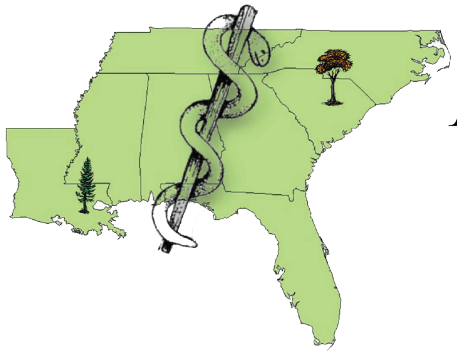

AUBURN UNIVERSITY
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SOIL PHYSIOCHEMICAL PROPERTIES AND FOLIAR NUTRIENT ANALYSIS PRIOR TO INOCULATING LOBLOLLY PINE STAND WITH *LEPTOGRAPHIUM TEREBRANTIS*

by
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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, 1971).

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).

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).

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).

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.

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 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 (cm3)

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 (pHH2O) and 0.01 M CaCl2 solution (pHsalt) (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 N_{tot} and S_{tot}, 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 N_{tot} and S_{tot} 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 C_{tot}, N_{tot}, and S_{tot}, 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).

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).

2.5. 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 N_{tot}, S_{tot}, 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 N_{tot} of the study site was 0.4 %. A significantly higher N_{tot} 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 N_{tot} in plot 12 (0.6%) compared to that in plot 13 (0.3%). Significantly higher S_{tot} 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 S_{tot} (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 N_{tot}, S_{tot}, 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.

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*
C_{tot}	4	2.48	0.0555	4	32.07	<0.0001	16	0.66	0.8217
N_{tot}	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								

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		

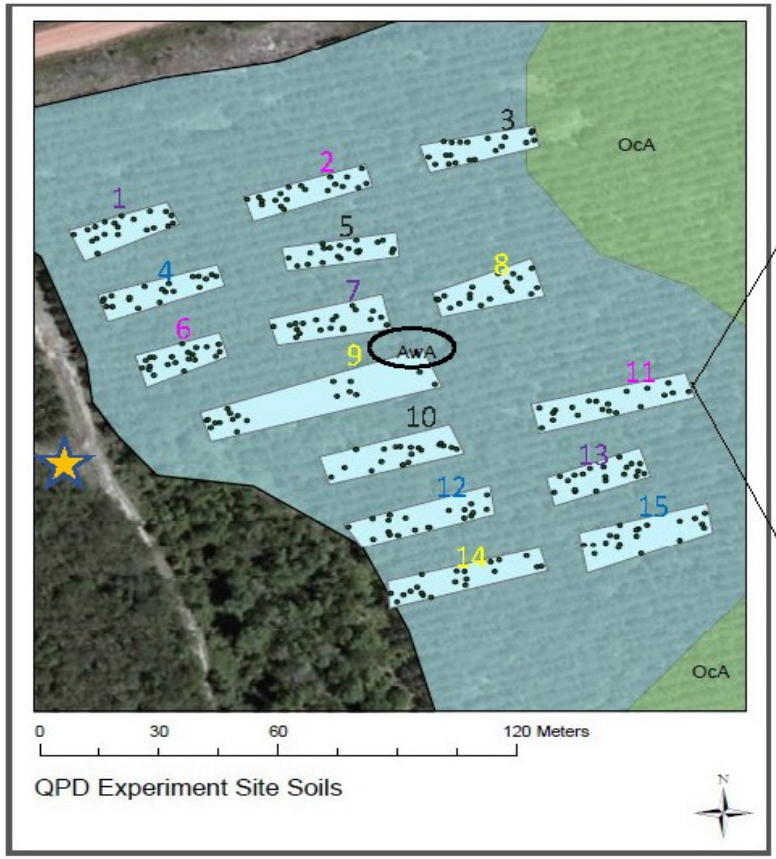

Figure 2.1. Map of the study site near Eufaula, Alabama. Treatment plots are represented by rectangles and soil series are represented by background color (AwA: Annemaine- Wahee complex). Small circles represent pre-thinning tree locations. Filled yellow star indicates the location of weather station.

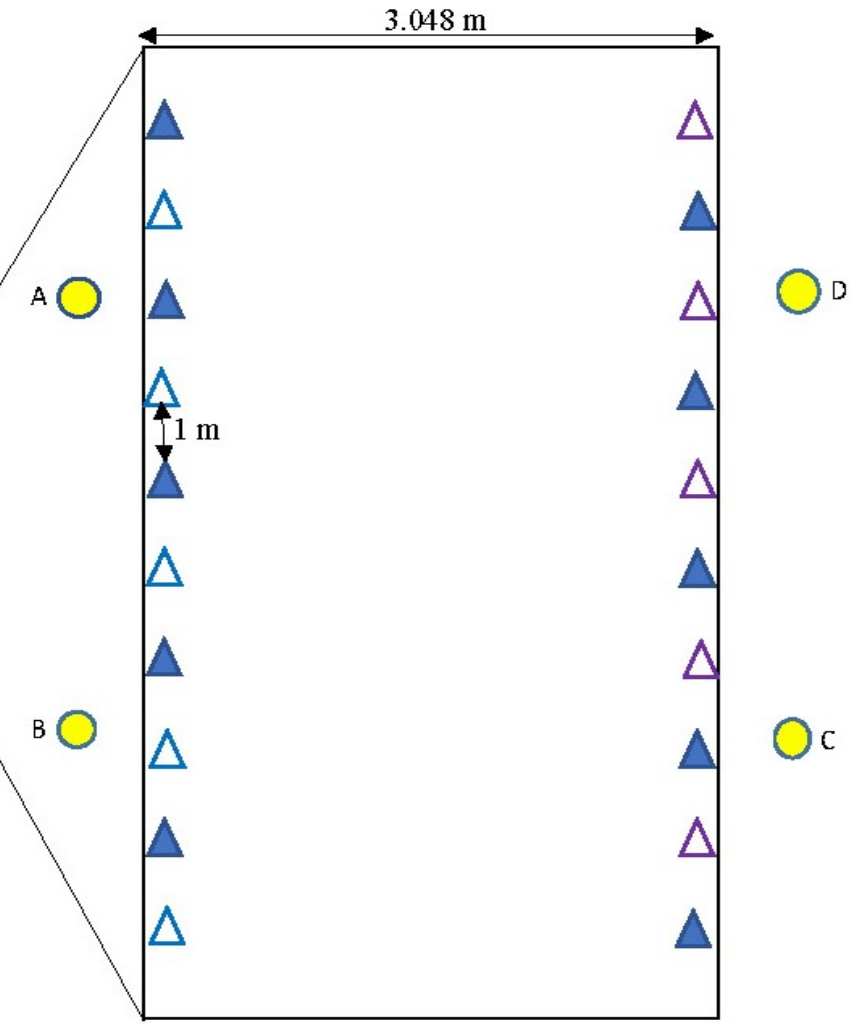

Figure 2.2. Post-thinning field layout of tree arrangement and distance between them in each treatment plot. Unfilled purple triangles indicate inoculated trees, unfilled blue traingles indicate control trees, and solid blue traingles indicate other trees. Letters (A-D) indicate the points from where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

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.

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

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

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.

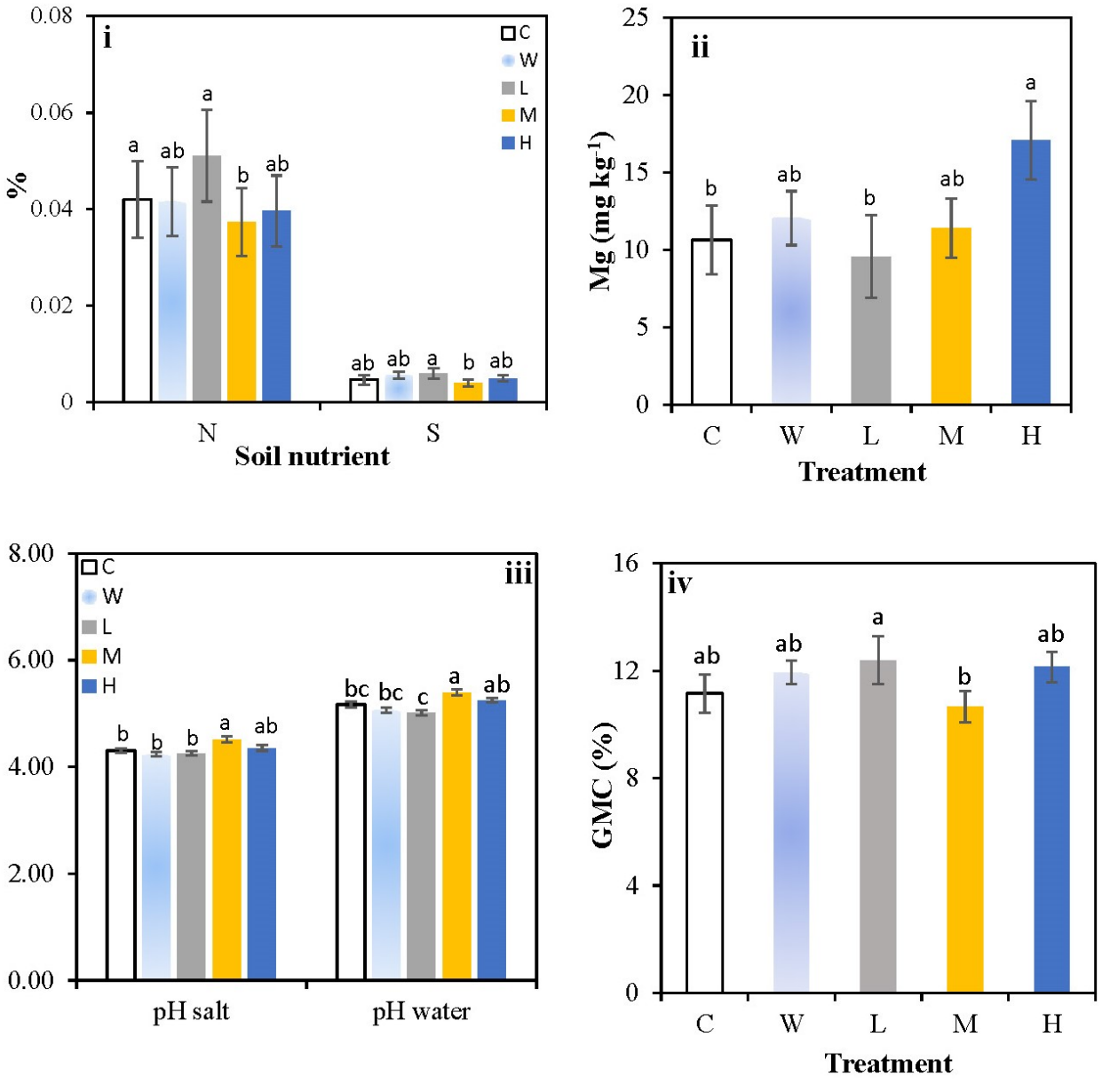

Figure 2.7. Average **(i)** total N and total S, **(ii)** exchangeable Mg, **(iii)** pH, and **(iv)** gravimetric soil moisture (%GMC) among treatments before the application of inoculation treatment on loblolly pine trees. Treatments were control (C), wound (L), low (L), medium (M), and high (H) inoculation. 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.

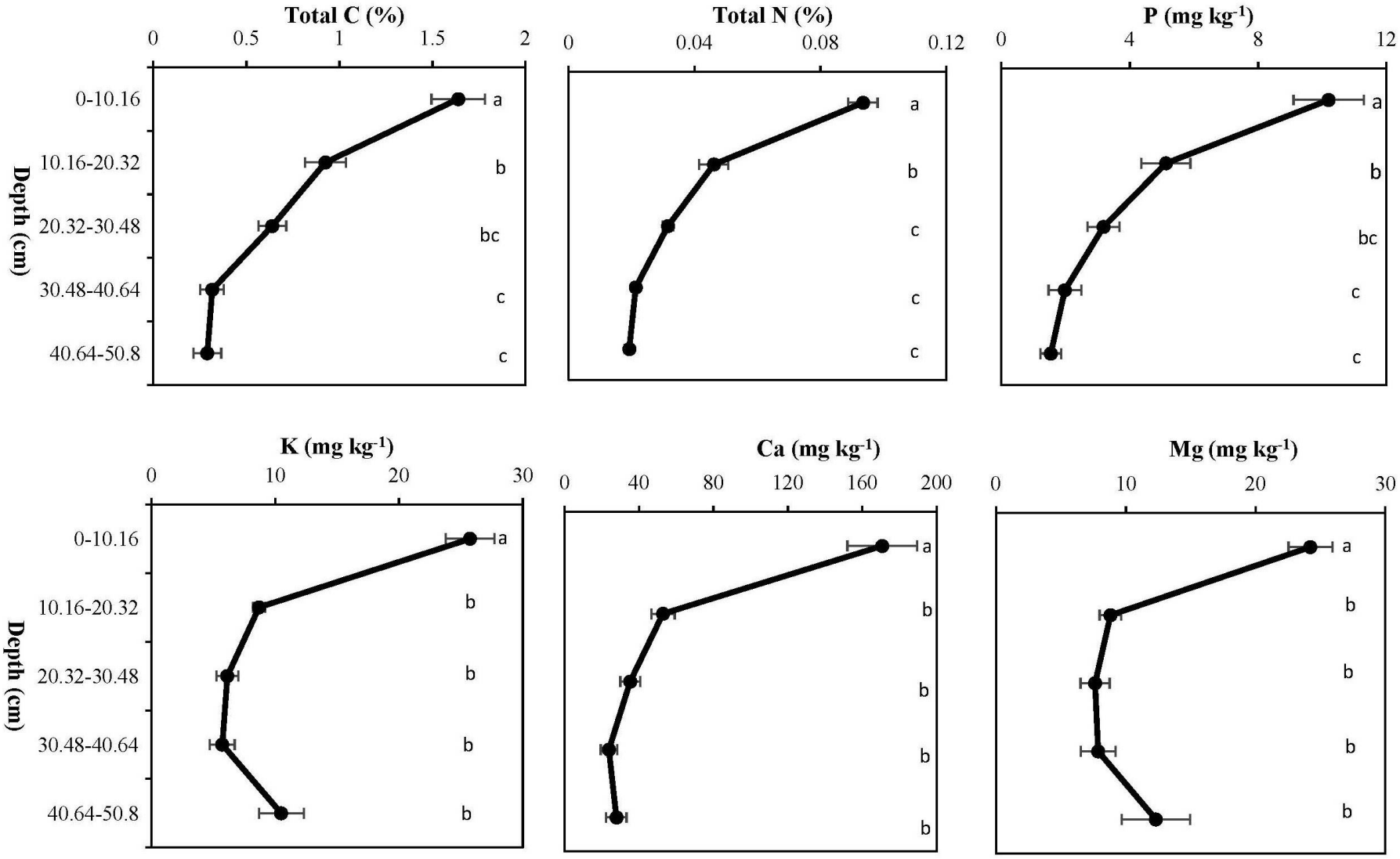

Figure 2.8. Soil nutrients comparison across top 50.8 cm depth in loblolly pine stand. The analyzed nutrients include total C, total N, available P, available K, available Ca, and available Mg. Error bars represent the standard error of the mean. In each figure, means associated with a different lower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

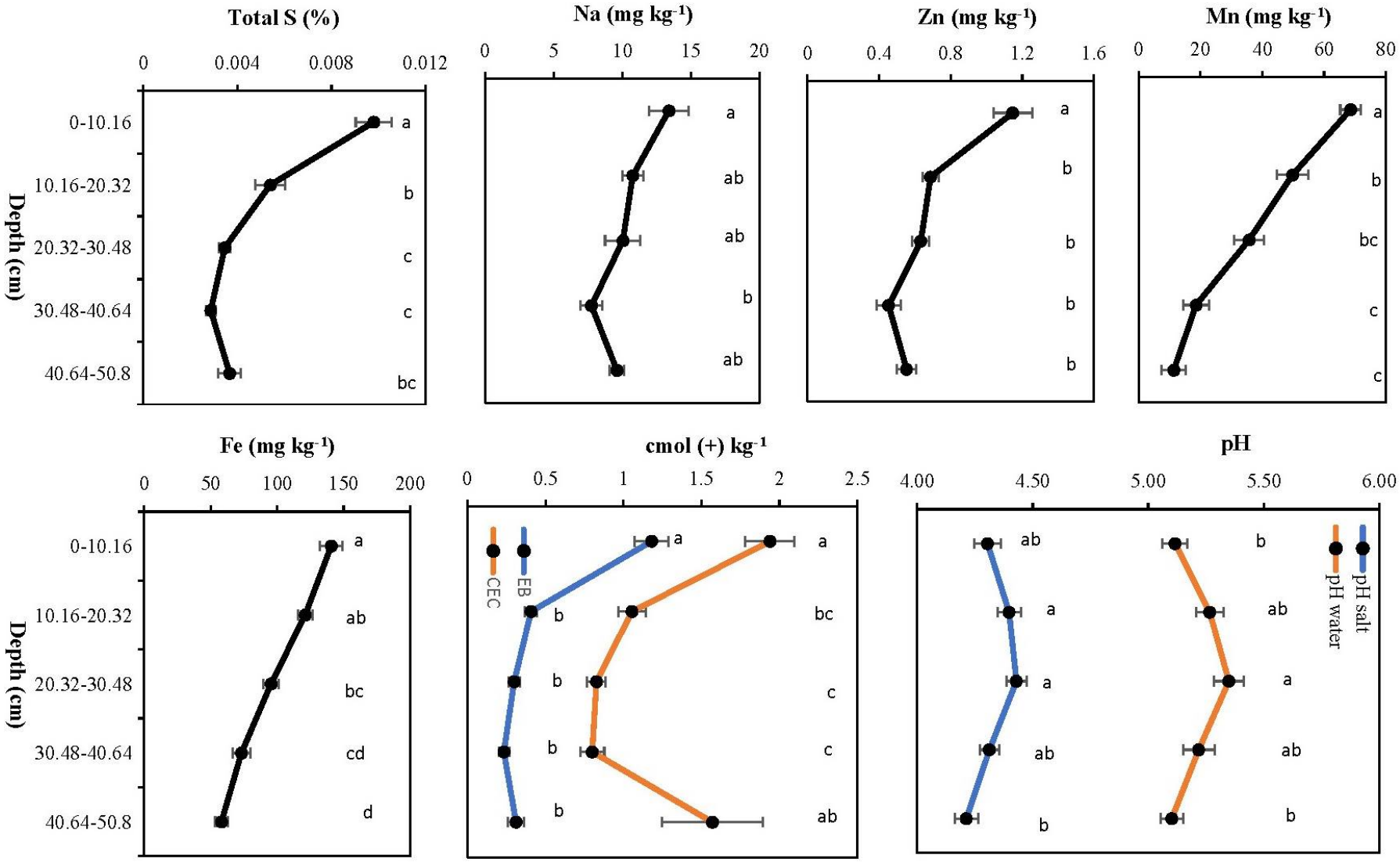

Figure 2.9. Soil properties comparison across top 50.8 depth in loblolly pine stand .The analyzed properties include total S, available Na, available Zn, extractable Mn, available Fe, exchangeable base, effective cation exchange capacity, pH_{salt}, and pH_{water}. Error bars represent the standard error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

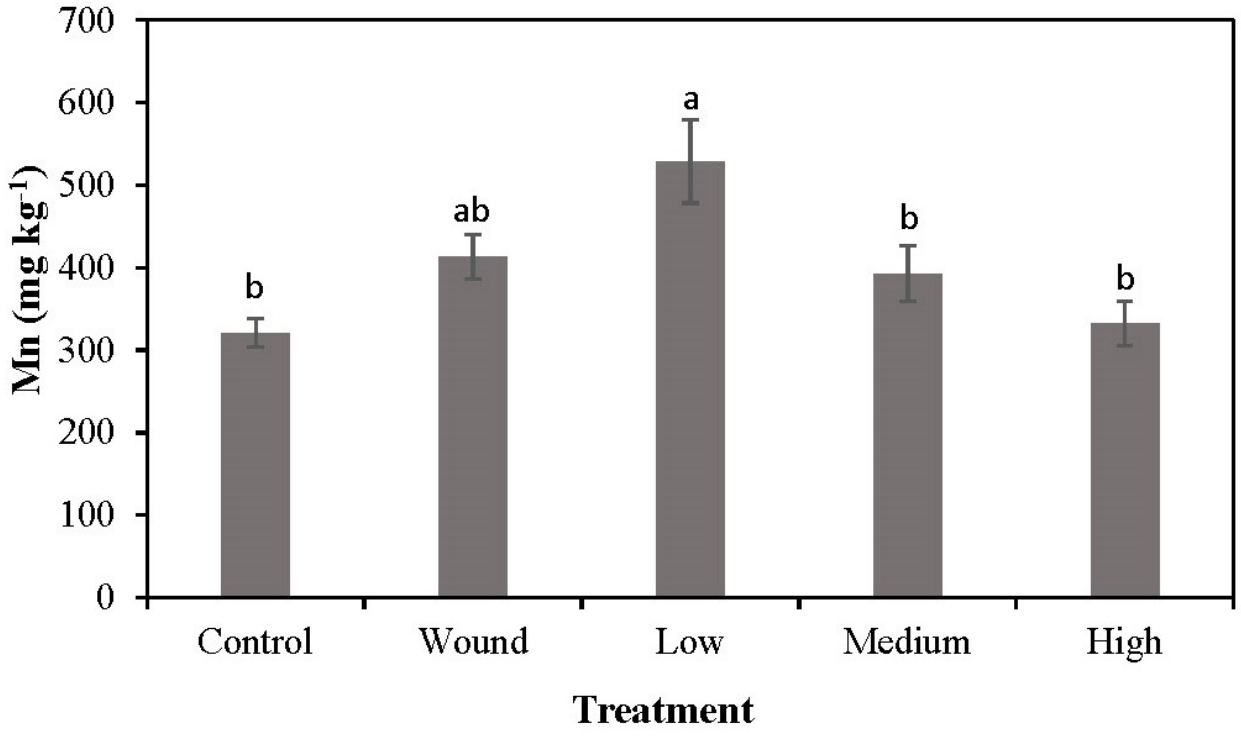

Figure 2.10. Average foliar manganese (Mn) concentration among trees assigned for control, wound, low, medium, and high inoculation treatments with *Leptographium terebrantis*. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are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

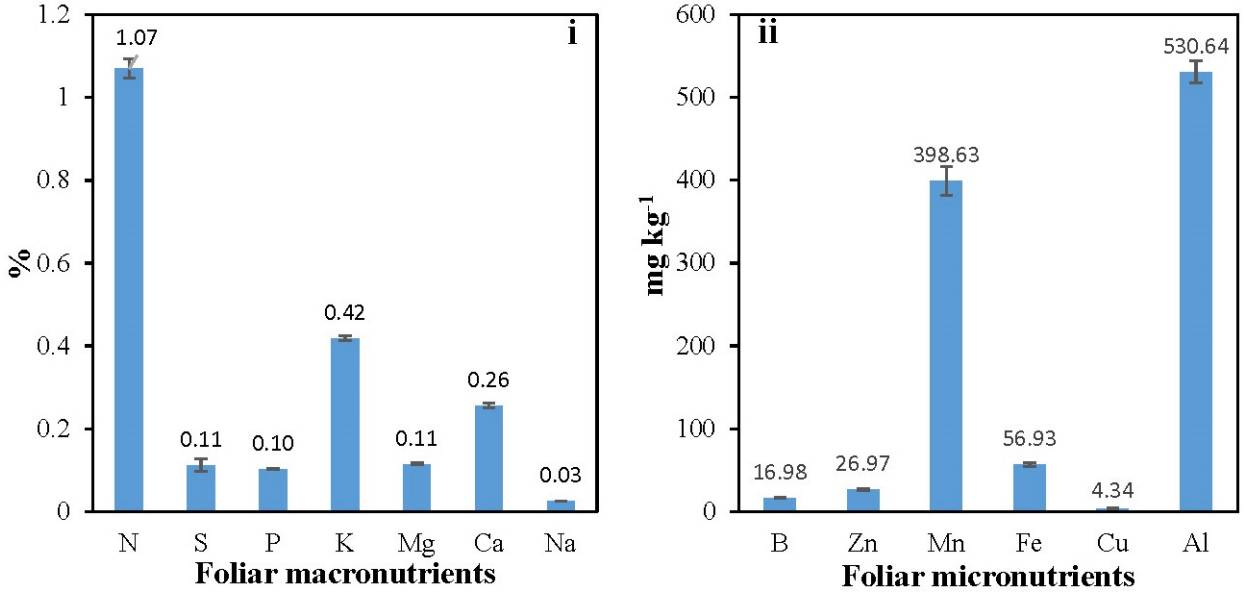

Figure 2.11. Average concentrations of foliar **(i)** nitrogen (N), sulphur (S), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), **(ii)** boron (B), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), and aluminum (Al) in the study site before the application of treatments to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