

**Belowground Carbon and Nitrogen Cycling in a Loblolly Pine Forest Managed for
Bioenergy Production**

Kevan Joseph Minick

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Brian D. Strahm, Co-Chair

Thomas R. Fox, Co-Chair

Eric B. Sucre

John F. Fike

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ABSTRACT

Concern over rising atmospheric CO₂ due to fossil fuel combustion has intensified research into carbon-neutral energy and fuel production. Therefore, bioenergy production has expanded during the last decade, increasing demand for forest-based bioenergy feedstocks. Millions of acres of privately and industrially owned pine plantations exist across the southeastern US, representing a vast area of land that could be utilized to produce bioenergy without significant land-use change or diversion of agricultural resources from food production. Furthermore, loblolly pine (*Pinus taeda* L.) plantations offer the unique opportunity to utilize space between rows of planted trees to grow an herbaceous bioenergy crop, such as switchgrass (*Panicum virgatum* L.). This novel forest management regime has the potential to provide positive environmental and economic services, but hinges in part on impacts to soil carbon (C) and nitrogen (N) cycling, availability of belowground resources, and potential negative impacts of competition between pine and switchgrass on plant productivity. Three specific objectives were addressed in this study: 1) compare different bioenergy management regimes in regards to temporal dynamics of N cycling and availability following forest establishment (*see* Chapter 2); 2) determine the impact of loblolly pine and switchgrass intercropping on microbial N cycling processes (*see* Chapter 3); and 3) evaluate chemical and physical mechanisms of soil organic matter (SOM) stabilization and test their sensitivity to pine-switchgrass intercropping (*see* Chapter 4).

The study site was located in the Lower Coastal Plain physiographic province in Lenoir County, North Carolina, USA (35-12'59" N; 077-26'13" W). Soils were mapped as Pantego (fine-loamy, siliceous, semiactive, thermic Umbric Paleaquults) or Rains (fine-loamy, siliceous, semiactive, thermic Typic Paleaquults) soil series, both of which are very poorly drained. However, previous site management in the late 1960's and early 1970's included installation of ditches to lower the water table and reduce saturation at the soil surface. Additionally, bedding of soil in rows was used to raise root systems of planted loblolly pine seedlings above the water table, increase soil aeration, and reduce competition. Space between bedded rows of pine trees was referred to as the interbed.

Results from Chapter 2 showed that switchgrass significantly reduced interbed soil NH₄⁺ and NO₃⁻ concentrations by 39% and 60%, respectively, over the course of the timeframe (30 months) of this study. Surprisingly, in beds of the pine-switchgrass treatment significant increases in NO₃⁻ concentration were measured from July - December 2011.

From Chapter 3, gross N mineralization rates ranged from 0.18 - 4.7 μg N g⁻¹ soil d⁻¹, while gross nitrification rates ranged from 0.02 - 0.47 μg N g⁻¹ soil d⁻¹. At the 0-5 cm depth in switchgrass interbeds, gross N mineralization was reduced from April to November potentially reflecting microbial C limitations due to reduced soil C concentrations. At the 0-5 cm depth in beds of the pine-switchgrass treatment, gross N mineralization rates were elevated by 1.29 μg N

g^{-1} soil d^{-1} in November and $1.02 \mu\text{g N g}^{-1}$ soil d^{-1} in February on average corresponding to a 305% and 193% increase, respectively.

From Chapter 4, total C content in beds and interbeds ranged from 15 to 88 Mg C ha^{-1} and was reduced by 27% in beds of the pine-switchgrass treatment. Average C concentration for aggregate fractions was significantly lower in beds of the pine-switchgrass treatment at 0-5, 15-30, and 30-45 cm depths, amounting to ~23%, ~28%, and ~34% reduction, respectively. Values of $\delta^{13}\text{C}$ for the $>2000 \mu\text{m}$ aggregate size fraction at the 0-5 cm depth were diluted, corresponding to estimates of 13 - 25% of the $>2000 \mu\text{m}$ C pool comprised of new pine-derived C. For SOM fractionated by density, elevated C concentrations were found in the occluded light fractions in both beds and interbeds of the pine-switchgrass treatment. Enriched $\delta^{13}\text{C}$ in occluded light fractions led to estimates of 2.5 - 12.5% of this C fraction comprised of new switchgrass-derived C. In the free light fraction, new pine-derived C accounted for 15% and 9% of C at the 5-15 and 15-30 cm depth, respectively.

Three overarching conclusions were generated from my research: 1) switchgrass grown between loblolly pine trees effectively utilized excess soil NH_4^+ and NO_3^- when N availability was high following harvesting of a mature plantation proceeded by establishment of a second rotation of loblolly pine (*see* Chapter 2); 2) gross N mineralization rates were reduced under switchgrass during the growing season when soil C availability was low, but were elevated under switchgrass and adjacent loblolly pines when switchgrass was dormant and C availability was likely higher (*see* Chapter 3); and 3) SOM stabilized by physical or chemical mechanisms responded differently to pine-switchgrass intercropping, with losses in aggregate-stabilized C and gains in occluded, mineral-stabilized C. Furthermore, losses of aggregate C was associated with a significant reduction in total soil C in beds of the pine-switchgrass treatment. Results from ^{13}C mass balance suggested incorporation of switchgrass-derived C into occluded light fractions of beds and interbeds. Finally, incorporation of new pine-derived C into the $>2000 \mu\text{m}$ aggregate size fraction and free light fraction indicate pine inputs of particulate organic matter into these SOM fractions in beds of the pine-switchgrass treatment (*see* Chapter 4). I hypothesize that loblolly pines have increased root growth in beds in response to competition with switchgrass for N in the interbed, thereby alleviating seasonal microbial C limitations and stimulating microbial N cycling processes and increasing plant-available N.

Overall, this research suggests that soil C and N cycling in pine plantations is altered by intercropping of pine and switchgrass. Through a mechanistic understanding of how C and N are cycled in forests and the impact of various forest management regimes on soil C and N cycling, effective management strategies can be implemented to utilize forests for intensive biomass production while limiting loss of soil C and N, and in some cases even enhancing soil C and N retention. Future research initiatives should seek to unravel the complex belowground interactions between roots of different plant species and soil microbial communities competing for limiting resources. Understanding how these interactions drive soil C storage, N cycling and availability, and forest productivity will ultimately improve resource utilization in these managed ecosystems as well as our basic understanding of how natural and managed ecosystems function.

Dedication

To my wife

Patricia Minick

for her love, friendship, support, and wisdom in all experiences we have shared together

My daughter

Jade Minick

who has brought such joy to my life and helped me to look deeper into myself, life, and science

And my immediate family

Doug, Rosanne, and Andy Minick

for providing the opportunities, support, friendship, and guidance necessary to achieve this goal

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Contributing Authorship

Chapter 2.

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Brian D. Strahm (Virginia Tech)
Thomas R. Fox (Virginia Tech)
Eric B. Sucre (Weyerhaeuser Company)
Zakiya H. Leggett (Weyerhaeuser Company)
Jose S. Zerpa (Virginia Tech)

Chapter 3.

Chapter 3 has been submitted to Ecological Applications as of September 10, 2014

Brian D. Strahm (Virginia Tech)
Thomas R. Fox (Virginia Tech)
Eric B. Sucre (Weyerhaeuser Company)
Zakiya H. Leggett (Weyerhaeuser Company)

Chapter 4 will be submitted to Plant and Soil by December 2014

Chapter 4.

Brian D. Strahm (Virginia Tech)
Thomas R. Fox (Virginia Tech)
Eric B. Sucre (Weyerhaeuser Company)
Zakiya H. Leggett (Weyerhaeuser Company)

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Chapter 1. Introduction

1.1. Justification

Concern over rising atmospheric CO₂ and other greenhouse gases has intensified research into carbon (C)-neutral energy and fuel production. This is largely due to increased fossil fuel combustion and other anthropogenically driven inputs of greenhouse gases to the atmosphere. Reduction of atmospheric greenhouse gases can be achieved by increasing soil C sequestration and reducing CO₂ emissions by supplementing fossil fuel production with renewable biofuels. Loblolly pine (*Pinus taeda* L.) plantations in the southeastern United States offer a unique opportunity to utilize interbed space for the production of lignocellulosic herbaceous crops that could be converted to biofuels. Switchgrass (*Panicum virgatum* L.) has been identified as an environmentally and economically promising crop for bioenergy production (McLaughlin and Kszos, 2005; Parrish and Fike, 2005). Approximately 32 millions of acres of privately and industrially owned pine plantations exist across the southeastern United States (Fox et al., 2007), representing a vast area of land that could be utilized to produce bioenergy without significant land-use change or diversion of agricultural resources from food production. Furthermore, on many southern loblolly pine plantations, a minimal amount of land is actually utilized for aboveground tree production, with the rest as interbed space in which switchgrass could be grown. Sustainability of this intercropping system will depend on meeting both species' requirements for light, water, and essential nutrients, while minimizing negative impacts on the surrounding environment. Competition for belowground resources, such as water, nutrients, and rooting space, may also influence the growth response of loblolly pine to the presence of switchgrass. The introduction of a highly productive grass to these forests may also influence soil C cycling and nutrient availability through various effects on soil organic matter (SOM) quality, quantity, and stabilization, and the release of nutrients through microbial-mediated decomposition processes. Nitrogen (N) is typically considered the most limiting nutrient to primary productivity in terrestrial ecosystems. Therefore, understanding soil N cycling and availability will be important to evaluate how intercropping of loblolly pine and switchgrass will influence plant-available N. The goal of my research was to investigate C and N cycling in soils

as influenced by pine-switchgrass intercropping in a southern pine forests in order to better understand the environmental sustainability of this system.

1.2. Literature Review

1.2.1. Rooting Dynamics and Belowground Competition

Different rooting characteristics of pine and switchgrass will likely influence C and nutrient biogeochemistry. Switchgrass produces extensive fine-root networks with up to 50% of root biomass in the top 20 cm of soil but can extend over 150 cm deep, possibly contributing substantial amounts of C to the soil (Frank et al., 2004; Garten Jr. and Wullschlegel, 1999; Liebig et al., 2005; Ma et al., 2000a) through root turnover and exudation. Loblolly pine rooting characteristics are different than an herbaceous grass such as switchgrass, with the production of large, deep penetrating tap roots and the majority of total root biomass being comprised of coarse roots (Adegbidi et al., 2004; Miller et al., 1996; Kapeluch and Van Lear, 1995). Rooting density for loblolly pine is concentrated in the top 60 cm of soil (Adegbidi et al., 2004; Kapeluch and Van Lear, 1995; Miller et al., 1996; Parker and Van Lear, 1996), with tap roots extending much deeper into the soil profile depending on soil physical conditions. Given these general differences in rooting dynamics between pine and switchgrass, the potential exists for each species to impact soil C and nutrient cycling differently and to compete for essential nutrients.

Competition for belowground resources may occur between pine and switchgrass due to rooting zone interactions. Many studies have shown the negative impact of competing vegetation on pine tree growth and nutritional status (Smethurst et al., 1993; Smethurst and Nambiar, 1993). Growth of panicum grasses in young slash pine (*Pinus elliotii* Engelm. var. *elliotii*) plantations has been shown to significantly reduce pine tree root extension and density and reduced potassium (K) and phosphorus (P) in pine tissues (Smethurst et al., 1993), implying that these grasses can have a negative impact on pine tree root development and nutrient uptake potential. Smethurst and Nambiar (1993) showed that weed growth on radiata pine (*Pinus radiata* D. Don) reduced soil mineral-N concentrations, pine foliar N concentrations, and amount of N in trees due to direct, belowground competition between pines and weeds. These studies highlighted the importance of competition for nutrients to explain effects of competing

vegetation on tree growth. Therefore, potential competition for nutrients between pine and switchgrass exists and may impact pine productivity.

Root dynamics and coupling between the C and nutrient cycling processes has been shown to be important in forests (Ross et al., 2001; Hart et al., 1994; Phillips et al., 2011). Many studies have focused on the comparison of soil C and nutrient dynamics in pure switchgrass fields and conventional agricultural or grassland (Zan et al., 2001; Bransby et al., 1998; Ma et al., 2000b; Garten and Wullschleger, 1999; Liebeg et al., 2005; Liebeg et al., 2008) and in aggrading forests (Johnson et al., 2003, Richter et al., 1999), but results of pine-switchgrass intercropping on soil C and nutrients dynamics are only now starting to be published (Minick et al., 2014; Strickland et al., 2014; Blazier et al., 2013). Introduction of grass into forest ecosystems can increase soil C stocks (Liebig et al., 2008; Ma et al., 2000b) due to fine root turnover and rhizodeposition, or decrease soil C stocks (Strickland et al., 2011; Strickland et al., 2014) due to losses of faster-cycling C pools via priming of microbial communities. Soils under grasslands and forests have inherently different C storage and C cycling dynamics due to differences in organic C inputs (Oades, 1988). Therefore, the production of switchgrass on existing pine tree plantations may affect soil C cycling (Oades, 1988; Strickland et al., 2011; Liebig et al., 2008), and in turn influence N cycling and availability.

1.2.2. Nitrogen Cycling and Availability

Of the major nutrients essential for plant growth, N has been identified as the most limiting nutrient to productivity in many terrestrial ecosystems (Vitousek and Howarth, 1991). Availability of N and other nutrients can limit growth and productivity of loblolly pine trees (Albaugh et al., 2004; Albaugh et al., 2008; Fox et al., 2007; Jokela and Martin, 2000). Loblolly pine responds positively to N fertilization (Albaugh et al., 1998; Amateis et al., 2000; Hynynen et al., 1998), as well as P fertilization on P-deficient soils in southeastern US coastal plain soils particularly at time of planting (Gent et al., 1986; Prichett and Comerford, 1983). Large growth responses to N-fertilization occur during early- and mid-rotation phases of stand development particularly following thinning. Furthermore, greater yield responses can occur when N + P are applied simultaneously on the right landforms (Amateis et al., 2000; Hynynen et al., 1998). Demand for essential nutrients [e.g., N, P, K, and boron (B)] by loblolly pine must be met in

order to optimize tree growth and meet biomass demands of the 21st century (Allen et al., 2005; Fox, 2000). Belowground competition between pine and switchgrass for N may have a negative effect on pine growth if switchgrass out-competes pine for these nutrients.

Current silviculture practices in southern loblolly pine plantations involve fertilization and mechanical and chemical suppression of competing vegetation (Fox et al. 2007). These practices can increase pine seedling survival and growth (Allen et al., 2005; Fox, 2000; Jokela and Martin, 2000). Suppression of hardwood and herbaceous vegetation at the early stages of stand development increases availability of essential soil nutrients (Gurlevik et al., 2004; Smethurst et al., 1993), which can lead to increased productivity at later stages of stand development (Fox et al., 2007; Lauer et al., 1993; Miller et al., 2003). Fertilization and vegetation control have been shown to increase net N mineralization in southern pine plantations (Gurlevik et al., 2004). Gurlevik et al. (2004) suggest that changes in net N mineralization are possibly due to changes in microbial N immobilization by microbes or through the alteration of labile C inputs to the soil. It is also plausible that changes in net N mineralization results from increases or decreases in gross N mineralization or microbial N immobilization, although documentation of gross N transformations in pine plantations appears limited (Xu et al., 2008). The influence of switchgrass on N availability to loblolly pine will be an important aspect in the sustainability of pine-switchgrass intercropping systems. Therefore quantifying microbially mediated N cycling processes in these systems is essential to fully understand management impacts on plant-available N.

Availability of inorganic N to plants is determined primarily by decomposition of SOM. Release of inorganic N via mineralization of SOM is the main process by which N is released into the soil in a plant-available form. Gross N mineralization is the process carried out by soil heterotrophic microorganisms which releases inorganic N from SOM. Microbial N immobilization in surface soil horizons acts as a large sink for soil N and is a N retention mechanism within ecosystems (Buchmann et al., 1996; Davidson et al., 1990; Groffman et al., 1993; Nadelhoffer et al., 1995; Vitousek and Matson, 1985). Plant-available N is driven by the balance between mineralization-immobilization-nitrification processes and is represented by net N pools. Measuring gross N transformations help to explain observed changes in net N transformations and ultimately the availability of inorganic N to plants (Davidson et al., 1992; Hart et al., 1994; Minick et al., 2011; Verchot et al. 2001), which provides important information

on processes affecting ecosystem N dynamics that net rates may not capture (Hart et al., 1994). Given the importance of N availability to plant productivity, the impact of switchgrass production on N cycling and availability may affect productivity of loblolly pine. On the other hand, inputs of labile organic matter to soils by switchgrass could positively influence N availability (McLaughlin and Kszos, 2005).

Inputs of labile SOM have been suggested as a driver of inorganic N availability (Gurlevik et al., 2004) and availability of C sources can increase N mineralization (Compton and Boone, 2002; Schimel, 1986). Strong linkages between substrate quality (e.g. C/N ratio, lignin:N ratio, etc.) and N transformations have been identified in forest soils (Finzi et al., 1998; Hart et al., 1994; Scott and Binkley, 1997), cropland (Schimel, 1986), and grassland (Hart et al., 1993), suggesting that changes in SOM quality or quantity may result in alterations of N cycling pathways due to changes in microbial activity. Therefore, switchgrass-driven changes in SOM may alter N cycling and have important implications for plant-available N, as well as soil C sequestration (McLaughlin and Kszos, 2005).

1.2.3. Carbon Cycling and Availability

Investigating mechanisms which stabilize SOM can help elucidate the influence of land-use change and forest management regimes on soil C sequestration. Measuring changes in total soil C usually involves direct combustion methods on bulk soil samples collected from various soil depth increments. Investigation of the impact of switchgrass (Ma et al., 2000b; Zan et al., 2001) and pine growth (Johnson et al., 2003; Richter et al., 1999) separately on total soil C has been used to predict the influence of these species on soil C sequestration. For example, Richter et al. (1999) found that total soil C increased in the surface soil horizons 40 years following reforestation of a loblolly pine on previously cultivated soils in the southern US. They also showed the usefulness of C stable isotope analysis to track changes in soil C in a more detailed way than bulk soil C measurements alone. To better understand the potential for soil C sequestration under different forest management regimes it is important to investigate mechanisms which protect SOM from decomposition.

Information gained from total soil C analysis can be informative, although separating SOM into various fractions based on decomposability and chemical and/or physical protection

from degradation may provide more detailed insight into soil C dynamics. This allows deeper understanding into finer-scale influences of different plant species on the susceptibility of organic C pools to decomposition. This may also provide more useful information about soil C dynamics over the short term compared to bulk soil C measurements. For instance, Strickland et al. (2011) found that the invasion of a grass into forests led to a reduction in the faster-cycling particulate organic matter (POM) C fraction, but not in total soil C.

Soil organic matter dynamics is often modeled by dividing SOM into three compartments based on mean residence time; including the active, passive, and slow cycling C pools (Smith et al., 1997). These three pools generally represent the susceptibility of SOM to decomposition by soil microorganisms, with the active pool being the most susceptible and the slow cycling pool being the least susceptible. Specific mechanisms of SOM stabilization occur in soils, including uncomplexed, biochemical recalcitrance (e.g., C/N, lignin content), spatial inaccessibility (e.g., aggregate-occluded), and organo-mineral complexes (Jastrow et al., 2007; Kögel-Knabner et al., 2008; Oades, 1988; Six et al., 2002; Stevenson, 1994). The mechanism of SOM protection from decomposition is important when determining if SOM is in an active, passive, or slow cycling pool. Many methods are used to measure SOM fractions and the various C constituents found in each fraction (von Lützow et al., 2007). Specific fractionation methods, such as density flotation (organo-mineral complexes), aggregate fractionation (spatial accessibility), and acid hydrolysis (biochemical recalcitrance), are employed in an attempt to separate homogenous functional groups of SOM. Individual fractionation methods typically do not isolate mutually exclusive SOM fractions and commonly fail to completely separate homogenous SOM fractions (von Lützow et al., 2007). Limitations posed by individual fractionation methods can be reduced if physical and chemical methods are combined (von Lützow et al., 2007). Despite these methodological limitations, fractionation of SOM can still provide valuable information on SOM stabilization mechanisms, susceptibility of SOM to decomposition, and soil C sequestration potential.

Studies investigating SOM primarily focus on separating particulate- versus mineral-associated organic matter. For example, one study investigated POM C (Ocumpaugh et al., 2003) and the other divided fractions into POM and mineral-associated organic matter (MOM) (Garten Jr. and Wullschleger, 2000). Ocumpaugh et al. (2003) found that the POM fraction was higher in surface soils under switchgrass compared to other cropland but much lower than

coastal and forested ecosystems. In a review by McLaughlin and Kszos (2005), inputs of POM by switchgrass was identified as a potential to readily incorporate organic matter into an active nutrient cycling pool, via stimulation of microbial activity, and possibly enhancing managed nutrient additions (i.e., reincorporating fertilizers into mineralizable SOM fractions). Garten and Wullschleger (2000) measured enriched $\delta^{13}\text{C}$ values of the POM pool under switchgrass compared to a tall fescue and fallow plots. They attributed this change in POM to root turnover primarily in the surface soil layers. Turnover rates of POM and MOM were estimated at 2.4 to 4.3 years and 26 to 40 year, respectively, and for switchgrass coarse roots at 1.4 to 2.1 years, indicating a faster-cycling, labile source of SOM (Garten and Wullschleger, 2000). These studies provide evidence that switchgrass production can influence SOM fractions, specifically in the light or active fractions of SOM and exemplify the utility of ^{13}C stable isotope analysis for detecting changes in C pools.

Changes in the sources of SOM can be detected by ^{13}C stable isotope analysis given differences in the isotopic signature of C compounds found in pine and switchgrass. Different photosynthetic pathways between loblolly pine trees (C3) and switchgrass (C4) lead to the fixation of biomass-C with distinct isotopic signatures. Contrasts in the $\delta^{13}\text{C}$ value between a C3 plant (-28.0 ‰) and a C4 plant (-12 ‰) (Garten Jr. and Wullschleger, 2000; Skjemstad et al., 1990) provide a useful tool in determining switchgrass- versus pine-derived C compounds in labile and recalcitrant SOM pools. The differences in isotopic signatures can be useful to understand how changes in SOM fractions relate to C turnover and sequestration. Detectable changes in the ^{13}C -signature of SOM due to the introduction of a C4 plant, such as switchgrass, may take as long as 5 to 10 years after establishment (Garten Jr. and Wullschleger, 1999; Garten Jr. and Wullshleger, 2000; Ma et al., 2000b). However, these studies investigated conversion of grass fields or agricultural land to switchgrass fields, which may induce less change in soil C compared to introducing an herbaceous plant with high rooting biomass and root turnover into a forested ecosystem. Furthermore, the prevalent abundance of C3 plants in loblolly pine forests may allow for a fine-scale assessment of soil $\delta^{13}\text{C}$ values and changes in SOM pools due to switchgrass intercropping. Changes in SOM have been used to determine management impacts and land-use change on SOM (Schoenholtz et al., 2000), even over short periods of time. Measuring SOM fractions, specifically active fractions combined with ^{13}C stable isotope

analysis, may provide a useful indicator of short-term effects of switchgrass intercropping on SOM stabilization.

1.3. Objectives

The overall objective of this dissertation research was to investigate how intensified production and removal of biomass from loblolly pine plantations influence soil C and N cycling in a loblolly pine-switchgrass intercropping system in the Lower Coastal Plain of North Carolina. Specifically, I was interested in testing SOM stabilization mechanisms and N cycling and availability in order to evaluate the environmental sustainability of intercropping of pine and switchgrass in young loblolly pine plantations. Three specific objectives were addressed: 1) compare different bioenergy management regimes in regards to temporal dynamics of N cycling and availability following forest establishment (*see* Chapter 2); 2) determine the impact of loblolly pine and switchgrass intercropping on microbial N cycling processes (*see* Chapter 3); and 3) evaluate the importance of SOM stabilized by chemical and physical mechanisms and test their sensitivity to pine-switchgrass intercropping (*see* Chapter 4).

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Chapter 2. Switchgrass intercropping reduces soil inorganic nitrogen in a young loblolly pine plantation located in coastal North Carolina

Abstract

As biofuel production continues to increase, so will demand on forests to provide sources of biomass feedstocks. Intensively managed pine (*Pinus* spp.) plantations cover 15.8 million ha of the southeastern United States. Intercropping of switchgrass (*Panicum virgatum* L.) within loblolly pine (*Pinus taeda* L.) stands offers an opportunity to use interbed space to produce an herbaceous biomass feedstock. Furthermore, removal of post-harvest woody residues could act as another forest-based biofuel feedstock. Understanding how managing forests for biomass production influences soil nitrogen (N) cycling and availability is crucial given the critical role N plays in terrestrial ecosystem productivity. Therefore, our objective was to study effects of harvest residue removal and pine-switchgrass intercropping on soil extractable NH_4^+ and NO_3^- . We used a randomized complete block design, consisting of four blocks of seven plots (0.8 ha) established in the summer of 2008 on a recently harvested 34-year-old loblolly pine plantation in the Lower Coastal Plain of North Carolina, USA. Ion exchange membranes were deployed in the top 10 cm of mineral soil starting in June 2009 and replaced continuously every 4-6 weeks through December 2011. Presence of switchgrass significantly reduced soil extractable NH_4^+ and NO_3^- , amounting to a total reduction of 39% and 60%, respectively, over the course of the timeframe (30 months) of this study. There was evidence that intercropping of switchgrass increased extractable NO_3^- in the adjacent pine bed, although this result was only found in the final six months of the study. Presence or absence of harvest residues and/or interbed pines in the interbeds generally had no effect on soil inorganic N pools. These results indicate that switchgrass effectively utilized inorganic N during a time when mineral N supply was greater than N demand by loblolly pines. Assessment of the long-term effects of switchgrass intercropping on soil nutrient cycling and availability and pine health and productivity will be essential to determine the environmental and economic sustainability of intercropping.

2.1. Introduction

Bioenergy production has expanded during the last decade (IEA, 2010), increasing demand for biomass feedstocks. Up to now, most biofuel has been produced from 1st generation feedstocks from agricultural crops, such as corn (USDA-DOE, 2005). Forested ecosystems can produce much of the needed biomass for biofuel production, especially in intensively managed forests. Although use of forest biomass for feedstocks is likely to increase, our understanding of the long-term sustainability of intensified bioenergy production from forests is not complete. As of 2010, there were 15.8 million ha of pine (*Pinus* spp.) plantations in the southern United States (Wear and Greis, 2012). Of these plantations, those that are more intensively managed may be able to sustainably produce needed biomass without significant land-use change or diversion of agricultural crops from food production. Forested ecosystems can provide multiple sources of biomass feedstocks, including post-harvest residues or biomass recovered from thinning operations. Furthermore, in southern pine plantations, the space between rows of planted trees could be utilized to grow a bioenergy crop, such as switchgrass (*Panicum virgatum* L.), to produce additional biomass for bioenergy feedstocks. Knowledge of long-term sustainability of this novel approach will be essential in order to realize this potential.

Switchgrass has been identified as an environmentally and economically promising feedstock for 2nd-generation biofuel production due to its high productivity, perennial life history and long lifespan, tolerance of a wide range of climatic conditions, and low nutrient and water requirements (McLaughlin and Kszos, 2005; Parrish and Fike, 2005; Schmer et al., 2008). Production of herbaceous biomass from forested ecosystems could diversify economic returns for land-owners and may lead to positive ecological impacts, including increased soil carbon (C) storage and ecosystem nitrogen (N) retention (McLaughlin and Kszos, 2005). Woody biomass from forests has also been considered, and extensively studied, as a potential renewable bioenergy source.

Removal of woody biomass, such as post-harvest residues, may alter short- and long-term soil nutrient availability, exacerbating potential plant nutrient limitations and long-term soil productivity. Positive effects of harvest residue retention or stem-only harvesting on soil nutrient stocks have been shown in many instances (Barber and Van Lear, 1984; Gosz et al., 1973; Hyvönen et al., 2000; Olsson et al., 1996; Tew et al., 1986). This is not always the case,

however (Sanchez et al., 2009; Smethurst and Nambiar, 1990) and results depend on the soil type, nutrient of interest, timescale, and intensity of biomass removal. Barber and Van Lear (1984) found that decomposing loblolly pine residues retained a large proportion of initial N and phosphorus (P) content, but not calcium (Ca), magnesium (Mg), and potassium (K) seven years post-cutting. Benefits of retaining harvest residues are likely to occur that may not be detectable if measuring soil and plant responses shortly after site establishment (Barber and Van Lear, 1984; Hyvönen et al., 2000; Smethurst and Nambiar, 1990). Impacts of woody biomass removal on long-term soil nutrient capital in successive rotations, and its relationship to the inherent site nutrient capital, must be considered to determine the sustainability of such practices (Squire et al., 1979; Squire et al., 1985).

Understanding how increased forest-based biomass extraction influences soil N cycling and availability is important for long-term viability of managed forests. Nitrogen has been identified as the most limiting nutrient to growth and productivity in many terrestrial ecosystems (Vitousek and Howarth, 1991), including loblolly pine forests (Albaugh et al., 2004; Albaugh et al., 2008; Fox et al., 2007; Jokela and Martin, 2000). Inherent site quality plays an important role in loblolly pine forest productivity, although fertilizers can be applied to help alleviate nutrient limitations and increase productivity. Loblolly pine often responds positively to N fertilization (Albaugh et al., 1998; Amateis et al., 2000; Hynynen et al., 1998), P fertilization (Gent et al., 1986; Pritchett and Comerford, 1983), and N + P additions (Amateis et al., 2000; Hynynen et al., 1998), but depends on soil characteristics of a given site. Demand for N and other essential nutrients (e.g., P, K, Ca, and Mg), by loblolly pine must be met in order to optimize tree growth (Allen et al., 2005; Fox, 2000).

Competition between planted pines and switchgrass for nutrients may lead to reduced productivity of one or both species without careful soil nutrient management. For example, belowground competition between pine and switchgrass for nutrients or rooting space may have a negative effect on pine growth if switchgrass out-competes pine for these nutrients, especially N (Woods et al., 1992). Nutrient limitations exist within most loblolly pine forests in the Southeast USA and are exacerbated by the presence of competing vegetation (Smethurst and Nambiar, 1989). Many studies have shown the negative impact of competing vegetation on pine tree growth and soil and/or foliar nutritional status (Borders et al., 2004; Carter et al., 1984; Gurlevik et al., 2004; Smethurst and Nambiar, 1989; Smethurst et al., 1993; Woods et al., 1992)

including studies in agroforestry systems (Jose et al., 2004). Growth of *Panicum* species in young slash pine (*Pinus elliottii* Engelm. Var. *elliottii*) plantations significantly reduced pine tree root extension and density and reduced K and P in pine tissues (Smethurst et al., 1993). Suppression of hardwood and herbaceous vegetation at the early stages of stand development increases availability of essential soil nutrients (Gurlevik et al., 2004; Smethurst et al., 1993), which can lead to increased productivity at later stages of stand development (Fox et al., 2007; Lauer et al., 1993; Miller et al., 2003). Therefore, the impact of switchgrass production on N cycling and availability may negatively affect pine productivity if N availability is limiting and utilization by each species overlaps spatially, temporally, or both.

Disparities between soil N availability and tree demand typically occur in young pine stands following harvest as soil N availability is in excess of pine tree demand (Allen et al., 1990; Fox et al., 1986; Fox et al., 2007). Any large disturbance resulting in removal of the overstory vegetation often leads to increased soil N availability (Allen et al., 1990; Fox et al., 1986; Likens et al., 1970; Vitousek and Matson, 1985). As stands develop over time, soil N availability decreases as favorable conditions (e.g., increased soil temperature and soil moisture) for N transformations subside and as plant N uptake increases, eventually leading to possible N limitations in pine stands (Miller et al., 1976; Miller et al., 1981). This imbalance between N availability and plant uptake has important implications for N cycling in relation to availability to plants and losses from the forest ecosystem. Therefore, it may be possible to utilize increased inorganic N availability to produce a bioenergy feedstock and reduce potential losses of N from the ecosystem during the early period of stand development.

Our main objective was to determine the effects of harvest residue removal and switchgrass intercropping on soil inorganic N availability. In particular, we were interested in the ability of switchgrass to reduce excess soil inorganic N in a young loblolly pine stand. We tested two main hypotheses: 1) soil inorganic N concentrations in the surface mineral horizon will be reduced by intercropping of switchgrass between rows of pine; and 2) short-term inorganic N concentrations in the surface mineral horizon are not impacted by the removal of harvest residues. To test these hypotheses, soil NH_4^+ and NO_3^- availability were measured over a 2.5-year period using ion exchange membranes deployed in a recently established field study with treatments reflecting various forest biomass removal and production scenarios.

2.2. Materials and Methods

2.2.1. Study Site and Treatments

The Lenoir I Intercropping Sustainability Study site was located in the Lower Coastal Plain physiographic province in Lenoir County, NC (35-12'59'' N; 077-26'13'' W). Soils were mapped as Pantego (fine-loamy, siliceous, semiactive, thermic Umbric Paleaquults) or Rains (fine-loamy, siliceous, semiactive, thermic Typic Paleaquults) soil series, both of which are classified as deep, very poorly drained soils (USDA Soil Survey, 2013). However, previous site management included installation of ditches (early 1970's) to lower the water table and reduce saturation at the soil surface. Additionally, soils were bedded to raise root systems of planted loblolly pine seedlings above the water table, increase soil aeration, and reduce competition. Mean annual temperature at the site ranged from 15 to 21 °C and mean annual precipitation ranged from 1,000 to 1,500 mm y⁻¹, with a frost-free period of 180 to > 320 days (Baker and Langdon, 1990; Barrett, 1995).

In summer 2008, four blocks of seven treatments (0.8 ha treatment plots with 0.4 ha measurement plots with a minimum 15 m outer buffer) were established on a recently harvested 34-year-old loblolly pine plantation with a site index of 21.3 m at age 25. A randomized complete block design (RCBD) was used in this study, in which each treatment plot was assigned at random within each of the four blocks. This design allowed for four replicates per treatment. Treatments included: 1) loblolly pine establishment (crop trees planted on bedded rows spaced approximately 6.1 m apart) with harvest residues left in place (i.e., all non-merchantable material remained on site) (Pine "+" Residues); 2) loblolly pine establishment with harvest residues removed (i.e., all woody debris > 5 cm in diameter removed) (Pine "- Residues); 3) loblolly pine establishment intercropped with switchgrass between bed rows with harvest residues left in place (Pine w/ Switchgrass "+" Residues); 4) loblolly pine establishment intercropped with switchgrass between bed rows with harvest residues removed (Pine w/ Switchgrass "- Residues); 5) loblolly pine establishment with an additional row of loblolly pine planted in the interbed with harvest residues left in place (Pine w/ Extra Pine "+" Residues); 6) loblolly pine establishment with an additional row of loblolly pine planted in the interbed with harvest residues removed (Pine w/ Extra Pine "- Residues); and 7) switchgrass only

(Switchgrass). Switchgrass planted at this research site was ‘Alamo’, a lowland variety which has been identified as the most promising for biomass production across the southeastern United States (Ocumpaugh et al., 2003).

2.2.2. Site Preparation

All treatments (except switchgrass only) were mechanically site prepared, including V-shearing to break up stumps and roots, and subsoiling. Bedding spaced at 6.1 m apart were installed, a common practice in Lower Coastal Plain loblolly pine plantations used to increase the survival and growth of seedlings (Fox et al., 2007). Treatments with harvest residues in place represented standard operating procedures under normal harvesting scenarios. Treatments requiring removal of harvest residues were accomplished using a grapple-claw excavator to remove all non-merchantable woody biomass approximately > 5 cm in diameter. While this does not represent what would happen operationally, this extreme removal took place to achieve a maximum amount of removal for this study ($\approx 85\%$ was removed, not including sheared stumps; Beauvais (2010)). The switchgrass only treatment plots were V-sheared and then root-raked, thereby removing all residues. Switchgrass was harvested once per year in the late fall. Treatments with additional row of pines (also being considered as a bioenergy feedstock) will be harvested at 5 to 7 years of age.

Herbicide was aerially applied to the whole site in November 2008 prior to pine establishment using imazapyr (3.51 L ha^{-1}) (Chopper®, BASF Corporation, Florham Park, NJ, USA) and a premium, buffered surfactant blend (0.94 L ha^{-1}) (Red River Supreme®, Red River Specialties Inc, Shreveport, LA, USA). Beds were initially fertilized in November 2008 directly after aerial application of herbicide and prior to pine establishment with a liquid-suspension fertilizer containing N (1.1 kg ha^{-1}), P (3.4 kg ha^{-1}), K (1.2 kg ha^{-1}), Ca (2.5 kg ha^{-1}), and Mg (1.3 kg ha^{-1}). Containerized loblolly pine seedlings were planted by hand in bedded rows in December 2008 at $1100 \text{ stems ha}^{-1}$. The extra row of pine trees were planted between bedded rows at the end of December 2008. Switchgrass was machine-seeded at a depth of 6 cm in June 2009 at $9 \text{ kg pure live seed ha}^{-1}$ in rows spaced 38 cm apart. Intercropped switchgrass was planted in a 3 m wide strip between bedded rows of pine trees. Switchgrass only and pine-switchgrass intercropping treatments received herbicide as a mixture of glyphosate and 2,4-

Dichlorophenoxyacetic acid (2,4-D) (4.68 L ha^{-1}) in May 2009 prior to switchgrass planting and with 2,4-D (4.68 L ha^{-1}) and a post-emergent herbicide (Basagran (Benzaton); 0.88 L ha^{-1}) in June 2010 to facilitate establishment of switchgrass over other competing herbaceous and woody vegetation. Intercropped switchgrass and switchgrass only treatments were fertilized in July 2010 with a coated urea fertilizer composed of N (65.6 kg ha^{-1}), P (6.6 kg ha^{-1}), and boron (B) (0.2 kg ha^{-1}), following switchgrass establishment. Fertilization of treatments containing switchgrass will occur as needed. Switchgrass was mowed in 2009 and subsequent cutting and baling (i.e., biomass removal) occurred in December 2010 and 2011, at the end of the second and third growing season, respectively.

2.2.3. Ion Exchange Membranes

Cation exchange membranes (CEM) and anion exchange membranes (AEM) (GE Power and Water, Trevose, PA, USA) were used to measure NH_4^+ and NO_3^- concentrations, respectively, and to provide an index of cumulative N availability in the soil solution over the course of their deployment (Cheesman et al., 2010; Skogley and Dobermann, 1996; Subler et al., 1995). Ion exchangeable soil NH_4^+ and NO_3^- concentrations were continuously measured from the time of switchgrass establishment in June 2009 through the end of the third growing season in December 2011. A laboratory procedure similar to Cooperband and Logan (1994) was followed, but extended to include NH_4^+ and NO_3^- . Cation and anion exchange membranes were cut into 5 x 10 cm strips with a 6.3 mm diameter hole punched near one end to tie nylon string for recovery. Total single-membrane area accounting for the hole was 49.7 cm^2 . New membrane strips were rinsed with deionized H_2O (dH_2O) and shaken for 10 min in 5% HCl (vol/vol). Membranes were rinsed again with dH_2O and shaken for 10 min in dH_2O . Membranes were stored in 1M NaCl for at least 24 h to occupy exchange sites on the membranes with either Na^+ or Cl^- ions. For storage greater than 72 h, membranes were placed in a 1M NaCl solution at 4°C . Membranes were rinsed with dH_2O prior to deployment in the field.

Cation and anion exchange membranes were deployed vertically within the top 10 cm of the A horizon in a manner to minimize soil disturbance and maximize soil-membrane contact (Cooperband and Logan, 1994). The CEM and AEM were located in four randomly selected bed and interbed landscape positions within each plot. Every 4-6 weeks, membranes were removed

from the soil and replaced with new membranes in the same general location. Membranes were combined by plot and landscape position prior to further processing and statistical analysis to provide data for beds and interbeds separately within each treatment plot. Membranes were stored at 4°C for transport back to the laboratory and processed within two days. Newly collected membranes were rinsed free of any soil with dH₂O in the lab, and extracted with 50 mL of 1M KCl per membrane (Cooperband and Logan, 1994). Combined membranes were shaken for 1 h after which a 20 mL aliquot of the extracting solute was transferred to a plastic scintillation vial and stored at 4°C until analysis. Extracts were analyzed for NH₄-N, and NO₃-N concentrations using a TRAACS 2000 analytical console (Bran & Luebbe, Norderstedt, Germany). When processing of collected membranes was completed, membranes were cleaned and recharged in the same fashion as the new membranes as described above.

2.2.4. Statistical Analysis

Treatment effects on monthly NH₄⁺ and NO₃⁻ data were analyzed using repeated-measures analysis of variance (ANOVA) (PROC MIXED package) with time as the repeated measure and using the autoregressive (ar(1)) covariate structure. Alternative spatial covariate structures were tested and the optimal structure was determined by proper conversion of the model matrix and Akaike's Information Criterion (AIC). Monthly raw data were natural log-transformed before analyses to establish homogeneity of variance. Statistical analysis occurred separately for bed and interbed data. Data from each of the seven treatments within the bed, or interbed, were statistically analyzed together and then separated by treatment for graphical presentation. Data were plotted as means of raw data with treatment means comparisons being estimated on transformed data. If significant treatment or treatment by time interactions were detected ($P < 0.1$) in the repeated measures ANOVA, data were analyzed for differences in treatment means on each individual sampling date using the slice option in the PROC MIXED package. Repeated-measures ANOVA was performed using Statistical Analysis System (SAS) software (SAS Institute, Cary, NC).

In order to evaluate the cumulative impact of intercropping treatments on soil NH₄⁺ and NO₃⁻ concentrations, an index of total NH₄⁺ and NO₃⁻ concentrations was calculated and analyzed using one-way ANOVA (PROC GLM package). Total NH₄⁺ and NO₃⁻ were calculated

as the sum of NH_4^+ or NO_3^- pools, respectively, for each treatment over the course of the entire study. Total NH_4^+ data were natural log-transformed before analysis in order to establish homogeneity of variance. Statistical analysis of bed and interbed data again were conducted separately. Data were plotted as means of raw data, while treatment comparisons were determined with transformed data. If a significant treatment effect was detected ($P < 0.1$), treatment means were compared using Tukey's honestly significant difference (HSD) test. One-way ANOVA was performed using Statistical Analysis System (SAS) software (SAS Institute, Cary, NC).

Negative and zero concentrations of extractable NH_4^+ and NO_3^- were assigned values for the minimal detectable limit (MDL) divided by two (Smith, 1991) to allow for log-transformation. Minimum detectable limits were calculated for the TRAACS 2000 analytical console as $0.0435 \mu\text{g L}^{-1}$ and $0.0044 \mu\text{g L}^{-1}$ for NH_4^+ and NO_3^- , respectively.

2.3. Results

Repeated measures analysis of NH_4^+ concentration in the bed showed no significant treatment by time or treatment effect (Table 2.1). For NO_3^- , there was a significant treatment by time interaction ($p = 0.06$) (Table 2.1). Treatment comparisons at each sampling date identified significant increases in NO_3^- concentration from July - December 2011 in bedded pine rows when switchgrass was intercropped in the interbed (Fig. 2.1). Inorganic N in pine beds was not affected by presence or absence of harvest residues nor by the additional row of loblolly pines.

A significant time effect for bed NH_4^+ and NO_3^- concentrations was found (Table 2.1), evident by high NH_4^+ and NO_3^- concentrations following site establishment in summer 2009 which tapered off by spring 2010 (Fig. 2.1). Differences in the concentration and temporal patterns of bed NH_4^+ and NO_3^- were also evident. Nitrate peaked at a much higher concentration than NH_4^+ and declined quickly to a fairly steady baseline level by February 2010, whereas NH_4^+ concentration rose to approximately one-third the concentration of NO_3^- and then declined more gradually up through fall 2010.

Repeated measures analysis of monthly interbed data revealed significant treatment by time, treatment, and time effects, for both NH_4^+ and NO_3^- (Table 2.1). Presence of switchgrass, whether in a pure stand or intercropped, significantly reduced NH_4^+ and NO_3^- concentrations

(Fig. 2.2; Table 2.1). This result was found primarily during the year following switchgrass establishment, when N concentrations in other treatments were at their highest. After the initial flush of inorganic N, interbed soil NO_3^- concentrations were significantly lower under switchgrass during Spring 2010 and 2011 (Fig. 2.2C and 2.2D), amounting to approximately 80% reduction in soil NO_3^- concentrations for each year. In 2010, a spike in NH_4^+ availability was measured from July through September due to the fertilization of switchgrass interbeds and fields (Fig. 2.2A and 2.2B), although no apparent effect of fertilizer application was found on soil NO_3^- concentration. In the absence of switchgrass, interbed N concentrations tended to follow a similar temporal pattern compared to that of the bed, except that concentrations of NH_4^+ and NO_3^- were nearly a half lower in the interbed.

Presence of post-harvest residues had no direct effect on soil NH_4^+ or NO_3^- concentrations in the interbed. Harvest residues did have a suppressive effect on soil NH_4^+ concentrations in the interbed, evident by fewer sampling dates with significant treatment differences and generally reduced NH_4^+ concentrations compared to treatments with harvest residues removed (Fig. 2.2A and 2.2B). However, no significant differences were found between total NH_4^+ concentrations between treatments with presence of harvest residues and corresponding treatments with harvest residues removed (Fig. 2.3).

A significant treatment effect was found for total NH_4^+ concentrations in the interbed ($F = 2.48$; $p = 0.06$) although no differences in treatment means were found (Fig. 2.3). It is likely that this result was driven by the approx. 40% reduction in total NH_4^+ concentrations in treatments with switchgrass present. Total NH_4^+ availability for data clumped into switchgrass versus non-switchgrass pools was not significant ($F = 2.08$; $p = 0.25$) (Fig. 2.3). Total NO_3^- concentrations in the interbed was significantly reduced in the presence of switchgrass, regardless of whether treatments were clumped ($F = 87.97$; $p = 0.003$) or kept separate ($F = 7.05$; $p < 0.001$) for analyses (Fig. 2.4).

2.4. Discussion

Our study evaluated the effects of various forest-based bioenergy management regimes on soil inorganic N concentrations in the surface mineral soil of a recently established loblolly pine plantation in the Lower Coastal Plain physiographic region of North Carolina. We focused

on N due to its known importance to forest productivity and potential negative environmental impacts. We found direct support for our first hypothesis, showing reduced soil NH_4^+ and NO_3^- concentrations under intercropped switchgrass. Our results suggest that switchgrass effectively utilized inorganic N at a time when N availability exceeds pine demand or uptake potential. Total inorganic N availability measured over 2.5 years was reduced by approximately 39% and 60% for NH_4^+ and NO_3^- , respectively, when switchgrass was present.

We found partial support for our second hypothesis which stated that inorganic N availability would not be influenced by presence or absence of harvest residues. There was a trend of reduced soil NH_4^+ concentrations when harvest residues were retained on site, although no significant differences in treatments were found. Presence of residues likely provided a buffer against extremes in soil temperature and thereby reduced soil N transformations (Smethurst and Nambiar, 1990), although this assumption was not tested in this study.

Intercropped switchgrass was effective at using the increased soil N availability. Following site establishment, a mismatch between supply and demand of mineral N was observed as a large ‘flush’ of soil inorganic N in beds and interbeds in the absence switchgrass. Soil N availability has been shown to increase following forest harvest or other disturbances (Fox et al., 1986; Likens et al., 1970; Vitousek and Matson, 1985), and typically exceeds plant demand in young loblolly pine stands (Allen et al., 1990, Fox et al., 2007). The imbalance between tree N demand and soil N supply in young forested stands is an environmental concern when managing large-scale forest disturbance or harvesting operations. Therefore, reduced soil N availability by switchgrass at a time when N competition should be low provides an effective management strategy to utilize excess available N to produce a biomass feedstock and control ecosystem N losses via leaching or denitrification.

Smethurst and Nambiar (1989) and Woods et al. (1992) showed that competing weedy vegetation reduced soil N levels in young Monterey pine (*Pinus radiata* D. Don) plantations resulting in reduced pine foliar N and pine tree growth. It is possible that sustained reductions in soil N by switchgrass could lead to N competition between pine and switchgrass, thereby impacting one or both species’ productivity. Reduced soil NO_3^- availability under switchgrass occurred during spring to early summer of 2010 and 2011 after the initial peak in N levels following site preparation had subsided in January 2010. Reduced NO_3^- availability in spring sampling dates coincides with switchgrass leaf emergence and peak switchgrass growth rates

(Gillin and McNew, 1987), reflecting that N demand to support vegetative growth is higher for switchgrass compared to interbeds containing non-switchgrass species and therefore reduces soil NO_3^- . This finding has important implications for long-term N availability if switchgrass production and removal continues in these forests. Harvesting switchgrass following senescence will be one important strategy to reduce ecosystem N losses, given that switchgrass retranslocates N from blades to roots during fall senescence (McLaughlin and Kszos, 2005).

Surprisingly, effects of intercropping were not isolated to the interbed. We observed an increase in soil NO_3^- concentration in bedded rows of pine trees when switchgrass was located in the interbed. From previous studies, it might be expected that some degree of belowground interactions, be they direct resource competition or changes in spatial rooting patterns, will occur between switchgrass and adjacent pines (Lehmann et al., 1998; Schroth, 1999; Woods et al., 1992). Changes in pine rooting behavior due to intercropped switchgrass may have important implications for N cycling and availability due to the various effects of fine root turnover, root exudation, and rhizosphere priming on breakdown of soil organic matter. Impacts of switchgrass intercropping on water availability may also occur, although this is unlikely at this site given that these soils are poorly drained and the water table is at or near the surface during wet seasons (USDA Soil Survey, 2013). The mechanism for observed increases in NO_3^- availability in pine beds adjacent to switchgrass remains unclear. Future research should address the effect of interbed crops on soil N dynamics in bedded rows of trees to fully understand larger scale soil N dynamics and potential implications for ecosystem productivity. The overall effect of intercropping on nutrient availability will depend on inherent site N status, proper nutrient management, and rooting characteristics (e.g., rooting depth, root length, plasticity).

Differences between the response of NH_4^+ and NO_3^- to site preparation and treatments were also observed. Soil NO_3^- tended to be more dynamic in its response to treatment and site preparation compared to NH_4^+ . In the interbed, reductions in soil NO_3^- concentrations were observed under switchgrass almost twice as often as NH_4^+ . Total soil NO_3^- availability over the entire study was significantly reduced in presence of switchgrass, while no treatment differences were found for total soil NH_4^+ . Finally, soil NO_3^- concentrations were 2-3 times higher in both beds and switchgrass-free interbeds up through February 2010 compared to NH_4^+ . Therefore, soil NO_3^- concentrations and N transformations which influence the quantity of NO_3^- in soil may be more sensitive to disturbance and land-use change than NH_4^+ . Furthermore, detection of

NH_4^+ in soils using ion-exchange membranes may have been hampered due to its reduced mobility within the soil profile compared to NO_3^- . Other studies have found that intensive site preparation led to higher soil NO_3^- concentrations compared to soil NH_4^+ (Vitousek and Matson, 1985; Vitousek et al., 1992), with nitrification rates being more affected than mineralization rates when comparing clear cuts versus uncut forests (Matson and Vitousek, 1981). Differentiating effects of forest management and large-scale disturbances on the different forms of soil inorganic N and the processes that effect their formation and turnover are essential to determine long-term ecosystem N dynamics.

Although we generally found no effects of retention or removal of harvest residues on inorganic N levels, the positive benefits of retention of these materials on short-term availability of non-N soil nutrients has been shown in other systems (Barber and Van Lear, 1984; Gosz et al., 1973; Hyvönen et al., 2000; Olsson et al., 1996; Tew et al., 1986). Long term effects of decomposing residues on soil N pools may become evident if N deficiencies develop later in the rotation (Miller et al., 1976; Miller et al., 1981). Other plant-essential cations, including Ca, Mg, and Zn, are easily leached from coarse woody debris and can contribute significant amounts of these nutrients to soil mineral horizons over a much shorter time span than inorganic N (Barber and Van Lear, 1984). Therefore, the importance of retention of harvest residues on soil quality extends beyond consideration of just soil N inputs. Furthermore, effects of decomposing woody debris on soil structure and nutrient availability may act on time scales of multiple successive forest generations and not necessarily within a single rotation (Squire, 1983; Squire et al., 1979; Squire et al., 1985).

2.5. Conclusion

This research highlights an effective forest-based bioenergy management strategy to utilize excess soil N in intensively managed, young loblolly pine stands. Production of an herbaceous biomass feedstock in these forests may provide positive benefits from both an environmental and bioenergy production standpoint. Positive environmental effects of reduced inorganic soil N due to switchgrass uptake may be expected (Bransby et al., 1998; Eghball et al., 2000), including reduced NO_3^- leaching and N gaseous losses. On the other hand, effects of

switchgrass on soil inorganic N levels will have important management implications if pine tree N deficiencies arise following long-term reduction in soil available N pools by switchgrass.

These results indicate the importance that proper nutrient management for both species will play in the sustainability of this system (Fox et al., 2000; Parrish and Fike, 2005), and how critical an understanding of the temporal dynamics of the species interactions during stand development will be. We expect that switchgrass will become less competitive for resources at pine crown closure, due to shading effects on switchgrass growth. This should reduce potential competitive effects, but the specific timing of this should be monitored. Furthermore, the potential to incorporate decomposing switchgrass roots into actively cycling soil organic matter and nutrient pools upon pine canopy closure may have a positive effect on soil N availability and pine productivity. A robust understanding of biotic and abiotic factors that influence soil N availability and the microbial processes that drive soil N transformations will be important to determine long-term soil productivity and develop sustainable forest management strategies.

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Table 2.1. Results (F-values and significance) of repeated measures ANOVA testing effects of treatment, time, and treatment*time interaction for soil NH_4^+ and NO_3^- concentrations in the beds and interbeds measured using ion exchange membranes.

Source	DF	NH_4^+ F-Value	NO_3^- F-Value
Bed			
Treatment	5	0.25	0.89
Time	20	38.37***	55.96***
Treatment*Time	100	0.59	1.27 ⁺
Interbed			
Treatment	6	4.57**	17.51***
Time	20	16.05***	24.69***
Treatment*Time	100	3.18***	1.50***

⁺ P < 0.1; * P < 0.05, ** P < 0.01, *** P < 0.0001

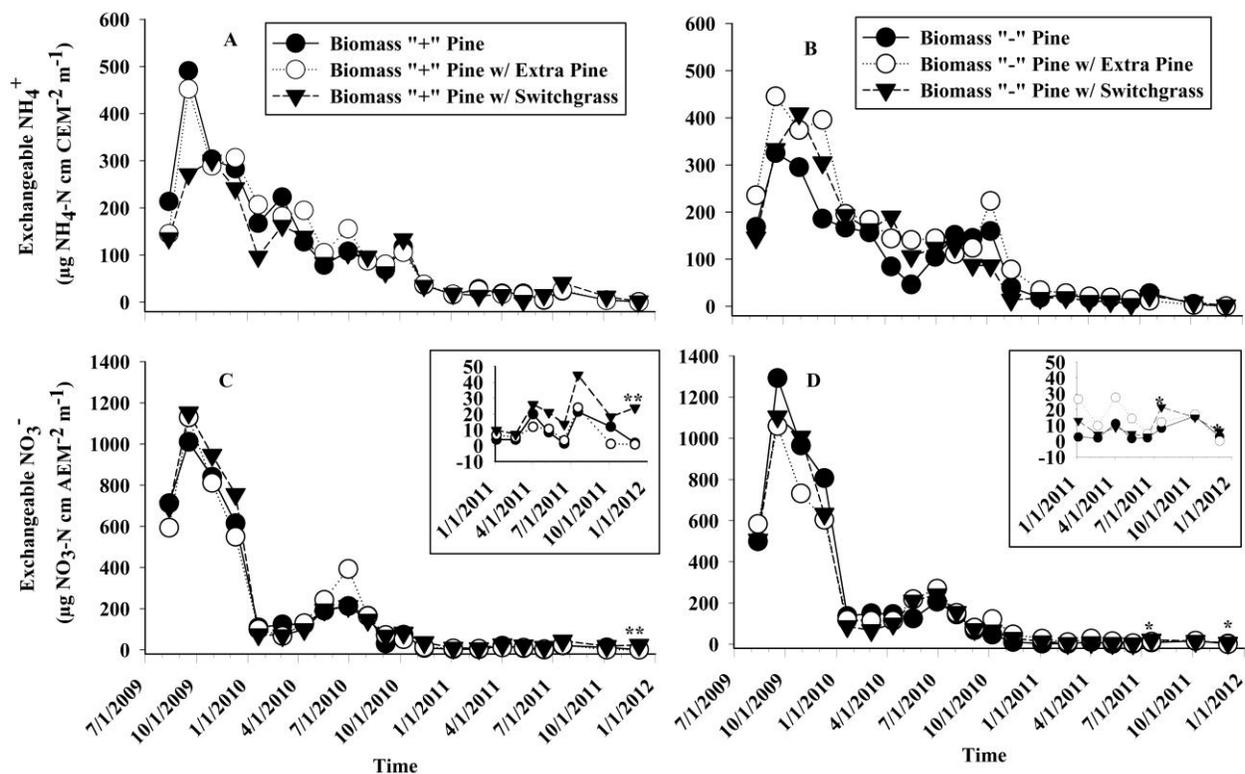


Figure 2.1. Soil exchangeable NH_4^+ (A and B) and NO_3^- (C and D) for samples obtained from beds of treatment plots. Panels A and C include treatments with harvest residues retained ("+" Residues) and panels B and D include treatments with harvest residues removed ("-" Residues). Insets in panels C and D represent an expanded view of the final year of measurements in order to highlight treatment differences. Symbols indicate significant differences at each time period ($^+ P < 0.1$; $*p < 0.05$; $**p < 0.01$; $***p < 0.0001$).

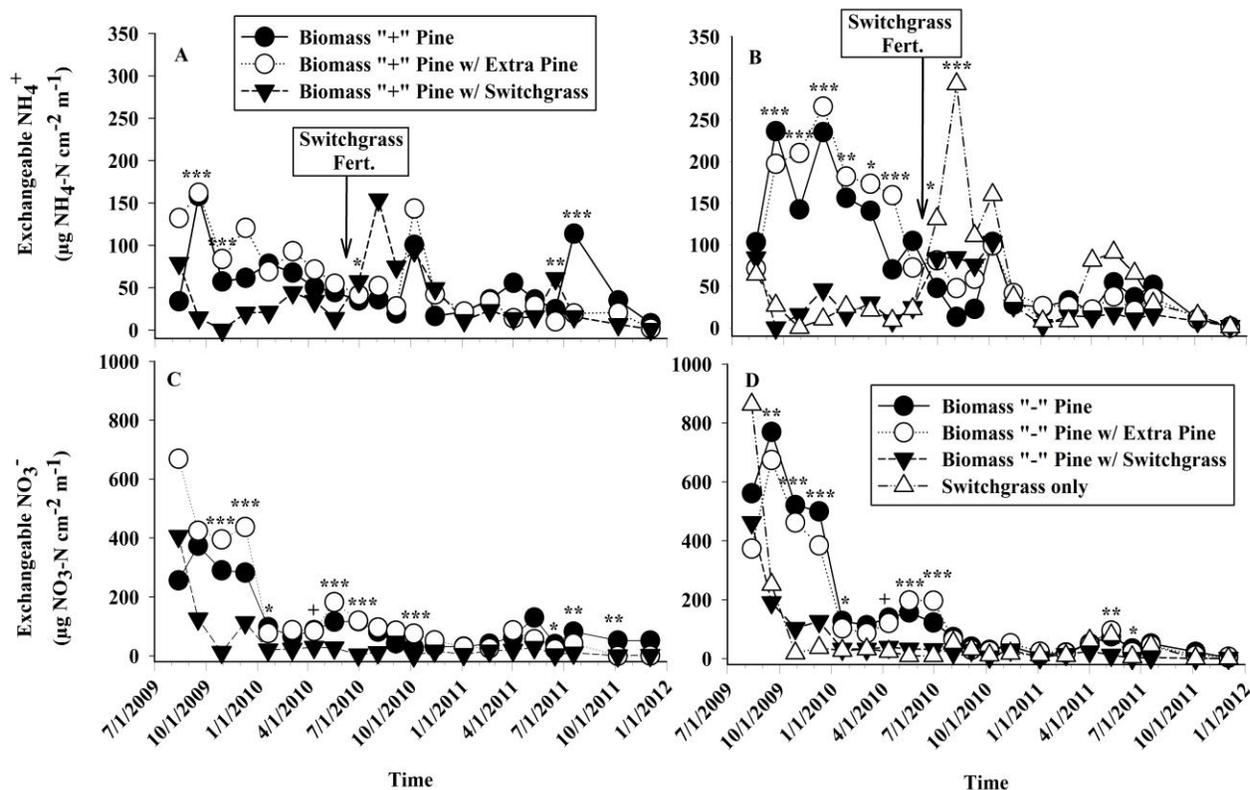


Figure 2.2. Soil exchangeable NH_4^+ (A and B) and NO_3^- (C and D) for samples obtained from interbeds of treatment plots. Panels A and C include treatments with harvest residues retained (“+“ Residues) and panels B and D include treatments with harvest residues removed (“-“ Residues). Switchgrass only treatment is plotted with panels B and D given that this treatment was most similar to other intercropped treatments with residues removed. Symbols indicate significant differences at each time period (+ $P < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$).

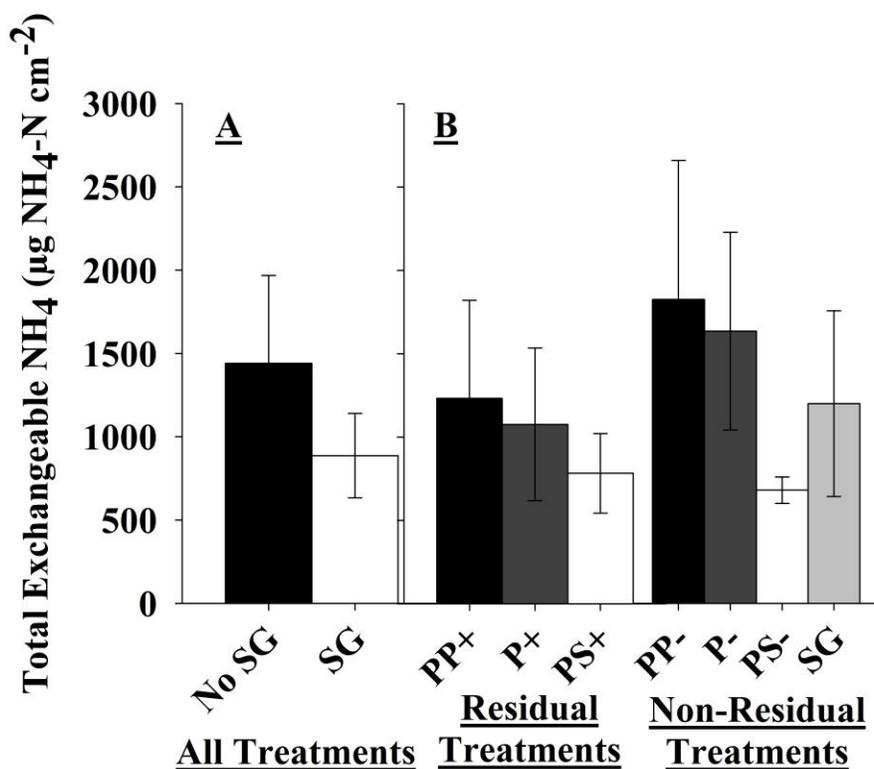


Figure 2.3. Total exchangeable NH_4^+ from interbed samples measured over the duration of the study. Total NH_4^+ availability for treatments without switchgrass (No SG) and treatments containing switchgrass (SG) is presented in panel 'A'. Total NH_4^+ availability for pine intercropped with an additional row of pine (PP), pine only (no intercropping) (P), or pine intercropped with switchgrass (PS), each with or without harvest residues (+/-), and the switchgrass only (SG) treatment is presented in panel 'B'. Error bars are standard error of the mean (n= 4). Statistical analysis for panels 'A' and 'B' were performed separately.

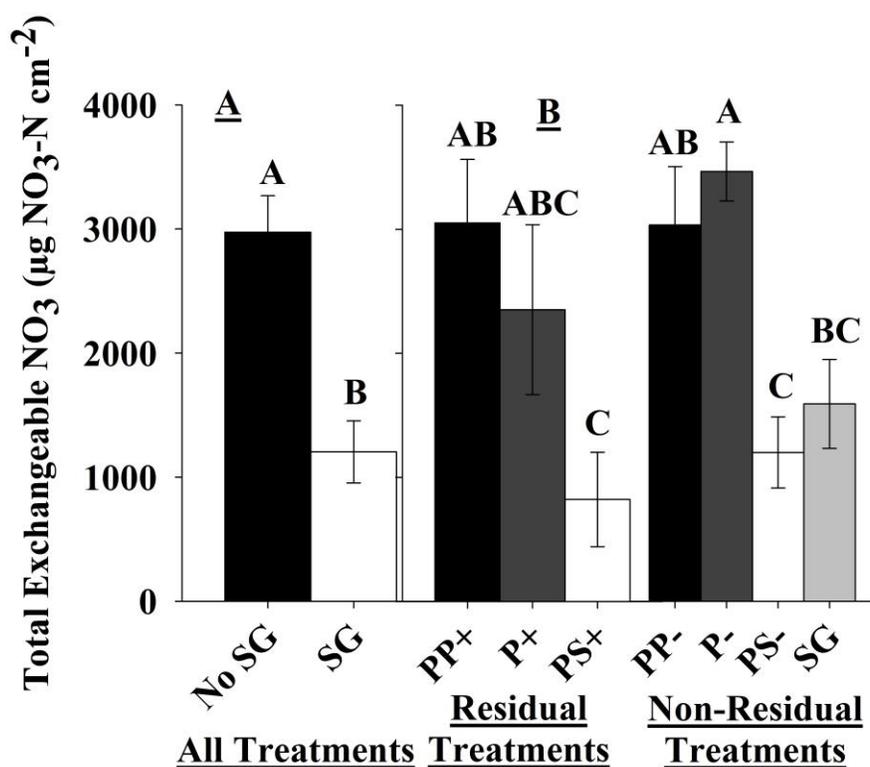


Figure 2.4. Total exchangeable NO₃⁻ from interbed samples measured over the duration of the study. Total NO₃⁻ availability for treatments without switchgrass (No SG) and treatments containing switchgrass (SG) is presented in panel 'A'. Total NO₃⁻ availability for pine intercropped with an additional row of pine (PP), pine only (no intercropping) (P), or pine intercropped with switchgrass (PS), each with or without harvest residues (+/-), and the switchgrass only (SG) treatment is presented in panel 'B'. Error bars are standard error of the mean (n= 4). Different letters indicate significant differences between treatment means (p<0.05). Statistical analysis for panels 'A' and 'B' were performed separately.

Chapter 3. Microbial Nitrogen Cycling Responses to Forest-Based Bioenergy Production

Abstract

Concern over rising atmospheric CO₂ and other greenhouse gases has intensified research into carbon-neutral energy and fuel production. This is largely due to increased fossil fuel combustion and other anthropogenically driven inputs of greenhouse gases to the atmosphere. Millions of acres of pine plantations exist across southeastern United States, representing a vast land area advantageous for bioenergy production without significant land-use change or diversion of agricultural resources from food production. Furthermore, intercropping of pine with bioenergy grasses could provide annually harvestable, lignocellulosic biomass feedstocks along with production of traditional wood products. The viability of such a system hinges in part on soil nitrogen (N) availability and effects of N competition between pine and grasses on ecosystem productivity. We investigated effects of intercropping loblolly pine (*Pinus taeda*) with switchgrass (*Panicum virgatum*) on microbial N cycling processes and N availability in the Lower Coastal Plain of North Carolina, USA. Soil samples were collected from bedded rows of pine and interbed space of two treatments, comprised of either volunteer native woody and herbaceous vegetation in the interbed (Pine-Native) or pure switchgrass in the interbed (Pine-Switchgrass). An *in vitro* ¹⁵N pool-dilution technique was employed to quantify gross N transformations at two soil depths (0-5 cm and 5-15 cm) and four dates in 2012-2013. At the 0-5 cm depth in beds of the Pine-Switchgrass treatment, gross N mineralization was 2-3 times higher in November and February, resulting in increased NH₄⁺ availability. Gross and net nitrification was also significantly higher in February in the same pine beds. In interbeds of the Pine-Switchgrass treatment, gross N mineralization was reduced from April to November, but increased in February, potentially reflecting positive effects of switchgrass root-derived C inputs during dormancy on microbial activity. These findings indicate soil N cycling and availability has increased under pine in the Pine-Switchgrass treatment, potentially alleviating any negative effects of N competition between pine and switchgrass. We expect that reduced soil C in the Pine-Switchgrass treatment, effects of pine and switchgrass rooting dynamics on soil C availability, and plant N demand are major factors influencing soil N transformations. Future

research should examine rooting dynamics in intercropped systems and the effects on soil microbial communities and function.

3.1. Introduction

Forest-based bioenergy production has received renewed attention over the last decade given increased energy production from renewable resources (IEA, 2010). Southern U.S. pine forests could play a particularly important role in providing biomass feedstocks given their rapid growth, existing infrastructure to facilitate biomass production and removal, and minimal land-use change or conversion of currently cultivated cropland. Furthermore, loblolly pine (*Pinus taeda* L.) plantations offer the unique opportunity to use space between rows of trees to grow an herbaceous bioenergy crop, such as native switchgrass (*Panicum virgatum* L.), and produce traditional wood and bioenergy products (Albaugh et al., 2012). This novel forest management regime has potential to provide positive environmental and economic services (Albaugh et al., 2012; Minick et al., 2014; Susaeta et al., 2012), but hinges in part on availability of belowground resources and potential effects of nitrogen (N) competition between pine and switchgrass on productivity. A fundamental understanding of processes impacting cycling of major elements through plants and soils provides a basis for implementing sustainable forest management practices, especially when considering potential negative effects of increased biomass removal on N cycling and availability.

Of the major nutrients essential for plant growth, N has been identified as the most limiting nutrient to productivity in many terrestrial ecosystems (Vitousek and Howarth, 1991), including that of intensively managed loblolly pine forests (Fox et al. 2007a). The amount of N available to loblolly pine trees is predominately mediated by mineralization-immobilization activities of soil microorganisms. The microbial processes which affect soil N cycling are impacted by various forest management practices, such as harvesting (Likens et al., 1970), site preparation (Fox et al., 1986; Vitousek et al., 1992), competing vegetation control (Gurlevik et al. 2004; Smethurst and Nambiar, 1989; Woods et al., 1992), and fertilization (Gurlevik et al., 2004). Therefore, we were interested in understanding the effect of intercropping loblolly pine and switchgrass on soil microbial N cycling processes, given the important role N cycling and availability plays in ecosystem function and productivity.

Size of the plant-available N pool is driven by the balance between mineralization-immobilization-nitrification processes, which are typically represented by changes in net N mineralization or nitrification (Binkley and Hart, 1989). Although net N transformations provide

beneficial insight into availability of N to plants over a given time frame, they do not capture important microbial processes that dictate the form and fate of soil N or how these processes may be impacted by forest management. Direct measurement of gross N transformations can help explain observed changes in net N transformations (Davidson et al., 1992, Hart et al., 1994, Verchot et al., 2001) and provide important information on processes affecting ecosystem N dynamics that net rates may not reveal (Hart et al., 1994). Immobilization of mineralized and nitrified N by microorganisms in surface soil horizons acts as a large sink for mineralized N (Buchmann et al., 1996; Davidson et al., 1990; Nadelhoffer et al., 1995; Vitousek and Matson, 1985) and is typically thought to govern short-term inorganic N availability to trees (Groffman et al., 1993; Kaye and Hart, 1997; Zak et al., 1990). Carbon (C) availability has also been suggested as a driver of inorganic N availability (Gurlevik et al., 2004; Hart et al., 1994; Zak et al., 1993), hence changes in C availability can alter microbial mineralization-immobilization dynamics (Barrett and Burke, 2000; Booth et al., 2005; Compton and Boone, 2002; Schimel, 1986).

Strong linkages between soil C availability and microbial N transformations have been identified in forest soils (Booth et al., 2005; Hart et al., 1994; Finzi et al., 1998; Melillo et al., 1989; Scott and Binkley, 1997; Zak et al., 1993) and grassland soils (Barrett and Burke, 2000; Booth et al., 2005; Elliot, 1986; Schimel, 1986). Changes in soil C quality or quantity due to switchgrass may result in alterations of microbial N cycling pathways and release of soil N into a plant-available N. Switchgrass produces large, surface rooting mats and extensive fine-root systems (Frank et al., 2004; Garten Jr. and Wullschlegel, 1999; Ma et al., 2000a; McLaughlin and Kszos, 2005), which could impact N cycling if fine-root turnover and rhizodeposition alters organic C pools, particularly in the rhizosphere (Dijkstra et al., 2009; Jackson et al., 2008; Phillips et al., 2011). Indeed, switchgrass grown in pure stands has been linked to increased C pools and particulate organic matter fractions (Dou et al., 2013; Frank et al., 2004; Liebig et al., 2005; Ma et al., 2000b; McLaughlin and Kszos, 2005), microbial biomass C and mineralizable C (Dou et al., 2013; Chatterjee et al., 2013; Ma et al., 2000c), and changes in N cycling and availability (Smith et al., 2013). It is important to note that most studies have investigated effects of switchgrass establishment on degraded lands or conversion from conventional annual crop production. Until recently, few studies have reported effects of switchgrass production on soil C and N cycling within traditional forested landscapes.

Positive growth impacts following N fertilization to both pine forests (Fox et al., 2007a ; Hynynen et al., 1998; Miller et al., 1976; Miller, 1981; Vose and Allen, 1988) and switchgrass fields (Muir et al., 2001; McLaughlin and Kszos, 2005; Newell, 1968; Parrish and Fike, 2005) have been documented. However, response to N fertilization by both trees (Fox et al., 2007a) and switchgrass (Parrish and Fike, 2005) depends upon site factors such as landscape position, geology, N cycling processes and availability, and availability of other potentially growth limiting nutrients. Therefore, quantifying gross and net N transformations will help elucidate effects on microbial activity and availability of N to pine and switchgrass when grown concurrently in an intercropping regime.

In our study system, previous research has been conducted examining the effect of pine-switchgrass intercropping on soil C cycling and inorganic N availability (Minick et al., 2014; Strickland et al., 2014), although basic questions still remain as to the effects of intercropping on microbial N cycling processes. Strickland et al. (2014) found that switchgrass grown between bedded rows of loblolly pine trees reduced mineralizable C, particulate organic matter C, and total C, but increased microbial biomass. In another study looking at inorganic N availability over the first 2.5 growing seasons of pine and switchgrass, Minick et al. (2014) showed that soil NH_4^+ and NO_3^- concentrations in the surface mineral soil were reduced under switchgrass. Nitrate was found to be particularly sensitive to switchgrass, evident by lower availability during spring in the second and third growing season when no effect was found on NH_4^+ availability. Interestingly, switchgrass intercropping also led to an increase in NO_3^- concentrations in adjacent pine beds (Minick et al., 2014), which suggests that switchgrass intercropping induced changes in N cycling and microbial activity under loblolly pine trees. However, these results did not negatively affect loblolly pine growth. In a similar study conducted in northwestern Louisiana, USA, Blazier et al. (2012) found that intercropping of switchgrass in loblolly pine stands of different age (e.g., 2 – 22 years old) was associated with increased labile C, C mineralization, and microbial biomass C, regardless of initial stand age. In light of these studies, we expect that altered C and N dynamics due to switchgrass growth will have subsequent impacts on gross and/or net N transformations.

Our objective was to assess potential impacts of pine-switchgrass intercropping on gross N transformations to gain a better understanding of cycling and availability of soil inorganic N in these forests during a potentially intense period of plant competition for this limited resource.

We were interested in testing three hypotheses: 1) gross and/or net N transformation rates are higher in pine beds adjacent to switchgrass, explaining previously observed increases in available NO_3^- (Minick et al., 2014); 2) gross nitrification rates are reduced under intercropped switchgrass, lending support to observed reductions in spring NO_3^- availability in previous years (Minick et al., 2014); and 3) gross N mineralization and immobilization rates are higher under intercropped switchgrass, due to expected stimulation of microbial activity via inputs of labile organic C by switchgrass. To address our objectives and specific hypotheses, we used an *in vitro* ^{15}N pool-dilution technique to quantify gross and net N transformations in mineral soils collected from a 4 year-old loblolly pine forest in the Lower Coastal Plain, North Carolina, USA, either with or without intercropped switchgrass.

3.2. Materials and Methods

3.2.1. Study Site and Treatments

The Lenoir I Intercropping Sustainability Study was located in the Lower Coastal Plain physiographic province in Lenoir County, North Carolina, USA (35-12'59" N; 077-26'13" W). Mean air temperature for 2010 was approximately 16.5°C and mean annual precipitation was approximately 1252 mm (Albaugh et al., 2012). Soils were mapped as Pantego (fine-loamy, siliceous, semiactive, thermic Umbric Paleaquults) or Rains (fine-loamy, siliceous, semiactive, thermic Typic Paleaquults) soil series, both of which are very poorly drained (USDA Soil Survey, 2013). However, previous site management in the late 1960's and early 1970's included installation of ditches to lower the water table and reduce saturation at the soil surface. Additionally, bedding of soil in rows was used to raise root systems of planted loblolly pine seedlings above the water table, increase soil aeration, and reduce competition. Bedding is a common practice in Lower Coastal Plain loblolly pine plantations to increase survival and growth of pine seedlings (Fox et al., 2007b) (Fig. 3.1). Space between bedded rows of pine trees is commonly referred to as the interbed (Fig. 3.1).

The study site was established during June-September 2008, following harvest of a 34-year-old loblolly pine plantation with a site index of 21.3 m at age 25. A randomized complete block design containing four blocks was used to help control for variability in soil

characteristics. For each treatment plot, a 0.4 ha measurement plot was nested within a total plot size of 0.8 with a minimum 15 m outer buffer between plots (Fig. 3.1A). Treatments included: 1) loblolly pine planted on beds with post-harvest residues removed (i.e., all woody debris > 5 cm in diameter removed) with the interbed region containing a mix of native woody and herbaceous vegetation (Pine-Native) (Fig. 3.1B); and 2) loblolly pine planted on beds with post-harvest residues removed and switchgrass planted in the interbed region (Pine-Switchgrass) (Fig. 3.1C).

Sites for both treatments were mechanically prepared by V-shearing to break up stumps and roots followed by bedding. Interbeds where switchgrass was planted were also V-sheared one week before switchgrass planting. This additional V-shearing prepared soil for better germination and mechanical planting. Post-harvest residues > 5 cm in diameter were removed using a grapple-claw excavator. Approximately 85% of non-merchantable woody biomass was removed, not including sheared stumps (Beauvais, 2010). Herbicide was aerially applied to the whole site in November 2008 prior to pine establishment using imazapyr (3.51 L ha^{-1}) (Chopper®, BASF Corporation, Florham Park, NJ, USA) and a premium, buffered surfactant blend (0.94 L ha^{-1}) (Red River Supreme®, Red River Specialties Inc, Shreveport, LA, USA). Beds were initially fertilized in November 2008 directly after aerial application of herbicide and prior to pine establishment with a liquid-suspension fertilizer containing N (1.1 kg ha^{-1}), P (3.4 kg ha^{-1}), K (1.2 kg ha^{-1}), Ca (2.5 kg ha^{-1}), and Mg (1.3 kg ha^{-1}). Containerized loblolly pine seedlings (improved 2nd generation genetic material) were hand planted in December 2008 at $1100 \text{ stems ha}^{-1}$ at a spacing of 6.1 m between bedded rows and 1.5 m within rows (Fig. 3.1A-C). Switchgrass planted was ‘Alamo’, a lowland variety which has been identified as the most promising for biomass production across the southeastern US (Ocumpaugh et al., 2003). In June 2009, switchgrass was machine-seeded at $9 \text{ kg pure live seed ha}^{-1}$ in a 3 m wide strip between bedded rows of pine trees at a depth of 6 cm and in rows spaced 38 cm apart (Fig. 3.1C). Switchgrass interbeds received herbicide as a mixture of glyphosate and 2,4-Dichlorophenoxyacetic acid (2,4-D) (4.68 L ha^{-1}) in May 2009 prior to switchgrass planting and with 2,4-D (4.68 L ha^{-1}) and a post-emergent herbicide [Basagran (Benzaton); 0.88 L ha^{-1}] in June 2010 to facilitate establishment of switchgrass over other competing herbaceous and woody vegetation. Switchgrass treatments were fertilized in June 2010 and April 2012 (after first sampling date) with a coated urea fertilizer composed of N (65.6 kg ha^{-1}), P (6.6 kg ha^{-1}), and

boron (B) (0.2 kg ha^{-1}). Switchgrass was only mowed in 2009 to facilitate establishment of a strong switchgrass stand, then mowed, raked and baled annually following senescence (December 2010 and 2011 and January 2013) at the end of the second, third, and fourth growing season, respectively. Native interbeds were characterized by presence of mixed grass, woody tree and shrub, vine, and forb species, including switchcane (*Arundinaria gigantea*), broomsedge (*Andropogon virginicus*), sweetgum (*Liquidambar styraciflua*), blackberry (*Rubus* spp.), bonesets (*Eupatorium* spp.), goldenrods (*Solidago* spp.), and pepperbush (*Clethra alnifolia*) (Lane, 2010).

3.2.2. Soil Collection and Processing

Soil samples from both the bed and interbed region were collected from each treatment at four sampling dates in 2012-2013 to capture potential seasonal plant phenological impacts on gross N transformations. Sampling dates were April 2012 (after switchgrass had broken winter dormancy and obvious signs of above-ground growth were present), July 2012 (peak switchgrass growth), October 2012 (switchgrass mid-senescence), and February 2013 (switchgrass dormancy and after switchgrass harvest). For ease of presentation and discussion, data obtained from soil samples collected in pine beds (B) from the two treatments were designated as B/Pine-Native and B/Pine-Switchgrass. Samples collected in interbeds (IB) from the two treatments were designated as IB/Pine-Native and IB/Pine-Switchgrass.

Soils from the bed and interbed were sampled separately by taking 6 cores from each location using a 5 cm PVC corer and divided into 0-5 and 5-15 cm depth increments (Fig. 3.1). Soils from each treatment plot were composited by depth separately for bed and interbed samples. Soil samples were stored on ice and transported back to the laboratory within 48 h of collection. Composited soil samples were gently mixed and all visible roots, litter, and identifiable organic matter were removed. Soil moisture was determined by drying a subsample for 24 h at 105°C to standardize incubations by soil dry weight and calculate gross N transformation based on soil dry weight.

A subsample from each composite soil sample was air dried, passed through a 2mm sieve and ground in a ball mill (Retsch MM-400, Haan, Germany). Carbon and N concentration, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ were measured on an IsoPrime 100 isotope ratio mass spectrometer (IRMS) interfaced

with an elemental vario MICRO cube dry-combustion elemental analyzer (Elementar, Hanau, Germany).

3.2.3. Gross N Transformations

Gross N transformations were measured on field moist soils using a ^{15}N pool-dilution model, described by Kirkham and Bartholomew (1954). This method quantifies changes in pools and ratios of ^{15}N and ^{14}N due to microbial consumptive and productive processes. Specifically, gross N mineralization, gross nitrification, and gross N immobilization were measured during a short-term (2 d) laboratory incubation experiment. *In situ* incubations were considered but would have been logistically challenging given our interest in measuring gross N transformations at multiple soils depths and dates. Additionally, laboratory incubations allowed for control of climatic variables due to standardized laboratory temperature and moisture conditions, thereby focusing analysis and interpretation of gross N data on changes in C and N substrate quantity.

Four replicate soil subsamples (each equivalent to 25 g fresh soil weight) were weighed out into 1 L glass jars and allowed to equilibrate to laboratory temperature (20°C) during a 10 h pre-incubation period. Following pre-incubation, ^{15}N was added as either $(^{15}\text{NH}_4)_2\text{SO}_4$ (99 atom% ^{15}N enrichment) or K^{15}NO_3 (99 atom% ^{15}N enrichment) to separate soil incubations in order to determine gross N mineralization rates and gross nitrification rates, respectively. Addition of ^{15}N solution was estimated to achieve approximately 2-5% enrichment of the existing soil NH_4^+ or NO_3^- pool. Concentration of added N ranged from 0.04 - 0.18 $\mu\text{g N g}^{-1}$ for NH_4^+ and 0.04 - 0.12 $\mu\text{g N g}^{-1}$ for NO_3^- , depending on soil inorganic N concentrations determined at each collection date. Soil NO_3^- concentrations were typically very low, if detectable at all, and therefore enrichment of existing soil NO_3^- pool was higher than desired for some samples. Labelled solutions were added dropwise to each incubated soil subsample and gently mixed.

The initial inorganic N and percent ^{15}N enrichment was quantified by extracting one subsample containing $^{15}\text{NH}_4^+$ and another containing $^{15}\text{NO}_3^-$ in 100 mL 2 M KCl 1h after ^{15}N addition, providing an initial (T_i) measure of extractable NH_4^+ and NO_3^- and ^{15}N enrichment. The other subsamples were incubated for 48 h at 20°C and subsequently extracted with 100 mL

2 M KCl, providing a final (T_f) measure of extractable NH_4^+ and NO_3^- and ^{15}N enrichment in order to calculate gross and net N transformation rates. After shaking for 1h, T_i and T_f samples sat for 12-24 h prior to filtering through a Whatman No. 1 filter. Concentration of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in KCl extracts was analyzed on a TRAACS 2000 analytical console (Bran & Luebbe, Norderstedt, Germany).

Recovery of the $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ from KCl extracts followed a diffusion procedure similar to that outlined by Brooks et al. (1989) and modified by Stark and Hart (1997) to include the Teflon [polytetrafluoroethylene (PTFE)] acid trap diffusion procedure. A subsample of the KCl extract (40 mL) was placed in a 140 mL specimen cup. To recover $^{15}\text{NH}_4^+$, approximately 0.2 g MgO was added to the KCl extract to facilitate conversion of NH_4^+ to NH_3 , along with one PTFE acid trap containing two KCl sterilized, Whatman No. 3, 7 mm filter disks acidified with 10 μL of 2.5 M KHSO_4 and sealed between two strips of PTFE tape. Diffusion vessels were tightly closed and mixed daily during a 6 d incubation period at 20°C. After the 6 d incubation, acidified filter disks containing trapped NH_3 were placed on a rack in a desiccator with a beaker containing 50 mL H_2SO_4 to dry for at least 4 hr. To recover $^{15}\text{NO}_3^-$, NH_4^+ was first removed to avoid contamination of the $^{15}\text{NO}_3^-$ pool by residual NH_4^+ using the procedure outlined above, except at the end of the 6 d incubation acidified filter disks containing trapped NH_3 were disposed. After removal of NH_4^+ , approximately 0.4 g Devarda's Alloy was added to the KCl extract to facilitate reduction of NO_3^- to NH_4^+ and an additional 0.2 g MgO to convert NH_4^+ to NH_3 . Upon addition of the PTFE acid trap, diffusion vessels were tightly closed and mixed daily for 6 d. Following diffusion, $\delta^{15}\text{N}$ atom percent enrichment (APE) and N concentrations of acidified filter disks were determined using an IsoPrime 100 stable IRMS interfaced with an elemental vario MICRO cube dry-combustion elemental analyzer (Elementar, Hanau, Germany).

Gross rates of N transformations ($\mu\text{g N g}^{-1}$ soil d^{-1}) were calculated using changes in $\delta^{15}\text{N}$ APE and NH_4^+ and NO_3^- pool sizes, following isotope dilution equations developed by Kirkham and Bartholomew (1954). Ammonium immobilization ($\mu\text{g N g}^{-1}$ soil d^{-1}) was calculated as the difference between gross NH_4^+ consumption and gross nitrification and NO_3^- immobilization ($\mu\text{g N g}^{-1}$ soil d^{-1}) was calculated as the difference between gross and net nitrification. Net N mineralization ($\mu\text{g N g}^{-1}$ soil d^{-1}) was calculated as the difference in total inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) between T_i and T_f incubations. Net nitrification ($\mu\text{g N g}^{-1}$ soil d^{-1}) was calculated as the difference in NO_3^- between T_i and T_f . Mean residence time (MRT) of NH_4^+ and NO_3^- was

calculated by dividing T_i NH_4^+ and NO_3^- concentrations by the gross N mineralization and gross nitrification rates, respectively, providing an estimate of internal N cycling rate (Davidson et al., 1992).

3.2.4. Statistical Analysis

Gross and net N transformation data were analyzed using repeated measure ANOVA with time as the repeated measures (PROC MIXED package), treatment as the fixed effect, and block as the random effect. Because time was a repeated measure we used the sp (pow) covariate structure in the analysis. Alternative spatial covariate structures were tested and the optimal structure was determined using Akaike's Information Criterion (AIC) obtained for each structure tested. Repeated measures analysis was performed separately for data obtained from each sampling location and depth. Soil C and N concentration, soil ^{13}C and ^{15}N natural isotopic abundance, initial NH_4^+ and NO_3^- concentrations, and NH_4^+ and NO_3^- MRT were averaged across all sampling dates and analyzed using one-way ANOVA (PROC GLM package). Raw data were natural log-transformed where necessary to establish homogeneity of variance. All data were plotted as means of raw data with treatment means comparisons being estimated on untransformed and transformed data where appropriate. Negative and zero concentrations of extractable NH_4^+ and NO_3^- were assigned values for the minimal detectable limit (MDL) divided by two (Smith, 1991) to allow for log-transformation. Minimum detectable limits were calculated for the TRAACS 2000 analytical console (Bran & Luebbe, Norderstedt, Germany) as $0.0435 \mu\text{g L}^{-1}$ and $0.0044 \mu\text{g L}^{-1}$ for NH_4^+ and NO_3^- , respectively. If significant treatment or treatment x time interactions were detected ($P < 0.1$) in the repeated measures ANOVA then differences in treatment means at each sampling date were tested using the 'slice' function and Fisher's LSD. If a significant treatment effect was detected ($P < 0.1$) in the one-way ANOVA, then differences in treatment means were tested using Fisher's LSD. All analyses were performed using Statistical Analysis System (SAS) 9.4 software (SAS institute, Cary, North Carolina, USA).

3.3. Results

3.3.1. Bulk Soil C and N Pools

Across all sampling depths and locations, soil C and N concentrations ranged from 48 - 99 g C kg⁻¹ and 1.9 - 4.1 g N kg⁻¹, with a C:N ratio of approximately 26 (Table 3.1). At the 0-5 cm depth in IB/Pine-Switchgrass, soil C and N concentrations were reduced by 27 % and 30 %, respectively, compared to the IB/Pine-Native (Table 3.1). Soil $\delta^{15}\text{N}$ values at 0-5 cm also increased from 0.54 ‰ to 1.38 ‰ in the IB/Pine-Switchgrass compared to the IB/Pine-Native (Table 3.1). To help determine potential effects of root-derived C on soil isotopic natural abundance and changes in soil $\delta^{15}\text{N}$, pine and switchgrass roots were collected in summer 2012 and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Pine roots had a $\delta^{13}\text{C}$ value of -28.1 ± 0.06 ‰ and $\delta^{15}\text{N}$ value of 0.6 ± 0.13 ‰, while switchgrass roots had a $\delta^{13}\text{C}$ value of -13.0 ± 0.47 ‰ and $\delta^{15}\text{N}$ value of -1.3 ± 0.26 ‰. Soil $\delta^{13}\text{C}$ values were unaffected by switchgrass, suggesting that after four growing seasons any direct inputs of switchgrass-derived C to this system has not manifested itself in changes in bulk soil ^{13}C signature. Furthermore, changes in the $\delta^{15}\text{N}$ value at the 0-5 cm depth in IB/Pine-Switchgrass were likely not a result of switchgrass-derived root inputs, given the low $\delta^{15}\text{N}$ values of switchgrass roots.

3.3.2. Gross N Transformations

On average, rates of gross N mineralization ranged from 0.18 - 4.7 $\mu\text{g N g}^{-1}$ soil d⁻¹, while gross nitrification ranged from 0.02 - 0.47 $\mu\text{g N g}^{-1}$ soil d⁻¹. A significant time effect was found for gross N mineralization and NH_4^+ immobilization (Table 3.2), with seasonal rates tending to be lowest in August (Fig. 3.2 - 3.5). Gross N mineralization rates were approximately 10- to 100-fold higher than those of gross nitrification (Fig. 3.2 - 3.5). Variability in gross N mineralization rates was remarkably low in both the bed and interbed, while gross nitrification exhibited high variability in the interbed (Fig. 3.2 - 3.5). Seasonal changes in gross nitrification were detected in pine beds at both sampling depths (Table 3.2), evident by increasing rates at each progressive sampling date (Fig. 3.2C and Fig. 3.3C). Ammonium and NO_3^- immobilization generally followed the same patterns as gross N mineralization and gross nitrification,

respectively, and therefore are not presented graphically. Overall, gross N mineralization and NH_4^+ immobilization were tightly coupled across all sampling dates and depths ($r = 0.93$; $p < 0.001$), while gross nitrification and NO_3^- immobilization were somewhat less correlated ($r = 0.60$; $p < 0.001$).

In pine beds, repeated measures analysis revealed a significant treatment x time interaction for gross N mineralization at the 0-5 cm depth (Table 3.2). We found that gross N mineralization rates were elevated in B/Pine-Switchgrass on average by $1.29 \mu\text{g N g}^{-1} \text{ soil d}^{-1}$ in November and $1.02 \mu\text{g N g}^{-1} \text{ soil d}^{-1}$ in February (Fig. 3.2A), which corresponded to a 305 % and 193 % increase during those sampling dates, respectively. Gross nitrification also increased by $0.31 \mu\text{g N g}^{-1} \text{ soil d}^{-1}$ in February (Fig. 3.2C), potentially reflecting the impact of more NH_4^+ substrate to nitrify. Given the tight relationships between gross N transformations and N immobilization, we observed increased NH_4^+ and NO_3^- immobilization in November and February in the B/Pine-Switchgrass compared to B/Pine-Native. At the 5-15 cm depth in pine beds, no treatment differences for gross N mineralization were found. A significant treatment effect was found for rates of gross nitrification and a treatment x time interaction for NO_3^- immobilization in beds at the 5-15 cm depth (Table 3.2), supported by higher rates in February in B/Pine-Switchgrass compared to B/Pine-Native (Fig. 3.3C and Fig. 3.3D).

In the interbed, presence of switchgrass had numerous impacts on gross N transformations. At the 0-5 cm depth, a significant treatment x time interaction for gross N mineralization and NH_4^+ immobilization and a significant treatment effect for gross nitrification and NO_3^- immobilization were found (Table 3.2). Specifically, gross N mineralization rates were reduced in the IB/Pine-Switchgrass in April, August, and November and were elevated in February compared to IB/Pine-Native (Fig. 3.4A). Gross nitrification (Fig. 3.4C) and NO_3^- immobilization were both lower in the IB/Pine-Switchgrass in April. There was generally no effect of treatment or time on gross N transformations at the 5-15 cm depth in interbeds (Table 3.2; Fig. 3.5A and Fig. 3.5C), except for a small increase in NO_3^- immobilization in IB/Pine-Switchgrass in February (data not shown).

3.3.3. Net N Transformations

In general, net N transformations were less impacted by presence of switchgrass compared to gross N transformations. No time effect was found for net N transformations at either soil depths or locations (Table 3.2), suggesting that this index of N availability was tightly constrained by microbial gross productive and consumptive processes across time. Net N mineralization tended to hover at or below zero, while net nitrification remained positive throughout much of the growing season. Net N mineralization rates were on average 10 times lower than gross N mineralization rates, while net nitrification rates were generally of the same order of magnitude as rates of gross nitrification.

In pine beds at the 0-5 cm depth, no treatment effects were found for net N mineralization or nitrification rates (Table 3.2; Fig. 3.2B and Fig. 3.2D). At 5-15 cm, we did find a significant treatment effect for net N transformations (Table 3.2) and further analysis showed elevated rates of net N mineralization and net nitrification in February in B/Pine-Switchgrass (Fig. 3.3B and 3.3D). Net N mineralization increased from $0.11 \mu\text{g N g}^{-1} \text{soil d}^{-1}$ to $0.32 \mu\text{g N g}^{-1} \text{soil d}^{-1}$ (Fig. 3.3B) and net nitrification from $-0.11 \mu\text{g N g}^{-1} \text{soil d}^{-1}$ to $0.07 \mu\text{g N g}^{-1} \text{soil d}^{-1}$ in these beds (Fig. 3.3D), indicating a switch from net negative to net positive availability of NH_4^+ and NO_3^- .

In the interbeds, switchgrass had relatively little impact on net N transformations. At the 0-5 cm depth, there was a significant treatment effect for net nitrification (Table 3.2). In April, net nitrification was reduced in IB/Pine-Switchgrass (Fig. 3.4D), demonstrating that reductions in gross nitrification and NO_3^- immobilization led to lower rates of net nitrification. There was no effect of treatment or time on net N transformations at the 5-15 cm depth in interbeds (Table 3.2; Figure 3.5B and 3.5D).

3.3.4 Inorganic N Pools and Mean Residence Time

Concentration of inorganic N from T_1 soil KCl extracts and MRT of NH_4^+ and NO_3^- were averaged across all sampling dates to provide an overall index of the effect of switchgrass intercropping on plant-available N pools and internal cycling rates of inorganic N. In pine beds, extractable NH_4^+ concentration at the 0-5 depth was significantly higher in B/Pine-Switchgrass compared B/Pine-Native (Table 3.3), an approximate 14 % increase. The MRT of NH_4^+ was

reduced from 2.8 to 2.2 d in these same beds (Table 3.3), indicating faster turnover of the NH_4^+ pools. In pine beds, NO_3^- concentration and MRT were unaffected by treatment at either sampling depth (Table 3.3). In IB/Pine-Switchgrass, NO_3^- concentrations were reduced by 78 % and 58 % at the 0-5 and 5-15 cm sampling depths, respectively (Table 3.3). Coupled with generally lower rates of gross nitrification, NO_3^- MRT in the IB/Pine-Switchgrass was reduced from 5.1 d to 1.4 d and 4.2 d to 1.6 d at the 0-5 and 5-15 cm depth, respectively, while MRT of NH_4^+ was increased at the 0-5 cm depth (Table 3.3). Although NO_3^- concentrations were quite low, NO_3^- comprised ~ 3 - 17% of total inorganic N.

3.4. Discussion

As of 2005, most bioenergy was produced from 1st generation biofuel feedstocks from agricultural crops, such as corn (USDA-DOE, 2005). Forested ecosystems can produce much of the needed biomass for bioenergy production, especially in intensively managed forests. Southern U.S. pine forests could provide multiple sources of biomass feedstocks, including post-harvest residues or biomass recovered from thinning operations. Furthermore, forest management regimes involving the intercropping of pine and highly productive perennial grasses, such as switchgrass, are being explored (Albaugh et al., 2012). Although use of forest-generated biomass feedstocks is likely to increase, our understanding of long-term sustainability of intensified bioenergy production from forests is not complete. Intensified strategies to extract biomass from forested landscapes will likely lead to greater removal of N, potentially jeopardizing future function and productivity of these systems. Therefore, we were interested in assessing soil N cycling and availability in a young loblolly pine forest intercropped with switchgrass, given the importance of soil N to plant productivity and impacts of potential losses of N on soil quality.

We found that intercropping of loblolly pine and switchgrass had a pronounced effect on soil N transformations and that these effects were not isolated to the interbed, where switchgrass was planted. Treatment differences in gross N transformations were primarily confined to the 0-5 cm soil depth in both beds and interbeds. Overall, rates of gross N transformations were much more sensitive to switchgrass than were those of net N transformations. Net N rates generally hovered around zero and no time effect was found in the repeated measures analysis, supporting

the idea that net N transformations were tightly regulated and constrained by mineralization-immobilization processes in these soils.

Gross N productive and consumptive processes have received less attention in southern U.S. pine forests, whereas net N mineralization and nitrification have been a routine metric for plant-available N for decades (Binkley and Hart, 1989; Coile, 1938; Fox et al., 1986; Gurlevik et al., 2004; Piatek and Allen, 1999). Net N mineralization was relatively low or negative in our system, while gross N mineralization was typically much higher. This is a common observation in studies reporting both gross and net N transformations in pine and temperate forest mineral soils (Vitousek and Andariese, 1986, Christenson et al. 2009). For example, Vitousek and Andariese (1986) found that loblolly pine forests in the Piedmont of North Carolina, USA exhibited higher gross N mineralization rates in the surface mineral soil of a young, intensively managed (~1.5 year old) loblolly pine forest compared to a 22 year-old reference stand and that gross N rates always exceeded net N rates. To further emphasize the magnitude of gross N mineralization in relation to other N inputs and fluxes, we used site bulk density data from beds and interbeds to estimate yearly gross N mineralization rate of ~182 - 365 kg N ha⁻¹ yr⁻¹ (~164 - 324 lbs ac⁻¹ yr⁻¹). In comparison to estimated atmospheric N deposition rates of ~3 - 15 kg N ha⁻¹ yr⁻¹ (Galloway et al., 2008) and periodic fertilization rates in southern pine forests of ~168 - 224 kg N ha⁻¹ (150 - 200 lbs ac⁻¹, Fox et al., 2007a), gross N mineralization rates exceed all natural and anthropogenic yearly N inputs to these forests. This in itself is important to understand as long-term sustainability of intensified land-use will rely on proper management and utilization of existing N stocks and not on externally derived inputs alone.

Gross N mineralization rates measured in our study (0.18 - 4.7 µg N g⁻¹ soil d⁻¹) were within the range of those observed in other pine forest surface mineral soils (0.4 - 4.0 µg N g⁻¹ d⁻¹; Dijkstra et al., 2009; Finzi and Schlesinger, 2003; Parfitt et al., 2003; Staelens et al., 2012; Zeller et al., 2007) and in other temperate forest mineral soils (1.0 - 4.0 µg N g⁻¹ d⁻¹; Staelens et al., 2012; Verchot et al., 2001; Zeller et al., 2007). Gross nitrification was generally lower in our study compared to numerous other studies (Parfitt et al. 2003; Stark and Hart, 1997; Verchot et al., 2001; Zeller et al. 2007), but comparable to those measured in other studies (Staelens et al., 2012; Venterea et al., 2004). Gross nitrification rates were comparable to net nitrification, an observation that has been found in other forested ecosystems (Christenson et al., 2007). Given the acidic nature of these soils and the suppressive effect of high soil moisture on nitrification

(Malhi and McGill, 1982; Miller and Johnson, 1964), low rates of nitrification are not that surprising. Differences in soil biotic and abiotic properties, particularly C and N concentrations (Booth et al., 2005), and above-ground plant species and productivity dynamics likely drive differences in observed rates of gross N transformations between different studies (Christenson et al., 2009; Zellar et al., 2007).

Rates of gross N transformations under switchgrass appear to have received even less attention than in pine forests. In a study on a well-drained Alfisol in central Tennessee, USA, Garten Jr. et al. (2010) observed gross N mineralization and gross nitrification rates under switchgrass ranging from 0.13 - 0.27 g m⁻² d⁻¹ and 0.11 - 0.15 g m⁻² d⁻¹, respectively, over the growing season. Similar to that of gross N transformations rates in other forested ecosystems, our data for gross N mineralization were fairly consistent with that of Garten Jr. et al. (2010) while gross nitrification rates were an order of magnitude lower in our study. Results from studies measuring net N transformation in association with switchgrass typically show that net N transformations are reduced by switchgrass compared to other perennial biofuels or annual row crops (Chatterjee et al., 2013; Davis et al., 2013; Hargreaves and Hofmockel, 2014; Smith et al., 2013).

In beds of the Pine-Switchgrass treatment, gross N mineralization and gross nitrification were higher and resulted in greater plant-available NH₄⁺ at the 0-5 cm sampling depth. These results partially support our first hypothesis stating that higher gross and/or net N transformation rates were responsible for previously observed increases in NO₃⁻ availability (Minick et al., 2014). In the current study, NO₃⁻ concentrations tended to be higher in these pine beds, although the differences were not statistically significant. Treatment differences in gross N transformations were isolated to the November and February sampling dates, possibly reflecting effects of seasonal plant N demand and fine-root turnover and root exudation on microbial activity. At the 5-15 cm depth, switchgrass intercropping resulted in higher net N mineralization and net nitrification in beds, indicating a positive impact of switchgrass on N availability at deeper soil depths. Positive effects of increased soil N availability on pine growth have long been recognized (Birk and Vitousek, 1986; Miller et al., 1976; Powers, 1980) and are fairly ubiquitous across a range of forested ecosystems. Hence, we expect that increased N transformations and inorganic N availability in pine beds will benefit pine growth, particularly if pines are competing with switchgrass for N in the interbed. The direct reasons for increased

gross N transformations in pine beds is unclear, but we speculate that changes in pine root growth and exudation in response to belowground competition has stimulated microbial N cycling processes.

Localized pine root proliferation in surface mineral soil in pine beds adjacent to switchgrass could occur in response to belowground space or resource competition between pine and intercropped switchgrass (Eissenstat and van Rees, 1994; Hodge, 2004; Jackson et al., 2008; Smethurst and Comerford, 1993; Vogt et al., 1986). A recent study at our site found the presence of pine and switchgrass roots in both beds and interbeds, with the greatest mixing of root biomass between each species found at the interface between beds and interbeds (Shrestha, 2013). This indicates the potential for N competition between pine and switchgrass exists. It has been shown that growth of *Panicum* grasses in young slash pine (*Pinus elliotii* Engelm. var. *elliotii*) plantations significantly reduced pine tree root extension (Smethurst and Comerford, 1993), suggesting that pine root growth is indeed altered in response to belowground competition. Preferential rooting in beds by loblolly pine in response to reduced interbed root extension could increase labile C inputs to the soil thereby ‘priming’ microbial activity and release of N into a plant-available form (Dijkstra et al., 2009; Kuzyakov et al., 2000; Phillips et al., 2011). In studies conducted on pine trees, higher gross N mineralization rates have been linked to increased rhizodeposition and fine root production and turnover (Dijkstra et al., 2009; Parfitt et al., 2003; Phillips et al., 2011). For example, Phillips et al. (2011) found that rhizosphere soils collected from a mature loblolly pine stand had higher rates of gross N mineralization under elevated CO₂ due to the positive impact of increased root C exudation on microbial N cycling processes. Furthermore, during sampling dates when we measured elevated gross N mineralization, pines exhibit substantial fine-root growth and turnover (Eissenstat and van Rees, 1994; Vogt et al., 1986). If increased pine root proliferation occurred within the B/Pine-Switchgrass then we would expect greater fine root turnover and inputs of C to the soil during this time of year compared to the B/Pine-Native, thereby enhancing microbial activity and N release. Therefore, stimulation of gross N transformations due to priming of soil microorganisms by loblolly pine provides a mechanism by which these trees could optimize root growth and N capture in beds in response to reduced access to interbed rooting space and/or N pools.

We hypothesized that presence of switchgrass in interbeds would increase mineralization rates, but found that was not true for either gross or net N transformations on three of the four sampling dates. Gross N mineralization and NH_4^+ immobilization rates in switchgrass interbeds were reduced during the growing season in 0-5 cm soil samples, while net N mineralization was unaffected at either sampling depth. One mechanism to explain this result is that soil microbes were more C-limited due to reduced C availability in IB/Pine-Switchgrass compared to IB/Pine-Native, thereby reducing microbial activity. Gross N transformations have been shown to correlate positively with soil C concentration (Barrett and Burke, 2000) and we found support of this in our system where a significant positive correlation existed between soil C concentration and gross N mineralization ($r = 0.37$; $p < 0.0001$). At the same study site, Strickland et al. (2014) sampled soils in 2011 from the 0-15 cm depth and found that presence of switchgrass in interbeds was associated with decreases in total C, particulate organic matter C, and mineralizable C. These results are in agreement with our data showing reduced total C and N at the 0-5 cm depth in IB/Pine-Switchgrass. Supporting the notion that switchgrass has induced losses of soil C and N due to decomposition, $\delta^{15}\text{N}$ values at the 0-5 cm depth under switchgrass were enriched which can be attributed to progressive isotopic fractionation during mineralization of soil organic matter (Högberg, 1997; Nadelhoffer and Fry, 1988). Therefore, we attribute seasonal declines in gross N mineralization under switchgrass to microbial C limitation induced by lower soil C availability. Changes in microbial community structure and function due to the introduction of switchgrass and associated changes in soil C inputs (Strickland et al., 2014) may also affect microbial N demand and therefore could alter N mineralization rates. Future studies should characterize microbial communities in response to the introduction of bioenergy grasses to forested systems and link these communities to indices of microbial function in order to gain a deeper understanding of how forest-based bioenergy production influences soil N biogeochemistry.

Interestingly, gross N mineralization was higher in February after switchgrass senescence, potentially signifying the impact of switchgrass fine root turnover and rhizodeposition on C availability and soil N cycling processes (Phillips et al., 2011). One plausible mechanism for increased gross N mineralization in the IB/Pine-Switchgrass was priming of the microbial community via increased switchgrass root C inputs due to root turnover during switchgrass dormancy. This explanation is founded in the same rationale used to interpret

data from pine beds showing increased gross N transformations in B/Pine-Switchgrass and agrees with expected patterns of N mineralization-immobilization upon alleviation of a microbial C limitation (Dijkstra et al., 2009; Phillips et al., 2011; Wardle, 1992).

Given that availability of C is the main factor considered limiting to soil microbial activity (Wardle, 1992), seasonal changes in C availability should alter microbial activity and lends support to our observation of increased gross N mineralization rates in February in IB/Pine-Switchgrass. By the time senescence occurs, switchgrass has likely experienced peak root growth with an overwinter decline in live roots (Garten Jr. et al., 2010; Tufekcioglu et al., 2003), providing a potentially substantial pool of labile C via root turnover during dormancy and after cutting (e.g., February sampling date). For example, Garten Jr. et al. (2010) and Tufekcioglu et al. (2003) showed that switchgrass increased fine- and coarse-root biomass and C and N content throughout the growing season and peaking in November, providing a significant pool of labile C compounds as those live roots turnover during dormancy. Based on results found in this study, microbial C limitation is likely present during the growing season in IB/Pine-Switchgrass due to reduced C availability but this limitation is alleviated during dormancy when switchgrass-derived root C inputs are higher under switchgrass compared to native vegetation.

In support of our third hypothesis, switchgrass led to reduced gross and net nitrification in interbeds in April. Enhanced utilization of NH_4^+ and NO_3^- by switchgrass during spring (Minick et al., 2014) may result in intense plant-microbe competition for inorganic N. Reduced availability of NH_4^+ substrate to nitrifying microorganisms can reduce gross and net N nitrification and NO_3^- immobilization (Kay and Hart, 1997; Zak et al., 1990), which was likely given reduced rates of gross N mineralization. Turnover of the NO_3^- pool at both interbed sampling depths was faster under switchgrass, which could lead to a leakier system relative to losses of N. Although, the low rates of nitrification and reduced size of an already small NO_3^- pool found in this study, suggest that this effect will not result in significant losses of N. Furthermore, increased NH_4^+ MRT indicate that N may be retained in a more environmentally favorable and stable form within the Pine-Switchgrass ecosystem.

3.5. Conclusion

We were able to develop a robust understanding of how managing young (4 year old) loblolly pine forests for bioenergy production influences N cycling and availability. Switchgrass intercropping had a significant impact on soil N cycling processes and inorganic N pools in both beds and interbeds. Rates of gross N transformations increased in beds of the Pine-Switchgrass treatment resulting in increased concentrations of plant-available N. During the period of this study, no difference in aboveground productivity of pine trees between the two treatments was observed (Eric Sucre *personal communication*), indicating that either pine productivity was not impacted by intercropped switchgrass or that increased N availability in pine beds was maintaining expected levels of productivity. We hypothesize that increased pine rooting in these beds, in response to switchgrass intercropping, has alleviated microbial C limitation thereby stimulating microbial N production and consumption and ultimately increasing plant-available N. Alternatively, microbial C limitation was likely present in IB/Pine-Switchgrass due to reduced soil C and N concentrations and likely explains observed reductions in gross N transformations during the growing season. Similar to that of B/Pine-Switchgrass, increased gross N transformations in February in IB/Pine-Switchgrass may have resulted from increased C inputs via switchgrass fine root turnover stimulating microbial activity during switchgrass dormancy.

This study lends support and clarification to patterns of N cycling and availability measured in a previous study at this same site, which showed that switchgrass intercropping led to increased NO_3^- availability in beds of the Pine-Switchgrass treatment and a reduction in NH_4^+ and NO_3^- availability in IB/Pine-Switchgrass (Minick et al. 2014). Our data also supports the positive effect that switchgrass has on ecosystem N retention (McLaughlin and Kszos, 2005; Smith et al., 2013; Tufekcioglu et al., 1999; Tufekcioglu et al., 2003), via reductions in soil NO_3^- and the potential losses of N due to leaching or denitrification. Widespread introduction of bioenergy grasses to southern pine plantations as a cellulosic biomass feedstock may lead to more efficient cycling and utilization of soil N, although longer-term effects on soil N cycling and availability must be considered when determining sustainability of intercropping systems. We expect that switchgrass will become less competitive for resources at pine crown closure, due to shading effects on switchgrass growth. This should reduce competitive interactions, but

the specific timing of this should be monitored. Furthermore, the potential to incorporate decomposing switchgrass roots into actively cycling soil C and N pools upon pine canopy closure may have a positive effect on soil C storage, soil N availability, and pine productivity. Understanding belowground feedbacks among the plant-soil-microbe communities is important to our understanding of ecosystem function and productivity as anthropogenically driven demands on forested lands continue to increase.

3.6. Acknowledgments

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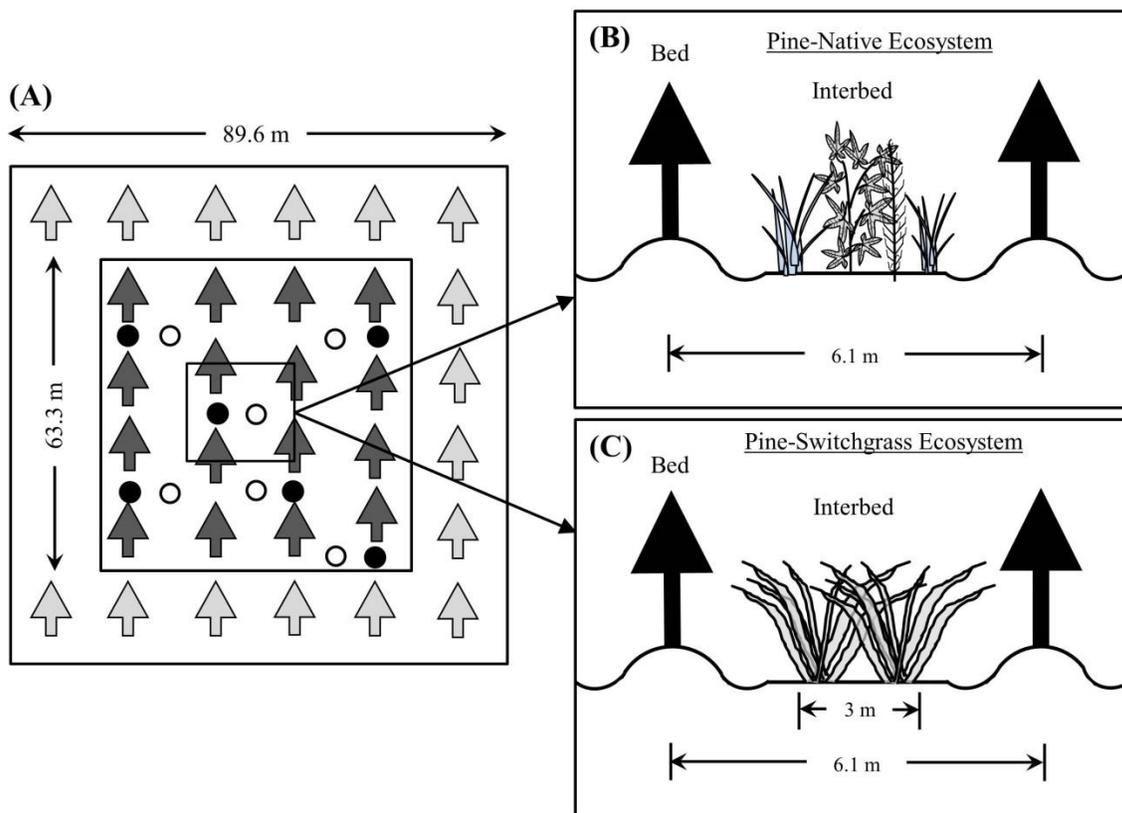


Figure 3.1. Diagram representing plot and treatment design. Panel (A) represents the 0.4 ha measurement plot nested within the 0.8 ha treatment plot. Open circles (○) represent interbed sampling points and closed circles (●) represent bed sampling points. Panel (B) represents a schematic of the Pine-Native treatment with bed and interbed regions identified and the associated interbed 'native' plant communities. Panel (C) represents a schematic of the Pine-Switchgrass intercropping treatment with bed and interbed regions identified and switchgrass located in a 3 m wide strip in the interbed region.

Table 3.1. Soil C and N concentrations, C:N ratio, and ^{13}C and ^{15}N natural abundance for soil samples collected from the 0-5 cm and 5-15 cm depth from beds and interbeds. Data represent means for all sampling dates with standard error in parenthesis (n=4). Significant differences between treatment means within each sampling depth and location are indicated by different letters ($P < 0.1$).

Treatment	Soil C (g C kg ⁻¹ soil)	Soil N (g N kg ⁻¹ soil)	C:N	Soil $\delta^{13}\text{C}$ (‰)	Soil $\delta^{15}\text{N}$ (‰)
Bed					
<u>0-5cm</u>					
Pine-Native	64 (5)	2.5 (0.3)	26.5 (2.3)	-28.0 (0.3)	1.6 (0.1)
Pine-Switchgrass	62 (5)	2.3 (0.2)	27.0 (0.8)	-28.2 (0.3)	1.4 (0.2)
<u>5-15cm</u>					
Pine-Native	74 (6)	2.8 (0.2)	27.5 (2.1)	-27.6 (0.4)	1.6 (0.2)
Pine-Switchgrass	69 (4)	2.5 (0.1)	27.2 (0.5)	-27.7 (0.2)	1.5 (0.1)
Interbed					
<u>0-5cm</u>					
Pine-Native	99 (7)a	4.1 (0.3)a	24.6 (1.3)	-27.1 (0.2)	0.5 (0.1)a
Pine-Switchgrass	72 (5)b	2.9 (0.2)b	25.3 (0.4)	-26.6 (0.3)	1.4 (0.1)b
<u>5-15cm</u>					
Pine-Native	60 (6)	2.3 (0.3)	27.4 (2.1)	-28.1 (0.5)	2.1 (0.2)
Pine-Switchgrass	50 (5)	1.9 (0.2)	26.2 (0.9)	-29.2 (0.6)	2.3 (0.2)

Table 3.2. Results (p-value) of repeated measures ANOVA testing effects of treatment, time, and treatment*time interaction for gross and net N transformations measured at two soil depths in the beds and interbeds using a short-term (2d) soil laboratory incubation method.

Source	DF	Gross N Min.	NH ₄ ⁺ Immob.	Net N Min.	Gross Nitr.	NO ₃ ⁻ Immob.	Net Nitr.
Bed							
<u>0-5cm</u>							
Treatment	1	0.008	0.031	0.353	0.001	0.016	0.052
Time	3	0.001	0.001	0.716	<0.0001	<0.0001	0.284
Treatment*Time	3	0.022	0.071	0.409	0.008	0.204	0.672
<u>5-15cm</u>							
Treatment	1	0.342	0.261	0.024	0.097	0.432	0.049
Time	3	0.001	0.001	0.194	<0.0001	0.001	0.147
Treatment*Time	3	0.994	0.822	0.318	0.765	0.104	0.457
Interbed							
<u>0-5cm</u>							
Treatment	1	0.023	0.141	0.502	0.100	0.079	0.059
Time	3	<0.0001	<0.0001	0.306	0.312	0.039	0.326
Treatment*Time	3	0.004	0.009	0.236	0.327	0.425	0.411
<u>5-15cm</u>							
Treatment	1	0.715	0.202	0.197	0.308	0.629	0.157
Time	3	0.001	0.001	0.885	0.140	0.029	0.896
Treatment*Time	3	0.958	0.130	0.333	0.266	0.080	0.471

Table 3.3. Inorganic N concentrations and mean residence time of NH_4^+ and NO_3^- pools. Data represent means for all sampling dates with standard error in parenthesis (n=4). Significant differences between treatment means within each sampling depth and location are indicated by different letters ($P < 0.1$).

	Initial NH_4^+ ($\mu\text{g N g}^{-1}$)	Initial NO_3^- ($\mu\text{g N g}^{-1}$)	NH_4^+ Mean Residence Time (d)	NO_3^- Mean Residence Time (d)
Bed				
<u>0-5cm</u>				
Pine-Native	2.33 (0.24) a	0.08 (0.01)	2.8 (0.1) a	2.1 (0.4)
Pine-Switchgrass	2.65 (0.29) b	0.19 (0.08)	2.2 (0.3) b	1.7 (0.4)
<u>5-15cm</u>				
Pine-Native	2.63 (0.39)	0.13 (0.05)	3.1 (1.2)	2.5 (0.1)
Pine-Switchgrass	2.92 (0.15)	0.30 (0.13)	2.0 (0.2)	5.2 (2.7)
Interbed				
<u>0-5cm</u>				
Pine-Native	5.71 (0.90)	0.91 (0.38) a	3.9 (0.7) a	5.1 (2.16) a
Pine-Switchgrass	4.38 (0.26)	0.20 (0.10) b	8.6 (1.4) b	1.4 (0.36) b
<u>5-15cm</u>				
Pine-Native	3.28 (0.55)	0.67 (0.27) a	2.4 (0.4)	4.2 (1.0) a
Pine-Switchgrass	3.03 (0.38)	0.28 (0.05) b	2.0 (0.2)	1.6 (0.9) b

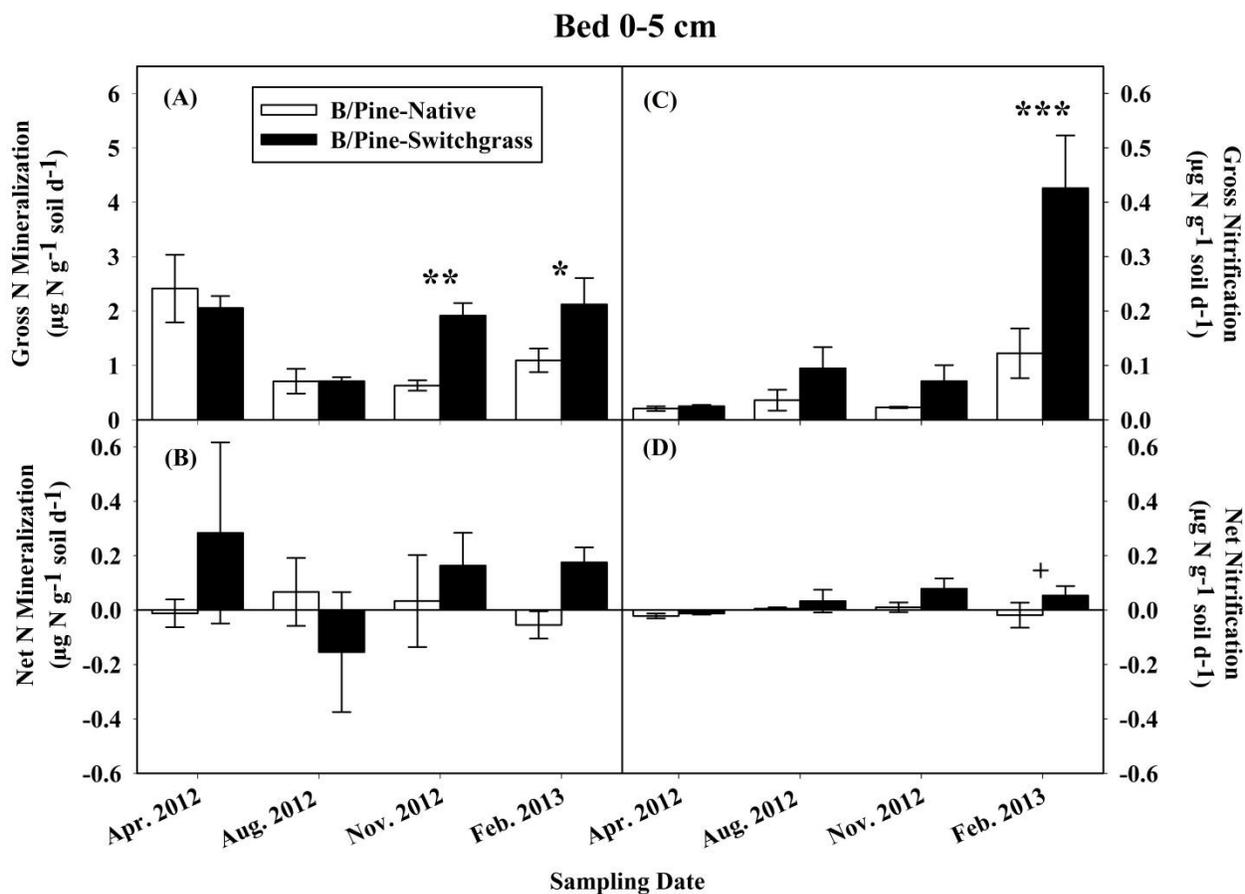


Figure 3.2. Gross (A) and net N mineralization (B) and gross (C) and net nitrification (D) for samples collected from pine beds at the 0-5 cm depth. Bars represent means with standard error ($n=4$). Symbols above bars indicate significant differences between B/Pine-Native and B/Pine-Switchgrass treatments at each sampling date ($^{\dagger} P < 0.1$; $* P < 0.05$, $** P < 0.01$, $*** P < 0.0001$).

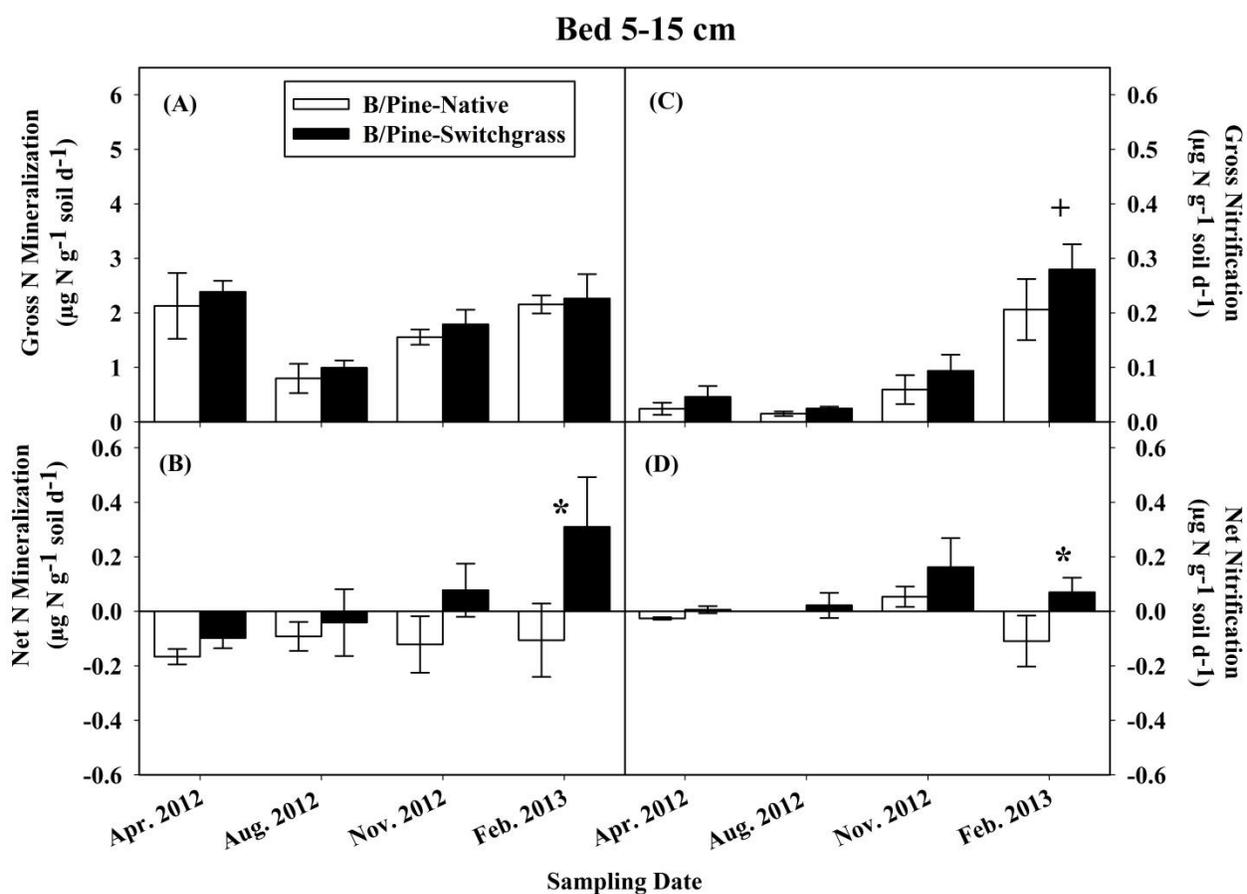


Figure 3.3. Gross (A) and net N mineralization (B) and gross (C) and net nitrification (D) for samples collected from pine beds at the 5-15 cm depth. Bars represent means with standard error (n=4). Symbols above bars indicate significant differences between B/Pine-Native and B/Pine-Switchgrass treatments at each sampling date ([†] P < 0.1; * P < 0.05, ** P < 0.01, ***P < 0.0001).

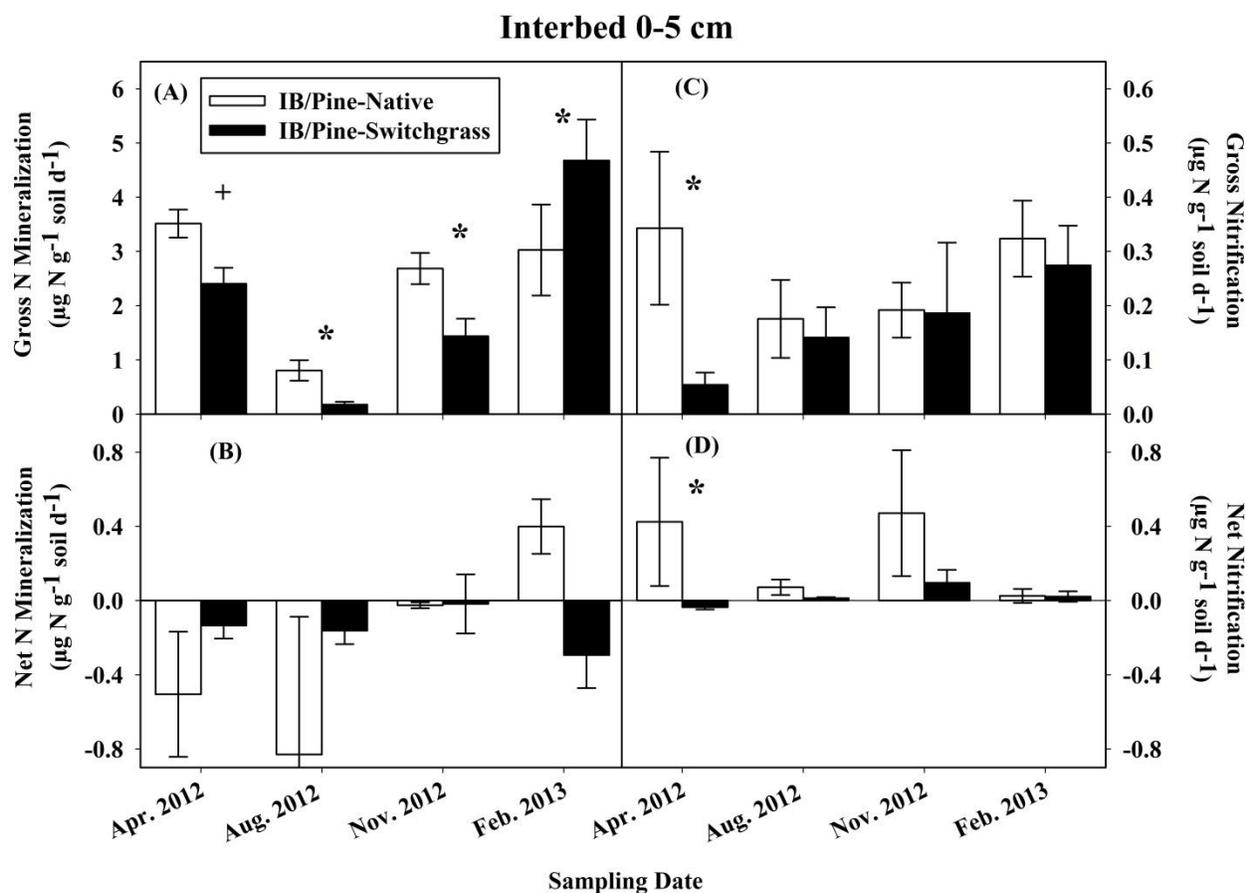


Figure 3.4. Gross (A) and net N mineralization (B) and gross (C) and net nitrification (D) for samples collected from interbeds at the 0-5 cm depth. Bars represent means with standard error ($n=4$). Symbols above bars indicate significant differences between IB/Pine-Native and IB/Pine-Switchgrass treatments at each sampling date ($^+ P < 0.1$; $* P < 0.05$, $** P < 0.01$, $*** P < 0.0001$).

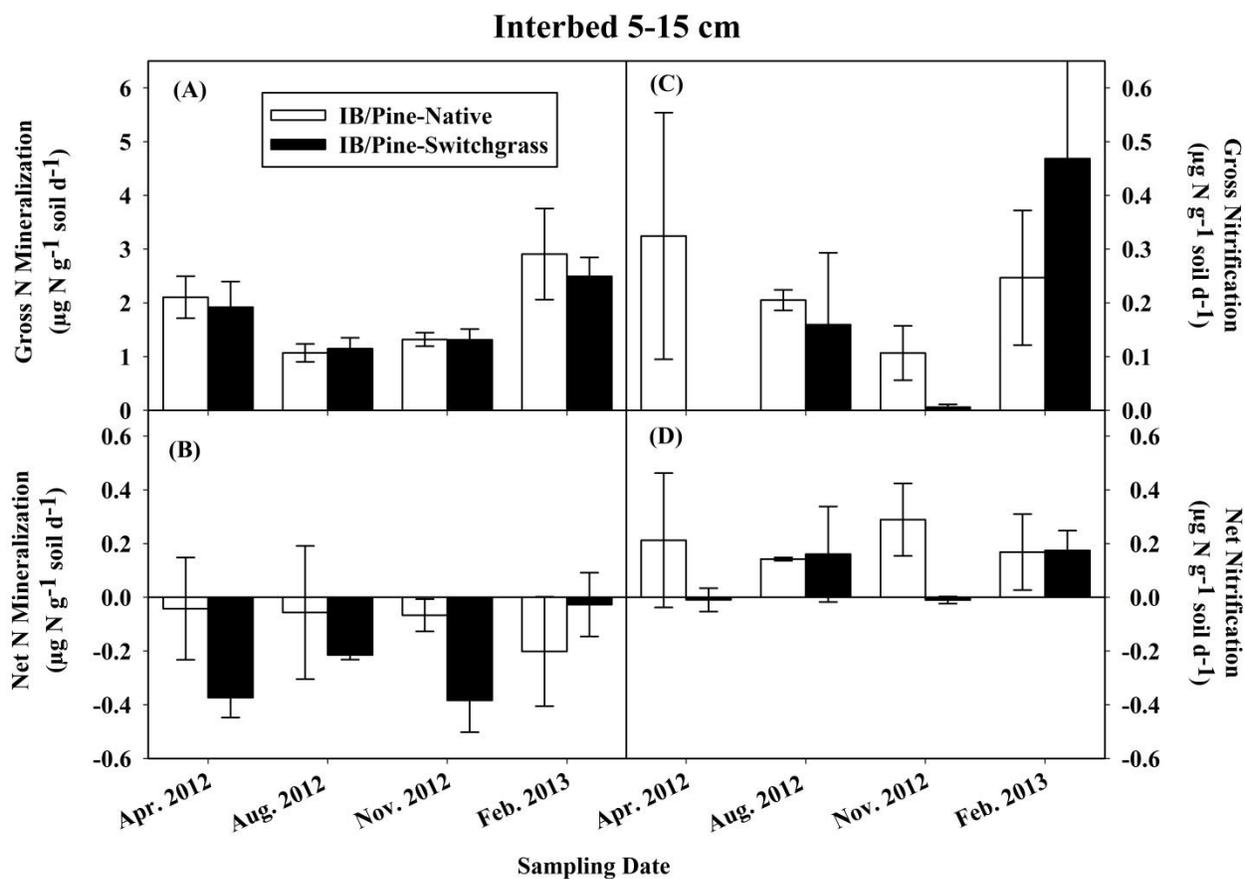


Figure 3.5. Gross (A) and net N mineralization (B) and gross (C) and net nitrification (D) for samples collected from interbeds at the 5-15 cm depth. Bars represent means with standard error (n=4). Symbols above bars indicate significant differences between IB/Pine-Native and IB/Pine-Switchgrass treatments at each sampling date ([†] P < 0.1; * P < 0.05, ** P < 0.01, ***P < 0.0001).

Chapter 4. Soil organic matter stabilization in loblolly pine forest mineral soils of coastal North Carolina

Abstract

Soil organic matter (SOM) stabilization largely results from physical or chemical mechanisms that protect organic carbon (C) compounds from microbial degradation. Understanding how forest management impacts these SOM stabilization mechanisms is needed to better manage soil for C storage, particularly those that aim increase the production and removal of biomass for bioenergy production. In the southern US, managed loblolly pine (*Pinus taeda* L.) forests have received recent attention as a potential system to provide increased biomass production, including interplanting of loblolly pine with a woody or herbaceous bioenergy crop. Our study site was located in coastal North Carolina in young loblolly pine stands (2.5 years old) that had either switchgrass (*Panicum virgatum* L.) planted in between bedded rows of pine trees (Pine-Switchgrass) or interbed space comprised of native, coastal successional vegetation (Pine-Native). Soil samples were collected from beds and interbeds of both treatments at four sampling depths (0-5, 5-15, 15-30, and 30-45 cm) and SOM was fractionated into three aggregate sizes (>2000, 2000-250, and 250-53 μm) and three organo-mineral densities (1.65, 1.85, and 2.00 g cm^{-3}). We utilized the unique ^{13}C signature of switchgrass vegetation (a C4 plant) compared to that of loblolly pine vegetation (a C3 plant) to investigate changes in ^{13}C isotopic composition of bulk soil and SOM fractions and estimate inputs of pine- versus switchgrass-derived C. Overall, switchgrass intercropping led to a reduction of plot-level bulk soil C concentrations and in total C content in pine beds of the Pine-Switchgrass treatment. Across all aggregate fractions and soil depths, aggregate C concentration was significantly reduced in beds of the Pine-Switchgrass treatment. Values of $\delta^{13}\text{C}$ for the >2000 μm aggregates at the 0-5 cm depth were diluted, suggesting the incorporation of 13-25% of the existing >2000 μm C pool with pine-derived C. Elevated C concentrations were found in the occluded light fractions (<1.65, 1.65-1.85, and 1.85-2.00 g cm^{-3}) in both beds and interbeds of the Pine-Switchgrass treatment. Enriched $\delta^{13}\text{C}$ in these fractions led to estimates of between 2.5-12.5% of existing soil C comprised of new switchgrass-derived C. In the free light fraction, pine-derived C contributed 15 and 9% on average of this C pool at the 5-15 and 15-30 cm depth,

respectively. The replacement of older C with new pine-derived C possibly reflects increased fine root growth and turnover in beds in response to switchgrass intercropping. Stabilization of SOM by aggregates or mineral adsorption responded differently to management, with pine and switchgrass having a variable impact on each mechanism. Overall, changes in aggregate-associated C appeared to better predict changes in bulk soil C pools than did mineral-associated C.

4.1. Introduction

Forested ecosystems store large amounts of atmospheric carbon (C) as plants incorporate CO₂ into organic compounds and deposit C into soil organic matter (SOM) pools. The degree of C accrual in forest soils is determined by inputs of plant- and animal-derived organic C compounds and outputs of CO₂ during decomposition. Detailed understanding of forest soil C cycling and storage are critical to determine the role of forests in the cycling of C between atmospheric and terrestrial pools. Forests can act as C sinks or sources dependent upon the impact of forest management on processes that influence soil C sequestration (Guo and Gifford, 2002; Huang et al., 2011; Jandl et al., 2007; Johnson and Curtis, 2001). However, our ability to predict changes in and effectively manage forests for soil C sequestration hinges on our understanding of more subtle changes in soil C dynamics revealed by fractionation techniques. Increasingly, demands have been put on forested ecosystems to supply biomass necessary for wood and energy production, while the impacts of intensified land-use on SOM is still relatively unknown. Southern U.S. pine plantations are of special interest given the large land area occupied by these highly productive forests and the opportunity to maximize biomass production through precision silviculture (Fox, 2000; Fox et al., 2007), including the potential to utilize space between pine trees to interplant a woody or herbaceous biomass feedstock (Albaugh et al., 2012). In this study, the impact of intercropping loblolly pine (*Pinus taeda* L.) with switchgrass (*Panicum virgatum* L.) on SOM dynamics was evaluated in a young loblolly pine forest (2.5 years old) located in the Lower Coastal Plain of North Carolina.

Complex interactions between organic C compounds and soil biological, physical, and chemical properties act in concert to drive soil C cycling and storage. Over the last three decades, mechanisms responsible for turnover and stability of SOM have received renewed attention due to advances in our understanding of stabilization of SOM within soil matrix and soil biogeochemical properties which influence soil C storage (Schmidt et al., 2011). Historically, the residence time of SOM has been viewed as a function of inherent molecular structure of C molecules and the formation of large, humic complexes (Stevenson, 1994). Current theories based on empirical evidence downplay the importance of molecular recalcitrance for long-term SOM stabilization and contend that long-term stabilization of soil C is driven by physical and chemical protection mechanisms which reduce microbial access to C

compounds (Dungait et al., 2012; Jastrow et al., 2007; Schmidt et al., 2011; von Lützow et al., 2006). Mechanisms of SOM stabilization that occur in forest soils include formation of uncomplexed, biochemically recalcitrant SOM (e.g., low-N C compounds, lignin, humified SOM), spatially inaccessible SOM (e.g., aggregate-occluded), and adsorption of C compounds to mineral surfaces (e.g., organo-mineral complexes) (Jastrow et al., 2007; Kögel-Knabner et al., 2008; Oades, 1988; Schmidt et al., 2011; Six et al., 2002; Sollins et al., 2006).

Many methods are employed to separate SOM fractions, and the various C constituents found in each fraction (von Lützow et al., 2007), in an attempt to isolate homogenous functional groups of SOM in regards to turnover rate and accessibility to microbial degradation. Two main methods have been developed to test for aggregate- and mineral-associated C, including sieving to separate SOM stabilized within and among soil aggregates and density flotation to isolate particulate and occluded organic matter associated with various clay and mineral surfaces. Methods used to fractionate SOM pools help tease apart finer-scale influences of forest management on the susceptibility of SOM to decomposition and provide useful information about soil C dynamics over the short-term that are not revealed through bulk soil C measurements alone. We were interested in comparing C protected via aggregate formation and through chemical associations with soil minerals in a loblolly pine forest in coastal North Carolina and in determining how these stabilization mechanisms are impacted by intensified forest biomass production and removal.

Changes in SOM pools can also be detected by ^{13}C stable isotope analysis given differences in the isotopic signature of organic C compounds found in pine and switchgrass. Distinct photosynthetic pathways of loblolly pine trees (C3-plant) and switchgrass (C4-plant) lead to the fixation of biomass-C with distinct isotopic signatures. Contrasts in the $\delta^{13}\text{C}$ value between a C3 plant ($\sim -28.0\text{‰}$) and a C4 plant ($\sim -12\text{‰}$) (Garten Jr. and Wullschleger, 2000; Skjemstad et al., 1990) provide an excellent marker to determine changes in SOM pools due to switchgrass-derived C inputs. Introduction of switchgrass to this system can be viewed as an *in situ* ^{13}C labelling of the SOM pool and can be leveraged to study C dynamics. Changes in the ^{13}C isotopic composition of SOM can arise as new C4 organic material is incorporated into existing SOM pools and replaces native C. Changes in the ^{13}C -signature of total SOM and SOM fractions have been measured following establishment of switchgrass on arable or marginal lands previously inhabited by C3 plants and typically show positive effects of switchgrass on soil C

storage (Garten Jr. and Wullschleger, 1999; Garten Jr. and Wullshleger 2000; Ma et al., 2000). Analysis of ^{13}C isotopic composition of SOM fractions will provide insight into which SOM fractions switchgrass-derived C is accumulating and which mechanism of SOM stabilization is most sensitive to the introduction of switchgrass into these forests.

Soil organic matter stabilization has been investigated in both southern pine plantations and switchgrass fields, although few studies have looked at the effects of intercropping on soil C dynamics. Stabilization of SOM in southern US pine forests has received detailed attention (Echeverría et al., 2004; Johnsen et al., 2013; Maier et al., 2012; Sarkhot et al., 2007a,b; Sarkhot et al., 2008), focusing on a basic understanding of factors influencing soil C storage and the impact of various forest management practices on belowground C storage. On degraded land, marginal lands, or in comparison to other arable lands switchgrass production typically increases soil C specifically that of the POM or labile C pools (Garten Jr. and Wullschleger, 2000; McLaughlin and Kszos, 2005; Ocumpaugh et al., 2003). Recent studies in which switchgrass has been introduced into young pine forests have found variable impacts on total soil C, but appear consistent in regards to the positive effect of switchgrass on labile C pools and microbial activity (Blazier et al., 2012; Strickland et al., 2014). These studies provide evidence that switchgrass production can influence SOM fractions, specifically those classified as labile. Most studies evaluate only one mechanism of SOM stabilization or utilize a combination of fractionation procedures to characterize SOM dynamics. Analysis aggregate and mineral stabilized SOM mechanisms rarely occur simultaneously, therefore understanding of how forest management affects SOM stabilization and soil C sequestration may be incomplete.

We were interested in testing multiple SOM stabilization mechanisms in our system in order to fully evaluate SOM stabilized by chemical and physical mechanisms and to test their sensitivity to pine-switchgrass intercropping. Developing a detailed understanding of soil C dynamics in these young loblolly pine forests will provide information on short-term C cycling and may be suggestive of future trends in soil C storage. Our objectives were to: 1) quantify and compare SOM stabilized by chemical (organo-mineral interactions) and physical (aggregate) mechanisms; and 2) determine the effect of pine-switchgrass intercropping on the concentration and distribution of C stabilized by soil aggregation and interactions with mineral surfaces.

4.2. Materials and Methods

4.2.1. Study Site and Treatments

The Lenoir I research site was located in the Lower Coastal Plain physiographic province near Dover, NC (35°15'N, 77°28'W). Soils are mapped as Pantego (fine-loamy, siliceous, semiactive, thermic Umbric Paleaquults) or Rains (fine-loamy, siliceous, semiactive, thermic Typic Paleaquults) soil series and are very poorly drained (USDA Soil Survey, 2013). However, previous site management in the 1970's included installation of ditches to lower the water table and reduce saturation at the soil surface. Mean air temperature was approximately 16.5°C and mean annual precipitation was approximately 1252 mm (Albaugh et al., 2012).

From June-September 2008, treatments were established in a randomized complete block design with four blocks (0.8 ha treatment plots with 0.4 ha measurement plots with a minimum 15 m outer buffer) following harvest of a 34-year-old loblolly pine plantation with a site index of 21.3 m at age 25. In order to address our specific objectives, two treatments were utilized for this study including: 1) loblolly pine planted on bedded rows with harvest residues removed (i.e., all woody debris > 5 cm in diameter removed) and native vegetation allowed to re-colonize the area between rows of loblolly pine (Pine-Native); and 2) loblolly pine planted on bedded rows with harvest residues removed and switchgrass interplanted between the rows of loblolly pine (Pine-Switchgrass).

Sites for both treatments were mechanically prepared, including V-shearing to break up stumps and roots, and bedding. Harvest residues > 5 cm in diameter were removed using a grapple-claw excavator. Approximately 85% of the non-merchantable woody biomass was removed, not including sheared stumps (Beauvais, 2010). The switchgrass treatment was subjected to an extra strip V-shear within the interbed, one week before switchgrass planting. This additional V-shearing prepared soil for better germination and mechanical planting. Containerized loblolly pine seedlings (improved 2nd generation genetic material) were hand planted in December 2008 at 1100 stems ha⁻¹ and spacing of 6.1 m between bed rows and 1.5 m within rows. Switchgrass planted at this research site was 'Alamo', a lowland variety which has been identified as the most promising for biomass production across the southeastern US (Ocumpaugh et al., 2003). In June 2009, switchgrass was machine-seeded at 9 kg pure live seed

ha⁻¹ in a 3 m wide strip between bedded rows of pine trees at a depth of 6 cm and in rows spaced 38 cm apart. Switchgrass was mowed in 2009 and mowed, raked, and baled in December 2010, at the end of the second growing season. Switchgrass treatments were fertilized in June 2010 with a coated urea fertilizer composed of N (65.6 kg ha⁻¹), phosphorus (P) (6.6 kg ha⁻¹), and boron (B) (0.2 kg ha⁻¹). Herbicide was originally applied to the whole site, before pine and switchgrass establishment. The switchgrass treatment received an extra herbicide application immediately prior to planting and in June 2010 to facilitate establishment of switchgrass over other competing vegetation, primarily nutsedge (*Cyperus* spp.). Interbeds with native vegetation were characterized by the presence of mixed grass, tree, woody shrub, vine, and forb species, including switchcane (*Arundinaria gigantea*), broomsedge (*Andropogon virginicus*), sweetgum (*Liquidambar styraciflua*), blackberry (*Rubus* spp.), bonesets (*Eupatorium* spp.), goldenrods (*Solidago* spp.), and pepperbush (*Clethra alnifolia*) (Lane, 2010).

4.2.2. Soil Collection and Processing

Soil samples were collected in June of 2011, in the third growing season for both loblolly pine and switchgrass. Four soil samples from both the beds and interbeds of each treatment were collected using a 7 cm diameter PVC corer. Soil cores were composited by location (e.g., bed and interbed) and by discrete depth increments at 0-5, 5-15, 15-30, and 30-45 cm (Fig. 4.1). Samples were stored on ice and transported back to the lab for further processing within one week from collection. Soils in beds were subject to mixing during site preparation and generally included mixed A and Btg horizons from 0-30 cm and a Btg horizon from the 30-45 cm depth (Fig. 4.1). The soil profile sampled in the interbeds included an A horizon from 0-15 cm and a Btg horizon from the 15-45 cm depth with clear signs of mottling. For data presentation and discussion, soil samples collected from pine beds (B) from the two treatments are identified as either Pine-Native/B or Pine-Switchgrass/B. Soil samples collected from interbeds (IB) from the two treatments are identified as either Pine-Native/IB or Pine-Switchgrass/IB.

Composited soil samples were air dried and passed through an 8 mm sieve to remove large organic matter. A 75 g subsample was removed for the soil aggregate analysis. The remaining air-dried soil was sieved through a 2 mm sieve to further homogenize the soil and used for bulk soil C analysis and the density fractionation procedure. Soil subsample masses

were corrected for soil moisture by drying a subsample of soil at 105°C for 24 h and were measured separately for each SOM fractionation method. Bulk soil samples were ball milled and analyzed for C and N concentrations and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signature on an IsoPrime 100 stable isotope ratio mass spectrometer (IRMS) interfaced with an elemental vario MICRO cube dry-combustion elemental analyzer (elementar, Hanau, Germany). Soil bulk density for beds and interbeds was used to estimate stand-level total soil C content. Soil bulk density was measured in July 2011 at 0-15, 15-30, and 30-45 cm depth in the beds and interbeds, with measurements for the 0-15cm depth being applied to both the 0-5 and 5-15 cm depths collected for the SOM analysis.

4.2.3. *Aggregate Fractionation*

Wet sieving methods developed by Elliot and Cambardella (1991) and Cambardella and Elliot (1993) were used to separate aggregates of various size. Soils were sequentially passed through three sieves to obtain large macroaggregates (>2000 μm size fraction), small macroaggregates (2000-250 μm size fraction), and microaggregates (250-53 μm size fraction). Air dried soils (50 g dry weight) were submerged in distilled water (dH_2O) for 5 min on a 2 mm sieve. Slaked aggregates were separated by moving the sieve 3 cm vertically 50 times over 2 min, breaking the surface of the water each time. Residual aggregates and other organic material remaining on the sieve were back washed into aluminum pans and dried at 55°C. Material which passed through the 2 mm sieve was gently transferred to the 250 μm sieve size and the previous steps were repeated. These steps were repeated once more using a 53 μm sieve to obtain the 250-53 μm size fraction. Particles passing through the < 53 μm sieve were not collected, but C concentration for the <53 μm fraction was calculated by multiplying the bulk soil C concentration by the estimated proportion of material passing through the 53 μm sieve. Dried aggregate fractions were ground by mortar and pestle and analyzed for C and N concentrations and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signature on an IsoPrime 100 stable IRMS interfaced with an elemental vario MICRO cube dry-combustion elemental analyzer (elementar, Hanau, Germany).

4.2.4. Density Fractionation

We hypothesized that switchgrass would more heavily influence the light fraction (LF) versus heavy fraction (HF), therefore multiple densities were chosen that were representative of lighter fraction SOM ($< 2.0 \text{ g cm}^{-3}$). Densities chosen to fractionate at for this study included 1.65 g cm^{-3} , 1.85 g cm^{-3} , and 2.0 g cm^{-3} (Golchin et al., 1994a; Sollins et. al., 2006). Light fraction (LF) and heavy fraction (HF) SOM were separated using a combination of sequential density fractionation methods (Golchin et al., 1994a; Gochin et. al., 1994b; Baisden et al., 2002; Sollins et. al., 2009) using sodium polytungstate ($\text{Na}_3\text{W}_0_4:9\text{W}_0_3:\text{H}_2\text{O}$; Sometu-Europe, Berlin, Germany) (SPT) dissolved in dH_2O as the density reagent (Baldock et al., 1990). A procedure developed by Six et al. (1999) was used to recycle the SPT to allow reuse of the reagent and avoid C contamination between samples. Methods presented by Golchin et al. (1994a) were used to isolate the free LF ($\text{fLF}_{<1.65}$) and the occluded LF ($\text{oLF}_{<1.65}$) at a density of 1.65 g cm^{-3} SPT. In order to separate the $\text{fLF}_{<1.65}$, 10 g of air dried soil for all samples were separately weighed into 50 mL centrifuge tubes after which 35 mL of $1.65 \pm 0.03 \text{ g cm}^{-3}$ SPT was added. Capped tubes were inverted 5 times to mix the sample thoroughly and was allowed to settle for 30 min before centrifugation at 2000 g for 1 h. Material floating after centrifugation was aspirated onto a $0.8 \mu\text{m}$ Whatman Treck-etched polycarbonate membrane and filtered under vacuum. The sample was rinsed with dH_2O under a vacuum and transferred to a pre-weighed aluminum boat and dried at 55°C for 24 h. To obtain the oLF , the soil remaining in the centrifuge tube was resuspended in 35 mL of $1.65 \pm 0.03 \text{ g cm}^{-3}$ SPT, placed in an ice bath, and sonicated for 5 min at 100 % power and 70 % pulse time using a UP100H sonicator with a 10 mm probe tip (Hielscher Ultrasonics GmbH, Teltow, Germany). The tube was then gently rotated by hand to resuspend the remaining soil sample at each density of SPT. The same steps to recover the $\text{fLF}_{<1.65}$ were repeated to recover the $\text{oLF}_{<1.65}$, as well as SOM fractions separated at densities of 1.85 and 2.00 g cm^{-3} SPT (e.g., $\text{oLF}_{1.65-1.85}$, $\text{oLF}_{1.85-2.00}$, and $\text{HF}_{>2.00}$). The final fraction that did not float in the 2.00 g cm^{-3} SPT was rinsed and centrifuged three times with 35 mL dH_2O to remove residual SPT and then transferred to an aluminum boat for drying. All fractions were ground by mortar and pestle and analyzed for C and N concentrations and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signature on an IsoPrime 100 stable IRMS interfaced with an elemental vario MICRO cube dry-combustion elemental analyzer (elementar, Hanau, Germany).

4.2.5. $\delta^{13}\text{C}$ Isotopic Calculations

We measured the ^{13}C natural abundance of bulk soil and SOM fractions and calculated the percent of C originating from switchgrass, utilizing an equation outlined by Fry (2006), as follows:

$$\% \text{ C} = (\delta^{13}\text{C}_{\text{switchgrass soil}} - \delta^{13}\text{C}_{\text{native soil}}) / (\delta^{13}\text{C}_{\text{switchgrass root}} - \delta^{13}\text{C}_{\text{native soil}}) * 100$$

Where $\delta^{13}\text{C}_{\text{switchgrass soil}}$ is the $\delta^{13}\text{C}$ value of the bulk soil or SOM fraction collected from the Pine-Switchgrass treatment, $\delta^{13}\text{C}_{\text{native soil}}$ is the $\delta^{13}\text{C}$ value of the corresponding bulk soil or SOM fraction collected from the Pine-Native treatment, and $\delta^{13}\text{C}_{\text{switchgrass root}}$ is the average $\delta^{13}\text{C}$ value of switchgrass roots collected from our study site. Switchgrass roots collected at this site had a $\delta^{13}\text{C}$ value of -13.0 ± 0.47 ‰. The average $\delta^{13}\text{C}$ value for switchgrass roots was used as the input for $\delta^{13}\text{C}_{\text{switchgrass root}}$ in the above equation. Due to the nature of the isotope mass balance approach, higher (less negative) $\delta^{13}\text{C}$ values in the Pine-Native samples compared with the corresponding Pine-Switchgrass samples resulted in negative values for those samples. Negative values were assumed to result from two scenarios: 1) loblolly pine (C3) inputs in the Pine-Switchgrass treatment diluting soil $\delta^{13}\text{C}$ values; or 2) non-switchgrass, C4-plant inputs in the Pine-Native treatment increasing soil $\delta^{13}\text{C}$ values; in which the former reasoning was invoked to explain negative values arising from this analysis.

4.2.6. *Statistical Analysis*

We were interested in testing how SOM stabilization mechanisms differed between beds and interbeds and with soil depth, as well as testing effects of pine-switchgrass intercropping on SOM stabilization. Therefore, soil C and $\delta^{13}\text{C}$ data for bulk soils and SOM fractions were analyzed using repeated measures ANOVA (PROC MIXED; SAS institute, Cary, NC), with soil depth as the repeated measure and treatment, sampling location, and SOM fraction as fixed effects and block as a random effect (Schabenberger and Pierce, 2001). Percent data were arc-sine transformed prior to statistical analysis in order to conform to normal distribution. All other raw data were natural log-transformed where necessary to establish homogeneity of variance.

All data were plotted as means of raw data with main effects and interactions being estimated on untransformed and transformed data where appropriate. If significant main effects or interactions were detected in the repeated measures ANOVA, then these differences were further tested using the Tukey-Kramer multiple comparison procedure. When all fixed effects were involved in significant interactions, analysis of treatment differences at each sampling location, depth, and fraction was carried out using the Tukey-Kramer multiple comparison procedure. Data were presented for all treatment, sampling locations, soil depths, and SOM fractions in order to facilitate comparison among fractionation methods and to address the multitude of interactions between various factors found in the repeated measures ANOVA. For analysis of switchgrass- and pine-derived C, the 95 % C.I. were calculated and any value with an interval crossing over zero was assumed to represent no new inputs of C to SOM pools by pine or switchgrass.

4.3. Results

4.3.1. Bulk Soil C

Bulk C concentration ranged from 11 to 60 g C kg⁻¹ soil and 6.9 to 94 g C kg⁻¹ soil across all soil depths sampled in the bed and interbed, respectively (Table 4.1). A significant location x depth interaction ($F = 20.04$; $p < 0.0001$) was found, implying that changes in soil C with depth were different in beds compared to interbeds. The impact of soil mixing due to bedding on bulk C concentration with depth was evident by the presence of high C concentrations well into the 15-30 cm depth and the overall greater concentrations compared to the interbed 15 - 30 and 30-45 cm depth (Table 4.1). Furthermore, the 5-15 cm depth in pine beds had the highest C concentration, followed by the 0-5 cm, 15-30 cm, and 30-45 cm depths. In the interbeds, soil C concentration decreased with soil depth as expected (Table 4.1). Carbon concentration in the 0-5 cm depth of the interbeds was significantly higher than that of the bed, for all other depths the bed was significantly higher (Table 4.1).

Total C content ranged from 15 to 88 Mg C ha⁻¹ (Table 4.1). For total C, there was a significant treatment x location interaction ($F = 4.19$; $p = 0.06$). Total C was 57 ± 8.4 Mg ha⁻¹ for Pine-Switchgrass/B and 42 ± 6.4 Mg ha⁻¹ for Pine-Native/B, amounting to 27% less total C in

Pine-Switchgrass/B compared to Pine-Native/B ($F = 4.19$; $p = 0.05$). In the interbed, total C content was $34 \pm 4.0 \text{ Mg ha}^{-1}$ for Pine-Switchgrass/B and $39 \pm 4.9 \text{ Mg ha}^{-1}$ for Pine-Native/B. A significant location \times depth interaction ($F = 7.89$; $p = 0.0005$) was also found for total C content and further analysis showed that total C content in beds decreased with soil depth in the following order: 5-15 cm = 15-30 cm > 0-5 cm = 30-45 cm and in interbeds where 5-15 cm = 0-5 cm = 15-30 cm > 30-45 cm (Table 4.1).

Bulk soil ^{13}C isotopic composition ranged from -26.5 to -28.0 (‰) (Table 1). A significant treatment \times location \times depth interaction ($F = 2.56$; $p = 0.07$) was found. Values of $\delta^{13}\text{C}$ were significantly higher at the 0-5 cm depth in Pine-Switchgrass/B and significantly lower at the 0-5 cm depth in Pine-Switchgrass/IB (Table 4.1).

4.3.2. Aggregate Fractionation

Multiple interactions were found for C data obtained from aggregate fractions. For the distribution of C among aggregate size fractions, a significant location \times depth \times fraction interaction ($F = 3.57$; $p = 0.0005$) was found. The distribution of C among aggregate fractions was concentrated in the 2000-250 μm aggregate fraction at all depths, except for the 0-5 cm depth in the interbed of both the Pine-Native and Pine-Switchgrass treatment where similar C concentrations were found in the >2000 μm size fraction (Fig. 4.2). Furthermore, a treatment \times fraction interaction ($F = 3.66$; $p = 0.02$) was found which corresponded to ~8% more C associated with the 2000-250 μm size fraction in the Pine-Switchgrass treatment compared to the Pine-Native treatment when averaged over each depth and sampling location. Specifically, distribution of C was significantly higher in the 2000-250 μm size fraction at the 0-5 and 5-15 cm depth in the Pine-Switchgrass/B by ~17 and ~21 %, respectively, compared to Pine-Native/B (Fig. 4.2).

For aggregate-associated C concentration, a significant location \times depth \times fraction interaction ($F = 2.01$; $p < 0.04$) was found. In both beds and interbeds, the 250-53 μm size fraction had significantly lower C concentration down to 30 cm compared to most other soil fractions (Fig. 4.3). At the 15-30 cm depth in beds, the >2000 μm fraction was significantly higher than all other fractions (Fig. 4.3). At the 30-45 cm depth in both beds and interbeds, the >2000 μm fraction exhibited higher C concentration (Fig. 4.3). It is important to note that most

data from the >2000 μm fraction had missing values (indicated by the lack of error estimates) due to the fact that aggregates of this size were generally not found at this soil depth (Fig. 4.3). At the 5-15 and 15-30 cm depth, the >2000 μm fraction was significantly higher than the <53 μm fraction (Fig. 4.3).

A treatment x location x depth interaction ($F = 2.58$; $p = 0.06$) was also found for aggregate-associated C concentration, indicating that the overall C concentration for aggregate fractions varied by treatment at each sampling depth and location. Average C concentration for all aggregate fractions was significantly lower in Pine-Switchgrass/B compared to Pine-Native/B at the 0-5, 15-30, and 30-45 cm depth amounting to ~23%, ~28% and ~34% reduction, respectively. Differences in aggregate C concentrations between Pine-Native/B and Pine-Switchgrass/B stemmed from numerous reductions in C concentration among aggregate fractions in the Pine-Switchgrass treatment (Fig. 4.3). In the Pine-Switchgrass/B at the 0-5 cm depth, C concentration was reduced in the > 2000 μm aggregate fraction by ~30 % (from $64 \pm 5.8 \text{ g kg}^{-1}$ to $45 \pm 5.0 \text{ g kg}^{-1}$), the 250-53 μm aggregate fraction by ~29 % (from $45 \pm 5.3 \text{ g kg}^{-1}$ to $32 \pm 6.0 \text{ g kg}^{-1}$), and the < 53 μm aggregate fraction by ~27 % (from $60 \pm 7.9 \text{ g kg}^{-1}$ to $44 \pm 6.6 \text{ g kg}^{-1}$) (Fig. 4.3). Carbon concentration was also reduced in the Pine-Switchgrass/B at 15-30 cm depth in the 2000-250 μm aggregate fraction (Fig.4.3). No treatment differences in aggregate C concentrations were found between interbed C concentrations.

Values of $\delta^{13}\text{C}$ for aggregate fractions were much higher than those measured for bulk soil, irrespective of switchgrass intercropping (Table 4.1; Table 4.2). Average $\delta^{13}\text{C}$ values for the >2000 μm , 2000-250 μm , and the 250-53 μm size fraction were -21.2 ± 0.21 (‰), -21.9 ± 0.28 (‰), and -21.7 ± 0.14 (‰). A significant treatment x location x depth x fraction interaction ($F = 2.58$; $p = 0.006$) was found with significant treatment differences in $\delta^{13}\text{C}$ values depending on sampling location, depth, and aggregate fraction (Table 4.2).

4.3.3. Density Fractionation

As with the aggregate fractionation data, multiple interactions were found for density fractionation data, although they tended to be a different set of interactions. Further, significant interactions in the density fraction data differed based on the response variable. For the distribution of C among density fractions (Fig. 4.4), a location x depth x fraction interaction ($F =$

11.03; $p < 0.0001$) was found. Carbon was primarily concentrated in the $fLF_{<1.65}$ at the surface and in the $HF_{>2.00}$ at depth (Fig. 4.4). For example, at the 0-5 cm depth in the interbed approximately 50% of total C was found in the $fLF_{<1.65}$ depth and only 12% in the $HF_{>2.00}$. At the 30-45 cm depth however, less than 10% of C was found in the $fLF_{<1.65}$ and approximately 65% was found in the >2.00 HF. Otherwise, distribution of C among the other density fractions was uniform and generally below 25% (Fig. 4.4). More uniform distribution of soil C among density fractions at 0-30 cm depths was observed in beds due to the effect of soil mixing to this depth during establishment of beds. Changes in C distribution among density fractions were evident at 15-30 cm depth in the interbeds, while the 5-15 cm depth appeared to be a transition zone between dominance of fLF to HF for C storage (Fig. 4.4).

Carbon concentrations in the LFs ranged from 5.2 to 467 $g\ kg^{-1}$ (Fig. 4.5). On average, C concentrations were $333 \pm 6.10\ g\ kg^{-1}$ in the $fLF_{<1.65}$, $419 \pm 6.45\ g\ kg^{-1}$ in the $oLF_{<1.65}$, $310 \pm 6.38\ g\ kg^{-1}$ in the $oLF_{1.65-1.85}$, $191 \pm 6.82\ g\ kg^{-1}$ in the $oLF_{1.85-2.00}$, and $11 \pm 0.87\ g\ kg^{-1}$ in the $HF_{>2.00}$. In summary, C concentration varied with density in the following order: $oLF_{<1.65} > fLF_{<1.65} = oLF_{1.65-1.85} > oLF_{1.85-2.00} > HF_{>2.00}$. These general patterns in C concentration at different organo-mineral densities were consistently observed in beds and interbeds and at each location (Fig. 4.5).

A significant treatment x location x sampling depth x fraction interaction ($F = 2.42$; $p = 0.0005$) was found for C concentrations of density fractions. In the Pine-Switchgrass treatment, C concentrations were higher in several oLF s in soils collected from both the bed and interbed at the 30-45 cm depth (Fig. 4.5). Average C concentration was almost always higher for oLF s in the Pine-Switchgrass treatment compared to the Pine-Native treatment (Fig. 4.5). Carbon concentration averaged across all oLF fractions ($oLF_{<1.65} + oLF_{1.65-1.85} + oLF_{1.85-2.00}$) and sampling locations was significantly higher in the Pine-Switchgrass treatment for all sampling depths (treatment x depth interaction; $F = 3.25$, $P = 0.03$), equating to a 9 to 53 % increase (or 27 to 89 $g\ C\ kg^{-1}$) in C across all depths compared to the Pine-Native treatment.

Values of $\delta^{13}C$ for density fractions were similar to those of bulk soil samples and were therefore more diluted than that of aggregate fractions (Table 4.3). A significant treatment x fraction interaction ($F = 8.76$; $p < 0.0001$) was found due to differences in $\delta^{13}C$ values of density fractions between the two treatments when averaged over location and sampling depth. Further analysis showed that $\delta^{13}C$ values were enriched in the $oLF_{<1.65}$ in the Pine-Switchgrass treatment

compared to the Pine-Native treatment averaging -28.2‰ and -29.2‰, respectively ($F = 11.06$; $p = 0.0013$). In the $\text{oLF}_{1.65-1.85}$, the $\delta^{13}\text{C}$ value was also enriched measuring -27.1‰ and -28.0‰ for the Pine-Switchgrass and the Pine-Native treatment, respectively ($F = 6.62$; $p = 0.0117$). In the $\text{HF}_{>2.00}$, we found an opposite effect wherein the Pine-Native treatment had enriched $\delta^{13}\text{C}$ values of -27.9‰ compared to -29.2‰ of the Pine-Switchgrass treatment ($F = 16.14$; $p = 0.0001$). A significant depth x fraction interaction was also found ($F = 2.09$; $p = 0.02$). Differences between $\delta^{13}\text{C}$ values of density fractions at each sampling depth were identified for the 0-5 cm ($F = 8.25$; $p < 0.0001$), 5-15 cm ($F = 5.90$; $p = 0.0002$), and 15-30 cm ($F = 9.07$; $p < 0.0001$) depths. Average $\delta^{13}\text{C}$ values for density fractions in the 0-5, 5-15 cm, and 15-30 cm depth were more dilute in the $\text{oLF}_{<1.65}$ (-28.9 to -28.5‰) and the $\text{HF}_{>2.00}$ (-28.8 to -27.9‰) while $\delta^{13}\text{C}$ values for the $\text{oLF}_{1.65-1.85}$ (-27.6 to -27.3‰) and the $\text{oLF}_{1.85-2.00}$ (-27.2 to -26.5‰) were generally more enriched. The $\text{fLF}_{<1.65}$ (-28.0 to -27.0‰) tended to be either more or less enriched than the other density fractions, except for the $\text{oLF}_{1.65-1.85}$ for which it was never significantly different. No significant differences in $\delta^{13}\text{C}$ values between density fractions were found for the 30-45 cm depth ($F = 1.50$; $p = 0.2$).

4.3.4. Pine- and Switchgrass-Derived C

Using $\delta^{13}\text{C}$ values, we calculated the proportion of C in bulk soil and SOM fractions comprised of recent pine- and switchgrass-derived C. In interbeds, we found that new inputs of switchgrass-derived C accounted for approximately 4.8% and 1.5% of bulk soil C at the 0-5 and 5-15 cm depth, respectively (Table 4.4). No inputs of switchgrass-derived C were calculated for any aggregate fraction in beds or interbeds. Surprisingly, ^{13}C calculations indicated a 7.5% contribution of pine-derived C to the bulk soil C pool at the 0-5 cm depth and 3.8% at the 15-30 cm depth (Table 4.4). Furthermore, at the 0-5 cm depth in Pine-Switchgrass/B we calculated a negative value ranging from 13.1 to 24.9% in the $> 2000 \mu\text{m}$ aggregate fraction.

In stark contrast to calculations for aggregate fractions, we calculated inputs of switchgrass-derived C into the $\text{oLF}_{<1.65}$ and $\text{oLF}_{1.65-1.85}$ at multiple sampling depths in both beds and interbeds of the Pine-Switchgrass treatment (Table 4.4). Incorporation of switchgrass-derived C accounted for 2.5 to 12.5% of the C associated with the $\text{oLF}_{<1.65}$ and $\text{oLF}_{1.65-1.85}$, respectively. At the 30-45 cm depth in Pine-Switchgrass/IB, new C inputs from switchgrass

accounted for upwards of 20% of C within the oLF_{1.85-2.00}. It was also surprising to find a shift towards lower $\delta^{13}\text{C}$ values for the fLF_{<1.65} in Pine-Switchgrass/B. Based on the ^{13}C calculations, approximately 9 - 18 % of C in the fLF_{<1.65} at the 5-15 and 15-30 cm depth could be attributed to recent inputs of pine-derived C (Table 4.4). Finally, we calculated inputs of pine-derived C into the HF_{>2.00}, which implies that this density fraction may also be susceptible to management induced changes over short-time periods, even though this fraction is typically considered more stable than the fractions <2.00 g cm⁻³ density fractions.

4.4. Discussion

4.4.1. General

Soil C storage results from a multitude of abiotic and biotic processes which stabilize C primarily via the formation of soil aggregates and organo-mineral complexes. Fractionating C based on these stabilization mechanisms was useful to test short-term changes in soil C and to gain a deeper understanding of how these soil C pools respond to pine-switchgrass intercropping. Switchgrass intercropping impacted stabilization of SOM in both aggregate and density fractions and these impacts were found in beds, where pines were planted, and interbeds, where switchgrass was planted. For example, aggregate-associated C was reduced in beds of the Pine-Switchgrass treatment, while mineral-associated C was higher in the interbeds of Pine-Switchgrass treatment. It appears that loss of aggregate-stabilized C in the beds of the Pine-Switchgrass treatment had a stronger impact on bulk soil C than did gains of mineral-stabilized C in the interbeds. That is, bulk C content was significantly reduced in the beds of the Pine-Switchgrass treatment, while no changes in bulk soil C were found in the interbeds. Furthermore, estimates of pine- and switchgrass-derived C supports the notion that the effect of pine-switchgrass intercropping was dependent on the given stabilization mechanism and was influenced differently by each plant species. This was reflected primarily by inputs of pine-derived C into the >2000 μm aggregate size fraction and the fLF and switchgrass-derived C inputs into oLF. While these general interpretations of our results are not necessarily surprising, it is clear that evaluation of only one stabilization mechanism may lead to different interpretations of how forest management impacts long-term soil C dynamics. Therefore, care

should be taken when considering which stabilization mechanism is most relevant in a given system and susceptible to change under different management scenarios.

4.4.2. *Aggregate Fractionation*

We found that aggregate-associated C was significantly reduced in Pine-Switchgrass/B, with estimates of ~20% of the C associated with the >2000 μm aggregate fraction being comprised of new pine-derived C inputs. Macroaggregates, particularly those > 2000 μm , are more susceptible to plant inputs and land-use change and generally have a faster turnover time than those of smaller macroaggregates or microaggregates (Balesdent et al., 1987; Jastrow et al., 1996; John et al., 2005). In support of this finding, Jastrow et al. (1996) used aggregate- ^{13}C natural abundance to show that macroaggregates receive greater inputs of recent C than those of microaggregates. Carbon associated with the macroaggregate fraction is typically considered the most accessible to soil microbial breakdown (Elliott, 1986; Puget et al., 2000; Yamashita et al., 2006) and in a less decomposed state compared to microaggregates (Gregorich et al., 2006; Golchin et al., 1994a, b; Sohi et al., 2001). Furthermore, C within microaggregates is considered more stable due to its inclusion within other aggregates and longer residence time compared to macroaggregate-associated C (John et al., 2005; Puget et al., 2000; von Lützow et al., 2007; Yamashita et al., 2006). Therefore, it is not surprising that new pine-derived C inputs were only found for the >2000 μm aggregate fraction. Together, our results suggest that recent inputs of C by pines and potential priming effects may be leading to loss of aggregate-associated C in Pine-Switchgrass/B.

The distribution of C was primarily concentrated in the 2000-250 μm fractions at all soil depths in both beds and interbeds, demonstrating the importance of this fraction for overall soil C storage in these forest soils. Other studies in southern pine plantations have found this aggregate fraction to be an important reservoir for soil C. For example, in a study in a 4-year-old loblolly pine plantation located in a sandy Florida spodosol, Sarkhot et al. (2007a) also found that the majority of organic C (40-46 %) in the 0-10 cm depth fell within the 2000-250 μm fraction and that intensive forest management reduced soil C content of aggregates in the 2000-53 μm range. Aggregate stability and C residence time increases with decreasing aggregate size, including >2000 μm and the 2000-250 μm aggregate fractions (Balesdent et al., 1987; Jastrow et al., 1996;

Puget et al., 2000; Six et al., 2002; Yamashita et al., 2006). Overall, ~8% more was C associated with the 2000-250 μm aggregate fraction in the Pine-Switchgrass treatment compared to the Pine-Native treatment. Therefore, reversal of soil C losses due to switchgrass intercropping may be ameliorated over the long-term if storage of C continues to shift toward the 2000-250 μm aggregate fraction.

The ^{13}C content of aggregates was much lower than those measured in bulk soil, indicating that either: 1) aggregate-associated C has been subject to microbial degradation and loss of lighter C from residual C substrates, or 2) relics of past vegetation previous to the establishment of pine plantations are still evident in aggregate-stabilized SOM. It seems unlikely that this finding resulted from treatment effects in the present study, since consistently lower $\delta^{13}\text{C}$ values were found regardless of sampling location, depth, or treatment. Given the nature of our study and our original objectives, we are unable to verify if this finding is representative of relics of past land-use, advanced degree of decomposition, or some other factor not considered in this study. Jastrow (1996) and Balesdent et al. (1987) both found similar ^{13}C values for aggregate fractions recovered in soils collected from ungrazed pastures comprised of C3 grasses and under long-term corn (C4) cultivation. John et al. (2005) found that aggregates separated from forest soils had $\delta^{13}\text{C}$ values closer to those expected in a C3-dominated system. They attribute differences in aggregate-associated ^{13}C to the nature of the predominant vegetation, namely C3 plants diluting ^{13}C content and C4 plants enriching ^{13}C content of aggregate-associated C.

4.4.3. Density Fractionation

The occluded fractions (e.g., oLF_{<1.65}, oLF_{1.65-1.85}, oLF_{1.85-2.00}) were most sensitive to detecting changes in C concentration due to switchgrass intercropping and had overall higher C concentration than density fractions from the Pine-Native treatment. Light fraction C concentrations from our study exceeded those measured by Echeverria et al. (2004) in the 0-10 cm A horizon in loblolly pine stands in Georgia and Florida, but were comparable to those measured by McFarlane et al. (2010) in three soil types supporting *Pinus ponderosa* stands in northern California. Soil organic matter fractionated at increasingly higher densities is commonly associated with increased stability and degree of transformation (John et al., 2005; von Lützow et al., 2007), as indicated by losses of O-alkyl C (e.g., carbohydrates) and increases

in mean residence time (Golchin et al., 1994a, b; John et al, 2005). Furthermore, C compounds isolated within the 1.65-2.00 g cm⁻³ oLF are composed of organic C compounds indicative of more highly decomposed organic matter than those found in the fLF (Golchin et al. 1994a, b; Sohi et al., 2001). Therefore, increased C concentrations in <2.00 g cm⁻³ occluded density fractions in the Pine-Switchgrass treatment suggests accumulation of more highly decomposed C compounds in these density fractions. Similar to the chemical structure of C compounds identified with decreasing aggregate size, C compounds at increasing density are of a more decomposed nature evident by greater proportion of aliphatic hydrocarbon (e.g., proteins), carboxylic anions, and aromatic C (Sohi et al., 2001). It is therefore possible that the observed increases in occluded LF C concentrations indicate accumulation of decomposing aggregate-associated C within these density fractions.

Distribution of C among density fractions varied with depth and was not impacted by switchgrass, but did exhibit significant changes in distribution with soil depth. Differences in C distribution among density fractions with soil depth likely relate to differences in the A horizon versus Btg horizons, with the Btg subsurface horizons receiving less POM inputs compared to that of the A horizon and characterized by more adsorptive clay and mineral surfaces. The 5-15 cm depth in the interbed and the 15-30 cm depth in the bed appear to represent a transition zone between SOM stabilized in LFs and HF. In a study of an 80 year-old *Picea abies* stand in Germany, John et al. (2005) measured a shift in the distribution of C among density fractions with depth, with 33.6% and 48.4% of C found in the fLF_{<1.6} and HF_{>2.00}, respectively, at the 0-7 cm depth and 14.6 % and 77.2% of C found in the fLF_{<1.6} and HF_{>2.00}, respectively, at the 25-40 cm depth. These findings reflect the decreasing importance of POM inputs and increasing importance of mineral- and clay-bound SOM to C storage with soil depth.

In the Pine-Switchgrass treatment, we measured enriched $\delta^{13}\text{C}$ values for C associated with the oLF_{<1.65} and oLF_{1.65-1.85}, regardless of sampling location or soil depth. Given that switchgrass biomass has $\delta^{13}\text{C}$ values of ~12 (‰), we expect that inputs of organic matter from switchgrass was likely driving the observed reductions in $\delta^{13}\text{C}$ value of interbeds. The proportion of switchgrass-derived C accounted for 5 to 10 % of C associate with these two density fractions, which was similar to the amount of switchgrass-derived C previously estimated to have been recently incorporated into the POM C pool at this site (Strickland et al., 2014). It is unclear though how measurable amounts of switchgrass-derived C are being incorporated into

density fractions of pine beds. We speculate that progressive ^{13}C fractionation by soil microorganisms during decomposition led to enriched $\delta^{13}\text{C}$ values in these fractions, especially if we attribute increased oLF C concentrations to accumulation of decomposing aggregate-associated C. However, we cannot rule out that this has resulted from inputs of switchgrass biomass to beds during switchgrass baling or encroachment of switchgrass roots into adjacent pine beds (Shrestha, 2013). Finally, in agreement with ^{13}C calculations for aggregate fractions, we measured inputs of pine-derived C into the fLF at the 5-15 and 15-30 cm depth. This result lends further support to the notion that pine-derived C inputs are increasing in SOM pools characterized by POM, potentially reflecting increased pine root growth and a labile C source to prime decomposing microorganisms in beds of the Pine-Switchgrass treatment.

4.4.4. *Comparison of Fractionation Procedures*

Techniques used to fractionate SOM into operationally defined pools are likely more sensitive to management-induced changes in soil C than bulk soil measurements, although a few notable changes in bulk soil C pools were found. Total soil C content was significantly lower in the Pine-Switchgrass/B compared to Pine-Native/B, with a small but meaningful proportion of this pool comprised of new pine-derived C. In contrast to the beds, estimates of C inputs into the interbed indicated incorporation of switchgrass-derived C into the bulk soil C pool. Soil C concentration in bulk mineral soil and SOM fractions far exceeded that of other studies conducted in many southern US loblolly pine forests and other forested ecosystems (Choi et al., 2005; Echeverria et al., 2004; Richter et al., 1999; Sarkhot et al. 2007a) due to the high organic matter content characteristic of drained Lower Coastal Plain soils. Therefore, it is noteworthy that detectable changes in bulk soil C pools and ^{13}C isotopic composition were identified over such a short time period indicating that bulk soil measurements can provide important information in regards to changes in soil C over the short-term. Utilization of SOM fractionation techniques is important though, in order to gain a deeper understanding of changes in soil C due to forest management.

Light or POM fractions are typically found to be most sensitive to disturbance, land-use change, and management and represent an easily accessible source of SOM for microbial breakdown and nutrient release (Compton and Boone, 2002; Magid and Kjaergaard, 2001;

McLaughlin et al., 2005). Consequently, fractionation methods that isolate POM or LF are the focus of studies interested in understanding management impacts on soil C dynamics and nutrient cycling and availability (Gregorich et al., 2006; Magid and Kjaergaard, 2001; Strickland et al., 2011; Strickland et al., 2014). Surprisingly, intercropping of switchgrass had less of an effect on C dynamics of aggregate and density fractions in the interbeds than expected. Hence, the notion that switchgrass would increase labile or POM soil C pools was only weakly supported by the density fractionation data. On the other hand, $\delta^{13}\text{C}$ calculations revealed significant accumulation of pine-derived C in the $> 2000 \mu\text{m}$ and $\text{fLF}_{<1.65}$, both of which are indicative of POM pools. Therefore, species effects on SOM fractions and stabilization mechanisms are important to consider when determining their impact on C storage.

It is not surprising that overlap among SOM stabilization mechanisms and fractionation methods contribute to the difficulty in generalizing the impact of pine-switchgrass intercropping on SOM dynamics. To date, methods used to separate SOM fractions are only operationally defined. Organic matter associated with aggregates is typically characterized by old roots, fungal hyphae, microbial byproducts, and other partially decomposed organic compounds (Tisdall and Oades, 1982), while organic matter separated by density represents a range of organic compounds and their interactions with different minerals and reactive surfaces (Golchin et al., 1994a,b; Sollins et al., 2006; Sollins et al., 2009). Neither method isolates mutually exclusive SOM fractions, with both mechanisms acting in concert to stabilize SOM. Therefore, our interpretation of soil C dynamics will likely depend on the stabilization mechanism.

Despite these complications, our results do indicate that the aggregate and density fractionation methods separate functionally dissimilar organic C compounds which are impacted differently by forest management. This was shown by the reduction in aggregate-associated C and the increase in mineral-associated C in the Pine-Switchgrass treatment. In many instances, soil C concentration and distribution of aggregate- and mineral-stabilized C were subject to different interactions between treatment, sampling location, soil depth, and SOM fraction. Regardless of the explanation for these results, it is clear that separating SOM into fractions using aggregate and density fractionation methods may respond differently to forest management. Combining fractionation methods may provide a more detailed analysis of SOM stabilization (Six et al., 1998), although these methods still produce heterogeneous SOM fractions (von Lützow et al., 2007).

Differences between ^{13}C natural abundance of SOM fractions separated by density and aggregates were not unexpected. Natural ^{13}C abundance of SOM fractionated by density was much closer to that of bulk soil, if not slightly lower, while that associated with aggregates was much higher. Soil organic matter stabilized by soil aggregation may be of a more highly decomposed nature, in which the lighter C isotope is preferentially utilized by microorganisms leading to retention of the heavier C isotope in SOM or result from incorporation of organic material with an enriched ^{13}C signature. On the other hand, SOM stabilized by organo-mineral complexes may represent C compounds associated with dissolved organic compounds, root exudates, or microbial byproducts which would likely exhibit lower ^{13}C isotopic composition. John et al. (2005) found that $\delta^{13}\text{C}$ values of density fractions from an 80 year old *Picea abies* stand in Germany were between -25.0 (‰) and -26.4 (‰) for free $\text{POM}_{<1.6}$, occluded $\text{POM}_{1.6}$ and $1.6-2.0$, and of the mineral fraction ($>2.00 \text{ g cm}^{-3}$). These values are generally less negative than those found in our study, in which $\delta^{13}\text{C}$ values ranged from -26.0 (‰) to -30.0 (‰). We expect these differences in aggregate and density fraction $\delta^{13}\text{C}$ values arise from variations in the C constituents separated by each fractionation method and the stabilization of different plant- and microbial-derived C compounds by each mechanism.

4.5. Conclusion

We were interested in testing different SOM stabilization mechanisms in a loblolly pine forest and potential changes in soil C storage due to pine-switchgrass intercropping. Separation of SOM stabilized by different mechanisms can provide information useful to predict longer-term patterns in C cycling and to gain a mechanistic understanding of changes in bulk soil C. Overall, our results suggest that the introduction of switchgrass to these pine plantations is leading to losses of total soil C, primarily in pine beds, as a result of reduced aggregate-associated C. In these soils, aggregates within the 2000-250 μm fraction are likely the most important in regards to storage of C and distribution. Interestingly, distribution of C in the 2000-250 μm aggregate size fraction was enhanced by switchgrass intercropping which may enhance C storage in the future. Interestingly, results from the density fractionation show increased C concentrations in the oLFs in response to switchgrass intercropping. Therefore, it is likely that C associated with the occluded light fractions represent a functionally different group of C

compounds than those associated with aggregates. Given the results of this study, we conclude that changes in soil C in these forest soils can be identified in as little time as 2.5 years since forest establishment and the different mechanisms of SOM stabilization may respond differently to forest management.

4.6. Acknowledgments

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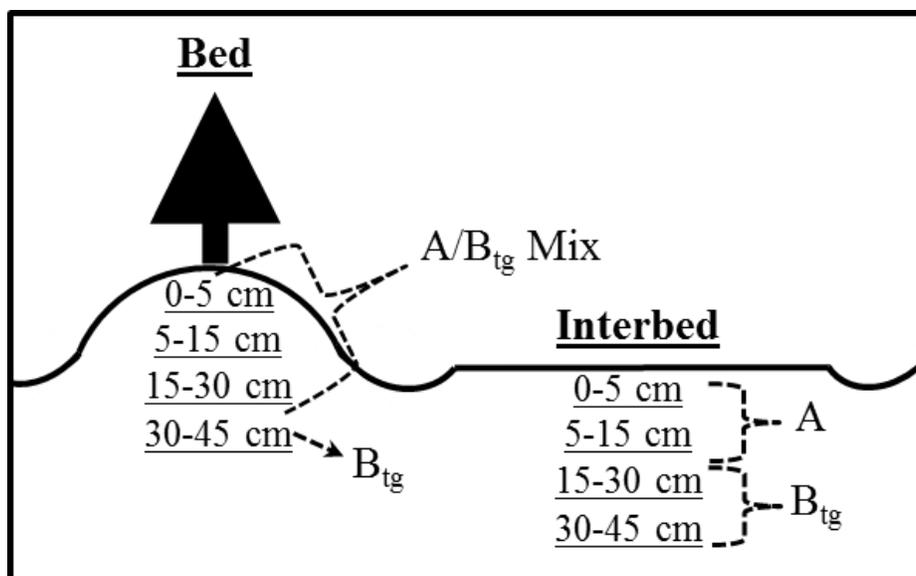


Figure 4.1. Diagram showing sampling locations in the beds and interbeds, discrete soil sampling depths, and associated soil horizon. The depression in soil surface between beds and interbeds represent the area where soil was mixed into the beds during the bedding process.

Table 4.1. Carbon concentration (g C kg^{-1}), total C content (Mg ha^{-1}), and $\delta^{13}\text{C}$ values (‰) of bulk soil samples collected from multiple depths in the bed and interbed from the Pine-Native and Pine-Switchgrass treatments. Data represent means with standard error in parenthesis ($n=4$). Differences in treatment means for $\delta^{13}\text{C}$ (‰) values at each sampling location and depth were analyzed using Tukey-Kramer adjustment with significant differences indicated by different lower case letters ($p < 0.1$).

Bed	<u>C Concentration</u> (g kg^{-1})		<u>Total C Content</u> (Mg ha^{-1})		<u>$\delta^{13}\text{C}$ (‰)</u>	
	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass
Depth (cm)						
0-5	60 (6.9)	44 (6.5)	33 (1.7)	25 (4.5)	-27.0 (0.2) a	-28.0 (0.3) b
5-15	69 (13)	69 (15)	75 (8.4)	67 (15)	-27.6 (0.2)	-27.6 (0.3)
15-30	50 (17)	34 (3.5)	88 (23)	51 (13)	-27.3 (0.2)	-27.5 (0.1)
30-45	13 (2.1)	11 (1.1)	33 (3.7)	23 (2.7)	-26.8 (0.5)	-26.5 (0.1)

Interbed	<u>C Concentration</u> (g kg^{-1})		<u>Total C Content</u> (Mg ha^{-1})		<u>$\delta^{13}\text{C}$ (‰)</u>	
	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass
Depth (cm)						
0-5	93 (12)	82 (11)	37 (7.1)	50 (8.8)	-27.2 (0.1) a	-26.5 (0.0) b
5-15	56 (8.0)	50 (4.4)	44 (8.4)	49 (7.4)	-27.8 (0.1)	-27.5 (0.1)
15-30	21 (2.7)	19 (2.7)	38 (6.4)	37 (11.2)	-27.7 (0.1)	-28.1 (0.3)
30-45	6.9 (0.4)	8.1 (1.4)	15 (1.2)	19 (4.5)	-27.2 (0.3)	-26.9 (0.5)

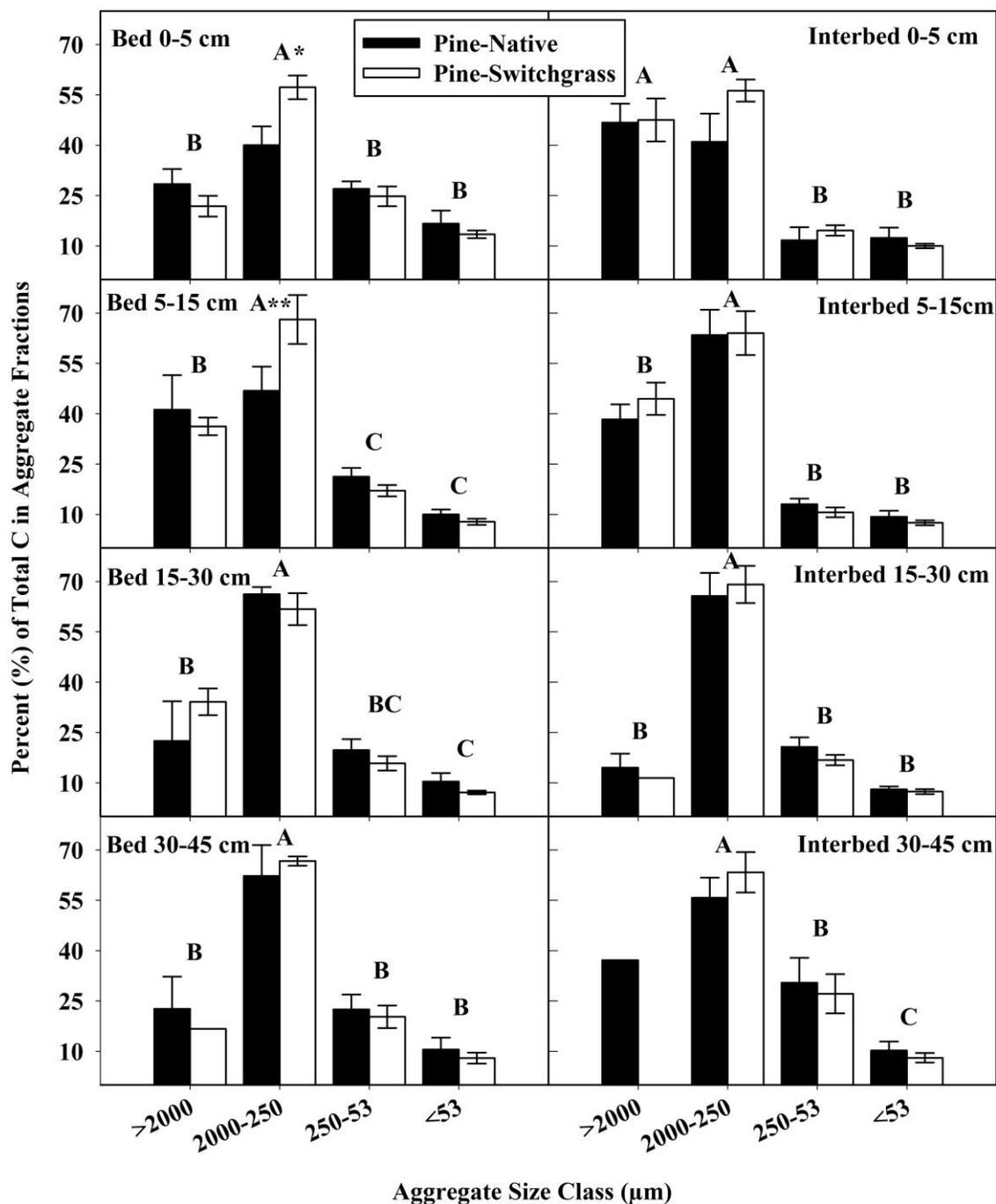


Figure 4.2. Distribution of C (%) among aggregate fractions for soil samples collected from multiple depths in the bed and interbed from the Pine-Native and Pine-Switchgrass treatments. Bars represent means with standard error (n=4). Post-hoc statistical analysis was performed for the location x depth x fraction with different letters representing significantly differently means between aggregate fractions, averaged over treatment within each location and depth and location ($p < 0.05$). Symbols represent significant differences between treatment means at each sampling location, depth, and aggregate fraction (* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$).

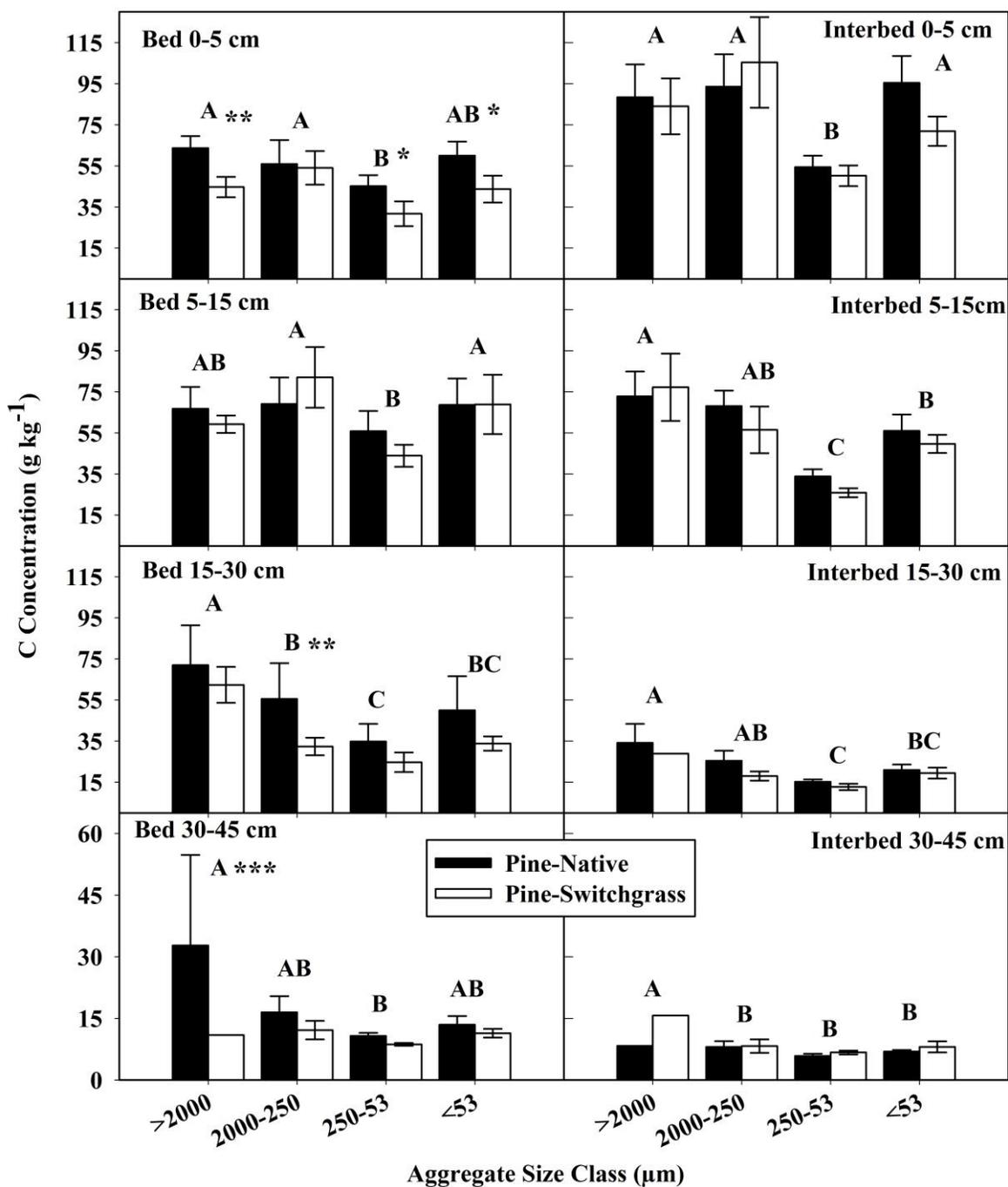


Figure 4.3. Carbon concentration (g kg⁻¹) of aggregate size fractions for soil samples collected from multiple depths in the bed and interbed from the Pine-Native and Pine-Switchgrass treatments. Bars represent means with standard error (n=4). Post-hoc statistical analysis was performed for the location x depth x fraction interaction with different letters representing significantly differently means between aggregate fractions, averaged over treatment, within each location and depth and location (p < 0.05). Symbols represent significant differences between treatment means at each sampling location, depth, and aggregate fraction (*P < 0.1; **P < 0.05; ***P < 0.01).

Table 4.2. Values of $\delta^{13}\text{C}$ (‰) of soil organic matter separated by aggregate fractionation for soil samples collected from multiple depths in the bed and interbed from the Pine-Native and Pine-Switchgrass treatments. Data represent means with standard error in parenthesis (n=4). Differences in treatment means at each sampling location, depth, and aggregate fraction were analyzed using Tukey-Kramer adjustment with significant differences indicated by different lower case letters ($p < 0.05$).

Bed						
$\delta^{13}\text{C}$ (‰)						
Depth (cm)	> 2000 μm		2000-250 μm		250-53 μm	
	Pine- Native	Pine- Switchgrass	Pine- Native	Pine- Switchgrass	Pine- Native	Pine- Switchgrass
0-5	-20.8 (0.6)	-22.2 (0.3)	-24.0 (1.1)	-23.2 (0.1)	-23.0 (0.3)	-22.9 (0.2)
5-15	-26.1 (3.0) a	-22.6 (0.1) b	-24.4 (1.5) a	-22.2 (0.3) b	-22.3 (0.2)	-22.5 (0.4)
15-30	-23.1 (1.1)	-23.0 (0.3)	-17.5 (2.6) a	-22.0 (0.3) b	-22.6 (0.3)	-22.8 (0.0)
30-45	-21.6 (0.1)	-21.7 (n.a.)	-21.9 (0.3)	-22.1 (0.2)	-21.9 (0.4)	-22.2 (0.2)

Interbed						
$\delta^{13}\text{C}$ (‰)						
Depth (cm)	> 2000 μm		2000-250 μm		250-53 μm	
	Pine- Native	Pine- Switchgrass	Pine- Native	Pine- Switchgrass	Pine- Native	Pine- Switchgrass
0-5	-18.7 (0.6)	-18.9 (0.1)	-18.5 (0.5) a	-25.7 (2.7) b	-19.5 (0.3)	-19.6 (0.1)
5-15	-19.1 (0.3) a	-19.8 (0.1) b	-21.4 (1.9)	-20.0 (0.1)	-21.7 (0.3)	-21.6 (0.1)
15-30	-20.6 (0.1)	-20.2 (n.a.)	-20.4 (0.3)	-20.6 (0.3)	-20.6 (0.2)	-20.6 (0.2)
30-45	-19.7 (n.a.)	-19.2 (n.a.)	-22.2 (0.3)	-22.1 (0.2)	-21.8 (0.3)	-21.5 (0.3)

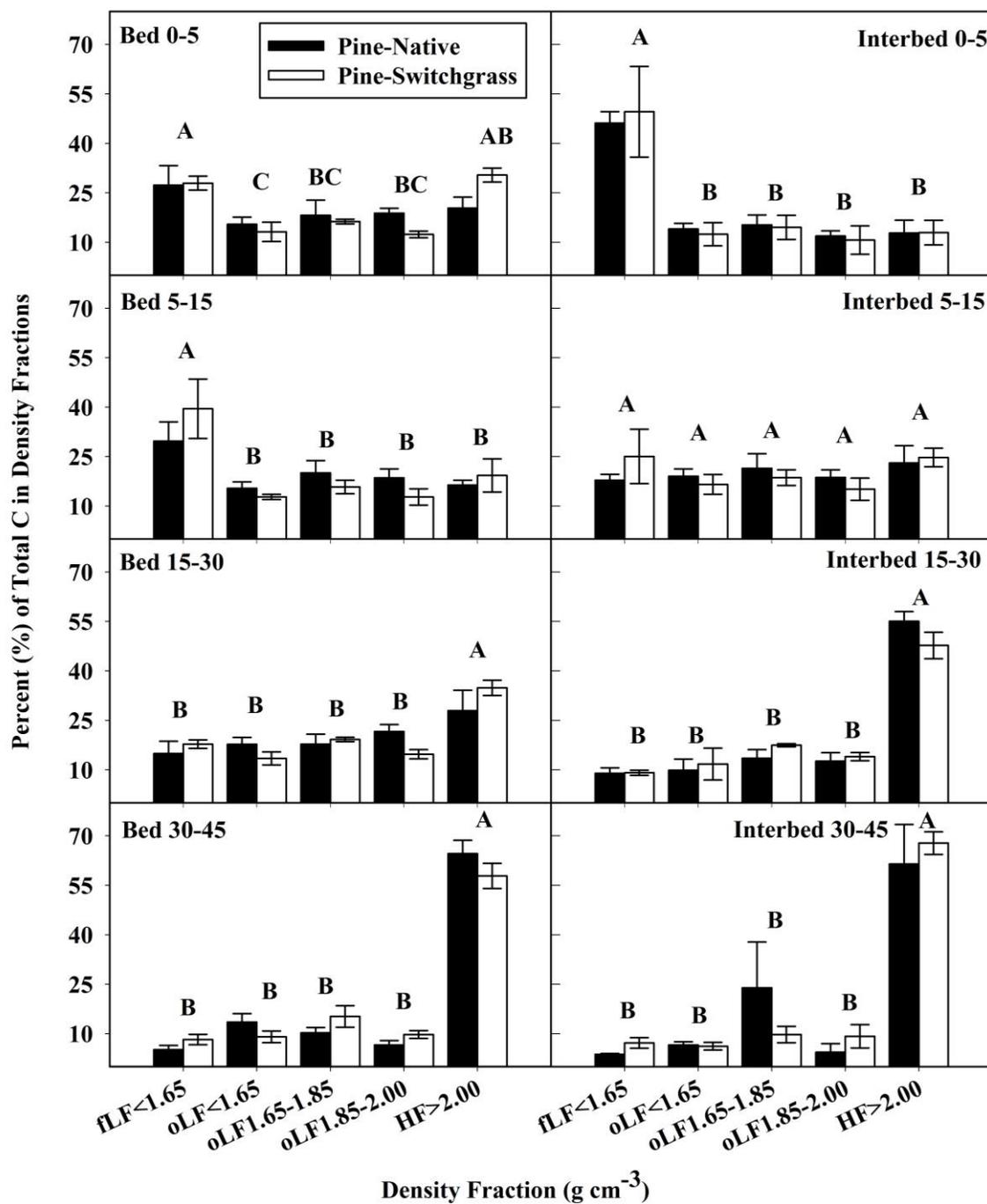


Figure 4.4. Distribution of C (%) among free and occluded light fractions (fLF and oLF, respectively) and heavy fraction (HF) for soil samples collected from multiple depths in the bed and interbed from the Pine-Native and Pine-Switchgrass treatments. Bars represent means with standard error (n=4). Post-hoc statistical analysis was performed for the location x depth x fraction interaction. Different letters represent significantly differently means between density fractions, averaged over treatment, within each sampling location and depth and location ($p < 0.05$).

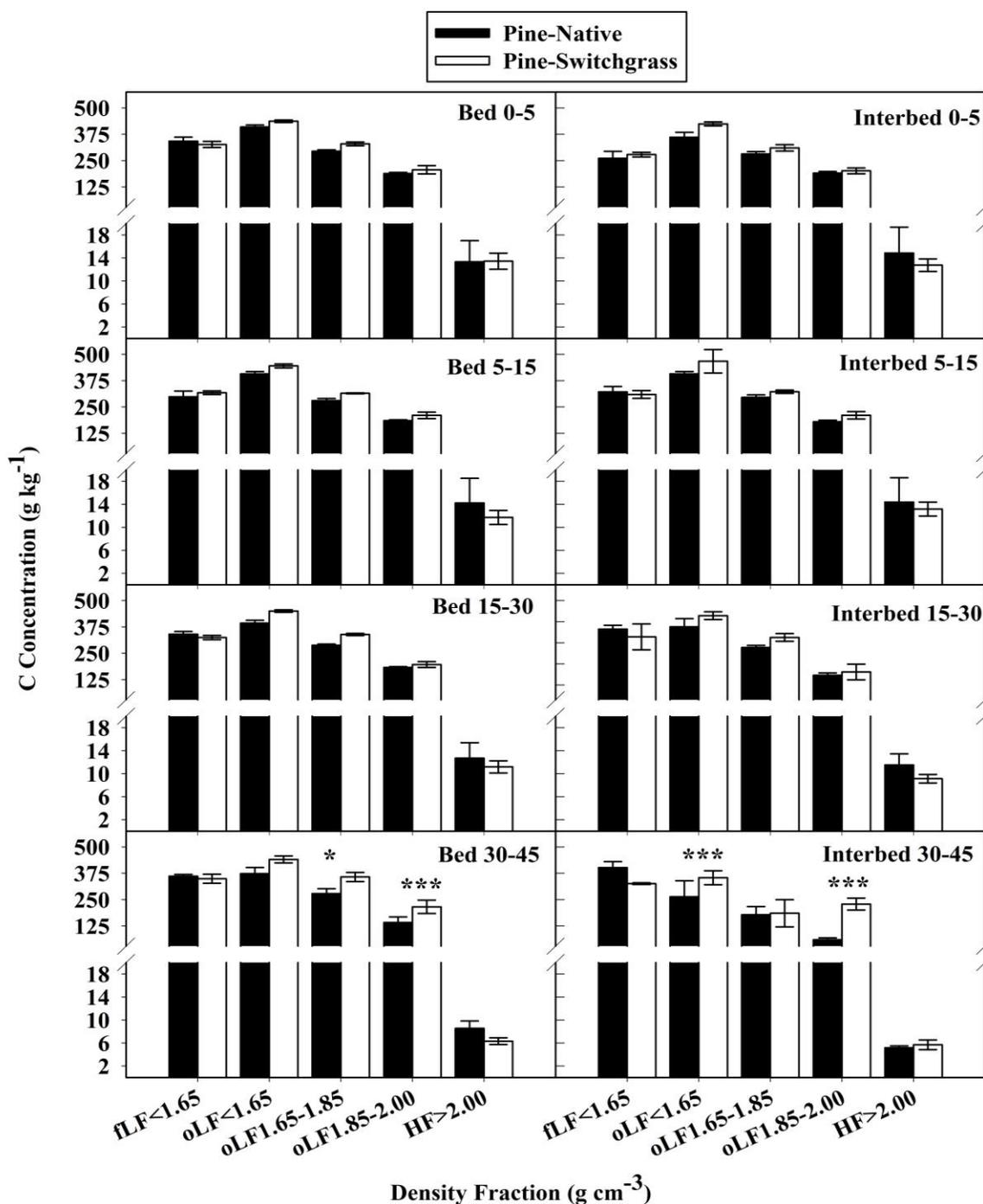


Figure 4.5. Carbon concentration (g kg^{-1}) of free and occluded light fractions (fLF and oLF, respectively) and heavy fraction (HF) for soil samples collected from multiple depths in the bed and interbed from the Pine-Native and Pine-Switchgrass treatments. Bars represent means with standard error ($n=4$). Post-hoc statistical analysis was performed for the treatment \times location \times depth \times fraction interaction. Symbols represent significant differences between treatment means at each sampling depth, location, and aggregate fraction (* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$).

Table 4.3. Values of $\delta^{13}\text{C}$ (‰) of soil organic matter separated by density fractionation for soil samples collected from multiple depths in the bed and interbed from the Pine-Native and Pine-Switchgrass treatments. Data represent means with standard error in parenthesis (n=4).

Bed		$\delta^{13}\text{C}$ (‰)									
Depth (cm)	fLF _{<1.65}		oLF _{<1.65}		oLF _{1.65-1.85}		oLF _{1.85-2.00}		HF _{>2.00}		
	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass	
0-5	-27.9 (0.6)	-28.8 (0.6)	-28.9 (0.1)	-28.5 (0.3)	-28.0 (0.3)	-27.2 (0.1)	-26.5 (0.4)	-26.7 (0.4)	-26.8 (1.2)	-28.7 (0.3)	
5-15	-26.8 (0.6)	-28.8 (0.6)	-29.2 (0.3)	-28.0 (0.1)	-27.8 (0.7)	-27.1 (0.1)	-26.8 (0.6)	-26.7 (0.3)	-28.0 (0.7)	-29.2 (0.4)	
15-30	-26.6 (0.3)	-27.8 (0.6)	-28.9 (0.4)	-28.3 (0.4)	-27.5 (0.6)	-27.1 (0.3)	-26.3 (0.6)	-27.2 (0.2)	-28.3 (0.5)	-29.3 (0.4)	
30-45	-28.6 (1.9)	-27.4 (0.3)	-29.3 (0.5)	-28.3 (0.3)	-27.6 (0.3)	-26.3 (0.3)	-27.8 (0.9)	-27.5 (0.4)	-28.1 (1.2)	-30.0 (0.4)	
Interbed		$\delta^{13}\text{C}$ (‰)									
Depth (cm)	fLF _{<1.65}		oLF _{<1.65}		oLF _{1.65-1.85}		oLF _{1.85-2.00}		HF _{>2.00}		
	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass	Pine-Native	Pine-Switchgrass	
0-5	-27.6 (0.9)	-27.7 (0.9)	-29.3 (0.3)	-28.7 (0.4)	-28.1 (0.4)	-27.0 (0.3)	-26.0 (0.4)	-26.7 (0.2)	-27.8 (0.7)	-28.1 (0.3)	
5-15	-27.2 (0.6)	-27.9 (0.8)	-28.8 (0.2)	-28.1 (0.4)	-27.9 (0.4)	-26.7 (0.2)	-26.9 (0.4)	-26.7 (0.1)	-27.7 (0.7)	-28.7 (0.2)	
15-30	-26.6 (0.5)	-27.1 (1.0)	-29.7 (0.5)	-28.5 (0.2)	-28.1 (0.4)	-26.5 (0.2)	-27.3 (0.5)	-27.7 (0.9)	-27.9 (0.4)	-29.7 (0.2)	
30-45	-28.8 (1.4)	-27.7 (0.5)	-29.9 (1.3)	-26.9 (2.1)	-28.9 (0.7)	-29.2 (1.4)	-29.8 (0.8)	-27.7 (1.0)	-28.1 (0.7)	-29.4 (0.6)	

Table 4.4. Percentage of pine- and switchgrass-derived C in bulk soil and soil organic matter separated using aggregate and density fractionation methods at multiple depths in the bed and interbed from the Pine-Native and Pine-Switchgrass treatments. Values represent means with standard errors (n=4). Data with 95 % confidence intervals overlapping zero were effectively considered to have no new inputs of pine- or switchgrass-derived C, and were labelled as such. Negative values are a result of the Pine-Native soil samples having a higher (less negative) $\delta^{13}\text{C}$ value than Pine-Switchgrass soil or fraction.

Bed		<u>Aggregate Fraction (μm)</u>			<u>Density Fraction (g cm^{-3})</u>				
Depth (cm)	Bulk Soil	>2000	250-2000	53-250	fLF_{<1.65}	oLF_{<1.65}	oLF_{1.65-1.85}	oLF_{1.85-2.00}	HF_{>2.00}
0-5	-7.5 ± 1.4	-19 ± 5.9	0	0	0	0	5.0 ± 1.6	0	-4.7 ± 2.2
5-15	0	0	0	0	-15 ± 8.2	7.2 ± 1.6	0	0	-8.3 ± 4.0
15-30	-3.8 ± 0.5	0	0	0	-9.1 ± 4.5	0	0	0	0
30-45	0	n.a.	0	0	0	5.7 ± 2.0	8.7 ± 3.1	0	0

Interbed		<u>Aggregate Fraction (μm)</u>			<u>Density Fraction (g cm^{-3})</u>				
Depth (cm)	Bulk Soil	>2000	250-2000	53-250	fLF_{<1.65}	oLF_{<1.65}	oLF_{1.65-1.85}	oLF_{1.85-2.00}	HF_{>2.00}
0-5	4.8 ± 0.6	0	0	0	0	0	8.1 ± 3.6	0	0
5-15	1.5 ± 0.4	-5.6 ± 0.1	0	0	0	4.2 ± 1.7	7.3 ± 2.6	0	0
15-30	0	n.a.	0	0	0	6.8 ± 3.1	10 ± 2.5	0	-12 ± 3.8
30-45	0	n.a.	0	0	n.a.	0	0	17 ± 2.8	0

Chapter 5. Conclusions

5.1. Introduction

Bioenergy production has expanded during the last decade (IEA, 2010), increasing demand for forest-based biomass feedstocks. Up to now, most bioenergy has been produced from 1st generation biofuel feedstocks from agricultural crops, such as corn (USDA-DOE, 2005). Forested ecosystems can produce much of the needed biomass for biofuel production, especially in intensively managed forests. Although use of forest biomass for feedstocks is likely to increase, our understanding of the long-term sustainability of intensified bioenergy production from forests is not complete. Millions of acres of privately and industrially owned pine plantations exist across the southeastern United States (Fox et al. 2007a), representing a vast area of land that could be utilized to produce bioenergy without significant land-use change or diversion of agricultural resources from food production. As of 2010, there were 15.8 million ha of pine (*Pinus* spp.) plantations in the southern United States (Wear and Greis, 2012). Of these plantations, those that are more intensively managed may be able to sustainably produce needed biomass without significant land-use change or diversion of agricultural crops from food production. Forested ecosystems could provide multiple sources of biomass feedstocks, including post-harvest residues or biomass recovered from thinning operations. Furthermore, loblolly pine (*Pinus taeda* L.) plantations offer the unique opportunity to utilize space between rows of trees to grow an herbaceous bioenergy crop, such as switchgrass (*Panicum virgatum* L.) (Albaugh et al., 2012). This novel forest management regime has the potential to provide positive environmental and economic services (Albaugh et al., 2012; Susaeta et al., 2012), but hinges in part on impacts to soil carbon (C) dynamics, availability of belowground resources, and potential impacts of competition between pine and switchgrass on productivity. A fundamental understanding of the processes impacting cycling of major elements through plants and soils provides a basis for implementing sustainable forest management practices.

Production of forest-based plant biomass for traditional wood products and bioenergy feedstocks has important implications for soil quality, including losses of soil C and N due to removal of previously retained organic material and other essential nutrients. In order for the sustainable production of wood and bioenergy from southern US pine forests, knowledge of the

soil factors that affect plant productivity must be obtained. A key question arising from previous research is how will the introduction of a highly productive grass, such as switchgrass, affect tree growth due to above- and below-ground competition. Specifically, competition for belowground resources, such as water, nutrients, and rooting space, will likely influence the growth response of loblolly pine to switchgrass. It is well known that competitive interactions between crop trees and non-commercial woody and herbaceous vegetation can reduce availability of key nutrients to pines and lead to reductions in productivity (Smethurst and Nambiar 1989, Smethurst and Nambiar, 1990; Smethurst et al., 1993; Gurlevik et al. 2004) and that management intensity can effect soil C stabilization (Sarkhot et al., 2007; Sarkhot et al., 2008; Echeverria et al., 2004; Maeir et al., 2012; Johnsen et al., 2013). Nitrogen (N) commonly limits loblolly pine productivity (Fox et al., 2007b) and soil microorganisms predominately mediate N supply to trees. The microbial processes which affect soil N cycling are impacted by various forest management practices, such as harvesting (Likens et al., 1970), site preparation (Fox et al., 1986; Vitousek et al., 1992), competing vegetation control (Smethurst and Nambiar 1989; Woods et al., 1992; Gurlevik et al., 2004), and fertilization (Gurlevik et al., 2004). Given the strong linkages between substrate quality (e.g. C/N ratio, lignin:N ratio, etc.) and N transformations in forest soils (Finzi et al. 1998, Hart et al. 1994, Scott and Binkley 1997), cropland (Schimel 1986), and grassland (Hart et al. 1993), changes in SOM quality and/or quantity may also result in alterations of N cycling pathways due to changes in microbial activity and ultimately plant-available N.

Reduction of atmospheric greenhouse gases can be achieved by increasing soil C sequestration and reducing CO₂ emissions by supplementing fossil fuel production with a renewable energy source. Forested ecosystems store large amounts of atmospheric C as plants incorporate CO₂ into organic compounds and deposit C into soil organic matter (SOM) pools. The degree of C accrual in forest soils is balanced by inputs of plant- and animal-derived organic C compounds and outputs of CO₂ during decomposition. Detailed understanding of forest soil C cycling and storage are critical to determine the role of forests in the cycling of C between atmospheric and terrestrial pools. Forests can act as C sinks or sources dependent upon the impact of forest management on processes that influence soil C sequestration (Guo and Gifford, 2002; Johnson and Curtis, 2001; Huang et al., 2011; Jandl et al., 2007). However, our ability to predict changes in and effectiveness of managing forests for soil C sequestration hinges on our

understanding of the more subtle changes in soil C dynamics as revealed by stabilization mechanisms.

The major objectives of this research were to investigate how intensified production and removal of biomass from loblolly pine plantations influence soil C and N cycling. In the three previous chapters, three specific objectives were addressed: 1) compare different bioenergy management regimes in regards to temporal dynamics of N cycling and availability following forest establishment (*see* Chapter 2); 2) determine the impact of loblolly pine and switchgrass intercropping on microbial N cycling processes (*see* Chapter 3); and 3) evaluate chemical and physical mechanisms of SOM stabilization and test their sensitivity to pine-switchgrass intercropping (*see* Chapter 4).

This research quantified temporal availability of mineral soil N, gross N transformations, and stabilization of SOM as influenced by loblolly pine and switchgrass intercropping. Ion exchange membranes were employed for the first 2.5 years (e.g., July, 2009 - January, 2012) following forest establishment to measure exchangeable inorganic N cycling and availability. Microbially-mediated N transformations (e.g., gross and net N mineralization, gross and net nitrification, and NH_4^+ and NO_3^- immobilization) were quantified the following year at four separate sampling dates in order to evaluate effects of loblolly pine and switchgrass on microbial activity and N availability. Finally, SOM stabilization mechanisms were measured in order to evaluate the impact of intensified forest biomass production on soil C storage.

5.2. Chapter Results and Discussion

The findings from this dissertation research illustrate the importance of belowground interactions between plants of different species and how these influence soil microbial activity and C and N cycling. Three overarching conclusions were generated from my research:

- 1) Switchgrass grown between loblolly pine trees effectively utilized excess soil NH_4^+ and NO_3^- when N availability was high following harvesting of a mature plantation proceeded by establishment of a second rotation of loblolly pine (*see* Chapter 2).

- 2) Gross N mineralization rates were reduced under switchgrass during the growing season when soil C availability was low, but were elevated under switchgrass and adjacent loblolly pines when switchgrass was dormant and C availability was likely higher (*see* Chapter 3).

- 3) Soil organic matter stabilized by physical or chemical mechanisms responded differently to pine-switchgrass intercropping, with losses in aggregate-stabilized C and gains in occluded, mineral-stabilized C. Furthermore, losses of aggregate C was associated with a significant reduction in total soil C in beds of the pine-switchgrass treatment. Results from ^{13}C mass balance suggested incorporation of switchgrass-derived C into occluded light fractions of beds and interbeds. Finally, incorporation of new pine-derived C into the $>2000\ \mu\text{m}$ aggregate size fraction and free light fraction indicate pine inputs of particulate organic matter into these SOM fractions in beds of the pine-switchgrass treatment (*see* Chapter 4).

A detailed understanding of how managing these young (4 year old) loblolly pine forests for bioenergy production influences C and N cycling was provided by this dissertation research. Overall, this research suggests that soil C and N biogeochemistry has been altered by intercropping of pine and switchgrass. Most surprising was the effect of switchgrass intercropping on soil C and N cycling in adjacent loblolly pine beds. To this end, I hypothesize that loblolly pines have increased root growth in beds in response to competition with switchgrass for N in the interbed, thereby alleviating seasonal microbial C limitations and stimulating microbial N cycling processes and increasing plant-available N. That is, one potential source of belowground competition between pine and switchgrass was for inorganic N, given reduced N availability under switchgrass (*see* Chapter 2). This belowground competition for inorganic N between pine and switchgrass may have driven pines to more heavily mine bed soils for N in order to increase N availability to support pine growth. This was supported by soil C and ^{13}C isotopic data indicating that loblolly pines altered soil C dynamics in beds (*see* Chapter 4). Most notably, intercropping of pine and switchgrass led to losses of total soil C in pine beds as a result of reduced aggregate-associated C. Delta- ^{13}C data indicated that pines

increased root growth in beds adjacent to intercropped switchgrass, potentially alleviating microbial C limitations and increasing microbial N cycling and plant-available N.

Through an enhanced understanding of the factors that influence soil C and N cycling, effective forest management strategies can be implemented to maximize forests biomass production while limiting loss of soil C and N. For example, introduction of switchgrass has reduced the potential for losses of inorganic N due to leaching or denitrification and supports previous work showing the positive impact that switchgrass has on ecosystem N retention (Tufekcioglu et al., 1999; 2003; McLaughlin and Kszos, 2005; Smith et al. 2013). Informed soil C and N management will increase the sustainability of such intercropping system (Fox et al., 2000; Parrish and Fike, 2005; Albaugh et al., 2012, Susaeta et al., 2012). A future consideration will be to understand the temporal dynamics of species interactions as stands develop. We expect that switchgrass will become less competitive for resources at pine crown closure, due to shading effects on switchgrass growth. This should reduce potential belowground competitive interactions, but the specific timing of this should be monitored. Furthermore, the potential to incorporate decomposing switchgrass roots into actively cycling SOM and N pools upon pine canopy closure may have a positive effect on soil C storage, soil N availability, and pine productivity. A robust understanding of biotic and abiotic factors that influence soil C and N availability and the microbial processes that drive soil C and N cycling will be important to determine long-term soil productivity of these forests and develop sustainable forest management strategies.

Future research initiatives should seek to unravel the complex belowground interactions between roots of different plant species and soil microorganisms competing for limiting resources, in order to better understand how these interactions impact soil C storage and N cycling and availability. Furthermore, more detailed analysis of changes in microbial community structure, indices of microbial activity such as enzyme activity, and plant-microbe rhizosphere interactions should be addressed. The intricate interactions between plant growth, soil C storage, N availability, and soil microorganisms drive cycling of major elements between the soil-plant-atmosphere continuum. Therefore, use of advanced analysis and holistically designed experiments are necessary to better understand these interactions in order to forward our understanding of terrestrial ecosystem function and productivity, especially in forested

ecosystems in which high levels of complexity and heterogeneity can hamper efforts to understand forest biogeochemical cycles.

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