Partitioning Soil Respiration in Response to Drought and Fertilization in Loblolly Pine: Laboratory and Field Approaches

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Abstract

An understanding of ecosystem-level carbon (C) sequestration, or net ecosystem production (NEP), requires the separation of heterotrophic, microbial respiration (RH) from autotrophic, root-derived respiration (RA) as the components of RS (i.e., NEP = NPP - RH). However, separating these two sources in situ has been problematic since they are closely coupled. This study utilizes two similarly aged Pinus taeda L. stands, 8 and 9 years-old, aimed at quantifying these two respiration components through in-situ root severing. In order to use root-severing treatments to separate RS into RH and RA components, confirmation of carbohydrate depletion coupled to RA decline is crucial. This study evaluated the changes in CO₂ flux rates and carbohydrate supply upon root severing in Pinus taeda L. using a controlled laboratory validating a two-part field study. The first field study used root-severing cores to test in-situ if respiration components can be attained based on the depletion of carbohydrate supply. The second field study was aimed at how future changes in climate might affect the ability of forests to store C and how modern forestry practices might affect changes and was conducted over the course of two installations, spring and summer 2012. In this study we examined the effects of fertilization (0 and 100.9 kg N ha⁻¹) and throughfall reduction (0 and -30%) on total soil respiration (RS) as well as the heterotrophic contribution to RS, in a fully replicated (n=4), 2x2 factorial design. In the controlled lab experiment RS and RA declined by 86% and 95% respectively by the end of an 86 day trial and NSC carbohydrates declined by 60% for soluble, 29% for insoluble, and 43% for total (soluble + insoluble). The decline of RA was highly correlated to with the decline of NSC’s at 0.90, 0.69 and 0.93 for soluble, insoluble and total, respectively. The companion field study revealed a mean decrease 21±0.5% of over the final three dates when severed root respiration stabilized. In the second study, testing throughfall reduction and fertilization levels there were no fertilization by throughfall reduction interactions on the contribution of RH to RS in either the spring or summer; however, the main effect of throughfall reduction was significant in the spring. During the spring, the mean contribution of RH to RS for ambient throughfall plots was 96±6.4%, while the mean contribution under throughfall reduction was 68±1.9%. During the summer, there were no differences among treatments and the overall contribution of RH to RS was 78±1.6%. Collectively, both of these studies revealed that the severing of roots from their primary energy source and the subsequent depletion of stored NSC that the use of in-situ methods allows for the quantification of soil respiration components RA and RH. Using these estimates to model NEP in the short-term can be variable by season, however, long-term monitoring may simplify future NEP modeling scenarios.
Dedication

For my parents who always encouraged and had faith in me to make the right decisions and supported me through all of my adventures.

For old friends at home without their support I would not have gone to grad school and for new friends who helped make grad school fun…three words not often seen together.
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1. Literature Review

1.1 Terrestrial carbon

The terrestrial biosphere is one of the main reservoirs in the global carbon (C) cycle (Schimel 1995); however, land use change since the mid 19th century has reduced the soil C pool size (Schlesinger 1977, Canadell et al. 2007, Pan et al. 2011). Soils are the largest terrestrial C pool (Schlesinger 1997) and soil respiration (R_S) is the source of the second largest biotic flux in the C cycle. Because of the size of this C pool and flux, even small perturbations have the potential to impact global C cycles. Currently, the terrestrial C pool is estimated at 3120 Pg C with live biomass ranging from 493 - 560 Pg C, and 1180 - 2500 Pg C in the first meter of soils (Lal 2010). The global terrestrial C sink is estimated in the range of 2.0 - 3.4 Pg C yr\(^{-1}\) (Pan et al. 2011), but estimates put the increase in global atmospheric C from 4 - 5 Pg yr\(^{-1}\) due to fossil fuel emissions (Raich and Schlesinger 1992, Canadell et al. 2007, Lal 2010). Therefore management impacts on the soil C pool that shift toward either storage or release could significantly influence this balance. Further, uncertainty about the effects of climate change also leads to ambiguity concerning the role of managed forests on the sequestration of atmospheric C. Yet, land use changes towards afforestation in general, can mitigate greenhouse gas emissions by sequestering C in biomass (IPCC 2000) and soil organic matter (SOM) (Richter et al. 1999). Forests are 90% of the net C sink in the U.S., sequestering approximately 12% of the annual U.S. greenhouse gas emissions (EPA, 2005).

There are 304 million ha of forestland in the continental U.S., with 208 million ha of that being timber forests (Smith et al. 2009). Timber forests are classified in the U.S. Forest and Rangeland Renewable Resources Planning Act of 1974 as “forests that can produce 20 cubic feet per acre of industrial wood annually and are not legally reserved from timber harvest” (Smith et al. 2009). The south and southeastern U.S. have 82 million ha of forest, with 22 million ha in loblolly pine (\textit{Pinus taeda} L.) and shortleaf pine (\textit{Pinus echinata} Mill.) (Smith et al. 2009) of which 13
million ha (Fox et al. 2007) are in intensively managed plantation style loblolly pine forests. Intensive management of loblolly forests have shown appreciable increases in productivity since widespread establishment of intensive silviculture practices beginning in the 1950’s and 60’s (Fox et al. 2007, Jokela et al. 2010). These increases in productivity, even while accounting for the use of fossil fuels during harvest and transport, make intensively managed plantations overall C sinks (EPA 2005, Gonzalez-Benecke et al. 2010).

Carbon dynamics are routinely modeled for intensively managed forests through the estimation of net primary production (NPP). Although aboveground NPP is generally a good indicator of belowground activity and correlates positively with $R_S$ (Schlesinger 1977, Raich and Schlesinger 1992), accurate understanding of ecosystem-level C sequestration, or net ecosystem production (NEP), requires the separation of heterotrophic, microbial respiration ($R_H$) from autotrophic, root-derived respiration ($R_A$) as the components of $R_S$ in order to calculate NEP from modeled NPP estimates (i.e. $NEP = NPP - R_H$). $R_H$ is a result of the decomposition of soil organic matter by soil microorganisms and the subsequent respiration of CO$_2$. $R_A$ in contrast, is root respiration that is defined here to include mycorrhizal respiration because symbiotic mycorrhizae acquire energy directly from their plant hosts to maintain metabolic activity (Högberg and Read 2006, Subke et al. 2006).

Experimental manipulations to understand $R_A$ and $R_H$ responses to fertilization and decreased precipitation are important in order to understand the interactive effects of forest management and climate variability on C fluxes and stocks across the southeastern U.S. Average temperatures are predicted to rise from 2.5° - 5° C by the 2080’s (Karl et al. 2009) and will effect soil temperatures and moisture content. The greatest temperature increase is predicted to occur in the summer with the number of hot days outpacing the average rise in temperatures. Rainfall is predicted to decrease between 10 and 30% (Solomon 2007, Karl et al. 2009). Increase in temperatures with a decrease in
precipitation will affect soil moisture content and potentially decrease the C sequestration ability of southern pine forests by 10% (Noormets et al. 2010). An $R_H$ estimate under such conditions will allow models to predict the C balance of southern pine forests to changing climate. Understanding how climate change will effect C sequestration in various ecosystems, such as a southern pine forest, will not only add to the body of work by other researchers but will aid in understanding global C sources and sinks (Raich and Schlesinger 1992).

Soil CO$_2$ efflux models have been developed using data from spot measurements together with soil temperature and moisture data (Vogel et al. 2008). Potentially, year round models can be developed using continuous soil moisture and temperature data that will help to predict NEP (Bond-Lamberty et al. 2011) from NPP field measurements, thus providing a more comprehensive view of the C sequestration potential of managed forest ecosystems. Regional models such as those developed by Gough et al. (2005) are able to quantify $R_S$ for intensively managed loblolly pine stands ranging from 1 – 25 years across the southeast. A logical next step is to model NEP of intensively managed pine stands in the southeast; however, this requires the ability to accurately and quickly separate $R_A$ and $R_H$ in situ which has yet to be accomplished.

1.2 Methods to quantify $R_H$

Högberg et al. (2001) referred to soil as a ‘black box’ because of the inability to disentangle the coupling of root and other soil processes. A variety of approaches have been taken to provide this resolution (Hanson et al. 2000, Kuzyakov 2006, Kutsch 2009), particularly with respect to separating soil respiration. Examples include component integration, trenching, artificial gaps and girdling, all of which have their own challenges, including disturbance/destruction and difficulties with replication. Component integration requires separating soil, roots and litter in order to measure each separately where $R_S$ is measured before and after roots are removed from the soil and $R_H$ is estimated by the difference (Hanson et al. 2000). Trenching is an *in situ* root exclusion approach where a small
vegetation free plot is made by removing living aboveground biomass and excavating a small trench around perimeter (Kutsch 2009). Removing vegetation will take away additional photosynthate being delivered to the roots, while the trench prevents roots from outside the vegetation free area from invading the plot. Ewel et al. (1987) found that it took four months for respiration rates in vegetation free 2.5 x 3.5 m trenched plots to stabilize because of fine root decomposition. Others have shown that up to a year is needed after trenched exclusions are installed (Vogel et al. 2005). Girdling blocks the supply of recent photosynthate to roots and is accomplished by removing the bark through the phloem around the entire circumference of the stem (Högberg et al. 2001) and as a result $R_A$ decreases. Artificial gaps, such as clear cuts, are similar to girdling and trenching where the aboveground biomass is removed while roots are left without a means of receiving new photosynthate (Kutsch 2009). However, artificial gaps expose the soil to disturbances such as erosion through decreased rainfall interception and a lost litter layer, and increases in soil temperature that alters microbial activity (Kutsch 2009). These methods of quantifying are all aimed at stopping the transport of recent photosynthate to roots for continued maintenance and storage, resulting in the depletion of stored non-structural carbohydrates (NSC).

1.3 Carbohydrate resources for $R_A$

NSC’s are low molecular weight soluble sugars (e.g., glucose, fructose and sucrose) as well as starch (Hoch et al. 2002). Starch is formed when C acquisition is greater than C demands of the plant that are needed for growth and maintenance (Chapin et al. 1990). Starch is composed of long chains of glucose but is not transportable so it remains at the site of formation for later use within the tissue. Starch is used when C assimilation is low or at a deficit, and is the most abundant stored energy reserve (Li et al. 2002). In essence, stored NSCs give long-lived plants the ability to survive during periods of physiological stress.
According to Chapin et al. (1990), it is more efficient for plants to store starch rather than mobile sugars, and plant growth declines when there are excess soluble sugars due to increased respiration (Schulze et al. 1991). In a study conducted by Wertin and Teskey (2008) assessing net photosynthesis, overall plant respiration and carbohydrates, NSC concentrations were measured in leaves and roots. They found that *Populus deltoides* relies on recent carbohydrate production (mobile sugars), for growth and maintenance of respiration (Wertin and Teskey, 2008). In order for plants to access stored energy, starch must be converted back to soluble forms enzymatically (Pallardy 2008). Because of this, stored NSCs in roots should be depleted when used for metabolism (Botelho and Vanden Heuvel 2005) in situations when newly assimilated photosynthate cannot be transported to the roots (Högberg et al. 2001). In a study by Li et al. (2002), where treatments of complete defoliation and pruning of 66% of the branches were applied, *Pinus cembra L.* showed a decline of NSCs in all tissues sampled, with buds, current needles, 2 year-old needles, stem wood, and roots being the most sensitive to the use of their stored NSC. These studies reinforce that severing roots from their energy supply will lead to eventual cessation of root respiration.

1.4 \( R_H \) estimates through root exclusion techniques

Root exclusion techniques have proved effective to estimating the relative contribution of \( R_A \) in various systems. Wiant et al. (1967) estimated that \( R_A \) was a third of total \( R_S \). In Ewel et al. (1987), trenched plots were used to estimate \( R_A \) in 9- and 29-year-old slash pine plantations in Florida. Estimates from this study were 51% and 62% (\( R_A/R_S \)), respectively. Vogel et al. (2005) found in a boreal black spruce (*Picea mariana*) forest that in trenched plots \( R_A \) contribution to \( R_S \) is 55%. Vogel and Valentine (2005) then compared differences between their trenched plots (i.e., large root exclusions) to small root exclusions (15.2 cm x 30cm diameter plastic pipe) and found that after 1-3 weeks \( R_H \) measurements made in small root exclusions were not significantly different from measurements done in large exclusion trenches after only 1-3 weeks. Work done by Högberg et al. (2001) who used girdling
techniques to partition $R_S$ reinforces the idea that preventing recent photosynthate transport to roots effectively decreases $R_A$ quickly and that respiration measurements for $R_H$ can then be taken the same season the treatment is applied. Hogberg et al. (2001) estimated that girdling treatments during the summer had a $R_S$ reduction of 52%, which was attributed to absence of $R_A$.

1.5 Effects of temperature and moisture on $R_S$

Temperature and moisture are two important abiotic factors that affect the rate of root growth and microbial decomposition, thus influencing $R_S$. The relationship of $R_S$ to temperature has been well established (Lloyd and Taylor 1994) and is often characterized by exponential relationship (Kirschbaum 2000, Fang and Moncrieff 2001, Janssens and Pilegaard 2003, Davidson et al. 2006). In temperate ecosystems typical respiration rates ranging from 2.5 – 3.9 (Raich and Schlesinger 1992, Davidson et al. 1998) and have been used to model seasonal cycles of soil respiration.

Soil moisture is found to also influence the rate of $R_S$ but tends to only have significant influence under extreme low (<15%) or high (>50%) soil moisture content while more moderate soil moistures do not influence $R_S$ greatly (Fang and Moncrieff 2001).

It has generally been demonstrated that the interaction of soil temperature and moisture are negatively correlated with each other (Davidson et al. 1998), while soil respiration is positively correlated to soil temperature. Wiseman and Seiler (2004) conducted respiration measurements on a loblolly plantation situated on the Virginia Piedmont and found that soil moisture was significantly related to the rate of respiration. During drought-like conditions, when soil temperatures were very high but soil moisture was low, $R_S$ was also very low. Similarly Tyree et al. (2008) conducted a study in the Virginia Piedmont in a 2-year-old loblolly plantation measured heterotrophic respiration and found that soil moisture contributed significantly to $R_H$ but explained little of the variation of heterotrophic respiration while temperature explained 30%
of the variation of total respiration. Wiseman and Seiler (2004) found that changes in respiration rates closely paralleled changes in soil temperatures throughout the year. In both studies, temperature is demonstrated to have greater influence on soil respiration than soil moisture. However, extremely low soil moisture can cause the soil respiration/temperature relationship to decouple. In an 18-year-old Douglas-fir stand Jassal et al. (2008) found that water matric potential ($\theta$) above $0.11 \text{ m}^3 \text{ m}^{-3}$, which corresponds to water potential ($\Psi$) of $-2 \text{ MPa}$, soil moisture and soil temperature are positively correlated to soil respiration but once $\theta$ dropped to $0.07 \text{ m}^3 \text{ m}^{-3}$ soil respiration became decoupled from soil temperature. The results of these studies provide evidence that the relationship of respiration to soil moisture is responding to processes occurring in the soil. Soil osmotic stress, diffusion, and oxygen limitation influencing the response of $R_s$ and can vary by individual soil types and their physical properties [e.g., soil pore space, bulk density and texture (Moyano et al. 2012) causing $R_s$ responses to soil temperature and moisture to be variable.

1.6 Nitrogen additions and soil respiration

Soil microbial biomass depends on organic matter inputs (Zak et al. 1990, Fisk et al. 1998), which themselves often scale with site quality/fertility (Maier and Kress 2000, Janssens et al. 2001). However, studies considering nitrogen (N) fertilization treatments and the effect on soil respiration and the subsequent heterotrophic and autotrophic responses have had variable results and are less straightforward (Söderström et al. 1983, Smolander et al. 1994).

Tyree et al. (2006) examined the long-term effects of fertilization on $R_s$ in a 33-year-old loblolly pine stand that had been fertilized 9 years after planting and found that $R_s$ was still increased 22 years after fertilization amendments. This increase was attributed to increased $R_A$ but not $R_H$. Short term effects of fertilization were studied by Gough et al. (2004) in 2-year-old loblolly pine seedlings and found that 49 days after fertilization that $R_A$ was 32% higher in fertilized plots than in
non-fertilized plots but eventually decrease until there was no difference from unfertilized plots 197 days after fertilization. They posited that higher $R_S$ due to $R_A$ is a result of increased metabolic activity as a result of nutrient uptake and assimilation that results in allocations to above ground biomass.

Recommended operational level fertilization in a loblolly pine plantation in the southeast by Gough et al. (2004) while Fisk and Fahey (2001) applied fertilizer that roughly tripled the site $N$ availability and both found that fertilization suppressed microbial biomass and thus soil respiration. Fisk and Fahey (2001) studied a young northern hardwood forest stand after eight years of fertilization; however, increases in specific respiratory activity (e.g., respiration to microbial biomass ratio) and $N$ turnover in the microbial pool were also observed, suggesting increased microbial activity (Wardle and Ghani 1995). While Gough et al. (2004) in 2-year-old loblolly pine seedlings found that fertilizer reduced $R_H$ by 42 and 32% at 49 and 197 days respectively after fertilizer was applied. A study by Tyree et al. (2008) measuring $R_S$, $R_H$, and $R_A$ during the first year of fertilization applied at an operational level for loblolly pine in the southeast in a 2-year-old loblolly pine stand in the Piedmont of Virginia reflected similar results to Fisk and Fahey (2001) and Gough et al. (2004). Overall, fertilization decreased $R_H$ by 32% but rates were variable by date with the strongest relative differences occurring 5 months after the fertilization treatment was applied. They also found that the highest $R_H$ rates were inverse to the amount of $N$ found in the soil. Additionally, Tyree et al. (2008) calculated that $R_A$ increased by 20% in fertilized plots over plots that were not treated with fertilizer when averaged over the year. In contrast, Gallardo and Schlesinger (1994) reported short-term increases in microbial biomass and $R_S$ in response to $N$ fertilization that doubled the annual input of $N$. Research done in forests with nutrient amendments have shown variability in soil respiration in response to fertilization however
the variability may depend on how long and how often $R_S$ is measured after fertilization (Tyree et al. 2008).

Changes in microbial responses to fertilization may also affect soil C pools. Fisk and Fahey (2001) did find that changes in nutrient regimes in the soil and litter affected the levels of microbial respiration, suggesting that it can also alter the amount of C stored in forest soils (Ågren et al. 2001). This sequestration may not be a result of the effects of fertilization on the microbial pool but on GPP (Raich and Schlesinger 1992) but fertilization may reduce the amount of C invested in belowground biomass (Raich and Schlesinger 1992) and eventually this reduces the amount of turnover in the rhizosphere and ultimately the soil C supply (Vogt et al. 1993, Wallenda et al. 1996). However, studies in loblolly pine plantations contrast this at both young and mature stands [e.g., seedlings (Pangle and Seiler 2002, Gough and Seiler 2004), 8- and 11-year-old stands (Albaugh et al. 1998, Maier and Kress 2000, King et al. 2002) increased $R_S$ is a due to increased root biomass as a result of fertilization. While in relative terms, the root to shoot ratio may decrease, the overall increase in above- and belowground biomass as a result of fertilization may increase $R_A$.

In summary, increasing $R_A$ indicates an increase in plant metabolic activity as a result of increasing C assimilation through photosynthesis and can lead to gains in aboveground biomass (Gough and Seiler 2004). Additionally, decreased $R_H$ as a result of fertilization ought to lead to overall gains in NEP or the amount of C stored in these forests. Thus, forest fertilization might serve to increase soil C stocks via two separate mechanisms.
2. Root non-structural carbohydrates and their relationship with autotrophic respiration of loblolly pine (*Pinus taeda* L.)

2.1 ABSTRACT

An understanding of ecosystem-level carbon (C) sequestration, or net ecosystem production (NEP), requires the separation of heterotrophic, microbial respiration ($R_H$) from autotrophic, root-derived respiration ($R_A$) as the components of $R_S$ (i.e., $NEP = NPP - R_H$). However, separating these two sources *in situ* has been problematic since they are closely coupled. Past studies have utilized root-severing treatments with the presumption that severing roots cuts off the supply of newly assimilated photosynthate, $R_A$ will approach zero as non-structural root carbohydrate supply diminishes. At that time, soil CO$_2$ efflux measurements inside root-severed areas will be derived only from $R_H$. In order to use root-severing treatments to separate $R_S$ into $R_H$ and $R_A$ components *in situ*, confirmation of carbohydrate depletion coupled to $R_A$ decline is crucial. We evaluated the changes in CO$_2$ flux rates and carbohydrate supply upon root severing in *Pinus taeda* L. using both a controlled laboratory and a field study. Total $R_S$ and $R_A$ were measured independently for an 86-day period. Following each measurement period, soluble and insoluble non-structural carbohydrates (NSC) were measured on the roots. By the end of an 86-day study period, soluble, insoluble and total NSC decreased by 60%, 29% and 43%, respectively. $R_S$ and $R_A$ declined asymptotically and after 86 days had declined by 86% and 95% of their original respiration, respectively. Both $R_S$ and $R_A$ were highly correlated with NSC variables. Correlation coefficients between $R_A$ and soluble sugars, insoluble carbohydrates and total carbohydrates were 0.90, 0.69 and 0.93, respectively. This suggests that root use of stored soluble sugars over time causes $R_A$ to decrease. In a companion study using root severing cores, respiration stabilized after 40 days with a mean respiration rates 21.±0.5% lower than total respiration rates with the difference presumably representing the drop in $R_A$. This relationship between total non-structural carbohydrates and $R_S$, and the steady decline in
$R_S$ and $R_A$ over time validates the observed time course of field-installed root-severing cores to stop contributions of $R_A$ within the collars.
2.2 INTRODUCTION

Carbon (C) dynamics are routinely modeled for forests through the estimation of net primary production (NPP). Although aboveground NPP is generally a good indicator of belowground activity and correlates positively with soil respiration ($R_S$) (Schlesinger 1977, Raich and Schlesinger 1992), quantifying ecosystem-level C sequestration, or net ecosystem production (NEP), from NPP estimates requires the separation of heterotrophic, microbial respiration ($R_H$) from autotrophic, root-derived respiration ($R_A$) as the separate components of $R_S$ (i.e., $\text{NEP} = \text{NPP} - R_H$).

Early methods to separate $R_S$, such as component integration, trenching (Hanson et al. 2000), or girdling (Högberg et al. 2001) typically resulted in large disturbances or difficulties of replication or repeated measures due to the destruction of trees. Root exclusion is a dominant method (Kutsch 2009) and is usually done by excavating a trench around a small area of several square meters. Trenching cuts roots from receiving new photosynthate and an estimate of $R_H$ can eventually be attained (Vogel and Valentine 2005). Girdling follows the same concept of ceasing the flow of recent photosynthate to roots by cutting tree bark around the circumference of the stem to the current xylem (Högberg et al. 2001). Girdling effectively diminishes $R_A$ contribution to $R_S$ within days to weeks; however, it is thought that C reserves, or non-structural carbohydrates (NSC), are responsible for continued root respiration despite girdling (Högberg et al. 2001). To minimize disturbance, labor, and time, Vogel and Valentine (2005) deployed small root exclusion cores in a boreal *Picea mariana* forest that would isolate tree roots from the flow of new photosynthate in small 15.2 cm diameter x 30 cm deep PVC tubes. They compared respiration measurements in small root exclusion cores to trenched plots and found that respiration in small root exclusion cores fell rapidly in 1 to 3 weeks after installation and that $R_H$ measurements were comparable to trenched exclusions. The decrease and eventual stabilization of $R_S$ associated with root severing is thought to be a result of the cessation of $R_A$ due to the depletion of NSCs. However, the timing of the NSC depletion and $R_A$ reduction is critical to informing *in situ* studies aimed at separating...
the components for $R_S$ required to model NEP from NPP estimates. Thus, the objectives of this current study are to determine the timing of NSC depletion and subsequent concentrations in loblolly pine roots as well as the relationship to $R_A$ of severed roots. Ultimately, field-testing this relationship in order to separate $R_S$ into $R_H$ and $R_A$ in situ using root-severing cores in a loblolly pine forest ecosystem.

2.3 METHODS

2.3.1 Laboratory incubation

From a 9-year old loblolly pine plantation in the Appomattox-Buckingham State Forest, located in the Piedmont of Virginia (Lat: 37.443 N, Lon: 78.664 W), soil and root samples were collected on June 11, 2012 from the upper 10 cm of the O and A Horizon. Soils of the plantation are mapped as Spears Mountain (fine, mixed, semiactive, mesic, Typic Hapludult) and Littlejoe (fine, mixed, subactive, mesic, Typic Hapludult) soil series (Web Soil Survey, accessed 04/25/2012). Samples were sieved to 2 mm and roots were hand picked before being separated into diameter classes. On June 13, 2012 homogenized soil and roots were separated into 80, 473 ml closed-cell extruded polystyrene foam containers. The same amount of soil and fresh roots were placed in each container by carefully weighing root fractions as follows: ~2 g of coarse roots (>3 mm diameter), 7-8 g of medium roots (1-3 mm diameter) and 2-3 g fine roots (< 1 mm diameter), totaling 12 g fresh roots and 450 g of soil per container, occupying a volume of 443 ml. Approximating the root lengths and diameters that were severed by the cores installed in the accompanying field study (see below). Sample units were allowed to equilibrate at constant temperature and soil moisture for 8 days. Soil moisture and temperature were closely monitored throughout incubation period and kept constant with temperatures between 20-21° C and gravimetric soil moisture between 6 – 10%.

A Li-Cor 6200 infrared gas analyzer (Li-Cor Biosciences, Lincoln NE, USA) and a dynamic closed cuvette constructed from PVC pipe (area 42.2 cm$^2$, volume 245 cm$^3$) was used to estimate CO$_2$ gas flux. Before measurements were taken, CO$_2$ in the chamber was allowed to equilibrate with ambient
air. Contact between the cuvette and the air-tight foam container allowed for an air-tight seal. Air was allowed to diffuse through perforated tubing inside the cuvette to facilitate even mixing. Once CO$_2$ concentrations began to increase consistently, the flux was measured over a 30 s period.

A component integration approach was used where total and $R_A$ were measured on 10 destructively sampled replicates on a weekly basis for the first 30 days of the incubation, and then every two weeks over the next 56 days, for a total of 8 harvests over a 86 day period. Total soil respiration measurements were taken to attain a baseline measurement prior to disturbance. Following a total soil respiration measurement, autotrophic respiration was measured independently by separating roots and soil using a 2 mm sieve. Roots were then placed in new 473 ml polystyrene containers where respiration was measured using the same methods described above, adjusted for the new container volume without soil. Following efflux measurements, root samples were immediately frozen at -80° C and stored for later NSC analysis.

### 2.3.2 Non-structural carbohydrate analysis

Modified methods of Poorter and Villar (1997) and Hansen and Møller (1975), were utilized to extract total NSC through the summation of sequential extractions of soluble and insoluble sugars. Roots were thawed and dried at 65° C for at least 48 hours then ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ) before being further pulverized using a Retsch MM200 ball mill (Retsch, Haan Germany) for 1 min at a rate of 500 rpm/min. Following the grinding procedure, root material was stored at 65°C until subsequent analysis. Extraction of NSC was done by incubating 100 mg samples of pulverized root tissue dissolved in 5 ml of 80% ethanol for 30 minutes in a 30° C water bath. Following incubation, samples were centrifuged in an Allegra 25R centrifuge (Beckman Coulter, Brea CA) at 2650g for 10 minutes. The supernatant was decanted and the process was repeated with an additional 2.5 ml of 80% ethanol. To remove compounds (e.g., lipids) that may interfere with the subsequent spectrophotometric determination of extracted carbohydrates, 5 ml of chloroform and 2.5 ml of
Deionized water were added to the supernatant that was then centrifuged for 10 minutes at 2650g.

Soluble NSC concentrations were determined by adding 5 ml of light and heat sensitive anthrone reagent to a 0.5 ml aliquot of sample (Dubois et al. 1956, Zill 1956) into a 13x100 mm round cuvette then placed in boiling water bath for 7.5 minutes after which they were cooled to room temperature. Once at room temperature, each sample was analyzed on a Spectronic 20D+ spectrophotometer (Thermo Scientific, Waltham, MA) at a wavelength of 675 nm. Following the soluble NSC extraction above, the plant residue remaining was further extracted by adding 20 ml of 3% hydrochloric acid (HCl) and digesting the mixture for three hours at 125° C. This process is reported to break down starch, fructans, pectins, and some hemicellulose (Bazzaz 1997). Once digestion was complete contents from digestion tubes were decanted into volumetric flasks then brought to 50 ml volume with deionized water, and transferred to a 50 ml test tube for centrifugation at 2650g for 10 minutes. After centrifuging the solution was ready for spectrophotometric analysis using the same methods used for soluble sugars. The combined soluble and insoluble sugars were used as the total non-structural carbohydrates.

Spectrophotometric determination of soluble and insoluble carbohydrates was done at the same time along with standards that were measured during sample analysis. Colorimetric wavelength data was converted to mg carbohydrates ml⁻¹ for each sample using the linear fit model for pure glucose standards (Hansen and Møller 1975) of increasing concentration prepared freshly each day.

2.3.3 Field study

From July - October 2011 an in situ study was conducted using root-severing cores to separate $R_H$ was located in an 8-year-old loblolly pine stand at the Reynolds Homestead Forest Resources Research Center located in Patrick County, Virginia (Lat:36°40’N, Lon: 80°10’W). Mean annual precipitation at the site is 1,279 mm, distributed evenly throughout the year. The 30
year mean annual and maximum temperature is 18.5° C and minimum temperature of 7° C with a high monthly temperature of 29.2° C, occurring in July, and a low average temperature of -4° C, occurring in January (Tyree et al. 2008). The site is a former tobacco plantation and was farmed heavily from the early 1800’s to the mid-1900’s that lead to severe erosion and loss of most of the A-horizon. Soils at the site are mapped as the Braddock series (fine, mixed, semiactive, Typic Hapludult) (Web Soil Survey, accessed 07/20/2013). Loblolly pines were planted in 2003 from container stock at a spacing of 3 m x 1.8 m. Eight replicates of the root-severing cores were installed in the stand using a completely randomized design. Root-severing cores were constructed of galvanized steel conduit (15.2 cm diameter and 35 cm length) and installed so that the upper rim of the core was flush with the mineral soil surface. It is assumed that 35 cm depth is sufficient for most of the root biomass, and has been shown that below 30 cm Rs is low (Warembourg and Paul 1973). For installation, pine leaf litter was temporarily displaced so as not to drag needles belowground, adding additional SOM inside the cores. Once cores were driven flush with the soil surface, leaf litter was replaced.

At each location, soil CO2 efflux was measured 9 times over an 86-day period in and immediately adjacent to the root-severing core using a LI-COR 6200 infrared gas analyzer (Li-Cor Biosciences, Lincoln NE, USA) and attached Li-Cor 6000-09S chamber with an area of 71.5 cm² and volume of 926 cm³. During measurements ambient CO2 concentrations were allowed to equilibrate within the cuvette near the soil surface before being pressed into a vegetation free area of the forest floor without cutting litter to create a seal. Once CO2 concentrations in the cuvette began to rise steadily a measurement was logged for 30 s and Rs was calculated as µmol m⁻² s⁻¹.

2.3.4 Statistical analysis

Correlation coefficients for Rs, Ra, soluble, insoluble, and total NSC using Spearman’s nonparametric rank correlation method with untransformed data as well as their corresponding P-
values (Table 4.1) were calculated using JMP Pro 10 statistical package (JMP®, Version 10.0. SAS Institute Inc., Cary, NC, 1989-2007).

2.4 RESULTS

2.4.1 Laboratory incubation

R_s declined asymptotically throughout the measurement period. By the end of the experiment (day 86), \( R_s \) had fallen 86±2.4% (Fig 4.1A). \( R_A \), as determined by soil-free root respiration, followed a similar pattern and fell 94±0.8% by the end of the experiment (Fig 4.1A).

Insoluble NSC follows a similar asymptotic pattern (Fig. 4.1B), whereas soluble NSC follows a more steady decrease over time (Fig. 4.1B). Soluble, insoluble and total NSC decreased by 60%, 29% and 43%, respectively by the end of the 90-day sampling period.

All NSC parameters were significantly correlated with both \( R_S \) and \( R_A \). Total NSC showed the strongest positive correlations, \( r = 0.98 \) and 0.93 with \( R_S \) and \( R_A \), respectively (Table 4.1 and Fig. 4.2). Insoluble NSC had the weakest correlations with both \( R_S \) and \( R_A \), 0.76 and 0.69, respectively.

2.4.2 Field study

Relative to measurements taken outside of cores, respiration inside the root-severing cores began to decrease rapidly over the first 13 days of installation before slowly decreasing and stabilizing on day 41. By the end of the measurement period (day 108) respiration inside the cores averaged 21±9.3% lower than outside the cores. Averaged across the last three measurement dates (when rates were relatively stable) (i.e., days 41, 65, and 108) rates inside cores were 21±0.5% lower than outside (Fig 4.3).

2.5 DISCUSSION

A very consistent drop in \( R_S \) occurred when loblolly pine roots were severed from their host tree both in the controlled laboratory root incubation and in the field using root-severing
cores. Incubation and laboratory analysis of $R_A$ and NSC revealed a strong correlation between $R_A$ and soluble sugars as well as $R_A$ and total NSCs suggesting declines in $R_A$ are due to a depletion of root energy supply. The field results suggest the percent contribution of $R_{HI}$ to total soil CO$_2$ efflux is 79±0.5%. There is evidence that respiration responds negatively with the depletion of NSC, with soluble sugars being the main source of energy for short-term root metabolic activity. Wertin and Teskey (2008) found that in *Populus deltoides*, $R_A$ was more strongly correlated with soluble sugar concentrations than with starch concentrations and results from roots that were incubated in this study confirmed their finding; starch, as an insoluble fraction of NSC, quickly declined shortly after roots were severed, and then stabilize along an asymptote for the remaining incubation period.

The asymptote for insoluble sugars could be a result of the HCl acid digestion method to quantify insoluble carbohydrates that may overestimate insoluble sugars because it dissolves fructans, pectins and some hemicellulose (Poorter and Villar 1997). This stabilization suggests (Fig. 4.1B) that portions of structural carbohydrates (i.e., hemicellulose) are being quantified. An alternative explanation is that after several weeks of not receiving new photosynthate, cellular processes begin to fail and as a result remaining starches are no longer accessible for conversion to soluble sugars for maintenance of metabolic processes within the root or both may be occurring. Sala et al. (2012) proposed that sugars play a role in maintaining vascular integrity, which suggests that the C demand of respiration, without resupply through assimilation, breaks down transport of nutrients and sugars to locations where they can be accessed for metabolism. As a result, respiration slowly decreases over time as soluble sugars are depleted until root respiration ceases, making the depletion of soluble NSC an important factor in root respiration.

Figure 4.1A shows that in lab trials respiration drops sharply within the first 17 days after sampling and thereafter begins to slowly decrease for the remaining sampling period. However,
soluble sugars and total NSC decline more gradually but are more variable than both insoluble sugars and $R_S$. Li et al. (2002) confirms this, in a complete defoliation of *Pinus cembra* L., they found that the significant reductions of NSC in the roots occurred by the end of the summer (September) with a 71% reduction ($P<0.01$) in starch, an 80% reduction ($P<0.05$) in soluble sugars and a 72% ($P<0.05$) reduction in total NSC. However throughout the summer there was little difference of total NSC concentrations of trees that were defoliated and trees that were intact. Coupling the decline of $R_A$ to stored resource use suggests that there is a threshold for resources where metabolic processes can continue. However, without resupply, resources drop below this threshold and metabolic processes become inefficient. Because of the scarcity of mobile C, a cascade of inefficiencies cause metabolic machinery to break down and may not able to access remaining reserves as efficiently as a result of this physiological or physical barrier. These inefficiencies cause respiration to decrease slowly before ceasing represented by an asymptote where root metabolism ceases to function properly.

The reduction of $R_S$ within root-severing cores tested in a loblolly pine stand suggests relatively small diameter root-severing cores can be used to estimate the relative contribution of $R_A$ to $R_S$ based on root carbohydrate depletion. Further, the strong relationship between TNC and $R_S$, and the steady decline in $R_S$ and $R_A$ over time validates the effectiveness of field-installed root-severing cores to stop contributions of $R_A$ within the collars.
3. The influence of fertilization and throughfall reduction on autotrophic and heterotrophic soil respiration in a loblolly pine (*Pinus taeda* L.) forest

3.1 ABSTRACT

Soils are the largest terrestrial carbon (C) pool and a source of the second largest biotic flux in the global C cycle. General circulation models have predicted up to a 30% decrease in precipitation in the southeastern U.S. that may lead to declines in productivity and may change regional forest C storage as a result. In this study we examine the effects of fertilization (0 and 100.9 kg N ha\(^{-1}\)) and throughfall reduction (0 and -30%) on total soil respiration (R\(_S\)) as well as the heterotrophic contribution to R\(_S\), in a fully replicated (n=4), 2x2 factorial design. In order to partition R\(_S\), root-severing cores were installed in a 9-year-old loblolly pine (*Pinus taeda* L.) plantation in the Piedmont of Virginia. Cores were installed during the spring and summer of 2012 to eliminate R\(_A\), leaving R\(_H\) as the sole source of soil CO\(_2\) flux from within the core. There were no fertilization by throughfall reduction interactions on the contribution of R\(_H\) to R\(_S\) in either the spring or summer; however, the main effect of throughfall reduction was significant in the spring. During the spring, the mean contribution of R\(_H\) to R\(_S\) for ambient throughfall plots was 96±6.4%, while the mean contribution under throughfall reduction was 68±1.9%. During the summer, there were no differences among treatments and the overall contribution of R\(_H\) to R\(_S\) was 78±1.6%. Lack of treatment effects on the R\(_H\) partitioning coefficient during the summer may suggest that the proportion of R\(_H\) is robust to nutrient and moisture manipulations in young intensively managed pine plantations, thereby simplifying future NEP modeling scenarios.
3.2 INTRODUCTION

Carbon (C) from the terrestrial biosphere is one of the main reservoirs in the global C cycle (Schimel 1995); however, land use change since the mid 19th century has reduced the soil C pool size (Schlesinger 1977, Canadell et al. 2007, Pan et al. 2011). Soils are the largest terrestrial C pool (Schlesinger 1997) and the source of the second largest flux in the terrestrial C cycle, soil respiration (R_S). Management impacts on the soil C pool that shift R_S toward decreased relative R_{H}, have the potential to impact atmospheric CO_2 through terrestrial C sequestration. Specifically, land use changes towards afforestation, particularly intensively managed forests, have been suggested as systems that can mitigate greenhouse gas emissions by sequestering C in biomass (Watson 2000) and soil organic matter (SOM) (Richter et al. 1999).

There are 304 million ha of forestland in the continental U.S., with 208 million ha of that is capable of producing timber (Smith et al. 2009). In the southeast there are 13 million ha (Fox et al. 2007) of loblolly pine (*Pinus taeda* L.) and shortleaf pine (*Pinus echinata* Mill.) forests, most of which are intensively managed loblolly pine plantations. Intensively managed forests have seen appreciable increases in productivity since widespread establishment of plantations in the 1950’s and 60’s (Fox et al. 2007, Jokela et al. 2010) and are overall C sinks (EPA 2005, Gonzalez-Benecke et al. 2010, Albaugh et al. 2012).

Carbon dynamics are routinely modeled for forests (Kutsch 2009) through the estimation of net primary production (NPP). Although aboveground NPP is generally a good indicator of belowground activity and correlates positively with R_S (Schlesinger 1977, Raich and Schlesinger 1992), an accurate understanding of ecosystem-level C sequestration, or net ecosystem production (NEP), requires the separation of soil heterotrophic, microbial respiration (R_{H}) from autotrophic, root-derived respiration (R_A) as the components of R_S (i.e., NEP = NPP - R_{H}). Quantifying NEP from modeled estimates of NPP through the equation above can be accomplished by severing roots from
their source of photosynthate, driving $R_A$ to zero, where $R_S$ then equals $R_H$ (Vogel and Valentine 2005, Bond-Lamberty et al. 2011). Methods to separate $R_S$ into $R_A$ and $R_H$ have caused disturbance to the soil, roots, and their interactions (Hanson et al. 2000, Kuzyakov 2006) and because of the close relationship with roots symbiotic mycorrhizal fungi are included into $R_A$ (Finlay 2005, Höggberg and Read 2006, Subke et al. 2006) along with microorganisms in the rhizosphere that depend on photosynthate (Wiant 1967).

Thus far the most widely used method to separate the components of soil respiration has been trenching (Kutsch 2009), which can cause great disturbance to the soil (Hanson et al. 2000), as well as take several months (Ewel et al. 1987) to a year (Vogel and Valentine 2005) to establish reliable $R_H$ measurements. While girdling (Höggberg et al. 2001) only takes several days to weeks to attain similar measurements as trenching, replication is difficult due to destruction of the trees. Small root exclusion collars act similar to girdling (Vogel and Valentine 2005) by terminating the flow of recently assimilated photosynthate to roots and thus driving $R_A$ to zero over time as roots deplete their storage of non-structural carbohydrates (Chapin et al. 1990, Höggberg et al. 2001, Sala et al. 2012).

Soil temperature (Lloyd and Taylor 1994) and moisture (Orchard and Cook 1983) are two important abiotic factors that influence the rate of $R_S$. Average temperatures are predicted to rise from 2.5° - 5° C by the 2080’s (Karl et al. 2009) and this will effect soil moisture availability. The greatest temperature increases are predicted to occur in the summer with the number of hot days outpacing the average rise in temperatures while rainfall is predicted to decrease between 10 and 30% (Solomon 2007, Karl et al. 2009). Increasing temperatures with a decrease in precipitation will affect soil moisture content and potentially decrease the C sequestration ability of southern pine forests (Noormets et al. 2010).
Responses of CO₂ efflux is generally closely coupled with temperature (Lloyd and Taylor 1994), however, soil moisture and fertilization can be a sources of variability depending on soil types and nutrient availability, specifically with respect to R₇ (Moyano et al. 2012). This variability can, as a result of soil moisture or fertilization, affect soil C pools (Söderström et al. 1983, Smolander et al. 1994). Over the long-term, a reduction in soil CO₂ efflux might be expected in response to fertilization because of smaller C allocation to belowground biomass (Raich and Schlesinger 1992), associated decreased R₇ ought to lead to overall gains in NEP or the amount of C stored in these forests.

Clarifying responses of R₇ and R₇ to the interaction of fertilization and decreased precipitation is important due to climate predictions for the southeastern United States. Methods to estimate R₇ derived from this study will allow models to predict the C storage ability of southern pine forests in a changing climate. Understanding how climate change will affect C pools in a variety of ecosystems, such as a southern pine forests, will aid in understanding global C dynamics in response to global and land use change (Raich and Schlesinger 1992). The objectives of this current study are to determine the effects of fertilization and throughfall reduction on R₅, and the partitioning of R₅ into R₇ and R₇.

3.3 METHODS

3.3.1 Study site

The study site is one of four large-scale manipulative (i.e., throughfall reduction and fertilization) studies in the Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP; http://pinemap.org/), a large coordinated agricultural project funded by the USDA National Institute of Food and Agriculture that includes 11 land grant universities and 8 cooperatives throughout the southeast focusing on the interactive effects of climate variability and forest management. The four locations were chosen in order to span the full range of climatic conditions where
loblolly pine is intensively managed in the southeastern U.S.

This study was installed in a 9-year-old loblolly pine plantation established on the Spears Mountain (fine, mixed, semiactive, mesic, Typic Hapludult) and Littlejoe (fine, mixed, subactive, mesic, Typic Hapludult) soil series (Web Soil Survey, accessed 04/25/2012) in the Appomattox-Buckingham State Forest located in the Piedmont of Virginia (Lat: 37.443 N, Lon: 78.664 W), at the northern most extent of the range of loblolly pine. The closest primary local climatological data site, Lynchburg, VA, reports that from 2001 to 2011 there was an average of 1055 mm of rainfall per year (278 mm as snowfall), and a mean annual temperature of 13.1° C with an average maximum of 19.6° C typically occurring in June – August and an average minimum of 6.5° C occurring December – February (NOAA, 2013 accessed on 08/06/2013).

3.3.2 Study design

The site is divided into 16 manipulated plots covering a total area of 1.3 ha with an average basal area of 13.2 m² ha⁻¹. Each plot has a buffer strip of 0.01 ha and a measurement area of 0.09 ha. The study design is a randomized complete block design with four replications of a 2 x 2 factorial of throughfall reduction (0 and 30% removed) and fertilization (0 and 100.9 kg N ha⁻¹ + P, K, and micronutrients) randomly assigned to each block. The estimated 30% reduction in throughfall is intercepted using throughfall exclusion structures constructed of dimensional lumber and reinforced plastic troughs approximately 1 m above the soil surface. The troughs span the length of the treatment plot (33m) and are 60 cm in width. The troughs are evenly distributed down alternate tree rows to cover 30% of the 0.10 ha plot. Intercepted throughfall is diverted downhill off the plot, a minimum of 3 m from the measurement plot boundary. Throughfall reduction was initiated during March of 2012. Fertilizer was applied once on March 15, 2012, broadcast with 100.9 kg N ha⁻¹, 12.7 kg P ha⁻¹, 25.4 kg K ha⁻¹, and 10.2 kg ha⁻¹ of “Southeast Mix” from Cameron Chemical Inc. (Portsmouth, VA) containing (5% B, 2% Cu, 6% S, 6% Mn, 5% Zn) with the goal of
eliminating any potential nutrient limitations across the network of sites. Sources for N and P were diammonium phosphate and urea coated with Agrotain Ultra nitrogen stabilizer (Agrotain, Wichita, KS) to reduce volatilization. K was added in the form of potassium chloride.

In order to account for spatial and temporal heterogeneity, three root-severing cores were installed as subsamples within each treatment measurement plot once in March and again in June. During each installation, 48 total cores were installed (3 subsample cores in each of 16 treatments). Cores were constructed of galvanized steel conduit (15.2 cm diameter and 35 cm length). During installation, pine leaf litter was temporarily displaced so as not to drag needles belowground and add additional soil organic matter (SOM) inside the cores. Cores were driven vertically into the soil so that the top of each core was flush with the soil surface. After installation, leaf litter was replaced over the installed core. Subsamples were randomly located in the treatment plots without throughfall reduction. In the throughfall reduction treatments, subsample cores were randomly placed along the inside edge of a throughfall exclusion structure.

R_s was measured immediately before installation of the root-severing core to attain base efflux rate and then again 6 to 7 days following installation (Bond-Lamberty et al. 2011) in order to give the soil time to equilibrate from the disturbance associated with installation. Throughout each installation period CO_2 efflux was measured approximately every 2 weeks between the hours 0900 and 1600 in order to consistently quantify R_s inside and outside of the root-severing cores. Replications were measured in the same order each day so that any daily temporal variation would be accounted for in the analysis. Measurements were taken from within and immediately adjacent to the root-severing core using a LI-COR 6200 infrared gas analyzer (Li-Cor Biosciences, Lincoln NE, USA) and attached Li-Cor 6000-09S chamber with an area of 71.5 cm^2 and volume of 926 cm^3. During measurements, ambient CO_2 concentrations were allowed to equilibrate within the cuvette near the soil surface before being pressed into a vegetation free area of the forest floor without cutting litter to create a seal. Once
CO₂ concentrations in the cuvette began to rise steadily, a measurement was logged for 30 s and \( R_S \) was calculated as \( \mu \text{mol m}^{-2} \text{s}^{-1} \).

Soil CO₂ efflux was measured within and adjacent to the root-severing core for each subsample location and then means for the subsamples were used as a single value for each treatment plot. These means were used to monitor the decline in soil CO₂ efflux within the cores over time until they reached an asymptote where \( R_A \) was assumed to have ceased and the \( R_S \) obtained inside the root-severing cores was used as a proxy for \( R_H \). For each subsample location, the proportion of \( R_S \) attributed to \( R_H \) was determined by dividing the CO₂ flux rate from within the root-severing cores (presumed to approach \( R_H \)) by the flux rate measured immediately adjacent to the core (\( R_S \)). This ratio is thought to be the heterotrophic partitioning coefficient and can be expressed as the percentage that \( R_H \) is contributing to \( R_S \). These partitioning coefficients are based on the stability of the respiration measurements taken within the core at a time roots are no longer contributing to \( R_S \). It has been demonstrated that root carbohydrates accessible for use in metabolic processes are no longer contributing to metabolism approximately 60 days after severing (Heim, chapter 2). Based on this depletion of root carbohydrates as a proxy for cessation of \( R_A \), subsample means across the final three measurement dates for each treatment (approximately 63+ and 65+ days in the spring for spring and summer) were used to calculate respiration estimates for both \( R_S \) and \( R_H \) and ratios of these estimates (\( R_H/R_S \)) were used for a grand partitioning coefficient mean for each treatment combination.

**3.3.3 Soil temperature and moisture**

Spot measurements of soil temperature (12 cm) and volumetric soil moisture (0-12 cm) were made adjacent to the core just after each efflux measurement so as not to disturb the soil prior to or during measurement. Soil temperature was measured using a digital thermometer to the nearest 0.1° C.
Percent volumetric soil moisture was measured using a hydrosense soil-water sensor (Campbell Scientific USA, Logan, UT) as whole percentage number.

### 3.3.4 Root morphology

At the conclusion of soil efflux measurements within the root-severing cores for each season, cores were excavated to collect soil and roots and transported to Virginia Tech where the roots were sorted out of the soil and washed. Roots were kept at (5° C) until they could be measured by WinRHIZO system (Regent Instruments, Québec, Canada) for root length (RL), Root Surface Area (SA), and root volume (V) by diameter classes of very fine (VF; < 1 mm), Fine (F; 1 – 2 mm), and medium (M; 3 – 5 mm). Roots were then dried for >72hrs at 65° C and weighed to the nearest 0.01 g.

### 3.3.5 Data and statistical analysis

A mixed effects ANOVA was used to test for main effects, interactions and block effects using JMP Pro 10 statistical package (JMP®, Version 10.0. SAS Institute Inc., Cary, NC, 1989-2007).

For each season of root collection, subsample means were calculated RL, SA, and V for each diameter class (very fine, fine and medium). Spearman’s nonparametric rank correlation coefficients were calculated for $R_S$, $R_H$, RL, SA and Vol.

### 3.4 RESULTS

#### 3.4.1 Treatment effects on $R_S$, $R_H$ and the partitioning coefficient

For both seasons, and most treatments, an asymptote for the decline in CO₂ efflux in the severing cores was attained approximately 63 days or later after the root-severing core installation (Fig. 4.4). Thus, the final three measurement dates (days 63, 76, and 83 in the spring and 65, 80, and 97 in summer installation) were used to calculate a single mean value for $R_S$, $R_H$ and the partitioning coefficient for both installation periods (Tables 4.2 and 4.3).
During the spring installation, efflux rates measured within root-severing cores decreased, demonstrated by lower means in root-severing core treatment than means measured adjacent to the core (Table 4.2). There were significant differences as a result of throughfall treatments; the mean efflux estimate within the core in reduced throughfall was $2.674 \pm 0.182 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$ while the mean value inside the core for ambient throughfall was $4.592 \pm 0.312 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$. Mean values for the partitioning coefficients of 68\% and 96\% were attained for reduced and ambient throughfall, respectively. The high coefficient for ambient throughfall was largely due to the ambient throughfall x fertilization treatment where even at the end of the study, efflux rates were higher in the severing cores than efflux rates measured adjacent to the core (Fig. 4.5B). During the spring installation there were no significant interaction or main effects on $R_S$ adjacent to the core with a mean of $4.825 \pm 0.22 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$ nor were there differences within the core due to fertilization regime with a mean of $3.633 \pm 0.338 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$ (Table 4.2), resulting in a mean partitioning coefficient of $82 \pm 0.1\%$ for fertilization regime (Table 4.3).

During the summer, respiration inside the cores initially increased to rates greater than those measured adjacent to the core; however, rates in cores quickly fell and remained below respiration rates measured adjacent (Fig. 4.4). $R_S$ and $R_H$ both were significantly affected by throughfall reduction and fertilization (Table 4.2). There were no significant interaction effects during the summer. Mean $R_S$ was $2.215 \pm 0.166 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$ and $1.612 \pm 0.083 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$ for unfertilized and fertilized, respectively and $2.153 \pm 0.176 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$ and $1.674 \pm 0.115 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$ for ambient and reduced throughfall, respectively. There were no effects of fertilization on respiration estimates inside the core and were less than $R_S$ values with a mean of $1.445 \pm 0.0997 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$ while throughfall reduction means were $1.652 \pm 0.135 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$ and $1.237 \pm 0.115 \, \mu\text{mol m}^{-2} \text{sec}^{-1}$ for ambient and reduced treatments, respectively. However, for the partitioning coefficient, there were no significant interaction
or main effects. As a result the overall $R_H$ partitioning coefficient across all treatments was $78\pm1.6\%$ (Table 4.3).

### 3.4.2 Temperature and moisture

There were no significant effects due to treatments on soil temperature (Fig. 4.6A and B). There were no significant interactions between treatments on soil moisture during either installation period. During the spring installation, reduced throughfall significantly decreased soil moisture on all measurement dates except for the first and third measurement dates (Fig 4.6C). Over all dates during the spring installation soil moisture was 28% lower in reduced throughfall treatments. Soil moisture measured inside the root-severing core on the final date was 43% higher than moisture measured adjacent to the core in the ambient throughfall treatment (Table 4.5) and had a mean of $31\pm5.4$ while the mean adjacent to the collar was $16\pm1.3$. During the summer, throughfall reduction significantly decreased soil moisture for all dates (Fig 4.6 D). Over all dates during the summer, soil moisture in the throughfall exclusion treatment was 33% lower than in the ambient throughfall treatments.

### 3.4.3 Root morphology correlation with $R_S$ and $R_H$

Few root parameters were significantly correlated (P<0.10) with $R_S$ or $R_H$ (or by extension, $R_A$). Notably though, most significant root correlations during spring were with $R_H$ and a few with $R_S$. The only significant root correlations during the summer are with $R_H$ (Table 4.6). Significant correlations with $R_H$ that were found during spring were associated with all of the very fine root measurements (root length, surface area and volume) as well as total root length. For $R_S$, correlated root parameters are the length of very fine root and the total root length. Roots collected for the summer measurements were only significantly correlated with two of the measurements for very fine roots, surface area and volume. During spring, root mass
was significantly correlated to both $R_H$ and $R_S$ but no significant correlations were found with root mass during the summer installation.

3.5 DISCUSSION

3.5.1 Treatment effects on partitioning coefficient

This study tested the effects of fertilization and throughfall reduction as well as their interactions on $R_S$, $R_H$ and the heterotrophic partitioning coefficient, that is, the percent contribution of $R_H$ to $R_S$. The partitioning coefficient estimates derived from this study, $68\pm4.7\%$ for spring throughfall reduction, $96\pm7.9\%$ for ambient throughfall treatments and $78\pm1.6\%$ for the site during the summer are higher than what is typically estimated but, with the exception of ambient throughfall during the spring, are still within the range of 50 - 80\% that are commonly reported. Ewel et al. (1987) estimated $R_H$ at 49 and 38\% in a 9 and 29-year-old slash pine stand while Maier and Kress (2000) estimated that roots in an 11-year old loblolly pine stand to a depth of 15cm accounted for 20-50\% of total soil respiration, thus leaving $R_H$ to account for the remaining 50-80\% of $R_S$. More recently Kim et al. (2012) reported that $R_H$ in a red pine forest to a depth of 30cm was 78\% and 62\% of $R_S$ for fertilized and unfertilized plots, respectively.

Throughfall reduction was the only treatment effect for the heterotrophic partitioning coefficient during the spring installation period (Fig. 4.5 Table 4.3). This difference could be a result of the root-severing method rather than the actual effect of the treatment on $R_H$. An obvious deficiency with this method is that transpiration in root-severing cores is stopped, thereby increasing soil moisture inside the cores that Bond-Lamberty et al. (2011) found to explain some of the variability in respiration within root exclusion cores. Soil moisture measurements were not collected within the root-severing collar throughout the course of the installation period; however, our own spring data on the last sampling date demonstrate that there is high variability of soil moisture within the root-severing core (Table 4.5). A plausible explanation for greater efflux estimates within the
core in plots with ambient throughfall treatment is that as a result of frequent spring rainfall elevated soil moisture may have impacted microbial populations within the collar (Moyano et al. 2013) or gas diffusion adjacent to the collar that would decrease CO$_2$ efflux rates (Davidson and Trumbore 1995).

### 3.5.2 Treatment effects on $R_S$ and $R_H$

Respiration rates estimated in this 9-year-old loblolly pine stand over the course of the two installation periods were consistent with rates that had been previously estimated (Table 4.2) (Maier and Kress 2000, Butnor et al. 2003, Gough et al. 2005) with the dominant driver of day-to-day fluctuations resulting from soil temperature (Lloyd and Taylor 1994). Summer efflux estimates were lower than would be normally expected given the soil temperatures and low efflux rates may have been a result of low soil moisture throughout most of the summer installation (Tables 4.2 and 4.4). However, all mean respiration rates within the throughfall reduction treatments were lower than estimates in plots with ambient throughfall. Borken et al. (2006) found that throughfall exclusion over the course of two season reduced soil respiration by 15-30% where $R_H$ served a greater role in this reduction than $R_A$.

We found that the response of throughfall reduction on respiration is apparent almost immediately following treatment installation; however, during the summer installation the fertilization effect began to manifest itself in $R_S$ (Table 4.2). Fertilization treatment effects may become more apparent with time, as trees respond with increased foliage and changes in below ground allocation patterns (Albaugh et al. 1998). Fertilization effects, in general, are highly variable (Maier and Kress 2000, Butnor et al. 2003, Tyree et al. 2006). Examining short-term fertilization effects, researchers have shown that fertilizers can suppress microbial respiration (Thirukkumaran and Parkinson 2000, Gough and Seiler 2004, Tyree et al. 2008) and is reflected in the results of this study (Table 4.2). However, unlike this present study researchers found a much quicker response in the drop in
R\textsubscript{H}. Gough and Seiler (2004) found that 49 days after fertilization, \( R\textsubscript{H} \) (measured in a sample of root free soil) had begun to decline and remained depressed 197 days after fertilization. Tyree et al. (2008) found that \( R\textsubscript{H} \) was immediately suppressed in plots that were fertilized and was significantly lower over the first five months. Other studies (Gallardo and Schlesinger 1994) have shown increases or no effect (Samuelson et al. 2009, Kim et al. 2012) to \( R\textsubscript{H} \) as a result of fertilization. Variations in the overall response or the length of response may be site specific. Although, fertilization had an effect on total soil CO\textsubscript{2} efflux in the summer, it did not affect the heterotrophic partitioning coefficient.

**3.5.3 Root morphology**

Fine root growth is seasonally dynamic, increasing with temperatures during the spring and summer. As a result, fine root parameters taken from within the core during the spring and summer installations are highly positively correlated to \( R\textsubscript{H} \). These correlations are not surprising since fine roots provide C to the soil through exudates and turnover that can provide a readily utilizable substrate for heterotrophic respiration (Wiant 1967).

**3.6 CONCLUSION**

Activity of the microbial pool can be indicative of the gain or loss of belowground C storage. Increasing \( R\textsubscript{A} \) as a result of increased heterotrophic activity would negatively impact belowground C storage, while decreasing \( R\textsubscript{H} \) would indicate a greater belowground C storage. At this early stage of an ongoing study throughfall and fertilization treatments do not appear to have significant effects on the partitioning of \( R\textsubscript{H} \). However the contribution of \( R\textsubscript{H} \) to overall soil respiration is higher than what has been previously reported in other studies using in situ methods to exclude \( R\textsubscript{A} \) resulting in a lower C storage during the course of this study than might be expected. Greater clarity estimating NEP in loblolly pine plantations will be attained by re-deploying these methods in a multi-year study across the range of loblolly pines throughout the southeastern U.S.
4. Figures and Tables

4.1 Laboratory incubation efflux and root non-structural carbohydrates

![Figure 4.1](image)

**Figure 4.1** - Mean non-structural carbohydrates (n=8) and mean respiration RS and RA (n=8) plotted over time for a period of 77 days from roots collected at the Appomattox-Buckingham state forest near Dwillyn, VA. Error bars represent one standard error.
Figure 4.2 - Mean non-structural carbohydrates (n=8) plotted against mean respiration RS and RA (n=8) for a period of 78 days from roots collected at PINEMAP Tier III site in the Appomattox-Buckingham state forest Dwillyn, VA. Error bars represent one standard error.
Figure 4.3 - Relative soil CO2 efflux (inside core/outside core) in an 8-year old loblolly pine stand located on the Virginia Piedmont following installation of 35 cm root-severing cores. Error bars are one standard error.

Table 4.1 - Correlation coefficients for total soil respiration (RS), autotrophic respiration (RA), soluble and insoluble non-structural carbohydrates. P-values are listed in parentheses significance measured at 0.05 level.

<table>
<thead>
<tr>
<th></th>
<th>RS</th>
<th>RA</th>
<th>Soluble NSC</th>
<th>Insoluble NSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>0.9762</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble</td>
<td>0.9524</td>
<td>0.9048</td>
<td>0.6905</td>
<td>0.6905</td>
</tr>
<tr>
<td>Insoluble NSC</td>
<td>0.6905</td>
<td>0.580</td>
<td>0.580</td>
<td></td>
</tr>
<tr>
<td>Total NSC</td>
<td>0.9762</td>
<td>0.9286</td>
<td>0.9762</td>
<td>0.7143</td>
</tr>
</tbody>
</table>

P-values are listed in parentheses. Significance measured at 0.05 level.
4.2 Soil CO$_2$ efflux estimates within and adjacent to root-severing cores and harvested root parameters

**Figure 4.4** - Relative soil CO$_2$ efflux (inside core/outside core) in a 9-year-old loblolly pine stand located on the Virginia Piedmont as influenced by the four treatment combinations. Each graph represents a treatment during an installation period. A) spring – unfertilized x ambient throughfall. B) spring – unfertilized x ambient throughfall, C) spring – 30% throughfall reduction, D) spring – 30% throughfall reduction x fertilization, E) summer – control F) summer – fertilization, G) summer – 30% throughfall reduction, and H) summer – 30% throughfall reduction x fertilization. Data points not connected by line indicate installation date of severing cores. Error bars represent one standard error.
Figure 4.5 – Mean soil CO$_2$ efflux estimated within root-severing core and adjacent to core in a 9-year-old loblolly pine stand located on the Virginia Piedmont as influenced by the four treatment combinations. Each graph represents a treatment during an installation period. A) spring – unfertilized x ambient throughfall. B) spring – fertilization. C) spring – 30% throughfall reduction. D) spring – 30% throughfall reduction x fertilization. E) summer – unfertilized x ambient throughfall. F) summer – fertilization. G) summer – 30% throughfall reduction. H) summer – 30% throughfall reduction x fertilization. Data points not connected by line indicate installation date of severing cores. Error bars represent one standard error.
Figure 4.6 – Spring (A, C) and summer (B, D) soil temperature (°C) and moisture (%) means by date. * indicate significant differences of reduced throughfall treatments, ‡ indicate significant differences in fertilization treatments, treatments with no designated symbols are not significantly different. Error bars indicate one standard error.
Table 4.2 – Respiration means (µmol CO2/m2*sec) taken adjacent and within the severing core for the final three dates measurement dates. Lower case letters indicate differences amongst treatments; uppercase letters indicate significant differences of main effects (P<0.05). Means connected by the same letter are not significantly different.

<table>
<thead>
<tr>
<th></th>
<th>Spring Installation</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RS (adjacent to cores)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unfertilized</td>
<td>Fertilized</td>
<td>Mean</td>
</tr>
<tr>
<td>Ambient Throughfall</td>
<td>5.242±0.499 a</td>
<td>5.064±0.565 ab</td>
<td>5.153±0.369 A</td>
</tr>
<tr>
<td>Reduced Throughfall</td>
<td>4.851±0.347 ab</td>
<td>4.142±0.382 b</td>
<td>4.497± 0.263 A</td>
</tr>
<tr>
<td>Mean</td>
<td>5.047±0.300 A</td>
<td>4.603±0.347 A</td>
<td></td>
</tr>
<tr>
<td>RH (within cores)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient Throughfall</td>
<td>4.223±0.3 48 a</td>
<td>4.957±0.351 a</td>
<td>4.592±0.254 A</td>
</tr>
<tr>
<td>Reduced Throughfall</td>
<td>3.040±0.262 b</td>
<td>2.307±0.189 b</td>
<td>2.674±0.176 B</td>
</tr>
<tr>
<td>Mean</td>
<td>3.633±0.246 A</td>
<td>3.632±0.338 A</td>
<td></td>
</tr>
</tbody>
</table>

|                | Summer Installation |               |               |
|                | RS (adjacent to cores) |               |               |
|                | Unfertilized        | Fertilized    | Mean          |
| Ambient Throughfall | 2.571±0.236 a      | 1.735±0.193 b | 2.153±0.173 A |
| Reduced Throughfall | 1.859±0.226 b   | 1.489±0.151 b | 1.674±0.115 B |
| Mean           | 2.215±0.176 A      | 1.612±0.123 B |               |
| RH (within cores) |                      |               |               |
| Ambient Throughfall | 1.925±0.220 a      | 1.378±0.190 b | 1.652±0.153 A |
| Reduced Throughfall | 1.332±0.183 b   | 1.142±0.146 b | 1.237±0.116 B |
| Mean           | 1.629±0.153 A      | 1.260±0.120 A |               |

Table 4.3 – Means of RH partitioning coefficients (%) for the spring and summer installations for final three measurement dates. Lower case letters indicate differences amongst treatments; uppercase letters indicate significant differences of main effects (P<0.05). Means connected by the same letter are not significantly different.

<table>
<thead>
<tr>
<th></th>
<th>Spring Installation</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfertilized</td>
<td>Fertilized</td>
<td>Mean</td>
</tr>
<tr>
<td>Ambient Throughfall</td>
<td>85±10 a</td>
<td>106±11 a</td>
<td>96±7.9 A</td>
</tr>
<tr>
<td>Reduced Throughfall</td>
<td>66±5.7 a</td>
<td>70±8.2 a</td>
<td>68±4.7 B</td>
</tr>
<tr>
<td>Mean</td>
<td>76±6.4 A</td>
<td>88±9.3 A</td>
<td></td>
</tr>
<tr>
<td>Summer Installation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient Throughfall</td>
<td>76±2.8 a</td>
<td>81±6.2 a</td>
<td>79±3.3 A</td>
</tr>
<tr>
<td>Reduced Throughfall</td>
<td>74±6.3 a</td>
<td>81±9.7 a</td>
<td>78±5.5 A</td>
</tr>
<tr>
<td>Mean</td>
<td>75±3.2 A</td>
<td>81±5.3 A</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.4 – Mean soil moisture (%) for all spring and summer installation measurement dates, differences amongst treatments; uppercase letters indicate significant differences of main effects (P<0.05). Means connected by the same letter are not significantly different.

<table>
<thead>
<tr>
<th></th>
<th>Spring Installation</th>
<th>Summer Installation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfertilized</td>
<td>Fertilized</td>
</tr>
<tr>
<td>Ambient Throughfall</td>
<td>17±0.7 a</td>
<td>16±0.6 a</td>
</tr>
<tr>
<td>Reduced Throughfall</td>
<td>11±0.6 c</td>
<td>13±0.6 b</td>
</tr>
<tr>
<td>Mean</td>
<td>14±0.5 A</td>
<td>15±0.5 A</td>
</tr>
</tbody>
</table>

Table 4.5 – Mean soil moisture (%) for the spring on the final day of installation measured adjacent and inside the collars. Lower case letters indicate differences amongst treatments; uppercase letters indicate significant differences of main effects (P<0.05). Means connected by the same letter are not significantly different.

<table>
<thead>
<tr>
<th></th>
<th>Spring Soil moisture adjacent to core</th>
<th>Soil Moisture within core</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfertilized</td>
<td>Fertilized</td>
</tr>
<tr>
<td>Ambient Throughfall</td>
<td>17±1.8 a</td>
<td>15±1.4 a</td>
</tr>
<tr>
<td>Throughfall Reduction</td>
<td>8.8±1.0 b</td>
<td>11±0.8 b</td>
</tr>
<tr>
<td>Mean</td>
<td>13±2.0 A</td>
<td>13±1.0 A</td>
</tr>
</tbody>
</table>
Table 4.6 - Correlation coefficients data of root morphology for June and September installations for root length (RL), surface area (SA), volume (vol.) for three root classifications very fine (VF), fine (F), and medium (M) tested against means of field measurements total soil respiration (RS) heterotrophic respiration (RH). * Indicate significant correlation (P<0.10).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spring installation</th>
<th>Summer installation</th>
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<tbody>
<tr>
<td></td>
<td>R_H</td>
<td>R_S</td>
</tr>
<tr>
<td>Mass</td>
<td>0.352</td>
<td>0.0141*</td>
</tr>
<tr>
<td>RL - VF</td>
<td>0.288</td>
<td>0.0474*</td>
</tr>
<tr>
<td>RL - F</td>
<td>-0.104</td>
<td>0.482</td>
</tr>
<tr>
<td>RL - M</td>
<td>-0.0160</td>
<td>0.914</td>
</tr>
<tr>
<td>RL - Total</td>
<td>0.281</td>
<td>0.0527*</td>
</tr>
<tr>
<td>SA - VF</td>
<td>0.305</td>
<td>0.0349*</td>
</tr>
<tr>
<td>SA - F</td>
<td>-0.117</td>
<td>0.431</td>
</tr>
<tr>
<td>SA - M</td>
<td>0.100</td>
<td>0.499</td>
</tr>
<tr>
<td>SA - Total</td>
<td>0.137</td>
<td>0.352</td>
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<tr>
<td>Vol.- VF</td>
<td>0.253</td>
<td>0.0826*</td>
</tr>
<tr>
<td>Vol. - F</td>
<td>-0.123</td>
<td>0.405</td>
</tr>
<tr>
<td>Vol. - M</td>
<td>0.112</td>
<td>0.450</td>
</tr>
<tr>
<td>Vol. - Total</td>
<td>0.0977</td>
<td>0.509</td>
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5. Literature Cited


