Temporal and spatial variation in longleaf pine soil respiration and its heterotrophic and autotrophic components

by

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A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
May 10, 2015

Keywords: *Pinus palustris* Mill., carbon, heterotrophic respiration, autotrophic respiration

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Abstract

Soil respiration (R_S) is the sum efflux of CO_2 from soil derived from the metabolic activity of autotrophs and heterotrophs in the litter layer, root-affected soil (rhizosphere), and bulk soil. Soil respiration exhibits a strong influence on the carbon balance of forests; specifically, the heterotrophic respiration (R_H) component can be weighed against all carbon assimilation, or net primary productivity (NPP), to estimate the carbon sink or source status of forested ecosystems. Soil respiration varies both temporally and spatially as environmental and ecological factors influence the individual components of R_S to varying degrees, and these dynamics of R_S have been studied in diverse ecosystems across the globe. However, R_S has been relatively understudied in longleaf pine (*Pinus palustris* Mill.) forests, which are being restored throughout the southeastern United States for a myriad of ecosystem services, including rare species habitat. This research project was conducted in diverse longleaf pine forests to increase our understanding of longleaf pine R_S dynamics, including the temporal and spatial variation in R_S and the ecological factors affecting said variation and the proportion of total R_S derived from heterotrophs. The intra-annual variation in R_S was measured from January 2012 through January 2013 across four diverse longleaf pine stands varying in age and structure, and the spatial variation of R_S in a 64-year-old longleaf pine forest was measured in July and August 2012. Concurrent measurements of the soil environment (soil temperature and moisture) and ecological factors (e.g. litter mass, distance to and diameter of nearby trees, root biomass) were made to determine how these factors influenced the temporal and spatial variation in R_S. Soil respiration was positively related to soil temperature on an intra-annual basis with a corresponding temperature sensitivity (Q_{10}) of 2.18; however, soil temperature did not influence the spatial variation in R_S in the 64-year-old longleaf pine stand. The value of Q_{10} was decreased during periods of drought-like soil

conditions, and soil moisture also influenced the spatial variation of R_S by homogenizing R_S variability in the wettest areas and decreasing R_S where soil conditions approached saturated levels. Litter mass and nearby trees increased R_S on both a temporal and a spatial basis; however, the influence of these variables on the intra-annual variation in R_S was marginal after first isolating the effect of soil temperature. Understory cover was correlated with the temporal variation in R_S, but confounded with the seasonal influence of soil temperature, and forb cover was the only cover category related to the spatial variation in R_S. Live fine root biomass was negatively related to the intra-annual variation in R_S and positively related to the spatial variation in R_S, and dead root biomass was negatively related to the spatial variation in R_S and not related to the temporal variation in R_S. Finally, R_S was partitioned into its autotrophic and heterotrophic components by means of small root exclusion tubes installed in three 26-year-old longleaf pine forests during the growing season of 2013. The presence of root exclusion tubes, when compared to adjacent control soil, significantly decreased R_S and live root biomass, and increased dead root biomass after 102–104 days of incubation. The corresponding estimates of the proportion of R_H to total R_S were 61 to 82 %, with the lowest ratio estimates after correcting for the initial within-block, pre-treatment variation in R_S and CO_2 lost due to root decay. This research provides a comprehensive view of the spatial and temporal variation in R_S and an estimate of the proportion of total R_S resulting from heterotrophic activity in longleaf pine forests located centrally within their native range.

Acknowledgments

This dissertation is dedicated to Uncle Jerry, who we lost too soon, but not before he taught me to appreciate life, nature, and a good laugh.

I would like to thank Dr. Samuelson for mentoring me throughout my PhD, pushing me to become a better scientist and writer, and supporting my personal research aspirations. I would also like to thank my advisory committee members for their support. In particular, I am thankful for Dr. Li and Dr. Feng for increasing my interest in spatial statistics and soil microbiology, respectively. I am also thankful that Dr. Chappelka jumped onto my committee at the (nearly) last minute, and for being the first person to make me feel welcome at Auburn long before I was accepted to the tree physiology lab. I have enjoyed getting to know Dr. Brockway, and appreciate being reminded of the bigger picture as I am often bogged down in the details. I also am thankful for all the hardworking research associates and undergraduate workers who provided hours of assistance in the laboratory and the field, and for my fellow graduate students for their camaraderie.

Mom, you have always been a role model for me, teaching me to work hard and to appreciate all that I have. My drive to learn something new every day started as a child because you nurtured my curiosity and taught me about the enjoyment that results from overcoming challenges. Thank you.

Finally, if it weren't for my incredibly patient, forgiving, and loving husband, Matt, this dissertation would not have been written. Thank you a thousand times over! mln

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Chapter 1

Dissertation Introduction

1.1 Background

Within a forested ecosystem, atmospheric carbon (as CO_2) is fixed through photosynthesis and released through respiration, oxidation, or organic transport (Fig. 1.1). Generally, photosynthesis is a biochemical process whereby CO_2 diffuses into mesophyll tissues within a leaf and is reduced into organic compounds (photosynthates) with the aid of enzymes and chemical energy harnessed from sunlight (Taiz and Zeiger, 2010). The total amount of carbon fixed through photosynthesis is called gross primary productivity (GPP) (Lovett et al., 2006). The recently fixed photosynthates follow source-sink pathways within the photosynthesizing vegetation, traveling from the location of production (source) to above- or belowground sink tissues (Lacointe, 2000). Photosynthate metabolism oxidizes organic carbon into CO_2 and releases energy, which is utilized for cellular growth or maintenance. The metabolic oxidation of organic carbon into CO_2 is referred to as respiration, a process common to all living organisms. Carbon fixed through GPP minus the CO_2 released from autotrophic respiration is net primary productivity (NPP) (Lovett et al., 2006). The CO_2 released by activity of heterotrophs, including grazers of live plant tissue, carnivores of live animal tissue, and detritivores of dead organic matter, is collectively referred to a heterotrophic respiration. Carbon that is not decomposed by heterotrophs can accumulate in above- and belowground biomass and in soil, be exported from the system, or be oxidized by fire. Net ecosystem productivity (NEP), which is used to estimate overall carbon balance of the forest, is thus calculated as above- and belowground NPP minus total heterotrophic respiration (Lovett et al., 2006; Irvine et al., 2007).

The environmental and ecological factors that affect the carbon balance of a forest are difficult to discern as these factors affect photosynthesis, heterotrophic respiration, and autotrophic respiration to varying degrees. Some ecological factors affect both photosynthesis and respiration. For instance, plant mortality directly decreases photosynthesis and autotrophic respiration, but stimulates heterotrophic respiration by detritivores (Harmon et al., 2011). On the other hand, plant growth and health stimulates both carbon fixation

through photosynthesis and carbon release through growth respiration (Reichstein et al., 2013). A study across a latitudinal gradient in Europe demonstrated that while latitude does not significantly affect photosynthesis directly, the role of respiration on ecosystem carbon balance does vary by latitude (Valentini et al., 2000). The authors suggested that the effect of latitude on carbon balance was due to: higher soil organic matter in boreal biomes; an accelerated effect of climate change in northern latitudes; more drought limitations to respiration in southern latitudes; and/or an increase in temperature sensitivity (Q_{10}, the rate of increase in respiration for a 10 °C increase in temperature). Net ecosystem productivity can also be affected by forest age. Humphreys et al. (2006) found that young *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) stands were always a carbon source (i.e., negative NEP); medium-aged forests were either sinks or sources of carbon depending on season; and old-growth forests were the strongest sink (attributed to high leaf area index).

Soil respiration (R_S) is the largest individual flux of CO_2 out of forested ecosystems and is a product of the combined activity of both heterotrophs and autotrophs (Raich and Nadelhoffer, 1989). Photosynthates that have been allocated to the roots may follow many fates, including being directly metabolized by root tissues, allocated to mycorrhizae associated with root tissues, released as exudates into the rhizosphere (i.e., root-affected soil), or translocated acropetally to other sink tissues (van Hees et al., 2005; Aubrey and Teskey, 2009). Autotrophic soil respiration (R_A) is composed of the respiration by root tissues and the relatively small autotrophic bacteria population within surface soils. Heterotrophic soil respiration (R_H) is derived from the metabolism of heterotrophs within the litter layer, rhizosphere, and the root-free bulk soil. Substrates for R_H include living tissue (including plant, microbial, and fungal), leaf litter, dry and wet deposition, buried course woody debris, detritus, recalcitrant soil organic matter, and labile carbon molecules released by roots into the rhizosphere (i.e., exudates) (van Hees et al., 2005; Taiz and Zeiger, 2010). The close association between plant roots and heterotrophs is complicated by the rhizosphere priming effect, whereby heterotrophic metabolism of labile photosynthates in the rhizosphere can

increase the rate of recalcitrant metabolism of soil organic matter by heterotrophs present in bulk soil (Zhu and Cheng, 2011).

Within a forest, R_S varies at both temporal (annual, seasonal, and diurnal) and spatial scales. Factors affecting belowground processes both directly (e.g., soil temperature, moisture, soil carbon quality, and root activity), and indirectly (e.g., litter mass, soil micronutrients, soil texture, aboveground productivity, and topography), all influence R_S and its heterotrophic and autotrophic components to varying degrees (van Hees et al., 2005; Maier et al., 2010). Field studies of R_S are designed to determine the individual influences of these environmental and ecological variables on the temporal and spatial variation of soil respiration. Total ecosystem respiration is often measured with eddy covariance flux systems, which provide continuous estimates of CO_2 fluxes across the canopy-atmosphere interface (Baldocchi, 2003). Soil respiration, on the other hand, is most often measured in the field with semi-portable or portable infrared gas analyzers. Specifically, for the studies described within this dissertation, R_S was measured with a LI-COR 6400-09 Soil CO_2 Flux Chamber attached to the infrared gas analysis sensor head of the LI-6400 system (LI-COR Biosciences, Lincoln, NE). A full description of the equations used to measure soil CO_2 efflux are reported in Appendix A.

Forested ecosystems are an important component of the global carbon budget; about 25 % of annual global carbon flux is to terrestrial land sinks with over half of terrestrial carbon stored within forested ecosystems (FAO, 2001; Reichstein et al., 2013; Le Quéré et al., 2014). In North America, 34 % of the land is forested (67.8 million ha) which composes 17 % of the world's forests by area (FAO, 2011). Soil carbon in forested ecosystems constitutes over 63 % of the total temperate forest carbon stock in the United States (FAO, 2001). Temperate forests, such as those in the southeastern US, account for approximately one-third of carbon accumulation globally (Pan et al., 2011). Longleaf pine (*Pinus palustris* Mill.) natively ranged from southeastern Virginia to eastern Texas (Fig. 1.2); however, degradation of native longleaf pine forests has occurred due to anthropogenic fire suppression, logging,

and turpentine harvesting (Noss, 1988). Although still a part of the landscape of the southeastern US, the ecophysiology of longleaf pine forests, particularly soil carbon processes, is understudied relative to other southeastern temperate conifers (Samuelson et al., 2014). Longleaf pine forests are characterized by a relatively open canopy structure (Hedman et al., 2000), a diverse understory of native grasses, shrubs, herbaceous vines and forbs (Brudvig and Damschen, 2011), and a reliance on fire for hardwood exclusion and seedling germination (Gilliam and Platt, 1999; McGuire et al., 2001). Due to the unique nature of longleaf pine forests, our understanding of the soil carbon dynamics of other southeastern conifers may have little bearing in these ecosystems. Thus, the overarching goal of this research was to comprehensively examine the dynamics of longleaf pine R_S.

1.2 Study Overview and Objectives

The overall objectives of this research were to: (1) quantify the temporal and spatial variation in R_S across diverse longleaf pine forests; (2) explore the relationships between environmental and ecological factors and R_S at temporal and spatial scales; and (3) estimate the heterotrophic and autotrophic proportions of longleaf pine R_S using a root exclusion technique. To meet these objectives, three studies were completed within longleaf pine forests of western Georgia and eastern Alabama, USA (Fig. 1.2; Table 1.1). The first two studies were conducted within Fort Benning Military base near Columbus, Georgia. In the first study, R_S and related environmental variables were measured monthly from January 2012 through January 2013 in four longleaf pine stands varying in age and structure in order to determine the drivers of the intra-annual variation of longleaf pine R_S. The second study was completed within a 64-year-old longleaf pine forest within the base and designed to isolate the spatial variation of R_S and related environmental variables while controlling for the effects of temperature and phenology. The final study was completed in three 26-year-old longleaf pine stands on The Nature Conservancy Land near Geneva, Georgia. This study

partitioned R_S into its autotrophic and heterotrophic components with the use of small-diameter root exclusion tubes, which were installed in May 2013. From May 10 to August 26, 2013, bi-weekly measurements of R_S were taken from within the root exclusion pipes and at nearby control soil to determine the basal heterotrophic respiration rate of the soil without live root activity.

Bibliography

Aubrey, D. P., and R. O. Teskey. 2009. Root-derived CO_2 efflux via xylem stream rivals soil CO_2 efflux. New Phytologist **184**:35–40.

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.

Brudvig, L. A., and E. I. Damschen. 2011. Land-use history, historical connectivity, and land management interact to determine longleaf pine woodland understory richness and composition. Ecography **34**:257–266.

FAO, 2001. State of the World's Forests 2001. Technical report, Food and Agriculture Organization of the United Nations.

FAO, 2011. State of the World's Forests 2011. Technical report, Food and Agriculture Organization of the United Nations.

Gilliam, F. S., and W. J. Platt. 1999. Effects of long-term fire exclusion on tree species composition and stand structure in an old-growth *Pinus palustris* (Longleaf pine) forest. Plant Ecology **140**:15–26.

Harmon, M. E., B. Bond-Lamberty, J. Tang, and R. Vargas. 2011. Heterotrophic respiration in disturbed forests: A review with examples from North America. Journal of Geophysical Research **116**:1–17.

Hedman, C. W., S. L. Grace, and S. E. King. 2000. Vegetation composition and structure of southern coastal plain pine forests: an ecological comparison. Forest Ecology and Management **134**:233–247.

Humphreys, E. R., T. A. Black, K. Morgenstern, T. Cai, G. B. Drewitt, Z. Nesic, and J. A. Trofymow. 2006. Carbon dioxide fluxes in coastal Douglas-fir stands at different stages of development after clearcut harvesting. Agricultural and Forest Meteorology **140**:6–22.

Irvine, J., B. E. Law, and K. A. Hibbard. 2007. Postfire carbon pools and fluxes in semiarid ponderosa pine in Central Oregon. Global Change Biology **13**:1748–1760.

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.

Le Quéré, C., G. P. Peters, R. J. Anders, R. M. Andrew, T. A. Boden, P. Ciasis, P. Friedlingstein, R. A. Houghton, G. Marland, R. Moriarty, S. Sitch, P. Tans, A. Arneth, A. Arvanitis, D. C. E. Bakker, L. Bopp, J. G. Canadell, L. P. Chini, S. C. Doney, A. Harper, I. Harris, J. I. House, A. K. Jain, S. D. Jones, E. Kato, R. F. Keeling, K. Klein Goldewijk, A. Körtzinger, C. Koven, N. Lefèvre, F. Maignan, A. Omar, T. Ono, G.-H. Park, B. Pfeil, B. Poulter, M. R. Raupach, P. Regnier, C. Rödenbeck, S. Saito, J. Schwinger, J. Segschneider, B. D. Stocker, T. Takahashi, B. Tilbrook, S. van Heuven, N. Viovy, R. Wanninkhof, A. Wiltshire, and S. Zaehle. 2014. Global carbon budget 2013. Earth System Science Data **6**:235–263.

Littel, Jr., E. L., 1971. Atlas of United States trees. Volume 1, conifers and important hardwoods. Miscellaneous publication 1146, United States Department of Agriculture.

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, M., H. Schack-Kirchner, E. E. Hildebrand, and J. Holst. 2010. Pore-space CO_2 dynamics in a deep, well-aerated soil. European Journal of Soil Science **61**:877–887.

McGuire, J. P., R. J. Mitchell, E. B. Moser, S. D. Pecot, D. H. Gjerstad, and C. W. Hedman. 2001. Gaps in a gappy forest: plant resources, longleaf pine regeneration, and understory

response to tree removal in longleaf pine savannas. Canadian Journal of Forest Research **31**:765–778.

Noss, R. F. 1988. The longleaf pine landscape of the Southeast: almost gone and almost forgotten. Endangered Species Update **5**:1–8.

Pan, Y., R. A. Birdsey, J. Fang, R. Houghton, P. E. Kauppi, W. A. Kurz, O. L. Phillips, A. Shvidenko, S. L. Lewis, J. G. Canadell, P. Ciasis, R. B. Jackson, S. W. Pacala, A. D. McGuire, S. Piao, A. Rautiainen, S. Sitch, and D. Hayes. 2011. A large and persistent carbon sink in the world's forests. Science **333**:988–993.

Raich, J. W., and K. J. Nadelhoffer. 1989. Belowground carbon allocation in forest ecosystems: global trends. Ecology **70**:1346–1354.

Reichstein, M., M. Bahn, P. Ciasis, D. Frank, M. D. Mahecha, S. I. Seneviratne, J. Zscheischler, C. Beer, N. Buchmann, D. C. Frank, D. Papale, A. Rammig, P. Smith, K. Thonicke, M. van der Velde, S. Vicca, A. Walz, and M. Wattenbach. 2013. Climate extremes and the carbon cycle. Nature **500**:287–295.

Samuelson, L. J., T. A. Stokes, J. R. Butnor, K. H. Johnsen, C. A. Gonzalez-Benecke, P. Anderson, J. Jackson, L. Ferrari, T. A. Martin, and W. P. Cropper Jr. 2014. Ecosystem carbon stocks in *Pinus palustris* forests. Canadian Journal of Forest Research **44**:476–486.

Taiz, L., and E. Zeiger. 2010. Plant Physiology. Sinauer Associates, Sunderland, MA.

Valentini, R., G. Matteucci, A. J. Dolman, E.-D. Schulze, C. Rebmann, E. J. Moors, A. Granier, P. Gross, N. O. Jensen, K. Pilegaard, A. Lindroth, A. Grelle, C. Bernhofer, T. Grünwald, M. Aubinet, R. Ceulemans, A. S. Kowalski, T. Vesala, Ü. Rannik, P. Berbigier, D. Loustau, J. Gudmundsson, H. Thorgeirsson, A. Ibrom, K. Morgenstern, R. Clement, J. B. Moncrieff, L. Montagnani, S. Minerbi, and P. G. Jarvis. 2000. Respiration as the main determinant of carbon balance in European forests. Nature **404**:861–865.

van Hees, P. A. W., D. L. Jones, R. Finlay, D. L. Godbold, and U. S. Lundström. 2005. The carbon we do not see–the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology & Biochemistry **37**:1–13.

Zhu, B., and W. Cheng. 2011. Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. Global Change Biology **17**:2172–2183.

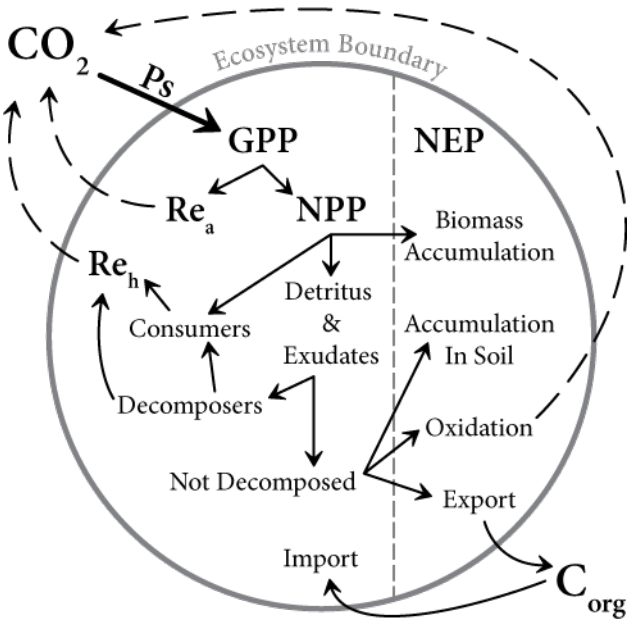

Figure 1.1: Schematic of the carbon cycle of a forested ecosystem adapted from Lovett et al. (2006). Carbon is brought into the ecosystem by photosynthesis (Ps) as gross primary productivity (GPP), then cycled within above- and belowground organisms, and finally released through respiration, oxidation, or export. NPP, net primary productivity; Re_a: autotrophic ecosystem respiration; Re_h: heterotrophic ecosystem respiration; NEP: net ecosystem productivity; C_{org}: organic carbon.

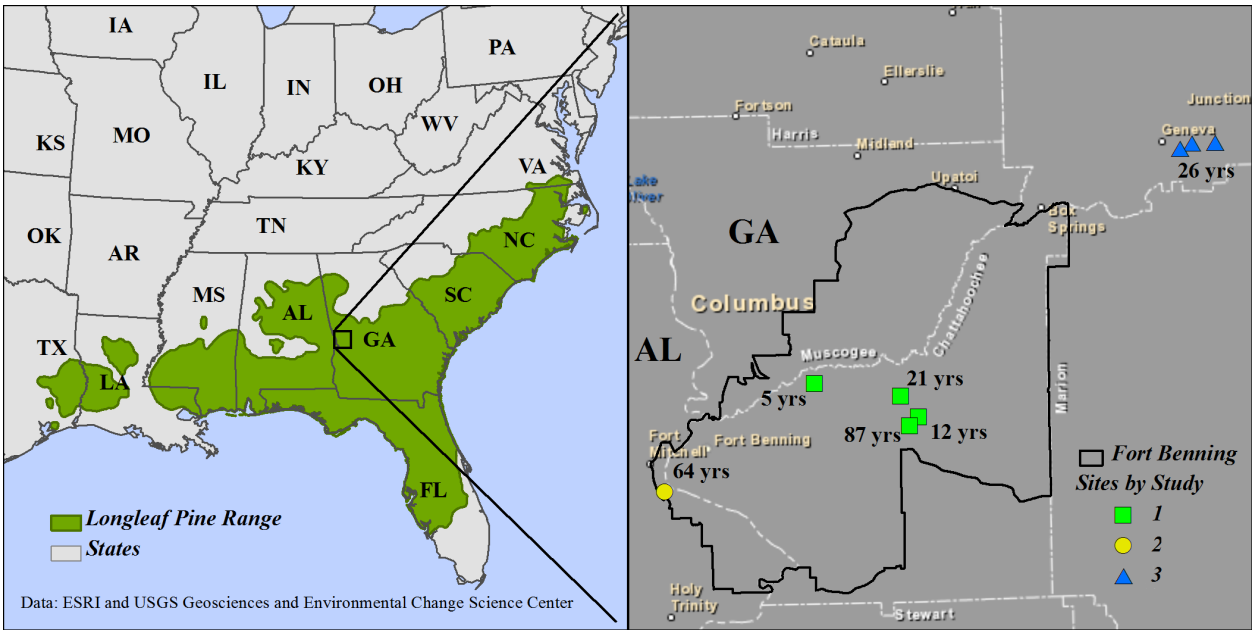

Figure 1.2: The extent of the native range of longleaf pine digitized by the USGS Geosciences and Environmental Change Science Center from Littel, Jr. (1971) (left). Study locations in respect to Fort Benning Military Base, labeled by stand age and symbolized by dissertation study (right).

Table 1.1: Overview of the eight longleaf pine forests used in these studies.

Age (yrs)	Coordinates W	Coordinates N	Managed by	Study Number	Chapter
5	-84° 52.265'	32° 23.999'	Fort Benning	1	2
12	-84° 46.622'	32° 22.353'	Fort Benning	1	2
21	-84° 47.550'	32° 23.373'	Fort Benning	1	2
87	-84° 47.112'	32° 21.967'	Fort Benning	1	2
64	-85° 0.514'	32° 19.142'	Fort Benning	2	3
26	-84° 36.039'	32° 34.469'	The Nature Conservancy	3	4
26	-84° 31.371'	32° 34.716'	The Nature Conservancy	3	4
26	-84° 30.117'	32° 34.706'	The Nature Conservancy	3	4

Chapter 2

Intra-annual variation of soil respiration across four heterogeneous longleaf pine forests in the southeastern United States

2.1 Abstract

Soil respiration (R_S) is the largest flux of CO_2 from forested ecosystems and is related to both soil climate and plant-driven substrate supply at various spatial and temporal scales. Relationships between the intra-annual variation in R_S and abiotic and biotic variables were examined across diverse longleaf pine (*Pinus palustris* Mill.) forests to better understand factors related to R_S in these low density, spatially heterogeneous forests. Soil respiration, soil temperature, soil moisture, litter mass, size and proximity of nearby trees, understory cover, and root biomass were measured over 13 months in four longleaf pine forests varying in age from 5 to 87 years of age and in forest structure. The exponential relationship between R_S and soil temperature accounted for over 70 % of the intra-annual variation in R_S with a corresponding temperature sensitivity (Q_{10}) of 2.18. Soil moisture affected the R_S-temperature relationship by dampening R_S and Q_{10} during times of extremely dry soil conditions, as defined by soil moisture ≤ 50 % of the texture-derived wilting point, but volumetric soil moisture did not directly correlate with R_S. The intra-annual variation in R_S was positively related to litter mass and understory cover and negatively related to distance to nearest tree and very fine non-pine root biomass, and these vegetation variables accounted for 6 to 15 % more variation in R_S beyond the R_S-temperature relationship. Annual R_S estimates ranged from 12.3 Mg C ha^{-1} in the 5-year-old stand with mostly grass stage seedlings to 13.9 Mg C ha^{-1} in the 87-year-old stand. This study contributes to our understanding of carbon fluxes across diverse longleaf pine ecosystems and indicates the importance of climate and net primary productivity in determining the carbon sink potential of southeastern longleaf pine forests.

2.2 Introduction

The efflux of CO_2 from soil, or soil respiration (R_S), is the dominant flux of CO_2 from forests and determines whether forests are carbon sources or sinks (Raich and Nadelhoffer, 1989; Gaumont-Guay et al., 2006). Intra-annual patterns of R_S generally follow soil temperature and the relationship between R_S and soil temperature has implications for carbon modeling of forested ecosystems, as it is the most commonly used model to calculate annual R_S (Bahn et al., 2010; Gomez-Casanovas et al., 2013). However, the temperature sensitivity of R_S (Q_{10}) has been shown to vary by forest type and age, latitude, and season (Knorr et al., 2005; Zhou et al., 2009; Mahecha et al., 2010; Subke and Bahn, 2010), and the R_S-temperature relationship may be affected by interactions with other biotic and abiotic factors that directly influence the autotrophic and heterotrophic components of R_S (Chen et al., 2011; Metcalfe et al., 2011). For example, soil moisture may affect R_S in a parabolic manner by limiting root and microbial activity in the soil at low soil moisture levels and restricting CO_2 diffusivity at high soil moisture levels (Orchard and Cook, 1983; Maier et al., 2010). But an effect of soil moisture is often only detected when field studies capture soil moisture levels low enough to be limiting to R_S or when R_S measurements are frequent enough to discern rapid responses of R_S to soil moisture fluctuations (Palmroth et al., 2005; Ford et al., 2012). In addition to soil temperature and moisture, R_S may also be affected by the proximity of nearby trees (Clinton et al., 2011), the amount and type of vegetation cover (Ma et al., 2005; Tjoelker et al., 2005; Fleming et al., 2006; Metcalfe et al., 2011), litterfall (Samuelson and Whitaker, 2012; Oishi et al., 2013), and amount and diversity of root functional groups (i.e., mycorrhizae-infected pine roots versus non-pine roots) (Tjoelker et al., 2005; van Hees et al., 2005; Metcalfe et al., 2011). The effect of many biotic variables, such as vegetation cover, litter mass, and root biomass, are coupled with seasonal increases in soil temperature; therefore, the temperature-independent influence of these variables on R_S can be difficult to quantify.

Soil respiration has been well studied in loblolly pine (*Pinus taeda* L.), the dominant plantation species in the southern United States, on a variety of sites throughout its range and under varying resource availability and forest management regimes (Samuelson et al., 2004; Wiseman and Seiler, 2004; Palmroth et al., 2005; Samuelson et al., 2009; Noormets et al., 2010; Oishi et al., 2013; Novick et al., 2014; Tyree et al., 2014; Heim et al., 2015). In contrast, relatively less is known of R_S in longleaf pine (*P. palustris* Mill.) forests. Longleaf pine forests were once common throughout the southeastern US (Noss, 1988), and are being actively restored throughout their native range (Hendricks et al., 2006; Brockway et al., 2014). In comparison to intensively managed southern pine plantations, longleaf pine forests are typically longer-lived, lower density stands that support high understory vegetation plant cover and species richness, and are managed with frequent prescribed fires to reduce hardwood succession, maintain spatial heterogeneity of the canopy, and promote natural regeneration and native plant diversity (Hedman et al., 2000; Hiers et al., 2003; Mitchell et al., 2006; Archer et al., 2007; Lavoie et al., 2012). Longleaf pine forests are also unique in that they have a relatively open coniferous canopy with a diverse grassland-like understory, and thus may be placed on the spectrum between conifer forests, which have relatively lower R_S than deciduous forests, and grasslands, which have relatively higher R_S than forests (Raich and Tufekcioglu, 2000). In previous studies of R_S in longleaf pine forests, R_S was shown to be related to: soil temperature and litterfall in 50-year-old stands varying in basal area (Samuelson and Whitaker, 2012); irrigation treatments in mature stands on excessively drained xeric soils (Ford et al., 2012); canopy scorching in a 22-year-old longleaf pine plantation (Clinton et al., 2011); and soil temperature and soil moisture in juvenile longleaf pine systems grown in control CO_2 ($365\,\mu$mol mol^{-1}) and elevated CO_2 ($720\,\mu$mol mol^{-1}) open-top chambers (Runion et al., 2012). Given the importance of R_S in quantifying net ecosystem productivity and forest carbon sequestration (Raich and Nadelhoffer, 1989; Lovett et al., 2006), a better understanding of R_S in longleaf pine forests across a range of

site and stand characteristics would improve efforts to quantify the carbon sink potential in these forests (Samuelson et al., 2014).

The overall objective of this study was to examine the intra-annual variation of R_S in longleaf pine forests in order to: (1) quantify R_S across a range of stand ages and forest structures; and (2) explore the relationships between R_S and factors related to forest abiotic and biotic factors. Longleaf pine stands ranging in age from 5 to 87 years were studied and represented a range in soil textures, stand structures, and management histories and thus allowed development of more robust longleaf pine R_S models than with a single study site. Although not a true chronosequence, measurement of R_S in different aged stands can contribute to identifying broad controls over ecosystem carbon exchange (Ryan and Law, 2005). We expected that soil temperature would account for the most intra-annual variation in R_S in an exponential manner, but hypothesized that soil moisture would affect the R_S-temperature relationship when at biologically limiting levels. Because of varying soil textures between stands, we predicted that the general effect of volumetric soil moisture on R_S may be confounded by soil textural differences (Balogh et al., 2011; Moyano et al., 2012). Therefore, to test for the limiting effect of soil moisture on R_S, the influence of both field-measured volumetric soil moisture and texture-derived soil water potential on the R_S-temperature relationship were explored. We also hypothesized that across these spatially heterogeneous, relatively open-canopied, diverse forests, biotic variables (specifically litter mass, proximity to nearby trees, understory cover, and pine and non-pine root biomass) would all contribute to variation in R_S independent of the influence of soil temperature.

2.3 Materials and Methods

2.3.1 Study sites and stand descriptions

Study sites were located at Fort Benning Military Base near Columbus, Georgia, USA (32.38° N, 84.88° W). The climate at Fort Benning is subtropical with 30-year-average minimum, mean, and maximum temperatures of 12.8 °C, 18.7 °C, and 24.6 °C, respectively (National Climatic Data Center, 2015*a*). The 30-year-average monthly temperatures range from highest in July (28.1 °C) to lowest in January (8.4 °C). The 30-year-average annual precipitation is 1180 mm, spread evenly throughout the year. The soils at Fort Benning are characteristic of highly weathered Ultisols of the southeastern US, with sandy and loamy sand soils in upland areas and sandy loam and sandy clay loams in lowland areas (Garten and Ashwood, 2004). Fort Benning is within the Southeastern Mixed Forest Preserve and is specifically positioned along the transition zone between the Southern Appalachian Piedmont Section in the northern two-thirds of the base and the Middle Section of the Coastal Plains in the southern one-third (Bailey, 1994).

Four longleaf pine stands were selected for this study, ranging in age from 5 to 87 years (Tables 2.1 and 2.2). The 5-, 12-, and 21-year-old stands were plantations and the 87-year-old stand was a naturally regenerated, even-aged forest. The 5- and 12-year-old stands were planted at a density of 1494 trees ha^{-1} and the 21-year-old stand was planted at 2235 trees ha^{-1}. Currently, longleaf pine densities are 123, 944, 1750, and 758 trees ha^{-1} in the 5-, 12-, 21-, and 87-year-old stands, respectively. The soil series dominating each stand were Nankin sandy clay loam, Nankin sandy loam, Troup loamy sand, and Troup loamy sand in the 5-, 12-, 21-, and 87-year-old stands, respectively (Soil Survey Staff, 2014). The 5-year-old stand was located in the Middle Section of the Coastal Plains, which is characterized by rolling to hilly topography with variable textured marine-based sediments (McNab et al., 2007). The other three stands were located within the Southern Appalachian Piedmont Section, characterized by highly weathered and eroded deep clayey soils and a mixture of conifer

forest cover types. Frequent, unrecorded burns occurred prior to 1981 due to live fire during military training. Stands were last burned before this study in the winter of 2010 and were on a 1-3 year burning cycle since 2002 (Table 2.1). No other records besides fire history were available for the 87-year-old stand. More specific information about stand characteristics, including total carbon stocks, can be found in Samuelson et al. (2014).

Circular plots one hectare in size were established in each stand. Within each plot, three 25 by 25 m subplots were placed 35 m from plot center to the northeast (45°), south (180°), and northwest (315°). Only two subplots (northeast and northwest locations) were created in the 12-year-old stand due to spacing restrictions from an adjacent study. Stand inventories of each subplot were conducted in February 2012 and included tree species and diameter at breast height (1.37 m, DBH). Trees were inventoried if they were taller than 2 m in height with at least 1 cm DBH and classified as saplings (DBH < 10 cm) or mature trees (DBH ≥ 10 cm). The four stands represented a range in age and forest structure (Table 2.2). The youngest stand was a juvenile forest with no mature trees, and a greater proportion of sapling species other than longleaf pine (e.g., *Quercus* spp., *Cornus* spp., and *Liquidambar styraciflua* L.); the 21-year-old stand had trees of a similar size as the 12-year-old stand but at a higher density and basal area; and the 87-year-old stand represented a more mature longleaf pine forest with fewer but larger mature trees and a large cohort of longleaf pine saplings. Understory longleaf pine seedlings and saplings less than 2 m in height were not recorded in this study, but were were cited in a related study at 625, 0, 0, and 500 trees ha^{-1} in the 5-, 12-, 21- and 87-year-old stands, respectively (Samuelson et al., 2014). Only one stand inventory was conducted due to limited site access by the military.

2.3.2 Soil respiration and soil environment

Each 25 by 25 m subplot was evenly divided into 625 1 m^2 sampling plots. Beginning in January 2012, five different sampling plots were randomly chosen from each subplot for measurement of R_S and related variables (55 randomly chosen 1 m^2 sampling plots per month).

Measurements were conducted monthly from January 2012 through January 2013. Soil respiration, soil temperature, soil moisture, understory vegetation cover, litter mass, and the distance and DBH of the nearest tree to R_S collar were measured at each 1 m^2 sampling plot. Soil respiration was measured with a soil respiration chamber head (LI-6400-09) attached to a portable infrared gas analyzer (LI-6400, LI-COR Biosciences, Lincoln, NE). Soil respiration collars (PVC, 10 cm diameter, 5 cm height) were installed in each 1 m^2 sampling plot at least 18 hours prior to R_S measurement in an area free from photosynthetically active vegetation. Soil respiration collars were pushed into the soil through standing litter with 2.5 cm of the collar remaining above mineral soil. Ambient CO_2 concentration (ppm) was measured at the first 1 m^2 sampling plot within each subplot and used for the target CO_2 concentration across that subplot. Soil temperature was measured concurrently with R_S at a 15 cm depth and within 10 cm of the collars using a soil temperature probe connected to the LI-6400 system. Volumetric soil moisture was measured to a 20 cm depth (Hydrosense II, Campbell Scientific, Inc., Logan, UT) within 10 cm of the collar location at the time of R_S and soil temperature measurement.

Soil temperature at a 10 cm depth was recorded half hourly from February 2012 through January 2013 using a data logger (HOBO U12-008, Onset Computer Co., Pocasset, MA) which was installed in the center of one subplot per stand and connected to four soil temperature probes placed 10 m from each data logger in the four cardinal directions.

2.3.3 Vegetation and root variables

Subsequent to the installation of the R_S collars, the percent of live vegetation cover (< 1 m in height) was ocularly estimated within the 1 m^2 sampling plots and included total cover and individual cover classes of woody plants, vines, forbs, legumes, and graminoids. Because of the overlapping vertical structure of the canopy layers, the cumulative percentage of individual cover classes could exceed total percent cover. The nearest tree (DBH > 1 cm and height > 2 m) to the R_S collar location was identified and its DBH and distance to the

collar were recorded. Subsequent to R_S measurement, standing litter down to mineral soil within the R_S collar was collected, dried at 70 °C for 48 hours and weighed.

Soil samples (10 cm diameter, 15 cm depth) were removed from directly below the R_S collars in February, May, and September 2012 and January 2013 for measurement of root biomass by type (live pine, live non-pine, and dead) and size class. Live root diameter classes included very fine ($\leq$ 1 mm), fine ($>$ 1 and $\leq$ 2 mm), coarse ($>$ 2 and $\leq$ 5 mm) and very coarse ($>$ 5 mm). Dead root diameter classes included coarse ($\leq$ 5 mm) and very coarse ($>$ 5 mm). Soil was air dried and sifted through a Number 10 sieve. Roots were removed from the soil, sorted, washed, dried at 70 °C for 72 hours, and weighed.

2.3.4 Data analysis

Each set of monthly measurements were averaged by subplot ($n = 11$ across all stands per month) and then correlation and regression analyses were performed to investigate the relationship between R_S and abiotic and biotic variables. All data analysis was completed in SAS (version 9.3, SAS Institute Inc., Cary, NC) with significance level of $\alpha = 0.05$. Since there was no true replication of stand ages, differences between stands were not tested.

The relationship between R_S and soil temperature was modeled using a first-order exponential relationship:

$$R_S = \beta_0 * \exp(\beta_1 T_{soil}) \tag{2.1}$$

where β_0 and β_1 are the fit parameters and T_{soil} is soil temperature. The temperature sensitivity of R_S was calculated as $Q_{10} = e^{10\,\beta_1}$ and basal R_S was estimated as the intercept (β_0) at $T_{soil} = 0$ °C. The coefficient of determination was calculated using a modified R^2 formula referred to as pseudo-R^2, which was calculated as $1 - (SSE/SST)$, where SSE and SST are the error sum of squares and the corrected total sum of squares, respectively.

To examine the effects of soil moisture on the relationship between soil temperature and R_S, we analyzed the change in nonlinear trends between R_S and soil temperature during

different water status periods (e.g., soil moisture at field capacity versus at wilting point). The soil water categories followed Ma et al. (2005) but were modified to relate volumetric soil moisture to soil texture properties, including wilting point (WP), field capacity (FC), and saturated soil (SS) from Table 2.1 and Oram and Nelson (2014). The final soil categories included: extremely dry soils ($0 \leq \theta \leq 0.5\text{WP}$); dry soils ($0.5\text{WP} < \theta \leq \text{WP}$); moderate soils ($\text{WP} < \theta \leq \text{FC}$); and wet soils ($\text{FC} < \theta \leq \text{SS}$). Soils were never measured at levels above saturation, so no soils were considered extremely wet. To compare trends in Eq. (2.1) between soil moisture levels, a sum of squares reduction test was completed using the following test statistic:

$$\text{F-statistic} = \frac{(\text{SSE}_{\text{red}} - \text{SSE}_{\text{full}})/(\text{df}_{\text{red}} - \text{df}_{\text{full}})}{\text{SSE}_{\text{full}}/\text{df}_{\text{full}}} \tag{2.2}$$

where SSE_{red} and SSE_{full} are the error sum of squares and df_{red} and df_{full} are the degrees of freedom of the reduced and full models, respectively. The simplest model (i.e., least parameters) that significantly reduced the error sum of squares to the same degree as the saturated model with individual parameters fit for each soil moisture category was retained.

The relationships between R_{S} and biotic variables were explored using Pearson's correlation coefficients and residuals analysis. First, Pearson's correlation coefficients were examined to determine both which variables correlated with R_{S} and the magnitude of multicollinearity. Then, the variables that were significantly correlated with R_{S} were regressed against the residuals from the exponential R_{S}-temperature model from Eq. (2.1) following DeForest et al. (2006).

Soil temperature data from the data loggers were averaged into hourly time steps. Gaps were filled from linear regression relationships between measured soil temperature and high resolution air temperature data (Station ID 13829/LSF, Fort Benning, Columbus: Lawson AAF Airport Version 2, Quality Controlled Local Climatological Data, National Climate Data Center). Annual soil respiration (January 1 to December 31, 2012) was calculated for

each stand by using the fitted exponential soil temperature model from Eq. (2.1) with the gap-filled hourly continuous soil temperature data.

2.4 Results

2.4.1 Variation in abiotic and biotic variables in longleaf pine stands

Mean monthly air temperature at Columbus, Georgia ranged from 9.9 °C to 28.8 °C over the measurement period with the highest temperature in July and lowest in January 2012 (Fig. 2.1A) (National Climatic Data Center, 2015*b*). Mean soil temperature exhibited typical seasonal patterns, closely paralleled with air temperature, with an average of 19.1 °C across stands and over the measurement period (Fig. 2.1B). Total monthly precipitation ranged from 12.4 mm month^{-1} to 138.7 mm month^{-1} (Fig. 2.1A). Palmer Drought Severity Index (PDSI) values were above severe drought only during two months, December 2012 and January 2013 (PDSI ranged from -5.23 to 0.26) (National Climatic Data Center, 2014), and monthly precipitation was below the 30-year-average monthly precipitation rates during every month except January, May, September, and December 2012 (data not shown) (National Climatic Data Center, 2015*a*). Mean monthly volumetric soil moisture varied from a low of 1.30 % in May 2012 to a high of 24.57 % in February 2012 and was generally lower in the 21-year-old stand (Fig. 2.1C).

The DBH of the nearest trees to R_S collars was in general smaller in the 5-year-old stand (range of 2.7 cm to 4.3 cm; Fig. 2.2A). The widest range in DBH and the largest trees were in the 87-year-old stand (range 2.4 cm to 18.7 cm). The mean distance to nearest trees was from 0.8 m to 1.1 m in the dense 21-year-old stand, and from 1.3 m to 3.7 m and 1.6 m to 3.7 m in the 5- and 87-year-old stands, respectively (Fig. 2.2B). The mean DBH and distance to the nearest tree in the 12-year-old stand ranged from 4.9 cm to 12.2 cm and 1.2 m to 1.9 m, respectively. Mean monthly litter mass was in general higher in the two oldest stands than in the two youngest stands and ranged from 3.0 Mg ha^{-1} to 15.2 Mg ha^{-1} across stands (Fig. 2.2C).

Seasonal variation in total percent vegetation cover was evident in all stands but less pronounced in the denser 21-year-old stand (Fig. 2.3). In the 21-year-old stand, peak total vegetation cover was generally lower than in the other stands and its understory was dominated by forb cover, such as *Pityopsis gramnifolia* (Michx.) Nutt., and woody cover (mainly *Toxicodendron* spp.). Grasses dominated the understory of the other three stands, followed by woody cover in the 12- and 87-year-old stands and woody and forb cover in the 5-year-old stand. Across all stands, annual mean vine and legume percent cover were 8.0 % and 3.8 %, respectively.

Mean root biomass values by type, size class, and sampling month are shown in Figure 2.4. Total live root biomass was as high as 20.4 $\mathrm{Mg\,ha^{-1}}$ in the 21-year-old stand, but in the other stands did not exceed 15.2 $\mathrm{Mg\,ha^{-1}}$. In the 5-year-old stand, non-pine root biomass was greater than pine root biomass during each sampling month, and in the 21-year-old stand, pine root biomass was greater than non-pine root biomass during each sampling month. In the 12-year-old stand, the relative contribution of pine and non-pine roots varied by sampling month, and in the 87-year-old stand, non-pine and pine roots had similar biomass quantities. On average, very fine, fine, coarse, and very coarse roots comprised 61, 16, 16, and 7 % of total non-pine root biomass, respectively, and 40, 17, 15, and 28 % of total pine root biomass, respectively.

2.4.2 Relationships between R_S and abiotic and biotic variables in longleaf pine stands

With all stands and seasons pooled together, mean monthly R_S ranged from 1.18 to 5.94 $\mu\mathrm{mol\,m^{-2}\,s^{-1}}$ (Fig. 2.5A). Soil respiration generally followed the seasonal trend in soil temperature and increased exponentially with increasing soil temperature (Figs. 2.1B; Table 2.3). Soil temperature explained 72 % of the variation in R_S. Across all the stands and on an annual basis, the temperature sensitivity (Q_{10}) of R_S was 2.18 ($\beta_1 = 0.078$) and basal R_S was 0.700 $\mu\mathrm{mol\,m^{-2}\,s^{-1}}$ (β_0). Annual R_S calculated from Eq. (2.1) and the continuous

soil temperature data was 12.3 ± 1.8, 13.6 ± 2.0, 13.3 ± 1.9, and $13.9 \pm 2.1\,\mathrm{Mg\,ha^{-1}\,yr^{-1}}$ in the 5-, 12-, 21-, and 87-year-old stands, respectively.

Categorization of soil moisture measurements based on stand-specific soil water status suggested that soil moisture limited R_S at levels below half the wilting point (extremely dry conditions, Fig. 2.5B). When soil moisture conditions were used to create separate models under the reduced sum of squares test, Q_{10} based on β_1 from Eq. (2.1), was significantly reduced under extremely dry soil conditions ($\beta_1 = 0.064$, $Q_{10} = 1.90$) compared to all other soil moisture conditions ($\beta_1 = 0.076$, $Q_{10} = 2.14$), while basal R_S based on β_0 was the same under all soil moisture conditions ($\beta_0 = 0.767\,\mu\mathrm{mol\,m^{-2}\,s^{-1}}$; Table 2.3). Of all iterations between the fully reduced model and the saturated model, this reduced model ($\mathrm{SSE_{red}} = 64.99, \mathrm{df} = 135$) was the simplest model that significantly accounted for the same variation as the saturated model ($\mathrm{SSE_{full}} = 61.29, \mathrm{df} = 130$) with a corresponding F-statistic of 1.57 ($p = 0.17$, Eq. 2.2). The coefficient of determination (pseudo-$R^2 = 0.78$) for the reduced model was calculated through dummy variables for extremely dry and non extremely dry soils from the reduced sum of squares test.

The linear relationships between R_S and vegetation and root variables were tested with Pearson's correlation coefficients (Table 2.4). Litter mass and cover classes were significantly and positively related with R_S, and distance to nearest tree was negatively related with R_S. Every cover category was positively correlated with R_S, with the strongest correlations between legume cover and R_S. Of all the root variables, only very fine and fine non-pine roots were significantly (negatively) related to R_S. Very fine non-pine root biomass was the only biotic (i.e., litter mass, cover, proximity to nearest tree, or root biomass) variable correlated with R_S but not also correlated with soil temperature.

Residuals regression analysis was performed on the remaining variation after isolating the effect of soil temperature with the R_S-temperature model (Eq. 2.1). Variation in the residuals was explained by linear relationships with litter mass (positive), distance to nearest tree (negative), and very fine non-pine root biomass (negative; Fig. 2.6 and Table 2.3).

The residuals analysis accounted for 6 to 15 % more of the intra-annual variation in soil respiration, with the most variation explained by the model with very fine non-pine root biomass, although with a reduced sample size. Multiple intercorrelations existed among abiotic and biotic variables, and of the three variables that were regressed against the R_S-temperature model residuals, litter mass was negatively correlated with both distance to nearest tree and very fine non-pine root biomass, but tree distance and very fine non-pine root biomass were not correlated (Table 2.4).

2.5 Discussion

Over the 13-month study period and across four heterogeneous longleaf pine stands, the temperature sensitivity of R_S was 2.18. Although estimating Q_{10} based on a first-order exponential function simplifies the phenological effects of substrate supply on R_S (Davidson et al., 2006; DeForest et al., 2006), it has also been shown to be adequate for evaluating the temperature sensitivity of R_S over a normal temperature profile (Lellei-Kovács et al., 2011), and in our study the range of monthly air temperature was similar to the 30-year-average air temperature range (National Climatic Data Center, 2015*a*,*b*). This value of Q_{10} is higher than those reported (0.77 to 1.18) for juvenile longleaf pine systems measured over a relatively narrow temperature range during 90 days in spring (Runion et al., 2012), but lower than the Q_{10} of 2.81 measured in 50-year-old longleaf pine stands by Samuelson and Whitaker (2012), who reported no measurable drought limitation on R_S at high temperatures. In support of our first hypothesis, Q_{10} was significantly lower during periods of drought-like soil conditions than during periods of non-limiting soil moisture, as also found under experimental drought treatments in mixed deciduous forests (Borken et al., 2006) and old-field grasslands (Chen et al., 2011). Even though 2012 was cited as the tenth driest year on record for Georgia (National Climatic Data Center, 2012), soil moisture did not affect basal R_S, indicating that drought stress had the largest impact on R_S when soil temperature was relatively high. This finding agrees with Concilio et al. (2006), who found that soil moisture limited R_S only

during periods of high soil temperature and low soil moisture in a fire-dependent old-growth mixed forest of the Sierra Nevada Mountains, and work by Davidson et al. (2006), who concluded that drought can reduce R_S during the growing season in temperate ecosystems when evapotranspiration may exceed precipitation, as it did in our study (i.e., negative 3-month Standardized Precipitation Evapotranspiration Index for May through August 2012, National Climatic Data Center, 2014).

Although the influence of soil moisture on R_S was apparent at high soil temperatures when related to the wilting point properties of the various soil textures across these stands, no linear relationship was detected when volumetric soil moisture was regressed directly against R_S. In a 85 to 95-year-old longleaf pine forest, continuous R_S measurements made after irrigation demonstrated that the response of R_S to volumetric soil moisture was complex and decoupled in time such that intermittent R_S measurements may not capture an effect of soil moisture on R_S (Ford et al., 2012). Volumetric soil moisture is easy to measure with portable devices and more commonly researched in relation to R_S than is texture-derived soil water potential (e.g. Borken et al., 2006; Balogh et al., 2011; Ceccon et al., 2011; Lellei-Kovács et al., 2011; Barron-Gafford et al., 2014), but water availability for root, mycorrhizae, and microbial uptake is dependent not only on the volumetric quantity of soil water, but also upon its sorption to soil particles (Saxton and Rawls, 2006). For instance, almost twice the volumetric soil moisture must be present in the relatively clay-rich 5-year-old stand to reach wilting point (13 %) compared to the sandier 21-year-old stand (7.0 %; Table **??**). Previous studies have stressed the importance of comparable, empirical models that describe the interactive roles of soil temperature and moisture on R_S (Dilustro et al., 2005; Lellei-Kovács et al., 2011; Moyano et al., 2012), and our results indicate the importance of relating volumetric soil moisture to soil-specific, texture-derived soil water potential, particularly when R_S is compared across a range of soil textures. Longleaf pine forests occupy a wide edaphic range (Burns and Honkala, 1990) and thus consideration of soil texture in future longleaf pine R_S studies is warranted.

The hypothesis that biotic variables would account for variation in R_S beyond the seasonal influence of soil temperature was supported, but residuals analysis showed that litter mass, distance to nearest tree, and very fine non-pine root biomass accounted for only 6 to 15 % of additional variation in R_S, and that understory cover was not related to R_S beyond the influence of soil temperature. In addition to soil temperature and moisture, substrate supply (i.e., quality, quantity and timing of carbon sources allocated to roots and microbes through photosynthesis, root exudation, and litter) is a large contributor to variation observed in R_S, and the effect of substrate supply on R_S is often evaluated through relationships between R_S and biotic variables (Davidson et al., 2006). Soil respiration has been shown to be positively related to litter mass (Oishi et al., 2013), ground cover (Fleming et al., 2006), proximity to nearby trees (Søe and Buchmann, 2005), and root biomass (Dore et al., 2014). But, in longleaf pine forests (Samuelson and Whitaker, 2012) and slash pine (*P. elliottii* Engelm.) forests (Fang et al., 1998), the effects of these vegetation variables on R_S were relatively small compared to the influence of soil temperature. Similarly, Vande Walle et al. (2007) found that adding biotic and abiotic factors (soil carbon, leaf area index, soil pH, and root biomass) in addition to soil temperature did not substantially improve R_S-temperature models in Belgium hardwood plantations. Despite variability in age, forest structure, soil textures, ground cover, and root biomass observed among stands, relationships between R_S and biotic variables were marginal after first isolating the seasonal influence of soil temperature, which is perhaps a function of tight coupling between the seasonal availability of substrate and soil temperature in these forests.

On an intra-annual basis, R_S was negatively related to very fine and fine non-pine root biomass and not related to pine root biomass, larger non-pine root biomass, or dead root biomass. The inverse relationship between R_S and non-pine roots perhaps reflects changes in herbaceous species through time and their associated specific root respiration rates. For example, mean specific root respiration rates vary amongst functional groups of herbaceous species, ranging from 5.7 for C4 grasses to 13.2 $\mathrm{nmol\,g^{-1}\,s^{-1}}$ for nitrogen-fixing legumes

(Tjoelker et al., 2005). In addition, drought may have had a greater impact on specific respiration rates of herbaceous roots relative to pine roots, which not only have access to water at deeper soil depths but also carbohydrates stored in coarse roots (Aubrey et al., 2012). Drought has been found to reduce specific root respiration of grasses and forbs by up to 44% (Hasibeder et al., 2015). Aubrey et al. (2012) determined that longleaf pine roots were able to rely upon stored carbohydrates for root respiration when photosynthate supply was reduced through foliage scorching treatments. The majority of the root biomass categories, including larger classes of non-pine roots and all sizes of pine and dead roots, did not correlate with R_S. Although root biomass directly contributes to R_S through autotrophic respiration, others have also found that it tends not to be a strong correlate with intra-annual variation in R_S (Samuelson et al., 2004; Wiseman and Seiler, 2004; Bréchet et al., 2009; Runion et al., 2012). But, root biomass does show better correlation with temperature-independent, within-forest spatial variation in R_S, as found in California mixed-conifer forests (Dore et al., 2014), mature German beech (*Fagus sylvatica* L.) forests (Søe and Buchmann, 2005), and Michigan poplar (*Populus* spp.) forests (Stoyan et al., 2000).

To our knowledge, no other study has comprehensively quantified R_S in longleaf pine ecosystems across such a range in stand structures. We found that annual R_S varied at most 14 % between stands (5- and 87-year-old stands) and among mature stands from 2 to 4 %. Across stands, average annual R_S was 13.3 $\mathrm{Mg\,C\,ha^{-1}\,yr^{-1}}$. Our annual R_S values are within the range of 11.0 to 17.9 $\mathrm{Mg\,C\,ha^{-1}\,yr^{-1}}$ reported for 50-year-old longleaf pine stands in southern Alabama varying in basal area from 7 to 36 $\mathrm{m^2\,ha^{-1}}$ (Samuelson and Whitaker, 2012), but higher than those estimated for longleaf pine forests across an edaphic gradient (4.6 to 6.8 $\mathrm{Mg\,C\ ha^{-1}\,yr^{-1}}$) (Hendricks et al., 2006) and for 85 to 95-year-old longleaf pine woodlands under control and irrigation treatments on xeric soils (6.5 to 7.1 $\mathrm{Mg\,C\ ha^{-1}\,yr^{-1}}$) (Ford et al., 2012). Samuelson and Whitaker (2012) found that annual R_S across stands related positively with mean annual litter mass, which varied from 6 to 18 $\mathrm{Mg\,ha^{-1}}$ in their study and from 5.6 to 9.5 $\mathrm{Mg\,ha^{-1}}$ in our study. In both Ford et al. (2012) and Hendricks

et al. (2006), which were completed at the same research center in southwest Georgia, litter mass and the organic horizon were described as being negligible due to frequent, 2-year burn intervals as well as being entrapped in wiregrass (*Aristida beyrichiana* Trin. & Rupr.) crowns above the soil surface. When present on the forest floor, decomposition of litter can contribute to total R_S. For instance, litter respiration was found to be 22 % of R_S in 20-year-old South Carolina longleaf pine stands (Reinke et al., 1981), up to 26 % of R_S in North Carolina loblolly pine plantations (Taneva and Gonzalez-Meler, 2011), and up to 17 % of R_S in 250-year-old Oregon ponderosa pine (*P. ponderosa* Lawson and C. Lawson) stands (Irvine and Law, 2002). Litter mass may contribute to some to degree to variation in annual R_S between forests throughout the native range of longleaf pine, particularly as litter mass is highly dependent upon interactions between aboveground productivity, leaf area index, and fire frequencies (Brockway and Lewis, 1997; Gonzalez-Benecke et al., 2012).

2.6 Conclusion

The goal of this study was to provide an comprehensive survey of R_S and related abiotic and biotic variables across four diverse longleaf pine stands. We found that mean monthly R_S was related most strongly to soil temperature and reduced during periods of drought stress. After isolating the effect of soil temperature, the relationship between biotic variables and intra-annual variation in R_S was marginal, despite a range in forest structure, soils, ground cover, and root biomass across stands. This study demonstrated that although plant-derived substrate supply may be important for belowground metabolism and R_S, in these forests, soil temperature, soil moisture, and biotic variables are interrelated in such a way that the relative importance of vegetation is overshadowed by the R_S-temperature relationship on an intra-annual basis. As longleaf pine forests are restored to the southern landscape, the low stand densities needed for restoration combined with higher temperatures associated with climate change (Karl et al., 2009) may influence their role in efforts to increase forest carbon sequestration in the southeastern US (Han et al., 2007). As such, the interaction between

climate change, prescribed burning, and R_S across the range of longleaf pine ecosystems warrants further examination.

2.7 Acknowledgements

This research was supported by the United States Department of Defense through the Strategic Environmental Research and Development Program (SERDP) and the School of Forestry & Wildlife Sciences, Auburn University. The authors thank Tom Stokes, Jake Blackstock, Justin Rathel, Michael Gunter, Ann Huyler, Joe Clark, and Lorenzo Ferrari for assistance in data collection and James Parker and Brian Waldrep from Fort Benning, Land Management Division for their support. We also appreciate the statistical assistance provided by Greg Somers.

Bibliography

Archer, J. K., D. L. Miller, and G. W. Tanner. 2007. Changes in understory vegetation and soil characteristics following silvicultural activities in a southeastern mixed pine forest. Journal of the Torrey Botanical Society **134**:489–504.

Aubrey, D. P., B. Mortazavi, J. J. O'Brien, J. D. McGee, J. J. Hendricks, K. A. Kuehn, R. O. Teskey, and R. J. Mitchell. 2012. Influence of repeated canopy scorching on soil CO_2 efflux. Forest Ecology and Management **282**:142–148.

Bahn, M., M. Reichstein, E. A. Davidson, J. Grünzweig, M. Jung, M. S. Carbone, D. Epron, L. Misson, Y. Nouvellon, O. Roupsard, K. Savage, S. E. Trumbore, C. Gimeno, J. Curiel Yuste, J. Tang, R. Vargas, and I. A. Janssens. 2010. Soil respiration at mean annual temperature predicts annual total across vegetation types and biomes. Biogeosciences **7**:2147–2157.

Bailey, R. G., 1994. Ecoregions of the United States. GIS shapefile. U.S. Department of Agriculture Forest Service.

Balogh, J., K. Pintér, S. Fóti, D. Cserhalmi, M. Papp, and Z. Nagy. 2011. Dependence of soil respiration on soil moisture, clay content, soil organic matter, and CO_2 uptake in dry grasslands. Soil Biology & Biochemistry **43**:1006–1013.

Barron-Gafford, G. A., J. M. Cable, L. P. Bentley, R. L. Scott, T. E. Huxman, G. D. Jenerette, and K. Ogle. 2014. Quantifying the timescales over which exogenous and endogenous conditions affect soil respiration. New Phytologist **202**:442–454.

Borken, W., K. Savage, E. A. Davidson, and S. E. Trumbore. 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Global Change Biology **12**:177–193.

Bréchet, L., S. Ponton, J. Roy, V. Freycon, M.-M. Coûteaux, D. Bonal, and D. Epron. 2009. Do tree species characteristics influence soil respiration in tropical forests? A test based on 16 tree species planted on monospecific plots. Plant and Soil **319**:235–246.

Brockway, D. G., and C. E. Lewis. 1997. Long-term effects of dormant season prescribed fire on plant community diversity, structure and productivity in a longleaf pine wiregrass ecosystem. Forest Ecology and Management **96**:167–183.

Brockway, D. G., E. F. Loewenstein, and K. W. Outcalt. 2014. Proporational basal area method for implementing selection silviculture systems in longleaf pine forests. Canadian Journal of Forest Research **44**:977–985.

Burns, R. M., and B. H. Honkala. 1990. Silvics of North America. United States Department of Agriculture Forest Service.

Ceccon, C., P. Panzacchi, F. Scandellari, L. Prandi, M. Ventura, B. Russo, P. Millard, and M. Tagliavini. 2011. Spatial and temporal effects of soil temperature and moisture and the relation to fine root density on root and soil respiration in a mature apple orchard. Plant and Soil **342**:195–206.

Chen, X., W. M. Post, R. J. Norby, and A. T. Classen. 2011. Modeling soil respiration and variations in source components using a multi-factor global climate change experiment. Climatic Change **107**:459–480.

Clinton, B. D., C. A. Maier, C. R. Ford, and R. J. Mitchell. 2011. Transient changes in transpiration, and stem and soil CO_2 efflux in longleaf pine (*Pinus palustris* Mill.) following fire induced leaf area reduction. Trees **25**:997–1007.

Concilio, A., S. Ma, S.-R. Ryu, M. North, and J. Chen. 2006. Soil respiration response to experimental disturbances over 3 years. Forest Ecology and Management **228**:82–90.

Davidson, E. A., I. A. Janssens, and Y. Luo. 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q_{10}. Global Change Biology **12**:154–164.

DeForest, J. L., A. Noormets, S. G. McNulty, G. Sun, G. Tenney, and J. Chen. 2006. Phenophases alter the soil respiration-temperature relationship in an oak dominated forest. International Journal of Biometeorology **1**:135–144.

Dilustro, J. J., B. Collins, L. Duncan, and C. Crawford. 2005. Moisture and soil texture effects on soil CO_2 efflux components in southeastern mixed pine forests. Forest Ecology and Management **204**:87–97.

Dore, S., D. L. Fry, and S. L. Stephens. 2014. Spatial heterogeneity of soil CO_2 efflux after harvest and prescribed fire in a California mixed conifer forest. Forest Ecology and Management **319**:150–160.

Fang, C., J. B. Moncrieff, H. L. Gholz, and K. L. Clark. 1998. Soil CO_2 and its spatial variation in a Florida slash pine plantation. Plant and Soil **205**:135–146.

Fleming, R. L., M. F. Laporte, G. D. Hogan, and P. W. Hazlett. 2006. Effects of harvesting and soil disturbances on soil CO_2 efflux from a jack pine forest. Canadian Journal of Forest Research **36**:589–600.

Ford, C. R., J. D. McGee, F. Scandellari, E. A. Hobbie, and R. J. Mitchell. 2012. Long- and short-term precipitation effects on soil CO_2 efflux and total belowground carbon allocation. Agricultural and Forest Meteorology **156**:54–64.

Garten, C. T., and T. L. Ashwood, 2004. Land cover differences in soil carbon and nitrogen at Fort Benning, Georgia. Doc. ORNL/TM-2004/14, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Gaumont-Guay, D., T. A. Black, T. J. Griffis, A. G. Barr, K. Morgenstern, R. S. Jassal, and Z. Nesic. 2006. Influence of temperature and drought on seasonal and interannual variations of soil, bole and ecosystem respiration in a boreal aspen stand. Agricultural and Forest Meteorology **140**:203–219.

Gomez-Casanovas, N., K. Anderson-Teixeira, M. Zeri, C. J. Bernacchi, and E. H. DeLucia. 2013. Gap filling strategies and error in estimating annual soil respiration. Global Change Biology **19**:1941–1952.

Gonzalez-Benecke, C. A., E. Jokela, and T. A. Martin. 2012. Modeling the effects of stand development, site quality, and silviculture on leaf area index, litterfall, and forest floor accumulations in loblolly and slash pine plantations. Forest Science **58**:457–471.

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

Hasibeder, R., L. Fuchslueger, A. Richter, and M. Bahn. 2015. Summer drought alters carbon allocation to roots and root respiration in mountain grassland. New Phytologist **205**:1117–1127.

Hedman, C. W., S. L. Grace, and S. E. King. 2000. Vegetation composition and structure of southern coastal plain pine forests: an ecological comparison. Forest Ecology and Management **134**:233–247.

Heim, B., J. R. Seiler, and B. D. Strahm. 2015. Root non-structural carbohydrates and their relationship with autotrophic respiration of loblolly pine (*Pinus taeda* L.). Communications in Soil Science and Plant Analysis **In Press**.

Hendricks, J. J., R. L. Hendrick, C. A. Wilson, R. J. Mitchell, S. D. Pecot, and D. Guo. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. Journal of Ecology **94**:40–57.

Hiers, J. K., R. J. Mitchell, L. R. Boring, J. J. Hendricks, and R. Wyatt. 2003. Legumes native to longleaf pine savannas exhibit capacity for high N_2-fixation rates and negligible impacts due to timing of fire. New Phytologist **157**:327–338.

Irvine, J., and B. E. Law. 2002. Contrasting soil respiration in young and old-growth ponderosa pine forests. Global Change Biology **8**:1183–1194.

Karl, T. R., J. M. Melillo, and T. C. Peterson, editors. 2009. Global climate change impacts in the United States. Cambridge University Press.

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

Lavoie, M., M. C. Mack, J. K. Hiers, and S. Pokswinski. 2012. The effect of restoration treatments on the spatial variability of soil processes under longleaf pine trees. Forests **3**:591–604.

Lellei-Kovács, E., E. Kovács-Láng, Z. Botta-Dukát, T. Kalapos, B. Emmett, and C. Beier. 2011. Thresholds and interactive effects of soil moisture on the temperature response of soil respiration. European Journal of Soil Biology **47**:247–255.

Lovett, G. M., J. J. Cole, and M. L. Pace. 2006. Is net ecosystem production equal to ecosystem carbon accumulation? Ecosystems **9**:152–155.

Ma, S., J. Chen, J. R. Butnor, M. North, E. S. Euskirchen, and B. Oakley. 2005. Biophysical controls on soil respiration in the dominant patch types of an old-growth, mixed-conifer forest. Forest Science **51**:221–232.

Mahecha, M. D., M. Reichstein, N. Carvalhais, G. Lasslop, H. Lange, S. I. Seneviratne, R. Vargas, C. Ammann, M. A. Arain, A. Cescatti, I. A. Janssens, M. Migliavacca, L. Montagnani, and A. D. Richardson. 2010. Global convergence in the temperature sensitivity of respiration at ecosystem level. Science **329**:838–840.

Maier, M., H. Schack-Kirchner, E. E. Hildebrand, and J. Holst. 2010. Pore-space CO_2 dynamics in a deep, well-aerated soil. European Journal of Soil Science **61**:877–887.

McNab, W. H., D. T. Cleland, J. A. Freeouf, J. E. Keys Jr, G. J. Nowacki, and C. A. Carpenter, 2007. Description of ecological subregions: sections of the conterminous United States. Technical Report WO-76B, Washington, DC: U.S. Department of Agriculture, Forest Service.

Metcalfe, D. B., R. A. Fisher, and D. A. Wardle. 2011. Plant communities as drivers of soil respiration: pathways, mechanisms, and significance for global change. Biogeosciences **8**:2047–2061.

Mitchell, R. J., J. K. Hiers, J. J. O'Brien, S. B. Jack, and R. T. Engstrom. 2006. Silviculture that sustains: the nexus between silviculture, frequent prescribed fire, and conservation of biodiversity in longleaf pine forests of the southeastern United States. Canadian Journal of Forest Research **36**:2714–2736.

Moyano, F. E., N. Vasilyeva, F. J. Cook, J. Craine, J. Curiel Yuste, A. Don, D. Epron, P. Formanek, A. Franzluebbers, U. Ilstedt, T. Kätterer, V. A. Orchard, M. Reichstein, A. Rey, L. Ruamps, J.-A. Subke, I. K. Thomsen, and C. Chenu. 2012. The moisture response of soil heterotrophic respiration: interaction with soil properties. Biogeosciences **9**:1173–1182.

National Climatic Data Center, 2012. Drought - Annual 2012. State of the Climate Report. Last accessed January 21, 2015. http://www.ncdc.noaa.gov/sotc/drought/2012/13.

National Climatic Data Center, 2014. NIDIS Map and Data Viewer. Last accessed January 10, 2014. http://gis.ncdc.noaa.gov/map/drought/.

National Climatic Data Center, 2015*a*. 1981-2010 Normals. Columbus Metropolitan Airport, GA. Station GHCND:USW00093842. Last accessed January 15, 2015. http://www.ncdc.noaa.gov/cdo-web/datatools/normals.

National Climatic Data Center, 2015*b*. Daily Summaries. Columbus Metropolitan Airport, GA. Station GHCND:USW00093842. Downloaded November 2014. http://www.ncdc.noaa.gov/cdo-web/datatools/findstation.

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.

Noss, R. F. 1988. The longleaf pine landscape of the Southeast: almost gone and almost forgotten. Endangered Species Update **5**:1–8.

Novick, K. A., A. C. Oishi, E. J. Ward, M. B. S. Siqueira, J.-Y. Juang, and P. C. Stoy. 2014. On the difference in the net ecosystem exchange of CO_2 between deciduous and evergreen forests in the southeastern United States. Global Change Biology .

Oishi, A. C., S. Palmroth, J. R. Butnor, K. H. Johnsen, and R. Oren. 2013. Spatial and temporal variability of soil CO_2 efflux in three proximate temperate forest ecosystems. Agricultural and Forest Meteorology **171-172**:256–269.

Oram, B., and R. Nelson, 2014. Soil texture triangle: hydraulic properties calculator. www.staffweb.wilkes.edu/brian.oram/soilwatr.htm. Sourced from Saxton et al. 1986. Soil Sci Soc Amer J 50(4):1031-1036.

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

Palmroth, S., C. A. Maier, H. R. McCarthy, A. C. Oishi, H.-S. Kim, K. H. Johnsen, G. G. Katul, and R. Oren. 2005. Contrasting responses to drought of forest floor CO_2 efflux in a loblolly pine plantation and a nearby oak-hickory forest. Global Change Biology **11**:421–434.

Raich, J. W., and K. J. Nadelhoffer. 1989. Belowground carbon allocation in forest ecosystems: global trends. Ecology **70**:1346–1354.

Raich, J. W., and A. Tufekcioglu. 2000. Vegetation and soil respiration: Correlations and controls. Biogeochemistry **48**:71–90.

Reinke, J. J., D. C. Adriano, and K. W. McLeod. 1981. Effects of litter alteration on carbon dioxide evolution from a South Carolina pine forest floor. Soil Science Society of America Journal **45**:620–623.

Runion, G. B., J. R. Butnor, S. A. Prior, R. J. Mitchell, and H. H. Rogers. 2012. Effects of atmospheric CO_2 enrichment on soil CO_2 efflux in a young longleaf pine system. International Journal of Agronomy **2012**:1–9.

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

Samuelson, L. J., K. H. Johnsen, T. A. Stokes, and W. Lu. 2004. Intensive management modifies soil CO_2 efflux in 6-year-old *Pinus taeda* L. stands. Forest Ecology and Management **200**:335–345.

Samuelson, L. J., R. Mathew, T. A. Stokes, Y. Feng, D. P. Aubrey, and M. Coleman. 2009. Soil and microbial respiration in a loblolly pine plantation in response to seven years of irrigation and fertilization. Forest Ecology and Management **258**:2431–2438.

Samuelson, L. J., T. A. Stokes, J. R. Butnor, K. H. Johnsen, C. A. Gonzalez-Benecke, P. Anderson, J. Jackson, L. Ferrari, T. A. Martin, and W. P. Cropper Jr. 2014. Ecosystem carbon stocks in *Pinus palustris* forests. Canadian Journal of Forest Research **44**:476–486.

Samuelson, L. J., and W. B. Whitaker. 2012. Relationships between soil CO_2 efflux and forest structure in 50-year-old longleaf pine. Forest Science **58**:472–484.

Saxton, K. E., and W. R. Rawls. 2006. Soil water characteristic estimates by texture and organic matter for hydrologic solutions. Soil Science Society of America Journal **70**:1569–1578.

Søe, A. R. B., and N. Buchmann. 2005. Spatial and temporal variations in soil respiration in relation to stand structure and soil parameters in an unmanaged beech forest. Tree Physiology **25**:1427–1436.

Soil Survey Staff, 2014. Web soil survey. Online. http://websoilsurvey.nrcs.usda.gov. Accessed January 2012.

Stoyan, H., H. De-Polli, S. Böhm, G. P. Robertson, and E. A. Paul. 2000. Spatial heterogeneity of soil respiration and related properties at the plant scale. Plant and Soil **222**:203–214.

Subke, J.-A., and M. Bahn. 2010. On the 'temperature sensitivity' of soil respiration: Can we use the immeasurable to predict the unknown? Soil Biology & Biochemistry **42**:1653–1656.

Taneva, L., and M. A. Gonzalez-Meler. 2011. Distinct patterns in the diurnal and seasonal variability in four components of soil respiration in a temperate forest under free-air CO_2 enrichment. Biogeosciences **8**:3077–3092.

Tjoelker, M. G., J. M. Craine, D. Wedin, P. B. Reich, and D. Tilman. 2005. Linking leaf and root trait syndromes among 39 grassland and savannah species. New Phytologist **167**:493–508.

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 & Biochemistry **69**:93–100.

van Hees, P. A. W., D. L. Jones, R. Finlay, D. L. Godbold, and U. S. Lundström. 2005. The carbon we do not see–the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology & Biochemistry **37**:1–13.

Vande Walle, I., R. Samson, B. Looman, K. Verheyen, and R. Lemeur. 2007. Temporal variation and high-resolution spatial heterogeneity in soil CO_2 efflux in a short-rotation pine plantation. Tree Physiology **27**:837–848.

Wiseman, P. E., and J. R. Seiler. 2004. Soil CO_2 efflux across four age classes of plantation loblolly pine (*Pinus taeda* L.) on the Virginia Piedmont. Forest Ecology and Management **192**:297–311.

Zhou, T., P. Shi, D. Hui, and Y. Luo. 2009. Global pattern of temperature sensitivity of soil heterotrophic respiration (Q_{10}) and its implications for carbon-climate feedback. Journal of Geophysical Research **114**:1–9.

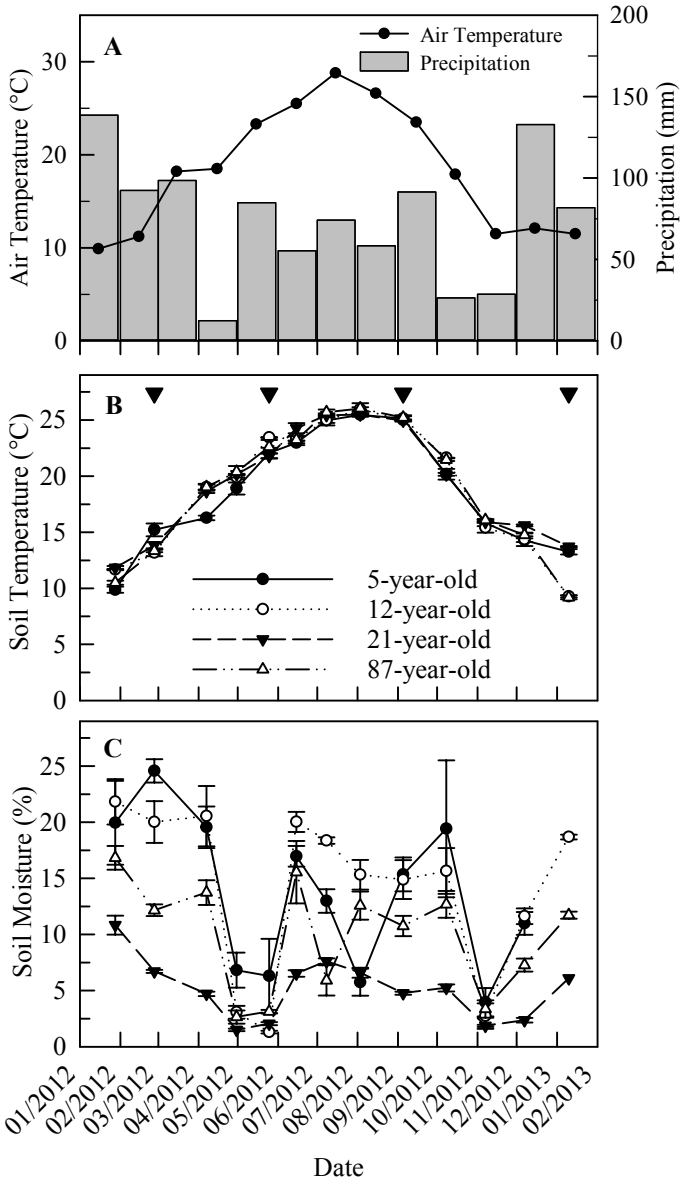

Figure 2.1: (A) Mean air temperature and total precipitation by month at a Columbus, Georgia weather station (National Climatic Data Center, 2015*b*). (B) Mean soil temperature and (C) soil moisture during soil respiration measurements in four longleaf pine stands varying in age and structure. Triangles represent dates of root biomass sampling and error bars are ± SE.

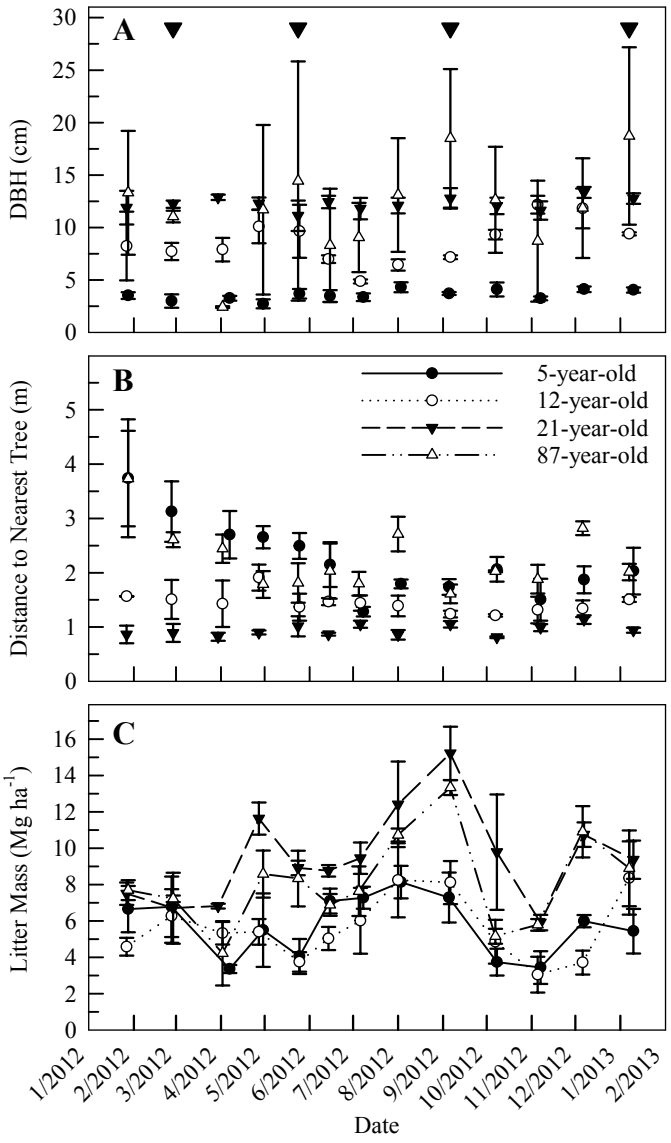

Figure 2.2: (A) Mean diameter at breast height (DBH) of the nearest tree to soil respiration (R_S) collars, (B) mean distance to nearest tree from R_S collars, and (C) litter mass by date in four longleaf pine stands varying in age and structure. Triangles represent dates of root biomass sampling and error bars are ± SE.

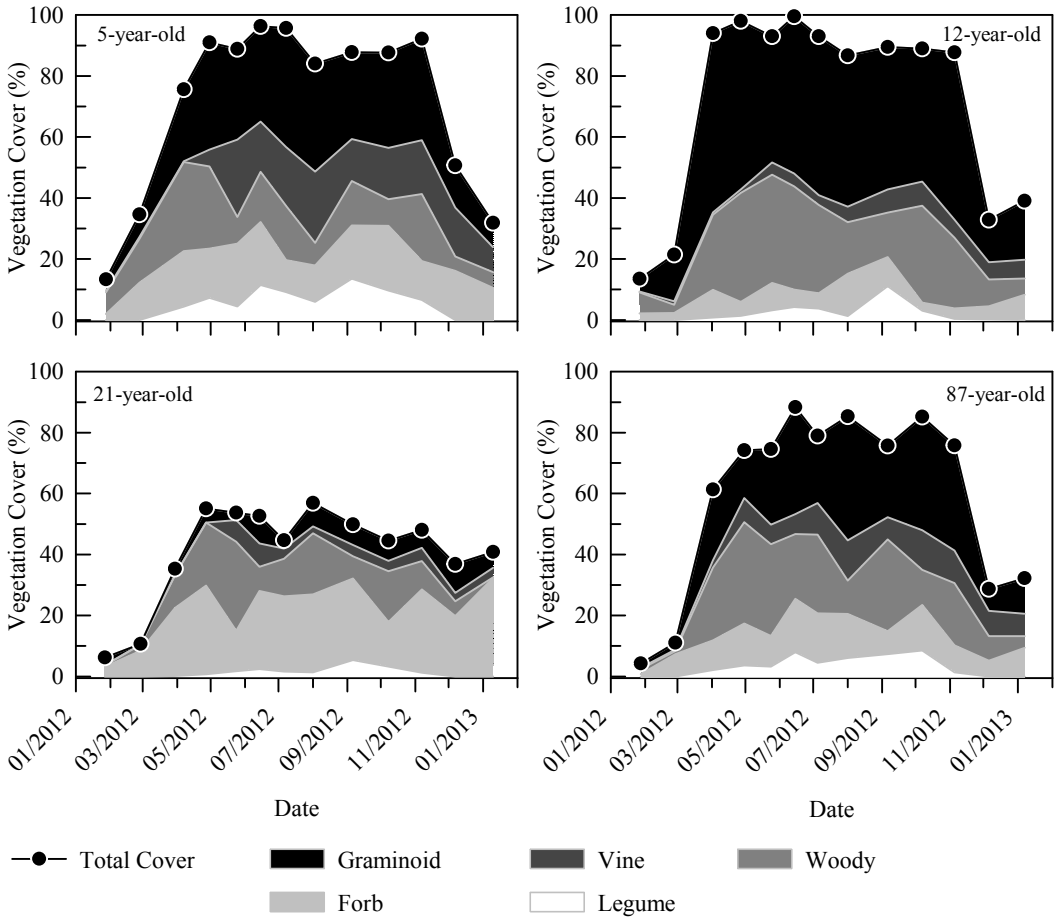

Figure 2.3: Mean total vegetation cover and proportions of individual cover classes to total cover by date in four longleaf pine stands varying in age and structure.

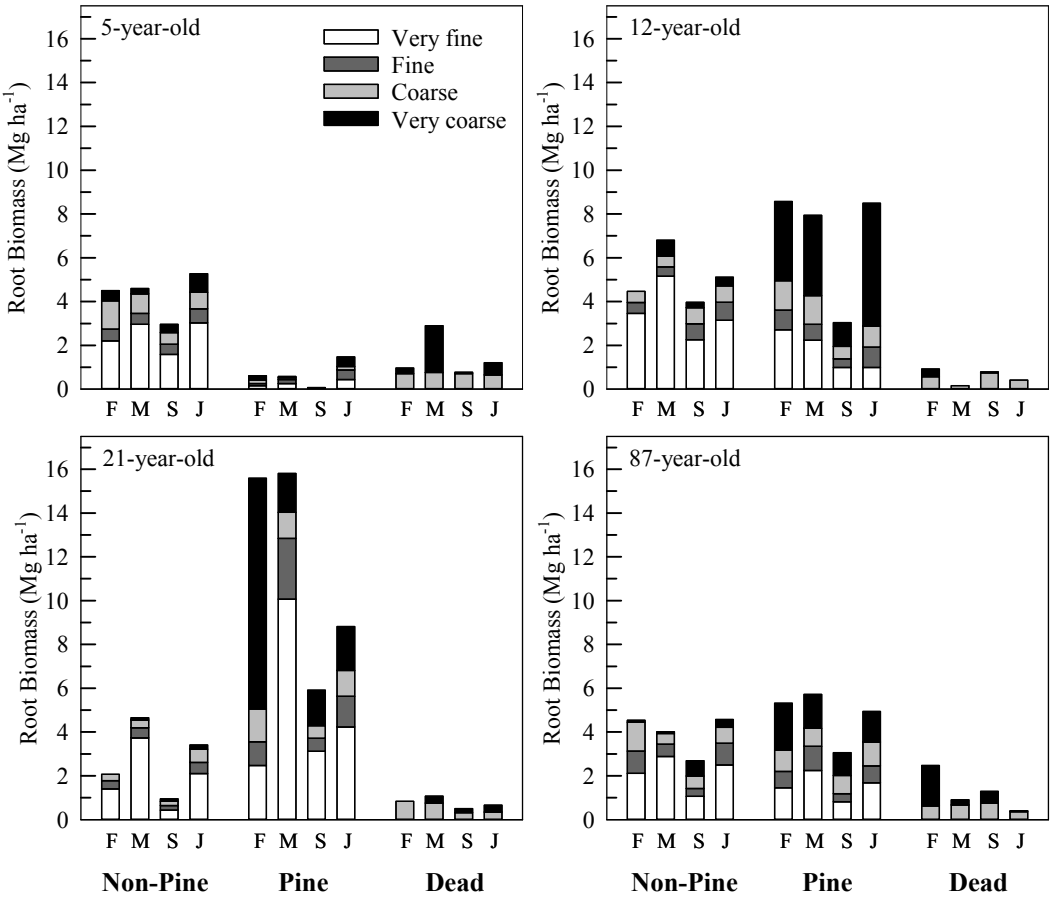

Figure 2.4: Mean root biomass during each sampling period by type and size class in four longleaf pine stands varying in age and structure. Size classes are determined by root diameter and include very fine roots (≤ 1 mm), fine (> 1 and ≤ 2 mm), coarse (> 2 and ≤ 5 mm), and very coarse (> 5 mm). Sampling periods include February 2012 (F), May 2012 (M), September 2012 (S), and January 2013 (J).

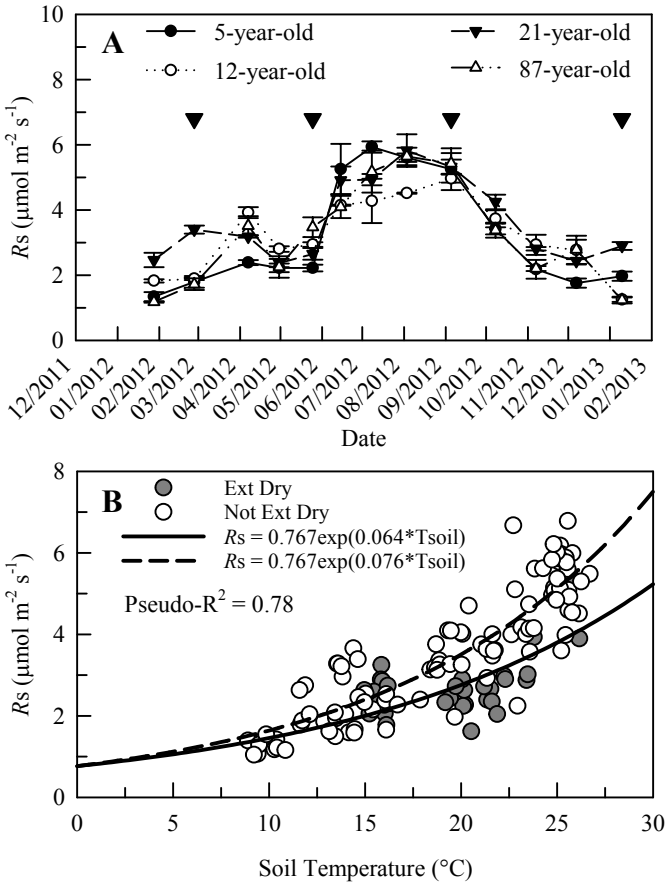

Figure 2.5: (A) Mean monthly soil respiration (R_S) by date in four longleaf pine stands varying in age and structure. Triangles represent dates of root biomass sampling and error bars are ± SE. (B) Soil respiration versus soil temperature. Ext Dry, extremely dry soils ($\theta \leq 0.5\,\mathrm{WP}$); Not Ext Dry, dry moderate or wet soils ($\theta > 0.5\,\mathrm{WP}$), where WP is wilting point.

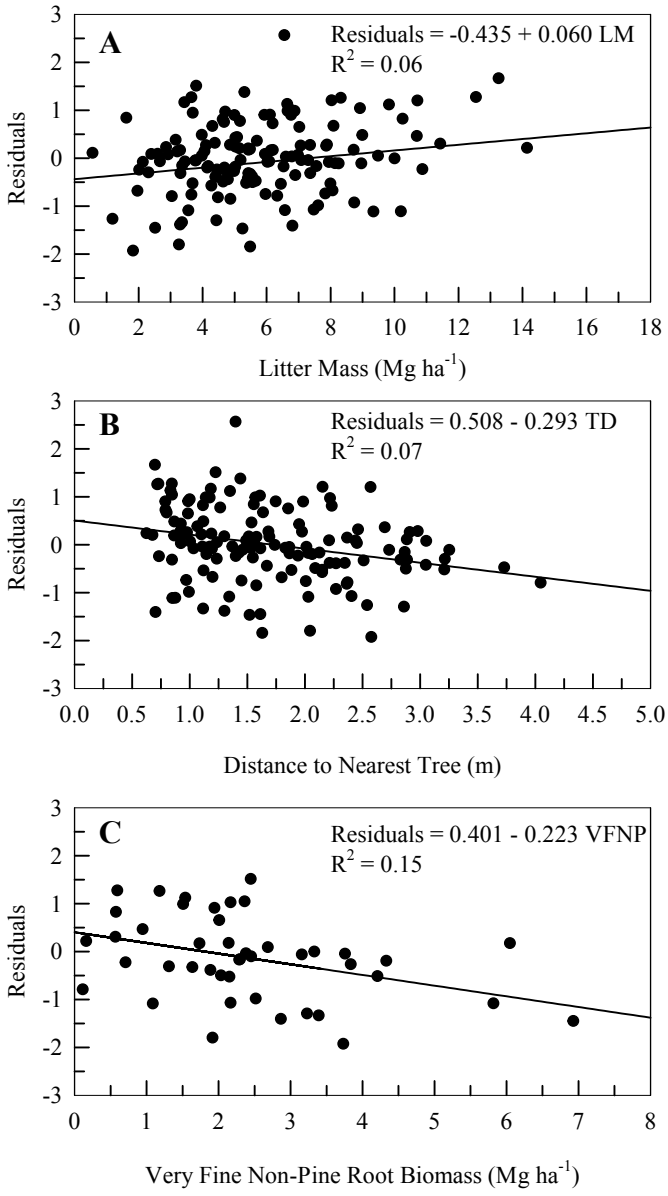

Figure 2.6: Residuals from the R_S-temperature model versus (A) litter mass (LM), (B) distance to nearest tree (TD), and (C) very fine non-pine roots (VFNP). Coefficients of determination are given as partial R^2 values. Full Pseudo-R^2 values for the complete exponential models are given in Table 2.3.

Table 2.1: The age, location, soil properties, planting density and burn history of four longleaf pine stands in western Georgia.

Stand Age	5 years	12 years	21 years	87 years
Longitude (°W)	-84° 52.265'	-84° 46.622'	-84° 47.550'	-84° 47.112'
Latitude (°N)	32° 23.999'	32° 22.353'	32° 23.373'	32° 21.967'
Soil Texture (%)[a]	73, 7, 20	80, 8, 12	85, 7, 8	80, 8, 12
Soil Water Properties (%)[b]	13, 21, 44	9, 17, 41	7, 16, 38	9, 17, 41
Soil Carbon ($Mg\,ha^{-1}$)[c]	61.0	76.0	52.9	84.8
Planting Density ($trees\,ha^{-1}$)	1494	1494	2235	Natural
Burn History[d]	2007, 2010	2002, 2005, 2008, 2010	1992, 1995, 1998, 2001, 2004[e], 2005, 2006[e], 2009, 2010[e]	1981, 1985[e], 1990, 1992, 1994, 1998, 2001, 2004, 2006, 2008, 2010

[a] Soil texture at 10-20 cm in sand, silt, clay.
[b] Soil water properties in wilting point, field capacity, saturation from Oram and Nelson (2014).
[c] Soil carbon to 1 m depth from Samuelson et al. (2014).
[d] Burn records began in 1981.
[e] Indicates wildfire.

Table 2.2: Mean stand characteristics of four longleaf pine forests in western Georgia ($n = 2$ to 3).

Stand age (years)	Size class[a]	Species[b]	Basal Area ($m^2\,ha^{-1}$)	Density ($trees\,ha^{-1}$)	DBH (cm)
5	Mature	LLP	0.0	0	-
		other	0.0	0	-
	Sapling	LLP	0.1	123	3.7
		other	0.2	475	1.94
12	Mature	LLP	2.1	176	12.1
		other	2.5	96	16.5
	Sapling	LLP	2.9	768	6.8
		other	0.2	248	1.9
21	Mature	LLP	17.7	1259	13.2
		other	2.2	165	12.9
	Sapling	LLP	2.5	491	7.9
		other	0.6	176	6.7
87	Mature	LLP	9.9	59	46.1
		other	0.8	11	26.0
	Sapling	LLP	0.8	699	3.6
		other	0.0	91	1.2

[a] Mature includes trees DBH $\geq 10\,cm$ and saplings include DBH $\geq 1\,cm$ and $< 10\,cm$. Height of all measured trees $> 2\,m$.
[b] LLP, longleaf pine.

Table 2.3: Soil respiration (R_S) models for four longleaf pine stands varying in age and forest structure.

Model Form[a]		Parameter Estimates(SE)				Fit[b]	
$R_S =$		β_0	β_1	β_2	β_3	n	R^2
$\beta_0 * \exp(\beta_1 T_{soil})$		0.700(0.07)	0.078(0.00)			143	0.72
$\beta_0 * \exp(\beta_1 T_{soil})$	if $\theta \leq 0.5$ WP	0.767(0.07)	0.064(0.00)			36	0.78
	if $\theta > 0.5$ WP	0.767(0.07)	0.076(0.00)			102	0.78
$\beta_0 * \exp(\beta_1 T_{soil}) + \beta_2 + \beta_3$ LM		0.700(0.07)	0.078(0.00)	-0.435(0.16)	0.060(0.02)	143	0.78
$\beta_0 * \exp(\beta_1 T_{soil}) + \beta_2 + \beta_3$ TD		0.700(0.07)	0.078(0.00)	0.508(0.16)	-0.293(0.09)	140	0.79
$\beta_0 * \exp(\beta_1 T_{soil}) + \beta_2 + \beta_3$ VFNP		0.700(0.07)	0.078(0.00)	0.401(0.24)	-0.223(0.08)	44	0.87

[a] R_S, soil respiration (μmol CO_2 m^{-2} s^{-1}); T_{soil}, soil temperature (°C); θ, volumetric soil moisture (%); WP, wilting point (%); LM, litter mass (Mg ha^{-1}); TD, distance to nearest tree (m); VFNP, very fine non-pine root biomass (Mg ha^{-1}); TC, total cover (%).

[b] Pseudo-R^2 value for the first equation, pseudo-R^2 based on combined models with soil water status dummy variables for the second and third equations, and pseudo-R^2 plus partial R^2 from residuals analysis for last three equations.

Table 2.4: Pearson's correlation coefficients (r) for soil respiration (R_S), abiotic, and biotic variables measured in four longleaf pine stands over 13 months. For these analyses, data were averaged across all stands and measurement dates. Bold r values are significant at $\alpha = 0.05$.

Variable[a]	R_S	T_{soil}	θ	DBH	TD	LM	TC	LC	FC	WC	VC	GC	VFNP	FNP	CNP	VCNP	VFP	FP	CP	VCP	CD	VCD
R_S	1.00																					
T_{soil}	**0.83**	1.00																				
θ	-0.06	**-0.17**	1.00																			
DBH	0.03	-0.02	**-0.29**	1.00																		
TD	**-0.33**	**-0.24**	**0.40**	-0.11	1.00																	
LM	**0.37**	**0.23**	**-0.25**	**0.37**	**-0.21**	1.00																
TC	**0.47**	**0.66**	-0.05	**-0.30**	-0.06	**-0.22**	1.00															
LC	**0.52**	**0.53**	0.13	**-0.26**	0.02	0.01	**0.61**	1.00														
FC	**0.29**	**0.39**	**-0.28**	0.02	**-0.20**	**0.18**	**0.29**	**0.23**	1.00													
WC	**0.24**	**0.40**	-0.10	**-0.19**	-0.12	-0.11	**0.66**	**0.23**	-0.09	1.00												
VC	**0.33**	**0.32**	-0.08	**-0.33**	0.05	-0.10	**0.54**	**0.51**	**0.17**	0.15	1.00											
GC	**0.35**	**0.47**	0.16	**-0.24**	0.08	**-0.28**	**0.81**	**0.47**	-0.06	**0.42**	**0.35**	1.00										
VFNP	**-0.38**	-0.14	-0.10	-0.20	-0.02	**-0.38**	0.16	-0.15	-0.25	0.23	0.14	0.12	1.00									
FNP	**-0.40**	**-0.48**	0.24	0.01	0.13	-0.09	-0.20	-0.13	-0.27	-0.09	-0.03	-0.08	**0.41**	1.00								
CNP	-0.28	-0.25	**0.34**	-0.18	**0.39**	-0.18	-0.08	-0.07	0.00	-0.02	0.08	-0.09	0.29	**0.50**	1.00							
VCNP	0.06	0.03	0.15	-0.08	0.13	-0.12	0.21	0.10	-0.11	0.26	0.18	0.20	0.09	0.09	0.22	1.00						
VFP	-0.03	0.07	**-0.50**	0.20	**-0.49**	0.18	-0.11	-0.25	0.02	0.17	-0.19	**-0.38**	0.21	-0.16	-0.24	-0.23	1.00					
FP	-0.16	-0.10	**-0.46**	0.24	**-0.45**	0.07	-0.17	**-0.33**	-0.04	0.08	-0.22	**-0.38**	0.24	-0.05	-0.23	-0.20	**0.88**	1.00				
CP	-0.13	-0.27	-0.28	0.25	**-0.43**	0.07	-0.27	**-0.35**	-0.27	0.12	**-0.34**	-0.27	0.23	0.02	-0.09	-0.21	**0.47**	**0.55**	1.00			
VCP	-0.09	**-0.30**	-0.12	0.12	**-0.40**	-0.08	**-0.38**	-0.27	-0.24	-0.14	-0.29	-0.25	-0.03	-0.07	-0.20	-0.15	0.16	**0.33**	**0.52**	1.00		
CD	0.10	0.13	0.05	0.03	-0.02	-0.03	0.01	0.15	-0.10	0.11	0.02	-0.07	-0.03	0.09	0.24	0.04	-0.04	-0.05	-0.21	-0.09	1.00	
VCD	-0.21	0.00	-0.10	-0.07	0.26	-0.16	0.00	-0.11	0.17	-0.07	0.16	-0.08	0.02	0.07	**0.38**	-0.13	-0.06	-0.08	-0.09	-0.07	0.26	1.00

[a] T_{soil}, soil temperature; θ, volumetric soil moisture; DBH, diameter at breast height of nearest tree; TD, distance to nearest tree; LM, litter mass; TC, total cover; HC, herbaceous cover; WC, woody cover; FC, forb cover; LC, legume cover; VC, vine cover; GC, graminoid cover; VFNP, FNP, CNP, VCNP, very fine, fine, coarse, and very coarse non-pine root biomass; VFP, FP, CP, VCP, very fine, fine, coarse, and very coarse pine root biomass; CD and VCD, coarse and very coarse dead root biomass.

Chapter 3

Spatial variation of soil respiration in a 64-year-old longleaf pine forest

3.1 Abstract

Soil respiration (R_S) demonstrates temporal and spatial variation that can affect the overall carbon balance of forested ecosystems. The objective of this study was to quantify the spatial variation in R_S and explore the relationships between R_S and ecological covariates in a mature longleaf pine (*Pinus palustris* Mill.) forest. Soil respiration, soil temperature, and soil moisture were measured systematically in three subplots within a 64-year-old longleaf pine forest during six days in the summer of 2012. Ecological covariates were also measured, including edaphic variables, forest floor variables, roots, and stand structural variables. Soil respiration demonstrated varying magnitudes, spatial patterns, and spatial autocorrelation in the three plots, and ranged from 2.4 to 8.6 μmol m^{-2}s^{-1} across plots. In Plot 1, R_S was highly spatially autocorrelated and related to soil bulk density, litter mass, the mean diameter at breast height (1.37 m) of trees within 8 m, 4 m, 2 m, and 1 m of the R_S collars, and total DBH of trees within 2 m and 1 m of the R_S collars. In Plot 2, R_S was spatially independent and related to soil moisture, soil bulk density, and total and mean DBH of trees within 4 m of the R_S collars. In Plot 3, R_S was spatially autocorrelated within a range of 11 m and was related to fine root biomass. Across all three plots, R_S was related to soil moisture, litter mass, forb percent cover, fine root biomass, coarse dead root biomass, the total DBH of trees within 8 m, mean DBH of trees within 1 m, and the number of trees within 1 m of R_S collars. This study illustrates complex spatial patterns and relationships between R_S and ecological covariates within a heterogeneous, mature longleaf pine forest.

3.2 Introduction

Soil respiration is the sum evolution of CO_2 respired from the activity of roots, mycorrhizae, and microorganisms within both the root-affected rhizosphere and the bulk soil (Raich and Nadelhoffer, 1989; Bahn et al., 2010). Autotrophic and heterotrophic activity

are affected by biotic and abiotic factors to varying degrees, thus causing temporal and spatial variability in R_S (Lavigne et al., 2003; Ruehr and Buchmann, 2010; Chen et al., 2011). Soil temperature is generally the most important factor influencing the seasonal changes in R_S as it increases both heterotrophic and autotrophic activity during the warmer growing seasons (Fang et al., 1998; Maier and Kress, 2000; Davidson et al., 2006; DeForest et al., 2006; Ruehr and Buchmann, 2010), but soil temperature has been found to have less control over the spatial variation in R_S within forests (Søe and Buchmann, 2005; Vande Walle et al., 2007; Geng et al., 2012). Plant and soil factors, on the other hand, can directly affect the spatial distribution of R_S. For instance, the allocation of photosynthates to roots increases autotrophic respiration; root exudates increase heterotrophic respiration within the rhizosphere; and litterfall quantity and quality increases heterotrophic respiration in surface soil layers (Bahn et al., 2010; Metcalfe et al., 2011). Belowground edaphic factors also exhibit spatial influence upon R_S, such as soil moisture, pH, bulk density, and soil carbon (Vande Walle et al., 2007; Luan et al., 2012; Fóti et al., 2014), but the influences of these edaphic variables are not independent of plant, root, and litter distribution in forests. For instance, the interaction between precipitation and trees, specifically throughfall and stemflow, affects the spatial variation of soil moisture in forests, and trees can affect soil nutrients, soil microbial community composition, and soil properties such as pH and bulk density (Zinke, 1962; Vetaas, 1992; Weber and Bardgett, 2011; Lavoie et al., 2012). A better understanding of the ecological variables that affect R_S within forests is a high research priority with the goal to develop mechanistic R_S models that include plant-soil interactive effects (Martin and Bolstad, 2009; Bahn et al., 2010; Chen et al., 2011; Martin et al., 2012; Mitra et al., 2014).

After historical degradation from logging, turpentine extraction, grazing and fire suppression (Noss, 1988), longleaf pine (*Pinus palustris* Mill.) ecosystems are now being restored throughout the southeastern United States to provide ecosystems services, such as rare species habitat and biodiversity conservation (Jose et al., 2006; Mitchell et al., 2006).

Mature, naturally regenerated longleaf pine forests are characterized by a spatially heterogeneous structure of the canopy, understory, and soils (Noss, 1988; Battaglia et al., 2002; Lavoie et al., 2012), which is the result of a complex mosaic of microhabitats. Longleaf pine forests depend upon fire to reduce succession by other trees, maintain herbaceous understory niches, and expose mineral soil to facilitate seedling regeneration (Noss, 1988; Brockway and Lewis, 1997). In unburned longleaf pine forests, trees exhibit a strong belowground influence on the spatial structure and chemistry of soil, but in frequently burned longleaf pine forests, the belowground influence of trees can become homogenized (Lavoie et al., 2012), and high intensity fires can also homogenize species composition, especially in the midstory (Lashley et al., 2014). Canopy gaps in longleaf pine forests affect light availability to the forest floor and increase understory plant biomass (McGuire et al., 2001), and the spatial variability of light available for understory species and seedling regeneration is optimized when uneven-aged, variable-retention silviculture is used and/or when larger aggregated canopy gaps are present (Brockway and Outcalt, 1998; Battaglia et al., 2002). Longleaf pine rooting zones extend beyond the extent of the canopy (Heyward, 1933; Hodgkins and Nichols, 1977), which creates complex spatial relationships between herbaceous roots of the continuous understory layer, pine roots within the tree rooting zones, and the influence of longleaf pine canopies (Vetaas, 1992; McGuire et al., 2001). Furthermore, the spatial distribution of lateral roots is positively related to tree age and competitive position within the forest, and the shape of the rooting zone outward from the stem is influenced by the presence of other trees (Hodgkins and Nichols, 1977). A final aspect of the spatial complexity of a mature longleaf pine forest results from the presence of nitrogen-fixing legumes, which affect the spatial distribution of soil nitrogen and decomposition rates of litter (Vetaas, 1992).

The main objectives of this study were (1) to quantify the spatial structure of R_S and (2) determine how ecological covariates (listed in Table 3.1) relate to R_S independent of the temporal effect of soil temperature. This study was conducted in a naturally-regenerated

64-year-old longleaf pine forest at Fort Benning, Georgia in which we established three, semi-independent plots that each included 25 gridded R_S measurement subplots. We expected that in this mature, spatially heterogeneous longleaf pine stand, above- and belowground ecological covariates would exhibit complex spatial patterns corresponding with the spatial distribution of R_S.

3.3 Methods

3.3.1 Site Description

The study site was located at Fort Benning Military Base near Columbus, Georgia (Fig. 3.1). The climate in this area has a mean maximum temperature of 24.6 °C, a mean minimum temperature of 18.7 °C, and a mean annual precipitation of 1187 mm (1981-2010 Normals) (National Climatic Data Center, 2015*a*). The stand used for this study was a 64-year-old longleaf pine stand located on the Alabama property of Fort Benning, near Fort Mitchell, Alabama and Lawson Army Airfield (32° 19.142' N, -85° 0.514' W, 97 m A.S.L.), and was a naturally-regenerated, even-aged longleaf pine forest. Soils at this stand were Troup-Springhill-Luverne complexes, which are typically very deep, well to excessively drained loamy fine sand or loamy sand soils on side slopes (10-30 % slopes) (Soil Survey Staff, 2014). Soil carbon in this stand was approximately 85.9 Mg C ha^{-1} to a 1 m depth, and soil texture consisted of 79 % sand, 8.5 % silt, and 12.5 % clay (Samuelson et al., 2014). These soil textures were associated with wilting point, field capacity, and saturation at 9.8, 18.2, and 41.5 % volumetric soil moisture, respectively (Oram and Nelson, 2014). This stand was last burned in the winter of 2010 and is maintained on an approximately three-year burning cycle. More information about this stand, including above and belowground carbon stocks, can be found in Samuelson et al. (2014).

The experimental design consisted of three 24 m by 24 m plots over a gradual 10 m topographical gradient (Fig. 3.1). Within each plot, 25 1 m^2 R_S sampling subplots were laid out evenly in a 5 by 5 grid with 6 m spacing between subplot centroids. At the center of

each R_S sampling subplot, a R_S collar (PVC, 10 cm diameter, 4.5 cm height) was installed into the ground through the standing litter and to a consistent 2.5 cm depth. In July 2012, all trees taller than 2 m in height with diameter at breast height greater than 1 cm were inventoried within 8 m of each R_S collar and classified as mature trees (DBH $\geq$ 10 cm) or saplings (DBH $<$ 10 cm; Figs. 3.1 and 3.2, Table 3.2). The three plots ranged in mature longleaf pine basal area from 8.0 to 10.3 $m^2\,ha^{-1}$, sapling longleaf pine basal area from 0.2 to 0.7 $m^2\,ha^{-1}$, and in total basal area from 11.5 to 15.1 $m^2\,ha^{-1}$ (Table 3.2). Density of mature and sapling longleaf pine trees ranged from 78.6 to 124.5 trees ha^{-1} and 85.2 to 373.4 trees ha^{-1}, respectively (Table 3.2). Mature longleaf pine trees ranged in DBH from 19.0 to 36.1 cm and sapling longleaf pine trees ranged in DBH from 4.5 to 5.0 cm (Table 3.2). Other pine species present in these plots included mature and sapling loblolly pine (*P. taeda* L.) and shortleaf pine (*P. echinata* Mill.) and hardwood species included oaks (*Quercus* spp.), hickories (*Carya* spp.), and sweetgum (*Liquidambar styraciflua* L.). No mature hardwood trees were observed.

3.3.2 Soil respiration measurements

Soil respiration was measured systematically on all 75 R_S collars in the morning (830-1100 hr EST) on six days in 2012 (July 14, July 24, July 26, August 2, August 14, and August 17). Soil respiration was measured using a LI-6400-09 Soil CO_2 Flux Chamber attached to a LI-6400 portable infrared gas analyzer (LI-COR Biosciences, Lincoln, NE, USA). Ambient atmospheric CO_2 concentration was measured at the first R_S collar in each plot and used as target CO_2 concentration for that entire plot. Soil temperature and soil moisture were measured within approximately 10-15 cm of each R_S collar at the time of R_S measurement. Soil temperature was measured with a 15-cm depth soil temperature thermocouple (6000-09, LI-COR Biosciences) and soil moisture was measured with a 20-cm depth soil moisture time domain reflectometry probe (Hydrosense II, Campbell Scientific, Logan, UT, USA).

3.3.3 Ecological covariate measurements

Prior to the installation of R_S collars, total percent live understory vegetation cover (< 1 m height) was ocularly estimated within the $1\,m^2$ R_S sampling subplots, as well as by plant functional group (woody, forb, legume, vine, and graminoid). Subsequent to all of the R_S measurements on August 17, 2012, litter was collected from within the R_S collars, dried to a constant weight at 70 °C, and weighed. After litter collection, the R_S collars were removed and soil samples (10 cm diameter, 15 cm depth) were collected from below the R_S collars, bagged, and kept cool until processing. Processing the soil samples consisted of dry sifting the soil through a 2 mm mesh sieve to retrieve roots. Roots were washed, sorted by type and size, dried at 70 °C for 48 hrs and weighed. Roots were sorted into very fine (diameter ≤ 1 mm), fine (1 mm $<$ diameter ≤ 2 mm), coarse (2 mm $<$ diameter ≤ 5 mm), and very coarse (diameter > 5 mm) categories and by live or dead based on texture, resiliency to bending, and coloration. Air dried soil samples were sent to the Auburn University Soil Testing Laboratory (Auburn, AL) for measurement of pH and concentrations of carbon (C), nitrogen (N), and organic matter (OM). Within 10 cm of each collar in undisturbed soil, bulk density samples were taken from 1-10 cm depth with a 5.7 cm diameter soil sampler (0200 Soil Core Sampler, Soil Moisture Equipment Corp., Goleta, CA, USA). Bulk density was calculated following the procedure of Law et al. (2008).

3.3.4 Data analysis

The locations of the plots and R_S collars were collected with a handheld, decimeter-accurate global positioning system (Trimble GeoXH, Trimble Navigation Limited, Sunnyvale, CA, USA) and downloaded to ArcGIS (Environmental Systems Research Institute, Inc., Redlands, CA, USA). The tree inventory data was digitized in ArcGIS and used to calculate mean DBH, total DBH, and number of trees within 8 m, 6 m, 4 m, 2 m, and 1 m of each R_S collar and the distance to the nearest tree from each R_S collar. Soil C, OM, and N concentrations were converted to a per area basis (Mg ha^{-1}) based on soil bulk density.

Repeated measures analysis with autoregressive moving average covariance structure was used to test for plot differences in R_S, soil temperature, and soil moisture within a mixed-model framework and then R_S, soil temperature, and soil moisture were averaged across the six measurement dates by R_S sampling subplot for geospatial and regression analysis.

Geospatial analysis was performed within each plot and across all three plots to determine the spatial extent of autocorrelation in R_S. Specifically, the spatial structure of R_S was determined with semivariogram analysis (package gstat; R Core Team, 2014). Semivariance (γ) measures the spatial relationship of R_S at two locations (x and $x+h$) separated by a lag distance h:

$$\gamma(h) = \frac{1}{2n(h)} \sum_{x=1}^{n} (Rs_x - Rs_{x+h})^2$$

where $n(h)$ is the number of pairs at distance h (Stoyan et al., 2000; Mitra et al., 2014). Semivariance was graphed versus h in a semivariogram and fit with nugget, linear, or exponential semivariogram models to determine the degree and scale of spatial autocorrelation of R_S. The fitted model for each semivariogram was chosen by minimizing the residual error sum of squares (SSE). A detailed description of this spatial data analysis is provided in Appendix B. No spatial autocorrelation (i.e., spatial independence) corresponded with a nugget model, moderate spatial autocorrelation corresponded with an exponential model, and strong spatial autocorrelation corresponded with the linear model.

The relationships between R_S and edaphic, forest floor, and root variables were evaluated with Pearson's correlation coefficients at $\alpha = 0.10$ within each plot and across all three plots. Because of high multicollinearity amongst stand structural variables, the relationship between R_S and stand structural variables was examined with principal components analysis and correlation coefficients following Søe and Buchmann (2005). Multiple linear regression models were developed through stepwise model selection for each plot and across all three

plots. The residuals of the multiple linear regression models were tested for spatial autocorrelation with semivariogram analysis and for multicollinearity with variance inflation factors (VIFs).

3.4 Results

3.4.1 Spatial variation in R_S and edaphic variables

Daily maximum air temperature varied from 27.8 to 38.3 °C during the measurement period from July 14, 2012 through August 17, 2012 at Columbus Metropolitan Airport Weather Station (Fig. 3.3) (National Climatic Data Center, 2015*b*). Daily minimum temperature ranged from 26.7 to 17.8 °C and daily precipitation ranged from 0.0 to 25.7 mm day^{-1} (Fig. 3.3). Daily mean soil temperature ranged from 25.2 to 27.1 °C and daily mean soil moisture ranged from 4.0 to 14.8 % across all three plots (Table 3.3). Daily coefficients of variation (CV) for soil temperature and soil moisture ranged from 1.4 to 3.6 % and 36.0 to 91.3 %, respectively (Table 3.3). Mean soil temperature and soil moisture were significantly different between plots ($F_{2,9} = 5.77$, $p = 0.0244$ and $F_{2,9} = 40.12$, $p < 0.0001$, respectively; Table 3.4). Plot means and CVs for other edaphic variables are given in Table 3.4.

Daily coefficients of variation for R_S ranged from 28.4 to 46.5 % (Table 3.3). Across the six measurement days, mean R_S in Plot 1 was 5.3 μmol m^{-2} s^{-1} (Fig. 3.4A) and showed highly autocorrelated spatial structure (linear semivariogram model; Fig. 3.4D, Table 3.4). In Plot 2, R_S averaged 3.9 μmol m^{-2} s^{-1} and showed spatial independence (nugget semivariogram model; Figs 3.4B and E). Soil respiration was 4.1 μmol m^{-2} s^{-1} on average in Plot 3 and demonstrated moderate spatial structure with a range of 10.9 m (exponential semivariogram model; Figs 3.4C and F). Soil respiration was significantly different between plots ($F_{2,9} = 215.54$, $p < 0.0001$; Table 3.4).

3.4.2 Spatial variation in forest floor and root variables

Mean ± SE (CV) litter mass for Plot 1, Plot 2, and Plot 3 were 7.2±1.4 (100.7), 4.6±0.4 (48.7), and 6.7 ± 0.8 (54.9), respectively. Total percent cover ranged from 68.7 to 85.6 % across the plots and was dominated by graminoid cover class in each plot (Fig. 3.5A). In Plots 1 and 3, the very fine size class dominated live root biomass, and in Plot 2, the very coarse size class dominated the live root biomass (Fig. 3.5B). Coarse dead root biomass ranged from 0.3 to 0.9 $\mathrm{Mg\,ha^{-1}}$ and very coarse dead root biomass ranged from 0.5 to 3.6 $\mathrm{Mg\,ha^{-1}}$.

3.4.3 Spatial variation in stand structural variables

In general, Plot 1 had the highest density of trees, a higher mean number of trees within each radius, and lower mean distance to nearest tree from R_S collar (Fig. 3.2, Tables 3.2 and 3.5). Plot 2 had the lowest total basal area and highest density of longleaf pine saplings, which tended to cluster in the east and north portions of the tree survey extent, which extended 8 m from each R_S collar (Fig. 3.2, Table 3.2). Plot 3 varied from an area of higher hardwood sapling density in the northeast to an area with few, larger longleaf pine trees in the southwest portion of the tree survey extent (Fig. 3.2).

3.4.4 Relationships between R_S and ecological covariates

The following variables were not significantly related to spatial variation in R_S in any plot or across all three plots: soil temperature; live very fine, coarse, or very coarse root biomass; dead very coarse root biomass; soil N, OM, or C; total, woody, legume, vine, or graminoid percent cover; number of trees within 8, 6, 4, or 2 m; mean or total DBH of trees within 6 m; and distance to nearest neighboring tree from R_S collar. Mean and total DBH of trees within 1 m were highly correlated (r = 0.99), and thus of these two variables, only the mean DBH of trees within 1 m was retained for further analysis.

In Plot 1, R_S was significantly and negatively related to soil bulk density and positively related to litter mass, mean DBH of trees within 4 m, mean and total DBH of trees within

2 m, and mean DBH of trees within 1 m (Figs. 3.6 and 3.7). The final multiple linear regression for Plot 1 included bulk density and mean DBH of trees within 1 m, and this model accounted for 37 % of the spatial variation in R_S in Plot 1 (Table 3.6). This model did not account for the spatial autocorrelation of R_S in Plot 1, as residuals were strongly autocorrelated (linear semivariogram model), but multicollinearity amongst regressors was not present (VIFs < 1.10).

In Plot 2, spatial variation in R_S was significantly and negatively correlated with soil moisture, bulk density, and total and mean DBH of trees within 4 m (Figs. 3.6 and 3.7). The final multiple linear regression model included soil bulk density and total DBH of trees within 4 m and accounted for 23 % of the spatial variation in R_S in Plot 2 (Table 3.6). Residuals exhibited spatial independence with semivariogram analysis (nugget semivariogram model) and VIFs were less than 1.01.

Fine live root biomass was the only variable that significantly correlated with R_S in Plot 3, and accounted for 13 % of the spatial variation of R_S in Plot 3 (Fig. 3.6, Table 3.6). Residuals from fine root biomass versus Plot 3 R_S were spatially independent (nugget semivariogram model).

Soil moisture and coarse dead roots negatively correlated with R_S across all three plots, and litter mass, forb cover, and fine live roots positively correlated with R_S across all three plots (Fig. 3.6). Soil respiration was closely related to mean DBH within 2 m, total DBH within 2 m, and mean DBH within 1 m when analyzed across all plots with principal components analysis (Fig. 3.8). Total DBH of trees within 8 m was also positively correlated with R_S across all three plots (Fig. 3.7). The final multiple linear regression model for all three plots combined included soil moisture, litter mass, coarse dead root biomass, and mean DBH of trees within 1 m (Table 3.6). This multiple linear regression model accounted for 33 % of the spatial variation in R_S across plots, had residuals that were spatially independent (nugget semivariogram model), and had VIFs less than 1.27.

3.5 Discussion

The range and strength of the spatial structure of R_S varied between plots, from being spatially independent in Plot 2, moderately spatially autocorrelated in Plot 3, and highly spatially autocorrelated in Plot 1. Management type and intensity is one factor that has been shown to affect spatial variation and autocorrelation in R_S (Dore et al., 2014). In this 64-year-old longleaf pine stand, prescribed fire is the only current management technique and is applied on a 3-year return interval. Fire intensity varies within a stand whereby low fire intensities are proximal to fire breaks and high fire intensities are more prevalent towards stand interiors (Lashley et al., 2014). Biennial or annual fires have been found to decrease litter mass compared with fire suppression (Brockway and Lewis, 1997), and higher fire intensities generally correspond with lower hardwood density (Lashley et al., 2014) and less spatial structure in soil edaphic properties (Lavoie et al., 2012). Based upon the spatial locations of the three plots relative to burn breaks and the relative differences in litter, hardwood saplings, and soil edaphic properties, perhaps fire intensity may have influenced the spatial variability in R_S between plots. However, lacking any direct information on fire intensity, this mechanism for between-plot differences in the structure of R_S is difficult to quantify.

Soil moisture, which varied between plots and is influenced by topography, soil depth, transpiration, and tree basal area (Tromp-van Meerveld and McDonnell, 2006), has also been shown to moderate the spatial structure of R_S. Specifically, high soil moisture conditions have low spatial variability (Tromp-van Meerveld and McDonnell, 2006), and can exhibit a concomitant homogenizing effect on R_S. For instance, high soil moisture reduced the spatial variation in R_S in sandy grasslands (Fóti et al., 2014), and heavy rainfall made R_S on bare soil plots spatially independent compared to highly autocorrelated on dryer days (La Scala, Jr. et al., 2000). In addition to affecting the overall spatial autocorrelation of R_S, soil moisture has also been shown to correlate with spatial variability in R_S in planted pine and secondary oak forests in China (Luan et al., 2012), in Sitka spruce (*Picea sitchensis*

(Bong.) Carrière) forests (Saiz et al., 2006), and in a beech (*Fagus sylvatica* L.) forest (Søe and Buchmann, 2005). In this study, soil moisture was significantly, negatively related to R_S within Plot 1, where it was the highest on average between plots. In particular, at least one R_S measurement subplot within Plot 2 was at or above field capacity (18.2 %) on every measurement day, and soil moisture was as high as 35.8 % in one subplot in Plot 2 on August 2, 2012. Soil moisture was least related to R_S in Plot 1, where soil moisture was the lowest with the least spatial variability, and negatively but not significantly related with R_S in Plot 3, which had intermediate soil moisture levels and variability. The presence of soil water decreases CO_2 diffusivity through soil pores, decreasing R_S (Maier et al., 2010). Soil moisture can also limit autotrophic and heterotrophic activity at very low levels (Orchard and Cook, 1983), however soil moisture was never measured below wilting point in these plots during this study, which suggests that the negative relationship observed between R_S and soil moisture is more likely due to high soil moisture conditions rather than drought-like soil conditions.

The variables that were related to R_S across all the plots included soil moisture, litter mass, forb cover, roots, and stand structural variables. However, because of the general differences in these variables between plots, it is difficult to determine at what scale these relationships apply (i.e., within-plot or between-plot variation). Plot variation in litter mass and forb cover may have been due to fire intensity (Brockway and Lewis, 1997) or interactions with overstory trees, including facilitation between herbaceous species and trees (Vetaas, 1992) and the accumulation of bark and leaf litter around trees (Zinke, 1962). Because litter mass can directly increase heterotrophic respiration and is approximately 15-25 % of total R_S in coniferous forests (Reinke et al., 1981; Irvine and Law, 2002; Taneva and Gonzalez-Meler, 2011), we expected litter mass to strongly relate to R_S within this longleaf pine forest. Furthermore, litter mass influenced intra-annual variation in R_S beyond the R_S-temperature relationship in four diverse longleaf pine stands (Chapter 2), and litter mass was positively related to temporal variation in R_S and annual R_S estimates in mature longleaf

pine stands varying in basal area (Samuelson and Whitaker, 2012). Litter has also been found to be specifically related to spatial variation in R_S in Florida slash pine plantations (Fang et al., 1998) and in unmanaged California mixed-conifer forests (Dore et al., 2014). However, although litter mass did exhibit a positive relationship with R_S within and across all three stands, this relationship was not always significant. Measuring standing litter mass within the collars may not have captured true spatial heterogeneity of litter quality and decomposition rates in these stands and thus the influence of litter mass on R_S may be confounded by varying decomposition rates and C:N ratios of leaf litter sources (Vetaas, 1992; Tjoelker et al., 2005).

Within a forest, soil bulk density exhibits spatial variability due, in part, to the presence of trees (Zinke, 1962), and soil bulk density was the only edaphic variable that was significantly related to R_S in more than one plot. Bulk density was inversely related to R_S such that as bulk density increased, R_S decreased, which is a fairly common relationship found in R_S studies and relates to the diffusion properties of CO_2 through soil (Raich and Schlesinger, 1992). For instance, in burned mixed-conifer forests in California (Dore et al., 2014) and in naturally regenerated oak forests and monoculture pine plantations in China (Luan et al., 2012), soil bulk density was inversely related to R_S.

Total understory cover was not related to spatial variation of R_S in any of these studies, perhaps due to the low spatial variability and high continuous herbaceous cover of this stand during the growing season when R_S measurements were made. Continuous herbaceous understories are indicative of woodland- and savanna-type ecosystems, and vegetation cover may have a weak influence on spatial heterogeneity in R_S in such systems (Vetaas, 1992). In Chapter 2, vegetation cover positively related to the intra-annual variation in R_S but followed seasonal increases in soil temperature such that cover did not account for any additional variation in R_S after accounting for the R_S-temperature relationship. Understory sagebrush (*Artemisia* spp.) and saw palmetto (*Serenoa repens* (W. Bartram) Small) have been shown to affect spatial variation in R_S in Wyoming shrublands (Mitra et al., 2014)

and a Florida slash pine plantation (Fang et al., 1998), respectively, but in both cases, the understory was reportedly patchy, compared to the relatively continuous understory of this study.

Live fine root biomass was generally positively related to R_S across these stands and was the only variable correlated with R_S in Plot 3. Fine roots were also positively related to spatial variation in R_S in control mixed-conifer forests (Dore et al., 2014), in an unmanaged beech forest (Søe and Buchmann, 2005), and in a slash pine plantation (Fang et al., 1998). Total root volume, but not mass, exhibited a positive influence upon the spatial, temperature independent variation in R_S measured in spring in loblolly pine plantations varying in age (Wiseman and Seiler, 2004). Dead coarse roots, on the other hand, were negatively related to R_S, perhaps due to the absence of autotrophic respiration and depleted labile carbon substrates for heterotrophic respiration. In Chapter 4, the higher presence of dead roots in root exclusion tubes corresponded with significantly lower R_S, where it was concluded that residual R_S in the tubes was mostly heterotrophic in origin and 18 to 39 % lower than total R_S from control soil.

Overall, R_S was positively linked with mean DBH of trees within 1 and 2 m of the R_S collars, based on the PCA analysis. Proximity to trees has been shown to increase R_S in other studies, such as across longleaf pine stands varying in age and structure (Chapter 2) and in a 22-year-old longleaf pine forest (Clinton et al., 2011). In loblolly pine plantations varying in age, R_S was significantly higher at the base of loblolly trees than 1.5 m away from trees, which was hypothesized to be due to higher root biomass and autotrophic respiration near tree bases (Wiseman and Seiler, 2004). However, in the current study the direction of the relationships between R_S and the size of trees varied between plots at intermediate distances (4 to 2 m), perhaps corresponding with the diverse stand structures of the three plots. For example, Plot 2 showed a depression of R_S in the center of the plot, in an area of more mature trees and less saplings, compared to Plot 1 which had highest R_S areas in the northern extent of the plot where relatively fewer saplings and more large mature longleaf

pine trees were present. The spatial influence of trees on R_S in longleaf pine trees may be further confounded by the complex spatial network of longleaf pine lateral roots that extend beyond the canopy boundaries and vary in shape and extent due to age, competitive status within the forest, and topography (Heyward, 1933; Hodgkins and Nichols, 1977).

3.6 Conclusion

Both the spatial patterns in R_S and the relationships between R_S and ecological covariates showed complex variation within this 64-year-old longleaf pine forest. Fang et al. (1998) concluded in a previous spatial R_S study that soil microbial respiration held the most control over the spatial variation in R_S, followed by litter microbial respiration, and least by autotrophic respiration (Fang et al., 1998). In agreement with Fang et al. (1998), we found that edaphic variables, such as soil moisture and bulk density, also correlated with spatial variation in R_S in this study, perhaps by directly affecting microbial respiration. However, forest floor and stand structural variables also affected R_S patterns in this forest, including litter mass, root biomass, and the size of trees within 1 m of R_S measurements. We suggest that the relatively simple spatial patterns in R_S observed in the monoculture slash pine plantation of Fang et al. (1998) allowed for more general plant-soil mechanisms of R_S to be developed, but that in this mature, spatially heterogeneous longleaf pine forest, above- and belowground interactions between plants, soil, topography, and perhaps fire intensity, have resulted in a much more complex profile of R_S. Therefore, we conclude that this study, which is the first of its kind in longleaf pine forest, proposes some potential mechanisms for the development of spatial R_S profiles within a mature longleaf pine forest, but also highlights the need for more direct, controlled studies of the plant-soil mechanisms driving R_S in heterogeneous longleaf pine forests.

3.7 Acknowledgements

This research was supported in part by the United States Department of Defense, through the Strategic Environmental Research and Development (SERDP), and by the School of Forestry and Wildlife Sciences, Auburn University, Auburn, Alabama. The authors would like to thank the following people for field and laboratory assistance: Tom Stokes, Jake Blackstock, Justin Rathel, Ann Huyler, Lorenzo Ferrari, and Michael Gunter.

Bibliography

Bahn, M., I. A. Janssens, M. Reichstein, P. Smith, and S. E. Trumbore. 2010. Soil respiration across scales: towards an integration of patterns and processes. New Phytologist **186**:292–296.

Battaglia, M. A., P. Mou, B. J. Palik, and R. J. Mitchell. 2002. The effect of spatially variable overstory on the understory light environment of an open-canopied longleaf pine forest. Canadian Journal of Forest Research **32**:1984–1991.

Brockway, D. G., and C. E. Lewis. 1997. Long-term effects of dormant season prescribed fire on plant community diversity, structure and productivity in a longleaf pine wiregrass ecosystem. Forest Ecology and Management **96**:167–183.

Brockway, D. G., and K. W. Outcalt. 1998. Gap-phase regeneration in longleaf pine wiregrass ecosystems. Forest Ecology and Management **106**:125–139.

Chen, X., W. M. Post, R. J. Norby, and A. T. Classen. 2011. Modeling soil respiration and variations in source components using a multi-factor global climate change experiment. Climatic Change **107**:459–480.

Clinton, B. D., C. A. Maier, C. R. Ford, and R. J. Mitchell. 2011. Transient changes in transpiration, and stem and soil CO_2 efflux in longleaf pine (*Pinus palustris* Mill.) following fire induced leaf area reduction. Trees **25**:997–1007.

Davidson, E. A., I. A. Janssens, and Y. Luo. 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q_{10}. Global Change Biology **12**:154–164.

DeForest, J. L., A. Noormets, S. G. McNulty, G. Sun, G. Tenney, and J. Chen. 2006. Phenophases alter the soil respiration-temperature relationship in an oak dominated forest. International Journal of Biometeorology **1**:135–144.

Dore, S., D. L. Fry, and S. L. Stephens. 2014. Spatial heterogeneity of soil CO_2 efflux after harvest and prescribed fire in a California mixed conifer forest. Forest Ecology and Management **319**:150–160.

Fang, C., J. B. Moncrieff, H. L. Gholz, and K. L. Clark. 1998. Soil CO_2 and its spatial variation in a Florida slash pine plantation. Plant and Soil **205**:135–146.

Fóti, S., J. Balogh, Z. Nagy, M. Herbst, K. Pintér, E. Péli, P. Koncz, and S. Bartha. 2014. Soil moisture induced changes on fine-scale spatial pattern of soil respiration in a semi-arid sandy grassland. Geoderma **213**:245–254.

Geng, Y., Y. Wang, K. Yang, S. Wang, H. Zeng, F. Baumann, P. Kuehn, T. Scholten, and J.-S. He. 2012. Soil respiration in Tibetan alpine grasslands: belowground biomass and soil moisture, but not soil temperature, best explain the large-scale patterns. PLoS ONE **7**:1–12.

Heyward, F. 1933. The root system of longleaf pine on the deep sands of Western Florida. Ecology **14**:136–148.

Hodgkins, E. J., and N. G. Nichols. 1977. Extent of main lateral roots in natural longleaf pine as related to position and age of the trees. Forest Science **23**:161–166.

Irvine, J., and B. E. Law. 2002. Contrasting soil respiration in young and old-growth ponderosa pine forests. Global Change Biology **8**:1183–1194.

Jose, S. J., E. Jokela, and D. L. Miller, editors. 2006. The Longleaf Pine Ecosystem: Ecology, Silviculture and Restoration. Springer, New York.

La Scala, Jr. N., J. Marques Jr., G. T. Pereira, and J. E. Cora. 2000. Short-term temporal changes in the spatial variability model of CO_2 emissions from a Brazilian bare soil. Soil Biology & Biochemistry **32**:1459–1462.

Lashley, M. A., M. C. Chitwood, A. Prince, M. B. Elfelt, E. L. Kilburg, C. S. Deperno, and C. E. Moorman. 2014. Subtle effects of a managed fire regime: a case study in the longleaf pine ecosystem. Ecological Indicators **38**:212–217.

Lavigne, M. B., R. Boutin, R. J. Foster, G. Goodine, P. 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.

Lavoie, M., M. C. Mack, J. K. Hiers, and S. Pokswinski. 2012. The effect of restoration treatments on the spatial variability of soil processes under longleaf pine trees. Forests **3**:591–604.

Law, B. E., T. Arkebauer, J. L. Campbell, J. Chen, O. Sun, M. Schwartz, C. van Ingen, and S. Verma, 2008. Terrestrial carbon observations: protocols for vegetation sampling and data submission. Available from http://www.fao.org/gtos, Global Terrestrial Observing System, Rome, Italy.

Luan, J., S. Liu, X. Zhu, J. Wang, and K. Liu. 2012. Roles of biotic and abiotic variables in determining spatial variation of soil respiration in secondary oak and planted pine forests. Soil Biology & Biochemistry **44**:143–150.

Maier, C. A., and L. W. Kress. 2000. Soil CO_2 evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability. Canadian Journal of Forest Research **30**:347–359.

Maier, M., H. Schack-Kirchner, E. E. Hildebrand, and J. Holst. 2010. Pore-space CO_2 dynamics in a deep, well-aerated soil. European Journal of Soil Science **61**:877–887.

Martin, J. G., and P. V. Bolstad. 2009. Variation of soil respiration at three scales: Components within measurements, intra-site variation, and patterns on the landscape. Soil Biology & Biochemistry **41**:530–543.

Martin, J. G., C. L. Phillips, A. Schmidt, J. Irvine, and B. E. Law. 2012. High-frequency analysis of the complex linkages between soil CO_2 fluxes, photosynthesis and environmental variables. Tree Physiology **32**:49–64.

McGuire, J. P., R. J. Mitchell, E. B. Moser, S. D. Pecot, D. H. Gjerstad, and C. W. Hedman. 2001. Gaps in a gappy forest: plant resources, longleaf pine regeneration, and understory response to tree removal in longleaf pine savannas. Canadian Journal of Forest Research **31**:765–778.

Metcalfe, D. B., R. A. Fisher, and D. A. Wardle. 2011. Plant communities as drivers of soil respiration: pathways, mechanisms, and significance for global change. Biogeosciences **8**:2047–2061.

Mitchell, R. J., J. K. Hiers, J. J. O'Brien, S. B. Jack, and R. T. Engstrom. 2006. Silviculture that sustains: the nexus between silviculture, frequent prescribed fire, and conservation of biodiversity in longleaf pine forests of the southeastern United States. Canadian Journal of Forest Research **36**:2714–2736.

Mitra, B., D. S. Mackay, E. Pendall, B. E. Ewers, and M. B. Clearly. 2014. Does vegetation structure regulate the spatial structure of soil respiration within a sagebrush steppe ecosystem? Journal of Arid Environments **103**:1–10.

National Climatic Data Center, 2015*a*. 1981-2010 Normals. Columbus Metropolitan Airport, GA. Station GHCND:USW00093842. Last accessed January 15, 2015. http://www.ncdc.noaa.gov/cdo-web/datatools/normals.

National Climatic Data Center, 2015*b*. Daily Summaries. Columbus Metropolitan Airport, GA. Station GHCND:USW00093842. Downloaded November 2014. http://www.ncdc.noaa.gov/cdo-web/datatools/findstation.

Noss, R. F. 1988. The longleaf pine landscape of the Southeast: almost gone and almost forgotten. Endangered Species Update **5**:1–8.

Oram, B., and R. Nelson, 2014. Soil texture triangle: hydraulic properties calculator. www.staffweb.wilkes.edu/brian.oram/soilwatr.htm. Sourced from Saxton et al. 1986. Soil Sci Soc Amer J 50(4):1031-1036.

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

R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raich, J. W., and K. J. Nadelhoffer. 1989. Belowground carbon allocation in forest ecosystems: global trends. Ecology **70**:1346–1354.

Raich, J. W., and W. H. Schlesinger. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus **44B**:81–99.

Reinke, J. J., D. C. Adriano, and K. W. McLeod. 1981. Effects of litter alteration on carbon dioxide evolution from a South Carolina pine forest floor. Soil Science Society of America Journal **45**:620–623.

Ruehr, N. K., and N. Buchmann. 2010. Soil respiration fluxes in a temperate mixed forest: seasonality and temperature sensitivities differ among microbial and root-rhizosphere respiration. Tree Physiology **30**:165–176.

Saiz, G., C. Green, K. Butterbach-Bahl, R. Kiese, V. Avitabile, and E. P. Farrell. 2006. Seasonal and spatial variability of soil respiration in four Sitka spruce stands. Plant and Soil **287**:161–176.

Samuelson, L. J., T. A. Stokes, J. R. Butnor, K. H. Johnsen, C. A. Gonzalez-Benecke, P. Anderson, J. Jackson, L. Ferrari, T. A. Martin, and W. P. Cropper Jr. 2014. Ecosystem carbon stocks in *Pinus palustris* forests. Canadian Journal of Forest Research **44**:476–486.

Samuelson, L. J., and W. B. Whitaker. 2012. Relationships between soil CO_2 efflux and forest structure in 50-year-old longleaf pine. Forest Science **58**:472–484.

Søe, A. R. B., and N. Buchmann. 2005. Spatial and temporal variations in soil respiration in relation to stand structure and soil parameters in an unmanaged beech forest. Tree Physiology **25**:1427–1436.

Soil Survey Staff, 2014. Web soil survey. Online. http://websoilsurvey.nrcs.usda.gov. Accessed January 2012.

Stoyan, H., H. De-Polli, S. Böhm, G. P. Robertson, and E. A. Paul. 2000. Spatial heterogeneity of soil respiration and related properties at the plant scale. Plant and Soil **222**:203–214.

Taneva, L., and M. A. Gonzalez-Meler. 2011. Distinct patterns in the diurnal and seasonal variability in four components of soil respiration in a temperate forest under free-air CO_2 enrichment. Biogeosciences **8**:3077–3092.

Tjoelker, M. G., J. M. Craine, D. Wedin, P. B. Reich, and D. Tilman. 2005. Linking leaf and root trait syndromes among 39 grassland and savannah species. New Phytologist **167**:493–508.

Tromp-van Meerveld, H. J., and J. J. McDonnell. 2006. On the interrelations between topography, soil depth, soil moisture, transpiration rates and species distribution at the hillslope scale. Advances in Water Resources **29**:293–310.

Vande Walle, I., R. Samson, B. Looman, K. Verheyen, and R. Lemeur. 2007. Temporal variation and high-resolution spatial heterogeneity in soil CO_2 efflux in a short-rotation pine plantation. Tree Physiology **27**:837–848.

Vetaas, O. R. 1992. Micro-site effects of trees and shrubs in dry savannas. Journal of Vegetation Science **3**:337–344.

Weber, P., and R. D. Bardgett. 2011. Influence of single trees on spatial and temporal patterns of belowground properties in native pine forest. Soil Biology & Biochemistry **42**:1372–1378.

Wiseman, P. E., and J. R. Seiler. 2004. Soil CO_2 efflux across four age classes of plantation loblolly pine (*Pinus taeda* L.) on the Virginia Piedmont. Forest Ecology and Management **192**:297–311.

Zinke, P. J. 1962. The pattern of influence of individual forest trees on soil properties. Ecology **43**:130–133.

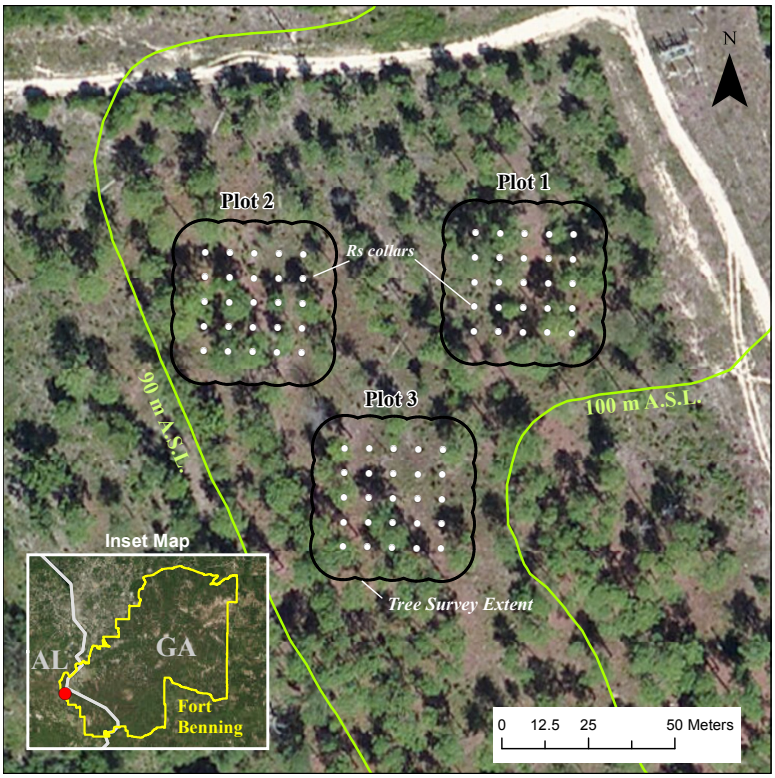

Figure 3.1: The study site was a 64-year-old longleaf pine forest located in eastern Alabama (Inset Map). Three plots were established within the stand and each contained a 5 by 5 grid of 25 R_S collars spaced 6 m apart. July 2012 tree inventory data was collected within the tree survey extent polygons, which extend 8 m from each R_S collar. Aerial imagery from Bing Maps and 10 m DEM contour lines from Geospatial Data Gateway (www.datagateway.nrcs.usda.gov).

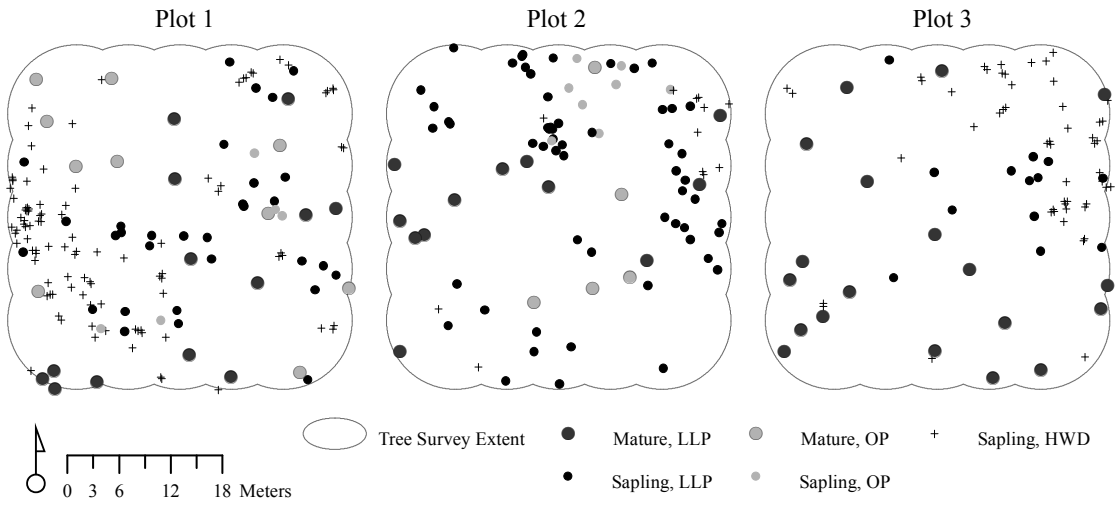

Figure 3.2: The location and diameter classes of longleaf pine (LLP), other pine (OP), and hardwood (HWD) trees within each plot in a 64-year-old longleaf pine forest. Inventoried trees were taller than 2 m in height with DBH greater than 1 cm. Diameter classes include mature (DBH $\geq$ 10 cm) and sapling (DBH $<$ 10 cm).

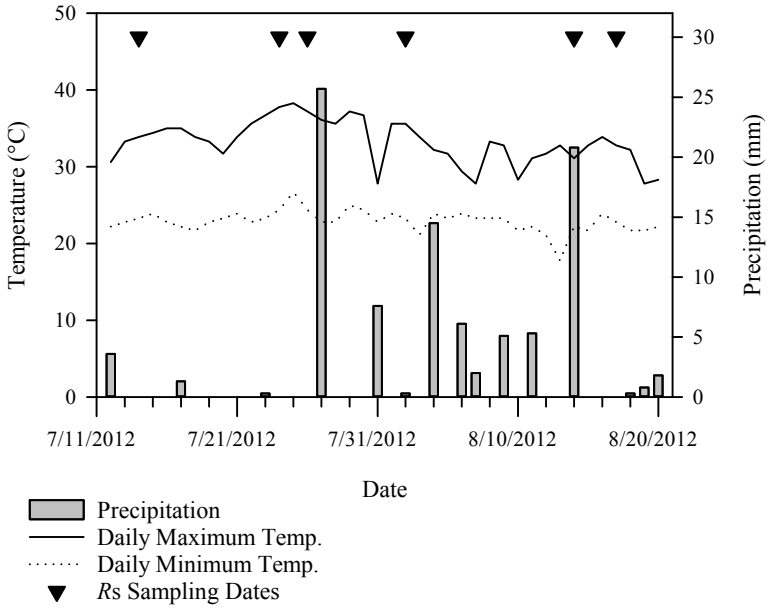

Figure 3.3: Daily maximum and minimum temperatures and total daily precipitation (National Climatic Data Center, 2015*b*). Triangles represent dates of soil respiration (R_{S}), soil temperature and soil moisture sampling.

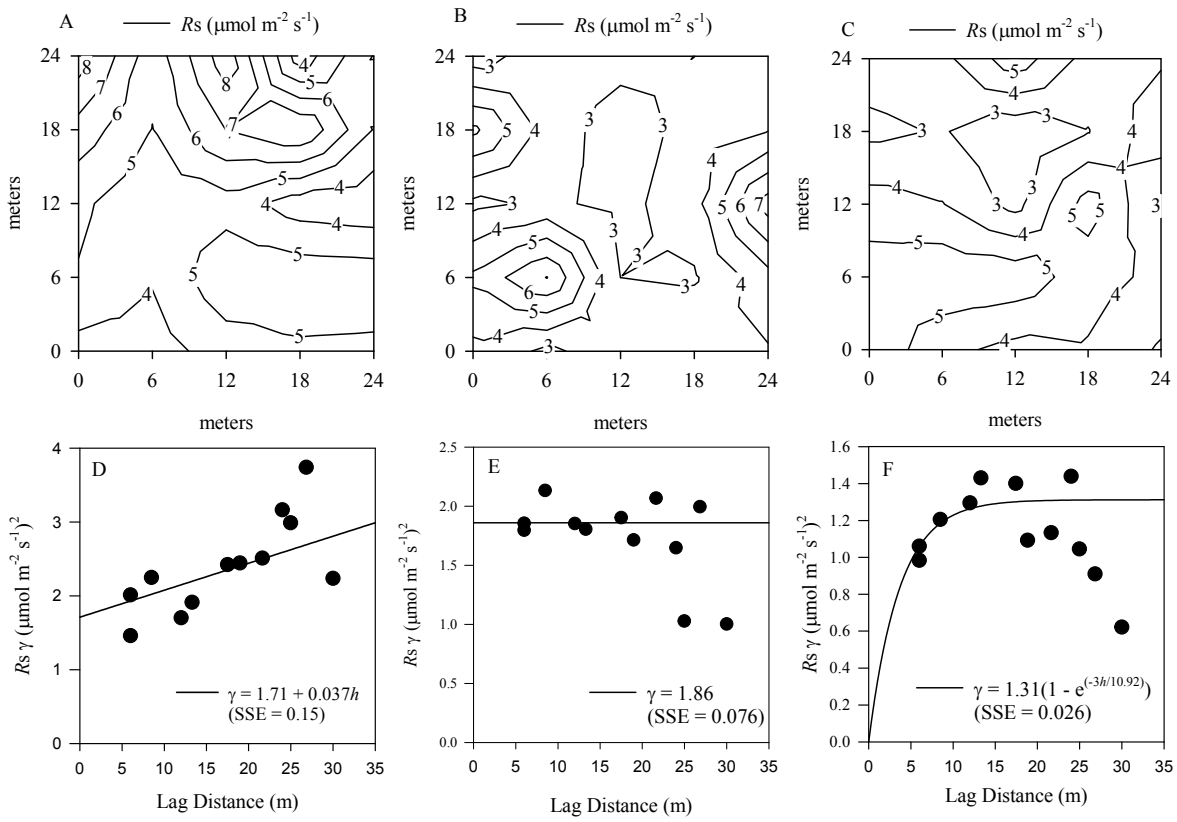

Figure 3.4: The spatial distribution of mean soil respiration (R_S) within Plot 1 (A), Plot 2 (B), and Plot 3 (C) and semivariance (γ) of R_S versus lag distance h in Plot 1 (D), Plot 2 (E), and Plot 3 (F) measured in a 64-year-old longleaf pine forest. Regression lines represent linear, nugget, and exponential semivariogram models in (D), (E), and (F), respectively.

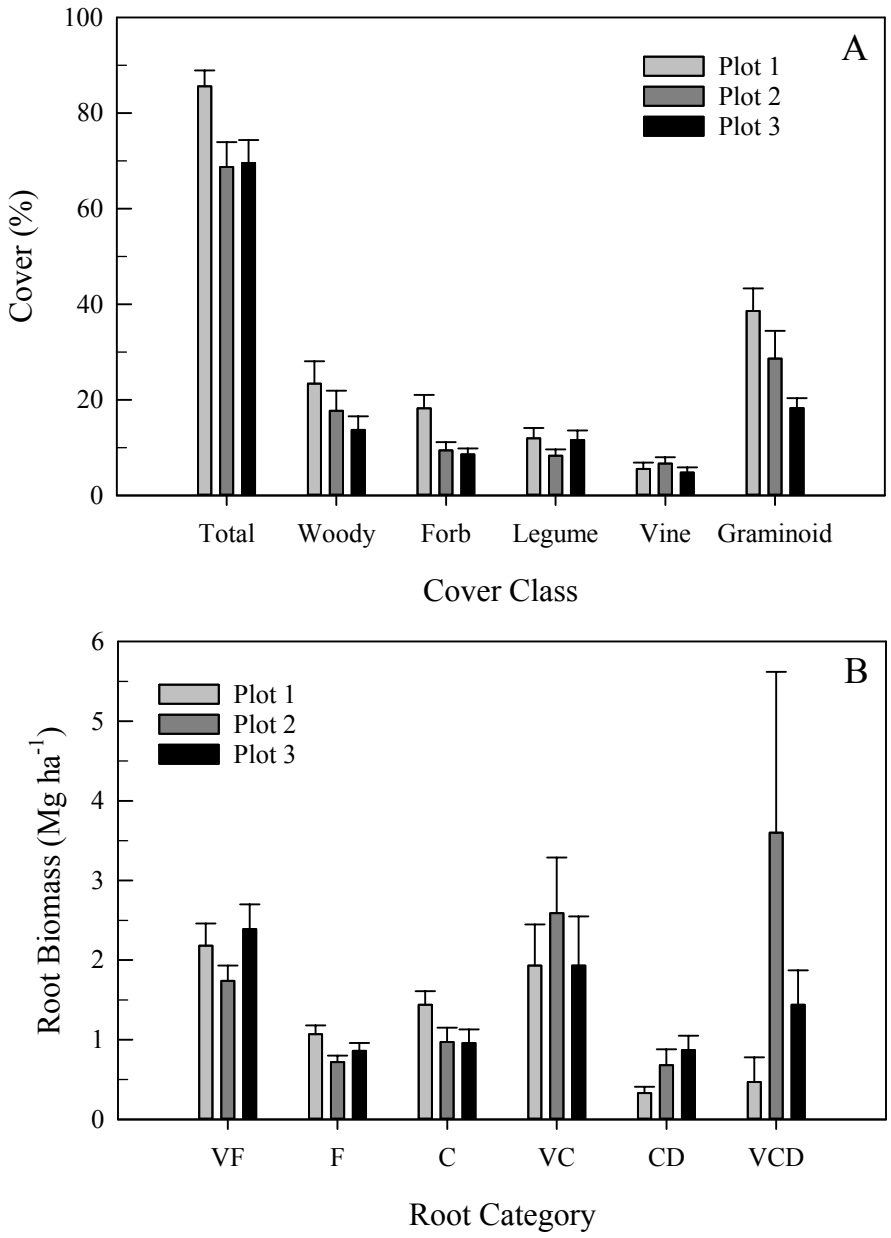

Figure 3.5: (A) Mean total cover and cover by class and (B) mean root biomass by size and type for each plot measured in a 64-year-old longleaf pine forest. Variable abbreviations and units are described in Table 3.1. Error bars are $\pm$ SE.

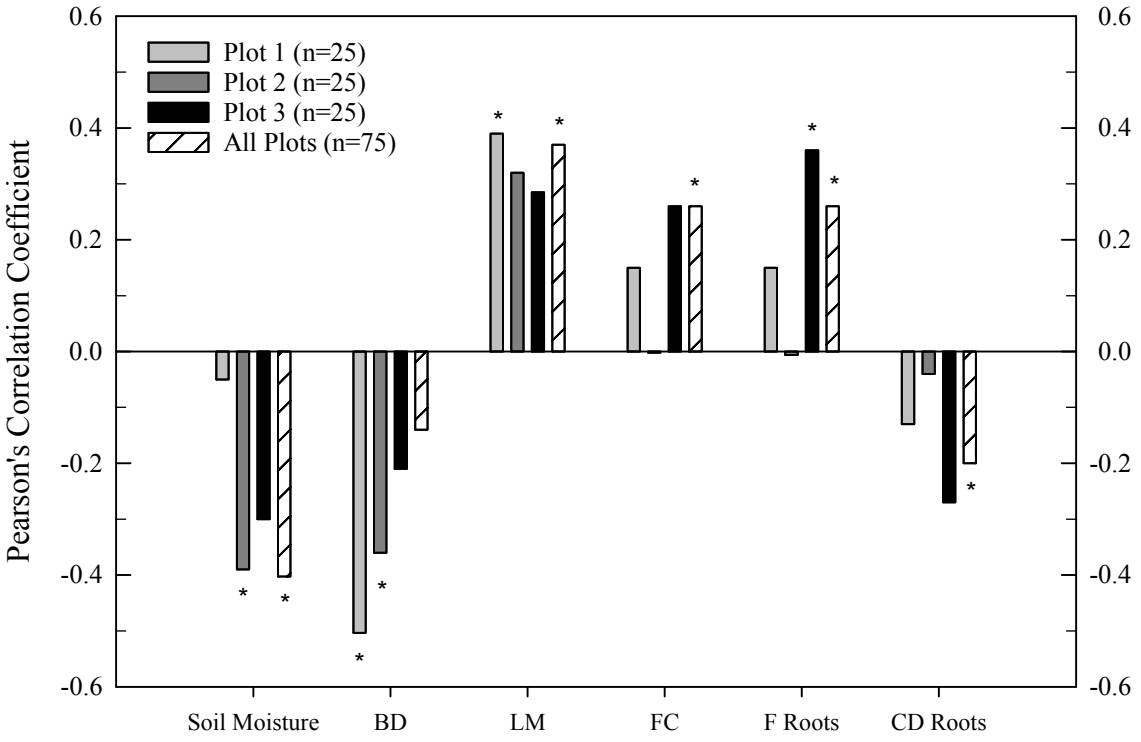

Figure 3.6: Pearson's correlation coefficients for edaphic, forest floor, and root variables versus soil respiration (R_S) in each plot and across all plots measured in a 64-year-old longleaf pine forest. Variable abbreviations and units are described in Table 3.1. Asterisks represent significant correlations at $\alpha = 0.10$.

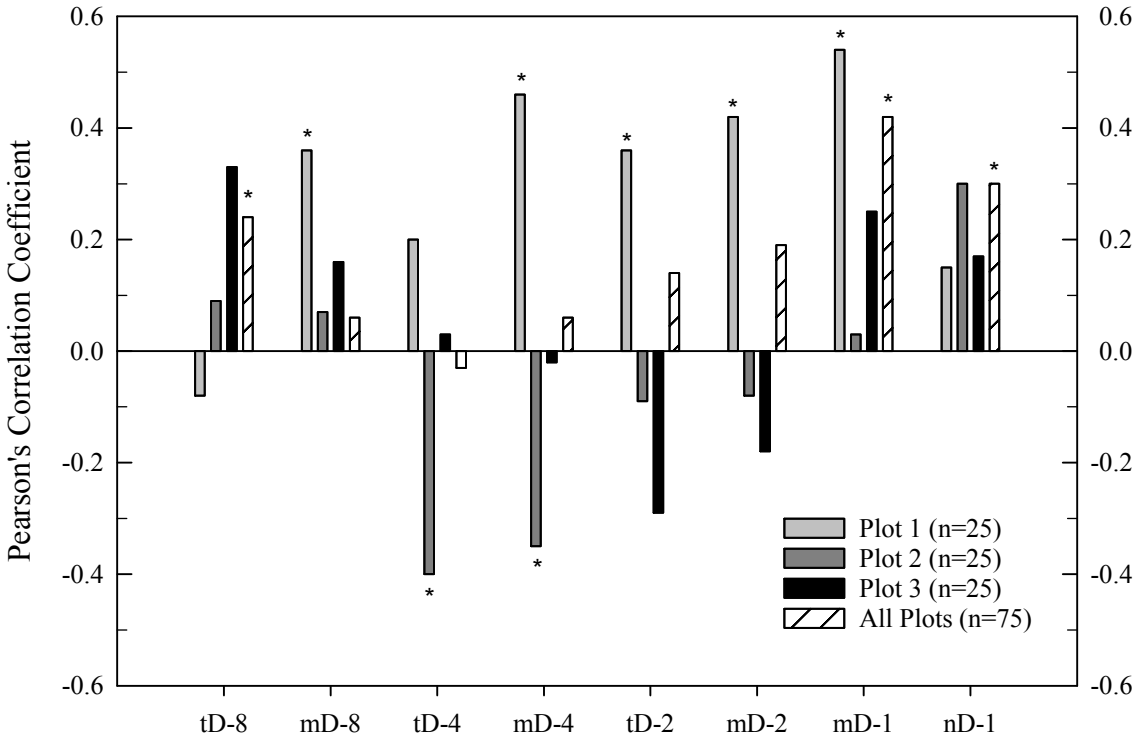

Figure 3.7: Pearson's correlation coefficients for stand structural variables versus soil respiration (R_S) in each plot and across all plots measured in a 64-year-old longleaf pine forest. Variable abbreviations and units are described in Table 3.1. Asterisks represent significant correlations at $\alpha = 0.10$.

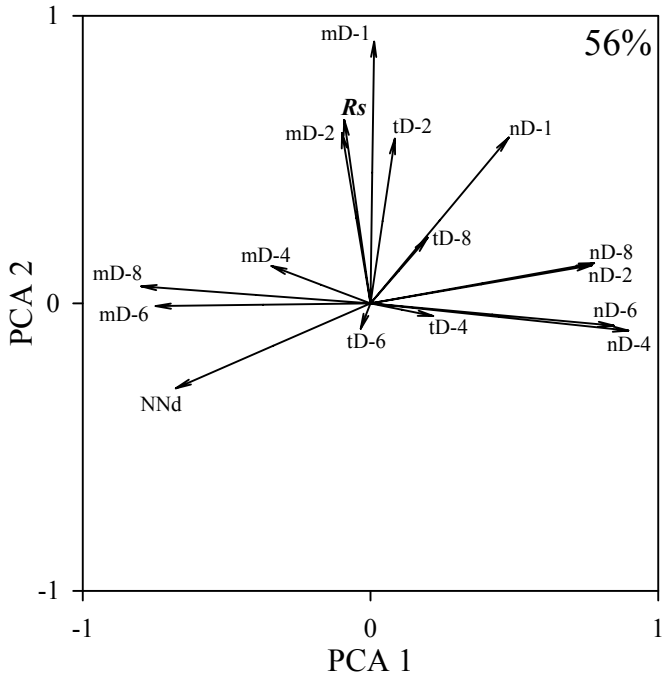

Figure 3.8: Principal components analysis for stand structural variables and soil respiration (R_S) across all three plots measured in a 64-year-old longleaf pine forest. Variable abbreviations and units are described in Table 3.1.

Table 3.1: Abbreviations and units of all measured ecological covariates by category.

Category	Abbr.	Units	Variable
Edaphic	T_{soil}	°C	soil temperature
	θ	%	soil moisture
	pH	–	soil pH
	BD	$g\,cm^{-3}$	soil bulk density
	C	$Mg\,ha^{-1}$	soil carbon
	OM	$Mg\,ha^{-1}$	soil organic matter
	N	$Mg\,ha^{-1}$	soil nitrogen
Forest Floor	LM	$Mg\,ha^{-1}$	litter mass
	TC	%	total cover
	WC	%	woody cover
	FC	%	forb cover
	LC	%	legume cover
	VC	%	vine cover
	GC	%	graminoid cover
Roots	VF	$Mg\,ha^{-1}$	very fine live roots
	F	$Mg\,ha^{-1}$	fine live roots
	C	$Mg\,ha^{-1}$	coarse live roots
	VC	$Mg\,ha^{-1}$	very coarse live roots
	CD	$Mg\,ha^{-1}$	coarse dead roots
	VCD	$Mg\,ha^{-1}$	very coarse dead roots
Structural	tD-r	cm	total DBH within r radius of R_S collar
	mD-r	cm	mean DBH within r radius of R_S collar
	nD-r	–	number of trees within r radius of R_S collar
	NNd	m	distance to nearest tree from R_S collar

Table 3.2: Basal area (BA, $m^2\,ha^{-1}$), tree density ($trees\,ha^{-1}$), and mean DBH (cm) of longleaf pine (LLP), other pine (OP), and hardwood (HWD) tree species by diameter class measured in a 64-year-old longleaf pine forest. Inventoried trees were taller than 2 m in height with DBH greater than 1 cm. Diameter classes include mature (DBH $\geq$ 10 cm) and sapling (DBH $<$ 10 cm).

		Plot 1			Plot 2			Plot 3		
Species	Size	BA	Density	DBH	BA	Density	DBH	BA	Density	DBH
LLP	Mature	10.3	85.2	19.0	8.0	78.6	35.1	13.2	124.5	36.1
	Sapling	0.4	203.1	5.0	0.7	373.4	4.6	0.2	85.2	4.5
OP	Mature	4.1	65.5	26.4	2.7	32.8	29.8	–	0.0	–
	Sapling	0.1	32.8	4.7	0.1	52.4	4.0	–	0.0	–
HWD	Mature	–	0.0	–	–	0.0	–	–	0.0	–
	Sapling	0.2	661.6	1.9	< 0.1	78.6	2.0	0.1	340.6	1.9
Total	–	15.1	1048	7.0	11.5	615.7	9.5	13.5	550.2	10.0

Table 3.3: Daily mean, standard error (SE), and coefficient of variation (CV, %) of soil temperature, soil moisture, and soil respiration across all three plots ($n = 75$) measured in a 64-year-old longleaf pine forest.

	Soil temperature		Soil moisture		Soil respiration	
Date	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV
July 14, 2012	25.67±0.08	2.6	5.04±0.40	68.6	5.36±0.20	32.9
July 24, 2012	26.08±0.10	3.3	3.95±0.34	73.9	3.75±0.18	41.5
July 26, 2012	27.12±0.11	3.6	4.15±0.44	91.3	3.15±0.17	46.5
August 2, 2012	26.66±0.06	2.1	12.66±0.65	44.3	4.82±018	31.4
August 14, 2012	25.23±0.05	1.4	11.84±0.62	36.4	5.22±0.21	28.4
August 17, 2012	25.65±0.05	1.5	14.80±0.62	36.2	4.46±0.17	32.5

Table 3.4: Mean, standard error (SE), and coefficient of variation (CV, %) of soil respiration (R_S) and edaphic variables by plot ($n = 25$) measured in a 64-year-old longleaf pine forest. Different lowercase letters indicate significant plot differences at $p < 0.05$ based on repeated measure mixed-model analysis. Variable abbreviations and units are described in Table 3.1.

	Plot 1		Plot 2		Plot 3	
Variable	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV
R_S	5.26±0.31^{a}	29.0	3.85±0.27^{b}	34.7	4.14±0.22^{b}	25.9
T_{soil}	26.02±0.10^{ab}	2.0	26.47±0.15^{a}	2.9	25.93±0.10^{b}	1.9
θ	6.52±0.40^{a}	30.5	10.39±0.84^{b}	40.5	9.20±0.67^{c}	34.7
pH	5.04±0.04	4.2	4.86±0.04	4.5	4.85±0.04	4.3
BD	1.34±0.03	11.9	1.19±0.02	8.8	1.25±0.02	9.2
C	20.21±1.23	30.4	34.05±1.53	22.5	25.66±1.49	27.8
OM	34.76±2.11	30.4	58.57±2.64	22.5	44.14±2.56	27.8
N	1.00±0.05	26.06	1.53±0.05	17.7	1.10±0.06	25.6

Table 3.5: Mean, standard error (SE), and coefficient of variation (CV, %) of stand structural variables by plot ($n = 25$) measured in a 64-year-old longleaf pine forest. Variable abbreviations and units are described in Table 3.1.

	Plot 1		Plot 2		Plot 3	
Variable	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV
nD-8	22.2±3.6	82.4	11.2±1.5	68.7	9.3±1.7	85.8
mD-8	10.3±1.4	66.2	13.4±1.5	56.5	17.7±2.3	62.7
tD-8	145.4±7.2	24.7	114.3±8.9	39.0	101.5±9.6	45.2
nD-6	10.8±1.8	83.9	6.7±0.9	68.5	4.8±1.0	102.0
mD-6	12.1±2.5	102.5	13.6±1.8	67.2	18.4±3.1	81.3
tD-6	69.7±5.4	39.1	71.3±7.2	50.4	54.1±8.3	73.6
nD-4	4.4±0.7	84.5	2.8±0.6	106.8	2.0±0.6	131.5
mD-4	10.7±2.6	122.3	9.8±2.3	115.9	12.9±3.3	123.7
tD-4	30.9±4.3	69.7	27.7±5.4	97.4	21.2±4.0	96.1
nD-2	1.2±0.3	129.5	0.7±0.2	141.9	0.6±0.2	183.6
mD-2	7.9±3.1	191.5	5.4±2.2	206.1	3.2±1.9	279.9
tD-2	10.4±3.2	152.6	6.7±2.3	171.4	4.8±2.4	240.0
nD-1	0.4±0.1	147.9	0.1±0.1	276.4	0.1±0.1	331.3
mD-1	5.9±2.9	242.9	1.3±0.9	355.8	0.2±0.1	344.6
tD-1	6.1±2.9	235.6	1.3±0.9	355.8	0.2±0.1	344.6
NNd	1.9±0.3	72.9	2.6±0.3	56.2	3.0±0.3	52.0

Table 3.6: Multiple linear regression models for R_S in a 64-year-old longleaf pine forest by plot ($n = 25$) and across all plots ($n = 75$) with coefficient estimates and standard error (Coef.±SE), p-values, and cumulative R^2 for each model parameter. Model summaries include model F-value, p-value, and adjusted R^2 (R^2_{adj}). Variable abbreviations and units are described in Table 3.1.

	Plot 1			Plot 2			Plot 3			All Plots		
Variable	Coef.±SE	p-value	R^2	Coef.±SE	p-value	R^2	Coef.±SE	p-value	R^2	Coef.±SE	p-value	R^2
Intercept	9.89±2.22	< 0.01	–	9.91±2.73	< 0.01	–	3.42±0.46	< 0.01	–	5.44±0.47	< 0.01	–
θ	–	–	–	–	–	–	–	–	–	-0.14±0.04	< 0.01	0.11
BD	-3.66±1.63	0.03	0.13	-4.61±2.27	0.05	0.13	–	–	–	–	–	–
LM	–	–	–	–	–	–	–	–	–	0.05±0.03	0.09	0.14
F Roots	–	–	–	–	–	–	0.84±0.48	0.09	0.13	–	–	–
CD Roots	–	–	–	–	–	–	–	–	–	-0.42±0.17	0.02	0.19
tD-4	–	–	–	-0.02±0.01	0.03	0.29	–	–	–	–	–	–
mD-1	0.05±0.02	0.02	0.42	–	–	–	–	–	–	0.04±0.02	0.02	0.36
Model	F-value	p-value	R^2_{adj}	F-value	p-value	R^2_{adj}	F-value	p-value	R^2	F-value	p-value	R^2_{adj}
Summary	8.67	< 0.01	0.37	4.60	0.02	0.23	3.11	0.09	0.13	9.71	< 0.01	0.33

Chapter 4

Partitioning longleaf pine soil respiration into its autotrophic and heterotrophic components through root exclusion

4.1 Abstract

Soil respiration (R_S) results from the metabolic activity of autotrophs and heterotrophs within the soil and litter profiles. Root exclusion techniques have been developed to remove autotrophic respiration sources from photosynthate supply, thus reducing R_S to its heterotrophic respiration (R_H) component. Small root exclusion tubes can be used to provide relatively inexpensive and rapid estimates of heterotrophic respiration compared to larger scale trenching techniques. Small root exclusion tubes were used in this study to partition R_S into its heterotrophic component in three 26-year-old longleaf pine stands in western Georgia. Heterotrophic respiration as measured on the root exclusion tubes was corrected for pretreatment R_S variation between the treatment and control collars and for CO_2 loss from root decay. The range in the ratio of R_H to R_S was 61 to 82 %, depending upon the estimation method that was applied to the R_H measurements.

4.2 Introduction

Anthropogenic emission of greenhouse gases and concomitant global climate changes have fostered an impetus for the modeling and quantifying of global carbon stocks and balances within terrestrial ecosystems. For instance, the United States Department of Defense has been examining the potential to offset its CO_2 emissions by increasing the carbon sinks of the forested ecosystems on its bases, such as the longleaf pine (*Pinus palustris* Mill.) forests in the southeastern US (Bush, 2007; Lopez, 2009; Zhao et al., 2010). The overall carbon balance of an ecosystem is dependent upon carbon assimilation (i.e., photosynthesis) being larger in magnitude than the carbon lost from ecosystem respiration, oxidation from fires, and carbon removal (e.g., biomass harvesting, herbivory, run-off, leaching) (Lovett et al., 2006). Soil heterotrophic respiration is the largest heterotrophic component of ecosystem respiration and is often compared with net primary productivity (NPP) to estimate whether forests are carbon sinks or sources (Lovett et al., 2006; Harmon et al., 2011). Net primary

productivity and related standing carbon stocks of longleaf pine forests have been previously examined (e.g. Mitchell et al., 1999; Hendricks et al., 2006; Samuelson et al., 2014); however, relatively less is known about the magnitude of R_{H} in longleaf pine forests, thus hindering accurate estimates of the carbon balance of longleaf pine forests. Specifically, the proportion of R_{H} to total soil respiration has been estimated only in one study (85–96 %) (Collins, 2005), and no known annual estimates of longleaf pine R_{H} have been published.

Partitioning R_{S} into autotrophic and heterotrophic components is difficult, and methods that have been developed to do so each have inherent strengths and weaknesses (Hanson et al., 2000; Kuzyakov, 2006). One such technique uses trenching to sever existing roots in order to cut off the photosynthetic carbon allocation pathway, which then theoretically leaves residual heterotrophic activity in the soil and allows for direct measurement of R_{H} (Subke et al., 2006). With this technique, plots that are trenched are often fairly large and in place for a year or more before R_{H} is measured (Vogel and Valentine, 2005). The downside of using a trenching method includes: (1) modification to the soil environment due to a changes in evapotranspirational demand from root-severed soil and in runoff and leaching patterns; (2) an increase in R_{H} due to the newly deceased root tissues in the root-severed soil; (3) residual autotrophic respiration (R_{A}) may continue in severed roots with large starch reserves; and (4) eventual changes in the microbial community composition as labile substrates diminish and microbes that decompose recalcitrant organic material become more dominant (Hanson et al., 2000; Vogel and Valentine, 2005; Kuzyakov, 2006; Subke et al., 2006; Díaz-Pinés et al., 2010). In order to mitigate these artifacts, a technique to measure R_{H} from root-excluded soils utilizing small diameter root exclusion tubes has been developed (Vogel and Valentine, 2005). Heterotrophic respiration can be more readily estimated after installation when using small root exclusion tubes compared to trenching plots, thus reducing potential changes in soil environment and microbial community composition (Vogel and Valentine, 2005). Also, since small root exclusion tubes are easier to install than the effort and expense necessary for trenching, they can be installed with higher replications and staggered over time to determine

how the profile of R_H changes seasonally or in response to disturbances. Finally, when small root exclusion tubes are coupled with root biomass measurements, the amount of root decay that has occurred post-treatment can be quantified and used to recalculate more accurate estimates of R_H (Subke et al., 2006; Bond-Lamberty et al., 2011).

The objective of this study was to estimate the proportion of R_H to R_S in longleaf pine plantations. Root exclusion tubes were installed in three 26-year-old longleaf pine stands in western Georgia and compared with paired control R_S measurements to determine the proportion of R_H to total R_S. We attempted to reduce the impacts of residual starch reserves in longleaf pine roots by installing the root exclusion treatment in mid-May during a period of starch depletion (Sword Sayer and Haywood, 2006), and we incorporated an estimation of fine root decay into R_H/R_S based upon measurements of root biomass in both the root-excluded and control soil profiles.

4.3 Methods

4.3.1 Study Site

This study was conducted in three 26-year-old longleaf pine stands located on property in a conservation easement held by The Nature Conservancy within the Chattahoochee Fall Line Wildlife Management area in Talbot County, Georgia (Fig. 4.1, Table 4.1). These stands were located within the Sand Hills Ecoregion, which is characterized by dry, deep sand and gently rolling hills that are excessively drained and nutrient poor (Griffith et al., 2001). All three stands were planted in 1988 at a density of 1793 seedlings ha^{-1} and spacing of 1.8 m by 3.0 m. Although no historical records are available for these specific stands, the presence of turpentine stumps in the area and historical records for the area indicate that these stands were previously native pine forests used for turpentine and rosin extraction (George Matusick, personal communication). Mean annual air temperature for this area is 24.6 °C with mean January and July air temperatures of 8.4 °C and 28.1 °C, respectively (National Climatic Data Center, 2015*a*). Mean precipitation is 1180 mm, spread evenly

throughout the year. Stands 1 and 2 were located in Lakeland sand soil (2 to 5 % slopes, excessively drained) and Stand 3 was located in a Troup loamy sand soil (2 to 5 % slopes, somewhat excessively drained) (Soil Survey Staff, 2014). Longleaf pine basal area ranged from 17.3 to 21.4 $m^2\,ha^{-1}$ and density ranged from 978 to 1567 trees ha^{-1} across the three stands (Table 4.1). Mean diameter at breast height (1.37 m, DBH) of longleaf pine trees ranged from 11.5 to 15.4 cm. Oak (*Quercus* spp.) basal area ranged from 0.0 to 0.2 $m^3\,ha^{-1}$, density ranged from 0 to 89 trees ha^{-1}, and mean DBH ranged from 4.0 to 4.4 cm.

4.3.2 Experimental Design

In this randomized complete block design, one 30 m by 30 m plot was established in each stand and five trees were randomly selected from within each plot from a gridded identification system based on row and tree number (Fig. 4.2). At each tree, one of four directions was randomly selected at a 45° angle to the rows. A temporary R_S collar (PVC, 10 cm diameter, 4.5 cm length) was placed at 1.0 m from the tree in that direction and an additional temporary R_S collar was placed 1.0 m away from a randomly selected adjacent tree (i.e., same row) in a parallel direction to the first temporary collar. Litter was cleared away from the location of collar placement and the collars were gently pounded into the soil to a depth of 2.0 cm. On May 10, 2013, these temporary collars were used for initial R_S measurements. Subsequently, the experiment was expanded to include five more trees per plot and the same protocol was followed to install two associated temporary collars at each tree. These temporary collars were measured for initial R_S rates on May 15, 2013.

After initial R_S measurements were made on the temporary collars, root exclusion tubes were installed at one randomly selected temporary collar from each pair. Root exclusion tubes were constructed from a 10 cm diameter stainless steel tube cut to 60 cm length with a sharp, beveled edge. Tubes were pounded into the ground with a rubber mallet and board until approximately 2 cm from the soil surface. A 2.5 cm long, 10 cm diameter PVC collar was glued to the exposed top of each root exclusion tube with instant epoxy and

waterproof silicone sealant to create an air-tight seal. After the epoxy and silicone had set, the root exclusion tubes were gently pushed down into the soil until the metal tube edge was flush with the adjacent undisturbed soil, leaving 2.5 cm of PVC collar aboveground. The remaining ten temporary collars per plot became permanent control treatment collars (i.e., no root exclusion). All root exclusion tubes were installed on May 14 or 16, 2013. Litter was removed and live vegetation was clipped from within all collars as needed to maintain bare soil.

4.3.3 Soil Respiration Measurements

Soil respiration was measured on both root exclusion collars and control collars by placing a LI-COR 6400-09 soil respiration chamber connected to a LI-COR 6400 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE) on the portion of PVC located above the ground. A styrofoam gasket was used on the soil respiration chamber to create an air tight seal with the collars and the depth to soil surface within each collar was used to calculate chamber volume for R_S measurements. Soil respiration was measured at approximately 2-week intervals from May 10, 2013 (first pretreatment measurement) through August 26, 2013; however, measurements could not be made in mid-August 2013 because of heavy rains. All R_S measurements were made from 830-1300 hr EST. Soil temperature and soil moisture were measured within 10 cm of the collars at the time of R_S measurements with a 15 cm-depth soil temperature probe connected to the LI-COR 6400 and a 20 cm-depth time domain reflectometry soil moisture probe (Hydrosense II, Campbell Scientific, Inc., Logan, UT), respectively. After the final R_S measurement was made at each collar on August 26, 2013, soil moisture and soil temperature were measured within each collar to test for treatment effects on the soil environment, albeit on only one date.

4.3.4 Soil Sampling

Following the final R_S measurements, soil samples were removed from directly underneath the control collars with a 10 cm diameter soil auger at depths of 0-15 cm, 15-30 cm, 30-45 cm, and 45-60 cm and placed in plastic bags for transport. Root exclusion collars and tubes were pulled out of the ground, sealed with plastic, and transported to the lab for processing. In the laboratory, soil was removed from root exclusion tubes in sections of 15 cm lengths to correspond with soil depths of 0-15 cm, 15-30 cm, 30-45 cm, and 45-60 cm. All soil was air dried and sifted through a 2 mm sieve. Roots were removed, washed, and sorted into fine and coarse size classes (diameter $\leq$ 2 mm or diameter $>$ 2 mm, respectively) and live versus dead based on texture, color and resiliency. Roots were oven dried at 70 °C and weighed.

4.3.5 Data Analysis

This randomized complete block design was replicated in each stand with temporary collar pairs as blocks, individual collars as experimental units, and two treatments: root exclusion tubes or control (R_S collar only, no root exclusion). To account for random variation between stands and between blocks, treatment effects were analyzed with a mixed-model approach at each measurement date ($n = 60$). The initial, pretreatment R_S measurements were pooled over May 10 and 15. All statistical analysis was done in SAS (version 9.3, SAS Institute Inc., Cary, NC) with a significance level of $\alpha = 0.05$.

The mean fine root decay rate (k) was calculated based upon the mean reduction in live fine root biomass in the root exclusion tubes compared to the control soil over the study duration. Total live fine root biomass over the entire sampled soil profile (0–60 cm depth) was used for these calculations. The total loss of live fine root biomass was stoichiometrically converted to μmol CO_2 m^2 s^{-1} assuming fine roots were 50 % carbon by mass (Woodbury et al., 2007).

Total R_S was measured from control collars directly, whereas R_H was estimated in four ways:

1. R_S from the root exclusion tubes (R_{exc}) was used as a direct proxy for R_H;
2. R_H was estimated by correcting R_{exc} by within-block, pretreatment R_S variation (Maier et al., 2013);
3. R_H was estimated by correcting R_{exc} for CO_2 loss due to fine root decay (k) (Díaz-Pinés et al., 2010); and
4. R_H was estimated by correcting R_{exc} for both within-block, pretreatment R_S variation and CO_2 loss due to k (methods 2 and 3 combined).

4.4 Results

During the duration of the experiment (May 10 through August 26, 2013), daily maximum temperature ranged from 22.2 to 35.0 °C and daily minimum temperature ranged from 9.4 to 25.6 °C (Fig. 4.3A) (National Climatic Data Center, 2015*b*). Daily precipitation ranged from 0.0 to 145.5 mm day^{-1} (Fig. 4.3A). Daily mean soil temperature near collars ranged from 19.9 to 26.2 °C, and on the final measurement day, August 26, 2013, mean soil temperature was 22.8 °C and 22.9 °C within the control collars and root exclusion tubes, respectively (Fig. 4.3B, Table 4.2). Soil temperature was not significantly different between measurements made within or near the collars for either treatment (Table 4.2). Daily mean soil moisture ranged from 2.9 to 7.1 % near the control collars and 3.1 to 6.8 % near the root exclusion tubes (Fig. 4.3C). On the final measurement day, mean soil moisture was 6.3 % and 7.8 % within the control collars and root exclusion tubes, respectively. Soil moisture was significantly higher within the root exclusion tubes than near the root exclusion tubes, but not significantly different between measurements made near or within control collars (Table 4.2).

Mean uncorrected R_{exc} ranged from 2.7 to 4.1 μmol m^{-2} s^{-1} (Fig. 4.4A). Mean control collar R_S ranged from 3.2 to 4.0 μmol m^{-2} s^{-1} over the 108-day study period and was significantly higher than uncorrected R_{exc} on the fourth, sixth, and final measurement dates (June 26, July 29, and August 26, 2013; Table 4.3). Mean R_{exc} corrected for within-block, pretreatment R_S variation ranged from 2.5 to 3.9 μmol m^{-2} s^{-1} and was also significantly lower than control collar R_S on the fourth, sixth, and final measurement dates (Fig. 4.4B; Table 4.3). The estimated ratio of R_H to R_S was 81.9 % and 76.9 % based on uncorrected R_{exc} and R_{exc} corrected for within-block, pretreatment R_S variation, respectively (Table 4.4).

Root biomass diminished with depth in the soil (Fig. 4.5). The majority of root biomass was within the top 15 cm of soil (55.1 %), followed by 15-30 cm, 30-45 cm, and 45-60 cm depths (24.6 %, 11.4 %, and 8.9 %, respectively). In the top 15 cm of soil, live fine and coarse root biomass were significantly lower in root exclusion tubes than under control collars (Table 4.5). In both 0-15 cm and 15-30 cm soil depths, dead root biomass was significantly higher in root exclusion tubes than under control collars (Table 4.5). Live fine root decay ranged from $k = 0.07$ to 0.24 (mean $k = 0.13 \pm 0.08$ yr^{-1}) and accounted for an additional flux of CO_2 from root exclusion collars of approximately 0.5 ± 0.3 μmol m^{-2} s^{-1}. Incorporation of root decay decreased the estimated ratio of R_H to R_S to 61.3 % and 66.3 % with and without additional correction for within-block, pretreatment R_S variation, respectively (Table 4.4).

4.5 Discussion

This study utilized small root exclusion tubes for relatively rapid estimation of R_H and total R_S in 26-year-old longleaf pine forests in western Georgia. After 41–43 days on the fourth measurement date (June 26, 2013), R_S from the root exclusion tubes was significantly lower than R_S from the control collars and corresponded with a ratio of R_H to R_S of 83.5 ± 5.5 % to 88.2 ± 4.8 % with and without correction for within-block, pretreatment R_S variation, respectively. This is higher, but not substantially different, than the ratio measured at the final measurement date, 102–104 days after root exclusion tube installation

(August 26, 2013), which suggests that an incubation period shorter than 102–104 days may be feasible for rapid estimates of R_H and R_S. Heim et al. (2015) similarly found that after 41 days, root exclusion tubes significantly reduced R_S compared to adjacent, undisturbed soil in a 9-year-old loblolly pine plantation with a corresponding ratio of R_H to R_S of 79.0 %. The results of these two studies indicate that 40 to 50 days may be an adequate time period to estimate R_H from root exclusion treatment collars. However, if we would have ended our experiment after 57–59 days on the fifth measurement date (July 12, 2013), we would not have discerned a treatment effect and our estimation of R_H/R_S would have been near 100 %. We suspect that the large rain event on July 10, two days prior to our fifth measurement date, confounded any treatment effects. Although more rapid (i.e., within one week) measurements of R_S from root severed or trenched soil are recommended by Sayer and Tanner (2010) to reduce methodological artifacts such as root decomposition or ingrowth, soil moisture alteration, and microbial community composition, we did not measure R_S from root exclusion collars until after two weeks of incubation. At that point, R_S from root exclusion tubes and control collars were not significantly different. Likewise, Vogel and Valentine (2005) suggest that measurements of R_S from small root exclusion tubes within three weeks of installation may mitigate effects of root exclusion on soil moisture. In this study, three weeks was too short of an incubation period to determine treatment effects, and because soil moisture was only directly measured within root exclusion tubes on the final measurement date, we cannot postulate whether treatment effects on soil moisture were immediate or cumulative over the study duration.

Only two known direct estimations of R_H or R_A in longleaf pine forests have been previously published, one with a novel device to measure R_A *in situ* (Cheng et al., 2005) and another that measured R_H with shallow (10 cm deep) root exclusion tubes (Collins, 2005). The former experiment, Cheng et al. (2005), cannot be compared to this study as they did not partition R_S into its autotrophic and heterotrophic components; rather, they measured direct R_A on a per root mass basis. On the other hand, Collins (2005) provided estimates of

the ratio of R_H/R_S based on comparison between root exclusion tubes to control soil after 45 days post-treatment. They found that R_H/R_S ranged from 90 to 96 % in stands with sandy soils and 85 to 88 % in stands with clayey soils. These estimates are generally higher than the range from the present study (61 to 82 %), which could be due to an overestimation of R_H in Collins (2005) from only severing the top 10 cm of roots or because CO_2 loss from fine root decay was not quantified. Our range of R_H to R_S compares well with estimates made for young (8-year-old or 9-year-old) loblolly pine plantations (68 % and 79 %, respectively) (Maier et al., 2013; Heim et al., 2015), but is higher than the ratio measured in loblolly forests under ambient CO_2 concentration and unfertilized conditions at the Duke FACE forest (54 %) (Drake et al., 2012). Subke et al. (2006) performed a global meta-analysis of the proportion of R_H to R_S based on ecosystem types, biomes, stand age, and R_H partitioning methods (e.g., root trenching). They found that the proportion of R_H to R_S varied with annual R_S levels. Thus, in order to make a comparison to their results, we assumed that annual R_S from these longleaf pine stands was similar to those measured in nearby longleaf pine stands varying in age and structure (12-14 $\mathrm{Mg\,C\,ha^{-1}\,yr^{-1}}$; Chapter 2). Unless root decay is incorporated, our range in R_H/R_S is higher than expected based on the meta-analysis results at this range of annual R_S; specifically, comparable temperate coniferous ecosystems with this range of annual R_S corresponded with an R_H/R_S ratio of approximately 60 ± 12 % (Subke et al., 2006).

A significant decrease in shallow live roots and increase in shallow dead roots were observed within root exclusion tubes, suggesting that death and decay of severed roots was present and may have caused R_H estimated from root exclusion tubes to be overestimated. Incorporating root decay estimates in this study resulted in an approximately 20 % decrease in the proportion of R_H to R_S. Estimating root decay caused 2 to 24 % reduction in R_H/R_S ratios in previous studies as reviewed in Subke et al. (2006); however, root decay rates cited in Subke et al. (2006) range from $k = 0.14$ to $0.96\,\mathrm{yr^{-1}}$ (mean $0.32\,\mathrm{yr^{-1}}$), and root decay rates were cited in two other R_S partitioning studies as $k = 0.5\,\mathrm{yr^{-1}}$ and $k = 0.3\,\mathrm{yr^{-1}}$ for a boreal

black spruce forest (Bond-Lamberty et al., 2011, 2012) and mountain Norway spruce forest (Díaz-Pinés et al., 2010), respectively. The lower mean root decay rate observed in this study ($k = 0.13\,\mathrm{yr}^{-1}$) may be due to a shorter time period of root exclusion incubation compared to other studies or because starch reserves in severed longleaf pine roots delayed root decay. Starch concentrations in longleaf pine roots peak between February and May and fine root elongation occurs after May (Sword Sayer and Haywood, 2006); as such, we believe that the timing of root exclusion core installation in May 2012 reduced the residual starch stores in severed roots while simultaneously excluding new spring root growth. However, other studies in longleaf pine have found evidence that longleaf pine roots maintain substantial carbohydrate stores that can be used to support autotrophic respiration under photosynthate interruption (e.g. canopy scorching; Clinton et al., 2011; Aubrey et al., 2012). Although, had there been significant carbohydrate stores in the longleaf pine roots in this study prior to severing, we would not have expected the significant decrease in R_S that we observed in the root-excluded soil.

4.6 Conclusion

In this study, we used small root exclusion tubes for relatively rapid assessment of R_H and highlighted the importance of corrections for initial, pretreatment variation in R_S and CO_2 loss from root exclusion tubes resulting from root decay. The ratio of R_H to R_S in these longleaf pine stands varied from approximately 61 to 82 %, depending upon our estimation method, with apparent overestimations of R_H occurring when root decay and pretreatment R_S variation were not considered. Although these stands had sandy soils with excessive drainage rates, the presence of small root exclusion tubes significantly increased soil moisture levels by only about 1 % as measured on the final measurement date. However, we cannot rule out treatment effects on soil moisture, which may have also affected respiration and decomposition rates with the root-excluded soil. The results of this study demonstrated that small root exclusion tubes may supply reasonable estimates of longleaf pine R_H as early

as 40 days post-treatment and up to at least 104 days post-treatment when measured at least three days after large rainfall events. A high replication of small root exclusion tubes staggered over time may provide a relatively inexpensive and reliable understanding of the profile of R_{H} through time in longleaf pine forests.

4.7 Acknowledgements

The authors appreciate the assistance of George Matusick at The Nature Conservancy for providing research access to these three longleaf pine stands. We also thank the following people for field, laboratory, and machine shop assistance: Tom Stokes, Jake Blackstock, Justin Rathel, Michael Gunter, and Ann Huyler.

Bibliography

Aubrey, D. P., B. Mortazavi, J. J. O'Brien, J. D. McGee, J. J. Hendricks, K. A. Kuehn, R. O. Teskey, and R. J. Mitchell. 2012. Influence of repeated canopy scorching on soil CO_2 efflux. Forest Ecology and Management **282**:142–148.

Bond-Lamberty, B., D. Bronson, E. Bladkya, and S. T. Gower. 2011. A comparison of trenched plot techniques for partitioning soil respiration. Soil Biology & Biochemistry **43**:2108–2114.

Bond-Lamberty, B., D. Bronson, E. Bladkya, and S. T. Gower. 2012. Corrigendum to "A comparison of trenched plot techniques for partitioning soil respiration" [Soil Biol. Biochem. 43 (10) (2011) 2108-2144]. Soil Biology & Biochemistry **47**:220.

Bush, G. W., 2007. "Executive Order 13423–Strenthening federal environmental, energy, and transportation management". Federal Registry Volume 72, Number 17.

Cheng, W., S. Fu, R. B. Susfalk, and R. J. Mitchell. 2005. Measuring tree root respiration using ^{13}C natural abundance: rooting medium matters. New Phytologist **167**:297–307.

Clinton, B. D., C. A. Maier, C. R. Ford, and R. J. Mitchell. 2011. Transient changes in transpiration, and stem and soil CO_2 efflux in longleaf pine (*Pinus palustris* Mill.) following fire induced leaf area reduction. Trees **25**:997–1007.

Collins, B., 2005. Thresholds of Disturbance: Land Management Effects on Vegetation and Nitrogen Dynamics. Technical Report, Oak Ridge National Laboratory.

Díaz-Pinés, E., A. Schindlbacher, M. Pfeffer, R. Jandl, S. Zechmeister-Boltenstern, and A. Rubio. 2010. Root trenching: a useful tool to estimate autotrophic soil respiration? A

case study in an Austrian mountain forest. European Journal of Forest Research **129**:101–109.

Drake, J. E., A. C. Oishi, M.-A. Giasson, R. Oren, K. H. Johnsen, and A. C. Finzi. 2012. Trenching reduces soil heterotrophic activity in a loblolly pine (*Pinus taeda*) forest exposed to elevated atmospheric [CO_2] and N fertilization. Agricultural and Forest Meteorology **165**:43–52.

Griffith, G. E., J. M. Omernik, J. M. Comstock, J. A. Lawrence, S. Martin, G. Goddard, V. J. Hulcher, and T. Foster, 2001. Ecoregions of Alabama and Georgia. Map, U.S. Geological Survey, Reston, Virginia.

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.

Harmon, M. E., B. Bond-Lamberty, J. Tang, and R. Vargas. 2011. Heterotrophic respiration in disturbed forests: A review with examples from North America. Journal of Geophysical Research **116**:1–17.

Heim, B., J. R. Seiler, and B. D. Strahm. 2015. Root non-structural carbohydrates and their relationship with autotrophic respiration of loblolly pine (*Pinus taeda* L.). Communications in Soil Science and Plant Analysis **In Press**.

Hendricks, J. J., R. L. Hendrick, C. A. Wilson, R. J. Mitchell, S. D. Pecot, and D. Guo. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. Journal of Ecology **94**:40–57.

Kuzyakov, Y. 2006. Sources of CO_2 from soil and review of partitioning methods. Soil Biology & Biochemistry **38**:425–448.

Lopez, C. T., 2009. Army aims to reduce greenhouse gases, 'carbon bootprint'. Online Article. http://www.army.mil/article/19315/army-aims-to-reduce-greenhouse-gases-carbon-bootprint/.

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., D. McInnis, and K. H. Johnsen, 2013. Partitioning root and heterotrophic respiration from soil CO_2 efflux in clonal loblolly pine plantations that differ in growth efficiency and carbon allocation. Poster at PINEMAP 2013 Annual Meeting.

Mitchell, R. J., L. K. Kirkman, S. D. Pecot, C. A. Wilson, B. J. Palik, and L. R. Boring. 1999. Patterns and controls of ecosystem function in longleaf pine–wiregrass savannas. I. Aboveground net primary productivity. Canadian Journal of Forest Research **29**:743–751.

National Climatic Data Center, 2015*a*. 1981-2010 Normals. Columbus Metropolitan Airport, GA. Station GHCND:USW00093842. Last accessed January 15, 2015. http://www.ncdc.noaa.gov/cdo-web/datatools/normals.

National Climatic Data Center, 2015*b*. Daily Summaries. Columbus Metropolitan Airport, GA. Station GHCND:USW00093842. Downloaded November 2014. http://www.ncdc.noaa.gov/cdo-web/datatools/findstation.

Samuelson, L. J., T. A. Stokes, J. R. Butnor, K. H. Johnsen, C. A. Gonzalez-Benecke, P. Anderson, J. Jackson, L. Ferrari, T. A. Martin, and W. P. Cropper Jr. 2014. Ecosystem carbon stocks in *Pinus palustris* forests. Canadian Journal of Forest Research **44**:476–486.

Sayer, E. J., and E. V. J. Tanner. 2010. A new approach to trenching experiments for measuring root–rhizosphere respiration in a lowland tropical forest. Soil Biology & Biochemistry **42**:347–352.

Soil Survey Staff, 2014. Web soil survey. Online. http://websoilsurvey.nrcs.usda.gov. Accessed November 24, 2014.

Subke, J.-A., I. Inglima, and M. F. Cotrufo. 2006. Trends and methodological impacts in soil CO_2 efflux partitioning: A meta-analytical review. Global Change Biology **12**:921–943.

Sword Sayer, M., and J. D. Haywood. 2006. Fine root production and carbohydrate concentrations of mature longleaf pine (*Pinus palustris* P. Mill.) as affected by season of prescribed fire and drought. Trees **20**:165–175.

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.

Woodbury, P. B., J. E. Smith, and L. S. Heath. 2007. Carbon sequestration in the U.S. forest sector from 1990 to 2010. Forest Ecology and Management **241**:14–27.

Zhao, S., S. Liu, Z. Li, and T. L. Sohl. 2010. Federal land management, carbon sequestration, and climate change in the southeastern U.S.: A case study with Fort Benning. Environmental Science Technology **44**:992–997.

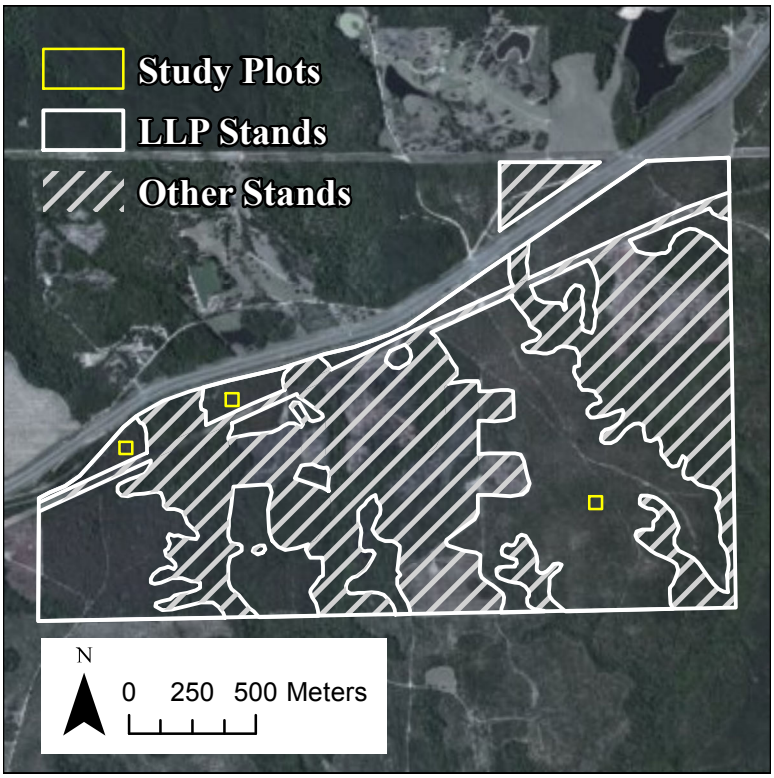

Figure 4.1: Location for study plots within three 26-year-old longleaf pine stands at Blackjack Crossing Tract, Talbot County, Georgia. Note: LLP, longleaf pine.

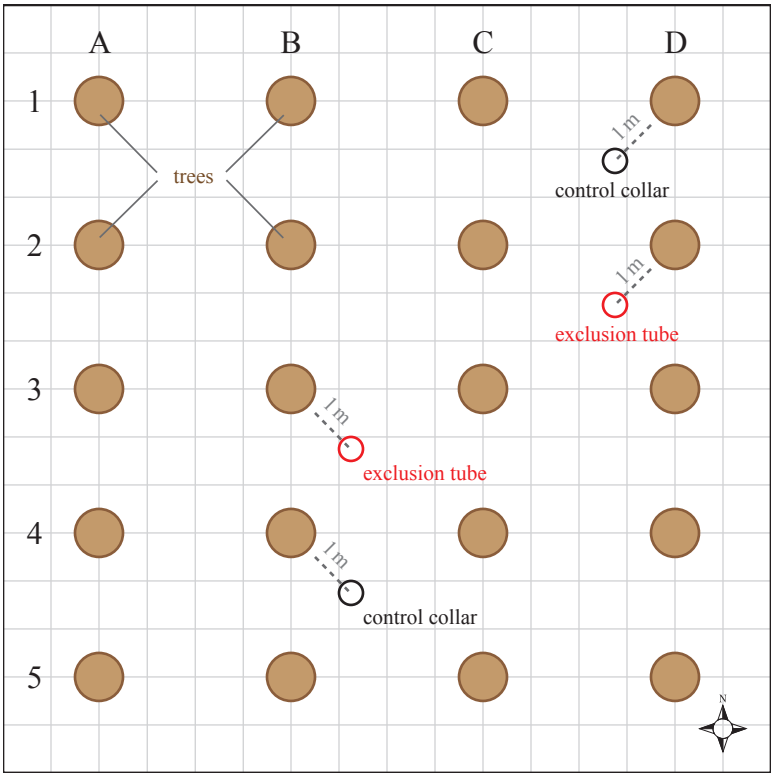

Figure 4.2: Schematic of experimental design (not to scale). First, a tree was chosen using the row-tree grid (e.g., B3), then a direction at 45° to row was randomly selected (e.g., SE) and a temporary R_S collar was placed 1 m from the tree in that direction. Secondly, an adjacent tree was randomly selected (e.g., B4) and another temporary collar was placed at 1 m from that tree in the same direction. Finally, after initial R_S measurements, the treatment factor was randomly applied to temporary collar locations; for example, a root exclusion tube was installed in the location of the collar at tree B3 and the temporary collar at tree B4 became the control treatment collar (i.e., no root exclusion).

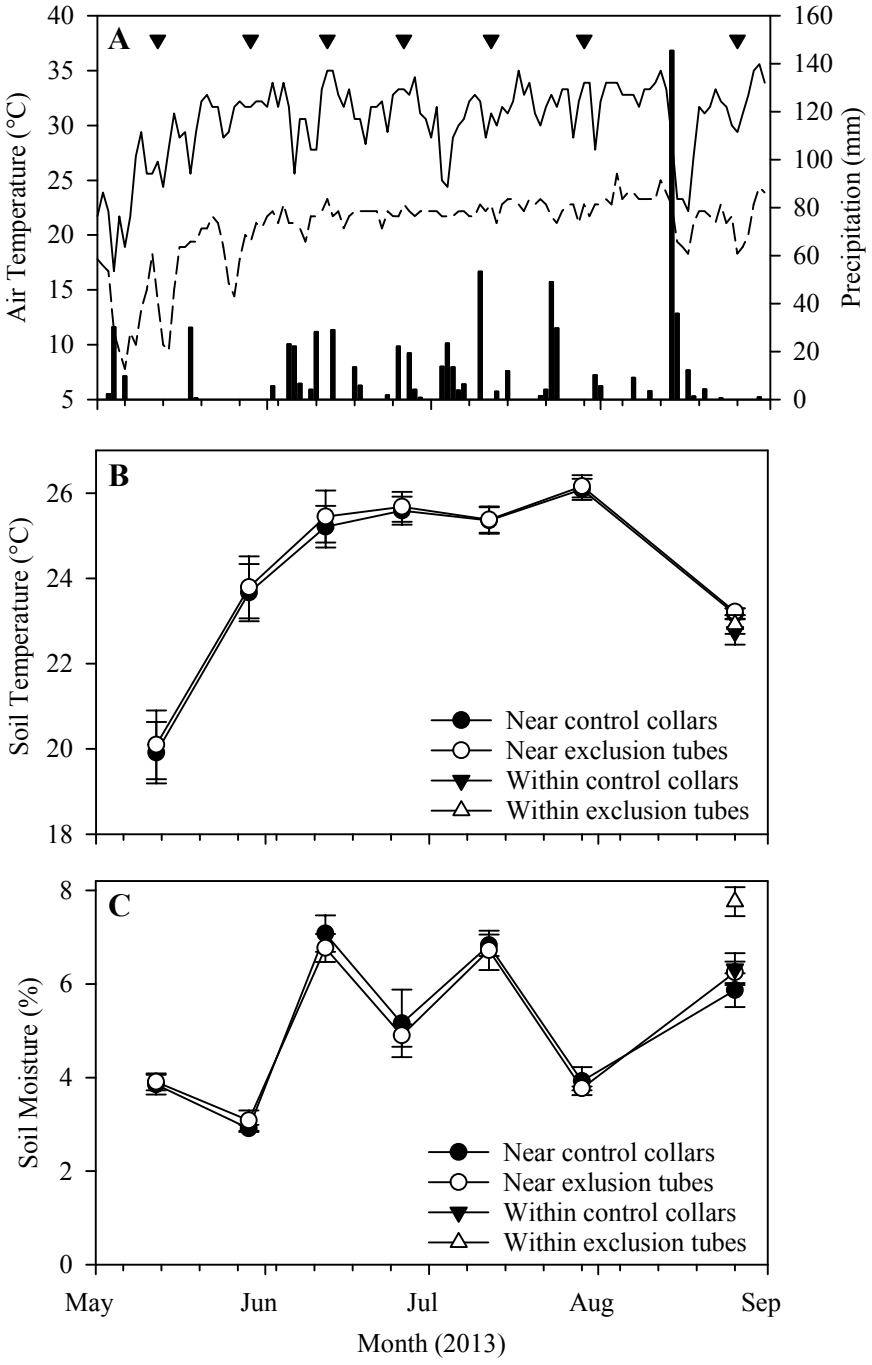

Figure 4.3: (A) Daily maximum (solid line) and minimum (dashed line) temperature and precipitation (black bars) for Columbus Metropolitan Airport Weather Station (National Climatic Data Center, 2015*b*). Triangles represent sampling dates. Soil temperature (B) and soil moisture (C) by sampling date and treatment measured in three 26-year-old longleaf pine stands. Note: Error bars are ± 1 SE. Initial R_S measurements were pooled from May 10 and May 15, 2013.

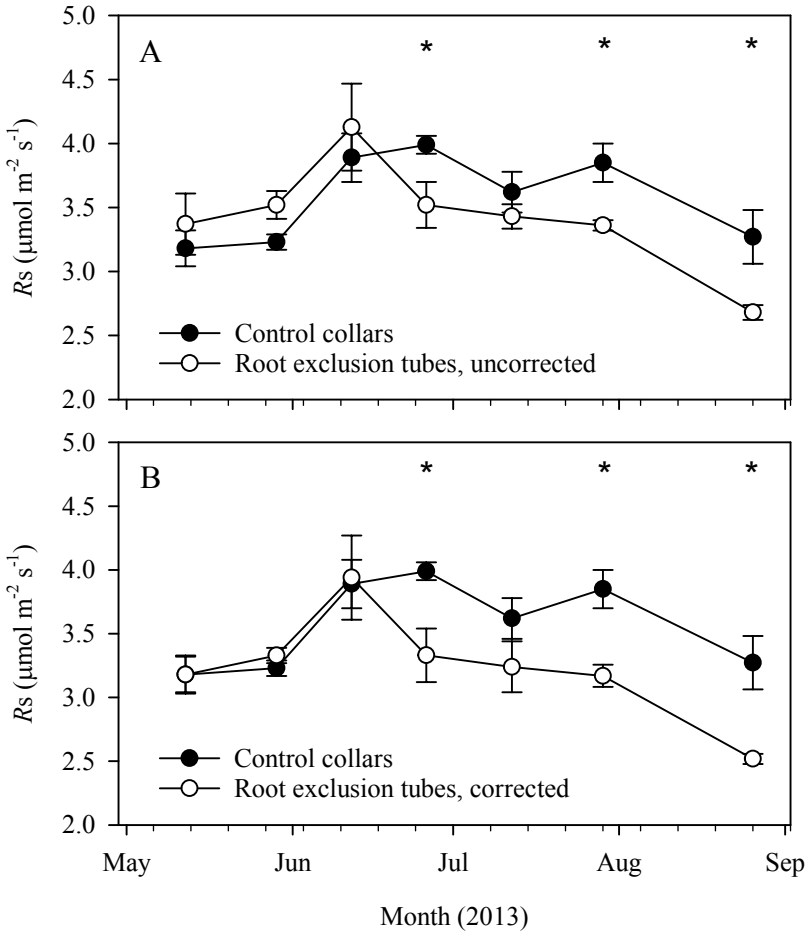

Figure 4.4: (A) Soil respiration (R_S) by control collar and root exclusion tube and (B) control collar and root exclusion tube corrected by the within-block, pretreatment R_S variation (Δ_{Rs}) by measurement date measured in three 26-year-old longleaf pine stands. Note: Asterisks (*) denote significant treatment effects at $\alpha = 0.05$. Error bars are ± 1 SE. Initial R_S measurements were pooled over May 10 and May 15, 2013.

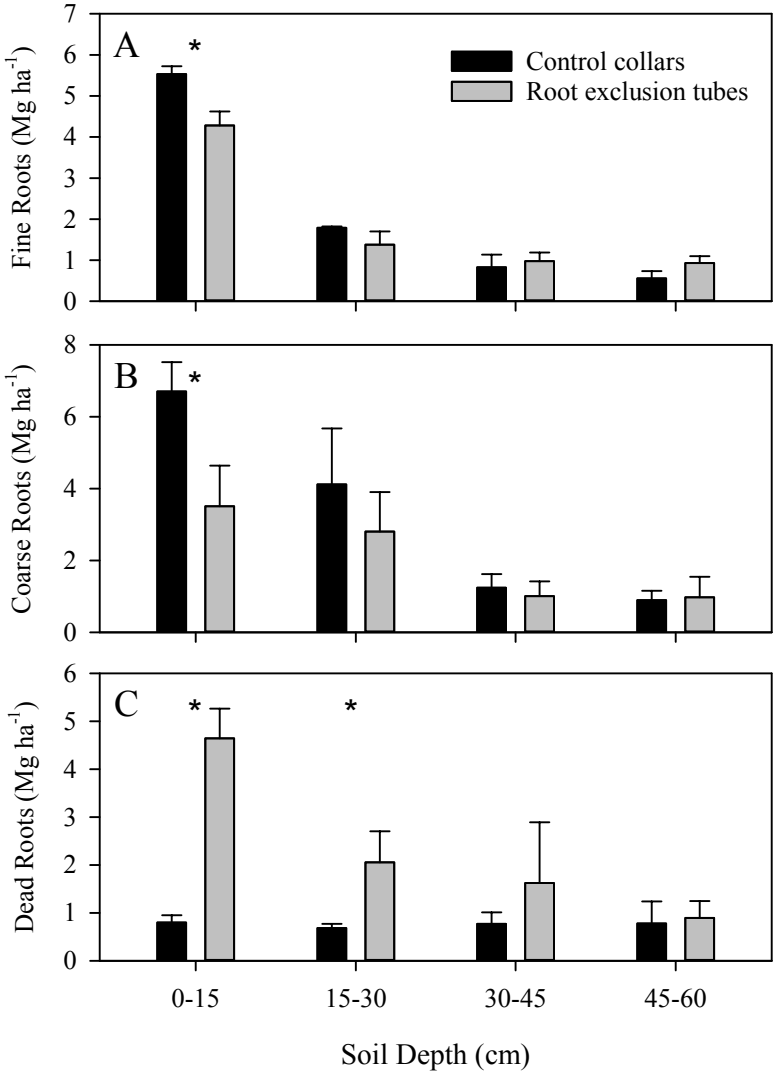

Figure 4.5: Root biomass by treatment and depth for live fine (A), live coarse (B), and dead (C) root categories measured in three 26-year-old longleaf pine stands. Note: Asterisks (*) denote significant treatment effects at $\alpha = 0.05$. Error bars are ± 1 SE.

Table 4.1: Location and forest structural characteristics for three 26-year-old longleaf pine stands at Blackjack Crossing Tract, Talbot County, Georgia. Note: LLP, longleaf pine; HW, hardwoods; DBH, diameter at breast height (1.37 m).

Variable	Stand 1	Stand 2	Stand 3
	Location & Size		
° N	32° 34.469'	32° 34.716'	32° 34.706'
° W	-84° 36.039'	-84° 31.371'	-84° 30.117'
Area (ha)	75.3	4.0	2.4
	Basal Area ($m^3\,ha^{-1}$)		
Total	18.82	21.48	17.34
LLP	18.60	21.40	17.34
HW	0.22	0.08	0.00
	Tree Density ($trees\,ha^{-1}$)		
Total	1222	1533	1922
LLP	978	1089	1567
HW	89	33	0
Dead	156	411	356
	Mean DBH ± SE (cm)		
LLP	14.89 ± 0.48	15.44 ± 0.35	11.50 ± 0.25
HW	4.39 ± 1.36	4.00 ± 2.70	na

Table 4.2: Influence of the location of soil temperature and moisture measurements (i.e. within or near R_S collars) for control collars and root exclusion tubes measured in three 26-year-old longleaf pine stands.

	Soil Temperature±SE (°C)		Soil Moisture±SE (%)	
Location	Control	Root Exclusion	Control	Root Exclusion
Near	23.2±0.1	23.2±0.1	5.9±0.4	6.3±0.2
Within	22.8±0.3	22.9±0.2	6.3±0.3	7.8±0.3
t-value	-1.30	-0.90	1.50	5.05
p-value	0.20	0.38	0.25	< 0.01

Table 4.3: Statistical summary of the influence of treatment on mean R_S measurements by date as measured in three 26-year-old longleaf pine stands. Summaries given for the comparison of soil respiration from control collars versus both uncorrected root exclusion tubes and root exclusion tubes corrected for within-block, pretreatment R_S variation.

Date (m/dd/2013)	5/10 & 5/15	5/29	6/12	6/26	7/12	7/29	8/26
Uncorrected							
$F_{1,27}$	1.64	2.10	0.71	5.94	0.76	4.68	7.63
p-value	0.21	0.16	0.41	0.02	0.39	0.04	0.01
Corrected for within-block, pretreatment R_S variation							
$F_{1,27}$	–	0.30	0.03	13.72	2.39	10.08	11.73
p-value	–	0.59	0.85	< 0.01	0.13	< 0.01	< 0.01

Table 4.4: Mean estimated soil respiration (R_S) and heterotrophic respiration (R_H) and the ratio of R_H to R_S (%) by R_H estimation method including: (1) uncorrected R_S measured from root exclusion collars (R_{exc}); (2) R_{exc} corrected by the within-block, pretreatment R_S variation; (3) R_{exc} corrected by the loss of CO_2 due to root decay (k); and (4) R_{exc} corrected by the combined effects of within-block, pretreatment R_S variation and k. Measurements made in three 26-year-old longleaf pine stands

R_H estimation method	$R_S \pm$ SE (μmol m^{-2} s^{-1})	$R_H \pm$ SE (μmol m^{-2} s^{-1})	R_H/R_S (%)
1. Uncorrected R_{exc}	3.27 ± 0.16	2.67 ± 0.16	81.87 ± 7.7
2. R_{exc} corrected for initial R_S variation	3.27 ± 0.18	2.52 ± 0.18	76.92 ± 9.0
3. R_{exc} corrected for k	3.27 ± 0.16	2.17 ± 0.37	66.28 ± 17.7
4. R_{exc} corrected for initial R_S variation & k	3.27 ± 0.18	2.01 ± 0.38	61.33 ± 20.0

Table 4.5: Statistical summary of the influence of treatment on mean root biomass by type and depth as measured in three 26-year-old longleaf pine stands. Summaries given for the comparison of root biomass from soil beneath control collars versus soil within root exclusion tubes.

Depth (cm)	0-15	15-30	30-45	45-60
Live Fine Root Biomass				
$F_{1,27}$	7.17	2.35	0.37	3.16
p-value	0.01	0.14	0.55	0.09
Live Coarse Root Biomass				
$F_{1,27}$	4.82	0.64	0.31	0.01
p-value	0.04	0.43	0.58	0.92
Dead Root Biomass				
$F_{1,27}$	8.42	7.26	0.69	0.11
p-value	< 0.01	0.01	0.42	0.74

Chapter 5

Conclusion

This research examined the dynamics of soil respiration (R_S) in longleaf pine (*Pinus palustris* Mill.) forests located centrally within their native range (Fig. **??**). Specifically, we examined the temporal and spatial variation of R_S in longleaf pine forests, and the environmental and ecological factors affecting the variation in R_S, and we partitioned longleaf pine R_S into its heterotrophic and autotrophic components (R_H and R_A, respectively).

The first study measured R_S and related environmental and ecological variables monthly from January 2012 through January 2013 in four longleaf pine stands varying in age (5 to 87 years old) and structure at Fort Benning, Georgia (Chapter 2). On an intra-annual basis, R_S ranged from 1.2 to 5.9 μmol m^{-2} s^{-1} and was controlled by seasonal fluctuations in soil temperature, peaking in the summer of 2012. Mean annual R_S was 13.3 Mg C ha^{-1} based upon the R_S-temperature relationship. Soil moisture influenced R_S by decreasing the temperature sensitivity (Q_{10}) of R_S during drought-like soil conditions (i.e., soil moisture at half the texture-derived wilting point), and litter mass, distance to nearest tree from R_S collar, and root biomass also affected R_S albeit by a marginal degree (i.e., partial R^2 values of 0.07 to 0.15) after first isolating the R_S-temperature relationship.

The second study measured the spatial variation in R_S in a 64-year-old longleaf pine stand at Fort Benning, Georgia in July and August 2012 (Chapter 3). Soil respiration ranged from 3.9 to 5.3 μmol m^{-2} s^{-1} and demonstrated varying degrees of spatial autocorrelation, perhaps because of the homogenizing effects of soil moisture and prescribed burning on R_S. Environmental and ecological variables influenced the spatial variation in R_S in complex ways, depending on the scale (within-plot versus between-plot) and location within the stand. For instance, the mean size of trees within 4 m of R_S collar was positively related to R_S in one plot with higher mean R_S and negatively related to R_S in another plot with the lowest mean R_S, and forb understory cover was only significantly related to R_S variation between plots but not within individual plots.

In the third study, R_H was estimated by comparing root-excluded soil to adjacent undisturbed soil in three 26-year-old longleaf pine stands in Talbot County, Georgia (Chapter 4).

The proportion of R_H to R_S ranged from 61 to 82 % and was lowest when the initial, pre-treatment variation in R_S and CO_2 loss from root decay were incorporated. Compared to the control soil, the presence of root exclusion tubes significantly decreased fine and coarse live root biomass and increased dead root biomass, especially in shallow soil layers (i.e., 0–15 cm depth)

This research has improved our collective understanding of the dynamics of R_S in longleaf pine forests. However, further research can be done to better quantify the overall carbon sink strength of longleaf pine forests. In particular, based on these studies, the range of the ratio of R_H to total R_S from Chapter 4 and the annual R_S estimates for the four diverse longleaf pine stands examined in Chapter 2 can be used to estimate annual R_H (8.11 to 10.9 $Mg\,ha^{-1}\,yr^{-1}$). Then, to estimate net ecosystem productivity (NEP), this value can be compared with net primary productivity (NPP) estimates for longleaf pine forests, such as the range of NPP from 5.2 to 13.2 $Mg\,ha^{-1}\,yr^{-1}$ for longleaf pine forests in southwest Georgia across an edaphic gradient (Hendricks et al., 2006). However, Chapter 3 demonstrated that within a relatively small area of one longleaf pine forest, R_S ranged widely, and this spatial variation of R_S increases the uncertainty of annual R_S measurements. We conclude that future work should focus on three specific areas: (1) quantification and incorporation of the spatial variation of R_S into annual R_S estimates; (2) studies that couple annual R_S and NPP measurements across the native range of longleaf pine; and (3) use of root exclusion tubes staggered over time to capture the proportion of R_H to R_S on an annual basis and in response to disturbances.

Bibliography

Hendricks, J. J., R. L. Hendrick, C. A. Wilson, R. J. Mitchell, S. D. Pecot, and D. Guo. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. Journal of Ecology **94**:40–57.

Appendices

Appendix A

LI-COR CO_2 efflux measurement equations

A.1 Overview of soil respiration measurements with LI-COR 6400

The following equations are derived from the LI-COR LI-6400-09 Soil Chamber manual and the Application Note #124 (LI-COR, Inc., 1997, 1998). In summary, the ideal gas law states that CO_2 efflux (f_c) will be dependent upon molar air density (ρ), chamber volume (V), soil area (s), the slope or rate that CO_2 increases over time ($\partial c/\partial t$), and the dilution factor from water vapor, as in this equation:

$$f_c = \frac{\rho V}{s}\left(\frac{\partial c}{\partial t} + \frac{c}{1-w}\frac{\partial w}{\partial t}\right) \tag{A.1}$$

where w is the molar concentration of water.

A.2 Derivation of Eq. (A.1)

The ideal gas law:

$$\begin{aligned} sf_c &= \frac{PV}{nRT}\left(\frac{\partial c}{\partial t}\right), \text{ or equivalently:} \\ sf_c &= \rho V\left(\frac{\partial c}{\partial t}\right) \end{aligned}$$

is used to measure soil CO_2 efflux, f_c. However, there is also some loss of air containing c concentration of CO_2 at a flow rate of u:

$$sf_c = \rho V\left(\frac{\partial c}{\partial t}\right) + uc \tag{A.2}$$

The loss of air through u is assumed to be from the evaporative loss of water vapor with w concentration of water, such that:

$$\begin{aligned}
sf_w &= u \text{ and} \\
sf_w &= \rho V \left(\frac{\partial w}{\partial t}\right) + uw, \text{ thus} \\
sf_w &= \rho V \left(\frac{\partial w}{\partial t}\right) + sf_w w, \text{ or solved for } sf_w: \\
sf_w &= \frac{\rho V}{1-w} \left(\frac{\partial w}{\partial t}\right), \text{ thus} \\
u &= \frac{\rho V}{1-w} \left(\frac{\partial w}{\partial t}\right)
\end{aligned}$$

Now, substituting this in for u in Eq. (A.2):

$$\begin{aligned}
sf_c &= \rho V \left(\frac{\partial c}{\partial t}\right) + \frac{\rho V}{1-w} \left(\frac{\partial w}{\partial t}\right) c \\
sf_c &= \rho V \left(\frac{\partial c}{\partial t}\right) + \frac{\rho V c}{1-w} \left(\frac{\partial w}{\partial t}\right) \\
sf_c &= \rho V \left(\frac{\partial c}{\partial t} + \frac{c}{1-w} \frac{\partial w}{\partial t}\right), \text{ and solved for } f_c: \\
f_c &= \frac{\rho V}{s} \left(\frac{\partial c}{\partial t} + \frac{c}{1-w} \frac{\partial w}{\partial t}\right), \text{ which is Eq. A.1.}
\end{aligned}$$

Bibliography

LI-COR, Inc., 1997. 6400-09 Soil CO_2 Flux Chamber. Publication No. 9720-119, LI-COR, Inc., Environmental Division, Lincoln, Nebraska.

LI-COR, Inc., 1998. Considerations for modeling ground CO_2 effluxes and chambers. Application Note #124.

Appendix B

Semivariogram analysis equations and script

B.1 Description of semivariogram analysis

The gstat procedure *fit.variogram* was used to fit the semivariogram with nugget, linear, and exponential models. The nugget model:

$$\gamma = n \tag{B.1}$$

represents no spatial autocorrelation; that is, the nugget n, which is the amount of semivariance that cannot be explained by spatial autocorrelation, represents the total semivariance and no additional semivariance is explained by spatial relationships between measurements. The linear model:

$$\gamma = n + \beta_1(h) \tag{B.2}$$

demonstrates that while some semivariance is not spatially related (n), the semivariance that is explained by spatial autocorrelation does not saturate within a lag distance of 30 m. Finally, the exponential model:

$$\gamma = n + s(1 - \mathrm{e}^{-3h/r}) \tag{B.3}$$

describes the amount of semivariance unexplained by spatial autocorrelation (n) as well as the range (r) and covariance level (sill, $n + s$) at which spatial autocorrelation of covariance is saturated. Past r, spatial autocorrelation between measurements ceases to increase. The nugget model represents spatial independence of the R_{S} sampling subplots; the linear model represents strong spatial autocorrelation that continues beyond the plot boundary; and the exponential model represents spatial autocorrelation within the bounds of the plot. The fitted model for each semivariogram was chosen by minimizing the residual error sum of squares (SSE). In the condition that the $r > 30$ m in a fitted exponential semivariogram model, linear was chosen because $r > 30$ m represents autocorrelation past the boundaries of the plots (effectively a linear semivariogram model).

B.2 R script for semivariogram analysis

```
rm(list=ls())
library(maptools)
library(gstat)

# import data
sp1 = readShapePoints('C:/Users/aaa0013/Documents/
ArcGIS/dissertation/spatial_study/R/process20141002/sp1n25')
names(sp1)

# make vectors matching the variables wanted to analyze
vars = c(9, 12, 14, 19:36, 38:43, 48:64)
varsName <- names(sp1)
varsName <- varsName[vars]
varnamesall <- names(sp1)

### NUGGET MODELS ###
#initialize psill and err vectors
nuggetPsill <- c(1:length(vars))
nuggetErr <- c(1:length(vars))
# calculate and fit variogram
print("Nugget Models")
for(i in vars){
    vario <- variogram(sp1[[i]]~1,sp1, cutoff=30)
    v.nug <- fit.variogram(vario,  model=vgm(model="Nug"))
    v.nugErr <- attr(v.nug, "SSErr")
    #print(varnamesall[[i]])
    #print(v.nug)
```

```
    #print(v.nugErr)
    nuggetPsill[[i]] <- c(v.nug$psill)
    nuggetErr[[i]] <- c(v.nugErr)
}
# output only for variables tested
print("Nugget model results")
nuggetPsill <- nuggetPsill[vars]
nuggetPsill
print("Nugget model SSErr")
nuggetErr <- nuggetErr[vars]
nuggetErr

### LINEAR MODEL NO INTERCEPT ###
#initialize psill and err vectors
lin0Psill <- c(1:length(vars))
lin0Err <- c(1:length(vars))
# calculate and fit variogram
print("Linear no Int Models")
for(i in vars){
     vario <- variogram(sp1[[i]]~1,sp1, cutoff=30)
     v.lin0 <- fit.variogram(vario,  model=vgm(model="Lin"))
     v.lin0Err <- attr(v.lin0, "SSErr")
     #print(varnamesall[[i]])
     #print(v.lin0)
     #print(v.lin0Err)
     lin0Psill[[i]] <- c(v.lin0$psill)
     lin0Err[[i]] <- c(v.lin0Err)
}
print("Linear noInt Slope as Psill")
```

```
lin0Psill <- lin0Psill[vars]
lin0Psill
print("Linear noInt SSErr")
lin0Err <- lin0Err[vars]
lin0Err

### LINEAR MODEL WITH INTERCEPT ###
#initialize data frame with 2 rows
linResults <- data.frame(one=c("nugget","slope"))
linResults
linErr <- c(1:length(vars))
# calculate and fit variogram
print("Linear w Int Models")
for(i in vars){
     vario <- variogram(sp1[[i]]~1,sp1, cutoff=30)
     v.lin <- fit.variogram(vario,  model=vgm(model="Lin", nugget=1))
     v.linErr <- attr(v.lin, "SSErr")
     #print(varnamesall[[i]])
     #print(v.lin)
     #print(v.linErr)
     linResults <- cbind(linResults,c(v.lin$psill))
     linErr[[i]] <- c(v.linErr)
}
# print results
print("Linear Nugget, Slope")
linResults
print("Linear SSErr")
linErr <- linErr[vars]
linErr
```

```
### SPHERICAL MODEL ###
# initialize output dataframes
sphResults <- data.frame(one=c("nugget","sill","null","range"))
sphResults2 <- data.frame(one=c("sill","range"))
sphErr <- c(1:length(vars))
# calculate and fit variograms
print("Spherical Models")
for(i in vars){
     #print(varnamesall[[i]])
     vario <- variogram(sp1[[i]]~1,sp1, cutoff=30)
     v.sph <- fit.variogram(vario,  model=vgm(model="Sph", range=100,
          nugget=1))
     v.sphSing <- attr(v.sph, "singular")
     if(v.sphSing == "FALSE") {
         #print("Intercept")
         v.sphErr <- attr(v.sph, "SSErr")
         #print(v.sph)
         #print(v.sphErr)
         sphResults <- cbind(sphResults,i,c(v.sph$psill,v.sph$range))
         sphErr[[i]] <- c(v.sphErr)
     }
     if(v.sphSing == "TRUE") {
         v.sph2 <- fit.variogram(vario,  model=vgm(model="Sph",
        range=100))
         v.sphErr2 <- attr(v.sph2, "SSErr")
         #print("No intercept")
         #print(v.sph2)
         #print(v.sphErr2)
```

```
          sphResults2 <- cbind(sphResults2,i,c(v.sph2$psill,v.sph2$range))
          sphErr[[i]] <- c(v.sphErr2)
     }
}
#two outputs of results this time, based on singular or not
print("Spherical results")
print("Nugget,sill, range")
sphResults
print("No nugget")
sphResults2
print("Spherical SSErr")
sphErr <- sphErr[vars]
sphErr

### EXPONENTIAL MODELS ###
# initialize output dataframes
expResults <- data.frame(one=c("nugget","sill","null","range"))
expResults2 <- data.frame(one=c("sill","range"))
expErr <- c(1:length(vars))
# calculate and fit variograms
print("Exponential Models")
for(i in vars){
     #print(varnamesall[[i]])
     vario <- variogram(sp1[[i]]~1,sp1, cutoff=30)
     v.exp <- fit.variogram(vario,  model=vgm(model="Exp",
        range=100, nugget=1))
    v.expSing <- attr(v.exp, "singular")
    if(v.expSing == "FALSE") {
          #print("Intercept")
```

```
        v.expErr <- attr(v.exp, "SSErr")
        #print(v.exp)
        #print(v.expErr)
        expResults <- cbind(expResults,i,c(v.exp$psill,v.exp$range))
        expErr[[i]] <- c(v.expErr)
    }
    if(v.expSing == "TRUE") {
        v.exp2 <- fit.variogram(vario,  model=vgm(model="Exp",
       range=100))
        v.expErr2 <- attr(v.exp2, "SSErr")
        #print("No intercept")
        #print(v.exp2)
        #print(v.expErr2)
        expResults2 <- cbind(expResults2,i,c(v.exp2$psill,v.exp2$range))
        expErr[[i]] <- c(v.expErr2)
    }
}
#two outputs of results this time, based on singular or not
print("Exponential results")
print("Nugget,sill, range")
expResults
print("No nugget")
expResults2
print("Exponential SSErr")
expErr <- expErr[vars]
expErr

### SSTot ###
# calculated for each variable
```

```
sstot <- c(1:length(vars))
for(i in vars){
     vario <- variogram(sp1[[i]]~1,sp1, cutoff=30)
     weight <- vario$np/vario$dist^2
     total <- sum(weight*vario$gamma)/sum(weight)
     sstot[[i]] <- c(total)
}
sstot <- sstot[vars]
sstot

### Variogram Output###
# for graphing purposes
varioFull <- data.frame(iter=c(1:12))
for(i in vars){
     vario <- variogram(sp1[[i]]~1,sp1, cutoff=30)
     varioFull <- cbind(varioFull,i,c(vario$gamma))
}
varioFull <- cbind(varioFull,c(vario$np),c(vario$dist))
varioFull

### export to Excel ###
# unless you want to make new CSV, use for every output you want
writeClipboard(as.character(nuggetErr))
writeClipboard(as.character(varioFull))
```