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Coarse Root, Forest Litter, and Soil Organic Matter Contributions to Carbon Sequestration in Loblolly Pine (Pinus taeda) Plantations in East Texas

William Wedge wedgewl@jacks.sfasu.edu

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Coarse Root, Forest Litter, and Soil Organic Matter Contributions to Carbon Sequestration in Loblolly Pine (*Pinus taeda)* **Plantations in East Texas**

By

WILLIAM WEDGE

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Coarse Root, Forest Litter, and Soil Organic Matter Contributions to Carbon Sequestration in Loblolly Pine (*Pinus taeda*) Plantations in East Texas

By

William Wedge, Bachelor of Science in Plant and Soil Science

APPROVED:

Kenneth Farrish, Ph.D. Thesis Director

Brian Oswald, Ph.D. Committee Member

____________________________________ Frantisek Majs, Ph.D. Committee Member

Jason Grogan, MS. Committee Member

Pauline M. Sampson, Ph.D. Dean of Research and Graduate Studies

ABSTRACT

Understanding ecosystem carbon dynamics is of increasing importance with atmospheric carbon dioxide $(CO₂)$ concentrations on the rise. Land management strategies, such as land use conversion, effect ecosystem carbon cycling dynamics and can alter the quantity of carbon sequestered in vegetation and soils. In East Texas and much of the southern United States, there has been a trend of converting marginal pastureland into loblolly pine (*Pinus taeda*) plantations. This afforestation, like all other land use conversions, leads to a redistribution of carbon in vegetation and soil carbon sinks. In 2003, five marginal pasturelands in East Texas were afforested with loblolly pine with the intent of quantifying the organic carbon sequestered as a result of this land use change. In 2003 and 2015, soils were sampled on three of the sites in East Texas to measure the change in soil organic carbon in the top 40 cm of soil, and the accumulated O horizons were sampled in 2015. In the summer of 2017, tap root systems and coarse roots on each of the three sites were excavated to quantify belowground biomass. All sites experienced increases in carbon sequestered belowground in coarse roots, tap roots, and also O horizons. Only one site had a statistically significant increase in soil organic carbon (SOC).

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INTRODUCTION

Carbon cycling occurs throughout all of the Earth's basic geospheres. The interfaces of the lithosphere, hydrosphere, atmosphere and biosphere produce variable and complex environments (Lal, 2008). One of the most common of these interfaces is the pedosphere, or soil environment. However, due to the difficulty and complexity of observing soil dynamics in-situ, there is still relatively little known on carbon dynamics of soil systems. What is known is that the pedosphere is a large component of the global carbon cycle, acting as both a major sink and source for atmospheric carbon (Weil and Brady, 2017).

It is well documented that localized disturbances of the pedosphere leads to redistribution of carbon. Some of the largest pedologic disturbances in the form of land use changes. Until the 1940s, land use change, primarily the conversion of natural ecosystems to alternative uses; accounted for more carbon dioxide $(CO₂)$ emissions than the combustion of fossil fuels; by 2008, approximately 18% of global $CO₂$ emissions still originated from deforestation in conversion to agricultural land use (Lal, 2008).

Anthropogenic influences on the global carbon cycle, such as land use change, have been one of the factors that have been linked to global climate change, and has made the importance of carbon accounting and modeling critical in determining

anthropogenic influences on climate change. Using afforestation as a mitigation method offers the opportunity of sequestering carbon in soil carbon and in forest biomass, especially when previous land use management strategies were destructive to soil organic carbon, e.g. intensive deep tillage.

In its simplest form, organic carbon is introduced to soil environments from biologic inputs, primarily through autotrophic organisms. Photosynthesizing organisms assimilate atmospheric CO_2 and convert it to glucose ($C_6H_{12}O_6$) which is used in cellular growth, maintenance, and respiration. Necrosed matter is then subject to decomposition where some of the carbon is oxidized to $CO₂$, and the remaining carbon is released as waste products of decomposer organisms. These organic residues then can be sorbed to soil particles or transformed into other organic molecules by soil organisms. Decomposition rates are affected by many factors, including moisture, temperature, oxygen availability, and the bioavailability of the carbon in substrates.

Soil carbon, including biomass, detritus, and humus, represents the largest terrestrial carbon pool (Lal, 2004; Scharlemann et al., 2014; Weil and Brady, 2017). In plant roots, biomass is one of the important pathways that organic introduces materials into soil systems. Roots can be characterized in different ways; one of the most common is based on the diameter of roots, with fine roots being classified as smaller than 2 mm in diameter and coarse roots being larger than 2mm in diameter.

In the southeastern United States, a popular species for afforestation projects is loblolly pine (*Pinus taeda*) because of its rapid growth, economic value as a timber source, and site adaptability. While there has been ample research on loblolly pine, most of the research has focused on the production of loblolly for fiber and timber. The research that has focused on belowground characteristics of loblolly pine has generally been centered on fine root dynamics and seedling root:shoot ratios.

In 2003, approximately 512 hectares on five sites of what had been previously pastureland in east Texas, was planted to loblolly pine as a part of a carbon sequestration project funded by STMicroelectronics in collaboration with the Arthur Temple College of Forestry and Agriculture at Stephen F. Austin State University. These operational carbon sequestration plantations offered an opportunity to evaluate changes in soil carbon storage, including the contribution of carbon from coarse roots, as a result of afforestation activity. This study examined the changes in coarse root, forest litter, and soil organic matter contributions to carbon sequestration after afforestation on three of the original five sites that together accounted for 460 hectares.

OBJECTIVES

The principle purpose of this study was to observe and quantify the amount of carbon sequestered in loblolly pine (*Pinus taeda*) plantations from woody coarse roots, forest litter layers (O horizons), and soil organic carbon after 16 years since afforestation in Eastern Texas. More specifically the objectives of this study were to quantify:

- 1. belowground coarse woody root biomass of loblolly pine for the purpose of carbon sequestration assessment.
- 2. carbon accumulation in forest litter (O horizons) for the purpose of carbon sequestration assessment.
- 3. accumulation of soil organic carbon in a loblolly plantation setting for the purpose of carbon sequestration assessment.

LITERATURE REVIEW

Carbon Dioxide and Climate Change

The global climate throughout Earth's history has been dynamic in nature. Within the past 40 years, changes in atmospheric gas composition has come to be understood as one of the sources of climate change, specifically the concentration of greenhouse gasses (GHGs), including methane (CH4), nitrogen oxides (NOx), and carbon dioxide $(CO₂)$. Incoming solar radiation warms the surface of the earth which, in turn, reemits this thermal energy that is absorbed by GHGs and reemitted back towards earth (Anderson et al., 2016). While the majority of GHGs are naturally occurring, the exponential increase of anthropogenic activity has led to an increase in GHGs atmospheric concentrations, with the combustion of fossil fuels and land use conversion as major contributors. Reducing the $CO₂$ produced from the combustion of fossil fuels is one of the primary targets of reducing net emissions in a global attempt to curb GHG emissions (IGBP, 1998).

Emissions of $CO₂$ from fossil fuel combustion have been projected to peak between the years 2029 and 2044, with emissions of 11.1Gt C yr^{-1} and 16.1GtC yr^{-1} , respectively, and not return to atmospheric concentration below 400 ppm $CO₂$ for at least two centuries (Tans, 2009).

Carbon Cycling

Carbon, like nitrogen and water, cycles throughout different pools on a global scale. Carbon pools, or reservoirs, can be grouped into five major classifications: atmospheric, geologic, oceanic, pedologic, and biotic (Lal, 2008). The latency of carbon in these pools varies and depends on many factors including bioavailability and reactivity. For example, carbon sequestered in pedologic pools may be sequestered for centuries in humus (Weil and Brady, 2017).

Carbon and Forest Ecosystems

Mitigating increasing levels of atmospheric carbon dioxide through forest management and conservation was debated as early as the 1970s (Montagnini and Nair, 2004). Forests represent a large proportion of the terrestrial carbon pool, storing large amounts of carbon in organic materials and woody biomass (Birdsey, 1992). The United States contains an estimated 295 million hectares of forests, which represent a large potential carbon sink (Birdsey, 1992). In a forested ecosystem, carbon is exchanged with the atmosphere in the form of $CO₂$, which is assimilated by trees and other plants through photosynthesis and stored in plant biomass, soil and litter components in the ecosystem. Respiration, both autotrophic and heterotrophic, release stored carbon back into the atmosphere, primarily as CO2.

It is best to quantify the flux of carbon in an ecosystem on a temporal scale. Photosynthesis is directly correlated with solar radiation, meaning carbon sequestration is correlated to solar radiation (IPCC, 2000). During periods of little to no solar radiation, when forests are not photosynthesizing, carbon stored in carbohydrates is released during cellular respiration; this uses about 50% of the carbon assimilated, while the remaining carbon is used in growth and maintenance (IGBP, 1998). The difference between gross photosynthesis and autotrophic respiration is referred to as net primary productivity (NPP) (IGBP, 1998; IPCC, 2000; Kinerson et al., 1977). As trees grow, necrosed matter falls to the forest floor, where a portion is decomposed by heterotrophic organisms, and is released as $CO₂$. The remaining carbon in biomass and organic residues is net environmental productivity (NEP), i.e., the difference between gross photosynthesis and the sum of heterotrophic and autotrophic respiration equals NEP (IGBP, 1998; IPCC, 2000; Kinerson et al., 1977). The remaining biomass, in a forested setting, is often harvested for wood and fiber production. The difference between NEP and disturbances in an ecosystem (e.g. harvest or fire) is net biome productivity (NBP) (IGBP, 1998; IPCC, 2000). Each component of a systems carbon flux is temporally based, with NPP ranging on an hourly to daily range, NEP on a monthly to yearly scale, and NBP on a decadal scale. While NBP has been thought to be zero across all natural ecosystems $(\pm 1$ Gt yr⁻¹), the modeling does not account for ex situ carbon sequestered, or changes due to anthropogenic land use conversion.

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Ex Situ Carbon Sequestration

Carbon sequestered during the life span of a tree in a forested ecosystem is not automatically released back into the atmosphere upon the removal of biomass from the ecosystem (e.g. harvest). Forests have the capacity to sequester carbon in situ (biomass, soil, litter) and ex situ (timber and wood products) (Johnsen et al., 2001). Carbon can remain sequestered long past the rotation of a stand in the products that are derived from the wood and fiber products produced. For example, timber used in the construction of single-family homes built before 1980 is estimated to have a half-life of 80 years (Skog and Nicholson, 1998). The half-life of sequestered carbon is the amount of time it takes for half of the carbon in wood and fiber products in use to be transformed into more mobile forms of carbon such as $CO₂$ or CH₄.

Ex situ sequestered carbon in wood and timber products no longer in use are usually deposited in landfills where they are buried with other wastes, which limits the amount of oxygen available for microorganisms to decay organic products. Aerobic respiration produces $CO₂$, but once oxygen is depleted and anaerobic respiration takes dominance, methane (CH_4) is the primary greenhouse gas byproduct. In the atmosphere, CH_4 is more effective at trapping heat compared to CO_2 by a factor of 25 (Skog and Nicholson, 1998).

In landfills that have been capped and closed, $CO₂$ emitted through microbial respiration represents about 40% of total carbon emitted, while CH₄ is roughly 60% (Skog and Nicholson, 1998). Half of all $CO₂$ emitted will occur in the first three years, while half of all CH⁴ will be released in the first 20 years (Micales and Skog, 1997). However, it is believed that less than 50% of carbon stored in timber or wood products in landfills is converted to $CO₂$ or $CH₄$ (Skog and Nicholson, 1998). This is important because the remaining carbon in wood products and timber in landfills potentially could be considered as a longer term carbon sink from a carbon accounting perspective. Not accounting for ex situ carbon sequestered can greatly underestimate the ability of forest systems to sequester carbon long term (Smith et al., 2006).

Soil Organic Matter

Globally, soils represent the largest terrestrial carbon pool at 3.3 times atmospheric concentrations and 4.5 times the size of the biotic pool (Lal, 2004). The three major components of soil organic matter (SOM) are biomass, detritus, and humus (Weil and Brady, 2017). Approximately 59% of carbon in forested ecosystems is contained in soils; with the addition of roots, it is estimated to be 64% (Birdsey, 1992).

Biomass

Soil biomass consists of all biotic organisms in soil environments, including, but not limited to, microbes, earthworms, and roots. Typically the most abundant organisms found in soils globally on a mass/area basis are either bacteria or fungi, depending on factors that influence soil conditions such as soil pH and climatic variables (Weil and Brady, 2017).

In addition to the biomass that roots contribute to soil organic matter, roots can contribute organic compounds to the soil in a number of ways, collectively known as rhizodeposition. Primarily, the additions of organic substances to the soil can come from the inputs of cellular materials and exudates. Carbon can be released in exudates as organic and inorganic carbon with the form of carbon depending on many factors including plant type, climate, and physical and chemical soil parameters (McNear Jr., 2013). Carbon mobilized from shoots of plants to the root system of plants can account for 2-30% of total dry matter production (Weil and Brady, 2017). Rhizodeposition decreases with plant age and the majority of rhisodeposition studies are conducted in laboratory settings with juvenile plants which might not reflect true field conditions (Weil and Brady, 2017). Nevertheless, carbon contributed from biomass and exudates are important in carbon cycling and humus production in soil environments.

Detritus

Detritus on the forest floor is primarily oxidized or modified through faunal and microbial activity, and factors that affect the respiration rates of these organisms will affect the latency of carbon stored in plant materials (Enriquez et al., 1993). One factor is the carbon/nitrogen ratio of plant materials. It is well understood that plant materials with low C:N ratios undergo faster decomposition than those with high C:N ratios (Enriquez et al., 1993; Weil and Brady, 2017). Those with high C:N ratios are more resistant to decomposition and will have longer latency compared to detritus with low C: N ratios.

Detritus originating from conifers have a median half-life higher than deciduous trees (Enriquez et al., 1993). Additionally, detritus originating from multiple sources has higher decomposition rates than detritus from a single species (Hättenschwiler, 2005). A monoculture would therefore generate detritus that has a longer latency than compared to natural stands or detritus produced by mixed forests.

Loblolly pine needles have been shown to decompose at a constant rate with 44% of needle dry weight remaining after one year of decomposition (Thomas, 1968). If it is assumed that this rate holds constant, remnants of existing loblolly pine needles would still be present after two years of decomposition.

Humus

Humification is the process in which organics from detritus and exuded organics are converted to humus through microbial decomposition. Monomers and polymers with defined, known structures such as amino acids, lipids, and carbohydrates are classified as nonhumic substances. Organic substances that do not fall into any one identifiable category of organic molecules are referred to as humic substances. Humic substances can be further divided into humin, humic acid, and fulvic acids depending on a compound's solubility in acidic and alkali conditions (Weil and Brady, 2017). Humic substances make up 60 to 80% of humus, while nonhumic substances make up 20 to 30% (Weil and Brady, 2017).

Biomass, detritus, and humus all contribute to total soil organic matter in pedologic environments. However, carbon sequestered in biomass and detritus is typically more labile than carbon sequestered in humus, making humus a large sink to net carbon sequestration. It was previously believed that the stability of humic substances came from the size of the molecules. Ranging from 2,000 to 300,000 g mol⁻¹, humic substances are very resistant to microbial decomposition, but not impervious (Weil and Brady, 2017). However, these macromolecules may be the result of polymerization of smaller organic monomers and polymers formed during the laboratory extraction process (Denef et al., 2009; Weil and Brady 2017). These smaller organic molecules that are now believed to exist in situ in soil are more bioavailable than previously believed.

Soil texture plays an important role in soil carbon dynamics; the clay and silt sized fraction of soils effects the potential of a soil for sequestering carbon in organic matter. Some of the micropores formed by clay particles are physically inaccessible to decomposer organisms, leaving organics trapped in these pores inaccessible (Weil and Brady, 2017). Additionally, organics can be sorbed to clay particles removing them from solution, rendering them inaccessible to decomposers. The allophane clays associated with Andisols are believed to be a contributing factor for their high organic matter contents (Weil and Brady, 2017).

Soil organic carbon (SOC) is derived from SOM and is more meaningful when estimating carbon storage in an ecosystem. To calculate SOC from SOM the accepted conversion is SOC is equal to half of SOM. Previous empirical studies have found this value to be closer to 0.52 while theoretical studies have found it closer to 0.5 (Pribyl, 2010; Weil and Brady, 2017). The inherent variability of soils means that the accepted 0.5 conversion factor will not hold true for all SOM, but is an acceptable value for simplified modeling on larger scales.

Loblolly Pine (*Pinus taeda***)**

Loblolly pine (*Pinus taeda*) is the most important timber species in the southern United States, constituting over 50% of the standing pine volume in the region (Baker

and Langden, 1990), which as of 2004 was the most intensively managed forested area in the world (Johnsen et al, 2004). In the southern United States, loblolly pine occupied some $11.7x10⁶$ ha, making it the most commercially significant timber species (Baker and Langden, 1990), and more recently, the US Forest Service estimated the total area of loblolly pine to encompass some $22x10^6$ ha in the United States (USFS, 2018). The westernmost expanse of the range of loblolly pine extends into eastern Texas, where it comprises 97% of all softwood volume with a volume of $2.36x10⁹ m³$ (Dooley and Brandeis, 2014).

Aboveground Biomass of Loblolly Pine

The significance of loblolly pine as one of the most commercially important timber species means it is also one of the most widely studied species. Most studies have focused on growth, yield, and responses to management practices for the production of timber. One of the most practical applications of research has been the development of taper equations for land managers to predict the volume of trees and thus stands. The earliest whole stand yield models were developed between 1937 and 1939 and have been continued to become more refined and species specific, as Coble (2009) used 987 observations to develop a model for total loblolly pine tree ft^3 ac⁻¹ specifically for stands in East Texas. Studies similar to this have been conducted across different loblolly pine sites to develop models for loblolly pine in different regions. Using the wood volume produced by these equations, carbon stored in aboveground biomass can be estimated.

Belowground Biomass of Loblolly Pine

The majority of studies regarding the belowground component of biomass in loblolly pine plantations have focused on singular aspects of the root system such as root ball, tap roots, or lateral roots. Others have separated roots based on diameter classifications.

Kinerson et al. (1977) excavated seven loblolly pine root systems and found that 70-75% of lateral root biomass existed in the top 20cm of the soil and that 50% of the total root biomass was attributed to the belowground stump component. Miller et al. (2006) found that 91.9% of the biomass in loblolly pine root systems occurs in the upper 50cm of a soil profile, reaffirming Kinerson et al. (1977), who did not describe the method of excavation, leaving the question of whether or not the roots can be properly attributed to the above ground biomass of individual trees, which was used to derive their results. They also made no mention of non-stump originating vertical (sinker) roots which could imply that excavation was done on a volume basis where roots were excavated and sieved, leading to no distinction from vertical and horizontal roots, and assumed all roots were laterally oriented. Despite these shortcomings, the authors construct one of the early models for NPP in loblolly pine plantations.

Albaugh et al. (2006) examined three different stands of loblolly pine across different stand development stages and site characteristics and found that coarse root biomass was approximately 50% of stem biomass on a per hectare basis.

Rooting density decreases with depth (Albaugh et al, 2006; Farrish, 1991; Johnsen et al, 2004; Kinerson et al, 1977; Parker and Van Lear, 1996), due to a large number of factors. Parker and Van Lear (1996) point out some of the soil factors that could lead to this trend, such as finer textures and higher mechanical resistance impair root development at greater depths. Additionally, decreases that are associated with increase in depth, such as decreases in organic matter, biologic activity, aeration, and fertility, could also discourage foraging behavior associated with fine roots (Parker and Van Lear, 1996). These assumptions mean that root development at increasing depths could be genetically based or could serve to provide water for the plant in times of water stress, serve as structural support for the plant, or a combination of these and other factors.

The largest problem with rhizology studies in a natural environment is the methodology in which they are carried out. Most methods are time consuming and labor intensive (Böhm, 1979). Fine roots are relatively homogenously distributed in comparison to coarse roots, making estimation via soil coring a much more practical method than excavation methods necessary for coarse roots (Johnsen et al., 2004). Mou et al. (1995) found no correlation between spatial distribution of aboveground biomass and fine root biomass, but there was a correlation between coarse roots and their proximity to the stems. In their experimental design, juvenile loblolly pine trees were planted at random on experimental plots and not in rows as would be expected to be seen in a plantation setting. In a row system, if the assumption that coarse root density is

correlated to the proximity of stems holds true, it would be expected that coarse roots are distributed more evenly than in natural stands. This could make sampling for coarse roots contribution to carbon sequestration a more manageable task, as well and lead to more accurate models of carbon dynamics in plantation style forested ecosystems.

The most often used definition of fine roots are roots that are less than 2 mm in diameter (Johnsen et al; 2004). Roots greater than 3 mm in diameter have undergone secondary xylem thickening along with developed phloem, meaning these roots are generally perennial in nature (McClaugherty and Aber, 1982). While the definition of fine roots being less than 3 mm is backed in physiology, the less than 2 mm definition of fine roots is more widely used in the classification of fine roots.

Fine roots compose only \sim 1% of standing biomass in loblolly pine trees but account for ~13% of annual biomass production (Albaugh et al., 1998). While fine roots represent a significant portion of carbon allocation, their importance in NEP is questionable. Relatively speaking, fine roots in forested ecosystems are short lived and decompose rapidly; therefore, much of the carbon in fine roots is released back into the atmosphere as $CO₂$.

Necromass, the portion of dead, recognizable mass, of loblolly pine tap root systems has been observed in situ in measurable quantities 60 years post-harvest (Ludovici et al., 2002), meaning coarse roots, including tap root systems, represent a multi-decade, if not century, sink for carbon in loblolly pine systems.

METHODS

Study Area

The study was conducted on three SFA Real Estate Foundation-owned properties, known collectively as the STMicroelectronics properties. Two of the three sites were located approximately 16km east of Crocket, Texas, near the western boundary of the Davy Crockett National Forest, approximately 10km apart in Houston County, Texas. The third site was located in Cherokee County, Texas, approximately 11km east southeast of Rusk, Texas. Each site contained 16 year old, thinned loblolly pine plantations. Prior to planting of loblolly in 2003, each site had previously been used as pastureland for forage production for several decades.

Study Sites

The property located near the western edge of the Davy Crockett National Forest at 31°12'53.56"N, 95°18'7.18"W, will be referred to as the Arbor Grove site. The second site located 10 km to the northeast of Arbor Grove is Hickory Creek (31°23'28.36"N, 95°15'52.21"W). Because of the proximity of the Arbor Grove and

Hickory Creek sites, the climate data was identical with a mean temperature of 18.5^oC with a mean annual precipitation of 106.8 cm year⁻¹ (Soil Survey Staff, 2018). Both Arbor Grove and Hickory Creek were pastureland prior to planting of loblolly pine in 2003. The third site was located in Cherokee County, Texas referred to as the Atoy site (31°15'38.12"N, 95°2'32.65"W). Atoy receives a mean annual precipitation of 125.9 cm year⁻¹ with a mean annual temperature of 18.2° C (US Climate Data, 2018). Prior to planting of loblolly pine in 2003, the Atoy site supported an improved costal bermudagrass pasture.

Arbor Grove occupied 190.2 ha, with 148.6 ha supporting a 16 year old thinned loblolly pine plantation. Hickory Creek was a 157.7 ha pine plantation with a small stand of planted hardwoods. Hickory Creek was predominantly alluvial floodplain that accounts for 85.3 ha of the property with 49.1 ha designated as upland. Both the upland and most of the alluvial floodplain portions of the site had a 16 year old thinned loblolly pine plantation. Similar to the Arbor Grove and Hickory Creek sites, Atoy had a 16 year old thinned loblolly pine plantation on 78.3 of the 154.1 ha. The remaining area consists of un-thinned and poorly stocked pine plantations.

Study Site Soils

Soils in the Arbor Grove tract were predominantly Alfisols with approximately 70% coverage with the remaining classified as Inceptisols. The dominant soil series were

the Lovelady series (Arenic Glossudalfs) that occupied roughly 39% of the Arbor Grove site. The remaining soils consisted of Fluvaquentic Endoaquepts, Glossic Natraqualfs, Oxyaquic Glossudalfs, Oxyaquic Eutrudepts, Aquic Glossudalfs, and Vertic Hapludalfs. Drainage classification ranged from well-drained to somewhat poorly drained with the Lovelady series classified as well-drained.

Hickory Creek, unlike Arbor Grove, was primarily composed of Inceptisols covering 54.3% of the site. The remainder was composed of Alfisols and a small proportion of Ultisols, constituting 38.4% and 3.2% of total land area, respectively. Laneville loam series (Fluvaquentic Eutrudept) was the most abundant soil at 34.8% land coverage. The remaining soils in order of land area were Vertic Hapludalfs, Fluvaquentic Dystrudepts, Aquic Glossudalfs, Fluvaquentic Endoaquepts, Glossic Paleudalfs, and Arenic Hapludults. Like Arbor Grove, drainage classification ranged from well-drained to somewhat poorly drained, however there was a smaller proportion of somewhat poorly drained soils compared to Arbor Grove. Moderately well-drained soils were the most abundant, including Laneville loam.

Over 70% of soils at Atoy were Ultisols with the remaining soils comprised of Alfisols and an Entisol. Sacul fine sandy loam (Aquic Hapludult) covered the majority of Atoy with 55.1% coverage. Like Hickory Creek, the predominant drainage classification was moderately well-drained covering 72% of the property.

Sampling

A total of nine trees, three each from Arbor Grove, Hickory Creek, and Atoy, were sampled in 2018 for belowground biomasses. Aboveground biomasses of the trees was also recorded to evaluate possible aboveground predictors for belowground biomass. Soils were sampled in 2003 and 2015 for soil organic carbon to evaluate if any significant change in organic carbon had occurred.

Aboveground Biomass Sampling

Basal area was calculated using a 10-m radius sample plot with the sample tree placed at the center. Additionally, trees counted within this sample plot were used to calculate trees per ha⁻¹. Before belowground biomass could be determined, sample trees were cut at ground line and their aboveground biomass determined. Diameter at breast height (DBH) is correlated with aboveground biomass and was recorded prior to tree felling. Aboveground biomass was defined as all biomass >5cm above ground level. Once felled, two limbs from the upper and the lower crown were randomly selected for a total of four limbs, that were separated into branch and needle components to be dried and weighed to develop a correction for moisture content. The remainder of crown green weight biomass was weighed using a large electronic platform scale in the field and

recorded to the nearest hundredth of a kilogram. Necromass was separated from biomass in order to avoid over estimation of biomass.

After all limbs were removed, the merchantable length of the stem was measured. Merchantable length was defined as the length between 5 cm above ground line to a 5 cm diameter top. The stem was cut into manageable segments to allow for weighing and mass lost to kerf during cutting was assumed to be negligible. The stem was weighed using the same large electronic platform scale used for weighing the crown of the tree and weight was recorded to the nearest hundredth of a kilogram. Three sub samples were cut from the stem, one at breast height, one at one-half of merchantable stem height and one at 90% merchantable stem height, and were oven-dried and weighed to develop a correction for stem moisture content.

Moisture lost in stem and crown samples between the time of sampling and initial weighing was assumed to be negligible. Sub-samples were weighed to determine initial weight and then placed in a forced draft drying oven at 60°C until a constant weight was achieved and oven-dry weight was recorded.

Belowground biomass

A destructive sampling strategy was used to sample belowground biomass. However, every effort was made to keep roots intact if possible, to assure minimal root biomass loss. A combination of an air spade that was operated between 90 and 100 psi, and mini excavator was used in order to extract coarse roots, stumps and taproots

For coarse roots, a 1-m² sample area was randomly selected along an imaginary grid system with the origin of the grid centered on the stump. Using the mini excavator, a trench was dug parallel and adjacent to one side of the $1-m^2$ sample area. The trench was necessary during operation of the air spade as a place for excess soil to be placed. Using the air spade system, the $1-m^2$ area was excavated in 20-cm increments to 1 m in depth, for an excavated volume of 1 m^3 with all loblolly pine coarse roots collected. A visual inspection of roots in the field was used to distinguish loblolly pine roots from other roots based on physical and morphological characteristics. Coarse root samples were then oven-dried until a constant weight was achieved. After which, sub samples were taken from coarse roots and cleaned of remaining soil to develop a correction for remaining adhering soil mass. From this, roots were scaled to the 10 m radius plot used in calculating basal area and subsequently divided by the number of trees per plot to determine the average contribution of an individual tree to carbon stored in coarse roots.

Loblolly pine taproots were defined as roots originating from the primary root ball with a vertical orientation that were greater than 2 mm in diameter. Removal of the taproot system began by excavating a "Y" shaped trench, with the stump and assumed diameter of the taproot system between the two arms of the "Y". The air spade system was used to remove remaining soil around the taproot system. Depending on soil characteristics and depth of tap root systems, determinations were made in the field to continue excavation with the air spade or excavator. Once the taproot system was removed, excess soil was removed using the air spade and non-taproots were removed

from the primary taproot system. The entire taproot system was then weighed and three sub-samples were cut from the most prominent taproot used to correct for remaining soil and moisture content. Sub-samples of the tap root originated near the end of the tap root, the middle, and the upper portion of the root.

Soil Samples

Soil samples were collected to a depth of 40 cm on a 1.7-ha grid. Collected in 2003 and 2015, soil samples were analyzed in the Soil, Plant and Water Analysis Laboratory (SPWAL) located at Stephen F. Austin State University for organic carbon content. Excess soil not used in analysis was oven-dried at 60°C and stored at 22°C.

Over the course of the multi-year sampling, the SPWAL used different C:N analyzers to determine soil carbon content of samples. Carbon content of samples collected in 2003 were measured using different analytical equipment than what is currently used at SPWAL that was used to determine carbon in samples taken in 2015. This created a potential source of error that was addressed by retesting 16 randomly selected samples from 2003 to determine if there is a source of error caused by machine differences that could be misinterpreted as a difference in soil organic carbon. It was assumed that after the initial drying and storage, carbon mineralization was negligible.

O Horizons

In 2015, the accumulated O horizons were sampled from a 27 cm diameter plot on the same 1.7-ha grid from which soil were sampled. Samples were oven-dried at 60°C until a constant weight was achieved. Organic matter was determined on subsamples by the loss on ignition method in a muffle furnace at 500°C. The organic matter concentration was converted to organic carbon by dividing organic matter mass by 2.

Statistical Analysis

Using paired t-tests, the average SOC in the mineral portion of the soil was compared by site between the years 2003 and 2015 with the null hypothesis being there was no change in SOC over time. An alpha value of 0.05 was used in testing the probability of significant differences in SOC in the mineral portion of soil. To determine outliers in the data set, Tukeys determination of outliers was used.

Initially, the O horizon, as well as coarse woody tree roots, would be assumed to be negligible due to the previous grass-only vegetation community present before tree planting, which does not produce coarse woody roots. In addition, before tree planting there was no significant accumulation of organic litter to form an O horizon. Therefore, any measurable amounts of O horizon and coarse roots were assumed to be a net increase in their respective categories.

Correlation analysis was run on all variables to determine if any aboveground variable (DBH, stem height, stem mass, and crown mass) was correlated with carbon stored belowground in coarse woody roots and taproot systems. Afterwards, regression analysis was performed on correlations that were significant to develop models for estimating belowground carbon in coarse woody roots using measured aboveground variables. All statistical tests were run using the statistical software package SAS version 9.4 and used an alpha value of 0.05.

RESULTS AND DISCUSSION

Belowground Carbon

CN analytical limitations corrections

There was a significant difference between the mean soil organic carbon measured in 2003 and retested in 2017 (p<0.0001) with those in 2017 consistently higher than the original values reported in 2003. There was however, one outlier in the data set that was removed due to the fact that it showed the sample gained 16,698 mg C kg⁻¹ dry soil. It is highly unlikely that the sample gained this much organic carbon in the years it was in storage and that it is most likely due to some extraneous circumstance, most likely laboratory error. Additionally, the sample size used for determining a machine correction factor was small enough that the outlier was unduly influencing the correction factor and needed to be removed in order to more accurately define the correction factor. After the outlier was removed, there was still a significant difference in original (2003) measurements and the rerun samples $(p<0.0001)$, with the rerun samples still consistently higher than the original values.

The increase in organic carbon would not be expected to come from additions of organic materials, but more likely a difference in the analytical equipment's ability to

quantify organic carbon concentrations. To correct for the differences in the laboratory's ability to quantify soil organic carbon due to technological limitations, a linear transformation was applied to the original values reported in 2003. After the linear transformation was applied, there was no significant difference between original sample values and the rerun samples. From this, it is assumed that values from 2003 read lower than analytical equipment used to run samples in 2015 would have read. To correct for this, the linear transformation was applied to each 2003 sample using equation [1] to compensate for the differences between the laboratory's technology used to calculate 2003 and 2015 soil organic carbon. It is assumed that the coefficient of the function [1] represents the actual change in soil organic carbon in samples and the intercept (4043.2) is the difference in the analytical equipment. The adjusted 2003 SOC (C03') and the original 2003 SOC (C03) readings, are both expressed in mg C kg⁻¹ dry soil.

$$
C03' = C03 + 4043.2 \tag{1}
$$

The differences could be due to changes in calibration technology, hardware and software technologies, or a combination of factors

Soil Organic Carbon

 After adjusting for the difference in analytical equipment used to evaluate soil organic carbon in 2003 and 2015, the 165 paired soil samples (n) for the Arbor Grove and the 150 paired samples from the Atoy sites, no significant difference was detected in

SOC located in the top 40 cm (Table 1). The Hickory Creek site had 154 paired soil samples that showed a statistically significant increase in carbon in the top 40 cm.

The Hickory Creek site, on average, has poorer drainage than the other two sites based on field observations. While the NRCS data may not reflect this observation, the alluvial floodplain on Hickory Creek holds more water than soils on the other two sites. This could create soil environment conditions that trend more towards anaerobic that would slow the decomposition of soil organic matter that leads to an accumulation of soil organic carbon.

Table 1. Results of paired t-tests between soil organic carbon (SOC) between 2003 and 2015 by site. Values expressed in mg C kg⁻¹ soil (α =0.05).

Site	n	Mean	Std. dev.	\pm 95% CL	P value
Arbor Grove	165	-497.6	3385.6	520.5	0.0608
Atoy	150	35.9	4392.2	708.6	0.9203
Hickory Creek	154	3039.7	5399.6	859.6	< 0.0001

Coarse Roots

Mou et al. (1995) concluded that coarse roots are distributed in greater quantities near the stem, combined with the uniformity of stem planting associated with the row planting of plantation production operations; the 1 $m³$ excavated areas would be representative samples of coarse root densities within the stand. With the assumption that there were no coarse roots present prior to tree planting, excavation of all 1 m^3 yielded coarse woody roots. On average, trees at the Arbor Grove site had 17.58 kg C in coarse

roots in the top 1.0 m, Atoy trees had 11.42 kg C in the top 1.0 m, and Hickory Creek trees had 14.50 kg C in the top 1.0 m. Atoy had less coarse root mass than Hickory Creek and Arbor Grove, this difference is believed to be a result of pedologic conditions. Soils where trees were excavated on the Atoy site were all mapped as Ultisols. This relatively higher clay content, compared to other locations where trees were measured, should be investigated in further research to determine if rooting depth is effected by clay content.

At each site, carbon stored in coarse roots in the top 20 cm accounted for over 30% of total carbon stored in coarse woody roots (Table 2). Carbon stored in root biomass in the top 40 cm accounted for the majority of carbon stored in lateral coarse roots, with Arbor Grove having the lowest proportion at 62.4%. The range from 0 to 60 cm contained over 75% of carbon stored in coarse woody root biomass for all sites; this is in contrast to Kinerson et al. (1977), who found $70 - 75\%$ of loblolly pine lateral roots were located in the top 20 cm of the soil profile. The latter study was located on sandy loam over sandy clay to clay subsoils, which is similar to the soils excavated in this study (Table A1), but produced different results. Retzlaff et al. (2001) showed that genetics between loblolly pines found in the Lost Pines region of Texas and Atlantic Coast Pines of the Piedmont region in North Carolina did not play a part in lateral root partitioning by depth, with similar results to Kinerson that over 70% of lateral roots were found in the upper 20 cm of the profile. Trees excavated by Kinerson et al. were 16 years old at the time of excavation and trees excavated by Retzlaff were four years old at the time of the

study; both studies found the same depth of rooting patterns of lateral roots. Additionally, Retslaff et al. (2001) conducted their study on sandy, siliceous, thermic Psammentic Hapludults, while excavations at the Atoy site were conducted on fine, mixed, active, thermic Aquic Hapludults. Coarse roots at Atoy should have had rooting patterns similar to the results in Retslaff et al., but roots were observed in greater proportions at depth compared to Retslaff. The difference between the two studies suggest that age plays a part in coarse root partitioning at depth.

Arbor Grove and Hickory Creek both had increases in carbon stored in coarse roots at certain depths. For Hickory Creek, the increase came at depths of 20-40 cm, while Arbor Grove experienced an increase at 60-80 cm in depth. Only Atoy had a decline in coarse roots concentrations at every depth interval (Figure 1). This again could be due to pedologic conditions. With successive Bt horizons associated with the Aquic Hapludult (Sacul fine sandy loam), meaning higher mechanical resistance with depth, there might have been a greater diminishing return for trees to increase rooting density at depths.

Few coarse roots were observed below 100 cm in depth. Observations of roots below 100 cm showed that there was an insignificant amount of coarse roots in relation to roots above 100 cm in depth. One sample collected from the Hickory Creek site had no roots, coarse or fine, below 80 cm in depth. Therefore, roots below 100 cm in depth were not sampled for their contribution to carbon sequestration.

Table 2. Cumulative percentage of total carbon by depth to 100 cm in coarse woody roots (not including tap root systems) at Arbor Grove, Atoy, and Hickory Creek study sites.

Depth (cm)	Arbor Grove	Atoy	Hickory Creek
$0 - 20$	32.6	38.3	38.1
$20 - 40$	62.4	71.8	78.5
$40 - 60$	75.9	85.9	92.6
$60 - 80$	92.5	98.0	98.3
$80 - 100$	100	100	100

Figure 1. Mean Kg C by depth in coarse roots sequestered at Arbor Grove, Atoy and Hickory Creek study sites.

Tap Roots

Excavated tap root systems had means of 25.75 kg C tree⁻¹, 32.10 kg C tree⁻¹, and 34.83 kg C tree⁻¹ for Arbor Grove, Atoy, and Hickory Creek, respectively. To convert to Mg C ha⁻¹, tap root mass was multiplied by trees per ha. Arbor Grove, Atoy, and

Hickory Creek had on average 10.17, 13.28, and 18.33 Mg C ha⁻¹, respectively, stored in tap root systems.

O Horizons

O horizon means were 6.56 Mg C ha⁻¹; 6.28, and 6.48 Mg C ha⁻¹ for Arbor Grove, Atoy, and Hickory Creek, respectively [\(Table 5\)](#page-45-1). With the assumption that at the time of planting there was no O horizon present, the data shows that loblolly pine trees contributed significantly to accumulation of an O horizon and the carbon sequestered in it. While carbon stored in the O horizon is subject to more rapid decomposition relative to other C sinks in forested systems, the O horizon should be considered a sink. Barring drastic changes in the equilibrium of the system (e.g. fire, removal, clear cutting, etc.) decomposition and mineralization rates will not outpace accumulation rates of the O horizon. The equilibrium of the system may shift over time with less accumulation and higher decomposition rates, but on a decadal scale, carbon will still be present in organic form within the O horizon.

Carbon Sequestration

Hickory Creek was the only site that had a statistically significant change in SOC from 2003 to 2015. The grassland ecosystems present prior to planting would have had carbon sequestered primarily in SOC, with the latency of carbon sequestered in biomass negligible due to the rapid decomposition associated with non-woody root structure of grasses.

Total belowground carbon was considered to be the sum of C in SOC, taproots, and coarse roots. All sites were able to sequester carbon belowground. In 2017, the Arbor Grove and Atoy sites had no significant difference in carbon stored in SOC, therefore only carbon stored in coarse roots and tap root systems contributed to carbon sequestered. The Arbor Grove site sequestered 16.60 Mg C ha⁻¹ and Atoy sequestered 18.01 Mg C ha⁻¹. Hickory Creek had an increase in carbon stored in SOC with 14.59 Mg C ha⁻¹ sequestered, and with the addition of coarse roots and tap root systems, Hickory Creek sequestered 40.10 Mg C ha⁻¹ (Table 3).

 The largest contributor to carbon sequestered on all sites was in tap root system biomass, with coarse roots contributing the least to carbon sequestered. Net carbon sequestered in SOC at Hickory Creek was another large contributor to total carbon sequestration. However, the Arbor Grove and the Atoy sites did not contain the same amount of total carbon sequestered belowground due to the lack of supporting statistical evidence that there was a change in carbon stored in SOM. The difference of SOC present in 2015 from 2003 at the Hickory Creek site significantly contributed to total belowground carbon sequestered compared to the other two sites (Table 3).

In addition to sequestering the most carbon, Hickory Creek also had the most carbon present belowground [\(Table 4\)](#page-44-1). Even without the carbon sequestered in SOC, Hickory Creek had more carbon sequestered belowground than Arbor Grove and Atoy. With the sites being in close proximity, the difference is most likely due to pedologic differences.

	Arbor Grove	Atoy	Hickory Creek
SOC	$\overline{}$	$\overline{}$	14.59
Coarse Roots	6.43	4.73	7.18
Tap Roots	10.17	13.28	18.33
Total	16.60	8.01	

Table 4. Carbon present belowground (Mg ha⁻¹) in 2017. Values in parenthesis are the percentage (%) of total carbon present belowground.

In all sites, carbon present in SOC made up over 70% of total belowground carbon with Atoy having the highest proportion of carbon in SOC to total belowground carbon (Table 4). The proportion of carbon stored by SOC, coarse roots, and tap roots by site were similar, differing only by 2.01 percentage points between carbon stored in coarse roots between Atoy and Arbor Grove.

With the addition of carbon sequestered in the O horizon, all sites were able to sequester over 6 Mg C ha⁻¹ in addition to what was sequestered belowground (Table 5).

Table 5. Net carbon sequestered $(Mg ha^{-1})$ belowground as well as in O horizons at Arbor Grove, Atoy, and Hickory Creek study sites.

Regression

To explore the relationship between DBH, stem height, basal area, trees per hectare, crown C, stem C, tap root system C, coarse root C, aboveground C, belowground C, and total tree C, correlation and regression were performed. Crown C, stem C, tap root system C, and coarse root C were the measured amounts of carbon stored in their respective category. Aboveground C was the sum of crown C and stem C, while belowground C was the sum of tap root C and coarse root C. The total tree C was the

sum of aboveground C and belowground C. All values were expressed in kg C tree⁻¹ with the exception of DBH and stem height, which were expressed in cm.

Analysis revealed that the most significant correlation was between belowground carbon sequestered in roots and carbon sequestered in tap roots. However, the inherent relationship between carbon belowground and carbon in tap roots, does not provide a suitable means of predicting belowground carbon sequestered in coarse roots. The correlation analysis did reveal that total carbon sequestered in trees was correlated with carbon sequestered in stems with an r value of 0.9455.

Linear regression analysis was performed on carbon sequestered in merchantable stems and total carbon sequestered which resulted in equation 2 with a p-value <0.0001, $r^2 = 0.8940$, and an RMSE = 12.8054.

$$
Total C = 74.6618 + 1.1350 (Stem C)
$$
 [2]

Where Total C is the total carbon sequestered in loblolly pine biomass and Stem C is the carbon sequestered in loblolly pine merchantable stems.

While not a direct predictor for carbon sequestered belowground in coarse root mass, equation 2 provides the option for estimating total carbon sequestered which is more applicable and relevant to foresters in real world scenarios. However, carbon in merchantable stems is not typically a metric that managers have readily available to them. Because producers are focused on predicting merchantable volume, the majority of the

models used to predict merchantable volume use DBH and length in calculations. Additionally, these metrics are easily measured in the field.

In order to produce a meaningful model for managers to assess carbon sequestered in loblolly pine mass, a non linear regression was performed using DBH and merchantable stem length as input parameters. This resulted in equation 3 below:

Total $C = 0.048$ **x** DBH^{1.1241} **x** SL^{0.6415} [3]

Where Total C is the total carbon sequestered in loblolly pine biomass, DBH is the diameter at breast height in cm, and SL is the merchantable stem length in cm.

With a slightly higher RMSE than equation 2, equation 3 had an $RMSE =$ 12.8475. However, equation 3 offers more standard approach to estimating total carbon sequestered by using parameters commonly measured in forest inventories; DBH and merchantable stem length. In comparison, equation 2 requires the user to calculate carbon sequestered in stems to then calculate total carbon sequestered. Equation 3 allows the direct calculation of carbon sequestered, both above and below ground, per tree, directly from direct tree measurements.

CONCLUSIONS

All sites had an increase in carbon sequestered belowground, with increases in carbon stored in tap roots, coarse roots, and O horizons, but only Hickory Creek experienced a significant increase in SOC. Coarse roots on all sites were recorded at higher concentrations at deeper depths than reported in previous studies with over 75% of carbon stored in coarse roots found between 0 and 60 cm. O horizons on all sites were able to sequester carbon. The latency of these horizons and the carbon within them is heavily dependent on management practices.

SOC in loblolly pine plantations is dependent on many soil factors, with only one site able to have a positive net sequestration of carbon. More research into soil parameters affecting the accumulation of SOC in loblolly pine plantations is needed in order to more accurately assess whether afforestation leads to an increase in SOC in loblolly pine plantations.

Using regression analysis, two equations were developed using aboveground variables to estimate total carbon sequestered in loblolly pine components. Derived from linear regression, equation 2 uses carbon sequestered in stems to calculate total carbon sequestered in loblolly pine biomass. Equation 3 was derived from nonlinear regression techniques and uses DBH and merchantable stem length to calculate total carbon

sequestered in loblolly pine biomass. Equation 3 was developed to be more useful in real-world applications by using parameters that are commonly measured during forest inventories; in contrast, carbon in stems, which cannot directly be calculated from field measurements, requires the use of additional equations to estimate above-ground carbon.

With all trees being 16 years in their first rotation and the resulting narrow range in DBH, future studies should examine trees on a wider age range as well as different soils to examine whether relationships are constant across age ranges and different soil conditions such as texture and drainage classification.

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APPENDIX

Figure A1. Graphic representation of differences between readings of CN analyzer in 2003, 2017, and adjusted 2003 values.

common names

common names

Table A1. Common and taxonomic classification of soils present at each study site. Asterisk marks denote soil mapping units Table A1. Common and taxonomic classification of soils present at each study site. Asterisk marks denote soil mapping units

Figure A3. Location of Atoy study site in Cherokee County, Texas. Figure A3. Location of Atoy study site in Cherokee County, Texas.

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Figure A6. Soils at the Hickory Creek study site. Data obtained from Houston County, Texas, Soil Survey. NRCS, 2002.

Vita

William Wedge was born to Kevin and Janean Wedge who helped him discover his appreciation for the sciences. After graduating from Plano Senior High, he attended Oklahoma State University where in 2016 he graduated with a Bachelor of Science in Plant and Soil Science with an emphasis in Soil and Water Resources. While obtaining his undergraduate degree he worked as a student worker under Dr. Tyson Ochsner in the Oklahoma State Soil Physics laboratory. Upon graduation he decided to return to Texas to study at Stephen F. Austin State University in the Environmental Science Program. While working as a graduate assistant, he pursued a Master of Science in Environmental Science.

Permanent Address: 3203 Canyon Creek Drive Richardson, TX 75080

Style Manual Designation: Soil Science Society of America

This thesis was typed by William L Wedge.