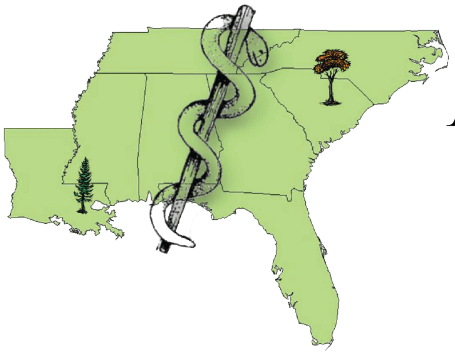

AUBURN UNIVERSITY
FOREST HEALTH COOPERATIVE

RESEARCH REPORT 19-02

FATE OF SOIL MICROBIAL BIOMASS IN THE
LEPTOGRAPHIUM TEREBRANTIS INOCULATED LOBLOLLY PINE STAND

by
Shrijana Duwadi and Lori Eckhardt

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 m2. 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).

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).

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.

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 K2SO4 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.

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).

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).

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.

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		

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		

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	.06934	<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		

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 x T	8	0.98	0.4868	Trt x T	8	0.93	0.9700
Trt x C/N	4	0.26	0.9015	Error	29		
T x C/N	2	4.10	0.0381				
Trt x T x C/N	8	0.93	0.5174				
Error	15						

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 x T	16	1.27	0.2530	Trt x T	8	0.18	0.9926
Error	50			Error	44		

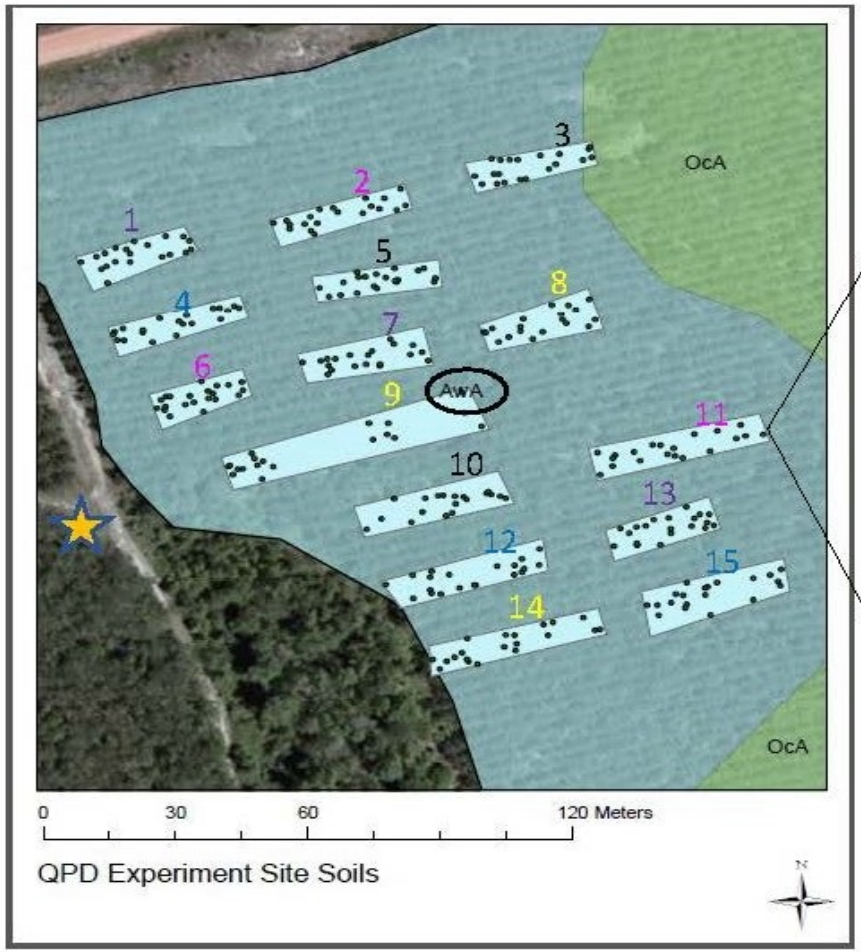

Figure 3.1. Map of the study site near Eufaula, Alabama. Treatment plots are represented by rectangles and soil series are represented by background color (AwA: Annemaine-Wahee complex). Small circles represent pre-thinning tree locations. Filled yellow star indicates the location of weather station.

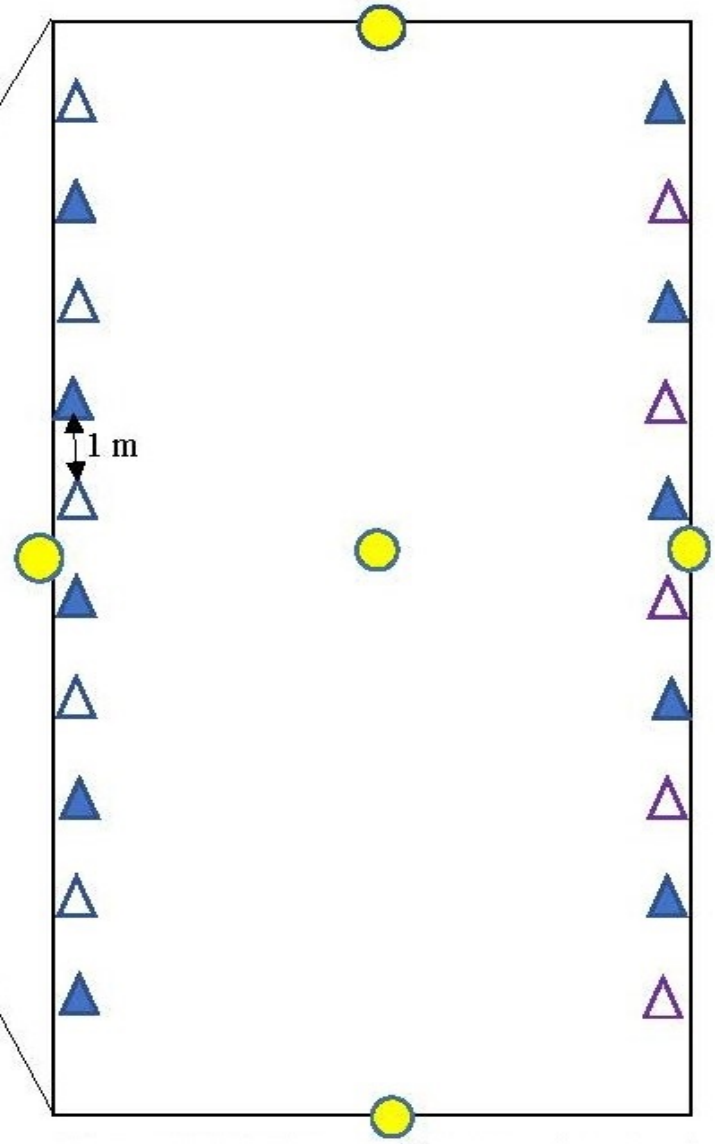

Figure 3.2. Post thinning field layout of tree arrangement and distance between them in each treatment plot. Unfilled purple triangles indicate inoculated trees, unfilled blue triangles indicate control trees, and solid blue triangles indicate other trees. Yellow circles represent microbial biomass collection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

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.

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

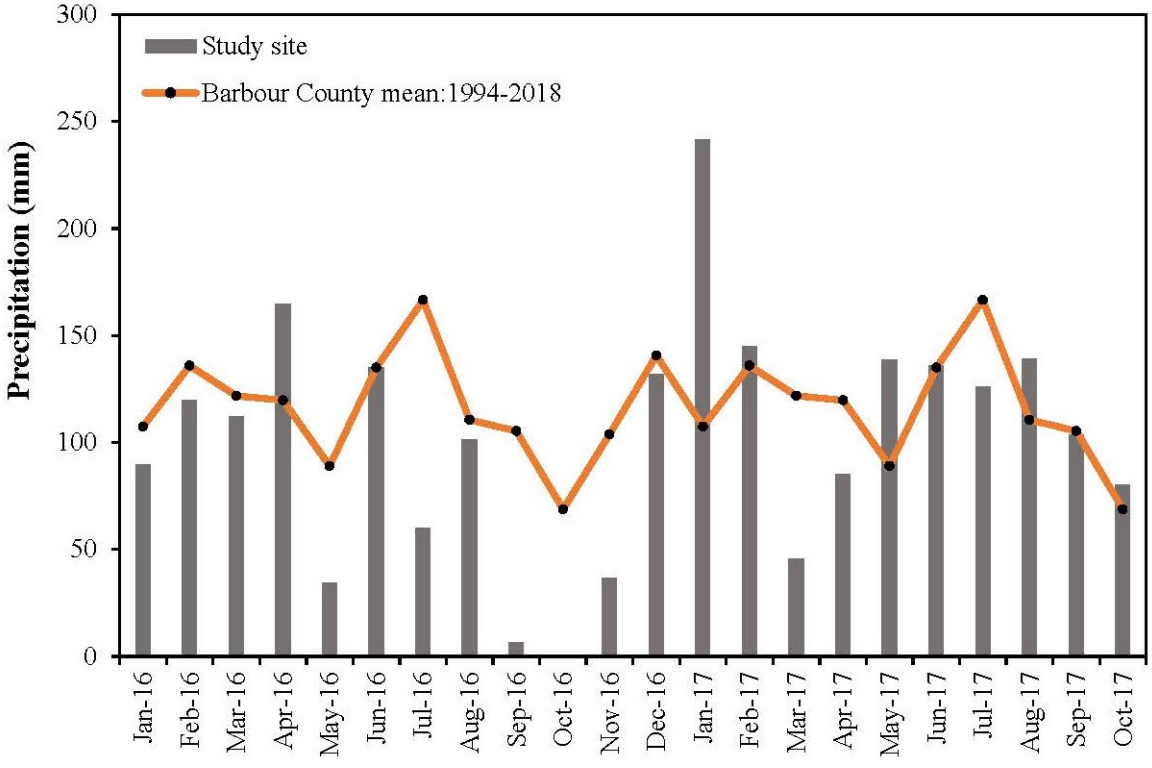

Figure 3.5. Mean monthly precipitation (mm) between 1994 and 2018 in Barbour County, Alabama and total monthly precipitation between January 2016 and October 2017 in a mature loblolly pine stand near Eufaula, Barbour County, Alabama.

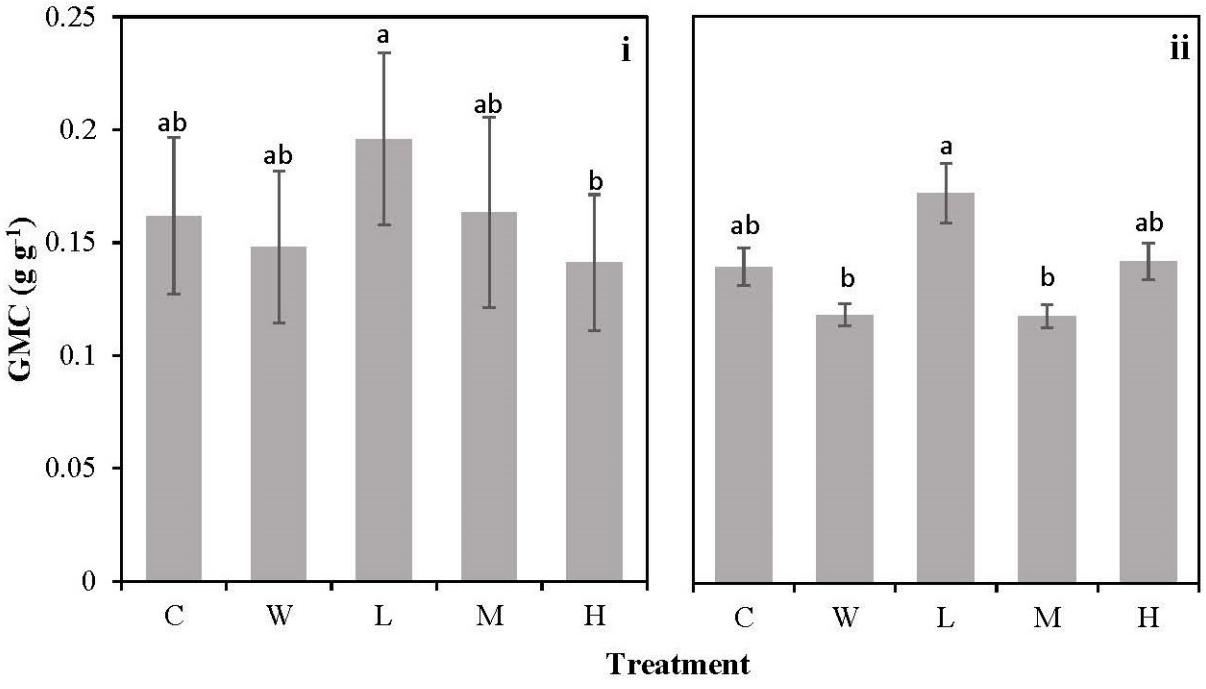

Figure 3.6. Average gravimetric soil moisture (GMC) among treatments **(i)** before and **(ii)** after the application of inoculation treatments on loblolly pine trees. Treatments were control (C), wound (L), low (L), medium (M), and high (H) inoculation. Error bars represent the standard error of the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

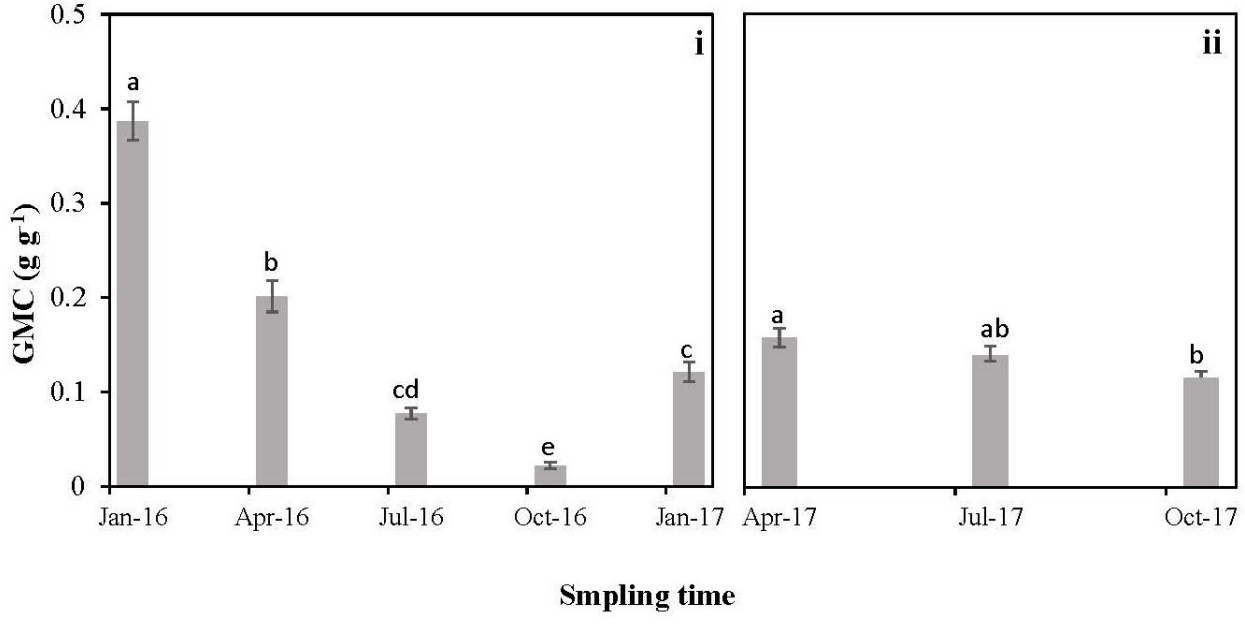

Figure 3.7. Average gravimetric soil moisture (GMC) among sampling time **(i)** before and **(ii)** after the application of inoculation treatments on loblolly pine trees. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter are significantly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

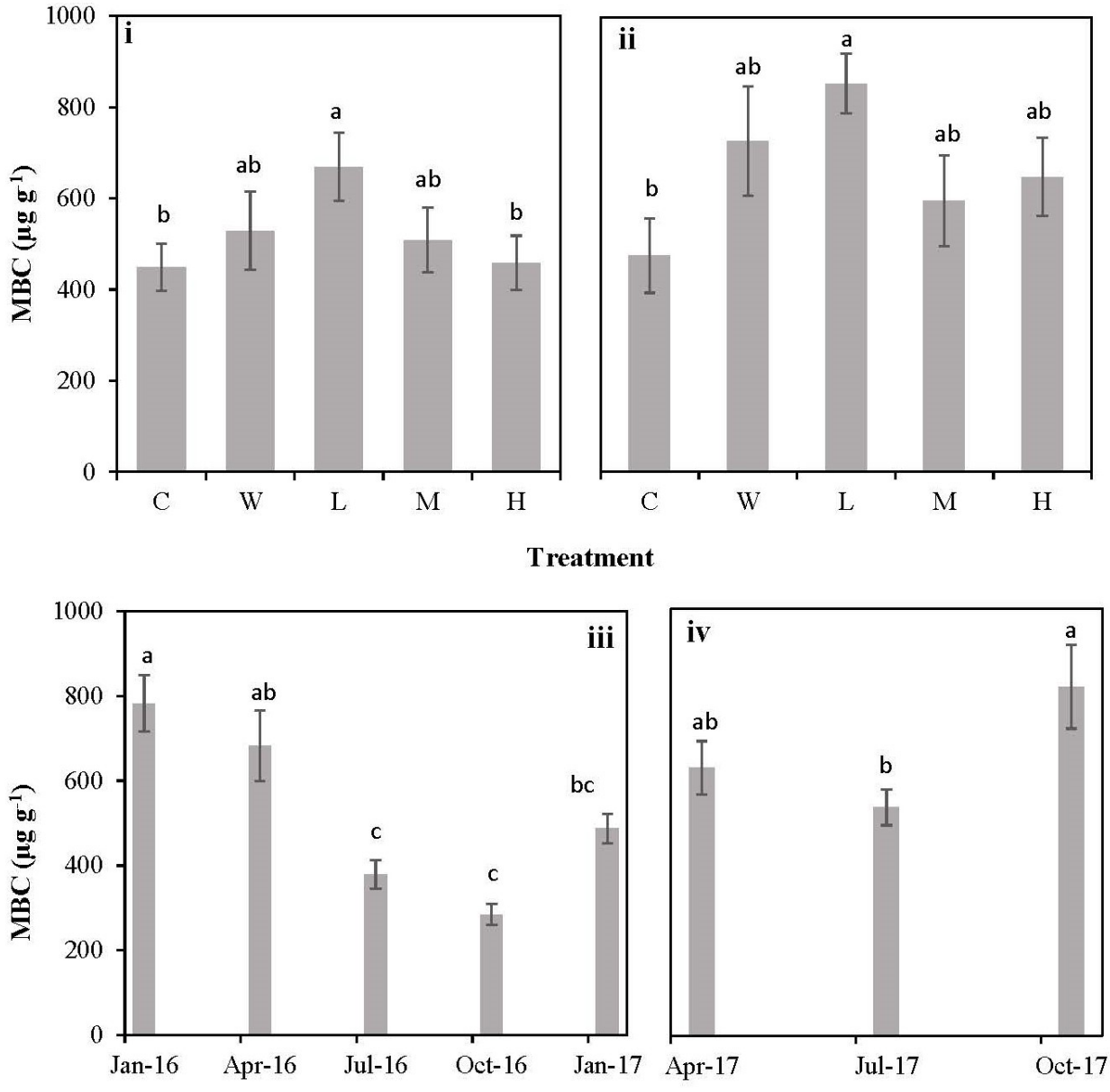

Figure 3.8. Average microbial biomass carbon (MBC) among treatments **(i)** before and **(ii)** after the application of inoculation treatments and among sampling time **(iii**) before and **(iv)** after the application of inoculation treatments on loblolly pine trees. Treatments were control (C), wound (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.

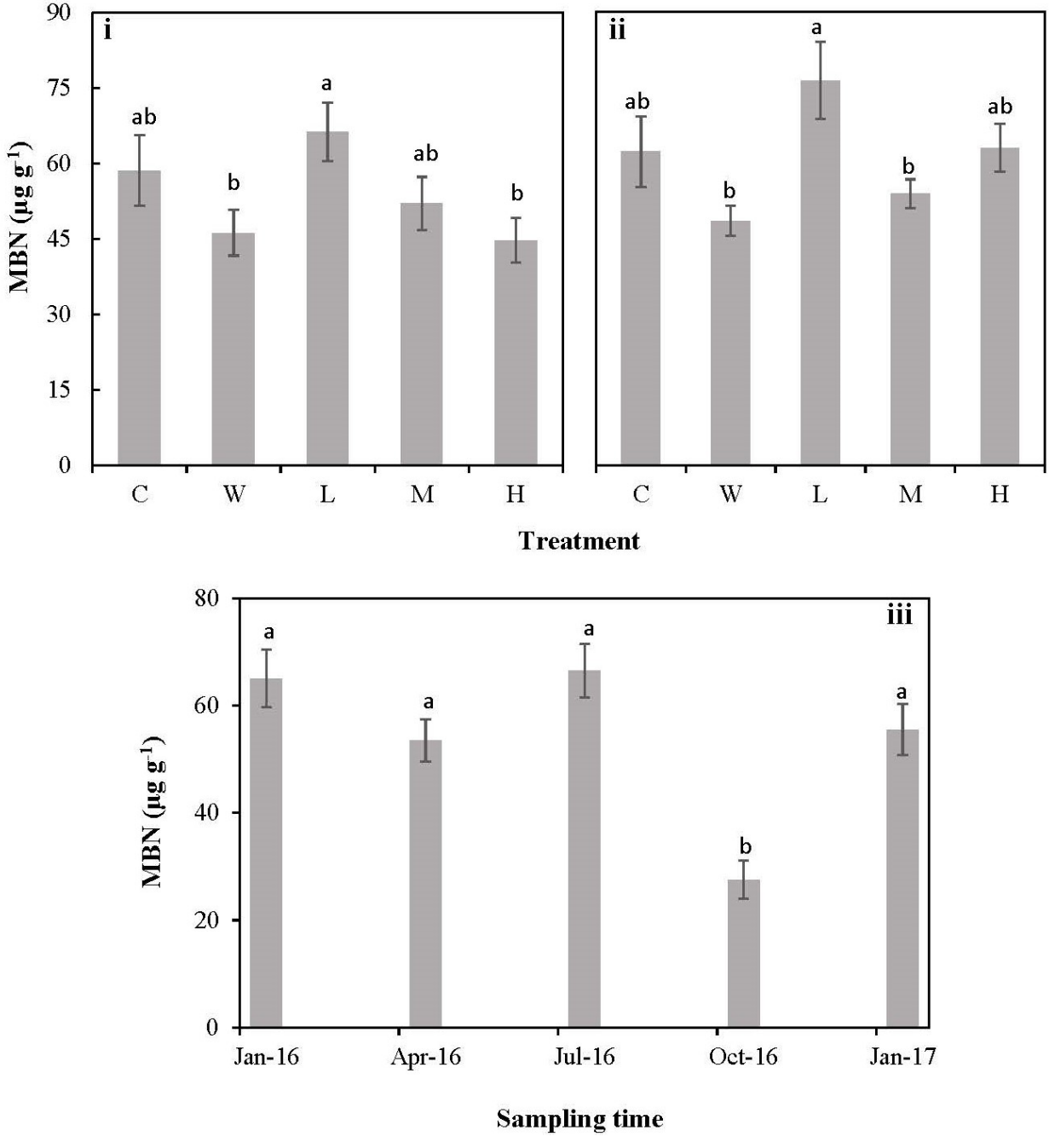

Figure 3.9. Average microbial biomass nitrogen (MBN) among treatments **(i)** before and **(ii)** after application of inoculation treatments and among sampling time **(iii)** before the application of inoculation treatments on loblolly pine trees. Treatments were control (C), wound (L), low (L), medium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

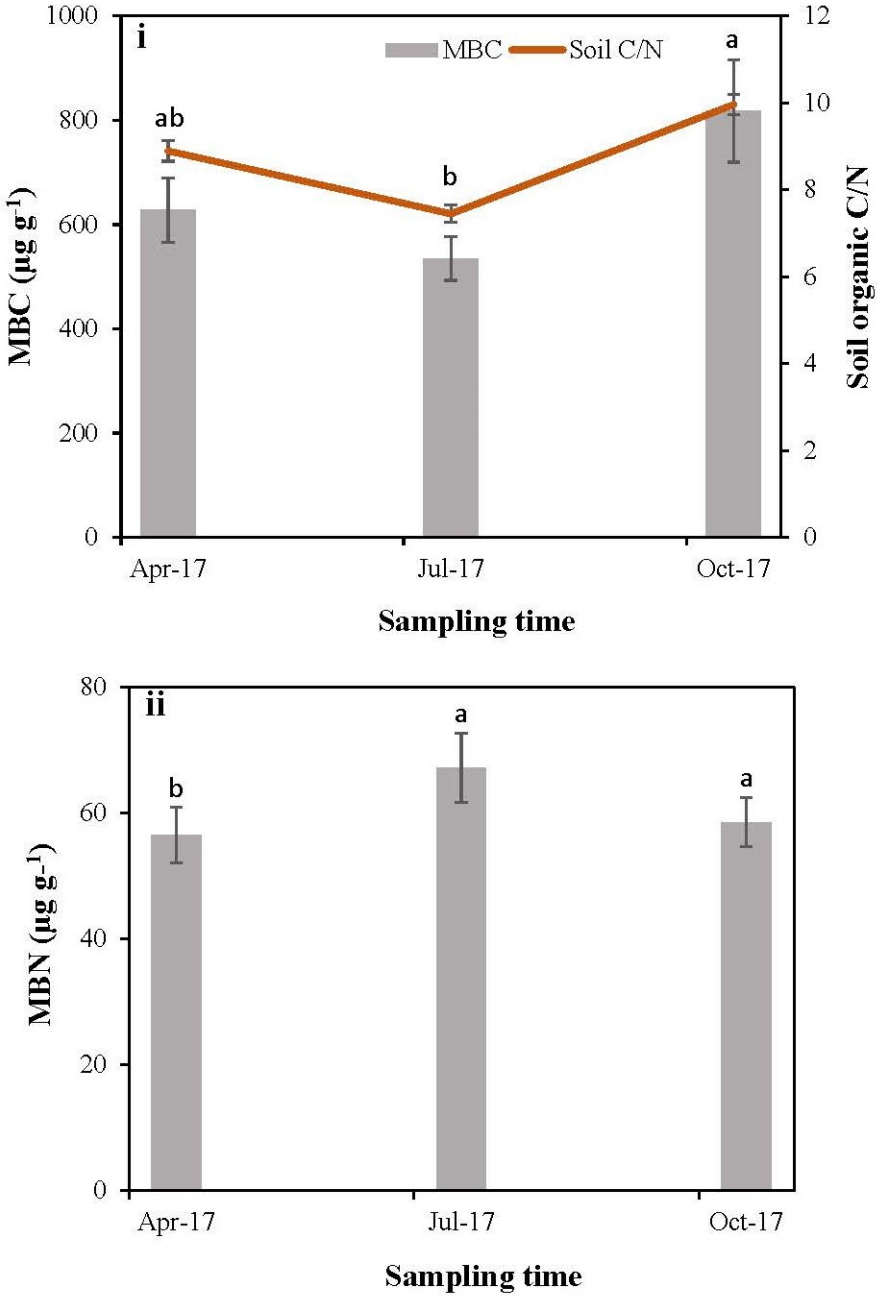

Figure 3.10. Average microbial biomass **(i)** carbon (MBC) and soil organic C/N ration and **(ii)** nitrogen (MBN) among sampling time after the application of inoculation treatments on loblolly pine trees. Error bars represent the standard error of the mean. In each figure, means associated with a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

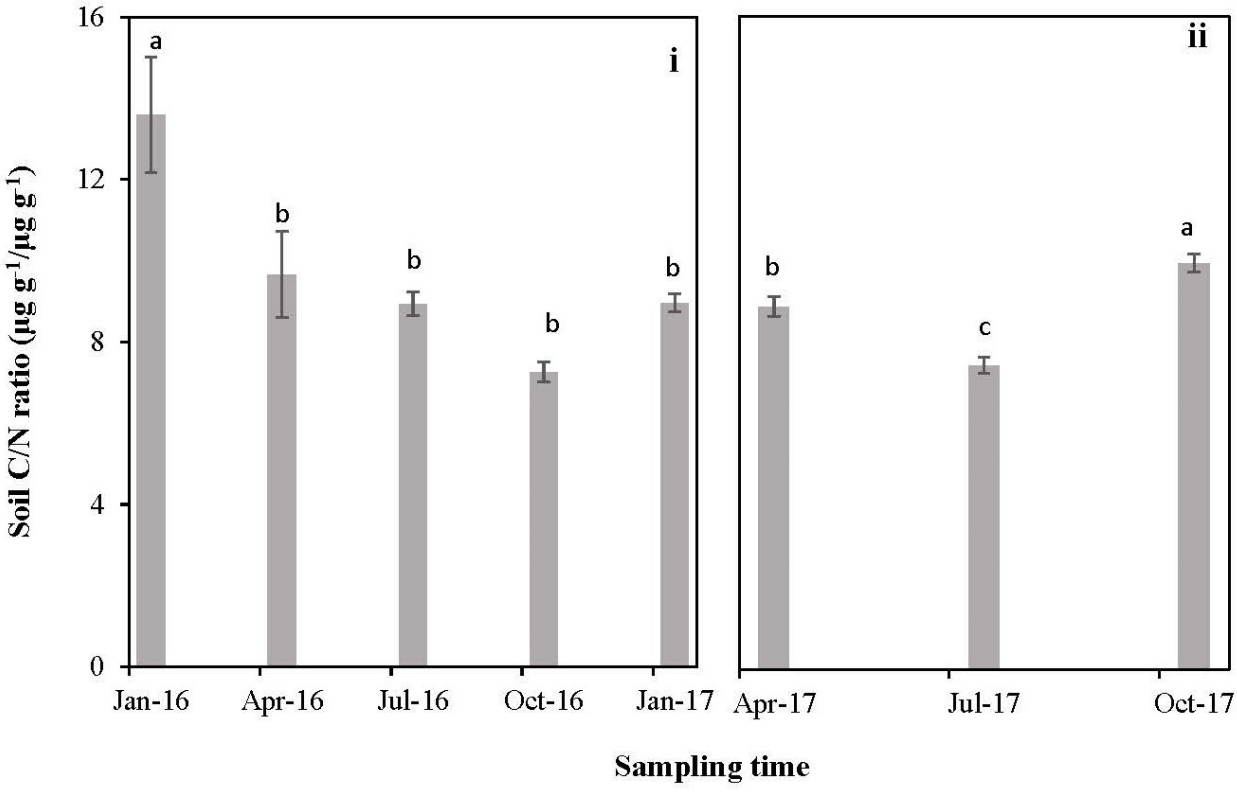

Figure 3.11. Average soil organic C/N ratio among sampling times **(i)** before and **(ii)** after the inoculation treatment of loblolly pine trees with *Leptographium terebrantis*. Error bars represent the standard error of the mean. In each figure, means associated with a different lower case letter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