Quantification and Physiology of Carbon Dynamics in Intensively Managed Loblolly Pine (*Pinus taeda* L.)

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(ABSTRACT)

Loblolly pine (*Pinus taeda* L.) occupies 13 million hectares in the United States and represents a critical component of the global carbon (C) cycle. Forest management alters C dynamics, affecting the C sequestration capacity of a site. Identifying drivers that influence C cycling, quantifying C fluxes, and determining how management alters processes involved in C cycling will allow for an understanding of C sequestration capacity in managed forests.

Objectives of the first study included (1) investigating environmental, soil C, root, and stand influences on soil CO_2 efflux on the South Carolina coastal plain and (2) quantifying soil CO_2 efflux over a rotation in loblolly pine stands located on the South Carolina coastal plain and the Virginia piedmont. In relation to the first objective, temporal variation in soil CO_2 efflux was most highly related to soil temperature. Spatial and temporal variability in soil CO_2 efflux was weakly related to soil C and root biomass, and not related to coarse woody debris, stand age, stand volume, or site index [Chapter 2]. Soil CO_2 efflux was not related to stand age on the South Carolina sites while efflux was positively related to age on the Virginia sites. Cumulative soil C efflux on the South Carolina sites over 20 years is an estimated 278.6 Mg C/ha compared with an estimated 210.9 Mg C/ha on the Virginia sites [Chapter 3].

Objectives of the second study were (1) to investigate short-term effects of fertilization on processes permitting enhanced growth in loblolly pine and (2) to determine the short-term effects of fertilization on autotrophic, heterotrophic, and soil respiration. Major results from the study include the finding that fertilization caused a transient rise in photosynthetic capacity, which paralleled changes in foliar nitrogen. Leaf area accumulation and enhanced growth following fertilization was partly due to enhanced C fixation capacity [Chapter 4]. Fertilization altered the contribution of autotrophic and heterotrophic respiration to total soil CO_2 efflux. Enhanced specific root respiration was short-lived while suppressed microbial respiration following fertilization was maintained over the course of the nearly 200-day study. Respiring root biomass growth increased total soil respiration over time [Chapter 5].

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CHAPTER 1

Introduction

Background and Context

Global warming is partly blamed on accumulating atmospheric greenhouse gases, including CO_2. The international Kyoto Protocol, which was not signed by the United States, outlined a plan in which developed nations would be required to reduce greenhouse gas emissions or enhance greenhouse gas sinks in order to reduce net greenhouse gas production (Rotter and Danish 2000). Although the Kyoto Protocol was not signed by the United States, measures at the national level are currently being taken to reduce net greenhouse emissions since, at the current rate, atmospheric CO_2 concentrations are projected to reach 700 ppm by the middle of this century (Allen 1994). The Kyoto Protocol introduced the concept of "carbon credits" (i.e. a quantifiable C sink) for practices that mitigate CO_2 build-up in the atmosphere. For these reasons, policy makers, forest industry, and the general public has become increasingly interested in understanding how forestry activities affect C dynamics of not only individual stands, but also an entire region (Rotter and Danish 2000). In fact, *the Chicago Climate Exchange* is a pilot program with the goal of implementing a feasible market-based C trading system (Walsh 2003). The program currently lists several members from forest industry and continues to grow. The success of programs such as *the Chicago Climate Exchange* and future implementation of large-scale, possibly international, C trading relies on quantitative information regarding the impact of forest management has on C sequestration capacity.

Intensively managed loblolly pine (*Pinus taeda* L.) stands account for over 13 million hectares of forested land in the southeast (Wear and Greis 2002). Management practices in the southeast play a significant role in global greenhouse gas levels by influencing the productivity of forest ecosystems and the ability of forests to sequester C in biomass and soils (Edwards and Ross-Todd 1983, Londo et al. 1999, Lee et al. 2002). Intensively managed loblolly pine stands may serve as an effective sink earlier in a rotation when growth is enhanced through various silvicultural practices (Gladstone and

Ledig 1990, Maier and Kress 2000). However, the overall impact of intensive forestry on C dynamics has not been explored fully. Collectively, a loblolly pine ecosystem serves as a C source when net primary productivity (NPP) is less than C losses due to heterotrophic respiration, and, conversely, as a C sink when NPP exceeds heterotrophic respiratory loss. Thus, a stand is a net source of C until NPP (i.e. biomass) exceeds microbial and soil organismal respiration. However, within any given management scenario the C flux varies spatially, temporally and with genotype. Additional quantitative data sets will provide critical information to improve process-based models that will be used by scientists and forest managers to estimate stand-level and regional C dynamics.

Soil respiration, which includes respiration from roots, soil organisms, and mycorrhizae, is a key source of CO_2 in terrestrial ecosystems and an important component of the global C cycle (Raich and Schlesinger 1992, Raich and Tufekcioglu 2000, Rustad et al. 2000, Schlesinger and Andrews 2000). However, belowground processes have not been adequately quantified and changes in physiologically processes in response to management have not been extensively characterized (Rustad et al. 2000). An estimated 10^8 Pg C exists in the earth's C pools, including the earth's crust, dissolved oceanic carbonates, gas hydrates, fossil fuels, the terrestrial biosphere, soils and the atmosphere (Rustad et al. 2000). Biological systems and biogeochemical cycles that normally keep the pools in dynamic equilibrium have been disrupted by anthropogenic activities such as the burning of fossil fuels and deforestation. Several factors affect soil respiration and the return of stored soil C to the atmosphere, including temperature, soil moisture, vegetation and substrate quality (e.g. type of organic matter), net ecosystem productivity, allocation of above- and belowground biomass, population and community interactions, and land use disturbances (Rustad et al. 2000). Currently, soil respiration is estimated to contribute approximately 75 x 10^{15} g C/yr back to the atmosphere (Schlesinger and Andrews 2000).

Although Raich and Tufekcioglu (2000) reported that vegetation type is not as important in determining soil respiration as environmental drivers, considerable debate continues over actual amounts of soil C released by various ecosystems. Based on a compilation of soil respiration rates (n=23) reported in the literature, Raich and

Schlesinger (1992) found that average effluxes were approximately 681 gC/m^2/yr in temperate coniferous forests. This figure is approximately half of the mean yearly rates reported for tropical moist forests and about double those reported for boreal forests. Raich and Potter (1995) used a semi-mechanistic, empirically based statistical model to predict mean soil CO_2 efflux rates among different vegetation types. They predicted much lower rates of 364 gC/m^2/yr for needleleaf evergreen forests. While these estimates provide a sense of the relative soil CO_2 efflux from various ecosystems, they offer a crude and oversimplified estimate that is based on data collected using different methodologies. The calculations also fail to adequately take into account intra-ecosystem heterogeneity (i.e. spatial and temporal site variation). The authors acknowledge that much more data are required to account for spatial and temporal variability on a larger scale and to improve the mechanistic understanding of soil respiration on a smaller scale. Rustad et al. (2000) similarly conclude that spatial and temporal considerations of soil CO_2 efflux need to be further addressed, so that results from small chamber studies can adequately be scaled-up to the regional level. Furthermore, variation among stands under different management regimes and of several locations and/or inherent site conditions within the region must be considered. Collectively, these data will provide the information needed to develop a comprehensive model that sufficiently represents the entire southeast loblolly pine community.

As alluded to, physiological mechanisms affecting C flux in loblolly pine have not been sufficiently addressed. Specifically, the physiological changes that may occur in gas exchange (both respiration and photosynthesis) when a stand is fertilized could affect the when a pine plantation changes from a source to a sink (Maier and Kress 2000) since fertilization often increases NPP and results in biomass allocation shifts (Albaugh et al 1998) and may reduce microbial respiration (e.g. Thiurkkumaran and Parkinson 2000). While there are no loblolly pine studies that examined above- and belowground gas exchange simultaneously after nutrient additions, collective evidence from the literature suggests that nutrient additions may enhance both biomass and soil C sequestration.

Literature Review

Overview of Soil Respiration: Components and Major Drivers

Soil Respiration and the Environment

Soil respiration is the sum of heterotrophic (i.e. non-rhizosphere and rhizosphere) respiration and autotrophic (i.e. root) respiration. The reported contribution of each component to total soil respiration varies within the literature and appears to be highly influenced by environmental conditions, including fertility, and also by genetics and ontogeny. These factors affect biomass partitioning, growth rates, and (both microbial and root) specific respiration rates. Maier and Kress (2000) attributed 52% and 73% of the total soil respiration to root respiration in non-fertilized and fertilized loblolly pine stands, respectively. In a recent review, Raich and Tufekcioglu (2000) noted that root respiration is the primary contributor to soil CO_2 efflux in forested land. They also cited differences among forest types. Generally, the proportional contribution of root respiration to soil respiration in forests is greater in colder climates (the arctic and boreal forests). Based on the literature surveyed by Raich and Tufekcioglu (2000), pine forest roots contribute 35 to 62% to total soil CO_2 efflux. Another review by Hanson et al. (2000) reported a much broader range for all forest-types, citing that root respiration may account for as little as 10% to over 90% of the total soil respiration. They attributed differences in the literature to variation in vegetation type, the time of year when measurements were taken, and varying methodologies.

Soil respiration is affected by several environmental factors that are both inherent to a given site and also influenced by practices common to intensive loblolly pine management. To a large extent, temporal (i.e. seasonal) and spatial (i.e. latitudinal and intersite) variation in soil respiration and its components is driven by differences in soil temperature and moisture (Raich and Tufekcioglu 2000). This is due to the fact that reaction velocities of enzymatic processes, which are highly temperature driven dramatically affect respiration rates. Typically, soil respiration rates double or even triple with an increase in temperature of 10°C. The relationship between temperature and respiration is typically considered exponential and usually expressed in terms of Q_{10}, which is defined as the ratio of the rate of respiration at temperature T to the rate at temperature T + 10°C. This relationship generally holds true across a range of temperatures, except at high temperatures (>35°C), when respiration often declines due to denaturation of associated enzymes (Kozlowski and Pollardy 1997). Howard and Howard (1993) reported Q_{10} values for soil respiration that ranged from 0.96 to 2.83 over a range of soil types and temperatures. Bouma et al. (1997) reported a Q_{10} of 2.0 for Douglas-fir (*Pseudotsuga menziessi* Franco) roots and Maier and Kress (2000) cited Q_{10} values of 2.2 to 2.4 for soils in both fertilized and non-fertilized loblolly pine stands. Other studies have also found high correlations between soil respiration and temperature (Kowalenko et al. 1978, Pajari 1995). Soil moisture affects respiration rates, especially under droughty conditions (Kowalenko et al. 1978, Howard and Howard 1993, Bouma et al. 1997, Pangle and Seiler 2002). Maier and Kress (2000) determined that soil water status significantly limited soil respiration rates in loblolly pine stands when soil moisture was low.

Soil Respiration Across Spatial and Temporal Scales

Variation in soil respiration within and between sites is directly related to spatial and temporal variability in the autotrophic and heterotrophic CO_2 sources. Spatial variation in soil respiration on a given site is partially related to the proximity of roots to the measurement chamber. Soil respiration rates observed at the base of seedlings on two piedmont sites in Virginia were consistently higher than those observed between planting rows (Popescu 2001, Pangle and Seiler 2002). These results indicate that small-scale spatial variation and the contribution of roots and the rhizosphere to total soil respiration (near the plant) may be fairly significant, even during the seedling stage. Spatial variability in heterotrophic respiration has not been directly addressed in the literature.

The impact stand age has on soil respiration is not well-defined in the literature. However, the few studies that examined soil respiration across different aged stands generally found an inconsistent relationship between soil respiration and stand age. Ewel et al. (1987) reported highest soil respiration rates in recently clear-cut slash pine (*Pinus elliottii* Engelm.) plantations. Rates were significantly lower in a 29-year-old stand, but lowest in a 9-year-old stand. Klopatek (2002) reported increasingly higher soil CO_2 efflux rates in 20-year-old, old-growth, and 40-year-old Douglas-fir stands, respectively. Pyker and Fredeen (2003) observed highest soil respiration rates 2 years after harvest in hybrid spruce stands. Rates were lowest the year of harvest and tended to show less of an age effect after the second year of growth. Irvine and Law (2002) reported similar soil respiration rates in 14-year-old and >50-year-old ponderosa pine (*Pinus ponderosa* Dougl.) stands located in Oregon. They determined that water availability was a significant influence on soil respiration on their sites. Collectively, the preceding authors conclude that harvest intensity, water availability, quantity of residual slash following harvest, belowground biomass accumulation, and changing site nutrition partially explain differences in soil respiration across ages.

The extent to which relative and total contributions of autotrophic and heterotrophic respiration change with stand age is poorly understood. However, total root respiration likely increases with age since total respiring root tissue (i.e. mostly fine root) biomass generally increases (Makkonen and Helmisaari 2001), which would proportionally increase soil respiration. The ontogeny and timing of root development depends on the forces of genetics and also on the environment. Environmental influences on root development most notably include water relations and nutrition, but also may include temperature, O_2 concentration, soil compaction, and competition among vegetation (Eissenstat and Van Rees 1994). Changes in specific root respiration with age have not been widely addressed. Specific microbial respiration (qCO$_2$) in a southern Australian mixed boreal forest generally increased with stand age in a report by Bauhus et al. (1998). (Specific microbial respiration or the respiratory quotient is defined as microbial respiration rate/unit microbial biomass.) The authors concluded that while qCO$_2$ increased with age, organic matter quality diminished. Other factors affecting root, microbial, and soil respiration include the succession of vegetation and microflora associated with stand development (Fritze et al. 1993).

Soil Respiration and Forest Management

Forest management plays a crucial role in soil C turnover and soil C sequestration (Woodwell et al. 1983, Turner et al. 1995, Field and Fung 1999, Banfield et al. 2002, Liski et al. 2002). Inherent site characteristics along with forest management practices that commonly alter microclimate and productivity may directly impact soil respiration. However, the literature provides no clear consensus concerning the effect of forest management on soil CO_2 efflux. Previous investigators observed an increase (Gordon et al. 1987, Lytle and Cronan 1998, Londo et al. 1999), decrease (Striegl and Wickland 1998), or no change (Edwards and Ross-Todd 1983, Fernandez et al. 1993, Toland and Zak 1994) in soil respiration rates following clear-cut harvesting. Differences among ecosystems and experimental designs may account for inconsistencies in literature; however, the distinct differences found among investigators support the idea that generalizations across all forest types are not appropriate. Frequently, soil CO_2 efflux and soil C turnover increase with harvest intensity (Edwards and Ross-Todd 1983, Londo et al. 1999, Lee et al. 2002). Also, Mallik and Hu (1997) showed that soil respiration rates corresponded to the amount of organic matter incorporated into the soil during site preparation in boreal mixedwood forest. Further, prescribed burning, which is a common tool in loblolly pine management, may impact soil respiration by altering the quality and quantity of C substrate available to microorganisms and by changing the soil microclimate (Fritze et al. 1993, Pietikainen and Fritze 1993, Pietikainen and Fritze 1995, Hernandez et al. 1997). Prescribed burning induces physical, chemical, and biological changes in soil, which are related to fire intensity and duration as well as soil type, moisture, vegetation, and climate (Chandler et al. 1983, Pietikainen and Fritze 1995).

Effects of Nutrient Additions on Pine Productivity

Fertilization and Photosynthesis

Fertilization generally increases leaf areas in loblolly pine (Albaugh et al. 1998), which logically enhances overall productivity. However, in order for higher leaf areas to be realized, one of the following must occur: (1) Fertilization may have a direct effect on photosynthetic efficiency (photosynthesis/unit leaf area) so that more C is fixed per leaf area. The “surplus” photoassimilate is used to build additional leaf area. (2) Assimilate allocation may shift from root to aboveground components (specifically leaf area). This effectively reduces sink tissue (i.e. roots) and increases source tissue (i.e. leaves). (3) Increased productivity is the result of both mechanisms. Enhanced photosynthetic efficiency combined with lower respiratory costs and allocation shifts may all contribute to productivity increases. However, current knowledge of the mechanisms and physiological processes leading to enhanced growth in loblolly pine remains unclear.

Previous studies that investigated fertilization effects on pine foliar gas exchange provide mixed results. Overall, plant photosynthesis and enhanced stem wood production in fertilized stands may be partially the result of increased leaf area (Teskey et al. 1987, Vose and Allen 1988, Teskey et al. 1994, Albaugh et al. 1998) and/or fertilization may directly improve photosynthesis rates per unit leaf area (Mitchell and Hinkley 1993, Murthy et al. 1996). In loblolly pine, Zhang et al. (1997) found that fertilization did not affect photosynthesis per unit leaf area in loblolly pine grown on an infertile site in Oklahoma. Tang et al. (1999) similarly concluded that fertilization of loblolly pine on a well-drained site in Louisiana did not significantly impact photosynthesis rates. Gough et al. (*in press*) found that optimum fertilization treatments in loblolly pine after 9 years had little influence on specific net photosynthesis. In fact, during much of the growing season, mean foliar photosynthesis rates were higher in non-fertilized stands.

Previous studies have conflictingly reported that foliar nitrogen (N) in conifers is highly correlated with photosynthesis, while others found little or no correlation. Mitchell and Hinckley (1993) found in Douglas-fir that photosynthetic rates and foliar N concentrations were positively correlated. In Scots pine seedlings, photosynthesis was strongly correlated with foliar N content only during the growing season (Vapaavuori 1995). Schoettle and Smith (1999) reported a weak relation between foliar N and photosynthesis in lodgepole pine (*Pinus contorta* Dougl.), except in young leaves. No differences in photosynthesis were found in mature slash pine foliage that had been fertilized (Teskey et al. 1994). Zhang et al. (1997) reported that, although N fertilization increased leaf N in loblolly pine, there was no increase in photosynthesis rates or quantum yield during the growing season. Foliar N concentration was not correlated with photosynthesis in a long-term loblolly pine fertilization study conducted in North Carolina (Gough *in press*). However, evidence from the same stand suggests that fertilization may increase specific net photosynthesis in the short-term (Murthy et al. 1997). Foliar N amendments, when limiting, probably allow for the greater production and maintenance of photosynthetic enzymes such as ribulose 1,5-bisphosphate carboxylase oxygenase (Rubisco), explaining why specific photosynthesis sometimes increases with fertilization (Field and Mooney 1986).

Microbial, Root and Soil Respiration

The literature generally shows that soil microbial respiration declines shortly after the application of N (Kawalenko et al. 1978, Soderstrom et al. 1983, Smolander et al. 1994, Lovell and Hatch 1998, Thiurkkumaran and Parkinson 2000). Specific root respiration on the other hand frequently increases with fertilization (Zogg et al. 1996, Griffin et al. 1997, Lu et al. 1998). Interestingly, several researchers measuring soil respiration have found no fertilization effect (Castro et al. 1994, Vose et al. 1995, Maier and Kress 2000), indicating that any decrease in microbial respiration could be offset by increases in root respiration.

Thiurkkumaran and Parkinson (2000) monitored microbial respiration in the laboratory from soil collected in a lodgepole pine forest over a 120-day period. They determined that ammonium nitrate and urea additions (of 188 kg N ha^{-1} and 300 kg N ha^{-1}) decreased microbial respiration. Ammonium nitrate produced only a temporary increase in specific respiration or the metabolic quotient (qCO$_2$), which was observed on the tenth day after fertilization. Lower values were recorded after day 40 in ammonium nitrate amended soils. They did not attribute the observed changes to alterations in soil pH, but rather speculated that osmotic changes or ammonium toxicity may be responsible. They suggested that the common fertilizer rates for lodgepole pine may reduce microbial activity. The qCO$_2$ was reduced in a long-term study performed on grazed swards receiving 200 N kg ha^{-1} as well (Lovell and Hatch 1998). A long-term study in Norway spruce (*Picea abies* L.) reported that cumulative N and phosphorus additions reduced microbial respiration in 40 to 60 year-old stands (Smolander et al. 1994). Stands were fertilized over a 20 to 30 year period, but had not been nutrient amended for at least 2 years prior to the study. Soil pH was not affected by fertilization. These findings indicate that fertilization may have long-term effects as well. N additions reduced microbial respiration and biomass within 1 week in a study performed on several coniferous forest soils (Soderstrom et al. 1983). The reduction persisted for 3 to 5 years after a single fertilization of 150 kg N ha^{-1} of ammonium nitrate. Similarly, Kowalenko et al. (1978) found that soil CO$_2$ evolution decreased in both a clay loam and sandy soil with fertilization over a 3-year period. In the previous two studies, a reduction in pH with fertilization may have partially explained the reduction in soil respiration.

Root respiration accounts for much of the total C efflux from plant respiration, which is reported to represent 30 to 60% of the C fixed via photosynthesis (Schlesinger and Andrews 2000). Increases in root respiration with fertilization are commonly reported in the literature. In 155-day-old loblolly pine and ponderosa pine seedlings, above optimal N additions increased specific root respiration rates (Griffin et al. 1997). Both species had significantly lower root biomass in comparison to the control. However, the authors found that ammonia toxicity caused reduced growth in plants receiving supra-optimal levels of N (a soil concentration of 7 mM ammonia). The authors concluded that root respiration was primarily driven by N uptake and detoxification of ammonia rather than growth since growth was actually stunted in plants receiving high N. In Douglas-fir seedlings grown in root boxes, specific root respiration and total root respiration increased with N additions of 10 and 50 mg L^{-1} (Lu et al. 1998). Specific root respiration leveled off at 200 mg L^{-1} N and total respiration actually declined due to a reduction in total root biomass and mycorrhizal growth, indicating that N toxicity occurred. Zogg et al. (1996) determined that fertilization did not significantly alter specific fine-root respiration rates in sugar maple (*Acer saccharum* L.); however, total root respiration was highly correlated with root tissue N concentration.

There are several potential reasons for increased specific root respiration with N fertilization, including the high metabolic (i.e. respiratory) costs associated with the conversion of NO_3^- to NO_2^- by nitrate reductase and the subsequent conversion of NO_2^- to NH_4^+ by nitrite reductase (Zogg et al. 1996). Also growth respiration costs of incorporating NH_4^+ into amino acids are significant (Vessey and Layzell 1987). Furthermore, maintenance costs increase with increasing protein concentration. When N additions increase whole-plant biomass, total maintenance respiration costs also increase (Ryan 1991).

The literature often reports that soil respiration remains unchanged with fertilization, which, as previously mentioned, may be the result of a decrease in microbial respiration offset by an increase in total root respiration. Maier and Kress (2000) measured soil respiration in 11-year-old loblolly pine stands fertilized for 4 years and found that fertilization reduced CO_2 evolution on several measurement days in stands when forest floor litter was included, but they observed no significant differences among fertilized and non-fertilized stands when litter was removed. The fact that soil respiration was lower in fertilized stands when litter was included implies that microbial respiration decreased relative to non-fertilized stands. Castro et al. (1994) similarly found that in mature slash pine 4 years of urea-N fertilization did not result in changes in soil CO_2 evolution. No changes in soil respiration were detected in 3-year old ponderosa pine fertilized with 10 and 20 g m^{-2} of ammonium sulfate (Vose et al. 1995). The authors concluded that the lack of response to N might be due to the counteracting effects of autotrophic and heterotrophic respiration. However, some studies have reported increases or decreases in soil CO_2 efflux when soils are fertilized. Johnson et al. (1994) reported an increase in soil respiration with N fertilization. Using correlative and quantitative comparisons, they determined that microbial respiration was unchanged, and therefore attributed soil respiratory increases to increased root respiration, which may have been partly due to increased root biomass in fertilized seedlings. Conversely, Haynes and Gower (1995) observed soil CO_2 evolution in a Wisconsin red pine (*Pinus resinosa* Ait.) forest and found a decrease in soil respiration with fertilization. In a recent study conducted in loblolly pine stands growing on the North Carolina piedmont, Butnor et al. (2003) reported a reduction in soil respiration following 3 years of annual fertilization of 11.2 g N/m^2.

Fertilization and Allocation

Theoretically, photoassimilate partitioning in the plant is directly influenced by the most limiting resource. When a resource is limiting, such as N, more resources are allocated to fine roots, for example, in order to enhance uptake until a balance is achieved and N is no longer the primary limiting factor. This theory however ignores multiple limiting factors that may influence allocation, including ontogeny and genetics (King et al. 1999). Ovington (1957 in King et al. 1999) reported ontogenetic changes in allocation when studying Scots pine (*Pinus sylvestris* L.), citing a maximum root:shoot ratio of 0.82 at age 7 and a reduction to 0.29 by age 55. Fertilization may affect allocation in two ways. Fertilization may simply enhance photosynthetic efficiency, resulting in higher overall plant productivity and shifts in allocation associated with these changes. Fertilization may also reduce biomass partitioning to the roots if nutrient resources become less limiting and the balance (i.e. the more limiting resource) discussed above is altered.

King et al. (1999) reported that 4 years of fertilization on a poor site increased partitioning to the perennial tissues, including coarse roots, taproots and branches, while decreasing partitioning to ephemeral tissues, including the fine roots and foliage in 12-year-old loblolly pine that had been fertilized for 4 years. Albaugh et al. (1998) cited similar results for the same stand, specifically reporting an average of 8% and 22% in total biomass allocation to fine roots in fertilized and non-fertilized stands, respectively. Measurements were performed over 3 consecutive years when the stand was 9 to 11 years of age and fertilized stands had received optimum nutrient additions treatments for one to 3 years. In both studies, overall biomass increased in all tissues except fine roots, indicating that fertilization reduced the plant’s fine root requirement for sufficient nutrient uptake. Thus, a proportional and absolute reduction in fine root allocation and production was observed. Interestingly, King and coworkers (1999) found that perennial root biomass increased more than perennial shoot biomass. They also determined that biomass partitioning to branches was greater than to the foliage, possibly due to the fact that lower branches often retain foliage longer in fertilized stands. Other studies have found both an absolute and relative decrease in root biomass production in fertilized pine stands (Axelsson and Axelsson 1986, Haynes and Gower 1995) or reductions in fine root:shoot biomass (Adams et al. 1989).

Loblolly pine seedlings subjected to a lower fertility have been shown to maintain an adequate uptake of nutrients by investing greater amounts of biomass in roots (Griffin et al. 1995, Gebuaer et al. 1996). However, King et al. (1999) found the opposite relationship between N and allocation in 7 and 9 year-old loblolly pine trees continuously fertilized for 2 and 4 years, respectively, which is in contrast to findings from seedling studies (Griffin et al. 1995, Gebuaer et al. 1996). Findings by King et al. (1999) in loblolly pine trees conflict with reports from long-term fertilization studies in 20-year-old Scots pine stands, in which significant reductions in root allocation were observed with fertilization. Therefore, shifting allocation patterns following fertilization may be species specific. Together, previous studies suggest that ontogenetic control may be equally as influential on biomass partitioning as the nutritional environment. The strong interaction between genetics and the environment indicates that allocation patterns in response to fertilization will also vary depending on timing of the fertilizer application. Further, the issue of whether or not shifting allocation patterns following fertilization are partly responsible for the establishment of greater leaf areas remains unclear since fertilization may increase root allocation in trees (King et al. 1999).

An increase in leaf area index (LAI) is often observed in fertilized stands, which could significantly enhance productivity, since LAI is often positively related to growth (Hay and Walker 1989). Vose and Allen (1988) reported an increase in LAI of up to 60% occurred with N fertilization in loblolly pine. Albaugh et al. (1998) reported an LAI increase of 101% in nutrient poor loblolly pine stands amended with optimum levels of fertilizer.

Collective Evidence from SETRES Studies

Several previously published reports based on data collected from a loblolly pine stand in North Carolina at the Forest Service's Southeastern Tree Research and Education Site (SETRES) suggest that fertilization may have an effect on photosynthetic capacity, root/soil respiration, and allocation. Since 1992 (stand age of 6 years), fertilized stands at SETRES received nutrient amendments that are considered optimal for loblolly pine (Allen 1987). Murthy et al. (1997) reported that foliage from fertilized stands had higher photosynthetic capacities than control foliage after 2 years of nutrient additions. During approximately the same time, Albaugh et al. (1998) found that leaf area indexes (LAI) increased from 1992 (pre-fertilization) to 1996 (fourth year fertilization) in the same fertilized stands. Nine months after nutrient additions, a more rapid increase in LAI was observed in fertilized plots when compared to control stands followed by a lesser relative increase during years 3 and 4 of fertilization. Initially fine root biomass was greater in fertilized stands, but absolute and relative biomass allocation to fine roots was greater in control stands when sampled from 1993 to 1996 (Albaugh et al. 1998, King et al. 1999, Maier and Kress 2000). Coarse root biomass consistently increased in fertilized stands for all years sampled after fertilization, largely due to the greater size of fertilized trees

(King et al. 1999). Maier and Kress (2000) observed an increase in specific root respiration following fertilization, probably reflecting an increase in N uptake and assimilation.

Albaugh et al. (1998) determined that increased partitioning to leaf area in fertilized stands only partly accounted for enhanced productivity. The authors concluded that fertilization must have enhanced photosynthesis, which is consistent with findings by Murthy et al. (1997). Thus, nutrient additions may have initiated shifts in allocation to foliage concurrently with increased specific net photosynthesis shortly after fertilization, providing the resources necessary to build additional leaf area in fertilized stands. Once fine roots were reduced in fertilized stands, total root maintenance respiration costs may have leveled or even decreased. Ultimately, these changes may explain the overall increases in productivity observed by Albaugh et al. (1998), who reported a 99% increase in total biomass in fertilized stands after the fourth year of nutrient additions. Interestingly, specific net photosynthesis was generally the same in fertilized and non-fertilized stands during the eighth year (age 14) of fertilization at SETRES (Gough et al. *in press*). Perhaps after maximum increases in leaf areas were achieved in fertilized stands, photosynthesis per unit leaf area was downregulated. Collectively, these studies from a single stand, suggest that the physiological mechanisms responsible for enhanced biomass production in fertilized plots were in place when photosynthesis was measured during the eighth year of fertilization and were visible shortly after fertilization.

Objectives

Objectives of research presented in this dissertation fall into two categories that have not been fully addressed in prior published research and include: (1) identifying drivers and quantifying spatial and temporal variation in soil respiration throughout a range of environments that are characteristic of intensively managed loblolly pine ecosystems and (2) determining short-term physiological changes in above- and belowground processes following nutrient additions. Specific objectives for the four papers presented in this dissertation are as follows:

The influence of environmental, soil carbon, root, and stand characteristics on soil CO_2 efflux in loblolly pine (*Pinus taeda* L.) located on the South Carolina coastal plain (Chapter 2)

1. To link environmental, soil C, root, and stand characteristics to spatial and temporal variability in soil respiration on South Carolina coastal plain sites. More specifically:
    a. To determined the influence of soil temperature and soil moisture on soil respiration.
    b. To assess the influence of soil C, coarse woody debris, and roots sampled from directly beneath the measurement chamber on soil respiration.
    c. To examine the relationship between stand characteristics including age, productivity, and biomass on soil respiration.

Soil CO_2 efflux over a 20-year rotation in loblolly pine (*Pinus taeda* L.) stands located on the Virginia piedmont and South Carolina coastal plain (Chapter 3)

1. To determine if spatial and temporal patterns in soil CO_2 efflux differ between loblolly pine stands located on the southeastern piedmont and the coastal plain, which differ in climate, productivity, and cultural practices.
2. To develop empirical models used to predict soil CO_2 efflux from loblolly pine stands across the region.
3. To estimate total soil C efflux from loblolly pine stands over a rotation.

Short-term effects of fertilization on loblolly pine (*Pinus taeda* L.) physiology (Chapter 4)

1. To elucidate the short-term sequence of physiological changes responsible for enhanced productivity following fertilization.
2. To examine the relationship between photosynthetic capacity and foliar N concentration.
3. To identify whether altered photosynthetic capacity following fertilization parallels changes in electron transport chain capacity and/or carboxylation capacity.

An intensive examination of belowground carbon dynamics in loblolly pine (*Pinus taeda* L.) immediately following diammonium phosphate fertilization (Chapter 5)

1. To examine short-term changes in specific autotrophic and heterotrophic respiration, soil respiration, and root biomass following fertilization of loblolly pine seedlings.
2. To investigate whether fertilization has the potential to enhance soil C storage.

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CHAPTER 2

The influence of environmental, soil carbon, root, and stand characteristics on soil CO_2 efflux in loblolly pine (*Pinus taeda* L.) located on the South Carolina coastal plain

Abstract. While the effect of soil temperature and moisture on soil CO_2 efflux (E_c) has been widely investigated, the relationship between E_c and soil carbon (C), root, and stand parameters has not been comprehensively examined or quantified across extensive spatial and temporal scales. We measured E_c in loblolly pine (*Pinus taeda* L.) stands located in South Carolina across sites, seasons, and ages. Concurrent with *Ec* measurements, we monitored soil temperature (top 10 cm) and soil moisture (top 10 cm) along with mineral soil C, coarse woody debris (CWD), root surface area, and root volume in the top 20 cm of the mineral soil below the measurement chamber. We also examined the effect of stand age, stand volume, and site quality on E_c. Using linear regression analysis, we determined that E_c was most highly correlated with soil temperature alone (R^2=0.263). Mineral soil C alone explained a small, but significant amount of E_c variance (R^2=0.026). When all variables were considered simultaneously, only soil temperature (R^2=0.249), mineral soil C (R^2=0.0378), and root surface area (R^2=0.0149) explained a significant amount of variance in E_c. Other variables tested were not significantly correlated with E_c. Mineral soil C concentration was greater in samples taken directly adjacent to trees (on beds) compared with samples between rows (interbeds), which partially explained why we observed greater E_c rates next to trees. With increasing stand age, CWD decreased and root surface area increased suggesting that opposite shifts in total root and microbial respiration over time are responsible for the lack of correlation between E_c and stand age.

Introduction

Soil CO_2 efflux (E_c), which includes respiration from roots (autotrophic) and soil organisms (heterotrophic), is a key source of CO_2 from terrestrial ecosystems and an important component of the global carbon cycle (Raich and Schlesinger 1992, Raich and Tufekkcioglu 2000, Rustad et al. 2000, Schlesinger and Andrews 2000). Several factors affect E_c and the return of stored soil carbon (C) to the atmosphere including soil temperature, soil moisture, vegetation type, substrate quality (e.g. type of organic matter), net ecosystem productivity, allocation of assimilate to above- and belowground biomass, population and community interactions, and land use disturbances (Rustad et al. 2000). While the influence of major E_c drivers including soil temperature and moisture have been extensively quantified, the effect soil C and root parameters have on E_c measurements from small chamber studies has not been adequately studied (Rustad et al. 2000). Quantifying the influence of factors driving E_c will assist in resolving uncertainties regarding spatial and temporal variability in soil CO_2 efflux across small and large scales (Raich and Potter 1995). Further, enhancing current knowledge of spatial and temporal E_c patterns will allow for results from small chamber studies to be scaled-up to the landscape level with greater certainty (Rustad et al. 2000). Environmental, soil C, and root measurements paired with R_s measurements may assist in explaining variability in soil CO_2 efflux since small chamber measurements are influenced by the immediate soil environment (Maier and Kress 2000, Pangle and Seiler 2002).

E_c is affected by environmental factors and soil characteristics that are both inherent to a location and also influenced by practices common to intensive forest management. To a large extent, temporal (i.e. daily and seasonal) and spatial (i.e. latitudinal and intrasite) variation in E_c and its components is driven by differences in soil temperature and moisture (Kowalenko et al. 1978, Howard and Howard 1993, Pajari 1995, Bouma et al. 1997, Maier and Kress 2000, Pangle and Seiler 2002). However, studies are lacking that simultaneously examine the influence of soil temperature and moisture on E_c along with other factors including root and soil C characteristics across spatial and temporal scales. Although the relative influence of roots and soil C on E_c is sparsely quantified in the literature, previous investigators have shown that spatial variation in E_c on a given site is partly related to the proximity of roots to the measurement chamber in loblolly pine stands (*Pinus taeda* L.). E_c rates observed at the base of seedlings in Virginia were consistently higher than those observed between planting rows (Popescu 2001, Pangle and Seiler 2002). These results indicate that small-scale spatial variation and the contribution of roots to total E_c (near the plant) may be fairly significant, even during the seedling stage. Other evidence suggests that factors including soil C percent and coarse woody debris (CWD) content of the mineral soil influence E_c rates (Trumbore et al. 1996, Progar et al. 2000, Wang et al. 2002).

In this study, we examined the soil environment, sources of soil C, roots, and stand characteristics concurrently with E_c in loblolly pine stands in an effort to determine the extent to which these factors influence E_c. Specifically, through regression, we addressed the relative influence of soil temperature, soil moisture, root volume, root surface area, CWD, mineral soil C percent, stand age, stand volume, and site productivity in explaining variance in E_c in South Carolina coastal plain stands. Quantifying the influence of less commonly addressed soil factors including soil C, CWD, and root volume on E_c will assist researchers in defining the utility of such measurements since they are often time consuming and expensive procedures.

Methods

Study Sites

Sites were located approximately 40 kilometers northwest of Charleston, South Carolina in Berkeley County (33.18°N, 79.95°W) on MeadWestvaco Corporation land located on upper coastal plain flats. Stands ranged in age from 1 to 24-years-old at the beginning of the study. The average annual temperature in Berkeley County is 17.7°C, with an average maximum of 28.2°C and an average minimum of 9.89°C. Average annual rainfall is 125 cm. Flooding is relatively common; however, severe drought frequently occurs during the summer and fall seasons as well (SCSCO 2000). Precipitation for the months coinciding with measurements was on average 15.5% lower than the 30-year mean (SRCC). Elevation ranges from 1.5 to 4.6 m above sea level with mild slopes of less than 2%. Soil parent material is generally Wicomico or Penholoway backberrier flats, former shoreline, or offshore deposits. Soils are generally acidic and low in phosphorus. Common soil series and taxonomic classifications for each block are: 1) Coxville series: fine, kaolinitic, thermic Typic Paleaquults, 2) Rains series: fine-loamy, siliceous, semiactive, thermic Typic Palequults, 3) Bonneau series: loamy, siliceous, thermic Arenic Paleudults, and 4) Lynchburg series: fine-loam, siliceous, semiactive, thermic Aeric Paleaquults. Inter-beds are frequently submerged during the cooler, wetter winter months. Site indices range from 20.0 to 22.3 meters at 25 years for loblolly pine. All sites were bedded prior to planting. The native forest cover type is a loblolly pine-hardwood mix.

Study Design

Four replications consisting of four age classes were selected from existing loblolly pine stands managed by MeadWestvaco. Stands were grouped according to the following age classes: 1, 4 to 6, 8 to 12, and 18 to 25 years-old since planting (at beginning of the data collection). Each block, containing one stand from each age class, had similar soil and drainage characteristics and were in close proximity (<1 km) to each other. However, stands within a block were not necessarily adjacent. Stand were accessed by road and all measurements were taken beyond the edge of the stand. Within each study plot, measurements described below were taken near the base of the tree and between rows (two measurement positions) in order to account for spatial variability described by Pangle and Seiler (2002). Measurements began in August 2001 and continued bimonthly through the following August. An additional measurement date in January 2003 was added in order to cover the range in temperature variability that is representative of the study location. A total of 32 measurements (on each parameter described below) were collected on a sampling date (4 replications x 4 age classes x 2 measurement positions). The resulting dataset contained 256 measurements of each variable over the course of the study.

Soil CO_2 Efflux Measurements

Soil CO_2 efflux was measured using the LiCor 6200 infrared gas analyzer (IRGA) (LiCor Inc., Lincoln, Nebraska) and a dynamic closed cuvette chamber system (Janssens et al. 2000). Measurements were taken on the surface of the forest floor where living plant material was not present. This was an effort to eliminate CO_2 efflux detection from aboveground plant tissues and respiring senescent tissue in the cuvette. The chamber was constructed from a 20.3-cm internal diameter PVC end cap assembled with a foam gasket around the base to provide a seal with the ground. The chamber height at the center was approximately 10 cm. A gas sampling line and a return port (from the LiCor) was attached to the chamber in order to provide both a gas input and output from the chamber to the IRGA. The internal volume of the chamber was 4105 cm^3 and the LiCor was calibrated accordingly. Soil CO_2 efflux rates were determined by measuring CO_2 evolution over a 30-s period and calculating the respiration rate per unit land area from the following equation:

$$E_c = [(\Delta C/\Delta t)(PV_t/RT)] \text{ / soil surface area covered by chamber}$$

Where $C = [CO_2]$, t = time, P = atmospheric pressure, V_t = system volume, R = universal gas constant, and T = temperature.

Soil Temperature and Moisture Measurements

Soil temperature and moisture were determined at each soil CO_2 efflux measurement location. Soil temperature at 10 cm was measured at each location using a Digi-sense temperature gauge (model no. 8528-20, Cole-Parmer Instrument Co., Niles, Illinois). Volumetric soil moisture was determined to a depth of 10 cm using a time domain reflectometer (Soil Moisture Equipment Corporation, 6050X1, Golena, CA).

Soil Excavation

After soil CO_2 efflux, temperature, and moisture measurements were completed at a location, a cylindrical corer with a 10 cm diameter by 20 cm depth was used to extract a 0.0157 m^3 soil sample from beneath the measurement location in order to evaluate soil parameters. The O layer was removed prior to the excavation of the mineral soil and associated roots.

Laboratory Analysis

Soil samples were sifted through a 6.4 mm screen to separate soil from live roots and CWD. No attempt was made to separate pine from non-pine roots. A subsample of soil was collected from each soil sample after manual homogenization. Soil subsamples were oven-dried at 65°C for 48 hours and sifted through a 2 mm sieve to remove coarser organic matter. Samples were analyzed for C percent by the USDA Forest Service Southern Research Station laboratory (RTP, NC) using a Carlo-Erba elemental analyzer (Model NA 1500, Fison Instruments, Danvers, MA). Live root surface area and root volume were determined using the WinRhizo 5.0A software (Regent Instruments Inc., Quebec, Canada). CWD was oven-dried at 65°C for 48 hours, weighed in the laboratory, and then ashed in a muffle furnace (Sybron/Thermolyne F-A1740, Debuque, IA) at 500°C for 24 hours. The ash weight was subtracted from the pre-ashed mass in order to correct for mineral content.

Aboveground Biomass Estimates

When CO_2 efflux measurements were completed (January 2003), standing stem volumes of the three oldest age classes were estimated in each plot based on a 1/50 hectare sampling of tree DBH and height using the following volume equation for loblolly pine:

$$\text{Stem Volume (cubic feet)} = 0.21949 + 0.00238D^2H$$

where D = diameter at breast height in inches and H = total tree height in feet (Tasissa et al. 1997). All volume estimates were converted to metric units. Seedling volume in 1-year-old stands was determined by multiplying ground-line diameter squared by seedling height.

Statistical Analysis

Simple and multiple linear regression analyses was used to assess the statistical relationship between E_c and potential explanatory variables including soil temperature, soil moisture, root volume, root surface area, CWD, coarse fragments, mineral soil C percent, stand age, and measurement position. Multiple linear regression analysis was performed using the SAS stepwise procedure (SAS Inst., Cary, NC) in order to determine the primary variables driving soil CO_2 efflux in the context of all possible variables. Simple regression analysis was also used to examine the relationship between E_c and parameters individually. Variables were transformed when necessary in order to allow for the best fit with the data. Standardized residuals and normality plots were examined and adjustments were made to minimize bias in models. All statistical analyses were performed using PROC REG in SAS.

Results

Soil Microclimate and Soil CO_2 Efflux

Simple linear regression indicated a relatively strong positive relationship between E_c and soil temperature ($R^2=0.262$, $P<0.0001$), while no significant relationship existed between E_c and the range of soil moistures observed on our sites ($R^2=0.009$, $P=0.1512$; Figure 2.1 A,B).

Soil Carbon, Coarse Woody Debris, Roots and Soil CO2 efflux

Root surface area ($R^2=0.010$, $P=0.1650$) and root volume ($R^2=0.006$, $P=0.3014$) in the top 20 cm of the mineral soil directly below the E_c chamber was not significantly related to E_c (Figure 2.1 C,D). There was no relationship between E_c and CWD in the top 20 cm of mineral soil ($R^2=0.006$, $P=0.2669$; Figure 2.1 E,F). However, a weak positive relationship existed between E_c and percent soil C in the top 20 cm of the mineral soil on our sites ($R^2=0.026$, $P=0.0161$).

Stand Characteristics and Soil CO_2 efflux

Stand age ($R^2=0.000$, $P=0.9965$), stand volume ($R^2=0.006$, $P=0.9179$), and site index ($R^2=0.004$, $P=0.3392$) were not significantly correlated with E_c on our sites (Figure 2.2).

Ranking Variables Simultaneously

Using the stepwise selection process in SAS, we tested all variables in an effort to determine the amount of variance in E_c explained by variables simultaneously. Results acquired from the stepwise procedure were similar to those obtained using simple linear regression. Temperature explained a majority of the variance in E_c and was positively related to E_c (partial $R^2=0.249$, $P<0.0001$). Soil C percent was weakly positively related to E_c in the context of other variables (partial $R^2=0.038$, $P=0.0017$). Unlike our simple linear regression results, root surface area was weakly positively related to E_c in the stepwise selection ($R^2=0.015$, $P=0.0450$). The additional significance of root surface area is probably due to the reduction in error when variance in E_c due to soil temperature and soil C is taken into account. Also, we tested whether accounting for variance in E_c due to temperature would change the significance of other potential explanatory variables by regressing both temperature and each variable individually against E_c. No additional variables became significant when variance in E_c due to soil temperature was removed.

Root Characteristics, Carbon and Spatial Variation

We observed a weak relationship between measurement location and E_c ($R^2=0.017$, $P=0.0564$). E_c rates were higher near the base of the tree in comparison to away from the tree. In order to test whether or not roots, CWD, and/or C percent in the top 20 cm below the measurement chamber explains spatial differences in E_c, we used simple linear regression to examine the relationship between measurement location and root volume, root surface area, CWD, and C percent. Root volume, root surface area, and CWD were not significantly correlated with measurement position ($P<0.05$). In contrast, simple linear regression analysis demonstrated that soil C was significantly greater in soil samples taken near the base trees on the beds compared with samples between rows on the interbeds ($R^2=0.043$, $P=0.0020$). No position x age interactions were significant ($P>0.05$).

Root Characteristics, Carbon and Stand Age

Since fluctuating relative contributions of autotrophic respiration and heterotrophic respiration over time may partially explain the lack of any trend between E_c and stand age, we investigated changes in the mineral soil components over stand ages that likely parallel trends in autotrophic and heterotrophic respiration. Specifically, we analyzed trends in root surface area and CWD from the upper 20 cm of mineral soil across stand ages (Figure 2.3). Root surface area density was positively related (R^2=0.2127, P<0.0001) to stand age while CWD was negatively correlated with stand age (R^2=0.1577, P<0.0001). No position x age interactions were significant (P>0.05).

Discussion

Our results indicate that soil temperature by far explains the greatest amount of variance in E_c observed within and across sites and over seasons on the South Carolina coastal plain (R^2=0.263, P<0.0001). Soil moisture was not a significant E_c driver despite ranging from 2 to nearly 33% soil moisture. Soil C percent, CWD, root volume, and root surface area in the top 20 cm of the soil explained little or no significant amount of variance in E_c. Soil C percent alone explained only 2.6% of the variance in E_c within and across plots. When simultaneously considered, soil temperature, soil C percent, and root surface area explain 24.9, 3.78, and 1.49% of the variance in E_c on our sites.

Our findings are consistent with previous reports that cite a strong relationship between soil temperature and E_c (Kowalenko et al. 1978, Howard and Howard 1993, Pajari 1995, Bouma et al. 1997, Maier and Kress 2000, Pangle and Seiler 2002). Soil moisture did not explain a significant amount of variance on our sites, but we may not have observed E_c above and below critical moisture levels. Frequently, soil is saturated on the coastal plain, which would prevent aerobic activity from microbes. However, we observed very little water between beds probably because average precipitation was 15.5% less than normal during the measurement months. Therefore, soil moisture on our sites may not have been representative of a typical year. Generally, soil moisture limits E_c at either extremely high or low levels (Kowalenko et al. 1978, Howard and Howard 1993, Bouma et al. 1997, Pangle and Seiler 2002).

Respiring roots directly below the measurement chamber should exert a significant influence on E_c since CO_2 efflux from roots comprises 10 to 90% of the total soil CO_2 efflux in forests (Hanson et al. 2000). However, our results indicate that root surface area and root volume directly below the chamber are weakly or not at all related to E_c. While these results are somewhat surprising, Pangle and Seiler (2002) reported only a minor influence of root biomass on E_c in a young loblolly pine stand. Specifically, they found fine root biomass explained 2.52% of the variance in E_c across a single site on one measurement date, attributing the minor influence of roots to non-vertical flow of CO_2 through the soil profile. CO_2 originating from root respiration will move along the path of least resistance, which may be vertical or lateral. Likewise, roots in the profile that are adjacent to the sampled area beneath the measurement chamber will impact E_c measurements when CO_2 moves laterally in the soil. The degree of lateral movement of CO_2 in soil depends on soil physical properties such as soil texture, strength, pore space, and tortuosity (Weerts et al. 2001, Susfalk et al. 2002).

Similarly, mineral soil C was weakly related to E_c, while CWD was not significantly correlated with E_c. Mineral soil C and CWD represent potential C substrate sources for microbes and should accordingly affect microbial activity (Trumbore et al. 1996, Progar et al. 2000, Wang et al. 2002). However, soil C and CWD in the top 20 cm of the soil below the measurement area may not be a good indicator of E_c for two reasons. First, as previously discussed with roots, CO_2 originating from microbial respiration may not move only vertically in the profile. Second, soil C quality may be equally important to quality when relating C to microbial activity (Giardina and Ryan 2000). Russell and Voroney (1998) reported that less than 2% of the observed variance in E_c in a boreal aspen forest (*Populus tremuloides*) was explained by soil organic matter (SOM) quantity sampled directly below measurement chambers. They conclude that SOM had little influence on E_c; however, they did not address issues of substrate quality or availability either. Pangle and Seiler (2002) found no relationship between E_c and mineral soil C sampled directly below their measurement chamber in a loblolly pine stand on a single day. Our results along with others cited above indicate that uncertainties regarding soil physical and chemical properties limit the value of soil sampling directly below the measurement cuvette using our methodology.

The fact that E_c was not related to stand age, stand volume, or site index implies that the accumulation of respiring root biomass over time was offset by reductions in microbial respiration on our sites. The effect of stand age on E_c has been shown to be inconsistent (Ewel et al. 1987, Klopatek 2002, Pyker and Fredeen 2003). However, E_c generally increases with age and stand volume, presumably due to greater respiring root biomass. Our observations are not typical among other reports in the literature, stressing the need for studies addressing autotrophic and heterotrophic shifts over time. Further, the inconsistent relationship between stand age, stand volume, and E_c reported in the literature may be the result of shifts in autotrophic and heterotrophic components guided by different management regimes. Multiple management activities have been shown to influence both root and microbial biomass and activity (Edwards and Ross-Todd 1983, Londo et al. 1999, Lee et al. 2002, Mallik and Hu 1997).

Root and CWD data from our study support the proposal that changes in autotrophic and heterotrophic respiration over time may explain why we did not observe a stand age or stand volume effect on E_c. Root surface area must have increased over time in our stands (Figure 2.3), resulting in enhanced autotrophic respiration over time (Ewel et al. 1987). At the same time, the declining amount of CWD in the mineral soil with increasing stand age (Figure 2.3) may parallel microbial activity since CWD may serve as a C substrate pool for microbes (Progar et al. 2000, Wang et al. 2002, Davis et al. 2003). Together, these data suggest that autotrophic and heterotrophic contributions to E_c shift inversely over the course of a loblolly pine rotation on the South Carolina coastal plain, which would explain the weak relationship between E_c and stand age. Other investigators concluded that inverse shifts in autotrophic and heterotrophic respiration prevented them from detecting a change in E_c over time (Edwards and Ross-Todd 1983, Toland and Zak 1994).

Spatial differences in E_c may be partly due to variability in the distribution of roots and C across and within sites. Similar to our findings, Pangle and Seiler (2002) reported a relationship between measurement position and E_c, citing higher rates near the base of trees in comparison to rates between rows. While the influence of roots near the base of the tree may partly explain the spatial variability of E_c observed in stands, we found no significant difference between root volume or root surface area and measurement position within a stand. However, as previously discussed, sampling soil directly below the measurement chamber may not adequately capture all roots contributing to the observed soil CO_2 efflux. The fact that we observed an effect due to measurement position may in fact be due to the greater influence of root respiration near the base of a tree; however, our sampling failed to fully capture influential roots if this is the case. For example, the influence of the taproot and major lateral roots were not accounted for in our cores. We also observed no spatial difference in CWD density. The distribution of CWD in the mineral soil, while highly variable across sites, did not vary between locations on the bed and between the bed.

In contrast, we observed a spatial pattern in relation to mineral soil content on our sites. Mineral soil C was significantly greater on the beds near trees in comparison to between rows on the interbeds. The fact that mineral soil C in the top 20 cm below our measurement location is a weak, yet significant E_c driver on our sites and the observation that measurement location affects E_c on our sites suggests that differences in mineral soil C are partly responsible for the spatial variation we observed. Higher mineral soil C near the base of trees may be the combined result of greater root exudates and root turnover near the tree base, which both contribute to the belowground C pool in loblolly pine forests (Andrews et al. 1999, Luan et al. 1999). However, our data do not suggest differences in root-related C additions to the mineral soil are responsible for higher mineral soil C near trees since we did not observe greater root volumes in soil samples taken near the tree base. A more likely explanation is that greater C concentrations in mineral soil sampled next to trees is due to site preparation utilized on the study sites. All of our sites were bedded, which incorporates residual C and slash (i.e. CWD) closer to the trees. Perhaps CWD incorporated into beds decomposes quickly (i.e. prior to planting), resulting in a greater pool of mineral soil C, which serves as an accessible substrate for microbes. CWD is an important input into the forest soil C pool in some forest systems, and decomposition rates have been shown to vary depending on management and environmental influences (Trumbore et al. 1996, Progar et al. 2000, Wang et al. 2002, Davis et al. 2003).

Overall, our results indicate that soil temperature is the best predictor of E_c across seasons. Additional information concerning soil C, CWD, root volume, and root surface area 20 cm below the measurement chamber explains a negligible amount of additional variance in E_c across time and space, at least on our sites. Further, stand parameters including stand age, stand volume, and site index were not useful in explaining variability in E_c probably due to opposite shifts in autotrophic and heterotrophic respiration.

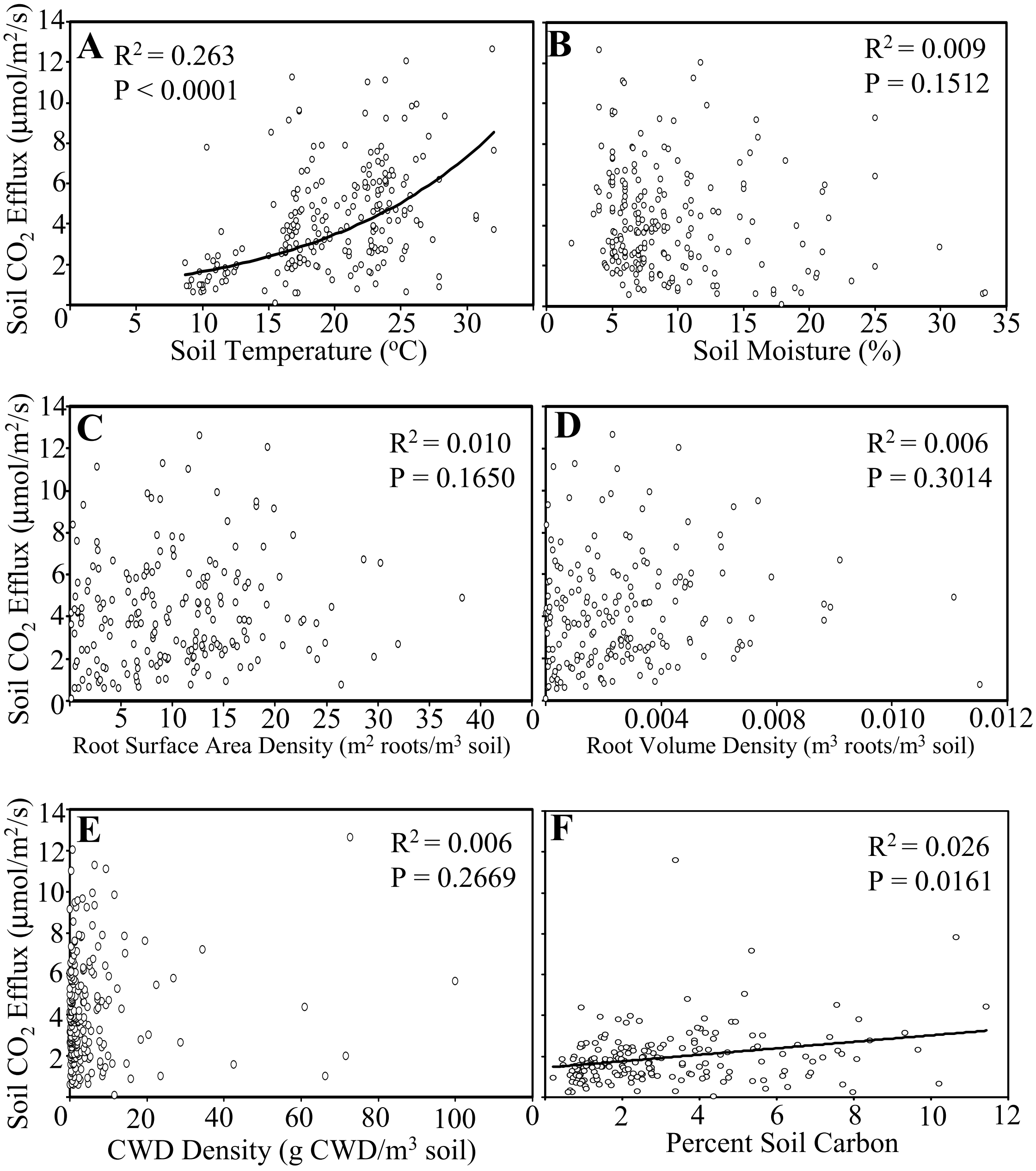

Figure 2.1. The relationship between soil CO_2 efflux and soil temperature (top 10 cm; A), soil moisture (top 10 cm; B), root surface area density (top 20 cm; C), root volume density (top 20 cm; D), CWD density (top 20 cm; E) and percent soil carbon (top 20 cm; F) on the South Carolina coastal plain. Data were collected concurrently with soil CO_2 efflux measurements. A 10-cm diameter soil core was used to extract the top 20 cm of mineral soil directly below the soil CO_2 measurement chamber. Trend lines shown only when the relationship is significant ($P<0.05$).

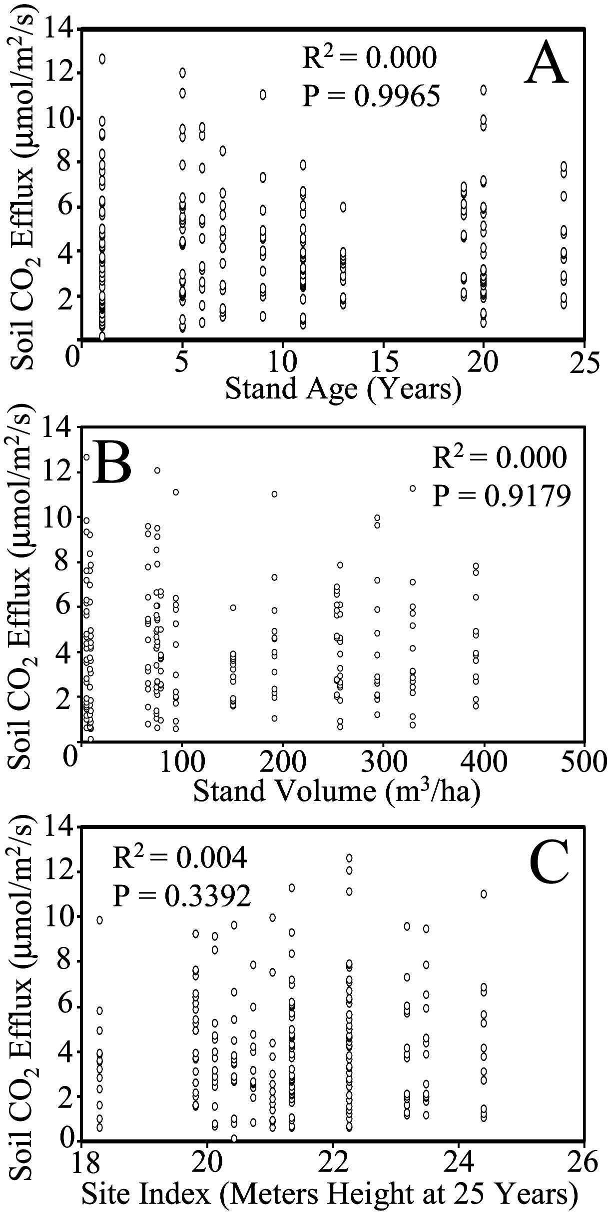

Figure 2.2. The relationship between soil CO_2 efflux and stand age (A), stand volume (B), and site index (C) in loblolly pine stands sampled on the South Carolina coastal plain.

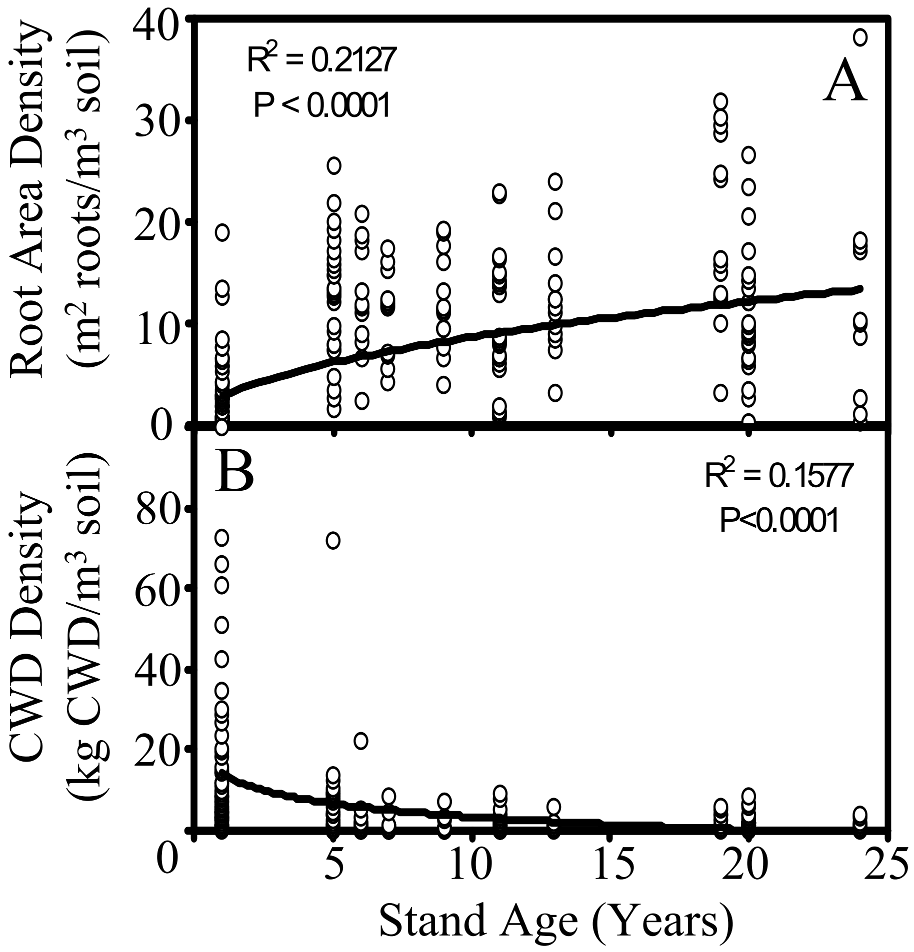

Figure 2.3. Relationship between stand age and root area density (A), and stand age and CWD density (B) in South Carolina coastal plain loblolly stands. Roots and CWD were sampled concurrently with soil respiration measurements in the top 20 cm of the mineral soil.

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CHAPTER 3

Soil CO_2 efflux over a 20-year rotation in loblolly pine (*Pinus taeda* L.) stands located on the Virginia piedmont and South Carolina coastal plain

Abstract. We measured soil CO_2 efflux (E_c) from loblolly pine stands (*Pinus taeda* L.) located on the Virginia piedmont and South Carolina coastal plain in an effort to quantify soil carbon (C) loss on sites differing in climate, productivity, and cultural practices. Patterns in E_c over a short rotation were examined using a replicated chronosequence approach. E_c was measured using a closed dynamic chamber over an entire year in four separate age classes at both measurement locations in order to capture spatial and temporal variation in E_c over a 20+ year rotation. Within each stand, spatial variability was also accounted for by taking measurements both near the base of the tree and between rows. We found that soil temperature (top 10 cm) was the major driver of E_c on both locations. We also observed a positive age effect on E_c in Virginia piedmont stands and no relationship between age and E_c on the South Carolina coastal plain stands. We estimate a total efflux rate of 278.6 Mg C/ha over a 20-year rotation for South Carolina stands and 210.9 Mg C/ha over the same time period in Virginia stands. Over a 20-year period, annual C efflux is expected to decline with stand age in South Carolina due to reductions in soil temperatures as crown closure occurs. Annual C efflux is expected to increase with age in Virginia stands because of the positive relationship between stand age and E_c we observed, which is appears to be directly related to an increase in the contribution of autotrophic respiration to total E_c over time.

Introduction

Recent trends in global warming may be due to accumulating atmospheric greenhouse gases, including CO_2. The international Kyoto Protocol outlined a plan in which developed nations would be required to reduce greenhouse gas emissions or enhance greenhouse gas sinks in order to reduce net greenhouse gas production (Rotter and Danish 2000). Although the Kyoto Protocol was not signed by the United States, it is likely that future measures will be taken to reduce net greenhouse emissions since, at the current rate, atmospheric CO_2 concentrations are projected to double by the middle of this century (Allen 1994). The Kyoto Protocol introduced the concept of "carbon credits" (i.e. a quantifiable C sink) for practices that mitigate atmosphere CO_2 accumulation. Soil C may account for over 70% of the stored C in a forest ecosystem (Schlesinger and Andrews 2000), making a quantitative understanding of soil C efflux imperative to our understanding of net ecosystem C sequestration. For these reasons, and because intensively managed forests may store more soil C than natural forests (Valentini et al. 2000), there is increasing interest in understanding how forestry activities affect C dynamics of not only individual stands, but also an entire region (Rotter and Danish 2000). An example is *The Chicago Climate Exchange* is a pilot program, which has the goal of implementing a feasible market-based C trading system (Walsh 2003). The program currently lists several members from forest industry and continues to grow. The success of programs such as *the Chicago Climate Exchange* and future implementation of large-scale, possibly international, C trading relies on quantitative information regarding the impact of forest management has on C sequestration capacity.

Intensively managed loblolly pine (*Pinus taeda* L.) stands account for over 13 million hectares of forested land in the southeast (Wear and Greis 2002). Management practices in the southeast may play a significant role in global greenhouse gas levels by influencing the development of terrestrial ecosystems that are either a net sink or source of CO_2. Intensively managed loblolly pine stands may serve as an effective sink earlier in a rotation when growth is enhanced through various silvicultural practices (Gladstone and Ledig 1990, Maier and Kress 2000). However, within any given management scenario, stand C dynamics vary spatially, temporally and with genotype. Empirical quantitative relationships between environmental data and soil CO_2 efflux data from across the region are necessary in order to provide critical information that will be used by scientists, forest managers, and politicians to predict stand-level and regional C dynamics.

Soil CO_2 efflux (E_c) or soil respiration includes respiration from roots (autotrophic respiration), and soil microorganisms (heterotrophic respiration). E_c is a key source of CO_2 in terrestrial ecosystems and an important component of the global C cycle (Raich and Schlesinger 1992, Raich and Tufekkcioglu 2000, Rustad et al. 2000, Schlesinger and Andrews 2000). E_c is affected by several environmental factors that are both inherent to a given site and also influenced by practices common to intensive loblolly pine management. To a large extent, temporal (i.e. seasonal) and spatial (i.e. latitudinal and intersite) variation in E_c and its components is driven by differences in soil temperature and moisture. Numerous studies have discussed the high correlation between E_c and temperature (Kowalenko et al. 1978, Howard and Howard 1993, Pajari 1995, Bouma et al. 1997, Maier and Kress 2000, Pangle and Seiler 2002). Soil moisture can also affect respiration rates, especially under droughty conditions (Kowalenko et al. 1978, Howard and Howard 1993, Bouma et al. 1997). Maier and Kress (2000) and Pangle and Seiler (2002) determined that soil water status significantly limited E_c rates in loblolly pine stands when soil moisture was low.

Researchers have cited a continued need for better understanding and quantifying the role forest management may play in soil C turnover over a range of stand ages (Woodwell et al. 1983, Turner et al. 1995, Field and Fung 1999, Banfield et al. 2002, Liski et al. 2002). The literature provides no consensus concerning the effect of forest management on E_c. Previous investigators observed an increase (Gordon et al. 1987, Lytle and Cronan 1998, Londo et al. 1999), decrease (Striegl and Wickland 1998), or no change (Edwards and Ross-Todd 1983, Fernandez et al. 1993, Toland and Zak 1994) in E_c rates following clear-cut harvesting. Differences among ecosystems and experimental designs may account for inconsistencies in literature; however, the distinct differences found among investigators support the idea that generalizations across all forest types are not appropriate when developing C budgets. Frequently, E_c and soil C turnover have been shown to increase with harvest intensity (Edwards and Ross-Todd 1983, Londo et al. 1999, Lee et al. 2002). Also, Mallik and Hu (1997) showed that E_c corresponded to the amount of organic matter incorporated into the soil during site preparation in boreal mixedwood forest. Further, prescribed burning, which is a common tool in loblolly pine management, may impact E_c by altering the quality and quantity of C substrate available to microorganisms and by changing the soil microclimate (Fritze et al. 1993, Pietikainen and Fritze 1993, Pietikainen and Fritze 1995, Hernandez et al. 1997). Stand age may also impact E_c rates and therefore is an important consideration when developing C budgets for forest stands (Ewel et al. 1987a, Klopatek 2002, Pyker and Fredeen 2003).

Across the southeast, loblolly pine is managed on sites varying in productivity, climate, and common cultural practices. Therefore, it is crucial to investigate multiple locations within the region in an effort to accurately quantify soil CO_2 efflux across the region. We present the trends in E_c observed on the Virginia piedmont and the South Carolina coastal plain across a range of stand age classes. We also present an estimation of yearly total E_c for each study location over the course of a 20-year rotation. Our objectives included the quantification of spatial and temporal variation in E_c over a range of environments that are characteristic of intensively managed loblolly pine. We hypothesized that E_c would be highly temperature and age related on both study locations and that major differences in E_c between locations would be due to climate differences.

Methods

Study Sites

The South Carolina coastal plain study sites are located approximately 40 kilometers northwest of Charleston, South Carolina in Berkeley County (33.18°N, 79.95°W) on MeadWestvaco Corporation land. Stands ranged in age from 1 to 23 years old at the beginning of the study (Table 3.1). The average annual temperature in Berkeley County is 17.7°C, with an average maximum of 28.2°C and an average minimum of 9.89°C. Average annual rainfall is 125 cm. Flooding is relatively common; however, severe droughts frequently occur during the summer and fall seasons. Precipitation for the months coinciding with measurements was on average 15.5% lower than the 30-year average (SRCC). Elevation ranges from 1.5 to 4.6 m above sea level with mild slopes of less than 2%. Inter-beds are frequently submerged during the cooler, wetter winter months. Soil parent material is generally Wicomico or Penholoway backberrier flats, former shoreline, or offshore deposits. Soils are generally deep to very deep, acidic, and low in phosphorus. Common soil series and taxonomic classifications for soil from each block are: 1) Coxville series: fine, kaolinitic, thermic Typic Paleaquults, 2) Rains series: fine-loamy, siliceous, semiactive, thermic Typic Palequults, 3) Bonneau series: loamy, siliceous, thermic Arenic Paleudults, and 4) Lynchburg series: fine-loam, siliceous, semiactive, thermic Aeric Paleaquults. All sites were bedded prior to planting. Site indices range from 20.0 to 22.3 meters at 25 years for loblolly pine. The native forest cover type is a loblolly pine-hardwood mix (MeadWestvaco Corporation, unpublished data).

Virginia piedmont sites were located on MeadWestvaco Corporation property in Buckingham County, Virginia (37.34°N, 78.26°W). Stands chosen for investigation ranged from 1 to 25 years of age at the beginning of the study (Table 3.1). Average annual precipitation for this region is 107 cm. Precipitation during the measurement months on the Virginia sites averaged 9.0% lower than the 30-year average (SRCC). Elevation ranges from approximately 40 to 55 m above sea level with broad ridges and moderate slopes ranging from 5% to 25%. The average annual temperature is 13°C, with an average maximum of 23.9°C and an average minimum of 1.98°C. In general, soil parent material is derived from stratifed, metasedimentary bedrock of the western piedmont geologic formation. Soils are moderately well-drained to well-drained and deep. Major soil series and associated taxonomic descriptions found on the study sites include: 1) Cecil series: fine, kaolinitic, thermic Typic Kanhapludult (moderately well-drained), and 2) Appling series: Fine, kaolinitic, thermic Typic Kanhapludult (well-drained). The Virginia sites were not blocked by soil type as they were in South Carolina since soils were similar in the area. Site indexes range from 15.8 to 18.4 meters at 25 years for loblolly pine. Site preparation prior to planting involved varying intensities of broadcast burning, chopping, and raking (MeadWestvaco Corporation, unpublished data).

Study Design

Four replications of four age classes were selected at each study location (South Carolina and Virginia) from existing loblolly pine stands managed by MeadWestvaco. Stands were grouped according to the following age classes: 1, 4 to 6, 8 to 12, and 18 to

25 years-old since planting (at beginning of the data collection). Replications had similar soil and drainage characteristics and were in close proximity (<1 km) to each other. Plots within replications were not necessarily adjacent. All stands were accessable via roads and efforts were made to take measurements beyond the edge of the stand in order to avoid potential edge effects. Within each study plot, E_c, temperature, and moisture measurements described below were taken near the base of the tree and between rows (two measurement positions) in order to account for spatial variability within a site described by Pangle and Seiler (2002). Three sets of measurements, considered subsamples, were taken at each replication x age class x measurement position. Subsamples were averaged for analysis. Measurements began in April 2000 on the Virginia location and continued monthly through March 2001. Measurements in South Carolina began in August 2001 and continued bimonthly through the following August. An additional South Carolina measurement date in January 2003 was added in order to cover the range in temperature variability that is representative of the study location. A total of 96 measurements (on each parameter described below) was collected on a sampling date (4 replications x 4 age classes x 2 measurement positions x 3 subsamples). The resulting datasets for the South Carolina and Virginia locations include 768 and 1152 paired measurements, respectively. Our sampling allowed us to capture variability due to location, season, temperature, moisture, stand age, and measurement position within a stand. Data from Virginia sites were provided by Wiseman (2001).

Soil CO_2 efflux Measurements

E_c was measured using the LiCor 6200 infrared gas analyzer (IRGA) (LiCor Inc., Lincoln, Nebraska) and a dynamic closed cuvette chamber system (Janssens et al. 2000). Measurements were taken on the surface of the forest floor where living plant material was not present. This was an effort to eliminate the measurement of CO_2 efflux from aboveground plant tissues and respiring senescent tissue. The chamber was constructed from a 20.3 cm internal diameter PVC end cap assembled with a foam gasket around the base to provide a seal with the ground. The chamber height at the center was approximately 10 cm. A gas sampling line and a return port (from the LiCor) was attached to the chamber in order to provide both a gas input and output from the chamber to the IRGA. The internal volume of the chamber was 4105 cm^3 with an area of 368 cm^2. The LiCor was calibrated to a CO_2 standard and zeroed prior to measurements. E_c rates were determined by measuring CO_2 evolution over a 30 s period and calculating the respiration rate per unit land area from the following equation:

$$E_c = [(\Delta C/\Delta t)(PV_t/RT)] \ / \ \text{surface area of soil}$$

Where C = [CO_2], t = time, P = atmospheric pressure, V_t = system volume, R = universal gas constant, and T = temperature.

Soil Temperature and Moisture Measurements

Soil temperature and moisture were determined at each E_c measurement location. Soil temperature at 10 cm was measured at each location using a Digi-sense temperature gauge (model no. 8528-20, Cole-Parmer Instrument Co., Niles, Illinois). Volumetric soil moisture was determined to a depth of 10 cm using a time domain reflectometer (Soil Moisture Equipment Corporation, 6050X1, Golena, CA).

Soil Excavation

In South Carolina a cylindrical corer was used to extract a 0.0157 m^3 soil sample from beneath the measurement location on each measurement date in order to evaluate soil parameters after E_c, temperature, and moisture measurements were completed. The O layer was removed prior to the excavation of the mineral soil and associated roots to a 20 cm depth. In Virginia, a 0.003814 m^3 soil sample was collected below the measurement area following E_c, temperature, and moisture measurements only on the last measurement date. Mineral soil and roots were excavated to a depth of 10.2 cm.

Laboratory Analysis

Soil samples were sifted through a 6.4 mm screen to separate soil from live roots and coarse woody debris (CWD). No attempt was made to separate pine from non-pine roots. Live roots surface area and root volume were determined using the WinRhizo 5.0A software (Regent Instruments Inc., Quebec, Canada). CWD was oven-dried at 65°C for 48 hours, weighed, and then ashed in a muffle furnace (Sybron/Thermolyne F-A1740, Debuque, IA) at 500°C for 24 hours. The ash weight was subtracted from the pre-ashed mass in order to correct for mineral content.

Statistical Analysis

Multiple linear regression analyses were used to assess the statistical relationship between E_c and candidate explanatory variables including soil temperature, soil moisture, stand age, site index, and measurement position. Initially, full models were developed that incorporated datasets from both the South Carolina and Virginia locations. Once a full model containing significant variables ($P<0.05$) was produced, we developed common reduced models for the South Carolina coastal plain and the Virginia piedmont locations, which contained the same parameters as the full model, but differed in coefficient values. Coefficients for each parameter were compared statistically between study locations to determine whether a full model or reduced model was statistically and biologically more appropriate. A full model is appropriate when the response of E_c to driving variables is statistically identical on both study locations. A reduced model is used when one or more driving variables influence E_c differently.

Regression analysis was initially performed using the SAS stepwise procedure (SAS Inst., Cary, NC) in order to determine the primary variables driving E_c. Standardized residuals and normality plots were examined and adjustments were made to minimize bias in models. Once primary variables were identified, a full model containing five variables was developed that explained the greatest variance in E_c for the South Carolina and Virginia study locations combined. A simpler full model was also constructed that marginally reduced the explained variance in E_c, but reduced the explanatory variable inputs from five to two. Following the development of a full model, reduced models were constructed. Parameter coefficients were compared statistically using indicator variables to determine whether a single full model or two reduced models for each study location were appropriate. All statistical analyses were performed using PROC REG in SAS.

20-Year Rotation Soil CO_2 Efflux Estimates

Yearly cumulative soil C efflux over a 20-year rotation was estimated for the South Carolina and Virginia study locations. E_c was scaled up using our simpler, two variable common regression model developed for each study location, which requires soil temperature and stand age inputs. Since we did not continuously monitor soil temperature on our study sites, we developed regression models for each study location in an effort to predict soil temperature based on air temperature and stand age. To do this, we obtained daily air temperature averages acquired from onsite meteorological stations at each study site location. Daily temperature averages were regressed against average soil temperature data collected on the same measurement date. Since canopy cover alters soil temperature, stand age was also included in the model. Air temperature and stand age explained 94 and 95% of the variability in soil temperature at 10 cm in the South Carolina and Virginia study sites, respectively (Figure 3.1). The following common model allowed us to predict soil temperature across a range of air temperatures and stand ages:

South Carolina: $T_s = -0.153 + 1.04T_a + 0.196A - 0.103\ (T_a{*}\ln A) + \varepsilon_i$

Virginia: $T_s = 1.58 + 0.988T_a + 0.0784A - 0.0812\ (T_a{*}\ln A) + \varepsilon_i$

Where T_s = estimated soil temperature at 10 cm, T_a = average daily air temperature, and A = stand age.

In order to scale up soil C efflux to the stand level, we obtained daily temperature averages (NOAA) for the cities of Charleston, SC, and Farmville, VA, which house meteorological stations closest to our study locations. We averaged daily air temperatures for a given Julian day from 1991 to 2001 for the two locations. Next, daily air temperature averages in combination with stand ages were used to estimate daily soil temperature at 10 cm over a 20-year period for each study location. Daily soil temperature averages estimated for each study location were then input into the simple two variable E_c predictive model along with stand age in order to estimate average daily E_c rates over 20 years. Estimated daily E_c rates were used to calculate cumulative E_c on a daily time step for a 20-year rotation. Daily E_c was summed over a year. In order to account for compounding error due to the estimation of soil temperature, standard error of E_c estimations was calculated by taking into account the variance in the soil temperature estimate using the following model:

$$E_c = B_0 + B_1(T_s - a_i) + B_2[\ln(T_s - a_i)] + B_3[\ln A(T_s - a_i)] + \varepsilon_i$$

Where E_c = estimate of soil CO_2 efflux, T_s = estimated soil temperature at 7 cm, A = stand age, a_i = the error of the soil temperature estimate, and ε_i = the error of the E_c estimate alone (Montgomery et al. 2001). All estimations of soil temperature and E_c, and associated standard errors were calculated using SAS. Annual E_c estimates were expressed in terms of C rather than CO_2.

Results

Ranges in Soil CO_2 Efflux, Soil Temperature, and Soil Moisture

While our primary means of analysis in this report is linear regression, we calculated the mean E_c, temperature, and moisture values on each measurement day by the different age classes to demonstrate the range in data collected. E_c rates on the South Carolina coastal plain ranged from a mean high of 8.5 μmol/m^2/s in 1-year-old stands on the August 2001 measurement date to a mean low of 1.1 in 1-year-old stands on the January 2003 measurement date (Figure 3.2). On the Virginia piedmont, E_c ranged from an average high of 6.5 μmol/m^2/s in the 24-year-old age class on the June 2000 measurement date to a low of 0.31 μmol/m^2/s in the 1-year-old stands on the December 2001 measurement date. As expected, the range in soil temperature at 10 cm on both locations varied mostly in the 1-year-old stands, which lacked the radiative buffering capacities of stands with crown closure. E_c rates generally paralleled soil temperature values, which ranged from 6.04^{o}C to 28.3^{o}C in 1-year-old South Carolina stands and 0.51^{o}C to 27.3^{o}C in 1-year-old stands in Virginia. The range in observed soil temperatures covers the average range in air temperature for both locations, implying that our measurement days captured a range of soil temperature conditions that are typical of each location. Soil moisture at 10 cm ranged from 5.8% volumetric water content in the 21-year-old age class to 20.5% water in 1-year-old South Carolina stands. In contrast, volumetric water content was a low of 8.8% in 9-year-old stands to 28.1% in 1-year-old stands in Virginia.

Modeling Results

Full and reduced common models were developed to identify and quantify the impact of major E_c drivers and to compare whether or not the relationship between explanatory variables and E_c differs between study locations (Table 3.2). A 'best' full model was developed, which explained the greatest amount of variance in E_c when datasets from both study locations were combined ($R^2 = 0.60$) and in which all parameters in the model were significant ($P<0.05$). The model includes seven parameters and the following five input variables: soil temperature, stand age, soil moisture, measurement position, and site index. The form of the common model is as follows:

$$E_c = B_o + B_1(T_s) + B_2(\mathrm{Ln}[T_s]) + B_3[(\mathrm{Ln}[A]) \times T_s] + B_4(P) + B_5(M_s) + B_6(A \times SI_{25}) + \varepsilon_i$$

where T_s = soil temperature (°C) at 10 cm; A = stand age in years; P = measurement location (dummy variable where 1 = near the base of tree and 2 = between rows); M_s = soil moisture at 10 cm; SI_{25} = site index in meters at 25 years; ε_i = error associated with estimate of E_c. Statistical comparisons between alike parameter coefficients for the South Carolina coastal plain and Virginia piedmont locations revealed that coefficients associated with the parameters T_s, $[(\mathrm{Ln}[A]) \times T_s]$, and M_s were different ($P<0.05$), indicating that the relationship between these variables and E_c differed between the two locations. The reduced model improved the amount of variance in E_c explained on the Virginia piedmont sites (R^2 VA = 0.77) and reduced the variance explained on the South Carolina coastal plain (R^2 SC = 0.54). Coefficient differences and distinct changes in the fit of the above model between locations indicated that the reduced common model was more appropriate than a single full model. Only T_s and P were highly significant in the reduced model for the South Carolina coastal plain ($P<0.05$), explaining 49% and 3.8% of the variance in E_c, respectively. However, we did not discard the remaining non-significant variables from the common model since all parameters were highly significant in explaining variance in E_c on the Virginia piedmont location ($P<0.05$). The following amount of variance in E_c, expressed as a partial R^2 value, was explained by each parameter on the Virginia piedmont site: T_s= 59.3%, Ln[T_s] = 0.28%, [(Ln[A]) x T_s] = 11.9%, P = 1.78%, M_s = 3.05%, A x SI_{25} = 0.80%. T_s provided the greatest explanation of the variance in E_c on both locations.

While the initial model explains the greatest variance in E_c on the two locations, we developed a 'simple' model that reduced the number of input variables from five to two. Explanatory variables and parameters for the 'simple' model were chosen that provided the best fit of the data from both study locations without greatly compromising the amount of variance in E_c explained. The model developed has the following form:

$$E_c = B_o + B_1(T_s) + B_2(\mathrm{Ln}[T_s]) + B_3[(\mathrm{Ln}[A]) \times T_s] + \varepsilon_i$$

where symbols are identical to the 'best' model presented above. While P was a significant variable in the 'best' common model for both locations, we chose not to include this variable in the 'simple' model since a dummy variable is difficult to apply to scaled-up estimates of ecosystem level CO_2 efflux. Although it is important to note that spatial variability exists within a stand, we could not reasonably define the amount of area considered near the base of the seedling and the area between rows; therefore, we chose to exclude the P variable from the simple model resulting in a slightly lower model R^2 for both locations. The reduced 'simple' model indicates that soil temperature and stand age alone explain 50% and 70% of the variance in E_c in the South Carolina and Virginia stands, respectively. In the reduced model, all parameters explain a significant amount of variance in E_c on the Virginia sites ($P<0.05$) and have similar partial R^2 values as the 'best' model. Only T_s is significant in explaining variance in South Carolina stands, however. Again, we did not remove the parameters Ln[T_s] and Ln[A]) x T_s from the common model since they were highly significant in explaining E_c on the Virginia location and we wished to compare whether the relationship between E_c and explanatory variables differed between the two locations. A comparison of alike model parameter coefficients associated with each location showed that the coefficient for the parameter Ln[A] x T_s differs for Virginia and South Carolina stands, suggesting that the response of E_c to stand age across soil temperatures differs between locations.

Trends in Soil CO_2 Efflux across Soil Temperatures and Stand Ages

We felt the reduced explanation of variance in E_c when using the ‘simple’ model rather than the ‘best’ model was offset by the benefit of a model with fewer input variables that is easily interpreted. Therefore, we used the ‘simple’ model for the remaining trend analysis and for the scaling-up of E_c to stand-level annual soil C efflux estimates. Trend lines generated using the ‘simple’ common model demonstrate a distinctly different response of E_c to stand age in the South Carolina stands in comparison to the Virginia sites (Figure 3.3). On the South Carolina coastal plain, E_c primarily increased linearly across soil temperatures. The effect of age on E_c was insignificant in the South Carolina stands; however, since Ln[A] x T_s does not significantly explain variance in E_c on the South Carolina piedmont ($P>0.05$), the effect of stand age on E_c is not statistically significant. In contrast, the temperature effect was mildly curvilinear in Virginia stands and there was a strong positive relationship between stand age and E_c. Overall, E_c rates across soil temperatures are higher on the South Carolina coastal plain relative to the Virginia piedmont, except in the oldest age class.

Root Volume and Coarse Woody Debris Densities

We hypothesized that changes in E_c over a rotation might be due to variation over time in respiring root volume and C substrate available to microbes. Although we are unable to make direct comparisons between measurement locations due to different sampling depths at each location, we observed similar trends across stand ages in South Carolina and Virginia. In our survey of root volume and CWD below E_c measurement locations, we found that mean root volume density, although highly variable, increased with age in both the South Carolina and Virginia soils sampled (Figure 3.4). Conversely, mean CWD density in the mineral soil fell with increasing stand age on the South Carolina coastal plain and the Virginia piedmont sites sampled.

Predicted Yearly Soil Carbon Loss Over a 20-Year Rotation

Using the ‘simple’ reduced model developed for each location and predicted soil temperature data estimated using historical air temperature data from the closest U.S.

National Weather Service weather station, we estimated C fluxes over a 20-year rotation for both the South Carolina coastal plain and Virginia piedmont study locations. Two distinctly different trends are apparent over time for the two study locations (Figure 3.5). On the South Carolina coastal plain, C lost on a site is greatest at the beginning of the rotation, followed by a decline and a more steady C loss from age 8 through 20 years. In contrast, Virginia piedmont stands show a trend of steady increase in C loss with increasing stand age. Soil C loss estimates in 1-year-old South Carolina stands are 17.4 Mg C/ha/yr compared to an estimated loss of 7.28 Mg C/ha/year lost from the Virginia piedmont site. In 10-year-old stands, an estimated 13.4 and 10.7 Mg C/ha/year are respired from the soil in South Carolina and Virginia stands, respectively. At 20 years, C efflux is most similar between the two locations with an estimated 13.6 and 12.4 Mg C/hectare lost from the soil in South Carolina and Virginia stands, respectively.

Discussion

Soil CO_2 Efflux Drivers

Statistical modeling results indicate that several variables we examined were significantly correlated with E_c and that the response of E_c to these variables differed with location within the region. Coefficient comparisons indicated that the response of E_c to some explanatory variables differed between the South Carolina coastal plain and the Virginia piedmont (Table 3.2). Our statistical modeling results show that most of the variance in E_c from both locations was explained by soil temperature, which is consistent with previous findings (Kowalenko et al. 1978, Howard and Howard 1993, Pajari 1995, Bouma et al. 1997, Maier and Kress 2000, Pangle and Seiler 2002). Soil temperature was strongly positively correlated with E_c on both the South Carolina coastal plain and the Virginia piedmont. Also, measurement location influenced E_c on both study locations. Specifically, E_c was consistently higher near the tree in comparison to measurements taken between rows. Pangle and Seiler (2002) attributed higher rates near the base of a tree to respiring root biomass in recently planted loblolly pine. We found no significant interaction between stand age and position indicating a similar spatial trend in E_c exists in older stands as well as recently planted stands. Other variables, including soil moisture, site index, and stand age were significantly related to E_c on the Virginia Piedmont, but not on the South Carolina coastal plain. Soil moisture on the Virginia site had a slight positive effect on E_c. Soil moisture has been repeatedly shown to impact E_c, especially under extreme flooding or drought (Kowalenko et al. 1978, Howard and Howard 1993, Bouma et al. 1997, Pangle and Seiler 2002). Soil moisture was not significantly correlated with E_c on the South Carolina coastal plain. While we observed a narrower range in mean volumetric moisture contents across measurement days on the South Carolina coastal plain in comparison to Virginia, we observed individual volumetric moisture contents ranging from 2 to 69% in South Carolina compared with a range of 2 to 35% in Virginia. Soil moisture was generally more variable on the South Carolina sites, especially in 1-year-old bedded stands in which microtopography highly influenced soil water content. Our inability to correlate E_c with soil moisture on the South Carolina sites suggests that soil water status was not a major driver of E_c, even across a broad span of soil moistures. Site index was not significant in explaining variance in E_c on the South Carolina coastal plain, while the variable only slightly explained variance in E_c on the Virginia piedmont in the context of the age by site index interaction (A x SI_{25}; partial R^2=0.008). Site index was only slightly related to E_c despite the fact that faster growing stands should have greater respiring root biomass at an earlier age (Ewel et al. 1987b). However, we may not have captured an adequate range of site indexes within a given age class on each study location, which would prevent us from detecting a strong stand age x site index interaction. A measure of productivity or biomass may be critical when using our models to predict E_c on other sites since growth and biomass production reflects both age and site productivity.

We observed a considerably different relationship between E_c and stand age on the two sites. Specifically, the Virginia sites displayed a strong positive effect of age on E_c, while E_c on the South Carolina location exhibited an insignificant response to stand age. Stand age generally has an inconsistent effect on E_c according to previous reports. Ewel et al. (1987a) reported highest E_c rates in recently clear-cut slash pine (*Pinus elliottii* Englem.) plantations. Rates were significantly lower in a 29-year-old stand, but lowest in a 9-year-old stand. Klopatek (2002) reported increasingly higher E_c rates in 20-year-old, old-growth, and 40-year-old Douglas-fir stands (*Pseudotsuga menziesii* Franco), respectively. Pyker and Fredeen (2003) observed highest E_c rates 2 years after harvest in hybrid spruce (*Piecea glauca* x *engelmannii*) stands. Rates were lowest the year of harvest and tended to show less of an age effect after the second year of growth. Collectively, the preceding authors conclude that harvest intensity, residual slash following harvest, belowground biomass accumulation, and changing site nutrition partially explain difference in observed E_c among the various ages.

Cultural practices on the coastal plain and piedmont differ considerably, which may provide a better explanation of the inconsistent age effect we observed between the two locations. Generally, E_c or C turnover increases as management intensity and soil disturbances increase (Edwards and Ross-Todd 1983, Londo et al. 1999, Lee et al. 2002). Specifically, the intensity of site preparation and the amount of organic matter incorporated into the soil have been shown to affect E_c (Ewel et al. 1987a, Mallik and Hu 1997). Bedding, which was performed on our sites located on the South Carolina coastal plain, incorporates slash into the soil and severely disturbs mineral soil structure. Bedding may have created a more favorable microenvironment for soil heterotrophs in part by providing accessible C substrate in the form of slash (Trumbore et al. 1996, Progar et al. 2000, Wang et al. 2002). Thus, the total contribution of microbial respiration to E_c was probably greatest early in the rotation in South Carolina stands. E_c was greater on the South Carolina sites relative to Virginia sites across temperatures in 1-year-old stands, supporting the idea that microbial respiration is elevated early in the rotation in South Carolina stands. The difference in E_c across soil temperatures between the two locations must primarily be the result of a greater contribution of microbial respiration in South Carolina stands since 1-year-old stands from both locations should have only minor contributions from respiring roots early in the rotation. With increasing stand age, CWD incorporated into beds during site preparation is probably subjected to microbial decomposition since CWD density sharply declined with stand age on the South Carolina sites. After initial inputs of CWD and other forms of C substrate are exhausted, microbial respiration rates probably drop. In fact, initial inputs of CWD on the South Carolina sites will turnover within 5 years given the estimated rates of soil C efflux. The 5-year turnover of initial inputs of mineral soil CWD corresponds to a stabilization in estimated C loss from the soil, supporting the idea that initial decomposition of the CWD accounts for most of CO_2 efflux from the soil early in the rotation. In contrast, the amount of E_c attributable to root respiration probably increases over time, which is consistent with the increase in root biomass we observed with stands age. Ewel et al. (1987b) reported increasing contributions of root respiration to E_c in slash pine plantations with increasing stand age. Therefore, it is likely that inverse shifts in contributions from root respiration and microbial respiration resulted in no significant change in E_c over time in the South Carolina stands. Our results are similar to those of previous authors who concluded that increases in microbial respiration following clear-cutting were offset by reductions in root respiration, resulting in no detectable difference in E_c between recently cut and intact stands (Edwards and Ross-Todd 1983, Toland and Zak 1994).

The strong positive relationship between E_c and stand age in Virginia was probably the combined result of both a low intensity mineral soil disturbance and the use of burning during site preparation. Respiring root biomass and recovering microbial populations impacted by burning may account for the positive trend in E_c we observed in Virginia stands with age. Investigators have shown that burning reduces microbial respiration in European coniferous forests by changing soil physical and chemical properties, by reducing C substrate availability, and also through surface sterilization (Fritze et al. 1993, Pietikainen and Fritze 1993, Pietikainen and Fritze 1995, Hernandez et al. 1997). Further, Chang et al. (1995) observed an increase in microbial biomass with stand age following clear-cutting and burning in northern Vancouver Island coniferous forests, again suggesting microbial contributions to E_c increase over time in burned stands. Further, the contribution of microbial respiration to E_c may be less in young stands since the mineral soil was less disturbed in the Virginia stands during site preparation. We did not observe identical CWD densities in the mineral soil sampled from both locations, probably in part because site preparation on the Virginia piedmont did not incorporate residual organic matter into the mineral soil. Also, some soil organic matter was probably released from combustion during burning. While CWD densities were not entirely comparable between the two locations due to the fact that we sampled to a shallower depth in Virginia, we expect that sampling deeper would actually reduce our CWD density values for Virginia since total C tends to decrease with depth (Jobbagy and Jackson 2000) and because no tillage occurred during site preparation. Root volume density was not as high on the Virginia piedmont in comparison the South Carolina coastal plain. However, the root volume densities we observed are not directly comparable between the Virginia and South Carolina locations due to the difference in sampling depth and because rooting depths may differ between locations. Rooting depths of pines vary according to genetics and the soil environment (Eissenstat and Van Rees 1994).

Overall, the 'best' model explained 54% and 77% of the variance in E_c on the South Carolina sites and the Virginia sites, respectively. The 'simple' model, including only soil temperature and stand age drivers, explains 50% and 70% of the variance in E_c on the South Carolina coastal plain and the Virginia piedmont, respectively. A distinct difference in R^2 values between study locations may be due to more intensive site preparation on the South Carolina coastal plain, which appeared to result in high site heterogeneity relative to the Virginia piedmont location. Specifically, other factors, which we did not measure, including soil physical and chemical properties may have explained some of the remaining variance in E_c. Several previous reports cite the influence of edaphic properties on E_c and its components (Thirukkumaran and Parkinson 2000, Burton et al. 2002, Pangle and Seiler 2002). Further, variability in E_c decreased with increasing stand age, implying that high heterogeneity due to site disturbance diminishes over time. The coefficient of variability (CV) was highest in the 1-year-old age class with a value of 52.2% and lowest in the 21-year-old age class, which had a CV of 33.4%, indicating that E_c was more variable in young stands. In comparison, the 1-year-old and 24-year-old Virginia age class had CV values of 47.4% and 33.4%, respectively. High site heterogeneity on the South Carolina coastal plain, especially in young stands, is likely due to high site disturbance from bedding. Bedding probably encouraged pockets of high microbial respiration, which were apparent in the elevated E_c values we observed in the 1-year-old stands. These pockets of high microbial activity would explain why we observed the highest variance in E_c across temperatures in 1-year-old stands. Again, previous reports attribute high site disturbance to a rise in E_c (Edwards and Ross-Todd 1983, Londo et al. 1999, Lee et al. 2002). The fact that CV values decrease with increasing stand age suggests that spikes in microbial activity following site disturbance are less common in older stands. Taken together, our results and the results of previous authors cited above imply that root and microbial contributions to E_c shift over time due to on multiple factors, resulting in an inconsistent relationship between stand age and E_c. Thus, an examination of autotrophic and heterotrophic components of E_c individually and concurrently is clearly important to our understanding of why soil C efflux varies with stand age, management regimes, and between locations.

Soil Carbon Efflux Over a 20-year Rotation

Projected patterns and the amount of annual soil C efflux varied considerably between the two locations within the region. Estimated annual soil C efflux rates were greatest in 1-year-old South Carolina stands, with a value of 17.4 Mg C/ha/yr. Annual rates were projected to slowly decline and level, reaching 13.6 Mg C/ha/yr when the stand is 20-years-old. Conversely, estimated annual C efflux is lowest in 1-year-old Virginia piedmont stands, emitting 7.28 Mg C/ha/yr, and reaching a maximum soil C efflux rate of 12.4 Mg C/ha/yr when stands are 20-years-old. Differences in annual C efflux are due to the differential effect of age on E_c described in detail above and in response to the changing relationship between stand age and soil temperature. Soil temperature is most variable in young stands, especially prior to crown closure, since less foliage is available to absorb and trap heat. In South Carolina, high annual soil C efflux in young stands is directly related to greater soil temperatures early in the rotation since we observed no significant effect due to stand age. In Virginia, where E_c was strongly positively correlated with stand age, the changes in soil temperature with stand age dampened the strong effect of age on annual C efflux rates, explaining why annual C efflux values do not simply parallel the relationship between E_c and stand age. Gordon and Van Cleve (1987) attributed increases in E_c following a clear-cut in Alaska white spruce (*Picea glauc* Voss) forests mainly to higher soil temperatures, which supports our findings that changes in soil temperature associated with crown cover exert a strong influence on C efflux.

Previously reported annual soil C efflux rates vary considerably. Our estimates are among the highest reported in the literature for North American forests. Ewel et al. (1987a) reported the highest values for soil C efflux expressed in the literature to our knowledge, estimating a loss of 13.0, 8.20, and 22.7 Mg C/ha/yr in recently clear-cut, 9-

year-old, and 29-year-old Florida slash pine, respectively. Maier and Kress (2000) measured E_c in 11-year-old fertilized and non-fertilized loblolly pine stands located in the Sandhills region of North Carolina. They determined that annual C efflux from the site averaged 14.1 Mg C/ha/yr, which is close to our estimated annual efflux of 13.3 Mg C/ha/yr from 11-year-old South Carolina coastal plain loblolly pine stands. Pyker and Fredeen (2003) reported annual soil C efflux rates ranging from 5.60 Mg C/ha/yr to 8.61 Mg C/ha/yr in recently cut and 2-year-old hybrid spruce stands, respectively, located in British Columbia. Klopatek (2002) estimated annual C efflux was 13.67, 8.83, and 11.94 Mg C/ha/yr from 20-year-old, 40-year-old, and old-growth Douglas-fir stands, respectively. Conant et al. (1998) estimated yearly soil C efflux rates were approximately 4 Mg C/ha/yr for ponderosa pine stands in Arizona while Law et al. (1999) reported slightly higher rates of 6.83 Mg C/ha/yr for mixed-aged ponderosa pine stands in Oregon. Haynes and Gower (1995) reported annual soil C efflux values ranging from 3.31 to 5.41 Mg C/ha/yr in 31-year-old red pine (*Pinus resinosa* Ait.) plantations located in northern Wisconsin. Similar annual estimated soil C efflux rates were reported for young and old-growth Oregon ponderosa pine (*Pinus ponderosa* Dougl.) stands (Irvine and Law 2002). The authors determined that the average annual rates of 483 Mg C/ha/yr in the 14-year-old stands and the 526 Mg C/ha/yr in >50-year-old stands were highly influenced by soil moisture availability rather than age or standing biomass. While latitudinal and topographic variation and associated variation in soil temperature and moisture explain some of the reported differences among annual soil C efflux reported in the literature (Conant et al. 1998, Burton et al. 2002), genetics, site productivity, soil characteristics, and cultural practices likely influence the rate of soil C efflux. The large range of soil C efflux rates reported in the literature, along with a poor understanding of the likely shift in contribution of root and microbial respiration components over time, demonstrates the need for quantitative inquiries into the two components contributing to E_c. Further, previous studies along with the current study demonstrate the need to better understand and quantify the role forest management plays in soil C turnover and belowground C sequestration.

Table 3.1. Mean stand age, mean site index (base age 25 years), soils description, and common site preparation methods associated with South Carolina coastal plain and Virginia Piedmont study plots. See text for more detailed soils description.

Location	**Mean Age (Years)**	**Mean Site Index$_{25}$ (m)**	**Soils Description**	**Site Preparation**
SC Coastal Plain	1	21.5	Deep, poorly drained, acidic, low P, sandy to sandy-loam derived from marine sediment	Bedding
	6	21.6		
	11	22.3		
	21	20.0		
VA Piedmont	1	15.8	Deep, moderately well- to well-drained, loam to sandy-loam derived from sedimentary bedrock	Chop and burn
	4	17.8		
	9	18.4		
	24	17.9		

Table 3.2. Model R^2 values, y-intercepts, and variable coefficients associated with common models developed for loblolly pine stands located on the South Carolina coastal plain (SC) and the Virginia piedmont (VA). Parameters are presented for the common model developed with the highest R^2 values (Best) and for the simplified model (Simple). Stars (*) indicate a significant difference between coefficients associated with alike variables in common models developed for both locations ($P<0.05$). Abbreviations: Y-int = y-intercept; T_s = soil temperature (^oC); A = stand age in years; P = measurement location; M_s = soil moisture; SI_{25} = site index in meters at 25 years.

				Parameters					
	Site	**R^2**	**Y-int.**	**T_s**	**$Ln(T_s)$**	**$Ln(A)xT_s$**	**P**	**M_s**	**$AxSI_{25}$**
Best	SC	0.54	1.48	0.320^{*}	-0.655	$-8.74E^{-3*}$	-0.875	$-1.35E^{-3*}$	$8.48E^{-5}$
	VA	0.77	-0.934	0.166^{*}	-0.281	0.0649^{*}	-0.560	0.0833^{*}	$-5.14E^{-5}$
Simple	SC	0.50	0.341	0.324	-0.752	$-6.75E^{-3*}$			
	VA	0.70	$7.25E^{-3}$	0.198	-0.432	0.0403^{*}			

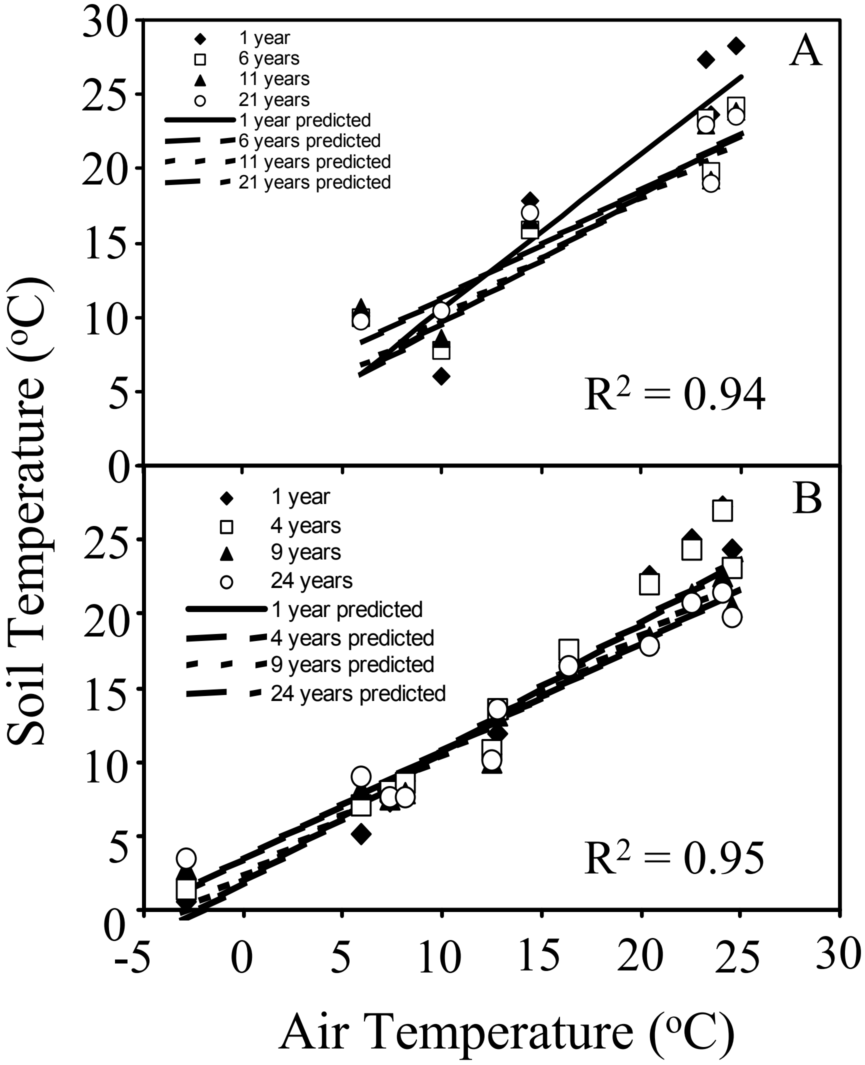

Figure 3.1. Relationship between mean soil temperature (top 10 cm) and mean air temperature on measurement days at the South Carolina (A) coastal plain and the Virginia piedmont (B) study locations for each age class. Soil temperatures presented in the figure are mean values for each age class on a given measurement day while air temperatures provided are mean values collected from an on-site meteorological station on measurement dates. Multiple linear regression was used to define the relationship between soil temperature and the continuous variables air temperature and stand age. Symbols alone represent actual data while lines are regression lines corresponding to estimated values for each age classes. Regression lines were generated by varying air temperatures and holding mean age constant for each age class.

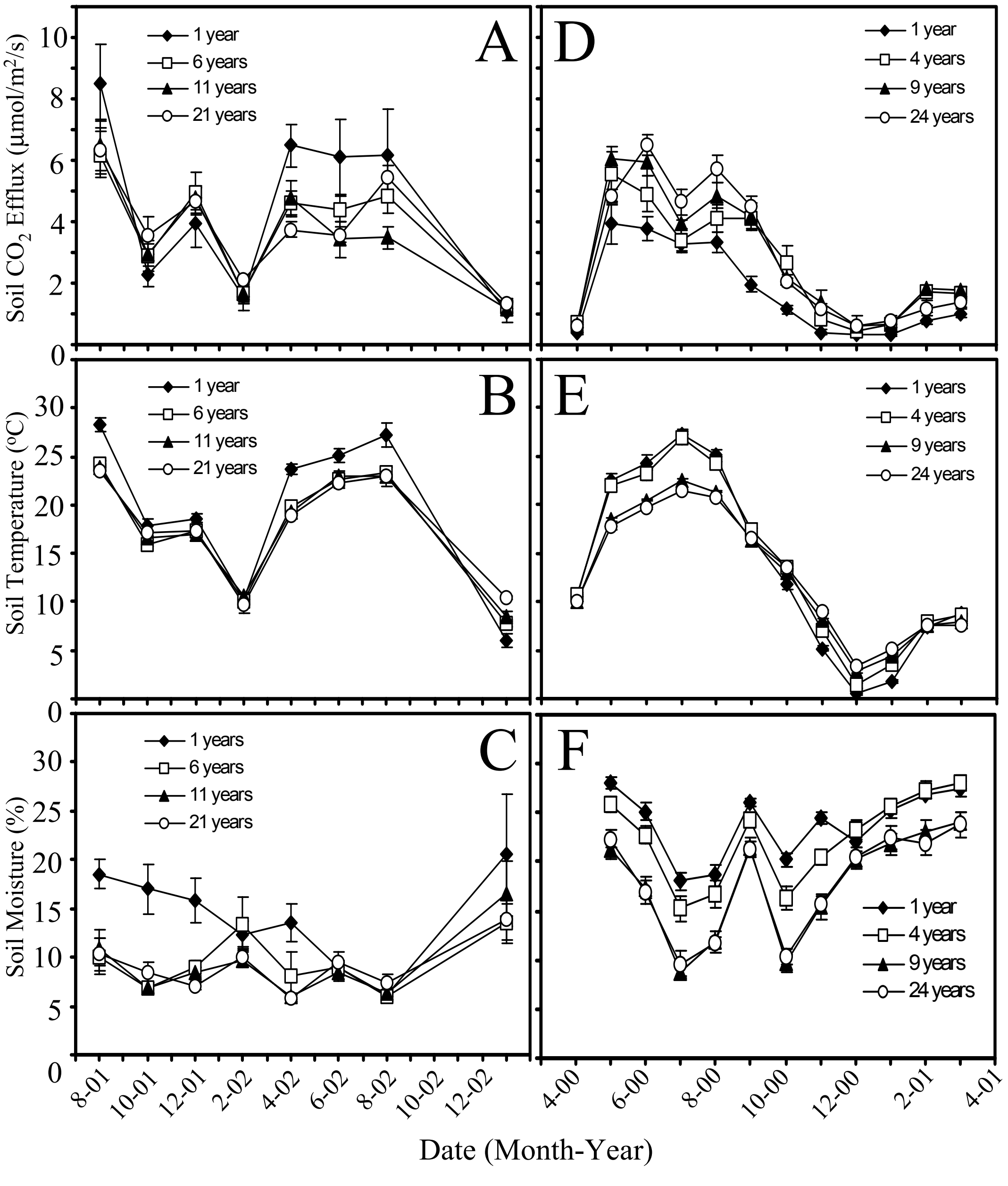

Figure 3.2. Mean soil CO_2 efflux rates (A,D), soil temperatures (top 10 cm; B,E), and soil moistures (top 10 cm; C,F) of loblolly pine stands during measurement days on the South Carolina coastal plain (A,B,C) and the Virginia piedmont (D,E,F). Means are presented for each age class investigated. Bars associated with means represent one standard error.

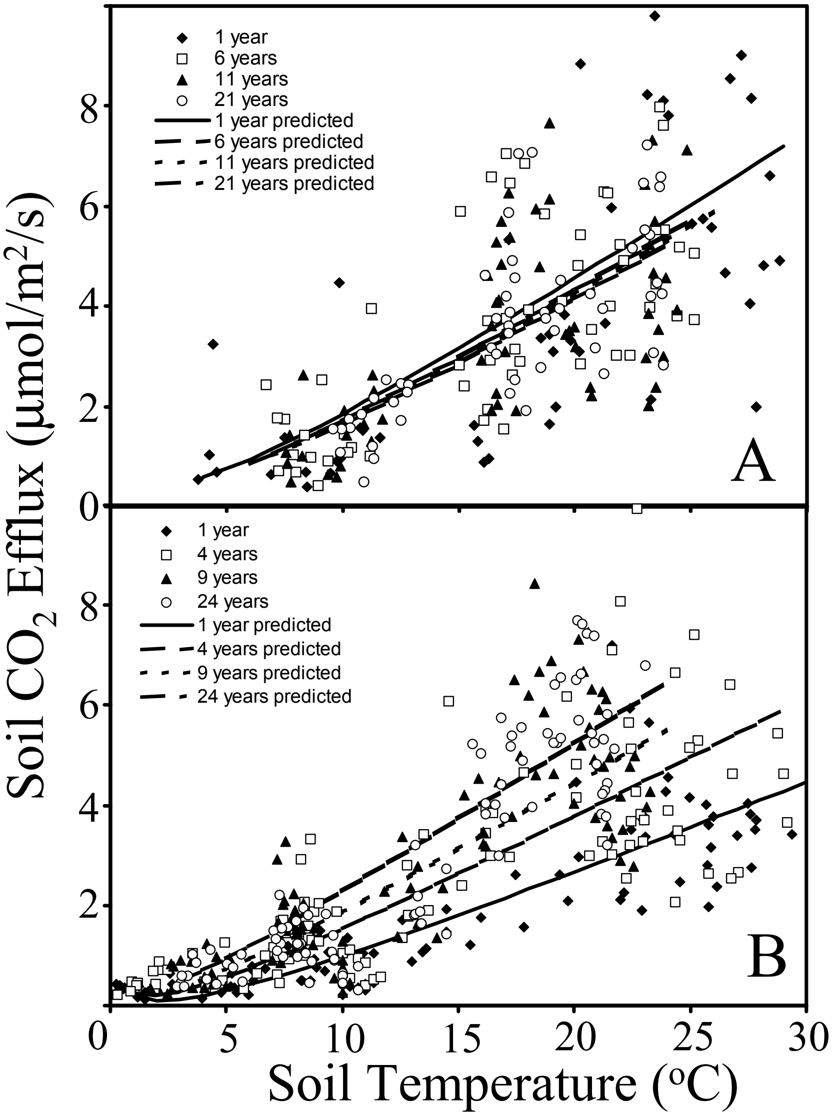

Figure 3.3. Relationship between soil temperatures (10 cm depth) and soil CO$_2$ efflux in four age classes of loblolly pine stands located on the South Carolina coastal plain (A) and the Virginia piedmont (B). Lines represent the linear regression line relating soil CO$_2$ efflux to soil temperature for a given stand age class calculated using the “simple” model, which contains soil temperature and stand age inputs.

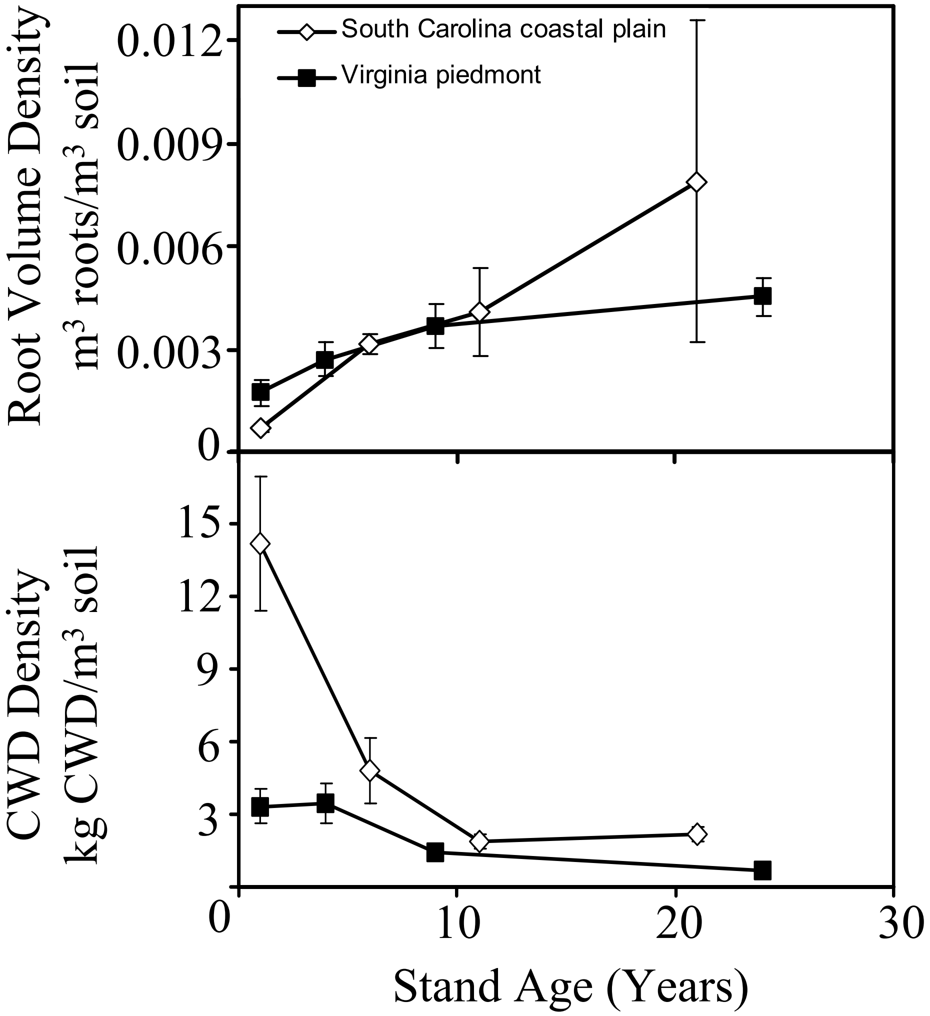

Figure 3.4. Mean root volume density and mean coarse woody debris (CWD) density in the mineral soil from loblolly pine stands on the South Carolina coastal plain and the Virginia piedmont varying in age. A mineral soil core was taken in the top 20 cm on the South Carolina coastal plain and top 10.2 cm on the Virginia piedmont. The densities shown above were calculated for the soil depths sampled at each location.

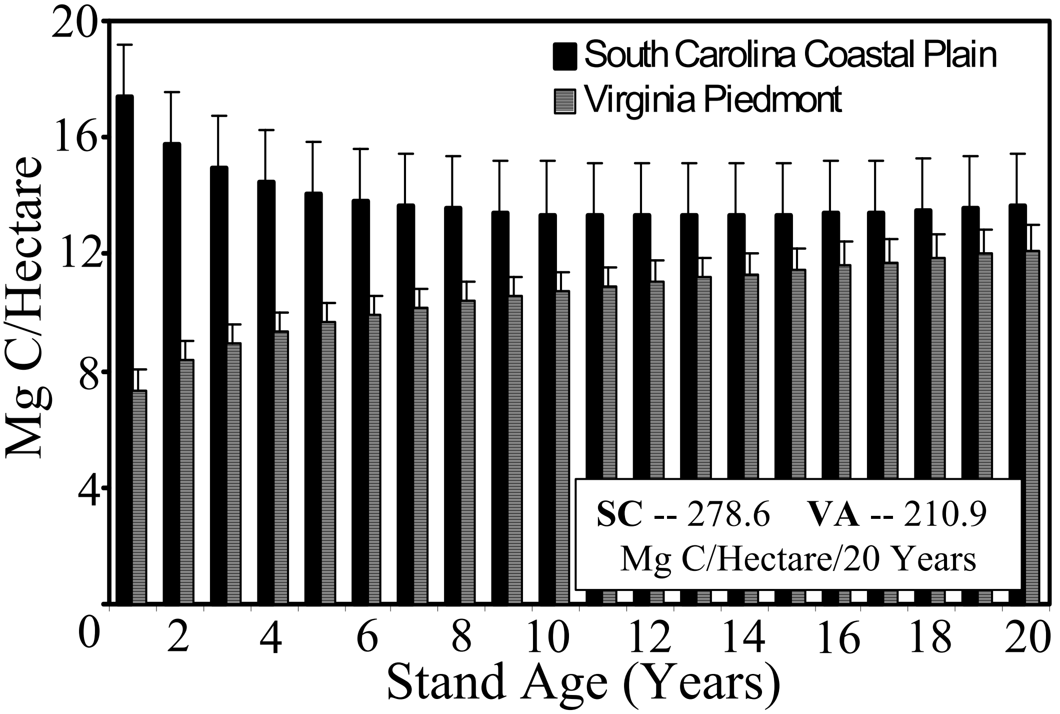

Figure 3.5. Estimated yearly soil carbon losses from loblolly pine stands located on the South Carolina coastal plain and the Virginia piedmont over a 20-year rotation. Estimates were derived by scaling up chamber based measurements using the “simple” model. Standard error bars take into account error of the efflux estimation and the error associated with soil temperature estimates, which were input into the “simple” efflux model.

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CHAPTER 4

Short-term effects of fertilization on loblolly pine (*Pinus taeda* L.) physiology

Abstract. Fertilization commonly increases biomass production in loblolly pine (*Pinus taeda* L.) and other plant species; however, the sequence of short-term physiological adjustments allowing for the establishment of leaf area and enhanced growth rates is not well understood. We intensively investigated effects of fertilization on loblolly pine seedling photosynthetic parameters, root respiration, and growth for over 200 days following the application of diammonium phosphate (DAP) in a greenhouse study in an effort to establish a relative sequence of events associated with improved growth. DAP fertilization resulted in the temporary elevation of specific root respiration (R_r), probably reflecting the uptake and metabolism of nitrogen (N). Foliar N concentrations were detectably greater 16 days following fertilization and corresponded to improved light saturated specific net photosynthesis (A_{sat}). A_{sat} was positively correlated with foliar N concentration when non-fertilized and fertilized treatments were pooled (R^2=0.47). Improved photosynthetic capacity following fertilization was due to both improved photochemical efficiency and capacity and enhanced carboxylation capacity of Rubisco. Positive effects of fertilization on growth were observed shortly after A_{sat} was increased. Fertilized seedlings had 36.5% more projected leaf area and 36.5% greater total dry weight biomass 211 days following fertilization. Dry matter partitioning at harvest did not differ between treatments. Elevated A_{sat} along with improved photochemistry and carboxylation capacities in fertilized treatments were temporary and corresponded to reductions in foliar N levels. Fertilization likely contributed to a temporary increase in photosynthetic capacity, which resulted in a pool of photoassimilate used to build greater leaf areas. N initially invested in photosynthetic structures and enzymes probably retranslocated to newly developing foliage, explaining the gradual reduction in N after peak levels were achieved following fertilization. We conclude that foliar N levels and timing of the fertilizer application may influence ability of investigators to detect associated changes in photosynthetic capacity. Further, a short-term improvement in photosynthetic capacity following fertilization is probably a mechanism used to build leaf area in loblolly pine, subsequently resulting in increased growth.

Introduction

Fertilization is commonly applied in forest management to enhance forest productivity. However, the initial physiological changes permitting increased biomass productivity following fertilization are not well characterized. Fertilization generally increases leaf areas in pine, which enhances overall productivity by increasing whole-plant carbon (C) fixation (Teskey et al. 1987, Vose and Allen 1988, Teskey et al. 1994, Albaugh et al. 1998). Vose and Allen (1988) reported an increase in leaf area index (LAI) of up to 60% with N fertilization in loblolly pine (*Pinus taeda* L.). Albaugh et al. (1998) reported a LAI increase of 101% in nutrient poor loblolly pine stands amended with optimum levels of fertilizer. However, in order for higher leaf areas to be realized, one of the following must occur: (1) Fertilization may have a direct effect on photosynthetic capacity (net photosynthesis per unit leaf area) resulting in more C being fixed per unit leaf area. The "surplus" photoassimilate is subsequently used to build additional leaf area; (2) Biomass allocation may shift from root to aboveground components (specifically leaf area). This effectively reduces C sink tissue (i.e. roots) and increases source tissue (i.e. leaves); (3) Increased productivity is the result of both mechanisms. While any of the above mechanisms could reasonably account for increased biomass productivity in response to N fertilization, the literature fails to clearly identify a single mechanism.

Previous studies that investigated fertilization effects on Pinaceae specific foliar net photosynthesis (P_n) provide mixed results and collectively present an uncertain sequence of physiological adjustments associated with productivity shifts. Fertilization has directly improved P_n in some cases (Mitchell and Hinkley 1993, Murthy et al. 1996). Other studies report that fertilization had little or no impact on P_n (Zhang et al. 1997, Tang et al. 1999, Gough et al. (*in press*), Samuelson et al. 2001, Maier et al. 2002). Similarly, previous studies report conflicting results concerning the relationship between foliar N status and P_n. Some reports indicate that foliar N in Pinaceae species is positively correlated with P_n (Mitchell and Hinckley 1993, Vapaavuori 1995, Murthy et al. 1997), while others report a weak or non-existent relationship between foliar N and P_n (Teskey et al. 1994, Zhang et al. 1997, Schoettle and Smith 1999, Gough et al. (*in press*), Maier et al. 2002). Generally, the literature suggests that P_n is enhanced over the short-term, possibly followed by a return to non-fertilized levels over the long-term. However, the implications of these results as they relate to mechanistic changes associated with fertilization are confounded due to the lack of uniformity in genetics, measurement timing, and study design. It is possible that any increase in P_n following fertilization is brief, providing the necessary photoassimilate for enhanced leaf areas. Once greater leaf areas are established following nutrient amendments, P_n may return to pre-fertilization levels. The preceding hypothesis could explain why the literature provides conflicting reports concerning effects of fertilizer on P_n, since measurement timing following fertilization may be critical to the detection of changes in photosynthetic capacity. This hypothesis has never been tested in a single study that intensively monitors changes in photosynthetic capacity.

Theoretically, shifts in biomass partitioning and allocation in plants are influenced by the most limiting resource according to the functional balance theory. When a resource is limiting, such as N, assimilate allocation to the roots may increase, for example, enabling enhanced N uptake until a balance is achieved and N is no longer the primary limiting factor. King et al. (1999) reported that 4 years of fertilization of a nutrient poor site increased partitioning to the perennial tissues, including coarse roots, taproots and branches, while decreasing partitioning to ephemeral tissues, including the fine roots and foliage in 12-year-old loblolly pine. A 6% shift in biomass partitioning was observed. Albaugh et al. (1998) cited similar results for the same stand, specifically reporting an average of 8% and 22% in total biomass allocation to fine roots in fertilized and non-fertilized stands, respectively. Other studies have found both an absolute and relative decrease in root biomass production in fertilized pine (Axelsson and Axelsson 1986, Griffin et al. 1995, Haynes and Gower 1995, Gebuaer et al. 1996).

Increases in specific root respiration (R_r) following fertilization are relatively common (Griffin et al. 1997, Lu et al. 1998). Zogg et al. (1996) determined that fertilization did not significantly alter specific fine-root respiration in sugar maple (*Acer saccharum* Marsh.); however, total root respiration was highly correlated with root tissue N concentration. N fertilization likely increases R_r due to metabolic costs associated with both uptake and assimilation (Zogg et al. 1996). Growth respiration costs of incorporating NH_4^+ into amino acids are significant (Vessey and Layzell 1987). When N additions increase whole-plant biomass, total maintenance respiration costs also increase (Ryan 1991).

Collectively, the studies cited above imply a sequence of events that leads to greater leaf areas, which permits faster growth rates. However, no study has exclusively monitored multiple physiological events that likely alter productivity immediately following fertilization. In this study, we present results from a study, in which seedlings were fertilized and intensively monitored for short-term physiological changes associated with productivity shifts. Specifically, our objectives included determining the short-term photosynthetic, root respiratory, and biomass allocation responses to nutrient additions. Further, we examined the photosynthetic components responsible for changes in photosynthetic capacity following fertilization through chlorophyll fluorescence (light reactions) and A/C_i (carboxylation reactions) measurements. In our discussion, we outline the likely sequence of physiological events resulting in enhanced whole-plant productivity following fertilization.

We hypothesized that specific R_r would increase immediately following fertilization due to increasing metabolic demands. Shortly after fertilization, we expected to observe an increase in photosynthetic capacity, which would parallel enhanced foliar N concentrations. We expected that photosynthetic capacity would eventually return to non-fertilized levels following the establishment of greater leaf areas. We also expected a return in foliar N levels to non-fertilized levels over time since N is 'diluted' in the expanded leaf areas. Finally, we hypothesized that a final harvest of seedlings would show that leaf areas were in fact greater in fertilized plants and shifts in relative biomass allocation would favor aboveground tissues rather than root formation.

Methods

Study Design

An infertile soil (sandy, siliceous, thermic Psammentic Hapludult [Wakulla series], USDA Forest Service, unpublished data) was collected from the Forest Service's Southeastern Tree Research and Education Site (SETRES) located in Scotland County, North Carolina (35°N lat., 79°W long.) in the Sandhills region. This soil was chosen for the experiment because of its relatively low fertility and the reported positive effects of fertilization on loblolly pine productivity when applied to this soil (Albaugh et al. 1998). The soil was sifted through a 6.4 mm screen to remove coarse woody debris and coarse roots, and homogenized by manually mixing the soil. Forty containerized 1-0 half-sibling (Westvaco Corp., family: WV-3) loblolly pine seedlings were planted in 56.8-liter containers (dimensions: 355.6 mm x 292.1 mm x 279.4 mm, Custom™ 2800C, Hummert International, Earth City, MO) in an effort to minimize physical restrictions on root growth. The total soil volume of each pot was approximately 25,000 cm^3. All pots initially received a low-level dose of fertilizer (equivalent to 15 kg/ha of elemental N) in order to prevent or alleviate nutrient deficiencies prior to actual fertilization treatments. Potted seedlings were randomly chosen and paired. A single pair of pots was placed next to each other on a greenhouse bench and served as one block. The study was arranged as a randomized complete block design with fertilized and non-fertilized treatments.

Growth Conditions

Seedlings were grown for approximately 1 year in a greenhouse prior to the fertilization application outlined below. Seedlings were watered frequently in order to prevent water stress. Excessive watering was avoided in efforts to prevent leaching of nutrients from soil. Plants were grown under a 16-hour photoperiod for the duration of the study.

Nutrient Additions and Prior Measurements

Within each block (two seedlings), seedlings were randomly assigned to either the fertilized or non-fertilized treatment. Seedling height and diameter at the seedling base were determined, and foliage samples were collected from each seedling for a foliar C and N analysis prior to fertilizer treatment application. Also, P_n, discussed below in detail, was measured prior to fertilization to ensure that both treatments had statistically equal physiological and biomass parameters prior to fertilizer application. Each seedling assigned to the fertilization treatment received 1.85 g (0.39 g N and 0.43 g P) of diammonium phosphate (DAP), which is the recommended operational level for seedling fertilization of loblolly pine (Jokela and Long 1999). The equivalent operational rate is 280 kg/ha or 59.4 kg/ha of elemental N and 65.8 kg/ha of elemental P.

Growth Measurements

Seedling growth between the two treatments was monitored over the course of the study by tracking changes in ground-line diameter and height. Seedling growth was generally monitored every week or every other week.

Specific Root Respiration

Specific root respiration was measured 49 and 197 days after fertilization using the LiCor 6200 with a 0.25-L cuvette chamber. Approximately 10 cm^2 of fine roots (< 2 mm) close to the soil surface were gently excavated, and loose soil was shaken free. Roots were placed on a moist paper towel positioned on the cuvette to prevent them from rapidly drying. Respiration was measured over a 30 s sampling period. Roots surface area was determined using the WinRhizo 5.0A software (Regent Instruments Inc., Quebec, Canada) and all measurements were expressed on a per unit area basis. A subsample of roots was sampled from each pot.

Photosynthesis Measurements

Net photosynthesis under saturating light levels (A_{sat}) was measured on newest fully expanded leaves using the LiCor 6400 Portable Photosynthesis System (LiCor Inc., Lincoln, Nebraska). A_{sat} was immediately measured on detached foliage prior to and immediately following fertilization for a period of 190 days. Measurements initially were taken daily and later continued at less frequent intervals until physiological changes resulting from fertilization stabilized. A_{sat} was measured in the same sequential blocking order during every measurement period. For this study, the following chamber conditions were set prior to all measurements: photosynthetically active radiation (PAR) = 1600 μmol/m^2/s; [CO_2] = 360 μmol mol^{-1}; temperature = 25°C; humidity = 50% or greater. The PAR level chosen represents a photosynthetically saturating light intensity in loblolly pine (Teskey et al. 1987). The other values, although somewhat arbitrary, served to minimize potentially confounding effects of environmental variation during measurements. A_{sat} was expressed on a per leaf area basis using the following equation (Ginn et al. 1991):

$$LA_l = (n * l * d) + (\pi * d * l)$$

where l = the length of the needle, d = fascicle diameter and n = number of needles in the fascicle.

Foliar Nitrogen Concentration

Following A_{sat} measurements, each detached fascicle was pooled by treatment into groups of four (i.e. four blocks were combined) on each measurement date, since one needle was not of sufficient mass for N analysis. Needles were oven-dried at 65°C for 48 hours and ground in a Wiley mill. Samples were analyzed for N percent by the USDA Forest Service Southern Research Station laboratory (RTP, NC) using a Carlo-Erba elemental analyzer (Model NA 1500, Fison Instruments, Danvers, MA).

Chlorophyll Fluorescence Measurements

We used chlorophyll fluorescence to examine the efficiency of the photosynthetic machinery and the light reactions. Chlorophyll fluorescence and A_{sat} of the youngest fully expanded attached needles were simultaneously measured 22 and 204 days following fertilization using the LiCor 6400-40 fluorescence attachment (LiCor Inc., Lincoln, Nebraska). The LiCor fluorescence attachment allows for the monitoring of gas exchange from the same tissue subjected to fluorescence. Chamber CO_2 concentration, temperature, and relative humidity parameters were controlled as described above. Needles were dark-adapted for 20 minutes prior to the measurement of dark-adapted or minimum fluorescence (F_o), which was measured using a weak measurement beam (<1 µmol photons/m^2/s). Maximum fluorescence of both dark-adapted (F_m) and light-adapted (F_m') foliage was determined following a red light saturating pulse >7000 µmol photons/m^2/s and centered at a wavelength of 630 nm. Steady state fluorescence of light-adapted leaves (F_s) was determined following the application of 1600 µmol/m^2/s of continuous actinic light. The minimal fluorescence of a light-adapted leaf (F_o') was also determined after a brief 1-s period of darkness. F_s, F_o', F_m', and A_{sat} can take several minutes to reach equilibrium due to the lagging activity of photochemical and non-photochemical reactions following initial exposure to actinic light after dark acclimation (Kooten and Snel 1990). Therefore, we measured F_s and F_m' sequentially five times at 3-minute intervals under continuous actinic light levels of 1600 μmol/m^2/s to obtain steady state measurements. Using the parameters F_o, F_o', F_m, F_s, and F_m', the following photochemical and non-photochemical parameters were calculated:

(1) Maximum photochemical efficiency of PSII (F_v/F_m) = $(F_m – F_o)/F_m$

(2) Quantum efficiency of PSII (Φ_{PSII}) = $\Delta F/F_m' = (F_m'-F_s)/F_m'$

(3) Photochemical quenching (qP) = $(F_m'-F_s)/(F_m'-F_o')$

(4) Non-photochemical quenching (qN) = $(F_m-F_m')/(F_m-F_o')$

(Butler 1978 [F_v/F_m], Genty et al. 1989 [Φ_{PSII}], Kooten and Snel 1990 [qP, qN]). F_v/F_m is the fraction of photons used for photochemistry by dark-adapted leaves while Φ_{PSII} represents the fraction of absorbed photons that are used for photochemistry by light-adapted leaves. Photochemical quenching involves the conversion of light energy to biochemical energy. In the photochemical quenching of light energy, light is principally used to drive the biochemical reactions of photosynthesis and photorespiration. Non-photochemical quenching of light energy occurs when excess light energy is converted to heat energy through processes such as xanthophyll epoxidation (Malkin and Niyogi 2000).

A/C_i Curves

We measured the response of A to internal CO_2 partial pressures (A/C_i response curves) *in situ* with a portable photosynthesis system (LiCor 6400, LiCor, Lincoln, NE, USA). Young, fully expanded leaves were selected from shoots adjacent to those measured for A_{sat}. Chamber conditions were maintained at 1600 μmol m^{-2} s^{-1} PPFD, 25 ^{o}C and >55% relative humidity. Foliage was sealed into the leaf chamber and allowed to equilibrate to chamber conditions and at an initial [CO_2] of 370 μmol mol^{-1}.

Measurements of A, C_i and stomatal conductance to water vapor were made at nine steps of external [CO_2] (70, 150, 230, 290, 370, 570, 890, 1500 and 1900 μmol mol^{-1}) ensuring that at each step chamber [CO_2] and A had stabilized before recording the measurement. Subsets of seedlings from eight blocks were measured three times, once before fertilization and then 22 and 175 days after fertilization. Leaf area and N concentration were measured for each leaf sampled. The photosynthetic response curve parameters $V_{c,max}$ (maximum carboxlyation rate of Rubisco) and J_{max} (RuBP regeneration capacity mediated by maximum electron transport rate) were calculated with the Farquhar biochemical model of photosynthesis (Farquhar et al. 1980; David Ellsworth, University of Michigan, personal communication).

Partitioning and Post-Harvest Measurements

Seedlings were harvested 211 days after fertilization, and dry weight biomass of the roots, shoots, and foliage was determined for the two treatments. Roots were thoroughly washed in an effort to remove soil following harvest. Differences in dry matter partitioning between treatments were examined using an allometric relationship between roots and shoots where shoot weight = a(root weight)^k (Ledig et al. 1970). Parameter coefficients were compared statistically in order to determine whether partitioning differed between fertilized and control plants. Projected leaf area of fresh foliage was determined using the LI-3100 Area Meter (LiCor Inc., Lincoln, Nebraska). Following leaf area measurements, organs were oven-dried at 65°C for 1 week and weighed to determine the biomass for each treatment.

Statistical Analysis

Variables measured repeatedly (over time) were analyzed using a time series and variables measured only at harvest were analyzed using a randomized complete block design (with 20 replications per treatment). A time series analysis was used to examine the effect of time, fertilization and time x fertilization on all variables measured on two or more dates over the study. All statistical analyses were performed using the PROC GLM procedure in SAS (SAS Institute, Cary, NC). Variables compared between fertilization treatments include A_{sat}, root respiration, foliar N concentration, chlorophyll fluorescence-derived parameters, A/C_i curve derived parameters, tree height growth, stem diameter growth, and root, shoot, and foliage biomass.

Results

Foliar Nitrogen Percent

Foliar N concentration was statistically greater in from the fertilized plants in 16 days following the treatment application. Foliar N concentrations in the fertilized treatment remained elevated over the control for approximately 50 days ($P<0.05$; Figure 4.1). Foliar N concentrations 2 days following fertilization were 0.87% in the non-fertilized treatment and 0.80% in the fertilized treatment and were not significantly different. The peak difference in foliar N concentration between treatments was observed 28 days following fertilization, when foliage from the fertilized treatment contained almost 1.7% N and controls contained only 0.76%. Foliar N concentrations measured 146 days following fertilization indicate that N levels returned to non-fertilized levels, at which time mean foliar N did not significantly differ between treatments.

Growth Measurements

Fertilized seedling heights and ground diameters were significantly greater than the controls beginning 32 days following fertilization and continuing for the duration of the study ($P<0.05$; Figure 4.2). The trajectory of growth and the fact that we observed a time x fertilizer treatment interaction indicates that growth rates were consistently greater for the fertilized seedlings in comparison to the controls. Mean heights and ground diameters of seedlings increased by 44.3% and 46.4%, respectively, in the control treatment over the course of the study. In comparison, mean seedling heights and diameters grew by 82.4% and 79.6% in the fertilized treatment over the study period.

Specific Root Respiration

Specific root respiration rates from fertilized plants were statistically higher 49 days after fertilization ($P<0.05$; Figure 4.3) with non-fertilized rates averaging 32% less than the average rate in fertilized plants. No significant difference between treatments was apparent 197 days following fertilization.

Photosynthesis Measurements

A_{sat} was statistically greater in fertilized treatments compared to the controls beginning 6 days after fertilization ($P<0.05$; Figure 4.4). A_{sat} generally remained significantly elevated in the fertilized foliage until the last two measurement dates. However, the difference in A_{sat} between the control and fertilized foliage was generally reduced over the last 100 days of the study, indicating a slow return of A_{sat} in the fertilized foliage to control rates.

Mean A_{sat} on a measurement day ranged from approximately 1.5 $\mu mol/m^2/s$ to nearly 6 $\mu mol/m^2/s$ from foliage in the fertilized treatment. While both the time effect and the time x fertilizer treatment interaction were significant ($P<0.05$), light and other environmental conditions on the day of measurements appeared to affect A_{sat} rates despite constant measurement chamber conditions. For example, 64 and 135 days following fertilization A_{sat} was depressed relative to other adjacent measurement days and non-fertilized and fertilized rates were not significantly different on those measurement days. These days were cloudy and PAR was substantially less than other measurement days, averaging close to 100 $\mu mol/m^2/s$. In comparison, PAR on sunny or partly cloudy days averaged greater than 1200 $\mu mol/m^2/s$ and had higher A_{sat} rates.

Light-Saturated Photosynthesis and Foliar Nitrogen Percent

The relationship between A_{sat} and foliar N concentration was examined using linear regression. Mean A_{sat} values for a measurement day were regressed against corresponding mean foliar N concentrations from each treatment for the analysis. A significant relationship between A_{sat} and foliar N concentration was observed ($P<0.05$), and N percent explained 47% of the variance in A_{sat} when non-fertilized and fertilized treatments data were pooled (Figure 4.5).

Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence was used to examine the competency of the photosynthetic machinery associated with the electron transport chain. Fluorescence-derived parameters indicate an increase in electron transport efficiency 22 days following fertilization ($P<0.05$; Table 4.1). Specifically, F_v/F_m, Φ_{PSII}, and qP were significantly greater in foliage from the fertilized treatment. A_{sat} measured concurrently with fluorescence measurements was also greater in the fertilized treatment on day 13 ($P<0.05$). qN was not significantly different between treatments 22 days following fertilization. Chlorophyll fluorescence parameters and simultaneously measured A_{sat} were not statistically different among treatments 204 days following fertilization.

A/C_i Curves

Foliage from fertilized seedlings displayed greater maximum electron transport rates (J_{max}), higher carboxylation rates of Rubisco ($V_{c,max}$), and increased electron transport capacity ($V_{c,max}/J_{max}$) in comparison to foliage from non-fertilized seedlings 22 days following fertilization ($P<0.05$; Table 4.2). Enhanced electron transport and carboxylation rates coincided with the temporary increase in foliar N concentrations that we observed in the fertilized treatment. J_{max}, $V_{c,max}$, and $V_{c,max}/J_{max}$ did not differ between treatments prior to fertilization. The increase in J_{max}, $V_{c,max}$, and $V_{c,max}/J_{max}$ in the fertilized treatment observed 22 days following fertilization was not apparent when the same parameters were measured 204 days following fertilization. The transient increase in the A/C_i derived parameters J_{max}, $V_{c,max}$, and $V_{c,max}/J_{max}$ in foliage from fertilized seedlings corresponded to enhanced electron transport efficiency determined through chlorophyll fluorescence and improved A_{sat}.

Biomass and Partitioning

Foliage, stem, and root dry weights at harvest (207 days following fertilization) were determined for the non-fertilized and fertilized treatments. Treatment differences in biomass were significant ($P<0.05$). There was no statistical difference in partitioning based on our analysis of the allometric relationship between roots and shoots for each treatment. Coefficients for the allometric relationship between roots and shoots where shoot weight = $a(\text{root weight})^k$ were not statistically different between treatments ($P>0.05$). However, we observed an insignificant relationship between root and shoot dry weight biomass in both treatments. The exponent coefficient k in the above function was not significant when estimated for each treatment ($P>0.05$), which prevented us from making meaningful statistical comparisons of root and shoot partitioning between treatments.

Projected leaf areas were 36.5% greater in fertilized seedlings compared to the controls at harvest ($P<0.05$; Table 4.3). Control seedlings had an average projected leaf area of 0.346 m^2 at harvest, while the fertilized seedlings averaged 0.545 m^2 of leaf area.

Discussion

Short-term Physiological Changes Associated with Fertilization

Collectively, our results provide an outline of the physiological events leading to enhanced growth following loblolly pine fertilization. The order of physiological changes associated with enhanced leaf area and growth observed in this study are summarized as follows. Mean specific root respiration was elevated 49 days after fertilization relative to the controls, suggesting that increased N uptake and assimilation occurred following fertilization (Vessey and Layzell 1987, Ryan 1991, Zogg et al. 1996, Griffin et al. 1997, Lu et al. 1998). Within 16 days following fertilization, we observed statistically greater foliar N concentrations in the fertilized treatment, which coincided with increased A_{sat}. N concentration was correlated with A_{sat} ($R^2=0.47$) suggesting that foliar N directly impacted photosynthetic capacity. Our findings are consistent with some previous reports that investigated the effects of foliar N and/or N fertilization on A_{sat} of Pinaceae species (e.g. Mitchell and Hinckley 1993, Vapaavuori 1995, Schoettle and Smith 1999), but not others (e.g. Teskey et al. 1994, Zhang et al. 1997). Increased A_{sat} was due to greater photosynthetic electron transport capacity and efficiency and enhanced carboxylation capacity of foliage from fertilized plants. Changes in both the photosynthetic apparatus, and carboxylation capacity have been positively related to N fertilization and foliar N in Pinaceae species (Kellomaki and Wang 1997, Strand 1997, Wang and Kellomaki 1997, Cheng et al. 2000, Lavigne et al. 2001, Livonen et al. 2001). Less than 4 weeks following the initial rise in photosynthetic capacity, fertilized seedlings had statistically higher ground diameters and heights, demonstrating a short lag between enhanced photosynthetic capacity and improved growth. Further, projected leaf areas were 36.5% greater in fertilized seedlings at the end of the study, implying that enhanced photosynthetic capacity allowed for the production of greater leaf areas.

Enhanced leaf area production following fertilization is consistent with stand-level loblolly pine studies (Vose and Allen 1988, Albaugh et al. 1998). Over time, the relative difference in A_{sat} between the fertilized and non-fertilized treatments narrowed, which corresponded to the return of foliar N concentration in the fertilized seedlings to non-fertilized levels. Finally, A_{sat} returned to non-fertilized levels in the fertilized seedlings, which coincided with the return of electron transport and carboxylation capacities to non-fertilized levels. Our results suggest that the establishment of greater leaf areas in fertilized seedlings coincided with the retranslocation and dilution of foliar N, which negatively impacted A_{sat}.

While we realize that the exact sequence observed in this study may vary in a field setting, combined evidence from previous studies suggest the relative sequence of events leading to greater productivity may be similar. Results from multiple studies conducted at the USDA Forest Service's Southeastern Tree Research and Education Site (SETRES) in North Carolina suggest that fertilization had an effect on both photosynthetic capacity and biomass allocation, which resulted in the establishment of greater leaf areas and enhanced growth. Since 1992 (stand age of 6 years), fertilized stands at SETRES have received nutrient amendments that are considered optimal for loblolly pine (Allen 1987). Murthy et al. (1996) reported that foliage from fertilized stands had higher N concentrations and greater photosynthetic capacities than non-fertilized foliage following 1 and 2 years of nutrient additions, which coincided with the establishment of greater leaf areas in fertilized stands (Albaugh et al. 1998). Investigators also observed shifts in biomass allocation with fertilization, including an initial reduction in relative root biomass (Albaugh et al. 1998, King et al. 1999). Ultimately, these changes increased total biomass by 99% in fertilized stands after the fourth year of nutrient additions at SETRES (Albaugh et al. 1998). Specific P_{n} was generally the same in fertilized and non-fertilized stands during the eighth year (stand age 14 years) of fertilization at SETRES. Thus, following the establishment of greater leaf areas in fertilized stands, P_{n} returned to pre-fertilization levels. Collectively, studies from SETRES suggest that several of the short-term physiological processes responsible for the establishment of greater leaf areas in loblolly pine trees following fertilization are consistent with our findings in seedlings, including our observation that enhanced photosynthetic capacity is transient following fertilization.

Photosynthesis and Foliar Nitrogen Concentration

Our findings are consistent with the general trend in the literature that indicates foliar N to P_n are often positively correlated. Of the literature we examined, 15 out of 19 studies reported a positive relationship between foliar N and P_n in Pinaceae species (Mitchel and Hinkley 1993, Teskey et al. 1994, Chandler and Dale 1995, Vapaavuori et al. 1995, Murthy et al. 1996, Kellomaki and Wang 1997, Strand 1997, Wang and Kellomaki 1997, Roberntz and Stockfors 1997, Schaberg et al. 1997, Zhang et al. 1997, Boucher et al. 1998, Schoettle and Smith 1999, Jach and Ceulemans 2000, Lavigne et al. 2001, Livonen et al. 2001, Samuelson et al. 2001, Maier et al. 2002, Meir et al. 2002). The exact correlation between foliar N and P_n reported in the literature varies considerably. We found that 47% of the variance in A_{sat} was explained by foliar N when non-fertilized and fertilized treatments were pooled. The variable relationship between foliar N and P_n reported in the literature may be due to differences in study design, genetics, foliage age, crown position, seasonal changes in foliar N, and the range of N examined. Meir et al. (2002) reported significant differences among species in relation to foliar N concentration and photosynthetic capacities. Maier et al. (2002) examined the relationship between foliar N and A_{sat} in a 13-year-old loblolly pine stand subjected to 'optimum' nutrient amendments for over 5 years. Maier and his coworkers found an inconsistent relationship between leaf N and A_{sat}, which ranged from highly significant to insignificant depending on the season and the needle age. Vapaavuori et al. (1995) reported both seasonal and needle age effects on the relationship between N and P_n in Scots pine, citing the strongest relationship between N and P_n in developing needles during the middle of the growing season. Similarly, Schoettle and Smith (1999) reported the highest correlation between N and P_n in young lodgepole pine needles. Zhang et al. (1997) showed that, while light levels affected foliar N concentrations in loblolly pine, changes in foliar N did not alter P_n.

The large range in foliar N concentrations we observed in the fertilized treatment over the course of the study may in part explain why we detected a relationship between foliar N and A_{sat}. Foliar N in both treatments combined varied considerably over the course of the study, ranging from less than 0.8% to nearly 1.6%. The lowest foliar N concentrations we recorded were well below the reported critical level for foliar N of 1.2% (Allen 1987, Colbert and Allen 1996). Thus, photosynthetic capacity of foliage from fertilized seedlings may have been especially impacted by N additions in our study due to the low foliar N concentrations prior to fertilization. While we found a significant relationship between A_{sat} and foliar N when all data were analyzed, we observed no significant relationship between A_{sat} and N when foliar N ranged from 1.23% to 1.66%, suggesting that there is no effective change in photosynthetic capacity above the suggested critical N level of 1.2% in loblolly pine. A lack of foliar N concentrations sampled below the critical level may have precluded some previous researchers from documenting a positive relationship between P_n and N in loblolly pine, even after fertilization. For example, Samuelson et al. (2001) found no relationship between foliar N and P_n in loblolly pine having 1.2 to 1.5% foliar N. Similarly, Zhang et al. (1997) reported an increase in foliar N following fertilization, but found no change in loblolly pine P_n when foliar N concentrations ranged from 1.2 to 1.8%. In a long-term fertilization study, Gough et al. (*in press*) reported no correlation between P_n when foliar N varied from 1.13% and 1.40% in loblolly pine. In contrast, Murthy et al. (1996) reported elevated A_{sat} 1 year following initial applications of ‘optimum’ nutrient additions in loblolly pine, which corresponded to foliar N concentrations ranging from less than 0.7% to greater than 1.3%. Five years later Maier et al. (2002), in the same stands, found a mixed relationship between A_{sat} and N when foliar N ranged from about 0.7% to nearly 1.5%, indicating that the range of N concentration does not exclusively dictate whether a relationship exists between P_n and N.

Our results suggest that measurement timing following fertilization may be important in efforts to detect the effect of N on P_n since the rise and subsequent return of N and P_n to non-fertilized levels following fertilization occurred within a time frame of just over 100 days. We observed almost a two-fold increase and peak in N only 28 days following fertilization, which corresponded to a peak in A_{sat}, followed by a relatively sharp decline and return to control foliar N levels 106 days following fertilization. Other investigators have found similar short-term responses to fertilization (Foyer et al. 1994, Livonen et al. 2001). Thus, the pulse of N concentrated in the foliage may have retranslocated to developing foliage or other organs. Zhang and Allen (1996) found that fertilized 13-year-old loblolly pine retranslocated 75% of its foliar N, which corresponded to simultaneous reductions of N in old foliage and increases in developing foliage. The redistribution or retranslocation of N to developing foliage probably occurred in the current study since we observed greater leaf areas in the fertilized seedlings at harvest and a concurrent return of foliar N concentrations to control levels. However, it is interesting that foliar N levels eventually returned to pre-fertilization levels in the fertilized seedlings, rather than stabilizing at the suggested critical foliar N level of 1.2%. The transient nature of foliar N accumulation and subsequent dilution of N in developing photosynthetic tissues may explain why some studies fail to link fertilization with changes in foliar N or P_n, but do link fertilization to increased leaf area production. For example, Tang et al. (1999) found no change in P_n 5 years following fertilization applications in a loblolly pine stand that had already established greater leaf areas than the controls.

The photosynthetic efficiency and capacity of the electron transport chain and the carboxylation capacity of Rubisco were temporarily improved by fertilization and paralleled changes in foliar N. Fertilization and greater foliar N concentrations coincided with improved maximum photochemical efficiency (F_v/F_m), quantum efficiency of PSII (Φ_{PSII}), maximum rate of electron transport (J_{max}), and electron transport capacity ($V_{c,max}/J_{max}$) which allowed for a temporary improvement in photochemical quenching (qP) following fertilization. Higher photosynthetic capacity also coincided with a temporary enhancement in the carboxylation capacity of Rubisco ($V_{c,max}$). Improved photosynthetic capacity in relation to foliar N concentration of Pinaceae species is frequently due to more efficient and enhanced capacity of electron transport (Kellomaki and Wang 1997, Strand 1997, Wang and Kellomaki 1997, Cheng et al. 2000, Lavigne et al. 2001, Livonen et al. 2001) and/or greater carboxylation capacity (Kellomaki and Wang 1997, Meir et al. 2002). Our results suggest that N provided from fertilizer was initially used in part to build and maintain proteins associated with both electron transport and carboxylation (i.e. Rubisco). The return of the photosynthetic capacity in fertilized seedlings to non-fertilized levels indicates that diminished production and greater turnover of photosynthetic proteins and enzymes likely contributed to the mobile N pool. Subsequently, the labile N was probably retranslocated to newly developing foliage, which ultimately resulted in the dilution of foliar N we observed after the initial peak in foliar N following fertilization. The contribution of N from protein turnover to the mobile N pool is well documented (Spremulli 2000).

Partitioning Shifts and Nitrogen

While enhanced biomass production in fertilized seedlings was clearly related to increased photosynthetic capacity and leaf area growth, we cannot rule out that temporary shifts in biomass allocation may have also contributed to the building of additional leaf area. Brief shifts in biomass partitioning immediately following fertilization may have contributed to leaf area growth in fertilized seedlings. We can draw limited conclusions from our data since we did not observe a strong relationship between root and shoot dry weight biomass within treatments and because we did not measure changes in partitioning over time. Other studies suggest that shifts in biomass partitioning account for some, but not all changes in leaf area and productivity associated with loblolly pine fertilization (Vose and Allen 1988, Albaugh et al. 1998, King et al. 1999).

Changes in allocation associated with fertilization are inconsistent across ages in loblolly pine (Griffin et al. 1995, Gebuaer et al. 1996, King et al. 1999). Loblolly pine seedlings subjected to a lower fertility sometimes maintain an adequate uptake of nutrients via enhanced assimilate allocation to roots (Griffin et al. 1995, Gebuaer et al. 1996). However, King et al. (1999) found the opposite relationship between N and allocation in 7- and 9-year-old loblolly pine trees continuously fertilized for 2 and 4 years, respectively, which is in contrast to findings from seedling studies (Griffin et al. 1995, Gebuaer et al. 1996). Whether or not shifting allocation patterns following fertilization are partly responsible for the establishment of greater leaf areas remains unclear since fertilization may increase root allocation in trees (King et al. 1999). However, if foliar biomass partitioning is initially reduced in favor of root production following fertilization, the establishment of greater leaf areas proceeding nutrient additions must be due to C gain from enhanced photosynthetic capacity rather than reallocation of resources from roots to foliage.

Summary of Physiological Sequence Following Fertilization

Our results and collective findings from other studies discussed above suggest the following sequence of physiological events occurs following fertilization in loblolly pine: (1) R_r temporarily increases following fertilization due to increased uptake and assimilation demands. (2) A_{sat} improves as N accumulates in foliage and contributes to larger pools of proteins associated with electron transport and carboxylation. (3) Greater C fixation per unit leaf area generates photoassimilate necessary to build additional leaf area and improve growth. (4) N from fertilizer that was initially incorporated into photosynthetic proteins is retranslocated to developing foliage, decreasing the pool of photosynthetic proteins per unit leaf area and effectively reducing A_{sat}. (5) Improved growth continues in fertilized plants despite the return of photosynthetic capacity per unit leaf area to control levels due to enhanced photosynthetic surface area. Allocation shifts from the roots to foliage may also occur concurrently with enhanced photosynthetic capacity, but we were unable to detect a change in partitioning in our study.

Table 4.1. Fluorescence-derived parameters and light-saturated net photosynthesis (A_{sat}) in non-fertilized (NF) and fertilized (F) treatments measured 22 and 204 days following fertilization. Mean maximum photochemical efficiency of PSII (F_v/F_m), quantum efficiency of PSII (Φ_{PSII}), photochemical quenching (qP), non-photochemical quenching (qN) were monitored simultaneously with A_{sat} on the newest, fully elongated intact loblolly pine foliage. P-values were obtained from pairwise comparisons of mean values for non-fertilized (NF) and fertilized treatments.

		Days After Fertilization			
		22		204	
Variable	**Treatment**	**Mean**	**P-value**	**Mean**	**P-value**
F_v/F_m	**NF**	0.756	0.0021	0.790	0.2638
	F	0.800		0.777	
Φ_{PSII}	**NF**	0.058	<0.0001	0.051	0.8601
	F	0.107		0.054	
qP	**NF**	0.204	0.0009	0.244	0.7128
	F	0.313		0.273	
qN	**NF**	0.911	0.1966	0.931	0.9502
	F	0.901		0.932	
A_{sat} ($\mu mol/m^2/s$)	**NF**	2.789	0.0365	3.191	0.6683
	F	3.937		2.404	

Table 4.2. Maximum carboxylation rate of Rubisco ($V_{c,max}$, μmol $CO_2/m^2/s$), maximum electron transport rate (J_{max}, μmol RuBP/m^2/s), electron transport capacity ($V_{c,max}/J_{max}$), and foliar N concentration (N%) of sampled leaves prior to fertilization and 22 and 204 days following fertilization. $V_{c,max}$, and J_{max} were calculated from A/C_i curves performed on fully elongated intact loblolly pine foliage. P-values were obtained from pairwise comparisons of mean values for non-fertilized (NF) and fertilized (F) treatments.

				Days After Fertilization			
		Pre-fertilization		**22**		**204**	
Variable	**Treatment**	**Mean**	**P-value**	**Mean**	**P-value**	**Mean**	**P-value**
$V_{c,max}$	**NF**	21.4	0.7530	21.1	0.0001	18.8	0.1188
	F	22.4		44.1		24.1	
J_{max}	**NF**	25.8	0.5441	29.5	<0.0001	27.5	0.0936
	F	28.1		52.0		32.4	
$V_{c,max}/J_{max}$	**NF**	1.24	0.5848	1.43	0.0029	1.58	0.3189
	F	1.29		1.22		1.38	
N%	**NF**	0.925	0.5712	0.796	<0.0001	0.618	0.0571
	F	0.819		1.40		0.751	

Table 4.3. Projected leaf area (LA) of fresh foliage along with biomass and percentage of total biomass allocated to foliage, stem, and roots 211 days following DAP fertilization application of non-fertilized (NF) and fertilized (F) loblolly pine seedlings. P-values are associated with treatment mean comparisons of LA and absolute foliage, stem, root, and total dry weight biomass. Trends in dry weight partitioning (or relative allocation) of roots and shoots were compared between treatments using a regression approach. Since the relationship between root and shoot biomass for treatments individually was insignificant (P<0.05), meaningful statistical comparisons between treatments were not possible and are not presented below.

	LA (cm^2)	**Foliage**		**Stem**		**Root**		**Total** (g)
		Actual (g)	Percent (%)	Actual (g)	Percent (%)	Actual (g)	Percent (%)	
NF	3461	63.14	34.90	63.69	35.20	54.11	29.90	180.4
F	5452	99.94	35.13	110.3	38.79	74.20	26.09	284.4
P	<0.0001	<0.0001		<0.0001		0.0155		<0.0001

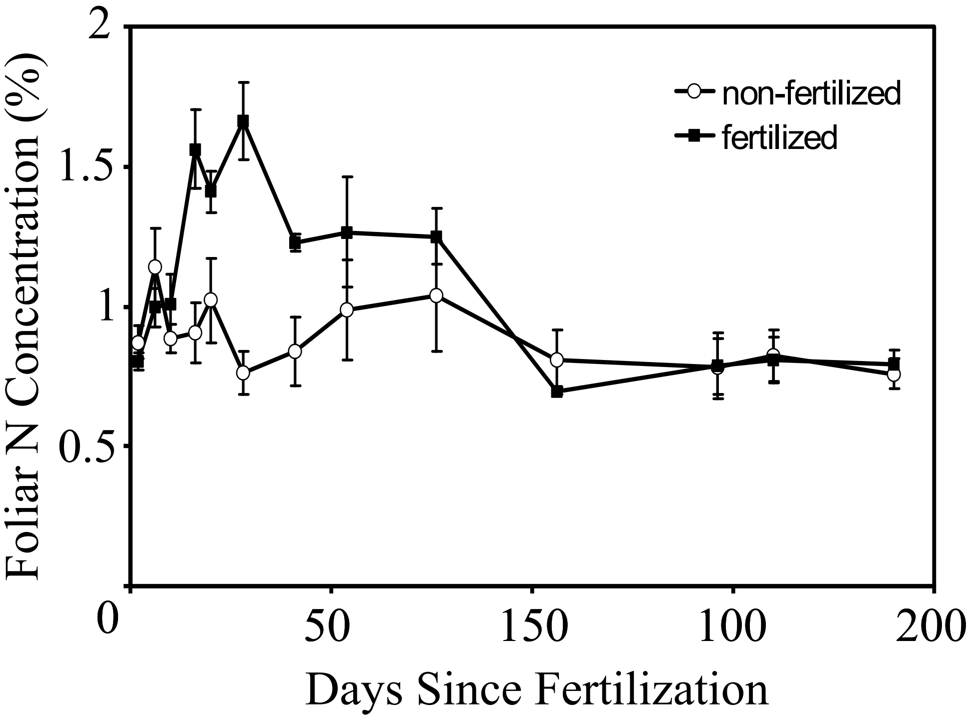

Figure 4.1. Foliar nitrogen concentration by percent in fertilized and non-fertilized loblolly pine seedlings following treatment application. Seedlings were grown in 56.8-L pots in sandy, unfertile soil. Fertilized seedlings received 1.85 g diammonium phosphate (DAP) per pot, which is an equivalent to 280 kg/ha. Nitrogen concentration was sampled from foliage collected for A_{sat} was measurements. Needles collected from four blocks were pooled for N analysis.

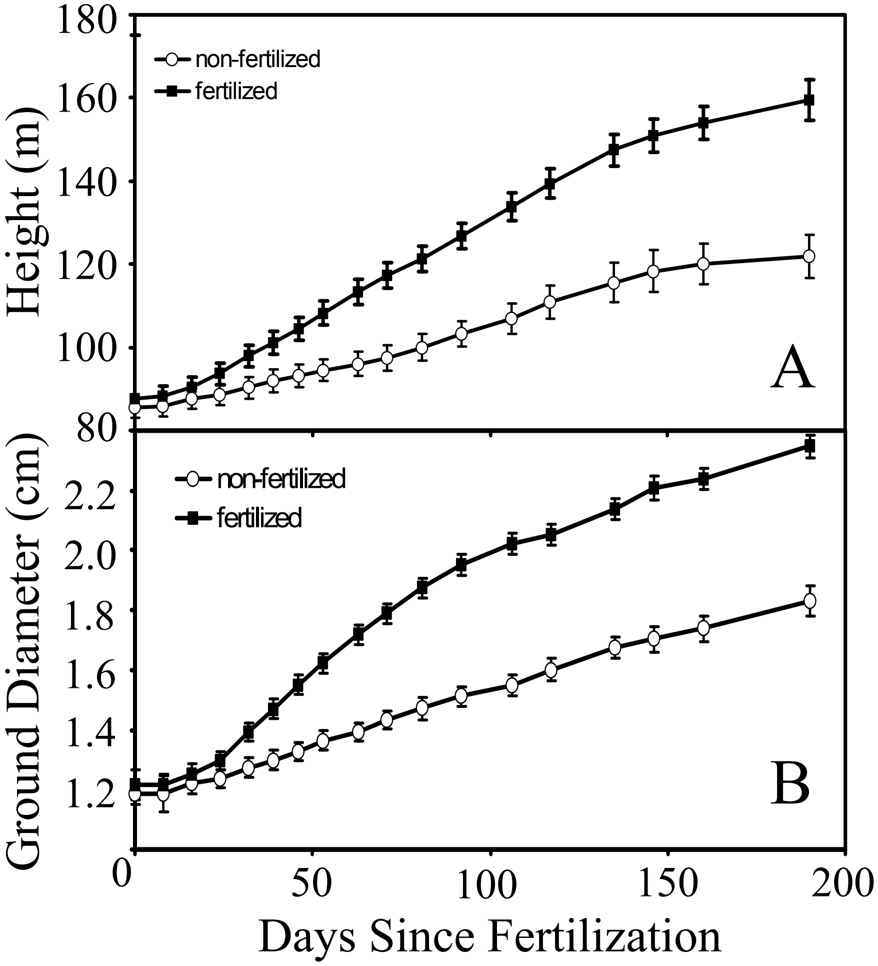

Figure 4.2. Height (A) and ground diameter (B) growth in fertilized and non-fertilized loblolly pine seedlings following treatment application. Seedlings were grown in 56.8-L pots in sandy, unfertile soil. Fertilized seedlings received 1.85 g diammonium phosphate (DAP) per pot, which is equivalent to 280 kg/ha.

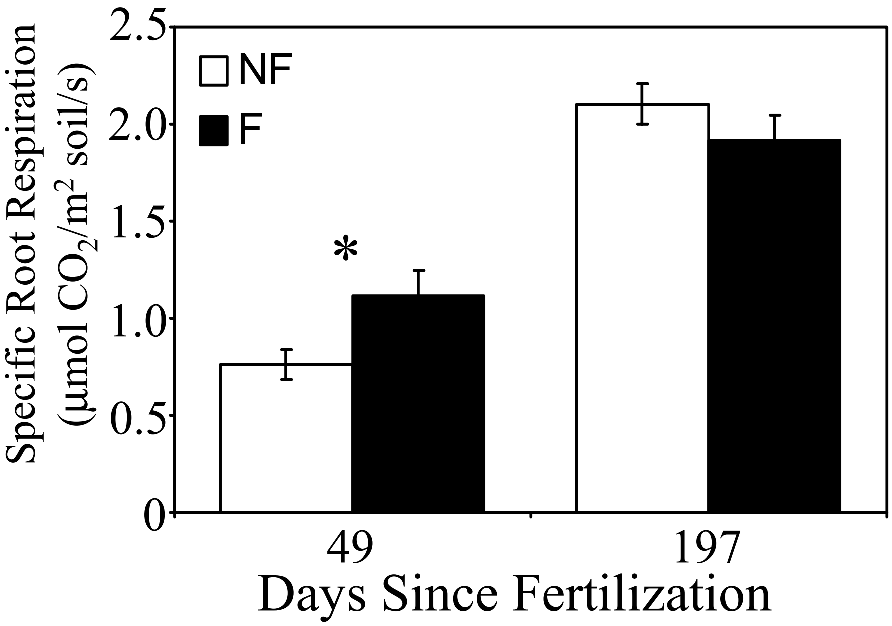

Figure 4.3. Specific root respiration in non-fertilized and fertilized loblolly pine observed on two measurement dates. Star above bars indicates significance between treatment means on the corresponding measurement date ($\alpha = 0.05$). NF=non-fertilized, F=fertilized.

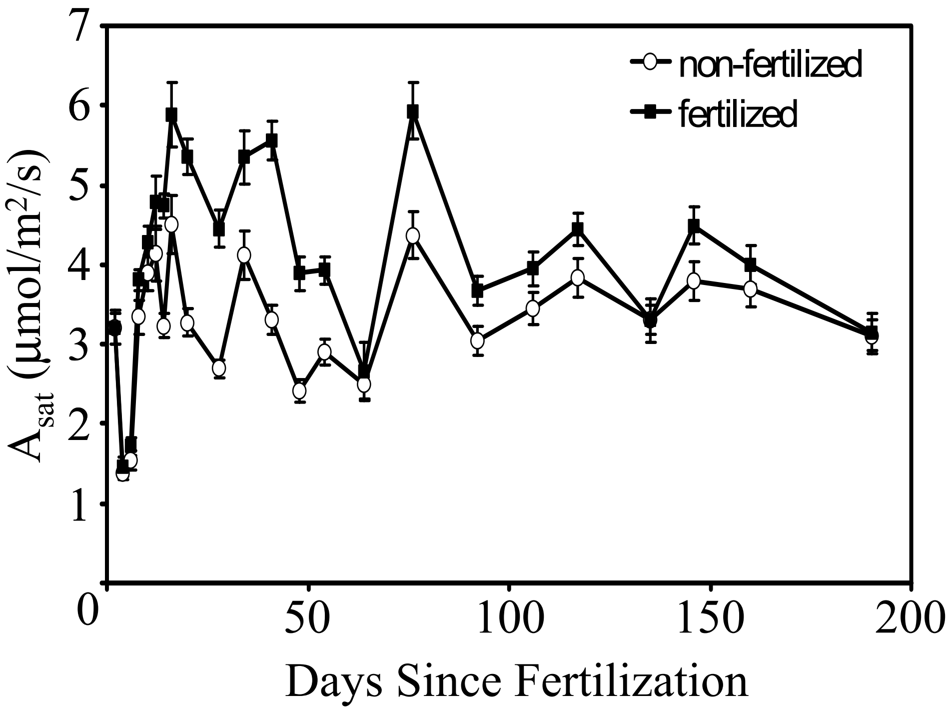

Figure 4.4. Light-saturated net photosynthesis (*A*$_{sat}$) in fertilized and non-fertilized loblolly pine seedlings following treatment application. Seedlings were grown in 56.8-L pots in sandy, unfertile soil. Fertilized seedlings received 1.85 g diammonium phosphate (DAP) per pot, which is an equivalent to 280 kg/ha. Foliar N concentration was measured on a subset of samples for which *A*$_{sat}$ was measured.

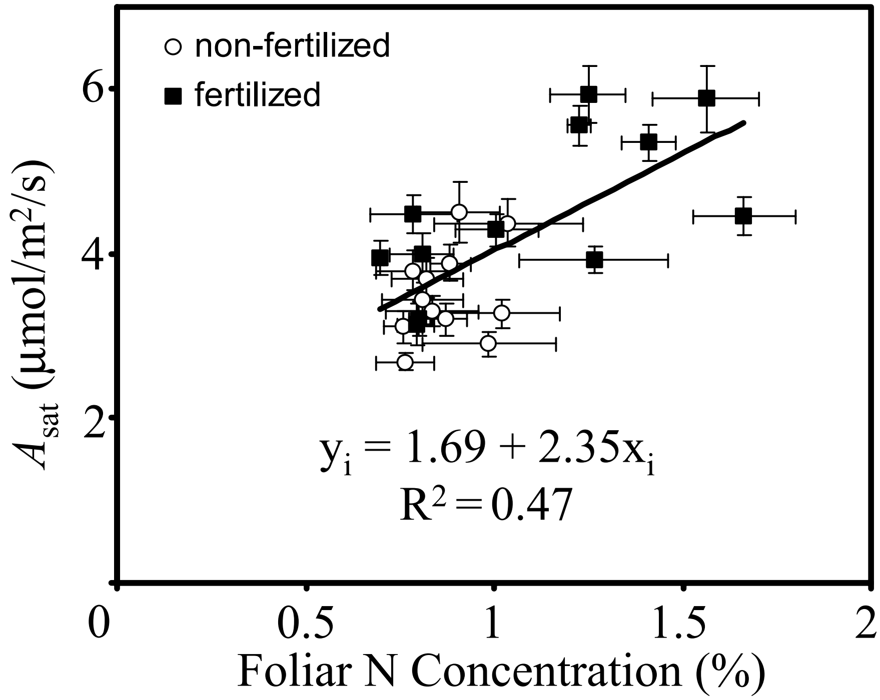

Figure 4.5. Relationship between foliar nitrogen concentration by percent and light-saturated photosynthesis (A_{sat}) in loblolly pine seedlings. Fertilized seedlings received a diammonium phosphate fertilization (DAP) rate of 280 kg/ha. Data are both non-fertilized and fertilized treatments. Foliar N concentration was measured on a subset of samples for which A_{sat} was measured.

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CHAPTER 5

An intensive examination of belowground carbon dynamics in loblolly pine (*Pinus taeda* L.) immediately following diammonium phosphate fertilization

Abstract. Forest soils serve as an immense global store of carbon (C) and a potential sink for atmospheric C. Forest management practices such as fertilization may enhance overall C storage in soils, yet changes in physiological processes following nutrient amendments have not been widely investigated. We intensively monitored belowground C dynamics for nearly 200 days following diammonium phosphate (DAP) fertilization of loblolly pine (*Pinus taeda* L.) seedlings grown in pots in an effort to examine the short-term effect fertilization has on processes involved in soil C sequestration. Soil respiration initially increased in fertilized pots relative to the controls. Subsequently, controls were temporarily became elevated, followed by a sustained increase in soil respiration in the fertilized treatment. Patterns in soil respiration over time reflected changes in autotrophic (root) and heterotrophic (microbial) components of soil respiration. Specific root respiration was greater in the fertilized treatment 49 day following fertilization and returned to control levels by the end of the study. In contrast, microbial respiration and microbial activity per soil C concentration remained depressed over the same time period. Total root biomass was greater in fertilized seedlings harvested at the end of the study. The elevated soil respiration levels we observed toward the end of the study were due to increased respiring root biomass. Collectively, our results indicate that fertilization, at least over the short-term, may increase soil C sequestration by increasing belowground biomass production and reducing microbial driven C turnover.

Introduction

Short-term changes in belowground carbon (C) dynamics associated with fertilization have not been intensively examined in loblolly pine (*Pinus taeda* L.). Yet in terms of C sequestration, belowground C storage may account for over 70% of the total C stored in forest ecosystems (Schlesinger et al. 2000). Common forest management practices, such as fertilization, may shift the C balance and enhance the total amount of C stored both above- and belowground (Valentini et al. 2000). Although aboveground C management in terms of biomass has been of prime importance to forest managers for decades, the potential implementation of C credits may present opportunities for C storage belowground. Thus, there is increasing interest in maximizing ecosystem C sink and storage strength via management (Woodwell et al. 1983, Turner et al. 1995, Field and Fung 1999, Banfield et al. 2002, Liski et al. 2002). Understanding how components of the belowground C cycle are altered when fertilization occurs will allow for greater understanding concerning the potential for enhanced belowground C storage through fertilization.

The literature generally states that soil microbial respiration, or heterotrophic respiration, declines shortly after nutrient additions (Kawalenko et al. 1978, Soderstrom et al. 1983, Smolander et al. 1994, Lovell and Hatch 1998, Thiurkkumaran and Parkinson 2000). Specific root respiration, or autotrophic respiration, on the other hand frequently increases with fertilization (Zogg et al. 1996, Griffin et al. 1997, Lu et al. 1998). Several researchers measuring soil respiration, the sum of autotrophic and heterotrophic respiration, have found no effect due to fertilization (Castro et al. 1994, Vose et al. 1995, Maier and Kress 2000), indicating that any decrease in microbial respiration could be offset by increases in root respiration. Other investigators report reductions in soil respiration following fertilization (Haynes and Gower 1995, Butnor et al. 2003). If fertilization has no effect or suppresses microbial driven C turnover, net belowground C sequestration may be enhanced when belowground biomass increases in fertilized stands. Increases in belowground biomass associated with fertilization have been shown to occur in loblolly pine (Albaugh et al. 1998, King et al. 1999).

While the literature collectively offers insight into the likely changes in belowground C dynamics following fertilization, there has been little investigation into autotrophic, heterotrophic, and total soil respiration simultaneously. In this study, we intensively examined changes in belowground C dynamics for over 190 days following diammonium phosphate (DAP) fertilization in potted loblolly pine. We monitored specific root and microbial respiration, and examined changes in belowground biomass at the end of the study. We hypothesized that soil respiration would reflect a changing balance between root respiration and microbial respiration following fertilization, initially increasing due to increased specific root respiration followed by a reduction due to suppressed microbial respiration rates. We expected that over the longer-term, soil respiration rates would climb in the fertilized pots relative to the controls since respiring root biomass would increase over time.

Methods

Study Design

An infertile soil (sandy, siliceous, thermic Psammentic Hapludult [Wakulla series], USDA Forest Service, unpublished data) was collected from the Forest Service's Southeastern Tree Research and Education Site (SETRES) located in Scotland County, North Carolina (35°N lat., 79°W long.) in the sandhills region. This soil was chosen for the experiment because of its relatively low fertility and the reported positive effects of fertilization on loblolly pine productivity when applied to this specific soil (Albaugh et al. 1998). The soil was sifted through a 6.4 mm screen to remove coarse woody debris and coarse roots, and homogenized by manually mixing the soil. Forty containerized 1-0 half-sibling (Westvaco Corp., family: WV-3) loblolly pine seedlings were planted in 56.8-liter containers (dimensions: 355.6 mm x 292.1 mm x 279.4 mm, Custom™ 2800C, Hummert International, Earth City, MO) in an effort to minimize physical restrictions on root growth. The total soil volume of each pot was approximately 25,000 cm^3. All pots initially received a low-level dose of fertilizer (equivalent to 15 kg/ha of elemental N) in order to prevent or alleviate nutrient deficiencies prior to actual fertilization treatments. Potted seedlings were randomly chosen and paired. A single pair of seedlings was placed next to each other on a greenhouse bench and served as one block. The study was arranged as a randomized complete block design with fertilized and non-fertilized treatments.

Growth Conditions

Seedlings were grown for approximately 1 year in a greenhouse prior to the fertilization application outlined below. Seedlings were watered frequently in order to prevent water stress. Excessive watering was avoided in efforts to prevent leaching of nutrients from soil. Plants were grown under a 16-hour photoperiod for the duration of the study.

Nutrient Additions and Prior Measurements

Within each block (two seedlings), seedlings were randomly assigned to either the fertilized or control treatment. Soil respiration, discussed below in detail, was measured prior to fertilization to ensure that both treatments were statistically identical prior to the fertilizer application. Each seedling assigned to the fertilization treatment received 1.85 g diammonium phosphate (DAP) (0.35 g N and 0.37 g P), which is the recommended operational level for seedling fertilization for loblolly pine (Jokela and Long 1999). The equivalent operational rate is 280 kg/ha or 50 kg/ha of elemental N and 106 kg/ha of elemental P.

Soil Respiration Measurements

Soil CO_2 efflux was measured using the LiCor 6200 infrared gas analyzer (IRGA) (LiCor Inc., Lincoln, Nebraska) and a dynamic closed cuvette chamber system (Janssens et al. 2000). A chamber was constructed from a plastic trash can lid, which was designed to provide a seal with the measurement pots in a way that covers and isolates the soil and entire seedling root system. The cuvette was constructed so that the chamber fit around and formed a seal with the seedling stem and minimized stem contributions to soil respiration measurements. A gas sampling line and a return port (from the LiCor) was attached to the chamber in order to provide both a gas input and output from the chamber to the IRGA. The internal volume of the chamber was 12000 cm^3 with a lid area of 1385 cm^2. Soil CO_2 efflux rates were determined by measuring CO_2 evolution over a 30-s period, which was used to calculate the respiration rate per unit ground area with the following equation:

$$\text{Soil } CO_2 \text{ efflux} = [(\Delta C/\Delta t)(PV_t/RT)] \,/\, \text{surface area of soil}$$

Where C = [CO_2], t = time, P = atmospheric pressure, V_t = system volume, R = universal gas constant, and T = temperature.

Soil respiration measurements were initially performed daily and later at less frequent intervals until physiological changes resulting from fertilization stabilized. Soil respiration was measured in the same sequential blocking order during every measurement period.

Specific Root Respiration

Specific root respiration was measured 49 and 197 days after fertilization using the LiCor 6200 with a 0.25-L cuvette chamber. A fine root (< 2 mm) area of approximately 10 cm^2 close to the soil surface were gently excavated and loose soil was shaken free. Roots were placed on a moist paper towel positioned on the cuvette to prevent them from rapidly drying. Respiration was measured over a 30-s sampling period as described above. Root surface area was determined using the WinRhizo 5.0A software (Regent Instruments Inc., Quebec, Canada) and all measurements were expressed on a per unit root surface area basis. A subsample of roots was sampled from each pot.

Specific Microbial Respiration

Specific microbial respiration was measured 49 and 197 days after fertilization using the LiCor 6200 with a 0.25-L leaf cuvette chamber. Approximately 350 cm^3 of soil was extracted from the soil profile of each pot using a 30-cm push-tube with a 2.5 cm diameter. All roots were removed and the soil was mixed and placed in an open aluminum weigh boat (10 cm x 2 cm), which was immediately positioned in the leaf cuvette chamber. After respiration measurements, soil was oven-dried for 2 days at 65°C and reweighed. Microbial respiration measurements were expressed on a per soil mass basis. Also, in order to provide an index of microbial activity in relation to soil C quantity, we compared absolute and relative microbial activity (i.e. soil respiration) per gram of soil C (Nohrstedt et al. 1989). Efflux was calculated as described above.

Soil Carbon Percent

C percent was determined for soil collected during specific microbial respiration measurements. Samples were analyzed for C percent by the USDA Forest Service Southern Research Station laboratory (RTP, NC) using a Carlo-Erba elemental analyzer (Model NA 1500, Fison Instruments, Danvers, MA).

Root Biomass Measurements

Seedlings were harvested 211 days after fertilization, and dry weight biomass allocation to the roots and aboveground organs were determined for the two treatments. Roots were thoroughly washed in an effort to remove intact soil following harvest. Organs were oven-dried at 65°C for 1 week and weighed in order to determine absolute and relative dry weight biomass for the two treatments.

Statistical Analysis

The effect of fertilization on soil respiration, specific root respiration, specific soil respiration, and soil C percent was examined using a time series analysis with 20 replications per treatment since all variables were measured at least twice over the study. The effect of fertilization on root and aboveground biomass was analyzed using a randomized complete block design. All statistical analysis was performed using the PROC GLM procedure in SAS (SAS Institute, Cary, NC).

Results

Soil Respiration Measurements

Soil respiration rates were significantly greater ($P<0.05$) in the fertilized pots relative to the controls beginning 4 days after the fertilizer application (Figure 5.1). This trend persisted for 13 days, followed by a reversal in which non-fertilized pots had signficantly higher soil respiration rates on days 26 and 40 ($P<0.05$). Soil respiration rates became significantly greater in the fertilized pots 47 days following the initial fertilization ($P<0.05$) and this trend persisted for the duration of the study. Soil respiration rates ranged among measurement dates from about 1 $\mu mol/m^2/s$ to approximately 6 $\mu mol/m^2/s$. However, the range in rates likely reflects changes in soil temperature over the study along with increasing root biomass and alterations in microbial activity making comparisons between dates less meaningful.

Specific Root Respiration and Specific Microbial Respiration

Specific root respiration rates of fertilized plants were statistically higher 49 days after fertilization ($P<0.05$; Figure 5.2) with rates in fertilized plants averaging 32% greater than the controls. No significant difference between treatments was apparent 197 days following fertilization. Microbial respiration rates were statistically less in the fertilized pots both 49 and 197 days after fertilization ($P<0.05$), with mean rates 42% and 32% less than the controls, respectively.

Percent Soil Carbon and Microbial Activity

Percent soil C (dry weight) from samples collected concurrently with root and microbial respiration measurements were not significantly different between the two dates or treatments (Table 5.1) and ranged from 0.483% to 0.726%. Relative microbial activity per soil C concentration was significantly lower in fertilized pots on both sampling dates ($P<0.05$; Table 5.1). Specifically, microbial activity per gram of soil C in fertilized treatments was 44 and 66% of the maximum observed in the non-fertilized treatments 49 and 197 days following fertilization, respectively. Comparisons are made in relative terms since absolute microbial activity is highly influenced by immediate environmental conditions such as temperature.

Root Biomass Measurements

Mean belowground dry weight biomass was 27% greater for fertilized seedlings (74.2 g) in comparison to non-fertilized seedlings (54.1 g) (Figure 5.3; $P<0.05$).

Discussion

Our results suggest that DAP fertilization may simultaneously reduce microbial-mediated C turnover and increase overall belowground biomass. If this pattern persists over the long-term, greater belowground C sequestration will occur by increasing the storage capacities of both the roots and soil. Initially, we observed a brief increase in soil respiration after fertilization followed by a temporary reduction in relative to the controls, which was likely linked to the depressed microbial respiration rates we observed 49 and 197 days following fertilization. The brief reduction in soil respiration was followed by a reversal in the observed trend in which soil respiration reached sustained higher rates in the fertilized pots for the remainder of the study. Initially, root respiration rates were greater in fertilized pots, which probably reflected increased uptake and metabolism of applied nutrients. However, root respiration rates returned to control levels when measured 197 days following fertilization.

Effects of Fertilization on Microbial Respiration

The reduction in microbial respiration we observed with fertilization is supported by previous reports, which found both short- and long-term reductions in microbial activity following N additions. Thiurkkumaran and Parkinson (2000) monitored microbial respiration in the laboratory from soil collected in a lodgepole pine (*Pinus contorta* Dougl.) forest over a 120-day period. They determined that ammonium nitrate and urea additions (of 188 kg N ha^{-1} and 300 kg N ha^{-1}) decreased microbial respiration, speculating that osmotic changes or ammonium toxicity were responsible. Microbial respiration was reduced in a long-term study performed on grazed swards receiving 200 N kg ha-1 as well (Lovell and Hatch 1998). A long-term study in Norway spruce (*Picea abies* L.) reported that cumulative N and P additions reduced microbial respiration in 40 to 60-year-old stands (Smolander et al. 1994). Stands were fertilized over a 20 to 30 year period, but had not been nutrient amended for at least 2 years prior to the study. Soil pH was not affected by fertilization. Thus, fertilization may have long-term effects as well. Similar to our findings, N additions reduced microbial respiration and biomass within weeks in a study performed on different coniferous forest podzols (Soderstrom et al. 1983). The reduction persisted for 3 to 5 years after a single fertilization of 150 kg N ha^{-1}

of ammonium nitrate. Similarly, Kowalenko et al. (1978) found that soil CO_2 evolution decreased in both a clay loam and sandy soil with fertilization over a 3-year period. Whether N additions reduce microbial respiration over the long-term in loblolly pine has not been investigated, but previously cited reports suggest that depressed microbial activity may be long-term and the effect may occur in multiple ecosystems. However, the duration of and extent of the effect of N on long-term soil C storage deserves attention.

Our results also indicate that microbial activity decreased relative to soil C concentration in fertilized pots. Our results are consistent with findings by Nohrstedt et al. (1989), who reported reduced microbial respiration per gram of soil C in Swedish Scots pine (*Pinus sylvestris* L.*)* forests receiving N amendments. In a review of the literature, Fog (1988) cited over 60 reports that found a reduction in organic matter decomposition following N additions to soils. Fog proposed the three following explanations for reductions in soil C turnover following fertilization cited in the literature: (1) N increases competition between microbes, which results in a net reduction of activity. (2) Biochemical changes occur in the enzymatic activity of microbes following N additions, which results the reduced activity of ligninases or other enzymes critical to decomposition. (3) N additions promote the formation of toxic or recalcitrant compounds. Our results do not provide insight into the specific mechanism responsible for the reduced microbial activity we observed.

Effects of Fertilization on Root Respiration

Our data are consistent with previous reports that indicate N fertilization increases root respiration, including in loblolly pine. In 155 day-old loblolly pine and ponderosa pine (*Pinus ponderosa* Dougl.) seedlings, above optimal ammonium additions increased specific root CO_2 efflux rates (Griffin et al. 1997). The authors concluded that root respiration was primarily driven by N uptake and detoxification of ammonia rather than growth since growth was actually stunted in plants receiving high N. Elevations in root respiration in response to N may depend on root size. In another loblolly pine study, Maier and Kress (2000) observed an increase in coarse root respiration, but not an increase in fine root respiration in response to N. In Douglas-fir (*Pseudotsuga menziesii*

Franco) seedlings grown in root boxes, specific root respiration and total root respiration increased with enhanced N (Lu et al. 1998). Zogg et al. (1996) determined that N fertilization did not significantly alter specific fine-root respiration rates in sugar maple (*Acer saccharum* L.); however, total root respiration was highly correlated with root tissue N concentration.

There are several potential reasons for increased specific root respiration with N fertilization, including the high metabolic (i.e. respiratory) costs associated with the conversion of NO_3^- to NO_2^- by nitrate reductase and the subsequent conversion of NO_2^- to NH_4^+ by nitrite reductase (Zogg et al. 1996). Also growth respiration costs of incorporating NH_4^+ into amino acids are significant (Vessey and Layzell 1987). Furthermore, maintenance costs increase with increasing protein concentration. When N additions increase whole-plant biomass, total maintenance respiration costs also increase (Ryan 1991).

Effects of Fertilization on Soil Respiration

Soil respiration became greater in fertilized pots relative to the controls due to an increase in total respiring root biomass, which is supported by our findings that fertilized seedlings had greater root biomass. Previous investigators found increases in loblolly pine seedling root biomass following fertilization (Griffin et al. 1997, Gebuaer et al. 1996). The fact that we observed a continued depression in microbial respiration rates over the study period along with the return of specific root respiration rates to control levels indicates that the increased root biomass associated with fertilization is responsible for enhanced soil respiration we observed during the second half of the study. Therefore, despite greater soil respiration rates, belowground C should accumulate following fertilization over the long-term if microbial rates remain depressed and belowground biomass remains greater in fertilized plants.

Our results also indicate that timing may be important in the detection of effects of fertilizer on soil respiration since we observed a reduction, an increase, and no difference in soil respiration rates following fertilization at different times over the course of the study. Some authors report that soil respiration remains unchanged with fertilization, which may be the result of a simultaneous decrease in microbial respiration and an increase in total root respiration. Maier and Kress (2000) measured soil respiration in l1-year-old loblolly pine stands fertilized for 4 years and found that fertilization reduced CO_2 evolution on several measurement days in stands when forest floor litter was included, but they observed no significant differences among fertilized and non-fertilized stands when litter was removed. Castro et al. (1994) similarly found that 4 years of urea-N fertilization in mature slash pine (*Pinus elliottii* Engelm.) forests did not result in changes in soil CO_2 evolution. No changes in soil respiration were detected in 3-year old ponderosa pine fertilized with 10 and 20 g m^{-2} of ammonium sulfate (Vose et al. 1995). The authors concluded that the lack of response to N might be due to the counteracting effects of autotrophic and heterotrophic respiration. Soil respiration rates in the current study were not significantly different between treatments for a short period of time centered around 25 days following fertilization. Previously discussed shifts in microbial and root respiration following fertilization in the current study indicate that opposing changes in the two soil respiration components resulted in no detectable difference in soil CO_2 efflux for a brief period. In particular, specific and total root respiration rates were elevated while microbial respiration was depressed.

Other studies report an increase or decrease in soil respiration when soils are fertilized. Johnson et al. (1994) reported an increase in soil respiration with N fertilization of chamber grown ponderosa pine seedlings and concluded that microbial respiration was unchanged following fertilization. Similar to our study, Johnson and coworkers attributed soil respiratory increases to increased respiring root biomass and enhanced specific root respiration following fertilization. In fact, Johnson and coworkers detected an increase in soil respiration beginning 6 months following nutrient applications, which is consistent with the timing of our observations. In contrast, Butnor et al. (2003) reported a reduction in soil respiration following 3 years of annual fertilization (11.2 g N/m^2) in loblolly pine stands located on the North Carolina piedmont. Haynes and Gower (1995) observed decreased soil respiration in continuously fertilized red pine (*Pinus resinosa* Ait.) stands. They attributed lower soil respiration rates in fertilized plots to decreased root and/or mycorrhizal growth or respiration rather than to a reduction in bacterial respiration.

The Double Benefit of Fertilization in Forest Management

In terms of soil C sequestration, N fertilization may have the dual benefits of increasing belowground biomass production and reducing soil C turnover. If fertilization has no effect or suppresses soil C turnover via a reduction in microbial activity, net belowground C sequestration will be enhanced when belowground biomass increases in fertilized stands (Figure 5.4). Our results offer only a short-term illustration of belowground C dynamics following fertilization, but they demonstrate the changing nature of soil C efflux following fertilization and further exemplify the need to simultaneously monitor the heterotrophic and autotrophic components of the C cycle over time. Further, a greater understanding concerning how N alters C substrate quality and/or affects microbial activity deserves attention. These studies will be crucial in understanding whether or not common forest management practices such as fertilization allow for soil C conservation or even increases in soil C capture and storage over multiple intensively managed rotations.

Table 5.1. Percent soil carbon and microbial respiration per gram of soil carbon in non-fertilized (NF) and fertilized (F) treatments 49 and 197 days following fertilization of loblolly pine seedlings. Standard error associated with the mean is provided. Superscripted letters represent multiple comparisons grouping (Tukey's HSD α=0.05).

Day	Treatment	Percent soil C	Microbial respiration per gram of soil C	
			absolute (μmol CO_2 g^{-1} soil C s^{-1})	**relative** to maximum (percent)
49	NF	0.551 ± 0.0282^{a}	2.88 ± 0.238^{a}	100 ± 8.25^{a}
	F	0.726 ± 0.0846^{a}	1.27 ± 0.113^{b}	44 ± 8.90^{c}
197	NF	0.483 ± 0.0131^{a}	1.90 ± 0.117^{ab}	100 ± 6.14^{a}
	F	0.499 ± 0.0157^{a}	1.26 ± 0.096^{b}	66 ± 7.68^{b}

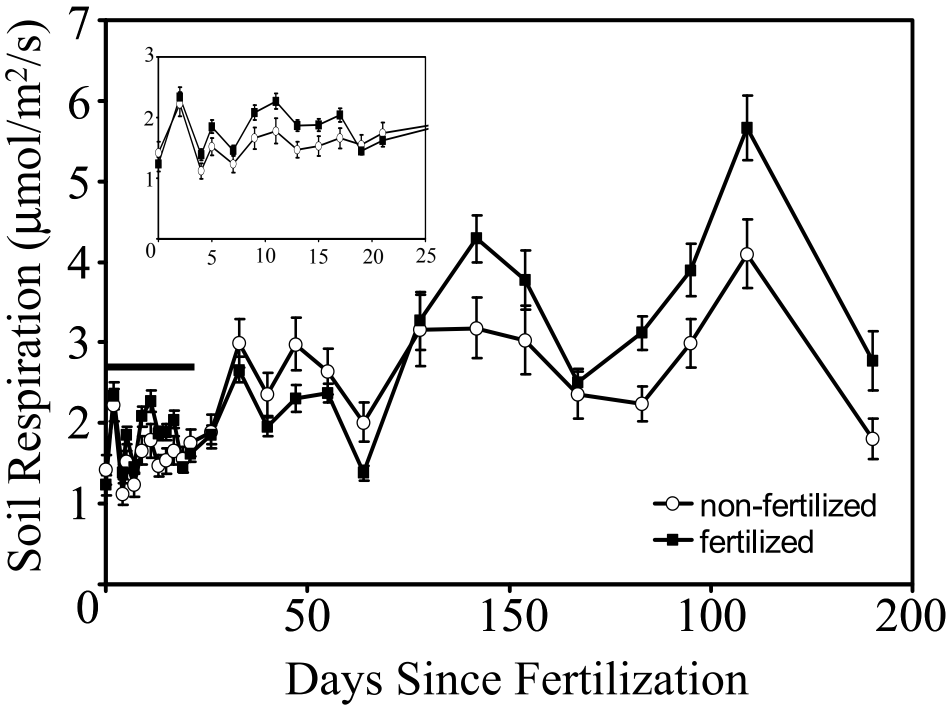

Figure 5.1. Soil respiration in fertilized and non-fertilized loblolly pine seedlings following treatment application. Seedlings were grown in 56.8-L pots in sandy, unfertile soil. Fertilized seedlings received 1.85 g diammonium phosphate (DAP) per pot, which is an equivalent operational rate of 280 kg/ha. The inset figure is soil respiration on measurement dates during the first 25 days following fertilization.

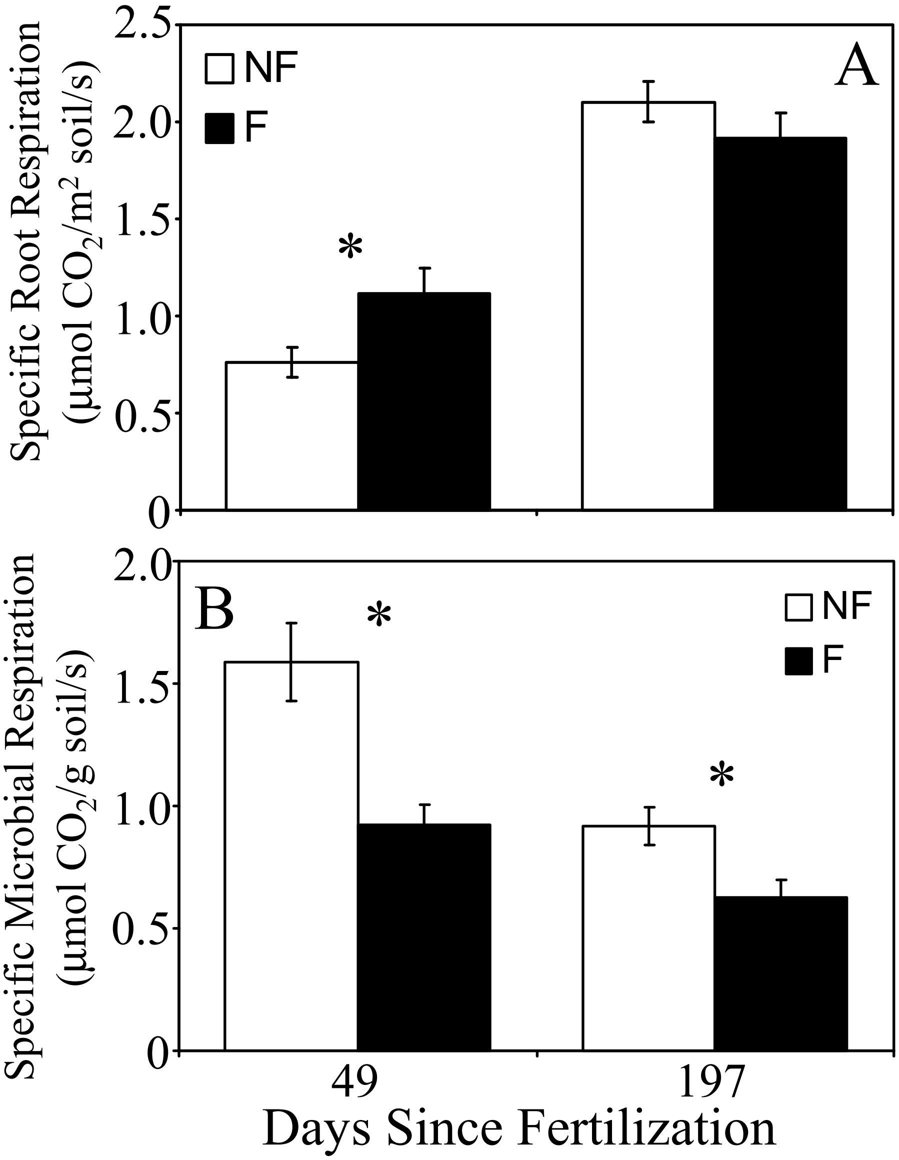

Figure 5.2. Mean specific root respiration (A) and mean microbial respiration (B) in non-fertilized and fertilized loblolly pine on two measurement dates. Star above bars indicates significance between treatment means on the given measurement date (α=0.05).

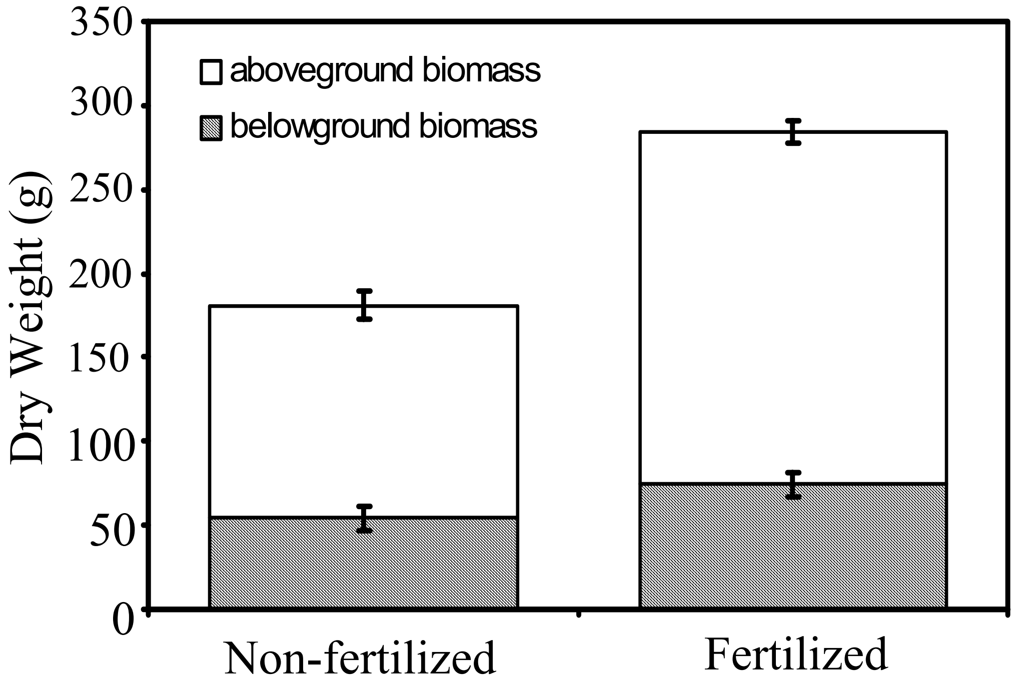

Figure 5.3. Aboveground and belowground dry weight biomass in fertilized and non-fertilized loblolly pine seedlings 207 days after treatment application. Seedlings were grown in 56.8-L pots in sandy, unfertile soil. Fertilized seedlings received 1.85 g diammonium phosphate (DAP) per pot, which is an equivalent operational rate of 280 kg/ha.

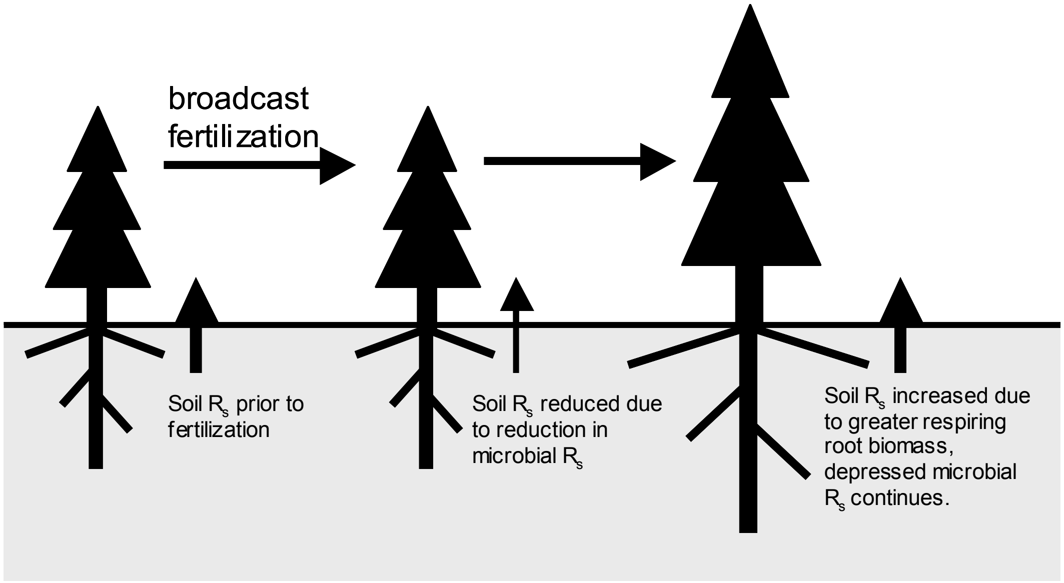

Figure 5.4. Belowground changes in carbon flux following fertilization. Fertilization increases belowground carbon storage by reducing microbial activity and simultaneously enhancing belowground biomass production. Note that although soil respiration is greater than prefertilization rates over the longer-term, overall belowground carbon sequestration will increase since carbon efflux is primarily due to root respiration and not microbial-mediated carbon turnover.

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CHAPTER 6

Synthesis

Novel Contributions to the Current Body of Knowledge

The major findings and contributions to the current body of scientific knowledge presented in this dissertation are summarized as follows. Chapter 2: Spatial and temporal variation in soil respiration is only weakly related to soil carbon (C), coarse woody debris (CWD), and root biomass sampled directly below the soil CO_2 efflux measurement chamber. Chapter 3: Patterns in soil CO_2 efflux over a rotation differed between the South Carolina sites and the Virginia piedmont sites sampled. Soil CO_2 was not related to stand age on the South Carolina plain sites and strongly positively related to age on the Virginia piedmont site to the degree that these sites are representative of their locations within the region. Further, cumulative soil C efflux on the South Carolina coastal plain over 20 years was estimated to be 24% greater than on the Virginia piedmont. Chapter 4: A sequence of events occurs following loblolly pine seedling fertilization that allows for the establishment of greater leaf areas and enhanced growth associated with nutrient amendments. Fertilization is followed by a brief increase in root respiration and a transient rise in photosynthetic capacity, which parallels changes in foliar nitrogen (N). Leaf area accumulation and subsequent growth following fertilization is at least partially related to temporarily enhanced C fixation per unit leaf area. Chapter 5: Diammonium phosphate (DAP) fertilization alters the contribution of autotrophic and heterotrophic respiration to total soil CO_2 efflux over the short-term. Enhanced specific root respiration is short-lived, while suppressed microbial respiration following fertilization was maintained over the course of the nearly 200-day study. Growth of respiring root biomass over time increased total soil respiration, but the sustained suppression of microbial activity following fertilization indicates that fertilizer could enhance soil C storage if the trend persists over the longer-term.

Implications of Findings and Recommendations

The findings outlined above have several ramifications in the context of both basic and applied science dealing with C cycling. In terms of more basic science, results presented here provide some understanding of sampling limitations associated with linking small chamber studies to soil characteristics. While spatial and temporal patterns of soil CO_2 efflux are highly variable within and across sites, modeling results presented in Chapters 2 and 3 indicate that soil respiration can be quantified using soil temperature and stand age as explanatory (or input) variables on the sites we examined. Multiple studies have shown that soil temperature is a major driver of soil respiration in loblolly pine stands (Maier and Kress 2000, Pangle and Seiler 2002); however, no prior studies in loblolly pine stands examined the effect of soil characteristics on soil respiration across broad spatial and temporal scales. Results presented in Chapter 2 indicate that soil characterization below the measurement cuvette resolves little additional variance in soil respiration, at least given the parameters measured and the methodology used. Soil C, CWD, and root biomass are probably only slightly related to soil respiration measured using small chambers because CO_2 originating in the profile does not necessarily travel vertically in the profile (Weerts et al. 2001, Susfalk et al. 2002). Also, variability in soil C substrate quality was not examined in the studies presented in this dissertation. Determination of substrate quality and availability may have resolved additional variance in soil respiration since not all soil C is readily decomposed by microbes (Progar et al. 2000, Wang et al. 2002, Davis et al. 2003). Another novel finding is the discovery that both above- and belowground C dynamics exhibit transitory patterns as demonstrated by the short-term greenhouse fertilization study (Chapters 4 and 5). The rapid and temporary changes in physiology observed following fertilization indicate the need for researchers to consider short-lived changes in physiological processes. Several authors have cited an inconsistent relationship between fertilization and physiological events such as enhanced photosynthetic capacity, possibly because investigators began monitoring processes either before or after physiological changes occurred (Tang et al. 1999, Samuelson et al. 2001). The brief, yet critical rise in photosynthetic capacity appears responsible for the establishment of leaf area, which permits enhanced growth.

Synthesis

Several more applied scientific questions were addressed that dealt with C cycling in the context of forest management and the broader topic of forest C sequestration as a tool to mitigate greenhouse gas emissions. Results presented in this dissertation further illustrate the extent to which forest management may impact both above- and belowground C sequestration processes. The Department of Energy (DOE) estimates that forest and agricultural management in the United States will play a critical role in C sequestration for the next 20 years. DOE estimates that biological CO_2 "scrubbers" will account for the sequestration of approximately 20% of the total greenhouse gas emissions (DOE 2003). DOE estimates that the reductions in greenhouse gas emissions from newer, cleaner energy technologies after 2020 will far outweigh the more modest contribution of forests and agriculture in offsetting rising atmospheric CO_2 levels. The results presented here express the sensitivity of the C balance to management, specifically through regional differences in management (i.e. bedding and burning) and common practices throughout the region such as forest fertilization. Because the loblolly pine ecosystem represents the largest managed forest ecosystem in the United States occupying over 13 million hectares (Wear and Greis 2002), intensively managed loblolly pine forests have the potential to play a significant role in C sequestration and offsetting greenhouse gas emissions. All of the papers presented in this dissertation directly or indirectly address the role forest management has on C dynamics and the topic remains relevant given the rising concern in global warming.

While foresters are typically concerned with aboveground biomass production, the role of belowground C storage may be equally as important in the context of mitigating atmospheric inputs of CO_2 (Schlesinger and Andrews 2000). Assuming the projections provided by DOE concerning the relative contribution of forest and agricultural C sequestration are correct, the role of forest management in greenhouse gas mitigation is most significant at the present. In order for loblolly pine forest management to be immediately effective as a mechanism to mitigate greenhouse gas emissions, findings presented here and other information from the current body of knowledge must be integrated in order to make immediate recommendations to forest managers, policymakers and economists. The effect common forest management practices have on both above- and belowground C sequestration must be considered. Specifically, common practices including fertilization, thinning, and competition control along with rotation length should be considered. Currently and over the short-term, simple qualitative recommendations should be made to forest managers regarding management procedures known to promote C storage on managed land. For example, fertilization generally improves production on a site for a given length of time and may reduce soil C turnover. However, the exact quantitative impact of these management regimes on belowground processes are poorly understood. Combined results from this dissertation (Chapter 5) and previous reports overwhelmingly suggest that fertilization reduces soil C turnover by suppressing microbial respiration, but long-term effects have not been quantified (Soderstrom et al. 1983, Fog 1988, Smolander et al. 1994). Over the longer-term, more sophisticated methods of screening and quantifying the effects of forest management on soil C sequestration may be instituted as C cycling predictions become more quantitatively comprehensive and accurate. Robust process models in combination with remote sensed input variables, for example, may allow managers to track above- and belowground C sequestration and possibly modify existing management practices in an effort to encourage maximum C storage for large land areas.

Results presented in this dissertation demonstrate that regional differences in soil CO_2 efflux exist due to the extensive range of climates, cultural practices, and inherent site productivities within the range of loblolly pine. The equations presented in Chapter 3 should be useful in estimating total C efflux from managed loblolly pine stands on the southeastern coastal plain and the piedmont since soil CO_2 efflux calculated using these models closely matches trends in soil respiration measured from other sites (Figure 6.1). Results presented in Chapter 3 indicate that quantitative C budget estimates for managed loblolly pine stands must take into account the distinct variability within the region. In the future, comprehensive remote sensed soil temperature and stand age/volume data collected across the region will be useful in providing model inputs and allowing for the prediction of C fluxes on a region-wide scale.

Future Work

Additional investigation is required to fully provide an understanding of how management affects autotrophic and heterotrophic respiration across spatial and temporal scales. In the context of forest soil C sequestration, soil respiration does not necessarily reflect mineral soil C turnover from microbial decomposition since microbial and root respiration change over time (Chapter 3 and 5). If one goal of the forest manager is to sequester more C in soils, an understanding of heterotrophic activity is crucial in efforts to reduce C turnover and thereby increase soil C storage. Understanding changes in heterotrophic respiration over time in the context of management will allow for a greater mechanistic understanding of C turnover, which is lacking in the current literature. For example, Fog (1988) cited over 60 studies that reported reductions in soil C turnover following N fertilization. However, Fog and more current authors cite the need to further understand processes and mechanisms responsible for impacting C cycling (Rotter and Danish 2000, Rustad et al. 2000). Thus, more information is required concerning the long-term impact of forest management practices on soil C storage and specifically how heterotrophic and autotrophic contributions to total soil CO_2 efflux change over time and with different management regimes. General questions that should be addressed through future work given findings presented in this dissertation are as follows:

1. What is the net soil CO_2 efflux from all managed loblolly pine forests in the region?

2. How does autotrophic and heterotrophic respiration vary across spatial and temporal scales?

3. How is autotrophic and heterotrophic respiration altered over the long-term by fertilization, fire, and bedding?

4. Can intensive management increase the soil C pool over the long-term?

5. How does C “quality” affect heterotrophic activity and how does forest management alter “quality”? For example, do fire and fertilization impact C quality?

6. How does fertilization affect physiological processes over the short-term and long-term when trees are fertilized? Are mechanisms of enhanced growth following forest fertilization identical for seedlings and trees?

7. What explains the inconsistent relationship observed between foliar N concentration and fertilization?

8. What is the role of allocation shifts in enhancing leaf area and subsequent growth following fertilization?

Several of the above questions are especially relevant due to current concerns related to global warming. Many of the above questions will require input from multiple specialists, including plant ecophysiologists, soil scientists, process modelers, microbiologists, GIS specialists, and forest management practitioners. Pertinent basic and applied scientific data are required to better resolve many of the uncertainties involving the effectiveness of forest management as a method of sequestering C belowground in an effort to offset greenhouse gas emissions.

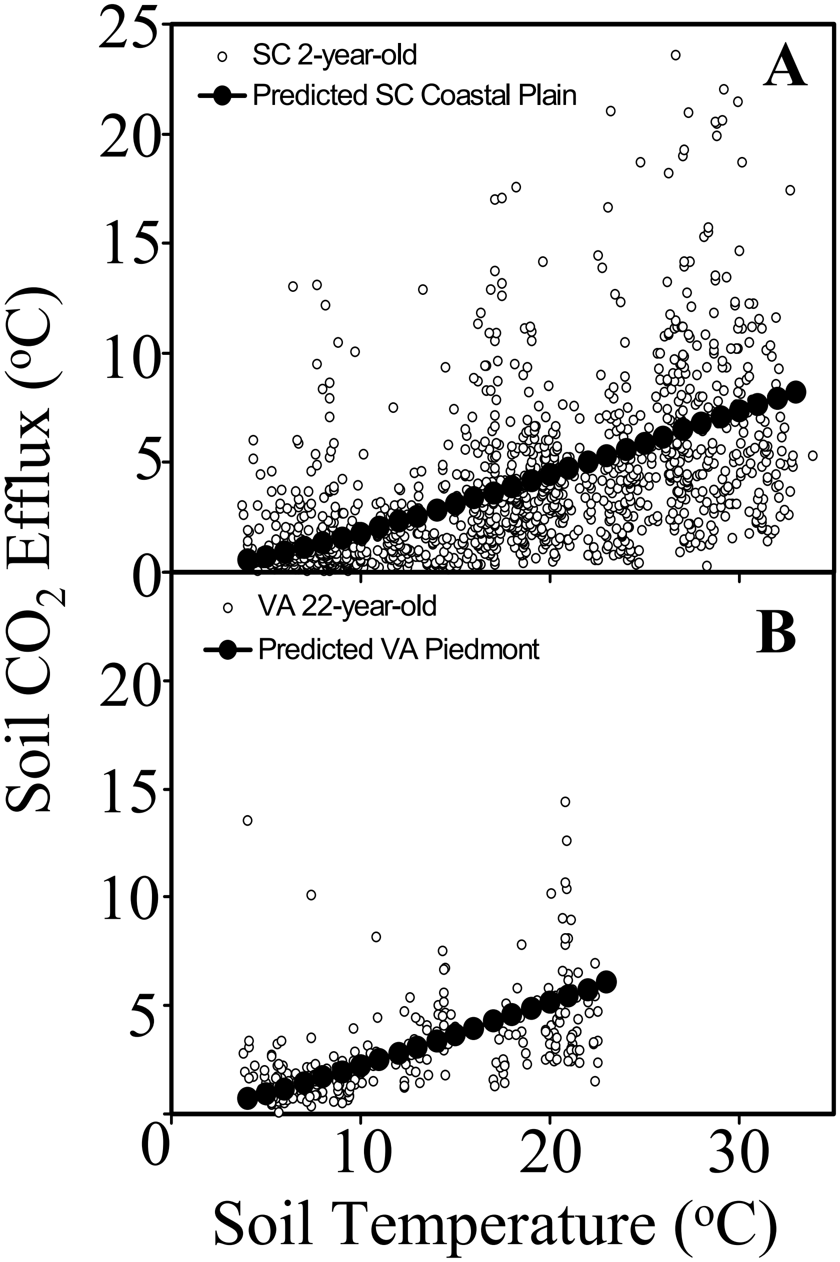

Figure 6.1. Soil CO_2 efflux plotted against soil temperature data collected from independent studies on the South Carolina coastal plain (A) and the Virginia piedmont and predicted lines generated for the corresponding area of the region using equations presented in Chapter 3. Predicted lines were generated using the “simple” model, which requires soil temperature and stand age inputs.

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Vita

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Christopher Michael Gough was born in Madison, Wisconsin in 1975 and attended primary and secondary schools in Oak Ridge, Tennessee and Fredericksburg, Virginia. While earning his B.S. in biology from James Madison University in 1997, Chris wrote an honors thesis based on his undergraduate research titled "The DNA sequence of an *Arabidopsis* α-glucosidase". After receiving his undergraduate degree, Chris worked in the biology department at JMU under Dr. Jon Monroe on the molecular and biochemical characterization of *Arabidopsis thaliana* α-glucosidases. Chris completed his M.S. in Forestry under the direction of Dr. John Seiler in 2000 with a focus in tree physiology. His M.S. thesis was titled "Environmental influences on gas exchange in fertilized and non-fertilized stands of loblolly pine". Upon completion of his Ph.D., Chris will become a post-doctoral researcher with` Dr. Peter Curtis at the Ohio State University in the department of Evolution, Ecology, and Organismal Biology. Chris will continue investigating carbon cycling in Northern Michigan hardwood forests.