DECADE-SCALE INFLUENCES OF ORGANIC MATTER REMOVAL ON FOREST SOIL BIOGEOCHEMISTRY AND MICROBIAL ECOLOGY

A Dissertation

by

RYAN MICHAEL MUSHINSKI

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Thomas W. Boutton
Co-Chair of Committee,	Terry J. Gentry
Committee Members,	Jason G. Vogel
	Jason B. West
Head of Department,	Kathleen L. Kavanagh

August 2017

Major Subject: Ecosystem Science and Management

ABSTRACT

One of the main goals of applied forest ecology is to utilize silvicultural practices that generate economic output from forest products while simultaneously maintaining the long-term sustainability of forest stand properties such as wildlife habitat, the ability to sequester carbon, soil nutrient stocks, and soil microbial community structure and function. To this regard, there are some forest management methods (such as clear-cutting, short rotations, whole-tree harvesting, and litter removal) that utilize intensive biomass removal techniques to increase economic output. These intensive methods could jeopardize the long-term sustainability of the forest ecosystem through the degradation and destabilization of soil biotic and abiotic properties. Because of this, the purpose of this study was to analyze specific soil biogeochemical and microbiological properties of *Pinus taeda* L. (loblolly pine) stands that were subjected to different harvest methods 18 years ago. Soil properties of intensively harvested stands (whole-tree harvest + forest floor removal) were compared to those of stands subjected to a less intensive method (bole-only harvest), and to unharvested control stands (tree age: 60-80 years old).

Results indicate that increasing organic matter removal intensity can lead to reduced tree size and reduced soil organic carbon and soil total nitrogen; furthermore, soils from whole-tree harvest + forest floor removal plots were less enriched in $\delta^{13}C$ and more enriched in $\delta^{15}N$ suggesting that increasing forest harvest intensity decreases long-term carbon mineralization and decomposition potential as well as increases N-losses through volatilization and leaching. Increasing organic matter removal intensity also reduced microbial biomass carbon and microbial biomass nitrogen, NH_4^+, and $NO_2^- + NO_3^-$ pools, increased the concentration of Mehlich-III extractable P, and altered the abundance of archaeal and bacterial *amoA*. Furthermore, intensive forest harvest led to decade-scale alterations in ammonia-oxidizer,

fungal, and bacterial and community structure as well as functional fungal and bacterial groups relative to unharvested stands. These results imply that more intensive harvest methods not only lead to reduced tree size, but also create decade-long alterations in physical, chemical, and biological properties in surface and subsurface soils, which could be inherited by future rotations.

ACKNOWLEDGEMENTS

I would first like to thank my committee co-chairs, Dr. Tom Boutton and Dr. Terry Gentry, for their support and allowing me the opportunity to work in their respective labs; it has been an absolute pleasure. Many thanks also go to Dr. Jason Vogel and Dr. Jason West for serving on my committee as well as their guidance and support throughout the course of this research. This work could not have been completed without the help of numerous individuals in the Stable Isotopes for Biosphere Sciences Laboratory, most notably, Dr. Ayumi Hyodo and Matt Smith as well as Heidi Mjelde and Dr. Pauline Wanjugi from the Soil and Aquatic Microbiology Laboratory. I would also like to thank Dr. Andy Scott and Brian Townsend from the USDA Forest Service for allowing me the opportunity to sample soil on the Davy Crockett National Forest. Many thanks to my lab mates (Yong Zhou, Julie Foote, Rachel Wellman, Darcy Moreland, Brandon Cawthon, and Rachel Adams) for lively discussion and timely jokes. Finally, and most importantly, thanks to my family, especially my mother, father, and brother, for their support and encouragement and to my beautiful wife, Bekah, for her patience and love.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supported by a dissertation committee consisting of Dr. Thomas Boutton (chair), Dr. Terry Gentry (co-chair), Dr. Jason West (committee member), and Dr. Jason Vogel (committee member). Bacterial *amoA* standard for qPCR assays, as noted in chapter III, was provided by Robert Dorosky, and archaeal *amoA* standards for qPCR were provided by the lab of Raina Maier (University of Arizona). Illumina sequencing was done by Molecular Research DNA Laboratory (www.mrdna.com, Shallowater, TX, USA). Mehlich-III extraction and subsequent analysis of macronutrients was conducted by the staff at Texas A&M's Soil, Water, and Forage Testing Laboratory. All other work conducted for this dissertation was completed by the student independently.

Funding Sources

This dissertation was funded in part by a Graduate Merit Fellowship from the Office of Graduate and Professional Studies at Texas A&M University, a McMillan-Ward Fellowship from the Department of Ecosystem Science and Management at Texas A&M University, and an Excellence Fellowship from the College of Agriculture and Life Sciences at Texas A&M University. Further support for graduate study was received the George Bush Presidential Library, and the Soil Science Society of America. Research was funded by USDA/NIFA Hatch Project 1003961. The contents of this dissertation are solely the responsibility of the authors and do not necessarily represent the official views of the funding offices.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

Page

LIST OF TABLES

Page

Page

CHAPTER I

INTRODUCTION

Forest ecosystems in the United States (US) have functioned as a strong carbon (C) sink in recent decades (Lal et al., 2005; Pan et al., 2011); sequestering an equivalent of 25 years' worth of US fossil fuel CO_2 emissions (Woodall et al., 2011). Southern pine forests represent a major component of this C sink (Han et al., 2007). In addition, these southern forests account for ≥40% of the annual US softwood production and consistently produce 19% and 12% of the worldwide pulpwood and industrial timber, respectively (McNulty et al., 1996; Hodges et al., 2011; Brandeis et al., 2012). Both the sustainability of the C sink strength and the economic output of forest products are contingent upon the maintenance of biogeochemical processes that sustain soil fertility and forest productivity (Woodall et al., 2013), as well as the microbial populations that carry out those processes. However, disturbances such as intensive organic matter removal associated with timber harvest have the potential to diminish the C sink strength and forest productivity by removing limiting nutrients in the harvested biomass and increasing the potential for nutrient losses via erosion, leaching, and trace gas fluxes (Kellman et al., 2014). Because of the multifaceted importance of southern pine forestlands, the purpose of this study is to evaluate the long-term implications of extreme levels of forest harvesting on the soil biogeochemical and microbiological properties and processes that maintain this ecosystem.

Soil Biogeochemistry and Microbial Ecology of Managed Forestlands

Terrestrial soil carbon pools account for roughly 1,200-1,500 Pg of the yearly carbon budget (Houghton, 2007). Soil organic carbon (SOC) accounts of two thirds of the total soil carbon pool, and is considered one of the most important indicators of soil quality due to its ability to influence soil structure, nutrient concentrations, water-holding capacity, and microbial

activity (Lal, 2004; Bationo et al., 2007; Batjes, 2014). Removal or redistribution of forest biomass during timber harvest has the potential to alter SOC stocks and modify rates of nutrient cycle processes (Chen et al., 2013; Dangal et al., 2014; Vario et al., 2014), potentially jeopardizing forest production and sustainability over the longer term. Recent studies have shown that there is a positive relationship between the amount of biomass removed and percentage of nutrients lost from the system (Hazlett et al, 2014; Kellman et al. 2014; Vario et al. 2014; Achat et al., 2015a). In addition to direct losses associated with forest harvest, indirect consequences may occur due to changes in the microclimate. For example, increases in soil temperature could influence the activity of extracellular carbon-degrading enzymes (Agren and Hyvonen, 2003) and the ability of the soil to physically protect nutrients (Anderson et al., 2010). In regards to southeastern US pine forests, previous studies have shown that intensive forest harvest practices can result in reductions in C pool size for surface mineral soil (Scott et al., 2014; Foote et al., 2015).

The majority of our understanding regarding the processes that govern soil carbon pools and fluxes in forest ecosystems is derived from studies that focus on the top 30-cm of the soil profile. This uppermost portion of the soil profile is the most biologically active region within any given soil profile, and stores a significant proportion of key nutrients. However, it has been reported that increased attention should be given to deeper soil horizons due to the realization that there can be significant stores of organic matter in deeper portions of the soil profile (Diochon et al., 2009; Rumpel and Kogel-Knabner, 2011; James et al., 2014; James et al., 2015; Karhu et al., 2016). The few forestry-related studies that have investigated deeper soil C pools have generally occurred in hardwood forest ecosystems. These studies have found that following disturbance, loss of soil C content deeper in the soil profile is consistent with reductions in soil C in more superficial soil strata (Zummo and Friedland, 2011; Egnell et al., 2015). Therefore, there

is a precedent that disturbances, such as forest harvest, can influence alterations of SOC stocks at depths of greater than 30-cm. Efforts should be made to investigate whether these assertions are consistent in southern pine forest systems that are subjected to intensive management techniques.

Primary production in southern US forests is commonly N limited (Richter et al., 1999; Piatek and Allen, 2000) which can be attributed to the exceedingly high demand for plant-available N, the relatively small pool of inorganic N in the soil at any one time, slow rates N turnover, and N losses due to leaching and gaseous volatilization (Binkley et al., 2000). In general, a change in soil N availability can alter the C:N ratio of above- and belowground litter inputs by changing plant tissue N concentrations. In ecosystems that are already N limited, a reduction in N inputs and modifications to the N-cycling community could be detrimental to subsequent plant growth. Forest harvest has the potential to increase N in soil by reducing plant uptake (Burns and Murdoch, 2005); however, this observed increase in soil N usually peaks 5 years after harvest and then decreases substantially (Bradley et al., 2001). Depending on the harvest method used, this N-deficit could have long-lasting impacts on the total soil N pool. Because of these reasons, it is particularly critical to understand the impacts of intensive forest harvest on surface and subsurface soil N storage.

In relation to microbial community composition, it has been shown that changes in inorganic N pools can alter community structure (Frey et al., 2004; Galloway et al., 2004) and inhibit activity (Fisk and Fahey, 2001; Burton et al., 2004). Forest disturbances, including harvest, fire, and fertilization, have the potential to modify the production and consumption of nitrate (NO_3^-) through the alteration of the microbial community. A meta-analysis conducted by Jerabkova et al. (2011) illustrated that in coniferous forests, clearcutting significantly altered the pool size of NO_3^-; hypothesizing that this observation was a result of a change in the nitrifying community. Hynes and Germida (2011) assessed ammonia oxidizing bacteria (AOB) community

composition and N bioavailability in a chronosequence of clear-cut boreal forest soils. They found that the AOB community composition differed along the chronosequence and associated changes in AOB community composition paralleled N bioavailability. Other studies have also shown that forest harvest can impact nitrification rates as well as the abundance of inorganic N-containing compounds (Kreutzweiser et al., 2008; Kellman et al. 2014). Because NO_3^- is extremely susceptible to losses via leaching and denitrification, as well as the potential effect that forest harvest may have on AOB and ammonia oxidizing archaea (AOA) communities, it is necessary to explore these concepts in a consistently N-depleted system such as a southern US pine forest.

Soil microbes (bacteria, archaea, fungi, viruses, and micro-fauna) provide vital maintenance ecosystem services such as the management of organic matter decomposition, the mediation of nutrient cycling, and the establishment of mutually beneficial symbiotic relationships with various flora (Andren and Balandreau, 1999; McGuire and Treseder, 2010). Research has shown that a loss of biodiversity (micro and macro) can lead to a loss of ecosystem function and stability (Cardinale et al., 2012). This poses the question of whether aboveground disturbance can have a broad effect on surface and subsurface microbial community composition and structure. Studies investigating the effect of intensive harvest methods on the relative size of the microbial populations, proxied by soil microbial biomass (SMB), have been inconsistent. Some studies show that harvest intensity has no influence on SMB (Busse et al., 2006; Mariani et al. 2006; Smolander et al. 2010), while others have found decreases in SMB with increasing forest harvest intensity (Hassett and Zak 2005; Tan et al. 2008). In studies that do observe differences, they hypothesize that the reduction of litter inputs and altered soil microclimate increases rates of decomposition and mineralization following harvest which eventually leads to reductions in C and N leading to an overall decrease in the size of the microbial community. This

poses the question of how resilient to intensive forest harvest is the size and composition of the microbial community as well as the relative abundance of specific taxa, especially deeper in the soil profile.

Knowledge Gaps in Forest Ecology Research

Despite the ecological and economic importance of southeastern pine forest ecosystems, studies have generally neglected investigating the long-term effects of overly intensive forest management techniques on some aspects of soil biogeochemistry and many aspects of soil microbial ecology. This is especially true for deeper soil horizons (i.e., the upper 1 meter of the soil profile). Microbial communities are integral components of the biogeochemistry, fertility, and structure of forest soils (Schlesinger et al., 2013), and management practices that modify the microbial environment could jeopardize the long-term sustainability and productivity of forestlands. In a broader context, very few studies of microbial community structure have been attempted in the southeastern US soils, mainly due to the challenge in successfully extracting high quality nucleic acid from these generally acidic soils (Burgmann et al. 2001; Sargova-Mareckova et al. 2008). However, new methodologies and technologies now can be utilized to successfully characterize these often difficult to work with soils (ISO, 2012).

Continent-Wide Long Term Soil Productivity Study

Most of the intensively managed United States forest plantations utilize bole-only harvest methods. These management techniques redistribute the branches and needles across the entirety of the site in order to retain some of the aboveground C; however, if this methodology is disregarded, higher levels of biomass removal could impact the long term soil productivity and soil carbon stores (Powers et al. 2005). As the demand for forest products, other than whole-wood, continues to increase, more intensive harvest methods will become more popular.

Biomass utilization, in the form of forest litter, for biofuel production and other applications, will continue to gain popularity due to the abundance and low price of the product (EISA, 2007). The Long Term Soil Productivity (LTSP) program was initiated in 1989 to address concerns over potential losses in soil productivity from forest management practices on National Forest lands (Powers, 2006). This program now consists of sites, across all of North America, which employs a common experimental design examining the effects of forest harvest intensity on forest productivity. The core responsibility of the LTSP initiative is to map the short- and long-term effect of harvest disturbance on a forest's ability to capture and retain carbon to produce biomass (Ponder et al., 2012). The full scale of the project constitutes a total of over 100 North American installations; of which above ground biomass will be harvested in three levels, bole-only harvest, full-tree harvest, and full-tree harvest + forest floor removal. Concurrently, three levels of soil compaction are applied to each level of organic matter removal which results in a three by three factorial design (nine treatment combinations). Chemical vegetation control, glyphosate herbicide, was also investigated on some sites.

Previous studies, across all climactic regions, have shown that increasing harvest intensity tends to decreases soil C concentrations (Scott et al., 2014) and reduces nutrient availability up to 20 cm (Powers, 2005) and 60 cm (Slesak et al., 2011) in the soil profile; as well as reduced growth, 5 years after planting, on productive, nutrient-limited, warm-humid sites (Fleming et al., 2006). However, 10-years post-harvest, no differences in tree biomass or foliar nutrition were observed on some sites (Ponder et al., 2012). Tan et al. (2005) found that on boreal forest soils, full tree + forest floor removal significantly reduced microbial biomass carbon (MBC) and microbial biomass nitrogen (N), which was hypothesized to be a result of the removal of organic substrates responsible for microbial growth. However, Busse et al. (2006) found that microbial biomass, respiration, and fungal phospholipid fatty acids were generally

unaffected by organic matter removal. Other studies have found that net rates of N mineralization are increased as a result of the whole-tree + forest floor removal treatment. Soil temperature increase, as a result losing the forest floor buffering capacity, may have led to this acceleration.

Description of Study Area

This study was carried out within a site associated with the Long-term Soil Productivity network (Powers et al., 2005; Powers et al., 2006; Ponder et al., 2012). Specifically, the study area is located in the Davy Crockett National Forest near Groveton, Texas, USA (31°06´ 32.48´´N, 95°09´ 59.15´´W) harvested in 1996 and replanted to *P. taeda* L. in 1997. The climate is subtropical with a mean annual temperature of 18.7°C and mean annual precipitation total of roughly 1100 mm (1950-2010). Rainfall is evenly distributed throughout the year with May and September as the wettest months. Potential annual evapotranspiration is approximately 1200 mm (Norwine et al., 2005). Because of a lack of climate sensors centralized at our study site, climate data will be obtained from the National Climatic Data Center (NOAA) weather stations in Crockett, TX and Lufkin, TX, 38 km northwest and 48 km northeast of the study site, respectively, and averaged to obtain a mean value for the general area. Topography is nearly flat with slopes of 1-3% and elevation ranging from 101-m to 110-m. Soils across the study area are fine-loamy, siliceous, thermic Oxyaquic Glossudalf in the Kurth series which developed in loamy coastal plain sediments of the Yegua and Whitset geological formations.

The experimental design includes: (i) unharvested control forest stands comprised of trees 60-80 years of age; (ii) merchantable bole/stem-only harvest stands; and (iii) whole-tree harvest + forest floor removal stands. The unharvested control stands were thinned intermittently. Each treatment is comprised of three 0.2 ha replicates. Trees were hand-felled and lifted off of the plots with a loader. Forest floor removal was accomplished by hand-raking

all aboveground organic matter from the whole-tree + forest floor removal treatment plots. Containerized *P. taeda* L. seedlings of 10-half sib families from the US Forest Service seed orchards were hand planted on a 2.5-m x 2.5-m spacing.

Research Objectives and Hypotheses

The contributing research for this dissertation has been divided into three main subject-areas, all aimed at unraveling the long-term influence of intensive forest management on soil biogeochemical and soil microbiological properties. Listed below are the objectives of each subject-area and associated hypotheses.

First, we seek to determine the influence of an intensive forest management technique on soil organic carbon (SOC) and soil total nitrogen (TN) for the uppermost one meter of the soil profile. We hypothesize that 18-years post-harvest, stands subjected to the most intensive management technique will have lower stocks of SOC and TN, at all soil depths analyzed. We can attribute this to a reduction in potential C and N inputs over time. Simultaneously, we will analyze the stable isotopes of SOC ($\delta^{13}C$) and TN ($\delta^{15}N$) to explore potential mechanisms underlying changes in soil C and N stores. We hypothesize that soils more enriched in $\delta^{13}C$ and $\delta^{15}N$ will be associated with the intensive harvest stands due accelerated losses of C and N followed by reduced inputs over time.

Secondly, because most soils in the southeastern US are limited by N, we seek to determine the long-term implications of intensive forest management on pools of inorganic N, rates of nitrification, and the abundance of microbial groups associated with these processes. Due to previous studies showing that more intensive management methods tend to result in reductions in TN (Foote et al., 2015), we hypothesize that pools of inorganic N will be reduced as well. From this proposed reduction in inorganic N (specifically, $NH_3 + NH_4^+$) we suspect that rates of nitrification will be reduced accordingly. Also, to coincide with the reduction in rates of

nitrification, the community size of ammonia oxidizing bacteria and archaea will be smaller in the intensively managed stands. Furthermore, although $NH_3 + NH_4^+$ is proposed to be smaller in the intensively managed stands, the ratio of $NH_3 + NH_4^+$ to $NO_2^- + NO_3^-$ will be larger due to the reduction in rates of nitrification. In a broader microbial ecology context, many studies have shown that in acidic forest soils archaea dominate the process of ammonia oxidation (Stempfhuber et al., 2015). This question has not been investigated in this region, and because of that, a component of this subproject will be focused on this question. We think that it appropriate to hypothesize that due to the previously reported soil conditions for this region, archaea will be the dominant ammonia oxidizer.

Finally, because of the importance of soil microbes to biogeochemical cycling and their ability to be altered by disturbance, we seek to determine the influence of intensive forest management on soil prokaryote and fungal community composition and structure. We hypothesize that stands subjected to intensive forest management techniques will show reductions in common microbial metrics such as biomass, diversity, and richness, relative to the other two stand types. In addition, we hypothesize that the differing treatments will result in distinct microbial populations which can be correlated to concurrent changes in physicochemical properties such as soil pH, SOC content, and TN content. We also predict that any observed differences will be less pronounced at deeper soil horizons due to niche differentiation and the buffering capacity of the soil.

Expected Results

This study will contribute to a better understanding of the long-term implications of intensive harvest management techniques on soil properties that influence the productivity of southern US forestlands. Given the global need for forest-based products, the results of this study are intended to help guide forest management practices that promote the sustainability of forest

resources. Furthermore, this will be one of the first studies to link biogeochemistry and microbial ecology to better understand the effect of intensive timber harvest in the southeastern US. Based on previous studies, I anticipate that soils in stands subjected to the more intensive forest management technique will possess lower SOC, TN, and inorganic N leading to lower microbial biomass and an altered microbial community composition with lower diversity and richness. I also anticipate that SOC, TN, and inorganic N will decrease with increasing soil depth, which, although at lower concentrations than that of surface soil, will help to better explain total C and N stocks in soils of the southeastern US.

CHAPTER II

DECADAL-SCALE CHANGES IN FOREST SOIL CARBON AND NITROGEN ARE INFLUENCED BY ORGANIC MATTER REMOVAL DURING TIMBER HARVEST*

This study investigates whether different intensities of organic matter removal associated with timber harvest influence decadal-scale storage of soil organic carbon (SOC) and total nitrogen (TN) in the top 1 m of mineral soil 18 years post-harvest in a *Pinus taeda* L. forest in the Gulf Coastal Plain. We quantified forest harvest-related changes in SOC, TN, microbial biomass carbon (MBC), and nitrogen (MBN) pools (0-100 cm) in unharvested control stands and in two organic matter removal treatment stands subjected to either (i) merchantable bole/stem-only harvest, or (ii) whole-tree harvest + forest floor removal. In addition, $\delta^{13}C$ of SOC and $\delta^{15}N$ of TN were measured in surface and subsurface mineral soil to provide insights regarding mechanisms that might explain changes in SOC and TN pool sizes. Soils were sampled seasonally for one year. Increasing organic matter removal intensity reduced SOC , TN, MBC, and MBN at all depths relative to the unharvested control. Furthermore, soils from whole-tree harvest + forest floor removal stands had lower $\delta^{13}C$ and higher $\delta^{15}N$ values suggesting that increasing organic matter removal may decrease heterotrophic activity as well as increase rates of N-loss. Seasonal variability in SOC and TN were correlated to changes in forest biological properties such as root and litter biomass. These results indicate that more intensive harvest methods may lead to decade-scale decreases in SOC and TN storage in surface and subsurface soils which could influence rates of biogeochemical processes, the availability of soil nutrients, and potential forest productivity.

*Reprinted with permission from "Decadal-scale changes in forest soil carbon and nitrogen storage are influenced by organic matter removal during timber harvest" by Mushinski RM, Boutton TW, Scott DA, 2017. Journal of Geophysical Research-Biogeosciences, 122, 846-862, Copyright 2017 by John Wiley & Sons.

Introduction

The global soil organic carbon (SOC) pool stores approximately 1,200-1,550 Pg C in the upper 1-m of soil, which represents about 75% of the terrestrial carbon (C) pool (Jobbagy and Jackson, 2000; Houghton, 2007; Batjes, 2014); congruently, soil total nitrogen (TN) stocks are estimated at 133-140 Pg N within that same depth interval (Batjes, 2014). In forest soils, the upper 1 m stores 353-413 Pg C which is nearly 30% of global SOC and accounts for more C than is stored in above and belowground live biomass, deadwood biomass, and litter biomass (Pan et al, 2011). Furthermore, it has been noted that most forest ecosystems store between 2-12 Mg N ha^{-1} in mineral soil (Johnson and Turner, 2014), which is equivalent to 8-48 Pg N across all forestlands (FAO, 2016). Hansen et al. (2010) recently showed that the global rate of gross forest cover loss due to natural and anthropogenic perturbations between the years 2000 to 2005 was >1x 10^6 km^2, equivalent to 3.1% of global forest cover in 2000. Given the geographic dimensions of forest disturbance, the magnitude of soil C and N stores in forest soils, and the important roles of these elements in global biogeochemistry and the climate system, it is important to understand how forest soils might respond to disturbance.

Responses of SOC and TN stocks in surface and subsurface mineral soils may be particularly relevant in the context of timber harvesting, whose legacy effects may last for decades to centuries (Chen et al., 2013; Kellman et al., 2014; Prest et al., 2014; Dean et al., 2016). Both SOC and TN are important indicators of soil quality due to their ability to influence soil structure, nutrient concentrations, water-holding capacity, and microbial activity (Lal, 2004; Bationo et al., 2007). Removal or redistribution of forest biomass during timber harvest has the potential to alter SOC and TN stocks and modify rates of nutrient cycling processes (Chen et al., 2013; Dangal et al., 2014; Vario et al., 2014), potentially jeopardizing forest production and sustainability. Furthermore, forest biomass removal may also substantially affect climate change

through the conversion of forests from carbon sinks to carbon sources (Pan et al., 2011; Chen et al., 2013). For these reasons, the accurate quantification of SOC and TN are essential for determining the long-term influence of intensive timber harvest regimes. Studies investigating the effect of forest disturbance on SOC and TN have generally been limited to the top 10 cm of the soil profile; however, it has become apparent that deeper (i.e., >10 cm depth) soil C and N stocks need to be quantified in order to more fully characterize the effects of forest harvest (Diochon et al., 2009; Slesak et al., 2011; Buchholz et al., 2014; James et al., 2014; James et al., 2015). Furthermore, SOC and TN temporal dynamics at depth are poorly understood and should be investigated to determine what factors maintain C and N in subsurface soil horizons.

Investigations on changes in SOC storage in response to differing timber harvest methods have been shown to result in divergent conclusions. Studies from single-locations or -regions sometimes report reductions in SOC with increasing timber harvest intensity (Li et al., 2003; Jones et al., 2011; Huang et al., 2013) while other report no differences (Johnson and Todd, 1998; Zerpa et al., 2010). However, most meta-analyses that incorporate studies from across a range of abiotic and environmental gradients consistently report that intensive timber harvest results in a general reduction in SOC (Johnson and Curtis, 2001; Nave et al., 2010; Achat et al., 2015a). Specifically, Achat et al. (2015a) showed that intensive timber harvest methods involving removal of harvest residues can reduce SOC content in mineral soil by 10%. These reductions have been shown to be positively correlated to both the amount of harvested biomass removed and the C and N stores in that biomass (Hazlett et al, 2014; Kellman et al. 2014; Vario et al. 2014; Achat et al., 2015a; Achat et al., 2015b). A recent literature review and modeling study has suggested that the observed wide range of SOC responses to forest harvest is likely due to variability in (a) the time interval between the harvest event and SOC sampling, and (b) the number of prior logging cycles at a given site (Dean et al. 2016). It should also be noted that soil

C has been shown to take several decades to recover to pre-harvest levels following harvest, with some soil orders taking upwards of 75 years to recover (James and Harrison, 2016); however, continued research is needed to further investigate the mechanisms governing soil C recovery, especially at depth.

In addition to SOC and TN stocks, differing timber harvest methods can also influence the size of the soil microbial biomass pool and the rates of the biogeochemical processes that they mediate. Microbial biomass C (MBC) and N (MBN) are fundamental components of SOC and TN as well as indicators of biogeochemical potential in surface and subsurface soil (Wardle, 1992; Gallardo and Schlesinger, 1994; Zak et al., 1994) and should be investigated when quantifying SOC and TN stocks. It has been shown that the magnitude of microbial biomass is highly correlated with pool sizes of SOC and TN (Wardle, 1992; Allen and Schlesinger, 2004); therefore, any changes in the size of SOC and TN pools following disturbance may negatively impact the microbial biomass, even at depth. Previous studies have shown that surface soil microbial biomass (i.e., 0-10 cm) can be affected by timber harvest (Busse et al., 2006; Foote et al., 2015); however, no study has investigated whether this trend persists in deeper portions of the soil profile.

$\delta^{13}C$ values of SOC and $\delta^{15}N$ values of TN can add insight into the relative magnitude of C and N inputs versus losses from the soil following disturbance (Ehleringer et al., 2000; Robinson, 2001; Pataki et al., 2003; Diochon and Kellman, 2008; Schlesinger, 2013). The few studies that have investigated changes in $\delta^{13}C$ values in response to different levels of timber harvest showed enrichment in $\delta^{13}C$ as harvest intensity increases (Diochon et al., 2008; Huang et al., 2011). Congruently, disturbances that accelerate ecosystem N-losses through higher rates of denitrification or nitrification can result in enrichment of bulk soil $\delta^{15}N$ (Nadelhoffer and Fry, 1994; Högberg, 1997; Bai et al., 2013). Few studies have utilized $\delta^{13}C$ and $\delta^{15}N$ values to better

understand the mechanisms that might lead to changes in soil C and N stores in either surficial or deep soils.

The purpose of this study was to quantify the long-term (decade-scale) consequences of different timber harvest methods on C and N pool sizes in mineral soil and in the soil microbial biomass throughout the upper 1 m of the profile. We hypothesized that: (1) SOC, TN, and microbial biomass would be lowest in harvest treatments with the highest levels of organic matter removal, and these decreases would be evident throughout the entire soil profile; (2) soil $\delta^{13}C$ and $\delta^{15}N$ values would be more enriched in the more intensively harvested treatment due to accelerated C and N losses; and (3) SOC, TN, and microbial biomass compartments would vary seasonally in the upper portions of the soil profile in response to intra-annual variation in litter and root biomass inputs, but not in deeper portions of the profile where organic matter inputs are more limited.

Materials & Methods

Study Site and Experimental Design

Research was conducted in Davy Crockett National Forest near Groveton, Texas, USA (31°06´ 32.48´´N, 95°09´ 59.15´´W) at a site that is part of the Long-term Soil Productivity (LTSP) network (Powers et al., 2006; Ponder et al., 2012). Topography is nearly flat with slopes of 1-3% and elevation ranging from 101-m to 110-m. Soil across the study area is classified as a fine-loamy, siliceous, thermic Oxyaquic Glossudalf in the Kurth series which developed in loamy coastal plain sediments of the Yegua and Whitset geological formations (USDA/NRCS, 2003). The climate is subtropical with a mean annual temperature of 18.7°C and mean annual precipitation total of 1107 mm (1950-2010). Rainfall is evenly distributed throughout the year with May and September as the wettest months. Potential annual evapotranspiration is approximately 1200 mm (Norwine et al., 2005). Climate data during the study period was

obtained from the NOAA National Climatic Data Center weather stations in Crockett, TX and Lufkin, TX, 38 km northwest and 48 km northeast of the study site, respectively, and averaged to obtain mean values for the study area.

The experimental design used in this study includes: (i) unharvested control forest stands comprised of trees 60-80 years of age; (ii) merchantable bole/stem-only harvest stands; and (ii) whole-tree harvest + forest floor removal stands. The unharvested control stands have been thinned intermittently; however the last thinning was before the treatment plots were established (i.e., >20 years ago). Each treatment was comprised of three replicates, and each replicated plot was approximately 0.2 ha (i.e., 63 m x 32 m). In 1996, trees in harvested plots were hand-felled and lifted off of the plots with a loader. Forest floor removal was accomplished by hand-raking all aboveground organic matter from the whole-tree + forest floor removal treatment plots. Containerized *P. taeda* L. seedlings of 10-half sib families from the US Forest Service seed orchards were hand planted on a 2.5-m x 2.5-m spacing in 1997.

Sample Collection

Soil cores were collected at approximately 4 month intervals from June 2014 through March 2015 using a JMC Environmentalist Sub-Soil Probe PLUS, 2.8 cm diameter x 120 cm length coring tube (Clements Associates Inc., Newton, IA, USA). Soil cores were taken at 1.8 m from the base of a randomly selected *P. taeda* L. individual with a diameter at breast height (DBH) between 18 and 24 cm. A three tree buffer from the outside of the plots was not sampled to avoid edge effects. At each sample point, forest floor materials (litter layer/O-horizon) were collected down to the mineral soil from a 0.25 x 0.25 m quadrat followed by the extraction of a soil core. Soil sampling followed a stratified random sampling design in which four cores were taken from each plot and pooled by depth increment to increase sample mass and reduce error introduced by environmental heterogeneity. Specifically, each soil core was partitioned into four

depth increments in the field (0-10, 10-30, 30-60, and 60-100 cm), pooled together with the other replicated cores, and individual depths were analyzed separately. Samples were transported on ice packs from the field to the lab on the same day they were taken from the ground. Soil samples were aseptically homogenized in the lab and stored at 4°C until analyzed.

Soil Chemical and Physical Characterization

Soil pH was determined using an Accumet Basic pH meter (Denver Instrument, Arvada, CO, USA) on a 1:2 solution of soil in a 0.01M $CaCl_2$ solution (Minasny et al., 2011). A 50-g aliquot of field-moist soil was dried at 105°C for 48-hours to calculate bulk density and volumetric soil moisture. The remaining soil was passed through a 2-mm sieve to homogenize the soil and to remove large organic fragments and roots. Roots were saved for biomass quantification. A 25-g aliquot of sieved soil was then dried at 60°C for 48 hours and finely ground into powder using a TE250 ring pulverizer (Angstrom, Inc., Belleville, MI, USA). The pulverized soil was used to determine the concentration and isotopic composition of C and N. An additional 125-g aliquot of sieved soil was dried for 48 hours at 105°C for texture analysis using the hydrometer method (Ashworth et al., 2001).

Carbon and Nitrogen Concentrations, Densities and Isotopic Composition

Soils were analyzed for SOC and TN concentrations, as well as their $\delta^{13}C$ and $\delta^{15}N$ values, in the Stable Isotopes for Biosphere Science Laboratory at Texas A&M University. Analyses were conducted on a Carlo Erba EA-1108 elemental analyzer (CE Elantech, Lakewood, NJ, USA) interfaced with a Thermo Fisher Delta Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) in continuous flow mode. Carbon and N isotope ratios were reported in delta notation:

$$\delta^{xx}E\ (‰) = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000$$

where *E* is the element (either C or N), R_{sample} is the ratio of either $^{13}C:^{12}C$ or $^{15}N:^{14}N$ in the sample, and $R_{standard}$ is the ratio of $^{13}C:^{12}C$ of the international standard V-PDB (Coplen et al., 2006) or $^{15}N:^{14}N$ of the international atmospheric N_2 standard (Mariotti 1983). Soil C and N stocks (g m^{-2}) were computed as the product of the elemental concentration and soil bulk density for each soil depth (Ellert and Bettany, 1995). Carbon to nitrogen ratios (C:N) were calculated as the proportion of SOC to TN on a g kg^{-1} basis.

Microbial Biomass

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined on homogenized soil subsamples using the chloroform fumigation extraction method (Vance et al., 1987). Two, 10-g, field fresh aliquots of each sample were placed into separate 50-ml glass beakers. One aliquot served as a non-fumigated control and was immediately extracted with 40 ml of 0.5M K_2SO_4, shaken for one hour, centrifuged at 700 x g for 10 minutes, filtered over pre-leached (0.5M K_2SO_4) #5 Whatman filter paper, and the filtrate was analyzed for dissolved organic C (DOC) and dissolved organic N (DON) using a Shimadzu TOC-V_{CSH} with a TNM-1 module (Shimadzu Corp., Kyoto, Japan) set for 5X dilution (Chen et al., 2005). The second 10-g aliquot was fumigated at field moisture in a dark vacuum desiccator for 24 hours in the presence of ethanol-free chloroform. Following the incubation, DOC and DON from each fumigated sample was extracted and analyzed using the same procedure used for the non-fumigated control. MBC and MBN were calculated using the following formula:

$$MBC = (C_{fumigated} - C_{control}) / k_{EC}; \text{ and}$$

$$MBN = (N_{fumigated} - N_{control}) / k_{EN}.$$

Because extraction efficiencies for DOC and DON are less than 100%, extraction coefficients for carbon (k_{EC}) of 0.45 (Potthoff et al., 2009; Joergensen et al. 2011) and nitrogen (k_{EN}) of 0.54

(Brookes et al., 1985) were used to calculate soil microbial biomass carbon and nitrogen, respectively.

Vegetation, Roots, and Litter

Diameter at breast height (DBH) and tree height were measured for 150 and 30 randomly selected individuals per treatment, respectively. Understory vegetation cover was measured using the line intercept method; specifically, 6 randomly placed parallel transects that measured 63-m in length were placed in each of the plots and the horizontal linear length of each understory plant that intercepts each line was noted as well as the identity of the plant. Those values were added together, divided by the total length of the transect and then multiplied by 100 to obtain an understory vegetation percentage. The understory percentages were averaged by plot and those values were used to compare treatments using a student's t-test. Roots collected during sieving and all litter materials were dried at 60°C until stable mass was achieved and then weighed.

Statistical Analyses

All data and statistical analyses were performed using JMP Pro 11 (SAS Institute, Inc., Cary, NC, USA) or OriginPro (OriginLab, Inc., Northhampton, MA, USA). All datasets were tested for normality using Shapiro-Wilk's test. When data were not of normal distribution, log transformations were applied. Edaphic variables were statistically analyzed using a linear mixed model ANOVA. Because of the inherent autocorrelation between differing soil depths, a split plot experimental design with repeated measures was employed with harvest treatment as the fixed main plot, soil depth designated as the fixed split plot, and time incorporated as the repeated measure. Replicated plots were nested within harvest treatment and were considered a random effect. Results from the mixed model ANOVA were compiled into Table 1. When differences were significant, Tukey's honest significant difference (HSD) test was performed to

assess post hoc contrasts with significance inferred at $\alpha \leq 0.05$. Spearman's correlation analysis for all sample points including time, harvest treatment, and soil depth was used to assess connections between physicochemical properties.

Results

Climate and Soil Characteristics

Precipitation in 2014 was 1136 mm which was similar to the 60-year average of 1107 ± 33 mm (mean ± standard error); however, the first six months (January-June) of 2015 recorded a total of 1152 mm, which was 50% higher than the 60-year average of 577 ± 50 mm over that same monthly interval (Figure 1). From January 2014 through June 2015, temperatures did not deviate appreciably from the 60-year average (Figure 1). Soil volumetric water content (VWC) varied significantly with time and depth but not harvest treatment (Figure 1). VWC was highest in June 2014 and March 2015, reflecting the distribution of rainfall during the duration of the study.

Soil texture in all treatments was a uniform sandy loam from 0-60 cm, consisting of 685.8 ± 14.2 g kg^{-1} sand, 179.5 ± 12.1 g kg^{-1} silt, and 134.7 ± 12.4 g kg^{-1} clay. From 60-100 cm, soil texture was a sandy clay loam consisting of 594.9 ± 17.5 g kg^{-1} sand, 146.8 ± 11.1 g kg^{-1} silt, and 258.3 ± 21.8 g kg^{-1} clay (Figure 2). When averaged across all depths, controls (227.1 ± 6.9 g kg^{-1}) possessed a significantly higher proportion of silt than the bole-only harvest treatment (124.7 ± 45.7 g kg^{-1}) or the whole-tree harvest + forest-floor removal treatment (162.3 ± 6.2 g kg^{-1}). Generally, soil pH decreased with depth and increased with increasing timber harvest intensity (Figure 2), but was not altered by time. Soil from the whole-tree harvest + forest floor removal plots were significantly higher (pH = 4.02 ± 0.07) than soil from the bole-only harvest stands (pH = 3.77 ± 0.09) and unharvested control stands (pH = 3.57 ± 0.07) over the entirety of

Table 1: Effects of harvest treatment, soil depth, time, and their interactions on soil chemical, physical, and biological properties. *p<0.05; **p<0.01; ***p<0.001.

	Source of Variation						
	Harvest Treatment (OMR)	Soil Depth (SD)	Sampling Time (T)	OMR x SD	OMR x T	SD x T	OMR x SD x T
	F-Ratio						
VWC (%)	3.5	**22.8*** **	**70.7*** **	**2.6***	2.0	1.1	0.6
Sand (%)	2.1	**25.3*** **	0.5	**4.3*** **	0.1	0.3	0.1
Silt (%)	**12.6****	**16.3*** **	**13.0*** **	1.8	0.5	0.4	0.4
Clay (%)	1.5	**58.3*** **	**9.9*** **	**3.0***	0.2	0.3	0.4
Soil pH	**4.6***	**24.2*** **	0.1	**8.3*** **	1.0	0.2	0.2
Bulk Density (g cm^{-3})	2.7	**113.9*** **	2.1	1.3	2.2	0.8	0.9
Roots (g m^{-2})	2.1	**46.5*** **	**5.7****	**2.2***	0.3	1.1	0.4
[a]Forest Floor (g m^{-2})	**1296.7*** **	-	**39.4*** **	-	**8.4*** **	-	-
SOC (g C m^{-2})	**14.1****	**378.5*** **	0.6	**3.6****	**2.9***	1.2	1.2
TN (g N m^{-2})	2.8	**128.9*** **	**3.8***	0.9	0.8	0.5	0.4
SOC (g C kg^{-1})	**9.2***	**207.5*** **	1.2	**2.7***	1.1	0.8	0.7
TN (g N kg^{-1})	2.4	**64.9*** **	**5.9****	0.9	0.4	0.4	0.4
C:N	0.3	**143.9*** **	**3.8***	2.1	**3.0***	0.6	0.9
$\delta^{13}C$ (‰)	1.1	**89.4*** **	0.4	1.1	0.3	0.1	0.4
$\delta^{15}N$ (‰)	4.5	**112.1*** **	**37.1*** **	0.8	0.4	1.6	0.6
MBC (µg g^{-1})	**9.2***	**258.6*** **	**39.4*** **	**4.8*** **	**2.7***	**9.5*** **	1.6
MBN (µg g^{-1})	1.9	**192.3*** **	**42.1*** **	1.3	0.8	**17.9*** **	0.9

[a]Forest floor mass was statistically analyzed for harvest treatment and time using a two-way ANOVA

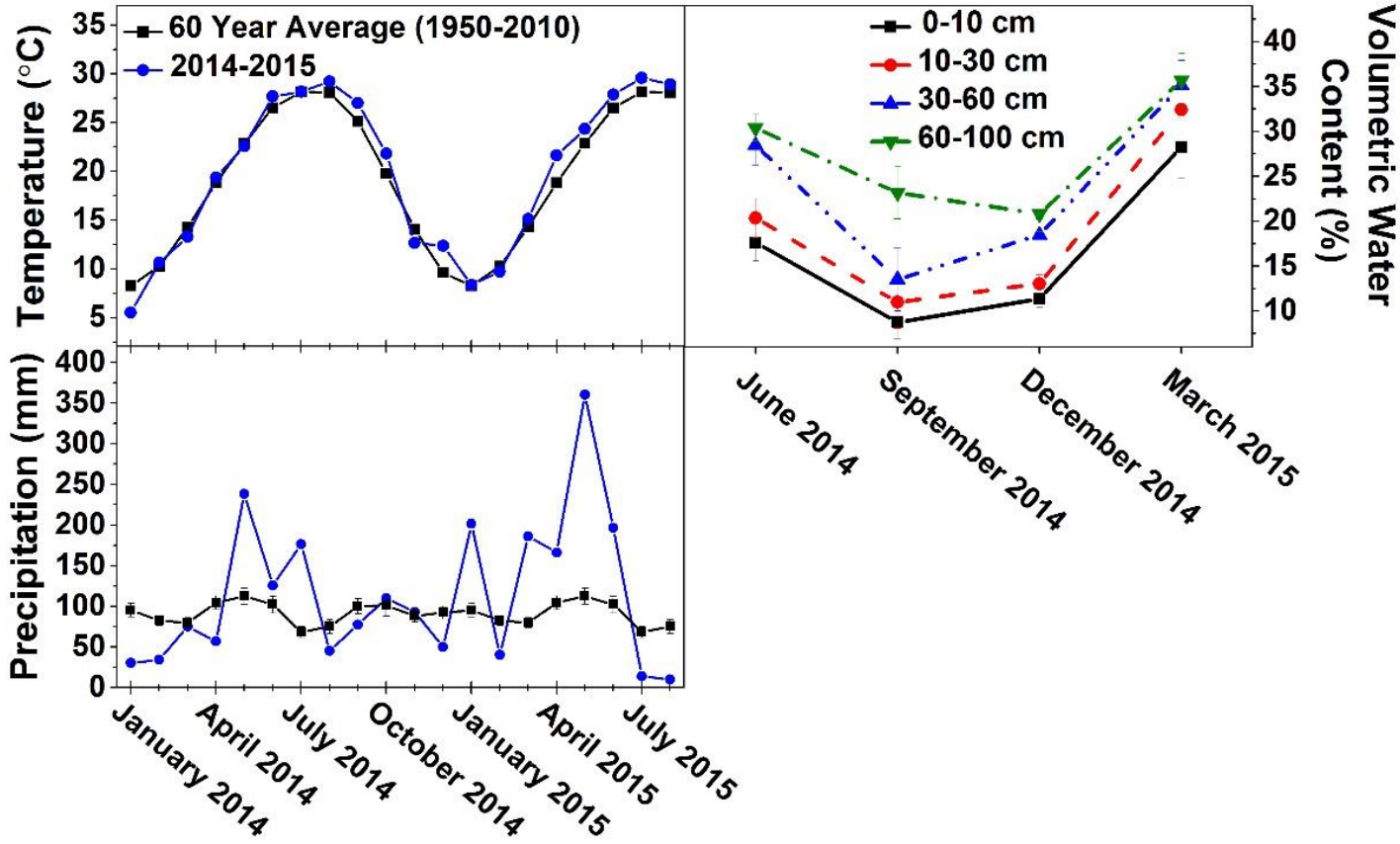

Figure 1: Monthly distribution and 60-year mean of air temperature and total precipitation from 5 months prior, during, and 5 months after the study as well as volumetric water content for each depth of interest during the four sampling points. Error bars for the 60-year average indicate standard error and due to low standard error for the temperature are not observable on this figure. Data for air temperatures and total precipitation are from the National Oceanic and Atmospheric Administration sites in Crockett, TX and Lufkin, TX. Temperature and precipitation were averaged from the two sites to obtain an overall mean for the entire area.

the 1 m soil core (Figure 2). Bulk density increased significantly with soil depth ($p < 0.001$), ranging from 1.03 ± 0.03 g cm^{-3} at 0-10 cm to 1.60 ± 0.03 g cm^{-3} at 60-100 cm (Figure 2).

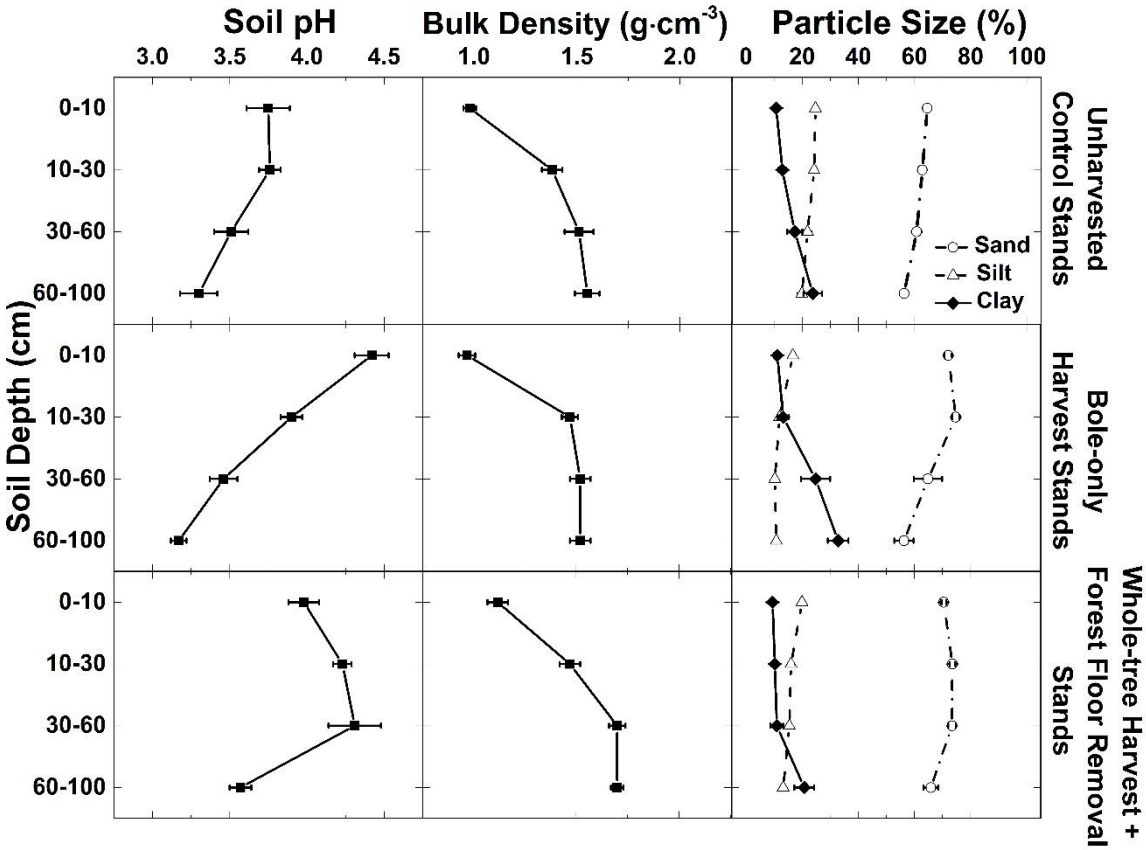

Figure 2: Physical and chemical characteristics of soil from different timber harvest methods over a range of soil depth increments (0-100 cm). Each point is the mean ± standard error of 12 replicates. Symbols used for particle size distribution: ○-sand, Δ-silt, ♦ -clay.

Vegetation Composition, Root and Litter Biomass

When the two harvest treatments were compared using a single factor ANOVA (i.e., bole-only harvest vs. whole-tree harvest + forest floor removal), mean DBH and tree height were statistically larger in bole-only harvest stands (Table 2). Unharvested control stands were not included in this analysis because of the differences in tree age; however, mean DBH and tree

height for the unharvested control stands were larger than either of the treatment plots (Table 2). Average understory cover per plot was 33.8 ± 1.4% and did not vary by treatment (Table 2). Nearly 90% of the understory was attributed to *Ilex vomitoria* (Table 2). Root biomass did not vary with respect to harvest intensity; however, significant differences occurred between soil depths (Figure 3). Specifically, root biomass was highest in the surface soil (1079.5 ± 52.6 g m^{-2}) and decreased to 166.8 ± 13.9 g m^{-2} at 1 m. Root biomass in the 10-30 cm increment (475.3 ± 55.5 g m^{-2}) was not significantly different than what was found in the 30-60 cm increment (611.4 ± 81.7 g m^{-2}). Litter biomass significantly varied with harvest intensity and time. When averaged across all time points, mean litter biomass was highest for the unharvested control stands (2369.4 ± 107.9 g m^{-2}) and lowest in the whole-tree harvest + forest floor removal stands (1056.2 ± 32 g m^{-2}). When averaged across all treatments, mean litter biomass was highest in the March (1902.4 ± 262.9 g m^{-2}) and lowest in the December (1520.4 ± 159.4 g m^{-2}).

SOC, TN, and C:N Ratio

Mean SOC concentrations, aggregated from all depths and sampling times, in unharvested control stands (8.1 ± 1.2 g kg^{-1}) and the bole-only harvest stands (7.4 ± 1 g kg^{-1}) did not differ from each other, but both possessed significantly larger concentrations of SOC than did the whole-tree harvest + forest floor removal stands (4.7 ± 0.8 g kg^{-1}) (Figure 4). SOC concentrations, averaged across all harvest treatments and sampling times, in the 0-10 cm (17.9 ± 1 g kg^{-1}) and 10-30 cm (4.4 ± 0.3 g kg^{-1}) increments were significantly different from all other depths. The 30-60 cm (2.3 ± 0.2 g kg^{-1}) and 60-100 cm (2.3 ± 0.1 g kg^{-1}) depth increments contained statistically smaller concentrations of SOC than the shallower depths ($p < 0.001$) (i.e., 0-10 and 10-30 cm); however, they did not vary from each other. When summed SOC stocks (g C m^{-2}) from all depth increments are averaged across all sampling time points and analyzed for treatment differences using a one-way ANOVA, we observe significantly higher stocks in the

Table 2: Forest vegetation and biological properties for each harvest treatment. A single factor ANOVA was used to compare the two harvest treatments with significance inferred at $\alpha \leq 0.05$. Values are listed as mean (standard error). Different letter following values indicate significant difference.

Harvest Treatment	*P. taeda* DBH (cm) N=150	*P. taeda* Height (m) N=30	Total Understory Cover (%) N=18	*I. vomitoria* Cover (%) N=18	Litter Biomass ($g \cdot m^{-2}$) N=12	Mean Root Biomass 0-100 cm ($g \cdot m^{-2}$) N=48
Unharvested Control Stands	27.89 (1.23)	22.92 (2.16)	34.07 (2.92)	31.04 (2.70)	2369.36 (107.94)	661.47 (69.80)
Bole-Only Harvest Stands	20.66 (0.36)a	17.43 (0.65)a	34.78 (2.18)a	32.06 (2.13)a	1633.42 (34.75)a	690.25 (73.27)a
Whole-Tree Harvest + Forest Floor Removal Stands	17.59 (0.30)b	14.34 (0.54)b	32.81 (2.01)a	29.91 (1.77)a	1056.17 (32.04)b	398.03 (51.56)b

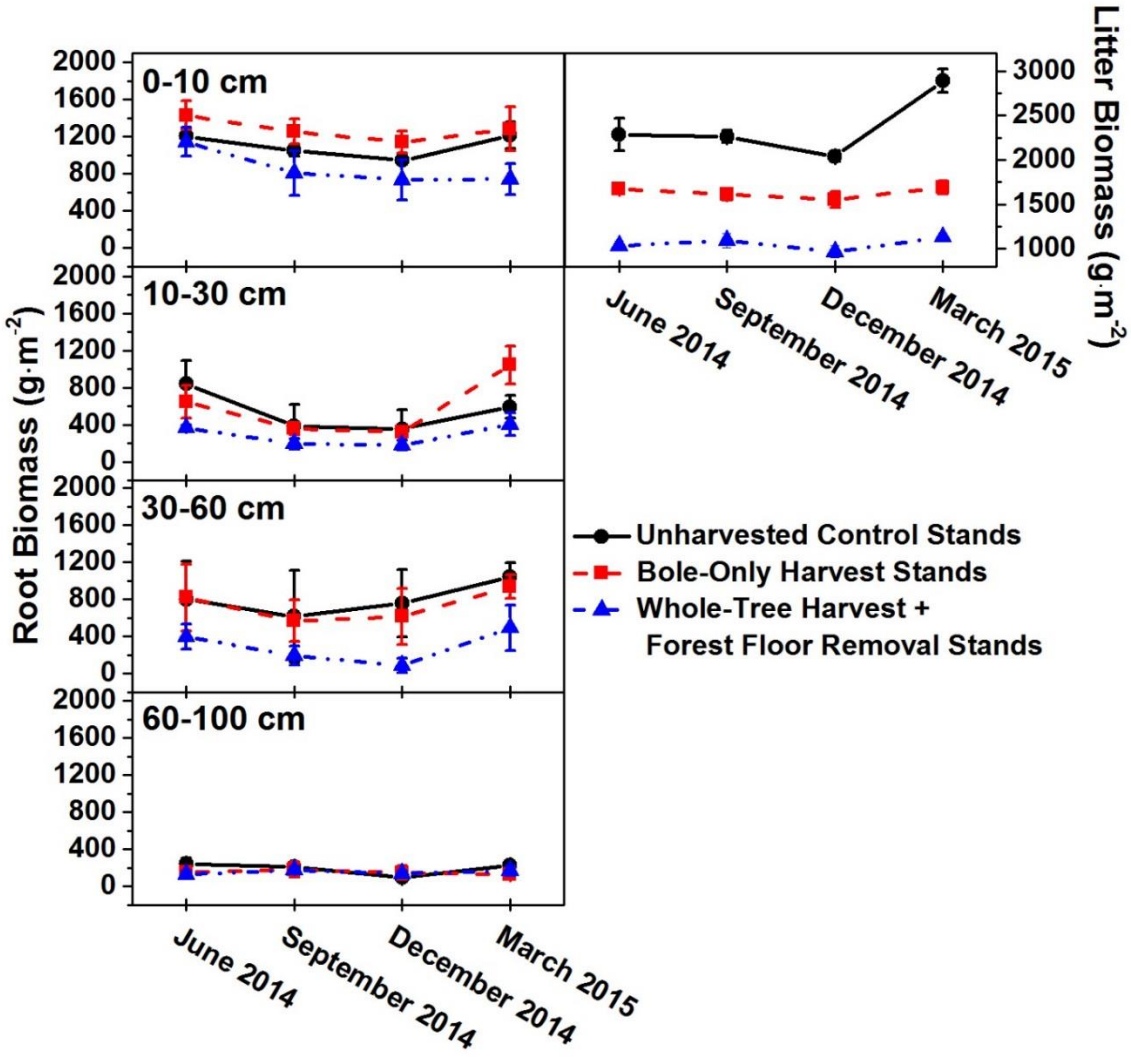

Figure 3: Variations in root and litter biomass (g m^{-2}) for each sampling point. Root biomass is separated into depth increments with each point being the mean ± standard error of 3 replicates. Singular points for litter biomass represent the mean ± standard error of 3 replicates

unharvested control (3681.5 ± 283.3 g C m^{-2}) and bole-only harvest (3366.7 ± 191.9 g C m^{-2}) than the whole-tree harvest + forest floor removal treatment (2417.8 ± 217.6 g C m^{-2}).

There was no statistical difference in TN concentration between the three treatments (Figure 4); however, TN was significantly altered by soil depth (0-10 cm: 0.78 ± 0.04 g kg^{-1}; 10-30 cm: 0.27 ± 0.02 g kg^{-1}; 30-60 cm: 0.23 ± 0.03 g kg^{-1}; 60-100 cm: 0.28 ± 0.02 g kg^{-1}) and significantly varied over time (June: 0.40 ± 0.04 g kg^{-1}; September: 0.38 ± 0.05 g kg^{-1}; December: 0.34 ± 0.04 g kg^{-1}; March: 0.44 ± 0.06 g kg^{-1}). Soil TN in the 0-10 cm increment contained a higher concentration of TN than any other depth ($p < 0.001$), but there were no significant differences between the deeper depths (i.e., 10-30, 30-60, 60-100 cm). When integrated over the entire 1 m depth, the unharvested control (217.9 ± 21.5 g N m^{-2}) possessed more TN that the bole-only harvest treatment (211.5 ± 14.7 g N m^{-2}) and whole-tree harvest + forest floor removal treatment (150.3 ± 14.7 g N m^{-2}).

Temporal variability in SOC and TN stocks were strongly correlated with concurrent changes in litter and root biomass; however, the extent to which either SOC or TN was correlated to root or litter biomass was depth dependent. Specifically, temporal variability in SOC and TN stocks in the 0-10 cm depth increment (for all treatments) was significantly correlated with temporal variability in litter biomass (Table 3). In contrast, SOC and TN stocks in the 30-60 cm and 60-100 cm depth increments were correlated with changes in root biomass (Table 3).

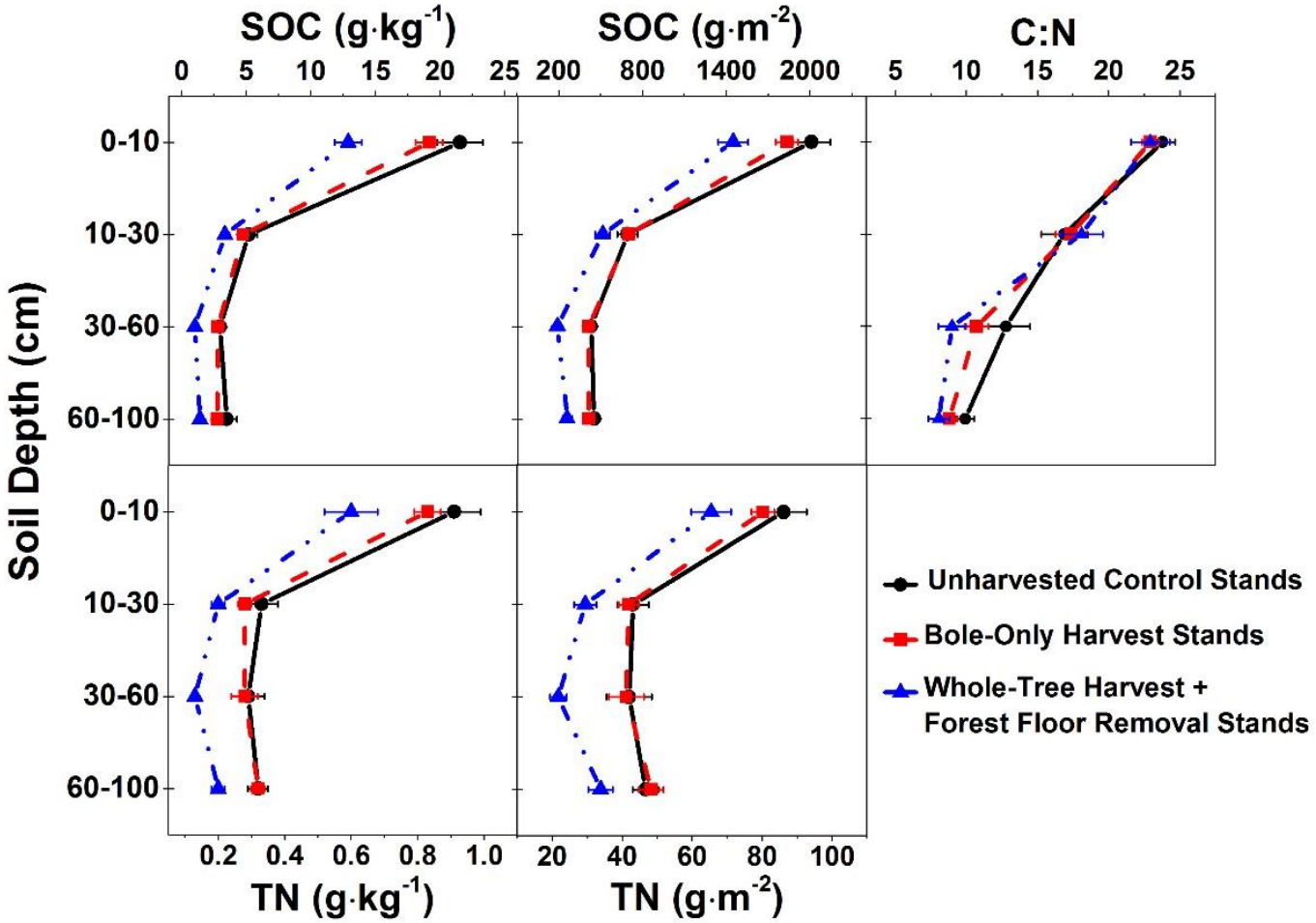

Figure 4: Soil organic carbon (SOC) and total nitrogen (TN) stocks and concentrations as well as ratios of SOC to TN (C:N) over the top 1 meter of the soil profile. Each time point is the mean ± standard error of 12 replicates without regard for seasonal differences.

Table 3: Spearman's correlation analysis among SOC and TN with root biomass, litter biomass, MBC, and MBN at different soil depths for each harvest treatment. Data from all treatments, soil depths, and sampling times were included in these calculations.

Harvest			Spearman's Rho			
Treatment	Soil Depth (cm)		Roots ($g \cdot m^{-2}$)	Litter ($g \cdot m^{-2}$)	MBC ($\mu g\text{-}C \cdot g^{-1}$)	MBN ($\mu g\text{-}N \cdot g^{-1}$)
Unharvested	0-10	SOC ($g \cdot m^{-2}$)	0.37	**0.84*** **	**0.72** **	0.46
Control		TN ($g \cdot m^{-2}$)	0.18	**0.64***	0.44	0.4
	10-30	SOC ($g \cdot m^{-2}$)	-0.24	0.51	0.56	0.34
		TN ($g \cdot m^{-2}$)	0.34	**0.62***	**0.80** **	**0.60***
	30-60	SOC ($g \cdot m^{-2}$)	**0.75** **	0.4	0.19	0.13
		TN ($g \cdot m^{-2}$)	**0.71** **	0.38	-0.03	0.08
	60-100	SOC ($g \cdot m^{-2}$)	**0.79** **	0.18	0.17	0.43
		TN ($g \cdot m^{-2}$)	**0.64***	0.25	0.08	0.23
Bole-Only	0-10	SOC ($g \cdot m^{-2}$)	0.53	**0.73** **	0.15	-0.05
Harvest		TN ($g \cdot m^{-2}$)	0.55	**0.75** **	0.29	0.1
	10-30	SOC ($g \cdot m^{-2}$)	0.02	0.13	0.22	0.09
		TN ($g \cdot m^{-2}$)	**0.76** **	**0.59***	**0.75** **	**0.65***
	30-60	SOC ($g \cdot m^{-2}$)	**0.83** **	0.22	0.43	0.08
		TN ($g \cdot m^{-2}$)	**0.89*** **	0.4	**0.64***	**0.66***
	60-100	SOC ($g \cdot m^{-2}$)	**0.79** **	-0.41	0.15	0.22
		TN ($g \cdot m^{-2}$)	**0.66***	-0.36	0.36	0.3
WT Harvest +	0-10	SOC ($g \cdot m^{-2}$)	0.1	**0.58***	0.11	-0.03
FF Removal		TN ($g \cdot m^{-2}$)	0.01	**0.67***	0.41	0.45
	10-30	SOC ($g \cdot m^{-2}$)	0.1	0.3	0.55	0.31
		TN ($g \cdot m^{-2}$)	0.29	0.54	**0.70***	**0.58***
	30-60	SOC ($g \cdot m^{-2}$)	**0.66***	0.06	0.2	0.29
		TN ($g \cdot m^{-2}$)	**0.63***	0.24	**0.71***	**0.57***
	60-100	SOC ($g \cdot m^{-2}$)	**0.71***	-0.35	0.23	0.3
		TN ($g \cdot m^{-2}$)	**0.71** **	0.18	0.41	0.28

*$p < 0.05$

**$p < 0.01$

*** $p < 0.001$

The carbon to nitrogen ratio (C:N) of mineral soil decreased significantly with respect to depth (0-10 cm: 23.2 ± 0.6; 10-30 cm: 17.5 ± 0.8; 30-60 cm: 10.8 ± 0.7; 60-100 cm: 9.2 ± 0.3) when averaged across all stands and time (Figure 4); furthermore, all depths were statistically different from one another. C:N was not impacted by harvest treatments. The C:N ratio varied significantly with time; however, post-hoc contrasts reveal that the only difference was between September 2014 (16.1 ± 1.1) and March 2015 (14.1 ± 1.1).

Soil $\delta^{13}C$ and $\delta^{15}N$ Analysis

Bulk soil $\delta^{13}C$ values were not statistically impacted by harvest treatment or time; however, they became significantly more enriched with soil depth (0-10 cm: -27.8 ± 0.1‰; 10-30 cm: -25.9 ± 0.2‰; 30-60 cm: -23.8 ± 0.3‰; 60-100 cm: -23.1 ± 0.3‰) (Figure 5). Most soil depth increments varied from each other with the exception of the 30-60 cm increment not being statistically different from the 60-100 cm increment. When averaged across all soil depths and sample times, soil $\delta^{13}C$ values in the whole-tree harvest + forest floor removal stands (-25.7 ± 0.3 ‰) were more depleted than the bole-only harvest treatment (-24.9 ± 0.4‰) and the unharvested control treatments (-24.8 ± 0.3‰). Although the effect of organic matter removal intensity had no effect on $\delta^{13}C$ in the initial mixed model ANOVA, a post hoc contrast comparing the combined effects of the unharvested control treatment and the bole-only harvest treatment against the whole-tree harvest + forest floor removal treatment, we do observe a significant difference ($p < 0.01$).

No statistical differences due to harvest treatment were observed in soil $\delta^{15}N$ ($p = 0.06$); however, mean values varied significantly through time (June: 4.9 ± 0.3‰; September: 4.5 ± 0.3‰; December: 2.9 ± 0.3‰; March: 4.9 ± 0.3‰) and with depth (0-10 cm: 1.9 ± 0.2‰; 10-30 cm: 4.6 ± 0.2‰; 30-60 cm: 5.6 ± 0.3‰; 60-100 cm: 5.0 ± 0.2‰) (Figure 5). Soil $\delta^{15}N$ values across all depths and sampling times were most depleted in the unharvested control stands

(mean: 3.7 ± 0.2‰). In contrast, whole-tree harvest + forest floor removal treatments (mean: 4.6 ± 0.3‰) were most enriched.

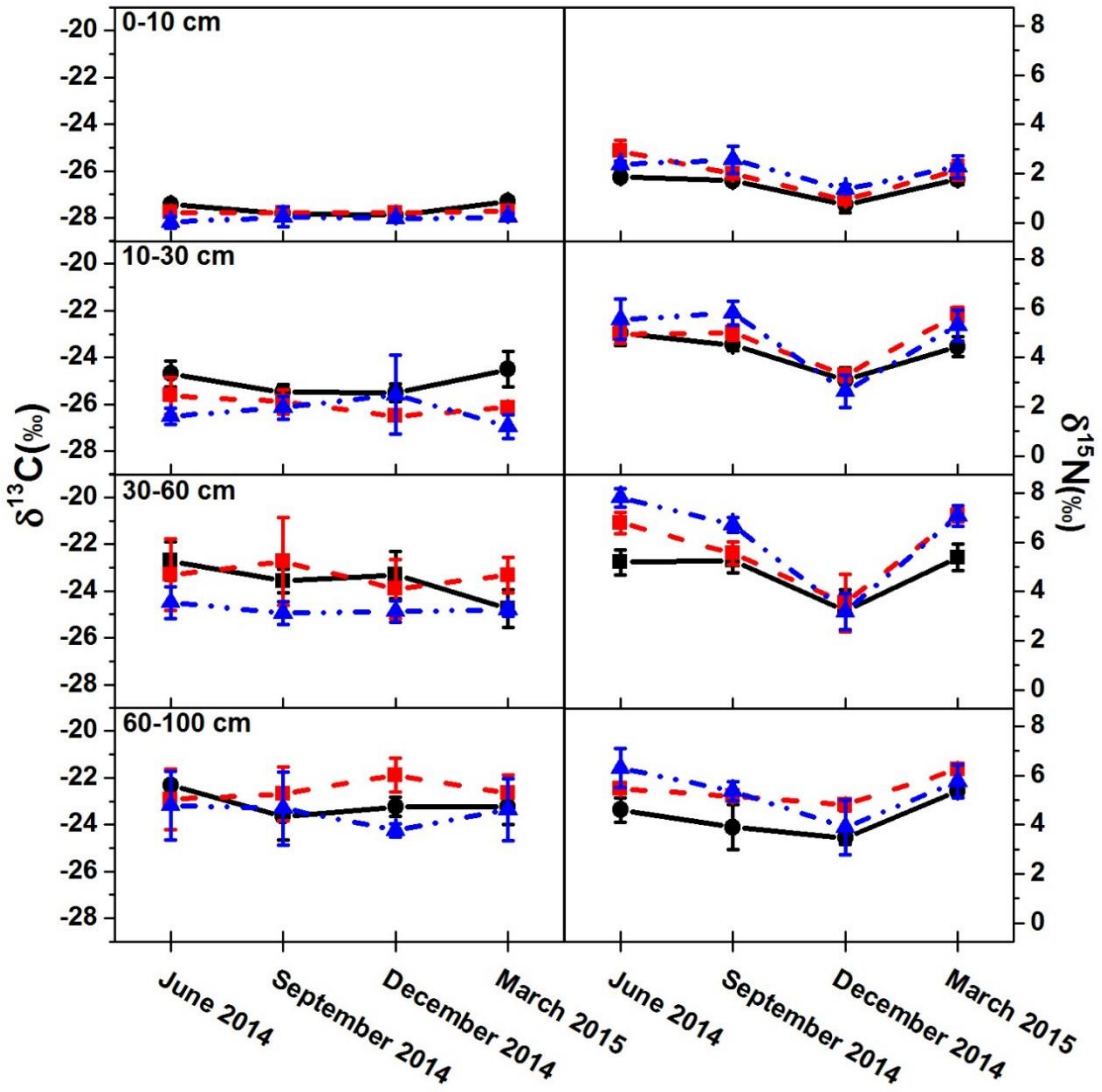

Figure 5: $\delta^{13}C$ of SOC and $\delta^{15}N$ of TN by treatment, separated into different depth increments and depicting seasonal trends. Each point is the mean ± standard error of 3 replicates. Symbols used for treatment differentiation: ●-Unharvested control stands, ■-Bole-only harvest stands, ▲-Whole-tree harvest + forest floor removal.

Microbial Biomass

When averaged across all soil depths and time points, microbial biomass carbon (MBC) was significantly higher in the unharvested control (113.7 ± 21.7 μg g^{-1}) and bole-only harvest treatment (103.5 ± 18.1 μg g^{-1}) than the whole-tree harvest + forest floor removal treatment (76.4 ± 14.9 μg g^{-1}); however, microbial biomass nitrogen (MBN) did not vary between harvest treatments. Mean MBC did vary with time (June: 109.9 ± 23.7 μg g^{-1}; September: 68.7 ± 16 μg g^{-1}; December: 54.4 ± 12.8 μg g^{-1}; March: 158.5 ± 26.1 μg g^{-1}) and decreased drastically with depth (0-10 cm: 275.6 ± 22.6 μg g^{-1}; 10-30 cm: 64.2 ± 8.7 μg g^{-1}; 30-60 cm: 24.9 ± 3.9 μg g^{-1}; 60-100 cm: 26.7 ± 4.4 μg g^{-1}) (Figure 6). The same statistical differences were observed for MBN in regards to both time (June: 13.1 ± 2.7 μg g^{-1}; September: 4.4 ± 0.9 μg g^{-1}; December: 7.9 ± 1.9 μg g^{-1}; March: 19.8 ± 3.9 μg g^{-1}) and depth (0-10 cm: 32.6 ± 3.4 μg g^{-1}; 10-30 cm: 6.6 ± 0.9 μg g^{-1}; 30-60 cm: 3.1 ± 0.4 μg g^{-1}; 60-100 cm: 3.1 ± 0.4 μg g^{-1}). Roughly 70% of the total MBC and MBN (0-100 cm) was observed in the 0-10 cm depth increment. When averaged across all time points and depth increments, MBC in the unharvested control stands (113.7 ± 21.7 μg g^{-1}) was 9% higher than in the bole-only stands (103.5 ± 18.1 μg g^{-1}) and 39% more than the whole-tree harvest + forest floor removal stands (76.4 ± 14.9 μg g^{-1}). MBN was 8% lower in unharvested controls stands (11.6 ± 2.3 μg g^{-1}) when compared to bole-only treatment stands (12.6 ± 2.4 μg g^{-1}); however, the whole-tree harvest + forest floor removal stands (9.7 ± 2.4 μg g^{-1}) possessed 17% less MBN than the control stands.

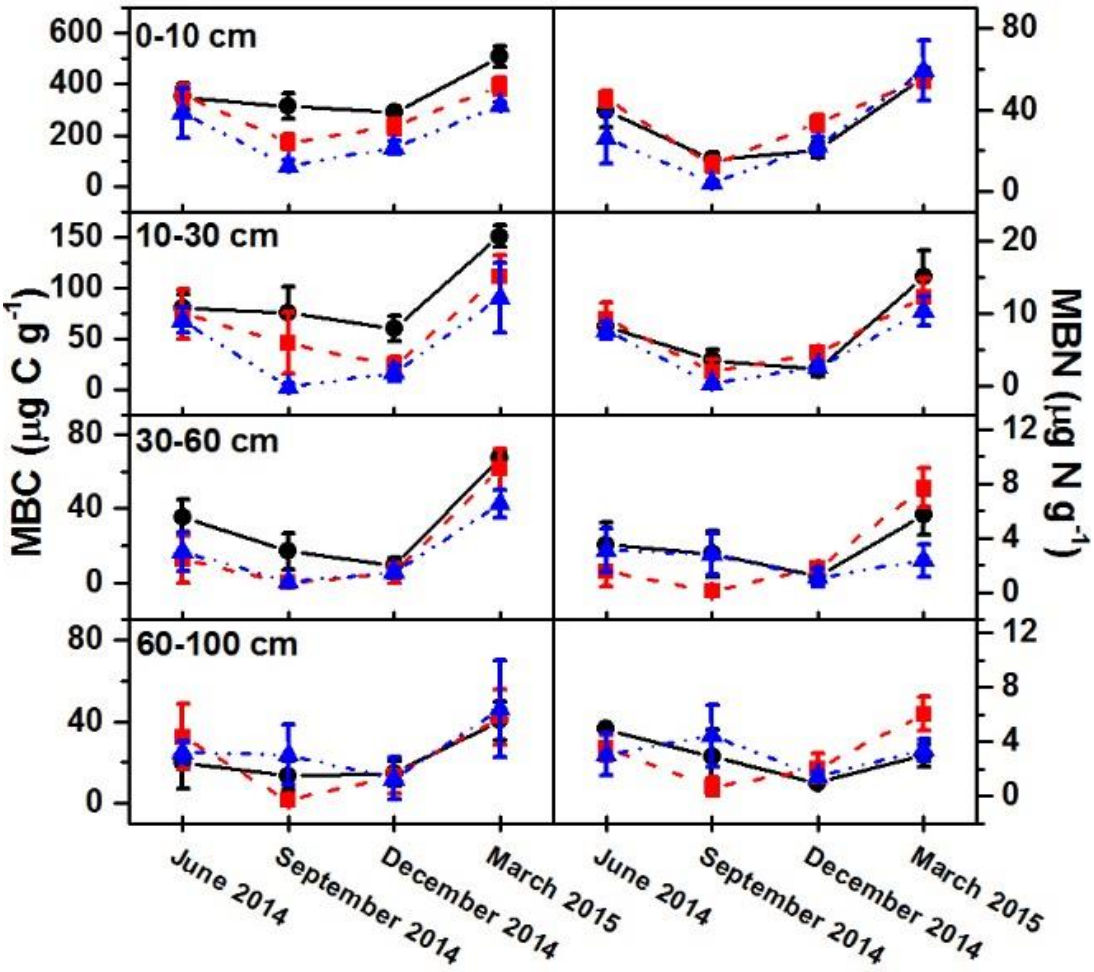

Figure 6: Microbial biomass carbon (MBC) and nitrogen (MBN) by treatment, separated into different depth increments and depicting seasonal trends. Each point is the mean ± standard error of 3 replicates. Symbols used for treatment differentiation: ●-Unharvested control stands, ■-Bole-only harvest stands, ▲-Whole-tree harvest + forest floor removal.

Variations in MBC and MBN across all treatments and soil depths were correlated with both SOC (MBC: r = 0.74, $p < 0.001$; MBN: r = 0.68, $p < 0.001$) and TN (MBC: r = 0.72, $p < 0.001$; MBN: 0.66, $p < 0.001$). However, when MBC and MBN are analyzed by harvest intensity and depth, these variables are correlated primarily with TN in the 10-30 and 30-60 cm depth

increments (Table 3). When we estimate the percent difference, based on samples from all treatments and depths, in MBC between December 2014 (lowest mean MBC) and March 2015 (highest mean MBC), we note an 80% difference that for the 0-10 cm compared to a 156% difference for the 30-60 cm increment. The other two depths fell in between these values; however, they both exceeded 100% difference. For each depth, MBC and MBN were correlated with soil water content (Table 4). The ratio of MBC to SOC (MBC/SOC) showed significant variation by time with the highest ratios, averaged from all treatments and soil depths, in March (2.9 ± 0.3) and the lowest in December (0.7 ± 0.1).

Table 4: Spearman's ranked correlation analysis between microbial biomass carbon (MBC) and nitrogen (MBN) and volumetric water content (VWC). *p<0.05; **p<0.01; p<0.001.

		MBC	MBN
		$\mu g\ g^{-1}$	
	Soil Depth (cm)	*Spearman's Rho*	
VWC (%)	0-10	0.64***	0.66***
	10-30	0.66***	0.81***
	30-60	0.55***	0.41*
	60-100	0.50***	0036*

Discussion

Organic Matter Removal Influences Soil C and N Storage on Decadal Time Scale

The impact of differing levels of organic matter removal associated with timber harvest on long-term soil C and N storage and forest productivity has been of great interest globally as reflected in the numerous publications over a wide range of forest ecosystems (Johnson and Curtis, 2001; Scott et al., 2004; Powers et al., 2005; Hansen et al., 2010; Nave et al, 2010; Slesak et al., 2011; Thiffault et al, 2011; Chen et al., 2013; Foote et al., 2015; Achat et al, 2015a; Achat

et al, 2015b). However, the extent to which these differing harvest methods influence decadal-scale storage, in surface and subsurface mineral soils, has not been studied extensively, or has been inconclusive (Jones et al., 2008; Slesak et al., 2011). This can be partially attributed to the expensive (monetarily and temporally) task of maintaining and sampling experimental sites.

Almost two decades after above-ground organic matter removal and replanting of *P. taeda*, SOC and TN in the upper 1 m of the profile remained lower in whole-tree harvest + forest floor removal compared to unharvested controls and bole-only harvest. SOC stocks, across all soil depths and sampling points, were 39% lower in the whole-tree harvest + forest floor removal treatment when compared to the unharvested control stands; congruently, the concentration of TN was 37% lower when comparing the same treatments. In the top 10 cm of the soil profile, SOC was 28% lower, and TN was 24% lower. This is consistent with Scott et al. (2004) and Foote et al. (2015) in which measurements were taken at 5 years and 15 years post-harvest, respectively, using the same study area. Our results are also similar to Mack et al. (2014) who reported that the removal of forest floor material can lead to long-term (15 years) general reductions in mineral soil C and N at another site along the Gulf Coastal Plain. This long-term evidence suggests that when intensive organic matter removal is employed (i.e., whole-tree harvest + forest floor removal), diminished SOC and TN occurs within the first 5 years of organic matter removal and can persist for decades, throughout the upper 1-m of the soil profile. In comparison, a 6% reduction in SOC and a 1% reduction in TN were observed when comparing bole-only harvest treatment to unharvested control treatment. The reduction in SOC is similar in scale as Laiho et al. (2003), Smaill et al. (2008), and Huang et al. (2011), and TN values are within ranges of studies by Thiffault et al. (2011), Zummo and Friedland (2011), Prest et al. (2014), and Kellman et al. (2014). Our values are also consistent with a meta-analysis by

Achat et al. (2015b) in which the effect of harvest intensity on soil organic matter and nutrient stocks was compiled from 140 articles and 168 experimental forest sites.

The decadal-scale decrease in SOC and TN in the whole-tree harvest + forest floor removal treatment may have multiple causes, some being categorized as initial (< 5 years after harvest) and others as sustained (5+ years after harvest). The main initial decrease in SOC and TN may be attributed to a large portion of potential SOC and TN inputs being removed in the form of aboveground biomass and litter during organic matter removal, coupled with relatively low rates of above and belowground organic matter inputs from regrowing forest compared to older forest. This original disturbance would also theoretically allow for increased radiant energy reaching the soil surface as well as increased moisture infiltration. These conditions would result in a situation that is conducive for increased rates of C- and N-cycle processes. Sustained decreases in SOC and TN are likely driven by long-term soil organic matter destabilization arising from alterations in the biophysical conditions that influence the stability of soil organic matter (i.e., aggregation), especially in sandy soils. As mentioned previously, similar studies have placed emphasis on environmental controls such as temperature and moisture availability (Bormann and Likens, 1979; Johansson et al., 1995; Paul et al., 2003; Kellman et al., 2014; Solly et al., 2014) that regulate decomposition rates, SOC and TN transport, and organomineral interactions. In regards to the loss of SOC and TN at depth, it has been shown that changes in the structure of the forest floor can increase infiltration of labile organic matter to deeper depths, possibly creating a priming effect and increasing decomposition rates of deep roots and other forms of recalcitrant organic matter (Fontaine et al., 2007; Blagodatskaya and Kuzyakov, 2008). This priming effect may have occurred shortly after harvest leading to the observed reduction at year 5 (Scott et al., 2004), and has since been unable to recover. The inability of SOC and TN in whole-tree harvest + forest floor removal stands to recover to pre-harvest conditions, even 18

years post-harvest, illustrates the importance of C and N stores in the forest floor and slash residues in maintaining SOC and TN in surface and subsurface mineral soil post-harvest.

Observed temporal variability in SOC and TN was correlated with concurrent variation in root and litter biomass. In the surface soil, variation in SOC and TN were significantly correlated with changes in litter biomass, while in subsurface soils (i.e., 30-60 and 60-100 cm) those variables were instead correlated with changes in root biomass. This trend was consistent regardless of harvest treatment. Both root and litter biomass were largest in March. Previous studies have noted similar patterns (Gill et al., 1999; Wuest, 2014). These depth-dependent correlations suggest that litter inputs are strong determinants of SOC and TN in the surface soil, while root inputs are the important drivers of those pool sizes in deeper portions of the profile.

C:N ratios were not statistically impacted by differing timber harvest intensities, and are similar to those reported by Smaill et al. (2008), Diochon et al. (2009), Zummo and Friedland (2011), and Prest et al. (2014). This could be explained by proportional SOC and TN losses following timber harvest where increased rates of biogeochemical cycling following harvest results in C loss through increased heterotrophic respiration while a proportional amount of N is simultaneously being mineralized, oxidized, and subsequently lost through leaching or volatilization.

Microbial Biomass Varies with Treatment, Time, and Depth

There were large reductions in MBC and MBN when the unharvested control treatment was compared to the whole-tree + forest floor removal treatment; however, only MBC was statistically affected. The magnitude of MBC and MBN for the 0-10 cm depth increment were similar to those found in a North Carolina pine plantation (Busse et al., 2006) and a boreal coniferous forest (Wardle, 1992). MBC and MBN values obtained at the Groveton-LTSP site 4 years earlier (Foote et al., 2015) indicated statistically significant differences in MBC and MBN;

however, this study only investigated the 0-10 cm depth increment. When we analyze only our 0-10 cm data, we also see a statistical difference in MBC and MBN between treatments; however, for MBN, this was not observed in any of the deeper increments which impacted the lack of significance in the overall model. We observed that roughly 30% of MBC and 28% of MBN in the upper 1 m of the soil profile occurred below 10 cm, which is similar in magnitude to Fierer et al. (2003), suggesting a high potential for biogeochemical activity at depth.

We initially hypothesized that MBC and MBN would show more pronounced seasonal variation in the surface soils vs. deeper soil due to larger seasonal organic matter inputs that are concentrated in upper portions of the profile. However, we observed that seasonal fluctuations in deeper portions of the profile were comparable in magnitude to those found throughout the entire 1 m of the profile. Within any given soil depth increment and treatment, MBC and MBN varied by approximately 2- to 8-fold across all time points in this one year study. This may be partly due to the availability and movement of pulses of substrate to deeper depths during the wetter, warm months and reductions of those substrates during the drier colder months. In contrast, the reduced variability in the surface soil may be attributed to the more sustained availability of substrates year-round; however, variation is still observed. We generally observed more microbial biomass during the warmer months (i.e., June, September, and March) than December which is consistent with Bååth and Söderström (1982) who showed fungal biomass is highest in the warmer, summer months. Contrary to our observation of variation in MBC and MBN over time, both Holmes and Zak (1994) and Blume et al. (2002) note that microbial population size is generally stable over time. Also, our temporal pattern of microbial biomass differs from Maithani et al. (1996) in which the highest values of microbial biomass were observed in the winter. Many studies have suggested that in subtropical forest soils, soil moisture is the major controlling factor of microbial biomass (Diaz-Ravina et al., 1995; Yang et al., 2010). Similar to

Yang et al. (2010), we observed that seasonal dynamics in microbial biomass were correlated with variation in soil water content; both of these variables were highest in spring and lowest in the winter. The seasonal variations in soil moisture may be responsible for the variation in MBC and MBN at each depth, which is similar to studies of microbial biomass in other pine plantations (Chen et al., 2003; Yang et al., 2010).

The MBC/SOC ratio or microbial quotient has been widely used as an indicator of the changes in organic matter status due to alterations of soil conditions (Sparling 1992). Although no differences were observed for harvest treatment or depth, we used seasonal differences to evaluate trends in substrate availability and the proportion of total SOC immobilized in microbial biomass. The ratio of MBC/SOC was lowest in December and highest in March, suggesting a possible decrease in microbial immobilization during the winter months, which is consistent with reports from a Chinese pine plantation (Yang et al, 2010).

Proposed Mechanisms of C and N Cycling Following Timber Harvest Inferred from Soil $\delta^{13}C$ and $\delta^{15}N$

Following timber harvest, accelerated biogeochemical transformations may occur resulting in altered stocks of SOC and TN (Thiffault et al, 2011; Achat et al, 2015a and 2015b). These processes may leave behind isotopic signatures which can be used to infer the mechanisms behind the associated gains or losses (Amundson et al, 2003; Garten et al, 2007; Templer et al, 2007; Diochon and Kellman, 2008; Hobbie and Quimette, 2009; Craine et al, 2015). Thus, $\delta^{13}C$ and $\delta^{15}N$ values may offer insights regarding forest ecosystem responses to organic matter removal during tree harvest events.

During the decomposition of soil organic matter, CO_2 released from soil due to heterotrophic respiration tends to be depleted in ^{13}C while the stabilized residual soil organic matter is enriched in ^{13}C, relative to the original substrate (Mary et al., 1992; Santruckova et al.,

2000). With timber harvest having the potential to increase rates of decomposition, $\delta^{13}C$ values could be used to indicate the relative influence of different timber harvest intensities on heterotrophic activity. We hypothesized that following timber harvest, bulk soil C in the whole-tree harvest + forest floor removal stands would have higher $\delta^{13}C$ values due to higher rates of C-cycling and heterotrophic activity. Contrary to our hypothesis, soil $\delta^{13}C$ values were more depleted in the whole-tree + forest floor removal treatment, suggesting more intensive organic matter removal methods may actually decrease rates of heterotrophic activity and carbon mineralization by reducing new and relatively labile organic matter inputs to the soil. Alternatively, in the whole-tree + forest floor removal treatment, the replanted forest may be delivering new litter inputs to the soil that are more ^{13}C-depleted relative to the old litter that was removed at the time of the harvest event. In forest ecosystems, $\delta^{13}C$ values generally increase in the order: leaves < fresh litter < older litter < soil (Balesdent et al, 1993; Garten et al, 2000). Thus, removing the old litter and replacing it with more recent and more ^{13}C-depleted litter could potentially cause a reduction in soil $\delta^{13}C$ values.

As we predicted, bulk soil $\delta^{15}N$ values of surface and subsurface soils were consistently most depleted in the unharvested control stands and most enriched in the whole-tree + forest floor removal stands. Specifically, whole-tree harvest + forest floor removal and bole-only harvest stands were 0.9‰ and 0.8‰ more enriched in $\delta^{15}N$, respectively, when compared to the unharvested control stands, over the entire 1 m profile. It is likely that the higher $\delta^{15}N$ values in the more severe organic matter removal treatments are at least in part attributable to higher rates of N-losses due to acceleration of nitrification and denitrification that result in higher $\delta^{15}N$ values for the residual ecosystem N. Increases in solar radiation reaching the soil surface, decreases in transpiration and rainfall interception, and increases in the amount of precipitation reaching and infiltrating the forest floor and into the soil would favor higher rates of N-losses during the time

interval between harvest and stand recovery. Significant temporal differences were observed at all depth increments, with some depth increments producing greater than 50% reduction in enrichment when comparing summer 2014 to winter 2014. Winter tends to be the least biologically active season and can result in the accumulation of ^{14}N-enriched organic matter which would reduce $\delta^{15}N$ values. Our results are consistent with other studies that have shown $\delta^{15}N$ values of soil total N can be increased for as long as several decades following tree harvesting (Pardo et al., 2002; Kellman et al., 2014).

CHAPTER III

FOREST HARVEST INTENSITY AND SOIL DEPTH ALTER INORGANIC NITROGEN POOL SIZES AND AMMONIA OXIDIZER COMMUNITY COMPOSITION

Intensive forest harvest techniques have the potential to alter soil carbon and nutrient stocks and biogeochemical processes. We investigated how differing levels of organic matter removal (OMR) during timber harvest influenced the long-term stability of nitrification and the microbes regulating this process. Nitrification is limited by the activity of ammonia oxidizing bacteria (AOB) and archaea (AOA); however, reports on the relative contribution of each of these groups to forest soil nitrification have varied and have not been investigated in response to OMR. The influence of soil depth on the structure and function of the ammonia-oxidizing community has also been underreported and was included in this study. We quantified soil physicochemical properties including concentrations of ammonium (NH_4^+) and nitrite (NO_2^-) + nitrate (NO_3^-), and also coupled next generation sequencing and qPCR of the *amoA* gene to a whole-soil assay that stimulates nitrification and allows for the discrimination of AOA- from AOB-activity using 1-octyne, which inhibits bacterial ammonia monooxygenase activity. Soils were collected (1 m depth) from replicated loblolly pine (*Pinus taeda* L.) stands subjected to three different intensities of OMR (i.e., unharvested control, bole-only harvest, and whole-tree harvest + forest floor removal). Increasing intensity of OMR and increasing soil depth lead to significant reductions in concentrations of in situ NH_4^+ and $NO_2^- + NO_3^-$. Sequencing and subsequent annotation of the ammonia oxidizing community revealed that AOA were dominated by Crenarchaeota and AOB were dominated by *Nitrosospira* spp. The abundance of both bacterial and archaeal *amoA* were influenced by OMR and soil depth; furthermore, archaeal *amoA* was more abundant than bacterial *amoA* across all soil depths and the ratio of AOA to

AOB increased with depth. Community structure of AOA and AOB were influenced by soil depth; however, only AOB were altered by OMR. Soil incubations revealed nitrification was N-limited in these forest soils. Furthermore, AOA- and AOB-contributions to total nitrification were nearly equivalent in surface soils; however, AOA contribution increased to 75% at 1 m. In general, the highest rates of nitrification occurred in the soils taken from unharvested control stands; however, OMR treatment differences were only significant when soils were amended with high levels of ammonia indicating that at ambient levels, intensive OMR may not lead to long-term alterations in nitrification potential.

Introduction

Coniferous forests of the southeastern USA comprise 9% of total North American forestlands (Oswalt et al., 2014) provide habitat for wildlife (Neu et al., 2014), contribute to carbon sequestration (Noormets et al., 2015), and provide economic output in the form of timber-related products (McNulty et al., 1996; Hodges et al., 2011; Brandeis et al., 2012). Recently there has been growing interest in utilizing intensive organic matter removal (OMR) techniques during timber harvest to increase economic output. Intensive techniques such as whole-tree harvest + forest floor removal result in the removal of all aboveground organic matter as well as forest byproducts such as downed woody debris, slash, sawdust, and forest litter. These byproducts have been utilized as substitute feedstocks in industrial processes, for bio-energy production, and sold as merchantable mulch (Janowiak and Webster, 2010; Dickens et al., 2012). Before being broadly adopted, the long-term biogeochemical consequences of these intensive OMR techniques should be investigated in order to determine if they are sustainable.

Nitrogen (N) is often the most limiting nutrient in terrestrial ecosystems (Binkley and Vitousek, 1989; Vitousek and Howarth, 1991; LeBauer and Treseder, 2008; Mitchell, 2011) and its availability is influenced by biogeochemical processes including plant-uptake, microbial-

immobilization, ammonification, nitrification, and denitrification (Schlesinger and Bernhardt, 2013). Intensive OMR associated with timber harvest has been shown to impart decade-scale reductions in soil carbon (C) and nutrient stocks (Johnson and Curtis, 2001; Hazlett et al., 2014; Vario et al., 2014; Foote et al., 2015; Dean et al., 2017) and alter nutrient transformation rates (Yanai, 1998; Burns and Murdoch, 2005; Kreutzweiser et al., 2008; Wilhelm et al., 2013). It has been shown that shortly after harvest (i.e., 1 yr.) soil nitrate (NO_3^-) can increase up to 8x pre-harvest conditions (Burn and Murdoch, 2005); however, the long-term effect of OMR on soil inorganic-N pool sizes and the processes that regulate these pool sizes has not been investigated, especially at soil depths that exceed 10-15 cm. Considering that intensive OMR can result in significant long-term reductions in soil total nitrogen (TN) (Kellman et al, 2014; Achat et al, 2015a, 2015b), it is conceivable that inorganic-N stocks and process rates will follow suit. Furthermore, OMR-induced loss of inorganic-N may be exacerbated in the southeastern US where soils are often sandy, highly weathered, acidic, and possess a low cation exchange capacity.

Nitrification has been extensively studied because of the influence of inorganic-N pool size on plant productivity, soil fertility, water quality, and the release of greenhouse gases into the atmosphere. Ammonia (NH_3) oxidation to nitrite (NO_2^-), the initial step in nitrification is carried out by both chemolithoautotrophic ammonia-oxidizing archaea (AOA) and bacteria (AOB) (De Boer and Kowalchuk, 2001) and is considered rate limiting. Growing evidence suggests that AOA frequently outnumber AOB in a multitude of ecosystems (Leininger et al., 2006; Prosser and Nicol, 2008; Hatzenpichler, 2012; Norman and Barrett, 2014) indicating that AOA may contribute more to nitrification than AOB (Chen et al., 2008; Leininger et al., 2006; Prosser and Nicol, 2008); however, diverging reports have led to questions regarding the mechanisms controlling ammonia oxidizer niche differentiation (Yarwood et al., 2010; Hu et al.,

2014a). Many have suggested that nitrogen availability and pH are the major determinants of the abundance and functionality of AOA versus AOB (Offre et al., 2009; Stopnišek et al., 2010). Forest disturbances have been shown to affect the community composition of AOA and AOB through modifications of the aforementioned soil properties. Disturbances such as fire (Webster et al., 2005; Yeager et al., 2005; Ball et al., 2010; Tourna et al., 2010), tree girdling (Rasche et al., 2011), and forest clear-cutting (Hynes and Germida, 2011) have been investigated; however, the decade-scale influence of differing intensities of forest harvest on AOA and AOB community structure and function has not been investigated nor has the vertical distribution of ammonia oxidizers.

Although molecular methods have made it easier to determine the relative abundance and community structure of AOA and AOB, coupling functionality to community metrics has been difficult and often relies on gene expression methods. Recently, Taylor et al. (2013) described an assay for discriminating between AOA and AOB activities, which is based upon AOB ammonia oxidization being irreversibly inactivated by 1-octyne. This method has subsequently been applied to agricultural (Giguere et al., 2015) and forest systems (Lu et al., 2015). We utilized this method to link AOA and AOB community metrics to ammonia oxidation functionality in soil.

In this study, we attempt to determine the decade scale influence of OMR on inorganic-N stocks as well as the composition and potential activity of the ammonia oxidizing archaeal and bacterial communities in the upper 1 m of the soil profile in a southeastern US loblolly pine forest. We hypothesized that (i) increasing OMR intensity would impose significant reductions in inorganic-N resulting in altered community structure and abundance of AOA and AOB, (ii) AOA would constitute a significantly larger proportion of the ammonia oxidizing community as proxied by *amoA* gene copy number, (iii) AOA abundance would not be altered with soil depth

while AOB *amoA* gene copy number would be reduced, (iv) AOA and AOB community composition would be altered by depth, and (v) rates of nitrification would be reduced by increasing intensity of OMR with AOA contributing a higher proportion to total nitrification potential than AOB.

Materials & Methods

Study Site Description and Experimental Design

Field sampling was conducted in April 2015 at the Long-Term Soil Productivity (LTSP) site (Powers, 2006; Ponder et al., 2012) in Davy Crockett National Forest near Groveton, TX, USA (31°06' 32.48"N, 95°09' 59.15"W). The climate is subtropical with a mean annual temperature of 18.7°C and mean annual precipitation of 1107 mm (1950-2010). Topography is relatively flat with slopes of 1-3% and elevation ranging from 101 to 110 m. Soil across the study area is a fine-loamy, siliceous, thermic Oxyaquic Glossudalf in the Kurth series which developed in loamy coastal plain sediments of the Yegua and Whitset geological formations (USDA/NRCS, 2003). The experimental design includes *Pinus taeda*-dominant unharvested control stands (tree age = 60-80 yrs.), and two harvest treatments differing in the extent of organic matter removal. The harvest treatments consisted of low-intensity treatment, bole-only (BO) harvest, where only the bole of the tree was removed, and a high-intensity treatment, whole-tree harvest + forest floor removal (WT+FF), where the entire tree (bole, branches, leaves) was removed and the forest floor litter was removed by hand-raking. During harvest, trees were hand-felled and lifted off the plots with a loader to reduce soil compaction. Control and both harvest treatments were replicated 3X and each replicate was 0.2 ha. All plots are located within a 1.5 km radius. Treatment plots were harvested in 1996 and then replanted in 1997 with containerized *P. taeda* L. (loblolly pine) seedlings at 2.5 m x 2.5 m spacing.

Soil Sampling

Soil cores were extracted with a JMC Environmentalist's Sub-Soil Probe PLUS (Clements Associates, Newton, IA, USA) (2.8 cm diameter x 120 cm depth). Cores were taken in both control and treatment plots at 1.8 m from the base of a randomly selected *P. taeda* individual with a diameter at breast height (DBH) between 18 and 24 cm. A 7.5 m buffer from the outside of the 0.2 ha plots was not sampled to avoid edge effects. In some of the WT+FF stands, the forest floor had not yet redeveloped; because of this, the organic soil horizon in all other plots (approximate thickness: < 3 cm) was removed prior to coring in order to investigate mineral soil horizons exclusively. Soil sampling followed a stratified random sampling design in which four cores were taken from each plot and homogeneously pooled by depth (i.e., 0-10, 10-30, 30-60, 60-100 cm) to increase sample mass and reduce error introduced by environmental heterogeneity. This resulted in 1 composited core per plot, separated into 4 depth increments, and replicated 3X per treatment. On the day in which soil cores were taken from the ground, samples were transported at 4°C from the field to the lab, aseptically homogenized by hand, and 6 g subsamples (3 $sample^{-1}$) were immediately stored at -80°C for future DNA extraction. The remaining soil was stored at 4°C for subsequent biogeochemical analysis.

Soil Physicochemical Analyses

Soil pH was analyzed using an Accumet Basic pH meter (Denver Instrument, Arvada, CO, USA) on a 1:2 solution of soil in a 0.01M $CaCl_2$ solution (Minasny et al., 2011). Bulk soil was passed through a 2-mm sieve to remove large organic material and roots. A 25-g aliquot of sieved soil was dried at 60°C for 48 hours and subsequently pulverized. The pulverized soil was used to determine soil organic carbon (SOC) and total nitrogen (TN) concentration via combustion elemental analysis on a Carlo Erba EA-1108 elemental analyzer (CE Elantech, Lakewood, NJ, USA).

Soil inorganic-N was extracted from 15 g of sieved, field moist, soil with 50 ml of 2M KCl within 36-hrs of soil being taken from the ground. The soil + KCl solution was shaken for 1 hour and then filtered over pre-leached (2M KCl) #40 Whatman filter paper and analyzed immediately for concentrations of NH_4^+ and $NO_2^- + NO_3^-$ on a Seal Analytical AQ2+ Discrete Chemistry Analyzer (SEAL Analytical, Ltd., Southhampton, UK). Colorimetric-based chemistry for the determination of NH_4^+ was based on indophenol-blue chemistry, and determination of $NO_2^- + NO_3^-$ was based on cadmium-reduction and subsequent diazotization. To assess the availability of NH_3 for ammonia oxidizer consumption, pH-adjusted NH_3 levels were calculated as described by Norman and Barrett (2016).

Whole Soil Nitrification Assay

A soil assay (Taylor et al., 2013) was used to measure total nitrification as well as the potential contributions of AOA and AOB to nitrification. Soil samples were incubated at three different NH_4^+ levels (equivalent to 3.5, 25, and 100 mg NH_4^+ kg^{-1} soil) achieved by adding sufficient anhydrous NH_3 gas to the headspace of a 125-ml Wheaton bottle fitted with a butyl stopper. NH_4^+ levels were verified by colorimetric analysis. The lowest level of NH_4^+ addition (3.5 mg NH_4^+ kg^{-1}) was selected because it represented the highest environmental level of NH_4^+ and was thereby the lowest possible normalized level for all samples. The moderate and high levels of NH_4^+ (i.e., 25 and 100 mg NH_4^+ kg^{-1}) were selected because they represented the initial stimulation of nitrification (i.e., 25 mg NH_4^+ kg^{-1}) and the maximum rate of nitrification (i.e., 100 mg NH_4^+ kg^{-1}) as evidenced by a preliminary experiment (Figure 7). Prior to initiation of the experiment, soils were pre-incubated at 25°C for 48-hrs to stimulate microbial activity. Three treatments were imposed to each sample at each NH_4^+ level: (i) acetylene amendment (6 μmol L^{-1}) to inhibit all autotrophic nitrification, (ii) 1-octyne amendment (4 μmol L^{-1}) to inhibit AOB nitrification, and (iii) positive control (no octyne or acetylene amendment) to determine

autotrophic + heterotrophic nitrification. Following amendment, soil samples were incubated at 25°C for 96 hrs. Subsequent NO_2^- + NO_3^- concentrations were determined using a Seal Analytical AQ2+ Discrete Chemistry Analyzer (SEAL Analytical, Ltd., Southhampton, UK) as previously noted. Total chemoautotrophic nitrification rates were calculated after subtracting NO_2^- + NO_3^- accumulation in the acetylene treatment and pre-incubation levels of NO_2^- + NO_3^-. Nitrification in the presence of 1-octyne (octyne-resistant) was attributed to AOA activity, with AOB activity (octyne-sensitive) calculated as the difference between total potential autotrophic nitrification and AOA potential nitrification.

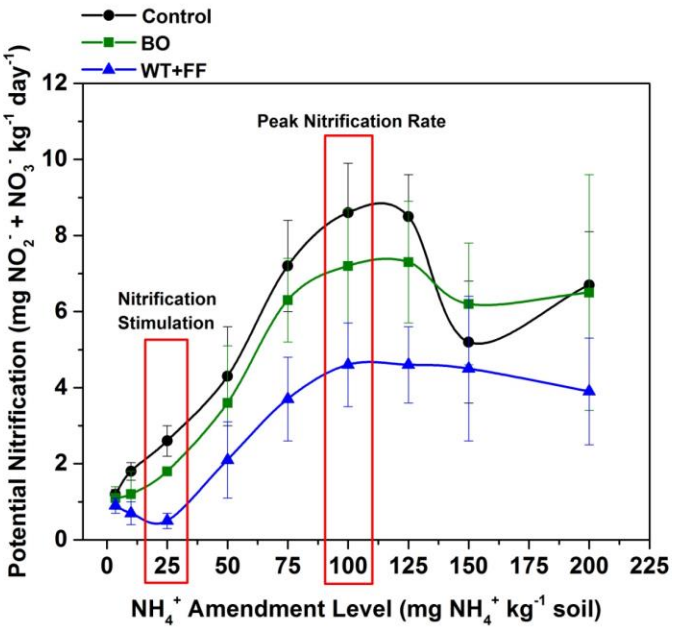

Figure 7: Total potential nitrification rates for each organic matter removal (OMR) treatment in the 0-10 cm depth increment. Prior to initiation of the experiment, soils were pre-incubated at 25°C for 48-hrs to stimulate microbial activity. Soils were then normalized with increasing amounts of NH_4^+ (3.5-200 mg NH_4^+ kg^{-1} soil) to determine the range of values over which nitrification increased exponentially and the maximum rate of nitrification. NH_4^+ levels were achieved by adding anhydrous NH_3 gas to the headspace of a 125-ml Wheaton bottle fitted with a butyl stopper. Three treatments were imposed to each sample at each NH_4^+ level: (i) acetylene amendment (6 µmol L^{-1}) to inhibit all autotrophic nitrification, (ii) 1-octyne amendment (4 µmol L^{-1}) to inhibit AOB nitrification, and (iii) positive control (no octyne or acetylene amendment) to determine autotrophic + heterotrophic nitrification. Following amendment, soil samples were incubated at 25°C for 96 hrs. NH_4^+ and NO_2^- + NO_3^- levels were verified by colorimetric analysis. Each point is the mean ± standard error of three biological replicates (N=3).

DNA Extraction, PCR Amplification, DNA Library Construction, and Sequencing

DNA extraction followed the modified version of the International Standard for the extraction of DNA from soil as described by Terrat et al. (2014). Further modification was made to extract DNA from 3 g of soil (dry weight equivalent) rather than the prescribed 1 g. DNA was extracted from 3 analytical replicates per sample and then pooled to increase mass and reduce environmental heterogeneity. DNA library preparation and sequencing of ammonia oxidizer communities was done by Molecular Research DNA Laboratory (www.mrdna.com, Shallowater, TX, USA) through target-based unidirectional amplification of the *amoA* gene with primers Arch amoA-1F (5'-STA ATG GTC TGG CTT AGA CG-3'; Francis et al., 2005) and Arch amoA-2R (5'- GCG GCC ATC CAT CTG TAT GT -3'; Francis et al., 2005) for AOA as well as amoA-1F (5'- GGG GTT TCT ACT GGT GGT -3'; Rotthauwe et al., 1997) and amoA-2R (5'- CCC CTC KGS AAA GCC TTC TTC -3'; Rotthauwe et al., 1997) for AOB. PCR amplification was accomplished by utilizing the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, USA) under the following conditions: an initial denaturation step at 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. PCR products were verified via gel electrophoresis (2% agarose gel). Samples were barcoded and subsequently pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified with calibrated AMPure XP beads (Agencourt Biosciences Co., Brea, CA, USA). The pooled and purified PCR products were then used to prepare an Illumina DNA library for each sample. Synthesis-based sequencing on an Illumina MiSeq followed the manufacturer's guidelines and resulted in single-end reads of 250.4 ± 25.9 (mean ± std. dev.) bp for AOA and 399.1 ± 135 bp for AOB.

Bioinformatic Analysis

Resulting .fasta and .qual files were demultiplexed, quality filtered, and analyzed using the QIIME 1.9.1 pipeline (Caporaso et al, 2010). Illumina sequences with <200 and >1000 bp, barcode or primer sequence errors, and those with homopolymers or ambiguous base calls that exceed six nucleotides were discarded. Raw sequences were deposited in NCBI's sequence read achieves under the accession number SRR5218290. Operational taxonomic units (OTUs) were defined by clustering at 97% sequence identity using the QIIME implementation of UCLUST (Edgar, 2010). Final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu).

Quantification of amoA Gene Copy Number

Quantitative-PCR (qPCR) targeting ammonia oxidizing bacteria and archaea were performed using primers amoA 1F/amoA 2R for bacteria (Rotthauwe et al., 1997) and Arch amoA 1F/Arch amoA 2R for archaea (Francis et al., 2005). The 25 µL reaction mixture contained 13 µL SYBR green real master mix (5Prime, Gaithersburg, MD), 0.5 µL of each primer (concentration 10 µM), 1 µL DNA template, and 10 µL molecular grade water. Each analysis run included a set of standards, negative controls, and replicated samples (n = 3) on a 96-well plate. For bacterial and archaeal *amoA*, the qPCR was run with the following conditions: 95°C for 5 min; 94°C for 45 sec, 56°C for 45 sec, and 72°C for 1.5 min (30 cycles). All qPCR assays were performed using an Eppendorf Mastercycler® ep realplex thermal cycler (Eppendorf, Hamburg, Germany). qPCR products were length-verified via gel electrophoresis (2% agarose gel). Amplification efficiencies of 89.7–99% were obtained for AOA and AOB, with r^2 values > 0.97.

AOB *amoA* standards were acquired from the lab of Raina Maier (Nelson et al, 2015). The archaeal *amoA* gene standard was prepared by amplifying soil DNA extracts using primers

Arch amoA 1F/Arch amoA 2R (Francis et al, 2005). The PCR reaction followed the same conditions as listed above and produced 635 bp amplicons that were cloned using the TOPO® TA Cloning Kit (Life Technologies) with pCR™2.1-TOPO® vector and transformed into chemically competent *Escherichia coli* DH5α. The sequence of *amoA* clones was verified via sequencing with an ABI 2700 PCR sequencing system (IPGB, Texas A&M University). Copy numbers are reported as *amoA* gene copies g^{-1} dry-weight soil.

Statistical Analysis

All statistical analyses on AOA and AOB communities were carried out using the sequence count within each OTU as an abundance value (Danzeisen et al., 2011). All datasets were tested for normality using Shapiro-Wilk's test. When data was not of normal distribution, non-parametric statistical tests or $\log_{10}$ transformations were applied. OTU data generated in QIIME were used to quantify the number of observed OTUs, richness, and diversity. Community metric calculations were analyzed using normalized sequence data set to 5,352 reads for AOA and AOB. Unless otherwise noted, physicochemical properties, AO community metric estimates, and OTU abundance values were statistically analyzed using a linear mixed model ANOVA. Because of the inherent autocorrelation between differing soil depths, a split plot repeated measures statistical design was employed with OMR as the fixed main plot and soil depth designated as the fixed split plot (Derner et al., 2006). Soil depth was also designated as a repeated measure. Replicated plots were nested within harvest treatment and considered a random effect (Dai et al., 2006). When differences were significant, Tukey's honest significant difference (HSD) test was performed to assess *post hoc* contrasts with significance inferred at $p < 0.05$. Non-metric multidimensional scaling (NMDS) based on a Bray-Curtis dissimilarity matrix (Bray and Curtis, 1957) was performed on normalized OTU data. A permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) using the Bray-Curtis

matrix listed above was employed to characterize differences in AOA and AOB community structure based on OMR treatment and soil depth. PERMANOVAs were run using 999 permutations. Correlation analyses was performed using JMP (SAS Institute, Inc., Cary, NC, USA).

Results

Soil Properties

Control stands possessed the highest mean SOC and TN concentration and the most acidic overall soil pH while the WT+FF stands possessed the lowest SOC and TN concentration and the least acidic soil pH (Table 5). BO stands fell between control and WT+FF stands in regards to SOC, TN, and soil pH. Regardless of treatment, SOC and TN decreased with depth; however, soil pH was not different with depth. Extractable NH_4^+ and $NO_2^- + NO_3^-$ were lowest in WT+FF stands and highest in control stands. Inorganic-N concentrations in BO stands were generally identical to the control stands (Table 5). Calculated NH_3 was statistically unaffected by harvest treatment, but decreased significantly with increasing soil depth ($p < 0.05$). On average, NH_4^+ concentrations were 4.5x higher than $NO_2^- + NO_3^-$ concentrations across all treatments and depths. NH_4^+ accounted for 0.45% of TN while $NO_2^- + NO_3^-$ accounted for 0.10% of TN on a g kg^{-1} basis. Extractable NH_4^+ ($p < 0.001$) and $NO_2^- + NO_3^-$ ($p < 0.05$) decreased significantly with depth; however, $NO_2^- + NO_3^-$ was less affected by depth than NH_4^+. Soil pH was negatively correlated to concentrations of NH_4^+ and $NO_2^- + NO_3^-$ while SOC, TN, NH_4^+, and $NO_2^- + NO_3^-$ were all significantly positively correlated with each other (Table 6).

Table 5: Edaphic parameters of the three organic matter removal treatments for each of the four soil depth increments *Post hoc* contrasts (Tukey-Kramer) were computed on values for each depth nested within each treatment and indicated by differing letters within each column. For each soil depth in each treatment, n=3. Statistical differences were inferred at $p<0.05$. SOC: soil organic carbon, TN: soil total nitrogen.

	Soil Depth (cm)	SOC	TN	Soil pH	NH_4^+	$NO_2^- + NO_3^-$	Calculated NH_3
Unharvested Control		(g kg^{-1} soil)			(mg N kg^{-1} soil)		(ng N kg^{-1} soil)
	0-10	27.6 (5.1)a	1.1 (0.2)a	3.3 (0.2)a	3.4 (0.2)a	0.7 (0.1)a	4.7 (2.1)a
	10-30	7.2 (1.3)bc	0.5 (0.2)bcd	3.6 (0.1)ab	2.7 (0.4)ab	0.5 (<0.1)abc	6.4 (0.5)a
	30-60	3.3 (0.6)cd	0.3 (0.1)de	3.5 (0.2)ab	1.8 (0.3)bcd	0.4 (<0.1)bcde	4.3 (2.0)a
	60-100	2.4 (0.1)d	0.3 (0.1)cde	3.4 (0.3)a	1.9 (0.3)bcd	0.3 (0.1)cde	3.5 (1.9)a
Bole-only Harvest							
	0-10	16.5 (2.1)a	0.8 (0.1)ab	4.2 (0.1)ab	3.1 (0.4)a	0.6 (0.1)ab	26.9 (7.9)a
	10-30	4.1 (0.3)cd	0.3 (<0.1)cde	3.7 (0.3)ab	2.8 (0.5)ab	0.5 (0.1)bcd	9.7 (3.8)a
	30-60	2.6 (0.7)d	0.3 (0.1)cde	3.4 (0.2)a	2.4 (0.4)abc	0.4 (0.1)bcd	3.5 (1.2)a
	60-100	2.4 (0.3)d	0.3 (<0.1)cde	3.3 (0.2)a	1.4 (0.4)cde	0.5 (0.1)bcd	1.6 (0.9)a
WT Harvest +FF Removal							
	0-10	12.9 (1.3)ab	0.6 (<0.1)abc	4.0 (0.3)ab	1.5 (0.3)cd	0.4 (0.1)bcde	10.3 (5.0)a
	10-30	4.0 (0.8)cd	0.2 (<0.1)de	4.4 (0.1)ab	1.1 (0.4)de	0.3 (0.1)cde	14.0 (5.2)a
	30-60	1.2 (0.4)e	0.2 (<0.1)e	4.3 (0.4)ab	0.8 (0.2)de	0.2 (0.1)de	23.2 (20.4)a
	60-100	1.3 (0.7)e	0.2 (0.1)e	4.7 (0.1)b	0.4 (0.1)e	0.2 (0.1)e	8.9 (1.3)a

Table 6: Spearman's ranked correlation analysis between soil physicochemical and biological properties. Bold values indicate significance with level of significance inferred by superscript symbol. AOA: ammonia oxidizing archaea, AOB: ammonia oxidizing bacteria. *p<0.05, †p<0.01, ‡p<0.001

								amoA copy No.		Unique OTUs		Chao 1		Simpson's Index	
		SOC	TN	Soil pH	Calculated NH_3	Environmental NH_4^+	Environmental $NO_2^- + NO_3^-$	AOA	AOB	AOA	AOB	AOA	AOB	AOA	AOB
	SOC														
	TN	**0.82‡**													
	Soil pH	-0.12	-0.30												
Calculated	NH_3	0.20	-0.04	**0.84‡**											
Environmental	NH_4^+	**0.57‡**	**0.52‡**	**-0.47†**	0.02										
Environmental	$NO_2^- + NO_3^-$	**0.62‡**	**0.55‡**	**-0.53‡**	-0.21	**0.68‡**									
amoA Copy No.	AOA	0.30	0.27	**-0.56‡**	-0.31	**0.59‡**	**0.55‡**								
	AOB	**0.66‡**	**0.45†**	-0.13	0.16	**0.53‡**	**0.55‡**	0.19							
No. Unique OTUs	AOA	-0.18	-0.17	0.03	0.12	-0.14	-0.10	-0.11	-0.08						
	AOB	0.20	0.06	**0.38***	**0.41***	-0.02	0.13	-0.31	0.32	-0.01					
Chao 1	AOA	0.02	0.09	-0.08	0.24	0.06	0.16	-0.07	0.21	**0.52‡**	-0.13				
	AOB	0.28	0.18	**0.39***	**0.47†**	0.01	**0.13**	-0.29	0.31	-0.01	**0.96‡**	-0.08			
Simpson's Index	AOA	**-0.45†**	**-0.48†**	0.27	0.17	-0.27	**-0.38***	-0.32	0.08	-0.05	**0.77‡**	-0.23	**0.74‡**		
	AOB	0.09	-0.02	0.27	0.23	-0.12	0.01	-0.32	**-0.38***	-0.03	-0.08	0.05	-0.14	-0.05	

AOA and AOB Community Composition

Sequencing revealed that richness ($p < 0.001$) and diversity ($p < 0.001$) metrics were statistically higher for AOA than AOB regardless of harvest treatment or soil depth. Counts of OTUs in AOA libraries were statistically lower in control treatment stands than BO and WT+FF treatment stands ($p < 0.05$), but did not vary with soil depth (Table 7). AOA and AOB OTU richness (Chao1) were statistically unaffected by treatment and depth; however, AOB OTU richness showed a general increase with increasing OMR and a reduction with depth. Furthermore, AOB richness was positively correlated to NH_3. Simpson's diversity for AOA and AOB generally increased with increasing OMR and AOA diversity was negatively correlated to increasing concentrations of SOC, TN, and $NO_2^- + NO_3^-$ (Table 6). AOB diversity was unaffected by depth; however, AOA diversity significantly increased with depth ($p < 0.01$).

Phylum-level annotation of OTUs revealed that the ammonia oxidizing community was dominated by Crenarchaeota (> 90% of all AOA sequences) and Thaumarchaeota (> 9% of all AOA sequences) for AOA and Proteobacteria (> 83% of all AOB sequences) for AOB (Figure 8). The majority of AOA sequences were annotated to Crenarchaeota spp. (representing > 64% of all AOA sequences) and AOB sequences were in the *Nitrosospira* lineages (representing >31% of all AOB sequences). Harvest intensity did not alter the relative abundance of AOA phyla; however, increasing depth did lead to significant decreases in OTUs annotated to the phylum Crenarchaeota ($p < 0.01$) and significant increases in Thaumarchaeota ($p < 0.01$). Likewise, the relative abundance of AOB OTUs annotated at the phylum-level illustrated no response to harvest treatment; however, OTUs annotated as Proteobacteria did significantly decrease with increasing depth ($p < 0.05$).

Table 7: Summary of ammonia-oxidizer operational taxonomic units (OTUs), and their diversity and richness estimates. Datasets were normalized by setting each sample to 5,352 sequences per sample. OTUs were defined as sequences sharing ≥97% similarity and served as the basis for number of unique OTUs, Chao1 richness estimate, and Simpson's Diversity Index. For each soil depth in each treatment, n=3; for mean values per treatment, n=12. AOA: ammonia oxidizing archaea, AOB: ammonia oxidizing bacteria. Mean values are bolded.

	No. Unique OTUs		Chao1 Richness Estimate		Simpson's Diversity Index	
Soil Depth (cm)	AOA	AOB	AOA	AOB	AOA	AOB
Unharvested Control						
0-10	470 (27)	248 (52)	1 718 (415)	424 (29)	0.84 (0.03)	0.69 (0.2)
10-30	494 (15)	219 (79)	2 644 (398)	343 (110)	0.91 (0.02)	0.87 (0.03)
30-60	452 (55)	162 (41)	2 429 (250)	282 (70)	0.92 (0.03)	0.74 (0.1)
60-100	326 (86)	120 (9)	2 079 (102)	224 (12)	0.89 (0.03)	0.66 (0.1)
Mean	**435 (30)**	**188 (27)**	**2 218 (172)**	**318 (36)**	**0.89 (0.02)**	**0.74 (0.1)**
Bole-only Harvest						
0-10	456 (39)	262 (44)	2 470 (176)	473 (81)	0.92 (0.01)	0.74 (0.1)
10-30	574 (46)	166 (24)	1 185 (713)	300 (59)	0.93 (0.01)	0.76 (0.04)
30-60	574 (4)	239 (25)	2 739 (668)	404 (70)	0.95 (0.01)	0.85 (0.04)
60-100	546 (29)	127 (33)	2 420 (350)	251 (75)	0.94 (0.01)	0.64 (0.2)
Mean	**537 (20)**	**199 (22)**	**2 203 (268)**	**357 (40)**	**0.94 (0.01)**	**0.75 (0.1)**
WT Harvest + FF Removal						
0-10	480 (72)	321 (14)	2 165 (289)	549 (40)	0.88 (0.03)	0.95 (<0.01)
10-30	619 (79)	308 (83)	2 646 (274)	497 (116)	0.94 (0.02)	0.84 (0.1)
30-60	546 (47)	201 (57)	1 799 (314)	335 (88)	0.95 (0.01)	0.84 (0.1)
60-100	465 (21)	174 (21)	1 941 (574)	295 (26)	0.93 (0.02)	0.85 (0.02)
Mean	**528 (31)**	**251 (29)**	**2 138 (190)**	**419 (46)**	**0.92 (0.01)**	**0.87 (0.03)**

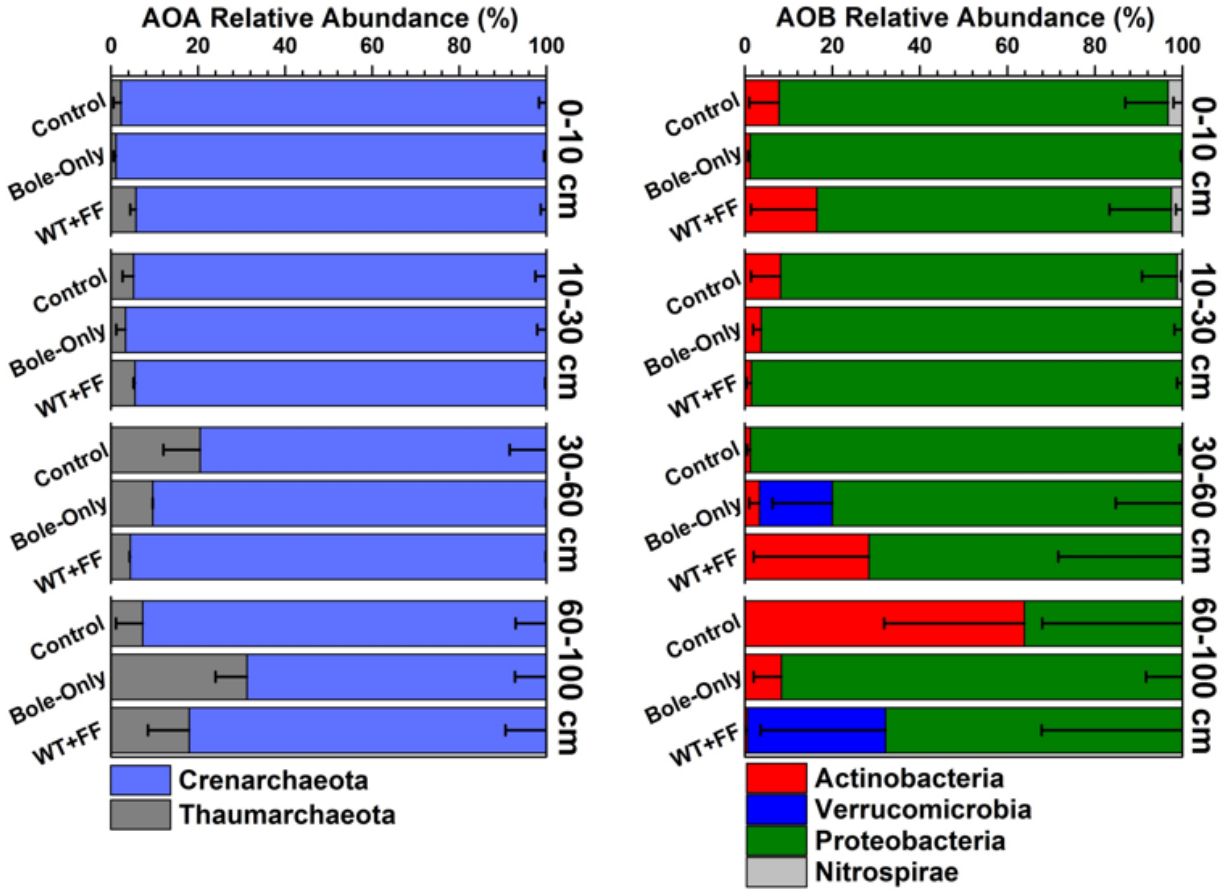

Figure 8: Phylum-level distribution and relative abundance of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) communities. Bars are the mean percentage of 3 biological replicates and error bars indicate standard error of the 3 replicates nested within each soil depth increment. (Control=Unharvested Controls; Bole-Only=Bole-Only Harvest; WT+FF=Whole-tree Harvest + Forest Floor Removal).

Non-metric multidimensional scaling (NMDS) plots, based on Bray-Curtis distance matrices, of OTUs resulted in no statistical separation for AOA based on harvest treatments; however, AOB community composition was significantly affected by treatment ($p < 0.05$) (Figure 9). AOA ($p < 0.01$) and AOB ($p < 0.001$) community composition was statistically altered by soil depth with unique clustering when soil depth was analyzed independent of OMR treatments; specifically, for AOA the 60-100 cm depth was statistically separated from the other 3 depths ($p < 0.01$), while for AOB the 0-10 cm and 10-30 cm increments were statistically different than the 30-60 and 60-100 cm increments ($p < 0.05$).

AOA and AOB amoA Gene Abundance

AOA *amoA* copy numbers were significantly higher than AOB *amoA* ($p < 0.001$) regardless of harvest treatment or depth (Figure 10). Increasing harvest treatment significantly reduced AOA *amoA* ($p < 0.01$) and AOB *amoA* ($p < 0.05$) copy numbers. Specifically, control treatment stands possessed a statistically larger amount of AOA and AOB *amoA* copies than BO and WT+FF stands. Soil depth drove significant linear reductions in copies of AOB *amoA* but not AOA *amoA*. The ratio of AOA:AOB *amoA* copy number ranged from 3.6 to 356 and did not vary by treatment; however, this ratio significantly increased linearly with depth ($p = 0.001$) (Figure 10). Copy number of AOA and AOB *amoA* were significantly positively correlated to concentrations of NH_3 and NH_4^+ (Table 10). Congruently, AOA *amoA* was negatively correlated to soil pH and AOB *amoA* was positively correlated to concentrations of SOC and TN.

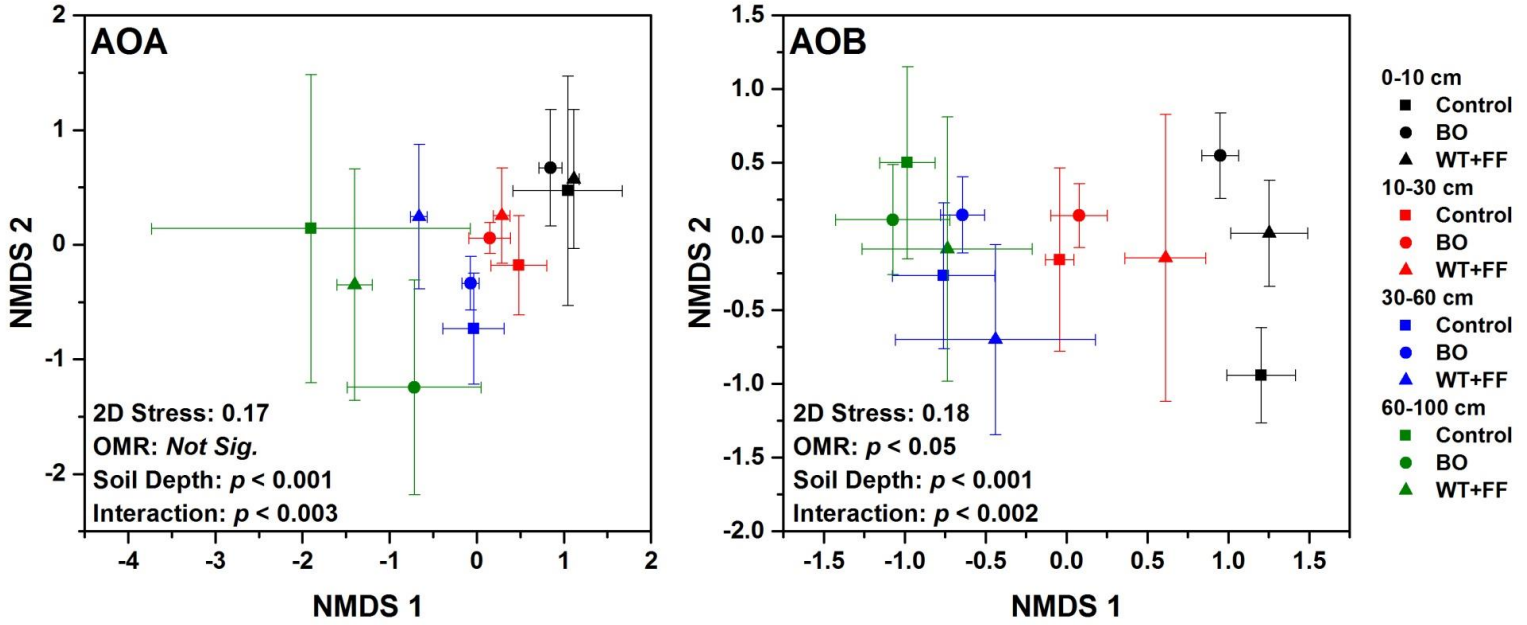

Figure 9: Nonmetric multidimensional scaling (NMDS) ordinations of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) communities based upon their OTU composition derived from Bray-Curtis distances matrices. Each point and corresponding bars represent mean ± standard deviation (n=3). Statistical differences in organic matter removal, soil depth, and their interaction were obtained using PERMANOVA. Control: unharvested control, BO: bole-only harvest, WT+FF: whole-tree harvest + forest floor removal.

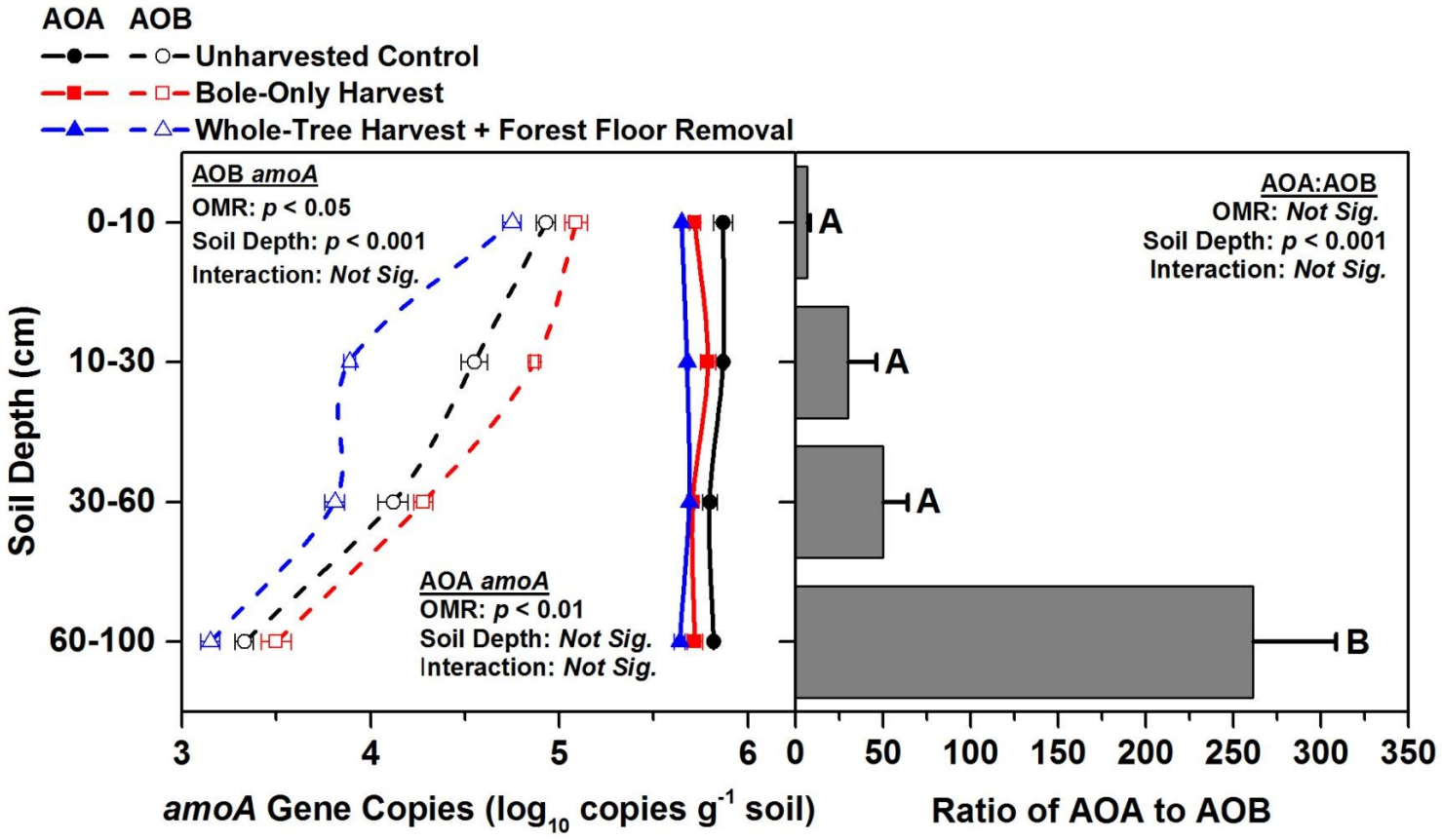

Figure 10: Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) *amoA* quantification based on organic matter removal (OMR) treatment and soil depth as well as ratios of AOA to AOB *amoA* gene copies in response to soils depth.

Whole-Soil Nitrification Assay

Incubation in the presence of acetylene led to no significant accumulation of NO_2^- + NO_3^- from soils at the Groveton-LTSP indicating that heterotrophic nitrification is not a major process in these soils. The small amount of NO_2^- + NO_3^- that did accumulate (< 0.1 mg N kg^{-1} soil) was subsequently subtracted before calculating potential autotrophic nitrification rates. Following incubation, accumulation of NO_2^- + NO_3^- was detected for every sample. Regardless of harvest treatment or depth, total rates of NO_2^- + NO_3^- accumulation were highest when soil was amended to 100 mg NH_4^+ kg^{-1} soil which was four-fold higher than the 25 mg NH_4^+ kg^{-1} normalization level ($p < 0.001$) and seven-fold higher than the 3.5 mg NH_4^+ kg^{-1} normalization level ($p < 0.001$) (Figure 11). Total nitrification potential in the presence of 3.5 mg NH_4^+ kg^{-1} was statistically unaffected by harvest treatment, but significantly decreased linearly with depth ($p < 0.001$). Significant total nitrification rate differences for harvest treatment and depth were observed for soils amended with 25 mg NH_4^+ kg^{-1} (harvest treatment: $p < 0.001$; depth: $p < 0.001$) and 100 mg NH_4^+ kg^{-1} (harvest treatment: $p < 0.001$; depth: $p < 0.001$). For both the 25 and 100 mg NH_4^+ kg^{-1} normalization levels, the control treatment possessed the highest accumulation rate which was significantly higher than the BO treatment (25 mg NH_4^+ kg^{-1}: $p < 0.001$; 100 mg NH_4^+ kg^{-1}: $p < 0.001$) and the WT+FF treatment (25 mg NH_4^+ kg^{-1}: $p < 0.001$; 100 mg NH_4^+ kg^{-1}: $p < 0.001$). As was observed for soils amended with 3.5 mg NH_4^+ kg^{-1}, total NO_2^- + NO_3^- accumulation decreased linearly for both the 25 and 100 mg NH_4^+ kg^{-1} normalization levels.

Potential nitrification rates in the presence of 1-octyne (octyne-resistant, AOA) statistically varied by treatment (3.5 mg NH_4^+ kg^{-1}: $p < 0.05$; 25 mg NH_4^+ kg^{-1}: $p < 0.001$; 100 mg NH_4^+ kg^{-1}: $p < 0.001$); however, only the 25 mg NH_4^+ kg^{-1} ($p < 0.01$) and the 100 mg NH_4^+ kg-1 ($p < 0.001$) varied by depth (Figure 12). Specifically, 1-octyne-resistant (AOA) nitrification

	NH_4^+ Normalized Level (mg NH_4^+ kg^{-1} soil)		
	3.5	25	100
	F-Ratio		
OMR	3.5	**224.5*****	**882.4*****
Soil Depth	**13.6*****	**14.8*****	**63.3*****
Interaction	2.1	2.6	2.2

**p*<0.05 ***p*<0.01 ****p*<0.001

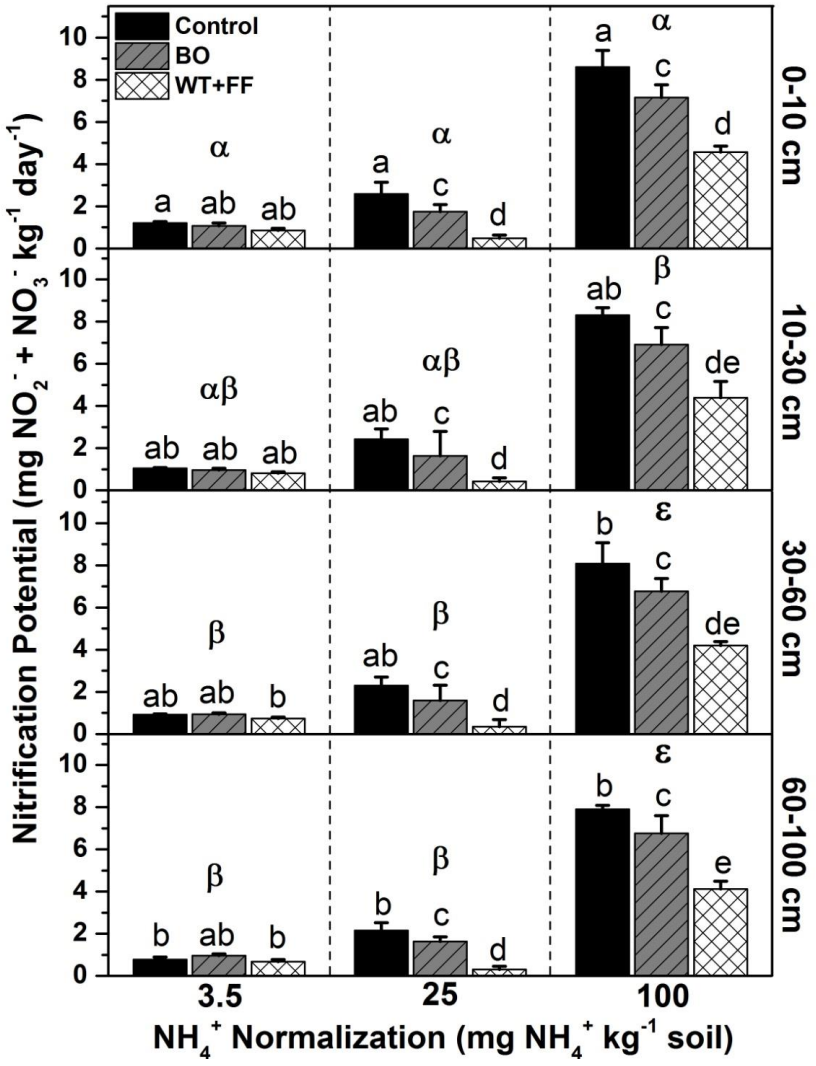

Figure 11: Potential total nitrification rates in response to organic matter removal (OMR) and differing levels of NH_4^+ addition within each soil depth. Data are means ± standard error (n=3). A repeated measures ANOVA table shows the statistical significance of OMR, soil depth, and their interaction for each NH_4^+ normalization level (i.e., 3.5, 25, 100 mg NH_4^+ kg^{-1} soil). *Post hoc* contrasts (Tukey-Kramer) were computed on values for each treatment within a depth profile (0-100 cm) for each NH_4^+ amendment level and significance is indicated by different letters above bars. *Post hoc* contrasts were also calculated for mean soil depth values and significance is indicated by different Greek letters in each box. Control: unharvested control, BO: bole-only harvest, WT+FF: whole-tree harvest + forest floor removal.

potential was highest in the control treatment which was significantly higher than the BO treatment ($p < 0.001$) and the WT+FF treatment ($p < 0.001$); furthermore, the BO treatment had significantly more $NO_2^- + NO_3^-$ accumulation than the WT+FF treatment ($p < 0.001$). In contrast, octyne-sensitive (AOB) nitrification was statistically unaffected by harvest treatment for the 3.5 mg NH_4^+ kg^{-1} normalization level; however, the 25 ($p < 0.01$) and 100 ($p < 0.001$) mg NH_4^+ kg^{-1} normalization levels were both statistically affected, with the control and BO treatments reporting higher rates of potential nitrification than the WT+FF treatment. Octyne-sensitive (AOB) nitrification was significantly reduced with increasing soil depth for all levels of NH_4^+ normalization (3.5 mg NH_4^+ kg^{-1}: $p < 0.001$; 25 mg NH_4^+ kg^{-1}: $p < 0.001$; 100 mg NH_4^+ kg^{-1}: $p < 0.001$).

Unlike total nitrification, octyne-resistant (AOA) accumulation of $NO_2^- + NO_3^-$ increased linearly with depth for the 25 mg NH_4^+ kg^{-1} ($p < 0.01$) and 100 mg NH_4^+ kg^{-1} ($p < 0.001$) normalization levels. Regardless of harvest treatment, the proportion of total nitrification attributed to octyne-resistant (AOA) activity at 0-10 cm decreased with increasing concentrations of NH_4^+ from 58% at 3.5 mg NH_4^+ kg^{-1} to 49% at 100 mg NH_4^+ kg^{-1}. Increasing depth resulted in an overall linear increase in the proportion of nitrification attributed to AOA activity. Regardless of treatment or NH_4^+ normalization level, AOA contributed from 51% at 0-10 cm to 76% at 60-100 cm. Octyne-resistant and -sensitive nitrification was significantly correlated to multiple biological and edaphic properties for each NH_4^+ normalization level. Specifically, octyne-resistant (AOA) nitrification was negatively correlated to soil pH and calculated NH_3 levels and positively correlated to AOA *amoA* copy numbers; congruently, octyne-sensitive (AOB) nitrification was positively correlated to SOC, TN, and AOB *amoA* levels (Table 4).

	Octyne-Resistant (AOA)			Octyne-Sensitive (AOB)		
	3.5	25	100	3.5	25	100
	F-Ratio					
OMR	**6.4***	**228.1*****	**97.2*****	0.5	**32.7*****	**35.6*****
Soil Depth	0.3	**7.0****	**18.8*****	**44.4*****	**69.6*****	**33.5*****
Interaction	1.6	2.0	**3.5***	1.0	**7.3*****	**2.9***

*p<0.05 **p<0.01 ***p<0.001

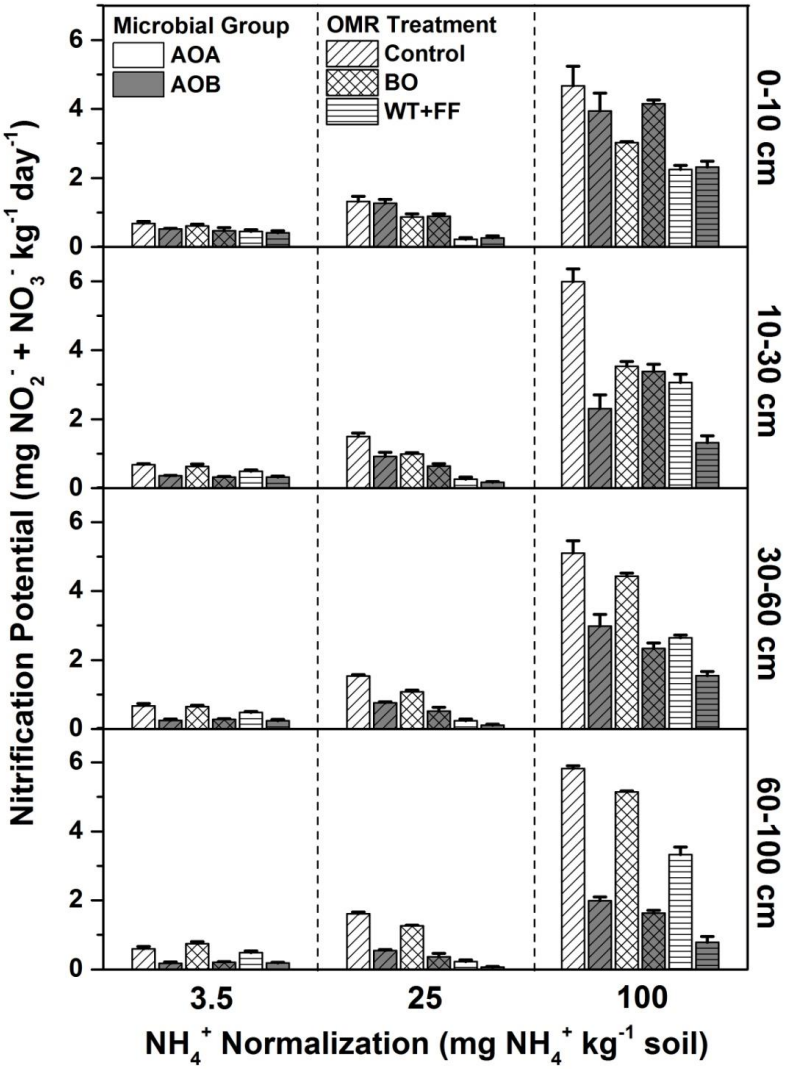

Figure 12: Potential octyne-resistant (ammonia-oxidizing archaea, AOA) and octyne-sensitive (ammonia-oxidizing bacteria, AOB) nitrification rates in whole soil assays amended with differing levels of NH_4^+. Data are means ± standard error (n=3). A repeated measures ANOVA table shows the statistical significance of organic matter removal (OMR), soil depth and their interaction for each NH_4^+ normalization level (i.e., 3.5, 25, 100 mg NH_4^+ kg^{-1} soil). Control: unharvested control, BO: bole-only harvest, WT+FF: whole-tree harvest + forest floor removal. AOA: ammonia oxidizing archaea, AOB: ammonia oxidizing bacteria

Table 8: Spearman's ranked correlation analysis between octyne-resistant (ammonia-oxidizing archaea, AOA) and –sensitive (ammonia-oxidizing bacteria, AOB) nitrification potential for each NH_4^+ addition level (3.5, 25, 100 mg NH_4^+ kg^{-1} soil) and select soil physicochemical and biological properties. Bold values indicate significance with level of significance inferred by superscript symbol. *p<0.01, †p<0.01, ‡p<0.001.

	Nitrification Potential (mg NO_2^- + NO_3^- kg^{-1} day^{-1})					
	Octyne-Resistant (AOA)			Octyne Sensitive (AOB)		
	3.5	25	100	3.5	25	100
SOC	0.15	0.11	-0.03	**0.78‡**	**0.65‡**	**0.67‡**
TN	0.18	0.09	-0.01	**0.58‡**	**0.57‡**	**0.61‡**
Soil pH	**-0.68‡**	**-0.67‡**	**-0.58‡**	-0.05	**-0.51‡**	**-0.35***
NH_3	**-0.47‡**	**-0.46†**	**-0.47†**	0.28	-0.10	0.09
NH_4^+	**0.51‡**	**0.48†**	0.31	**0.59‡**	**0.81‡**	**0.78‡**
NO_2^- + NO_3^-	**0.77‡**	**0.47†**	0.29	**0.70‡**	**0.80‡**	**0.66‡**
AOA *amoA* Copy No.	**0.58‡**	**0.76‡**	**0.65‡**	0.23	**0.73‡**	**0.53†**
AOB *amoA* Copy No.	0.19	0.06	-0.11	**0.68‡**	**0.58‡**	**0.67‡**

Discussion

In this study we illustrate how differing intensities of OMR associated with timber harvest can influence concentrations of SOC, TN, and inorganic N as well as community structure, distribution, abundance, and activity of AOA and AOB. We found that intensive forest harvest led to decade-scale reductions in concentrations of SOC and TN which is consistent with what has been observed previously in surface soils at this site (Foote et al., 2015) and other sites (Johnson and Curtis, 2001; Li et al., 2003; Nave et al., 2010; Jones et al., 2011; Huang et al., 2013; Achat et al., 2015a, 2015b). However, our results indicate that forest harvest-induced losses can occur throughout the upper 1 m of the profile and persist for decades. Furthermore, concentrations of soil NH_4^+ and NO_2^- + NO_3^- were also significantly altered by increasing harvest intensity; specifically, the WT+FF treatment resulted in significantly lower concentrations of soil inorganic-N than the control and BO treatments. It is likely that lower concentrations of

inorganic-N in the more severe organic matter removal treatment are at least in part attributable to higher rates of N-losses. For example, changes in microclimate conditions following harvest such as increases in solar radiation reaching the soil surface, decreases in transpiration and rainfall interception, and increases in the amount of precipitation reaching and infiltrating the forest floor and into the soil would favor higher rates of leaching (Vitousek et al., 1997; Carlyle et al., 1998; Holmes and Zak, 1999; Marchman et al., 2015) and denitrification (Brumme, 1995) following treatment application. Sustained reductions in inorganic-N may be attributable to reduced N-inputs (i.e., leaf litter, woody debris, root exudates, etc.) in the WT+FF treatment stands. To that point, the observed reduction in NH_4^+ and $NO_2^- + NO_3^-$ concentrations were significantly correlated to the concurrent reduction of TN. Increasing soil depth lead to significant reduction in both NH_4^+ and $NO_2^- + NO_3^-$. This is consistent with most studies of inorganic-N in forest soils where ammonification typically decreases with depth (Liu et al., 2015; Tanner et al., 2016) and $NO_2^- + NO_3^-$ concentrations decrease because of the mobility of NO_3^- in well drained soils.

Both AOA and AOB *amoA* genes were detected in all treatments and at all depths. Based on *amoA* quantification, AOA were 95X more abundant than AOB throughout the soil profile which is consistent with what has been previously reported in arid ecosystems (Adair and Schwartz, 2008), agricultural plots (Nicol et al., 2008; Hai et al., 2009) and forest soils (Lu et al., 2015). AOB *amoA* gene abundance declined significantly with depth in all treatments while AOA *amoA* gene abundance remained relatively constant, resulting in a significantly larger AOA to AOB ratio at 60-100 cm. As postulated by Leininger et al. (2006), the high numbers of AOA (relative to AOB) at depth indicate that AOA are adapted to a broad range of growth conditions and may possess a more versatile metabolism than AOB. Furthermore, the small cell size, small genome, and oligotrophic lifestyle associated with AOA (Hatzenpichler et al., 2008;

Tourna et al., 2011; Hatzenpichler, 2012) may contribute to their high abundance deeper in the soil profile where energy sources are likely to be more limited. It has also been shown that some archaeal groups lack an enzyme homologous to hydroxylamine oxidoreductase (Schleper and Nicol, 2010) and therefore may oxidize NH_3 via a nitroxyl intermediate (Walker et al., 2010) instead of hydroxylamine as seen in AOB. This alternate nitroxyl pathway requires less oxygen than the hydroxylamine pathway, which may allow AOA to occur and function in soil horizons and microsites where oxygen concentrations may be low (Schleper and Nicol, 2010). Abundance of AOB *amoA* was significantly positively correlated to SOC and TN with the highest abundances being found in surface soils as well as control stands. It has been shown that AOB are likely to be more abundant in soils with high nutrient and substrate availability (Wessén et al., 2010; Rasch et al., 2011). Control and BO treatment stands possessed significantly higher AOA and AOB *amoA* gene copy numbers than WT+FF treatment stands which may be attributed to the harvest-induced differences in physicochemical soil properties. It has been hypothesized that soil pH drives niche differentiation of AOA and AOB (Nicol et al., 2008; Stempfhuber et al., 2014) with archaea being more competitive at low pH due to their high affinity for NH_3 (Martens-Habbena et al., 2009; Verhamme et al., 2011) and AOB's physiological inability to function at low pH (Frijlink et al., 1992; Gubry-Rangin et al., 2011; Zhang et al, 2012; Hu et al, 2014b). Although it has been theorized that AOB are physiologically unable to oxidize NH_3 at pH <5.5 (Hankinson and Schmidt, 1988; Jiang and Bakken, 1999) their presence in acidic soils is generally observed and leads to the hypothesis that they may contribute to nitrification in acidic soils, perhaps in less acidic microsites. AOA *amoA* abundance increased with increasing soil acidity while AOB *amoA* abundance was statistically unaffected; congruently, we observed the least acidic conditions in WT+FF treatment stands. From these results, we would expect the ratio of AOA:AOB would be lowest for the WT+FF

treatment due to the less acidic pH; however, it is actually the highest. This suggests that harvest-induced reductions in SOC and TN are more detrimental to AOB abundance than concurrent increases in soil pH are to AOA abundance.

Results show that AOB community structure is significantly different among the three OMR treatments with clear separation in the 0-10 cm increment. This indicates that the AOB community was less resistant and/or resilient to perturbation and has not yet recovered to pre-harvest conditions represented by the unharvested control, most likely because of reduced substrate availability. In contrast, AOA community structure was not statistically affected by harvest treatment, indicating community resistance and/or resilience to increasing harvest intensity. The lack of treatment differences for AOA is most likely related to their physiological ability to maintain functionality in nutrient-depleted conditions. This is similar to Pereira e Silva et al. (2012) who showed that ammonia oxidizer community structure can change in response to seasonal differences in substrate availability with AOB variability being higher than AOA. Regardless of harvest treatment, we observed distinct community composition clustering for both AOA and AOB in response to soil depth. This is similar to what was observed by Gan et al. (2016), who demonstrated that soil depth has more influence on AOA and AOB community structure than forest type.

We also observed that the diversity and richness of AOA OTUs were significantly higher than AOB regardless of OMR or soil depth which is similar to previous studies (Pester et al., 2012; Stahl et al., 2012). Although AOA richness was unaffected by depth, we did observe that OTU numbers were lowest in controls suggesting that disturbance promotes the cohabitation of AOA lineages with differing ecological strategies. Similar to what was observed with AOB *amoA* quantification, AOB OTU richness decreased with depth. Congruently, AOB OTU richness was positively correlated to calculated levels of NH_3. This implies that only select AOB

lineages are able to function in soil horizons with lower resource levels. In contrast, AOA diversity increased with depth indicating that there is more AOA intraspecific competition when resources are low. This hypothesis is bolstered by the observation that AOA diversity was negatively correlated to SOC, TN, and $NO_2^- + NO_3^-$.

Potential total nitrification rates measured across all harvest treatments and soil depths fall within the range of 0.57-1.35 mg $NO_2^- + NO_3^-$ kg^{-1} soil day^{-1} (for soils amended with 3.5 kg NH_4^+ kg^{-1} soil) and 4.01-8.72 mg $NO_2^- + NO_3^-$ kg^{-1} soil day^{-1} (for soils amended with 100 kg NH_4^+ kg^{-1} soil). These values are consistent those observed previously in forest soils (Vitousek et al., 1982; Wertz et al., 2012; Lu et al., 2015). We found that both AOA and AOB activity was stimulated by the addition of anhydrous NH_3, indicating that both populations were N-limited in these soils. Although many studies dealing with acidic soils have reported the presence of AOB (Nicol et al., 2008; Gubry-Rangin et al., 2010; Stopnišek et al., 2010; Yao et al., 2011; Zhang et al., 2011, Lu et al., 2015), it has not been entirely clear if these populations are actively oxidizing NH_3. Similar to Lu et al. (2015), we observed that NH_4^+-stimulated activity was octyne sensitive in all treatments and at all depths indicating that AOB are potentially active in this system. Furthermore, regardless of harvest treatment, AOB contributed roughly 47% to total nitrification at 0-10 cm with 3.5 mg NH_4^+ kg^{-1}; however, this percentage increased to 51% contribution when 100 mg NH_4^+ kg^{-1} was applied. This indicates that AOB become more competitive when higher substrate concentration becomes available which is consistent with Giguere et al. (2015). AOB contribution decreased linearly to 23-25% at 60-100 cm which is similar to the trend that was observed with *amoA* gene copy number. Considering the acidity of this system it was surprising that AOB were not only functionally active but also contributed substantially to total nitrification. It is not entirely clear why the AOB contributed so greatly to nitrification in these soils; however, AOB might persist and be locally active in acidic forest soils via protective (less-

acidic) aggregates and microsites as was maintained in this whole-soil assay. Slurry assays in which soil structure is lost have reported lower AOB contributions to nitrification than whole-soil assays (Lu et al., 2015). It is also possible that AOA phylotypes exist in the acidic forest soils that are more sensitive to inhibition by octyne, which could potentially alter the relative contributions of AOA and AOB to total nitrification (Lu et al., 2015).

Increasing OMR intensity did not result in significant potential total nitrification difference for the 3.5 mg NH_4^+ kg^{-1} normalization level; however differences were observed with higher levels of NH_4^+. This indicates that at lower NH_4^+ conditions (similar to what is observed in the environment), increasing OMR intensity may not lead to long-term changes in total rates of nitrification which is somewhat surprising considering that we observed significant treatment differences in both AOA and AOB *amoA* quantification; however, *amoA* quantification is only an estimate of potential activity. Coupling this nitrification assay to gene expression analysis may yield more accurate estimates of actual activity; however this was not done in this study because of the difficulty in extracting quality mRNA from these acidic, humic soils. The observed treatment differences in total, octyne-resistant (AOA), and octyne-sensitive (AOB) nitrification at high levels of NH_4^+ may be an indication that high levels of AMO enzyme synthesis (mirroring *amoA* quantification levels) are activated only when substrate levels reach a certain threshold which exceeds environmental levels in this system. Contrary to total nitrification potential and AOB (octyne-sensitive) nitrification potential, AOA (octyne-resistant) nitrification was significantly reduced by increasing OMR intensity at 3.5 mg NH_4^+ kg^{-1}. Considering that AOA are dominant (especially at depth) in this system, increasing OMR intensity may be a solution to reducing total nitrification rates, and the subsequent loss of nitrogen from the soil system; however, the associated loss of total nitrogen with increasing OMR intensity most likely outweighs any positive contribution of reduced nitrification.

CHAPTER IV

INCREASING FOREST HARVEST INTENSITY LEADS TO DECADE-SCALE ALTERATIONS IN SURFACE AND SUBSURFACE SOIL FUNGAL COMMUNITIES

Forest harvest has the potential to impart long-term alterations to soil properties. This study investigated how intensive organic matter removal (OMR) during forest harvest may result in long-term alterations to soil fungal community structure to a depth of 1 meter in the soil profile. Using a replicated pine forest field experiment in the Gulf Coastal Plain, USA, we assessed the impact of OMR on soil fungal communities 18 years post-harvest. Soils were collected from replicated (n = 3) loblolly pine (*Pinus taeda* L.) plots including unharvested control stands and 2 harvest treatments differing in OMR intensity. Forest harvest treatments altered diversity and structure of the fungal community relative to unharvested controls. The ratio of Ascomycota to Basidiomycota increased as OMR intensity increased and was positively correlated to concurrent changes in soil pH. Community composition of fungal functional taxa was also altered by OMR. The most abundant genus, *Russula* exhibited significant reductions in response to increasing intensity of OMR. Results illustrate strong linkage between aboveground perturbation, edaphic factors, and belowground fungal communities. Furthermore, tree harvesting effects on soil fungal communities can persist throughout the soil profile for decades post-harvest, with potential implications for soil functional characteristics and the sustainability of forest production systems.

Introduction

Soils are vital components of forest ecosystems as they help regulate necessary ecosystem processes, such as nutrient cycling, decomposition, and water availability. In addition, these soils provide habitats for a vast array of microorganisms including archaea, bacteria, fungi,

and protozoa. Of these, fungi are the most abundant group by mass, accounting for approximately 2,750 kg ha^{-1} of microbial biomass (Metting, 1992) and are considered one of the most diverse eukaryotic groups with only a small fraction of the estimated worldwide fungal diversity having been reported (Hibbett et al., 2011). In addition, soil fungi directly influence ecosystem dynamics as plant pathogens, predators, and soil organic matter (SOM) decomposers (de Boer et al., 2005; Baldrian and Lindahal, 2011), as well as acting as mutualists in assisting with plant uptake of key nutrients (Rudgers et al., 2015; Wagg et al., 2015; Zeilinger et al., 2016). Despite their ecological significance, soil fungal communities have been greatly understudied relative to soil prokaryotic communities and should be investigated more thoroughly.

Fungal community dynamics have been shown to be defined by abiotic and edaphic factors including elevation (Meng et al., 2013; Thébault et al., 2014), precipitation regimes (Sorensen et al., 2013), fires (Sun et al., 2011; Brown et al, 2013), substrate composition (Bååth and Anderson, 2003; Zinger et al., 2009; Rousk et al., 2010; Dai et al., 2013b; Anderson et al., 2016; Martínez-Garcia et al., 2015; Mayor et al., 2015), and soil pH (Rousk et al., 2010; Wang et al., 2015). The composition of above-ground vegetation may also play an important role in shaping fungal community structure through endophytic, pathogenic, and mycorrhizal association (Wallenstein et al., 2007; Toberman et al., 2008), as well as saprotrophic differences induced by litter quality and source (Bottomley et al., 2006; Artz et al., 2007; Wallenstein et al., 2007; Buée et al., 2009; Lewandowski et al., 2016). Because soil fungi respond strongly to environmental variables, it is conceivable that anthropogenic disturbances that alter physicochemical properties will lead to changes in fungal community composition.

Over the past century, coniferous forests in the southern USA have been extensively managed for timber-related products, such as merchantable and industrial timber as well as

pulpwood. This region covers >275,000 km^2 and represents 9% of the total US forest area (Oswalt et al., 2014), approximately half of which is utilized for plantation forestry (Fox et al., 2007). These managed and natural forests are vital to the regional economy, yielding ≥40% of annual US softwood and consistently producing 19% and 12% of the worldwide pulpwood and industrial timber, respectively (McNulty et al., 1996; Hodges et al., 2011; Brandeis et al., 2012). Recently there is growing interest in the removal of non-merchantable woody debris, including slash, sawdust, and forest litter, to be used as substitute feedstocks in industrial processes and for bio-energy production (Janowiak and Webster, 2010). Furthermore, commercial timber corporations and individual land owners are beginning to utilize forest litter for the production of merchantable mulch (Dickens et al., 2012). Management techniques that lead to increased biomass removal may affect fungal community structure and lead to long-term reductions in forest productivity (Wei et al., 2000; Walmsley et al., 2009; Achat et al., 2015a, 2015b). Intensive OMR associated with timber harvest has been shown to negatively affect SOM stocks, including soil organic carbon (SOC) (Laiho et al., 2003; Smaill et al., 2008; Huang et al., 2011; Foote et al., 2015) and total nitrogen (TN) (Thiffault et al., 2011; Zummo and Friedland, 2011; Kellman et al., 2014; Prest et al., 2014; Foote et al., 2015), as well as microbial biomass (Busse et al., 2006; Foote et al., 2015) and SOM decomposition potential (Cardenas et al., 2015; Leung et al., 2015); however, other than Hartmann et al. (2012), few have investigated the decadal-scale effect of intensive forest OMR on the ecology of soil fungi, especially at depth.

In this study, we explored the decade-scale response of the soil fungal community to intensive OMR in surface and subsurface mineral soils of the southeastern US. We used high-throughput fungal internal transcriber spacer (ITS) sequencing and soil physicochemical analyses to document changes in fungal community structure resulting from soil disturbance during timber harvest that occurred 18 years ago. The objectives of this study were to (i) assess

the long-term impact of forest OMR on fungal community structure, (ii) investigate how differences in environmental factors influence the diversity and distribution of soil fungi, and (iii) investigate whether the distribution of functional fungal groups have being influenced by OMR. We hypothesized that (a) increasing forest OMR will result in reduced substrate and resources (i.e., SOC and TN) and lead to reduced fungal diversity and altered community structure, (b) these changes in fungal community structure would be more evident in surface rather than subsurface portions of the soil profile, and (c) fungal species that form mycorrhizal associations would be more abundant in plots that were subjected to the most severe harvest treatment due to the reduction in plant-available nutrient resources.

Materials & Methods

Study Site and Experimental Design

Research was conducted in Davy Crockett National Forest near the town of Groveton, TX, USA (31° 06’ 32.48’’ N, 95° 09’ 59.15’’ W). The study site is part of the Long-term Soil Productivity (LTSP) network aimed at understanding the effects of forest management on soil properties and processes (Powers et al., 2005; Powers, 2006; Ponder et al., 2012; Scott et al., 2016). Soils across the study area are uniform (fine-loamy siliceous, thermic Oxyaquic Glossudalf in the Kurth series), and topography is relatively flat. The experimental design includes unharvested control stands (tree age = 60-80 yrs.), and two harvest treatments differing in the extent of organic matter removal. Harvest treatments consisted of a low intensity treatment, bole-only (BO) harvest, where only the bole of the tree was removed, and a high-intensity treatment, whole-tree harvest + forest floor removal (WT+FF), where the entire tree (bole, branches, leaves) was removed along with the forest floor debris being removed by hand-raking. During harvest, trees were hand-felled and lifted off of the plots with a loader to reduce soil compaction. Controls and both harvest treatments were replicated 3X and each replicate was

0.2 ha. All plots are located within a 1.5 km radius. Plots were harvested in 1996 and then replanted in 1997 with containerized *P. taeda* L. (loblolly pine) seedlings of 10-half sib families from the US Forest Service seed orchards using 2.5-m x 2.5-m spacing.

Soil Sampling

Field sampling took place in late April 2015. Soil cores were extracted using a JMC Environmentalist's Sub-Soil Probe PLUS (Clements Associates Inc., Newton, IA, USA) (2.8 cm diameter x 120 cm length). For control and treatment plots, soil cores were taken at 1.8 m from the base of a randomly selected *P. taeda* individual with a diameter at breast height (DBH) between 18 and 24 cm. A 7.5 m buffer from the outside of the 0.2 ha plots was not sampled to avoid edge effects. In some of the WT+FF stands, the forest floor had not yet redeveloped; because of this, the organic soil horizon in all other plots (approximate thickness: < 3 cm) was removed prior to coring in order to investigate mineral soil horizons exclusively. Soil sampling followed a stratified random sampling design in which four cores were taken from each plot and homogeneously pooled by depth (i.e., 0-10, 10-30, 30-60, 60-100 cm) to increase sample mass and reduce error introduced by environmental heterogeneity. This resulted in 1 composited core per plot, separated into 4 depth increments, and replicated 3X per treatment. On the day in which soil cores were taken from the ground, samples were transported at 4°C from the field to the lab, aseptically homogenized, and 5-g subsamples (3 sample^{-1}) were immediately stored at -80°C for future DNA extraction and subsequent analysis. The remaining soil was stored at 4°C for physicochemical analysis.

Physicochemical Soil Analysis

Soil pH was determined using an Accumet Basic pH meter (Denver Instrument, Arvada, CO, USA) on a 1:2 solution of soil in a 0.01M $CaCl_2$ solution (Minasny et al., 2011). A 50-g aliquot of field-moist soil was dried at 105°C for 48-hours to calculate bulk density and soil

moisture. The remaining soil was then passed through a 2-mm sieve to remove large organic material and roots. A 25-g aliquot of sieved soil was then dried at 60°C for 48 hours and finely ground using a TE250 ring pulverizer (Angstrom, Inc., Belleville, MI, USA). The pulverized soil was used to determine SOC and TN stocks. Carbon (C) and nitrogen (N) analyses were conducted on a Carlo Erba EA-1108 elemental analyzer (CE Elantech, Lackwood, NJ, USA). Soil C and N stocks (g m^{-2}) were computed as the product of the elemental concentration and soil bulk density for each depth (Ellert and Bettany, 1995). The C to N ratio was calculated as the proportion of SOC to TN on a g kg^{-1} basis. Pulverized soil was also used to quantify Mehlich-III extractable phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and sodium (Na) using inductively coupled mass spectrometry at the Texas A&M Soil, Water, and Forage Testing Laboratory (Mehlich, 1978).

DNA Extraction, PCR Amplification, DNA Library Construction, and Sequencing

Because of the difficulty in extracting environmental DNA from soil with the potential for high humic co-extractant (He et al., 2005; Wang et al., 2007) as well as an overall low microbial biomass, DNA extraction followed the modified version of the International Standard for the extraction of DNA from soil as described by Terrat et al. (2014). Further modification was made to extract DNA from 3 g of soil rather than the prescribed 1 g. DNA was extracted from 3 analytical replicates per sample and then pooled to increase mass and reduce environmental heterogeneity. DNA library preparation and sequencing of soil fungal communities was done by Molecular Research DNA Laboratory (www.mrdna.com, Shallowater, TX, USA) through target-based amplification of the entire fungal internal transcribed spacer (ITS) region (ITS1, 5.8s, ITS2) with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3'; White et al., 1990) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White et al., 1990). These primers (ITS1 and ITS4) were selected because of sequence variability and representation in

public data repositories and reference databases (Nilsson et al., 2009). PCR amplification was accomplished by utilizing the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, USA) under the following conditions: an initial denaturation step at 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. After amplification, PCR products were verified via gel electrophoresis (2% agarose gel), barcoded, pooled together in equal proportions based on their molecular weight and DNA concentrations, and purified with calibrated AMPure XP beads (Agencourt Biosciences Co., Brea, CA, USA). Synthesis-based sequencing on an Illumina MiSeq followed the manufacturer's guidelines and resulted in single-end ITS reads of 343 ± 67.5 (mean ± standard deviation) bp.

Bioinformatic Analysis

Raw Illumina reads were reoriented so that all amplicons were oriented 5'-3'. Resulting .fasta and .qual files were demultiplexed, quality filtered, and analyzed using the QIIME 1.9.1 pipeline (Caporaso et al., 2010). Illumina sequences with <200 and >900 bp, barcode or primer sequence errors, and those with homopolymers or ambiguous base calls that exceed six nucleotides were discarded. Sequences were trimmed to contain only the ITS1 region using the ITSx 1.0.11 software (Nilsson et al., 2010; Bengtsson-Palme et al., 2013). The trimming of the ITS1 region allowed for the extraction of a defined gene segment that is better suited for community comparisons and also provides further quality control by confirming the authenticity of the target. Raw sequences were deposited in NCBI's sequence read achieves under the accession number SRR5202284. Operational taxonomic units (OTUs) were defined by clustering at 97% sequence identity (Nilsson et al., 2008; Tedersoo et al., 2010b) using the QIIME implementation of UCLUST (Edgar, 2010). Subsequent taxonomic annotations, refer to assignments based on >97% sequence identity for species, 95-97% sequence identity for genera,

90-95% for families, and 77-80% for phyla using the UNITE fungal database (Lemos et al., 2011). Chimera checking was performed on remaining sequences using the QIIME-based ChimeraSlayer (Haas et al., 2011). OTUs annotated to the genus-level were putatively categorized as either ectomycorrhizal (ECM), arbuscular mycorrhizal (AM), ericoid mycorrhizal (ErM), or saprotrophic from a variety of published sources that comment on the ecological status of specific fungal genera (Miller and Miller, 1988; Roberts, 1999; Redecker et al., 2000; Sylvia et al., 2005; Redecker and Rabb, 2006; Rice and Currah, 2006; Cannon and Kirk, 2007; Kirk et al., 2008; Rinaldi et al. 2008; Buée et al., 2009; Desjardin et al., 2010; Tedersoo et al., 2010a). The ecological role of all remaining genera were undefined.

Statistical Analysis

All statistical analyses on fungal communities were carried out using the sequence count within each OTU as an abundance value for the fungal community (Danzeisen et al., 2011). All datasets were tested for normality using Shapiro-Wilk's test. When OTU data were not of normal distribution, non-parametric statistical tests or log transformations were applied. OTU data generated in QIIME were used to quantify the number of observed species (S_{obs}), richness (Chao1: Chao, 1984), diversity (Simpson's 1-D: Simpson, 1949), and Good's coverage (Good, 1953). Community metric calculations and rarefaction curves were analyzed using normalized OTU data set to 11,620 reads sample^{-1}. Physicochemical properties, fungal community metric estimates, and OTU abundance values were statistically analyzed using a linear mixed model ANOVA. Because of the inherent autocorrelation between differing soil depths, a repeated measures experimental design was employed with OMR as the fixed main plot and soil depth as the repeated measure (Derner et al., 2006). Replicated plots were nested within harvest treatment and considered a random effect (Dai et al., 2006). When differences were significant, Tukey's honest significant difference (HSD) test was performed to assess post hoc contrasts with

significance inferred at $p \leq 0.05$. Non-metric multidimensional scaling (NMDS) based on a Bray-Curtis dissimilarity matrix (Bray and Curtis, 1957) was performed on normalized fungal ITS data. A permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) using the Bray-Curtis matrix listed above was employed to characterize differences in the soil fungal community based on OMR treatment and soil depth. PERMANOVAs were run using 999 permutations. Spearman's correlations were calculated using JMP (SAS Institute, Inc., Cary, NC, USA).

Results

Soil Physicochemical Properties

SOC and TN were significantly reduced by increasing intensity of OMR (SOC: $p < 0.001$; TN: $p < 0.05$) (Table 9). Control stands possessed 1191 g C m^{-2} more SOC and 20 g N m^{-2} more TN than BO stands and 1954 g SOC m^{-2} more SOC and 69.1 g N m^{-2} more TN than the WT+FF stands for the entire 0-100 cm depth profile. Increasing soil depth significantly reduced SOC ($p < 0.001$) and TN ($p < 0.001$) (Table 9). The ratio of carbon to nitrogen (C:N) was statistically unaffected by differing intensities of OMR; however, C:N ratios tended to be higher in the control stands than either of the harvest treatments. Increasing soil depth significantly impacted C:N ($p < 0.001$) which decreased linearly from 21.2 ± 1.6 (mean ± standard error) at 0-10 cm to 7.7 ± 1.1 at 60-100 cm. Bulk density significantly increased with depth ($p < 0.001$). Soil pH was not significantly altered by differing depths; however, it did become less acidic with increasing harvest intensity ($p < 0.05$). The concentration of Mehlich-III extractable P was significantly higher ($p < 0.05$) in the WT+FF (5.7 ± 1.1 mg P kg^{-1}) than the control (3.9 ± 1.0 mg P kg^{-1}) or BO treatment (4.6 ± 0.6 mg P kg^{-1}) (Table 10).

Table 9: Edaphic parameters of the three organic matter removal treatments for each of the four soil depth increments. Cumulative values (0-100 cm) for SOC and TN are also reported. Post hoc contrasts (Tukey-Kramer) were computed on values for each depth nested within each treatment and indicated by differing letters within each column. For each soil depth in each treatment, N=3. Statistical differences were inferred at $p \leq 0.05$.

Soil Depth (cm)	SOC (g m^{-2})	TN (g m^{-2})	C:N	pH (0.01M CaCl)	Bulk Density (g cm^{-3})	Water Content (%)
Unharvested Control				*Mean (Std. Error)*		
0-10	2 498 (233)a	107 (14)a	23.9 (2.5)a	3.32 (0.2)a	1.09 (0.1)a	38.65 (4.9)a
10-30	963 (96)cd	57.3 (11)abc	18.1 (3.7)abc	3.64 (0.1)ab	1.43 (0.3)a	37.76 (2.9)a
30-60	445 (69)de	39.8 (13)bc	14.3 (4.8)abc	3.54 (0.2)ab	1.41 (0.3)a	31.94 (4.4)a
60-100	427 (60)de	45.9 (6.2)bc	9.6 (1.8)bc	3.41 (0.3)a	1.76 (0.2)a	33.47 (1.7)a
Cumulative	**4 333 (458)**	**250 (44.2)**	-	-	-	-
Bole-only Harvest						
0-10	1 724 (180)b	84 (8.2)ab	20.5 (1.2)ab	4.15 (0.1)ab	1.08 (0.2)a	22.8 (3.3)a
10-30	653 (42)de	48 (2.5)bc	13.6 (1.1)abc	3.70 (0.3)ab	1.61 (0.1)a	34.7 (2.4)a
30-60	382 (52)de	44 (9.3)bc	8.9 (0.6)bc	3.36 (0.2)a	1.57 (0.2)a	41.9 (5.5)a
60-100	383 (48)de	54 (3.9)bc	7.08 (0.4)c	3.25 (0.2)a	1.63 (0.2)a	40.9 (5.2)a
Cumulative	**3 142 (322)**	**230 (23.9)**	-	-	-	-
WT+FF Removal						
0-10	1 344 (182)bc	75.9 (14)abc	19.1 (4.3)abc	3.97 (0.3)ab	1.02 (0.1)a	23.4 (5.2)a
10-30	592 (141)de	34.5 (6.5)c	17.0 (2.1)abc	4.36 (0.1)ab	1.46 (0.1)a	24.9 (4.9)a
30-60	208 (69)e	29.6 (6.1)c	6.7 (1.2)c	4.26 (0.4)ab	1.77 (0.03)a	31.5 (2.7)a
60-100	235 (127)e	40.9 (11)bc	6.3 (2.7)c	4.67 (0.1)b	1.80 (0.1)a	32.7 (7.5)a
Cumulative	**2 379 (519)**	**180.9 (37.6)**	-	-	-	-

Table 10: Mehlich-III extractable phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and sodium (Na) in each of the three organic matter removal treatments and for each of the four soil depth increments within those treatments. Mean values (0-100 cm) are also reported. For each soil depth in each treatment, N=3.

Soil Depth (cm)	P	K	Ca	Mg	S	Na
Unharvested Control	Mehlich III-Extractable (mg kg^{-1} soil)					
0-10	7.7 (3.3)	230.1 (43.8)	545.2 (268.3)	136.0 (60.1)	20.7 (2.4)	66.9 (17.7)
10-30	3.8 (0.8)	286.7 (73.7)	334.5 (164.9)	153.2 (104.1)	15.9 (2.0)	56.4 (5.2)
30-60	2.7 (0.9)	300.4 (88.3)	167.4 (24.1)	176.8 (127.1)	14.8 (2.6)	63.5 (7.5)
60-100	1.5 (0.4)	297.6 (86.3)	207.1 (114.4)	190.5 (56.5)	24.1 (2.9)	143.6 (62.2)
Mean	**3.9 (1.0)**	**278.7 (33.2)**	**313.6 (84.3)**	**164.1 (39.7)**	**18.9 (1.6)**	**82.6 (17.6)**
Bole-only Harvest						
0-10	8.5 (0.4)	201.1 (23.1)	756.7 (143.2)	130.1 (21.0)	16.6 (2.3)	54.9 (5.3)
10-30	5.0 (0.2)	195.6 (18.7)	290.1 (28.3)	79.0 (18.9)	11.6 (1.4)	49.1 (5.4)
30-60	3.4 (0.4)	326.7 (104.1)	240.8 (51.5)	235.5 (124.9)	15.7 (3.7)	52.6 (4.2)
60-100	1.5 (0.4)	361.5 (76.5)	185.6 (59.8)	273.4 (60.1)	23.9 (2.9)	91.1 (6.0)
Mean	**4.6 (0.8)**	**271.2 (36.0)**	**368.3 (77.1)**	**179.5 (38.3)**	**17.0 (1.8)**	**61.9 (5.6)**
WT Harvest + FF Removal						
0-10	11.1 (1.0)	179.4 (14.8)	350.7 (24.5)	54.3 (0.9)	14.9 (1.4)	48.5 (2.2)
10-30	6.5 (0.2)	203.2 (13.5)	170.1 (23.9)	36.2 (6.6)	10.1 (1.1)	53.9 (7.0)
30-60	3.4 (1.3)	213.3 (5.9)	155.2 (55.8)	53.1 (16.1)	11.9 (3.2)	56.1 (15.3)
60-100	1.8 (0.5)	276.5 (27.7)	253.8 (156.3)	188.6 (63.6)	30.2 (8.9)	109.1 (50.0)
Mean	**5.7 (1.1)**	**218.1 (13.1)**	**232.4 (43.0)**	**83.0 (23.2)**	**16.8 (3.1)**	**66.9 (13.5)**

Fungal Community Structure and Metrics

Of the initial 1,098,145 sequences, a total of 724,669 high-quality sequences were retained following filtering. Sequences were distributed evenly throughout all 36 samples with 20,130 ± 1,915 sequences per sample, which did not statistically vary between OMR or differing soil depths. These sequences corresponded to a diverse soil fungal community of 936 ± 51 unique OTUs per sample. Counts of OTUs in individual ITS libraries did not vary by OMR treatment; however, they were significantly reduced by increasing soil depth ($p < 0.001$). Fungal libraries captured a wide variety of taxa as exemplified by summiting rarefaction curves (Figure 13), suggesting that the breadth of sequencing was appropriate for this system; furthermore, coverage estimates were very high (0.97 ± 0.01), indicating an overall excellent OTU coverage afforded by the level of sequencing employed in this study, which did not statistically differ amongst OMR or soil depths.

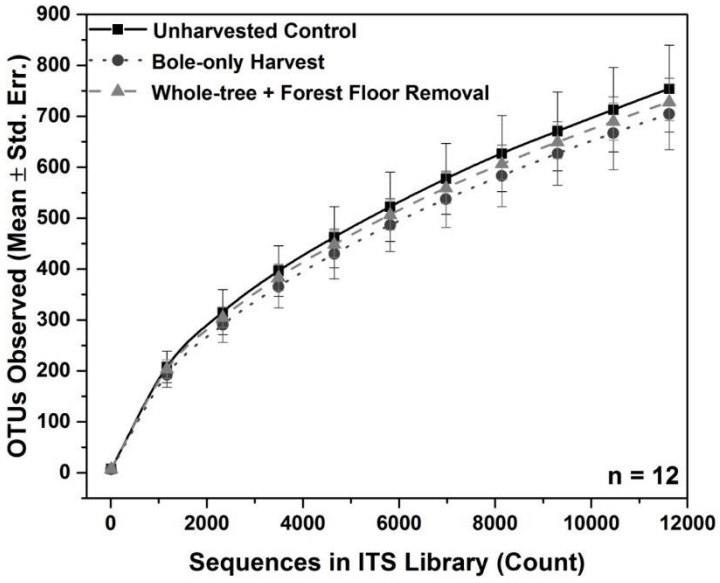

Figure 13: Rarefaction analysis of fungal ITS gene sequences associated with each of the three organic matter removal treatments. The mean number of sequences in each library is plotted against the mean number of OTUs observed in the same library. OTU data was normalized at 11 620 sequences per sample. OTUs were defined at the 97% similarity level.

Richness (Chao1) was not influenced by OMR treatments; however, diversity (Simpson's 1-*D*) showed significant reductions with respect to increasing OMR (Table 11). ANOVA followed by Tukey-Kramer *post hoc* comparison showed that when all soil depths were pooled, the WT+FF treatment had the lowest mean diversity values, which was significantly lower than the control stands (Simpson's 1-D: $p < 0.05$). BO stands did not significantly differ from control or WT+FF treatments. Control plots presented the highest mean Chao1 estimate; however, when contrasted to the two OMR treatments, no statistical difference was observed. Increasing soil depth significantly reduced richness ($p < 0.001$) and diversity ($p < 0.01$). When post hoc contrasts were computed on diversity metrics, there were statistical differences in OMR treatment for the most superficial depths (0-30cm); however, no differences occurred below 30 cm. SOC, TN, and C:N were all strongly positively correlated with number of unique OTUs, Chao1, and Simpson's diversity (Table 12). However, bulk density was negatively correlated with these same indices, while soil water content and pH were unrelated.

Table 11: Summary of fungal operational taxonomic units (OTUs), and their diversity and richness estimates. Post hoc contrasts (Tukey-Kramer) were computed on values for each depth nested within each treatment and indicated by differing letters within each column. Post hoc contrasts were also calculated for mean treatment values and differences are indicated by differing Greek letters in each column. For each soil depth in each treatment, N=3; for mean values per treatment, N=12. Statistical differences were inferred at $\alpha \leq 0.05$.

Soil Depth (cm)	No. Unique OTUs	Chao1 Richness Estimate	Simpson's Diversity Index (1-*D*)
Unharvested Control		*Mean (Std. Error)*	
0-10	1 295 (185)a	2 209 (175)a	0.96 (0.01)a
10-30	1 030 (33)ab	1 749 (59)ab	0.94 (0.02)a
30-60	942 (55)ab	1 661 (123)ab	0.80 (0.03)b
60-100	570 (137)b	1 095 (212)b	0.52 (0.04)c
Mean	**959 (93)α**	**1 678 (136)α**	**0.90 (0.02)α**
Bole-only Harvest			
0-10	1 230 (72)a	2 131 (95)a	0.92 (0.01)a
10-30	1 090 (51)ab	1 879 (60)ab	0.92 (0.01)a
30-60	693 (126)b	1 357 (304)ab	0.71 (0.14)bc
60-100	625 (165)b	1 165 (196)b	0.42 (0.29)c
Mean	**910 (91)α**	**1 633 (142)α**	**0.74 (0.08)αβ**
WT+FF Removal			
0-10	1 147 (208)ab	2 015 (310)ab	0.83 (0.02)b
10-30	1 090 (117)ab	1 933 (163)ab	0.73 (0.03)bc
30-60	863 (130)ab	1 456 (181)ab	0.65 (0.07)bc
60-100	651 (136)ab	1 234 (285)ab	0.45 (0.06)c
Mean	**938 (88)α**	**1 660 (142)α**	**0.66 (0.05)β**

Table 12: Spearman's rank order correlation coefficients for fungal community metrics and edaphic parameters. Asterisks indicate a significant relationship with: *p<0.05, **p<0.01, ***p<0.001.

	No. Unique OTUs	Chao1 Richness Estimate	Simpson's Diversity Index (1-*D*)
		Spearman's Rho	
SOC (g C·m^{-2})	**0.69*** **	**0.71*** **	**0.72****
TN (g N·m^{-2})	**0.35***	**0.38***	**0.49***
C:N	**0.71*** **	**0.71*** **	**0.66*** **
pH (0.01M CaCl)	0.35	0.12	-0.26
Bulk Density (g·cm^{-3})	**-0.40****	**-0.38***	**-0.31***
Water Content (%)	-0.15	-0.09	0.03

Non-metric multidimensional scaling (NMDS) plots, based on Bray-Curtis distance matrices, of OTUs showed distinct separation of the three OMR treatments, across multiple depth increments (Figure 14); furthermore, when soil depth was analyzed without regard for OMR treatments, the two shallowest depth increments (i.e., 0-10 and 10-30 cm) tended to cluster tightly, with lower standard deviation, while the 30-60 and 60-100 cm depth increments were more variable (Figure 15). Based on PERMANOVA, fungal community composition was statistically altered by OMR across all depth increments except 60-100 cm.

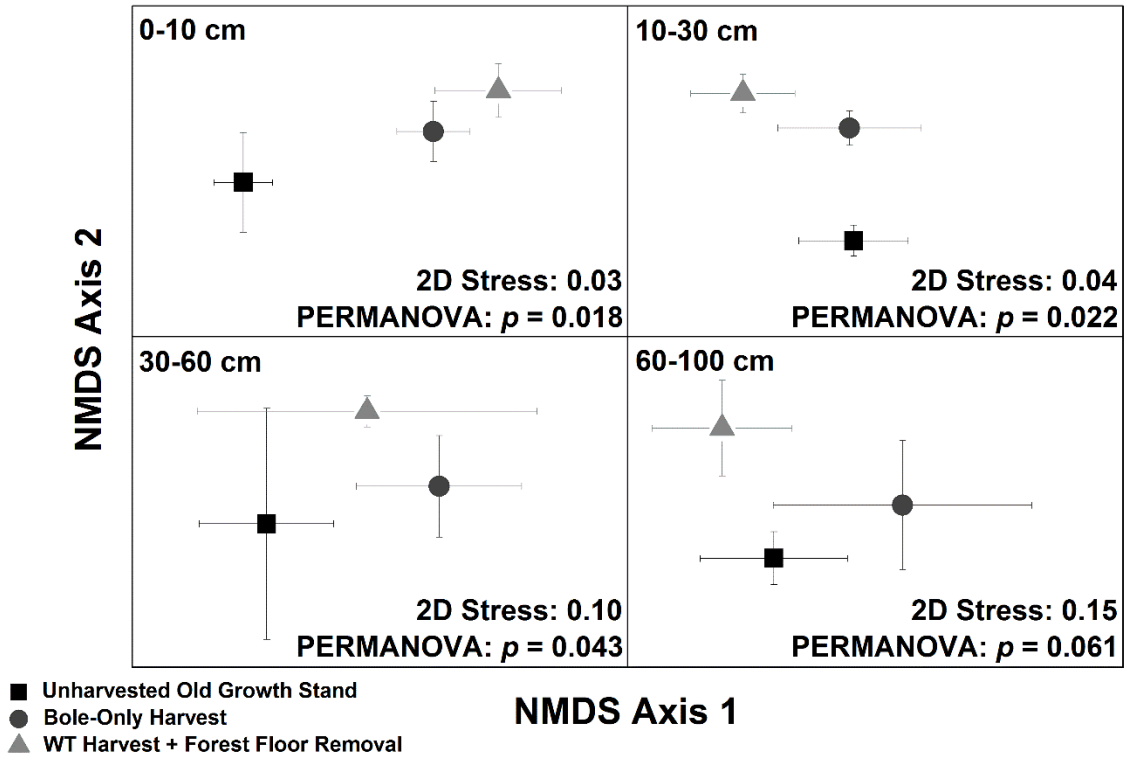

Figure 14: Nonmetric multidimensional scaling (NMDS) ordinations of the fungal communities based upon their OTU composition derived from Bray-Curtis distances matrices. Plots are differentiated by organic matter removal treatment (Square=Unharvested Control; Circle=Bole-Only Harvest; Triangle=Whole-tree Harvest + Forest Floor Removal) at each soil depth. Each point and corresponding bars represents mean ± standard deviation (N=3). Statistical differences in organic matter removal were inferred using PERMANOVA at each soil depth.

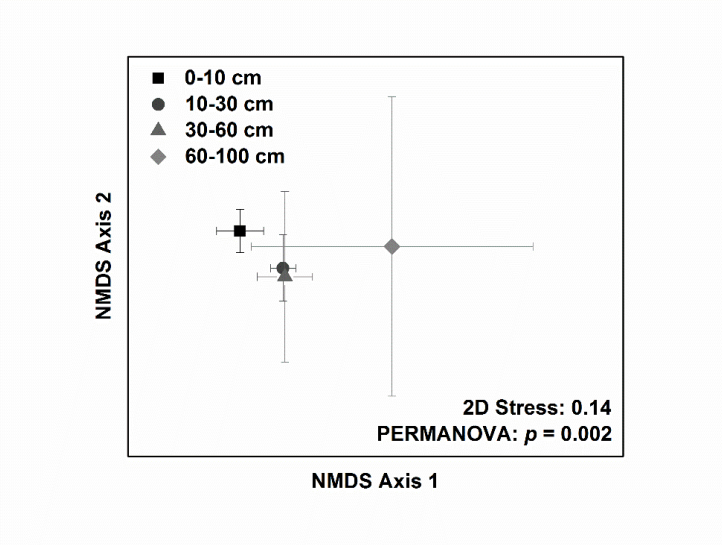

Figure 15: NMDS ordinations based on Bray-Curtis dissimilarity matrices for the comparison of differing soil depths (Square=0-10cm; Circle=10-30cm; Triangle=30-60cm; Diamond=60-100cm). Each point and corresponding bars represent the mean ± standard deviation (N=9).

Taxonomic Characterization

Phylum-level OTU characterization indicated that communities were dominated by Basidiomycota (61 ± 2.9%) and Ascomycota (35 ± 2.6%) across all treatments and soil depths (Table 13). To a lesser extent, libraries were composed of Zygomycota (3.5 ± 0.6%). The remaining sequences were annotated to either Glomeromycota, Chytridiomycota, or Neocallimastigomycota. Neither OMR treatment nor soil depth statistically impacted the abundance of OTUs annotated to Basidiomycota or Ascomycota, the two most dominant phyla. However, noteworthy trends did emerge. The number of sequences annotated to Ascomycota increased by 20% when comparing the control treatment to WT+FF treatment; simultaneously, Basidiomycota decreased by 17% when comparing the same stands (Figure 16). We observed a significant positive relationship between the ratio of Ascomycota to Basidiomycota and soil pH (Figure 17). As soil pH increased, we observed a greater proportion of Ascomycota which corresponds to the WT+FF treatment.

Table 13: The 20 most abundant genera composited from all samples (n=36) at the Groveton-LTSP site. A linear mixed model was used to analyze the influence of organic matter removal and soil depth on OTU abundance values and Spearman's rank order correlation analysis was used to analyze relationship between OTUs and certain edaphic properties. Asterisks indicate a significant relationship with: *p<0.05, **p<0.01, ***p<0.001. ‡: relative abundance increased with increasing harvest intensity or soil depth; Φ: relative abundance decreased with increasing harvest intensity or soil depth.

Phylum	Genus	Relative Abundance	OMR Treatment	Soil Depth	Soil pH	SOC	TN
			F-Ratio		*Spearman's Rho*		
Ascomycota		35.01	0.54	2.98	0.21	**0.40***	0.20
	Aspergillus	1.36	1.09	**5.69**Φ**	0.09	0.09	-0.24
	Beauveria	0.95	0.99	**3.09*Φ**	0.02	**0.48****	0.22
	Cladophialophora	3.81	3.35	**17.06***Φ**	0.26	**0.50****	**0.49****
	Elaphomyces	0.94	3.17	0.97	0.02	**0.38***	0.18
	Leptodontidium	2.86	0.3	**4.58**Φ**	0.24	**0.64*****	0.27
	Penicillium	3.76	0.04	**4.75*Φ**	0.16	**0.47****	0.22
	Raffaelea	2.03	0.6	**4.33*Φ**	-0.09	**-0.42***	-0.05
	Talaromyces	1.07	1.51	1.22	0.17	**0.45****	-0.01
	Trichoderma	1.41	0.19	1.76	**0.49****	**0.56*****	0.17
	Other Ascomycete Genera	16.82	2.24	1.64	**0.35***	0.26	-0.02
Basidiomycota		60.70	0.36	0.60	**-0.44****	0.02	-0.04
	Amanita	3.11	2.95	2.87	0.18	**0.38***	0.19
	Amphinema	1.49	0.84	0.26	**0.63*****	0.32	0.08
	Cryptococcus	0.97	0.29	**4.34*Φ**	0.24	**0.43****	-0.01
	Inocybe	14.14	0.05	2.12	-0.16	-0.05	-0.18
	Laccaria	3.16	0.34	0.84	0.27	0.22	-0.07
	Lactarius	0.94	1.25	1.31	0.15	**0.42***	0.29
	Rhizopogon	6.02	2.86	0.77	**0.36***	0.03	0.07
	Russula	23.07	**11.42**Φ**	2.25	**-0.54*****	0.05	0.03
	Tomentella	1.31	3.19	**3.05*Φ**	0.08	**0.51****	0.29
	Trechispora	2.14	0.02	1.09	0.2	0.2	0.08
	Other Basidiomycete	4.35	2.24	**4.28*Φ**	0.08	**0.61*****	0.32
Zygomycota		3.51	1.36	**5.25**Φ**	**0.49****	**0.43****	0.04
	Mortierella	2.15	1.22	**3.83*Φ**	**0.40***	0.31	-0.09
	Other Zygomycete Genera	1.36	1.63	**10.99***Φ**	**0.43****	**0.63*****	0.30
Other Fungal Phyla		0.78	2.36	0.70	**0.42***	**0.35***	0.11

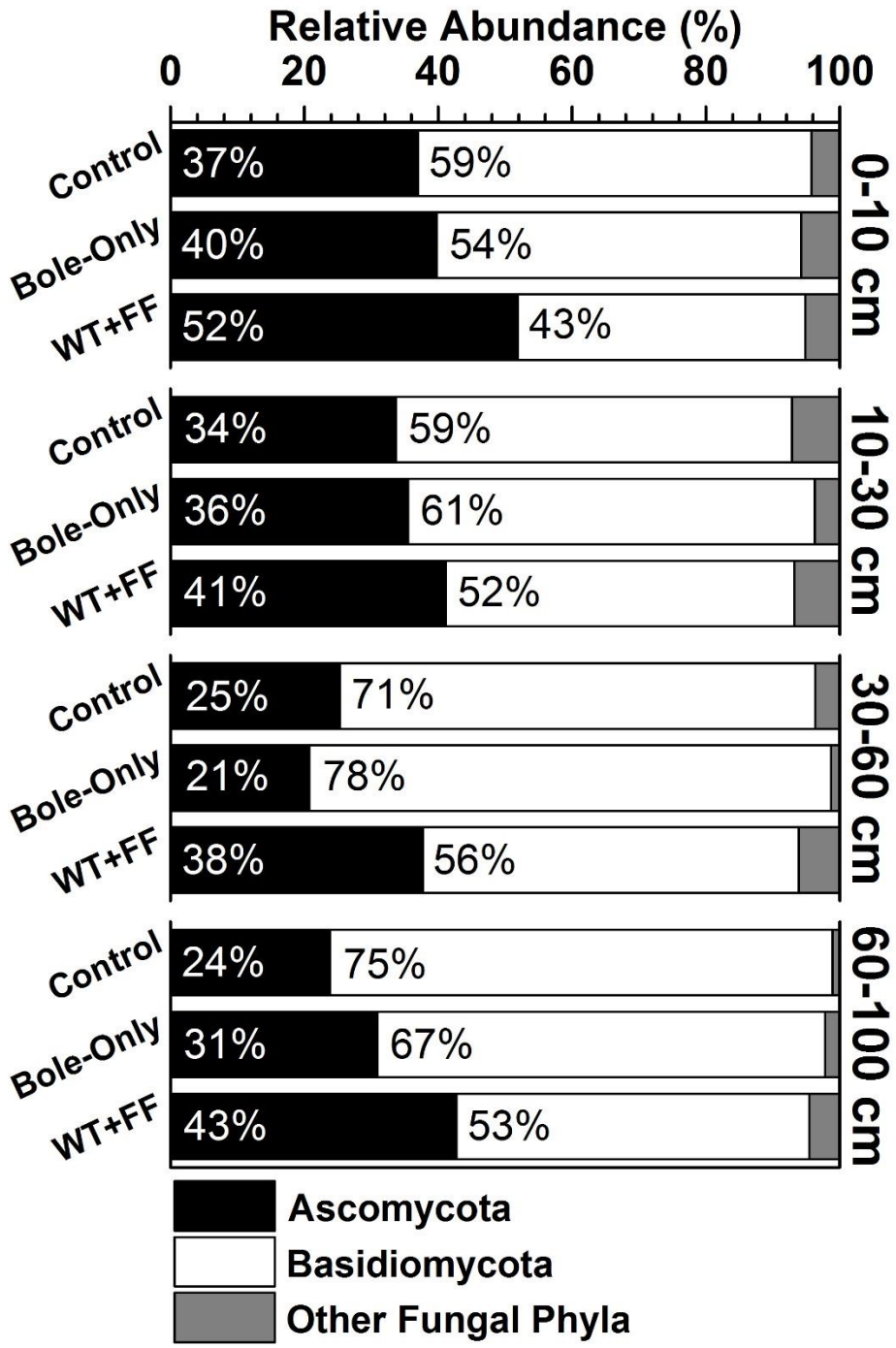

Figure 16: Distribution and relative abundance of major fungal phyla for each treatment (Control=Unharvested Controls; BO=Bole-Only Harvest; WT+FF=Whole-tree Harvest + Forest Floor Removal) nested within soil depth increment.

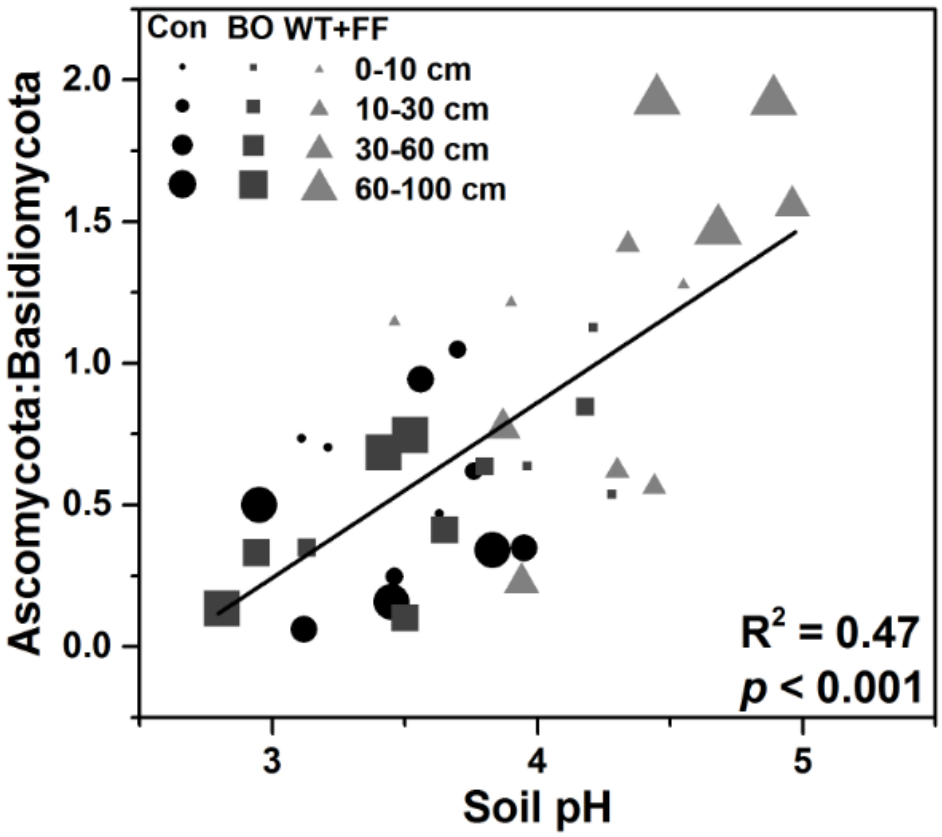

Figure 17: The relationship between the ratio of Ascomycota:Basidiomycota and soil pH. Sample points are separated by harvest treatment and soil depth (size dependent).

The three most abundant genera, *Russula*, *Inocybe*, and *Rhizopogon* comprised over 43% of all OTUs (Table 13) and belong to Basidiomycota. The fourth and fifth most abundant genera, *Cladophialophora* and *Penicillium*, were associated with Ascomycota and account for roughly 8% of all annotated OTUs. *Russula* (>23% of all OTUs) was significantly reduced with increasing OMR intensity, across all sample depths ($p < 0.01$). Of the 20 most abundant genera, none besides *Russula* were statistically influenced by OMR treatment. Of the less abundant genera, the relative abundance of 4 were influenced by OMR (i.e., *Chaetosphaeria, Craterellus, Meliniomyces, and Pycnidiophora*). Numerous genera showed significant variation with respect

to soil depth. Of note, the abundance of saprobic genera such as *Penicillium* ($p < 0.05$) and *Aspergillus* ($p < 0.01$) significantly decreased with depth. Based on relative abundance data, 7 of the 10 most abundant Basidiomycete genera were found to be correlated to either variations in soil pH or SOC; however, none were correlated to TN (Table 13). Congruently, Ascomycete taxa were significantly correlated to soil pH, SOC, and TN; notably, 7 of the 9 most abundant Ascomycetes were positively correlated to SOC (Table 13).

Distribution and Diversity of Functional Fungal Groups

We were able to putatively classify 89 (14%) fungal OTUs annotated to the genus level (95-97% sequence identity) as being similar to known mycorrhizal fungi. This includes 72 ectomycorrhizal (ECM) genera, 16 arbuscular mycorrhizal (AM) genera, and 1 ericoid mycorrhizal (ErM) fungal genus based on literature (Table 14). Basidiomycota (67%) dominated the ECM communities representing 28 families and 48 genera, Ascomycota (32%) accounted for 13 families and 23 genera, and Zygomycota (1.4%) consisted of 1 genus. Of the 549 non-mycorrhizal genera, 60 were classified as saprophytic.

Table 14: The impact of organic matter removal treatment and soil depth on functional fungal groups. Spearman's rank order correlation analysis was used to analyze relationship between fungal groups and certain edaphic properties. Asterisks indicate a significant relationship with: *p<0.05, **p<0.01, ***p<0.001.

	No. Genera	Treatment	Soil Depth	SOC	TN	Soil pH
Fungal Group		*F-Ratio*		*Spearman's Rho*		
True Saprotrophs	60	0.65	**6.41**Φ**	**0.54*** **	**0.29***	0.30
Ectomycorrhizal	72	0.40	0.50	0.04	-0.03	**-0.43****
Arbuscular Mycorrhizal	16	1.91	0.19	0.21	0.13	**0.33***
Ericoid Mycorrhizal	1	1.07	**3.40***‡	**0.36***	0.04	0.24

‡ Indicates that relative abundance significantly increased with increasing harvest intensity or soil depth
Φ Indicates that relative abundance significantly decreased with increasing harvest intensity or soil depth

As a group, ECM fungi were unaffected by increasing harvest intensity; however individual species with mycorrhizal potential such as *Inocybe muricellata* ($p < 0.05$), *Elaphomyces granulatus* ($p < 0.05$), *Russula cyanoxantha* ($p < 0.05$), *Astraeus hygrometricus* ($p < 0.01$), and *Tylopilus felleus* ($p < 0.05$) were all significantly reduced with increasing OMR intensity. No ECM OTUs were statistically affected by differing soil depth increments. AM fungi were not statistically impacted by either OMR or soil depth; however, they showed highest abundance in the control treatment. The ericoid mycorrhizal fungi, represented singularly by *Rhizoscyphus ericae*, showed significant increases with respect to increasing harvest intensity. In regards to depth, *R. ericae* was concentrated at the 10-30 cm depth increment compared to all other soil depths ($p < 0.05$). Saprotrophic OTU abundance decreased significantly with depth and was highest in the WT+FF treatment, although not significant.

Analysis of ECM, AM, and saprophytic community structure using PERMANOVA revealed significant variations amongst harvest treatments across all depths (ECM: $p < 0.05$; AM: $p < 0.05$; saprophytic: $p < 0.01$); furthermore, saprophytic community structure changed significantly with soil depths ($p < 0.01$). Correlation analysis of mycorrhizal and saprophytic communities with soil physicochemical properties using Spearman's ranked correlation analysis show that there are certain edaphic factors that may potentially influence the distribution of fungal communities (Table 14). Notably, saprotrophs and ErM were positively correlated with SOC while ECM was negatively correlated with soil pH and AM were positively correlated with soil pH.

Discussion

As has been shown in previous studies, intensive OMR methods associated with timber harvest can lead to long-term alterations in above- and belowground properties (Johnson and Curtis, 2001; Li et al., 2003; Nave et al., 2010; Jones et al., 2011; Huang et al., 2013; Achat et

al., 2015a, 2015b; Foote et al., 2015); however, there is still a pressing need to analyze the combined effects of these management practices on soil physical, chemical, and biological properties in order to better understand the sustainability of intensive OMR techniques, especially at depth. Previous studies on the effect of OMR have led to the conclusion that soil microbial communities possess a high resilience following intensive OMR (Li et al., 2004; Busse et al., 2006; Hannam et al., 2006; Mariani et al., 2006); however, others have challenged this assumption of resilience and shown that intensive OMR can strongly affect microbial communities for decades following harvest (Hartmann et al., 2012). Our results agree with the later, and we have shown that OMR can result in long-term reductions in SOC and TN as well as increased soil pH and Mehlich-III extractable P. Furthermore, this disturbance can significantly alter soil fungal community structure as well as the relative abundance of predominant taxa and ecologically-relevant functional groups.

Fungal Community Structure: A Long-Term Response to Forest Harvest

In this study we found that timber harvest has the potential to impart long-term alterations in overall fungal community structure and modify the relative abundance of specific fungal taxa. Measures of fungal diversity and richness (0-10 cm) were similar to other southeastern coniferous forests in Georgia (USA) (Brown et al., 2013) and slightly higher than that reported for eastern coniferous forests in North Carolina (USA) (O'Brien et al., 2005) and boreal forests in Alaska (Allison et al., 2007). In addition, observed richness and OTU abundance were greater than grassland soils in Alaska (Penton et al., 2013), New Mexico (Porras-Alfaro et al., 2011), Oklahoma (Penton et al., 2013), Texas (Hollister et al., 2010), and arctic tundra soils (Wallenstein et al., 2007).

The highest mean fungal diversity and richness values (0-100cm) were observed in control stands; however, richness was found to be statistically unaffected by increasing harvest

intensity. The majority of treatment-induced biodiversity differentiation was observed within the 0-10 cm depth increment. This may be related to the removal of potential substrate during tree harvesting at the beginning of this study. Furthermore, we noted that in the WT+FF stands, some of the sampling locations had not developed a true litter layer which may also contribute to the reduced diversity in surface mineral soils. Although fungal richness and diversity in deeper portions of the profile did not differ between treatments, it is difficult to infer whether these fungal communities are resistant to tree harvest, or if they are resilient. That is, were the deep soil fungal communities initially perturbed by tree harvest and are now fully recovered (resilient), or were they simply not affected by tree harvest (resistant)?

At other LTSP sites, increased harvest intensity reduced fungal biomass (Busse et al., 2006) and altered fungal community structure (Hartmann et al., 2012). Furthermore, fungal populations respond strongly to changes in aboveground vegetation composition (Wu et al., 2011) and concurrent variability in soil physicochemical properties. At the Groveton-LTSP, the differences observed in SOC and TN with respect to differing intensities of timber harvest correlate strongly with variable levels of fungal richness, diversity, and differences in the proportion of specific fungal groups. In contrast to our results, Goldmann et al. (2015) observed higher diversity and richness in young managed beech forests subjected to recurrent thinning as opposed to older unmanaged beech forests. They argue that this observation is a function of the reduction in dominant plant-associated fungi, such as ECM, which would lead to the colonization of a diverse assemblage of opportunistic fungal species (Lazaruk et al., 2005; Gömöryová et al., 2013). We would suspect that a similar result would be observed at the Groveton-LTSP site if soils had been sampled within a year of harvest; however, after 2 decades of reduced SOC and TN, the fungal community has been altered to the point where biodiversity metrics exhibit significant reductions throughout the upper 1 m of the soil profile.

In addition to differences in common community metrics, our results indicate that fungal community structure is significantly different among the three OMR treatments of interest. Within the top 60 cm, NMDS ordination plots exhibited tight clustering of samples in accordance with specific harvest treatments. PERMANOVA results corroborate NMDS finding with significant differences in OMR treatment at every soil depth except 60-100 cm. This is consistent with our hypothesis that deeper soil fungal communities may be more resistant and/or resilient to alterations in aboveground biomass structure. This resistance and/or resiliency may be a function of the large variability in community structure observed in deeper soil. Increased fungal community variability with depth has been previously reported by Jumpponen et al. (2010) in prairie soils. It is conceivable that observed differences between the control stands and the two treatment stands may be due to other variables such as tree age and complexity of root networks; however, when communities from the two treatment stands were compared independently of the control stands, we still observed community difference in the 0-60 cm portion of the profile. These results are consistent with studies of Chinese pine plantations (Nie et al., 2012), aspen-dominated forests of Minnesota (Lewandowski et al., 2016), and spruce-dominated forests of Finland (Toivanen et al., 2012).

Influence of Organic Matter Removal on Specific Fungal Taxa and Functional Groups

Much like other temperate forests, Groveton-LTSP soils were dominated by Basidiomycota and Ascomycota, with many of the 20 most common fungal genera having been reported extensively in soils from other forest ecosystems (O'Brien et al., 2005; Allison et al., 2007; Buée et al., 2009 Wubet et al., 2012). The ratio of Ascomycota to Basidiomycota was unaffected by soil depth; furthermore, when samples from all depths are pooled by treatment, the abundance of Ascomycota, relative to Basidiomycota, increased with respect to increasing soil pH. As noted, soils in the WT+FF treatment tend to have a higher soil pH. Soil pH may directly

affect fungal community structure by imposing physiological constraints on fungal survival and growth where some taxa may be unable to cope with soil pH outside of a certain range (Warcup, 1951; Fries, 1956; El-Abyad and Webster, 1968; Yamanaka et al., 2003; Zhang et al., 2016). Although no relationship was found between the ratio of Ascomycota to Basidiomycota and SOC, we did observe that the relative abundance of Ascomycota was significantly positively correlated to SOC. This is consistent with cellulolytic ability being widespread among Ascomycota (Weber et al., 2011).

In regards to the putatively characterized saprotrophic taxa, we found no significant difference with respect to harvest treatment; however, the highest abundance values were observed in the WT+FF stands, and trends did emerge with specific saprobic groups. Results indicate a significant increase in the abundance of *Aspergillus* and *Penicillium*, which are widely considered to be ruderals and germinate rapidly in response to readily available substrate. Because of the reduced organic layer in WT+FF stands, pulses of available substrate may occur unexpectedly which would be much more suitable for opportunistic fungi than other functional taxa. Increasing soil depth significantly reduced the abundance of saprotrophs, which is to be expected considering their lifestyle is predicated on the abundance of substrate, which is further validated by pairwise correlations showing that saprobic taxa are positively correlated with SOC and TN. ECM fungi were unaffected by treatment and soil depth; however, as was with the saprotrophic fungi, trends emerged for specific taxa. All things considered, caution must be used when elucidating significance on ecologically-significant fungal taxa solely on the basis of published information.

With respect to increasing organic matter removal intensity, we observed significant reductions in the relative abundance of *Russula* across all depths. This is consistent with Dickie et al. (2009), who showed that timber harvest can lead to decreased abundance of *Russula* in

Nothofagus rainforests of New Zealand. The genus *Russula* is considered one of the most abundant, diverse, and widely distributed fungal genera (Miller and Buyck, 2002; Buyck et al., 2008), accounting for roughly 750 known species worldwide (Kirk et al, 2008). In this system, *Russula* was the dominant genus accounting for greater than 23% of all OTUs and can be considered the dominant driver of observed fungal differentiation is this system. It has been hypothesized that SOM drives the majority of change in fungal communities (Wubet et al., 2012); however, *Russula* abundance was not correlated to SOC or TN. Potentially, the major edaphic factor responsible for the distribution of *Russula* is soil pH. As soil pH increases we observe a significant decrease in the abundance of *Russula*. It has been theorized that within acidic soil, the availability of phosphorus (P) becomes more limited due to mobilized Al^{3+} binding to inorganic P and rendering it less available to plants. Forest trees can compensate for reduced mineral P availability by mycorrhizal colonization. ECM have the ability to produce extracellular enzymes that hydrolyze organic P, making it available for plant uptake. Previous studies have shown that increasing timber harvest can lead to increased inorganic soil P pool size (Dickie et al., 2009). Whether this increase is related to the change in soil pH is unknown; however, the increase in inorganic soil P may lead to the reduced abundance of *Russula* due to the removal of P limitation on the associated plant species. It has been shown that total P is significantly reduced with respect to increasing OMR intensity at the Groveton LTSP (Foote, 2014); however, we have shown that Mehlich-III P, which is generally considered *plant-available P*, increases significantly with increasing OMR, which coincides with our hypothesis regarding the decreased relative abundance of *Russula*.

Ericoid mycorrhizae, illustrated singularly by fungi similar to *Rhizoscyphus ericae* in our system, showed significant increase in relative abundance with respect to increasing OMR intensity and reduced with increasing soil depth. Vegetation surveys of the experimental plots

and surrounding natural forest have not lead to the discovery of any plant species in the Ericaceae family; however, all species in this plant family are well suited for acidic soils and have been reported throughout the southeastern US. It is likely that *R. ericae* is forming associations with the loblolly pine (Villarreal-Ruiz et al., 2004). Heinonsalo et al. (2007) showed that following clear-cut, Scots pine (*Pinus sylvestris*) seedlings had a higher percentage of root-associated *R. ericae* than the control forest. It is possible that this species colonized loblolly pine seedlings at the onset of these treatment plots, and may be well adapted to persisting in environments of low SOC and TN as evidenced by its high relative abundance in the WT+FF treatment.

CHAPTER V

SOIL BACTERIAL COMMUNITY COMPOSITION IS ALTERED BY TIMBER HARVEST INTENSITY

Forest ecosystems in the southern United States are substantially altered by events such as hurricane, fire, timber harvest, and land conversion, with effects being observed in plant community composition as well as soil nutrient cycling and storage. Furthermore, the desire to develop renewable energy sources in the form of biomass extraction from logging residues may result in alterations in soil community structure and function. While the impact of forest management on the biogeochemistry of the region has been studied, its long-term effect on soil bacterial community composition is unknown. This study investigates how different, forestry-related, above-ground organic matter removal intensities associated with timber harvest influence decadal-scale alterations in bacterial community structure in the upper 1-m of the soil profile, 18 years post-harvest in a *Pinus taeda* L. forest in the Gulf coastal plain region of the southeastern USA. Amplicon sequencing of the 16S rRNA gene was used in conjunction with soil chemical analyses to evaluate differences in community composition as well as potential environmental drivers of associated change. Results indicate that increasing organic matter removal intensity leads to altered community composition. Although no differences were observed for relative abundance of bacterial phyla, the relative abundance of *Burkholderia* increased with increasing while that of *Aciditerrimonas* decreased with increasing organic matter removal. Correlation analyses revealed that treatment-based differences for these two genera could be driven by concurrent alterations in soil pH and phosphorus availability. Furthermore, the relative abundance of ammonia-oxidizing bacteria decreased with increasing harvest intensity while the relative abundance of denitrifying bacteria increased. These changes were

correlated to reductions in NH_4^+, and $NO_2^- + NO_3^-$ as well as altered microclimatic conditions following harvest. Our results imply that intensive organic matter removal may create long-term alterations in bacterial community structure which are potentially driven by concurrent changes soil physicochemical properties.

Introduction

Soil bacterial communities are vital ecosystem components as they help regulate nutrient availability, influence the formation of soil structure, and facilitate energy flow to higher trophic levels (de Boear et al., 2005; Schneider et al., 2012); however, there is a great deal that we do not understand regarding the structure and functioning of the bacterial microbiome (Little et al., 2008) especially in regards to aboveground disturbance. It has been shown that multiple factors including vegetation composition (Hart et al., 2005; Cong et al., 2015) and soil physicochemical properties (Kaiser et al., 2016) can directly influence bacterial community composition and functionality. Furthermore, natural and anthropogenic disturbances have been shown to alter soil properties (Kurth et al., 2014; Foote et al., 2015; Oliver et al., 2015; James and Harrison, 2016) potentially affecting soil bacterial community structure and function. Forest management activities such as timber harvest can result in decade-scale reductions in forest floor and soil carbon (C) and nutrient pools. At some sites, these reductions have been shown to last for decades to centuries (Chen et al., 2013; Kellman et al., 2014; Prest et al., 2014; Dean et al., 2016; Menegale et al., 2016). Because of the potential for timber harvest to impart long-term impacts on soil physicochemical properties and the connectedness of these soil properties to the soil bacterial community, it is important to investigate how different intensities of organic matter removal (OMR) associated with timber harvest influence bacterial community structure in surface and subsurface soils, especially in a long-term context. Previous studies have shown that intensive OMR can lead to long-term effects on the composition of soil microbial communities

in boreal forests of western Canada (Hartman et al., 2012) as well as reductions in organic matter decay rates (Cardenas et al., 2015; Leung et al., 2016); however, these studies were confined to the top 30 cm of the soil profile.

Southern coniferous forests in the USA comprise over 275,000 km^2 and represent approximately 9% of the total US forest area (Oswalt et al., 2014). A majority of these southern forests have been converted to commercial plantations and are intensively managed for timber-related products, such as merchantable and industrial timber as well as pulpwood (Fox et al., 2007). These forests account for greater than 40% of annual US softwood and consistently produce 19% and 12% of the worldwide pulpwood and industrial timber, respectively (McNulty et al., 1996; Hodges et al., 2011; Brandeis et al., 2012). There has recently been growing interest in the removal of non-merchantable woody debris, including slash, sawdust, and forest litter, to be used as substitute feedstocks in industrial processes and for bio-energy production (Janowiak and Webster, 2010). Furthermore, commercial timber corporations and individual land owners are beginning to utilize forest litter for the production of merchantable mulch (Dickens et al., 2012). Forest management practices that lead to increased biomass removal during harvest may not only affect bacterial community structure but also lead to long-term reductions in forest productivity (Wei et al., 2000; Walmsley et al., 2009; Achat et al., 2015a, 2015b).

In this study, we explored the decade-scale response of the soil bacterial community to intensive OMR in surface and subsurface mineral soils of the southeastern US. We used high-throughput bacterial 16S sequencing to document changes in bacterial community structure and relative abundance of specific taxa resulting from soil disturbance during timber harvest that occurred 18 years ago. The objectives of this study was to evaluate the response of bacterial community structure and function to timber harvest, and identify environmental drivers that might be driving these responses. We hypothesized that (a) increasing forest OMR will result in

reduced substrate and nutrient pools which would lead to reduced bacterial diversity and altered community structure, (b) these changes in bacterial community structure would be more evident in surface rather than subsurface portions of the soil profile due to the direct influence of aboveground biomass on this portion of the soil profile, and (c) specific bacterial groups that gain energy from nitrogen cycling processes would be altered in plots that were subjected to the most severe harvest treatment due to the reduction in energy-gaining resources.

Materials and Methods

Study Site and Sampling Design

Research was conducted in Davy Crockett National Forest near the town of Groveton, TX, USA (31° 06' 32.48'' N, 95° 09' 59.15'' W). The study site is part of the Long-Term Soil Productivity (LTSP) network aimed at understanding the effects of common forest management practices on soil properties and processes (Powers et al., 2005; Powers, 2006; Ponder et al., 2012). The Groveton LTSP site was established in 1997 and includes differing intensities of organic matter removal associated with timber harvest. These treatments include a low intensity harvest treatment (merchantable bole/stem only; BO) where only the bole of the tree was removed and a high intensity harvest treatment (whole tree harvest + forest floor removal; WT+FF) where the entire tree (bole, branches, leaves) was removed along with the forest floor debris being removed by hand-raking. In addition, the experimental design includes unharvested control stands (tree age = 60-80 yrs.). During harvest, trees were hand-felled and lifted off of the plots with a loader to reduce soil compaction. Controls and both harvest treatments were replicated 3X and each replicate was 0.2 ha. All plots are located within a 1.5 km radius. Plots were harvested in 1996 and then replanted in 1997 with containerized *P. taeda* L. (loblolly pine) seedlings of 10-half sib families from the US Forest Service seed orchards using 2.5-m x 2.5-m

spacing. Soils across the study area are uniform fine-loamy siliceous, thermic Oxyaquic Glossudalf in the Kurth series.

Soil samples were collected in April 2015 using a JMC Environmentalist Sub-Soil Probe PLUS (2.8 cm diameter x 120 cm length) (Clements Associates Inc., Newton, IA, USA). Soil cores were taken 1.8 m from the base of a randomly selected *P. taeda* L. individual with a diameter at breast height (DBH) between 18 and 24 cm. A three-tree buffer from the outside of the plots was not sampled to avoid edge effects. In some of the WT+FF stands, the forest floor had not yet redeveloped; because of this, the organic soil horizon in all other plots (approximate thickness: < 3 cm) was removed prior to coring in order to investigate mineral soil horizons exclusively. Soil sampling followed a stratified random sampling design in which four cores were taken from each plot and pooled by depth increment to increase sample mass and reduce error introduced by environmental heterogeneity. Each soil core was partitioned into four depth increments in the field (0-10, 10-30, 30-60, and 60-100 cm), pooled together with the other replicated cores, and individual depths were analyzed separately. On the day in which soil cores were taken from the ground, samples were transported at 4°C from the field to the lab, aseptically homogenized, and 5-g subsamples (3 sample^{-1}) were immediately stored at -80°C for future DNA extraction and subsequent analysis. The remaining soil was stored at 4°C for soil chemical analysis.

Soil Physical and Chemical Parameters

Soil was passed through a 2-mm sieve to remove large organic material and roots. A 25-g aliquot of sieved soil was then dried at 60°C for 48 hours and finely ground using a TE250 ring pulverizer (Angstrom, Inc., Belleville, MI, USA). Using pulverized soil, organic carbon (SOC) and total nitrogen (TN) were analyzed conducted on a Carlo Erba EA-1108 elemental analyzer (CE Elantech, Lackwood, NJ, USA). Soil total P concentrations were determined using the

lithium fusion method (Lajtha et al., 1999). Soil pH was determined on field-fresh soil using an Accumet Basic pH meter (Denver Instrument, Arvada, CO, USA) on a 1:2 solution of soil in a 0.01M $CaCl_2$ solution (Minasny et al., 2011). Soil inorganic-N was extracted from 15 g of sieved, field moist, soil with 50 ml of 2M KCl within 36-hrs of soil being taken from the ground. The soil + KCl solution was shaken for 1 hour and then filtered over pre-leached (2M KCl) #40 Whatman filter paper and analyzed immediately for concentrations of NH_4^+ and $NO_2^- + NO_3^-$ on a Seal Analytical AQ2+ Discrete Chemistry Analyzer (SEAL Analytical, Ltd., Southhampton, UK). Colorimetric-based chemistry for the determination of NH_4^+ was based on indophenol-blue chemistry, and determination of $NO_2^- + NO_3^-$ was based on cadmium-reduction and subsequent diazotization. Mehlich III extractable phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), and sulfur (S) were analyzed from pulverized soil with inductively coupled plasma mass spectrometry at the Texas A&M Soil, Water, and Forage Testing Laboratory (Mehlich, 1978).

DNA Extraction

DNA extraction followed the modified version of the International Standard for the extraction of DNA from soil as described by Terrat et al. (2014). Further modification was made to extract DNA from 3 g of soil (dry weight equivalent) rather than the prescribed 1 g. DNA was extracted from 3 analytical replicates per sample and then pooled to increase mass and reduce environmental heterogeneity. DNA library preparation and sequencing of bacterial communities was done by Molecular Research DNA Laboratory (www.mrdna.com, Shallowater, TX, USA). Bacterial sequencing targeted the 16S rRNA coding region using primers 1100F (5'-GGCAACGAGCGMGACCC-3'; Lane et al., 1985; Dorsch and Stackerbrandt, 1992) and 1492R (5'-GGTTACCTTGTTACGACTT-3'; Turner et al., 1999). PCR amplification was accomplished by utilizing the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, USA)

under the following conditions: an initial denaturation step at 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. PCR products were verified via gel electrophoresis (2% agarose gel). Samples were barcoded and subsequently pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified with calibrated AMPure XP beads (Agencourt Biosciences Co., Brea, CA, USA). The pooled and purified PCR products were then used to prepare an Illumina DNA library for each sample. Synthesis-based sequencing on an Illumina MiSeq followed the manufacturer's guidelines and resulted in single-end reads of 416 ± 58 bp.

Sequencing and Bioinformatic Analysis

Resulting .fasta and .qual files were demultiplexed, quality filtered, and analyzed using the QIIME 1.9.1 pipeline (Caporaso et al., 2010). Illumina sequences with <200 and >1000 bp, barcode or primer sequence errors, and those with homopolymers or ambiguous base calls that exceed six nucleotides were discarded. Raw sequences were deposited in NCBI's sequence read achieves under the accession number SRR5218289. Operational taxonomic units (OTUs) were defined by clustering at 97% sequence identity using the QIIME implementation of UCLUST (Edgar, 2010). Subsequent taxonomic annotations refer to assignments based on >97% sequence identity for species, 95-97% sequence identity for genera, 90-95% for families, and 77-80% for phyla using the Greengenes 16S database (McDonald et al., 2012). Bacterial OTUs annotated to the genus-level were putatively categorized into either nitrogen-cycling processes (nitrogen-fixation, ammonia-oxidation, nitrite-oxidation and denitrification), sulfur-cycling processes (sulfate-reduction and sulfur-oxidation), or iron-cycling processes (iron reduction and iron oxidation) from a variety of published sources that comment on the ecological status of specific bacterial genera (Belsar, 1979; Cabello et al., 2009; Lens, 2009; Sánchez et al., 2011; Strous,

2011; van Lis et al., 2011; Andrews et al., 2013). The ecological role of all remaining bacterial genera were undefined.

Statistical Analysis

All statistical analyses on bacterial communities were carried out using the sequence count within each OTU as a relative abundance value (Danzeisen et al., 2011). All datasets were tested for normality using Shapiro-Wilk's test. When OTU data were not normally distributed, non-parametric statistical tests or log transformations were applied. OTU data generated in QIIME were used to quantify the number of unique OTUs, richness (Menhinick), and diversity (Shannon's Diversity Index). Community metric calculations and rarefaction curves were analyzed using normalized OTU data set to 40,814 reads sample^{-1}. Physicochemical properties, bacterial community metric estimates, and OTU relative abundance values were statistically analyzed using a linear mixed model ANOVA. Because of the inherent autocorrelation between differing soil depths, a repeated measures experimental design was employed with OMR as the fixed main plot and soil depth as the repeated measure (Derner et al., 2006). Replicated plots were nested within harvest treatment and considered a random effect (Dai et al., 2006). When differences were significant, Tukey's honest significant difference (HSD) test was performed to assess post hoc contrasts with significance inferred at $p \leq 0.05$. Non-metric multidimensional scaling (NMDS) based on a Bray-Curtis dissimilarity matrix (Bray and Curtis, 1957) was performed on normalized bacterial 16S data. A permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) using the Bray-Curtis matrix listed above was employed to characterize differences in soil bacterial community composition based on OMR treatment and soil depth. PERMANOVAs were run using 999 permutations. Spearman's correlations were calculated using JMP and aggregated across all sample points (SAS Institute, Inc., Cary, NC, USA).

Results

Soil Geochemical Parameters

Mean Soil pH (0-100 cm) was significantly higher in the WT+FF treatment (4.32 ± 0.10) (mean ± standard error) than the control (3.48 ± 0.11) or the BO treatment (3.62 ± 0.12) (Tables 15 and 16). SOC and TN were significantly reduced by increasing intensity of OMR; however, TP was not (Table 15 and 16). Increasing soil depth also led to significant reductions in SOC (0-10 cm: 18.9 ± 2.8 g C kg^{-1}; 10-30 cm: 5.1 ± 0.7 g C kg^{-1}; 30-60 cm: 2.3 ± 0.4 g C kg^{-1}; 60-100 cm 2.0 ± 0.3 g C kg^{-1}). Total N and P in the 0-10 cm increment (827.8 ± 107.1 mg N kg^{-1}; 94.5 ± 10.1 mg P kg^{-1}) were significantly higher than the 10-30 cm (333.3 ± 62.4 mg N kg^{-1}; 65.2 ± 6.9 mg P kg^{-1}), 30-60 cm (255.6 ± 50.3 mg N kg^{-1}; 63.6 ± 8 mg P kg^{-1}), and 60-100 cm (277.8 ± 27.8 mg N kg^{-1}; 74.5 ± 11.2 mg P kg^{-1}) depth increments (Table 15). Concentrations of soil NH_4^+ and $NO_2^- + NO_3^-$ were statistically equivalent in the control (NH_4^+: 2.4 ± 0.2 g N kg^{-1}; $NO_2^- + NO_3^-$: 0.5 ± 0.1 g N kg^{-1}) and BO treatment (NH_4^+: 2.4 ± 0.3 mg N kg^{-1}; $NO_2^- + NO_3^-$: 0.5 ± 0.1 mg N kg^{-1}), but decreased significantly in the WT+FF treatment (NH_4^+: 1.0 ± 0.2 mg N kg^{-1}; $NO_2^- + NO_3^-$: 0.3 ± < 0.1 mg N kg^{-1}) (Table 15). Both NH_4^+ and $NO_2^- + NO_3^-$ decreased significantly with depth. Of the Mehlich-III extractable nutrients, only P was significantly affected by timber harvest (Table 16). Specifically, Mehlich-III P concentrations were significantly higher in the WT+FF treatment (5.7 ± 1.1 mg P kg^{-1}) than the control (3.9 ± 1.0 mg P kg^{-1}) or BO treatment (4.6 ± 0.6 mg P kg^{-1}) (Table 15). Increasing soil depth lead to significant reductions Mehlich-III P and Ca as well as significant increases in K and S (Table 16).

Table 15: Soil chemical properties for each depth increment within each harvest treatment

Soil Depth (cm)	Soil pH	SOC	TN	TP	NH_4^+	NO_3^-	P	K	Ca	Mg	S	Na
Unharvested Control	(0.01 CaCl)	(g kg^{-1} soil)	(mg kg^{-1} soil)		(mg kg^{-1} soil)		Mehlich III-Extractable (mg kg^{-1} soil)					
0-10	3.32 (0.2)	27.6 (5.1)	1133.3 (218.6)	109 (30.7)	3.4 (0.2)a	0.7 (0.1)a	7.7 (3.3)	230.1 (43.8)	545.2 (268.3)	136.0 (60.1)	20.7 (2.4)	66.9 (17.7)
10-30	3.64 (0.1)	7.2 (1.3)	466.7 (176.4)	76.1 (19.1)	2.7 (0.4)ab	0.5 (0.03)abc	3.8 (0.8)	286.7 (73.7)	334.5 (164.9)	153.2 (104.1)	15.9 (2.0)	56.4 (5.2)
30-60	3.54 (0.2)	3.3 (0.6)	300 (115.5)	71.5 (17.8)	1.8 (0.3)bcd	0.4 (0.04)bcde	2.7 (0.9)	300.4 (88.3)	167.4 (24.1)	176.8 (127.1)	14.8 (2.6)	63.5 (7.5)
60-100	3.41 (0.3)	2.4 (0.1)	266.7 (33.3)	80.9 (29.4)	1.9 (0.3)bcd	0.3 (0.1)cde	1.5 (0.4)	297.6 (86.3)	207.1 (114.4)	190.5 (56.5)	24.1 (2.9)	143.6 (62.2)
Mean	**3.48 (0.1)**	**10.1 (3.3)**	**541.7 (124)**	**84.4 (11.5)**	**2.4 (0.2)**	**0.5 (0.1)**	**3.9 (1.0)**	**278.7 (33.2)**	**313.6 (84.3)**	**164.1 (39.7)**	**18.9 (1.6)**	**82.6 (17.6)**
Bole-only Harvest												
0-10	4.15 (0.1)	16.5 (2.1)	800 (57.7)	94.8 (4.3)	3.1 (0.4)a	0.6 (0.1)ab	8.5 (0.4)	201.1 (23.1)	756.7 (143.2)	130.1 (21.0)	16.6 (2.3)	54.9 (5.3)
10-30	3.70 (0.3)	4.1 (0.3)	300 (0)	69.6 (4.7)	2.8 (0.5)ab	0.5 (0.1)bcd	5.0 (0.2)	195.6 (18.7)	290.1 (28.3)	79.0 (18.9)	11.6 (1.4)	49.1 (5.4)
30-60	3.36 (0.2)	2.6 (0.7)	300 (100)	74.2 (13)	2.4 (0.4)abc	0.4 (0.1)bcd	3.4 (0.4)	326.7 (104.1)	240.8 (51.5)	235.5 (124.9)	15.7 (3.7)	52.6 (4.2)
60-100	3.25 (0.2)	2.4 (0.3)	333.3 (33.3)	87.5 (16.8)	1.4 (0.4)cde	0.5 (0.1)bcd	1.5 (0.4)	361.5 (76.5)	185.6 (59.8)	273.4 (60.1)	23.9 (2.9)	91.1 (6.0)
Mean	**3.62 (0.1)**	**6.4 (1.8)**	**433.3 (68.9)**	**81.5 (5.6)**	**2.4 (0.3)**	**0.5 (0.1)**	**4.6 (0.8)**	**271.2 (36.0)**	**368.3 (77.1)**	**179.5 (38.3)**	**17.0 (1.8)**	**61.9 (5.6)**
WT Harvest + FF Removal												
0-10	3.97 (0.3)	12.9 (1.3)	550 (28.9)	79.6 (6.9)	1.5 (0.3)cd	0.4 (0.1)bcde	11.1 (1.0)	179.4 (14.8)	350.7 (24.5)	54.3 (0.9)	14.9 (1.4)	48.5 (2.2)
10-30	4.36 (0.1)	4.0 (0.8)	233.3 (33.3)	50 (2.7)	1.1 (0.4)de	0.3 (0.1)cde	6.5 (0.2)	203.2 (13.5)	170.1 (23.9)	36.2 (6.6)	10.1 (1.1)	53.9 (7.0)
30-60	4.26 (0.4)	1.2 (0.4)	166.7 (33.3)	45.2 (4.5)	0.8 (0.2)de	0.2 (0.1)de	3.4 (1.3)	213.3 (5.9)	155.2 (55.8)	53.1 (16.1)	11.9 (3.2)	56.1 (15.3)
60-100	4.67 (0.1)	1.3 (0.7)	233.3 (66.7)	55.2 (8.1)	0.4 (0.1)e	0.2 (0.1)e	1.8 (0.5)	276.5 (27.7)	253.8 (156.3)	188.6 (63.6)	30.2 (8.9)	109.1 (50.0)
Mean	**4.32 (0.1)**	**4.8 (1.5)**	**295.8 (48.6)**	**57.5 (4.7)**	**1.0 (0.2)**	**0.3 (0.04)**	**5.7 (1.1)**	**218.1 (13.1)**	**232.4 (43.0)**	**83.0 (23.2)**	**16.8 (3.1)**	**66.9 (13.5)**

Table 16: ANOVA table for soil chemical properties. M-III = Mehlich-III extraction.

	Harvest Treatment	Soil Depth	HT x SD
		F-Ratio	
Soil pH	**16.17*** **	0.34	**2.52***
SOC	**9.86*** **	**64.58*** **	**3.79****
TN	**6.32****	**23.01*** **	1.67
TP	1.19	**17.59*** **	0.98
NH_4^+	**23.5*** **	**9.5*** **	0.62
$NO_2^- + NO_3^-$	**9.48*** **	**5.24****	0.68
P (M-III)	**6.51***	**19.76*** **	0.43
K (M-III)	0.53	**3.92***	0.78
Ca (M-III)	1.33	**5.95****	0.59
Mg (M-III)	2.22	2.04	0.41
S (M-III)	0.44	**9.04*** **	0.81
Na (M-III)	0.78	**4.37***	0.21

*$p < 0.05$

**$p < 0.01$

***$p < 0.001$

Bacterial Community Composition

A total of 2,642,414 high-quality 16S sequences were retained following filtering. Prior to normalization, samples consisted of 73,400 ± 3,849 sequences, which did not statistically vary between OMR treatments but did increase with increasing soil depths (0-10 cm: 58,097 ± 4,574; 10-30 cm: 64,617 ± 3,323; 30-60 cm: 77,467 ± 7,014; 60-100 cm: 93,421 ± 9,329). Following normalization to 40,814 sequences per sample, we observed a diverse bacterial community of 8,727 ± 221 unique OTUs per sample. Counts of OTUs in individual ITS libraries did not vary by OMR treatment or soil depth. Coverage estimates were very high (0.95 ± 0.01), indicating an overall excellent OTU coverage afforded by the level of sequencing employed in this study, which did not statistically differ amongst OMR or soil depths. OTU Richness (Menhinick) was not influenced by OMR treatments; however, statistical differences were between depth

increments. Specifically the 0-10 cm (35.6 ± 0.3) and 10-30 cm (37.6 ± 0.9) increments were significantly higher than 60-100 cm (26.7 ± 2.2) increment, but did not differ from each other (Table 17). Richness in the 30-60 cm (32.5 ± 1.3) increment was not statistically different than any other depth (Table 17). Similarly, OTU diversity (Simpson's) was statistically unaffected by timber harvest intensity, but did vary with soil depth. As was observed for richness, diversity in the 0-10 cm (0.96 ± < 0.01) and 10-30 cm (0.96 ± 0.01) increments were significantly higher than 60-100 cm (0.88 ± 0.02) increment, but did not differ from each other. Diversity in the 30-60 cm (0.91 ± 0.01) increment was not statistically different than any other depth (Table 17). Non-metric multidimensional scaling (NMDS) plots of OTUs, based on Bray-Curtis distance matrices, resulted in statistical separation of bacterial communities based on harvest treatments (Figure 18). Post hoc analyses revealed that statistically significant treatment differences were only observed at 0-10 cm. Furthermore, bacterial community composition was statistically altered by soil depth with unique clustering when soil depth was analyzed independent of OMR treatments; specifically, the 0-10 and 10-30 cm increments were significantly different than the 30-60 and 60-100 cm increments.

Table 17: Summary of 16S operational taxonomic units (OTUs), and their diversity and richness estimates. Datasets were normalized by setting each sample to 40,814 sequences per sample. OTUs were defined as sequences sharing ≥97% similarity and served as the basis for Menhinick's richness estimate, and Simpson's Diversity Index. *Post hoc* contrasts (Tukey-Kramer) were computed on values for each depth nested within each treatment and indicated by differing letters within each column. *Post hoc* contrasts were also calculated for mean treatment values and differences are indicated by differing Greek letters in each column. For each soil depth in each treatment, N=3; for mean values per treatment, N=12. Statistical differences were inferred at $p<0.05$.

Soil Depth (cm)	No. Unique OTUs	Menhinicks Index (Richness)	Simpson's Index (Diversity)
Unharvested Old-Growth	*Mean (Standard Error)*		
0-10	8920.3 (652.6)a	35.1 (0.6)ab	0.96 (0.01)ab
10-30	9272.7 (316.7)a	35.7 (0.3)ab	0.95 (0.01)ab
30-60	9487.3 (1306.2)a	32.3 (2.9)ab	0.91 (0.03)ab
60-100	6687.7 (942.6)a	23.3 (4.7)b	0.86 (0.04)b
Mean	**8592 (505.4)α**	**31.6 (1.9)α**	**0.92 (0.02)α**
Bole-only Harvest			
0-10	8015 (511.9)a	36.0 (0.7)ab	0.96 (0.01)ab
10-30	9331.7 (643.1)a	37.5 (1.9)a	0.95 (0.02)ab
30-60	8618 (382.5)a	31.8 (3.4)ab	0.90 (0.03)ab
60-100	8040.3 (644.2)a	27.0 (4.5)ab	0.86 (0.04)b
Mean	**8501.3 (287.1)α**	**33.1 (1.8)α**	**0.92 (0.02)α**
WT+FF Removal			
0-10	8646.7 (527.9)a	35.6 (0.5)ab	0.96 (<0.01)ab
10-30	10033.7 (942.2)a	39.7 (1.3)a	0.98 (0.01)a
30-60	8704 (203.6)a	33.5 (1.0)ab	0.92 (0.01)ab
60-100	8962.7 (783.2)a	29.9 (2.7)ab	0.91 (0.02)ab
Mean	**9086.8 (333.5)α**	**34.7 (1.3)α**	**0.94 (0.01)α**

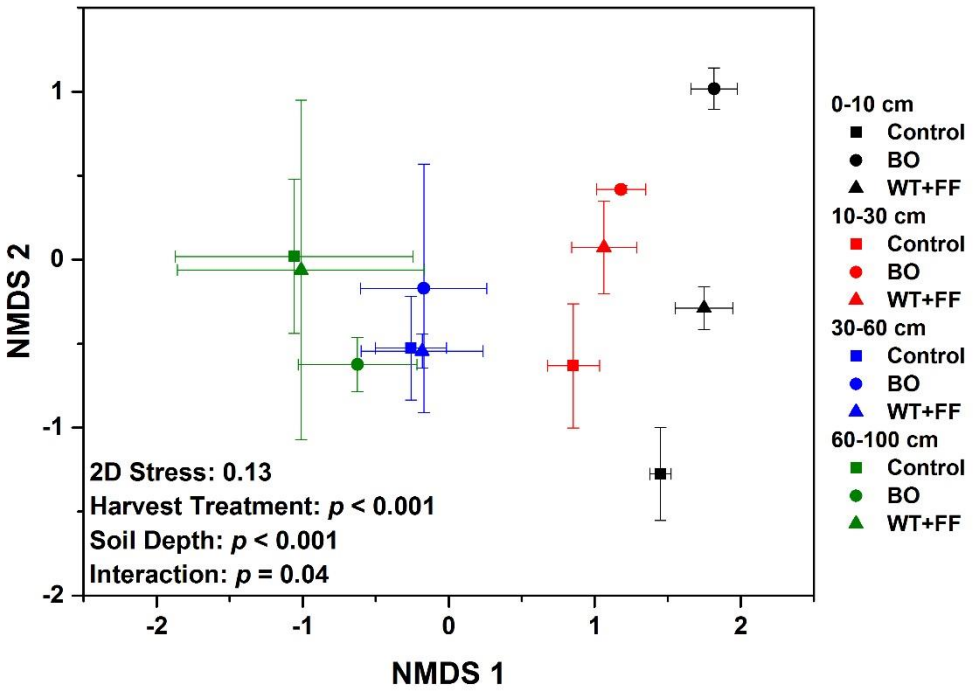

Figure 18: Nonmetric multidimensional scaling (NMDS) ordinations of soil bacteria communities based upon their OTU composition derived from Bray-Curtis distances matrices. Each point and corresponding bars represent mean ± standard deviation (n=3). Statistical differences in organic matter removal, soil depth, and their interaction were obtained using PERMANOVA. Control: unharvested control, BO: bole-only harvest, WT+FF: whole-tree harvest + forest floor removal.

Bacterial Taxonomic Classification

Phylum-level OTU characterization indicated that communities were dominated by Acidobacteria (43.5 ± 1.2%), Proteobacteria (20.7 ± 1.1%), Verrucomicrobia (11.2 ± 0.8%), Firmicutes (6.8 ± 0.8%), Actinobacteria (6.3 ± 0.2), Chloroflexi (4.4 ± 0.9%), and Bacteroidetes (4.3 ± 0.4%) across all treatments and soil depths (Figure 19). The remaining 2.7% of sequences were annotated to 29 other phyla. OMR treatment did not statistically alter the relative abundance of any of the phyla analyzed; however, depth did lead to significant differences. Specifically, the relative abundance of Acidobacteria, Firmicutes, Chloroflexi, and Nitrospirae

increased with depth while Bacteroidetes, Armatimonadetes, and Saccharibacteria decreased with increasing soil depth.

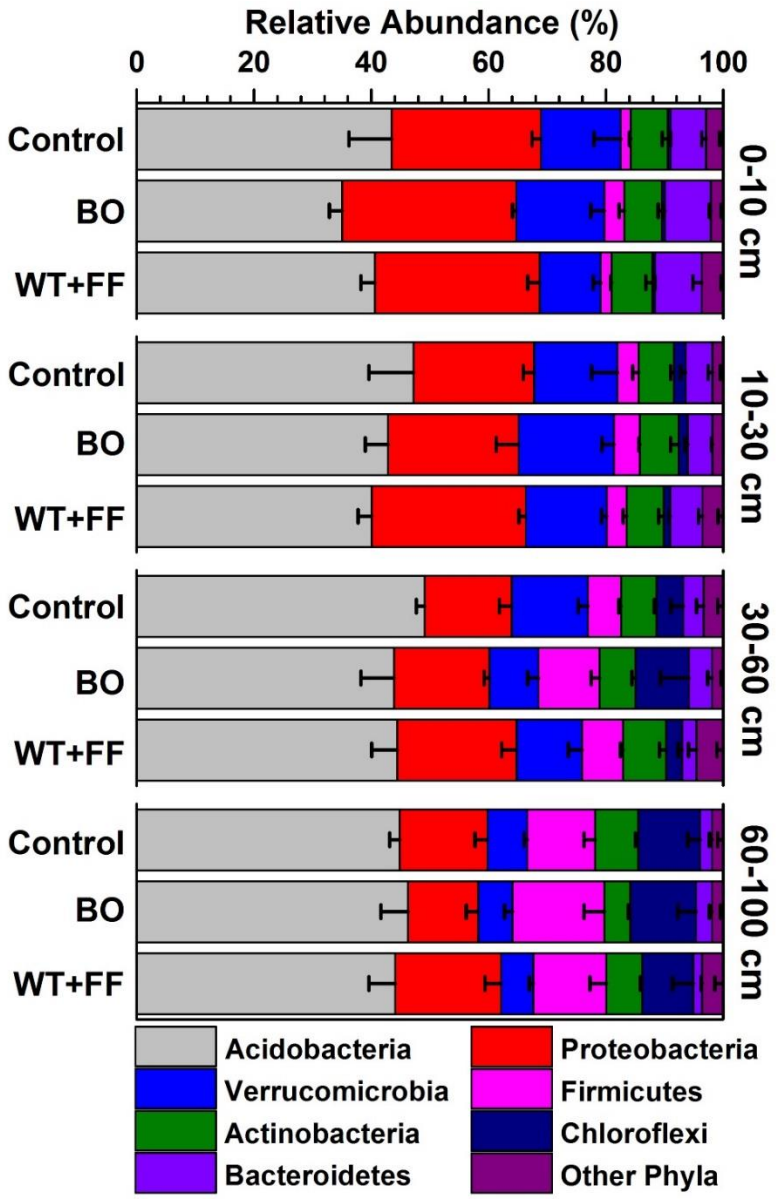

Figure 19: Distribution and relative abundance of major bacterial phyla for each treatment (Control=Unharvested Controls; BO=Bole-Only Harvest; WT+FF=Whole-tree Harvest + Forest Floor Removal) nested within soil depth increment. Error bars indicate standard error of 3 biological replicates.

The three most abundant bacterial genera, *Acidobacterium*, *Holophaga*, and *Candidatus Solibacter* accounted for 38.8% of all 16S sequences and belong to the phylum Acidobacteria (Table 18). The relative abundance of *Acidiobacterium*, *Holophaga*, *Ktedonobacter*, *Thermoflavimicrobium*, *Stella*, *Candidatus Koribacter*, *Pseudomonas*, and *Aciditerrimonas* increased significantly with depth while the relative abundance of *Prosthecobacter*, *Verrucomicrobium*, *Pseudolabrys*, *Burkholderia*, *Steroidobacter*, and *Bradyrhizobium* decreased significantly with depth (Table 18). Of the bacterial genera that comprised over 1% of all 16S reads, only *Burkholderia* and *Aciditerrimonas* were statistically affected by differences in OMR. Specifically, the relative abundance of *Burkholderia* increased with increasing intensity of organic matter removal while *Aciditerrimonas* decreased with increasing organic matter removal (Table 4). Spearman's correlation analyses revealed that *Burkholderia* was significantly positively correlated to soil pH and P and negatively correlated to K, Mg, S, and Na (Table 19). Congruently, *Aciditerrimonas* was positively correlated to K, Mg, S, and Na and negatively correlated to soil pH, P, and Ca (Table 19). Of the less abundant genera, 53 were significantly affected by timber harvest.

Table 18: Bacterial genera that comprise > 1% of all sequences composited from all samples (N=36) at the Groveton-LTSP site. A linear mixed model was used to analyze the influence of organic matter removal and soil depth on OTU abundance values. Spearman's rank order correlation analysis was used to analyze relationship between OTUs and certain edaphic properties. Asterisks indicate a significant relationship with: *p<0.05, **p<0.01, ***p<0.001.

Genus	Relative Abundance	Harvest Treatment	Soil Depth	HT x SD
	(% of all 16S Sequences)		F-Ratio	
Acidobacterium	23.75	0.27	**44.06*****‡**	1.96
Holophaga	8.82	2.72	**6.02****‡**	1.48
Candidatus Solibacter	6.24	0.09	1.99	1.41
Ktedonobacter	4.48	1.13	**48.16*****‡**	0.82
Thermoflavimicrobium	4.07	1.01	**90.81*****‡**	0.5
Prosthecobacter	4.04	0.01	**3.16*Φ**	1.74
Verrucomicrobium	3.60	0.33	**9.06*****Φ**	1.43
Stella	3.31	3.46	**4.57***‡**	0.95
Rhodothermus	2.92	0.64	1.73	0.88
Candidatus Koribacter	2.32	1.07	**29.63*****‡**	2.18
Chthoniobacter	1.89	1.68	2.6	1.98
Pseudolabrys	1.82	4.05	**10*****Φ**	1.02
Burkholderia	1.57	**7.13***‡**	**5.32****Φ**	0.48
Pseudomonas	1.54	3.82	**5.06***‡**	1.31
Conexibacter	1.50	0.98	2.27	0.77
Steroidobacter	1.39	0.01	**21.92*****Φ**	0.94
Bradyrhizobium	1.35	0.65	**25.83*****Φ**	1.01
Aciditerrimonas	1.29	**6.67*Φ**	**7.47****‡**	1.08
Actinoallomurus	1.10	2.22	2.23	0.97
All Other Genera	23.00	0.35	**4.77***‡**	0.07

‡ Relative abundance significantly increased with increasing harvest intensity or soil depth
Φ Relative abundance significantly decreased with increasing harvest intensity or soil depth

Table 19: Spearman's rank order correlation analysis was used to analyze relationship between abundant genera as well as functional groups and certain edaphic properties.

	Soil pH	SOC	TN	TP	NH_4^+	$NO_2^- + NO_3^-$	P	K	Ca	Mg	S	Na
							Mehlich-III Extractable					
Genera												
Acidobacterium	-0.23	**-0.71*** **	**-0.54*** **	-0.27	**-0.42***	-0.32	**-0.73*** **	**0.40***	**-0.65*** **	0.14	0.30	**0.47****
Holophaga	**-0.37***	-0.21	-0.11	-0.08	-0.16	-0.01	**-0.54*** **	0.28	**-0.37***	0.19	**0.48****	**0.51****
Candidatus Solibacter	**-0.35***	-0.2	-0.14	-0.05	-0.13	-0.07	-0.20	0.01	**-0.40***	-0.08	0.11	0.00
Ktedonobacter	-0.29	**-0.69*** **	**-0.36***	-0.04	**-0.38***	-0.31	**-0.80*** **	**0.58*** **	**-0.44****	**0.54*** **	**0.46****	**0.43****
Thermoflavimicrobium	-0.25	**-0.75*** **	**-0.37***	0.01	**-0.37***	**-0.40***	**-0.70*** **	**0.59*** **	**-0.45****	**0.54*** **	**0.41***	0.29
Prosthecobacter	0.16	0.07	-0.06	-0.04	0.22	0.01	0.25	-0.20	0.23	-0.10	**-0.44****	-0.32
Verrucomicrobium	0.13	-0.16	-0.3	-0.25	0.08	-0.16	0.03	-0.09	-0.17	-0.32	-0.32	**-0.41***
Stella	**-0.46****	-0.2	0.04	0.18	0.00	-0.06	**-0.49****	0.33	-0.16	**0.53****	**0.55*** **	0.29
Rhodothermus	-0.26	0.21	0.25	0.24	0.10	0.04	0.16	-0.11	-0.12	0.02	0.04	-0.29
Candidatus Koribacter	-0.1	**-0.61*** **	**-0.47****	**-0.36***	**-0.49****	**-0.52****	**-0.57*** **	0.27	**-0.51****	0.15	0.31	**0.53*** **
Chthoniobacter	**-0.53*** **	**0.34***	**0.54*** **	**0.65*** **	0.26	0.26	0.07	0.02	0.00	0.28	0.29	-0.18
Pseudolabrys	-0.04	**0.66*** **	**0.38***	0.12	**0.41***	0.39*	**0.58*** **	**-0.43****	0.29	**-0.35***	-0.17	-0.17
Burkholderia	**0.64*** **	0.22	-0.003	-0.10	-0.03	-0.14	**0.55*** **	**-0.40***	0.19	**-0.50****	**-0.53****	**-0.71*** **
Pseudomonas	**0.47****	**-0.66*** **	**-0.63*** **	**-0.42***	**-0.48****	**-0.55*** **	-0.26	0.15	-0.26	-0.08	-0.25	-0.13
Conexibacter	**0.49****	**-0.36***	**-0.38***	-0.25	-0.24	-0.32	0.00	0.05	-0.05	-0.10	-0.21	**-0.41***
Steroidobacter	0.05	**0.57*** **	0.27	0.04	0.21	0.28	**0.61*** **	**-0.53*** **	0.13	**-0.53****	-0.26	**-0.45****
Bradyrhizobium	0.21	**0.72*** **	**0.52****	0.32	0.31	0.24	**0.86*** **	**-0.50****	**0.45****	-0.30	-0.28	**-0.56*** **
Aciditerrimonas	**-0.51****	-0.27	-0.05	0.11	-0.15	-0.02	**-0.55*** **	**0.36***	**-0.37***	**0.41***	**0.65*** **	**0.45****
Actinoallomurus	**-0.45****	-0.04	0.23	0.31	-0.06	0.10	-0.28	0.30	-0.08	**0.48****	**0.64*** **	**0.46****
All Other Genera	0.32	-0.02	0.09	0.09	-0.33	-0.15	0.07	-0.03	-0.10	-0.09	0.14	-0.15
Functional Groupings												
N-Fixing (Free Living)	0.09	0.003	-0.25	**-0.35***	0.00	-0.09	0.16	**-0.34***	-0.12	**-0.50****	-0.31	-0.16
N-Fixing (Symbiotic)	0.23	**0.72*** **	**0.50****	0.29	0.31	0.23	**0.87*** **	**-0.51****	**0.45****	-0.33	-0.32	**-0.56*** **
Ammonia-Oxidizing	**-0.33***	**0.48****	**0.33***	0.29	**0.38***	**0.40***	0.18	-0.23	0.00	-0.04	0.01	-0.01
Nitrite-Oxidizing	**0.36***	**-0.82*** **	**-0.75*** **	**-0.50****	**-0.53*** **	**-0.62*** **	**-0.48****	0.24	**-0.43****	0.00	-0.04	0.12
Denitrifying	**0.54*** **	**-0.47****	**-0.45****	**-0.34***	-0.32	**-0.45****	-0.07	0.07	-0.09	-0.14	-0.27	-0.17
Iron-Oxidizing	0.29	**0.53****	0.25	0.07	-0.02	0.16	**0.64*** **	**-0.48****	**0.37***	**-0.40***	-0.31	**-0.39***
Iron-Reducing	0.27	-0.23	-0.28	**-0.50****	**-0.45****	**-0.36***	-0.20	-0.04	-0.31	-0.28	-0.02	-0.50
Sulfur-Reducing	**0.45****	**-0.46****	**-0.55*** **	**-0.52****	**-0.48****	**-0.53*** **	-0.22	-0.04	-0.26	**-0.35***	-0.13	-0.07
Sulfate-Oxidizing	**0.46****	-0.3	**-0.40***	**-0.61*** **	-0.35	**-0.54*** **	-0.22	-0.03	-0.31	**-0.33***	-0.04	0.16

*p <0.05

**p <0.01

***p<0.001

Distribution of Functional Bacterial Taxa

The effects of timber harvest intensity and soil depth on putatively characterized functional bacterial groups, based on literature, are summarized in Table 20. Free-living N-fixing genera which account for 0.5% of all 16S sequences (*Azonexus*, *Azospira*, *Azospirillium*, *Beijerinckia*, and *Leuconostoc*) and symbiotic N-fixing genera which account for 1.5% of all 16S sequences (*Azorhizobium*, *Bradyrhizobium*, *Frankia*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*) were unaffected by timber harvest; however, the relative abundance of symbiotic N-fixers did decrease significantly with depth. Ammonia-oxidizing bacteria (*Nitrosomonas*, *Nitrosospira*, and *Nitrosovibrio*) accounted for <0.01% of all 16S sequences and their relative abundance was significantly reduced with increasing harvest intensity. Nitrite-oxidizing genera (*Nitrobacter, Nitrolancea, Nitrospina*, and *Nitrospira*) accounted for 0.8% of all 16S sequences and was unaffected by harvest treatment; however, their relative abundance did increase with increasing soil depth. Denitrifying genera (*Pseudomonas* and *Bacillus*) were significantly more abundant than any other functional group analyzed, and increased significantly with increasing OMR intensity. The cumulative relative abundance of iron-oxidizing genera, which accounted for <0.1% of all 16S sequences (*Acidithiobacillus*, *Gallionella*, *Leptospirillum*, *Leptothrix*, *Mariprofundus*, *Rhodopseudomonas*, *Thiobacillus*) significantly decreased with depth and were significantly less abundant that iron-reducing genera (*Desulfovibrio*, *Geobacter*, and *Shewanella*) which accounted for 0.4% of all 16S reads. Sulfate-reducing genera (*Desulfobacca, Desulfobulbus, Desulfocaldus, Desulfocella, Desulfococcus, Desulfocurvus, Desulfofaba, Desulfomicrobium, Desulfomonile, Desulfonatronum, Desulfonauticus, Desulforegula, Desulforhabdus, Desulfosporosinus, Desulfotalea, Desulfotomaculum, Desulfovibrio, Desulfovirga, Thermodesulfobacterium*, and *Thermodesulfovibrio*) accounted for roughly 0.7% of all 16S sequences and significantly increased with increasing soil depth. In contrast, sulfur-

oxidizing genera (*Alkalilimnicola, Chloroherpeton, Halothiobacillus, Ignavibacterium, Marichromatium, Melioribacter, Thioalkalivibrio, Thiobacillus, Thiodictyon*, and *Thiorhodospira*) accounted for <0.1% of all 16S sequences and were unaffected by either timber harvest or soil depth. The two functional groups that were significantly affected by timber harvest (ammonia-oxidizing and denitrifying) were significantly correlated to multiple soil chemical properties. Specifically, the ammonia-oxidizing group was significantly positively correlated to SOC, TN, NH_4^+, and $NO_2^- + NO_3^-$ and negatively correlated to soil pH (Table 19). Congruently, the denitrifying group was positively correlated to soil pH and negatively correlated to SOC, TN, and $NO_2^- + NO_3^-$ (Table 19).

Table 20: The impact of organic matter removal treatment and soil depth on functional bacterial groups. These results are the function of a mixed model ANOVA and reported as F-ratios. **p*<0.05, ***p*<0.01, ****p*<0.001. ‡: relative abundance increased with increasing harvest intensity or soil depth; Φ: relative abundance decreased with increasing harvest intensity or soil depth.

	Percentage of 16S Reads	No. Genera	Harvest Treatment	Soil Depth	HT x SD
Functional Group				F-Ratio	
N-Fixing (Free Living)	0.50	5	0.14	2.20	0.67
N-Fixing (Symbiotic)	1.51	6	2.37	**25.07***Φ**	0.98
Ammonia-Oxidizing	< 0.01	3	**9.03**Φ**	0.84	0.43
Nitrite-Oxidizing	0.83	4	1.30	**10.9**‡**	1.00
Denitrifying	1.95	2	**3.7*‡**	1.20	1.80
Iron-Oxidizing	0.03	7	0.90	**4.7*Φ**	0.30
Iron-Reducing	0.43	3	0.40	0.50	0.50
Sulfur-Oxidizing	0.07	10	3.10	1.50	1.80
Sulfate-Reducing	0.71	20	2.60	**3.8*‡**	1.20

Discussion

Intensive OMR methods associated with timber harvest can significantly modify belowground properties (Johnson and Curtis, 2001; Li et al., 2003; Nave et al., 2010; Jones et al.,

2011; Huang et al., 2013; Achat et al., 2015a, 2015b; Foote et al., 2015); however, currently there are knowledge gaps regarding the effect of these practices on soil bacterial community composition, especially at depth. Results from previous studies on the effect of OMR have led to divergent conclusion. Some studies have shown that soil microbial consortia possess high resilience to intensive OMR (Li et al., 2004; Busse et al., 2006; Hannam et al., 2006; Mariani et al., 2006); however, others have challenged this assumption of resilience and shown that intensive OMR can strongly affect microbial communities for decades following harvest (Hartmann et al., 2012). We have shown that OMR can result in long-term alterations in chemical properties as well as significant modify soil bacterial community structure, the relative abundance of predominant genera, and ecologically-relevant functional groups.

Our results indicate that increasing harvest intensity has led to reductions in concentrations of SOC, TN, NH_4^+, and $NO_2^- + NO_3^-$ while soil pH and the concentration of Mehlich-III P have increased. The reduction in SOC and TN is most likely attributed to a large portion of potential SOC and TN inputs being removed during treatment application, coupled with relatively low rates of C and N inputs following forest regrowth. Lower concentrations of inorganic-N in the more severe organic matter removal treatment are potentially attributable to higher rates of N-losses following microclimate changes induced by OMR application. Specifically, increases in solar radiation reaching the soil surface, decreases in transpiration and rainfall interception, and increases in the amount of precipitation reaching and infiltrating the forest floor and into the soil would result in warmer, wetter soil conditions that favor higher rates of nitrification, leaching, and denitrification. Following the acceleration of N-cycle processes immediately after harvest, there may be an observed decrease a soil nitrogen over time due to reduced inputs. The overall reduction in the concentration of $NO_2^- + NO_3^-$ may also be a sign that nitrification has been significantly altered, which may explain treatment-based differences in soil

pH and Mehlich-III P. Specifically, it has been shown that increased rates of nitrification can result in more acidic soil conditions due to the release of hydrogen cations into the soil environment during the first step of nitrification, ammonia-oxidization (de Boer and Kowalchuk, 2001). Along with the decrease in the concentration of $NO_2^- + NO_3^-$, we observe an overall increase in soil pH which is consistent with the hypothesis that rates of nitrification are slowed following intensive timber harvest. Differences in soil pH may also be attributable to lower inputs of generally acidic fresh loblolly pine litter. Furthermore, the increase in soil pH may have increased the availability of Mehlich-III P by reducing the potential for metals such as aluminum, iron, and magnesium to immobilize P.

The soil bacterial community associated with the WT+FF treatment was generally more diverse and had greater estimated richness than those associated with the BO treatment and unharvested control; however, these differences did not lead to statistically significant findings. These larger estimates in the WT+FF suggests that disturbance promotes the coexistence of bacterial taxa with differing ecological strategies. When we investigate bacterial community structure using a PERMANOVA on normalized Bray-Curtis distance matrices, we see that harvest treatment has statistically affected bacterial community structure. Specifically we observe significant separation of the three treatments in the 0-10 and 10-30 cm increments; however, there were no statistical harvest-induced differences in the 30-60 or 60-100 cm increments. Furthermore, soil depth led to significant variations in community structure with the 0-10, 10-30, and 30-100 cm increments being statistically different from each other. Considering the depth-induced differences in bacterial community structure as well as treatment-based differences in the 0-10 and 10-30 cm increments, it is possible that taxa found at 0-30 cm are more susceptible to large changes in the volume of plant-based inputs such as woody debris and litter. These alterations in community structure would have taken place at some point following

harvest, and have been maintained over time by the concurrent alterations in soil chemical properties.

Sequences similar to the phylum Acidobacteria dominated our plots and accounted for >43% of all 16S sequences. This phylum is one of the most widespread and abundant bacterial groups on the planet; however, relatively little information in known regarding the actual activities and ecology of member taxa (Chow et al., 2002; Kuske et al., 2002; Gremion et al., 2003; Fierer et al., 2005; Janssen, 2006; Kim et al., 2007; Jesus et al., 2009; Kielak et al., 2009; Navarrete et al., 2010, Zhang et al., 2014). From culture-based experiments, Acidobacteria type species have been shown to be heterotrophic (Pankartov et al., 2012), with some subdivisions being quite versatile in carbohydrate utilization. Genomic and metagenomic data predict a number of ecologically relevant capabilities for some Acidobacteria, including the ability to use nitrite as an N source, respond to soil nutrients and pH, express multiple active transporters, degrade gellan gum, and produce exopolysaccharide (Janssen et al., 2002; Liles et al., 2003; Quaiser et al., 2003; Fierer et al., 2007; Pankratov et al., 2008; Riaz et al., 2008; Lauber et al., 2009; Ward et al., 2009; Kielak et al., 2010; Eichhorst et al., 2011; Parsley et al., 2011; Faoro et al., 2012 Foesel et al., 2013; Navarrete et al., 2013; Huber et al., 2014; Mendes et al., 2014; Garcia-Fraile et al., 2016). Although the relative abundance of Acidobacteria was statistically unaffected by harvest intensity, its type-genus (*Acidobacterium*) was significantly correlated to multiple soil chemical factors. Specifically, the relative abundance of sequences similar to *Acidobacterium*, which was the dominant genus in this system, was positively correlated to K and Na and negatively correlated to SOC, TN, NH_4^+, P, and Ca. This indicates that *Acidobacterium* may be more competitive in low-resource environments. Considering that *Acidobacterium* is regarded as acidophilic (Fierer et al., 2007), it was surprising that the relative

abundances of this genus was not positively correlated to soil pH; however, this may be due to the relatively small pH gradient observed at this site (3.25 ± 0.10 to 4.67 ± 0.20).

Of the genera that comprised >1% of all 16S sequences, only *Burkholderia* (Proteobacteria) and *Aciditerrimonas* (Actinobacteria) were statistically affected by harvest intensity. Specifically, the relative abundance of sequences similar to *Burkholderia* increased with increasing harvest intensity while *Aciditerrimonas* decreased. *Burkholderia* occupy remarkably diverse ecological niches, ranging from contaminated soils to the respiratory tract of humans (Parke and Gurian-Sherman, 2001; Coenye and Vandamme, 2003). Furthermore, this genus possesses remarkable metabolic versatility, and have been exploited for biocontrol, bioremediation, and plant growth promotion purposes (Parke and Gurian-Sherman, 2001; Coenye and Vandamme, 2003). It is possible that their high abundance in the WT+FF is a result of their functional versatility in which this group was better suited to deal with changing carbon and nutrient availability as opposed to other taxa who may possess more narrow metabolic capabilities. The ecology of *Aciditerrimonas* is much less characterized; however members of this genus exhibit both heterotrophy and autotrophy (Itoh et al., 2011). Specifically, this genus is capable of reducing ferric ions (Fe^{3+}) to facilitate autotrophic growth under anaerobic conditions (Itoh et al., 2011). We observed that the relative abundance of *Aciditerrimonas* was negatively correlated to soil pH. In less acidic conditions, as was observed in the WT+FF treatment, it is possible that the availability of Fe^{3+} was reduced which may explain the decreased relative abundance of an iron-reducing taxa such as *Aciditerrimonas*.

In regards to the relative abundance of functional groups, only ammonia-oxidizing bacteria and denitrifying bacteria were significantly altered. The ammonia-oxidizing bacteria were significantly reduced while the denitrifying group significantly increased. Considering that concentrations of TN, NH_4^+, and $NO_2^- + NO_3^-$ were significantly modified by timber harvest, it is

not surprising that the microbial populations regulating these pools were also affected. Sequences similar to known ammonia-oxidizing bacteria were relatively rare and comprised < 0.01% of all 16S reads. This group was positively correlated to SOC, TN, NH_4^+, and NO_2^- + NO_3^-, which may help in explaining their reduction in abundance with increasing harvest intensity. Furthermore, we hypothesized that the soil pH increase with increasing harvest intensity may be a function of reduced nitrification. This theory coincides with the overall reduction in ammonia-oxidizing bacteria as well as the observed reduction bacterial *amoA* as was shown in chapter 3 of this dissertation; however, it should be mentioned that ammonia-oxidation can also be carried out by archaea which were not quantified in this study. The denitrifying group was statistically the most abundant functional group analyzed (1.95% of all 16S reads). Considering we observed a reduction of NO_2^- + NO_3^- in the WT+FF treatment, it is somewhat surprising that the relative abundance of denitrifying bacteria increased with increasing harvest intensity. It is possible that microclimate conditions following harvest in which solar radiation reaching the surface increased as well as water infiltration selected for an increase in denitrifying taxa which has persisted for 18 years post-harvest.

CHAPTER VI

CONCLUSIONS

This research examined the long-term effect of intensive organic matter removal (OMR) associated with timber harvest practices on soil properties, biogeochemical processes, and the structure and function of soil microbial communities. We compared soil physical, chemical, and biological properties in the upper 1 m of the soil profile associated with a low-intensity harvest treatment, bole-only harvest (BO), and a high-intensity harvest treatment, whole-tree harvest + forest floor removal treatment (WT+FF), to an unharvested secondary-growth control. Soil texture, pH, and bulk density were measured to characterize basic soil attributes. We also quantified the elemental composition of soil carbon (SOC), nitrogen (TN), and phosphorus (TP) as well as Mehlich-III extractable phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and sodium (Na). Additionally, we measured the natural abundance of soil $\delta^{13}C$ and $\delta^{15}N$. In regards to the microbial community, we quantified microbial biomass carbon (MBC), nitrogen (MBN), and the abundance of the marker gene *amoA*. We also sequenced 16S, ITS and *amoA* amplicons in order to annotate the bacterial, fungal, and ammonia-oxidizing communities, respectively.

We found that 18 years after timber harvest, soil pH increased with increasing harvest intensity; however, bulk density and texture were not altered. SOC and TN have not recovered to pre-harvest conditions in the upper 1 m of the soil profile in the organic matter removal treatment. Furthermore, alterations in the natural abundance of $\delta^{13}C$ and $\delta^{15}N$ from bulk soil indicate that rates of key biogeochemical processes may be affected for a large portion of the mineral soil profile. $\delta^{13}C$ decreased with increasing harvest intensity indicating a decrease in heterotrophic activity while $\delta^{15}N$ increased with increasing harvest intensity indicating higher

rates of N loss following the implementation of harvest treatments. The loss of N resulting in higher $\delta^{15}N$ values may be linked to higher rates of nitrification and denitrification. MBC was also significantly higher in the control and bole-only harvest stands indicating a higher potential for microbial activity. It is important to note that these significant changes in soil biogeochemical properties are still evident more than 18 years following the timber harvest events.

Additionally, our findings indicate that differing intensities of OMR associated with timber harvest can impart long-term reductions in concentrations of inorganic N such as ammonium (NH_4^+) and nitrite (NO_2^-) + nitrate (NO_3^-), and alter the abundance and community structure of ammonia-oxidizing archaea (AOA) and bacteria (AOB) throughout the soil profile. The abundance of AOB *amoA* gene copy number was significantly positively correlated to SOC and TN, while AOA *amoA* was negatively correlated with soil pH indicating that the abundances of these two functional taxonomic groups are influenced by soil physicochemical properties which may be modified by different OMR treatments. Soil depth also strongly shaped AOA and AOB abundances and community composition, with an increasing ratio of AOA:AOB with increasing depth. Furthermore, total-, octyne-resistant (AOA), and octyne-sensitive (AOB) nitrification potential were all affected by increasing OMR intensity; however, only octyne-resistant (AOA) nitrification potential was significantly affected at low levels of NH_4^+ indicating that differing levels of OMR may not lead to significant differences in total rates of nitrification.

Furthermore, our findings indicate that differing intensities of above-ground OMR associated with timber harvest can impart substantial decade-scale changes to fungal community structure and functional potential across multiple soil depth increments. Fungal diversity generally decreased with increasing harvest intensity with the greatest differences being observed in shallower depths most likely a result of altered SOC and TN stocks. Deeper fungal

communities were extremely variable and did not seem to be affected in the long-term by harvest. The fungal community was dominated by Basidiomycetes; however, the abundance of Ascomycetes increased with increasing harvest intensity which was mirrored by increasing soil pH. The ectomycorrhizal genus *Russula* appeared to be the dominant taxa in this system and was significantly reduced by increasing OMR, possibly driven by host-plant response to altered physicochemical conditions including soil pH and Mehlich-III extractable phosphorus. The lone ericoid mycorrhizal fungal species, *Rhizoscyphus ericae*, showed significant increase in relative abundance with respect to increasing OMR intensity most likely attributed to its colonization of loblolly pine seedlings at the onset of treatment application.

Finally, timber harvest significantly altered bacterial community composition through concurrent alterations in soil physicochemical parameters. Bacterial communities associated with the WT+FF treatment were generally more diverse and had greater estimated richness than those associated with the BO treatment and unharvested control; however, these differences did not lead to statistically significant differences. The phylum Acidobacteria dominated our plots; specifically, its type-genus (*Acidobacterium*) was significantly positively correlated to K and Na and negatively correlated to SOC, TN, NH_4^+, P, and Ca, indicating that *Acidobacterium* may be more competitive in low-resource environments. Of the dominant genera, the relative abundance of sequences similar to *Burkholderia* increased with increasing harvest intensity while *Aciditerrimonas* decreased. The increase in the abundance of *Burkholderia* is most likely attributed to their metabolic versatility in which this group was better suited to deal with changing carbon and nutrient availability whereas the decrease in *Aciditerrimonas* can be attributed to their strong positive correlation to soil pH and its ability to regulate metal redox states, specifically Fe. In regards to the relative abundance of functional groups, the relative

abundance of ammonia-oxidizing bacteria decreased with increasing harvest intensity and denitrifying bacteria significantly increased.

Understanding the influence of OMR on physical, chemical and biological properties and processes can better explain how forest disturbance can impart alterations in biogeochemical cycling as well as forest productivity. Our results suggest that harvest practices aimed at retaining the forest floor should be employed to conserve SOC, key nutrients such as N and P, and to maintain the microbial community composition. Additionally, the WT+FF treatment should not be used if the goal is to maximize tree size of the subsequent rotation following harvest. Future research directed more specifically at the functional potential of the microbial community in this forest ecosystem will likely yield valuable insights into how microbial functionality is shaped by OMR.

REFERENCES

Abarenkov K, Nilsson RH, Larsson K, Alexander IJ, Eberhardt U, et al. 2010. The UNITE database for molecular identification of fungi- recent updates and future perspectives. *New Phytologist* 186, 281-285.

Achat DL, Deleuze C, Landman G, Pousse N, Augusto L. 2015a. Quantifying consequences of removing harvesting residues on forest soils and tree growth - A meta-analysis. *Forest Ecology and Management* 348, 124-141.

Achat DL, Fortin M, Landmann G, Ringeval B, Augusto L. 2015b. Forest soil carbon is threatened by intensive biomass harvesting. *Scientific Reports* 5, doi:10.1038/srep15991.

Adair KL, Schwartz E, 2008. Evidence that ammonia-oxidizing archaea are more abundant than ammonia-oxidizing bacteria in semiarid soils of northern Arizona, USA. *Microbial Ecology* 56, 420-426.

Agren GI, Hyvonen R. 2003. Changes in carbon stores in Swedish forest soils due to increased biomass harvest and increased temperatures analyzed with a semi-empirical model. *Forest Ecology and Management* 174, 25-37.

Allen AS, Schlesinger WH. 2004. Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. *Soil Biology and Biochemistry* 36, 581–589.

Allison SD, Hanson CA, Treseder KK. 2007. Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biology and Biochemistry* 39, 1878-1887.

Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, et al. 2003. Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochemical Cycles* 17, doi:10.1029/2002GB001903.

Anderson CJ, Lockaby BG. 2010. Research gaps related to forest management and stream sediment in the United States. *Environmental Management* 47, 303-313.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32-46.

Anderson OR, Lee JM, McGuire K. 2016. Experimental evidence that fungi are dominant microbes in carbon content and growth response to added soluble organic carbon moss-rich tundra soil. *The Journal of Eukaryotic Microbiology* 63, 363-366.

Andren O, Balandreau J. 1999. Biodiversity and soil functioning-from black box to can of worms? *Applied Soil Ecology* 13, 105–108.

Andrews S, Norton I, Salunkhe AS, Goodluck H, Aly WSM, et al. 2013. Controls of iron metabolism. In: Banci L. (Ed.). *Metallomics and the Cell*. Springer, Netherlands, pp. 203-239.

Artz RRE, Anderson IC, Chapman SJ, Hagn A, Schloter M, et al. 2007. Changes in fungal community composition in response to vegetational succession during the natural regeneration of cutover peatlands. *Microbial Ecology* 54, 508-522.

Ashworth J, Keyes D, Kirk R, Lessard R. 2001. Standard procedure in the hydrometer methods for particle size analysis. *Communications in Soil Science and Plant Analysis* 32, 633-642.

Bååth E, Anderson AH. 2003. Comparison of fungal/bacterial ratios in a pH gradient using physiological and PLF-based techniques. *Soil Biology and Biochemistry* 35, 955-963.

Bååth E, Söderström B. 1982. Seasonal and spatial variation in fungal biomass in a forest soil. *Soil Biology and Biochemistry* 14, 353–358.

Bai E, Boutton TW, Liu F, Wu XB, Archer SR. 2013. ^{15}N isoscapes in a subtropical savanna parkland: spatial-temporal perspectives. *Ecosphere* 4, doi:10.1890/ES12-00187.1.

Baldrian P, Lindahal B. 2011. Decomposition in forest ecosystems: After decades of research still novel findings. *Fungal Ecology* 4, 359-361.

Balesdent J, Girardin C, Mariotti A. 1993. Site-related $\delta^{13}C$ of tree leaves and soil organic matter in a temperate forest. *Ecology* 74, 1713-1721.

Ball PN, MacKenzie MD, DeLuca TH, Holben WE. 2010. Wildfire and charcoal enhance nitrification and ammonia-oxidizing bacterial abundance in dry montane forest soils. *Journal of Environmental Quality* 39, 1243-1253.

Bationo A, Kihara J, Vanlauwe B, Waswa B, Kimetu J. 2007. Soil organic carbon dynamics, functions and management in West African agro-ecosystems. *Agricultural Systems* 94, 13–25.

Batjes NH. 2014. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* 65, 4-21.

Belser LW. 1979. Population ecology of nitrifying bacteria. *Annual Review of Microbiology* 33, 309-333.

Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, et al. 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution* 4, 914–919.

Binkley D, Vitousek P. 1989. Soil nutrient availability. In: Pearcy R, Ehleringer J, Mooney H, Rundel P. (Eds.), *Plant Physiological Ecology*. Chapman and Hall Ltd.: New York, NY, USA pp. 75-96.

Binkley D, Son Y, Valentine DW. 2000. Do forests receive occult inputs of nitrogen? *Ecosystems* 3, 321-331

Blagodatskaya E, Kuzyakov Y. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: A critical review. *Biology and Fertility of Soils* 45, 115-131.

Blume E, Bischoff M, Reichert JM, Moorman T, Konopka, et al. 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. *Applied Soil Ecology* 20, 171-181.

Bormann FH, Likens GE. 1979. Pattern and process in a forested ecosystem. Springer-Verlag, New York.

Bottomley PJ, Yarwood RR, Kageyama SA, Waterstripe KE, Williams MA, et al. 2006. Response of soil bacterial and fungal communities to reciprocal transfers of soil between adjacent coniferous forest and meadow vegetation in the Cascade Mountains of Oregon. *Plant and Soil* 289, 35-45.

Bradley RL, Kimmins JP, Martin WL. 2001. Post-clearcutting chronosequence in the B.C. Coastal Western Hemlock Zone-II. Tracking the assart flush. *Journal of Sustainable Forestry* 14, 23-43.

Brandeis TJ, Hartsell AJ, Bentley JW, Brandeis C. 2012. Economic dynamics of forests and forest industries in the southern United States. e-General Technical Report, SRS-152. USDA Forest Service, Southern Research Station, Asheville, North Carolina, USA, pp. 77.

Bray JR, Curtis JT. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* 27, 325-349.

Brookes PC, Landman A, Pruden G., Jenkinson DS. 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry* 17, 837-842.

Brown SP, Callaham Jr. MA, Oliver AK, Jumpponen A. 2013. Deep Ion Torrent sequencing identifies soil fungal community shift after frequent prescribed fires in a southeastern US forest ecosystem. *FEMS Microbiology Ecology* 86, 557-566.

Brumme R. 1995. Mechanisms of carbon and nutrient release and retention in beech forest gaps. *Plant and Soil* 168, 593-600.

Buchholz T, Friedland AJ, Horning CE, Keeton WS, Zanchi G, et al. 2014. Mineral soil carbon fluxes in forests and implications for carbon balance assessments. *Global Change Biology Bioenergy* 6, 305-311.

Buée M, Reich M, Murat C, Morin E, Nilsson RH, et al. (2009). 454 pyrosequencing analyses of forest soils reveals unexpectedly high fungal diversity. *New Phytologist* 184, 449-456.

Burgmann H, Pesaro M, Widmer F, Zeyer J. 2001. A strategy for optimizing quality and quantity of DNA extracted from soil. *Journal of Microbiological Methods* 45, 7-20.

Burns DA, Murdoch PS. 2005. Effects of clearcut on the net rates of nitrification and N mineralization in a northern hardwood forest, Catskill Mountains, New York, USA. *Biogeochemistry* 72, 123-146.

Burton AJ, Pregitzer KS, Crawford JN, Zogg GP, Zak DR. 2004. Simulated chronic nitrate deposition reduces soil respiration in northern hardwood forests. *Global Change Biology* 10, 1080–1091.

Busse MD, Beattie SE, Powers RF, Sanchez FG, Tiarks AE. 2006. Microbial community responses in forest mineral soil to compaction, organic matter removal, and vegetation control. *Canadian Journal of Forest Research* 36, 577–588.

Buyck B, Hofstetter V, Eberhardt U, Verbeken A, Kauff F. 2008. Walking the thin line between Russula and *Lactarius*: The dilemma of *Russula* subsect. Ochricompactae. *Fungal Diversity* 28, 15-40.

Cabello P, Roldán MD, Castillo F, Moreno-Vivián C. 2009. Nitrogen Cycle. In: Schaechter M (Ed.), Encyclopedia of Microbiology (3rd ed.). Elsevier, NY, pp. 299-321.

Cannon PF, Kirk PM. 2007. Fungal Families of the World. CAB International: Wallingford, UK.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7, 335-336.

Cardenas E, Kranbetter JM, Hope G, Maas KR, Hallam S, et al. 2015. Forest harvesting reduces the soil metagenomics potential for biomass decomposition. *The ISME Journal* 9, 2465-2476.

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, et al. 2012. Biodiversity loss and its impact on humanity. *Nature* 486, 59-67.

Carlyle JC, Bligh MW, Nambiar EKS. 1998. Woody residue management to reduce nitrogen and phosphorus leaching from sandy soil after clear-felling *Pinus radiata* plantations. *Canadian Journal of Forest Research* 28, 1222-1232.

Chao A. 1984. Non-parametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics* 11, 265-270.

Chen G, Tian H, Huang C, Prior SA, Pan S. 2013. Integrating a process-based ecosystem model with landsat imagery to assess impacts of forest disturbance on terrestrial carbon dynamics: Case studies in Alabama and Mississippi. *Journal of Geophysical Research* 118, 1208-1224.

Chen TH, Chiu CY, Tian GL. 2005. Seasonal dynamics of soil microbial biomass in coastal sand dune forest. *Pedobiologia* 49, 645–53.

Chen XW, Li BL. 2003. Change in soil carbon and nutrient storage after human disturbance of a primary Korean pine forest in Northeast China. *Forest Ecology and Management* 186, 197-206.

Chen X, Zhu Y, Xia Y, Shen J, He J. 2008. Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? *Environmental Microbiology* 10, 1978-1987.

Chen G, Tian H, Huang C, Prior SA, Pan S. 2013. Integrating a process-based ecosystem model with landsat imagery to assess impacts of forest disturbance on terrestrial carbon dynamics: Case studies in Alabama and Mississippi. *Journal of Geophysical Research* 118, 1208-1224.

Chow ML, Radomski CC, McDermott JM, Davies J, Axelrood PE. 2002. Molecular characterization of bacterial diversity in Lodgepole pine (*Pinus contorta*) rhizosphere soils from British Columbia forest soils differing in disturbance and geographic source. *FEMS Microbiology Ecology* 42, 347–357.

Coplen TB, Brand WA, Gehre M, Groning M, Meijer HAJ, et al. 2006. New guidelines for $\delta^{13}C$ measurements. *Analytical Chemistry* 78, 2439-2441.

Coenye T, Vandamme P. 2003. Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environmental Microbiology* 5, 719-729

Craine JM, Brookshire ENJ, Cramer MD, Hasselquist NJ, Koba K, et al. 2015. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant and Soil* 396, 1-26.

Dai J, Hu J, Lin X, Yang A, Wang R, et al. 2013. Arbuscular mycorrhizal fungal diversity, external mycelium length, and glomalin-related soil protein content in response to long-term fertilizer management. *Journal of Soils and Sediments* 13, 1-11.

Dai X, Boutton TW, Ansley RJ, Hailemichael M, Jessup KE. 2006. Soil carbon and nitrogen storage in response to fire in a temperate mixed-grass savanna. *Journal of Environmental Quality* 35, 1620-1628.

Dangal SRS, Felzer BS, Hurteau MD. 2014. Effects of agriculture and timber harvest on carbon sequestration in the eastern US forests. *Journal of Geophysical Research* 119, 35-54.

Danzeisen JL, Kim HB, Isaacson RE, Tu ZJ, Johnson TJ. 2011. Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. *PLoS ONE* 6, doi:10.1371/journal.pone.0027949.

Dean C, Kirkpatrick JB, Friedland AJ. 2017. Conventional intensive logging promotes loss of organic carbon from the mineral soil. *Global Change Biology* 23, 1-11.

de Boer W, Folman LB, Summerbell RC, Boddy L. 2005. Living in a fungal world; impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* 29, 795-811.

de Boer W, Kowalchuk GA. 2001. Nitrification in acid soils: micro-organisms and mechanisms. *Soil Biology and Biochemistry* 33, 853-866.

Derner JD, Boutton TW, Briske DD. 2006. Grazing and ecosystem carbon storage in the North American Great Plains. *Plant and Soil* 280, 77-90.

Diaz-Raviná M, Acea MJ, Carballas T. 1995. Seasonal changes in microbial biomass and nutrient flush in forest soils. *Biology and Fertility of Soils* 19, 220-226.

Dean C, Kirkpatrick JB, Friedland AJ. 2016. Conventional intensive logging promotes loss of organic carbon from the mineral soil. *Global Change Biology* 23, doi:10.1111/gcb.13387.

Dickens ED, Moorehead DJ, Bargeron CT, Morris LA, Ogden LA, et al. 2012. A summary of pine straw yields and economic benefits in loblolly, longleaf and slash pine stands. *Agroforestry Systems* 86, 315-321.

Dickie IA, Richardson SJ, Wiser SK. 2009. Ectomycorrhizal fungal communities and soil chemistry in harvested and unharvested temperate *Nothofagus* rainforests. *Canadian Journal of Forest Research* 39, 1069-1079.

Diochon A, Kellman L. 2008. Natural abundance measurements of ^{13}C indicate increased deep soil carbon mineralization after forest disturbance. *Geophysical Research Letters* 35, doi:10.1029/2008GL034795.

Diochon A, Kellman L, Beltrami H. 2009. Looking deeper: An investigation of soil carbon losses following harvesting from a managed northeastern red spruce (*Picea rubens* Sarg.) forest chronosequence. *Forest Ecology and Management* 257, 413-420.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460-2461.

Egnell G, Jurevics A, Peich M. 2015. Negative effects of stem and stump harvest and deep soil cultivation on the soil carbon and nitrogen pools are mitigated by enhanced tree growth. *Forest Ecology and Management* 338, 57-67.

Ehleringer JR, Buchmann N, Flanagan LB. 2000. Carbon isotope ratios in belowground carbon cycle processes. *Ecological Applications* 10, 412–422.

Eichorst SA, Kuske CR, Schmidt TM. 2011. Influence of plant polymers on the distribution and cultivation of bacteria in the phylum acidobacteria. *Applied and Environmental Microbiology* 77, 586-596.

EISA. 2007. Energy Independence and Security Act. H.R. 6. 110th Congress, 1st Session.

El-Abyad MSH, Webster J. 1968. Studies of pyrophilous discomycetes. I. Comparative physiological studies. *Transactions of the British Mycological Society* 51, 353-367.

Ellert BH, Bettany JR. 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Canadian Journal of Soil Science* 75, 529-538.

FAO. 2015. Global forest resources assessment 2015. Food and Agricultural Organization of the United Nations, Rome.

Faoro H, Glogauer A, Couto GH, de Souza EM, Rigo LU, et al. 2012. Characterization of a new Acidobacteria-derived moderately thermostable lipase from a Brazilian Atlantic Forest soil metagenome. *FEMS Microbiology Ecology* 81, 386–394.

Fierer N, Schimel JP, Holden PA. 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry* 35, 167-176.

Fierer N, Jackson JA, Vilgalys R, Jackson RB. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology* 71, 4117-4120.

Fierer N, Bradford MA, Jackson RB. 2007. Toward an ecological classification of soil bacteria. *Ecology* 88, 1354-1364.

Fisk MC, Fahey TJ. 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young hardwood forests. *Biogeochemistry* 53, 201–223.

Fleming RL, Powers RF, Foster NW, Kranabetter JM, Scott DA, et al. 2006. Effects of organic matter removal, soil compaction, and vegetation control on 5-year seedling performance: a regional comparison of Long-Term Soil Productivity sites. *Canadian Journal of Forest Research* 36, 529-550.

Foesel BU, Rohde M, Overmann J. 2013. *Blastocatella fastidiosa* gen. nov., sp nov., isolated from semiarid savanna soil–the first described species of *Acidobacteria* subdivision 4. *Systematic and Applied Microbiology* 36, 82–89.

Fontaine S, Barot S, Barré P, Bdioui N, Mary B, et al. 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450, 277-280.

Foote JA. 2014. Biogeochemistry of the western Gulf Coastal Plain as impacted by forest management. (Texas A&M University Doctoral dissertation). Available electronically from http://hdl.handle.net/1969.1/152521.

Foote JA, Boutton TW, Scott DA. 2015. Soil C and N storage and microbial biomass in US southern pine forests: Influence of forest management. *Forest Ecology and Management* 355, 48-57.

Fox TR, Jokela EJ, Allen HL. 2007. The development of pine plantation silviculture in the southern United States. *Journal of Forestry* 105, 337-347.

Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences* 102, 14683-14688.

Frey SD, Knorr M, Parrent JL, Simpson RT. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management* 196, 159–171.

Frijlink MJ, Abee T, Laanbroek HJ, de Boer W, Konings WN. 1992. The bioenergetics of ammonia and hydroxylamine oxidation in *Nitrosomonas eurpoea* in acid and alkaline pH. *Archives of Microbiology* 157, 194-199.

Gan XH, Zhang FQ, Gu JD, Guo YD, Li ZQ, et al. 2015. Differential distribution patterns of ammonia-oxidizing archaea and bacteria in acidic soils of Nanling National Nature Reserve forests in subtropical China. *Antoine van Leeuwenhoek* 109, 237-251.

Garcia-Fraile P, Benada O, Cajthaml T, Baldrian P, Llado S. 2016. *Terracidiphilus gabretensis* gen. nov., sp nov., an abundant and active forest soil acidobacterium important in organic matter transformation. *Applied and Environmental Microbiology* 82, 560-569.

Garten Jr CT, Cooper LW, Post WM, Hanson PJ. 2000. Climate controls on forest soil C isotope ratios in the southern Appalachian Mountains. *Ecology* 81, 1108-1119.

Garten Jr CT, Hanson PJ, Todd Jr DE, Lu BB, Brice DJ. 2007. Natural ^{15}N- and ^{13}C-abundance as indicators of forest nitrogen status and carbon dynamics In: Lajtha K, Michener RH (Eds.), *Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific Publishing: Malden, MA, USA pp. 75-96.

Gallardo A, Schlesinger WH, 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. *Soil Biology and Biochemistry* 26, 1409–1415.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, et al. 2004. Nitrogen cycle: past, present, and future. *Biogeochemistry* 70, 153-226.

Giguere AT, Taylor AE, Myrold DD, Bottomley PJ. 2015. Nitrification responses of ammonia-oxidizing archaea and bacteria to ammonium concentrations. *Soil Science Society of America Journal* 79, 1366-1374.

Gill R, Burke IC, Milchunas DG, Lauenroth WK. 1999. Relationship between root biomass and organic matter pools in the shortgrass steppe of eastern Colorado. *Ecosystems* 2, 226-236.

Goldmann K, Schöning I, Buscot F, Wubet T. 2015. Forest management type influences diversity and community composition of soil fungi across temperate forest ecosystems. *Frontiers in Microbiology* 6, doi:10.3389/fmicb.2015.01300.

Gömöryová E, Ujházy K, Martinák M, Gömöry D. 2013. Soil microbial community response to variation in vegetation and abiotic environment in a temperate old-growth forest. *Applied Soil Ecology* 68, 10-19.

Good IJ. 1953. The population frequencies of species and the estimation of population parameters. *Biometrika* 40, 237-264.

Gremion F, Chatzinotas A, Harms H. 2003. Comparative 16S rDNA and 16S rRNA sequence analysis indicates that Actinobacteria might be a dominant part of the metabolically active bacteria in heavy metal-contaminated bulk and rhizosphere soil. *Environmental Microbiology* 5, 896–907.

Gubry-Rangin C, Nicol GW, Prosser JI. 2010. Archaea rather than bacteria control nitrification in two agricultural acidic soils. *FEMS Microbiology Ecology* 74, 566-574.

Gubry-Rangin C, Hai B, Quince C, Engel M, Thomson BC, et al. 2011. Niche specialization of terrestrial archaeal ammonia oxidizers. *Proceedings of the National Academy of Sciences* 108, 21206-21211.

Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, et al., The Human Microbiome Consortium, Petrosino JF, Knight R, Birren BW. 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Research* 21, 494-504.

Hai B, Diallo NH, Sall S, Haesler F, Schauss K, et al. 2009. Quantification of key genes steering the microbial nitrogen cycle in the rhizosphere of sorghum cultivars in tropical agroecosystems. *Applied and Environmental Microbiology* 75, 4993-5000.

Han FX, Plodinec MJ, Su Y, Monts DL, Li Z. 2007. Terrestrial carbon pools in the southeast and south-central United States. *Climate Change* 84, 191-202.

Hankinson TR, Schmidt EL. 1988. An acidophilic and neutrophilic *Nitrobacter* strain isolated from the numerically predominant nitrite-oxidizing population of an acidic forest soil. *Applied and Environmental Microbiology* 54, 1536-1540.

Hannam KD, Quideau SA, Kishchuk BE. 2006. Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta. *Soil Biology and Biochemistry* 38, 2565–2575.

Hansen MC, Stehman SV, Potapov PV. 2010. Quantification of global gross forest cover loss. *Proceedings of the National Academy of Sciences* 107, 8650-8655.

Hartmann M, Howes CG, VanInsberghe D, Yu H, Bachar D, et al. 2012. Significant and persistent impact of timber harvesting on soil microbial communities in northern coniferous forests. *The ISME Journal* 6, 2199-2218.

Hassett JE, Zak DR. 2005. Aspen harvest intensity decreases microbial biomass, extracellular enzyme activity, and soil nitrogen cycling. *Soil Science Society of America Journal* 69, 227-235.

Hatzenpichler R, Lebedeva EV, Spieck E, Stoecker K, Richter A, et al. 2008. A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. *Proceedings of the National Academy of Sciences* 105, 2134-2139.

Hatzenpichler R. 2012. Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. *Applied and Environmental Microbiology* 78, 7501-7510.

Hazlett PW, Morris DM, Fleming RL. 2014. Effects of biomass removal on site carbon and nutrients and jack pine growth in boreal forests. *Soil Science Society of America Journal* 78, S183-S195.

Heinonsalo J, Koskiahde I, Sen R. 2007. Scots pine bait seedling performance and root colonizing ectomycorrhizal fungal community dynamics before and during the 4 years after forest clear-cut logging. *Canadian Journal of Forest Research* 37, 415-429.

He JZ, Xu ZH, Hughes J. 2005. Pre-lysis washing improves DNA extraction from forest soil. Soil Biology and Biochemistry 37, 2337-2341.

Hibbett DS, Ohman A, Glotzer D, Nuhn M, Kirk P, et al. 2011. Progress in molecular and morphological taxon discovery in fungi and options for formal classification of environmental sequences. *Fungal Biology Reviews* 25, 38-47.

Hobbie EA, Quimette AP. 2009. Controls of nitrogen isotope patterns in soil profiles. *Biogeochemistry* 95, 355-371.

Hodges DG, Hartsell AJ, Brandeis C, Brandeis TJ, Bentley JW. 2011. Recession effects on forests and forest products industries of the south. *Forest Productivity Journal* 61, 614-624.

Högberg P. 1997. ^{15}N natural abundance in soil-plant systems. *New Phytologist* 137, 179–203.

Hollister EB, Schadt CW, Palumbo AV, Ansley RJ, Boutton TW. 2010. Structural and functional diversity of soil bacterial and fungal communities following woody plant encroachment in the southern Great Plains. *Soil Biology and Biochemistry* 42, 1816-1824.

Holmes WE, Zak DR. 1994. Soil microbial biomass dynamics and net nitrogen mineralization in Northern hardwood ecosystems. *Soil Science Society of America Journal* 58, 238–243.

Houghton RA. 2007. Balancing the global carbon budget. *Annual Review of Earth and Planetary Sciences* 35, 315-347.

Huang Z, Clinton PW, Davis MR. 2011. Post-harvest residue management effects on recalcitrant carbon pools and plant biomarkers within the soil heavy fraction in *Pinus radiata* plantations. *Soil Biology and Biochemistry* 43, 404-412.

Huang Z, He Z, Wan X, Hu Z, Fan S, et al. 2013. Harvest residue management effects on tree growth and ecosystem carbon in a Chinese fir plantation in subtropical China. *Plant and Soil* 364, 303-314.

Huber KJ, Wust PK, Rohde M, Overmann J, Foesel BU. 2014. Aridibacter famidurans gen. nov., sp nov and *Aridibacter kavangonensis* sp nov., two novel members of subdivision 4 of the Acidobacteria isolated from semiarid savannah soil. *International Journal of Systematic and Evolutionary Microbiology* 64, 1866–1875.

Hu HW, Xu ZH, He JZ. 2014a. Ammonia-oxidizing archaea play a predominant role in acid soil nitrification. *Advances in Agronomy* 125, 261-302.

Hu Z, He Z, Huang Z, Fan S, Yu Z, et al. 2014b. Effects of harvest residue management on soil carbon and nitrogen processes in a Chinese fir plantation. *Forest Ecology and Management* 326, 163-170.

Hynes HM, Germida JJ. 2012. Relationship between ammonia oxidizing bacteria and bioavailable nitrogen in harvested forest soils of central Alberta. *Soil Biology and Biochemistry* 46, 18-25.

ISO. 2012. Soil quality – Method to directly extract DNA from soil samples. ISO Standard 11063.

Itoh T, Yamanoi K, Kudo T, Ohkuma M, Takashina T. 2011. *Aciditerrimonas ferrireducens* gen. nov., sp. nov., an iron-reducing *Thermoacidophilic actinobacterium* isolated from a solfataric field. *International Journal of Systematic and Evolutionary Microbiology* 61, 1281–1285.

James J, Devine W, Harrison R, Terry T. 2014. Deep soil carbon: Quantification and modeling in subsurface layers. *Soil Science Society of America Journal* 78, S1-S10.

James J, Knight E, Gamba V, Harrison R. 2015. Deep soil: Quantification, modeling, and significance of subsurface nitrogen. *Forest Ecology and Management* 336, 194-202.

James J, Harrison R. 2016. The effect of harvest on forest soil carbon: A meta-analysis. *Forests* 7, doi:10.3390/f7120308.

Janowiak M, Webster C. 2010. Promoting ecological sustainability in woody biomass harvesting. *Journal of Forestry* 108, 16–23.

Janssen PH, Yates PS, Grinton BE, Taylor PM, Sait M. 2002. Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. *Applied and Environmental Microbiology* 68, 2391-2396.

Janssen PH. 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Applied and Environmental Microbiology* 72, 1719-1728.

Jerabkova L, Prescott CE, Titus BD, Hope GD, Walters MB. 2011. A meta-analysis of the effects of clearcut and variable retention harvesting on soil nitrogen fluxes in boreal and temperate forests. *Canadian Journal of Forest Research* 41, 1852-1870.

Jesus ED, Marsh TL, Tiedje JM, Moreira FMD. 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils. *The ISME Journal* 3, 1004-1011.

Jiang QQ, Bakken LR. 1999. Comparison of *Nitrosospira* strains isolated from terrestrial environments. *FEMS Microbiology Ecology* 30, 171-186.

Jobbagy EG, Jackson RB. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* 10, 423–436.

Joergensen RG, Wu J, Brookes PC. 2011. Measuring soil microbial biomass using an automated procedure. *Soil Biology and Biochemistry* 43, 873–876.

Johansson M, Berg B, Meentemeyer V. 1995. Litter mass-loss rates in late stages of decomposition in a climate transect of pine forests. Long-term decomposition in a Scots pine forest. *Canadian Journal of Botany* 73, 1509-1521.

Johnson DW, Curtis PS. 2001. Effects of forest management on soil C and N storage: Meta-analysis. *Forest Ecology and Management* 140, 227-238.

Johnson DW, Todd DE. 1998. Harvesting effects on long-term changes in nutrient pools of mixed oak forests. *Soil Science Society of America Journal* 62, 1725-1735.

Johnson DW, Turner J. 2014. Nitrogen budgets of forest ecosystems: A review. *Forest Ecology and Management* 318, 370-379.

Jones HS, Beets PN, Kimberley MO, Garrett LG. 2011. Harvest residue management and fertilisation effects on soil carbon and nitrogen in a 15-year-old *Pinus radiata* plantation forest. *Forest Ecology and Management* 262, 339-347.

Jones HS, Garrett LG, Beets PN, Kimberley MO, Oliver GR. 2008. Impacts of harvest residue management on soil carbon stocks in a plantation forest. *Soil Science Society of America Journal* 72, 1621-1627.

Jumpponen A, Jones KL, Blair J. 2010. Vertical distribution of fungal communities in tallgrass prairie soil. *Mycologia* 102, 1027-1041.

Karhu K, Hilasvuori E, Fritze H, Biasi C, Nykanen H, et al. 2016. Priming effect increases with soil depth in a boreal forest soil. *Soil Biology and Biochemistry* 99, 104-107.

Kellman L, Kumar S, Diochon A. 2014. Soil nitrogen dynamics within profiles of a managed moist temperate forest chronosequence consistent with long-term harvesting-induced losses. *Journal of Geophysical Research* 119, 1309-1321.

Kielak A, Pijl AS, van Veen JA, Kowalchuk GA. 2009. Phylogenetic diversity of Acidobacteria in a former agricultural soil. *The ISME Journal* 3, 378–382.

Kielak AM, van Veen JA, Kowalchuk GA. 2010. Comparative analysis of Acidobacterial genomic fragments from terrestrial and aquatic metagenomics libraries, with emphasis on Acidobacteria subdivision 6. *Applied and. Environmental Microbiology* 76, 6769-6777.

Kim JS, Sparovek G, Longo RM, DeMelo WJ, Crowley D. 2007. Bacterial diversity of terra preta and pristine forest soil from the Western Amazon. *Soil Biology and Biochemistry* 39, 684-690.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (Eds.). 2008. Ainsworth & Bisby's Dictionary of the Fungi. CAB International: Wallingford, UK.

Kreutzweiser DP, Hazlett PW, Gunn JM. 2008. Logging impacts on the biogeochemistry of boreal forest soils and nutrient export to aquatic systems: A review. *Environmental Review* 16, 157-179.

Kuske CR, Ticknor LO, Miller ME, Dunbar JM, Davis JA, et al. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Applied and Environmental Microbiology* 68, 1854-1863.

Lajtha K, Driscoll CT, Jarrell WM, Elliott ET. 1999. Soil phosphorus: characterization and total element analysis. In: Roberts GP, Coleman DC, Bledsoe CS, Sollins P (Eds.), *Standard Soil Methods for Long-Term Ecological Research*, Oxford University Press, New York, NY, USA, pp. 115-142.

Lal R. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science* 304, 1623-1627.

Lal R. 2005. Forest soils and carbon sequestration. Forest Ecology and Management 220, 242-258.

Laiho R, Sanchez F, Tiarks A, Dougherty PM, Trettin CC. 2003. Impacts of intensive forestry on early rotation trends in site carbon pools in the southeastern US. *Forest Ecology and Management* 174, 177-189.

Lauber CL, Hamady M, Knight R, Fierer N. 2009. Pyrosequencing- based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75, 5111-5120.

Lazaruk LW, Kernaghan G, Macdonald SE, Khasa D. 2005. Effects of partial cutting on the ectomycorrhizae of *Picea glauca* forests in northwestern Alberta. *Canadian Journal of Forest Research* 35, 1442-1454.

LeBauer DS, Treseder KK. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89, 371-379.

Leininger S, Urich T, Schloter M, Schwark L, Qi J, et al. 2006. Archaea predominate among ammonia oxidizing prokaryotes in soils. *Nature* 442, 806-809.

Lemos LN, Fulthrope RR, Triplett EW, Roesch LFW. 2011. Rethinking microbial diversity analysis in the high throughput sequencing era. *Journal of Microbiological Methods* 86, 42-51.

Lens P. 2009. Sulfur Cycle. In: Schaechter M (Ed.), Encyclopedia of Microbiology (3rd ed.). Elsevier, NY, pp. 299-321.

Leung HTC, Maas KR, Wilhelm RC, Mohn WW. 2016. Long-term effects of timber harvesting on hemicellulolytic microbial populations in coniferous forest soils. *The ISME Journal* 10, 363-375.

Lewandowski TE, Forrester JA, Mladenoff DJ, D'Amato W, Palik BJ. 2016. Response of the soil microbial community and soil nutrient bioavailability to biomass harvesting and reserve tree retention in northern Minnesota aspen-dominated forests. *Applied Soil Ecology* 99, 110-117.

Liles MR, Manske BF, Bintrim SB, Handelsman J, Goodman RM. 2003. A census of rRNA genes and linked genomic sequences with in a soil metagenomics library. *Applied and Environmental Microbiology* 69, 2684-2691.

Li Q, Allen HL, Wilson CA. 2003. Nitrogen mineralization dynamics following the establishment of a loblolly plantation. *Canadian Journal of Forest Research* 33, 364-374.

Li QC, Allen HL, Wollum AG. 2004. Microbial biomass and bacterial functional diversity in forest soils: effects of organic matter removal, compaction, and vegetation control. *Soil Biology and Biochemistry* 36, 571-579.

Liu X, Chen C, Wang W, Hughes JM, Lewis T, et al. 2015. Vertical distribution of soil denitrifying communities in a wet sclerophyll forest under long-term repeated burning. M*icrobial Ecology* 70, 993-1003.

Lu X, Bottomley PJ, Myrold DD. 2015. Contributions of ammonia-oxidizing archaea and bacteria to nitrification in Oregon forest soils. *Soil Biology and Biochemistry* 85, 54-62.

Mack J, Hatten J, Sucre E, Roberts S, Leggett Z, et al. 2014. The effect of organic matter manipulations on site productivity, soil nutrients, and soil carbon on a southern loblolly pine plantation. *Forest Ecology and Management* 326, 25-35.

Marchman SC, Miwa M, Summer WB, Terrell S, Jones DG, et al. 2015. Clearcutting and pine effects on nutrient concentrations and export in two mixed use headwater streams: Upper Coastal Plain, Southeastern USA. *Hydrological Processes* 29, 13-28.

Mariotti A. 1983. Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. *Nature* 303, 685-687.

Mary B, Mariotti A, Morel JA. 1992 Use of ^{13}C variations at natural abundance for studying the biodegradation of root mucilage, roots and glucose in soil. *Soil Biology and Biochemistry* 24, 1065–1072.

Maithani K, Tripathi RS, Arunachalam A, Pandey HN. 1996. Seasonal dynamics of microbial biomass C, N and P during regrowth of a disturbed subtropical humid forest in north-east India. *Applied Soil Ecology* 4, 31–37.

Mariani L, Chang SX, Kabzems R. 2006. Effects of tree harvesting, forest floor removal, and compaction on soil microbial biomass, microbial respiration, and N availability in a boreal aspen forest in British Columbia. *Soil Biology and Biochemistry* 38, 1734-1744.

Martínez-Garcia LB, Ochoa-Hueso R, Manrique E, Pugnaire FI. 2015. Different mycorrhizal fungal strains determine plant community response to nitrogen and water availability. *Journal of Plant Nutrition and Soil Science* 178, 146-154.

Mayor JR, Mack MC, Schuur EAG. 2015. Decoupled stoichiometric, isotopic, and fungal responses of an ectomycorrhizal black spruce forest to nitrogen and phosphorus additions. *Soil Biology and Biochemistry* 88, 247-256.

McGuire KL, Treseder KK. 2010. Microbial communities and their relevance for ecosystem models: decomposition as a case study. *Soil Biology and Biochemistry* 42, 529–535.

McNulty SG, Vose JM, Swank WT, 1996. Potential climate change effects on loblolly pine forest productivity and drainage across the southern United States. *Ambio* 25, 449-453

Mehlich A. 1978. New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese and zinc. Communications in Soil Science and Plant Analysis 9, 477–492.

Mendes LW, Kuramae EE, Navarrete AA, van Veen JA, Tsai SM. 2014. Taxonomical and functional microbial community selection in soybean rhizosphere. *The ISME Journal* 8, 1577-1587.

Meng H, Li K, Mie M, Wan JR, Quan ZX, et al. 2013. Responses of bacterial and fungal communities to an elevation gradient in a subtropical montane forest of China. *Applied Microbiology and Biotechnology* 97, 2219-2230.

Martens-Habbena W, Berube PM, Urakawa H, de la Torre JR, Stahl DA. 2009. Ammonia oxidation kinetics determine niche separation of nitrifying archaea and bacteria. *Nature* 461, 976-979.

Metting FB. 1992. Structure and physiological ecology of soil microbial communities. In: Metting FB (Ed.), *Soil Microbial Ecology*. Marcel Dekker, Inc.: New York, NY, USA, pp 3-25.

Miller OK, Miller H. 1988. Gasteromycetes: Morphological and developmental features, with keys to the orders, families, and genera. Mad River Press: Eureka, CA, USA.

Miller L, Buyck B. 2002. Molecular phylogeny of the genus *Russula* in Europe with a comparison of modern infragenetic classifications. *Mycological Research* 106, 259-276.

Minasny B, McBratney AB, Brough DM, Jacquier D. 2011. Models relating soil pH measurements in water and calcium chloride that incorporate electrolyte concentration. *European Journal of Soil Science* 62, 728-732.

Mitchell MJ. 2011. Nitrate dynamics of forested watersheds: spatial and temporal patterns in North America, Europe and Japan. *Journal of Forest Research* 16, doi:10.1007/s10310-011-0278-1.

Nadelhoffer KJ, Fry B. 1994. Nitrogen isotope studies in forest ecosystems. In: Lajtha K, Michener RH (Eds.), *Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific Publishing: Malden, MA, USA pp. 22-44.

Navarrete AA, Cannavan FS, Taketani RG, Tsai SM. 2010. A molecular survey of the diversity of microbial communities in different Amazonian agricultural model systems. *Diversity* 2, 787-809.

Navarrete AA, Kuramae EE, de Hollander M, Pijl AS, van Veen JA, et al. 2013. Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *FEMS Microbiology Ecology* 83, 607–621.

Nave LE, Vance ED, Swanston CW, Curtis PS. 2010. Harvest impacts on soil carbon storage in temperate forests. *Forest Ecology and Management* 259, 857-866.

Nelson KN, Neilson JW, Root RA, Chorover J, Maier RM. 2015. Abundance and activity of 16S rRNA, *amoA*, *nifH* bacterial genes during assisted phytostabilization of mine tailings. *International Journal of Phytoremediation* 17, 493-502.

Neu J, Jones PD, Demarais S, Ezell AW, Riffell SK, et al. 2014. Retained woody structure in 1- to 2-year-old loblolly pine (*Pinus taeda* L.) plantations in Mississippi, Louisiana, and Arkansas: Implications for wildlife conservation. *Journal of Sustainable Forestry* 33, 152-172.

Nicol GW, Leininger S, Schleper C, Prosser JI. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. E*nvironmental Microbiology* 10, 2966-2978.

Nie M, Meng H, Li K, Wan JR, Quan ZX, et al. 2012. Comparison of bacterial and fungal communities between natural and planted pine forests in subtropical China. *Current Microbiology* 64, 34-42.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson KH. 2008. Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics* 4, 193-201.

Nilsson RH, Ryberg M, Abarenkov K, Sjokvist E, Kristiansson E. 2009. The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. *FEMS Microbiology Letters* 296, 97-101.

Nilsson RH, Veldre V, Hartmann M, Unterseher M, Amend A, et al. 2010. An open source package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. *Fungal Ecology* 3, 284-287.

Noormets A, Epron D, Domec JC, McNulty SG, Fox T, et al. 2015. Effects of forest management on productivity and carbon sequestration: A review and hypothesis. *Forest Ecology and Management* 355, 124-140.

Norman JS, Barrett JE. 2014. Substrate and nutrient limitation of ammonia oxidizing bacteria and archaea in temperate forest soil. *Soil Biology and Biochemistry* 69, 141-146.

Norman JS, Barrett JE. 2016. Substrate availability drives spatial patterns in richness of ammonia-oxidizing bacteria and archaea in temperate forest soils. *Soil Biology and Biochemistry* 94, 169-172.

Norwine J, Giardino JR, Krishnamurthy S. 2005. Water for Texas. Texas A&M University Press, College Station, TX.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R. 2005. Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* 71, 5544-5550.

Offre P, Prosser JI, Nicol GW. 2009. Growth of ammonia-oxidizing archaea in soil microcosms is inhibited by acetylene. *FEMS Microbiology Ecology* 70, 99–108.

Oswalt SN, Smith WB, Miles PD, Pugh SA. 2014. Forest Resources of the United States, 2012: a technical document supporting the Forest Service 2015 update of the RPA Assessment. Gen. Tech. Rep. WO-91. Washington, DC: U.S. Department of Agriculture, Forest Service, Washington Office. 218 p.

Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, et al. 2011. A large and persistent carbon sink in the world's forests. *Science* 333, 988-993.

Pankratov TA, Serkebaeva YM, Kulichevskaya IS, Liesack W, Dedysh SN. 2008. Substrate-induced growth and isolation of Acidobacteria from acidic Sphagnum peat. The *ISME Journal.* 2, 551-560.

Pankratov TA, Kirsanova LA, Kaparullina EN, Kevbrin VV, Dedysh SN. 2012. *Telmatobacterbradus* gen. nov., sp nov., a cellulolytic facultative anaerobe from subdivision 1 of the Acidobacteria, and emended description of *Acidobacterium capsulatum* Kishimoto et al., 1991. *International Journal of Systematic and Evolutionary Microbiology* 62, 430–437.

Pardo LH, Nadelhoffer KJ. 2010. Using nitrogen isotope ratios to assess terrestrial ecosystems at regional and global scales. In: West JB, Bowen GJ, Dawson TE, Tu KP (Eds.), *Isoscapes: Understanding Movement, Pattern, and Process on Earth through Isotope Mapping*. Springer, New York, NY, USA pp. 221-249.

Parke JL, Gurian-Sherman D. 2001. Diversity of the Burkholderia cepacia complex and implications for risk assessment of biological control strains. *Annual Reviews of Phytopathology* 39: 225-258.

Parsley LC, Linneman J, Goode AM, Becklund K, George I, et al. 2011. Polyketide synthase pathways identified from a metagenomics library are derived from soil Acidobacteria. *FEMS Microbiology Ecology* 78, 176–187.

Pataki DE, Ellsworth DS, Evans RD, Gonzalez-Meler M, King J, et al. 2003. Tracing changes in ecosystem function under elevated carbon dioxide conditions. *Bioscience* 53, 805-818.

Paul KI, Polglase PJ, O'Connell AM, Carlyle JC, Smethhurst PJ, et al. 2003. Defining the relation between soil water content and net nitrogen mineralization. *European Journal of Soil Science* 54, 39-47.

Paulson JN, Stine OC, Bravo HC, Pop M. 2013. Differential abundance analysis for microbial marker-gene surveys. *Nature Methods* 10, 1200-1202.

Penton CR, St. Louis D, Cole JR, Luo Y, Wu L, et al. 2013. Fungal diversity in permafrost and tallgrass prairie soils under experimental warming conditions. *Applied and Environmental Microbiology* 79, 7063-7072.

Pereira e Silva MC, Poly F, Guillaumaud N, van Elsas JD, Salles JF. 2012. Fluctuations in ammonia oxidizing communities across agricultural soils are driven by soil structure and pH. *Frontiers in Microbiology* 3, doi:10.3389/fmicb.2012.00077.

Pester M, Rattei T, Flechl S, Gröngröft A, Richter A, et al. 2012. *amoA*-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of amoA genes from soils of four different geographic regions. *Environmental Microbiology* 14, 525-539.

Piatek KB, Allen HL. 2000. Site preparation effects on foliar N and P use, retranslocation, and transfer to litter in 15-years old Pinus taeda. *Forest Ecology and Management* 129, 143-152.

Porras-Alfaro A, Herrera J, Natvig DO, Lipinski K, Sinsabaugh RL. 2011. Diversity and distribution of soil fungal communities in a semiarid grassland. *Mycologia* 103, 10-21.

Ponder F, Fleming RL, Berch S, Busse MD, Elioff JD, et al. 2012. Effects of organic matter removal, soil compaction and vegetation control on 10th year biomass and foliar nutrition: LTSP continent-wide comparisons. *Forest Ecology and Management* 278, 35-54.

Potthoff M, Jackson LE, Solow S, Jorgensen RG. 2009. Below and above ground responses to lupine and litter mulch in a California grassland restored with native bunchgrasses. *Applied Soil Ecology* 42, 124 –133.

Powers RF, Scott DA, Sanchez FG, Voldseth RA, Page-Dumroese D, et al. 2005. The North American long-term soil productivity experiment: findings from the first decade of research. *Forest Ecology and Management* 220, 31-50.

Powers RF. 2006. Long term soil productivity: genesis of the concept and principles behind the program. *Canadian Journal of Forest Research* 36, 519-528.

Prest D, Kellman L, Lavigne MB. 2014. Mineral soil carbon and nitrogen still low three decades following clearcut harvesting in a typical Acadian Forest stand. *Geoderma* 214-215, 62-69.

Prosser JI, Nicol GW. 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environmental Microbiology* 10, 2931-2941.

Quaiser A, Ochsenreiter T, Lanz C, Schuster SC, Treusch AH, et al. 2003. Acidobacteria form a coherent but highly diverse group within the bacterial domain: evidence from environmental genomics. *Molecular Microbiology* 50, 563–575.

Rasche F, Knapp D, Kaiser C, Koranda M, Kitzler B, et al. 2011. Seasonality and resource availability control bacterial and archaeal communities in soil of a temperate beech forest. *The ISME Journal* 5, 389-402.

Redecker D, Morton JB, Bruns TD. 2000. Ancestral Lineages of Arbuscular Mycorrhizal Fungi (Glomales). *Molecular Phylogenetics and Evolution* 14, 276-284.

Redecker D, Raab P. 2006. Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* 98, 885-895.

Riaz K, Elmerich C, Moreira D, Raffoux A, Dessaux Y, et al. 2008. A metagenomics analysis of soil bacteria extends the diversity of quorum- quenching lactonases. *Environmental Microbiology* 10, 560–570.

Rice AV, Currah RS. 2006. Two new species of Pseudogymnoascus with Geomyces anamorphs and their phylogenetic relationship with Gymnostellatospora. *Mycologia* 98, 307-318.

Richter DD, Markewitz D, Trumbore SE, Wells CG. 1999. Rapid accumulation and turnover of carbon in a re-establishing forest. *Nature* 400, 56-58.

Rinaldi AC, Comandini O, Kuyper TW. 2008. Ectomycorrhizal fungal diversity: Separating the wheat from the chaff. *Fungal Diversity* 33: 1-45.

Roberts P. 1999. Rhizoctonia-forming fungi: a taxonomic guide. Royal Botanical Gardens, Kew, England.

Robinson D. 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. *TRENDS in Ecology and Evolution* 16, 153-162.

Rotthauwe JH, Witzel KP, Liesack W. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and Environmental Microbiology* 63, 4704-4712.

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, et al. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal* 4, 1340-1351.

Rudgers JA, Bell-Dereske L, Crawford KM, Emery SM. 2015. Fungal symbiont effects on dune plant diversity depend on precipitation. *Journal of Ecology* 103: 219-230.

Rumpel C, Kogel-Knabner I. 2011. Deep soil organic matter-a key but poorly understood component of the terrestrial C cycle. *Plant and Soil* 338, 143-158.

Sánchez C, Bedmar EJ, Delgado MJ. 2011. Denitrification in legume-associated endosymbiotic bacteria. In: Moir JWB (Ed.), Nitrogen Cycling in Bacteria – Molecular Analysis. Caister Academic Press, UK, pp. 197-210.

Santruckova H, Bird MI, Lloyd J. 2000. Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. *Functional Ecology* 14, 108–114.

Sargova-Mareckova M, Cermak L, Novotna J, Plhackova K, Forstova J, et al. 2008. Innovative methods for soil DNA purification tested in soils with widely differing characteristics. *Applied Environmental Microbiology* 74, 2902-2907.

Schleper C, Nicol GW. 2010. Ammonia-oxidising archaea – Physiology, ecology and evolution. *Advances in Microbial Physiology* 57, 1-41.

Schlesinger WH, Bernhardt ES. 2013. Biogeochemistry: An analysis of global change. Elsevier, NY.

Scott DA, Tiarks AE, Sanchez FG, Elliott-Smith M, Stagg R. 2004. Forest soil productivity on the southern Long-term Soil Productivity sites at age 5. In: Connor KF (Ed.), Proceedings of the 12th Biennial Southern Silvicultural Research Conference, Gen, Tech. Rpt. SRS – 71, Asheville, N.C.: USDA Forest Service, Southern Research Station.

Scott DA, Eaton RJ, Foote JA, Vierra B, Boutton TW, et al. 2014. Soil ecosystem services in loblolly pine plantation 15 years after harvest, compaction, and vegetation control. *Soil Science Society of America Journal* 78, 2032-2040.

Scott DA. 2016. A brief overview of the 25-year-old long-term soil productivity study in the south. In: Schweitzer CJ, Clatterbuck WK, Oswalt CM (Eds.). *Proceedings of the 18th Biennial Southern Sivicultural Research Conference*, pp 18-26.

Seidl R, Schelhaas MJ, Rammer W, Verkerk PJ. 2014. Increasing forest disturbance in Europe and their impact on carbon storage. *Nature Climate Change* 4, 806-810.

Simpson EH. 1949. Measurement of diversity. *Nature* 163: 688.

Slesak RA, Schoenholtz SH, Harrington TB, Meehan NA. 2011. Response of soil carbon and nitrogen to harvest intensity and competing vegetation control in Douglas-fir (*Pseudotsuga menziesi*i) plantations of the Pacific Northwest. *Forest Science* 57, 26-35.

Smaill SJ, Clinton PW, Greenfield LG. 2008. Postharvest organic matter removal effects on FH layer and mineral soil characteristics in four New Zealand *Pinus radiata* plantations. *Forest Ecology and Management* 256, 558-563.

Smolander A, Kitunen V, Tamminen P, Kukkola M. 2010. Removal of logging residue in Norway spruce thinning stands: long-term changes in organic layer properties. *Soil Biology and Biochemistry* 42, 1222-1228.

Solly EF, Schöning I, Boch S, Kandeler E, Marhan S, et al. 2014. Factors controlling decomposition rates of fine root litter in temperate forests and grasslands. *Plant and Soil* 382, 203-218.

Sorensen PO, Germino MJ, Feris KP. 2013. Microbial community response to 17 years of altered precipitation are seasonally dependent and coupled to co-varying effects of water content on vegetation and soil C. *Soil Biology and Biochemistry* 64, 155-163.

Sparling GP. 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. *Australian Journal of Soil Research* 30, 195–207.

Stahl DA, de la Torre JR. 2012. Physiology and diversity of ammonia-oxidizing archaea. *Annual Review of Microbiology* 66, 83-101.

Stempfhuber B, Engel M, Fischer D, Neskovic-Prit D, Wubet T, et al. 2015. pH as a driver for ammonia-oxidizing archaea in forest soils. *Microbial Ecology* 69, doi:10.1007/s00248-014-0548-5.

Stopnišek N, Gubry-Rangin C, Höfferle Š, Nicol GW, Mandič-Mulec I, et al. 2010. Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment. *Applied and Environmental Microbiology* 76, 7626–7634.

Strous M. 2011. Beyond denitrification: Alternative routes to dinitrogen. In: Moir JWB (Ed.), Nitrogen Cycling in Bacteria – Molecular Analysis. Caister Academic Press, UK, pp. 123-135.

Sun Y, Wu J, Shao Y, Zhou L, Mai B, et al. 2011. Responses of soil microbial communities to prescribed burning in two paired vegetation sites in southern China. *Ecological Restoration* 26, 669-677.

Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (Eds.). 2005. *Principles and Applications of Soil Microbiology*. Pearson Education Inc., Upper Saddle River, NJ, USA.

Tan X, Chang SX, Kabzems R. 2005. Effects of soil compaction and forest floor removal on soil microbial properties and N transformations in a boreal forest long-term soil productivity study. *Forest Ecology and Management* 217, 158-170.

Tan X, Chang SX, Kabzems R. 2008. Soil compaction and forest floor removal reduced microbial biomass and enzyme activities in a boreal aspen forest soil. *Biology and Fertility of Soils* 44, 471-479.

Tanner EVJ, Sheldrake MWA, Turner BL. 2016. Changes in soil carbon and nutrients following 6 years of litter removal and addition in a tropical semi-evergreen rain forest. *Biogeosciences* 13, 6183-6190.

Taylor AE, Vajrala N, Giguere AT, Gitelman AL, Arp DJ, et al. 2013. Use of aliphatic n-alkynes to discriminate soil nitrification activities of ammonia-oxidizing Thaumarchaea and bacteria. *Applied and Environmental Microbiology* 79, 6544-6551.

Tedersoo L, May TW, Smith ME. 2010a. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* 20, 217-263.

Tedersoo L, Nilsson RH, Abarenkov K, Jairus T, Sadam A, et al. 2010b. 454 pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytologist* 188, 291-301.

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, et al. 2014. Global diversity and geography of soil fungi. *Science* 346, 1256688.

Templer PH, Arthur MA, Lovett GM, Weathers KC. 2007. Plant and soil natural abundance $\delta^{15}N$: indicators of relative rates of nitrogen cycling in temperate forest ecosystems. *Oecologia* 153, 399-406.

Terrat S, Plassart P, Bourgeois E, Ferreira S, Dequiedt S, et al. 2014. Meta-barcoded evaluation of the ISO standard 11063 DNA extraction procedure to characterize soil bacterial and fungal community diversity and composition. *Microbial Biotechnology* 8, 131-142.

Thébault A, Clément JC, Ibanez S, Geremia RA, Pérez CA, et al. 2014. Nitrogen limitation and microbial diversity at the treeline. *Oikos* 123, 729-740.

Thiffault E, Hannam KD, Pare D, Titus BD, Hazlett PW, et al. 2011. Effects of forest biomass harvesting on soil productivity in boreal and temperate forests – A review. *Environmental Reviews* 19, 278-309.

Toivanen T, Markkanen A, Kotiaho JS, Halme P. 2012. The effects of forest fuel harvesting on fungal diversity of clear cuts. *Biomass and Bioenergy* 39, 84-93.

Tourna M, Freitag TE, Prosser JI. 2010. Stable isotope probing analysis of interactions between ammonia oxidizers. *Applied and Environmental Microbiology* 76, 2468-2477.

Tourna M, Stieglmeier M, Spang A, Könneke M, Schintlmeister A, et al. 2011. *Nitrososphaera viennensis*, an ammonia oxidizing archeon from soil. *Proceedings of the National Academy of Sciences* 108, 8420-8425.

USDA/NRCS. 2003. Soil Survey of Trinity County, Texas. US Department of Agriculture, Natural Resource Conservation Service, Washington DC, USA.

Vance ED, Brookes PC, Jenkinson DS. 1987. Microbial biomass measurements in forest soils: the use of the chloroform fumigation-incubation method in strongly acid soils. *Soil Biology and Biochemistry* 19, 691-702.

Vanderwel MC, Coomes DA, Purves DW. 2013. Quantifying variation in forest disturbance, and its effect on aboveground biomass dynamics, across the eastern United States. *Global Change Biology* 19, 1504-1517.

VanInsberghe D, Maas KR, Cardenas E, Strachan CR, Hallam SJ, et al. 2015. Non-symbiotic *Bradyrhizobium* ecotypes dominate North American forest soils. *The ISME Journal* 9, 2435-2441.

van Lis R, Ducluzeau AL, Nitsche W, Schoepp-Cothenet B. 2011. The nitrogen cycle in the archaean: an intricate interplay of enzymatic and abiotic reactions. In: Moir JWB (Ed.), Nitrogen Cycling in Bacteria – Molecular Analysis Caister Academic Press, UK, pp. 1-23.

Vario CL, Neurath RA, Friedland AJ. 2014. Response of mineral soil carbon to clear-cutting in a northern hardwood forest. *Soil Science Society of America Journal* 78, 309-318.

Verhamme DT, Prosser JI, Nicol GW. 2011. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *The ISME Journal* 5, 1067-1071.

Villarreal-Ruiz L, Anderson IC, Alexander IJ. 2004. Interaction between an isolate from *Hymenoscyphus ericae* aggregate and roots of *Pinus* and *Vaccinium*. *New Phytologist* 164, 183-192.

Vitousek PM, Gosz JR, Grier CC, Melillo JM, Reiners WA. 1982. A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. *Ecological Monographs* 52, 155-177.

Vitousek PM, Howarth RW. 1991. Nitrogen Limitation on land and in the sea: How can it occur? *Biogeochemistry* 13, 87-115.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, et al. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications* 7, 737-750.

Wagg C, Barendregt C, Jansa J, van der Heijden GA. 2015. Complementarity in both plant and mycorrhizal fungal communities are not necessarily increased by diversity in the other. *Journal of Ecology* 103, 1233-1244.

Walker CB, de la Torre JR, Klotz MG, Urakawa H, Pinel N, et al. 2010. *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. *Proceedings of the National Academy of Sciences* 107, 8818-8823.

Wallenstein MD, McMahon S, Schimel J. 2007. Bacterial and fungal community structure in Arctic tundra tussock and shrub soils. *FEMS Microbiology Ecology* 59, 428-435.

Walmsley JD, Jones DL, Reynolds B, Price MH, Healy JR. 2009. Whole-tree harvesting can reduce second rotation forest productivity. *For Ecology and Management* 257, 1104-1111.

Wang JT, Zheng YM, Hu HW, Zhang L, Li J, et al. 2015. Soil pH determines the alpha diversity but not beta diversity of soil fungal community along altitude in a typical Tibetan forest ecosystem. *Journal of Soils and Sediments* 15, 1224-1232.

Wang QK, Wang SL. 2007. Soil organic matter under different forest types in Southern China. *Geoderma* 142, 349-356.

Warcup JH. 1951. The ecology of soil fungi. *Transactions of the British Mycological Society* 34, 376-399.

Wardle DA. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biological Reviews* 67, 321-358.

Ward NL, Challacombe JF, Janssen PH, Hentrissat B, Coutinho B, et al. 2009. Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Applied and Environmental Microbiology* 75, 2046-2056.

Weber C, Zak D, Hungate B, Jackson R, Vilgalys R, et al. 2011. Responses of soil cellulolytic fungal communities to elevated atmospheric CO_2 are complex and variable across five ecosystems. *Environmental Microbiology* 13, 2778-2793.

Webster G, Embley TM, Freitag TE, Smith Z, Prosser JI. 2005. Links between ammonia oxidizer species composition, functional diversity and nitrification kinetics in grassland soils. *Environmental Microbiology* 7, 676-684.

Wei X, Liu W, Waterhouse J, Armleder M. 2000. Simulations on impacts of different management strategies on long-term site productivity in lodgepole pine forests of the central interior of British Columbia. *Forest Ecology and Management* 133, 217-229.

Wertz S, Leigh AKK, Grayston SJ. 2012. Effects of long-term fertilization of forest soils on potential nitrification and on the abundance and community structure of ammonia oxidizers and nitrite oxidizers. *FEMS Microbiology Ecology* 79, 142-154.

Wessén E, Nyberg K, Jansson JK, Hallin S. 2010. Responses of bacterial and archaeal ammonia oxidizers to soil organic and fertilizer amendments under long-term management. *Applied Soil Ecology* 45, 193-200.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds.), *PCR protocols: A guide to methods and applications*. Academic Press, New York, NY, USA, pp. 315–322.

Wilhelm K, Rathsack B, Bockheim J. 2013. Effects of timber harvest intensity on macronutrient cycling in oak-dominated stands on sandy soils of northwest Wisconsin. *Forest Ecology and Management* 291, 1-12.

Woodall CW, Skog K, Smith JE, Perry CH. 2011. Criterion 5: Climate change and global carbon cycles. In: Robertson G, Gaulke P, McWilliams R, LaPlante S, Guldin R, (Eds.), *National Report on Sustainable Forests – 2010*. U.S. Department of Agriculture, Forest Service, Washington DC, USA.

Woodall CW, Domke GM, Riley KL, Oswalt CM, Crocker SJ, et al. 2013. A framework for assessing global change risks to forest carbon stocks in the United States. *PLoS ONE* 8, doi:10.1371/journal.pone.0073222.

Wubet T, Christ S, Schöning I, Boch S, Gawlich Met al. 2012. Differences in soil fungal communities between European beech (*Fagus sylvatica* L.) dominated forests are related to soil and understory vegetation. *PLoS ONE* 7, doi:10.1371/journal.pone.0047500.

Wuest S. 2014. Seasonal variation in soil organic carbon. *Soil Science Society of America Journal* 78, 1442-1447.

Wu J, Liu Z, Wang X, Sun Y, Zhou L, et al. 2011. Effects of understory removal and tree girdling on soil microbial community composition and litter decomposition in two Eucalyptus plantations in South China. *Functional Ecology* 25, 921-931.

Yamanaka T. 2003. The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi in vitro. *Mycologia* 95, 584-589.

Yanai RD. 1998. The effect of whole-tree harvesting on phosphorus cycling in a northern hardwood forest. *Forest Ecology and Management* 104, 281-295.

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

Yang ZH, Xiao Y, Zeng GM, Xu ZY, Liu YS. 2007. Comparison of methods for total community DNA extraction and purification from compost. *Applied Microbiology and Biotechnology* 74, 918-925.

Yao H, Gao Y, Nicol GW, Campbell CD, Prosser JI, et al. 2011. Links between ammonia oxidizer community structure, abundance, and nitrification potential in acidic soils. *Applied and Environmental Microbiology* 77, 4618-4625.

Yarwood SA, Bottomley PJ, Myrold DD. 2010. Soil microbial communities associated with Douglas-fir and red alder stands at high- and low-productivity forest sites in Oregon, USA. *Microbial Ecology* 60, 606-617.

Yeager CM, Northup DE, Grow CC, Barns SM, Kuske CR. 2005. Changes in nitrogen-fixing and ammonia-oxidizing bacterial communities in soil of a mixed conifer forest after wildfire. *Applied and Environmental Microbiology* 71, 2713-2722.

Zak DR, Tilman D, Parmenter RR, Rice CW, Fisher FM, et al. 1994. Plant production and soil microorganisms in late successional ecosystems: a continental-scale study. *Ecology* 75, 2333–2347.

Zeilinger S, Gupta VK, Dahms TES, Silva RN, Singh HB, et al. 2016. Friends of foes? Emerging insights from fungal interactions with plants. *FEMS Microbiology Reviews* 40, 182-207.

Zerpa JL, Allen HL, Campbell RG, Phelan J, Duzan H. 2010. Influence of variable organic matter retention on nutrient availability in a 10-year-old loblolly pine plantation. *Forest Ecology and Management* 259, 1480-1489.

Zhang JB, Muller C, Zhu TB, Cheng Y, Cai ZC. 2011. Heterotrophic nitrification is the predominant NO_3^- production mechanism in coniferous but not broad-leaf acid forest soil in subtropical China. *Biology and Fertility of Soils* 47, 533-542.

Zhang LM, Hu HW, Shen JP, He JZ. 2012. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. *The ISME Journal* 6, 1032-1045.

Zhang YG, Cong J, Lu H, Li GL, Qu YY, et al. 2014. Community structure and elevational diversity patterns of soil Acidobacteria. *Journal of Environmental Sciences* 26, 1717–1724

Zhang T, Wang NF, Liu HY, Zhang YQ, Yu LY. 2016. Soil pH is a key determinant of soil fungal community composition in the Ny-Ålesund region, Svalbard (High Arctic). Frontiers in Microbiology 7, doi:10.3389/fmicb.2016.00227.

Zinger L, Shahnavaz B, Baptis F, Geremia RA, Choler P. 2009. Microbial diversity in alpine tundra soils correlate with snow cover dynamics. *The ISME Journal* 3, 850-859.

Zummo LM, Friedland AJ. 2011. Soil carbon releases along a gradient of physical disturbance in a harvested northern hardwood forest. *Forest Ecology and Management* 261, 1016-1026.