Soil Respiration and Decomposition Dynamics of Loblolly Pine (*Pinus taeda* L.) Plantations in the Virginia Piedmont

Kristin Mae McElligott

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Forest Resources and Environmental Conservation

John R. Seiler, Co-Chair
Brian D. Strahm, Co-Chair
Thomas R. Fox
Christopher A. Maier
Michael S. Strickland

February 13, 2017
Blacksburg, Virginia

Keywords: *CO_2 efflux, fertilization, microbial activity, carbon sequestration, throughfall reduction*

Soil Respiration and Decomposition Dynamics of Loblolly Pine (*Pinus taeda* L.) Plantations in the Virginia Piedmont

Kristin M. McElligott

Abstract

Forests of the southeastern U.S. play an important role in meeting the increasing demand for forest products, and represent an important carbon (C) sink that can be managed as a potential tool for mitigating atmospheric CO_2 concentrations and global climate change. However, realizing this potential depends on full accounting of the ecosystem carbon (C) budget. The separate evaluation of root-derived, autotrophic (R_A) and microbially-derived heterotrophic (R_H) soil respiration in response to management and climate change is important, as environmental and ecological factors often differentially affect these components, and R_H can be weighed against net primary productivity (NPP) to estimate the C sink or source status of forest ecosystems. The objective of this research was to improve the quantitative and mechanistic understanding of soil respiratory fluxes in managed loblolly pine (*Pinus taeda* L.) plantations of the southeastern U.S. To achieve this overall objective, three studies were implemented to: 1) estimate the proportion and seasonality of R_H:R_S in four stand age classes, and identify relationships between R_H:R_S and stand characteristics 2) evaluate the effects of forest nutrient management and throughfall reduction on factors that influence R_H and decomposition dynamics, including litter quality, microbial biomass, and enzyme activity and 3) evaluate the sensitivity of sources of R_H (mineral soil-derived heterotrophic respiration; R_{HM}, and leaf litter-derived heterotrophic respiration; R_{HL}) to varying soil and litter water content over the course of a dry down event, and assess whether fertilization influences R_H.

Stand age and measurement season each had a significant effect on R_H:R_S ($P < 0.001$), but there were no interactive effects ($P = 0.202$). Mean R_H:R_S during the 12-month study declined with stand age, and were 0.82, 0.73, 0.59, and 0.50 for 3-year-old, 9-year-old, 18- year-old, and 25-year-old stands, respectively. Across all age classes, the winter season had the highest mean R_H:R_S of 0.85 while summer had the lowest of 0.55. Additionally, there were highly significant ($P < 0.001$) and strong ($r > 0.5$) correlations between R_H:R_S and peak LAI, stem volume, and understory biomass. Fertilization improved litter quality by significantly decreasing lignin:N and lignin:P ratios, caused a shift in extracellular enzyme activity from mineral soil N- and P-acquiring enzyme activity to litter C-acquiring enzyme activity, and increased microbial biomass pools. Throughfall reduction decreased litter quality by increasing lignin:N and lignin:P, but also increased C-acquiring enzyme activity. R_{HL} was more sensitive to water content than R_{HM}, and increased linearly with increasing litter water content ($R^2 = 0.89$). The contribution of R_{HL} to R_H was greatest immediately following the wetting event, and decreased rapidly to near-zero between three – 10 days. R_{HM} also had a strong relationship with soil water content ($R^2 = 0.62$), but took between 200 – 233 days to attain near-zero R_{HM} rates. Fertilization had no effect on R_{HM} ($P = 0.657$), but significantly suppressed R_{HL} rates after the wetting event ($P < 0.009$).

This research provides estimates of R_H:R_S in managed loblolly pine systems that can be used to improve regional ecosystem C modeling efforts, and demonstrates the need to consider the impact of stand age and seasonal patterns to identify the point at which plantations switch from functioning as C sources to C sinks. Additionally, it demonstrates that the controls over R_H are dynamic and influenced in the short-term by fertilization and changed precipitation regimes, with the greatest impact on properties affecting litter R_H compared to mineral soil. Future research should work to improve the mechanistic understanding of the seasonal and spatial variability of R_H and related controlling biotic and abiotic parameters to remedy the variability in existing R_S and ecosystem C models. Understanding how management and climate change may impact factors that control R_H will ultimately improve our understanding of what drives changes in forest C fluxes.

Soil Respiration and Decomposition Dynamics of Loblolly Pine (*Pinus taeda* L.) Plantations in the Virginia Piedmont

Kristin M. McElligott

Abstract (public)

Quantification of the heterotrophic component of total soil respiration is important for estimating forest carbon (C) pools and fluxes, and for understanding how silvicultural management and climate change may influence forest C dynamics. The separate evaluation of root-derived, autotrophic (R_A) and microbially-derived heterotrophic (R_H) soil respiration is necessary, as environmental and ecological factors often differentially affect these components, and R_H can be weighed against net primary productivity (NPP) to estimate the C sink or source status of forest ecosystems. This research examined the dynamics of R_H in loblolly pine plantations of the southeastern U.S., and the drivers of R_H and organic matter decomposition in response to forest management (fertilization) and reduced precipitation (throughfall reduction) to improve the quantitative and mechanistic understanding of this important C flux. This work provided estimates of R_H in managed loblolly pine systems that can be used to improve regional ecosystem C modeling efforts, and demonstrates the need to consider the impact of stand age and seasonal patterns to identify the point at which plantations switch from functioning as C sources to C sinks. Additionally, it demonstrates that the controls over R_H, such as substrate quality and microbial community activity and biomass, are dynamic and influenced in the short-term by fertilization and altered moisture availability, with the greatest impact on properties affecting forest floor R_H compared to mineral soil R_H. Future research should work to improve the mechanistic understanding of the seasonal and spatial variability of R_H and related controlling biotic and abiotic parameters to remedy the variability in existing R_S and ecosystem C models. Understanding how management and climate change may impact factors that control R_H will ultimately improve our understanding of what drives changes in forest C fluxes.

Acknowledgements

I am especially grateful to my advisors, Brian Strahm and John Seiler, for their mentorship, guidance, and patience throughout this process. They fostered an atmosphere of support but allowed me to find my way, pursue my personal research interests, and challenged me to be a better scientist, communicator, and educator. Thank you for being role models, both professionally and personally, and for the many professional development opportunities you have made possible throughout this program. I am thankful to my committee members, Mike Strickland, Tom Fox, and Chris Maier, for their support and willingness to help in any capacity. I am grateful to have worked with and learned from scientists with diverse perspectives that provided me with new ways to think about science and complex questions. Thanks to all the colleagues and friends who provided their assistance in the field and laboratory. John Peterson, thanks for your energy and positivity in the field, your willingness to help in any scenario, and for the many insightful discussions we've shared in the field and on the farm. You and Jen made us feel like family. Thanks to Dave Mitchem and Bobbie Niederlehner for the time, energy, and guidance they provided in the lab. A special thank you to Kathie Hollandsworth, Sue Snow, and Mary Williams for helping me navigate through departmental and graduate school procedures. I am extremely grateful to have been a part of the PINEMAP project, whose mission, funding, and collaboration made this research possible. It was truly a transdisciplinary experience. My family's encouragement and understanding has allowed me to not only find my passions, but pursue them. Finally, this work would not have been possible if it were not for the love, support, and extreme amount of patience shown by my husband, Adam Foehringer. He kept me balanced, gave great pep talks when I doubted myself, and was always there to help me adjust my focus when I lost sight of the goal. Lastly, thank you to my son, Max, for every single moment you've shared with us. You give the best indiscriminate, toothless smiles to every stranger you meet, and they smile back. We need more of that.

Table of Contents

Abstract ..... **ii**
Abstract (public) ..... **i**
Acknowledgements ..... **iv**
Table of Contents ..... **v**
List of Tables ..... **viii**
List of Figures ..... **ix**
Chapter 1. Introduction **1**
1.1 Background 1
1.2 Research Overview and Objectives 7
Literature Cited 9
Chapter 2. Partitioning soil respiration across four age classes of loblolly pine (Pinus taeda L.) on the Virginia Piedmont **16**
Abstract 16
2.1 Introduction 18
2.2 Materials and Methods 21
2.2.1 Study Location 21
2.2.2 Experimental Design 22
2.2.3 Study Installation 22
2.2.4 R_S and R_H Measurements 23
2.2.5 Soil temperature and moisture measurements 24
2.2.6 Stand Measurements 24
2.2.7 Soil Sampling 25
2.2.8 Laboratory Analysis 26
2.2.9 Statistical Analysis 27
2.3 Results 27
2.3.1 Site Characteristics 27
2.3.2 Environmental Conditions 28
2.3.4 Relationship among site characteristics and R_H:R_S 30
2.4 Discussion 30
2.4.1 Stand age effects on R_H:R_S 30
2.4.2 Seasonal variation in partitioning R_H:R_S 32
2.4.3 Regional comparison of R_H:R_S estimates 32
2.5 Conclusions 33

Acknowledgements 34
Literature Cited 35
Chapter 3. Fertilization and throughfall reduction alter litter quality and extracellular enzyme activity 46
Abstract 46
3.1 Introduction 48
3.2 Materials and Methods 51
3.2.1 Study Location & Experimental Design 51
3.2.2 Soil and Litter Sampling 53
3.2.3 Soil and Litter Chemical Analysis 54
3.2.4 Microbial Biomass 56
3.2.5 Enzyme Activity 57
3.2.6 Statistical Analysis 58
3.3 Results 58
3.3.1 Treatment effects on soil and litter biochemical properties 58
3.3.2 Patterns of soil enzyme activities 60
3.3.3 Patterns of litter enzyme activities 61
3.3.4 Relationships between biochemical parameters and extracellular enzyme activities 62
3.4 Discussion 63
3.4.1 Litter and mineral soil biochemical properties 63
3.4.2 Enzyme activities 67
3.4.3 Relationships between biochemical parameters and enzyme activity 71
3.5 Conclusions 73
Acknowledgements 73
Literature Cited 75
Chapter 4. The impact of water content on sources of heterotrophic soil respiration 91
Abstract 91
4.1 Introduction 93
4.2 Materials and Methods 96
4.2.1 Site Description 96
4.2.2 Soil and Litter Sampling 97
4.2.3 R_{HM} and R_{HL} laboratory measurements 99
4.2.4 Statistical Analysis 101
4.3 Results 102
4.4 Discussion 103

4.5 Conclusion 108
Acknowledgements 109
Literature Cited 110
Chapter 5. Synthesis 119
Literature Cited 124

List of Tables

Table 2.1. Summary of stand characteristics for four stand age classes of loblolly pine. …40

Table 2.2. Summary of soil characteristics for four stand age classes of loblolly pine at three depth increments. 41

Table 2.3. Pearson's correlation coefficients for relationships between R_H:R_S and stand characteristics by season. 42

Table 3.1. Extracellular enzymes assayed in mineral soil (A horizon) and litter (Oi horizon) in an 11-year-old loblolly pine plantation, their abbreviations, functions, corresponding substrates, and incubation length. 85

Table 3.2. Analysis of variance for mineral soil (A horizon) and litter (Oi) horizon biochemical parameters collected from an 11-year-old loblolly pine plantation. .....……...86

Table 3.3. Mineral soil (A) horizon and litter (Oi) horizon biochemical properties collected from an 11-year-old loblolly pine plantation. Data presented in bold represent significant treatment main effects or significant interactions. Data represent plot means (n=4) and standard errors in parentheses. Lowercase letters denote significant differences ($P < 0.05$). 87

Table 3.4. Analysis of variance for mineral soil (A horizon) and litter (Oi) horizon extracellular enzyme activity collected from an 11-year-old loblolly pine plantation. …… 88

Table 3.5. Potential extracellular enzyme activities in mineral soil (A) horizon and litter (Oi) horizon collected from an 11-year-old loblolly pine plantation. Data presented in bold represent significant treatment main effects or significant interactions. Data represent plot means (n=4) and standard errors in parentheses. Lowercase letters denote significant differences ($P < 0.05$). ……89

Table 3.6. Significance of relationships between mineral soil (A horizon) and litter (Oi horizon) enzyme activities and biochemical parameters collected from an 11-year-old loblolly pine plantation. ……90

List of Figures

Figure 2.1. Mean heterotrophic soil respiration (R_H) proportion to total soil respiration (R_S) by stand age (a) and by season (b), and mean total soil respiration (R_S) by season. R_H:R_S was measured across five seasons and in four age classes of loblolly pine stands (*Pinus taeda* L.) in Virginia. Different letters within each category denote significant differences using Tukey's HSD at $P < 0.05$. Error bars represent ± 1 SE. ……43

Figure 2.2. Mean total soil respiration (RS) by season. RS was measured across five seasons and in four age classes of loblolly pine stands (*Pinus taeda* L.) in Virginia. Different letters within each category denote significant differences using Tukey's HSD at $P < 0.05$. Error bars represent ± 1 SE. 44

Figure 2.3. Mean soil moisture inside (a) and outside (b) root-exclusion cores and soil temperature at 2 cm depth (b), and at 10 cm depth (c) measured across five seasons and in four age classes of loblolly pine stands (*Pinus taeda* L.) in Virginia. Error bars represent ± 1 SE. …45

Figure 4.1. Change (A) mineral soil heterotrophic respiration (R_{HM}) and water content of the root-free mineral soil (B) over the duration of the dry down (days) in unfertilized (closed symbols) and fertilized samples (open symbols). The line represents the mean fit of the exponential decay function. Data represent actual values for each replicated treatment. …….116

Figure 4.2. Change in (A) litter heterotrophic respiration (R_{HL}) and litter water content (B) following the initial wetting event over the duration of the dry down (days) in unfertilized (closed symbols) and fertilized samples (open symbols). The line represents the fit of the exponential decay function. Data represent actual values for each replicated treatment. …….117

Fig 4.3. Relationships between (A) mineral soil heterotrophic respiration (R_{HM}) and soil gravimetric water content (GWC), and (B) leaf litter heterotrophic respiration (R_{HL}) and leaf litter gravimetric water content (GWC) in unfertilized (closed symbols, solid line) and fertilized samples (open symbols, dashed line). ……118

Chapter 1. Introduction

1.1 Background

Global atmospheric concentrations of carbon dioxide (CO_2) have increased markedly worldwide from the use of fossil fuels and land use changes since the late 1700's (Bernstein et al. 2008, WMO 2008, IPCC 2013). Increasing concentrations of CO_2 and other greenhouse gases have been correlated with regional and global increases in temperature and altered precipitation regimes (Pachauri and Reisinger 2007, Bernstein et al. 2008). These changes have increased dialogue among stakeholders and policy makers to develop carbon (C) mitigation strategies to reduce the impacts of climate variability associated with increasing atmospheric CO_2 concentrations (WMO 2008, Stocker et al. 2013), which include managing forested land for ecosystems services such as increased C sequestration (Ryan et al. 2010, Ashton et al. 2012) while maintaining biomass production. Thus, there is an increasing need to define forest C budgets, as the use of forests for climate mitigation depends on accurate ecosystem C accounting.

The C budget of a forest is dependent on the balance between the biogeochemical processes of C acquisition (e.g., photosynthesis, biomass production, belowground allocation), and C release (e.g., respiration of living biomass, microbial decomposition of detritus and soil organic matter (SOM). The total amount of C fixed (the reduction of CO_2 to organic compounds) by plants in the process of photosynthesis is defined as gross primary productivity (GPP) (Lovett et al. 2006). The products of photosynthesis (i.e., photosynthates) are distributed to above-and belowground sinks (e.g., foliage, stems, and roots). The metabolic oxidation of photosynthates oxidizes organic C into CO_2, and is referred to as autotrophic respiration (respiration derived

from plants, roots, and associated rhizosphere organisms for maintenance, growth, or ion uptake; R_A) (Steffen et al. 1998, Lacointe 2000). Net primary production (NPP) is quantified as the difference between GPP and R_A (Lovett et al. 2006). Estimates of productivity, or NPP, are relatively straightforward and a routinely modeled component of forest C inventories (e.g., 3-PG, SECRETS) (Landsberg and Waring 1997, Sampson et al. 2001, Sampson et al. 2008). However, to estimate the overall C balance of a forest (i.e., source or sink status), a measure of net ecosystem productivity (NEP) is necessary. NEP is defined as the net C accumulation by an ecosystem, is regarded as a metric for evaluating forest health, and is used in empirical models to evaluate effects of various climate, management, and disturbance regimes on fundamental ecosystem processes (Randerson et al. 2002). NEP is quantified as the difference between above- and belowground NPP and CO_2 released back to the atmosphere by heterotrophic respiration (R_H) (Randerson et al. 2002, Lovett et al. 2006). R_H generally refers to the CO_2 released by the metabolic activity of bacteria and fungi involved in the decomposition of detritus and soil organic matter (SOM), but also includes CO_2 released from other soil animals such as grazers, carnivores, and detritivores, which constitute a minor component (Kirschbaum 1995). Other pathways of C loss (e.g., erosion, fire, harvest, leaching of dissolved organic C (DOC), methane (CH_4) gains or losses), may also influence estimates of NEP, but are generally excluded from NEP calculations (Randerson et al. 2002).

Total soil respiration (R_S) or soil-surface CO_2 efflux, accounts for roughly two-thirds of the total ecosystem respiration in temperate forests (Davidson et al. 1998, Gaumont-Guay et al. 2008), and is the sum of CO_2 derived from autotrophic and heterotrophic activity in the litter, rhizosphere, and bulk soil. R_S is the second largest terrestrial flux of C in the global C cycle next to photosynthesis (Schlesinger and Andrews 2000, Högberg and Read 2006) producing 68-80 Pg

of CO_2-C annually, of which approximately 90% comes from forests (Schlesinger and Andrews 2000, Luo and Zhou 2006). Thus, even small changes in R_S can influence atmospheric CO_2 concentrations (Tang et al. 2003), and the C sequestration capacity of soils and forest ecosystems (Bowden et al. 1993, Kelting et al. 1998).

Quantitatively separating the components of forest soil respiration and analyzing the effects of important biotic and abiotic factors on each component is a challenging issue and a large impediment in efforts to fully quantify the C budget of a forest (Kuzyakov 2006, Subke et al. 2006). The distinction between R_A and R_H components of R_S is essential for interpreting and modeling C budgets (Ryan and Law 2005). While estimates of R_S can be used to calculate the amount of CO_2-C released annually from a system, and has been used for the development of respiration models and regional forest C budgets (Gough et al. 2005, Fenn et al. 2010, Templeton et al. 2015), it alone cannot be used to evaluate the fate of C within soil and the forest ecosystem, or be used to determine whether a forest is a source or sink of CO_2. Specifically, the separation of, and accurate estimate of R_H from R_S is needed, as R_H is weighed against all C assimilation (NPP) to estimate the C accumulation status of forested ecosystems (NEP). Additionally, the separate evaluation of these components in response to management and climate change is important, as environmental and ecological factors often differentially affect photosynthesis, R_H and R_A (Ryan and Law 2005, Vargas et al. 2010). The controls for NPP, such as light, temperature, moisture, and nutrient supply are well understood (Kloeppel et al. 2007). The same is not true for belowground C components, as the controls over C allocation and loss are highly varied in importance among respiratory components (R_H vs R_A) and poorly understood (Litton et al. 2007, Reichstein and Beer 2008). Many biotic and abiotic factors (e.g., microbial community activity and composition, substrate type and supply, stand dynamics, temperature, moisture, soil

pH, soil texture and mineralogy) control soil respiration, and interactively affect components and contributions of R_A and R_H to R_S at differing temporal (annual, seasonal, and diurnal) and spatial scales (Melillo et al. 2002, Heinemeyer et al. 2007, Fenn et al. 2010).

Soil C losses from R_H represent the largest source of uncertainty and variability in estimating total ecosystem respiration, and exerts the strongest control over annual NEP in forests (Melton et al. 2014, Rowland et al. 2014). This uncertainty is in no small part to methodological challenges and inconsistencies in partitioning R_H from R_S (Hanson et al. 2000, Baggs 2006), but is also a result of the dynamic seasonality of this flux, the greater sensitivities to soil water availability and substrate supply, and the controlling factors of soil microbial properties [e.g., microbial biomass, community composition, and enzyme kinetics](Wang et al. 2003, Lawrence et al. 2009, Davidson et al. 2012). Averaged on a global scale, R_H accounts for 54% of R_S in forests, however this contribution ranges widely from being a minor to a principal component (10-90%) of R_S depending on forest type, region, and partitioning methods (Hanson et al 2000). Accurate estimates of R_H are also important for quantifying changes in soil C stocks resulting from the difference between litter production and inputs and R_H (Hanson et al. 2000). Therefore, separating components of R_S contributes significantly to our understanding the C sequestration capacity of forests.

R_S models are generally developed using empirical data from weekly to seasonal soil surface spot measurements using chamber-based methods (Vogel et al. 2008, Kutsch et al. 2009). These models also rely upon field evaluation of controlling environmental and ecological variables on the spatial and temporal variation of R_S. The partitioning of R_S in these studies can improve our understanding of the different environmental controls that drive the two components (Bond-Lamberty et al. 2004), and allow the estimation of NEP when combined with NPP

estimates. The most common methods of partitioning R_S in the field include root-exclusion techniques (e.g. trenching, girdling, cores) that result in various levels of assumptions, limitations, and site disturbances (Kutsch et al. 2009). These methods, along with other isotopic, statistical, and laboratory techniques, have been reviewed extensively by Hanson et al. (2000), Kuzyakov (2006), and Subke et al. (2006). An alternative field-method to estimate NEP is achieved using eddy covariance technologies (Norman et al. 1997, Baldocchi 2003), that provide continuous estimates of CO_2 fluxes across the forest canopy-atmosphere interface (see Baldocchi 2003).

In the U.S., forested land represents approximately 90% of the net C sink and offsets approximately 12% of the annual U.S. greenhouse gas emissions (Murray et al. 2005). Southeastern U.S. forests currently have among the highest potential for C storage as they represent 30% of the forested land in the U.S (Han et al. 2007) and nearly 60% of the regional land area (Chen et al. 2006). Additionally, they are aggrading biomass with age and intensive management practices (Dale et al. 2001, Jokela et al. 2010), and have high rates of NPP (Wear and Greis 2002, Mitchell et al. 2014, Rocca et al. 2014). Southeastern forests reportedly store 12 Pg of C, which amounts to approximately 36% of the sequestered forest C in the contiguous U.S. (Turner et al. 1995), and have been sequestering C at an average rate of 3.5 Mg C ha^{-1} yr^{-1} from 1992 to the near-present (Binford et al. 2006, Zhao et al. 2013). Furthermore, these forests play an important role in meeting the increasing demand for forest products. Southeastern forests are comprised of more than 25 million hectares of pine plantations (Wear and Greis 2013) from which the U.S. harvests approximately 60% of total domestic wood products (Prestemon and Abt 2002, Wear and Greis 2002), accounting for 15.8% of the global industrial wood supply (Wear and Greis 2013). Loblolly pine (*Pinus taeda* L.) is the dominant plantation pine species in the

southeastern U.S. and represents approximately one-half of the standing pine volume in the region (Wear and Greis 2012). Its productivity has tripled over the last 50 years with enhanced seedling genetics and improved resource management and nutrient availability (Albaugh et al. 2004, Fox et al. 2007).

The predicted changes in climate for the Southeast regions could enhance forest growth and increase productivity, yet the forest C sink is expected to decline as forested land is lost to land use change and other disturbances (USDA 2012). In the southeastern U.S., average annual temperatures have increased 1-2°C over the last 30 years (Karl 2009) and this warming trend is projected to continue with predicted increases of 2.5–3.5°C by 2060 (Kunkel et al. 2013). Regional precipitation predictions are more uncertain, as an increase in annual precipitation of up to 6 % is expected (Mearns et al. 2009, Ingram et al. 2013, Wear and Greis 2013), along with exacerbated wet and dry periods, increases in the frequency of summer droughts, and larger rainfall events between drought periods (Groisman et al. 2004, Angert et al. 2005, Laseter et al. 2012). Rising atmospheric CO_2 concentrations (IPCC 2013), warmer temperatures, and increased precipitation for much of the South would likely increase tree growth and enhance C sequestration in standing biomass and in forest products; however, these gains may be offset by accelerated decomposition and thus C loss from soil (Vose et al. 2012) and declines in forested land resulting from urbanization, land use change, and extreme disturbances. As a result of urbanization alone, the Southeast is predicted to lose between 7 and 13 % (11 - 23 million acres) of forested land by the year 2060 (Wear and Greis 2013). Of the five southeastern forest subregions (Coastal Plain, Piedmont, Appalachian-Cumberland, Mid-South, and the Mississippi Alluvial Valley), the Piedmont region is predicted to lose between 19 and 24 % of its forest area, the greatest proportion of regional forest loss. Shifts in land-use, climate patterns, and associated

changes in temperature and soil water availability may have important impacts on forest productivity and therefore C sequestration in the southeastern U.S. (Noormets et al. 2010, Wear and Greis 2012).

1.2 Research Overview and Objectives

This research is part of the National Institute of Food and Agriculture (NIFA) funded, Pine Integrated Network: Education, Mitigation and Adaptation Project (PINEMAP) through an Agriculture and Food Research Initiative (AFRI) grant. The mission of PINEMAP was and to enable southern pine landowners to manage forests to increase C sequestration and mitigate atmospheric CO_2; increase efficiency of nitrogen (N) and other fertilizer inputs; and adapt forest management approaches to increase forest resilience and sustainability under variable climates. To achieve this goal, PINEMAP developed six primary aims: (1) ecophysiology and silviculture, (2) modeling, (3) genetics, (4) economics and management policy, (5) education, and (6) extension. The specific research presented here is a component of the ecophysiology and silviculture aim, which has established a three-tiered monitoring network based on existing cooperative research trials, with the goal of developing standardized research methods to quantify C, water, and nutrient storage and flux baselines in response to climate and management. The three-tiered monitoring network consists of Tier I "legacy", Tier II "active", and Tier III "Fertilization and throughfall exclusion" sites (see www.pinemap.org). Four experimental Tier III sites were established at the edges of the loblolly pine distribution that span the full range in precipitation, soil, and potential productivity gradients. In these studies, mineral nutrients and water were manipulated through fertilization and diversion throughfall. This approach enabled PINEMAP scientists to quantify the response of biogeochemical processes

controlling loblolly pine productivity and consequently C sequestration capacity associated with the interactive effects of climate variability and fertilization.

The overall objective of this dissertation research was to improve the quantitative and mechanistic understanding of soil respiratory fluxes in managed loblolly pine plantations of the southeastern U.S. These forests are tasked to serve not only as the principal producers of national forest products, but as long-term C sinks to meet burgeoning political, economic, and biological demands. Improving our mechanistic understanding of the relationships among abiotic and biotic controls of soil C loss in these systems, and the interactive effects of management and environmental change on these processes, will allow us to more accurately quantify and predict changes in ecosystem C pools and fluxes. Of particular interest was improving our annual estimates of R_H, and our understanding of the controls over R_H. R_H is a major flux of CO_2 from managed southeastern pine plantations, and is an important parameter in ecosystem C accounting. R_H demonstrates the difference between NPP and NEP, and represents a large source of uncertainty in NEP estimates. Specifically, the objectives were to: **(1)** Partition microbially-derived heterotrophic respiration (R_H) from total soil respiration (R_S), and evaluate the influence of stand age and seasonality on the proportion of R_H to R_S (R_H:R_S) in loblolly pine plantations **(2)** Evaluate the effects of forest nutrient management and throughfall reduction on factors that influence R_H, decomposition dynamics, and thus C storage; including litter quality, microbial biomass, and extracellular enzyme activity **(3)** Evaluate the sensitivity of sources of R_H (mineral soil-derived heterotrophic respiration; $R_{HM,}$ and leaf litter-derived heterotrophic respiration; R_{HL}) of a loblolly pine plantation to varying soil and litter water, with and without fertilization, over the course of a dry down event.

Literature Cited

Albaugh, T. J., H. L. Allen, P. M. Dougherty, and K. H. Johnsen. 2004. Long term growth responses of loblolly pine to optimal nutrient and water resource availability. Forest Ecology and Management 192:3-19.

Angert, A., S. Biraud, C. Bonfils, C. Henning, W. Buermann, J. Pinzon, C. Tucker, and I. Fung. 2005. Drier summers cancel out the CO2 uptake enhancement induced by warmer springs. Proceedings of the National Academy of Sciences of the United States of America 102:10823-10827.

Ashton, M. S., M. L. Tyrrell, D. Spalding, and B. Gentry. 2012. Managing forest carbon in a changing climate. Springer Science & Business Media.

Baggs, E. M. 2006. Partitioning the components of soil respiration: a research challenge. Plant and Soil 284:1-5.

Baldocchi, D. D. 2003. Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present and future. Global Change Biology 9:479-492.

Bernstein, L., P. Bosch, O. Canziani, Z. Chen, R. Christ, and K. Riahi. 2008. IPCC, 2007: climate change 2007: synthesis report. IPCC.

Binford, M., H. Gholz, G. Starr, and T. Martin. 2006. Regional carbon dynamics in the southeastern US coastal plain: Balancing land cover type, timber harvesting, fire, and environmental variation. Journal of Geophysical Research: Atmospheres 111.

Bond-Lamberty, B., C. Wang, and S. T. Gower. 2004. A global relationship between the heterotrophic and autotrophic components of soil respiration? Global Change Biology 10:1756-1766.

Bowden, R. D., K. J. Nadelhoffer, R. D. Boone, J. M. Melillo, and J. B. Garrison. 1993. Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperate mixed hardwood forest. Canadian Journal of Forest Research 23:1402-1407.

Chen, H., H. Tian, M. Liu, and J. Melillo. 2006. Effect of land-cover change on terrestrial carbon dynamics in the southern United States. Journal of environmental quality 35:1533.

Dale, V. H., L. A. Joyce, S. McNulty, R. P. Neilson, M. P. Ayres, M. D. Flannigan, P. J. Hanson, L. C. Irland, A. E. Lugo, and C. J. Peterson. 2001. Climate change and forest disturbances: climate change can affect forests by altering the frequency, intensity, duration, and timing of fire, drought, introduced species, insect and pathogen outbreaks, hurricanes, windstorms, ice storms, or landslides. BioScience 51:723-734.

Davidson, E., E. Belk, and R. D. Boone. 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Global Change Biology 4:217-227.

Davidson, E. A., S. Samanta, S. S. Caramori, and K. Savage. 2012. The Dual Arrhenius and Michaelis–Menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. Global Change Biology 18:371-384.

Fenn, K. M., Y. Malhi, and M. D. Morecroft. 2010. Soil CO 2 efflux in a temperate deciduous forest: environmental drivers and component contributions. Soil Biology and Biochemistry 42:1685-1693.

Fox, T. R., H. L. Allen, T. J. Albaugh, R. Rubilar, and C. A. Carlson. 2007. Tree nutrition and forest fertilization of pine plantations in the southern United States. Southern Journal of Applied Forestry 31:5-11.

Gaumont-Guay, D., T. Black, A. Barr, R. Jassal, and Z. Nesic. 2008. Biophysical controls on rhizospheric and heterotrophic components of soil respiration in a boreal black spruce stand. Tree Physiol.

Gough, C. M., J. R. Seiler, P. E. Wiseman, and C. A. Maier. 2005. Soil CO2 efflux in loblolly pine (Pinus taeda L.) plantations on the Virginia Piedmont and South Carolina Coastal Plain over a rotation-length chronosequence. Biogeochemistry 73:127-147.

Groisman, P. Y., R. W. Knight, T. R. Karl, D. R. Easterling, B. Sun, and J. H. Lawrimore. 2004. Contemporary changes of the hydrological cycle over the contiguous United States: Trends derived from in situ observations. Journal of hydrometeorology 5:64-85.

Han, F. X., M. J. Plodinec, Y. Su, D. L. Monts, and Z. Li. 2007. Terrestrial carbon pools in southeast and south-central United States. Climatic Change 84:191-202.

Hanson, P. J., N. T. Edwards, C. T. Garten, and J. A. Andrews. 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochemistry 48:115-146.

Heinemeyer, A., I. P. Hartley, S. P. Evans, J. A. Carriera de la Fuente, and P. Ineson. 2007. Forest soil CO2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. Global Change Biology 13:1786-1797.

Högberg, P., and D. J. Read. 2006. Towards a more plant physiological perspective on soil ecology. Trends in Ecology & Evolution 21:548-554.

Ingram, K. T., K. Dow, L. Carter, and J. Anderson. 2013. Climate of the Southeast United States: variability, change, impacts, and vulnerability. Springer.

IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, TF, D Qin, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley pm (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

Jokela, E. J., T. A. Martin, and J. G. Vogel. 2010. Twenty-five years of intensive forest management with southern pines: Important lessons learned. Journal of Forestry 108:338-347.

Karl, T. R. 2009. Global climate change impacts in the United States. Cambridge University Press.

Kelting, D. L., J. A. Burger, and G. S. Edwards. 1998. Estimating root respiration, microbial respiration in the rhizosphere, and root-free soil respiration in forest soils. Soil Biology and Biochemistry 30:961-968.

Kirschbaum, M. U. 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biology and Biochemistry 27.

Kloeppel, B. D., M. E. Harmon, and T. J. Fahey. 2007. Estimating aboveground net primary productivity in forest-dominated ecosystems. Principles and standards for measuring primary production. Edited by TJ Fahey and AK Knapp. Oxford University Press, New York:63-81.

Kunkel, K. E., L. E. Stevens, S. E. Stevens, L. Sun, E. Janssen, D. Wuebbles, S. Hilberg, M. Timlin, L. Stoecker, and N. Westcott. 2013. Regional climate trends and scenarios for the US national climate assessment. Part 3:142-143.

Kutsch, W. L., M. Bahn, and A. Heinemeyer. 2009. Soil carbon dynamics: an integrated methodology. Cambridge University Press.

Kuzyakov, Y. 2006. Sources of CO 2 efflux from soil and review of partitioning methods. Soil Biology and Biochemistry 38:425-448.

Lacointe, A. 2000. Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. Annals of Forest Science 57:521-533.

Landsberg, J., and R. Waring. 1997. A generalised model of forest productivity using simplified concepts of radiation-use efficiency, carbon balance and partitioning. Forest Ecology and Management 95:209-228.

Laseter, S. H., C. R. Ford, J. M. Vose, and L. W. Swift. 2012. Long-term temperature and precipitation trends at the Coweeta Hydrologic Laboratory, Otto, North Carolina, USA. Hydrology research 43:890-901.

Lawrence, C. R., J. C. Neff, and J. P. Schimel. 2009. Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. Soil Biology and Biochemistry 41:1923-1934.

Litton, C. M., J. W. Raich, and M. G. Ryan. 2007. Carbon allocation in forest ecosystems. Global Change Biology 13:2089-2109.

Lovett, G. M., J. J. Cole, and M. L. Pace. 2006. Is Net Ecosystem Production Equal to Ecosystem Carbon Accumulation? Ecosystems 9:152-155.

Luo, Y., and X. Zhou. 2006. Chapter 4 - Processes of CO2transport from soil to the atmosphere. Pages 61-76 *in* Y. Luo and X. Zhou, editors. Soil Respiration and the Environment. Academic Press, Burlington.

Mearns, L. O., W. Gutowski, R. Jones, R. Leung, S. McGinnis, A. Nunes, and Y. Qian. 2009. A regional climate change assessment program for North America. Eos 90:311.

Melillo, J., P. Steudler, J. Aber, K. Newkirk, H. Lux, F. Bowles, C. Catricala, A. Magill, T. Ahrens, and S. Morrisseau. 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science 298:2173-2176.

Melton, J., R. Shrestha, and V. Arora. 2014. The influence of soils on heterotrophic respiration exerts a strong control on net ecosystem productivity in seasonally dry Amazonian forests. Biogeosciences Discussions 11:12487-12529.

Mitchell, R. J., Y. Liu, J. J. O'Brien, K. J. Elliott, G. Starr, C. F. Miniat, and J. K. Hiers. 2014. Future climate and fire interactions in the southeastern region of the United States. Forest Ecology and Management 327:316-326.

Murray, B., B. Sohngen, A. Sommer, B. Depro, K. Jones, B. McCarl, D. Gillig, B. DeAngelo, and K. Andrasko. 2005. Greenhouse gas mitigation potential in US forestry and agriculture. Environmental Protection Agency. EPA.

Noormets, A., M. J. Gavazzi, S. G. McNulty, J. C. Domec, G. Sun, J. S. King, and J. Chen. 2010. Response of carbon fluxes to drought in a coastal plain loblolly pine forest. Global Change Biology 16:272-287.

Norman, J., C. Kucharik, S. Gower, D. Baldocchi, P. Crill, M. Rayment, K. Savage, and R. Striegl. 1997. A comparison of six methods for measuring soil surface carbon dioxide effluxes. J. Geophys. Res. I 2:28,771-728,777.

Pachauri, R. K., and A. Reisinger. 2007. Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change. IPCC, Geneva, Switzerland 104.

Prestemon, J. P., and R. C. Abt. 2002. Timber Products Supply and Demand. In: Wear, D.N.; Greis, J.G., The Southern Forest Resource Assessment. General Technical Report GTR-SRS-53. . U.S. Department of Agriculture Forest Service, Southern Research Station, Asheville, North Carolina.

Randerson, J., F. Chapin III, J. Harden, J. Neff, and M. Harmon. 2002. Net ecosystem production: a comprehensive measure of net carbon accumulation by ecosystems. Ecological Applications 12:937-947.

Reichstein, M., and C. Beer. 2008. Soil respiration across scales: the importance of a model–data integration framework for data interpretation. Journal of Plant Nutrition and Soil Science 171:344-354.

Rocca, M. E., C. F. Miniat, and R. J. Mitchell. 2014. Editorial. Introduction to the regional assessments: Climate change, wildfire, and forest ecosystem services in the USA.

Rowland, L., T. C. Hill, C. Stahl, L. Siebicke, B. Burban, J. Zaragoza-Castells, S. Ponton, D. Bonal, P. Meir, and M. Williams. 2014. Evidence for strong seasonality in the carbon storage and carbon use efficiency of an Amazonian forest. Global Change Biology 20:979-991.

Ryan, M. G., M. E. Harmon, R. A. Birdsey, C. P. Giardina, L. S. Heath, R. A. Houghton, R. B. Jackson, D. C. McKinley, J. F. Morrison, and B. C. Murray. 2010. A synthesis of the science on forests and carbon for US forests.

Ryan, M. G., and B. E. Law. 2005. Interpreting, measuring, and modeling soil respiration. Biogeochemistry 73:3-27.

Sampson, D. A., I. A. Janssens, and R. Ceulemans. 2001. Simulated soil CO $ _2 $ efflux and net ecosystem exchange in a 70-year-old Belgian Scots pine stand using the process model SECRETS. Annals of Forest Science 58:31-46.

Sampson, D. A., R. H. Wynne, and J. R. Seiler. 2008. Edaphic and climatic effects on forest stand development, net primary production, and net ecosystem productivity simulated for Coastal Plain loblolly pine in Virginia. Journal of Geophysical Research: Biogeosciences 113.

Schlesinger, W. H., and J. A. Andrews. 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48:7-20.

Steffen, W., I. Noble, J. Canadell, M. Apps, E. Schulze, P. Jarvis, D. Baldocchi, P. Ciais, W. Cramer, and J. Ehleringer. 1998. The terrestrial carbon cycle: implications for the Kyoto Protocol. Science 280:1393-1394.

Stocker, T., D. Qin, G. Plattner, M. Tignor, S. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. Midgley. 2013. IPCC, 2013: summary for policymakers in climate change 2013:

the physical science basis, contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, New York, USA.

Subke, J.A., I. Inglima, and M. Francesca Cotrufo. 2006. Trends and methodological impacts in soil CO2 efflux partitioning: A metaanalytical review. Global Change Biology 12:921-943.

Tang, J., D. D. Baldocchi, Y. Qi, and L. Xu. 2003. Assessing soil CO 2 efflux using continuous measurements of CO 2 profiles in soils with small solid-state sensors. Agricultural and Forest Meteorology 118:207-220.

Templeton, B. S., J. R. Seiler, J. A. Peterson, and M. C. Tyree. 2015. Environmental and stand management influences on soil CO 2 efflux across the range of loblolly pine. Forest Ecology and Management 355:15-23.

Turner, D. P., G. J. Koerper, M. E. Harmon, and J. J. Lee. 1995. A carbon budget for forests of the conterminous United States. Ecological Applications 5:421-436.

Vargas, R., M. S. Carbone, M. Reichstein, and D. D. Baldocchi. 2010. Frontiers and challenges in soil respiration research: from measurements to model-data integration. Biogeochemistry 102:1-13.

Vogel, J. G., B. P. Bond-Lamberty, E. A. Schuur, S. T. Gower, M. C. Mack, K. E. O'Connell, D. W. Valentine, and R. W. Ruess. 2008. Carbon allocation in boreal black spruce forests across regions varying in soil temperature and precipitation. Global Change Biology 14:1503-1516.

Vose, J. M., D. L. Peterson, and T. Patel-Weynand. 2012. Effects of climatic variability and change on forest ecosystems: a comprehensive science synthesis for the US.

Wang, W., R. Dalal, P. Moody, and C. Smith. 2003. Relationships of soil respiration to microbial biomass, substrate availability and clay content. Soil Biology and Biochemistry 35:273-284.

Wear, D. N., and J. G. Greis. 2002. Southern forest resource assessment: technical report. Journal of Forestry 100(7): 6-14.

Wear, D. N., and J. G. Greis. 2012. The southern forest futures project: summary report. General Technical Report SRS-168. USDA Forest Service Southern Research Station, Asheville, NC. 103 p.

Wear, D. N., and J. G. Greis. 2013. The Southern Forest Futures Project: technical report. Gen. Tech. Rep. SRS-GTR-178. Asheville, NC: USDA-Forest Service, Southern Research Station. 542 p. 178: 1-542

WMO. 2008. Greenhouse Gas Bulletin–The State of Greenhouse Gases in the Atmosphere Using Global Observations through 2008. World Meteorological Organization Geneva,, Switzerland.

Zhao, S., S. Liu, T. Sohl, C. Young, and J. Werner. 2013. Land use and carbon dynamics in the southeastern United States from 1992 to 2050. Environmental Research Letters 8:044022.

Chapter 2. Partitioning soil respiration across four age classes of loblolly pine (Pinus taeda L.) on the Virginia Piedmont

Published in Forest Ecology and Management

McElligott, K. M., J. R. Seiler, and B. D. Strahm. 2016. Partitioning soil respiration across four age classes of loblolly pine (Pinus taeda L.) on the Virginia Piedmont. Forest Ecology and Management 378:173-180.

Abstract

Quantification of the heterotrophic component of total soil respiration is important for estimating forest carbon pools and fluxes, and for understanding carbon dynamics associated with stand development and silvicultural management. We measured the proportion of heterotrophic respiration (R_H) to total soil respiration (R_S) in extensively managed loblolly pine (*Pinus taeda* L.) stands of four age classes in the Piedmont physiographic province of Virginia. Our objectives were to evaluate the influence of stand age and seasonality on the proportion of R_H to R_S (R_H:R_S). R_H was partitioned using root exclusion cores, and both R_S and R_H were measured 90 days following installation of cores for five seasons. Repeated measures analysis revealed that stand age and measurement season each had a significant effect on R_H:R_S ($P < 0.001$), but that there were no interactive effects ($P = 0.202$). Mean R_H:R_S during the 12-month study declined with stand age, and were 0.82, 0.73, 0.59, and 0.50 for 3-year-old, 9-year-old, 18-year-old, and 25-year-old stands, respectively. Across all age classes, the winter season had the highest mean R_H:R_S of 0.85 while summer had the lowest of 0.55. This study provides estimates of R_H:R_S in managed loblolly pine systems, and demonstrates the need to consider the impact of stand age and seasonal patterns when estimating net annual carbon (C) budgets of forest ecosystems and to identify the point at which plantations switch from functioning as C sources to C sinks.

Keywords: Carbon fluxes; Heterotrophic respiration; Loblolly pine plantations; Soil respiration

2.1 Introduction

Southeastern planted pine forests play an important role in meeting the increasing demand for forest products (Prestemon and Abt 2002) and in future carbon (C) sequestration strategies (Johnsen et al. 2001, Wear and Greis 2002, Han et al. 2007). Understanding how management, climate, and disturbances influence productivity and C sequestration in forests requires that mechanistic controls of both above-and below-ground terrestrial C pools and fluxes are well-understood, and current C stocks can be accurately quantified (Gorte 2009).

The net C balance of a forest ecosystem is dependent upon C uptake and storage being greater than C lost from plant and soil respiration (ecosystem respiration), and other sources [e.g., erosion, fire, harvest, leaching of dissolved organic C (DOC), methane (CH_4) emissions] (Lovett et al. 2006, Chapin et al. 2011). Forests influence the transfer of C between the atmosphere and soil as trees fix large amounts of CO_2 from the atmosphere through photosynthesis that is then allocated as organic C both aboveground and belowground. Large amounts of CO_2 are returned to the atmosphere through respiration by vegetation and heterotrophic soil organisms. A suite of existing models are commonly used to estimate C fixation and respiration of the trees themselves (e.g., 3-PG, Landsberg and Waring 1997; SECRETS, Sampson et al. 2001, 2008), or to assess the effects of soil C and nutrient cycling on long-term ecosystem C storage (e.g., CENTURY, Parton et al. 1988). However, to analyze and model C accumulated by the ecosystem, including the C stored in the soil, the separation of total soil respiration (R_S) into components of heterotrophic, microbial respiration (R_H) and autotrophic, root respiration (R_A) is necessary (Hanson et al. 2000, Subke et al. 2006)

A measure of net ecosystem production (NEP) represents the potential for organic C to be stored within or lost from an ecosystem, and helps predict how C budgets of forest ecosystems

will respond to changes in climate, management, or disturbance regimes. Estimates of NEP can be derived by subtracting R_H from estimates of net primary production (NPP) (Arneth et al. 2010), and used to identify whether a forest functions as a net sink or source of CO_2 (Randerson et al. 2002). Considerable effort has been devoted to quantifying fluxes of CO_2 from southeastern pine forests of various management intensities and stand ages in an attempt to understand the impacts of forest management and stand dynamics on belowground C dynamics, (Gough and Seiler 2004, Wiseman and Seiler 2004, Gough et al. 2005); however, quantifying contributions from R_H and co-located R_S to estimate NEP is challenging and has resulted in large variation in estimates (Singh and Gupta 1977, Hanson et al. 2000, Kuzyakov 2006).

Despite recent efforts in partitioning R_S, there still exists great uncertainty and variability among estimates within forest ecosystems (Hanson et al. 2000, Högberg et al. 2001, Lee et al. 2002, Rey et al. 2002, Lavigne et al. 2003). Previous attempts to partition R_S suggest the component values in forests range widely, for example, from 5 to 100% for R_H:R_S, depending on experimental methods, forest type and age, season, and time step of the analysis [e.g., seasonal, annual, or stand rotation length] (Hanson et al. 2000), while the majority of studies show R_A and R_H are roughly evenly partitioned between 50 and 60% (Bond-Lamberty et al. 2004b). However, in southeastern forests, recent attempts to partition R_S in loblolly pine (*Pinus taeda* L.) and longleaf pine (*Pinus palustris* Mill.) stands suggest R_S is dominated by R_H, with annual R_H:R_S estimates from 79 to 96% (Heim et al. 2015, ArchMiller and Samuelson 2016). Additionally, these studies were conducted in either mid- or late-rotation, intensively managed plantations with considerable competition control. Stand age, land use history, site characteristics, management intensity, and seasonality will likely influence R_H:R_S, and variation in R_H:R_S with stand age and by season has not been reported. It is uncertain if existing annual estimates are applicable to

other stands under various management regimes and with differing site characteristics. Uncertainty in the applicability and range of existing estimates that can be used to parametrize ecosystem C models have important and large impacts on the estimates of C storage in managed forest ecosystems. A higher proportion of R_H would result in lower estimates of NEP, whereas a lower proportion of R_H would result in higher estimates of NEP and ecosystem C storage.

Various methods have been attempted to partition R_S and all have a range of indirect and direct effects of disturbance to the soil (Kuzyakov 2006, Subke et al. 2006). Root exclusion techniques that prevent roots from receiving new photosynthate, such as trenching or girdling, are the most widely used method in forest ecosystems for partitioning R_S (Epron 2009). Recently, installation of root exclusion cores that function similarly to trenching, as they isolate tree roots from the flow of new photosynthate but minimize disturbance, cost, and time comparatively, have been utilized in partitioning studies to eliminate R_A and allow for direct measurements of R_H (Vogel and Valentine 2005, Strahm et al. 2014, Heim et al. 2015, ArchMiller and Samuelson 2016). Core installation severs existing roots, prevents ingrowth of new roots, and over time, non-structural carbohydrate stores in the severed roots are depleted thereby exhausting R_A inside the core (Heim et al. 2015). Comparing respiration measurements inside the core (R_H) and outside the core (R_S) allow for the necessary partitioning of R_S that can be used to more accurately estimate NEP of forest ecosystems.

The objective of this study was to estimate the proportion and seasonality of R_H:R_S in four age classes (2-3, 7-9, 16-18, and 23-25 year-old stands) of managed loblolly pine (*Pinus taeda* L.) plantations for one year using root severing cores. We hypothesized that the relative value and proportion of R_H will decrease with increasing stand age and will vary by season. We expected higher proportions of R_H:R_S in younger stands to reflect 1) higher rates of

decomposition of residues from the previous harvest and associated disturbances, and 2) lower root biomass and productivity of young trees. These estimates, in concert with existing estimates of R_H:R_S in mid-rotation, intensively managed loblolly pine plantations across the Southeast (Strahm et al. 2014) will ultimately be incorporated into current ecosystem C models to evaluate the effects of management intensity and stand age on NEP in southern pine ecosystems.

2.2 Materials and Methods

2.2.1 Study Location

This study was conducted on the Appomattox-Buckingham State Forest located in the Piedmont physiographic province of VA (37°26´19´´N, 78°39´52´´W). The mean elevation of the study area is 185 m, with minimum and maximum elevations of 133 m and 225 m, respectively. Local topography can be described as gentle rolling slopes and flat terrain. Vegetation is composed of various coniferous (*Pinus taeda*, *Pinus virginiana*, *Pinus echinata*, and *Pinus strobus*), deciduous (*Quercus alba*, *Quercus coccinea*, and *Liriodendron tulipifera*), and mixed forest stands. Land use-history consists of intensive agricultural use until the 1930's, followed by the establishment of mixed-hardwood forests and pine plantations (VDOF 2010). Average annual precipitation for this region is 109 cm. The average growing season temperature (April through September) is 22.9 °C and the average winter temperature (December to February) is 3.8 °C (NCDC.NOAA.gov accessed November 27, 2015).

The managed loblolly pine stands chosen for this investigation had similar land-use history and had undergone similar management practices since establishment. Stands were even-aged and ranged from 2 to 25 years of age at the beginning of the study. Site preparation prior to planting involved roller drum chopping followed by broadcast burning. All stands were

established by hand planting containerized loblolly pine seedlings on 2 m × 3 m spacing and received herbicide treatment for control of hardwood and herbaceous competition shortly after establishment. No stands under investigation were previously fertilized, but those stands in the 16-18 and 23-25 year-old age class were previously commercially thinned. Competing understory vegetation is predominant in all age classes. Common understory species include *Andropogon virginicus*, *Quercus alba*, *Acer rubrum*, *Pinus virginiana*, *Nyssa sylvatica*, *Sassafras albidum*, and *Quercus coccinea*. Soils are moderately well-drained to well drained and deep, formed in residuum from metamorphic rocks (sericite schist, graphitic schist, and/or phyllite). Specific soil series and taxonomic classifications for all sites were mapped as Spears Mountain series: fine, mixed, semiactive, mesic Typic Hapludults, or Tatum series: fine, mixed, semiactive, thermic Typic Hapludults (Soil Survey Staff, accessed Nov. 27, 2015).

2.2.2 Experimental Design

To assess the effects of forest age and season on R_H:R_S, stands representing a range of extensively managed loblolly pine ages and stand characteristics were selected (Table 2.1). Four age classes were chosen for this study and included 2- to 3-, 7- to 9-, 16- to 18-, and 23- to 25-year-old stands. Classes were based on the age of stands during the 2014 growing season. The study was designed as a randomized complete block, where each of the four stand age classes was replicated three times, for a total of 12 stands dispersed across four geographical blocks.

2.2.3 Study Installation

In March 2014, permanent fixed area measurement plots (radius=12.62 m; area=500 m^2) representative of the forest stand were installed in each replicate stand. To account for within-stand variability, three measurement subsamples were randomly installed within each measurement plot. To partition R_S, root exclusion cores constructed from steel conduit pipe (11.43 cm diameter x 35 cm length) were installed to sever roots in each subsample location within each treatment plot. Root biomass below 35 cm is assumed to be minimal at these sites and R_S is assumed negligible below depths of 30 cm (Warembourg and Paul 1973). Cores were driven vertically into the soil until the top was flush with the mineral soil surface. Prior to core installation, the O horizon (forest floor) was temporarily removed from the installation location to avoid driving organic matter into the soil profile or inside the core, and carefully replaced after installation. To measure seasonal variation of efflux components, root exclusion cores were installed 90-days prior to R_S and R_H measurements during five separate periods (March, June, September, and November of 2014, and March of 2015). R_H measurements were made 90 days post installation, when carbohydrate supply to roots is depleted, and R_A efflux inside the pipe is assumed to be zero (Heim et al. 2015), thereby providing an independent measure of R_H.

2.2.4 R_S and R_H Measurements

R_S and R_H measurements occurred once, seasonally from June 2014 through July 2015. Measurements were taken between 9:00 and 16:00, moving systematically from experimental blocks 1-3, using a LiCor 6200 (LiCor Inc., Lincoln, Nebraska) closed dynamic system with a LiCor 6250 infrared gas analyzer (IRGA) and attached chamber (6400-09) with an area of 71.5 cm^2 and volume of 926 cm^3. Measurements were logged for 30s following CO_2 equilibration within the chamber. Three subsample measurements were taken in each plot for R_S and R_H, for a

total of six measurements per treatment plot. A vegetation-free location was chosen adjacent to the root exclusion core for measurements of R_S. The LiCor chamber was placed directly over the soil surface for R_S measurements, and over the root exclusion core for R_H measurements. A single plot mean for each efflux component was calculated from the three subsample measurements. The relative contribution of R_H to R_S, or partitioning coefficient, was determined by dividing the R_H rate by R_S to represent the percentage contribution to R_S.

2.2.5 Soil temperature and moisture measurements

Individual spot measurements of soil temperature (2 and 10 cm) and soil volumetric water content (VWC) (0-12 cm) were made inside and adjacent to the root-exclusion core at each subsample location following the set of R_S and R_H measurements. Soil temperature was measured using a digital thermometer. Percent volumetric soil moisture was measured using a Hydrosense soil-water sensor (Campbell Scientific USA, Logan, UT). Mean air temperature and relative humidity (RH) were measured directly above the CO_2 efflux measurement locations using an Amprobe THWD-5 RH and temperature meter (Danaher Corporation USA, Everett, WA).

2.2.6 Stand Measurements

Standard site characteristics were measured for each measurement plot. Measurements included stem density, tree height, and diameter at breast height (DBH at 1.3 m) for all stems >1 cm DBH, tree species, understory vegetation species and biomass, and peak leaf area index (LAI). Leaf area index data were estimated for each measurement plot using the LiCor LAI-

2200 Plant Canopy Analyzer positioned 1.0 m above the ground during late summer (September 2014). Stem biomass estimates were determined using the National Biomass Estimator (NBE) model (Jenkins et al. 2003) for Pinus species (equation 1) and mixed hardwood species (equation 2), where aboveground stem biomass (B_D) is oven dry weight (kg) and DBH is in centimeters:

$$B_D = e^{-2.5356+2.4349(\ln(DBH))} \quad (1)$$

$$B_D = e^{-2.4800+2.4835(\ln(DBH))} \quad (2)$$

The NBE is a conservative and well-documented national model and is generally considered the standard biomass equation used nationwide by researchers and agencies to estimate tree- and stand-level forest biomass, including the official greenhouse gas inventories for the United States (EPA 2008).

Woody and herbaceous understory vegetation was sampled at three subsample locations in each treatment plot using a 0.25 m^2 quadrat and composited. Samples were oven dried at 65 °C until a constant mass was achieved, and dry biomass weights for each treatment plot was scaled to biomass in kg ha^{-1}.

2.2.7 Soil Sampling

Mineral soil samples were collected within each treatment plot in October, 2014. Soils were sampled from depths of 0-10 cm, 10-20 cm, and 20-30 cm from three subsample locations in each treatment plot and composited by depth and plot. Soil samples were stored on ice and transported back to the laboratory within 24 h of collection. Composited soil samples were

mixed and all visible roots, litter, and identifiable organic matter were removed. Bulk density was estimated using a 5-cm-diameter slide hammer with core sampler. Sequential soil cores (0-10 cm, 10-20 cm, and 20-30 cm depths) were collected from three points in each treatment plot. The O horizon (forest floor) was sampled at the same three points within each of the 12 treatment plots using a $0.1m^2$ quadrat. The collected O horizon samples were placed in paper bags, returned to the laboratory, and oven-dried at 65 °C to a constant mass.

2.2.8 Laboratory Analysis

Bulk density soil samples were first oven-dried at 105 °C for 24 h, then passed through a 2-mm sieve to remove coarse fragments and woody debris. Total mass of soil and coarse fragments (>2 mm) were recorded and divided by the sampled volume to calculate bulk density. Following determination of bulk density, organic matter content (% OM) was determined by loss on ignition (LOI), where approximately 10 g of oven-dry, sieved soil was placed in a muffle furnace and 380 °C for 24 hours, then reweighed. The difference in weight before and after ignition represents the amount of the OM present in the sample (Ben-Dor and Banin 1989).

The composited soil subsamples were air-dried for 24 h and passed through a 2-mm sieve. A 1g air-dry sample was added to a graphite crucible and analyzed for total C and N on a CNS Elemental analyzer (LECO Corporation, St. Joseph, MI). Results are reported on an oven-dry (105 ºC) basis. C content (t ha^{-1}) was then determined by C concentration (%) and soil bulk density (g cm^{-3}) for each depth (cm) (Lal et al. 2001). An aliquot of each composited subsample from each depth was sent to the Virginia Tech Soil Testing Laboratory for analysis of pH, cation exchange capacity, soluble salts, and extractable nutrients using the Mehlich-1 (0.05N HCl +

0.025N H_2SO_4) extraction procedure (Maguire and Heckendorn 2014). Soil pH and nutrient concentrations at installation are summarized in Table 2.2.

2.2.9 Statistical Analysis

All statistical analyses were performed using JMP® 11 software system (SAS Institute, Cary, NC, USA). This study was analyzed as a randomized complete block design (n=3). One treatment plot per stand represents the experimental unit, and subsamples were averaged as the estimate for each experimental unit. All variables were transformed as appropriate to meet assumptions of normality. To assess the effect of stand age on CO_2 efflux components, a repeated measures analysis of variance was performed and blocked for random effects, using restricted maximum likelihood (REML) estimations. Treatment differences were determined from the 95% confidence intervals about the least square (LS) means (α=0.05), and treatment means were tested at a significance level of $\alpha = 0.05$ using Tukey-Kramer Honestly Significant Difference (HSD). Analyses were conducted using plot level means for all measurements and for data separated by season. Pearson correlation analysis was performed to analyze the relationships between site characteristics and CO_2 efflux components.

2.3 Results

2.3.1 Site Characteristics

Many stand characteristics were significantly different ($P < 0.05$) across stand ages (Table 2.1), while few soil characteristics differed significantly by stand age (Table 2.2). Peak

LAI, stem density, basal area, stem volume, stem biomass and forest floor biomass differed among stand age classes, while understory biomass did not significantly differ among age classes due to the high variability among plots within stand age classes (Table 2.1).

Soil characteristics that differed among age classes were limited to soil pH, C:N, and organic matter percent (Table 2.2). Soil pH in the 0-10 cm depths was highest in the three-year age class, and significantly lower in the 25 year-age class. Soil pH in 10-20 cm depths was highest in the nine-year age classes and significantly lower in the 25 age class. Organic matter percent significantly differed among age classes in the 20-30 cm depth only, and was highest in the 18-year age class and lowest in the three-year age class.

2.3.2 Environmental Conditions

During the duration of the experiment (June 2014 - July 2015) measurements of mean soil temperature near measurement cores ranged from 3.04 to 28°C at 2 cm depth and 3.3 to 26°C at 10 cm depth (Figure 3). Soil moisture inside the root exclusion core ranged from 6.8 to 20.1 percent VWC and from 2.8 to 19.9 percent VWC adjacent to measurement cores (Figure 3). Mean air temperature near measurement cores ranged from 3.6 to 38.1°C, and relative humidity ranged from 18.3 to 83.7 percent. Mean soil moisture, soil temperature, air temperature, and relative humidity did not covary in the analysis with R_H:R_S ($P > 0.10$ for all variables). Soil moisture measurements taken inside and outside the root exclusion pipes were not significantly different ($P = 0.125$).

2.3.3 R_H:R_S

A repeated measures ANOVA analysis revealed a significant stand age effect on R_H:R_S ($P < 0.0001$), but no stand age effect on R_S ($P = 0.678$). The analysis also revealed a significant effect of season on R_H:R_S ($P < 0.001$) and R_S ($P < 0.001$). The interaction between stand age and season was not significant ($P = 0.202$).

Our results demonstrate a stepwise decrease in R_H:R_S with increasing stand age. Mean R_H:R_S by age class ranged from a high of 0.82 ± 0.06 for the three-year-old age class to a low of 0.50 ± 0.03 in the 25-year-old age class (Figure 1). The R_H proportion was 0.73 ± 0.05 in the nine-year-old age class and 0.59 ± 0.05 in the 18-year-old age class. Age class three significantly differed from age class 18 and 25 ($P < 0.05$), but did not differ from age class nine (Figure 1a). The mean R_H proportion of all measured stand ages and seasons was 0.67.

R_H:R_S ranged from a high of 0.85 ± 0.06 in the winter season, to a low of 0.56 ± 0.04 and 0.55 ± 0.05 in the summer 2014 and 2015 seasons, respectively. The R_H proportions for fall 2014 was 0.59 ± 0.06 and 0.75 ± 0.04 for spring 2015. Winter R_H proportions were significantly different from fall and summer measurements ($P < 0.05$), but not from spring measurements (Figure 1b). It is important to note that while the winter and spring seasons exhibited the highest proportion of R_H to R_S, they also had the lowest rate of R_S and relative value of R_H with rates of 0.05 µmol m^{-2} s^{-1}, and 0.03 µmol m^{-2} s^{-1}, respectively (Figure 2). Summer (averaged between the two summer sample years) and Fall seasons had the highest total soil CO_2 efflux rates of 7.00 µmol m^{-2} s^{-1} and 7.44 µmol m^{-2} s^{-1}, respectively, despite having the lowest R_H:R_S proportion of 0.55 for the averaged summer seasons and 0.59 for the fall season (Figure 1b).

2.3.4 Relationship among site characteristics and R_H:R_S

We analyzed relationships between R_H:R_S and site characteristics using Pearson's correlation. Many site characteristics were significantly correlated with the proportion of R_H to R_S in all seasons (Table 2.3). There were highly significant ($P < 0.001$) and strong ($r > 0.5$) correlations between R_H:R_S and peak LAI, stem volume, and understory biomass in spring, summer, and winter seasons, stem biomass in summer and winter seasons, basal area in summer seasons, and in forest floor biomass in the winter season. All of these correlations were negative except for understory biomass. Other correlations were significant at higher alpha levels as indicated in Table 2.3.

2.4 Discussion

2.4.1 Stand age effects on R_H:R_S

Our results indicate that R_H:R_S change with loblolly pine stand age, which could influence the attainment of a positive, annual net carbon (C) balance (NEP). R_H:R_S decreased with increasing stand age, which supports our hypothesis. In proportion to R_S, R_H decreased from 73 to 82% in young, open-canopy stands to 50 to 59% in older, closed-canopy stands. This pattern is consistent with previous studies assessing the change in R_A and R_H with stand development of various forest types, that report decreases in R_H from 70 to 95% in young, regenerating stands, to 20 to 40% in mature forests (Bond-Lamberty et al. 2004a, Epron et al. 2006, Noormets et al. 2012).

The pattern of decreasing R_H:R_S with increasing stand age is attributed to major structural changes resulting from stand development that alter the balance between C fixation and respiration. Young loblolly pine stands are typically C sources due to high soil CO_2 efflux rates relative to gross CO_2 canopy assimilation rates (Maier et al. 2004, Gough et al. 2005, Sampson et al. 2006) and higher R_H:R_S proportions associated with decomposition of harvest residues, inputs of dead organic matter into the soil, and subsequent changes in the soil microclimate and substrate availability following harvests (Noormets et al. 2012). Higher R_H in younger stands has also been attributed to higher substrate availability (Parmelee et al 1989 and Bhupinderal-Singh et al 2003). Mature loblolly pine stands are generally C sinks (Hamilton et al. 2002, Lai et al. 2002, Schafer et al. 2003) that decline in strength with decreases in stand LAI (Albaugh et al. 2004) and NPP (Sampson et al 2006, 2008) as stands mature. Additionally, the relative contribution of R_A has been shown to increase with stand age reflecting an increase in NPP and root biomass (Ewel et al. 1987), resulting in a decrease in the proportion of R_H to R_S with increasing stand age. The strong, negative correlation between R_H:R_S and peak LAI, stem volume, and stem biomass stand variables reported in Table 2.3, support the notion that as aboveground, and presumably belowground biomass increases with stand age, the proportion of R_A increases thereby resulting in a decrease in R_H:R_S with stand development. Conversely, understory biomass was strongly, negatively correlated with R_H:R_S. This may be a result of competition between understory and overstory vegetation, as understory vegetation and stem biomass are moderately, negatively correlated (r = -0.34, P = 0.033). Therefore, as understory biomass increases, the proportion of R_H:R_A may increase as a result of reduced overstory above- and belowground biomass. Alternatively, greater understory biomass may increase fine root

biomass, turnover, and inputs, thereby increasing substrate availability and stimulating microbial activity with consequent increases in R_H.

2.4.2 Seasonal variation in partitioning R_H:R_S

We observed significant seasonality of the proportion of R_H to R_S over the duration of this study. The lowest proportion of R_H:R_S was in the summer seasons, when the contribution of R_A to R_S is often higher (Bond-Lamberty et al. 2004a, Wieser and Bahn 2004). This is assumed to reflect a higher temperature sensitivity for R_A than R_H (Boone et al. 1998, Bond-Lamberty et al. 2004), or higher root biomass in the growing season (Bahn et al. 2006, Saiz et al. 2006). Our highest observed proportion of R_H to R_S were in winter and spring months. This could reflect the lower temperature sensitivity of R_H (Bond-Lamberty et al. 2004), or reflect increased substrate availability for soil microbial communities (Davidson and Janssens 2006), or the seasonal decline in R_A (Wieser and Bahn 2004).

2.4.3 Regional comparison of R_H:R_S estimates

The reported R_H:R_S in this study are lower than the reported proportions for other forest ecosystems in the southeast that used comparable root exclusion core methods (Strahm et al. 2014, Heim et al. 2015, Archmiller and Samuelson 2016). Additionally, this study evaluated R_H across various stand ages, while previous regional studies evaluated R_H to R_S in one age class. We report a range of 0.83 to 0.50 of decreasing R_H:R_S with loblolly pine stand age, with a mean R_H proportion of all measured stand ages and seasons of 0.67. This is comparable to the mean ratio of R_H to R_S of 0.60 ± 0.12 across temperature coniferous forests (Subke et al. 2006).

Archmiller and Samuelson (2016) evaluated the proportion of R_H to R_S in 26-year-old longleaf pine (*Pinus palustris* Mill.) stands in western Georgia and reported a range of 0.66 to 0.82 dependent upon whether R_H was corrected for root decay inside root exclusion cores. Heim et al. (2015) reported a R_H:R_S proportion of 0.79 ± .005 in in an eight-year-old loblolly pine plantation in VA. Similarly, Strahm et al (2014) reported a Southeast region-wide estimate for mid rotation (nine-10-year old) loblolly pine stands of 0.840 ± 0.026. In this study, our mid-rotation loblolly pine stands (age nine) had a mean R_H to R_S proportion of 0.73 ± 0.03.

The higher proportion of R_H observed in similarly aged loblolly pine stands is hypothesized to be a result of management intensity and competition control. The stands investigated for the Southeast region-wide R_H:R_S estimate received intensive competition control one year prior to study installation and the start of respiration measurements. Both mechanical clearing and broadcast herbicide treatments were applied to control hardwood, volunteer pine, and herbaceous competition. Subsequent, annual chemical control applications were applied as needed to keep the understory clear of competing vegetation. Conversely, the stands in this study received no competition control following the initial mechanical clearing and broadcast herbicide treatment at stand initiation, resulting in prevalent woody and herbaceous competing vegetation in all age classes. Implementation of competition control after stand establishment reduces competing vegetation volume and understory biomass, likely resulting in lower relative R_A contributions to R_S in intensively managed systems, compared to extensively managed systems without competition control.

2.5 Conclusions

The proportion of R_H to R_S in loblolly pine varied from approximately 0.83 to 0.55, dependent upon the stand age and season. The results of this study, when combined with other

region-wide estimates of the contribution of R_H to R_S in managed loblolly pine stands, demonstrate the need to account for site- and stand-specific characteristics, such as stand age, season, and management intensity, when modeling R_H in ecosystem C models. The range in available R_H:R_S estimates suggest C sequestration capacities of these systems are highly varied and dependent upon site-specific conditions. Existing efforts to model ecosystem C or NEP assume that soil respiration is evenly partitioned between R_H and R_A, while regional estimates of loblolly pine suggest R_H comprises a greater proportion of R_S in most age-classes. Over- or under-estimating R_H:R_S in ecosystem C models has important implications for estimates of C sequestration, and for identifying when forests switch from functioning as a C source to a C sink. Larger estimates of R_H suggest more fixed C is released back to the atmosphere than stored in the system. Incorporation of these results into existing models will enable more accurate estimates of C sequestration potential of planted pine in the southeastern US.

Acknowledgements

We would like to thank John Peterson, Ilia Donner, and Brian Parr for providing field and laboratory assistance, and Michael Womack and Thomas Zaebst of the Virginia Department of Forestry for logistical assistance with this study. Additionally, we thank the thoughtful reviews from anonymous reviewers on the final draft of this manuscript. This research was supported in part by the Virginia Agricultural Experiment Station and the McIntire-Stennis Program of the National Institute of Food and Agriculture (NIFA), U.S. Department of Agriculture (USDA), and by the Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP), a Coordinated Agricultural Project funded by the USDA NIFA (Award No. 2011-68002-30185).

Literature Cited

Albaugh, T. J., H. L. Allen, P. M. Dougherty, and K. H. Johnsen. 2004. Long term growth responses of loblolly pine to optimal nutrient and water resource availability. Forest Ecology and Management 192:3-19.

ArchMiller, A., and L. Samuelson. 2016. Partitioning Longleaf Pine Soil Respiration into Its Heterotrophic and Autotrophic Components through Root Exclusion. Forests 7:39.

Arneth, A., S. P. Harrison, S. Zaehle, K. Tsigaridis, S. Menon, P. J. Bartlein, J. Feichter, A. Korhola, M. Kulmala, D. O'Donnell, G. Schurgers, S. Sorvari, and T. Vesala. 2010. Terrestrial biogeochemical feedbacks in the climate system. Nature Geosci 3:525-532.

Bahn, M., M. Knapp, Z. Garajova, N. Pfahringer, and A. Cernusca. 2006. Root respiration in temperate mountain grasslands differing in land use. Global Change Biology 12:995-1006.

Bhupinderpal-Singh, A. N., M. O. Löfvenius, M. N. Högberg, P. E. Mellander, and P. Högberg. 2003. Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest, extending observations beyond the first year. Plant, Cell and Environment 26: 1287–1296.

Bond-Lamberty, B., C. Wang, and S. T. Gower. 2004a. Contribution of root respiration to soil surface CO2 flux in a boreal black spruce chronosequence. Tree Physiology 24:1387-1395.

Bond-Lamberty, B., C. Wang, and S. T. Gower. 2004. A global relationship between the heterotrophic and autotrophic components of soil respiration? Global Change Biology 10:1756-1766.

Boone, R. D., K. J. Nadelhoffer, J. D. Canary, and J. P. Kaye. 1998. Roots exert a strong influence on the temperature sensitivityof soil respiration. Nature 396:570-572.

Collins, B. 2005. Thresholds of Disturbance: Land Management Effects on Vegetation and Nitrogen Dynamics. Technical Report. Oak Ridge National Laboratory: Oak Ridge, TN, USA. pp. 1–63.

Chapin III, F. S., P. A. Matson, and P. Vitousek. 2011. Principles of terrestrial ecosystem ecology. Springer Science & Business Media.

Davidson, E. A., and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165-173.

Epron, D. 2009. Separating autotrophic and heterotrophic components of soil respiration: lessons learned from trenching and related root exclusion experiments. Soil carbon dynamics—an integrated methodology. Cambridge University Press, Cambridge:157-168.

Epron, D., Y. Nouvellon, P. Deleporte, S. Ifo, G. Kazotti, A. Thongo M'Bou, W. Mouvondy, L. S. Andre, O. Roupsard, and C. Jourdan. 2006. Soil carbon balance in a clonal Eucalyptus plantation in Congo: effects of logging on carbon inputs and soil CO2 efflux. Global Change Biology 12:1021-1031.

Ewel, K. C., W. P. Cropper. Jr, and H. L. Gholz. 1987. Soil CO2 evolution in Florida slash pine plantations. II. Importance of root respiration. Canadian Journal of Forest Research 17:330-333.

Gorte, R. W. 2009. Carbon sequestration in forests. CRS Report for Congress. Wasington, DC: Congressional Research Service.

Gough, C. M., and J. R. Seiler. 2004. The influence of environmental, soil carbon, root, and stand characteristics on soil CO2 efflux in loblolly pine (Pinus taeda L.) plantations located on the South Carolina Coastal Plain. Forest Ecology and Management 191:353-363.

Gough, C. M., J. R. Seiler, P. E. Wiseman, and C. A. Maier. 2005. Soil CO2 efflux in loblolly pine (Pinus taeda L.) plantations on the Virginia Piedmont and South Carolina Coastal Plain over a rotation-length chronosequence. Biogeochemistry 73:127-147.

Hamilton, J. G., R. B. Thomas, and E. H. Delucia. 2001. Direct and indirect effects of elevated CO_2 on leaf respiration in a forest ecosystem, Plant Cell Environ 24:975 – 982.

Han, F. X., M. J. Plodinec, Y. Su, D. L. Monts, and Z. Li. 2007. Terrestrial carbon pools in southeast and south-central United States. Climatic Change 84:191-202.

Hanson, P., N. Edwards, C. Garten, and J. Andrews. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48:115-146.

Heim, B. C., J. R. Seiler, and B. D. Strahm. 2015. Root Nonstructural Carbohydrates and Their Relationship with Autotrophic Respiration of Loblolly Pine (Pinus taedaL.). Communications in Soil Science and Plant Analysis 46:888-896.

Heim, Brett Christopher. Partitioning soil respiration in response to drought and fertilization in loblolly pine: laboratory and field approaches.Thesis. Virginia Tech, 2014. Web. 3 Feb. 2016.

Högberg, P., A. Nordgren, N. Buchmann, A. F. Taylor, A. Ekblad, M. N. Högberg, G. Nyberg, M. Ottosson-Löfvenius, and D. J. Read. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789-792.

Jenkins, J.C., Chojnacky, D.C., Heath, L.S., Birdsey, R.A. 2003. National-scale biomass estimators for United States tree species. Forest Science 49: 12-35.

Johnsen, K. H., D. Wear, R. Oren, R. O. Teskey, F. Sanchez, R. Will, J. Butnor, D. Markewitz, D. Richter, and T. Rials. 2001. Meeting global policy commitments: carbon sequestration and southern pine forests. Journal of Forestry 99:14-21.

Kuzyakov, Y. 2006. Sources of CO2 efflux from soil and review of partitioning methods. Soil Biology and Biochemistry 38:425-448.

Lai, C.T., G. Katul, J. Butnor, D. Ellsworth, and R. Oren. 2002. Modelling night-time ecosystem respiration by a constrained source optimization method. Global Change Biology 8:124 – 141.

Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A., eds. 2001.Assessment methods for soil carbon.Boca Raton, FL: Lewis Publishers.Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. 1999. Standard methods for long-term ecological research. Oxford, U.K: Oxford University Press.

Landsberg, J., and R. Waring. 1997. A generalised model of forest productivity using simplified concepts of radiation-use efficiency, carbon balance and partitioning. Forest ecology and management 95:209-228.

Lavigne, M. B., R. Boutin, R. J. Foster, G. Goodine, P. Y. Bernier, and G. Robitaille. 2003. Soil respiration responses to temperature are controlled more by roots than by decomposition in balsam fir ecosystems. Canadian Journal of Forest Research 33:1744-1753.

Lee, M. s., K. Nakane, T. Nakatsubo, W. h. Mo, and H. Koizumi. 2002. Effects of rainfall events on soil CO2 flux in a cool temperate deciduous broad-leaved forest. Ecological Research 17:401-409.

Lovett, G. M., J. J. Cole, and M. L. Pace. 2006. Is Net Ecosystem Production Equal to Ecosystem Carbon Accumulation? Ecosystems 9:152-155.

Maier, C. A., T. J. Albaugh, H. Lee Allen, and P. M. Dougherty. 2004. Respiratory carbon use and carbon storage in mid-rotation loblolly pine (Pinus taeda L.) plantations: the effect of site resources on the stand carbon balance. Global Change Biology 10:1335-1350.

Maguire, R.; Heckendorn, S. Explanation of Soil Tests; Virginia Cooperative Extension Publication 452–701. Available online: http://pubs.ext.vt.edu/452/452–701/452–701.html (accessed on 13 November 2015).

Noormets, A., S. G. McNulty, J.-C. Domec, M. Gavazzi, G. Sun, and J. S. King. 2012. The role of harvest residue in rotation cycle carbon balance in loblolly pine plantations. Respiration partitioning approach. Global Change Biology 18:3186-3201.

Parmelee, R. W., M. H. Beare, and J. M. Blair. 1989. Decomposition and nitrogen dynamics of surface weed residues in no-tillage agroecosystems under drought conditions: influence of resource quality on the decomposer community. Soil Biol. Biochem. 21:97-103.

Parton, W. J., J. W. Stewart, and C. V. Cole. 1988. Dynamics of C, N, P and S in grassland soils: a model. Biogeochemistry 5:109-131.

Prestemon, J. P., and R. C. Abt. 2002. Timber Products Supply and Demand. In: Wear, D.N.; Greis, J.G., The Southern Forest Resource Assessment. General Technical Report GTR-SRS-53. U.S. Department of Agriculture Forest Service, Southern Research Station, Asheville, North Carolina. pp. 299-325.

Randerson, J., F. Chapin III, J. Harden, J. Neff, and M. Harmon. 2002. Net ecosystem production: a comprehensive measure of net carbon accumulation by ecosystems. Ecological applications 12:937-947.

Rey, A., E. Pegoraro, V. Tedeschi, I. De Parri, P. G. Jarvis, and R. Valentini. 2002. Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Global Change Biology 8:851-866.

Saiz, G., K. Byrne, K. Butterbach-Bahl, et al. 2006. Stand-age related effects on soil respiration in a first rotation Sitka spruce chronosequence in central Ireland. Global Change Biology, 12:1007-20.

Sampson, D., R. Waring, C. Maier, C. Gough, M. J. Ducey, and K. Johnsen. 2006. Fertilization effects on forest carbon storage and exchange, and net primary production: A new hybrid process model for stand management. Forest Ecology and Management 221:91-109.

Sampson, D. A., I. A. Janssens, and R. Ceulemans. 2001. Simulated soil CO_2 efflux and net ecosystem exchange in a 70-year-old Belgian Scots pine stand using the process model SECRETS. Annals of Forest Science 58:31-46.

Sampson, D. A., R. H. Wynne, and J. R. Seiler. 2008. Edaphic and climatic effects on forest stand development, net primary production, and net ecosystem productivity simulated for Coastal Plain loblolly pine in Virginia. Journal of Geophysical Research: Biogeosciences 113.

Singh, J., and S. Gupta. 1977. Plant decomposition and soil respiration in terrestrial ecosystems. The Botanical Review 43:449-528.

Strahm, B., Seiler, J., Heim, B., Will, R., Ausmus, C., Vogel, J. Lokuta, G. Jokela, E. 2014. Partitioning Soil Respiration to Quantify Carbon Sequestration: A Regional Synthesis of Fertilization and Throughfall Reduction. PINEMAP Annual Report 3:20-21.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil Series Classification Database. Available online. Accessed [November/27/2015].

Subke, J.A., I. Inglima, and M. Francesca Cotrufo. 2006. Trends and methodological impacts in soil CO2 efflux partitioning: A metaanalytical review. Global Change Biology 12:921-943.

U.S. Environmental Protection Agency [US EPA]. 2008. Inventory of U.S. greenhouse gas emissions and sinks: 1990–2006. EPA 430-R-08-005. Washington, DC: U.S. Environmental Protection Agency, Office of Atmospheric Program.

Vogel, J. G., and D. W. Valentine. 2005. Small root exclusion collars provide reasonable estimates of root respiration when measured during the growing season of installation. Canadian Journal of Forest Research 35:2112-2117.

Warembourg, F. R. and E. A. Paul. 1973. The use of C1402 canopy techniques for measuring carbon transfer through the plant-soil system. Plant and Soil 38:331-345.

Wear, D. N., and J. G. Greis. 2002. Southern forest resource assessment: summary of findings. Journal of Forestry 100:6-14.

Wieser, G., and M. Bahn. 2004. Seasonal and spatial variation of woody tissue respiration in a Pinus cembra tree at the alpine timberline in the central Austrian Alps. Trees 18:576-580.

Wiseman, P. E., and J. R. Seiler. 2004. Soil CO2 efflux across four age classes of plantation loblolly pine (Pinus taeda L.) on the Virginia Piedmont. Forest Ecology and Management 192:297-311.

Table 2.1. Summary of stand characteristics for four stand age classes of loblolly pine.

Stand age	Peak LAI	Stem density (stems ha^{-1})	Basal area (m^2 ha^{-1})	Stem volume (m^3 ha^{-1})	Stem biomass (kg ha^{-1})	Understory biomass (kg ha^{-1})	Forest floor biomass (kg ha^{-1})
3	0.32 (0.32) [b]	229.32 (22.08) [b]	0.21 (0.13) [b]	9.03 (0.58) [b]	43.62 (38.57) [b]	3948.18 (521.59) [a]	1406.10 (307.56) [c]
9	2.18 (0.05) [a]	801.28 (114.55) [a]	18.32 (2.46) [a]	98.08 (5.53) [ab]	9471.38 (1645.73) [ab]	2353.69 (729.98) [a]	3196.38 (348.88) [b]
18	2.52 (0.14) [a]	609.72 (116.38) [ab]	26.34 (3.37) [a]	201.30 (28.38) [a]	14618.49 (2471.42) [a]	1103.20 (553.97) [a]	4037.18 (364.10) [ab]
25	3.11 (0.07) [a]	380.41 (85.27) [b]	29.65 (4.96) [a]	248.17 (58.32) [a]	13991.88 (2954.03) [a]	3759.51 (1790.37) [a]	5326.13 (380.79) [a]

Data represent plot means (n=3) and standard errors in parentheses. Stem density and biomass includes all stems greater than 1 cm DBH.
Lowercase letters denote significant differences ($P < 0.05$) among stand age classes.

Table 2.2. Summary of soil characteristics for four stand age classes of loblolly pine at three depth increments.

Depth (cm)	Stand age	pH	Bulk density <2mm (g cm^{-3})	N (%)	C (%)	C:N	C (Mg ha^{-1})	OM (%)	CEC ($cmol_c$ kg^{-1})	Base saturation (%)
0-10										
	3	4.88 (0.08) [a]	0.78 (0.07) [a]	0.10 (0.008) [a]	2.59 (0.12) [a]	25.49 (1.52) [b]	20.18 (2.56) [a]	5.70 (0.73) [a]	5.40 (0.28) [a]	19.33 (3.01) [a]
	9	4.76 (0.09) [ab]	0.85 (0.05) [a]	0.12 (0.037) [a]	2.86 (0.76) [a]	24.73 (1.12) [b]	23.97 (5.46) [a]	5.80 (0.50) [a]	5.27 (0.73) [a]	16.13 (4.08) [a]
	18	4.70 (0.03) [ab]	0.67 (0.06) [a]	0.06 (0.016) [a]	1.98 (0.24) [a]	33.01 (4.30) [a]	12.03 (2.76) [a]	7.09 (2.08) [a]	5.47 (0.53) [a]	13.03 (3.29) [a]
	25	4.28 (0.18) [b]	0.81 (0.07) [a]	0.12 (0.024) [a]	3.58 (0.86) [a]	29.94 (2.41) [ab]	28.92 (5.80) [a]	5.43 (0.30) [a]	6.63 (0.88) [a]	9.27 (2.32) [a]
10-20										
	3	4.99 (0.06) [a]	1.20 (0.07) [a]	0.04 (0.007) [a]	0.91 (0.15) [a]	25.51 (1.97) [a]	11.04 (2.44) [a]	3.05 (0.43) [a]	3.73 (0.60) [a]	16.07 (3.64) [a]
	9	5.03 (0.08) [a]	1.12 (0.04) [a]	0.04 (0.005) [a]	1.10 (0.11) [a]	25.27 (0.71) [a]	12.25 (1.04) [a]	2.71 (0.16) [a]	3.37 (0.34) [a]	15.97 (2.49) [a]
	18	4.87 (0.03) [ab]	1.13 (0.20) [a]	0.04 (0.002) [a]	1.15 (0.10) [a]	29.20 (2.46) [a]	12.33 (3.32) [a]	3.40 (0.73) [a]	4.67 (0.18) [a]	12.97 (3.17) [a]
	25	4.68 (0.03) [b]	1.32 (0.26) [a]	0.06 (0.008) [a]	1.70 (0.29) [a]	30.04 (1.33) [a]	23.96 (8.16) [a]	4.09 (0.43) [a]	4.80 (0.85) [a]	10.60 (1.55) [a]
20-30										
	3	4.92 (0.07) [a]	1.43 (0.03) [a]	0.03 (0.003) [a]	0.75 (0.05) [a]	22.46 (2.81) [a]	10.69 (0.62) [a]	1.78 (0.07) [b]	4.47 (0.84) [a]	11.50 (1.90) [a]
	9	4.96 (0.08) [a]	1.28 (0.07) [a]	0.03 (0.001) [a]	0.71 (0.04) [a]	23.25 (0.96) [a]	9.01 (0.50) [a]	2.66 (0.18) [ab]	3.67 (0.86) [a]	12.87 (2.39) [a]
	18	4.80 (0.03) [a]	1.32 (0.13) [a]	0.03 (0.002) [a]	0.82 (0.01) [a]	25.09 (1.29) [a]	10.82 (0.70) [a]	4.06 (0.43) [a]	5.40 (0.40) [a]	10.17 (1.77) [a]
	25	4.69 (0.04) [a]	1.26 (0.04) [a]	0.04 (0.003) [a]	0.91 (0.11) [a]	23.97 (1.09) [a]	11.33 (1.14) [a]	3.52 (0.72) [ab]	4.83 (0.58) [a]	9.13 (0.78) [a]

Data represent plot means (n=3) and standard errors in parentheses. Lowercase letters denote significant differences ($P < 0.05$) among stand ages within a given soil depth.

Table 2.3. Pearson's correlation coefficients for relationships between R_H:R_S and stand characteristics by season.

Season	Peak LAI		Stem density (stems ha^{-1})		Basal area (m^2 ha^{-1})		Stem volume (m^3 ha^{-1})		Stem biomass (kg ha^{-1})		Understory biomass (kg ha^{-1})		Forest floor biomass (kg ha^{-1})	
Fall	-0.55	**	-0.36	**	-0.33	**	-0.34	**	-0.30	*	0.53	**	-0.49	**
Spring	-0.63	***	-0.04	ns	-0.47	**	-0.64	***	-0.47	**	0.66	***	-0.57	**
Summer	-0.65	***	-0.33	*	-0.60	***	-0.70	***	-0.61	***	0.72	***	-0.51	**
Winter	-0.77	***	0.11	ns	-0.54	**	-0.61	***	-0.55	***	0.73	***	-0.87	***

Note: * $P < 0.1$, ** $P < 0.05$, *** $P < 0.0001$, ns = not significant. R_H, heterotrophic soil respiration; R_S, total soil respiration.

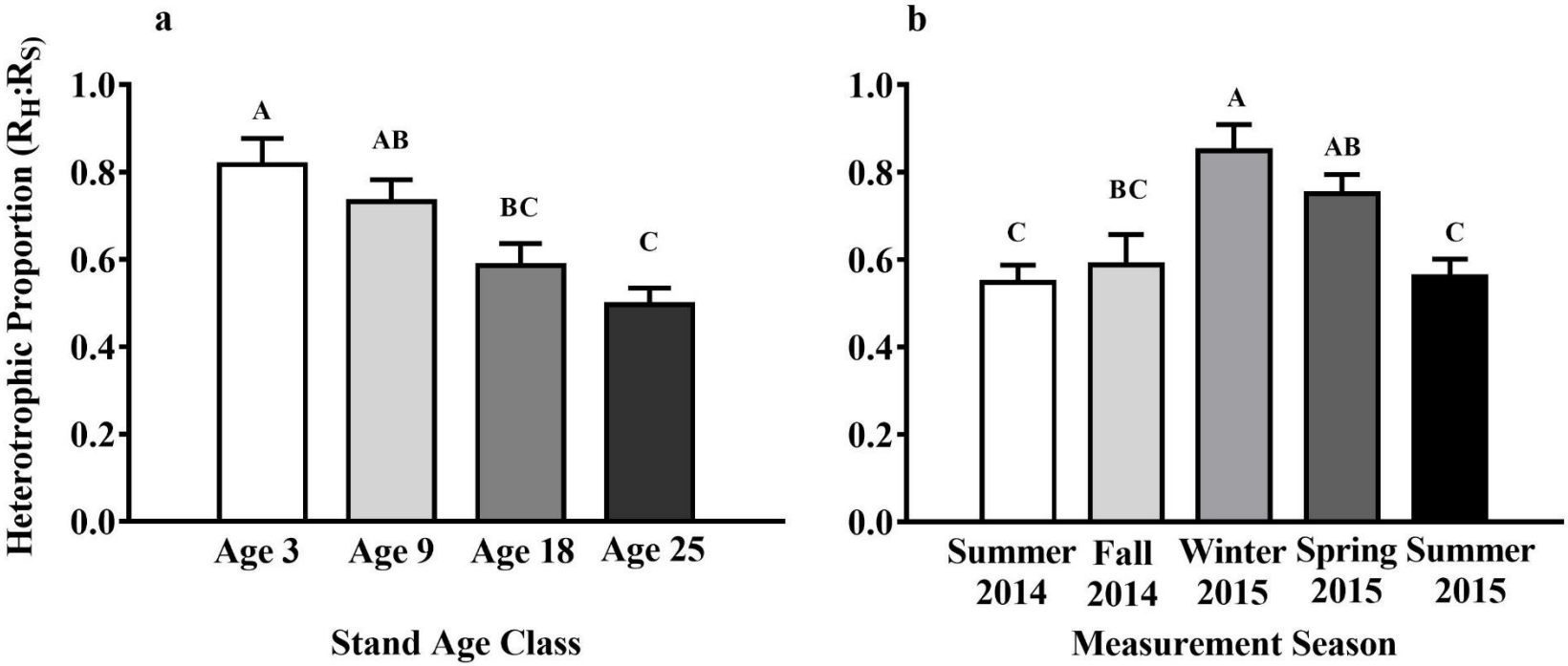

Figure 2.1. Mean heterotrophic soil respiration (R_H) proportion to total soil respiration (R_S) by stand age (a) and by season (b), and mean total soil respiration (R_S) by season. R_H:R_S was measured across five seasons and in four age classes of loblolly pine stands (*Pinus taeda* L.) in Virginia. Different letters within each category denote significant differences using Tukey's HSD at $P < 0.05$. Error bars represent ± 1 SE.

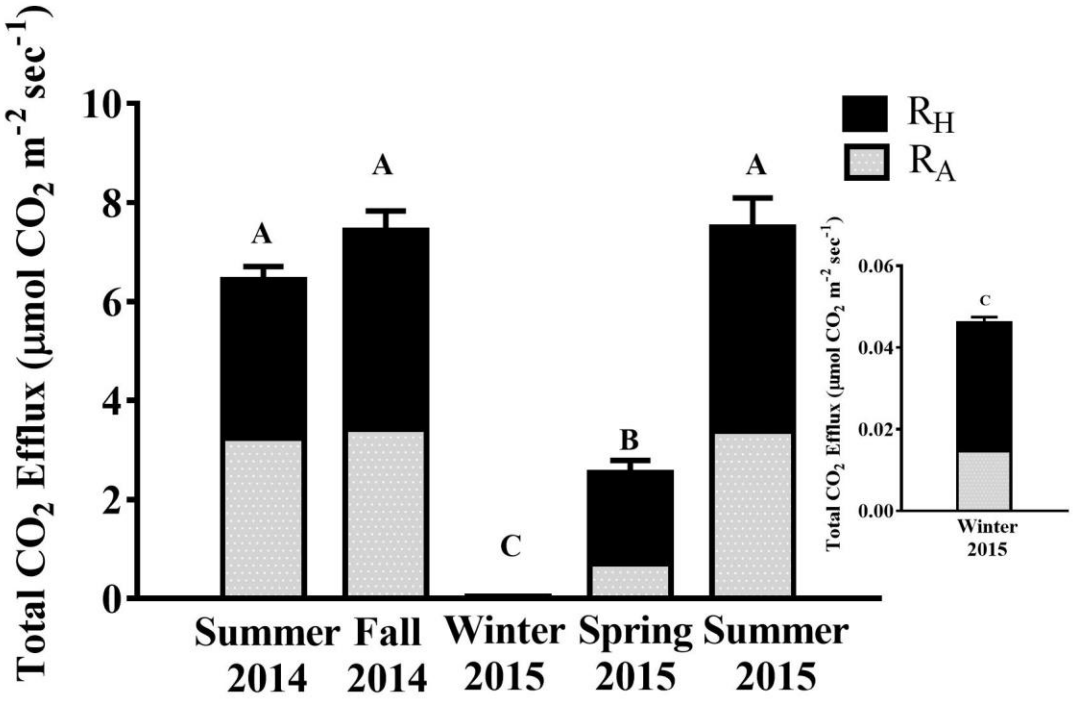

Figure 2.2. Mean total soil respiration (R_S) by season. R_S was measured across five seasons and in four age classes of loblolly pine stands (*Pinus taeda* L.) in Virginia. Different letters within each category denote significant differences using Tukey's HSD at $P < 0.05$. Error bars represent ± 1 SE.

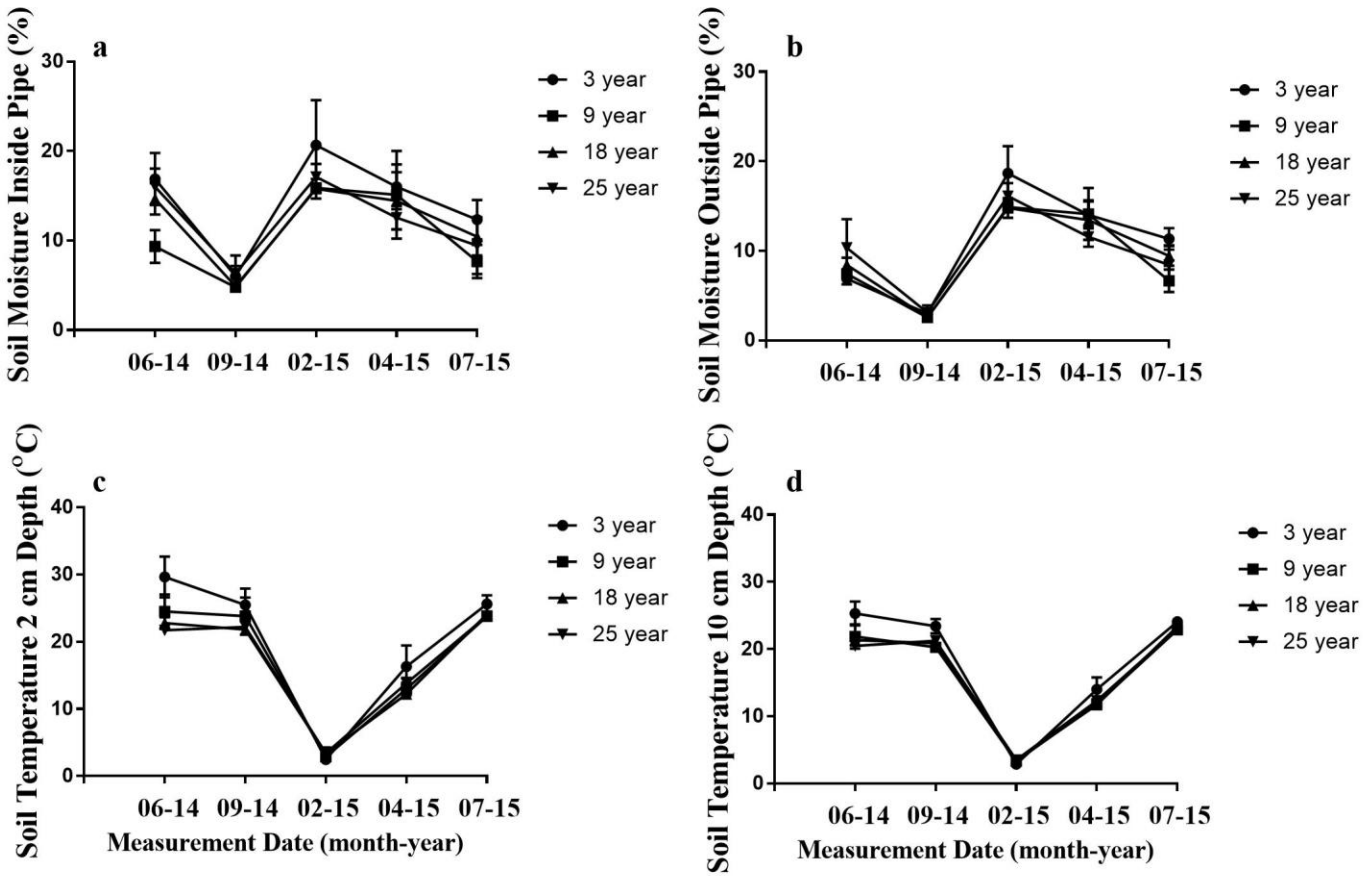

Figure 2.3. Mean soil moisture inside (a) and outside (b) root-exclusion cores and soil temperature at 2 cm depth (b), and at 10 cm depth (c) measured across five seasons and in four age classes of loblolly pine stands (*Pinus taeda* L.) in Virginia. Error bars represent ± 1 SE.

Chapter 3. Fertilization and throughfall reduction alter litter quality and extracellular enzyme activity

Abstract

Forest nutrient management and altered precipitation regimes are expected to affect the net carbon (C) balance of forest ecosystems by influencing microbial processes like decomposition. The objectives of this study were to evaluate how mineral (A horizon) and organic (Oi horizon; litter) soil quality (i.e., nutrient content and chemical composition), and extracellular enzyme activities, are influenced by the interactive effects of forest fertilization and throughfall reduction in a mid-rotation loblolly pine (*Pinus taeda* L.) plantation. Litter and mineral soil (0 – 10 cm) samples were collected in July 2014 from a field study installed in 2012 which consisted of a factorial combination of 30 % throughfall reduction and fertilization (224 kg nitrogen (N) ha^{-1}, 27 kg phosphorus (P) ha^{-1}, 56 kg potassium (K) ha^{-1} plus a micronutrient mix) treatments. Litter quality was evaluated using nutrient, structural carbon (C) and non-structural C components as determined by extraction and acid hydrolysis. Litter and mineral soil were assayed for extracellular enzyme activity using fluorometric and colorimetric microplate analysis. Microbial biomass C, N, and P was determined using the chloroform fumigation-extraction method. The results demonstrate that fertilization improved litter quality by decreasing lignin:N and lignin:P ratios, caused a shift in extracellular enzyme activity from mineral soil N- and P-acquiring enzyme activity to litter C-acquiring enzyme activity, and increased microbial biomass pools. Throughfall reduction decreased litter quality by increasing lignin:N and lignin:P, but also increased C-acquiring enzyme activity. Results from this study suggest that forest

nutrient management and changed precipitation regimes could increase rates of nutrient cycling in the forest floor by increasing litter quality, microbial biomass, and enzyme activities.

Keywords: Decomposition; Microbial communities; Heterotrophic respiration; *Pinus taeda* L.

3.1 Introduction

The interactive effects of forest management and climate change could have important consequences for forest ecosystem health, productivity, and C storage. Planted pine forests make up more than 65 % of the forested land in the southeastern U.S. (Wear and Greis 2002), play a key role in the regional economy, and are a significant portion of the national C budget (Turner et al. 1995, Wear and Greis 2002). The sustained productivity of these ecosystems and their capacity to sequester C depend on the rate of inputs exceeding the rate of losses of existing above- and below-ground C pools. Predicting these relationships in response to management and climate change requires that mechanistic controls of both above-and below-ground C pools and fluxes are well-understood. Given that soil microorganisms mediate many important ecosystem processes influencing C flows in forest ecosystems (Chapin et al. 2011), incorporating observations of belowground microbial dynamics with stratified measures of soil quality, nutrient pools, and fluxes is central to enhancing our understanding of processes influencing long-term C dynamics of forest ecosystems.

Fertilization of forest plantations in the southeastern U.S. is a common management practice to improve productivity, as most soils supporting forest plantations are often both nitrogen (N) and phosphorus (P) limited (Allen 1987, Fox et al. 2007). Changes in nutrient availability as a result of forest fertilization can alter the balance between net primary productivity (NPP) and decomposition (Prescott et al. 1999). Stimulatory, inhibitory, and neutral effects of fertilizer additions on decomposition have been observed (Knorr et al. 2005), and are largely explained by altered activities of substrate-specific extracellular enzymes involved in C, N, and P cycling (Carreiro et al. 2000, Matocha et al. 2004, Treseder 2008, Bautista-Cruz and Ortiz-Hernández 2015). Enzymes are synthesized and excreted by bacteria and fungi to

breakdown insoluble, complex organic matter into soluble products available for microbial assimilation or plant uptake. Assays of potential extracellular enzyme activities have long been used as indicators of change in soil and substrate quality, and nutrient cycling due to their rapid response to management- and climate-induced changes in their environment (Skujiņš and Burns 1976, Dilly and Nannipieri 2001, Waldrop et al. 2004, Sinsabaugh et al. 2009).

Fertilization can alter enzyme activity and decomposition through changes in microbial substrate quality (Carreiro et al. 2000, Sinsabaugh et al. 2002, Frey et al. 2004, Waldrop et al. 2004, Waldrop and Zak 2006, Sinsabaugh et al. 2008), microbial biomass and community composition (Ajwa et al. 1999, Compton et al. 2004), and site characteristics [e.g., nutrient availability, productivity, species composition] (Olander and Vitousek 2000, Treseder and Vitousek 2001, Acosta-Martinez et al. 2007, Štursová and Baldrian 2011). Substrate quality may change as a result of altering plant nutrient availability and uptake with fertilization (Polglase et al 1992; Minocha et al 2000; Kiser et al 2013), with consequent changes in substrate chemical composition (i.e., C:nutrient, and lignin:nutrient ratios; Miller and Miller 1976, Berg and Staaf 1980). Enzyme activity has been found to increase with long-term fertilization, resulting in accelerated hydrolysis of more labile C compounds and cellulose in forest, grassland, and agricultural soils (Ajwa et al. 1999, Carreiro et al. 2000, Saiya-Cork et al. 2002, Zak et al. 2008, Keeler et al. 2009, Tiemann and Billings 2011). Other studies have found that nutrient additions with fertilization or organic amendments inhibit the activity of enzymes that mineralize N, and those responsible for the oxidation of lignin, such as phenol oxidase (Fog 1988, Berg and Matzner 1997, Magill and Aber 1998, Hobbie 2000, Sinsabaugh et al. 2002, DeForest et al. 2004, Waldrop and Zak 2006). These changes have the potential to alter decomposition processes, which could impact C turnover and storage in soils.

The balance between NPP and decomposition may also be effected by forecasted changes in climate of increased rates of warming and increased year-to-year variability in precipitation regimes for the southeast region (McNulty et al. 2013, USGCRP 2009). Changes in precipitation patterns are expected to influence decomposition dynamics and extracellular enzyme activity, as water availability is a key factor controlling microbial activity and access to substrate (Sinsabaugh et al. 2008, Henry 2012). This is particularly relevant for forests of the southeastern U.S. as predicted changes in climate could result in precipitation declines of 30% (Christensen et al. 2007, Karl 2009), increased frequency of drought (Laseter et al. 2012), and increased soil water deficits (Noormets et al. 2010).

Extracellular enzyme response to experimental manipulations of moisture availability in desert, agricultural, and forest ecosystems has been widely varied, with reports of large decreases in enzyme activity (Li and Sarah 2003, Sardans and Peñuelas 2005, Sardans et al. 2008, Sardans and Peñuelas 2010, Steinweg et al. 2012), or little to no change in activity (Yavitt et al. 2004, Kreyling et al. 2008, Bell et al. 2009). Altered enzyme kinetics, substrate availability, and physiology of microbial communities have been proposed as possible causes of shifts in enzyme activity in response to changes in moisture availability (Collins et al. 2008, Stone et al. 2012); however, the magnitude of response will likely hinge on resource availability as enzyme activity is suggested to be decoupled from moisture availability when other resources are limiting (Geisseler et al. 2011). Water stress may also indirectly influence enzyme activity and decomposition by altering soil quality through a variety of mechanisms, including changes in soil nutrient availability and consequent changes in plant growth, and altered nutrient retranslocation strategies prior to leaf abscission (Entry et al 1998; Runion et al 1999, Sardans and Peñuelas, 2007; Sardans et al. 2008).

The complexities of site, substrate, and microbial interactions that control decomposition dynamics highlight the importance of identifying mechanisms responsible for such changes and their drivers. Integrating measures of soil biotic variables, such as estimates of extracellular enzyme activity, with measures of chemical properties will allow for a greater understanding of mechanisms responsible for changes in decomposition dynamics in response to management-and climate-induced changes. To examine potential mechanisms influencing decomposition dynamics in managed systems, we tested the effects of fertilization and altered precipitation (throughfall reduction) on soil and litter chemistry, microbial biomass, and extracellular enzyme activities in an 11-year-old loblolly pine plantation. Additionally, we characterized variation in enzyme activity among treatments and tested for mineral soil and litter biochemical characteristics (C:N, C:P, lignin concentration, microbial biomass) in an attempt to explain the observed variation in enzyme activity. We hypothesized that fertilization would increase enzyme activity of cellulose and labile C degrading enzymes, and decrease activities of lignin-degrading and N- and P-acquiring enzymes. Second, we hypothesized that throughfall reduction would decrease extracellular enzyme activity, but to a lesser extent in fertilized treatment plots. Finally, we hypothesized that the observed variation in enzyme activities would be best explained by litter quality metrics (e.g., lignin:nutrient ratios).

3.2 Materials and Methods

3.2.1 Study Location & Experimental Design

This research is a component of the Pine Integrated Network: Education, Mitigation, and Adaptation project (PINEMAP; http://pinemap.org/). This regional study was designed to evaluate the effects of decreased rainfall and nutrient additions on loblolly pine plantation

productivity and physiology (Will et al. 2015). Four locations were established to span the full temperature and precipitation range of the species. This study was located in a mid-rotation, 11-year-old loblolly pine forest located in the Appomattox-Buckingham State Forest, VA (37°27´37´´N, 78°39´50´´W), and was conducted on an existing 1.3 ha, long-term throughfall reduction x fertilization study installed during spring of 2012 (Will et al. 2015).

Mean annual precipitation for this region is 109 cm. The average growing season temperature (April through September) is 22.9 °C and the average winter temperature (December to February) is 3.8 °C (NCDC.NOAA.gov accessed November 27, 2015). Soils are well drained, formed in residuum from metamorphic rocks (sericite schist, graphitic schist, and/or phyllite). Soils are mapped as Spears Mountain (fine, mixed, semiactive, mesic Typic Hapludult) and Littlejoe (fine, mixed, subactive, mesic, Typic Hapludult) series (Soil Survey Staff, accessed June 28, 2016).

The loblolly pine plantation was operationally established in 2003 at a stand density of 1200 trees ha^{-1}, and planted with a local seed orchard mix of half-sib families representing genetically adapted sources. At the time of study installation (2012), no thinning or fertilization treatments had been previously performed; the stand density was 789 ± 68 trees ha^{-1}, mean tree size was 8.79 ± 0.19 m tall and 14.5 ± 0.52 cm dbh (diameter at breast height), with a mean BA (basal area) of 13.20 ± 0.71 m^2 ha^{-1} and site index (SI; base age 25) of 21.0 ± 0.3 m. Prior to study installation, all competing vegetation was eradicated with a combination of manual clearing and directed spraying of glyphosate (2 % solution) and metsulfuron methyl (140 g ha^{-1}). Additional site information and a description of the study installation can be found in Will et al. (2015).

Experimental treatments consisted of a factorial combination (n=4) of throughfall reduction (TR; 0 and 30% reduction), corresponding to ambient conditions and the driest predictions for the region (Christensen et al. 2007), and fertilization (Fert) with either no addition or a complete suite of essential nutrients to represent optimum nutrition. Treatment plots measured 14.6 x 16.8 m, surrounded by a 6.1 m buffer on all sides.

The throughfall reduction treatment consisted of covering approximately 30 % of the plot area with 1.5 m wide troughs at 0.3 m spacing constructed at approximately 1.5 m above the soil surface, to collect and divert throughfall away from the treatment plots. The elemental fertilization treatment was representative of operational application rates in loblolly pine plantations (Fox et al. 2007) and was broadcast applied in spring of 2012. N was applied at a rate of 224 kg N ha^{-1} as urea, and P was applied at a rate of 27 kg P ha^{-1} as diammonium phosphate. Elemental K was applied at a rate of 56 kg K ha^{-1} as potash, and a granular oxysulfate micronutrient mix (Southeast Mix, Cameron Chemicals, Inc., Virginia Beach, VA, USA) consisting of 6 % sulfur (S), 5 % boron (B), 2 % copper (Cu), 6 % manganese (Mn), and 5 % zinc (Zn) was applied at a rate of 22.4 kg ha^{-1}.

3.2.2 Soil and Litter Sampling

Soil and litter samples were collected within each treatment plot in July 2014. Litter from the Oi horizon and mineral soil (0-10 cm) from the A horizon were collected from six stratified random subsample locations in each treatment plot, and composited into one sample from each of the 16 treatment plots. Samples collected from the TR treatment plots were collected from underneath the exclusion troughs to minimize potential effects from moisture banding associated with the location of the troughs. Litter from the Oi horizon was sampled from a 10 cm × 10 cm

area, and homogenized. Mineral soil was sampled using a 5.7 cm diameter push-tube soil sampler to a depth of 10 cm from the six locations where litter samples were collected. Composited soil samples were mixed and all visible roots, litter, and identifiable organic matter were removed. Part of each litter and soil sample were placed in a plastic bag, transported in a cooler on dry ice, and stored at -20 °C until subsequent analysis of microbial activity. The remaining samples were stored at 4 °C for chemical analysis.

3.2.3 Soil and Litter Chemical Analysis

The composited soil subsamples were air-dried in a greenhouse (temperature ranged between 20° to 35°C) and passed through a 2-mm mesh stainless steel sieve. A 1g air-dry subsample was analyzed for total C and N on Elementar VarioMax CNS analyzer (Elementar Americas, Mt. Laurel, NJ). A portion of each composited subsample from each depth was sent to the Virginia Tech Soil Testing Laboratory for analysis of pH, cation exchange capacity, soluble salts, and extractable nutrients using the Mehlich-1 (0.05N HCl + 0.025N H_2SO_4) extraction procedure (Maguire and Heckendorn 2014).

Prior to chemical analysis, litter was dried at 65 °C for 48 h or until a constant mass was achieved, then ground using a Wiley Mill (Thomas Scientific Model 4 Miley Mill, Swedesboro, NJ) to pass through a 1-mm mesh sieve. Total C and N concentrations were determined using a CNS Elemental analyzer (LECO Corporation, St. Joseph, MI). A portion of the ground samples were dry-ashed at 500 °C for 24 h and then digested using 6 M HCl (Robarge and Fernandez 1986). Digested litter samples were analyzed for elemental nutrient concentrations (total P, K, Ca, Mg, Al, Fe, Zn, Cu, B, and Mn) by inductively coupled plasma spectroscopy (Varian Vista-MPX ICP-OES, Agilent Technologies, Santa Clara, CA). Litter pH was determined by adding 10

ml distilled H_2O to 1 g dry weight ground sample, shaking for 30 min, allowing to settle for 60 min, then measuring using a digital benchtop pH meter (Thermo *Orion pH* meter, model 420, Waltham, MA; Koide and Shumway 2000).

Compounds of leaf litter biomass used as litter quality indicators (soluble C extractives, cellulose, hemicellulose, and lignin) were determined using the analytical procedures of Van Soest et al. (1991) and the National Renewable Energy Laboratory analytical procedure for determination of extractives, structural carbohydrates, and lignin in biomass (Sluiter et al. 2005; Sluiter et al. 2011). This analysis involved the sequential digestions of fibers. Briefly, litter samples were air dried to a moisture content below 10%, and milled to pass through a 1-mm mesh-size screen. Soluble C extractives (non-structural carbohydrates such as sugars, nitrogenous material, chlorophyll, waxes, or other minor components) were measured from 5 g of each sample following a two-step water and ethanol extraction. Cellulose, hemicellulose, and lignin concentrations were measured from 300 mg of each sample that underwent extraction using acid detergent and hydrolysis. A two-step 72% sulfuric acid (H_2SO_4) hydrolysis procedure was performed to remove the cellulose and fractionate the lignin into acid insoluble material and acid soluble material. The acid soluble lignin was measured at a wavelength of 240 nm using a UV-Visible spectrophotometer (Genesys 10S UV-Vis Spectrophotometer). The acid insoluble lignin (Klason lignin) was analyzed gravimetrically through the mass difference before and after heating the acid-hydrolyzed residue at 575 °C. During hydrolysis the polymeric carbohydrates were hydrolyzed into the monomeric forms, which are soluble in the hydrolysis liquid. The structural sugars in the filtrate were analyzed in duplicates using Metrohm Ion Chromatography (IC; Metrohm Inc., USA). Monosaccharides (referred to herein as sugars%) in the filtrate were separated by a Hamilton RCX -30 (250 × 4.6 *mm*) column with DI-water as eluent. Eluent flow

rate was 1 ml min^{-1} and the column temperature was 32 °C. NaOH (350 mmol/L) was introduced after column separation, with a flow rate of 0.43 ml min^{-1}, to aid the signal generation in PAD at 35 °C. Five sugars including arabinose, galactose, glucose, xylose, and mannose were quantified in the Mag IC Net software. All samples were analyzed in duplicate and means are reported on a dry weight basis, as a weight percentage of the biomass.

3.2.4 Microbial Biomass

Microbial biomass (MB) C, N, and P of mineral soil and litter samples were determined using the chloroform fumigation-extraction method (Brookes et al. 1982, 1985; Vance et al. 1987; Horwath and Paul 1994). C, N, and P were extracted from two replicated 25 g field-moist soil samples and 5 g field-moist litter samples. Soil and litter subsample masses were corrected for soil moisture by drying a subsample at 105ºC for 24 h. One replicate of each soil sample was fumigated with chloroform ($CHCl_3$) in a vacuum desiccator for 24 hours, while the second replicate was not fumigated. Following fumigation, both replicates were shaken with 100 mL 0.5 *M* K_2SO_4 for 1 hour at 200 rev m^{-1}, and filtered through Whatman #2 filter paper into scintillation vials. Extracts were analyzed for total dissolved organic C and N using a Shimadzu TOC-N analyzer (Shimadzu Scientific Instruments, Inc., Columbia, Maryland, USA). The inorganic P content was measured with a Lachat QuikChem 8500 colorimetric analysis system following an alkaline persulfate digestion (Patton and Kryskalla 2003; USGS Method I-4650-03). The difference between the fumigated and non-fumigated extracts were calculated to determine the MBC, MBN, and MBP concentrations.

3.2.5 Enzyme Activity

We measured the potential activity of nine soil and litter enzymes that degrade a range of substrates that are common components of organic matter (Table 3.1). These enzymes are important for the cycling of C (β-glucosidase, β-xylosidase, α-glucosidase, and β-D-cellubiosidase), N (N-acetyl-β-glucosaminidase and leucine aminopeptidase), P (acid phosphatase), and the oxidation of phenolic compounds (polyphenol oxidase and peroxidase) (Sinsabaugh et al. 2009; Li et al. 2015). Enzyme assays were conducted following published protocol (Sinsabaugh et al. 2000) with modifications from Allison (2012) and DeForest (2009). Enzymes were assayed in duplicate at 25 °C using 0.5 g of field moist soil and litter homogenized in a slurry with a pH 4.5, 50 *mM* sodium acetate buffer to approximate the bulk soil pH of the system from which they were collected. The slurry was continuously mixed on a stir plate while aliquots were distributed onto a 96-well black microplate for preparing the blank, quench standard, and assay wells. The buffer, sample suspension, 1 *mM* reference stock solution (4-methylumbelliferone, MUB or 7-amino-4-methylcoumarin, AMC) and 200 *mM* substrate (Table 3.1) were dispensed into the wells of a black 96-well microplate according to the volume and order described by Sinsabaugh et al (2000a, 2000b) and DeForest (2009). The microplates were covered and incubated in the dark at 25 °C for up to 24 h (Table 3.1), and the fluorescence quantified using a microplate reader with fluorescence and absorbance detection (Tecan Infinite M200Pro) with 365 nm excitation and 450 nm emission filters. Fluorescence was measured one minute following addition of 10 µL of 0.5 N NaOH to each well to terminate the reaction. The non-fluorometric enzymes, phenol oxidase and peroxidase, were measured colorimetrically in clear 96-well microplate using the substrate of L-3, 4-dihydroxyphenylalanine (L-DOPA). The dispensed volume and the order of buffer, sample suspension, 25 mM L-DOPA and 0.3% H_2O_2

were the same as for the fluorometric enzymes. Potential enzyme activities were assayed by measuring the absorbance at 460 nm using the microplate reader.

3.2.6 Statistical Analysis

This study was analyzed as a randomized complete block design (n=4). A mixed-effects analysis of variance (ANOVA) in which block was considered a random effect while throughfall reduction and fertilization treatments were considered fixed effects, was used to test for main effects and interactions on multiple response variables (soil and litter chemistry, microbial biomass, and extracellular enzyme activity). All variables were log or square root transformed as appropriate to achieve assumptions of normality and equal variance. Treatment differences were determined from the 95% confidence intervals about the least square (LS) means (α=0.05), and treatment means were tested at a significance level of $\alpha = 0.05$ using Tukey-Kramer Honestly Significant Difference (HSD). Linear regression analyses were performed to establish the strength and significance of relationships between soil and litter quality parameters and enzyme activities and (n=16). All statistical analyses were performed using JMP® 11 software system (SAS Institute, Cary, NC, USA).

3.3 Results

3.3.1 Treatment effects on soil and litter biochemical properties

Soil biochemical characteristics were not strongly influenced by fertilization and throughfall reduction, with the exception of soil P concentrations and microbial biomass (Table 3.2 and 3.3). The Fert×TR interaction had a significant effect on extractable soil P

concentrations (P = 0.006; Table 3.2), with significantly higher concentrations in the Fert×TR treatment plots (6.50 ± 0.65 mg kg^{-1}; Table 3.3) relative to control (3.75 ± 0.48 mg kg^{-1}). Fert (4.50 ± 0.29 mg kg^{-1}) and TR (3.25 ± 0.25 mg kg^{-1}; Table 3.3) plots. The soil C:P ratio, calculated on a dry mass basis, was significantly lower in Fert plots (13853 ± 1553) relative to unfertilized plots (8729 ± 1199) as a result of increased soil P concentrations with Fert (Table 3.3). Fert significantly increased both MBC (µg microbial C per g dry $soil^{-1}$) and MBN (µg microbial N per g dry $soil^{-1}$) by 43% and 91%, respectively (Table 3.3). There were no measureable changes in 0-10 cm soil organic matter%, pH, N% and C%, C:N ratio, and extractable Mn due to Fert or TR treatments (P > 0.05; Table 3.2). Mean values and standard errors across treatment plots for each variable not significantly affected by treatments were as follows: organic matter%, 5.77 ± 0.97; pH, 4.50 ± 0.05; C%, 4.61 ± 0.30; N% 0.16 ± 0.01; C:N ratio 28.10 ± 0.61; Mn concentration, 11.18 mg kg^{-1} ± 1.01.

Numerous litter biochemical parameters were influenced by both Fert and TR treatments, but generally contrasting treatment responses were observed. A Fert treatment effect (P < 0.05) was observed for soluble C extractives%, structural sugars%, organic matter% and Mn%, lignin:N and lignin:P ratios, and MBN (Table 3.2). Significant decreases in soluble C extractives %, structural sugars%, and lignin:N, lignin:P ratios were observed in litter collected from Fert treatment plots relative to unfertilized plots. Fert treatment litter had 21.1% lower soluble C and 6.1% lower structural sugar concentrations compared to litter from unfertilized plots (Table 3.3). Decreased lignin:nutrient ratios were a result of significant increases in litter N and P concentrations with Fert; N increased by 56.4% and P by 66.7% (Table 3.3). There were no measureable differences in litter lignin concentrations with any treatment (Table 3.2); the cross site mean of litter lignin% was 37.68 ± 0.46. A significant increase in organic matter was

observed for Fert treatment litter, but the change is negligible (Table 3.3). Litter MBN (µg microbial N per g dry soil^{-1}) was significantly higher in Fert plots (6.76 ± 2.60) relative to unfertilized plots (1.96 ± 0.44; Table 3.3), but there was no treatment effect on MBC or MBP (Table 3.2).

Litter from TR plots had 3.9% lower cellulose, but 4.9% higher hemicellulose concentrations relative to litter from ambient throughfall plots (Table 3.3). Contrary to the effects of the Fert treatment, lignin:N and lignin:P ratios were significantly higher as a result of TR, and due to significant decreases in litter N and P concentrations (Table 3.3). Lignin:N increased by 16.4% and lignin:P increased by 26.9%, while N and P concentrations decreased by 14.8, and 25.0%, respectively. Litter Mn concentrations were lower in TR plots by 4.6% relative to ambient throughfall litter. There was no treatment effect on litter pH, C%, MBC, and MBP values. Mean litter pH across treatment plots was 4.04 ± 0.05, and mean C% across plots was 51.39 ± 0.25. MBC values of leaf litter were 17 times greater than soil values, with a site mean of 4396.56 ± 788.04 (µg C per g dry soil^{-1}). MBP values for mineral soil were not obtainable, but MBP of litter had a cross site mean of 11.59 ± 2.43 (µg P per g dry soil^{-1}).

3.3.2 Patterns of soil enzyme activities

A main Fert effect was observed for AP, XYL, and POX activities ($P < 0.05$, Table 3.4), with significant decreases observed in the P-acquiring enzyme AP, and the lignin-degrading enzyme POX of 22 and 77%, respectively (Table 3.5). Conversely, Fert increased C-acquiring XYL activities in mineral soil by 43% (Table 3.5). The N-aquiring enzyme LAP (P = 0.04, Table 3.4), activity was reduced by 117% in the TR treatment (Table 3.5). There was a significant Fert×TR interaction for mineral soil NAG and PER activities ($P < 0.05$, Table 3.4). N-acquiring

NAG activities were significantly decreased with Fert×TR, Fert, and to a lesser extent, TR treatments. Fert alone had the strongest effect, resulting in a 56% decrease in activity relative to the control plots. Conversely, PER activities were significantly increased with both Fert and TR treatments, with the greatest increase of 436% observed in the Fert×TR treatment plots relative to the control plots (Table 3.5). There were no significant treatment effects on the activities of BG, AG, and CHB in mineral soil ($P > 0.05$, Table 3.4); mean activity values across treatment plots were 2.93 ± 0.19, 0.20 ± 0.02, and 1.00 ± 0.09 μmol g^{-1} hr^{-1}, respectively.

3.3.3 Patterns of litter enzyme activities

A significant Fert and TR main effect was observed for the C-acquiring enzymes BG, AG, XYL, and CHB ($P < 0.05$, Table 3.4). BG activities increased by 49.0% under Fert treatments and 46.3% under TR treatments. Fert and TR treatments increased AG activities by 63.2 and 45.7%, respectively. XYL activities increased by 65.2% with Fert, and 41.6% with TR treatments. CHB activities increased by 66.2% with Fert and 33.7% with TR treatments (Table 3.5). LAP activities were significantly influenced by the Fert treatment only ($P = 0.004$, Table 3.4), resulting in a 40.0% increase in activities relative to unfertilized treatment plots (Table 3.5). The activities of the lignin-degrading enzyme PER were significantly reduced by 86.2% under TR treatment plots relative to ambient throughfall plots (Table 3.5). A significant Fert×TR interaction was observed for the activity of the N-cycling NAG enzyme ($P = 0.009$; Table 3.4). NAG activity was reduced by 46.5% only in the Fert×TR. The Fert, TR and control treatments did not differ significantly from each other (Table 3.5). There was no significant treatment effect of any kind on the activities of AP and POX. The mean litter AP activity across treatment plots was 47.12 ± 4.31 μmol g^{-1} hr^{-1}, and the mean POX values was 0.94 ± 0.18 μmol g^{-1} hr^{-1}. In

general, litter enzyme activity rates were proportionally greater than those of the mineral soil, with the exception of enzymes POX and PER.

3.3.4 Relationships between biochemical parameters and extracellular enzyme activities

Many of the litter and mineral soil biochemical variables explain small but statistically significant amounts of variation in enzyme activity rates when considered individually. The strongest of the relationships observed in mineral soil was the positive relationship between the C:P ratio and the activity of the phosphorus-acquiring enzyme AP ($P < 0.01$, $R^2 = 0.50$). C:P also had a significant positive relationship with NAG ($P < 0.01$, $R^2 = 0.35$), and a marginally significant negative relationship with PER ($P < 0.10$, $R^2 = 0.21$). Soil P had a significant negative relationship with AP activity ($P < 0.05$, $R^2 = 0.28$) and AG activity ($P < 0.05$, $R^2 = 0.31$), but significant positive relationships with XYL ($P < 0.05$, $R^2 = 0.29$) and LAP ($P < 0.01$, $R^2 = 0.42$). Soil N and extractable Mn had only marginally significant positive relationships with NAG activity ($P < 0.10$, Table 3.6), and soil C had only a marginally significant positive relationship with AP activity ($P < 0.10$, $R^2 = 0.22$). Soil organic matter had a significant positive relationship with AG activity ($P < 0.05$, $R^2 = 0.24$), and a marginally significant positive relationship with LAP activity ($P < 0.10$, $R^2 = 0.21$). Soil pH had no significant relationship with any soil enzymes assayed (Table 3.6). Soil MBC had a significant positive relationship with both AG and LAP ($P < 0.05$, $R^2 = 0.30$), while soil MBN had significant positive relationships with AG ($P < 0.01$, $R^2 = 0.43$), XYL ($P < 0.05$, $R^2 = 0.27$), and a marginally significant positive relationship with CHB ($P < 0.1$, $R^2 = 0.22$).

The litter chemical parameters that individually explained the most variation in the greatest number of litter enzyme activities were lignin:N, lignin:P and litter N concentration.

Significant negative relationships were observed between lignin:N and lignin:P, and the activities of AG, XYL, CHB, and LAP. Accordingly, these same enzymes had significant positive relationship with litter N, while only CHB and LAP had a significant positive relationship with litter P concentrations (Table 3.6). Litter lignin had differing relationships with the lignin-degrading enzymes POX and PER; there was a significant negative relationship with POX ($P < 0.01$, $R^2 = 0.34$) and significant positive relationship with PER ($P < 0.01$, $R^2 = 0.35$). Litter pH had a significant negative relationship with POX activity ($P < 0.01$, $R^2 = 0.45$) and a marginally significant negative relationship with NAG ($P < 0.10$, $R^2 = 0.20$). Mn% had a significant positive relationship with NAG activity ($P < 0.05$, $R^2 = 0.26$), but a significant negative relationship with CHB ($P < 0.05$, $R^2 = 0.25$), and LAP ($P < 0.05$, $R^2 = 0.25$). The concentration of structural sugars had a significant negative relationship with BG activity only ($P < 0.05$, $R^2 = 0.28$). Litter MBP had a significant negative relationship with NAG activity ($P < 0.05$, $R^2 = 0.30$), though there were no significant relationships between litter MBC or MBN and any litter enzyme activities. The only similarities in relationships between litter and soil were the positive relationships for both organic matter and P with litter and soil LAP activity, and the positive relationship between Mn and litter and soil NAG activity (Table 3.6).

3.4 Discussion

3.4.1 Litter and mineral soil biochemical properties

Management- and climate-induced changes in litter and mineral soil biochemical properties can influence the process of decomposition and release of nutrients (Melillo et al. 1982, Purahong et al. 2014), thereby influencing C turnover and storage in forests (Currie et al.

2002). Common metrics of litter quality, including lignin:nutrient ratios, have been shown to influence decomposition rates through changes in extracellular enzyme activity and microbial community composition (Allison and Vitousek 2004, Hobbie 2008). The results of this study suggest that three years following treatment installation, interactions between Fert and TR generally did not influence litter and mineral soil biochemical properties, but independently, both Fert and TR treatments affected loblolly pine leaf litter biochemical properties in similar magnitude, with only minor changes observed in mineral soil biochemical properties.

Fert altered litter quality by decreasing lignin:N and lignin:P ratios. Lowering of these ratios resulted mainly from a significant increase in litter N and P, as expected with exogenous nutrient additions (Miller and Miller 1976, Berg and Staaf 1980), but was not accompanied by a significant decrease in lignin. However, we observed a significant decrease of 15% in litter Mn concentrations despite inclusion of micronutrients in the fertilization application. Previous studies have found decreases in pine and hardwood foliar and litter Mn and other inorganic nutrient concentrations in response to N additions (Minocha et al. 2000, Turlapati et al. 2012, van Diepen et al. 2015). This response is suggested to result from the development of nutrient imbalances associated with gains in productivity following fertilization (Thelin et al. 1998, Minocha et al. 2000, van Diepen et al. 2015). Fert had only minor effects on mineral soil chemical properties, but resulted in higher soil P concentrations with the highest P concentrations occurring in the combination Fert×TR plots. This is consistent with reports of P accumulation in acidic forest soils of the southeastern U.S. with fertilization, (Comerford et al. 2002, Everett and Palm-Leis 2009, Kiser et al. 2013), and with increased soil P concentrations under drought conditions due to lower water availability that in turn may reduce microbial mineralization and decreased nutrient uptake by roots (Bradford and Hsiao 1982, Sardans and Peñuelas 2004).

Contrary to the effects of Fert, TR altered litter quality by increasing lignin:N and lignin:P ratios as a result of significant decreases in litter N and P, and increased litter Mn concentrations. Previous studies have shown that drought or water stress can alter litter quality, but no generalizable responses have been reported and results are inconsistent across tree species, biomass components, and geographic regions (Penuelas et al. 2004, Sardans et al. 2008, LeRoy et al. 2014, Brunner et al. 2015). Decreased soil water content associated with experimentally- or naturally-induced drought has resulted in leaf litter with higher levels of recalcitrant fractions (lignin, tannins) and other structural C components [e.g., cellulose, hemicellulose, structural sugars] (Bussotti et al. 1998, Entry et al. 1998, Peñuelas and Estiarte 1998), increased and decreased concentrations of N and P (Runion et al. 1999, Penuelas et al. 2004, LeRoy et al. 2014), and decreased levels of soluble C extractives [e.g., non-structural carbohydrates, lipids, chlorophyll, waxes] (Runion et al. 1999). We observed small but significant changes in structural C components with both TR and Fert treatments, making it difficult to generalize possible mechanisms responsible for altered C allocation strategies of loblolly pine, but could be attributed to species-specific responses to altered nutrient availability and environmental stress (Penuelas et al. 2004, Brunner et al. 2015). It is possible that the observed decrease in litter N and P and increase in Mn concentrations in TR treatment plots could have resulted from several physiological responses, such as altered retranslocation of nutrients prior to leaf senescence, internal remobilization of nutrients dependent on the water availability (Sardans et al. 2008, Sardans et al. 2012), or decreased absorption capacity and uptake by the trees because of decreased rates of decomposition or mineralization (Sardans and Peñuelas 2005, 2007).

Our results suggest that the interactions between Fert and TR did not influence litter and mineral soil properties (with the exception of mineral soil P concentration), but instead, Fert and TR independently altered these variables. The contradictory Fert and TR effects on litter lignin:nutrient ratios reported in this study could result in confounding effects on rates of decomposition and nutrient cycling under future management and climate change scenarios. Higher litter Mn concentrations have been shown to accelerate late stage decomposition rates, when litter lignin concentrations are highest (Berg et al. 2007, Davey et al. 2007, Berg and McClaugherty 2008). Whereas higher litter N concentrations generally accelerates early stage decomposition rates (Melillo et al. 1982, Hobbie 2005, Hobbie et al. 2012). The potential scenario of higher litter N and Mn could accelerate multiple stages of decomposition, leading to losses of forest floor organic matter and increased nutrient availability, potentially increasing productivity. Conversely, lower litter N and Mn may lead to slower decomposition rates, and with the absence of disturbances, result in an accumulation of recalcitrant forest floor material and greater immobilization of N and P, which could limit forest productivity.

Rates of decomposition and nutrient cycling could also be influenced by changes in the microbial biomass pool due to the dominant role microbes play in nutrient cycling and transformations. In this study, only Fert significantly influenced microbial biomass, increasing both MBC and MBN in mineral soil, and MBN in litter. Fertilization has commonly been found to increase MBN (Liang and MacKenzie 1995, Shi et al. 2015), and both increase (Parham et al. 2002, Luo et al. 2015), and decrease MBC (Wallenstein et al. 2006, He et al. 2013). Changes in microbial biomass have important consequences for C storage, and immobilization and retention of N (Treseder 2008, Yuste et al. 2011). Positive relationships have been demonstrated between soil microbial biomass and soil C and N content (Allen 1987, Li et al. 2004); therefore,

fertilization may enhance the size of the microbial biomass pool, which in turn could increase soil C storage and nutrient availability.

3.4.2 Enzyme activities

Previous studies have shown that environmental changes and nutrient amendments can alter enzyme activity potentials in soil and plant litter (Saiya-Cork et al. 2002, Waldrop et al. 2004, Keeler et al. 2009), which can lead to altered ecosystem processes (Caldwell 2005). In this study, both Fert and TR treatments significantly altered extracellular enzyme activity, and in some cases, the greatest magnitude of change resulted from the interaction between Fert and TR. In general, we observed cumulative increases in hydrolytic C-acquiring enzyme activity with Fert and TR, with the magnitude of response being greatest in the litter. This partially supports our hypothesis that C-acquiring enzyme activities would increase with Fert, but refutes our hypothesis that TR would suppress enzyme activity. Instead of observing a common decrease in N- and P-acquiring and oxidative lignin-degrading enzyme activities with Fert and TR as we predicted, we observed enzyme-specific increases and decreases with the greatest response observed in the mineral soil. These results suggest a shift in microbial resource allocation, generally from N and P acquisition to C acquisition, thereby increasing C-acquiring enzyme activities relative to others.

Consistent with our findings, several other studies from a variety of ecosystems have reported increases in C-acquiring enzymes with fertilization, particularly with N additions (Carreiro et al. 2000, Saiya-Cork et al. 2002, Cusack et al. 2010, Cusack et al. 2011, Zhang et al. 2015). Soil microorganisms produce extracellular enzymes according to their nutrient requirements and elemental stoichiometry (Sterner and Elser 2002, Allison et al. 2010);

therefore, increasing the availability of exogenous nutrients with fertilization may alleviate microbial N and P limitation, facilitating increased microbial synthesis of C-acquiring enzymes due to increased microbial C demand compared to N and P (Sinsabaugh and Moorhead 1994). All four C-acquiring enzymes assayed (AG, BG, CHB, and XYL) in leaf litter were sensitive to nutrient additions and activity potentials significantly increased with Fert, while only XYL significantly increased in the mineral soil. The disproportionate increase in C-acquiring enzyme activity in litter relative to mineral soil is likely due to the availability and utilization of differing substrates for microbial metabolism (Fioretto et al. 2000, Sall et al. 2003). Of the four C-acquiring enzymes evaluated, litter BG activity appeared to be most sensitive to nutrient additions and had the highest rates of activities in Fert treatment plots (12.37 ± 1.45 µmol g dry soil $^{-1}$). This is consistent with reports from other studies that use BG as an early indicator of change in soil and substrate quality in response to management (Das and Varma 2010). These results suggest that increasing the availability of N and P through fertilization can stimulate enzyme production, which may lead to increased rates of decomposition and C loss, since enzyme activity and mass loss are generally strongly correlated (Waring 2013).

The responses of N- and P-acquiring and lignin-degrading enzyme activities to Fert were varied. We expected both N- acquiring enzymes activities to decrease with Fert due to the increased availability of N, but only NAG activities were significantly suppressed whereas LAP activities were stimulated with Fert. Litter LAP activity increased by 48% in response to fertilization, and while soil LAP activity showed a similar pattern and increased by 30%, it was not significant. Conversely, NAG activities showed a significant decrease of 41% in mineral soil, and a marginally significant decrease in litter of 25%. It is unclear whether differences in microbial community composition or differing substrate availability in litter and mineral soil

cause these divergent responses. While both LAP and NAG contribute to N uptake, they utilize different substrates which could influence responses in litter and mineral soil to nutrient additions. Additionally, some groups of decomposer microorganisms have preferences for different N forms (Bago et al. 1996, Thirukkumaran and Parkinson 2000, Gallo et al. 2004), and as such, respond differently to inorganic N fertilization. Other studies have also reported mixed responses in N-acquiring enzyme activities to fertilization. For example, Saiya-Cork et al. (2002) found that with N additions, LAP activity decreased in forest soil but increased in litter, while NAG activity increased in mineral soil but decreased in litter. Conversely, Waldrop et al. (2004) and DeForest et al. (2004) found that NAG activity declined with N fertilization in forest soil, while others have found that fertilization had no effect on N-acquiring enzyme activity (Wang et al. 2011, Zhu et al. 2015). As expected, AP activities were significantly suppressed with Fert, but only in the mineral soil; there was no effect of Fert on AP activity in litter. Suppression or inhibition of AP activities in soil following inorganic P additions has been demonstrated in previous studies (Clarholm 1993, Garcıa-Gil et al. 2000, Olander and Vitousek 2000, Ai et al. 2012), and reflects a fertilizer-induced shift in microbial nutrient acquisition, as concentrations of available nutrients often negatively correlate with the activities of those nutrient-releasing enzymes (Sinsabaugh and Moorhead 1994).

We observed mixed responses of oxidative lignin-degrading enzyme activity to fertilization. Similarly, a number of previous studies have reported equivocal effects on lignin-degrading enzyme activities following N additions (Carreiro et al. 2000, Saiya-Cork et al. 2002, DeForest et al. 2004, Zeglin et al. 2007). The range in oxidative enzyme response to Fert may be explained, in part, by the differences in available substrate quality [e.g., labile or recalcitrant] (Weand et al. 2010). In this study, nutrient additions significantly decreased POX activity in

mineral soil, but increased PER activity in mineral soil and litter. Previous studies show that adding N to soil increases PER activity, indicating that N limitation may inhibit PER activity or production (Saiya-Cork et al. 2002, Henry et al. 2005). The suppressing effects of Fert on the activity of POX may be a result of shifts in the decomposer community or suppression of fungi that synthesize POX (Matocha et al. 2004), or abiotic stabilization of aromatic components with N (Fog 1988, Saiya-Cork et al. 2002, Matocha et al. 2004).

Contrary to our results, several studies have detected declines in hydrolytic and oxidative enzyme activities under drought or reduced precipitation conditions (Sardans et al. 2008, Toberman et al. 2008, Sardans and Peñuelas 2010), as soil moisture is generally positively correlated with enzyme activity, at least until anaerobic conditions are reached (Henry 2012). We reported significant increases in enzyme activities in response to TR, with the greatest treatment effect on litter enzymes. All four C-acquiring enzymes and the lignin-degrading PER increased in response to TR in litter, while NAG activity decreased, but only when combined with Fert. Only N-acquiring and lignin degrading enzymes were affected by TR in the mineral soil. Soil LAP and PER activities increased while NAG decreased in response to TR. However, when combined with Fert, PER and NAG responses were amplified relative to TR only. This likely reflects the complex interactions among oxygen, water, and nutrient availability on microbial activity (Freeman et al. 1996, Fenner et al. 2005), and suggests there may be important consequences for decomposition and nutrient cycling associated with the combined effects of nutrient additions and altered soil moisture status.

Stimulated activities of enzymes under TR treatments suggests soil moisture was likely not limiting enzyme synthesis. Alternatively, enhanced enzyme activity, particularly C-acquiring enzyme activity, could be attributed to higher root turnover or enhanced microbial

turnover (Kreyling et al. 2008), and from stimulation of fungi (Parham et al. 2002, Kreyling et al. 2008) under moisture-limited conditions. Increased fungal activity may stimulate the production of PER (Sinsabaugh 2010), or greater fungal turnover may stimulate C-aquiring enzymes that break down fungal biomass (Chung et al. 2006). Given its role in protein degradation, observed increases in LAP activity in response to TR could suggest increased protein input from higher root and microbial turnover.

Changing enzyme activities in soil and litter in response to Fert and TR, and their interaction, may indicate that both treatments change substrate availability through potential changes in rhizodeposition, root and microbial turnover, and litter quality, and that these changes may be amplified by simultaneous changes in nutrient and moisture status. Enzymes involved in the C cycle responded more strongly to both treatments compared with enzymes involved in the N cycle, which may illustrate a high C demand due to increased microbial biomass, or increases in the presence of complex resources, thereby stimulating microbial enzyme production (Harder and Dijkhuizen 1983, Allison and Vitousek 2005).

3.4.3 Relationships between biochemical parameters and enzyme activity

Biochemical parameters, such as substrate quality (e.g., C:N, lignin:N), have been found to partially regulate microbial enzyme activity and decomposition rates (Melillo et al. 1982, Waldrop et al. 2004, Zeglin et al. 2007, Prescott 2010). This study suggests that biochemical parameters may regulate the production and activity of enzymes through a variety of mechanisms, as evident by the large number of significant, but differing relationships observed among litter and soil enzymes (Table 3.6). In general, enzyme activity appears to most strongly reflect nutrient concentrations (N, P, Mn) and ratios (C:P, lignin:N, lignin P), and is consistent

with findings from previous studies (Güsewell 2005, Güsewell and Verhoeven 2006, Keeler et al. 2009). The strongest relationship observed was between soil C:P ratio and activity of AP (R^2 = 0.50). Soil AP activity was positively related to C:P supporting the assumption that P-acquiring enzyme activity increases when P is limiting (Sinsabaugh and Moorhead 1994). This is further supported by the significant negative relationship between soil P concentrations and AP activity, which has been demonstrated in other studies linking AP activity to P availability (Sinsabaugh and Linkins 1993, Güsewell 2005, Nannipieri et al. 2011). The most consistent pattern we observed among multiple enzymes were the negative relationships between lignin:nutrient ratios with the activities of AG, XYL, CHB and LAP in litter, which individually explained between 27 and 43 % of the variance. This generally suggests that as N and P limitation increases relative to C, microbial allocation to production of C- acquiring enzymes decreases (McDaniel et al. 2013). This concept has been supported by previous studies demonstrating that higher quality substrate with low C:N or lignin:N ratios are often associated with increased activity of C- acquiring enzymes (Allison and Vitousek 2005, Tiemann and Billings 2011).

The production and activity of soil and litter enzymes are likely regulated by interactions among many factors, such as substrate quality, resource availability, and microbial biomass. The relationships observed in this study support the notion that stoichiometric constraints largely control microbial production and activity of enzymes (Zechmeister-Boltenstern et al. 2015), and suggest that microorganisms produce enzymes to target the most limiting nutrient, or that enzyme activity is dependent upon microbial demands (Sinsabaugh and Moorhead 1994, Allison and Vitousek 2005). As such, changes in substrate quality and the microbial biomass pool through management practices or altered precipitation regimes, as observed in this study, will

likely influence decomposition dynamics with potential implications for forest C storage and productivity.

3.5 Conclusions

This study provides evidence that forest nutrient management and changed precipitation regimes have the potential to independently alter litter quality, microbial biomass pools, and enzyme activities. Further, changes in nutrient availability and litter quality appear to directly influence enzyme activities across treatments. However, observed horizon-specific enzymatic responses of various magnitudes to Fert and TR, and their interaction, along with differential relationships with biochemical parameters make it difficult to generalize potential implications for forest C storage and nutrient cycling. The forest floor appears to be impacted by Fert and TR treatments more strongly, and generally before mineral soil. Litter enzyme activities exhibited an overall trend of increased hydrolytic, C- acquiring enzyme activity under both treatments. Increased enzymatic activity along with increased litter quality of litter could result in accelerated mineralization rates in the forest floor and lead to greater C loss from this system. However, with increased rates of nutrient release, an increase in NPP is likely. Conversely, inhibited decomposition due to decreased litter quality with TR treatments could decrease nutrient cycling rates and increase nutrient immobilization, but increase C storage. Given that both substrate quality and enzyme activity are important controls over belowground processes influencing forest productivity and C storage, further insight into potential mechanisms influencing observed changes across a greater temporal scale is warranted.

Acknowledgements

Thanks to Ilia Donner and Brian Parr for providing field and laboratory assistance, and Ann Norris, Chip Frazier, and Wei Zhang for providing laboratory resources and assistance. This research was supported in part by the Virginia Agricultural Experiment Station and the McIntire-Stennis Program of the National Institute of Food and Agriculture (NIFA), U.S. Department of Agriculture (USDA), and by the Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP), a Coordinated Agricultural Project funded by the USDA NIFA (Award No. 2011-68002-30185).

Literature Cited

Acosta-Martinez, V., L. Cruz, D. Sotomayor-Ramirez, and L. Pérez-Alegría. 2007. Enzyme activities as affected by soil properties and land use in a tropical watershed. Applied Soil Ecology 35:35-45.

Ai, C., G. Liang, J. Sun, X. Wang, and W. Zhou. 2012. Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulk soil to long-term fertilization practices in a fluvo-aquic soil. Geoderma 173:330-338.

Ajwa, H., C. Dell, and C. Rice. 1999. Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. Soil Biology and Biochemistry 31:769-777.

Allen, H. L. 1987. Forest fertilizers: nutrient amendment, stand productivity and environmental impact. J. For. 85, 37-46.

Allison, S. D., and P. M. Vitousek. 2004. Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. Biotropica 36:285-296.

Allison, S. D., and P. M. Vitousek. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biochemistry 37:937-944.

Allison, S. D., M. N. Weintraub, T. B. Gartner, and M. P. Waldrop. 2010. Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. Pages 229-243 Soil enzymology. Springer.

Bago, B., H. Vierheilig, Y. Piché, and C. Azcon-Aguilar. 1996. Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus Glomus intraradices grown in monoxenic culture. New Phytologist 133:273-280.

Bautista-Cruz, A., and Y. D. Ortiz-Hernández. 2015. Hydrolytic soil enzymes and their response to fertilization: a short review/Enzimas hidrolíticas do solo e sua resposta à adubação: uma breve revisão. Comunicata Scientiae 6:255.

Bell, C. W., V. Acosta-Martinez, N. E. McIntyre, S. Cox, D. T. Tissue, and J. C. Zak. 2009. Linking microbial community structure and function to seasonal differences in soil moisture and temperature in a Chihuahuan desert grassland. Microbial Ecology 58:827-842.

Berg, B., and E. Matzner. 1997. Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. Environmental Reviews 5:1-25.

Berg, B., and C. McClaugherty. 2008. Plant litter. Decomposition, humus formation, carbon sequestration. 2nd Ed Springer.

Berg, B., and H. Staaf. 1980. Decomposition rate and chemical changes of Scots pine needle litter. II. Influence of chemical composition. Ecological Bulletins:373-390.

Berg, B., K. Steffen, and C. McClaugherty. 2007. Litter decomposition rate is dependent on litter Mn concentrations. Biogeochemistry 82:29-39.

Bradford, K., and T. Hsiao. 1982. Physiological responses to moderate water stress. Pages 263-324 Physiological plant ecology II. Springer.

Brunner, I., C. Herzog, M. A. Dawes, M. Arend, and C. Sperisen. 2015. How tree roots respond to drought. Frontiers in plant science 6.

Bussotti, F., E. Gravano, P. Grossoni, and C. Tani. 1998. Occurrence of tannins in leaves of beech trees (Fagus sylvatica) along an ecological gradient, detected by histochemical and ultrastructural analyses. New Phytologist 138:469-479.

Caldwell, B. A. 2005. Enzyme activities as a component of soil biodiversity: a review. Pedobiologia 49:637-644.

Carreiro, M., R. Sinsabaugh, D. Repert, and D. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359-2365.

Chapin, F. S., P. A. Matson, and P. Vitousek. 2011. Principles of terrestrial ecosystem ecology. Springer Science & Business Media.

Christensen, J., B. Hewitson, A. Busuioc, A. Chen, X. Gao, I. Held, R. Jones, R. Kolli, W. Kwon, and R. Laprise. 2007. Climate change 2007: the regional climate projections. IPCC.

Chung, H., D. R. Zak, and E. A. Lilleskov. 2006. Fungal community composition and metabolism under elevated CO2 and O3. Oecologia 147:143-154.

Clarholm, M. 1993. Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers. Biology and fertility of soils 16:287-292.

Collins, S. L., R. L. Sinsabaugh, C. Crenshaw, L. Green, A. Porras-Alfaro, M. Stursova, and L. H. Zeglin. 2008. Pulse dynamics and microbial processes in aridland ecosystems. Journal of Ecology 96:413-420.

Comerford, N., M. McLeod, and M. Skinner. 2002. Phosphorus form and bioavailability in the pine rotation following fertilization: P fertilization influences P form and potential bioavailability to pine in the subsequent rotation. Forest Ecology and Management 169:203-211.

Compton, J. E., L. S. Watrud, L. A. Porteous, and S. DeGrood. 2004. Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. Forest Ecology and Management 196:143-158.

Currie, W. S., R. D. Yanai, K. B. Piatek, C. E. Prescott, and C. L. Goodale. 2002. Processes affecting carbon storage in the forest floor and in downed woody debris. The potential for US forest soils to sequester carbon and mitigate the greenhouse effect. Boca Raton (FL): CRC Press. p:135-137.

Cusack, D. F., W. L. Silver, M. S. Torn, S. D. Burton, and M. K. Firestone. 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. Ecology 92:621-632.

Cusack, D. F., M. S. Torn, W. H. McDOWELL, and W. L. Silver. 2010. The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. Global Change Biology 16:2555-2572.

Das, S. K., and A. Varma. 2010. Role of enzymes in maintaining soil health. Pages 25-42 Soil enzymology. Springer.

Davey, M. P., B. Berg, B. A. Emmett, and P. Rowland. 2007. Decomposition of oak leaf litter is related to initial litter Mn concentrations. Botany 85:16-24.

DeForest, J. L., D. R. Zak, K. S. Pregitzer, and A. J. Burton. 2004. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biology and Biochemistry 36:965-971.

Dilly, O., and P. Nannipieri. 2001. Response of ATP content, respiration rate and enzyme activities in an arable and a forest soil to nutrient additions. Biology and fertility of soils 34:64-72.

Entry, J. A., G. B. Runion, S. A. Prior, R. J. Mitchell, and H. H. Rogers. 1998. Influence of CO2 enrichment and nitrogen fertilization on tissue chemistry and carbon allocation in longleaf pine seedlings. Pages 3-18 Root Demographics and Their Efficiencies in Sustainable Agriculture, Grasslands and Forest Ecosystems. Springer.

Everett, C. J., and H. Palm-Leis. 2009. Availability of residual phosphorus fertilizer for loblolly pine. Forest Ecology and Management 258:2207-2213.

Fenner, N., C. Freeman, and B. Reynolds. 2005. Hydrological effects on the diversity of phenolic degrading bacteria in a peatland: implications for carbon cycling. Soil Biology and Biochemistry 37:1277-1287.

Fioretto, A., S. Papa, E. Curcio, G. Sorrentino, and A. Fuggi. 2000. Enzyme dynamics on decomposing leaf litter of Cistus incanus and Myrtus communis in a Mediterranean ecosystem. Soil Biology and Biochemistry 32:1847-1855.

Fog, K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews 63:433-462.

Fox, T. R., H. L. Allen, T. J. Albaugh, R. Rubilar, and C. A. Carlson. 2007. Tree nutrition and forest fertilization of pine plantations in the southern United States. Southern Journal of Applied Forestry 31:5-11.

Freeman, C., G. Liska, N. Ostle, M. Lock, B. Reynolds, and J. Hudson. 1996. Microbial activity and enzymic decomposition processes following peatland water table drawdown. Plant and Soil 180:121-127.

Frey, S. D., M. Knorr, J. L. Parrent, and R. T. Simpson. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196:159-171.

Gallo, M., R. Amonette, C. Lauber, R. Sinsabaugh, and D. Zak. 2004. Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. Microbial Ecology 48:218-229.

Garcıa-Gil, J., C. Plaza, P. Soler-Rovira, and A. Polo. 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biology and Biochemistry 32:1907-1913.

Geisseler, D., W. R. Horwath, and K. M. Scow. 2011. Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. Pedobiologia 54:71-78.

Güsewell, S. 2005. High nitrogen: phosphorus ratios reduce nutrient retention and second-year growth of wetland sedges. New Phytologist 166:537-550.

Güsewell, S., and J. T. Verhoeven. 2006. Litter N: P ratios indicate whether N or P limits the decomposability of graminoid leaf litter. Plant and Soil 287:131-143.

Harder, W., and L. Dijkhuizen. 1983. Physiological responses to nutrient limitation. Annual Reviews in Microbiology 37:1-23.

He, Y., Y. Qi, Y. Dong, S. Xiao, Q. Peng, X. Liu, and L. Sun. 2013. Effects of nitrogen fertilization on soil microbial biomass and community functional diversity in temperate grassland in inner Mongolia, China. CLEAN–Soil, Air, Water 41:1216-1221.

Henry, H. A. 2012. Soil extracellular enzyme dynamics in a changing climate. Soil Biology and Biochemistry 47:53-59.

Henry, H. A., J. D. Juarez, C. B. Field, and P. M. Vitousek. 2005. Interactive effects of elevated CO2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. Global Change Biology 11:1808-1815.

Hobbie, S. E. 2000. Interactions between litter lignin and nitrogenitter lignin and soil nitrogen availability during leaf litter decomposition in a Hawaiian montane forest. Ecosystems 3:484-494.

Hobbie, S. E. 2005. Contrasting effects of substrate and fertilizer nitrogen on the early stages of litter decomposition. Ecosystems 8:644-656.

Hobbie, S. E. 2008. Nitrogen effects on decomposition: A five-year experiment in eight temperate sites. Ecology 89:2633-2644.

Hobbie, S. E., W. C. Eddy, C. R. Buyarski, E. C. Adair, M. L. Ogdahl, and P. Weisenhorn. 2012. Response of decomposing litter and its microbial community to multiple forms of nitrogen enrichment. Ecological Monographs 82:389-405.

Karl, T. R. 2009. Global climate change impacts in the United States. Cambridge University Press.

Keeler, B. L., S. E. Hobbie, and L. E. Kellogg. 2009. Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: implications for litter and soil organic matter decomposition. Ecosystems 12:1-15.

Kiser, L. C., T. R. Fox, and C. A. Carlson. 2013. Foliage and litter chemistry, decomposition, and nutrient release in Pinus taeda. Forests 4:595-612.

Knorr, W., I. Prentice, J. House, and E. Holland. 2005. Long-term sensitivity of soil carbon turnover to warming. Nature 433:298-301.

Kreyling, J., C. Beierkuhnlein, M. Elmer, K. Pritsch, M. Radovski, M. Schloter, J. Wöllecke, and A. Jentsch. 2008. Soil biotic processes remain remarkably stable after 100-year extreme weather events in experimental grassland and heath. Plant and Soil 308:175-188.

Laseter, S. H., C. R. Ford, J. M. Vose, and L. W. Swift. 2012. Long-term temperature and precipitation trends at the Coweeta Hydrologic Laboratory, Otto, North Carolina, USA. Hydrology research 43:890-901.

LeRoy, C. J., A. S. Wymore, R. Davis, and J. C. Marks. 2014. Indirect influences of a major drought on leaf litter quality and decomposition in a southwestern stream. Fundamental and Applied Limnology/Archiv für Hydrobiologie 184:1-10.

Li, X., and P. Sarah. 2003. Enzyme activities along a climatic transect in the Judean Desert. Catena 53:349-363.

Li, Y., M. Xu, O. J. Sun, and W. Cui. 2004. Effects of root and litter exclusion on soil CO2 efflux and microbial biomass in wet tropical forests. Soil Biology and Biochemistry 36:2111-2114.

Liang, B. C., and A. F. MacKenzie. 1995. Effect of fertilization on organic and microbial biomass nitrogen using15N under corn (Zea mays L.) in two Quebec soils. Fertilizer research 44:143-149.

Luo, P., X. Han, Y. Wang, M. Han, H. Shi, N. Liu, and H. Bai. 2015. Influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure in a brown soil of northeast China. Annals of microbiology 65:533-542.

Magill, A. H., and J. D. Aber. 1998. Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. Plant and Soil 203:301-311.

Matocha, C. J., G. R. Haszler, and J. H. Grove. 2004. Nitrogen fertilization suppresses soil phenol oxidase enzyme activity in no-tillage systems. Soil Science 169:708-714.

McDaniel, M., J. Kaye, and M. Kaye. 2013. Increased temperature and precipitation had limited effects on soil extracellular enzyme activities in a post-harvest forest. Soil Biology and Biochemistry 56:90-98.

Melillo, J. M., J. D. Aber, and J. F. Muratore. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621-626.

Miller, H., and J. Miller. 1976. Analysis of needle fall as a means of assessing nitrogen status in pine. Forestry 49:57-61.

Minocha, R., S. Long, A. H. Magill, J. Aber, and W. H. McDowell. 2000. Foliar free polyamine and inorganic ion content in relation to soil and soil solution chemistry in two fertilized forest stands at the Harvard Forest, Massachusetts. Plant and Soil 222:119-137.

Nannipieri, P., L. Giagnoni, L. Landi, and G. Renella. 2011. Role of phosphatase enzymes in soil. Pages 215-243 Phosphorus in action. Springer.

Noormets, A., M. J. Gavazzi, S. G. McNulty, J. C. DOMEC, G. Sun, J. S. King, and J. Chen. 2010. Response of carbon fluxes to drought in a coastal plain loblolly pine forest. Global Change Biology 16:272-287.

Olander, L. P., and P. M. Vitousek. 2000. Regulation of soil phosphatase and chitinase activityby N and P availability. Biogeochemistry 49:175-191.

Parham, J., S. Deng, W. Raun, and G. Johnson. 2002. Long-term cattle manure application in soil. Biology and fertility of soils 35:328-337.

Peñuelas, J., and M. Estiarte. 1998. Can elevated CO 2 affect secondary metabolism and ecosystem function? Trends in Ecology & Evolution 13:20-24.

Penuelas, J., C. Gordon, L. Llorens, T. Nielsen, A. Tietema, C. Beier, P. Bruna, B. Emmett, M. Estiarte, and A. Gorissen. 2004. Nonintrusive field experiments show different plant responses to warming and drought among sites, seasons, and species in a north–south European gradient. Ecosystems 7:598-612.

Prescott, C., R. Kabzems, and L. Zabek. 1999. Effects of fertilization on decomposition rate of Populus tremuloides foliar litter in a boreal forest. Canadian Journal of Forest Research 29:393-397.

Prescott, C. E. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101:133-149.

Purahong, W., D. Kapturska, M. J. Pecyna, E. Schulz, M. Schloter, F. Buscot, M. Hofrichter, and D. Krüger. 2014. Influence of different forest system management practices on leaf litter decomposition rates, nutrient dynamics and the activity of ligninolytic enzymes: a case study from Central European forests. PloS one 9:e93700.

Runion, G., J. Entry, S. Prior, R. Mitchell, and H. Rogers. 1999. Tissue chemistry and carbon allocation in seedlings of Pinus palustris subjected to elevated atmospheric CO2 and water stress. Tree Physiology 19:329-335.

Saiya-Cork, K., R. Sinsabaugh, and D. Zak. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology and Biochemistry 34:1309-1315.

Sall, S. N., D. Masse, F. Bernhard-Reversat, A. Guisse, and J.-L. Chotte. 2003. Microbial activities during the early stage of laboratory decomposition of tropical leaf litters: the effect of interactions between litter quality and exogenous inorganic nitrogen. Biology and fertility of soils 39:103-111.

Sardans, J., and J. Peñuelas. 2004. Increasing drought decreases phosphorus availability in an evergreen Mediterranean forest. Plant and Soil 267:367-377.

Sardans, J., and J. Peñuelas. 2005. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. Soil Biology and Biochemistry 37:455-461.

Sardans, J., and J. Peñuelas. 2007. Drought changes phosphorus and potassium accumulation patterns in an evergreen Mediterranean forest. Functional Ecology 21:191-201.

Sardans, J., and J. Peñuelas. 2010. Soil enzyme activity in a Mediterranean forest after six years of drought. Soil Science Society of America Journal 74:838-851.

Sardans, J., J. Peñuelas, and M. Estiarte. 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Applied Soil Ecology 2:223-235.

Sardans, J., A. Rivas-Ubach, and J. Peñuelas. 2012. The C: N: P stoichiometry of organisms and ecosystems in a changing world: a review and perspectives. Perspectives in Plant Ecology, Evolution and Systematics 14:33-47.

Shi, P., S. Wang, S. Jia, and Q. Gao. 2015. Effect of 25-year fertilization on soil microbial biomass and community structure in a continuous corn cropping system. Archives of Agronomy and Soil Science 61:1303-1317.

Sinsabaugh, R., M. Carreiro, and D. Repert. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60:1-24.

Sinsabaugh, R., and D. Moorhead. 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biology and Biochemistry 26:1305-1311.

Sinsabaugh, R. L., B. H. Hill, and J. J. F. Shah. 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795-798.

Sinsabaugh, R. L., C. L. Lauber, M. N. Weintraub, B. Ahmed, S. D. Allison, C. Crenshaw, A. R. Contosta, D. Cusack, S. Frey, and M. E. Gallo. 2008. Stoichiometry of soil enzyme activity at global scale. Ecology letters 11:1252-1264.

Sinsabaugh, R. L., and A. E. Linkins. 1993. Statistical modeling of litter decomposition from integrated cellulase activity. Ecology 74:1594-1597.

Skujiņš, J., and R. Burns. 1976. Extracellular enzymes in soil. CRC critical reviews in microbiology 4:383-421.

Steinweg, J., J. Dukes, E. Paul, and M. Wallenstein. 2012. Microbial responses to multi-factor climate change: effects on soil enzymes. Frontiers in microbiology 4:146-146.

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press.

Stone, M. M., M. S. Weiss, C. L. Goodale, M. B. Adams, I. J. Fernandez, D. P. German, and S. D. Allison. 2012. Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate forests. Global Change Biology 18:1173-1184.

Štursová, M., and P. Baldrian. 2011. Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity. Plant and Soil 338:99-110.

Thelin, G., U. Rosengren-Brinck, B. Nihlgard, and A. Barkman. 1998. Trends in needle and soil chemistry of Norway spruce and Scots pine stands in South Sweden 1985-1994. Environmental Pollution 99:149-158.

Thirukkumaran, C. M., and D. Parkinson. 2000. Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. Soil Biology and Biochemistry 32:59-66.

Tiemann, L. K., and S. A. Billings. 2011. Indirect effects of nitrogen amendments on organic substrate quality increase enzymatic activity driving decomposition in a mesic grassland. Ecosystems 14:234-247.

Toberman, H., C. Freeman, C. Evans, N. Fenner, and R. R. Artz. 2008. Summer drought decreases soil fungal diversity and associated phenol oxidase activity in upland Calluna heathland soil. FEMS microbiology ecology 66:426-436.

Treseder, K. K. 2008. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. Ecology letters 11:1111-1120.

Treseder, K. K., and P. M. Vitousek. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. Ecology 82:946-954.

Turlapati, S., R. Minocha, P. Bhiravarasa, L. Tisa, W. Thomas, and S. Minocha. 2012. Chronic N-amended soils exhibit an altered bacterial community structure in Harvard Forest, MA, USA. FEMS microbiology ecology.

Turner, D. P., G. J. Koerper, M. E. Harmon, and J. J. Lee. 1995. A carbon budget for forests of the conterminous United States. Ecological Applications 5:421-436.

van Diepen, L. T., S. D. Frey, C. M. Sthultz, E. W. Morrison, R. Minocha, and A. Pringle. 2015. Changes in litter quality caused by simulated nitrogen deposition reinforce the N-induced suppression of litter decay. Ecosphere 6:1-16.

Waldrop, M. P., and D. R. Zak. 2006. Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. Ecosystems 9:921-933.

Waldrop, M. P., D. R. Zak, R. L. Sinsabaugh, M. Gallo, and C. Lauber. 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecological Applications 14:1172-1177.

Wallenstein, M. D., S. McNulty, I. J. Fernandez, J. Boggs, and W. H. Schlesinger. 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. Forest Ecology and Management 222:459-468.

Wang, C., G. Han, Y. Jia, X. Feng, P. Guo, and X. Tian. 2011. Response of litter decomposition and related soil enzyme activities to different forms of nitrogen fertilization in a subtropical forest. Ecological Research 26:505-513.

Waring, B. G. 2013. Exploring relationships between enzyme activities and leaf litter decomposition in a wet tropical forest. Soil Biology and Biochemistry 64:89-95.

Weand, M. P., M. A. Arthur, G. M. Lovett, R. L. McCulley, and K. C. Weathers. 2010. Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. Soil Biology and Biochemistry 42:2161-2173.

Wear, D. N., and J. G. Greis. 2002. Southern forest resource assessment-technical report. Gen. Tech. Rep. SRS-53. Asheville, NC: US Department of Agriculture, Forest Service, Southern Research Station. 635 p. 53.

Yavitt, J. B., S. J. Wright, and R. K. Wieder. 2004. Seasonal drought and dry-season irrigation influence leaf-litter nutrients and soil enzymes in a moist, lowland forest in Panama. Austral Ecology 29:177-188.

Yuste, J. C., J. Penuelas, M. Estiarte, J. GARCIA-MAS, S. Mattana, R. Ogaya, M. Pujol, and J. Sardans. 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Global Change Biology 17:1475-1486.

Zak, D. R., W. E. Holmes, A. J. Burton, K. S. Pregitzer, and A. F. Talhelm. 2008. Simulated atmospheric NO3− deposition increases soil organic matter by slowing decomposition. Ecological Applications 18:2016-2027.

Zechmeister-Boltenstern, S., K. M. Keiblinger, M. Mooshammer, J. PENuelas, A. Richter, J. Sardans, and W. Wanek. 2015. The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. Ecological Monographs 85:133-155.

Zeglin, L. H., M. Stursova, R. L. Sinsabaugh, and S. L. Collins. 2007. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. Oecologia 154:349-359.

Zhang, L., W. Chen, M. Burger, L. Yang, P. Gong, and Z. Wu. 2015. Changes in soil carbon and enzyme activity as a result of different long-term fertilization regimes in a greenhouse field. PloS one 10:e0118371.

Zhu, B., K. Panke-Buisse, and J. Kao-Kniffin. 2015. Nitrogen fertilization has minimal influence on rhizosphere effects of smooth crabgrass (Digitaria ischaemum) and bermudagrass (Cynodon dactylon). Journal of Plant Ecology 8:390-400.

Table 3.1. Extracellular enzymes assayed in mineral soil (A horizon) and litter (Oi horizon) in an 11-year-old loblolly pine plantation, their abbreviations, functions, corresponding substrates, and incubation length.

Enzyme	Abbreviation	Function	Substrate	Incubation length (h)
Acid Phosphatase	AP	Organic P mineralization to phosphate	4-MUB phosphate	0.5
N-acetyl-β-glucosaminidase	NAG	Chitin degradation; N mineralization	4-MUB-N-acetyl-β-D-glucosaminide	0.5
β-glucosidase	BG	Cellulose and sugar degradation	4-MUB -β-D-glucopyranoside	3
α-glucosidase	AG	Starch and sugar degradation	4-MUB-α-D-glucopyranoside	3
β-xylosidase	XYL	Hemicellulose degradation	4-4-MUB-β-D-xylopyranoside	3
β-D-cellubiosidase	CHB	Cellulose degradation	4-MUB-β-D-cellobioside	3
Leucine aminopeptidase	LAP	Protein and peptide degradation	L-leucine-7-amido-4-methylcoumarin hydrochloride	18
Polyphenol oxidase	POX	Lignin and aromatic polymer degradation	L-DOPA	24
Peroxidase	PER	Lignin degradation, catalyzes oxidation reactions	L-DOPA + 0.3 % H_2O_2	24

MUB = methylumbelliferyl; LDOPA = L-dihydroxy phenylalanine

Table 3.2. Analysis of variance for mineral soil (A horizon) and litter (Oi) horizon biochemical parameters collected from an 11-year-old loblolly pine plantation.

	Fert		**TR**		**Fert×TR**	
	F	*P*	*F*	*P*	*F*	*P*
Soil						
Organic matter (%)	0.015	0.905	0.089	0.773	0.303	0.595
pH	0.278	0.611	4.873	0.055	2.034	0.188
N (%)	0.159	0.700	1.984	0.193	0.506	0.490
C (%)	0.890	0.674	1.726	0.218	0.362	0.562
P (mg kg $^{-1}$)	32.000	**<0.001**	4.520	0.063	12.510	**0.006**
Mn (mg kg $^{-1}$)	0.098	0.760	0.106	0.753	0.991	0.346
C:N	0.156	0.702	0.035	0.856	0.022	0.887
C:P	12.360	**0.007**	0.156	0.109	0.511	0.493
MBC	5.201	**0.048**	0.002	0.967	1.929	0.195
MBN	6.218	**0.034**	2.231	0.169	0.557	0.474
Litter						
Soluble C extractives (%)	7.684	**0.022**	3.105	0.112	0.720	0.417
Sugars (%)	5.706	**0.041**	1.847	0.207	1.652	0.231
Cellulose (%)	0.220	0.628	6.438	**0.032**	0.430	0.529
Hemicellulose (%)	1.599	0.238	5.455	**0.044**	3.522	0.093
Lignin (%)	0.954	0.354	0.293	0.602	2.309	0.163
Organic matter (%)	25.385	**0.001**	1.895	0.202	3.370	0.099
pH	0.283	0.621	4.335	0.059	2.134	0.197
N (%)	59.926	**<0.001**	8.128	**0.019**	0.011	0.446
C (%)	0.173	0.688	0.539	0.482	1.774	0.216
P (%)	18.969	**0.002**	9.298	**0.014**	0.018	0.897
Mn (%)	16.997	**0.003**	9.072	**0.015**	4.603	0.061
Lignin:N	61.882	**0.000**	8.899	**0.015**	0.025	0.878
Lignin:P	20.257	**0.002**	11.631	**0.008**	0.049	0.83
MBC	3.368	0.0996	0.347	0.571	1.567	0.242
MBN	5.372	**0.045**	1.643	0.232	0.006	0.939
MBP	3.27	0.104	4.731	0.057	1.628	0.308

Treatment abbreviations are noted Fert, fertilization; TR, throughfall reduction; Fert×TR, fertilization x throughfall reduction interaction. MB; microbial biomass

Table 3.3. Mineral soil (A) horizon and litter (Oi) horizon biochemical properties collected from an 11-year-old loblolly pine plantation. Data presented in bold represent significant treatment main effects or significant interactions. Data represent plot means (n=4) and standard errors in parentheses. Lowercase letters denote significant differences ($P < 0.05$).

	Treatment Main Effects								Treatment Interaction			
Parameter	No Fert		Fert		No TR		TR		C	Fert	TR	Fert×TR
Soil												
Organic Matter (%)	5.91 (1.60)	a	5.64 (1.23)	a	5.45 (1.14)	a	6.09 (1.65)	a				
N (%)	0.17 (0.14)	a	0.16 (0.01)	a	0.17 (0.01)	a	0.15 (0.01)	a				
C (%)	4.73 (0.45)	a	4.49 (0.41)	a	4.98 (0.48)	a	4.24 (0.33)	a				
P (mg kg $^{-1}$)									**3.75 (0.48) b**	**4.50 (0.29) b**	**3.25 (0.25) b**	**6.50 (0.65) a**
Mn (mg kg $^{-1}$)	1.15 (0.01)	a	1.08 (0.01)	a	1.16 (0.01)	a	1.08 (0.02)	a				
C:N	28.32 (0.72)	a	27.88 (1.03)	a	28.00 (0.96)	a	28.21 (0.83)	a				
C:P	**1.39×10^4 (1.55×10^3) a**		**8.73×10^3 (1.20×10^3) b**		1.25×10^4 (1.64×10^3) a		1.00×10^4 (1.60×10^3) a					
pH	4.51 (0.05)	a	4.47 (0.04)	a	4.41 (0.07)	a	4.56 (0.04)	a				
MBC (µg N per g dry soil^{-1})	**187.90 (29.08) b**		**269.31 (32.86) a**		200.04 (25.60) a		234.12 (64.32) a					
MBN (µg N per g dry soil^{-1})	**11.78 (3.48) b**		**22.51 (5.34) a**		13.32 (3.00) a		16.99 (5.00) a					
Litter												
Soluble C extractives (%)	**17.34 (1.36) a**		**13.68 (1.02) b**		14.35 (1.48) a		13.08 (1.81) a					
Sugars (%)	**53.48 (1.07) a**		**50.23 (1.45) b**		53.78 (1.37) a		52.03 (1.38) a					
Cellulose (%)	29.92 (0.46) a		29.16 (0.63) a		**30.64 (0.30) a**		**29.44 (0.32) b**					
Hemicellulose (%)	32.57 (0.74) a		31.75 (0.21) a		**31.40 (0.23) b**		**32.93 (0.65) a**					
Organic matter (%)	**97.33 (0.10) b**		**97.93 (0.08) a**		97.33 (0.10) a		97.93 (0.08) b					
N (%)	**0.39 (0.02) b**		**0.61 (0.04) a**		**0.54 (0.05) a**		**0.46 (0.05) b**					
P (%)	**0.03 (0.00) b**		**0.05 (0.00) a**		**0.04 (0.00) a**		**0.03 (0.00) b**					
Mn (%)	**0.09 (0.00) a**		**0.08 (0.00) b**		**0.087 (0.00) b**		**0.091 (0.00) a**					
Lignin:N	**95.97 (3.10) a**		**64.00 (4.18) b**		**73.93 (6.45) b**		**86.05 (6.93) a**					
Lignin:P	**1.23×10^3 (87.98) a**		**894.67 (87.93) b**		**934.99 (94.62) b**		**1.19×10^3 (99.38) a**					
MBC (µg N per g dry soil^{-1})	4.12×10^3 (5.45×10^2) a		4.67×103 (6.19×10^2) a		4.87×10^3 (4.34×10^2) a		5.3×10^3 (5.20×10^2) a					
MBN (µg N per g dry soil$^{-1)}$	**1.96 (0.44) b**		**6.76 (2.58) a**		4.03 (1.75) a		4.72 (2.31) a					

Treatment abbreviations are noted by C, control; Fert, fertilization; TR, throughfall reduction; FertxTR, fertilization x throughfall reduction

Table 3.4. Analysis of variance for mineral soil (A horizon) and litter (Oi) horizon extracellular enzyme activity collected from an 11-year-old loblolly pine plantation.

	Fert		**TR**		**Fert×TR**	
Enzyme	*F*	*P*	*F*	*P*	*F*	*P*
Soil						
AP	5.992	**0.037**	0.022	0.885	0.658	0.438
NAG	14.289	**0.004**	8.059	**0.019**	9.371	**0.014**
BG	1.762	0.217	0.022	0.884	1.658	0.230
AG	2.096	0.182	0.044	0.838	0.964	0.352
XYL	11.029	**0.009**	2.149	0.177	0.846	0.382
CHB	1.417	0.264	0.002	0.999	0.112	0.746
LAP	1.364	0.273	5.587	**0.042**	2.528	0.146
POX	5.042	**0.051**	0.603	0.457	2.465	0.151
PER	7.291	**0.024**	7.813	**0.021**	6.473	**0.032**
Litter						
AP	0.874	0.374	2.514	0.147	0.690	0.428
NAG	5.757	**0.039**	5.328	**0.046**	10.733	**0.009**
BG	17.732	**0.002**	16.206	**0.003**	3.784	0.083
AG	8.625	**0.017**	5.069	**0.050**	1.749	0.219
XYL	10.958	**0.009**	5.326	**0.046**	2.887	0.124
CHB	19.120	**0.002**	5.957	**0.037**	3.820	0.082
LAP	15.273	**0.004**	3.694	0.087	0.216	0.653
POX	0.155	0.703	0.781	0.400	1.661	0.230
PER	1.293	0.285	5.239	**0.048**	0.008	0.933

Treatment abbreviations are noted Fert, fertilization; TR, throughfall reduction; Fert×TR, fertilization x throughfall reduction interaction

Table 3.5. Potential extracellular enzyme activities in mineral soil (A) horizon and litter (Oi) horizon collected from an 11-year-old loblolly pine plantation. Data presented in bold represent significant treatment main effects or significant interactions. Data represent plot means (n=4) and standard errors in parentheses. Lowercase letters denote significant differences ($P < 0.05$).

	Treatment Main Effects				Treatment Interaction			
Enzyme	No Fert	Fert	No TR	TR	C	Fert	TR	Fert×TR
Soil								
AP	**13.88 (1.44) a**	**10.81 (0.86) b**	12.44 (1.14) a	12.254 (1.48) a				
NAG					**4.13 (0.54) a**	**1.80 (0.28) b**	**2.12 (0.06) b**	**1.88 (0.22) b**
XYL	**0.73 (0.06) b**	**1.05 (0.10) a**	0.96 (0.09) a	0.82 (0.10) a				
LAP	0.02 (0.01) a	0.03 (0.01) a	**0.02 (0.00) b**	**0.04 (0.01) a**				
POX	**3.50 (1.19) a**	**0.81 (0.33) b**	2.62 (1.14) a	1.69 (0.84) a				
PER					**1.48 (1.60) b**	**7.35 (0.47) a**	**7.56 (3.14) a**	**7.93 (1.82) a**
Litter								
NAG					**11.30 (1.85) a**	**12.69 (1.10) a**	**12.85 (1.60) a**	**6.04 (1.46) b**
BG	**8.30 (0.64) b**	**12.37 (1.45) a**	**8.38 (0.67) b**	**12.26 (1.48) a**				
AG	**0.98 (0.10) b**	**1.60 (0.22) a**	**1.05 (0.10) b**	**1.53 (0.24) a**				
XYL	**0.92 (0.07) b**	**1.52 (0.21) a**	**1.01 (0.08) b**	**1.43 (0.23) a**				
CHB	**4.26 (0.26) b**	**7.08 (0.81) a**	**4.86 (0.34) b**	**6.50 (0.82) a**				
LAP	**0.15 (0.04) b**	**0.21 (0.01) a**	0.16 (0.01) a	0.19 (0.02) a				
PER	0.70 (0.21) a	0.95 (0.22) a	**0.58 (0.15) b**	**1.08 (0.01) a**				

EEA units are µmol g dry soil^{-1} hr^{-1}. Treatment abbreviations are noted by C, control; Fert, fertilization; TR, throughfall reduction; FertxTR, fertilization x throughfall reduction.

Table 3.6. Significance of relationships between mineral soil (A horizon) and litter (Oi horizon) enzyme activities and biochemical parameters collected from an 11-year-old loblolly pine plantation.

	AP	NAG	BG	AG	XYL	CHB	LAP	POX	PER
Soil									
OM (%)	-	-	-	0.24 (+) *	-	-	0.21 (+) †	-	-
pH	-	-	-	-	-	-	-	-	-
N (%)	-	0.20 (+) †	-	-	-	-	-	-	-
C (%)	0.22 (+) †	-	-	-	-	-	-	-	-
P (mg kg $^{-1}$)	0.28 (-) *	-	-	0.31 (-) *	0.29 (+) *	-	0.42 (+) **	-	-
Mn (mg kg $^{-1}$)	-	0.23 (+) †	-	-	-	-	-	-	-
C:N	-	-	-	-	-	-	-	-	-
C:P	0.50 (+) **	0.35 (+) **	-	-	-	-	-	-	0.21 (-) †
MBC	-	-	-	0.30 (+) *	-	-	0.30 (+) *	-	-
MBN	-	-	-	0.43 (+) **	0.27 (+) *	0.22 (+) †	-	-	-
Litter									
Soluble C extractives (%)	-	-	-	-	-	-	-	-	-
Sugars (%)	-	-	0.28 (-) *	-	-	-	-	-	-
Cellulose (%)	-	-	-	-	-	-	-	-	0.20 (-) †
Hemicellulose (%)	-	-	-	-	-	-	-	-	-
Lignin (%)	-	-	-	-	-	-	-	0.34 (-) **	0.35 (+) **
OM (%)	-	0.20 (-) †	0.21 (+) †	-	-	0.35 (+) **	0.28 (+) *	-	-
pH	-	0.20 (-) †	-	-	-	-	-	0.45 (-) **	-
N (%)	-	-	-	0.24 (+) *	0.34 (+) *	0.33 (+) *	0.44 (+) **	-	-
C (%)	-	-	-	-	-	-	-	-	-
P (%)	-	-	-	-	-	0.25 (+) *	0.29 (+) *	-	-
Mn (%)	-	0.26 (+) *	-	-	-	0.25 (-) *	0.24 (-) *	-	-
Lignin:N	-	-	-	0.28 (-) *	0.36 (-) *	0.38 (-) **	0.43 (-) **	-	-
Lignin:P	-	-	-	0.27 (-) *	0.30 (-) *	0.27 (-) *	0.27 (-) *	-	-
MBC	-	-	-	-	-	-	-	-	-
MBN	-	-	-	-	-	-	-	-	-
MBP	-	0.30 (-) *	-	-	-	-	-	-	-

Significant individual linear regressions with enzyme activity are indicated by the R^2 value, significance, and sign of the relationship (in parentheses); *P < 0.05, **P < 0.01, ***P < 0.001, †P < 0.10, -, not significant. n=16. MB; microbial biomass

Chapter 4. The impact of water content on sources of heterotrophic soil respiration

Abstract

Heterotrophic respiration (R_H) is a major flux of CO_2 from forest ecosystems and represents a large source of uncertainty in estimating net ecosystem productivity (NEP) using regional soil respiration (R_S) models. R_H from leaf litter (R_{HL}) may contribute greatly to annual R_H estimates, but its contribution may be misrepresented due to the logistical and technical challenges associated with chamber-based field measurements of R_{HL}. The purpose of this study was to evaluate the sensitivity of sources of R_H (mineral soil-derived heterotrophic respiration; R_{HM}, and leaf litter-derived heterotrophic respiration; R_{HL}) of a loblolly pine plantation (*Pinus taeda* L.) to varying soil and litter water content over the course of a dry down event. Additionally, we investigated whether fertilization influenced R_{HL} and R_{HM} to understand how forest nutrient management may impact forest soil carbon (C) dynamics. R_{HL} was measured under dry conditions and at field capacity to evaluate water content controls on R_{HL}, determine the duration of increased CO_2 release following wetting, and evaluate the potential contribution to total R_H. We also measured R_{HM} inside collars that excluded plant roots and litter inputs, from field capacity until near-zero R_{HM} rates were attained. We found that R_{HL} was more sensitive to water content than R_{HM}, and increased linearly with increasing litter water content ($R^2 = 0.89$). The contribution of R_{HL} to R_H was greatest immediately following the wetting event, and decreased rapidly to near-zero between three – 10 days. R_{HM} also had a strong relationship with soil water content ($R^2 = 0.62$), but took between 200 – 233 days to attain near-zero R_{HM} rates. Fertilization had no effect on R_{HM} ($P = 0.657$), but significantly suppressed R_{HL} rates after the wetting event ($P < 0.009$). These results demonstrate that there is great temporal variability in

both CO_2 released and the water content of differing sources of R_H, and forest fertilization may largely impact forest floor C stocks. This variability may not be captured reliably using conventional weekly to monthly chamber-based field sampling efforts and could lead to over-or under-estimation of R_H. In the context of climate change, changes in the frequency and intensity of wetting and drying events will likely alter R_{HL} and its contribution to R_S. Separate consideration of R_H sources and controls, along with increased field sampling frequency using chamber-based methodology under a broader range of specific environmental conditions, are likely needed to reduce variability in R_H estimates and improve the accuracy of forest NEP predictions.

Keywords: Carbon cycle, Fertilization; Soil CO_2 efflux; Leaf litter; Loblolly pine plantations

4.1 Introduction

Current climate models predict a change in annual precipitation regimes for the southeastern United States and it is important to understand how these changes will affect the carbon (C) balance of forest ecosystems in this region (Mitchell et al. 2014, Rocca et al. 2014). Southeastern forests currently have among the highest potential for C storage as they represent nearly 60% of the regional land area, are aggrading biomass with age and with intensive management practices (Dale et al. 2001, Jokela et al. 2010), and have high rates of net primary productivity (NPP) (Wear and Greis 2002, Mitchell et al. 2014, Rocca et al. 2014). Altered regional precipitation and evapotranspiration, or hydroclimate, is posited to have the largest consequences for productivity and C sequestration potential. Water balance of a site can attenuate or exacerbate effects on NPP as a result of elevated atmospheric CO_2, warming temperatures, and consequently longer growing seasons (McNulty et al. 1996, Rocca et al. 2014). While increases in annual temperature of 1- 4 ° C (Mearns et al. 2009, Mitchell et al. 2014), along with increases in annual precipitation of up to 6 percent are generally predicted for this region (Mearns et al. 2009, Ingram et al. 2013, Rocca et al. 2014), intensification of annual precipitation regimes are expected, meaning exacerbated wet and dry periods, increases in the frequency of summer droughts, and larger rainfall events between drought periods (Groisman et al. 2004, Angert et al. 2005, Laseter et al. 2012). Drier summers can counter early season growth in the southeast by increasing evapotranspiration and decreasing soil moisture, which is a limiting factor controlling NPP, the decomposition of organic matter pools, and thus C and nutrient cycling (Angert et al. 2005). The interaction of increasing growing season temperatures and decreasing growing season precipitation may potentially decrease the C sequestration

capacity of southern pine forests by 10% (Noormets et al. 2010), and increase the variability of ecosystem C flows.

To understand how changes in moisture dynamics may influence the net C balance or net ecosystem production (NEP) of southeastern forests, the separation of total soil respiration (R_S) into components of heterotrophic, microbial respiration (R_H; i.e., decomposition) and autotrophic, root respiration (R_A) is necessary (Hanson et al. 2000, Subke et al. 2006). NEP is estimated from the balance between net primary production (NPP) and R_H (Woodwell and Whitaker 1968). Recently, considerable effort has been devoted to quantifying fluxes of CO_2 from southeastern pine forests to develop regional respiration models (Templeton et al. 2015), and to improve predictions of NEP by quantifying the R_H contribution to R_S (Heim 2014, Noormets et al. 2015, ArchMiller and Samuelson 2016, McElligott et al. 2016). These efforts have yielded highly variable predicted contributions of R_H to R_S in these ecosystems, ranging from of 55 - 84 percent (ArchMiller and Samuelson 2016, Brown 2016, McElligott et al. 2016, Brown). This variability may be attributed in part to the fact that that sources of R_S have different sensitivities to controlling factors such as water content, soil temperature, and substrate availability and composition (Luo and Zhou 2006, Suseela et al. 2012). Heterotrophic sources in particular have been shown to be more sensitive to water content than R_A (Inglima et al. 2009, Wang et al. 2014). Within heterotrophic sources, R_{HL} is more dynamic than R_{HM}, as surface litter layers are more susceptible to extremes and rapid changes in temperature and water content than subsurface mineral layers, and have a greater dependency on moisture than temperature (Hanson et al. 2003, Wang et al. 2014). Additionally, R_{HL} and R_{HM} rates differ temporally due to differences in substrate type and availability, and microbial community abundance and composition (Tewary et al. 1982, Boone et al. 1998, Chen et al. 1999).

R_{HL} may be a large source of uncertainty when estimating R_S and NEP due to the transient nature of this C pool and its properties. R_{HL} may contribute from 11 – 51 % of R_S (Hanson et al. 2003, Cisneros-Dozal et al. 2007, DeForest et al. 2009, Ataka et al. 2014, Xiao et al. 2014), suggesting the forest floor could be a primary source of C loss from forest ecosystems. However, the contribution of R_{HL} to R_S is largely dependent upon litter water content and the frequency and amount of precipitation (Hanson et al. 2003, Ataka et al. 2014). Our understanding of the controls over R_{HL} is limited by the lack of direct R_{HL} measurements and the inability to non-destructively and continuously measure leaf litter water content. Attempted seasonal, *in situ* spot measurements of R_{HL} using chamber-based methods have yielded near zero rates in loblolly pine forests of VA (McElligott et al. 2016 unpublished data). These near zero rates may be a result of the dynamic nature of litter moisture content, and the rapid and transient CO_2 pulse following surface wetting events (i.e. Birch effect; Birch 1958, Jarvis et al. 2007, Inglima et al. 2009), that may be missed with non-continuous spot measurements. The difference in temporal resolution of moisture control over litter compared to mineral soil is important to rectify to improve estimates of R_H.

To understand how R_{HL} and R_{HM} respond independently to water content, we measured these variables on samples collected from a mid-rotation loblolly pine forest over the course of a dry down event. Additionally, we compared R_{HL} and R_{HM} from samples collected from fertilized and unfertilized plots, as forest fertilization is a common management practice in southeastern pine forests (Fox et al. 2007), with the potential to affect C turnover and respiration rates with separate studies showing everything from increases, decreases, or even no effect (Knorr et al. 2005). This ambiguity has been attributed in part to the divergent and potentially counteracting response of sources of R_A and R_H to mineral nutrient additions, with R_A often increasing with

fertilization due to increased biomass (Lu et al. 1998, Gough et al. 2004) and R_H decreasing due to the decline in microbial activity (Thirukkumaran and Parkinson 2000). R_{HL} was measured first under dry conditions and then at field capacity to evaluate the temporal patterns occurring as a result of a wetting event. We hypothesized that R_{HL} would be more sensitive to water content than R_{HM}, in part due to greater availability of labile C and greater microbial biomass in litter relative to mineral soil (Van Gestel et al. 1993, Borken et al. 2003), and that fertilization would result in suppressed rates of respiration in both the mineral soil and leaf litter. Partitioning R_H into R_{HM} and R_{HL} can enhance our understanding of controls over these components, and identify sources of variability in ecosystem R_S and NEP estimates.

4.2 Materials and Methods

4.2.1 Site Description

This research is a component of the Pine Integrated Network: Education, Mitigation, and Adaptation project (PINEMAP; http://pinemap.org/). This regional study was designed to evaluate the effects of decreased rainfall and nutrient additions on loblolly pine plantation productivity and physiology (Will et al. 2015). Four locations were established to span the full temperature and precipitation range of the species. Leaf litter samples and soil cores used in this incubation study were collected from a 12-year-old loblolly pine (*Pinus taeda* L.) plantation located in the Appomattox-Buckingham State Forest, VA (37°27´37´´N, 78°39´50´´W); an existing 1.3 ha, long-term throughfall reduction x fertilization study installed during spring of 2012. Additional information on study installation and site characteristics can be found in Will et al. (2015).

Mean annual precipitation for this region is 109 cm. The average growing season temperature (April through September) is 22.9 °C and the average winter temperature (December to February) is 3.8 °C (NCDC.NOAA.gov accessed November 27, 2015). Soils are well drained, formed in residuum from metamorphic rocks (sericite schist, graphitic schist, and/or phyllite). Soils are mapped as Spears Mountain (fine, mixed, semiactive, mesic Typic Hapludult) and Littlejoe (fine, mixed, subactive, mesic, Typic Hapludult) series (Soil Survey Staff, accessed June 28, 2016).

4.2.2 Soil and Litter Sampling

The field study was design as a randomized complete block with four replications of a 2 x 2 factorial of throughfall reduction (0 and 30% reduction) and fertilization with either no addition or a complete suite of essential nutrients to represent optimum nutrition, randomly assigned to each block. However, for this incubation study, litter and soil samples were collected from the four control and fertilization treatment plots only. The fertilization treatment was broadcast applied once in spring, 2012. Nitrogen (N) was applied at a rate of 224 kg N ha^{-1} as urea, and phosphorus (P) was applied at a rate of 27 kg P ha^{-1} as diammonium phosphate. Elemental potassium (K) was applied at a rate of 56 kg K ha^{-1} as potash, and a granular oxysulfate micronutrient mix (Southeast Mix, Cameron Chemicals, Inc., Virginia Beach, VA, USA) consisting of 6 percent sulfur (S), 5 percent boron (B), 2 percent copper (Cu), 6 percent manganese (Mn), and 5 percent zinc (Zn) was applied at a rate of 22.4 kg ha^{-1}. Treatment plots measured 14.6 x 16.8 m, surrounded by a 6.1 m buffer on all sides.

To facilitate independent measures of R_{HM}, root exclusion cores constructed from steel conduit pipe (11.43 cm diameter x 35 cm length) were installed in the summer of 2013 in three locations within each treatment plot to sever roots for a preceding study (see Heim 2014). Cores

were driven vertically into the soil until the top was flush with the mineral soil surface. Prior to core installation, the O horizon (forest floor) was temporarily removed from the installation location to avoid driving organic matter into the soil profile or inside the core, and replaced after installation. Carbohydrate supply to roots is assumed to be depleted 90 days post installation, and root-derived, autotrophic respiration (R_A) inside the pipe is assumed to be zero thereby providing an independent measure of R_{HM} (Heim et al. 2015). This laboratory study utilized the existing in-tact root-severing field cores to isolate R_{HM} from R_A in a controlled environment. Cores were extracted from the field locations on four separate occasions from June through July, 2015, loosely wrapped with plastic to prevent soil and moisture loss, and transported to a temperature-controlled lab within four hours of collection. Mean air temperature and relative humidity (RH) were measured daily throughout the duration of the experiment using an Amprobe THWD-5 RH and temperature meter (Danaher Corporation USA, Everett, WA). Mean lab air temperature was 22.5 ± 0.07 and mean RH was 48.7 ± 0.04. On each of the four field sampling occasions, three replicate cores were collected from one control and one fertilization treatment plot located within one block, with the exception of block two where only two replicates were collected due to the inability to locate one previously installed root-severing cores. This sampling resulted in a total of 11 replicate cores from each treatment. Cores remained root-free while in the field, as no visible root ingrowth from the bottom up occurred, and any vegetation growth inside the pipe was clipped in advance of extraction. Once in the lab, cores were placed upright with their bottoms in 10 cm of oven-dried (105 °C), sterilized sand to maintain their upright position and minimize the likelihood of ambient air entering through the bottom of the core during subsequent respiration measurements. Cores were allowed to equilibrate for 48 hours prior to initiating respiration measurements.

Leaf litter from the Oi horizon was collected concurrently with the root-exclusion core extractions, for a total of 11 replicate samples from each treatment. The Oi horizon at this site was approximately 0.04 m thick. Litter was collected adjacent to each of the three root-severing cores from a 10 cm × 10 cm area, homogenized, and composited into one sample from each of the eight (2 fertilized treatments x 4 blocks) treatment plots. Litter samples were placed into paper bags and transported to the lab, then removed from bags and placed on trays to air dry for 48 hours before initiating the wetting event and respiration measurements.

4.2.3 R_{HM} and R_{HL} laboratory measurements

Eleven replicate microcosms for each treatment, three from blocks one, two, and four, and two from block three, were constructed using the extracted root-severing cores and PVC collars placed atop the cores to contain corresponding litter samples. Air-dry litter from each treatment plot was homogenized and 20 g was added to a 10 cm diameter × 10 cm height PVC collar to simulate the quantity and depth of the Oi horizon from the field site. One layer of 2 mm mesh size, gray fiberglass screen was applied to the bottom of each collar to prevent litter loss and allow for water drainage. Microcosms (litter collar + soil core) remained intact during the duration of the incubation, except when independent respiration measurements were being made. The initial weight of the litter collars and soil cores were measured before the start of the dry down, and immediately after each respiration measurement during the course of the experiment for later determination of gravimetric water content (GWC) at the time of each measurement. Soil and litter water content was expressed by weight as the ratio of the mass of water present to the dry weight of the samples. The water mass was determined by drying the soil core or litter microcosm after the incubation was completed, to a constant weight and measuring the sample

before and after drying. Soil cores were dried to a constant weight in a drying oven at 105 °C and litter was dried to a constant weight at 65 °C. Respiration measurements were made using a LI-8100A automated soil gas flux system and LI-8100-102 10 cm Survey Chamber (LI-COR Bioscience Inc., Lincoln, NE, USA). Measurements were logged for 90 s following CO_2 equilibration within the chamber. Laboratory air temperature and relative humidity was controlled for the duration of the incubation; air temperature remained between 21-23 °C and relative humidity remained between 46 and 49 percent.

One wetting event was initiated for R_{HL} measurements over the course of the dry down to bring litter to field capacity. Prior to wetting, mass of the sample and an initial respiration measurement was taken to confirm the respiration rates were zero or near-zero (i.e., rates less than 0.20 µmol m^{-2} s^{-1}) prior to the wetting event. Litter was brought to field capacity by layering the bottom of a 6 L capacity polypropylene container with 50 ml of deionized (DI) water (2 cm depth), then placing the collars in the container and irrigating the top of the collars with 200 ml of DI water to fully saturate the litter. The container was sealed to bring the container relative humidity to 100 percent, as measured using the Amprobe THWD-5 RH meter, and litter was allowed to equilibrate for five minutes. The collars were then removed from the container and allowed to drain and the weight to stabilize. Once excess water was removed, the starting weights were recorded and sequential respirations measurements were initiated. The bottom of the collar was sealed during active respiration measurements using closed cell rubber pipe insulation. Two samples, one from each treatment, were irrigated at a single time, and respiration measurements and sample weights were recorded systematically for 12 hours each day until the respiration rate reached 0, or reached a 95 percent rate-reduction from the initial peak rate. The

duration of the incubation varied by sample and ranged from three to 10 days to achieve near zero rates.

Soil cores did not receive a wetting event as they were near field capacity [between 32 and 45 percent volumetric water content] (*see* Saxton et al. 1986, van Genuchten et al. 1991) at the start of the dry down. Volumetric water content was estimated using a 12 cm HydroSense soil-water sensor (Campbell Scientific USA, Logan, UT) at the time of core extraction, and by converting GWC to VWC by multiplying GWC by the bulk density of soil (site-wide mean bulk density from 0-30 cm depth was 1.32 ± 0.16 g cm^{-3}) divided by the density of water (assuming 1.0 g cm^{-3}; Pikul 2003). R_{HM} and weight measurements began after the initial 48-hour equilibration period following sample collection, and continued until the respiration rates were near zero, or reduced by 95 percent from the peak measurement rate. The duration of the R_{HM} dry down to achieve near 0 rates varied by core but ranged from 200-223 days.

4.2.4 Statistical Analysis

The strength of the relationships between R_{HL} and R_{HM} and gravimetric water content for litter and mineral soil for each treatment were analyzed using linear regression with a significance level of $\alpha = 0.05$ (n=11). Variables were log transformed for fit or as necessary to achieve normal distribution before the regressions, and log(x+1) was applied to the efflux values due to the presence of values of zero in the dataset. The difference of the slopes and intercepts of R_{HM} or R_{HL} and moisture between the two treatment types (unfertilized and fertilized) was compared using ANOVA. An exponential decay function was fit to the relationship between R_{HL} and R_{HM}, and GWC over time to derive the decay constant, or *k*. The three parameter exponential decay equation is fit using the formula:

$$y = a + be^{kt} \quad (1)$$

All statistical analyses were performed using JMP® 11 software system (SAS Institute, Cary, NC, USA).

4.3 Results

R_{HL} and R_{HM} decreased over the course of the dry down experiment, and with decreasing GWC (Figures 4.1 and 4.2). Prior to the wetting event, R_{HL} of all treatments were near-zero and reached a peak rate in the first measurement following the wetting event that brought the litter water content to field capacity (Figure 4.2). GWC of litter and soil was a significant predictor of R_{HL} and R_{HM} ($P < 0.0001$), but had greater predictive value and a stronger linear relationship for R_{HL} than R_{HM}. Across all samples, litter GWC explained 89 percent of the variance in cumulative R_{HL} (i.e., R^2=0.89) using the equation:

$$\log(CO_2 + 1) = -0.318 + 0.570 \times \log(GWC) \quad (1)$$

The regression of R_{HL} on GWC differs between fertilized and unfertilized plots (Fig. 4.3) as the slopes of the regression equations differed significantly ($P < 0.0001$; e.g., 0.619 for unfertilized and 0.517 for fertilized R_{HL}), and the mean response for fertilization differs at zero (P<0.0001; e.g. intercepts of -0.375 for unfertilized and -0.259 for fertilized R_{HL}). The mean R_{HL} differs by treatment ($P = 0.009$), with lower mean R_{HL} in fertilized treatments (0.93 ± 0.040 μmol CO_2 m^{-2} s^{-1}) than unfertilized treatments (1.27 ± 0.042 μmol m^{-2} s^{-1}).

Soil GWC explained 62 percent of the variance in cumulative R_{HM} using the equation:

$$\log(CO_2 + 1) = 0.154 + 0.058 \times \log(GWC + 1) \quad (2)$$

There was no significant difference between the slopes of fertilized and unfertilized regression equations for R_{HM} ($P = 0.825$) and the mean response for fertilization did not differ at

zero (P = 0.604). Fertilization had no effect on R_{HM} (P = 0.657), with an overall mean R_{HM} of 1.705 ± 0.054 μmol CO_2 m^{-2} s^{-1}.

An exponential decay function was fit to quantify and compare the rate (k) at which R_{HL} and R_{HM}, and litter GWC and mineral soil GWC, declined over the duration of the dry down. (Figures 4.1 and 4.2). Initial CO_2 was deemed to be the first respiration measurement at R_{HL} or R_{HM} field capacity. In the case of R_{HL}, the function was fit from the first efflux measurement following the wetting event, as the first measurement of the dry litter was effectively zero. Dry down measurements were terminated once all efflux rates reached 95% of the initial peak efflux rate at the start of the study. The mean decay rate, defined as *k*, is commonly used in decomposition experiments as the decomposition constant (Gholz et al. 2000). The mean *k* for R_{HL} and R_{HM} were significantly different from each other (P < 0.0001), with a R_{HL} *k* mean of 0.78 ± 0.04 and a R_{HM} *k* mean of 0.01 ± 0.003 (Figures 4.1.A and 4.2.A). The *k* values did not vary with fertilization treatment in either R_{HL} (P = 0.649) or R_{HM} (P = 0.262). Similarly, litter and mineral soil mean GWC *k* values were significantly different from each other (P<0.0001), with a litter GWC *k* of 1.011 and a mineral soil GWC *k* of 0.009 (Figure 4.1.A, and 4.1.B), but did not differ by fertilization treatment in litter (P = 0.622) or mineral soil (P = 0.379). The change in R_{HL} and litter GWC over time was more rapid than mineral soil, resulting in higher *k* values; near-zero R_{HL} rates were reached between three and 10 days, while the dry down of mineral soil to near-zero R_{HM} rates under the same conditions required between 200 - 233 days.

4.4 Discussion

This study demonstrates that water content is a controlling factor for both R_{HL} and R_{HM}, but more strongly influences the magnitude of R_{HL}. Prior to the addition of water, dry leaf litter from fertilized and unfertilized litter samples had R_{HL} rates of zero or near zero. R_{HL}

immediately increased following the wetting event bringing the litter layer to field capacity, to an average rate of 2.65 μmol m^{-2} s^{-1} in unfertilized samples and 1.89 μmol m^{-2} s^{-1} in fertilized samples, and decreased to pre-wetting levels between 3-10 days following the decline in litter water content (Fig. 4.2). Based on our results, R_{HL} increases linearly with litter water content (Fig 3). The rapid decline in R_{HL} as litter water content decreased can be attributed to the osmotic stress experienced by microorganisms within a short time period as litter becomes dry (Fierer et al. 2003). These results are consistent with previous findings that show dry litter contributed proportional little to R_S, but following wetting, can contribute 11 – 51 percent of R_S, demonstrating the regulatory role of water content on R_{HL} (Borken et al. 2003, Cisneros-Dozal et al. 2007, Ataka et al. 2014). While the contribution of R_{HL} to R_S was not investigated in this study, we can approximate the R_{HL} contribution to R_H (R_{HL}+R_{HM}) when both sample types were at field capacity. Immediately following litter wet up, R_{HL} contributed between 38 and 69 percent to total R_H. Peak R_{HM} values ranged from 1.63 to 4.80 μmol m^{-2} s^{-1}. Unlike R_{HL}, peak R_{HM} values did not always occur at the highest water content, and in some cases, occurred in days following the start of the dry down. This may be due to the displacement of CO_2 from soil pores and aggregates as the soil begins to dry. While the contribution of R_{HL} to R_H declined markedly within a day and over the course of the dry down, it does suggest that R_{HL} is an important and dynamic source of C loss from forest soils after wetting events, and the contribution may be important to understanding spatial and temporal variability of R_H estimates in forest ecosystems.

The increase in respiration rates following a wetting event, as observed in R_{HL}, has been attributed to a rapid increase in microbial activity and availability of previously unavailable substrates with increased water availability (Schnürer et al. 1986, Wu and Brookes 2005, Borken and Matzner 2009). These substrates can be made available by the release of osmolytes from

microorganisms that accumulated during the dry period as a result of microbial stress prior to a wetting event, from release of some of the cytoplasmic solutes accumulated during the dry period into the soil solution from lysed cells upon wetting (Kieft 1987, Van Gestel et al. 1993, Fierer and Schimel 2003, Unger et al. 2010), and from a change in the kinetics of enzyme transport and microbial uptake with increased water availability (Fierer et al. 2003). The duration and magnitude of elevated respiration rates is strongly related to water content (Wu and Brookes 2005, DeForest et al. 2009), microbial physiology and community structure as influenced by the physical and chemical characteristics of the environment (Fierer et al. 2003), and therefore likely differs between organic and mineral soil horizons (Wu and Lee 2011). In mineral soil, the structural alterations in soil aggregates upon wetting may also cause the release of previously physically protected organic matter (Griffiths and Birch 1961, Inglima et al. 2009).

While the mineral soil in our study did not receive a wetting event as it was initially within the range of VWC values determined to be field capacity for the soil textural class [i.e., 32 – 45%] (Saxton et al. 1986, van Genuchten et al. 1991), we did observe marked differences in sensitivity to water content, and in the duration of the dry down. R_{HM} was more stable over the course of the dry down and less sensitive to decreasing soil water content than R_{HL}, which supports our initial hypothesis. Soil water content explained less of the variation in R_{HM} than litter water content in R_{HL} (R^2 0.62 and 0.89, respectively), and as expected, took far longer (> 200 days) to dry down to water contents that induced microbial stress, yielding a significantly lower mean *k* values than R_{HL}. However, the duration of this dry down was influenced by the controlled lab environment, and there were no roots present, no transpiration, and no lateral drainage in the mineral soil microcosms. Previous studies have also found respiration from organic horizons to be more sensitive to rainfall and moisture than mineral soil respiration in

temperate forests (Borken et al. 2003, Hanson et al. 2003, Cisneros-Dozal et al. 2007, Wang et al. 2012). The reason may be that compared with the mineral soil, litter physical and chemical properties differ, and the water conditions of litter layer are more dynamic (Hanson et al. 2003). Litter and mineral soil heterotrophic community composition and biomass also differ, which may result in varying physiological responses to wetting and drying events (Van Gestel et al. 1993, Šnajdr et al. 2008), and C utilization strategies (Butterly et al. 2009). Additionally, the C supply in the soil may remain active at lower water potentials, and microbes can persist in microsites where conditions are more suitable (Foster 1988, Lado-Monserrat et al. 2014). These results demonstrate the need for separate measurements of R_H sources and environmental controls (water content, temperature, etc.), as mineral soil is protected from extreme changes in environment by the litter layer (Chen et al. 1999), and there is large spatial and temporal variability of water content and respiration rates.

We expected fertilization to suppress R_{HL} and R_{HM}, as previous studies have reported reduced R_H with mineral nutrient additions (Thirukkumaran and Parkinson 2000, Gough et al. 2004, Tyree et al. 2014). These reductions may be associated with a direct influence on the soil microbial community, such as decreases in fungal biomass following fertilization (Lilleskov et al. 2002, Nilsson and Wallander 2003, Bittman et al. 2005). Fertilization lessened the magnitude of the pulse of CO_2 released after wetting and resulted in suppressed R_{HL} compared to unfertilized samples. Additionally, the slopes and intercepts of the linear relationship between R_{HL} and litter water content were significantly different (Fig. 2.3). There was no effect of fertilization on R_{HM} or the duration of increased CO_2 release following wetting for R_{HM} and R_{HL}, as the exponential curve function values were not significantly different. Previous studies have reported declines in R_H with fertilization in temperate ecosystems (Thirukkumaran and Parkinson

2000, Burton et al. 2004, Gough et al. 2004, Swanston et al. 2004, Olsson et al. 2005), but increases have also been reported in soils with inherently low nutrient status (Gallardo and Schlesinger 1994). Declines have been attributed to direct effects on the microbial community associated with osmotic changes and reduced metabolic activity per unit biomass (Thirukkumaran and Parkinson 2000). The variable response to fertilization observed in the litter and mineral soil in this study may be a result of multiple factors as reported in previous studies, such as differences in substrate quality (Lagomarsino et al. 2006, Sinsabaugh et al. 2008), nutrient availability, microbial community composition, and microbial biomass (Ajwa et al. 1999, Cusack et al. 2011, Luo et al. 2015). However, in a previous study conducted at this same study location (McElligott et al. 2015 unpublished data), the fertilized treatment resulted in improved litter quality (lower C:N ratios), increased potential extracellular enzyme activities, and increased microbial biomass, which does not directly support the suppressed R_{HL} rates reported in this study. Based on the results in this study, reduced R_{HL} with fertilization could lead to the accumulation of litter and higher soil C concentrations as a result of reduced microbial C turnover (Nohrstedt et al. 1989, Cusack et al. 2010).

Improved temporal resolution in relationships between litter water content and R_{HL} measurements are needed to improve estimates of R_H in soil respiration models. Field measurements of soil respiration may fail to adequately capture the annual R_H contribution to R_S due to dynamic moisture conditions, logistical constraints associated with sampling under wet conditions, and the shortage of direct measurements of R_{HL} and litter water content (Ataka et al 2014). To remedy this deficiency in current ecosystem C accounting efforts, measurements of heterotrophic respiration components should occur at greater frequencies, and at times that capture a range of moisture conditions and time following precipitation events. Respiration from

litter has been demonstrated to have a greater dependency on moisture than temperature, but R_{HL} contributions depend on the frequency and amount of precipitation (Hanson et al. 2003, Wu and Lee 2011). The southeastern region is predicted to experience greater frequency of summer droughts, and more intense precipitation events between drought periods (Groisman et al. 2004, Angert et al. 2005, Laseter et al. 2012). Drier soils have been shown to have a greater magnitude of C loss following wetting events (Orchard and Cook 1983, Lado-Monserrat et al. 2014), as the pulse of CO_2 is dependent upon pre-wetting water content. These findings suggest that more severe drought may increase the magnitude of CO_2 losses (Unger et al. 2010, Carbone et al. 2011). When under wet conditions, however, CO_2 losses could be depressed by subsequent rainfall by bringing soils to oxygen-limited levels that inhibit respiration (Liu et al. 2002, McIntyre et al. 2009). Additionally, the intensity of rainfall events could alter microbial biomass and activity with long-term effects on R_H, as rapid changes in water content could contribute to greater incidence of cell lysis (Lado-Monserrat et al. 2014). Given the range of predicted changes in regional hydroclimate and the varying sensitivities of R_H sources to water content, a better understanding of the mechanisms that influence the control of water content on R_H is needed to understand how changes in precipitation regimes will influence the C balance of these ecosystems.

4.5 Conclusion

The results of this study suggest that routine chamber-based measurements of forest soil respiration components performed in the field on a weekly to monthly sampling regime may not adequately capture the contribution of R_{HL} to $R_{H,}$ and may contribute largely to the variability in soil respiration models used to estimate NEP, particularly under future precipitation scenarios. Established regional respiration models exhibit large amounts of variability (~40%) that cannot

be explained by soil temperature, moisture, and other site parameters (Templeton et al. 2015). Sampling during periods when litter is either wet or dry, and failure to parameterize models with litter water content in addition to soil moisture, may influence this variability. We found that R_H sensitivity to water content and fertilization varies among heterotrophic sources (R_{HL} vs. R_{HM}), resulting in great vertical and temporal heterogeneity among R_H fluxes. R_{HL} rapidly increased with increases in litter water content, but this response was short-lived and may be missed during field sampling efforts without direct and more frequent measures of R_{HL} and litter water content. Further, failure to directly measure R_{HL} and litter water content could mask management effects on R_H, as fertilization led to suppressed R_{HL} but not R_{HM}. These results indicate that greater sampling frequency under varying moisture and environmental conditions is needed to improve our estimates of R_H in ecosystem C accounting models.

Acknowledgements

This research was supported in part by the Virginia Agricultural Experiment Station and the McIntire-Stennis Program of the National Institute of Food and Agriculture (NIFA), U.S. Department of Agriculture (USDA), and by the Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP), a Coordinated Agricultural Project funded by the USDA NIFA (Award No. 2011-68002-30185). Thanks to Brian Parr for providing field and lab assistance.

Literature Cited

Ajwa, H., C. Dell, and C. Rice. 1999. Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. Soil Biology and Biochemistry 31:769-777.

Angert, A., S. Biraud, C. Bonfils, C. Henning, W. Buermann, J. Pinzon, C. Tucker, and I. Fung. 2005. Drier summers cancel out the CO2 uptake enhancement induced by warmer springs. Proceedings of the National Academy of Sciences of the United States of America 102:10823-10827.

ArchMiller, A., and L. Samuelson. 2016. Partitioning Longleaf Pine Soil Respiration into Its Heterotrophic and Autotrophic Components through Root Exclusion. Forests 7:39.

Ataka, M., Y. Kominami, M. Jomura, K. Yoshimura, and C. Uematsu. 2014. CO2 efflux from leaf litter focused on spatial and temporal heterogeneity of moisture. Journal of forest research 19:295-300.

Birch, H. 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10:9-31.

Bittman, S., T.A. Forge and C.G. Kowalenko 2005. Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. Soil Biology & Biochemistry. 37:613-623.

Boone, R. D., K. J. Nadelhoffer, J. D. Canary, and J. P. Kaye. 1998. Roots exert a strong influence on the temperature sensitivityof soil respiration. Nature 396:570-572.

Borken, W., E. Davidson, K. Savage, J. Gaudinski, and S. E. Trumbore. 2003. Drying and wetting effects on carbon dioxide release from organic horizons. Soil Science Society of America Journal 67:1888-1896.

Borken, W., and E. Matzner. 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology 15:808-824.

Burton, A. J., K. S. Pregitzer, J. N. Crawford, G. P. Zogg, and D. R. Zak. 2004. Simulated chronic NO3− deposition reduces soil respiration in northern hardwood forests. Global Change Biology 10:1080-1091.

Butterly, C., E. Bünemann, A. McNeill, J. Baldock, and P. Marschner. 2009. Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biology and Biochemistry 41:1406-1416.

Carbone, M. S., C. J. Still, A. R. Ambrose, T. E. Dawson, A. P. Williams, C. M. Boot, S. M. Schaeffer, and J. P. Schimel. 2011. Seasonal and episodic moisture controls on plant and microbial contributions to soil respiration. Oecologia 167:265-278.

Chen, J., S. C. Saunders, T. R. Crow, R. J. Naiman, K. D. Brosofske, G. D. Mroz, B. L. Brookshire, and J. F. Franklin. 1999. Microclimate in forest ecosystem and landscape ecology variations in local climate can be used to monitor and compare the effects of different management regimes. BioScience 49:288-297.

Cisneros-Dozal, L. M., S. E. Trumbore, and P. J. Hanson. 2007. Effect of moisture on leaf litter decomposition and its contribution to soil respiration in a temperate forest. Journal of Geophysical Research: Biogeosciences 112.

Cusack, D. F., W. L. Silver, M. S. Torn, S. D. Burton, and M. K. Firestone. 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. Ecology 92:621-632.

Cusack, D. F., M. S. Torn, W. H. McDOWELL, and W. L. Silver. 2010. The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. Global Change Biology 16:2555-2572.

Dale, V. H., L. A. Joyce, S. McNulty, R. P. Neilson, M. P. Ayres, M. D. Flannigan, P. J. Hanson, L. C. Irland, A. E. Lugo, and C. J. Peterson. 2001. Climate change and forest disturbances: climate change can affect forests by altering the frequency, intensity, duration, and timing of fire, drought, introduced species, insect and pathogen outbreaks, hurricanes, windstorms, ice storms, or landslides. BioScience 51:723-734.

DeForest, J. L., J. Chen, and S. G. McNulty. 2009. Leaf litter is an important mediator of soil respiration in an oak-dominated forest. International journal of biometeorology 53:127-134.

Fierer, N., A. S. Allen, J. P. Schimel, and P. A. Holden. 2003. Controls on microbial CO2 production: a comparison of surface and subsurface soil horizons. Global Change Biology 9:1322-1332.

Fierer, N., and J. P. Schimel. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67:798-805.

Foster, R. 1988. Microenvironments of soil microorganisms. Biology and fertility of soils 6:189-203.

Fox, T. R., H. L. Allen, T. J. Albaugh, R. Rubilar, and C. A. Carlson. 2007. Tree nutrition and forest fertilization of pine plantations in the southern United States. Southern Journal of Applied Forestry 31:5-11.

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

Gholz H.L., D.A. Wedin, S.M. Smitherman, M.E. Harmon, W.J., Parton. 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Glob Chang Biol 6:751-765.

Gough, C., J. Seiler, and C. A. Maier. 2004. Short-term effects of fertilization on loblolly pine (Pinus taeda L.) physiology. Plant, Cell & Environment 27:876-886.

Griffiths, E., and H. Birch. 1961. Microbiological changes in freshly moistened soil.

Groisman, P. Y., R. W. Knight, T. R. Karl, D. R. Easterling, B. Sun, and J. H. Lawrimore. 2004. Contemporary changes of the hydrological cycle over the contiguous United States: Trends derived from in situ observations. Journal of hydrometeorology 5:64-85.

Hanson, P., N. Edwards, C. Garten, and J. Andrews. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48:115-146.

Hanson, P. J., E. G. O'Neill, M. L. S. Chambers, J. S. Riggs, J. D. Joslin, and M. H. Wolfe. 2003. Soil respiration and litter decomposition. Pages 163-189 North American temperate deciduous forest responses to changing precipitation regimes. Springer.

Heim, B. C. 2014. Partitioning soil respiration in response to drought and fertilization in loblolly pine: laboratory and field approaches. Virginia Tech.

Heim, B. C., J. R. Seiler, and B. D. Strahm. 2015. Root Nonstructural Carbohydrates and Their Relationship with Autotrophic Respiration of Loblolly Pine (Pinus taedaL.). Communications in Soil Science and Plant Analysis 46:888-896.

Inglima, I., G. Alberti, T. Bertolini, F. Vaccari, B. Gioli, F. Miglietta, M. Cotrufo, and A. Peressotti. 2009. Precipitation pulses enhance respiration of Mediterranean ecosystems: the balance between organic and inorganic components of increased soil CO2 efflux. Global Change Biology 15:1289-1301.

Ingram, K. T., K. Dow, L. Carter, and J. Anderson. 2013. Climate of the Southeast United States: variability, change, impacts, and vulnerability. Springer.

Jarvis, P., A. Rey, C. Petsikos, L. Wingate, M. Rayment, J. Pereira, J. Banza, J. David, F. Miglietta, and M. Borghetti. 2007. Drying and wetting of Mediterranean soils stimulates decomposition and carbon dioxide emission: the "Birch effect". Tree Physiology 27:929-940.

Jokela, E. J., T. A. Martin, and J. G. Vogel. 2010. Twenty-five years of intensive forest management with southern pines: Important lessons learned. Journal of Forestry 108:338-347.

Kieft, T. L. 1987. Microbial biomass response to a rapid increase in water potential when dry soil (Kutsch et al. 2009)is wetted. Soil Biology and Biochemistry 19:119-126.

Knorr, W., I. Prentice, J. House, and E. Holland. 2005. Long-term sensitivity of soil carbon turnover to warming. Nature 433:298-301.

Kutsch, W. L., M. Bahn, and A. Heinemeyer. 2009. Soil carbon dynamics: an integrated methodology. Cambridge University Press.

Lado-Monserrat, L., C. Lull, I. Bautista, A. Lidón, and R. Herrera. 2014. Soil moisture increment as a controlling variable of the "Birch effect". Interactions with the pre-wetting soil moisture and litter addition. Plant and Soil 379:21-34.

Laseter, S. H., C. R. Ford, J. M. Vose, and L. W. Swift. 2012. Long-term temperature and precipitation trends at the Coweeta Hydrologic Laboratory, Otto, North Carolina, USA. Hydrology research 43:890-901.

Lilleskov, E.A., T.J. Fahey, T.R. Horton and G.M. Lovett 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology. 83:104-115.

Liu, X., S. Wan, B. Su, D. Hui, and Y. Luo. 2002. Response of soil CO2 efflux to water manipulation in a tallgrass prairie ecosystem. Plant and Soil 240:213-223.

Lu, S., K. G. Mattson, J. B. Zaerr, and J. D. Marshall. 1998. Root respiration of Douglas-fir seedlings: effects of N concentration. Soil Biology and Biochemistry 30:331-336.

Luo, P., X. Han, Y. Wang, M. Han, H. Shi, N. Liu, and H. Bai. 2015. Influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure in a brown soil of northeast China. Annals of microbiology 65:533-542.

Luo, Y., and X. Zhou. 2006. Soil respiration and the environment.

McElligott, K. M., J. R. Seiler, and B. D. Strahm. 2016. Partitioning soil respiration across four age classes of loblolly pine (Pinus taeda L.) on the Virginia Piedmont. Forest Ecology and Management 378:173-180.

McIntyre, R. E., M. A. Adams, D. J. Ford, and P. F. Grierson. 2009. Rewetting and litter addition influence mineralisation and microbial communities in soils from a semi-arid intermittent stream. Soil Biology and Biochemistry 41:92-101.

McNulty, S., J. Vose, and W. Swank. 1996. Loblolly pine hydrology and productivity across the southern United States. Forest Ecology and Management 86:241-251.

Mearns, L. O., W. Gutowski, R. Jones, R. Leung, S. McGinnis, A. Nunes, and Y. Qian. 2009. A regional climate change assessment program for North America. Eos 90:311.

Mitchell, R. J., Y. Liu, J. J. O'Brien, K. J. Elliott, G. Starr, C. F. Miniat, and J. K. Hiers. 2014. Future climate and fire interactions in the southeastern region of the United States. Forest Ecology and Management 327:316-326.

Nilsson, L.O. and H. Wallander. 2003. Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. New Phytologist. 158:409-416.

Nohrstedt, H.-Ö., K. Arnebrant, E. Bååth, and B. Söderström. 1989. Changes in carbon content, respiration rate, ATP content, and microbial biomass in nitrogen-fertilized pine forest soils in Sweden. Canadian Journal of Forest Research 19:323-328.

Noormets, A., D. Epron, J. Domec, S. McNulty, T. Fox, G. Sun, and J. King. 2015. Effects of forest management on productivity and carbon sequestration: A review and hypothesis. Forest Ecology and Management 355:124-140.

Noormets, A., M. J. Gavazzi, S. G. McNulty, J. C. DOMEC, G. Sun, J. S. King, and J. Chen. 2010. Response of carbon fluxes to drought in a coastal plain loblolly pine forest. Global Change Biology 16:272-287.

Olsson, P., S. Linder, R. Giesler, and P. Högberg. 2005. Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Global Change Biology 11:1745-1753.

Orchard, V. A., and F. Cook. 1983. Relationship between soil respiration and soil moisture. Soil Biology and Biochemistry 15:447-453.

Pikul Jr, J. 2003. Gravimetric measurement of soil water. Encyclopedia of Water Science. New York, NY: Marcel Dekker, Inc:879-881.

Rocca, M. E., C. F. Miniat, and R. J. Mitchell. 2014. Editorial. Introduction to the regional assessments: Climate change, wildfire, and forest ecosystem services in the USA.

Rowland, L., T. C. Hill, C. Stahl, L. Siebicke, B. Burban, J. Zaragoza-Castells, S. Ponton, D. Bonal, P. Meir, and M. Williams. 2014. Evidence for strong seasonality in the carbon storage and carbon use efficiency of an Amazonian forest. Global Change Biology 20:979-991.

Saxton, K., W. J. Rawls, J. Romberger, and R. Papendick. 1986. Estimating generalized soil-water characteristics from texture. Soil Science Society of America Journal 50:1031-1036.

Schnürer, J., M. Clarholm, S. Boström, and T. Rosswall. 1986. Effects of moisture on soil microorganisms and nematodes: a field experiment. Microbial Ecology 12:217-230.

Šnajdr, J., V. Valášková, V. r. Merhautová, J. Herinková, T. Cajthaml, and P. Baldrian. 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. Soil Biology and Biochemistry 40:2068-2075.

Subke, J.-A., I. Inglima, and M. Francesca Cotrufo. 2006. Trends and methodological impacts in soil CO2 efflux partitioning: A metaanalytical review. Global Change Biology 12:921-943.

Suseela, V., R. T. Conant, M. D. Wallenstein, and J. S. Dukes. 2012. Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. Global Change Biology 18:336-348.

Swanston, C., P. S. Homann, B. A. Caldwell, D. D. Myrold, L. Ganio, and P. Sollins. 2004. Long-term effects of elevated nitrogen on forest soil organic matter stability. Biogeochemistry 70:229-252.

Tewary, C., U. Pandey, and J. Singh. 1982. Soil and litter respiration rates in different microhabitats of a mixed oak-conifer forest and their control by edaphic conditions and substrate quality. Plant and Soil 65:233-238.

Thirukkumaran, C. M., and D. Parkinson. 2000. Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. Soil Biology and Biochemistry 32:59-66.

Tyree, M. C., J. R. Seiler, and C. A. Maier. 2014. Contrasting genotypes, soil amendments, and their interactive effects on short-term total soil CO 2 efflux in a 3-year-old Pinus taeda L. plantation. Soil Biology and Biochemistry 69:93-100.

Unger, S., C. Máguas, J. S. Pereira, T. S. David, and C. Werner. 2010. The influence of precipitation pulses on soil respiration–Assessing the "Birch effect" by stable carbon isotopes. Soil Biology and Biochemistry 42:1800-1810.

Van Genuchten, M. T., F. Leij, S. Yates, and J. R. Williams. 1991. The RETC Code for Quantifying the Hydraulic Functions of Unsaturated Soils.

Van Gestel, M., R. Merckx, and K. Vlassak. 1993. Microbial biomass responses to soil drying and rewetting: the fate of fast-and slow-growing microorganisms in soils from different climates. Soil Biology and Biochemistry 25:109-123.

Wang, X., L. Liu, S. Piao, I. A. Janssens, J. Tang, W. Liu, Y. Chi, J. Wang, and S. Xu. 2014. Soil respiration under climate warming: differential response of heterotrophic and autotrophic respiration. Global Change Biology 20:3229-3237.

Wang, Y., Z.-L. Wang, H. Wang, C. Guo, and W. Bao. 2012. Rainfall pulse primarily drives litterfall respiration and its contribution to soil respiration in a young exotic pine plantation in subtropical China. Canadian Journal of Forest Research 42:657-666.

Wear, D. N., and J. G. Greis. 2002. Southern forest resource assessment-technical report. Gen. Tech. Rep. SRS-53. Asheville, NC: US Department of Agriculture, Forest Service, Southern Research Station. 635 p. 53.

Wu, H.-J., and X. Lee. 2011. Short-term effects of rain on soil respiration in two New England forests. Plant and Soil 338:329-342.

Wu, J., and P. Brookes. 2005. The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biology and Biochemistry 37:507-515.

Xiao, W., X. Ge, L. Zeng, Z. Huang, J. Lei, B. Zhou, and M. Li. 2014. Rates of litter decomposition and soil respiration in relation to soil temperature and water in different-aged pinus massoniana forests in the three gorges reservoir area, China. PloS one 9:e101890.

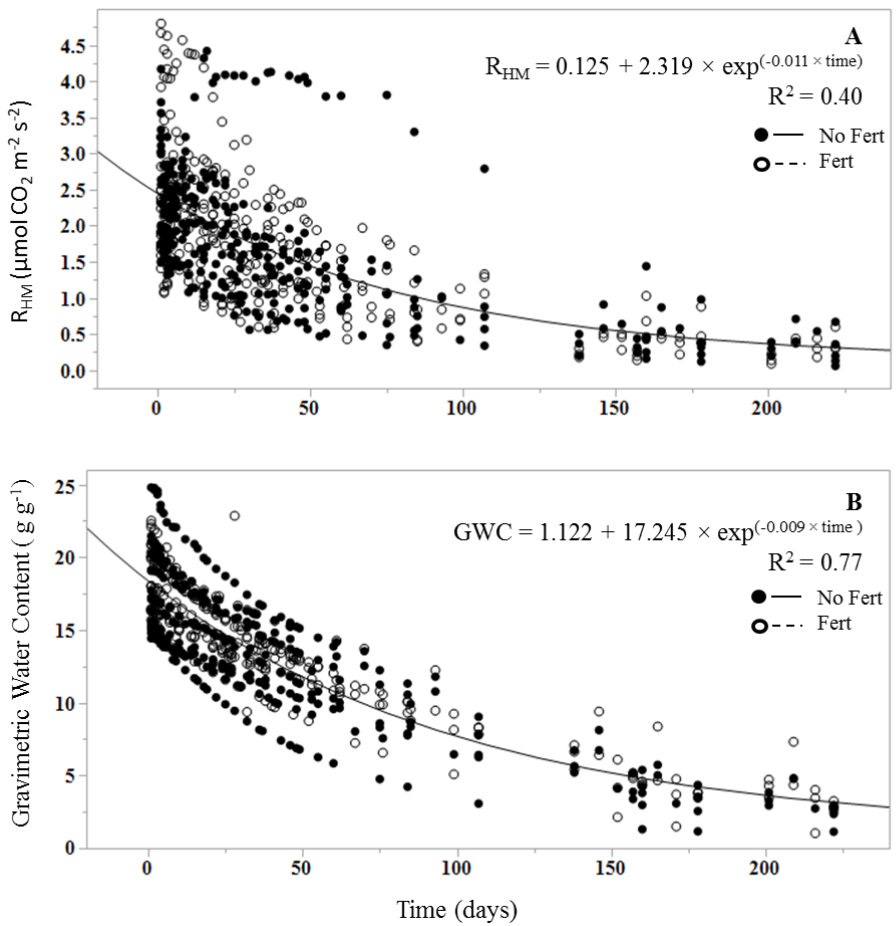

Figure 4.1. Change (A) mineral soil heterotrophic respiration (R_{HM}) and water content of the root-free mineral soil (B) over the duration of the dry down (days) in unfertilized (closed symbols) and fertilized samples (open symbols). The line represents the mean fit of the exponential decay function. Data represent actual values for each replicated treatment.

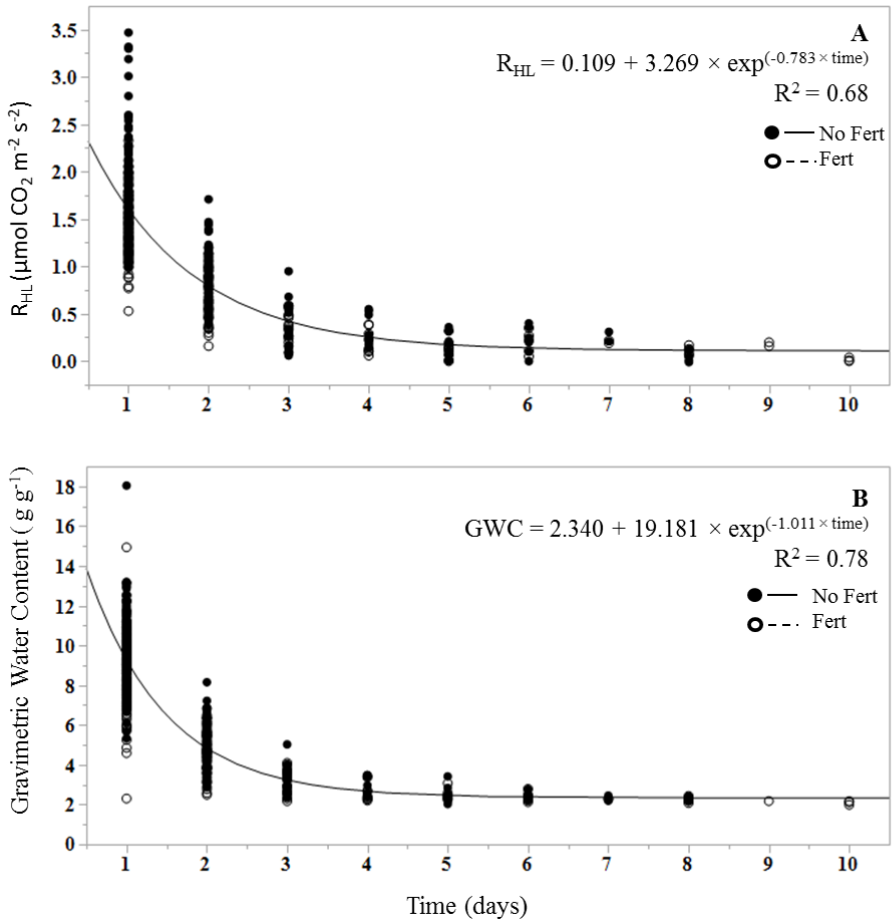

Figure 4.2. Change in (A) litter heterotrophic respiration (R_{HL}) and litter water content (B) following the initial wetting event over the duration of the dry down (days) in unfertilized (closed symbols) and fertilized samples (open symbols). The line represents the fit of the exponential decay function. Data represent actual values for each replicated treatment.

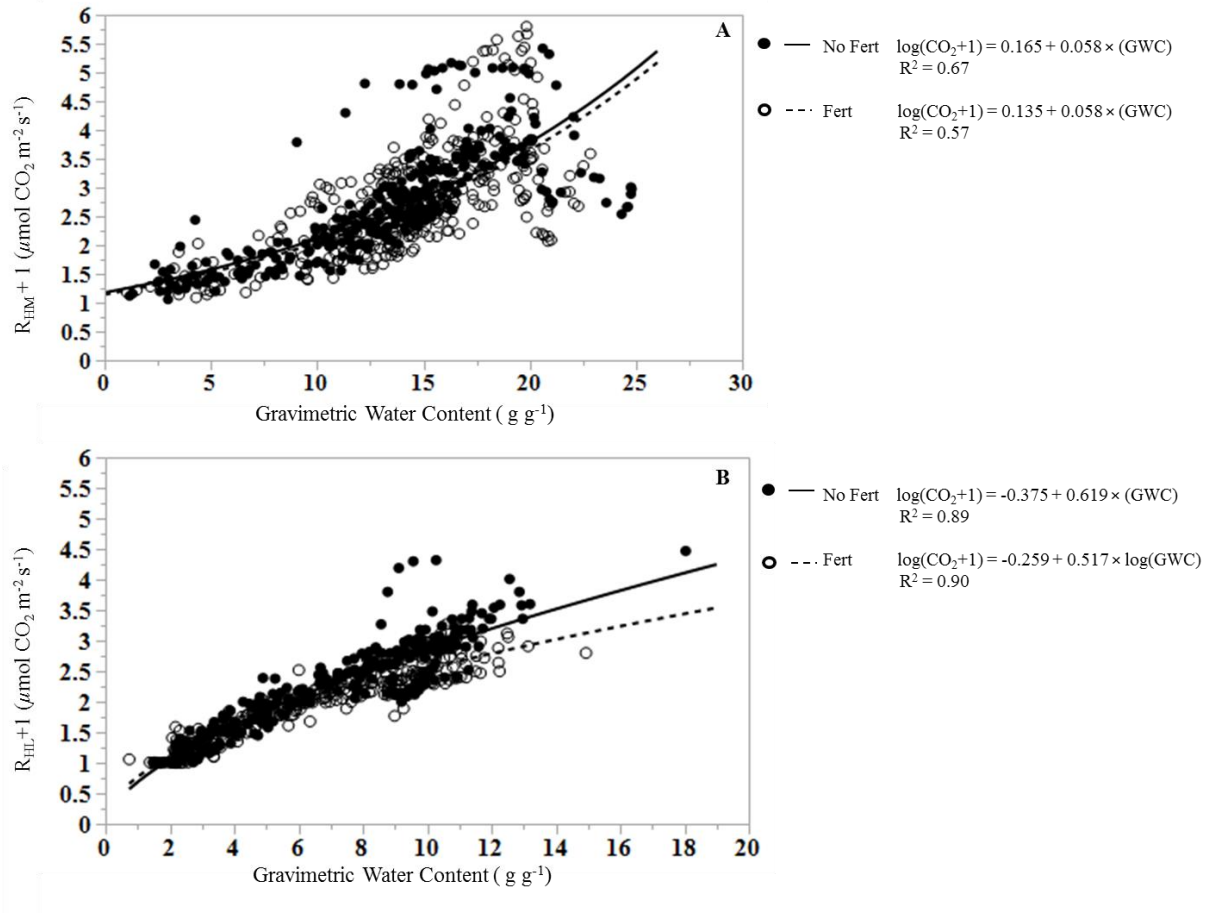

Fig 4.3. Relationships between (A) mineral soil heterotrophic respiration (R_{HM}) and soil gravimetric water content (GWC), and (B) leaf litter heterotrophic respiration (R_{HL}) and leaf litter gravimetric water content (GWC) in unfertilized (closed symbols, solid line) and fertilized samples (open symbols, dashed line).

Chapter 5. Synthesis

This research examined the dynamics of heterotrophic soil respiration (R_H) in loblolly pine plantations of the southeastern U.S., and the drivers of R_H and decomposition in response to forest management and climate change. I sought to improve the quantitative and mechanistic understanding of this important carbon (C) flux needed to quantify the overall C accumulation status of a forest by implementing three studies; one field-study deployed across four age classes of loblolly pine in the Appomattox Buckingham State Forest of VA, and two laboratory studies leveraging the VA PINEMAP Tier III site described in Chapter 1. In Chapter 2, R_H was partitioned from R_S across 3 - 25 year-old stands using an established root-exclusion technique, and each component and related environmental variables were measured seasonally from June 2014 - July 2015. This study provided seasonal estimates of R_H:R_S across stand ages to be used in regional, annual net ecosystem productivity (NEP) modeling efforts to quantify the C sequestration capacity of these forests. The proportion of R_H to R_S varied from approximately 0.83 to 0.50, dependent upon the stand age. Across all age classes, the winter season had the highest mean R_H:R_S of 0.85 while summer had the lowest of 0.55. By scaling seasonal R_H measurements to an annual C flux, the results from Chapter 2 demonstrate that R_H contributions range from 7.3 – 13.6 Mg C ha^{-1} yr^{-1} depending on stand age. Taken together with estimates of NPP for loblolly pine of similar age classes that range from roughly 2 to 14 Mg C ha^{-1} yr^{-1} (McNulty et al. 1996, Sampson et al. 2008, He et al. 2012), we can determine whether a forest functions as a C source or sink (i.e., NEP = NPP – R_H). The results of this study, when combined with other region-wide estimates of R_H:R_S that range from 0.50 – 0.84, suggest R_H represents a

principle component of soil respiration in most age classes of loblolly pine plantations, thereby influencing their potential to store C. Further, the range in R_H estimates demonstrates the need to account for seasonality and site- and stand-specific characteristics, such as stand age and management intensity, in efforts to model R_H. Over- or under-estimating this flux in ecosystem C models has important implications for estimates of NEP, and for identifying when forests switch from functioning as a C source to a C sink.

Due to the large contribution of R_H to R_S in loblolly pine forests demonstrated in Chapter 2, and the importance of accurate R_H estimates in NEP models, the objectives of Chapter 3 and 4 were to improve our mechanistic understanding of factors that influence R_H and the drivers of R_H (e.g., litter quality, microbial biomass and activity), specifically in response to nutrient and moisture manipulations. In Chapter 3 we found that, on a relatively short time-scale (i.e., three years following treatment installation), both fertilization and throughfall reduction influenced parameters known to regulate decomposition, and thus R_H, C turnover, and nutrient cycling. The forest floor appears to be impacted more strongly by altered nutrient and water availability, as litter enzyme activities exhibited an overall trend of increased hydrolytic, C- acquiring enzyme activity, and litter quality (e.g., C:nutrient ratios) was impacted in contrary ways by both treatments (litter quality increased with fertilization but decreased with throughfall reduction). Increased enzymatic activity along with increased litterquality with fertilization could result in accelerated mineralization rates in the forest floor and lead to greater C loss from this system. However, with increased rates of nutrient release, an increase in NPP is likely. Additionally, greater microbial biomass and activity associated with fertilization as reported in Chapter 3, could increase soil C pools through inputs of microbial necromass and decomposition-resistant microbial products (i.e., “microbial funnel”; Crowther et al. 2015, Bradford et al. 2016).

However, Chapter 4 reports a confounding fertilization response, as fertilization significantly reduced litter R_H rates, despite Chapter 3 results reporting an increase in litter microbial activity in samples collected from the same location the year prior. There was no fertilization effect on mineral soil R_H, further demonstrating the sensitivity of litter parameters to management-induced changes, and the need to understand processes responsible for altered R_H following fertilization.

Results from Chapter 4 demonstrate a deficiency in conventional weekly to monthly chamber-based field sampling efforts R_S and R_H, and implies that litter-derived sources of R_H (R_{HL}) may be a large source of uncertainty when estimating R_S and NEP. Field measurements of soil respiration may fail to adequately capture the annual R_H contribution to R_S due to dynamic moisture conditions, logistical constraints associated with sampling under wet conditions, and the shortage of direct measurements of R_{HL} and litter water content. Previous studies have demonstrated that R_{HL} is a large and important contribution to annual R_S in forests, ranging from 11 – 51% of R_S (Hanson et al. 2003, Cisneros-Dozal et al. 2007, DeForest et al. 2009, Ataka et al. 2014), suggesting the forest floor could be a primary source of C loss from forest ecosystems. This research showed that R_{HL} had a strong linear relationship with litter water content and is more sensitive to water content than mineral soil-derived sources (R_{HM}). Additionally, the contribution of R_{HL} to R_H was greatest immediately following the wetting event, and decreased rapidly, between three – 10 days. Other studies have highlighted the necessity to simulate the response of R_H to water content to accurately model both the annual and seasonal changes in NEP, as the seasonality of R_H and its drivers largely control estimates of NEP in forests (Rowland et al. 2014, Melton et al. 2015).

Collectively, these results highlight the dominant role that substrate quality and quantity, and resource limitation have on R_H in loblolly pine plantations. Furthermore, these results demonstrate that forest management practices can directly influence these drivers, and thus the ability of a forest to store C, by altering stand dynamics and manipulating nutrient availability. Chapter 2 supports the theory that R_H increases following forest disturbance (in this case, time from forest harvest as indicated by stand age) (Odum 1969, Chapin et al. 2011), as R_H contributions were highest in the younger stand age-classes. Greater R_H proportions in young stands, and thus lower C storage capacity, reflects increased substrate availability for soil microbial communities following a harvest operation, and therefore stimulated microbial activity and greater microbial biomass. Chapter 3 further supports the controlling role of resource limitation and substrate quality and quantity on R_H dynamics, as evident by the reported shifts in microbial nutrient acquisition strategies and increased microbial biomass with exogenous nutrient additions. Chapter 4 results are inconsistent with the observed trends in Chapter 3, in which we might have expected stimulated litter R_H with increased enzyme activity and microbial biomass, but also demonstrates the controlling role of resource availability, albeit through alternative mechanisms. The decreased R_H in fertilized litter could be attributable to changes in C-use efficiency (the ratio of C allocated to growth relative to C consumed for respiration) of the microbial community present in the litter (Manzoni et al. 2012). When nutrient availability is increased, the microbial community may increase their efficiency leading to a decrease in R_H. Additionally, these results demonstrate the importance of making the distinction between R_H in the litter layer and the underlying soil due to differing sensitivities to altered resource availability and substrate quality.

While these results represent relatively short-term responses of R_H and decomposition dynamics to forest management and moisture manipulations, they underscore the important influence forest management will have on forest C dynamics in a changing climate. Forest nutrient management in particular may have the greatest capacity to increase NEP, but can alter forest soil C dynamics through multiple potential pathways that warrant further investigation on multiple scales, and demonstrate the need to more critically evaluate the sources and controls of R_S components. These pathways include: increased litter nutrient concentration through altered nutrient resorption strategies following fertilization, and thus altered rates of nutrient cycling; increased allocation of photosynthates to belowground sinks that may stimulate both R_A and R_H through priming; and suppressed R_H through increased microbial C use efficiency or altered microbial community composition.

Literature Cited

Ataka, M., Y. Kominami, M. Jomura, K. Yoshimura, and C. Uematsu. 2014. CO2 efflux from leaf litter focused on spatial and temporal heterogeneity of moisture. Journal of forest research 19:295-300.

Bradford, M. A., W. R. Wieder, G. B. Bonan, N. Fierer, P. A. Raymond, and T. W. Crowther. 2016. Managing uncertainty in soil carbon feedbacks to climate change. Nature Climate Change 6:751-758.

Chapin, F. S., P. A. Matson, and P. Vitousek. 2011. Principles of terrestrial ecosystem ecology. Springer Science & Business Media.

Cisneros-Dozal, L. M., S. E. Trumbore, and P. J. Hanson. 2007. Effect of moisture on leaf litter decomposition and its contribution to soil respiration in a temperate forest. Journal of Geophysical Research: Biogeosciences 112.

Crowther, T. W., S. M. Thomas, D. S. Maynard, P. Baldrian, K. Covey, S. D. Frey, L. T. van Diepen, and M. A. Bradford. 2015. Biotic interactions mediate soil microbial feedbacks to climate change. Proceedings of the National Academy of Sciences 112:7033-7038.

DeForest, J. L., J. Chen, and S. G. McNulty. 2009. Leaf litter is an important mediator of soil respiration in an oak-dominated forest. International journal of biometeorology 53:127-134.

Hanson, P. J., E. G. O'Neill, M. L. S. Chambers, J. S. Riggs, J. D. Joslin, and M. H. Wolfe. 2003. Soil respiration and litter decomposition. Pages 163-189 North American temperate deciduous forest responses to changing precipitation regimes. Springer.

He, L., J. M. Chen, Y. Pan, R. Birdsey, and J. Kattge. 2012. Relationships between net primary productivity and forest stand age in US forests. Global Biogeochemical Cycles 26:3.

Manzoni, S., P. Taylor, A. Richter, A. Porporato, and G. I. Ågren. 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist 196:79-91.

Melton, J. R., R. K. Shrestha, and V. K. Arora. 2015. The influence of soils on heterotrophic respiration exerts a strong control on net ecosystem productivity in seasonally dry Amazonian forests. Biogeosciences 12:1151-1168.

McNulty, S., J. Vose, and W. Swank. 1996. Loblolly pine hydrology and productivity across the southern United States. Forest Ecology and Management 86:241-251.

Odum, E. P. 1969. The strategy of ecosystem development. Science.164:262.

Rowland, L., T. C. Hill, C. Stahl, L. Siebicke, B. Burban, J. Zaragoza-Castells, S. Ponton, D. Bonal, P. Meir, and M. Williams. 2014. Evidence for strong seasonality in the carbon storage and carbon use efficiency of an Amazonian forest. Global Change Biology 20:979-991.

Sampson, D. A., R. H. Wynne, and J. R. Seiler. 2008. Edaphic and climatic effects on forest stand development, net primary production, and net ecosystem productivity simulated for Coastal Plain loblolly pine in Virginia. Journal of Geophysical Research: Biogeosciences 113.