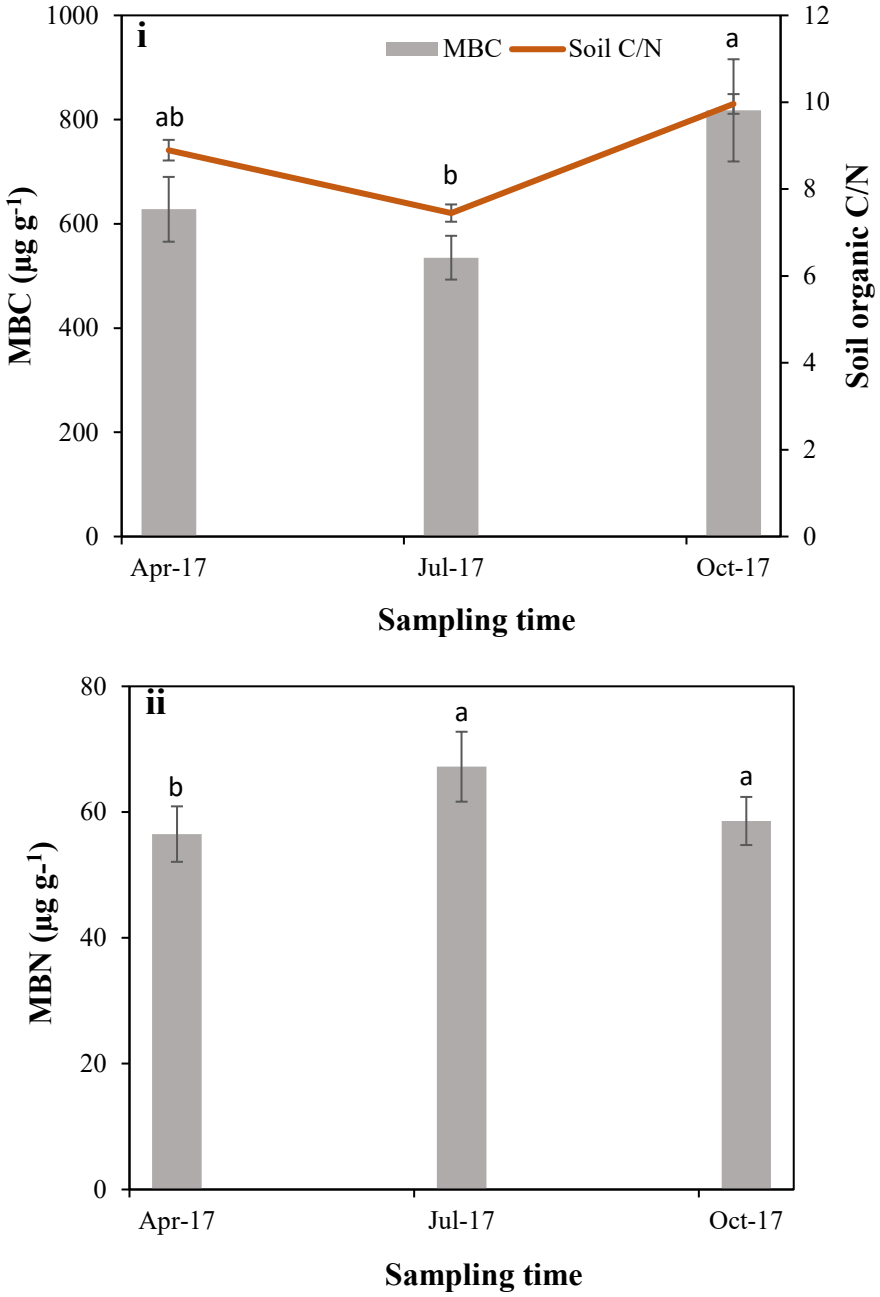


Figure 3.10. Average microbial biomass **(i)** carbon (MBC) and soil organic C/N ration and **(ii)** nitrogen (MBN) among sampling time after the application of inoculation treatments on loblolly pine trees. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

3.4.3. Soil organic C/N ratio

Soil organic C/N ratio was significantly different among treatments and sampling time both before and after treatments were applied (Table 3.8). The pre-inoculation soil organic C/N ratio was significantly higher in January 2016 than in April 2016 (P=0.0092), July 2016 (P=0.0013), October 2016 (P<0.0001), and January 2017 (P=0.0014). The post-inoculation soil organic C/N ratio was significantly higher in April 2017 than July 2017 (P=0.0003), and higher in October 2017 than April 2017 (P=0.0070) and July 2017 (P<0.0001) (Figure 3.11).

Table 3.8. Probabilities of a greater F-value from a two-way ANOVA for soil organic C/N ratio of a mature loblolly pine stand near Eufaula, Alabama before and after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Pre-inoculation				Post-inoculation			
Source	df	F-value	$P>F$	Source	df	F-value	$P>F$
Treatment (Trt)	4	0.72	0.5791	Trt	4	2.01	0.1188
Time (T)	4	8.68	<0.0001	T	2	30.41	<0.0001
Trt× T	16	1.27	0.2530	Trt× T	8	0.18	0.9926
Error	50			Error	44		

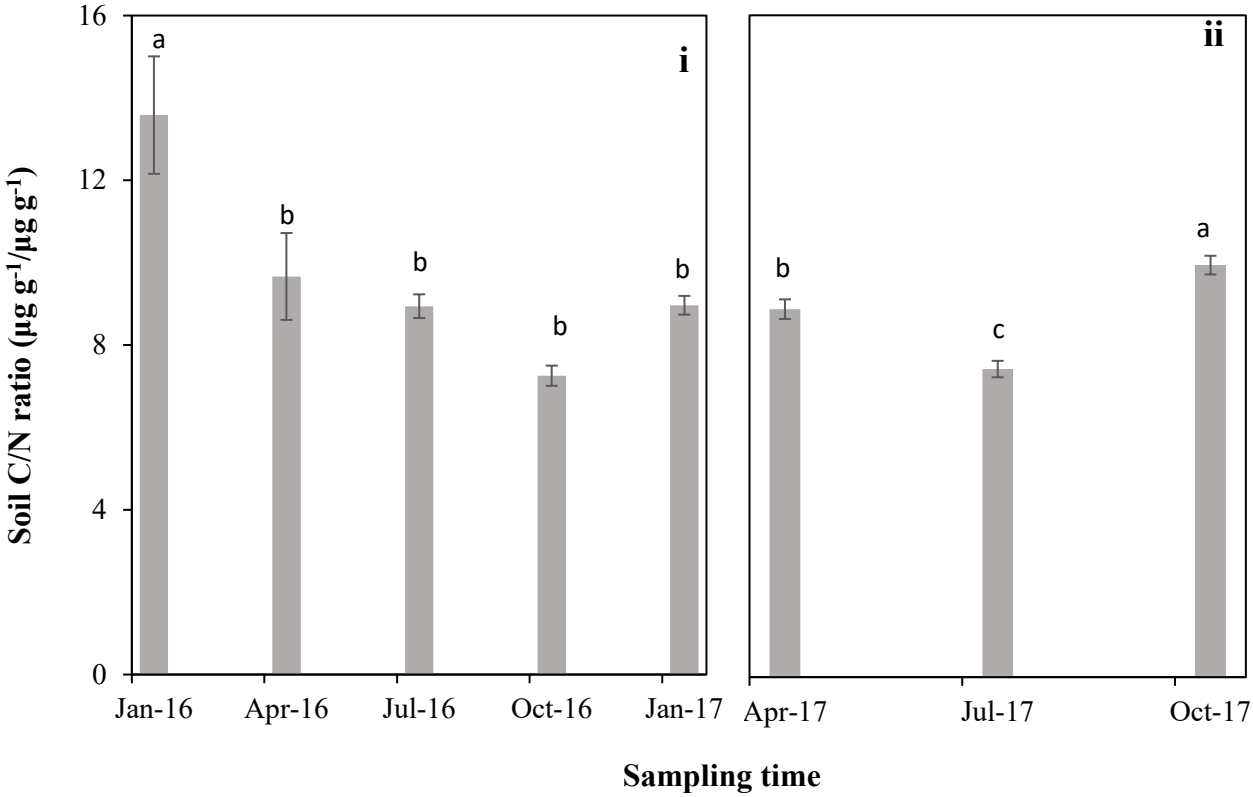


Figure 3.11. Average soil organic C/N ratio among sampling times **(i)** before and **(ii)** after the inoculation treatment of loblolly pine trees with *Leptographium terebrantis*. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

3.5. Discussion

This research was designed to study if an on-site inoculation treatment of loblolly pine trees with *L. terebrantis* affects MB in the upper portion of the mineral soil. Before the inoculation treatments were applied, differences in MBC and MBN among treatments was not expected. It was found that environmental or plot level factors were contributing to treatment difference. A probable reason for these observations is the significant correlations found among pre-inoculation MBC and MBN and either soil organic C/N ratio or GMC. Our results are supported by past studies that suggested the amount of MB in soil is related to soil moisture (Wardle, 1992; Devi & Yadava, 2006) and soil organic C (Hoyle et al., 2018). This also explains why pre-inoculation MBC and MBN were highest in the Low inoculation treatment plots as GMC was highest in these plots.

It appears that *L. terebrantis* did not affect the MBC or MBN during the post-inoculation period of observation in this study. Our results are supported by Holden & Treseder (2013), who reported that abiotic disturbances have a stronger effect on MB than biotic factors such as pests and pathogens. Post-inoculation MBC was significantly affected by soil organic C/N ratio and also by the interaction of soil organic C/N ratio with the sampling time. This explains similarities in the pattern of post-inoculation MBC and soil organic C/N ratio (Figure 3.10i). Similarly, GMC significantly affected post-inoculation MBN.

Consistent with the results of Devi & Yadava (2006), seasonal variation in MBC and MBN was evident both before and after the inoculation treatment. The lowest amounts of GMC, MBC, and MBN were recorded in fall 2016. Since moisture stress affects soil microbial communities (Devi & Yadava, 2006), a rainfall deficit from September 2016 through October 2016 (Figure 3.5), which was 15 times less than normal may have limited MB. It was interesting

to find that the pre-inoculation MBC and MBN were significantly correlated with GMC and soil organic C/N ratio, while the post-inoculation MBC was only correlated with soil organic C/N ratio and MBN was only correlated with GMC. These observations demonstrate the dynamic nature of MB.

Soil organic C/N ratio varied across seasons. During each season, the C/N ratio of the mineral soil at the 0-10 cm depth at our study site was lower than 14:1 (Figure 3.11), which is different from optimal C/N ratio of 24:1 preferred by soil microbes (USDA NRCS, 2018). Therefore, our results indicate that during the study period, rapid SOM decomposition (Benbi & Khosa, 2014, Sauvadet et al., 2017) yielded excess N in the soil that was available for plant uptake (USDA NRCS, 2018). Additionally, soil organic C/N ratio was significantly correlated with MB. This result is supported by Yang et al. (2010) who reported that mineralization of nutrients can take place with a decrease in MB and immobilization of nutrients can take place with an increase in MB.

In conclusions, the absence of a treatment effect on MBC and MBN indicated that determining the effect of loblolly pine stem inoculation by *L. terebrantis* on soil microbial communities might be difficult. Additionally, MB may not be affected before changes in the root system and soil physiochemical properties have occurred due to the inoculation treatments. The study period was probably insufficient to monitor a MB response to *L. terebrantis* inoculation. A longer study time is required to ensure pathogen establishment and pathogen effects before it interferes the downward supply of C to the root system and its exudates. Values of MB exhibited a strong seasonality and were influenced by soil organic C/N ratio and GMC.

Chapter 4

New root growth and ectomycorrhizal colonization of fine roots in loblolly pine as affected by the inoculation of *Leptographium terebrantis*

4.1. Abstract

Leptographium terebrantis is reported to affect the roots of loblolly pine trees throughout the southeastern United States. However, it is unknown if this fungus can affect fine roots that are ectomycorrhizal. From 2017 to 2019, a field study in a mature loblolly pine stand in Eufaula, AL was conducted to investigate root growth and ectomycorrhizal colonization responses to an on-site stem inoculation of loblolly pine trees with *L. terebrantis*. The post-inoculation analyses accounted for variations due to the inoculation treatment, measurement dates, and depths of observation. Root growth was studied for a period of 23 months using minirhizotrons and a root periscope and expressed as root length density (RLD). Fine roots were collected in September 2017 to determine percentage ectomycorrhizal colonization by the gridline intercept method. Though the treatment effect on analyzed parameters was insignificant till December 2018, seasonal and depth variation in cumulative RLD was evident. Significant differences in cumulative root length density among treatments was found in February 2019. In 2017 and 2018, root growth was rapid from April to October and February to October respectively. Over the entire study period, minimum amount of roots were observed near the soil surface and a maximum amount was observed at the 28.3 cm depth. Difference in cumulative RLD between the treatment and control pairs of trees within treatment plots were mostly insignificant. Ectomycorrhizal colonization varied by depth and was highest at the 20-30 cm depth. Our study showed that loblolly pine ectomycorrhizae thrive in acidic soil.

4.2. Introduction

In the 1950s, Brown & McDowell (1968) observed premature decline and death of loblolly pine (*Pinus taeda* L.) trees on the Oakmulgee District of the Talladega National Forest, AL. The infected trees exhibited fine root mortality and presence of stress cones for one year before they died. The results were consistent with the observations of Hess et al. (2002) who found that loblolly pine experienced a decrease in fine root biomass that was highly correlated with the occurrence of tree decline in Central Alabama, suggesting that fine root mortality is a sign of this problem. Yet it was reported that a reduction in the number of fine roots of declining loblolly and longleaf pine infected with *Leptographium terebtantis* Barras & Perry was followed by tree deterioration and death (Eckhardt & Menard, 2009). Eckhardt et al. (2007) observed that root mortality was higher on sites with declining loblolly pine trees than on sites with healthy loblolly pine trees.

Throughout the world, ophiostomatoid fungi have affected the root system and overall tree health of pine species resulting in tree death (Otrosina et al., 2002). These fungi with *Leptographium* anamorphs have been isolated from the woody roots of declining pines throughout the southeastern United States (Otrosina et al., 2002; Menard et al., 2006; Eckhardt et al., 2007). The ophiostomatoid fungal root pathogen, *L. terebrantis*, is one of the causal agents associated with declining loblolly pine trees (Eckhardt, 2003). Eckhardt et al. (2007) reported the isolation of abundant *L. terebrantis* from the woody roots of declining loblolly pine which indicates that in the future, this fungus may compromise tree health and productivity. This pathogen causes xylem occlusions and phloem lesions in woody roots of loblolly pine trees (Matusick & Eckhardt, 2010). Pathogenic infection in the xylem may subsequently decrease the basipetal supply of carbon (C) in the phloem (Oliva et al., 2014). Without the regular supply of C

to the root system and symbiotic fungi, mycorrhizal function is likely to be hindered (Högberg et al., 2002).

Correlation between forest decline and root system deterioration among coniferous and hardwood species has been reported around the world. Together, moisture stress and *Leptographium* and *Armillaria* root disease were determined as major factors contributing to the death of fine roots and subsequent decline of western white pine (*Pinus monticola* Douglas ex D. Don) in the Inland Empire (Auclair et al., 1990; Hennon, 1990; Manion, 1991). Poorly drained soils at low elevations, together with more than 50 species of fungi and a bark beetle species (Coleoptera: Scolytidae) were associated with fine root mortality in declining Alaskan yellow cedar (*Chamaecyparis nootkatensis* D.Don) trees (Hennon et al., 1990a,b). Mosca et al. (2017) reported that pedunculate oak (*Quercus robur* L.) trees experiencing severe decline had decreased ectomycorrhizal diversity and abundance. These results are consistent with those of Corcobado et al. (2014) who found that declining holm oak (*Quercus ilex* L.) trees affected by *Phytophthora cinnamomi* Rands were colonized by a less diverse ectomycorrhizal population and had fewer ectomycorrhizal root tips compared to non-declining trees.

Roots can be affected directly by various root diseases and indirectly by adverse environmental and anthropogenic factors (Atkinson, 1991; Smucker, 1993; Eissenstat et al., 2000). Root disease pathogens such as *L. terebrantis* inhibit the vascular transport of water, mineral nutrients, and carbohydrates which leads to root deterioration (Oliva et al., 2014). Also, because maintenance of a forest tree root system requires approximately 20-47% of photosynthetically fixed C (Smucker, 1993), factors that reduce C fixation in the tree crown have the potential to restrict C allocation to the tree root system. The ectomycorrhizal network that includes colonized fine roots, extramatrical hyphae, and rhizomorphs contributes to water and

nutrients uptake, protection of the root system from unfavorable environmental conditions and biotic stress, and maintenance of soil physical structure (Amaranthus, 1998). Ectomycorrhizae in return receive nearly 20-25% of the C fixed in tree crowns (Söderström & Read, 1987; Högberg & Högberg, 2002; Hobbie, 2006). Due to the mutualistic association of forest trees with ectomycorrhizal fungi, the impact of tree decline doubtlessly influences the fungal partner (Sapsford et al., 2017). Past studies have focused on either tree decline or mycorrhizal processes but not on the mycorrhizal processes of declining trees (Sapsford et al., 2017). Though there is substantial review on the potential effects of climate change on mycorrhizal fungal diversity (Bellgard & Williams, 2011), we have limited information to assess how forest health affects mycorrhizal communities (Egli, 2011).

A field study was installed in 2016 to investigate how the crown and root system of plantation loblolly pines respond to on-site stem inoculation with *L. terebrantis*. Over the course of five years, it is anticipated that pathogen spread in the stem xylem will adversely affect the translocation of soil resources to the crown. Subsequent reductions in leaf area and whole-crown C fixation will result followed by a decrease in carbohydrate supply to the root system and ectomycorrhizal network. We hypothesize that after the inoculation treatments and the appearance of significant inoculation treatment effects on tree leaf area or stemwood growth, new root growth expressed as root length density as well as the ectomycorrhizal colonization of fine roots will decrease in response to *L. terebranitis* infection. The present objectives are to (i) describe the installation of minirhizotron root tubes, (ii) document the efficacy of each tube for continued study, (iii) summarize root length density observations that were made twelve times at a 2 month interval after the inoculation treatment, and (iv) assess if ectomycorrhizal colonization differed among the treated trees 6 months after the inoculation treatment.

4.3. Materials and methods

4.3.1. Site description

The research site was located near Eufaula, Alabama, in Barbour County in a loblolly pine plantation managed by Rayonier Inc. (32°1'13.10"N, 85°12'31.76"W). The plantation is located within the east Gulf coastal plain physiographic region of Alabama. The study site was dominated by fine sandy loam soil. Loblolly pine seedlings were planted in January 2003 to establish the plantation which was intensively managed until the time of study establishment (Alan Wilson, personal communication).

Fifteen treatment plots were established in 2015 (Figure 4.1). The average area of each plot was 76.38 m^2. At the time of study establishment, the trees were 14 years old and when the inoculation treatments were established, the trees were 16 years old. In 2014, prior to the study establishment, thinning was done so each plot retained one pair of trees in parallel rows, approximately 1 m apart within the planting row and 3.048 m apart between planting rows (Figure 4.2). A metal tag was attached to each tree denoting the tree number and a diameter band was attached to the tree at breast height. A weather station (WatchDog 2000, Spectrum Technologies Inc.) was installed to record air temperature, solar radiation, relative humidity, precipitation, and wind speed of the study area.

The experimental design consisted of 15 plots arranged in a completely randomized design (CRD) with 5 treatments and 3 replicates (Figure 4.1). The treatments for the study were: control (no inoculum or wound), wound (no inoculum), low inoculation, medium inoculation, and high inoculation which were randomly assigned to five trees in one plantation row of each 15 pair-lined plots (Figure 4.1, Figure 4.2).

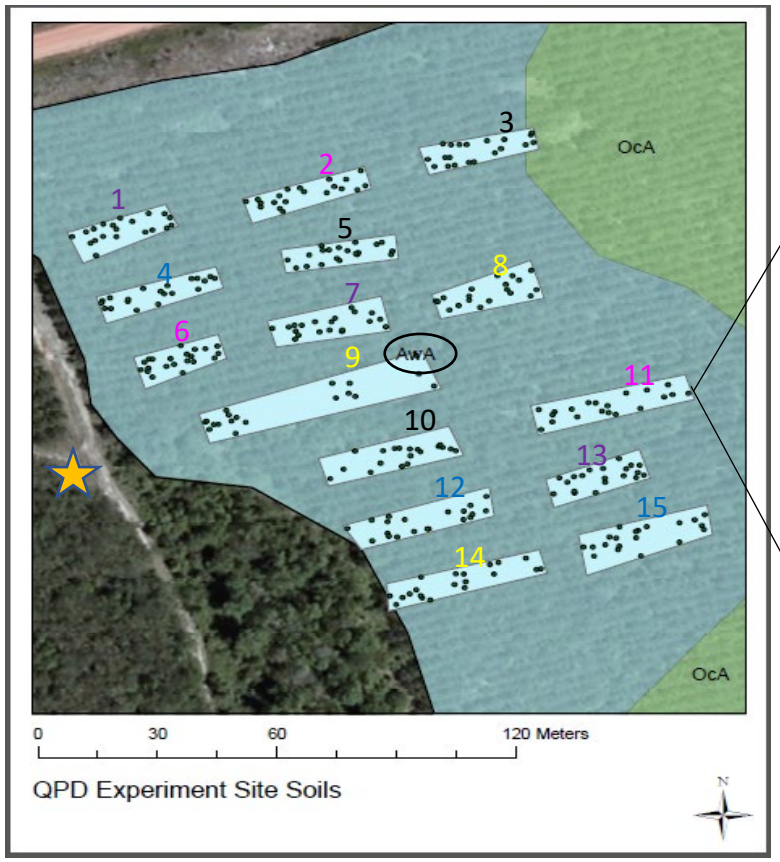


Figure 4.1. Map of the study site near Eufaula, Alabama. Treatment plots are represented by rectangles and soil series are represented by background color (AwA: Annemaine-Wahee complex). Small circles represent pre-thinning tree locations. Filled yellow star indicates the location of weather station.

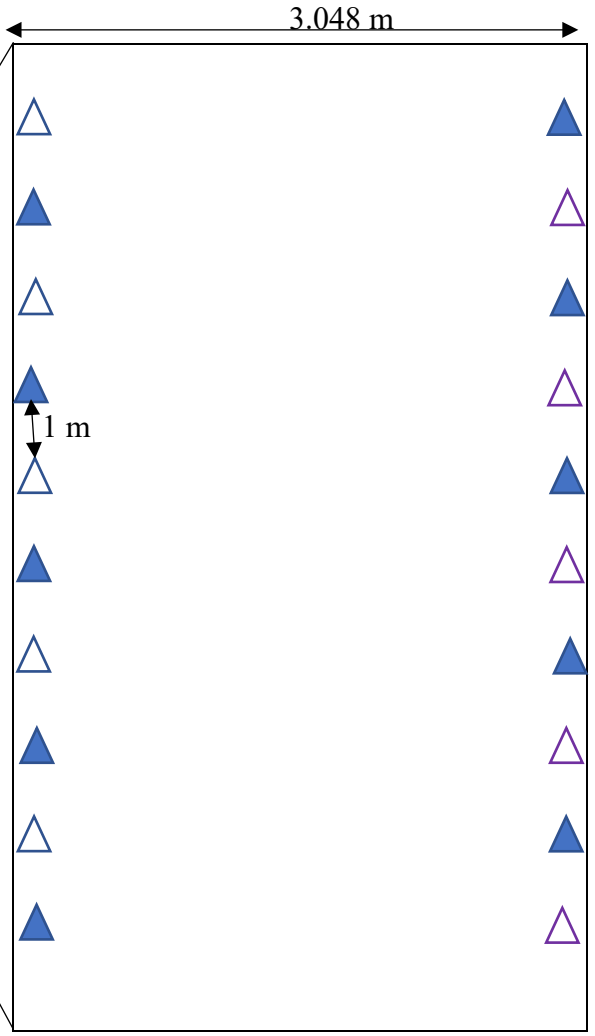


Figure 4.2. Post-thinning field layout of tree arrangement and distance between them in each treatment plot. Unfilled purple triangles indicate inoculated trees, unfilled blue traingles indicate control trees, and solid blue traingles indicate other trees.

4.3.2 Experiment design and inoculation treatment

Two preliminary small-scale experiments were conducted before inoculating the trees near Eufaula, Alabama primarily, to (i) select an appropriate virulent fungal isolate of *L. terebrantis* and (ii) to determine the levels of low, medium, and high inoculation treatment. To select the most virulent *L. terebrantis* isolate from 42 isolates, an extensive seedling inoculation experiment was done according to Devkota & Eckhardt (2018). From the study, ATCC accession no. MYA-3316 was found as the most virulent *L. terebrantis* isolate to loblolly pine in comparison to 41 other isolates.

To identify the levels of inoculation treatment, a preliminary field inoculation experiment was carried out at the Solon Dixon Forestry Education Center, Andalusia, Alabama. Following the sterilization of toothpicks at 121°C and 0.103 MPa for 60 minutes, *L. terebrantis* isolate (ATCC accession no. MYA-3316) was grown in toothpicks imbibed with malt extract agar for approximately 24 days at 23°C in the dark (Devkota et al., 2018).

Figure 4.3. Toothpicks with *Leptographium terebrantis* inserted at 16 radial points of four rows in loblolly pine saplings at the Solon Dixon Center, Andalusia, Alabama. Toothpicks were clipped to facilitate covering the inoculation zone with a duct tape.

The inoculation treatment of trees near Eufaula, Alabama was carried out on March 13 and 14, 2017. The treatments were applied in accordance with Devkota et al. (2018), and based on the response of loblolly pine to different densities of virulent *L. terebrantis* from the Andalusia, Alabama study (Devkota et al., 2018).

Figure 4.4. Toothpicks with *Leptographium terebrantis* inserted in loblolly pine tree at the study site near Eufaula, Alabama.

The treatments for the study were: control (no inoculum or wound), wound (no inoculum), low inoculation, medium inoculation, and high inoculation. Five randomly chosen trees in one plantation row of each pair-lined plot received one of the five inoculation treatments. Control trees were left untouched. To apply rest of the treatments, selected trees were wrapped in plastic transparencies before drilling the holes. Plastic transparencies were marked with pre-determined inoculation points for different inoculation treatments with a permanent marker. A 5 mm deep hole was drilled at each predetermined inoculation point. The holes were drilled perpendicular to the surface of the stem using a sterilized 1.5 mm drill bit. One toothpick was inserted per hole. The toothpick insertion method simulated fungal transfer from maturation feeding activities of root-feeding bark beetles. Toothpicks were left inserted, clipped down to the bark, and covered with duct tape. Wounded trees had one fungus-free toothpick inserted per 1.2 cm ground-line diameter. Trees that received low, medium, or high inoculation treatments had one toothpick infected with *L. terebrantis* inserted per 10.0 cm, 2.4 cm, and 1.2 cm of ground-line diameter, respectively. The inoculation points were radially equidistant from each other. Each inoculation point was replicated 4 times vertically and equally spaced.

4.3.3. Root growth study

4.3.3.1. Tube installation

Understory vegetation was removed manually to facilitate easier root tube installation and to reduce the interference of roots of competing vegetation with pine root growth. Clear acrylic tubes were chosen for installation as they have higher transparency and durability, and less effect on pigmentation and survival rates of white roots compared to other plastics, e.g. butyrate (Withington et al., 2003). For the minirhizotron tube placement, four trees (2 inoculated trees and 2 control trees) with a comparable diameter at breast height (dbh) were chosen in each plot (Figure 4.5) to minimize the influence of tree size on root growth. The tubes were installed on plots 1 and 2 on October 25, 2016, plots 3 and 8 on October 26, 2018 and on plots 7, 5 and 8 on October 27, 2016. Tubes were installed on the remaining eight plots on November 17 and 22, 2016.

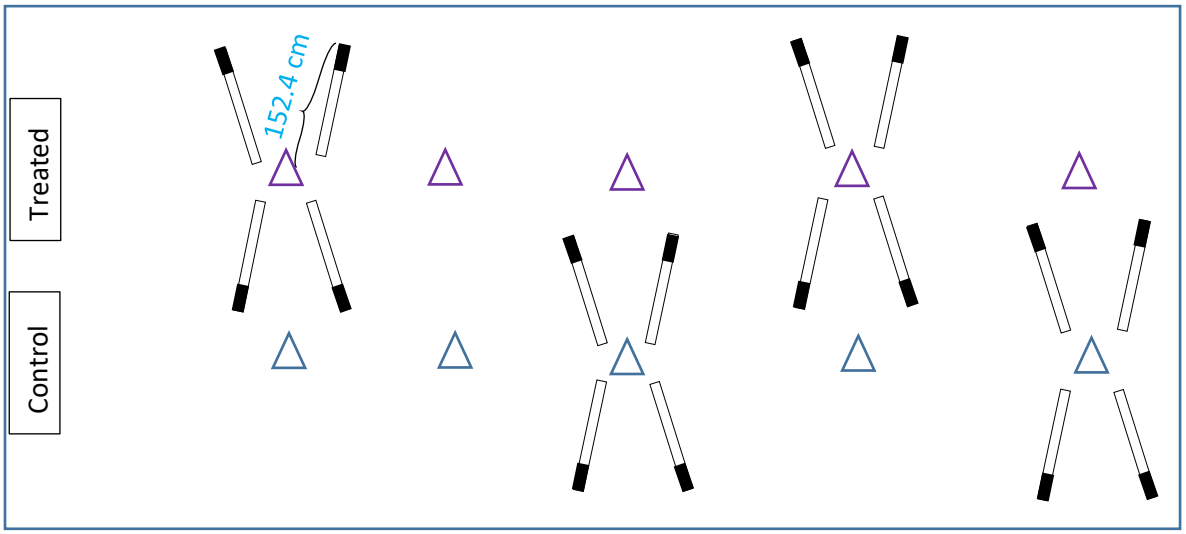
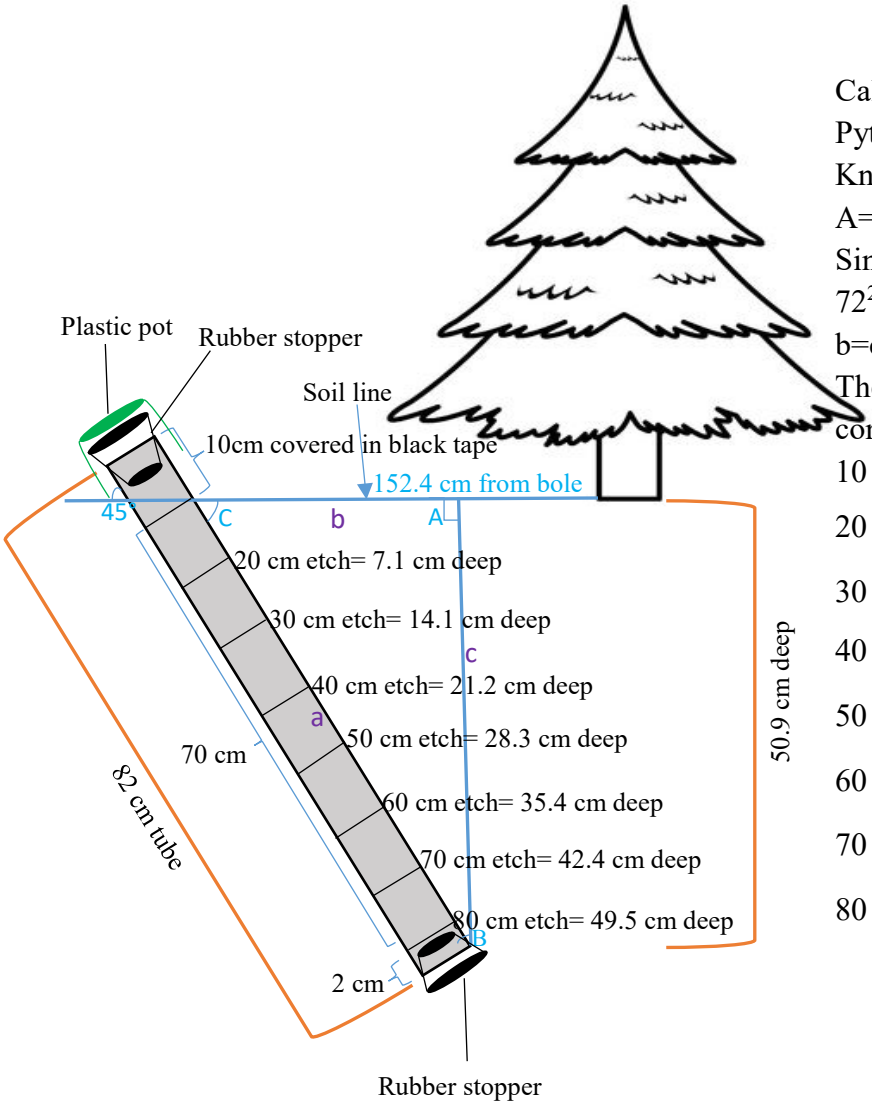


Figure 4.5. Field layout of minirhizotron tubes in each plot. The distance from the upper portion of the tube to the base of the tree is 152.4 cm. Unfilled purple triangles indicate inoculated trees and unfilled blue traingles indicate control trees.

Two pairs of holes were drilled on either side of each tree, outside the planting row, and 152.4 cm away from the base of the bole. Augured hole were drilled toward the tree at a 45° angle relative to the soil surface with a drill bit, extension bar, and gas powered auger. Prepared tubes were inserted into the augured holes (Figure 4.5, Figure 4.6). Each tube was 82 cm in length and had a 3.81 cm inner diameter. Their circumference was scored at eight 10 cm increments with 10 cm of tube length beyond the first scored line on the upper end of the tubes, and 2 cm of the tube length beyond the scored line on the bottom end of the tubes. Before installation, the end of the tube that went into the ground was sealed by a rubber plug to prevent water from seeping into the tube. Scored lines corresponded to 0, 7.1, 14.1, 21.2, 28.3, 35.4, 42.4 and 49.5 cm below the ground level after tube installation. To prevent light from entering the tube, the 10 cm length at the upper end of the tube was covered in black tape (Figure 4.7).

Figure 4.6. (i) Drilling holes using an extension bar and drill bit, **(ii)** Numbers (1-4) indicate the point where tubes were installed around a single tree.

Once installed, the taped end protruding from the soil surface was plugged with a black, size 7 rubber stopper, which was 25 mm in length and had a 37 mm top diameter and a 30 mm bottom diameter (VWR Catalog no. 59580-262). The stopper was used to prevent the entry of dust, water, and insects inside the tube. The tubes were then covered with a green plastic pot of 17.8 cm bottom diameter (Kord Plastics, Toronto, Canada) (Figure 4.7). The tube insertion areas were redressed with pine straw to avoid interference with insect activity and prevent erosion.



Calculation according to the Pythagorean Theorem:
Knowns:
A=90°, B=C=45°, a=72 cm
Since, $a^2=b^2+c^2$
$72^2=2b^2$
b=c=50.9 cm
Therefore, belowground depth corresponding to:
10 cm etch=0 cm
20 cm etch=$50.9-\sqrt{(\frac{62^2}{2})}=7.1$ cm
30 cm etch=$50.9-\sqrt{(\frac{52^2}{2})}=14.1$ cm
40 cm etch=$50.9-\sqrt{(\frac{42^2}{2})}=21.2$ cm
50 cm etch=$50.9-\sqrt{(\frac{32^2}{2})}=28.3$ cm
60 cm etch=$50.9-\sqrt{(\frac{22^2}{2})}=35.4$ cm
70 cm etch=$50.9-\sqrt{(\frac{12^2}{2})}=42.4$ cm
80 cm etch=$50.9-\sqrt{(\frac{2^2}{2})}=49.5$ cm

Figure 4.7. Field layout of minirhizotron tubes showing the belowground depth corresponding to each scored line on the tubes.

4.3.3.2. Root growth measurements

New root (<2mm diameter) measurements started approximately six months after minirhizotron root tube installation and one month post-inoculation. Root growth measurements started on April 13, 2017, and were repeated approximately at 2-month intervals until February 10, 2019. Root initiation was monitored using an optical root periscope (JRD Merrill Speciality Equipment, Logan, UT) that had a fiber optic light powered by a battery. Specifically, for each of the eight scored lines around the circumference of root tubes, numbers of pine root intersections were counted. Loblolly pine roots were visually identified and differentiated from the roots of other plants on the basis of color (white when first visible which changed to reddish-brown and then to brown as the root aged) (Sword et al., 2000), diameter, branching pattern, lifespan, and presence of ectomycorrhizal infection on distal root tips (Pregitzer et al., 2002, Sword & Haywood, 2006).

Figure 4.8. (**i)** Observing root intersections with an optical periscope. The black rectangular object on the ground is a battery which is connected to the periscope to power the light, **(ii)** the faint line noted by the red arrow is a new root.

Root data were expressed as root length density (RLD) according to the method described by Newman (1996). The Newman equation, $R= (\pi \times N \times A)/ (2 \times H)$ was applied to the new root data, where R is the total root length (cm), N is the number of root intersections with

scored lines, A is the area of tube being assessed, and H is the length of the scored line which is the tube circumference. RLD was calculated as: RLD=R/A, where RLD is root length density (cm/cm^2), R is root length (cm), and A is the area of tube being assessed.

4.3.4. Ectomycorrhizae study

4.3.4.1. Ectomycorrhizae sampling

Soil samples for assessing ectomycorrhizal colonization were collected on September 21 and 27, 2017. Ten soil cores, 6 cm × 50 cm, were removed from each plot with a pneumatic soil core sampler (Figure 4.9). The cores were taken from the planting row approximately 76 cm from either side of the stem of the five intensively measured treated trees in each treatment plot (Figure 4.10). The samples were sealed, placed in a cooler, and transported to the USDA Forest Service, Auburn, Alabama where they were stored at 4°C until being processed.

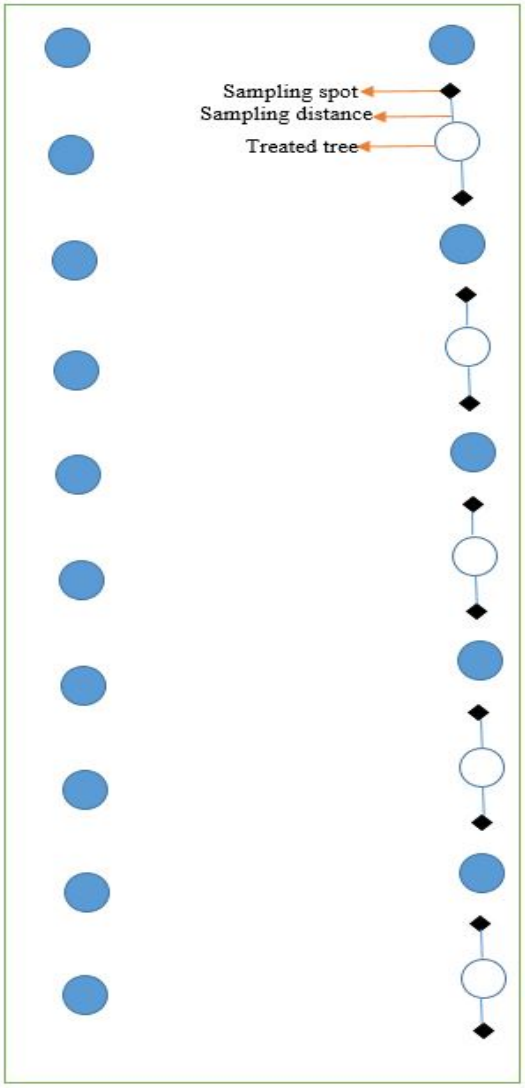


Figure 4.9. Field protocol for ectomycorrhizae sampling. Diamond shapes indicate points of soil core collection from either side of treatment trees in each plot.

Figure 4.10. Soil core with approximately 50 cm deep soil sample.

4.3.4.2. Ectomycorrhizae Processing

Each soil core was cut into 10 cm increments. From our previous minirhizotron observations on April-August, 2017, we found that an insignificant amount of new roots were present in the top 10 cm of the soil. In most plant species, the presence of mycorrhizae is related to the abundance of roots and this suggests that few ectomycorrhizae were present at the 0-10 cm depth (Jentschke & Godbold, 2001). Additionally, there was a lot of debris that interfered with the wet sieving of surface soil. Hence, the top 10 cm of each soil sample was discarded from the analysis. Using a soil sieve with 0.5 mm openings (No. 35), roots were washed free of soil by running tap water (Horton & Bruns, 1998; Roberts & Anderson, 2001) in the Forest Health Dynamics Laboratory (FHDL), Auburn University, Auburn, Alabama. The washed roots were compostited by depth (10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm) in each treatment plot for a total of 60 samples. Composited samples were stored in Ziploc bags at 4ºC until being processed.

To assess the percentage of fine root colonization by ectomycorrhizal fungi, one hundred 1 cm root segments were randomly selected from each of the 60 composited root samples (4 depth intervals × 15 plots). When 100 1-cm root segments were not available in a sample, all the roots were analyzed. Roots were cleared with 10% KOH, rinsed and stored in 50% glycerol until being analyzed (Brundrett et al., 1996). Ectomycorrhizal and non-ectomycorrhizal root tips were visually identified and quantified with a dissecting microscope at a magnification of 10-40X, and using the gridline intercept method (Brundrett et al., 1996). The percentage ectomycorrhizal colonization was calculated according to Danielsen et al. (2013) as:

EC (%) = (ET / ENT) × 100, where, EC is percentage ectomycorrhizal colonization, ET is number of ectomycorrhizal root tips, and ENT is the number of ectomycorrhizal plus non-ectomycorrhizal root tips.

4.3.5. Statistical analysis

4.3.5.1 New root growth

Initially, per-tube data were analyzed to check if the minirhizotron tubes were behaving normally. The tubes that had no root counts at any depth could not be used and therefore were deleted during the analysis. From a total of 240 tubes, 120 and 115 tubes were deleted during the month of February 2018 and month of February 2019 analyses, respectively. The tubes were deleted either due to death of the tree, an absence of fine roots at any depths, or due to the presence of water inside the tube. The tubes that were tight or had a tight rubber stopper which obstructed periscope insertion were also deleted (Table 4.1). From the remaining tubes, the legitimate outliers were deleted on a tube-by-tube basis during each measurement date. The scatterplot function of MS Excel was used to plot the data of each tube by depth. Observation of an abnormally high number of root counts at a certain depth was determined as an outlier and removed before data analysis.

The statistical analyses were completed using SAS version 9.4 (SAS Institute Inc. 2010, Cary, NC). For each measurement date, a two-way ANOVA (PROC GLM) was used to test if the cumulative RLD among trees that received different inoculation treatments varied significantly at different depths (Table 4.2). Cumulative RLD was averaged by inoculation treatment and measurement interval and also by inoculation treatment and depths to test the variation in cumulative RLD among depths and measurement dates, respectively by one-way ANOVA (PROC GLM). The differences among treatments and depths were considered significant at $P \leq 0.05$ unless otherwise noted. Tukey's Multiple Range test (PROC GLM) was used to compare the means of inoculation treatments, across depths, and measurement dates.

Net RLD among measurement dates were calculated by the difference between pairs of data points in two consecutive measurement intervals. Since, we took measurements bimonthly, RLD differences were divided by 2 and reported as cm cm^{-2} $month^{-1}$. Negative net RLD values were reported as 0.

The cumulative RLD of untreated trees (Figure 4.5, unfilled blue symbols) was compared with that of treated trees (Figure 4.5, unfilled purple symbols) within the treatment plots by a paired *t*-test (PROC TTEST). The trees that received low inoculation treatment in plot 4 were dead due to *Ips* sp. infestation. So, all plot 4 data was excluded while performing the paired *t*-test. The cumulative RLD of treated trees and control trees were averaged by depth within each treatment plots prior to the analysis.

4.3.5.2. Ectomycorrhizal colonization

Initially, a two-way ANOVA (PROC GLM) was used to test if ectomycorrhizal colonization among trees varied significantly by inoculation treatment, soil depth, or interaction between inoculation treatment and soil depth. These results and soil chemical properties results in Chapter 2 indicated that soil chemical properties may have impacted the response variable. As a result, soil pH (pH_{salt} from Chapter 2) was used in correlation (PROC CORR) analyses to determine the relationship between ectomycorrhizal colonization and soil pH at different soil depths. Correlation analyses were conducted among average ectomycorrhizal colonization and soil pH at 10-50 cm (4 depth intervals), and then by depth interval (10-20 cm, 20-30 cm, 30-40 cm, and 40-50 cm).

Because ectomycorrhizal colonization was significantly correlated with soil pH, soil pH was used as a covariate during data analysis to increase the precision in determining the effect of inoculation treatment on ectomycorrhizal colonization. A two-way ANCOVA (PROC GLM)

with soil pH as the covariate was used to test the main effects of inoculation treatment and soil depth, and the interaction effect of treatment × depth on ectomycorrhizal colonization. The correlations, differences among treatments, and across depths were considered significant at $P \leq 0.05$ unless otherwise noted. Tukey's Multiple Range test (PROC GLM) was used to compare the means of treatments.

4.4. Results

4.4.1. Stand environment and new root growth

Stand environment data from our on-site weather station for July 2018, August 2018, December 2018, January 2019, and February 2019 were not available and therefore are not included in comparisons between the climate during this study and average climatic conditions over the past 25 years. Compared to the 25-year average between 1994 and 2018 (NOAA, 2019) of Barbour County, Alabama, precipitation and air temperature from March 2017 through November 2018 were reduced by 19.8% and increased by 0.7%, respectively. The majority of this rainfall deficit occurred during the period of less root growth between October 2017 and February 2018 when precipitation was 50.6% less than normal (Figure 4.11).

From a total of 240 minirhizotron tubes, 120 and 125 tubes were used during the month of February 2018 and the month of February 2019 analyses, respectively (Table 4.1). During each measurement date between April 2017 and February 2019, cumulative RLD was significantly affected by depth (Table 4.2). Cumulative RLD in June 2017 was significantly different among treatments with a significantly higher value associated with trees that received the medium inoculation treatment compared to the low (P=0.0165) or high inoculation treatments (P=0.0278) (Table 4.2, Figure 4.12i). Cumulative RLD in February 2019 was significantly different among treatments with a significantly higher value associated with

control trees compared to the wound (P=0.0222) or high inoculation treatments (P=0.0379) (Table 4.2, Figure 4.12ii). A significant treatment × depth interaction was observed during June 2018 ($P \leq 0.05$) and December 2018 ($P \leq 0.1$) measurements (Table 4.2, Figure 4.13).

New roots were not observed at the soil surface during April 2017, June 2017, August 2017, and February 2019. During April 2017, June 2017, and October 2017, maximum cumulative RLD was observed at the 14.1 cm depth. During August 2017, December 2017, February 2018, April 2018, October 2018, and December 2018, maximum cumulative RLD was observed at the 28.3 cm depth. During December 2017, June 2018, August 2018, and February 2019, maximum cumulative RLD was observed at the 35.4 cm depth (Figure 4.14, Figure 4.15). Statistically significant differences in cumulative RLD among depths during each measurement date from April 2017 to February 2019 are shown in figures 4.14 and 4.15 and presented in tables from 4.3 to 4.6.

The sum of RLD among depths, averaged by inoculation treatment, varied significantly across measurement dates (F=10.84, P<0.0001), with peaks in October 2017 and 2018. These RLD values increased from April to October 2017 and again from February to October 2018 respectively. A plateau in cumulative RLD took place from June 2018 to February 2019 representing the return of root system to an equilibrium condition (Figure 4.16i). During 2017 and 2018, net RLD decreased from June to August in addition to October 2017 to February 2018 and October 2018 to February 2019 (Figure 4.16ii).

When averaged by inoculation treatment and measurement interval, cumulative RLD varied significantly across depths (F=66.32, P<0.0001). Differences across depths are shown in Figure 4.17, and statistically significant differences between depth pairs are presented in Table 4.7. Minimum cumulative RLD was observed at the soil surface and maximum cumulative RLD

was observed at the 28.3 and 35.4 cm depths with a gradual increase in cumulative RLD from 0 cm to the 28.3 cm depth and then a gradual decrease in cumulative RLD from 35.4 cm to the 49.5 cm depth (Figure 4.17).

Except for the within-plot comparison of wound and control trees in February 2019, the difference in cumulative RLD was not significant for within-plot comparisons of treated and control trees (Table 4.8). In February 2019, a significantly lower value of cumulative RLD was associated with wound trees compared to the control trees (P=0.0045) within the treatment plot (Figure 4.18).

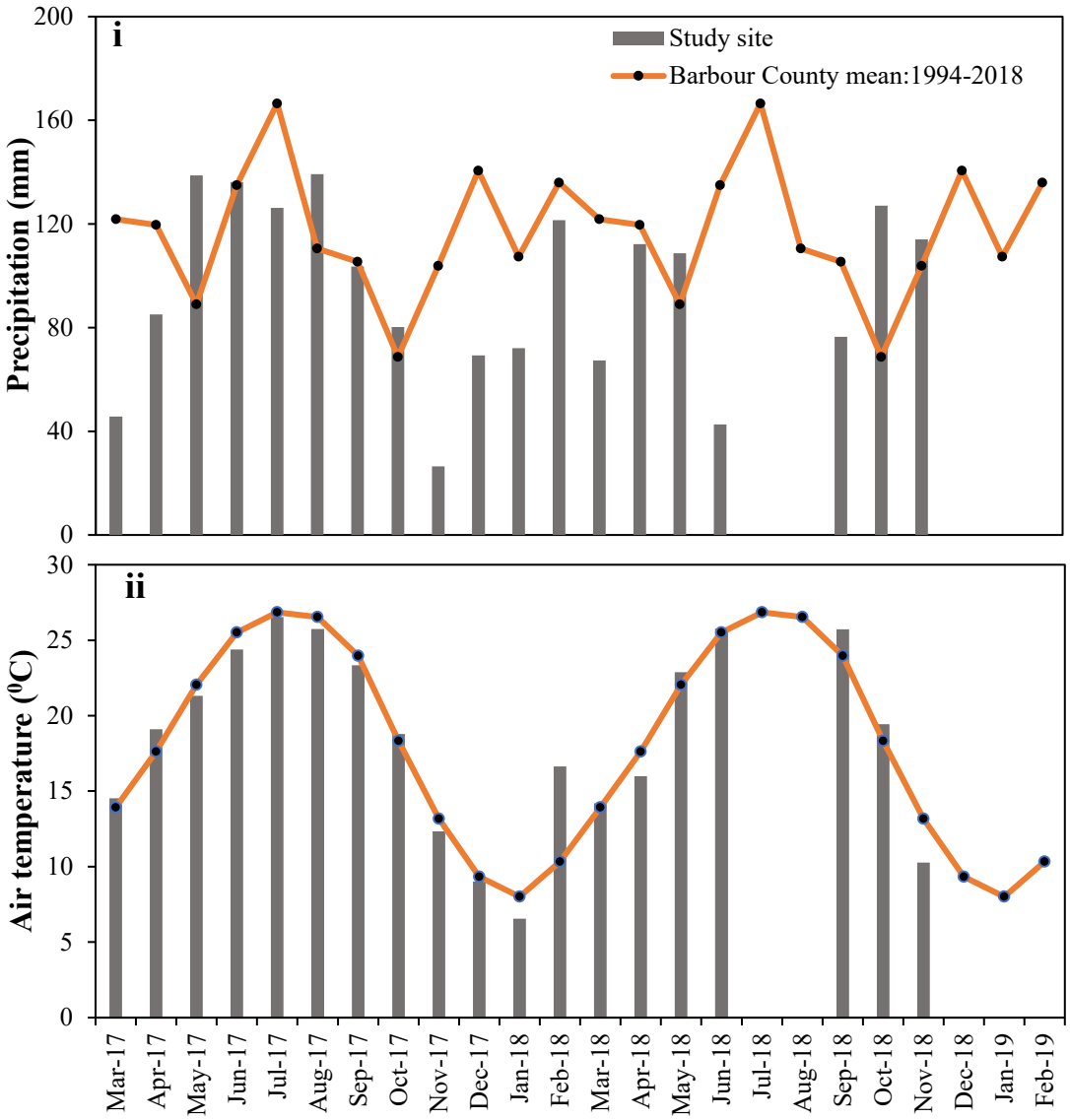


Figure 4.11. (i) Mean monthly precipitation (mm) between 1994 and 2018 in Barbour County, Alabama and total monthly precipitation between March 2017 and November 2018 in a mature loblolly pine stand near Eufaula, Barbour County, Alabama, and **(ii)** Mean monthly air temperature (^{0}C) between 1994 and 2018 in Barbour County, Alabama and monthly temperature (^{0}C) between March 2017 and November 2018 in a mature loblolly pine stand near Eufaula, Barbour County, Alabama. Data were not collected in July 2018, August 2018, December 2018, January 2019 and February 2019.

Table 4.1. Number of minirhizotron tubes used and deleted during analyses at the end of the first and second year of the new root growth study in a stand of mature loblolly pine.

Time	Minirhizotron tubes			
	Total	Used for analysis	Deleted	Cause for deletion
February 2018	240	120	120	No roots at any depth (109 tubes) Two dead trees (8 tubes) Stuck rubber stopper (3 tubes)
February 2019	240	125	115	No roots at any depth (98 tubes) Two dead trees + one tree harvested (12 tubes) Stuck rubber stopper (4 tubes) Water in the tube (1 tube)

Table 4.2. Probabilities of a greater *F*-value from a two-way ANOVA for the cumulative root length density of a mature loblolly pine near Eufaula, Alabama after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Time	Effect	df	*F*-value	*P>F*
April 2017	Treatment (T)	4	0.66	0.6173
	Depth (D)	7	5.02	<0.0001
	T×D	28	0.57	0.9630
June 2017	Treatment (T)	4	3.40	0.0091
	Depth (D)	7	6.35	<0.0001
	T×D	28	0.82	0.7328
August 2017	Treatment (T)	4	1.77	0.1329
	Depth (D)	7	10.36	<0.0001
	T×D	28	1.00	0.4715
October 2017	Treatment (T)	4	1.92	0.1052
	Depth (D)	7	2.73	0.0084
	T×D	28	1.13	0.2947
December 2017	Treatment (T)	4	0.96	0.4307
	Depth (D)	7	3.29	0.0019
	T×D	28	1.14	0.2846
February 2018	Treatment (T)	4	0.82	0.5147
	Depth (D)	7	3.27	0.0021
	T×D	28	0.99	0.4765
April 2018	Treatment (T)	4	0.32	0.8616
	Depth (D)	7	4.55	<0.0001
	T×D	28	0.84	0.7040

June 2018	Treatment (T)	4	1.15	0.2864
	Depth (D)	7	5.57	<0.0001
	T×D	28	1.50	0.0396
August 2018	Treatment (T)	4	1.19	0.3140
	Depth (D)	7	5.16	<0.0001
	T×D	28	1.01	0.4524
October 2018	Treatment (T)	4	1.73	0.1406
	Depth (D)	7	5.19	<0.0001
	T×D	28	1.35	0.1106
December 2018	Treatment (T)	4	1.21	0.3055
	Depth (D)	7	6.53	<0.0001
	T×D	28	1.38	0.0954
February 2019	Treatment (T)	4	4.01	0.0032
	Depth (D)	7	4.53	<0.0001
	T×D	28	0.79	0.7781

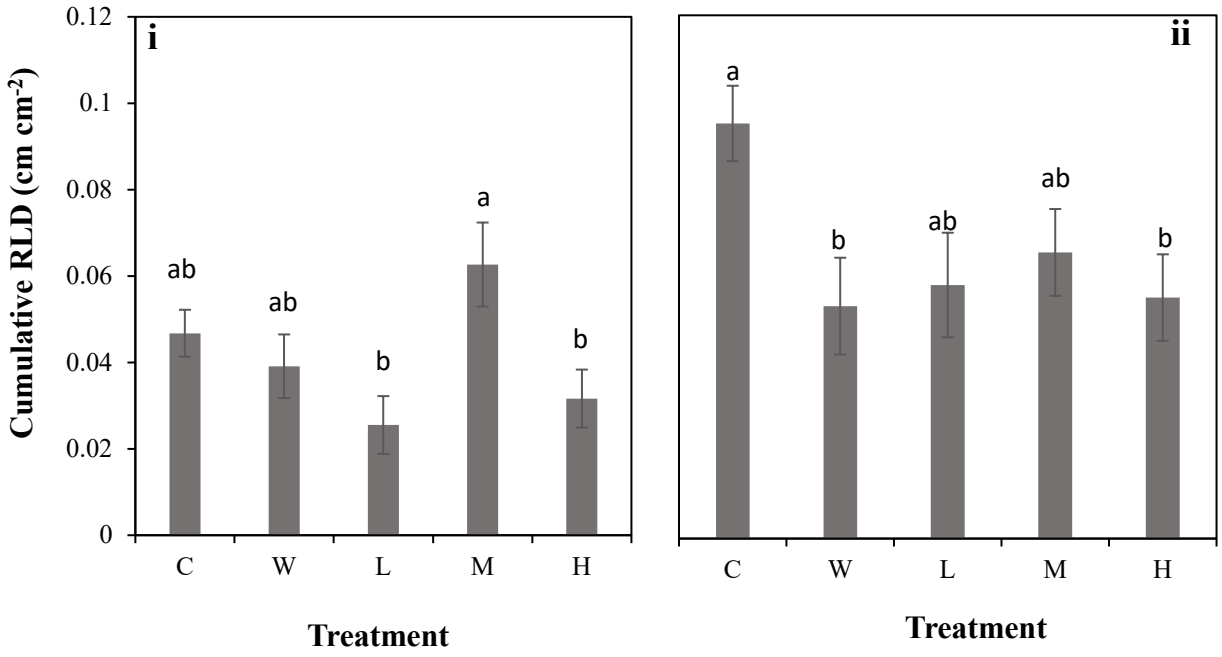


Figure 4.12. Average cumulative root length density (RLD) among treatments during the **(i)** June 2017 and **(ii)** February 2019 measurement intervals. Treatments were control (C), wound (L), low (L), medium (M), and high (H) inoculation. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

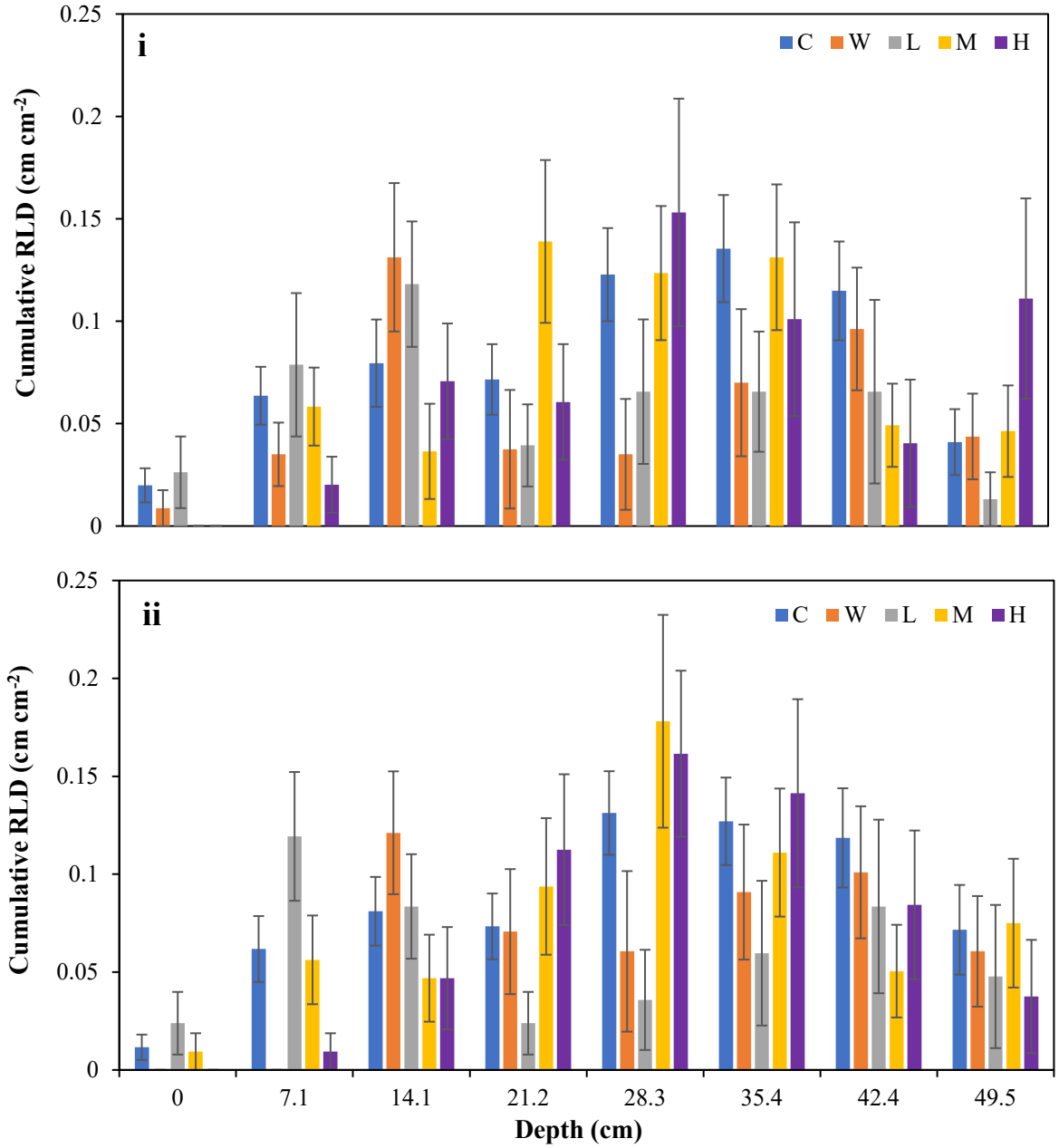


Figure 4.13. Average cumulative root length density (RLD) among treatments at different depths during the **(i)** June 2018 and **(ii)** December 2018 measurement intervals. Treatments were control (C), wound (L), low (L), medium (M), and high (H) inoculation. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

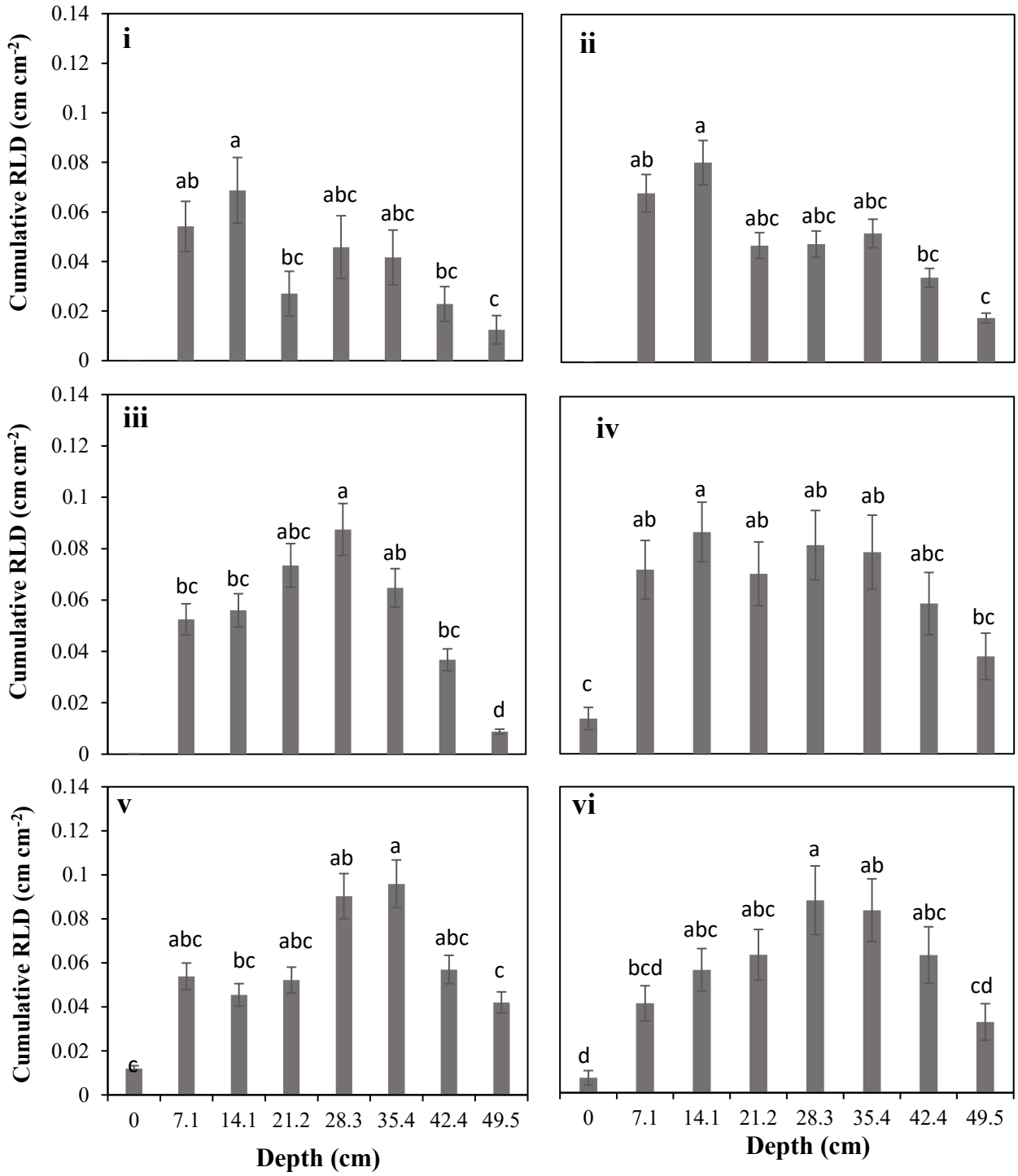


Figure 4.14. Average cumulative root length density (RLD) by depths during the **(i)** April 2017, **(ii)** June 2017, **(iii)** August 2017, **(iv)** October 2017, **(v)** December 2017, and **(vi)** February 2018 measurement intervals. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

Table 4.3. *P*-value for significantly different cumulative root length densities among depths of a mature loblolly pine stand near Eufaula, Alabama during April 2017, June 2017, and August 2017 after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Depth pairing			April 2017	June 2017	August 2017
7.1 cm	vs.	49.5 cm	0.0424	0.0506	0.0194
14.1 cm	vs.	21.2 cm	0.0424		
14.1 cm	vs.	42.4 cm	0.0160	0.0141	
14.1 cm	vs.	49.5 cm	0.0009	0.0007	0.0564
21.2 cm	vs.	49.5 cm			0.0004
28.3 cm	vs.	7.1 cm			0.0123
28.3 cm	vs.	14.1 cm			0.0478
28.3 cm	vs.	42.4 cm			0.0001
28.3 cm	vs.	49.5 cm			<0.0001
35.4 cm	vs.	49.5 cm			0.0067

Table 4.4. *P*-value for significantly different cumulative root length densities among depths of a mature loblolly pine stand near Eufaula, Alabama during October 2017, December 2017, and February 2018 after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Depth pairing			October 2017	December 2017	February 2018
7.1 cm	vs.	0 cm	0.0167		
14.1 cm	vs.	0 cm	0.0006		0.0423
14.1 cm	vs.	49.5 cm	0.0824		
21.2 cm	vs.	0 cm	0.0228		0.0106
28.3 cm	vs.	0 cm	0.0025	<0.0001	<0.0001
28.3 cm	vs.	7.1 cm			0.0653
28.3 cm	vs.	49.5 cm		0.0651	0.0125
35.4 cm	vs.	0 cm	0.0044	<0.0001	<0.0001
35.4 cm	vs.	14.1 cm		0.0430	
35.4 cm	vs.	49.5 cm		0.0228	0.0306
42.2 cm	vs.	0 cm			0.0117

Significance tested at $P< 0.05$ and $P<0.1$ by Tukey's Multiple Range test

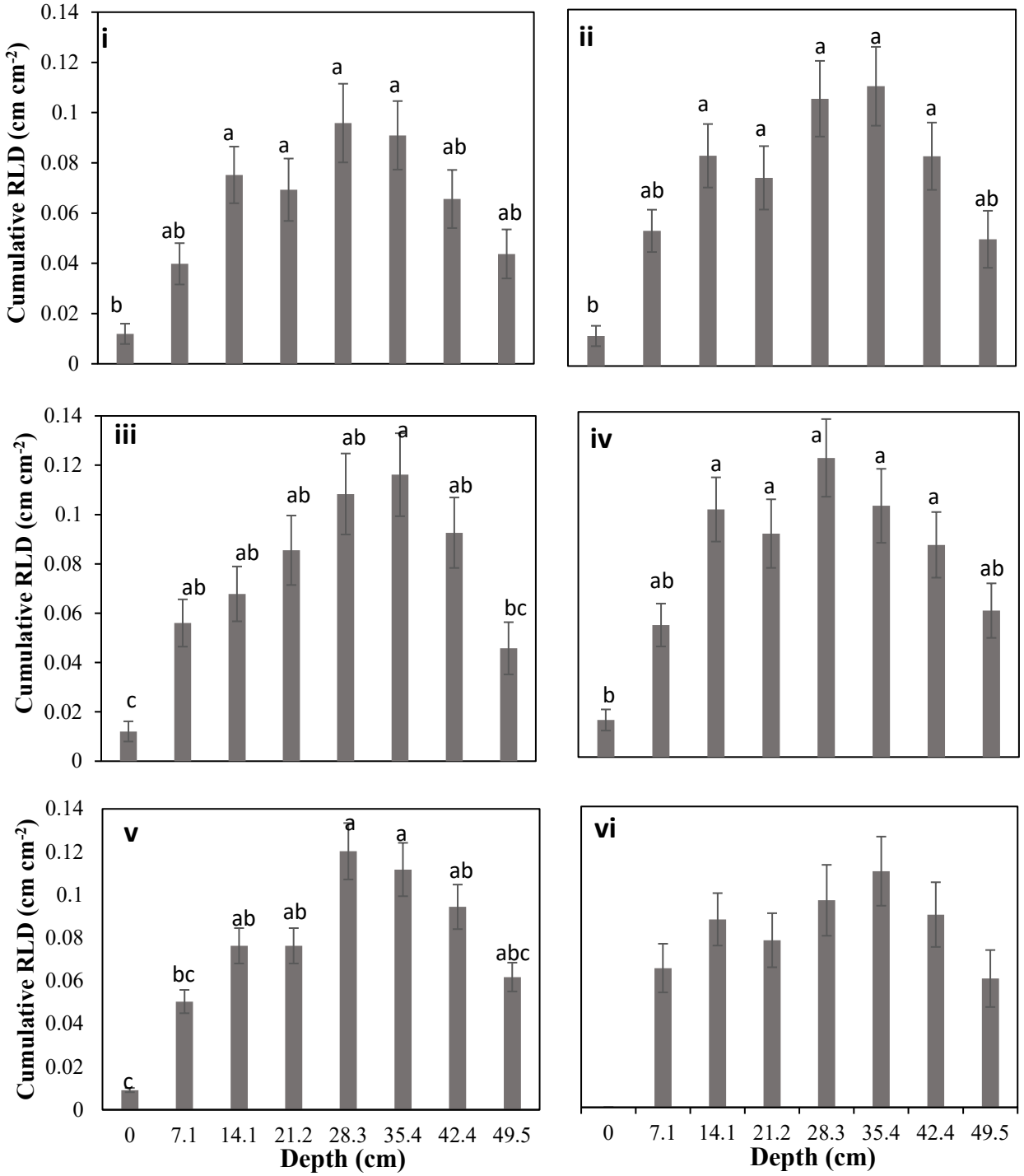


Figure 4.15. Average cumulative root length density (RLD) by depths during the **(i)** April 2018, **(ii)** June 2018, **(iii)** August 2018, **(iv)** October 2018, **(v)** December 2018, and **(vi)** February 2019 measurement intervals. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

Table 4.5. *P*-value for significantly different cumulative root length densities among depths of a mature loblolly pine stand near Eufaula, Alabama during April 2018, June 2018, and August 2018 after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Depth pairing			April 2018	June 2018	August 2018
14.1 cm	vs.	0 cm	0.0047	0.0006	0.0653
21.2 cm	vs.	0 cm	0.0180	0.0263	0.0060
28.3 cm	vs.	0 cm	0.0005	<0.0001	0.0008
35.4 cm	vs.	0 cm	0.0010	<0.0001	<0.0001
35.4 cm	vs.	49.5 cm			0.0163
42.2 cm	vs.	0 cm		0.0135	0.0027

Table 4.6. *P*-value for significantly different cumulative root length densities among depths of a mature loblolly pine stand near Eufaula, Alabama during October 2018 and December 2018 after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Depth pairing			October 2018	December 2018
14.1 cm	vs.	0 cm	0.0002	0.0071
21.2 cm	vs.	0 cm	0.0039	0.0088
28.3 cm	vs.	0 cm	<0.0001	<0.0001
28.3 cm	vs.	7.1 cm		0.0129
35.4 cm	vs.	0 cm	0.0005	<0.0001
35.4 cm	vs.	7.1 cm		0.0488
42.2 cm	vs.	0 cm	0.0043	0.0006

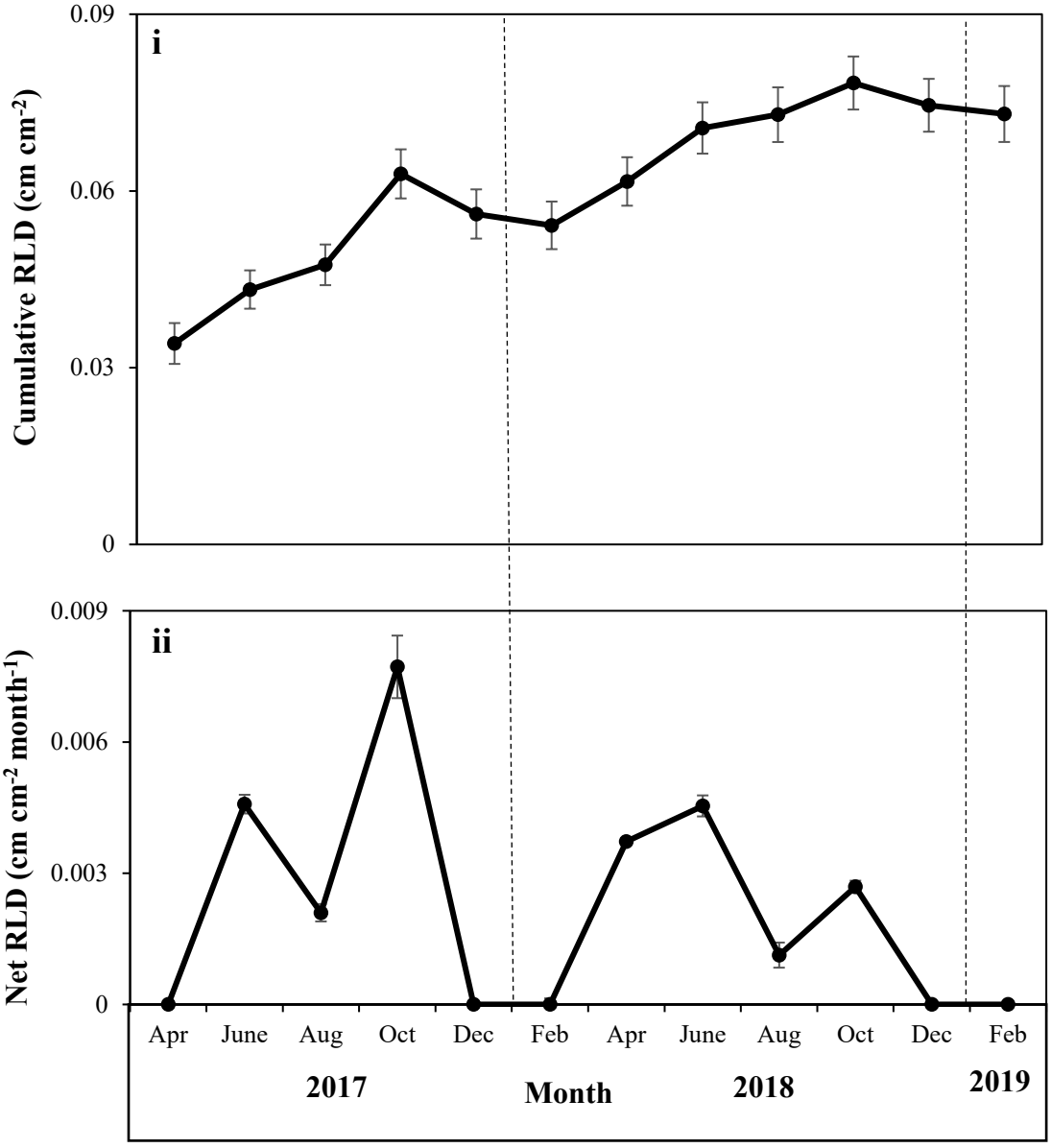


Figure 4.16. Average **(i)** cumulative root length density (RLD) and **(ii)** net root length density (RLD) of mature loblolly pine from April 2017 through February 2019 at the 0 cm to 49.5 cm depth. Error bars represent the standard error of the mean.

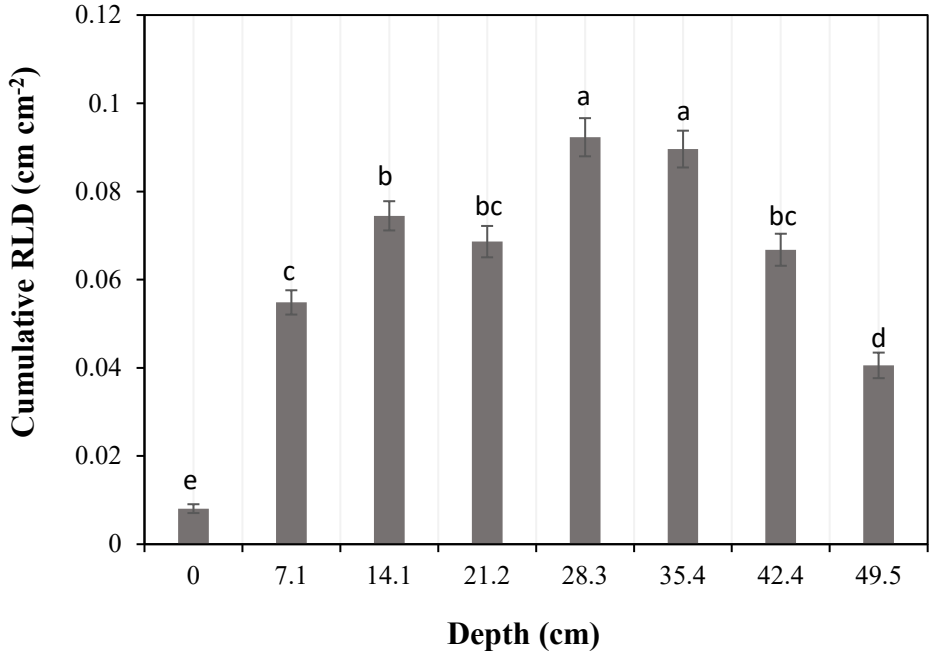


Figure 4.17. Average cumulative root length density (RLD) of a mature loblolly pine at different depths during the study period from April 2017 through February 2019. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

Table 4.7. *P*-value for significantly different cumulative root length densities among depths of a mature loblolly pine stand near Eufaula, Alabama during the study period from April 2017 through February 2019 after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Depth pairing			P-value
7.1 cm	vs.	0 cm	<0.0001
7.1 cm	vs.	49.5 cm	0.0560
14.1 cm	vs.	0 cm	<0.0001
14.1 cm	vs.	7.1 cm	0.0010
14.1 cm	vs.	49.5 cm	<0.0001
21.2 cm	vs.	0 cm	<0.0001
21.2 cm	vs.	49.5 cm	<0.0001
28.3 cm	vs.	0 cm	<0.0001
28.3 cm	vs.	7.1 cm	<0.0001
28.3 cm	vs.	14.1 cm	0.0049
28.3 cm	vs.	21.2 cm	<0.0001
28.3 cm	vs.	42.4 cm	<0.0001
28.3 cm	vs.	49.5 cm	<0.0001
35.4 cm	vs.	0 cm	<0.0001
35.4 cm	vs.	7.1 cm	<0.0001
35.4 cm	vs.	14.1 cm	0.0333
35.4 cm	vs.	21.2 cm	0.0003
35.4 cm	vs.	42.4 cm	<0.0001
35.4 cm	vs.	49.5 cm	<0.0001
42.4 cm	vs.	0 cm	<0.0001
42.4 cm	vs.	49.5 cm	<0.0001
49.5 cm	vs.	0 cm	0.0230

Table 4.8. Within-plot comparison of cumulative root length density by paired *t*-test for mature loblolly pine near Eufaula, Alabama after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.The cumulative root length density of trees that received the control (C) treatment was compared with the cumulative root length density of trees that received either wound (W), low (L), medium (M), or high (H) inoculation treatments.

Date	Treatment-Control pair	df	*t*-value	*P*>|*t*|
April 2017	W-C	23	-0.140	0.1739
	L-C	15	-1.34	0.2004
	M-C	23	0.36	0.7187
	H-C	23	-0.26	0.7944
June 2017	W-C	23	-1.54	0.1379
	L-C	15	-1.37	0.1896
	M-C	23	1.23	0.2327
	H-C	23	-1.28	0.2122
August 2017	W-C	23	-0.71	0.4867
	L-C	15	0.71	0.4860
	M-C	23	1.11	0.2793
	H-C	23	0.71	0.4860
October 2017	W-C	23	-0.149	0.1490
	L-C	15	-0.80	0.4366
	M-C	23	1.79	0.0865
	H-C	23	-1.82	0.0826
December 2017	W-C	23	-1.27	0.2168
	L-C	15	-0.87	0.3965
	M-C	23	0.74	0.4695
	H-C	23	-0.23	0.8188
February 2018	W-C	23	-0.06	0.9543
	L-C	15	-0.00	1.0000
	M-C	23	1.53	0.1384
	H-C	15	0.52	0.6104
April 2018	W-C	23	-0.81	0.4267
	L-C	15	1.47	0.1623
	M-C	23	0.07	0.9418
	H-C	23	1.01	0.3222
June 2018	W-C	23	-1.61	0.1212
	L-C	15	1.47	0.1623
	M-C	23	0.65	0.5199
	H-C	23	0.20	0.8453

August 2018	W-C	23	-1.43	0.1661
	L-C	15	-0.12	0.9030
	M-C	23	1.03	0.3123
	H-C	23	1.60	0.1235
October 2018	W-C	23	-0.22	0.8313
	L-C	15	1.16	0.2648
	M-C	23	0.21	0.8366
	H-C	23	1.24	0.2267
December 2018	W-C	23	-1.03	0.3125
	L-C	15	0.14	0.8915
	M-C	23	1.61	0.1205
	H-C	23	1.48	0.1516
February 2019	W-C	23	-3.15	0.0045
	L-C	15	0.98	0.3422
	M-C	23	0.94	0.3570
	H-C	23	1.35	0.1904

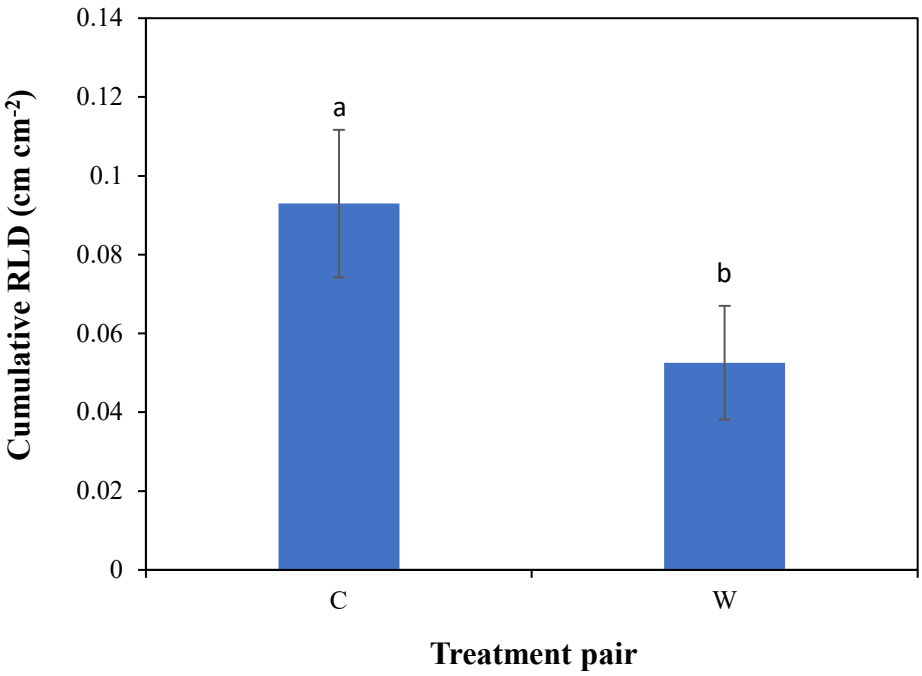


Figure 4.18. Within plot comparison of average cumulative root length density (RLD) of control (C) and wound (W) treatments during the February 2019 measurement. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

4.4.2. Ectomycorrhizal colonization of loblolly pine fine roots

Ectomycorrhizal colonization was significantly different among treatments (Table 4.9). Ectomycorrhizal colonization was significantly lower in the plots with trees inoculated with the medium inoculation treatment compared to plots with wounded trees (P=0.0397) and plots with trees inoculated with the low inoculation treatment (P=0.0033) (Figure 4.19). However, ANCOVA results indicated that ectomycorrhizal colonization was significantly affected by soil pH as a covariate, as well as depth, but not inoculation treatment or its interaction with depth (Table 4.11). Ectomycorrhizal colonization responses to depth resulted in significantly greater values at the 20-30 cm depth compared to the 40-50 cm depth (P=0.0109) (Figure 4.21). Ectomycorrhizal colonization across treatments and soil depths was 34.6%.

The ANCOVA results indicate that the ectomycorrhizal colonization response to inoculation treatment was not due to inoculation treatment but was due to covariance between ectomycorrhizal colonization and soil pH. These results are supported by a negative correlation between ectomycorrhizal colonization and soil pH (Table 4.10). When analyzed by depth, significant negative correlations between ectomycorrhizal colonization and soil pH were found at the 10-20 cm and 30-40 cm depths at a 0.05 significance level, and at the 20-30 cm depth at a 0.1 significance level (Table 4.10, Figure 4.20). Significant negative correlations between ectomycorrhizal colonization and soil pH and ANCOVA results with a significant effect of soil pH but not inoculation treatment on ectomycorrhizal colonization indicate that ANOVA results of ectomycorrhizal colonization risked misinterpretation of the treatment effect.

Table 4.9. Probabilities of a greater *F*-value from a two-way ANOVA for ectomycorrhizal colonization of fine roots in a mature loblolly pine stand near Eufaula, Alabama after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017.

Source	df	*F*-value	*P>F*
Treatment (T)	4	4.3156	0.0054
Depth (D)	3	0.9947	0.4051
T × D	12	1.1861	0.3256
Error	40		

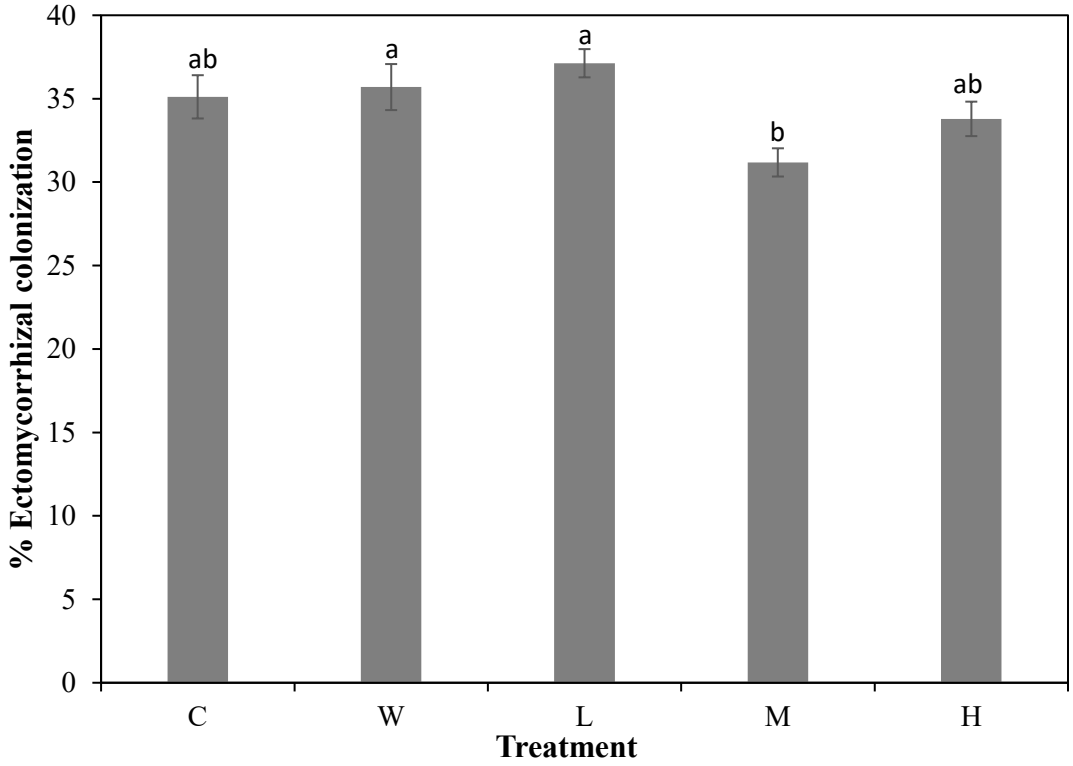


Figure 4.19. Average percentage (%) ectomycorrhizal colonization among treatments during September 2017. Treatments were control (C), wound (L), low (L), medium (M), and high (H) inoculation. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

Table 4.10. Pearson's coefficient of correlation between ectomycorrhizal colonization and soil pH along with their respective *P*-value at different soil depths.

Soil depth	n	r	*P*-value
10-50 cm	60	-0.3659	0.0040
10-20 cm	15	-0.6539	0.0082
20-30 cm	15	-0.4824	0.0686
30-40 cm	15	-0.6563	0.0079
40-50 cm	15	-0.3239	0.2389

Significance tested at $P< 0.05$ and $P<0.1$

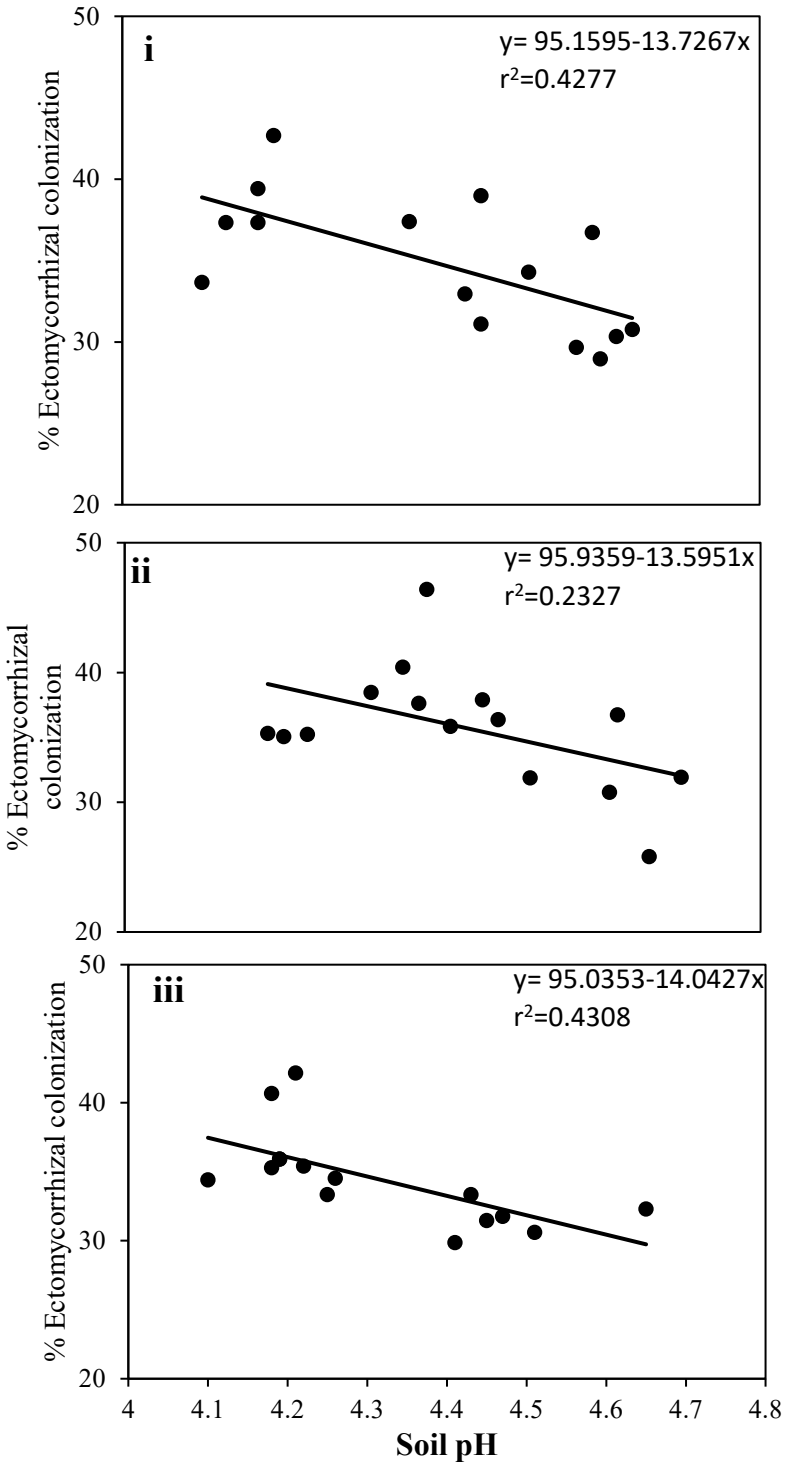


Figure 4.20. Relationship between ectomycorrhizal colonization of loblolly pine fine roots and soil pH at the **(i)** 10-20 cm, **(ii)** 20-30 cm, and **(iii)** 30-40 cm depths near Eufaula, Alabama (n=15, i.e. five treatments× three replicates) during September 2017.

Table 4.11. Probabilities of a greater *F*-value from a two-way ANCOVA for ectomycorrhizal colonization of fine roots in a mature loblolly pine stand near Eufaula, Alabama after an onsite stem inoculation treatment with *Leptographium terebrantis* in March 2017. Soil pH was used as a covariate in the model.

Source	df	*F*-value	*P>F*
Soil pH	1	10.35	0.0026
Treatment (T)	4	1.27	0.2989
Depth (D)	3	3.77	0.0182
T × D	12	1.63	0.1220
Error	39		

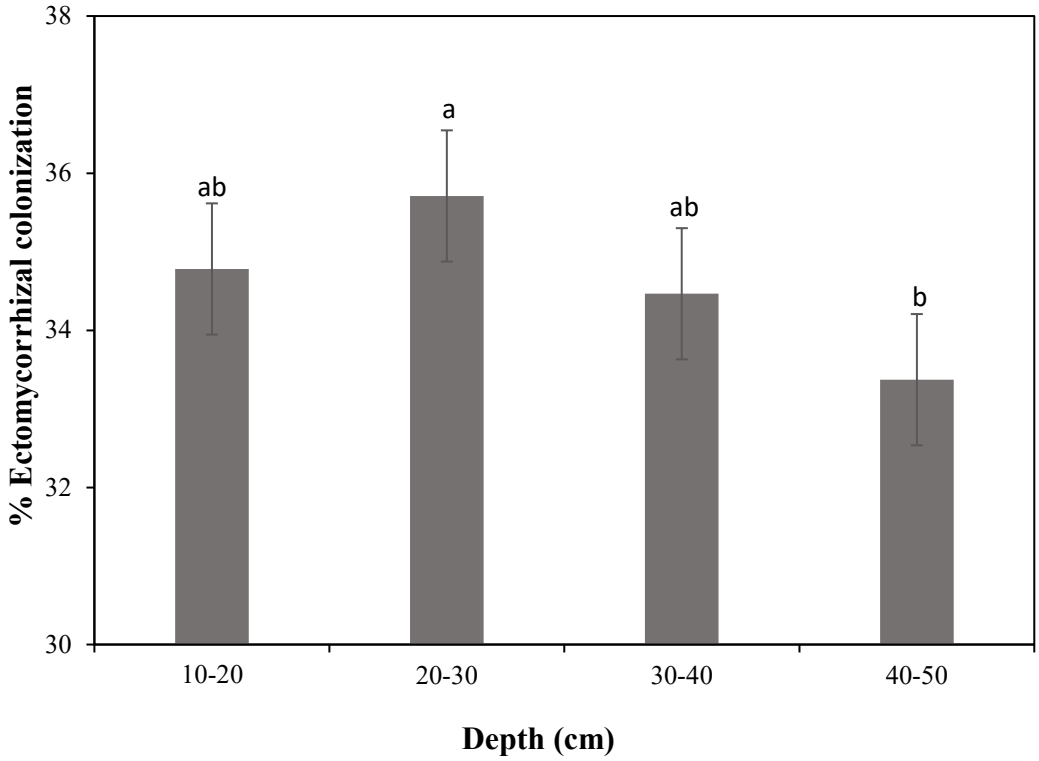


Figure 4.21. Average percentage (%) ectomycorrhizal colonization among depths during September 2017. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly different by Tukey's Multiple Range test.

4.5. Discussion

This research was designed to study if artificial stem inoculation with *L. terebrantis* affects the root growth or ectomycorrhizal colonization of mature loblolly pine trees. However, it appeared that stem inoculation with *L. terebrantis* did not affect loblolly pine new root growth or ectomycorrhizal colonization within 2 years of inoculation. Cumulative RLD in June 2017 was significantly higher among trees inoculated with the medium inoculation treatment compared to the low or high inoculation treatments. Nevertheless, this observation is probably not because of an inoculation treatment effect as the difference among treatment levels was insignificant among other measurement dates until February 2019. Specifically, significantly lower values of cumulative RLD in wounded trees and trees inoculated with high inoculation treatment were found compared to the control trees in February 2019. Also in February 2019, a within-plot comparison of cumulative RLD between control and either wounded or inoculated trees revealed significantly lower cumulative RLD associated with wounded trees compared to control trees. Based on ectomycorrhizal colonization responses to soil pH in this study, perhaps these RLD responses are also attributed to a microsite variable. Significant treatment × depth interaction was found during June and December 2018 representing a trend towards a decrease in cumulative RLD with an increase in inoculum load at the 42.4 cm depth.

New roots were not observed at the soil surface during the first three measurement dates or in February 2019. Interestingly, maximum numbers of new roots were observed at either the 14.1, 28.3, or 35.4 cm depths during different measurement dates. It is, however, uncertain if the diverse pattern of root growth among depths during different measurement dates is typical for our site. Regardless, a large proportion of new roots were observed at the 0-28.3 cm depth (approximately 72%, 70%, 71%, 65%, 57%, 59%, 59%, 57%, 76%, 60% 72%, and 56% in April

2017, June 2017, August 2017, October 2017, December 2017, February 2018, April 2018, June 2018, August 2018, October 2018, December 2018, and February 2019, respectively). Averaged over the entire study period, maximum cumulative RLD values were observed at the 28.3 and 35.4 cm depths, with 60% and 78% of cumulative RLD occurring at or above the 28.3 and 35.4 cm depths, respectively. Our results are consistent with those of Hendricks et al. (2006) who found that a higher percentage of fine roots in a longleaf pine (*Pinus palustris* Mill.) forest was present in the upper 30 cm of the soil profile.

New pine root growth in the southeastern Unites States is characterized by accelerated root elongation and persistence in spring through early fall followed by a period of low root elongation in fall through early spring (Sword et al., 1998; King et al., 2002; Coleman & Aubrey, 2018). Simultaneously, the timing of fine root mortality is variable and may be correlated to water and mineral nutrient availabilities (King et al., 2002). In the present study, there were four periods of interest with regard to the relationship between RLD and climate. These were the periods of high new root growth and maintenance between March and October 2017 and 2018 and the periods of less new root growth between October 2017 and February 2018 and 2019.

New root production was rapid from April 2017 to October 2017, decreased from October 2017 to February 2018, rose again from February 2018 to October 2018, and decreased afterward suggesting that new root growth occurred in two distinct phases. The first phase was when new root growth was rapid from April 2017 to October 2017 and February 2018 to October 2018, with a peak in October. This observation is supported by King et al. (2002) who reported maximum root growth in a loblolly pine plantation in spring through fall. Sword & Haywood (2006) reported similar results in a longleaf pine stand where maximum root elongation took place from May through October.

The second phase corresponds to a period of reduced root growth that occurs naturally during late fall through early spring (Sword et al., 1998; King et al., 2002; Coleman & Aubrey, 2018). In addition to the natural occurrence of this period of low root growth, three conditions may have contributed to reduced new root growth. First, measurements between April 2017 and October 2018 were during the equilibration period when the root system and soil were recovering after destructive installation of minirhizotron tubes. Pritchard et al. (2008) suggested that this pulse of root growth might be due to the production of a large number of new roots to compensate for the death of roots during destructive minirhizotron tube installation. Philips et al. (2006) also reported that root production was rapid for the first six months after minirhizotron tube installation and then decreased afterward in a 2-year minirhizotron study.

Second, since moisture stress affects root growth (Kaufmann, 1968; Torreano & Morris, 1998), a rainfall deficit from October 2017 through February 2018 resulted in 50.6% less rainfall than normal which may have decreased soil moisture and subsequently, limited new root growth (Sword et al., 1996, Torreano & Morris, 1998). A third possible factor contributing to slow root growth after October 2017 is the stand temperature which was 6.6% higher than normal from October 2017 through February 2018. This condition may have contributed to an increase in soil evaporation (Pritchett, 1979; Wells et al., 1979; Neary et al., 1999), thus aggravating low soil moisture in the rooting zone.

Nineteen months after minirhizotron tubes installation, new root growth was steady by June 2018, thus representing the return of tree root systems to an equilibrium condition (Rewald & Ephrath, 2013). Following the minirhizotron tube installation, the tree root system might take anywhere between 6 months to 3 years to return to an equilibrium condition (Johnson et al., 2001; Strand et al., 2008). A study by Ruess et al. (2003) in a black spruce (*Picea mariana* L.)

forest reported that the root distribution along minirhizotron tubes took two years or more to equilibrate. As equilibration is achieved, the magnitude of difference between RLD over time during phases one and two of the new root growth cycle will likely be less.

A significant difference in ectomycorrhizal colonization was observed between the 20-30 cm and 40-50 cm depths representing a trend towards a decrease in ectomycorrhizal colonization as soil depth increased. This observation is supported by Trautwig et al. (2017) who suggested that fine root colonization with ectomycorrhizal fungi in loblolly pine stands decreases with soil depth. Maximum ectomycorrhizal colonization at the 20-30 cm depth may be due to a higher prevalence of new root surface area for ectomycorrhizal colonization at the 21.2 - 35.4 cm depth. The present results are supported by Jentschke & Godbold (2001) who suggested that the presence of mycorrhizae is related to the abundance of roots.

More acidic soil favored ectomycorrhizal colonization in our commercial loblolly pine stand. Soil pH in plots that received the wound treatment or the low inoculation treatment averaged 4.2, and the soil pH in plots that received the medium inoculation treatment averaged 4.5. Ectomycorrhizal colonization was greater among plots receiving the wound or low inoculation treatment compared to those receiving the medium inoculation treatment. The present results are supported by those of Mikola (1973) who proposed that most ectomycorrhizal fungi thrive in acidic soil. Furthermore, Marx (1990) reported that ectomycorrhizal development at pH 5.8 was more than four times higher than that at pH 6.7.

In conclusion, since significant differences among inoculation treatments were not observed until February 2019, a longer observation period may have been required before detection of root responses to artificial *L. terebrantis* inoculation. Also, ectomycorrhizal colonization may not be affected until root system damage partially manifested as a decrease in

new root growth has occurred due to the inoculation treatment. The 42.4 cm depth may be the most reliable depth to observe an inoculation treatment effect on new root growth in the future since the trend of decrease in cumulative RLD with an increase in inoculum load was observed at this depth during June and December 2018. Regardless of whether the effect of a root pathogen on new root growth and ectomycorrhizal colonization is direct or indirect, knowledge of the interaction between root pathogens and the growth and maintenance of the root system and mycorrhizal network is critical to understanding how root pathogens affect tree productivity.

Chapter 5

Summary and Conclusions

Although the results from this study suggest no significant differences occurred in microbial biomass (MB) among the treatments in each sampling season, it is expected that over time, an impact might be seen. We suspect that the timeframe is too short to see a difference that may only be subtle at this time in terms of its magnitude. Changes in foliage inputs to the forest floor and subsequent changes in soil chemistry due to the inoculation treatments may require more time before they hinder with microbial processes. Even then any changes would be buffered by the older forest floor that had accumulated since stand establishment. In the future, taking MB samples closer to the tree stem for both inoculated and control trees might show some differences. However, in the longer term, any tree mortality, not necessarily tree mortality associated with *L. terebrnatis* infection would have changed MB and decomposition rates significantly due to shifts in temperature and moisture relations in the upper soil profile. Since microbial processes are notoriously variable, both spatially and temporally, changes in MB and the soil C/N ratio will be difficult to detect unless the study is very carefully designed.

Root growth was not affected by the inoculation treatment until December 2018. Some trees inoculated with the high inoculation treatment were found symptomatic at the end of December 2018. During the February 2019 measurement, we found that root growth in the wounded trees and trees inoculated with the high inoculum load was significantly lower than that of the control trees. Therefore, a longer study time may be advantageous to monitor the root system damage due to the inoculation treatment. New root growth varied by season and depth and was affected by air temperature and precipitation. Minirhizotron tubes were often obscured

after rainy days thus making it difficult for us to properly observe the roots. Therefore, changing the material of the tubes may be helpful in the future.

Ectomycorrhizal colonization of fine roots were not affected by the inoculation treatment. Acidic soil promoted ectomycorrhizal colonization of fine roots. Surprisingly, ectomycorrhizal colonization was highest at the 20-30 cm soil depth and not the 10-20 cm depth. It is suspected that this observation is attributed to the presence of sandy loam soil in our study site. Sandy loam soils are reported to be vulnerable to compaction and can lead to adverse soil physical and hydraulic properties that are unfavorable for proper root growth. Ectomycorrhizal fungi are known to protect the fine roots of forest trees from pathogenic infection (Zak, 1964). Therefore in the future, it may be vital studying ectomycorrhizal diversity in loblolly pine stands in an attempt to identify ectomycorrhizal fungi that are most tolerant of pine decline settings.

The fact that soil total N and S, available Mg, pH, and foliar Mn varied among treatments before we applied treatments showed that plot level variance in some soil chemical properties existed. Therefore, variation in soil properties should be taken into consideration while doing post-inoculation analyses.

References

Alabama Forestry Commission. 2008. Pine decline. URL: http://www.forestry.alabama.gov/Pages/Informational/Diseases/Pine_Decline.aspx

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova,N., Lim, J.H., Allard, G., Running, S.W., Semerci, A., Cobb, N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manage. 259:660-684

Allen, H.L. 1987. Forest fertilizers: nutrient amendment, stand productivity, and environmental impact. J For. 85:37-46

Allison, S.D., Treseder, K.K. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Glob Change Biol 14(12):2898-2909

Allmaras, R.R., Kraft, J. M., Smucker, J. M. 1988. Soil compaction and crop management effects on root diseases of annual food legumes. In: Summerfield, R.J. (ed) World Crops: Cool Season Food Legumes. 627-647p

Amaranthus, M.P. 1998. The importance and conservation of ectomycorrizal fungal diversity in forest ecosystems: lessons from Europe and the Pacific Northwest. Gen Tech Rep. PNW-GTR-431. Portland, OR: USDA For Ser, PNRS. 15p

Anderegg, W.R.L., Kane, J.M., Anderegg, L.D.L. 2012. Consequences of widespread tree Mortality triggered by drought and temperature stress. Nat Clim Change. 3:30-36

Atkinson D. 1991. Plant root growth an ecological perspective. Blackwell Science Publication, Oxford

Auclair, A.N.D., Martin, H.C., Walker, S.L. 1990. A case study of forest decline in western Canada and the adjacent United States. Water Air Soil Pollut. 53:13-31

Auclair, A.N.D., Worrest, R.C., Lachance, D, Martin, H.C. 1992. Climatic perturbation as a general mechanisms of forest dieback. In: Manion, P.D., Lachance, D. (eds) Forest Decline Concepts. APS Press, St paul, Minnesota. 38-58p

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol. 57:233-266

Baker, J.B., Langdon, O. G. 1990. *Pinus taeda* L. Loblolly Pine. In: R. M. Burns & B. H. Honkala (eds.), Silvics of North America. Conifers. Agric Handb. Washington, DC: USDA For Ser.1:497-512

Ballard, R. 1984. Fertilization of plantations.In: Bowen G.D., Nambiar, E.K.S. (eds) Nutrition of Plantation Forests, Academic Press, London. 327-360

Barras, S.J., Perry, T. 1971. *Leptographium terebrantis* sp. nov. associated with *Dendroctonus terebrans* in loblolly pine. Mycopathol Mycol Appl. 43:1-10

Battles, J.J., Fahey, T.J. 1996. Spruce decline as a disturbance event in the subalpine forests of the northeastern United States. Can J For Res. 26(3):408-421

Bellgard, S.E., Williams, S.E. 2011. Response of mycorrhizal diversity to current climatic changes. Diversity 3:8-90

Benbi, D.K., Khosa, M.K. 2014. Effects of temperature, moisture, and chemical composition of organic substrates on C mineralization in soils. Commun Soil Sci Plan. 45: 2734-2753

Berg, G., Smalla, K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1-13

Binkley, D. 1986. Forest Nutrition Management.Wiley, New York

Birk, E.M., Matson P.A. 1986. Site fertility affects seasonal carbon reserves in loblolly pine. Tree Physiol. 2:17-27

Blume, H.P., Brümmer, G.W., Fleige, H., Horn, R., Kandeler, E., Kögel-Knabner, I., Kretzschmar, R., Stahr, K., Wilke, B.M. 2015. Soil Science. Springer

Bonsen, K.J.M., Scheffer, R.J., Elgersma, D.M. 1985. Barrier zone formation as a resistance mechanism of elms to dutch elm disease. IAWA Bulletin. 6:71-76

Bowen, H.J.M. 1979. Environmental chemistry of the elements. Academic Press, London

Bratton, S. P. 1975. The effect of European wild boar (*Sus serofa*) on Gray Beech Forest in the Great Smoky Mountains. Ecol. 56:1356-1366

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem. 17 (6):837-842

Brouwer, R. 1962. Nutritive influences on the distribution of dry matter in the plant. Neth J Agric Sci. 10:399-408

Brown, M., Black, T.A., Nesic, Z., Foord, V.N., Spittlehouse, D.L., Fredeen, A.L., Trofymow, J.A. 2010. Impact of mountain pine beetle on the net ecosystem production of lodgepole pine stands in British Columbia. Agri For Meteorol. 150:254-264

Brown, H.D., McDowell, W.E. 1968. Status of loblolly pine die-off on the Oakmulgee District, Talladega National Forest, Alabama. Rep. 69-2-28. Pineville, LA: USDA For Ser, Forest Insect and Disease Management Group. 21 p

Brown, A.A., Davis, K.P. 1973. Forest fire control and use. 2nd ed. New York: McGraw-Hill

Bruce, E., Allen, D.C. 1991. Etiology of sugar maple decline. Can J For Res. 21(5):686-693

Brundrett M., Bougher N., Dell B., Grove T., Malajczuk N. 1996. Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research.

Camarero, J.J., Padró, A., Martín-Bernal, E., Gil-Pelegrín, E. 2002. Aproximación 582 dendroecológica al decaimiento del abeto (*Abies alba* Mill.) en el Pirineo 583 aragonés. Montes 70: 26-33

Campbell, W.A., Copeland, O.L. 1954. Littleleaf disease of shortleaf and loblolly pines. Circular No. 940, Washington, DC:USDA For Ser

Candau, J.N., Fleming, R.A., Hopkin, A. 1998. Spatiotemporal patterns of large-scale defoliation caused by the spruce budworm in Ontario since 1941. Can J For Res. 28:1733-1741

Capretti, P., Battisti, A. 2007. Water stress and insect defoliation promote the colonization of *Quercus cerris* by the fungus *Biscogniauxia mediterranea*. For Pathol. 37(20):129-135

Chai, D. D., Guo, A.J., Sun X.B., Qin, T.T. 2013. The major factors affecting ectomycorrhizal fungi diversity in the forest ecosystem. Advance J Food Sci Tech. 5(7): 879-890

Choat, B., Jansen, S., Brodribb, T., Cochard, H., Delzon, S., Bhaskar, R. 2012. Global convergence in the vulnerability of forest to drought. Nature. 491:752-755

Coleman, M.D., Aubrey, D.P. 2018. Stand development and other intrinsic factors largely control fine-root dynamics with only subtle modifications from resource availability. In Press at Tree Physiol

Comeau, P.G., Kimmins, J.P. 1989. Above- and below-ground biomass and production of lodgepole pine on the sites with different soil moisture regimes. Can J For Res. 19:447-454

Corcobado, T., Vivas, M., Moreno, G., Solla, A. 2014. Ectomycorrhizal symbiosis in declining and non-declining *Quercus ilex* trees infected with or free of *Phytophthora cinnamomi*. For Ecol Manage. 324:72-80

Coyle D.R., Klepzig, K.D., Koch, F.H., Morris, L.A., Nowak, J.T., Oak, S.W., Otrosina, W.J., Smith W.D., Gandi, K.J.K. 2015. A review of southern pine decline in North America. For Ecol Manag. 349:134-148

Dale, V.H., Joyce, L.A., McNulty, S., Neilson, R.P., Ayres, M.P., Flannigan, M.D., Wotton, B.M. 2001. Climate change and forest disturbances. BioScience**.** 51:723-734

Danielsen, L., Lohaus, G., Sirrenberg, A., Karlovsky, P., Bastein, C., Pilate, G., Polle, A. 2013. Ectomycorrhizal colonization and diversity in relation to tree biomass and nutrition in a plantation of transgenic poplars with modified lignin biosynthesis. PLoS One. 8(3): e59207

Devi, N.B., Yadava, P.S. 2006. Seasonal dynamics of soil microbial biomass C, N and P in a mixed oak forest ecosystem of Manipur, North-east India. App Soil Ecol. 31:220-227.

Devkota, P., Eckhardt, L.G. 2018. Variation in pathogenicity of different *Leptographium terebrantis* isolates to Pinus taeda L. For Pathol. 48: e12469

Devkota, P., Mensah, J.K., Nadel, R.L., Matusick, G., Eckhardt, L.G. 2018. *Pinus taeda* L. response to differential inoculum density of *Leptographium terebrantis* colonized toothpicks. For Pathol. 49: e12474

Dymond, C.C., Neilson, E.T., Stinson, G., Porter, K., MacLean, D.A., Gray, D.R., Kurz, W.A. 2010. Future spruce budworm outbreak may create a carbon source in eastern Canadian forests. Ecosystems.13:917-931

Eckhardt, L.G. 2003. Biology and Ecology of Leptographium species and their vectors as a component of loblolly pine decline. Ph. D Dissertation, Louisiana State University, Baton Rouge, LA

Eckhardt, L.G., Hess, N.J., Menard, R., Goddard, A.J. 2003. Assessment of loblolly pine decline on the Oakmulgee District, Talladega National Forest, Alabama. Report No. 2004-02-01, Forest Health Evaluation, Alexandria, LA: USDA For Ser, SRS. 42p

Eckhardt, L.G., Menard, R.D. 2008. Topographic features associated with loblolly pine decline in central Alabama. For Ecol Manag. 255:5-6

Eckhardt, L.G., Menard, R.D. 2009. Declining loblolly pine stands: symptoms, causes, and management options. AL Treasured Forest Magazine, xxxv111, 10-12

Eckhardt, L.G., Menard, R.D., Ditchkoff, S.S. 2016. Wild Pigs: inciting factor in southern pine decline? In: Proceedings of the 18th biennial southern silvicultural research conference. e-Gen Tech Rep SRS-212. Asheville, NC: USDA For Ser, SRS. 641p

Eckhardt, L.G., Weber, A.M., Menard, R.D., Jones, J.P., Hess, N.J. 2007. Insect-fungal complex associated with loblolly pine decline in central Alabama. For Sci. 53(1): 84-92

Edmonds, R.L., Agee, J.K., Gara, R.I. ed. 2000. Forest Health and Protection. Waveland Press, Inc. 554-556

Egli, S. 2011. Mycorrhizal mushroom diversity and productivity-an indicator of forest health? Ann For Sci. 230:184-190

Eissenstat, D.M., Wells, C.E., Yani, R.D., Whitbeck, J.L. 2000. Building roots in a changing environment: implications for root longevity. New Phytol. 147:33-42

Elkinton, J.S., Liebhold, A.M. 1990. Population dynamics of gypsy moth in North America. Annu Rev Entomol. 35:571-596

Evans J. 1992. Plantation Forestry in the Tropics. 2nd edn. New York: Oxford University Press

Foster, N.W., Bhatti, J.S. 2012. Forest Ecosystems: Nutrient Cycling. Encyclo Soil Sci. 718-721

Fowells, H. A. 1965. Silvics of forest trees of the United States. Washington, DC: USDA Forest Service: Agric Handbook. 271p

Frey, S.D., Drijber, R., Smith, H., Melillo, J. 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biol Biochem 40(11):2904-2907

Goetz S.J., Bond-Lamberty B., Law B.E., Hicke J.A., Huang C., Houghton R.A. 2012. Observations and assessment of forest carbon dynamics following disturbance in North America. J Geophys Res Biogeosci. 117:1-17

Graham, J.H. 2002. What do root pathogens see in mycorrhizas? New Phytol. 149(3):357-359

Graham, S.A., Knight, F.B. 1965. Principles of forest entomology. New York: McGraw-Hill. 417p

Haichar, F.Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T., Achouak, W. 2008. Plant host habitat and root exudates shape soil bacterial community structure. ISME J. 2:1221-1230

Halls, Lowell K., ed. 1977. Southern fruit-producing woody plants used by wildlife. Gen Tech Rep SO-16. New Orleans, LA: USDA For Ser, SFES. 235p

Hansen, E.M., Stone, J.K., Capitano, B.R. Rosso, P., Sutton, W., Winton, L., Kanaskie, A., McWilliams, M.G. 2000. Incidence and impact of Swiss needle cast in forest plantations of Douglas-fir in coastal Oregon. Plant Dis. 84:773-778

Harley, J.L., Smith, S.E. 1983. Mycorrhizal Symbiosis. Academic Press Inc., N.Y. and London, 483p

Harrington, T.C., Cobb, F.W. 1983. Pathogenicity of *Leptographium* and *Verticicladiella* spp. isoloated from roots of western North American conifers. Phytopathol.73:596-599

Harrington, T.C. 1988. *Leptographium* species, their distributions, hosts and insect vectors. In: Harrington, T.C., Cobb, F.W. Jr. (eds) *Leptographium* Root Diseases on Conifers. APS Press, St. Paul, MN, USA, l-40p

Hawkes, C.V., Kivlin, S.N., Rocca, J.D., Huguet, V., Thomsen, M.A., Suttle, K.B. 2011. Fungal community responses to precipitation. Glob Change Biol. 17:1637-1645

Hendricks, J.J., Hendrick, R.L., Wilson, C.A., Mitchell, R.J., Pecot, S.D., Guo, D. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. J Ecol 94:40-57

Hennon, P.E. 1990. Etiologies of forest declines in western North America. Proceedings of the Society of American Foresters National Convention, Washington DC, 154-159p

Hennon, P.E., Hansen E.M., Shaw III, C.G. 1990a. Dynamics of decline and mortality of *Chamaecyparis nootkatensis* in southeast Alaska. Can J Botany. 68:651-662

Hennon, P.E., Shaw III, C.G., Hansen, E.M. 1990b. Symptoms and fungal associations of declining *Chamaecyparis nootkatensis* in Southeast Alaska. Plant Dis. 74:267-273

Hess, N. J., Otrosina, W.J., Carter, E.A., Steinman, J.R., Jones, J.P., Eckhardt, L.G., Weber, A M., Walkinshaw, C.H. 2002. Assessment of loblolly pine decline in Central Alabama. Gen Tech Rep. SRS–48. Asheville, NC: USDA For Ser, SRS. 558-564p

Hess, N.J., Otrosina, W.J., Jones, J.P., Goddard, A.J., Walkinshaw, C.H. 1999. Reassessment of loblolly pine decline on the Oakmulgee District, Talladega National Forest, Alabama. Report No. 99-2-03, Forest Health Protection, Pineville, LA: USDA For Ser, SRS. 12p

Hicke, J.A., Allen, C.D., Desai, A.R., Dietze, M.C., Hall, R.J., Hogg, E.H., Kashian, D.M., Moore, D., Raffa, K.F., Sturrock, R.N., Vogelmann, J. 2012. Effects of biotic disturbances on forest carbon cycling in the United States and Canada. Glob Change Biol. 18:7-34

Highley L., Tattar, T.A. 1985. *Leptographium terebrantis* and black turpentine beetles associated with blue stain and mortality of black and scots pine on capecod, Massachusetts Plant Dis. 69:528-530

Hobbie, E.A. 2006. Carbon allocation to ectomycorrhizal fungi correlates with below-ground allocation in culture studies. Ecol. 87: 563-569

Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., Högberg, P. 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytol. 187:485-493

Högberg, M.N., Högberg, P. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytol. 154(3):791-795

Högberg, P., Nordgren, A., Aren, G. 2002. Carbon allocation between tree root growth and root respiration in boreal pine forest, Oecologia. 132: 579-581

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature. 411:789-792

Holden, S.R., Treseder, K.K. 2013. A meta-analysis of soil microbial biomass responses to forest disturbances. Front Microbiol. 4:163

Hooper, R.J., Sivasithamparam, K. 2005. Characterization of damage and biotic factors associated with the decline of Eucalyptus wandoo in southwest Western Australia. Can J For Res. 35(11):2589-2602

Horton, T.R., Bruns, T.D. 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). New Phytol. 139:331-339

Houston, D.R. 1992. A host-stress-saprogen model for forest dieback-decline diseases. In: Manion, P.D., Lachance, D. (eds) Forest Decline Concepts. APS Press, St paul, Minnesota, pp 3-25

Hoyle, F., Murphy, D., Sheppard, J. 2018. Microbial Biomass. URL: http://soilquality.org.au/factsheets/microbial-biomass

Huggett, R., Wear, D.N., Coulston, L.R., Liu, J.S. 2013. Forest forecasts. In: Wear, D.N., Greis, J.G. (eds) The southern forest future project: technical report. Gen Tech Rep No. USDA For Ser. 178p

Ilorker, V.M, Totey, N.G. 2001. Floristic diversity and soil studies in Navegaon National Park, Maharashtra. Indi J For. 24(4): 442-447

Jacobs, K., Wingfield, M.J. (eds). 2001. *Leptographium* species: Tree pathogens, insect associates, and agents of blue-stain. APS Press, St. Paul, MN USA. 1-207

Jenkins, M.A., Pallardy, S.G. 1995. The influence of drought on red oak group species growth and mortality in the Missouri Ozarks. Can J For Res. 25(11):1119-1127

Jentschke, G., Godbold, D.L. 2001. Metal toxicity and ectomycorrhizas. Int J Plant Biol. 109: 107-116

Jobbagy, G., Jackson, R.B. 2001. The distribution of soil nutrients with depth: global patterns and the imprint of plants. Biogeo. 53:51-77

Johnson, A.H., Friedland, A.J., Dushoff, J.G. 1985. Recent and historic red spruce mortality: Evidence of climatic influence. Water Air Soil Pollution. 30 (1986): 319-330

Johnson, D., Leake, J.R., Ostle, N., Ineson, P., Read, D.J. 2002. In situ $^{13}CO_2$ pulse labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. New Phytol. 153:327-334

Johnson, M.G., Tingey, D.T., Phillips, D.L., Storm M.J. 2001. Advancing fine root research with minirhizotrons. Environ Exp Bot. 45:263-289

Johnston, A.E. 1986. Soil organic matter, effects on soil and crops. Soil Use Manage. 2:97-105

Jokela, E.J. 2004. Nutrient management for southern pines. In: Dickens, E.D., Barnett, J.P., Hubbard, W.G., Jokela, E.J. (eds) Slash pine: still growing and growing! Proceedings of the slash pine symposium. Gen Tech Rep. SRS-76. Asheville, NC: USDA For Ser, SRS. 27-35p

Jokela, E.J., Martin, T.A., Vogel, J.G. 2010. Twenty-five years of intensive forest management with southern pines: important lessons learned. J For. 108:338-347

Jones, E.A., Reed, D.D., Mroz, G.D., Liechty, H.O., and Cattelino, P.J. 1993. Climate stress as a precursor to forest decline: paper birch in northern Michigan, 1985–1990. Can J For Res. 23(2): 229-233

Jorgensen, J.R., Wells, C.G., Metz, L.J. 1980. Nutrient changes in decompsing loblolly pine forest floor. SSSA J. 44(6):1307-1314

Joseph, G., Kelsey, R. G., Thies, W. G. 1998. Hydraulic conductivity in roots of ponderosa pine infected with black-stain (*Leptographium wageneri*) or annosus (*Heterobasidion annosum*) root disease. Tree Physiol. 18(5):333-339

Kachurina, O.M., Zhang, H., Raun, W.R., Krenzer, E.G. 2008. Simultaneous determination of soil aluminum, ammonium- and nitrate-nitrogen using 1 M potassium chloride extraction. 2008. Commun Soil Sci Plant Analy. 31(7-8):893-903

Kalra, Y.P., Maynard, D.G. 1991. Methods manual for forest soil and plant analysis. For Can. N For Cent, NWR, Edmonton, Alberta. Inf Rep NOR-X-319. 125p

Kaufmann, M.R. 1968. Water relations of pine seedlings in relation to root and shoot growth. Plant Physiol 43:281-288

Kaupenjohann, M., Zech, W., Hantschel, R., Horn, R. Schneider, B.U. 1989. Mineral nutrition of forest trees: A regional survey. In: Schulze, E.D., Lange, O.L., Oren, R. (eds) Forest decline and air pollution. A study of spruce (*Picea abies*) on acid soils. Ecol Stud. 77: 280-296

Keane, R.E., Stephen, F.A.1993. Rapid decline of whitebark pine in Western Montana: Evidence from 20-year remeasurements. West J Appl For. 8 (2):44-47

Keen, F.P. 1958. Progress in bark beetle control through silviculture in the United States. Tenth Inti Congr Entomol Proc. 4(1956): 171-180

Kessler, K.J. Jr. 1992. Oak decline on public lands in the central forest region. Res. Note NC-362. USDA For Ser

Keyes, M.R., Grier, C.C. 1981. Above- and below- ground net production in 40-year-old Douglas-fir stands on low and high productivity sites. Can J For Res. 11:599-605

King, J.S., Albaugh, T.J., Allen, H.L., Buford, M., Strain, B.R., Dougherty, P. 2002. Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. New Phytol. 154:389-398

Kirk, P.M., Cannon, P.F., David, J.C., Stalpers, J. 2001. Ainsworth and Bisby's Dictionary of the Fungi (9th ed.). Wallingford, UK: CAB International

Klepzig, K.D., Raffa, K.F., Smalley, E.B. 1991. Association of an insect- fungal complex with red pine decline in Wisconsin. For Sci. 37:1119-1139

Klepzig, K.D., Smalley, E.B., Raffa, K.F. 1995. *Dentroconus valens* and *Hylastes porulus* (Coleoptera: Scolytidae): Vectors of pathogenic fungi associated with red pine decline disease. Great Lakes Entomol. 28:81-87

Klepzig, K.D., Smalley, E.B., Raffa, K.F. 1996. Interaction of ecological similar saprogenic fungi with healthy and abiotically stressed conifers. For Ecol Manag. 86:163-169

Kowalenko, C.G. 2006. Assessment of Leco CNS-2000 analyzer for simultaneously measuring total carbon, nitrogen, and sulphur in soil. Comm Soil Sci Plant Anal. 32(13-14):2065-2078

Kumar, M., Sharma, C.M., Rajwar, G.S. 2004. Physico-chemical properties of forest soil along altitudinal gradient in Garhwal Himalaya. J Hill Res. 17(2):60-64

Lacki, M.J., Lancia, R.A. 1986. Effects of wild pigs on beech growth in Great Smoky Mountains National Park. J Wild Manage. 50:655-659

Landesberg, J.J., Gower, S.T. 1997. Carbon balance of forests. In: Applications of physiological ecology to forest management. 125-160p

Lapeyrie, F. 1990. The role of ectomycorrhizal fungi in calcareous soil tolerance by 'symbiocalcicole' woody plants. Ann Sci For. 21:579-589

Lawson, E.R. 1990. Shortleaf pine. Silvics of North America. In: Burns, R.M., Honkala, B.H. (eds) Conifers. Washington: U.S. Government Printing Office. (1):316-326

Linder, S. 1987. Responses to water and nutrition in coniferous ecosystems. In: Schulze, E.D., Zwolfer, H. (eds) Potentials and limitations of ecosystem analysis. Ecol Stud. 61:180-202

Litton, C.M., Raich, J.W., Ryan, M.G. 2007. Carbon allocation in forest ecosystems. Glob Change Biol. 13(10):2089-2109

Londo, A., Ezell, A.W. 2011. Planting southern pines: A guide to species selection and planting techniques. Mississippi State University Extension Service, POD

Lorio, P.L. 1966. *Phytophthora cinnamomi* and *Pythium* species associated with loblolly pine decline in Louisiana. Plant Dis Repr. 50:596-597

Lovett, G.M., Christenson, L.M., Groffman, P.M., Jones, C.G., Hart, J.E., Mitchell, M.J. 2002. Insect defoliation and nitrogen cycling in forests. Bioscience. 52: 335-341

Lucash, M.S., Joslin, J.D., Yanai, R. 2005. Temporal variation in nutrient uptake capacity by intact roots of mature loblolly pine. Plant and Soil. 272(1):253-262

Manion, P.D. 1991. Tree Disease Concepts, 2nd ed. Prentice Hall, Inc., Englewood Cliffs, NJ. 402p

Manion, P.D., Lachance, D. 1992. Forest decline concepts. APS Press, St. Paul, MN. 1p

Marks, G.C., Foster, R.C. 1973. Structure, morphogenesis and ultrastructure of ectomycorrhizae. In: Marks,G.C. & Kozlowski, T.T. (eds) Ectomycorrhizae: their ecology and physiology

Martin, T.A., Jokela E.J. 2004. Stand development and production dynamics of loblolly pine under a range of cultural treatments in north-central Florida USA. For Ecol Manage. 192(1):39-58

Marx, D.H. 1990. Soil pH and nitrogen influence *Pisoiithus* ectomycorrhizal development and growth of loblolly pine seedlings For Sci. 36(2):224-245

Mason, P.A., Wilson, J., Deacon, J.W., Fleming, L.V., Fox, F.M. 1986. Fruting and sucession of ectomycorrhizal fungi. In: Pegg G.F., Ayres, P.G. (eds.) Fungal Infection of plants. Symposium BMS

Mather, W.J., Simard, S.W., Heineman, J.L., Sachs, D.L. 2010. Decline of planted lodgepole pine in the southern interior of British Columbia. For Chron. 86(4):484-497

Matusick, G., Eckhardt, L.G. 2010. The pathogenicity and virulence of four ophiostomatoid fungi on young longleaf pine trees. Can J Plant Path. 32:170-176

Matusick, G., Menard, R.D., Zeng, Y., Eckhardt, L.G. 2013. Root-inhabiting bark beetles (Coleoptera; Curculionidae) and their fungal associates breeding in dying loblolly pine in Alabama. Flor Entomol. 96 (1):238-241

McDowell, N., Pockman, W., Allen, C., Breshears, D., Cobb, N., Kolb, T., Plaut, T., Sperry, J., West, A., Williams, D.G., Yepez, E.A. 2008. Mechanism of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol. 178:719-739

McMurtrie, R.E., Wolf, L.J. 1983. Above- and below-ground growth of forest stands: a Carbon budget model. Anna Bot. 52(4):437-448

Mead, D.J., 2013. Sustainable management of *Pinus radiata* plantations. FAO Forestry Paper No. 170. Rome, FAO

Mehlich, A. 1984. Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. Commun. In Soil Sci. Plant Anal. 15(12): 1409-1416

Menard, R., Eckhardt, L., Hess, N. 2006. Assessment of loblolly Pine on Fort Benning Military Reservation, Fort Benning, Georgia. Forest Health Protection Report No. 2006-02-01. USDA For Ser

Meyerpeter, M.B., 2012. Mapping loblolly pine decline hazard and risk across the Southeastern United States. MS Thesis, Auburn University, Auburn, AL, 88p

Mikola, P. 1973. Application of mycorrhizal symbiosis in forestry practice. In: Marks G.C., Kozlowski, T.T. (eds) Ectomycorrhizae: Their ecology and physiology. Academic Press, New York, 383-411p

Mitchell, R.J., Garrett, H.E., Cox, G.S., Atalay, A. 1990. Boron and ectomycorrhizal influences on mineral nutrition of container-grown *Pinus echinata* Mill. J Plant Nutr. 13:1555-1574

Moore, R., Williams, T., Rodriguez, E., Hepinstall-Cymmerman, J. 2013. Using non-market valuation to target conservation payments: An example involving Georgia's private forests. J For 111: 261-270

Morrison, D.J., Hunt, R.S. 1988. *Leptographium* species associated with root disease of conifers in British Columbia. In: Harrington, T.C., Cobb, F.W. (eds) *Leptographium* root diseases on conifers. APS, St. Paul, MN

Mosca, E., Montecchio, L., Barion, G., Dal Cortivo, C., Vamerali, T. 2017. Combined effects of thinning and decline on fine root dynamics in a *Quercus robur* L. forest adjoining the Italian Pre-Alps. Annals Botany. 119 (7):1235-1246

Moya, L., Laguarda, M.F., Cagno, M., Cardoso, A., Gatto, F., O'Neill, H. 2013. Physical and mechanical properties of loblolly and slash pine wood from Uruguayan plantations. For Prod J. 63:128-137

Nadelhoffer, K.J., Aber, J.D., Melillo, J.M. 1985. Fine roots, net primary production, and soil nitrogen availability: a new hypothesis. Ecol. 66(4):1377-1390

Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F. 1999. Fire effects on belowground sustainability: a review and synthesis. For Ecol Manage. 122:51-71

Newman, E.I. 1996. A method of estimating the total length of root in a sample. J Appl Ecol. 3:139-145

Nisbet, T.R., Mullins, C.E. 1986. A comparison of live and dead fine root weights in stands of Sitka spruce in contrasting soil water regimes. Can J For Res. 16(2):394-397

Nkongolo, N.V., Plassmeyer, C.J. 2010. Effect of vegetation type on soil physical properties at Lincoln University living laboratory. Res J For. 4:1-13

Nobel, P.S. 1991. Ecophysiology of roots of desert plants, with special emphasis on Agavies and Cacti. 839-66pp

Oak, S.W., Tainter, F.H. 1988. Risk prediction of loblolly pine decline on littleleaf disease sites in South Carolina. Plant Dis. 72:289 -293

Olivia, J., Boberg, J.B., Hopkins, A.J.M., Stenlid, J. 2013. Concepts of epidemiology of forest diseases. In: Gonthier, P., Nicolotti, G. (eds) Infectious forest diseases. CABI International, Oxfordshire, UK. 1-28p

Oliva, J., Stenlid, J., Martinez-Vilalta, J. 2014. The effect of fungal pathogen on the water and carbon economy of trees: implications for drought-induced mortality. New Phytol. 203(4):1028-1035

Omoro L.M.A., Laiho, R., Starr, M., Pellikka, P.K.E. 2011. Relationships between native tree species and soil properties in the indigenous forest fragments of the Eastern Arc Mountains of the Taita Hills, Kenya. For Stud China. 13(3):198-210

Otrosina, W.J., Bannwart, D., Roncadori, R.W. 1999. Root-infecting fungi associated with a decline of longleaf pine in the southeastern United States. Plant and Soil. 217(1-2):145-150

Otrosina, W.J., Hess, N.J., Zarnoch, S.J., Perry, T.J., and Jones, J.P. 1997.Blue-stain fungi associated with roots of southern pine trees attacked by the southern pine beetle, *Dendroctonus frontalis*. Plant Dis. 81: 942-945

Otrosina, W.J., Walkinshaw, C.H. Zarnoch, S.J., Sung, S., Sullivan, B.T. 2002. Root disease, longleaf pine mortality, and prescribed burning. Outcalt K.W. (ed) Proceedings of the Eleventh Biennial Southern Silvicultural Research Conference. Gen Tech Rep SRS-48. Asheville, NC:USDA For Ser, SRS

Paine T.D., Raffa K.F., Harrington T.C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annu Rev Entomol. 42:179–206

Parmeter, J.R.Jr., Vega, R.V., Neff, T. 1962. Chlorotic decline of ponderosa pine in southern California. Plant Dis. 48:4

Paul, E.A., Harris, D., Klug, M.J., Ruess, R.W. 1999. The determination of microbial biomass. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (eds) Standard soil methods for long-term ecological research. Oxford University Press, New York, 291-317

Pfeifer, E.M., Hicke, J.A., Meddens, A.J.H. 2011. Observations and modeling of above-ground tree carbon stocks and fluxes following a bark beetle outbreak in the western United States. Glob Change Biol.17: 339-350

Phillips, D.L., Johnson, M.G., Tingey, D.T., Catricala, C.E., Hoyman, T.L.,Nowak, R.S. 2006. Effects of elevated CO_2 on fine root dynamics in a Mojave Desert community: a FACE study. Glob Change Biol. 12:61-73

Pregitzer, K. S, DeForest, J. L., Burton A.J., Allen M.F., Ruess, R.W., Hendrik, R.L. 2002. Fine root architecture of nine North American trees. Ecol Monogr. 72:293-309

Pritchard, S.G., Strand, A.E., McCormack, M., Davis, M.A., Finzi, A.C., Jackson, R.B., Matamala, R., Rogers, H.H., Oren, R. 2008. Fine root dynamics in a loblolly pine forest are influenced by free-air-CO2-enrichment: a six-year-minirhizotron study. Glob Chang Biol. 14:588-602

Pritchett, W.L. 1979. Properties and management of forest soils. Wiley, New York, 500p

Pritchett, W.L., Comerford, N.B. 1983. Nutrition and fertilization of slash pine. In: Stone, E.L., (ed) The managed slash pine ecosystem. School of Forest Resources and Conservation, Univ FL, Gainesville, FL. 69-90p

Pritchett, W.L., Smith W.H. 1975. Forest fertilization in the U.S. southeast. In: Bernier, B., Winget, C.H. (eds) Forest soils and forest land management. Laval University Press, Quebec, C. 467-476

Raffa, K. E, Smalley, E. B. 1988. Host resistance to invasion by lower stem and root infesting insects of pine: Response to controlled inoculations with the fungal associate Leptographium terebrantis. Can J For Res. 18:675-681

Ralston, C.W. 1978. The southern pine: forests, physiography, and soils. Proceedings: a symposium on principles of maintaining productivity on prepared sites. 21-22p

Rauscher, H.M. 2004. A history of southern forest science, management, and sustainability issues. Gen Tech Rep USDA For Ser, SRS. Chapter, 1: 3-4p

Rewald, B., Ephrath, J.E. 2013. Minirhizotron techniques. In: Eshel, A., Beeckman, T. (eds) Plant roots: the hidden half. CRC Press, FL. 42:1-15

Richardson, B., Skinner, M.F., West, G. 1999. The role of productivity in defining the sustainability of plantation forests in New Zealand. For Ecol Manage. 122(1-2): 125-137

Roberts, K.J., Anderson, R.C. 2001. Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara and Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. American Midland Naturalist.146:146-152

Romme, W., Knight, D.H., Yavitt, J.B. 1986. Mountain pine beetle outbreaks in the Rocky Mountains: regulators of primary productivity. American Naturalist. 127: 484–494

Ruess, R.W., Hendrick, R.L., Burton, A.J., Pregitzer, K.S., Sveinbjornssön, B., Allen, M.F., Maurer, G.E. 2003. Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska. Ecol Monogr. 73:643-662

Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., Cornelissen, J., Gurevitch, J. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental warming. Oecologia. 126:543-562

SAS Institute, Inc. 2010. Version 9.4. Cary, NC, USA

Salamanca, E.F., Kaneko, N., Katagiri, S. 2003. Rainfall manipulation effects on litter decomposition and the microbial biomass of the forest floor. Appi Soil Ecol. 22(3):271-281

Santantonio, D., Grace, J.C. 1987. Estimating fine-root production and turnover from biomass and decomposition data: a compartment–flow model. Can J For Res. 17(8): 900-908

Sapsford, S.J., Paap, T., Hardy, G.E.St.J., Burgess, T.I. 2017. The ‘chicken or the egg’: which comes first, forest decline or loss of mycorrhizae? Plant Ecol. 218:1093-1106

Sauvadet, M., Chauvat, M., Brunet, N., Bertrand, I. 2017. Can changes in litter quality drive soil fauna structure and functions? Soil Biol Biochem. 107:94-103

Saxe, H. 1993. Triggering and predisposing factors in the "red" decline syndrome of Norway spruce (*Picea abies*). Trees. 8:39-48

Schoenholtz, S.H., Van Miegroet, H., Burger, J.A. 2000. A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. For Ecol Manage.138: 335-356

Schultz, R.P. 1997. Loblolly pine: the ecology and culture of loblolly pine (Pinus taeda L.). USDA Forest Service, Agric Handb. 713p

Sevanto, S., McDowell, N., Dickman, L., Pangle, R., & Pockman, W. 2014. How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. Plant Cell Environ. 37:153-161

Shigo, A., Tippett, J.T. 1981. Compartimentalization of American elm tissues infected by *Ceratocystis ulmi*. Plant Dis. 65: 715-718

Siccama, T.G., Margaret, B., Vogelmann, H.W. 1982. Decline of Red Spruce in the Green Mountains of Vermont. Bulletin of the Torrey Botanical Club. 109 (2):162-168

Six, D.L., Wingfield, M.J. 2011. The role of phytopathogenicity in bark beetle-fungus symbioses: A challenge to the classic paradigm. Annu Rev Entomol. 56:255-272

Skelly, J.M., Innes, J.L., 1994. Waldsterben in the forests of Central Europe and Eastern North America: fantasy or reality? Plant Dis. 78:1021-1032

Smeltzer, R.H., Mott, R.L., Mehra-Polta, A. 1977. Influence of parental tree genotype in the potential for in vitro cloning progenation from loblolly pine embryos. In: Forest biology wood chemical conference, Tappi Press, Atlanta, GA. 5-8p

Smucker, A.J.M. 1984. Carbon utilization and losses by plant root systems. In: Barber, Boulden., (eds) Roots, nutrient and water influx, and plant growth, SSSA. 27-46p

Smucker, A.J.M. 1993.Soil environmental modifications of root dynamics and measurement Annu Rev Phytopathol. 31:191-216

Söderström, B., Read, D.J. 1987. Respiratory activity of intact and excised ectomycorrhizal mycelial systems growing in unsterilized soil. Soil Biol Biochem. 19:231-236

Steinman, K., Siegwolf, R.T.W., Saurer, M., Körner, C. 2004. Carbon fluxes to the soil in a mature temperate forest assessed by ^{13}C isotope tracing. Oecologia. 141:489-501

Strand, A.E., Pritchard, S.G., McCormack M.L., Davis M.A., Oren R. 2008. Irreconcilable differences: fine root life spans and soil carbon persistence. Science. 319:456-458

Swift, M., Heal, O., Anderson, J. 1979. Decomposition in terrestrial ecosystems. Blackwell Scientific Publications, Oxford

Sword, M.A., Haywood J.D. 2006. Fine root production and carbohydrate concentrations of mature longleaf pine (*Pinus palustris* P. Mill.) as affected by season of prescribed fire and drought. Trees. 20:165-175

Sword, M.A., Haywood J.D., Dan, A.C., 1998. Seasonal lateral root growth of juvenile loblolly pine after thinning and fertilization on gulf coastal plain site. Proceedings of the Ninth Biennial Southern Silvicultural Research Conference

Sword, M.A., Gravatt, D.A., Faulkner, P.L., Chambers, J.L. 1996. Seasonal branch and fine root growth of juvenile loblolly pine five growing seasons after fertilization. Tree Physiol 16:899-904

Sword, Mary A., Kuehler, E.A., Tang, Z. 2000. Seasonal fine root carbohydrate relations of plantation loblolly pine after thinning. J of Sustain For. 10 (3-4): 295-305

Sypert, R.H. 2006. Diagnosis of loblolly pine (*Pinus taeda* L.) nutrient deficiencies by foliar method. Master's thesis. Dep For, VT Uni

Tamm, C.O. 1991. Nitrogen in terrestrial ecosystems: questions of productivity, vegetational changes and ecosystem stability. Ecol Stud. 81:1-115

Tetreault, J.P., Bernier, B., Fortin, J.A. 1978. Nitrogen fertilization and mycorrhizae of balsam fir seedlings in natural stands. Naturaliste Canadien (Quebec). 105:461-466

Thompson, K., Parkinson, J.A., Band, S.R., Spencer, R.E. 1997. A comparative study of leaf nutrient concentration in a regional herbaceous flora. New Phytol. 136:679-689

Thornley, J.H.M. 1972a. A model to describe the partitioning of photosynthate during vegetation growth. Annals of Botany. 36:419-430

Thornley, J.H.M. 1972b. A balanced quantitative model for root: shoot ratios in vegetative plants. Annals of Botany. 36:431- 441

Tkacz, B.M., Hansen, E.M. 1982. Damage by laminated root rot in two succeeding stands of Douglas-fir. J For. 80:788-791

Torreano, S.J., Morris, L.A. 1998. Loblolly pine root growth and distribution under water stress. Soil Sci Soc Am J. 62:818-827

Trautwig, A. N., Eckhardt, L.G., Loewenstein, N.J., Hoeksema, J.D., Carter, E.A., Nadel, R.L. 2017. Cogangrss (*Imperata cylindrica* (L.) Beauv.) affects above-and belowground processes in commercial loblolly pine (*Pinus taeda* L.) stands. For Sci. 63(1):10-16

Urban, J., Holušová, K., Menšík, L., C˘ermák, J., Kantor, P., 2013. Tree allometry of Douglas fir and Norway spruce on a nutrient-poor and nutrient-rich site. Trees. 27:97-110

USDA NRCS. 2019. Carbon to nitrogen ratios in cropping systems. URL: https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcseprd331820.pdf

Vance, E.D., Brookes, P.C., Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol Biochem. 19:703-707

van der Heijden, M.G.A., Bardgett, R.D., van Straalen, N.M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Letters. 11(3): 296-310

van Lear, D.H., Goebel, N.B. 1976, Leaf fall and forest charactersistics in loblolly pine plantations in the South Carolina Piedmont. SSSA J. 40(1):116-119

van Noordwijk, M., Purnomosidhi, P. 1995. Root architecture in relation to tree-soil-crop interaction and shoot pruning in agroforestry. Agrofor Syst. 30:161-173

Vitousek, P. 2004. Nutrient Cycling and Limitation. Princeton University Press

Vogt, K.A., Vogt, D.J., Fatuga, B.A., Redlin, M.R., Edmonds, R.L. 1987. Conifer and angiosperm fine root biomass in relation to stand age and site productivity in Douglas fir forests. J Ecol. 75:857-870.

Wahlenberg, W.G. 1960. Loblolly pine, its use, ecology, regeneration, protection, growth and management. Durham, NC: Duke University, School of Forestry.

Wade, D.D. 1985. Survival in young loblolly pine plantations following wildfire. Proceedings, 8th conference on fire and forest meteorology. SESAF, Detroit, MI. 52-59p

Wardle, D.A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol Rev. 67:321-358

Wareing, P.F., Patrick, J. 1975. Source-sink relations and the partition of assimilates in the plant. In: Cooper, J.P. (ed) Photosynthesis and productivity in different environments. Cambridge University Press, Cambridge, England. 481-500p

Waring, R.H. 1983. Estimating forest growth and efficiency in relation to canopy leaf area. Advance Ecol Res 13:327-354

Watson, G. 1991. Attaining root: crown balance in landscape trees. J Arbor. 17(8): 211-216

Wear, D.N., Gries, J.G. 2012. The southern forest futures project: Summary report. General Technical Report, USDA Forest Service, Ashevillle, NC. 54p

Wells, C.G., Campbell, R.E., DeBano, L.F., Lewis, C.E., Fredriksen, R.L., Franklin, E.C., Froelich, R.C., Dunn, P.H. 1979. Effect of fire on soil, A state-of-knowledge review. Gen Tech Rep WO-7. Washington, DC: USDA For Ser. 34p

Wells, C.G., Crutchfield, D.M., Berenyi, N.M., Davey, C.B. 1973. Soil and foliar guidelines for phosphorus fertilization of loblolly pine. Res Pap SE-110. Asheville, NC: USDA For Ser, SFES. 17 p

Wickland, K. 2014. *Sus scrofa*, wild boar. Animal Diversity Web. University of Michigan. Museum of Zoology. URL: http:// animaldiversity.org/accounts/Sus_scrofa/

Withington, J.M., Elkin , A.D., Bulaj, B., Olesinski, J., Tracy, K.N., Bouma,T.J., Oleksyn , J., Anderson, L.J., Modrzynski, J., Reich, P.B., Eissenstat, D.M. 2003. The impact of material used for minirhizotron tubes for root research. New Phytol. 160: 533-544

Woods, A., Coates, K.D., Hamann, A.H. 2005. Is an unprecedented Dothistroma needle blight epidemic related to climate change? BioScience. 55:761-769

Wood, D.L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. Annu Rev Entomol. 27: 411-446

Wu, J., Brookes, P.C., Jenkinson, D.S. 1996. Evidence for the use of a control in the fumigation-incubation method for measuring microbial biomass carbon in soil. Soil Biol Biochem. 28(4-5):511-518

Yadeta, K., Thomma, B. 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. Front Plant Sci. 4:97

Yang, K., Zhu, J., Zhang, M., Yan, Q., Sun, O.J. 2010. Soil microbial biomass carbon and nitrogen in forest ecosystems of Northeast China: a comparison between natural secondary forest and larch plantation. J Plant Ecol. 3(3):175-182

Zak, B. 1964. Role of mycorrhizae in root disease. Ann. Rev. Phytopathol. 2:377-92