The Short-term Effects of Fertilization on Total Soil CO_2 Efflux, Heterotrophic, and Autotrophic Respiration of Loblolly Pine (*Pinus taeda* L.)

By

Michael Christopher Tyree

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John R. Seiler, Chair

Thomas R. Fox

Wallace M. Aust

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ABSTRACT

Fertilization is a common, cost effective treatment for increasing forest productivity within managed forests of the southeastern United States. However, little is known about how fertilization affects the below-ground processes that drive soil CO_2 efflux in loblolly pine (*Pinus taeda* L.). A thorough understanding of below-ground carbon dynamics is necessary for the estimation of net ecosystem productivity and the carbon storage potential of these managed systems.

In April 2004, we began monitoring total soil CO_2 efflux (E_C), heterotrophic (R_H), and root respiration (R_R) in response to fertilization with diammonium phosphate (DAP). Respiratory components were measured prior to fertilization, weekly following fertilization, and bi-weekly after respiratory components stabilized using a dynamic closed chamber and an infrared gas analyzer. We found that E_C differed significantly ($P<0.0001$) between fertilized and unfertilized plots, but the direction was dependent on date. In the early period of the study, fertilized plot values were lower than control plots. However, by the latter periods fertilized plot values returned to control levels except for one sampling date in March 2005 when fertilized plot values were greater then control plots. Heterotrophic respiration was consistently and significantly ($P=0.0002$) lower in fertilized plots. Root respiration was significantly ($P=0.0597$) increased in fertilized plots when analyzed over the study and showed a 20% increase due to fertilization. We concluded that an increase in R_R and possibly root biomass was enough to balance the decrease in R_H leading to no difference in E_C later in the growing season.

We performed a pair of greenhouse studies to observe the effects of fertilization in the form of diammonium phosphate (DAP) on R_R. The objectives were to determine how nutrient additions initially affect R_R in one-year-old loblolly pine seedlings. Secondly, we wanted to determine if Captan [N-(trichloromethylthio) cyclohex-4-ene-1, 2-dicarboximide], a mild fungicide, could be used to reduce or eliminate ecto-mycorrhizae upon visual inspection. Both studies showed that initially, at a high rate (100 ppm N and 49 ppm P) of fertilization, R_R was significantly ($P\leq0.10$) increased relative to seedlings that did not receive fertilization. This increase was only temporary with rates returning to, or decreasing below, control levels by the end of the study. No consistent trend was found between low (25 ppm N and 13 ppm P) and moderate (50 ppm N and 25 ppm P) rates of fertilization. Captan was shown to generally have no affect on R_R. Captan and fertilization both showed (visual inspection) a decrease in fine-roots and mycorrhizae, which could explain the reduction in respiration rates observed in these treatments by the end of the studies.

Dedication

This document is dedicated to my loving wife Jennifer Tyree. Thank you for your never ending support and patience, without which, none of this could be possible.

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CHAPTER 1
Introduction

Background

Greenhouse gases, such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), have steadily increased in the last 150 years. Carbon dioxide, the major component of these gases is currently blamed for 60% of global climate change, [Intergovernmental Panel on Climate Change (IPCC) 1995]. From the start of the industrial revolution the concentration of atmospheric CO_2 has increased from approximately 280 parts per million by volume (ppmv) to about 365 ppmv (IPCC 1995, Schlesinger 1997, DeLucia *et al*. 1999, Lal 2003).

Since the late 19th century, the planet has experienced a rise in mean temperature of about 0.3 to 0.6°C, and a rise of 10 to 25-cm in sea level (IPCC 1995). The tendency of greenhouse gases to trap long-wave radiation as it is radiated back from the earth's surface makes it highly likely that we will continue to see future increases in global temperatures. The Intergovernmental Panel on Climate Change (IPCC) has developed a number of scenarios based on general circulation models (IS92 models a thru f). These models predict future greenhouse gas emissions based on future population growth, land use, technological advances, and economic growth. Depending on the model, the IPCC (1995) projects mean global temperature to increase by 1.0 to 3.5°C by the year 2100. Increasing temperature and CO_2 concentrations have the potential to cascade into further global changes. The IPCC's (1995) general circulation models project an additional rise in sea level of 19 to 95-cm due to thermal expansion and melting of ice-sheets. This could result in disturbances of weather patterns, in particular, the hydrologic cycle. Accompanying these changes could be shifts in global distributions of flora and fauna. Some scientists believe a rise in temperature and CO_2 concentrations will have eco-physiological effects such as increased: N-mineralization, decomposition of organic matter, fine-root turnover, and ultimately plant productivity (DeLucia *et al*. 1999, Gill and Jackson 2000, Rustad *et al*. 2001, Hamilton *et al*. 2002). Rustad (2001) speculated

that ecosystems in higher latitudes would show these effects to a greater degree because they are more limited by temperature.

In 1992, world governments concerned about the trend of rising greenhouse gas concentrations adopted the United Nations Framework Convention on Climate Change (UNFCCC), with the objective to "... stabilize atmospheric concentrations of greenhouse gases that would prevent dangerous human interference with the climate system..." (UNFCCC 2002). The 186 participating governments comprise two main groups: industrialized (Annex I Parties) and developing countries (non-Annex I Parties), which account for 41 and 145 countries, respectively. The United States as well as 23 other Annex I Parties are further classified into a group called Annex II Parties. Annex II Parties are obligated to financially assist non-Annex I parties and countries with economies in transition, to make the transfer to more environmentally friendly technologies; so long as these developing countries are able to report measures taken to address climate change. Although not-legally-bound, Annex I Parties were striving to reduce their greenhouse gas emission to 1990 levels by the year 2000 (UNFCCC 2002).

In 1997, in an effort to strengthen the UNFCCC, over 150 governments negotiated a more stringent, and legally-binding, set of requirements called the Kyoto Protocol. Industrialized countries that voluntarily ratified the protocol could satisfy these requirements by two means: (i) reducing their greenhouse gas emissions by at least 5% under 1990 levels by the years 2008 to 2012, and/or (ii) by obtaining carbon credits by increasing the amount of atmospheric carbon captured by carbon sinks as described by Articles 3.3 and 3.4 of the protocol (Murray *et al*. 2000).

The earth has historically stayed in a relative balance with carbon constantly moving from source to sink. Rustad (2000) estimated that the earth contains about 10^8 Pg of carbon, which is constantly being transferred among seven major pools. The vast majority is stored within the earths crust as geologic formations (90,000,000 Pg). An additional 38,000 Pg of carbon is stored in the ocean as dissolved carbonates, and another 10,000 Pg as gas hydrates. Fossil fuels, total biomass of terrestrial plants, and soils contain 4,000, 560, and 1,600 Pg C, respectively. This leaves a small fraction of the earth's carbon (750 Pg) in the atmosphere. It has not been until recently (last 200 yrs) that anthropogenic sources of CO_2 have begun to increase atmospheric concentrations

beyond geologically recent natural cyclical levels. Changes in land-use and the burning of fossil fuels for energy have contributed about 5 to 8 and 1.8 Pg C yr^{-1} to the atmospheric pool, respectively (Raich and Schlesinger 1992, Lal 2003). Raich and Schlesinger (1992) estimate soils emit 50 to 75 Pg C yr^{-1}, making CO_2 flux from soils the second largest flux in the global carbon cycle, an order of magnitude greater then CO_2 emissions from fossil fuel burning.

Tans *et al*. (1990) proposed, based on box and 3-D ocean circulation models, that 26 to 44% of CO_2 emitted by burning of fossil fuels is absorbed by oceans, leaving absorption by terrestrial sinks to balance the carbon budget. Schlesinger (1997) estimates that forests account for up to 75% of all carbon stored in terrestrial ecosystems, and are responsible for about 40% of the carbon flux between the atmosphere and the biosphere. Forests have the capability to act as giant carbon sinks by removing inorganic carbon from the atmosphere and fixing it as plant biomass. Carbon finds its way to the forest floor as fallen plant and animal material where it is either released back into the atmosphere by soil microbes and fire, or incorporated into the soil for long-term sequestration. Forests and forest soils can act as either carbon sinks or carbon sources, and only by understanding the different mechanisms that determine the shift between source and sink can forests be managed to sequester carbon long-term.

Delcourt and Harris (1980) reported the forests of the southeast have generally served as carbon sources from 1750-1960 due to deforestation of virgin forests during the industrial revolution. Large-scale reforestation of agricultural fields and the intensive management of secondary forests have made the southeast function largely as a carbon sink. With 13 million hectares of loblolly pine stands intensively managed (e.g. fertilization and controlling competing vegetation) in the southeastern United States there is the potential to sequester large amounts of carbon by increasing plant biomass and organic matter (e.g. litter fall, root biomass, exudates, etc.) in forest soils (Maier and Kress 2000, Jokela and Long 2003).

Fertilization in southeastern pine forests has increased approximately 800% since 1990 to just over a half million hectares of planted pine being fertilized in 2000 and 2001 [North Carolina State Forest Nutrition Cooperative (NCSFNC) 2002, Wear and Greis 2002]. Wear and Greis (2002) estimated that the use of fertilizer in American forests

exceeds total forest fertilization of the rest of the world. Fertilization has proven to increase plant productivity, and can shift carbon allocation from the fine-roots to aboveground tissue (Axelsson and Axelsson 1986, Ryan *et al.* 1996). The aboveground responses, to nutrient additions, are well understood (Albaugh *et al*. 1998, Albaugh *et al.* 2004, and Gough *et al.* 2004), but there are still questions concerning the impact of fertilization on below-ground carbon evolution.

Objectives

1. The overall objective of this research was to examine how fertilization initially impacts below-ground carbon fluxes in a two-year-old loblolly pine (*Pinus taeda* L.) clonal plantation, and if differences exist between clones. Specifically we tried to:

- Determine the one-year effects of fertilization with diammonium phosphate, supplemented with ammonium nitrate, on total soil respiration.

- Determine the relative contributions of heterotrophic and autotrophic respiration, over one year, as impacted by fertilization.

2. Attempt to isolate specific root respiration effects by comparing root respiration of hydroponically grown seedlings with and without mycorrhizae, and at different rates of fertilization.

Literature Review

Total CO_2 Efflux at Soil Surface – Components

Schlesinger (1997) estimated that soils are responsible for the largest movement of CO_2 between the biosphere and the atmosphere, accounting for approximately 68 Pg C yr^{-1} (Raich and Schlesinger 1992). CO_2 emissions from soils are estimated to be an order of magnitude greater then that released by the burning of fossil fuels (Raich and Schlesinger 1992), and approximately three times greater then that being emitted by the aboveground terrestrial biosphere (Rustad *et al.* 2000). Bolstad *et al.* (2004) provide further evidence supporting the importance of forest soils. This research found cumulative annual carbon efflux in northern deciduous forests of northern Wisconsin to be dominated by soil respiration as compared to respiration derived from aboveground biomass (stem and leaves). They estimated soil respiration accounted for more then 60 and 90% during the growing and dormant season, respectively.

Total CO_2 efflux at the soil surface is a combination of: (i) root (autotrophic) respiration resulting from maintenance and growth, and (ii) heterotrophic respiration produced during the decomposition of organic matter by soil organisms found within the soil profile. Currently, the relative contributions of these components to total soil CO_2 efflux are not well understood. This is further complicated by differences in ecosystems and changes of specific components within and between sites due to phenology, developmental, and forest management changes.

Autotrophic Respiration

Total root respiration is the sum of growth and maintenance respiration as well as rhizomicrobial respiration. The ratio of growth to maintenance respiration can shift in response to stressors, the developmental stage of the plant, as well as environmental changes such as season and CO_2 concentration (George *et al.* 2003). Growth respiration is the CO_2 that is emitted during the synthesis of new tissue, and maintenance respiration is CO_2 that is emitted during the repair and replacement of plant tissues. One component that is sometimes included, because of the inherent difficulty in separating it, is rhizomicrobial respiration, which is respiration originating from organisms that form a symbiotic relationship with roots (e.g. mycorrhizae) as well as other organisms found

within the rhizosphere. Rhizomicrobial respiration can makeup a significant portion of root respiration leading to large reported differences in respiration rates depending on whether included or separated during respiration measurements. Harley and Smith (1983) estimated that mycorrhizae associated with conifer roots accounts for 23-30%, and Ek (1997) estimated mycorrhizae (*Paxillus involutus*) associated with European white birch (*Betula pendula* Roth) accounts for 11-25% of the root respiration.

ATP and substrate supply (photosynthate) work in concert to drive the rate of root respiration (Dwivedi 2000). Processes, such as the active uptake, and reduction of ions, to repair and synthesize proteins require the use of ATP. The primary source of ATP for below-ground plant organs is derived from the electron transport pathways, which occurs inside the mitochondria during respiration. Dwivedi (2000), in a review of current literature, recognized that substrate supply (e.g. sucrose) influences root respiration by providing the C-skeletons and energy required for the synthesis and maintenance of tissues as well as acting as a signal to directly or indirectly affect gene expression (e.g. encoding nitrate reductase), which may play a part in regulating respiratory metabolism.

Raich and Schlesinger's (1992) estimated 30-70% of total CO_2 efflux at the soil surface to be derived from root respiration. Other researchers have found values that fall within this range with variation due to spatial, developmental, and methodological differences. Pangle and Seiler (2002), based on direct measurements, estimated root respiration to contribute 30% of total soil CO_2 efflux (taken near the seedling) in a two-year-old loblolly pine (*Pinus taeda* L.) plantation. Andrews *et al*. (1999) used δC^{13} labeling during free-air carbon dioxide enrichment (FACE) at the Duke University experimental forest and found that rhizomicrobial respiration (roots plus the microbes within the rhizosphere) made up 55% of the total soil respiration at the surface in a fifteen-year-old loblolly pine forest. Maier and Kress (2000) estimated that, in the top 15-cm of soil, root respiration was responsible for 20-50% of the total soil respiration, depending on the time of year. They also estimated that in the top 200-cm of the soil profile, root respiration was responsible for 50-70% of soil respiration annually in a loblolly pine plantation.

Plant roots can be categorized based on their diameter class as: fine-, medium-, and coarse-roots. Fine-roots are the most physiologically active and the sites of highest

respiration in below-ground tissue. Widén and Majdi (2001) estimated, from direct measurements of excised Norway spruce (*Picea abies* L.) and Scots pine (*Pinus sylvestris* L.) roots, that 12-62% of total soil respiration was derived from root respiration, and that fine-root respiration accounted for 5-58%. The authors cited an estimate by Ryan *et al*. (1996) that coarse-root respiration constituted 30% of the total soil respiration in Monterey pine (*Pinus radiata* D. Don).

Heterotrophic Respiration

There is a large diversity of microorganisms found within the soil matrix such as: bacteria, saprophytic fungi, protozoa, nematodes, mycorrhizae, and microarthropods (Ingham and Coleman 1984). Bacteria and fungi are the two predominant types of soil microbes responsible for carbon turnover, but these two organisms behave differently in their ability to acquire, store, and metabolize soil C (Bailey *et al*. 2002). Holland and Coleman (1987), in a review of the literature, reported that carbon assimilation efficiencies are significantly higher in fungi (40-73%) then bacteria (20-51%), meaning more carbon is retained as biomass and less is respired. Fungal composition (cell walls made up of polymers of melanin and chitin) is more resistant to decomposition than bacteria (membranes made up mostly of phospholipids), allowing for greater long-term storage of carbon as microbial biomass (Brady 1984, Holland and Coleman 1987, Bailey *et al*. 2002).

In general, microorganisms are not distributed evenly within the soil matrix. The concentration of microorganisms is highest near the plant in the volume of soil intimately associated with the roots (rhizosphere), where roots have a direct effect over microbial populations as compared to the surrounding bulk soil (Fisher and Binkley 2000). Soil microorganisms concentrate within the rhizosphere because roots deposit chemicals into the soil such as: root exudates, sloughed off root cap cells, dead root cells, and mucigel that is produced by the root cap (Fitter and Hay 1987), and are an important source of carbon and nutrients (e.g. P, K^{+}, Mg^{+2}, and Ca^{+2}) for soil microbes. Roots also affect soil chemical properties such as pH, redox reactions, as well as O_2 and CO_2 concentrations

Specifically, fungi and bacteria occupy different regions within the soil profile. Holland and Coleman (1987) found fungal biomass was greatest at the surface where the

organic matter was located and decreased with distance from the carbon source. They hypothesized that fungi are able to use their extensive hyphal networks to take advantage of organic matter located at the soil surface, while still being in contact with soil nitrogen, but deeper in the soil where moisture is greater, and the carbon and nitrogen sources are in close proximity conditions are more favorable for bacteria. Certini *et al.* (2003) also found that microbial biomass carbon decreased with soil depth.

The microbial composition is often expressed as a fungal-to-bacterial (F:B) activity ratio with 1.0 representing and equal active microbial contribution of fungi and bacteria (Bailey *et al*. 2002). F:B ratios are important in predicting how carbon will evolve in a soil because these two organisms have marked differences in their behavior. Microbial composition can be determined by analyzing phospholipid fatty acids (PLFA) within the soil. PLFA are found in membranes of all living cells. They degrade quickly after death and are unique to each organism (chain length, saturation, and branching) (Bailey *et al*. 2002). Community-level physiological profiling (CLPP) is a technique described by Leckie *et al.* (2004) by which the potential function of microbial communities can be measured.

Factors Affecting Rate of CO_2 Evolution

Numerous factors can affect the rate of CO_2 efflux from the soil surface, and interaction between these factors creates difficulty in modeling respiration rates. In addition, these factors may affect specific components (e.g. autotrophic and/or heterotrophic) differently. It is well established in the literature that the two greatest controls of CO_2 efflux are temperature and soil moisture.

Temperature

Both heterotrophic and autotrophic components show a strong positive correlation between respiration rates and temperature (Haynes and Gower 1995, Boone *et al*. 1998, Davidson *et al*. 1998, Niklińska *et al*. 1999, Chen *et al*. 2000, Fang and Moncrieff 2001, Qi and Xu 2001, Widén and Majdi 2001, Pangle and Seiler 2002, Gough and Seiler 2004b, Wieser 2004). The exponential relationship between temperature and respiration is expressed as a Q_{10} value, which is a rise in soil respiration in response to a rise in

temperature of 10°C. Care should be taken in the use of Q_{10} values; they are a good index of individual reactions, but are not especially good at measuring metabolic activity involving multiple reactions.

There is considerable variation among Q_{10} values depending on location and season making the use of a single average Q_{10} value, as is the case with global CO_2 models, inappropriate. Atkin and Tjoelker (2003), in a review of the literature, recognized Q_{10} decreases linearly with short-term increases in temperature at which measurements are taken. For example, plants grown under cold conditions (5°C) and measured at room temperature (20°C) will have a higher Q_{10} then plants that are grown in warmer temperatures and measured at room temperature (Chen *et al.* 2000). This is supported by higher Q_{10} values reported in colder climates, and, perhaps, explains the decrease in sensitivity of some models at low temperatures as observed by Fang and Moncrieff (2001) in incubated, intact soil cores.

At low temperatures, plant respiration is limited by the maximum enzyme activity (V_{max}) of the respiration enzymes, for example, NADH dehydrogenases and ATP-synthase (Atkin and Tjoelker 2003). This could be due to direct effects of cold temperature on potential enzyme activity, or by limiting the function of enzymes embedded in the membrane that has transitioned into the gel state. The authors also hypothesized that at warmer temperatures the limiting factor to respiration may be substrate supply or adenylates concentration. As the temperature increases past the transition temperature membrane leakiness could become more limited by substrate availability due to a difficultly in maintaining the concentration gradient (Atkin and Tjoelker 2003). Chen *et al.* (2000) attributed the observed decrease in root respiration at temperatures of 50 and 60°C to the breakdown of decomposer protein.

Haynes and Gower (1995) found soil temperature explained 54% of the variation of total soil respiration giving a Q_{10} value of 1.685 in a Wisconsin red pine (*Pinus resinosa* Ait.) plantation. Davidson *et al*. (1998) found in combining data from six sites on the Harvard forest that temperature explained 80% of the seasonal variation in CO_2 efflux giving a Q_{10} value of 3.9 ± 1.1. Janssens *et al.* (2001) found similar results and estimated that temperature explained 80% of the temporal variation on a 69-year-old Scots pine stand located in the Belgian Campine region. Qi and Xu (2001) reported 82%

of the temporal variation of CO_2 efflux was explained by temperature when soil moisture was held constant. When temperature and soil moisture were combined they explained 89% of the temporal variation of soil CO_2 efflux in a seven to eight-year-old ponderosa pine (*Pinus ponderosa* P.& C. Lawson) plantation located in the Sierra Nevada mountains (Qi and Xu 2001). Wieser (2004) found an average Q_{10} value of 4.0 in a 95-year-old *Pinus cembra* L. forest at the alpine timberline in the Central Austrian Alps. The author recognized that while the Q_{10} value fell within the range of 2.0 to 6.3 it was almost twice as high at the global median. Wieser found that temperature at 5-cm soil depth accounted for 65 to 75% of the total variation in soil respiration.

Studies have shown autotrophic and heterotrophic components react in different degrees to changes in temperature. Widén and Majdi (2001) found both total CO_2 efflux and root respiration increased exponentially with temperature, but root respiration was twice as sensitive. Boone *et al*. (1998) observed Q_{10} values for roots (including rhizosphere) and soil with no roots to be 4.6 and 2.5, respectively. In contrast Singh *et al.* (2003) suggested that root respiration was not as sensitive to temperature as heterotrophic respiration. Niklińska *et al*. (1999) in an incubation study found average Q_{10} values ranging from 1.0 to 5 in humus samples over seven Scots pine stands across the European continent. The authors found 71% of the variability in respiration rates of the humus was explained by a positive correlation in incubation temperature, soil pH, and C:N ratio and a negative correlation with soil total N. A short-term, incubation study conducted by Chen *et al*. (2000) to study the effects of temperature and moisture on respiration rates of decomposing conifer roots found that respiration rates increased to a maximum temperature of 30-40°C and then decreased. They obtained Q_{10} values of 3.99 and 2.4 between temperatures of 5-10°C and 10-15°C, respectively.

Soil Moisture

There seems to be an optimal level of soil moisture with excesses and deficiencies having negative effects on soil respiration rates (Davidson *et al*. 1998), but between these extremes Fang and Moncrieff (2001) observed no obvious effect on soil respiration rates. It has been shown that excess soil moisture will decrease microbial respiration (Roberge 1976, Kowalenko *et al*. 1978). Bouma and Bryla (2000) found that after irrigation there

was an immediate decrease in soil respiration. They hypothesized one reason for the decrease could be the limitation of gas diffusion to the soil surface, in wet soils, causing an accumulation of CO_2 in the soil. Chen *et al*. (2000) found that respiration rates of decomposing roots of five conifer species (Sitka spruce, Douglas-fir, western hemlock, ponderosa pine, and lodgepole pine) were very low when moistures were 20-50% due to the unavailability of water for metabolic activity of microbes. Root respiration rates increased with an increase in root moisture content to the optimum moisture range (125-225% for lodgepole pine, 125-275% for Sitka spruce, and > 275% for the three remaining) then decreased due to slowing decomposition rates.

Qi and Xu (2001) found that when temperature was held constant 84% of the variation was explained by soil moisture. Gough and Seiler (2004b) found no significant effect on CO_2 efflux due to soil moisture (R^2 0.009, P = 0.1512) in 1, 6, 11, 21-year-old loblolly pine stands on the Upper Coastal Plain of South Carolina. Lavigne *et al.* (2004) observed in a 40-year-old balsam fir (*Abies balsamea* (L.) Mill.) ecosystem in New Brunswick, Canada that when soil temperatures were relatively constant (summertime variation in soil temperature of 3-6°C) that plots that were exposed to moderate water stress (-0.6 to -0.2 MPa) showed a 25-50% decline in soil respiration compared to rates observed at field capacity.

Soil CO_2 Concentration

Soil CO_2 concentrations are generally an order of magnitude greater then ambient CO_2 concentrations giving the potential for an overestimation of root respiration when measured at ambient atmospherics CO_2 concentration (Rakonczay *et al*. 1997). Some researchers have found that measuring root respiration at lower CO_2 concentration results in a higher root respiration rate (Qi *et al*. 1994, Clinton and Vose 1999, McDowell *et al*. 1999), while others found no change or a decrease in respiration rates (Bouma *et al*. 1997, Burton and Pregitzer 2002).

Qi *et al*. (1994) found total and basal root respiration (minimum rate necessary for the survival of a "starving" plant), in undisturbed roots, decreased exponentially as soil CO_2 concentration rose from 130 to 7015 μmol mol^{-1} in one-year-old Douglas-fir [*Pseudotsuga menziesii* (Mirbel) Franco] seedlings planted in root boxes. The authors

found that doubling soil CO_2 concentration caused a decrease in both total and basal root respiration rates by 4-5 nmol CO_2 g^{-1} $s^{-1}$and suggest that soil CO_2 concentrations be accounted for in models of below-ground carbon budgets.

McDowell *et al*. (1999) planted western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] seedlings into root boxes so that total root respiration (R_t) could be measured on intact, undisturbed seedlings under increasing CO_2 concentrations (96, 151, 395, 1585, and 7009 μmol mol^{-1}). The authors found that R_m (measured by manipulating light conditions) showed an exponential decline (≈ 37%) for every doubling of CO_2 concentration, while there was no significant change in R_g at increased CO_2 concentrations.

Clinton and Vose (1999) suggest that soil CO_2 concentration has a direct effect on root respiration. Root respiration rates were consistently lower when measured at soil CO_2 concentrations than when measured at ambient CO_2 concentration. Also, when temperature was held at a constant 15°C, soil CO_2 concentration accounted for 73% of the variability of fine root respiration of mature eastern white pine (*Pinus strobus* L.). Bouma *et al*. (1997) did not find a significant (P=0.52) change in respiration rates of bean and citrus plants at different CO_2 concentrations (200 and 2000 μmol mol^{-1}). Similarly, Burton and Pregitzer (2002) found no different in root respiration in nine tree species (*Acer saccharum, Juniperus monosperma, Picea glauca, Pinus edulis, P. elliottii, P. resinosa, Populus balsamifera, Quercus alba, and Q. rubra*) when measured at 350 and 1000 ppm CO_2 concentrations.

Tissue Nitrogen Content

Ryan *et al.* (1996) found a positive relationship between tissue N content and maintenance respiration which they hypothesized was related to protein (e.g. Rubisco) repair and replacement. Pregitzer *et al*. (1998) found that root tissue N concentration explained 70% of the observed variation in respiration rates across diameter class, depth class, and site in sugar maple (*Acer saccharum* Marsh.) in two hardwood forests in Michigan. Both root diameter, soil depth, and diameter x depth interaction had highly significant (P<0.001) differences in root respiration and root N concentration. Finer lateral roots (<0.5-mm) found at distal ends of the roots system had greater tissue N

concentration and a significantly higher rate of respiration (2.4 to 3.4 times higher) as larger roots found at the same soil depth (0 to 10-cm).

Widén and Majdi (2001) found that fine-root respiration increased significantly ($P \leq 0.01$) with N concentration in a mixed pine and spruce forest, but the relationship was very weak ($R^2=0.08$). The authors believed that root N concentration would have accounted for more of the variation if the roots were separated by species and diameter class. Burton *et al.* (2002) found that fine-root tissue N concentrations were lower in gymnosperms than in angiosperms, which explained the lower respiration rates observed in gymnosperms. The authors used N concentration as a covariate and found that root respiration rates between these two taxonomic groups were no longer statistically significant ($P=0.214$). In a field experiment Burton *et al.* (2002) found that N concentration explained 60% of the variation in root respiration between species and sites. Clinton and Vose (1999) also found a weak, but significant, correlation ($R=0.30$; $P \leq 0.05$) between fine-root respiration and N concentration in eastern white pine when measured at ambient soil CO_2 concentration and normalized to 15ºC.

Seasonal and Diurnal Variation

Seasonal variation in CO_2 efflux rates are closely correlated with seasonal temperature and moisture regimes peaking in July, August, and September (eastern US) when soil temperatures were at their highest (Zogg *et al.* 1996, Maier and Kress 2000, Widén and Majdi 2001, Pangle and Seiler 2002, Yuste *et al.* 2003, Wieser 2004). Widén and Majdi (2001) found that fine-root respiration did not follow a seasonal pattern based on temperature, and proposed that root respiration may follow a diurnal pattern based on photosynthesis.

Photosynthesis

Supply of carbon to below-ground tissues can be positively affected by increasing photosynthesizing tissue (fertilization), or can be negatively affected by processes that decrease photosynthesis such as: girdling, removing limbs, shading leaves, or stress. Experiments on lettuce (*Lactuca sativa* L.), which used $^{14}CO_2$ pulse labeling of shoots

have shown that fixed carbon can be assimilated and translocated to below-ground tissue very quickly (Kuzyakov *et al.* 2002).

Evidence supporting this hypothesis comes from Högberg *et al.* (2001) who used large-scale tree girdling in a Scots pine dominated forest in northern Sweden to separate the contributions of root and heterotrophic respiration. The authors found a 27% drop in soil respiration compared to control plots (not girdled) 5 days after girdling trees early in the growing season, and a 37% drop in soil respiration rate in trees girdled later in the growing season. Both early and late girdled trees showed approximately 54% reduction in soil respiration 1-2 months after girdling relative to control plots. Ekblad and Högberg (2001) showed through natural ^{13}C abundance that it takes 1-4 days for fixed carbon to become available for roots and rhizomicrobial respiration in 20-25 meter Scots pine trees, which is approximately the time it took for Högberg *et al.* (2001) to see the decrease in respiration rates. Differences between early and late girdled trees 5-days after treatment application is thought to be due to larger starch reserves early in the growing season making the roots less dependent on recently fixed C (Högberg *et al.* 2001). Further, Singh *et al.* (2003) looked at the girdled trees beyond the first year and observed a decline in heterotrophic respiration in response to a 20-day long drop in temperature, but found no decline in calculated root respiration. Singh *et al.* (2003) proposed that root respiration may be less dependent on temperature than previously believed, and that future models of forest C balance to global climate change should utilize photosynthetic activity and carbon allocation.

Spatial Variation

Researchers have shown that soil CO_2 efflux rates vary within sites. Pangle and Seiler (2002) have shown that soil respiration was higher at the base of the plant than in between rows in a 2-year-old loblolly pine plantation in the Virginia Piedmont. Gough *et al.* (2005) found increased respiration rates at the base of the plant compared to between row measurements in a 1, 6, 11, and 21-year-old stands on the South Carolina Coastal Plain and on the Virginia Piedmont. Wieser (2004) found a decrease in soil surface respiration of approximately 20% for every meter increase in distance from the trunk. The authors attribute this difference in CO_2 efflux near plants to an increased root mass,

which resulted in higher autotrophic respiration. Also, increases in C inputs to the rhizosphere may lead to increased heterotrophic respiration. Plant roots are a big source of C, which in some cases may be more limiting to soil microorganisms than nitrogen availability (Ekblad and Nordgren 2002).

Effects of Fertilization

Nitrogen and phosphorous are frequently the most limiting nutrients in temperate forest soils (Fisher and Binkley 2000). Nutrient additions can increase productivity and carbon storage capacity of aboveground biomass, but their effect on long-term soil carbon storage is not well understood. Researchers have found an increase (Gough and Seiler 2004a), a decrease (Haynes and Gower 1995, Mattson 1995, Maier and Kress 2000), and no change (Pangle and Seiler 2002, Lee and Jose 2003) in CO_2 evolved from the soil surface in response to fertilization. This difference in responses could be due to a number of things. One proposed by Gough and Seiler (2004a), and by Lee and Jose (2003) is that a decrease in heterotrophic respiration combined with an increase in total autotrophic respiration may have a cancellation effect on CO_2 efflux. Other possible reasons are the rate of nitrogen fertilization, the vegetation type (Lee and Jose 2003), and the time from initial fertilization that soil respiration measurements were taken (Gough and Seiler 2004a).

Mattson (1995) measured three forested stands that had received three different nitrogen fertilization treatments (control, single, and double fertilized sites). The author measured CO_2 efflux to be 29% lower in the 2X-fertilized site when compared to the control. Differences between the control and 2X site were greatest in the 0-10 cm mineral layer initially, but after one-year differences diminished. Maier and Kress (2000) also found a significant decrease ($P \leq 0.10$) in total CO_2 efflux rates in 11-year-old loblolly pine trees that had been fertilized (to 1.3% optimum foliar nutrition; in the form of urea) relative to controls.

Gough and Seiler (2004a) found a significant ($P \leq 0.05$) increase in soil respiration in fertilized pots compared to controls 4 to 13 days after applying fertilizer in one-year-old loblolly pine seedlings grown in a greenhouse. From day 13 to 47 (post treatment) soil respiration rates reversed and were significantly ($P \leq 0.05$) lower in fertilized pots

compared to controls, and after day 47 fertilized pots became significantly ($P \leq 0.05$) higher again. From these findings the authors concluded that the time from initial treatment could be an important factor in determining the effect of fertilization on soil respiration.

Lee and Jose (2003) found that hardwoods and conifers behave very differently in regard to their below-ground responses (soil respiration, fine-root production, soil pH, and microbial biomass) to nitrogen fertilization. In the cottonwood (*Populus deltoides* Marsh.) stand the authors found a significant ($P=0.008$) decrease in soil respiration, but did not see a significant change in loblolly pine. Both showed a significant ($P=0.048$) reduction in microbial biomass, but while not significant the cottonwood stand showed a decrease in fine-root production while there was a slight increase in loblolly pine along a nitrogen gradient. The authors concluded that, in loblolly pine, the lack of change in soil respiration was due to a rise in root respiration counteracting the decrease in microbial respiration.

Bowden *et al*. (2004) have shown that chronic nitrogen additions to a red pine stand in the Harvard experimental forest showed a 20% decrease ($P<0.001$) in plots which received high nitrogen inputs relative to control plots in the first year following fertilization. By year two both high and low nitrogen fertilized plots had E_C that were significantly ($P<0.001$) lower then control plots which persisted 13 years later. The authors found a decrease in root biomass after 13 years, decrease in mycorrihizal fungal community, and a decrease in soil pH, which the authors hypothesized may have contributed to a lower R_H and ultimately lower rates of E_C.

Effects on Heterotrophic Respiration

There are several possible explanations for reduction in respiration rates at the soil surface. It has been well documented in the literature (Roberge 1976, Kowalenko *et al*. 1978, Bååth *et al*. 1981, Söderström *et al*. 1983, Nohrstedt *et al*. 1989, Smolander *et al.* 1994, Thirukkumaran and Parkinson 2000, Gough and Seiler 2004a, Lee and Jose 2003) that the addition of nitrogen to the soil affects heterotrophic respiration. The direction and degree to which nitrogen fertilization will affect soil respiration is always consistent and may be dependent on soil pH (Kowalenko *et al*. 1978, Thirukkumaran and Parkinson

2000), the application rate (Bååth *et al*. 1981, Nohrstedt *et al*. 1989), shifts in microbial population (Bååth *et al*. 1978), soil type (Kowalenko *et al*. 1978), and/or changes due to re-allocation of C from roots to shoots (Haynes and Gower 1995).

The addition of N fertilizer to soils has been shown to have an effect on soil pH. Urea [$(NH_2)_2CO$] has been shown to increase soil pH, accompanied by an initial increase in soil respiration, which is maintained long (10 years) after fertilization (Roberge 1976), or with time (175-days) decreased below control levels (Söderström *et al*. 1983, Thirukkumaran and Parkinson 2000). This effect could partially be due to increased C availability in more alkaline soils (Thirukkumaran and Parkinson 2000). Ammonium nitrate (NH_4NO_3), and other ammonium salts have been shown to lower soil pH, accompanied by a decrease in microbial activity (Kowalenko *et al*. 1978, Thirukkumaran and Parkinson 2000, Bowden *et al.* 2004).

Bååth *et al*. (1981) observed a decrease in soil respiration rate, FDA-active fungal biomass, and bacterial biomass with increasing rates of N fertilization. While the authors were able to still detect pH differences between types of fertilizers, they found no significant difference in respiration rates, five years after application, between ammonium nitrate or urea when both were applied at equivalent application rates. Nohrstedt *et al*. (1989) also, observed a decrease in soil respiration rates as the rate of fertilization increased (from 150 to 600 kg N ha^{-1}) regardless of the type of fertilizer used. Roberge (1976) observed a decrease in respiration rate at very high levels (2187 ppm) of N in the form of urea. Smolander *et al.* (1994) found a significant decrease in microbial biomass and respiration rate in a Norway spruce stand in southern and central Finland 30 years after the start of N and P fertilization. Nitrogen fertilization ceased 2 to 5 years, and phosphorous fertilization ceased 11 to 13 years prior to the start of the study. The authors observed no clear effects due to P fertilization.

A study by Leckie *et al.* (2004) suggests that microbial composition and activity in the long-term may be influenced by fertilization to a greater extent by indirect effects on plant growth and litter input as opposed to direct effects on microbial populations. When nitrogen is added to the soil, plants could shift carbon allocation from roots to shoots, negatively impacting heterotrophic activity by reducing their food supply, and possibly causing other nutrients, such as phosphorous, to become limiting. Another

possibility is a shift in population dominance within the soil community after the addition of nitrogen, and/or changes in available carbon may have contributed to an increase in microbial activity (Bååth *et al* 1978). Ekblad and Nordgren (2002) found in a boreal Scots pine forest that soil microbes were more limited by the availability of carbon then nitrogen. One hour after treatment, soil respiration rates doubled for ammonium, sucrose, and ammonium + sucrose treatments. On day 5, plots treated with sucrose continued to increase while those treated with ammonium alone decreased.

Wallander and Nylund (1992) studied the effect of excess nitrogen and phosphorous starvation on mycorrhizal development on Scots pine grown in a semi-hydroponics system. The authors found when nitrogen was increased (from 10-20 to 100-200mg l^{-1}) the result was a reduction in mycorrhizal biomass and an increase in shoot biomass. An increase in fungal growth when nitrogen levels later fell; indicated that the effects of excess nitrogen are short lived. The authors also found that phosphorous deficiencies resulted in an increase in mycorrhizal biomass regardless of the nitrogen status. Nilsson and Wallander (2003) showed that N-additions affected ectomycorrhizae the same in the field as in laboratory settings. On average the N-treated plots had only 50% ectomycorrhizae of that found in nonfertilized plots, and the addition of phosphorous (apatite) caused an increase in ectomycorrhizal mycelial production in fertilized plots. Ek (1997) found in a laboratory experiment that a particular species of ectomycorrhizae (*Paxillus involutus*) associated with European white birch increased respiration rates with the addition of nitrogen in the form of ammonium or nitrate. Respiration rates were higher in fungal compartments fertilized with nitrate then in compartments fertilized with ammonium. This is due to the extra energy requirements needed to assimilate nitrate (19-21 moles vs. 4-5 moles of ATP equivalents per mole of glutamate generated for nitrate and ammonium, respectively) (Ek 1997).

Recent studies in loblolly pine found decreases in microbial biomass C (Lee and Jose 2003), microbial respiration, and activity (Gough and Seiler 2004a) in response to nitrogen fertilization. Thirukkumaran and Parkinson (2000) found significant decreases in basal respiration ($P<0.001$) and substrate induced respiration ($P<0.01$) during the entire experiment with N fertilization in the form of ammonium nitrate, but found significant decreases ($P<0.01$) only after 40 days in substrate induced respiration rates in response to

N fertilization in the form of urea. The authors found increases in basal respiration at 10 days in incubated soils due to N fertilization in the form of urea. Thirukkumaran and Parkinson (2000) found at high levels, P fertilization decreased microbial respiration and biomass, but showed no significant effect on soil pH leading the authors to conclude suppression was due to osmotic effects. Gough and Seiler (2004a) found that microbial respiration was significantly ($P \leq 0.05$) depressed through out the entire 197 days in potted loblolly pine seedlings grown in the greenhouse. The authors found that microbial activity per gram of soil C was only 44 and 66% of that measured in control pots at 49 and 197 days following fertilization, respectively. In contrast Haynes and Gower (1995) found no difference in heterotrophic respiration between fertilized and control trenched plots in a 31-year-old red pine plantation in northern Wisconsin fertilized with equal proportions of NH_4^+ and NO_3^-. However, the authors did observe below-ground C allocation to be significantly lower in fertilized plots two out of three years.

Effects on root respiration

There are two mechanisms by which root respiration can be affected by fertilization. First, is by a change in specific root respiration (respiration per gram or per unit area of root). Nitrogen metabolism is energetically expensive for plants requiring both ATP generated from respiration and C-skeletons from stored carbohydrates or recent photosynthesis to actively take up, reduce, and assimilate nitrogen ions into amino acids. Plants can take up nitrogen in two forms: ammonium and nitrates. In conifers, ammonium is the dominant source of N owing to its greater availability in predominantly acid forest soil. Bedell *et al.* (1999) found that Douglas-fir seedlings had a preference for NH_4^+ as a nitrogen source as compared to NO_3^-. Zogg *et al.* (1996) observed a similar phenomenon in sugar maple, concluding NO_3^- was not an important source of nitrogen in these stands, but may in the long run be converted to NH_4^+ and then taken up by plants. Woody plants will take up nitrates, but they require additional energy costs to the plant by the reduction of nitrate to ammonia. Nitrates are easily stored in vacuoles during time of surplus, while NH_4^+ions must be assimilated immediately to prevent toxicity to the plant (Lasa *et al.* 2002).

The literature shows that nitrogen fertilization has mixed effects on specific root respiration. Lu *et al.* (1998) observed specific root respiration increased significantly ($P \leq 0.05$) with an increase from 10 to 50 mg N l^{-1}, but remained steady with a further increase to 200 mg N l^{-1}. The authors concluded that the negative effect on total root respiration was due to the significant ($P \leq 0.05$) decrease in root biomass at the high level (200 mg N l^{-1}) of fertilization. Similarly, Gough and Seiler (2004a) found 49 days after fertilization (DAP) that specific root respiration rates were significantly ($P \leq 0.05$) higher in pots that received fertilizer. After 197 days specific root respiration rates returned to control levels leading the author to conclude that timing may play an important role in detecting changes in specific root respiration in loblolly pine seedlings. In contrast, Maier and Kress (2000) found no significant increase in specific root respiration in loblolly pine seedlings due to fertilization except for the month of May, and Pangle and Seiler (2002) found no significant differences in root respiration when measured during the final harvest one year after fertilization. Zogg *et al.* (1996) also found no significant increase in fine-root respiration in sugar maple (*Acer saccharum* Marsh.) one growing season following fertilization in the form of NO_3^-.

A second mechanism that can increase total root respiration is by an increase in total root biomass. This has been observed in conifers by a number of researchers (Lu *et al.* 1998, Maier and Kress 2000, King *et al.* 2002, Pangle and Seiler 2002, Gough and Seiler 2004a, Lee and Jose 2003). However, the opposite effect has been observed in cottonwood (Lee and Jose 2003) and red pine (Haynes and Gower 1995) where researchers found a decrease in root biomass after fertilization.

Lu *et al.* (1998) measured the response in root respiration of Douglas-fir seedlings grown in root boxes to three levels (10, 50, and 200 mg l^{-1}) of nitrogen in the form of NH_4NO_3 and found that at low to medium levels, root growth is enhanced by fertilization, but at high levels fertilization had a negative affect on root growth. King *et al.* (2002) used minirhizotrons to observe a significant ($P \leq 0.05$) increase in fine-roots and net production of mycorrhizal roots in an eight-year-old loblolly pine plantation in response to fertilization. Pangle and Seiler (2002) also found a significant ($P \leq 0.05$) increase in fine-root (38%), medium-root (39%), coarse-root (46%), stem (43%), branch (58%), and foliage (47%) due to fertilization in two-year-old loblolly pine seedlings.

Gough and Seiler (2004a) found that after 197 days, dry weight root biomass was 27% greater in fertilized loblolly pine seedlings then in non-fertilized pots ($P \leq 0.05$). While not statistically significant, Lee and Jose (2003) found a slight increase of root biomass in loblolly pine after fertilization, but a decrease in cottonwood. In contrast, studies have shown a decrease in fine-root production, while increasing total root biomass by increasing the amount of mature (suberized) roots after nitrogen fertilization in loblolly pine (Albaugh *et al.* 1998, Maier and Kress 2000, Samuelson 2000).

Soil Carbon Storage

Nitrogen fertilization can potentially increase long-term soil carbon storage by two mechanisms: (i) increasing carbon inputs, and (ii) decreasing the rate of decomposition by soil microbes. Alternatively, some researchers believe, particularly in the tropics, this is only a transient effect and the long-term state of soil carbon will show no change or even a decline due to nutrient additions (Giardina *et al.* 2004). There are a number of ways nitrogen fertilization may increase carbon inputs. Studies have shown fertilization may result in increased root growth (Albaugh *et al.* 1998, Maier and Kress 2000, Samuelson 2000, King *et al.*, 2002, Pangle and Seiler 2002, Lee and Jose 2003, Gough and Seiler 2004a), or an increase in aboveground biomass in Scots pine, Norway spruce (Axelsson and Axelsson 1986), and loblolly pine (Albaugh *et al.* 1998, Pangle and Seiler 2002, Gough *et al.* 2004). An increase in aboveground biomass would result in more carbon being deposited into the soil as litter fall (Haynes and Gower 1995, Maier and Kress 2000) and root exudates.

A second way is by decreasing the rate at which organic matter (e.g. roots and litter) in the soil are decomposed. Thirukkumaran and Parkinson (2000) found that the rate of decomposition could be dependent on the type of fertilizer used. After 40 days fertilization with ammonium nitrate decreased decomposition while fertilization with urea and P did not suppress decomposition of aspen leaves. Other researchers have suggested that increases in soil C in fertilized stands must be partly due to decreased rates of decomposition (Nohrstedt *et al*. 1989, Smolander *et al.* 1994). Berg (2000) stated that high concentrations of nitrogen could decrease litter decomposition by suppressing decomposers such as white-rot fungi from producing lignin-degrading enzymes or by the

formation of highly recalcitrant compounds formed by the reaction of ammonium or ammonia and products from lignin degradation. This is supported by a decrease in heterotrophic respiration observed after N fertilization.

Isolating Root Respiration Using Biocides

The major problem in determining the relative contributions of roots and microorganisms to soil respiration is that the respiring roots and microorganisms are so intimately associated in the soil matrix. In order to obtain accurate estimations of the relative contributions of heterotrophic and autotrophic respiration towards total soil respiration, a system must be used to separate their effects. One method of removing, or reducing the effects of a specific group of organisms is by using biocides. There are numerous biocides that remove a whole host of organisms, but the key to selecting the correct biocide is that it effectively reduces the target organism without inhibiting other organisms (Ingham 1985). The biocide chosen must be considered for direct (e.g. reduction of a target species) and indirect effects (e.g. an increase of tolerant organisms in the absence of competition).

Captan® [preferred IUPAC name: N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide] is a mild fungicide that, at high concentrations has been shown to reduce ectomycorrhizae (Pawuk *et al*. 1980, Ingham and Coleman 1984, Colinas *et al*. 1994) and vesicular-arbusculus mycorrhizal (VAM) development (Kough *et al*. 1987) without negatively affecting higher plants (Pawuk *et al* 1980, Marx and Rowan 1981, Wigand and Stevenson 1997).

Pawuk *et al*. (1980) found that Captan significantly ($P \leq 0.05$) reduced ectomycorrhizal (*Pisolithus tictorius*) formation, when compared to control, on longleaf pine (*Pinus palustris* Mill.) seedlings when applied at a rate of 5 mg active ingredient per seedling in 50 mL of water after transplanting and at 4-week intervals. The authors found Captan reduced, but not significantly, the formation of airborne symbiont infection (*Thelephora terrestris* made up 95%). Finally, the authors found that Captan had no negative effect on seedling growth or stem diameter.

Ingham and Coleman (1984) found a reduction of active hyphal lengths, compared to the control, when Captan was added at a rate of 25 $\mu g\ g^{-1}$ of dry soil (50%

active ingredient), but the reduction was only significant ($P \leq 0.05$) on days 1 and 3. There was no change in total hyphal length, bacterial plate count, or protozoan estimates. Fungal plate count estimates increased on day 7 returning to control levels by day 14. The authors postulated that the Captan-treated soils may have undergone a change in fungal dominance, explaining the rise at day 7. Estimates of active and total hyphal lengths were made by direct observation (x 1000 magnification) after fluorescein diacetate (FDA) staining.

Colinas et al. (1994) performed and incubation experiment in which Captan had been applied at a rate of 25 $\mu g\ g^{-1}$ of dry soil resulting in a reduction of active hyphal length to 10% of that in control. Total hyphal length was not affected. The authors attributed this to the lack of time (only 48 hrs) for the dead hyphae to decompose. They also showed a reduction of total bacteria and bacteria-feeding nematodes of 57 and 30%, respectively, of those found in the control. Estimates of active and total hyphal lengths were made by direct observation (x 1000 magnification) after fluorescein diacetate (FDA) staining.

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CHAPTER 2

The effects of fertilization on heterotrophic, autotrophic, and total soil CO_2 efflux in a two-year-old loblolly pine (*Pinus taeda* L.) clonal plantation located on the Virginia Piedmont

Abstract. Fertilization is a common, cost effective treatment within managed forests of the southeastern United States. However, little is known about how fertilization will affect the below-ground processes that drive soil CO_2 efflux. A thorough understanding of below-ground carbon dynamics is necessary for the estimation of net ecosystem productivity and the carbon storage potential of these managed systems. In April 2004, we began monitoring total soil CO_2 efflux, heterotrophic, and root respiration in response to fertilization with diammonium phosphate (DAP). Respiratory components were measured prior to fertilization, weekly following fertilization, and bi-weekly after respiratory components stabilized using a dynamic closed chamber and an infrared gas analyzer (Li Cor 6200). We found that total soil CO_2 efflux differed significantly ($P<0.0001$) between fertilized and unfertilized plots, but the direction was dependent on date. In the early portion of the study fertilized plots were lower then control plots, but by the latter half fertilized plots returned to control levels except for one sampling date in March 2005 when fertilized plot rates were increased relative to control plot rates. Heterotrophic respiration was consistently and significantly ($P=0.0002$) lower in fertilized plots. When averaged over the study, fertilized plots were 32% lower then plots that did not receive fertilization. When analyzed separately by date we observed a consistent decrease in heterotrophic respiration starting 8 days following fertilization and remaining throughout the study. Root respiration was significantly ($P=0.0597$) increased in fertilized plots when analyzed over the study and showed a 20% increase due to fertilization. We concluded that an increase in specific root respiration and possibly root biomass was enough to balance the decrease in heterotrophic respiration leading to no observed differences in total soil CO_2 efflux later in the growing season.

Introduction

Schlesinger (1997) estimates that forests account for up to 75% of all carbon stored in terrestrial ecosystems, and are responsible for about 40% of the carbon flux between the atmosphere and the biosphere. CO_2 efflux from soils is the second largest flux in the global carbon cycle, making it an order of magnitude greater then CO_2 produced from the burning of fossil fuels (Raich and Schlesinger 1992), and approximately three times greater then that being emitted by the aboveground terrestrial biosphere (Rustad *et al.* 2000). Further, Bolstad *et al.* (2004) estimated soil respiration to account for more then 60 and 90% of total biosphere respiration during the growing and dormant season, respectively, in deciduous forests of northern Wisconsin.

Total CO_2 efflux (E_C) at the soil surface is predominantly made up of: (i) 'root' (autotrophic) respiration (R_R) resulting from maintenance and synthesis of root tissue and associated microorganisms within the plants rhizosphere that cannot be easily separated e.g. mycorrhizae, and (ii) heterotrophic respiration (R_H), which is CO_2 emitted during the decomposition of organic matter by soil organisms. There is some debate among researchers on the relative contributions that each of these components makes to E_C, but the overwhelming consensus is that between 30-70% comes from R_R (Raich and Schlesinger's 1992, Andrews *et al*. 1999, Maier and Kress 2000, Widén and Majdi 2001, Pangle and Seiler 2002).

Forests have the capability to remove inorganic carbon from the atmosphere and fix it as plant biomass. Fixed carbon can then find its way to the forest floor by a variety of avenues such as: fallen litter, increasing root mass, root exudates, and decomposing plants and animals. Organic carbon can be quickly released back into the atmosphere as CO_2 by combustion, but the vast majority is consumed by soil microbes and released into the atmosphere during decomposition of organic matter. Still, some organic carbon remains in the soil as microbial biomass or as recalcitrant carbon incorporated into the soil for long-term sequestration. Each of these stages can be influenced by climate, stand age, species composition, and human changes.

Forests and forest soils have the capability to act as either carbon sinks or carbon sources, and only by understanding the different mechanisms that determine the shift between source and sink can forests be managed to sequester carbon long-term. Delcourt

and Harris (1980) reported that forests of the southeast have acted as carbon sources from 1750-1960 due to the deforestation of virgin forests during the industrial revolution. Reforestation of agricultural fields and the intensive management of secondary forests has made the southeast function largely as a carbon sink. With 13 million hectares of loblolly pine stands intensively managed in the southeastern United States there is the potential to sequester large amounts of carbon in both plant biomass and as soil organic matter (Jokela and Long 2003).

Fertilization in southeastern pine forests has increased approximately 800% since 1990 to just over a half million hectares of planted pine being fertilized in 2000 and 2001 [North Carolina State Forest Nutrition Cooperative (NCSFNC) 2002, Wear and Greis 2002]. Wear and Greis (2002) estimated that the use of fertilizer in the United States exceeds that used in the rest of the world. The effects of aboveground responses, to nutrient additions, are fairly well understood. Fertilization has been shown to increase aboveground biomass (Axelsson and Axelsson 1986, Albaugh *et al.* 1998, Albaugh *et al.* 2004, Gough *et al.* 2004) therefore leading to increased carbon inputs as litter fall (Haynes and Gower 1995, Maier and Kress 2000), root exudates, and/or increased root biomass (Albaugh *et al.* 1998, Maier and Kress 2000, Samuelson 2000, King *et al.*, 2002, Pangle and Seiler 2002, Lee and Jose 2003, Gough and Seiler 2004a).

There are still questions concerning the impact of fertilization on below-ground carbon evolution that must be resolved before we can properly estimate what effects fertilization will have on net ecosystem productivity (NEP) as well as the carbon storage potential of forests in the southeastern United States. Researchers have found increases (Gough and Seiler 2004a), decreases (Haynes and Gower 1995, Mattson 1995, Maier and Kress 2000, Bowden *et al.* 2004), and no change (Pangle and Seiler 2002, Lee and Jose 2003) in E_C in response to fertilization. Nitrogen fertilizations has been shown to decrease R_H (Kowalenko *et al*. 1978, Bååth *et al*. 1981, Söderström *et al*. 1983, Nohrstedt *et al*. 1989, Smolander *et al.* 1994, Thirukkumaran and Parkinson 2000, Bowden *et al.* 2004, Gough and Seiler 2004a), and has shown mixed effects on specific root respiration. Few studies have intensively investigated the response of R_H and R_R to fertilization and how they impact E_C in the field.

The overall objective of this research is to examine how fertilization initially impacts below-ground CO_2 efflux in the field, and if differences exist between clones. Specifically, we will investigate the short-term effects of fertilization with diammonium phosphate (DAP), supplemented with ammonium nitrate on E_C, and its two major components (i) heterotrophic respiration and (ii) 'root respiration' within the rooting zone of a two-year-old clonal plantation of loblolly pine (*Pinus taeda* L.) located on the Virginia Piedmont. We hypothesize that an increase in R_R will not be enough to makeup for an overwhelming decrease in R_H leading to an overall decrease in E_C in response to fertilization.

Materials and Methods

Site description

This study was installed at Reynolds Homestead Forest Resources Research Center (FRRC) located in Patrick County, Virginia (latitude 36º40' N, longitude 80º10' W). The Reynolds plantation was intensively farmed with row crops and tobacco from the early 1800's to the mid 1900's. The soils are mapped as Hiwassee loam (very-fine, kaolinitic, thermic Rhodic Kanhapludults) and Louisa loam (loamy, micaceous, thermic, shallow Ruptic-Ultic Dystrudepts) series. Past farming practices lead to erosion and the removal of most of the A horizon, resulting in a truncated soil profile with clayey B horizons incorporated into surface Ap horizons. The climate is warm and humid receiving 1279-mm of precipitation spread evenly throughout the year peaking in July and dipping in January. The 30 year mean annual maximum temperature is 18.5ºC with an average monthly high temperature of 29.2ºC, occurring in July. The 30 year mean annual minimum temperature is 7ºC with an average monthly low temperature of -4ºC, occurring in January (Hoare 2005).

Experimental design

This study was a randomized complete block design with a split plot and repeated measures. Each of four blocks received two levels of fertilizer (present and not present) as the whole plot treatment, split with 25 unique loblolly clones (experimental unit) randomly repeated within each plot. The site was prepared by spraying with

glyphosphate (Round-Up®, Monsanto Co., St. Louis, MO) followed by ripping and a shallow cultivation of planting rows. The clones were planted on May 19, 2003 at 3.2 (row) x 2.6 (seedling) meters spacing with 25 clones per plot and a buffer strip of stock seedlings surrounding each plot. The clones were donated by the Forest Biology Research Cooperative, University of Florida, Gainesville for use in this project. Spring 2004, prior to fertilization, all vegetation was removed (chemically and mechanically) and plots kept free of all vegetation except for desired loblolly clones. After each sampling date any emerging vegetation was mechanically removed to insure a clean surface for the following sampling date.

We randomly pre-selected six clones per plot to be measured (Table 2.1), prior to fertilization, to determine a baseline respiration rate. On May 6, 2004 one randomly chosen plot within each of the four blocks received a single application of fertilizer, spread by hand, in the form of diammonium phosphate [$(NH_4)_2PO_4$] supplemented with ammonium nitrate (NH_4NO_3), at a rate of 225 kg ha^{-1} and 186.5 kg ha^{-1}, respectively, banded. This rate is equivalent to 112 kg N ha^{-1} and 23 kg P ha^{-1}. Following fertilization, measurements were repeated weekly on the same clones. When the response to fertilization leveled off, we scaled the measurements back to bi-weekly and eventually monthly until our last measurement on April 18, 2005.

Total soil CO_2 efflux measurements

Total soil CO_2 efflux was measured at the soil surface using the Li-Cor 6200 infrared gas analyzer (Li-Cor Inc., Lincoln, Nebraska) with a dynamic closed cuvette chamber constructed from a PVC pipe for walls, a plexi-glass top (25.5-cm internal diameter, height at center 13.5-cm) with a total system volume of 6744-cm^3. The bottom of the chamber was fitted with a stainless steel edge that was shallowly pressed (a few mm) in to the soil to create a seal. The gas input and output was provided by a gas sampling line (0.32-cm diameter plastic tubing) with return port connecting the chamber to the IRGA. Air was diffused, through a perforated hose lining the inside of the chamber, to allow even air flow and mixing (Selig 2003).

The Li-Cor 6200 was recalibrated before each sampling date by running a known CO_2 concentration and making necessary adjustments to the IRGA. Between blocks the

system was zeroed to account for any changes in temperature. Respiration measurements were made in the same sequential blocking order at approximately the same time of day for each sampling date. The CO_2 concentration in the cuvette chamber was brought down to ambient atmospheric CO_2 concentration then placed on the soil surface next to the seedling stem on a spot free of living, photosynthesizing vegetation. After efflux rates began to steadily rise CO_2 evolution was measured over a 30-second period and respiration rates (μmol m^{-2}_{ground} sec^{-1}) calculated on a per unit land area with the following equation:

$$[1] \qquad \text{Soil CO2 efflux} = \left[\left(\frac{\Delta C}{\Delta t}\right)\left(\frac{PV_t}{RT}\right)\right] \div \text{surface area of soil}$$

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

Heterotrophic respiration

Immediately following E_C measurements, R_H was measured using the Li-Cor 6200 with a 0.25-L cuvette chamber, with a total system volume of 429-cm^3. Soil was extracted to 10-cm in depth with a 2.5-cm diameter push-tube at the base of each seedling sampled. Roots were carefully removed from the soil sample by hand, and the soil placed into an aluminum weigh boat (10-cm x 2-cm), which was placed into the 0.25-L cuvette chamber. Once CO_2 began to steadily rise (typically within one minute) R_H was measured over a 30-second period. Soil was oven-dried for 48 hours at 105ºC and weighed gravimetrically to the nearest 0.01-g, and R_H was calculated and expressed on a per soil mass basis (μmol g^{-1}_{soil} sec^{-1}).

Autotrophic respiration

'Root' respiration was also measured with the Li-Cor 6200 system with a 0.25-L cuvette chamber. Approximately 10-cm^2 of fine-root (< 2 mm in diameter) was gently excavated and removed from near the soil surface for each clone. Fine particles of dirt were carefully shaken off and the 'root' immediately placed inside the cuvette and

measured over a 30-second period (Gough and Seiler 2004a). Immediately following measurements, 'roots' were sealed in a plastic bag and transported back to the lab where root surface area was determined using the WinRhizo 5.0A software (Regent Instruments Inc., Quebec, Canada). All R_R measurements were expressed on a per unit root area ($\mu mol\ CO_2\ m^{-2}_{root\ area}\ sec^{-1}$).

Temperature and moisture measurements

Soil temperature and percent volumetric soil water content (θ_v) were taken, concurrently with E_C measurements, next to the cuvette, to be used as covariates to normalize respiration rates to a common temperature and θ_v. Soil temperature was measured to the nearest 0.1 degree Celsius at 15-cm using a Digi-sense temperature gauge (model no. 8528-20, Cole-Parmer Instrument Co., Niles, IL). Percent volumetric soil water content was measured at a depth of 13-cm using a time domain reflectometer (Soil Moisture Equipment Co., 6050X1, Golena, CA) to the nearest percent. Meteorological data was collected by a National Resources Conservation Service (NRCS) weather station located on the Reynolds Homestead (site 2089, http://www.wcc.nrcs.usda.gov/scan/) and used to calculate average atmospheric temperatures and yearly precipitation.

Soil properties

Total soil nitrogen was analyzed by dry combustion at 900°C using a vario MAX CNS elemental analyzer (elementar Americas, Inc.) on the same soil samples that were used to measure R_H. Following oven drying, soil samples were mixed into a single composite sample for each plot. Tests showed no significant ($P \leq 0.05$) differences between air- and oven drying for our soils (unpublished data); allowing an analysis of total soil N for every sampling date. Soil samples were taken within the rooting zone of the seedling and values reported as mg N kg_{soil}^{-1}. Soil samples were not collected from the bulk soil, making it inappropriate to scale up total C and N values to a kg ha^{-1} basis.

Bulk density was determined by using the short core method (Grossman and Reinsch 2002) with a bulk density hammer to a depth of 10-cm at the base of three randomly chosen seedlings in each plot that were used for respiration measurements.

Cores were oven dried at 105°C and measured gravimetrically to the nearest 0.1-g. We determined active soil acidity, on soil samples collected on January 18, 2005, to the nearest 0.01 pH unit in a soil-water ratio of 1:2.

Data analysis

The effects of fertilization and clone on total CO_2 efflux, root respiration, and microbial respiration, were analyzed using an analysis of covariance with repeated measures. Total CO_2 efflux, root respiration, and microbial respiration were transformed by their natural log (ln) to meet the assumption of constant variance. All values are presented as non-transformed averages. Temperature squared and θ_v were used as covariates to normalize respiration rates to a constant temperature and moisture. If one or both covariates were not significant at the 0.05 alpha level they were dropped from the model. We performed an ANOVA on total soil N for each sampling date, and on soil pH from samples collected on January 18, 2005. August 19, 2004 and October 25, 2004 E_C data was dropped from the data set due to recent heavy precipitation. We measured unreasonably high respiration rates, which we hypothesized was due to bursts of air released as soil capillaries drained. These dates remained in the data set for R_H and R_R since both measurements are performed after the soil was disturbed eliminating any drainage problems.

Linear regression was used to determine what variation in total CO_2 efflux, heterotrophic, and root respiration was explained by soil temperature and θ_v (Table 2.2). The ln was used to transform y-variables to meet the assumption of equal variance, and θ_v was transformed by the ln of the arcsine (lnarcsin). All statistical analyses were performed using the PROC GLM and PROC REG in SAS® version 9 (SAS Institute, Cary, NC).

Results

Weather data

The average yearly air temperature was 13.7°C, with a maximum of 32.9°C in the month of July and a minimum of -15.1°C in January. Soil temperature at a depth of 15-cm peaked to a maximum daily average of 26.0°C in early August and reached a low of

1.6°C in late January (Figure 2.1A). Precipitation totaled 1289-mm for the year. With exception of a few large events, precipitation was evenly distributed throughout the year (Figure 2.1B). Percent volumetric soil water content fluctuated reaching an average daily maximum of 32% in August, 2004 and a minimum daily mean of 16% in April, 2005 (Figure 2.1B).

Effect of fertilization on total soil CO_2 efflux

When analyzed by repeated measures we found highly significant ($P<0.0001$) differences in the date by fertilizer interaction. We found no significant differences between clones or clonal interaction terms (Table 2.3). An analysis by date showed E_C rates did not initially differ due to fertilizer until approximately two months after fertilization. By late June E_C rates in fertilized plots decreased relative to control plots, and was significant ($P\leq0.05$) on four sampling dates. This trend remained until early September, at which point, E_C rates in fertilized plots returned to control levels and remained with the exception of one sampling date in mid March when rates in fertilized plots increased significantly ($P<0.05$) relative to control rates (Figure 2.2A).

Daily average total CO_2 efflux rates ranged from 6.8 to 0.6 μmol CO_2 m^{-2} sec^{-1} occurring in mid August and January, respectively. CO_2 Efflux rates tracked soil temperature (Figure 2.2A; figure 2.1A) with the greatest rates occurring in summer and lowest rates occurring in winter months. Temperature alone explained 30% of the variation ($R^2=0.3043$) in E_C when treatments were bulked together (Table 2.2).

Effect of fertilization on heterotrophic respiration

When R_H was analyzed by repeated measures, there was a significant ($P=0.0002$) fertilizer by date interaction. Clone and the clone by fertilizer interaction were found to be not statistically significant at the 0.05 alpha level (Table 2.3). When analyzed by date, R_H showed an immediate decrease in fertilized plots relative to control plots and this trend remained constant over the entire study (Figure 2.2B). Heterotrophic respiration rates decreased significantly ($P\leq0.05$) on three separate sampling dates and, in addition, were found to be slightly significant ($P\leq0.10$) on five sampling dates. When R_H rates

were averaged over the entire study, R_H in fertilized plots was 32% lower then control plots.

Daily average R_H ranged from $8.79e^{-4}$ in mid August to $1.96e^{-4}$ μmol CO_2 g^{-1}_{soil} sec^{-1} in mid January. Microbial respiration followed the same trend as soil temperature, with the largest values occurring in August and early September and the lowest values occurring during the winter months (Figure 2.2B; figure 2.1A). Temperature accounted for only 11% of the variation and θ_v accounted for an additional 6% of the variation R_H (Table 2.2).

Effect of fertilization on specific root respiration

Results from the time-series analysis indicate R_R increased significantly ($P=0.0597$) in the fertilized plots as compared to control plots (Table 2.3). Neither clone nor the interaction of clone by fertilizer was found to be statistically significant at the 0.05 alpha level. When all post fertilization respiration rates were averaged over the entire year fertilized plots were 20% greater then non-fertilized plots. Fertilization did not have an immediate affect on R_R for approximately two months, at which point R_R rates increased, though not significantly ($P<0.05$), relative to controls (Figure 2.2C). With exception to a measurement taken in mid November, when the roots were not physiologically active, R_R remained increased in fertilized plots throughout the duration of the study. Temperature only accounted for 14% of the variation in R_R, and θ_v was found to be non-significant at the 0.05 alpha level (Table 2.2).

Soil properties

The addition of nitrogen fertilizer showed an immediate increase in total soil nitrogen content which quickly fell below control levels. Surprisingly, on two sampling dates, plots that received nitrogen fertilization were significantly ($P\leq0.05$) lower relative to plots that did not receive fertilization (Figure 2.3A). When analyzed as percent change from pre-fertilization rates it became apparent fertilized plots simply fell back to pre-fertilization levels illustrating the transient nature of inorganic nitrogen in soils (Figure 2.3B). Soil pH was found to decrease significantly ($P\leq0.05$) by approximately 0.11 pH

units from 4.21 in the control plots to 4.10 in fertilized plots. As expected, we found no statistical difference between treatments in bulk density.

Discussion

Effects on total soil CO_2 efflux

Our daily average E_C rates ranged from 6.8 to 0.6 μmol CO_2 m^{-2} sec^{-1}. These values are similar to those found by others in loblolly pine (Maier and Kress 2000, Pangle and Seiler 2002, Lee and Jose 2003, Selig 2003, and Gough and Seiler 2004a) and other conifers (Haynes and Gower 1995, Mattson 1995). When we performed an analysis over the entire study we found a significant difference in E_C between fertilized and unfertilized plots, but the degree and direction was dependent on the sampling date. Contrary to our original hypothesis, that E_C would decrease after fertilization (Maier and Kress 2000, Bowden *et al.* 2004), our findings showed that early in the study E_C rates were lower in fertilized plots due to depressed R_H rates, but returned to control levels by the end of the first year. We speculate the lack of difference in E_C between fertilized and unfertilized plots was due to a combination of increasing specific R_R, which we reported, and increasing root biomass that was able to balance the decrease in R_H, which was also hypothesized by others (Gough and Seiler 2004a, Lee and Jose 2004).

Gough and Seiler (2004a) found similar results in a greenhouse study conducted on one-year-old potted loblolly pines. The authors found a significant ($P \leq 0.05$) increase in E_C compared to control pots 4 to 13 days following fertilization. From 13 to 47 days following fertilization E_C rates reversed and were significantly ($P \leq 0.05$) lower in fertilized pots, and after day 47 E_C in fertilized pots were, again, significantly ($P \leq 0.05$) greater. In contrast Lee and Jose (2003) found no significant ($P \leq 0.05$) difference in E_C due to fertilization in a 7-year-old loblolly pine stand located in Florida. The authors observed a significant ($P \leq 0.05$) decrease in microbial biomass and found a trend, though not statistically significant, of increasing fine-root production with increasing rates of N fertilization. Likewise, Pangle and Seiler (2002) found that fertilization did not significantly ($P \leq 0.05$) effect E_C in a two-year-old loblolly pine stand located on the Virginia Piedmont.

Effects on heterotrophic respiration

When averaged over the entire year we found a 32% reduction in R_H in fertilized plots relative to the controls. Eight days following fertilization we found a decrease in R_H rates that remained depressed over the entire study (Figure 2.2B). When heterotrophic respiration was analyzed over the entire year we found that respiration was significantly different between fertilized and unfertilized plots, but because of the large amount of variation, was dependent on date.

In support of our findings, other studies have found a decrease in R_H rates after fertilization with ammonium nitrate (Roberge 1976, Bååth *et al*. 1981, Nohrstedt *et al*. 1989, Smolander *et al.* 1994, Bowden *et al.* 2004). Gough and Seiler (2004) found that microbial respiration was significantly ($P \leq 0.05$) depressed throughout the entire 197 days in potted loblolly pine seedlings grown in a greenhouse. The authors found that microbial activity per gram of soil C was only 44 and 66% of that measured in control pots at 49 and 197 days following fertilization, respectively. In contrast, Haynes and Gower (1995) found no difference in heterotrophic respiration between fertilized and control trenched plots in a 31-year-old red pine (*Pinus resinosa* Ait.) plantation in northern Wisconsin.

One possible explanation for decreased R_H rates is a reduction in soil pH. This explanation is unlikely since soil pH was reduced on average by only 0.11 pH units in the fertilized plots relative to control plots. Though not biologically significant, our findings are consistent with other studies that have shown ammonium nitrate (NH_4NO_3), and other ammonium salts lowered soil pH, accompanied by a decrease in microbial activity (Kowalenko *et al.* 1978, Thirukkumaran and Parkinson 2000, Bowden *et al.* 2004). Diammonium phosphate (DAP) and ammonium nitrate are considered neutral fertilizers, but small decreases in pH can occur by a number of processes. The addition of NH_4^+ can decrease pH within the rooting zone by the release of protons from the roots necessary to maintain a neutral charge. Also, NH_4^+ can replace Al^+ on exchange sites, which when hydrolyzed, results in decreased soil pH. The process of nitrification results in the release of protons into the soil solution. An increase in R_R, which we observed, could result in higher CO_2 concentration in the soil rhizosphere, which could also lead to a decrease in soil pH.

A study by Leckie *et al.* (2004) suggests that microbial composition and activity in the long-term may be influenced by fertilization to a greater extent by indirect effects on plant growth and litter input as opposed to direct effects on microbial populations. The addition of N may cause shifts in microbial population dominance within the soil community such as a decrease in ecto-mycorrhizae (Wallander and Nylund 1992, Nilsson and Wallander 2003), or a shift in carbon allocation to aboveground organs (Haynes and Gower 1995, Ryan *et al.* 1996). However, it is unlikely that either of these long-term effects explain our rapid decrease after just eight days.

Effects on specific root respiration

Specific root respiration was increased by 20% in fertilized plots, and was found to be significantly greater when analyzed over the entire year. The daily averages ranged from a high of 1.72 to 0.51 μmol CO_2 m^{-2}_{root} sec^{-1}, which is similar to respiration rates found in loblolly pine grown in pots (Gough and Seiler 2004a). As Rakonczay *et al.* (1997) hypothesized these rates could reflect the upper limits due to a reduction in CO_2 concentration when roots were removed from a much higher ambient soil CO_2 concentration and measured at ambient atmospheric CO_2 concentrations. Some researchers have found that measuring root respiration at lower CO_2 concentration results in a higher root respiration rate (Qi *et al*. 1994, Clinton and Vose 1999, McDowell *et al*. 1999), while others found no change or a decrease in respiration rates (Bouma *et al*. 1997, Burton and Pregitzer 2002, Lipp and Andersen 2003).

Our findings are supported by other studies that have found increased R_R in response to fertilization. Lu *et al.* (1998) observed R_R increased significantly ($P \leq 0.05$) with an increase from 10 to 50 mg N l^{-1}, but remained steady with a further increase to 200 mg N l^{-1} in Douglas-fir [*Pseudotsuga menziesii* (Mirbl) Franco]. Gough and Seiler (2004a) found a significant ($P \leq 0.05$) increase in R_R 49 days after fertilization with DAP, but after 197 days specific root respiration rates returned to control levels leading the authors to conclude that timing may play an important role in detecting changes in specific root respiration. In contrast, Maier and Kress (2000) found no significant increase in specific root respiration in loblolly pine seedlings due to fertilization except for the month of May. Similarly, Pangle and Seiler (2002) found no significant ($P \leq 0.05$)

increase in specific root respiration in two-year-old loblolly pine seedlings in response to nitrogen one year after fertilization, and Zogg *et al.* (1996) found no increase during the first growing season in specific fine-root respiration of sugar maple (*Acer saccharum* Marsh.).

Increases in specific respiration rates in response to N fertilization can be attributed to the energy expended during uptake and assimilation of N. Increases in tissue N concentrations have been correlated with increased R_R (Widén and Majdi 2001). Ryan *et al.* (1996) found a positive relationship between tissue N content and maintenance respiration which they hypothesized was related to protein (e.g. Rubisco) repair and replacement. It requires both ATP generated from respiration and C-skeletons from stored carbohydrates or recent photosynthesis to actively absorb, reduce, and assimilate nitrogen ions, predominately ammonium (Bedell *et al.* 1999), into amino acids, nucleic acids, and other nitrogen containing compounds.

Clonal effects

There was no effect of clone as a main effect or as an interaction on CO_2 efflux, root, or heterotrophic respiration (Table 2.1; table 2.3). This is not surprising, since E_C and R_H, although influenced by the clones, are not measurements taken directly on the clones. We have not ruled out clonal differences in carbon use efficiency of below-ground plant organs. There is the possibility that differences haven't yet developed in the first year after fertilization, and may manifest themselves later in stand development.

Effects of temperature and soil moisture

Many studies have shown a strong relationship between soil temperature and respiration rates (Haynes and Gower 1995, Boone *et al*. 1998, Davidson *et al*. 1998, Niklińska *et al*. 1999, Chen *et al*. 2000, Fang and Moncrieff 2001, Qi and Xu 2001, Widén and Majdi 2001, Pangle and Seiler 2002, Gough and Seiler 2004b, Wieser 2004). In our study, temperature was a highly significant variable, but explained only 30% of the total variation in E_C and a very small percentage of the variation for R_H and R_R (Table 1). Moisture was only a significant regressor in R_H and explained very little of the variation. Fang and Moncrieff (2001) found that when soil moisture stayed within an optimal range

it had little to no effect on E_C, as did Gough *et al.* (2004) on loblolly pine stands that ranged in age on the Upper Coastal Plain of South Carolina. The low explanatory power of our models could be partly a result of not accounting for the variation due to fertilization. We found that temperature explained a larger percent of the variation in E_C and R_H when only data from the plots that did not receive nitrogen were used in the regression. In contrast, temperature explained less of the variation in R_R when control seedlings were analyzed separately. We found a significant ($P \leq 0.01$) increase in the slope estimate for E_C when we regressed respiration rates by temperature in the plots that did not receive fertilization indicating that E_C became less sensitive to temperature when fertilizer was introduced.

Effects of fertilization on soil carbon

How will fertilization influence carbon storage potential of forest soils? Our results suggest that if the trends we observed in the first year continue, in the long-term, soil carbon content could potentially increase due to fertilization. Fertilization has been shown to result in increased coarse and overall root biomass (Albaugh *et al.* 1998, Maier and Kress 2000, Samuelson 2000, King *et al.*, 2002, Pangle and Seiler 2002, Lee and Jose 2003, Gough and Seiler 2004a), or increased aboveground biomass in Scots pine, Norway spruce (Axelsson and Axelsson 1986), and loblolly pine (Albaugh *et al.* 1998, Pangle and Seiler 2002, Albaugh *et al.* 2004, Gough *et al.* 2004). An increase in aboveground biomass may results in more carbon being deposited into the soil as litter fall (Haynes and Gower 1995, Maier and Kress 2000), root exudates, and root turnover. Another way fertilization may increase soil carbon is by decreasing the rates at which organic matter (e.g. roots and litter) in the soil is decomposed, which our data supports by decreased heterotrophic respiration rates in fertilized plots. High N concentrations have been shown to suppress lignin-degrading enzymes produced by white-rot fungi and other decomposers, or from the formation of highly recalcitrant compounds formed by the reaction of low molecular weight N compounds and products from lignin degradation (Berg 2000). Thirukkumaran and Parkinson (2000) found that after 40 days fertilization with ammonium nitrate decreased decomposition while fertilization with urea and P did not suppress decomposition of aspen leaves. Others have found increases in soil carbon

could not be explained by increased inputs alone and that there must be a decrease in the rate of decomposition (Nohrstedt *et al*. 1989, Smolander *et al.* 1994).

Table 2.1. Average total CO_2 efflux (E_C), heterotrophic (R_H), and root respiration (R_R) by clone. Full sib clones are arranged in pairs. There were no significant ($P \leq 0.05$) differences between clones. This study took place at the Reynolds Homestead Forest Resources Research Center (Patrick Co., Virginia). Loblolly pine clones were donated by the Forest Biology Research Cooperative for use in this study.

Clone	**Parent 1**	**Parent 2**	**Avg E_C (SE)** ($n \geq 191$)	**Avg R_H (SE)** ($n \geq 184$)	**Avg R_R (SE)** ($n \geq 75$)
41021	10-10	22-44	3.94 (0.27)	3.93 E^{-4} (0.23 E^{-4})	1.06 (0.08)
41089	10-10	22-44	4.08 (0.33)	4.85 E^{-4} (0.27 E^{-4})	1.00 (0.06)
43671	7-56	10-5	4.09 (0.29)	3.82 E^{-4} (0.23 E^{-4})	1.11 (0.07)
43694	7-56	10-5	4.23 (0.31)	3.85 E^{-4} (0.24 E^{-4})	1.09 (0.07)
43903	10-10	7-1011	3.96 (0.26)	4.35 E^{-4} (0.30 E^{-4})	1.05 (0.08)
43976	10-10	7-1011	4.28 (0.30)	3.33 E^{-4} (0.17 E^{-4})	1.06 (0.06)

Table 2.2. Linear models and coefficient of determination values for total CO_2 efflux, heterotrophic respiration, and 'root' respiration. Only significant ($p \leq 0.05$) regressors were retained in the model.

Dep. variable	**Regression equation**	**R^2**
Total CO_2 efflux	$\ln E_C = -0.24 + 0.07$ (temp)	0.3045
Microbial respiration	$\ln R_H = -7.97 + 0.03$ (temp) + 1.06 (lnarcsin moist)	0.1670
Root respiration	$\ln R_R = -0.88 + 0.04$ (temp)	0.1352

Table 2.3. Partial ANOVA's for time-series analysis for total CO_2 efflux, heterotrophic respiration, and root respiration. Analysis performed using PROC GLM in SAS .

Source	Numerator df	Denominator df	F-value	P-value
Total CO_2 efflux				
fert	1	3	0.15	0.7247
clone	5	30	0.75	0.5927
clone*fert	5	30	1.93	0.1190
date	20	710	59.22	<.0001
date*fert	20	710	2.85	<.0001
date*clone	100	710	0.74	0.9688
date*clone*fert	100	710	0.98	0.5512
Heterotrophic respiration				
fert	1	3	3.12	0.1753
clone	5	30	1.09	0.3852
clone*fert	5	30	0.39	0.8487
date	22	783	12.27	<.0001
date*fert	22	783	2.51	0.0002
date*clone	110	783	0.91	0.7249
date*clone*fert	110	783	0.85	0.8502
tempsq	1	783	7.11	0.0078
Root respiration				
fert	1	3	8.74	0.0597
clone	5	30	0.91	0.4851
clone*fert	5	30	1.87	0.1286
date	8	279	10.72	<.0001
date*fert	8	279	1.48	0.1645
date*clone	40	279	0.70	0.9126
date*clone*fert	40	279	0.81	0.7856
Tempsq	1	279	5.39	0.0209

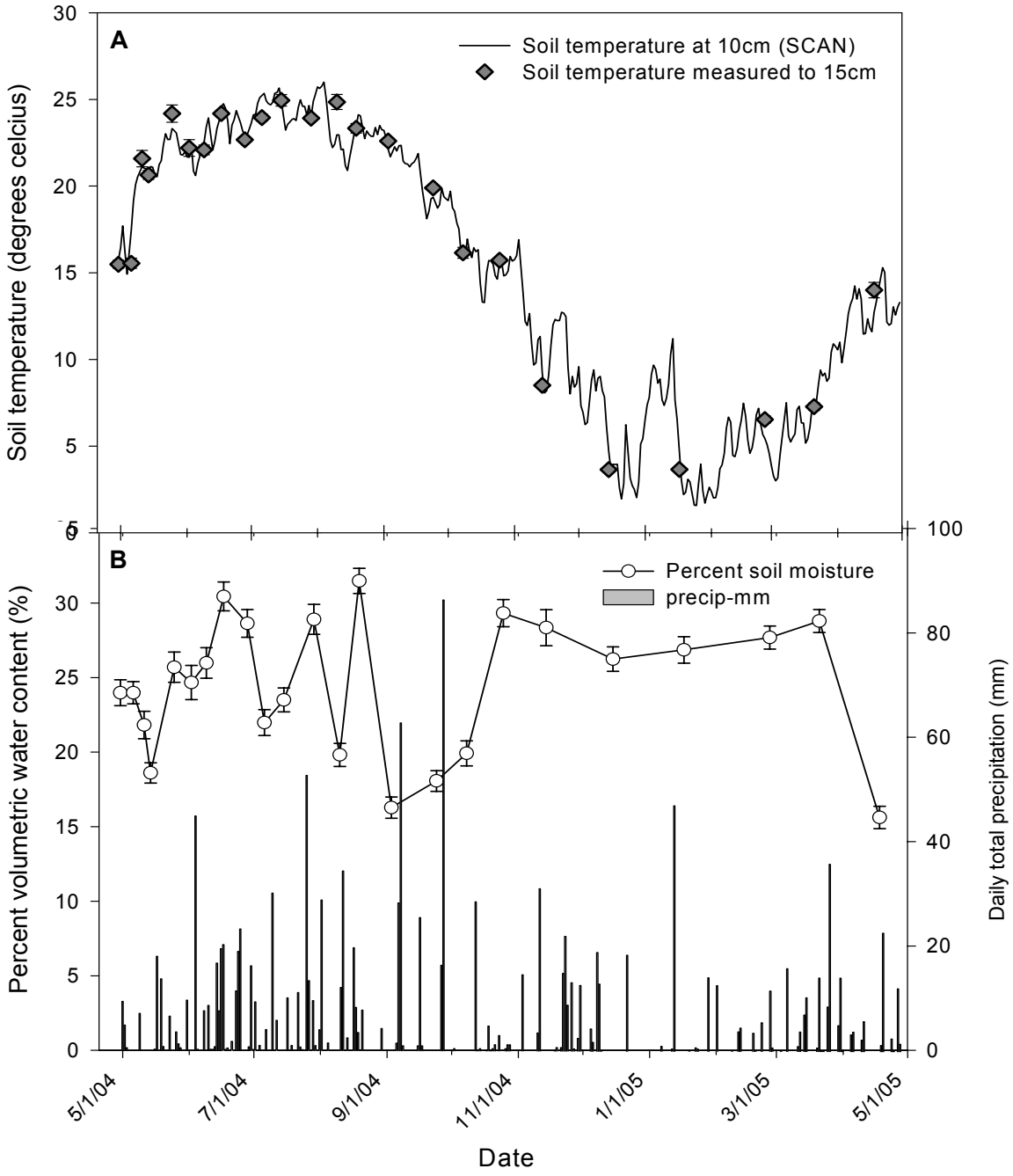

Figure 2.1. Soil temperature (A), percent volumetric water content and total precipitation (B) over a one year period at Reynolds Homestead Forest Resources Research Center (Patrick County, Virginia). Real time soil temperature at 10-cm depth was collected by (SCAN) weather station, United States Department of Agriculture, Natural Resources Conservation Service, site 2089. Soil temperature and percent soil moisture were measured at a depth of 15-cm and 13-cm, respectively, during 24 sampling periods (Error bars show $\pm$ 1 standard error from the mean; n=48).

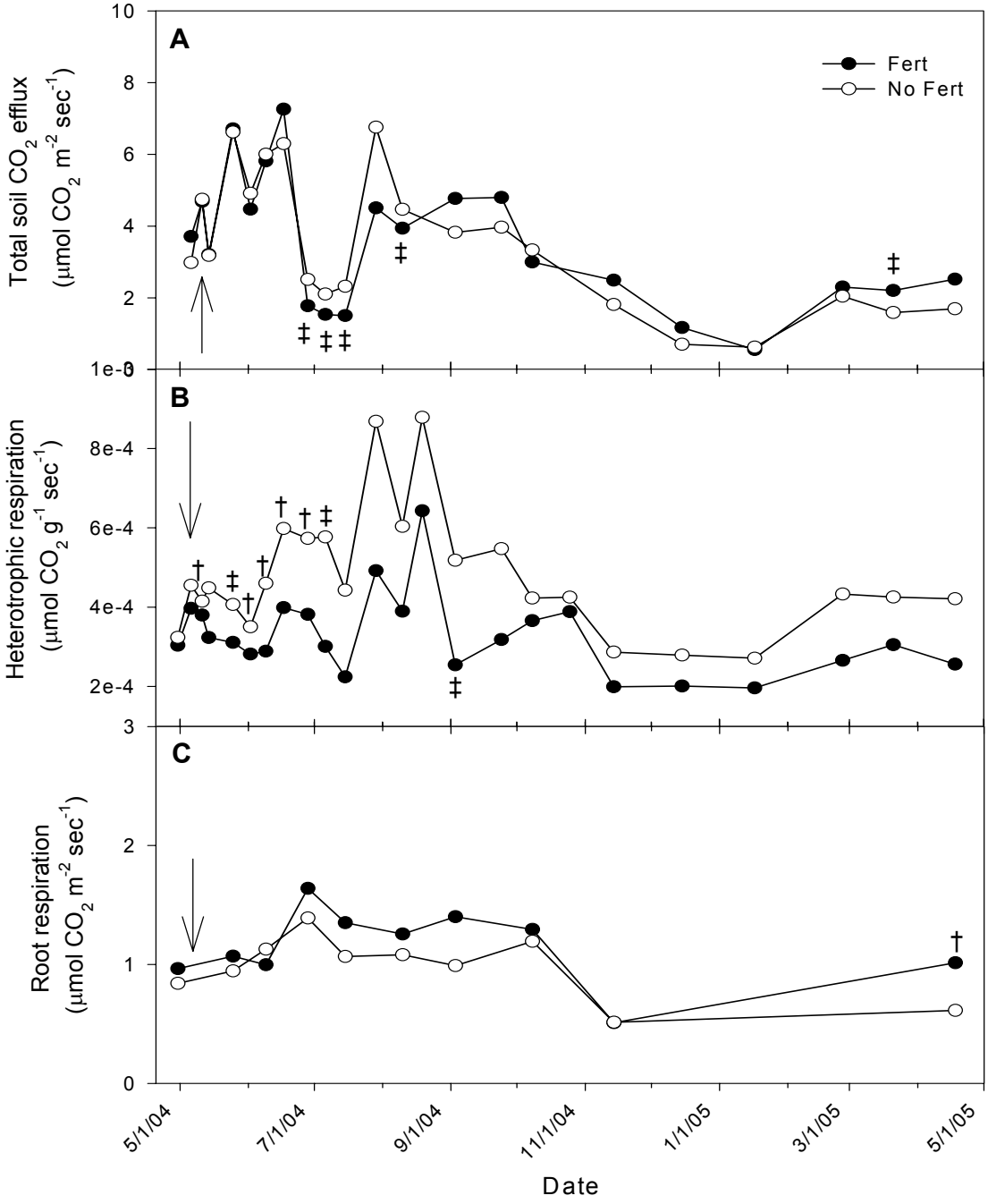

Figure 2.2. Total CO_2 efflux (A), heterotrophic (B), and root respiration (C) measured over one year in a two-year *Pinus taeda* plantation located at Reynolds Homestead Forest Resources Research Center (Patrick County, Virginia). Arrow indicates time of fertilization. Significant differences between treatments are represented by (‡) at the 0.05 and (†) at the 0.10 alpha level (n = 24). Fertilized plots received 112 kg N ha^{-1} and 23 kg P ha^{-1} in the form of diammonium phosphate and ammonium nitrate (225 kg ha^{-1} and 186.5 kg ha^{-1}, respectively).

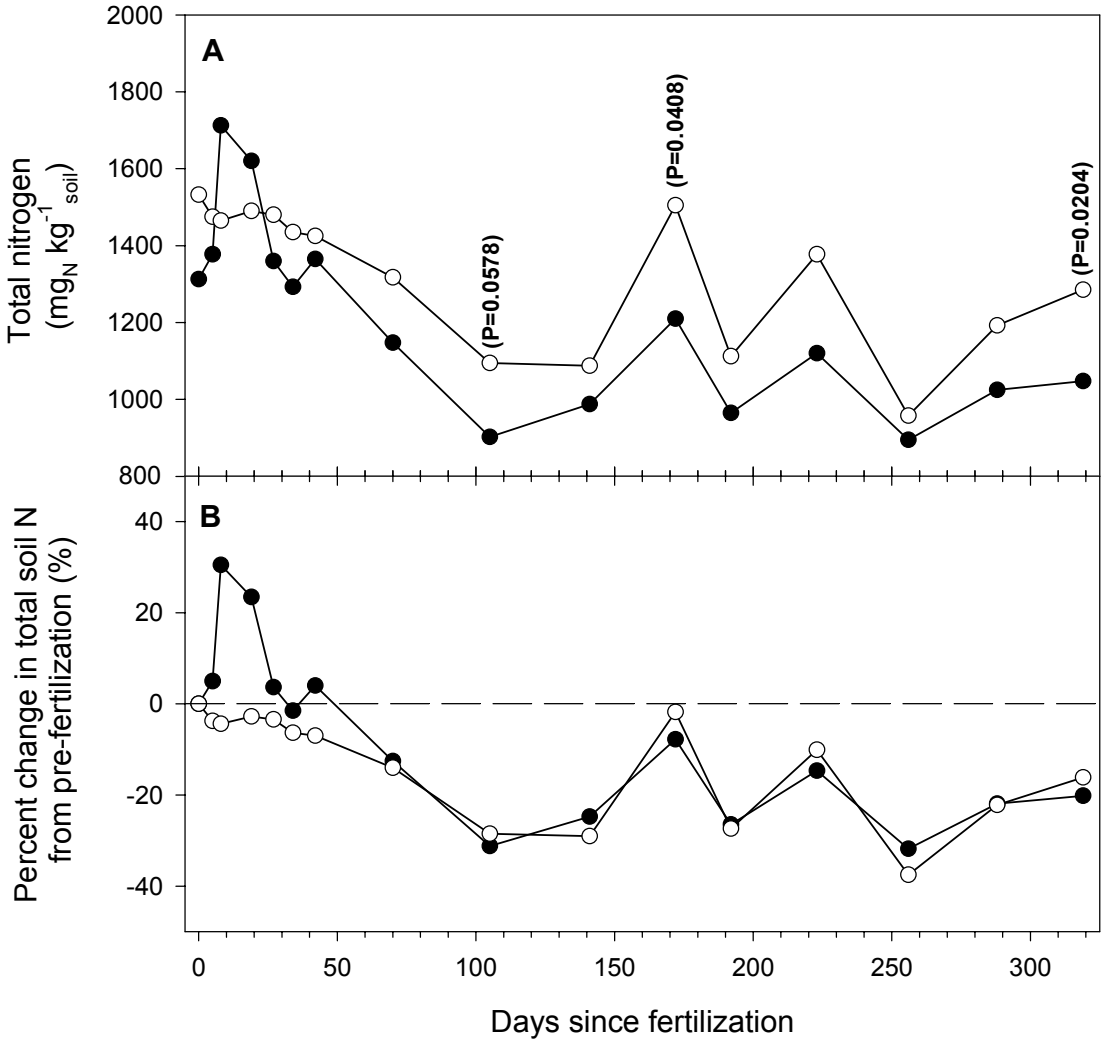

Figure 2.3. Total soil nitrogen (A) and percent difference in total soil N from pre-fertilization rates (B) measured over one year in a two-year *Pinus taeda* plantation located at Reynolds Homestead Forest Resources Research Center (Patrick County, Virginia). Fertilized plots received 112 kg N ha^{-1} and 23 kg P ha^{-1} in the form of diammonium phosphate and ammonium nitrate (225 kg ha^{-1} and 186.5 kg ha^{-1}, respectively). Plots that received fertilizer are shown as closed circles (●) and control plots are shown as open circles (○). Significant differences between treatments are shown as p-values (n=4).

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CHAPTER 3

Short-term effect of fertilization and Captan on specific root respiration in hydroponically grown one-year-old loblolly pine (*Pinus taeda* L.) seedlings

Abstract. Root respiration (R_R) can make up 30-70% of the total soil CO_2 efflux (E_C). To properly model the effects of fertilization on net ecosystem productivity (NEP) we need to understand the direction and degree to which fertilization affects R_R. Separating R_R from respiring microorganisms living within the soil matrix has proven to be a major obstacle. Growing seedlings hydroponically is one of many methods for isolating R_R while at the same time allowing measurements to be taken repeatedly with minimal disturbance to the seedling. We performed a pair of greenhouse studies to observe the effects of fertilization in the form of diammonium phosphate (DAP) on R_R. Root respiration was measured using a Li-Cor 6200 infrared gas analyzer with a 0.25-L closed cuvette. The objectives were to determine how nutrient additions initially affect R_R in one-year-old loblolly pine seedlings. Secondly, we wanted to determine if Captan [N-(trichloromethylthio) cyclohex-4-ene-1, 2-dicarboximide], a mild fungicide, could be used to reduce or eliminate ecto-mycorrhizae upon visual inspection and how this might influence the R_R response to fertilizer. In experiment 1 we observed significant differences in the date x fertilizer (P=0.0003) and the date x Captan (P=0.0458) interactions. An analyses by date showed that, initially, at a high rate (100 ppm N, 49 ppm P) of fertilization R_R was significantly ($P \leq 0.10$) increased relative to seedlings that did not receive fertilization. This increase was only temporary with rates returning to control levels by the end of the study. Experiment 2 also showed an initial increase in respiration rates with an eventual decline relative to control seedlings. No consistent trend was found between low (25 ppm N, 13 ppm P) and moderate (50 ppm N, 25 ppm P) rates of fertilization. Captan was shown to generally have no effect on R_R. Captan and DAP both showed (visual inspection) a decrease in fine-roots and mycorrhizae, which could explain the reduction in respiration rates observed by the end of the studies in these treatments. We concluded that further research to quantify changes in mycorrhizal biomass need to be conducted to determine Captan's effectiveness at reducing ecto-mycorrhizae without negatively affecting seedlings since we observed a 5% greater mortality in seedlings treated with Captan compared to seedlings that were not. In experiment 1, fertilized seedlings experienced high mortality rates. Water temperature (°C) was found to be a highly significant ($P<0.0001$) variable, but explained very little of the variance in R_R leading us to conclude that something else, such as solar irradiance, was responsible for daily differences in respiration rates.

Introduction

Root respiration contributes from 30-70% of total soil CO_2 efflux (E_C) (Raich and Schlesinger 1992, Andrews *et al*. 1999, Maier and Kress 2000, Ekblad and Högberg 2001, Widén and Majdi 2001, Pangle and Seiler 2002, Ruess *et al.* 2003) making it a significant component of the global carbon cycle. Total root respiration (R_R), as defined in this paper, is the sum of growth respiration (R_G), which is CO_2 emitted during the synthesis of new tissue, and CO_2 emitted during the repair and replacement of plant tissues referred to as maintenance respiration (R_M). While it is useful to partition R_R into R_G and R_M respiration for purposes of understanding, there is no biochemical difference in the products of the two processes. Another component that is sometimes included in R_R, because of its inherent difficulty in separating it, is rhizomicrobial respiration, which is respiration originating from organisms within the rhizosphere such as mycorrhizae and other organisms that are dependent on the plant for carbon and nutrients. Rhizomicrobial respiration can make up a significant portion of root respiration leading to large differences in reported respiration rates depending on whether included or separated during respiration measurements. Harley and Smith (1983) estimated that mycorrhizae associated with conifer roots accounts for 23-30% of respiration and Ek (1997) estimated mycorrhizae (*Paxillus involutus*) associated with European white birch (*Betula pendula* Roth) accounts for 11-25% of the root respiration. Biocides have been used to remove or significantly reduce mycorrhizae, which could allow for a better understanding of the effects of nutrient additions on root respiration. Captan® [preferred IUPAC name: N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide] is a mild fungicide, that at high concentrations, has been shown to reduce ectomycorrhizae (Pawuk *et al*. 1980, Ingham and Coleman 1984, Colinas *et al*. 1994) and vesicular-arbusculus mycorrhizal (VAM) development (Kough *et al*. 1987) without negatively affecting higher plants (Pawuk *et al* 1980, Marx and Rowan 1981, Wigand and Stevenson 1997).

Nitrogen is one of the most limiting elements to plant growth. The absorption and assimilation of nitrogen either as NH_4^+ or NO_3^- is energetically expensive for the plant, which can be observed by an increase in fine-root respiration (Bloom *et al.* 1992). Increases in root N concentrations have been shown to be positively correlated with R_R (Ryan *et al.* 1996, Zogg *et al.* 1996, Widén and Majdi 2001, Burton *et al.*2002). It is

hypothesized that increased nitrogen concentration increases R_M. It requires both ATP generated from respiration and C-skeletons from stored carbohydrates or recent photosynthate to actively take up, reduce, and assimilate nitrogen ions into amino acids, nucleic acids, and other nitrogen containing compounds. In conifers, ammonium is the dominant source of N owing to its greater availability in acidic forest soils (Bedell *et al.* 1999). There is no extra energy costs involved in reducing ammonium as with nitrate, but unlike nitrates, ammonium must be metabolized immediately to avoid reaching toxic levels.

One problem with accurately estimating root respiration is separating it from CO_2 that is being respired by soil organisms during decomposition of soil organic matter. There are a number of methods for trying to separate R_R from heterotrophic respiration (R_H) each with their own strengths and weaknesses. Methods that employ root exclusion such as trenching (Haynes and Gower 1995, Boone *et al.* 1998, Lavigne *et al.* 2004) and tree girdling (Högberg *et al.* 2001, Singh *et al.* 2003) prevent disturbance of root-fungi associations and fine-roots, but deprive soil organisms of a much needed energy source (e.g. root exudates). Others have measured root respiration directly on excised roots in greenhouse (Gough and Seiler 2004) and field experiments (Zogg *et al.* 1996, Rakonczay *et al.* 1997b, Maier and Kress 2000, Widén and Majdi 2001, Ruess *et al.* 2003), or *in situ* by placing a sections of root in a cuvette and reburying it in the ground (Rakonczay *et al.* 1997a), these methods allow measurements to be taken in the field, but preventing disturbance of fine-roots and fungal mycelium is unavoidable. Using carbon-13 (Andrews *et al.* 1999, Ekblad and Högberg 2001, Singh *et al.* 2003) or carbon-14 (Kuzyakov *et al.* 2002) labeling is a useful tool for separating root from R_H, but is quite expensive. Growing seedlings hydroponically (Bloom *et al.* 1992, Cramer and Lewis 1993, BassiriRad *et al.* 1997, Lasa *et al.* 2002), or in sterilized soils (Lu *et al.* 1998) is a good method for gaining insight into how R_R responds to a specific treatment, but as pointed out by Kuzyakov *et al.* (2002) is not necessarily representative of how a plant will react under natural conditions.

We have conducted a pair of greenhouse studies to explore the short-term effect of fertilization, in the form of diammonium phosphate (DAP), on specific fine-root respiration in one-year-old loblolly pine seedlings grown hydroponically. Specifically,

we wanted to observe how R_R responded to nutrient additions. We hypothesize that R_R will increase as the rate of fertilization increases eventually decreasing as seedlings become nitrogen saturated. Secondly, we wanted to determine, upon visual inspection, if Captan, a mild fungicide, was effective in reducing or eliminating ectomycorrhizae and what effect this had on the R_R response to fertilizer. These studies serve as pilot studies to determine to what direction and degree DAP will affect R_R. We recognize no attempt was made to measure nitrogen or phosphorous uptake (root tissue N and P concentration), plant growth, or to quantify mycorrhizal biomass.

Materials and Methods

Experiment 1

The design was a completely randomized design with a 2x2 complete factorial with fertilization and fungicide. One-year-old improved loblolly pine clones, produced by somatic embryogenesis, were donated by Plum Creek Timber Co. on February 9, 2004 for use in this study. However, differences between clones were not investigated in this study. Twenty-eight seedlings were randomly assigned to each of 4 tanks and grown hydroponically in a greenhouse, under ambient light and temperature, for 10-weeks. On February 17, 2004 seedlings were washed and roots trimmed to approximately 15-cm length. Seedlings were refrigerated until February 27, 2004, at which point they were placed into tanks.

Each 38 L (10 gal) tank was wrapped in aluminum foil to keep out light, stabilize temperature, and minimize algae growth. Seedlings were floated using 5-cm thick high density construction foam, and oriented so that their tops were exposed to light and roots submerged in water. Foam also served to limit movement within the tanks and to prevent light from penetrating the surface (Figure 3.1). Tanks were filled with 28-L of tap water and aerated with a pump (1 per tank) split with a T-connector linking two air stones positioned at opposite ends of the tank. Each tank received a base rate of fertilizer weekly using water soluble 20-20-20 all purpose plant food with micronutrients (Peters Professional®, Spectrum Group Div. of United Industries Corp., St. Louis, MO) at a rate of 2 ppm N and 1 ppm P. Every week, the seedlings were re-randomized and relocated to an identical set of tanks allowing tanks to be sterilized, and seedlings to be re-randomized

within their treatment group. By re-randomizing seedlings, between tanks and location within the greenhouse, we were able to treat this experiment as a completely randomized design as has been done in studies utilizing growth chambers (Teskey and Will 1999).

On March 19, 2004 tanks were randomly assigned one of four treatments: fertilizer (F), fertilizer and fungicide (FCAP), fungicide (CAP), and control (C). Treatments receiving fertilizer were brought to 100 ppm N and 49 ppm P in the form of diammonium phosphate (DAP) at the rate of 462 mg L^{-1}, the equivalent of 98 ppm N and 48 ppm P, in addition to the base fertilization rate. From this point on fertilization rates will be referred as 100 and 0 ppm N. Seedlings receiving fungicide were dipped in a 1% solution of N-(trichloromethylthio) cyclohex-4-ene-1, 2-dicarboximide (Captan-50% a.i. WP, Bonide Products, Inc., Oriskany, NY) for 60-min. Captan treatments were applied on March 19, 2004 (10 days prior to pre-fertilization measurements) and each week (using the same solution) immediately following respiration measurements.

Pre-fertilization measurements were taken on March 29, 2004 to determine base respiration rates. Fertilizer treatments were applied on March 29, 2004 following pretreatment measurement. On April 1, 5, 12, and 23, 2004 the respiration measurements were taken on 80 seedlings using the Li-Cor 6200 infrared gas analyzer (IRGA) (Li-Cor Inc., Lincoln, Nebraska) with a 0.25-L cuvette chamber as a closed system, with a total system volume of 429-cm^3. The instrument was zeroed before each sample date and recalibrated by running a known CO_2 concentration through the system. R_R was taken by removing a seedling from its tank, roots were patted dry with a paper towel, and approximately 10-cm^2 section of root was immediately placed on a damp piece of paper towel in the cuvette (BassiriRad *et al.* 1997, Gough and Seiler 2004). After rates stabilized (usually within a couple minutes) CO_2 evolution was measured over a 30-second period and respiration rate (μmol m^{-2}_{root} sec^{-1}) calculated on a per unit root area using the following equation:

$$[1] \qquad \text{Soil CO2 efflux} = \left[\left(\frac{\Delta C}{\Delta t}\right)\left(\frac{PV_t}{RT}\right)\right] \div \text{root surface area}$$

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

Measured roots were marked with thin copper wire (so same section of root could be remeasured) and scanned with a USB-scanner at 300 dpi then immediately placed back in its tank. The scanned image was isolated using image software (Adobe Photoshop® 6.0, Adobe Systems Inc., San Jose, CA) and the root surface area (cm^2) determined using WinRhizo 5.0A software (Regent Instruments Inc., Quebec, Canada). Water temperature was taken concurrently with respiration measurements using a Digi-sense temperature gauge (model no. 8528-20, Cole-Parmer Instrument Co., Niles, IL) to the nearest 0.1ºC.

A multivariate analysis of variance (MANOVA) with repeated measures was used to test the effects of fertilization and Captan on R_R. Seedlings that died during the course of the study were completely removed from the dataset so that only seedlings repeatedly measured on every sampling date remained. The effects of temperature on R_R were analyzed using simple linear regression. All analyses were performed using SAS version 9 (SAS Institute, Cary, NC) with an alpha level of 0.10.

Experiment 2

This study was designed as a completely randomized design with six replications of four levels of fertilization 2 ppm N and 1 ppm P (none), 25 ppm N and 13 ppm P (low), 50 ppm and 25 ppm P (mod), and 100 ppm N and 49 ppm P (High) applied to one-year-old loblolly pine seedlings grown hydroponically in a greenhouse at ambient temperature and light. From this point on fertilization rates will be referred to by their N concentrations. Open pollinated, improved seedlings were donated by Virginia Department of Forestry for use in this project. The design consisted of 38-L tanks (two per treatment) wrapped in aluminum foil filled with 28-L of tap water each containing 30 loblolly pine seedlings (as described above). On May 02, 2005 pre-fertilization measurements were taken to obtain a base root respiration rate and treatments applied immediately following. Fertilizer was applied in the form of DAP at a rate of 0, 23, 48, 98 ppm N, in addition to the 2 ppm N base solution, to bring tanks to their respective fertilization rates.

Root respiration rates were measured on six randomly selected seedlings (three per tank; 24 total per sampling period) using the Li-Cor 6200 with a 0.25-L closed cuvette chamber (as described above). Unlike the previous experiment, R_R was measured using approximately 10-cm^2 of excised root (<5-mm) and the seedling was discarded. A second change from the previous study involved determination of root surface area. Root area (cm^2) was directly (e.g. roots were not pre-scanned) determined using the WinRhizo 5.0A software and root scanner (Regent Instruments Inc., Quebec, Canada). Measurements were taken 1, 2, 4, 8, and 23 days following fertilization. Every week seedlings were re-randomized within tanks, and tanks were randomly assigned a new position on the greenhouse bench allowing for tanks to be sterilized and the replacement of nutrient solutions. Differences between treatments for each measuring date were determined with an analysis of variance (ANOVA) using general linear model in SAS version 9 (SAS Institute, Cary, NC). An alpha level of 0.10 was considered significant. Analysis of repeated measures could not be utilized since seedlings were discarded after each measure.

Results

Experiment 1

The date by fertilizer interaction was found to be highly significant (P=0.0003). When data were analyzed by date, respiration rates were significantly ($P \leq 0.10$) increased in fertilized relative to control seedlings by 33 and 35% on days 3 and 7 following fertilization, respectively. By the end of the experiment, rates were no longer statistically significant between fertilized and non-fertilized seedlings. Mean R_R rates were found to decrease by 16 and 8% in fertilized seedlings relative to non-fertilized seedlings 14 and 25 days after fertilization, respectively (Table 3.1; figure 3.2A). When analyzed over the course of the experiment the fungicide x date interaction was found to be significant (P=0.0458). Seven days following fungicide treatment Captan treated seedlings had significantly (P=0.0402) lower respiration rates by 22% (Table 3.1, figure 3.2B). The date x Captan x fertilizer interaction was found to be slightly significant (P=0.0713) when analyzed over the study (Table 3.1). Seedlings treated with both fertilizer and Captan were slightly, but not statistically significantly, increased relative to non-fertilized

seedlings and decreased relative to fertilizer only treated seedlings (Figure 3.3). Temperature was found to be a highly significant ($P<0.0001$) variable, but only accounted for 3% of the variation in R_R.

Mortality was quite high is some of the treatments. The FCAP treatment had the highest mortality rate at 65% of seedlings that died during the course of the study. The second highest mortality was 55% in the F treatment. There was 35 and 30% seedling mortality in CAP and C treatments, respectively. When treatments that received fungicide were bulked together, there was 48% seedling mortality. Likewise, when treatments that received fertilizer were bulked together, seedling mortality was 58%.

Experiment 2

On two separate sampling dates (4 and 16 days after fertilization) there were significant ($P\leq0.10$) differences between treatments. Four days following fertilization seedlings that received a high rate of fertilization (100 ppm N) showed increased R_R relative to control (2 ppm N) seedlings (Figure 3.4). Sixteen days following fertilization we found a reversal with seedlings receiving a high dose of fertilization showing a significant ($P\leq0.10$) decrease relative to control seedlings (Figure 3.4). We found no consistent trend or differences in R_R in seedlings that received low and moderate rates of fertilization during the course of this study. Temperature only explained 19% of the variation in R_R, but when date was included in the model both temperature and date explained 45% of the variation in R_R.

Discussion

Our daily average R_R ranged from 0.65 to 2.35 μmol CO_2 m^{-2}_{root} sec^{-1} for the two greenhouse studies, which are consistent with values we found in the field (Chapter 2) and in a greenhouse experiment (Gough and Seiler 2004) on excised loblolly pine roots. Lipp and Andersen (2003) showed that excising roots of 3-year-old ponderosa pine (*Pinus ponderosa* Laws.) did not impact R_R rates for up to six hours, although Rakonczay *et al.* (1997a) found that respiration rates dropped significantly ($P\leq0.05$) for the first 30 minutes after excision in eastern white pine (*Pinus strobus* L.), but then remained stable for 16 hours. Studies have shown that R_R rates measured at ambient atmospheric CO_2

concentrations were higher than when measured at ambient soil CO_2 concentrations (Qi *et al*. 1994, Clinton and Vose 1999, McDowell *et al*. 1999). Since our main objective was to observe the response of R_R to fertilization and not compare our rates with rates observed in the field no attempt was made to account for differences in CO_2 concentration.

Temperature was a highly significant ($P<0.0001$) variable in both experiments 1 and 2, but explained only 3 and 19% of the variance, respectively. Due to the lack of explanatory power of temperature on R_R, temperature was not used as a covariate. In contrast to our findings, others have shown that temperature exhibits a positive exponential relationship with R_R (Ryan *et al.* 1996, Boone *et al.*1998, Atkin and Tjoelker 2003). The lack of correlation we observed between temperature and R_R that we hypothesized was at least partly due to the small range in temperatures that the seedlings experience over the short duration of the experiment. Daily fluctuations in solar irradiance may have influenced differences in R_R between sampling dates more so than temperature. Photosynthesis is necessary to provide plants and associated mycorrhizae with energy to carry out maintenance and growth, and has been shown to be closely linked with root respiration using carbon-13 labeling (Ekblad and Högberg 2001) and tree girdling (Högberg *et al.* 2001) experiments. As Lipp and Andersen (2003) have pointed out, the increased carbohydrate storage in woody plants may be enough to offset daily changes in solar irradiance.

Both greenhouse experiments showed an initial increase in R_R between seedlings that received a high rate (100 ppm N) of fertilizer compared to seedlings that did not receive fertilization. This increase was found to be temporary with rates returning to, or decreasing below control levels within 30 days from the time of fertilization (Figures 3.2A and 3.4). Other researchers have also observed the transient nature of fertilization on R_R (Maier and Kress 2000, Gough and Seiler 2004). Gough and Seiler (2004) studied R_R in potted loblolly pine seedlings grown in a greenhouse over the course of one year, and found a significant ($P\leq0.05$) increase in R_R on day 49 with rates returning to control levels by day 197 following fertilization in the form of DAP. Maier and Kress (2000) measured R_R following three years of nutrient additions to an optimal foliar nutrition of 1.3% as urea in an 11-year-old loblolly pine stand located at the Southeast Tree Research

and Education Site (SETRES) in North Carolina. The authors found no increase in R_R due to fertilization except for the month of May when R_R was significantly ($P \leq 0.05$) higher in fertilized treatments relative to control treatments. Pangle and Seiler (2002) found no difference due to fertilization in R_R one year after fertilization in two-year-old loblolly pine seedlings. Lu *et al.* (1998) grew 6-month-old Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings in root boxes under three levels of N fertilization (10, 50, and 200 mg l^{-1} N; in the form of NH_4NO_3). The authors found that R_R in root boxes treated with the low level of N was significantly ($P \leq 0.05$) less then seedlings grown at higher rates of fertilization.

Increases in R_R could be due to increased maintenance costs associated with higher protein turnover (Bouma *et al.*1994, Ryan *et al.* 1996). Although, the short-term increase in R_R that we observed is more likely a result of energy expended to absorb and assimilate nitrogen. The decrease in R_R observed at the end of experiments 1 and 2 was unexpected. One explanation is after the initial increase we observed in R_R following fertilization, seedlings became nitrogen saturated and were unable to metabolize the NH_4^+ fast enough resulting in ammonium toxicity since excess ammonium cannot be stored (Lasa *et al.* 2002). Support of this hypothesis comes from the high mortality rates observed in experiment 1 with seedlings that received fertilization. Interestingly, we did not see any mortality in the second experiment. We believe that by repeatedly measuring R_R on the same plant, which we employed in experiment 1, may have stressed the seedlings, leaving them vulnerable to mortality. A second explanation for the observed decrease in respiration rates as the study progressed is that there were fewer fine-roots, and associated mycorrhizae, present (visual inspection) in seedlings that received high fertilization rates forcing us to use larger, more suberized roots sometimes referred to as brown roots, which are less physiologically active then white roots, resulting in lower respiration rates (Pregitzer *et al.* 1998, Widén and Majdi 2001, Lipp and Andersen 2003). Fertilization has been shown to decrease the proportion fine- to coarse-root biomass (Ryan *et al.* 1996, Albaugh *et al.* 1998, Maier and Kress 2000) and ectomycorrhizae (Wallander and Nylund 1992, Nilsson and Wallander 2003).

Upon visual inspection, seedlings that were treated with Captan showed less mycorrhizae and fine-roots then those which were not treated. Specific root respiration

was significantly ($P \leq 0.10$) decreased 7 days following a 1 hour Captan dip at 1% strength (Figure 3.2B). Although, this was the only day that significant differences were observed between treatments possibly caused by a dilution of the Captan with subsequent dips reducing its effectiveness. Reasons for the initial decrease can be attributed to a reduction in mycorrhizae or more likely a reduction of fine-roots. Captan has been shown to reduce ecto-mycorrhizae in longleaf pine (*Pinus palustris* Mill.) seedlings (Pawuk *et al*. 1980), but was not effective in reducing *Pisolithus tinctorius* or *Thelephora terrestris* (two common ecto-mycorrhizae used in nursery application) in loblolly pine (Marx and Rowan 1981). When applied as a soil drench, Captan has been shown to have no negative effects on seedling height or survival in pine (Pawuk *et al.* 1980, Marx and Rowan 1981). Conversely, we found our greatest mortality in seedlings that had been treated with both Captan and DAP, and found 5% greater mortality in Captan versus control treatments, indicating that Captan had a negative impact on seedling survival. Differences may have been attributed to the methods of application (drench versus dip), or the extra handling involved in Captan treated seedlings. Although we did not attempt to quantify ecto-mycorrhizal biomass, visually there was no difference between seedlings that were treated with Captan and seedlings that received fertilizer. Both reduced mycorrhizal formation and fine-roots. Interestingly, the significant ($P=0.0713$) date x Captan x fertilization interaction (Table 3.1) and differences between (F) and (FCAP) seedlings R_R means, although not statistically significant, seven days following fertilization (Figure 3.3) suggests that mycorrhizae may be partially responsible for the increased respiration rates in response to fertilization. Specific root respiration in treatments which received fertilizer (presumably seedlings + mycorrhizae) increased to a greater extent than the seedlings that had received fertilizer and Captan on day seven. This decreased fertilizer response in Captan treated seedlings does not appear to be due to any negative effect of Captan since R_R in Captan only treated seedlings did not differ from controls on day seven (Figure 3.3).

More work is needed to assess how Captan impacts mycorrhizal formation as well as any negative affects on the roots or root respiration rates. At this point we would conclude that use of Captan is not a good method for separating root and associated mycorrhizal respiration due to lack of knowledge of what effects it will have on

respiration. Hydroponic studies are a good way to separate R_R from R_H as well as allow measurements to be repeated with minimal disturbance to the plant, but as with all modified environments care must be taken when trying to apply results to field situations.

Table 3.1. Partial multivariate analysis of variance (MANOVA) table with repeated measures of specific root respiration for one-year-old loblolly pine (*Pinus taeda* L.) seedlings grown hydroponically in a greenhouse. Wilks' λ multivariate test was used to test the within subject effects at the 0.10 alpha level using PROC GLM in SAS version 9.

Source	Numerator df	Denominator df	Wilks' λ	F-value	P-value
Between subject effects					
Captan	1	40	---	1.27	0.2660
Fert	1	40	---	1.22	0.2751
Captan*Fert	1	40	---	2.10	0.1549
Within subject effects					
Date	4	37	0.2174	33.29	<.0001
Date*Captan	4	37	0.7746	2.69	0.0458
Date*Fert	4	37	0.5746	6.85	0.0003
Date*Captan*Fert	4	37	0.7969	2.36	0.0713

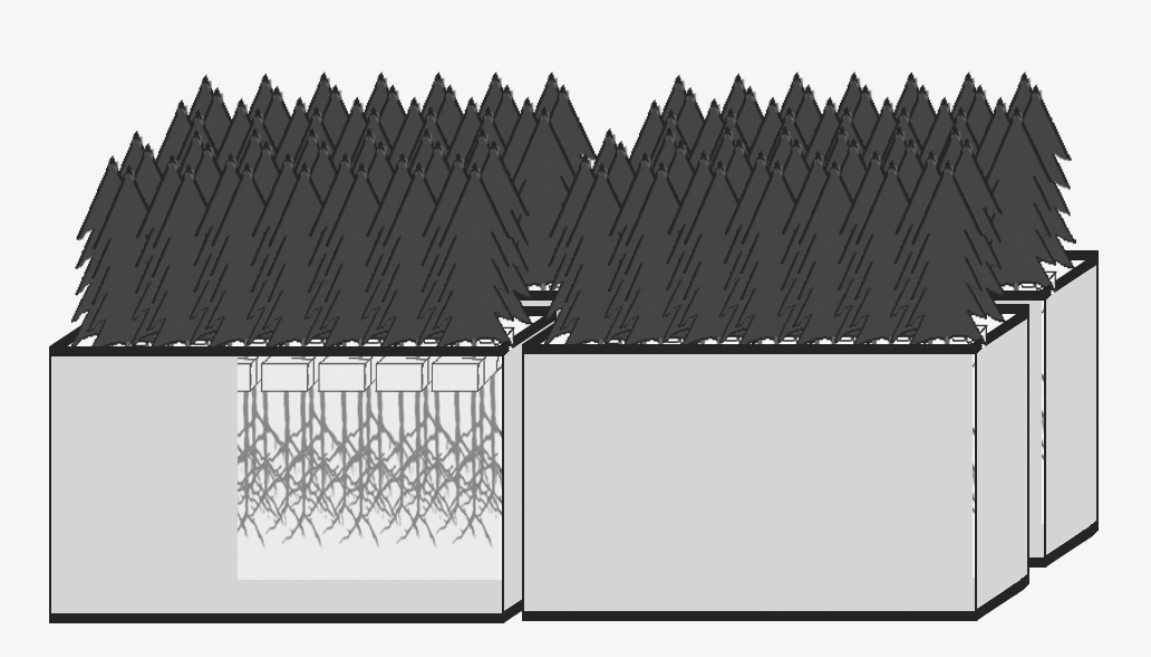

Figure 3.1. Side view of the greenhouse study. Four to eight 38-L tanks were randomly assigned a treatment, and a location on the greenhouse bench. Loblolly pine (*Pinus taeda*) seedlings were floated on 5-cm thick high density construction foam oriented so that roots are suspended in the nutrient solution with tops exposed to light. Tanks were aerated using pumps and two air-stones positioned at opposite ends of the tank.

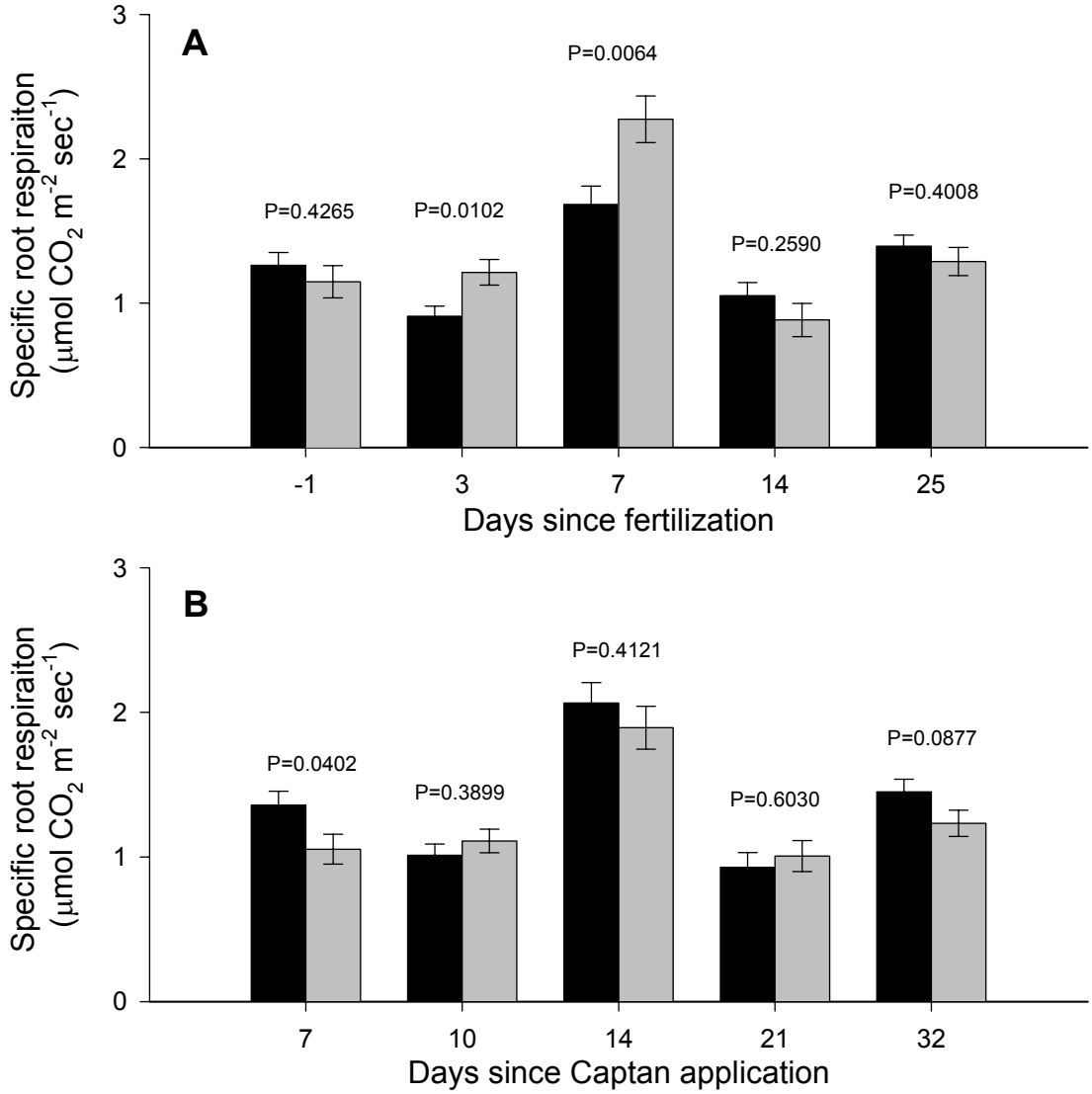

Figure 3.2. Main effect of fertilization on mean specific root respiration rates by sampling date in one-year-old *Pinus taeda* seedlings grown hydroponically in a greenhouse. Black bars represent seedlings that received no fertilization (n=27), and gray bars represent seedlings that were fertilized at a rate of 100 ppm N and 48 ppm P in the form of DAP (n=17) (A). Main effects of Captan on mean specific root respiration. Black bars represent seedlings that received no fungicide (n=23), and gray bars represent seedlings that were dipped for 60-min in 1% solution of Captan fungicide (n=21) (B). P-values are presented above each set and error bars represent $\pm$ 1 standard error.

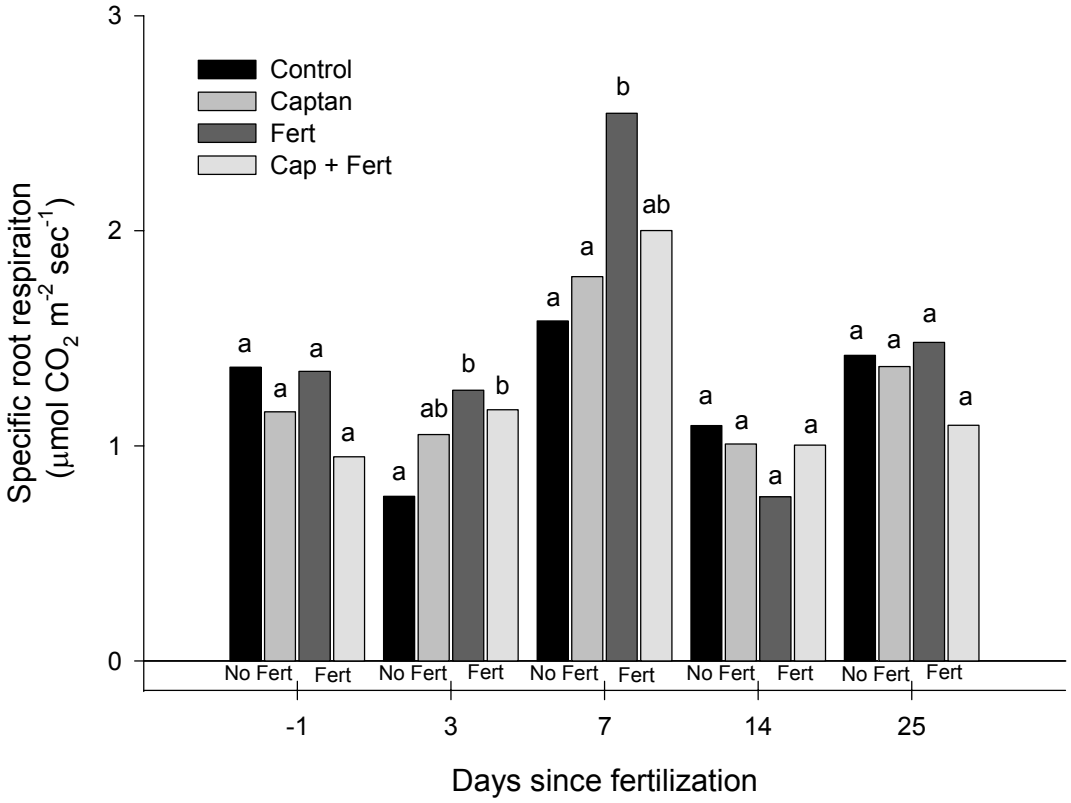

Figure 3.3. Mean specific root respiration rates by sampling date in one-year-old *Pinus taeda* seedlings grown hydroponically in a greenhouse. Fertilizer was applied at a rate of 100 ppm N and 48 ppm P in the form of DAP, and Captan was applied as a 60-min dip at a 1% concentration immediately following each measurement period. Different letters indicate significant ($P \leq 0.10$) differences in mean respiration rates within a sampling date (Tukey's HSD; $n \leq 17$).

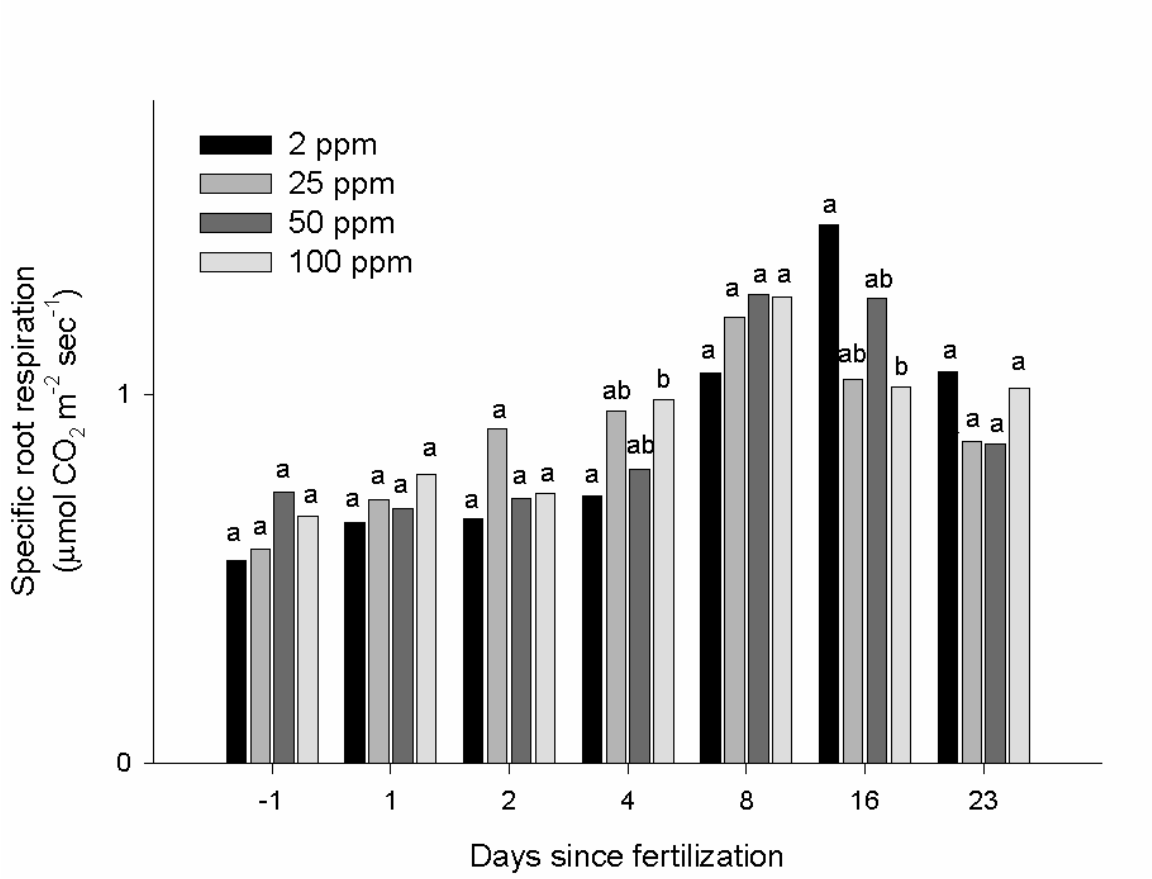

Figure 3.4. Mean specific root respiration rates by sampling date in one-year-old *Pinus taeda* seedlings grown hydroponically in a greenhouse with different levels of fertilization in the in the form of DAP. Different letters indicate significant ($P \leq 0.10$) differences in mean respiration rates for a specific sampling date (Tukey's HSD; n=6).

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CHAPTER 4
Conclusion

Findings and Implications

We found that heterotrophic respiration (R_H) was reduced immediately, and consistently after the addition of fertilizer in the form of DAP, but the degree of reduction was dependent of date as indicated by a highly significant (P=0.0002) date x fertilizer interaction (Chapter 2). There are a number of possible reasons for our observed decreases in R_H. Fertilization with ammonium has been shown to decrease soil pH, and consequently, microbial respiration (Kowalenko *et al.* 1978, Thirukkumaran and Parkinson 2000, Bowden *et al.* 2004). We observed a statistically significant (P<0.05) decrease in soil pH following fertilization, but the difference was only 0.11 pH units bringing into question the biological significance of pH difference between treatments. Other reasons could be: that fertilization may cause shifts in microbial population dominance within the soil community such as a decrease in ecto-mycorrhizae (Wallander and Nylund 1992, Nilsson and Wallander 2003), a shift in carbon allocation to aboveground organs (Haynes and Gower 1995, Ryan *et al.* 1996). However, it is unlikely that either of these long-term effects explain our rapid decrease after just eight days.

Specific root respiration (R_R) increased significantly (P=0.0597) over the first year in response to fertilization (Chapter 2). In the field study, increased R_R appeared two months following fertilization then remained steadily increased throughout the study. Reasons for this increase could be due to increased root tissue nitrogen concentrations, resulting in greater maintenance respiration in fertilized seedlings (Bouma *et al.* 1994, Ryan *et al.* 1996). The time it would take to increase protein content in the root could explain the delay we observed in a change in R_R (Chapter 2). In our two hydroponics studies we also found significant ($P \leq 0.10$) increases in R_R in response to fertilization with DAP, but rates quickly returned to control levels by the end of both one month experiments (Chapter 3). The transient response we observed in the hydroponics studies could be due to energy required to absorb and metabolize ammonium and not an increase

in maintenance respiration. Our greater sampling intensity in the hydroponics studies as compared to our field studies may have enabled us to pick up this immediate (< 1 week) increase associated with nitrogen metabolism.

When we performed an analysis over the entire study we found highly significant ($P<0.0001$) difference in total soil CO_2 efflux (E_C) between fertilized and unfertilized plots, but the degree and direction was dependent on the sampling date. An analysis by date showed E_C rates did not initially differ due to fertilizer until approximately two months after fertilization. By late June E_C rates in fertilized plots decreased relative to control plots, and was significant ($P\leq0.05$) on four sampling dates. This trend remained until early September, at which point, E_C rates in fertilized plots returned to control levels and remained with the exception of one sampling date in mid March when rates in fertilized plots increased significantly ($P<0.05$) relative to control rates. We speculate the observed increase in E_C in fertilized plots was due to a combination of increasing specific R_R, which we reported, and increasing root biomass that was able of balance the decrease in R_H, which was also hypothesized by others (Gough and Seiler 2004, Lee and Jose 2004).

There was no effect of clone as a main effect or included in any interaction on CO_2 efflux, root, or heterotrophic respiration (Chapter 2). This is not surprising, since E_C and R_H, although influenced by the clones, are not measurements taken directly on the clones. We have not ruled out clonal differences in carbon use efficiency of below-ground plant organs. There is the possibility that differences haven't yet developed in the first year after fertilization, and may manifest themselves later in stand development.

With 13 million hectares of intensively managed loblolly pine stands in the southeastern United States, small changes to the carbon cycle of these ecosystems can have profound effects on our global carbon cycle (Jokela and Long 2003). Fertilization in southeastern pine forests has increased approximately 800% since 1990 to just over a half million hectares of planted pine being fertilized in 2000 and 2001 [North Carolina State Forest Nutrition Cooperative (NCSFNC) 2002, Wear and Greis 2002]. Our results suggest that if the trends we observed in the first year continue, in the long-term, soil carbon content could potentially increase due to fertilization. Fertilization has been shown to result in increased coarse and overall root biomass (Albaugh *et al.* 1998, Maier

and Kress 2000, Samuelson 2000, King *et al.*, 2002, Pangle and Seiler 2002, Lee and Jose 2003, Gough and Seiler 2004), or increased aboveground biomass in Scots pine, Norway spruce (Axelsson and Axelsson 1986), and loblolly pine (Albaugh *et al.* 1998, Pangle and Seiler 2002, Albaugh *et al.* 2004, Gough *et al.* 2004). An increase in aboveground biomass may results in more carbon being deposited into the soil as litter fall (Haynes and Gower 1995, Maier and Kress 2000), root exudates, and root turnover. Secondly, fertilization may increase soil carbon by decreasing the rates at which organic matter (e.g. roots and litter) in the soil is decomposed, which our data supports as shown by decreased heterotrophic respiration rates in fertilized plots one year after fertilization (Chapter 2).

Future Research

A logical next step is to measure the effects of fertilization on carbon evolution throughout the range of loblolly pine by installing plots throughout the southeast. In addition more research needs to be done on the long-term effects of fertilization, specifically nitrogen and phosphorous fertilization, on soil carbon storage.

It is well established in the literature that temperature and moisture are the abiotic factors strongly influencing respiration rates. Generally, the relationship between respiration rate, temperature, and moisture in loblolly pine, although useful at explaining a large portion of the variation (40-70%), still leaves a large portion of the variance unaccounted for in field studies (Maier and Kress, Pangle and Seiler 2002, Lee and Jose 2004, Gough *et al.* 2005, Chapter 2). Likewise, a pair of greenhouse experiments (Chapter 3) with loblolly pine seedlings, showed that temperature only explained 3 and 19% of the variance in specific root respiration leading us to hypothesize that there must be another variable explaining a large portion of the variance. In a review of the literature Dwivedi (2000) recognized that substrate supply influences root respiration by providing the C-skeletons and energy required to synthesize and maintain plant tissues. Research is needed to address what influence recently fixed photosynthate has on specific root respiration in loblolly pine and what is the amount of time between carbon fixation and respiration. Photosynthetically available radiation (PAR) and vapor pressure deficit (VPD) are highly correlated with photosynthesis, and are two easily measured parameters

that may help in explaining a portion of the variation not explained by temperature. Studies that utilized ^{13}C (Ekblad and Högberg 2001) and large scale tree girdling (Högberg *et al.* 2001) have shown that recently fixed carbon takes from 1-5 days to become available to roots. Singh *et al.* (2003) suggested that photosynthetic activity should be included in future forest carbon balance models.

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Vita

Michael Christopher Tyree

The author was born in Alexandria, Virginia on February 9, 1979. At six-months-old he was moved to Dillsburg, Pennsylvania and lived there until the age of 21. He graduated from Northern High School in June 1997 and casually took classes at Harrisburg Area Community College for the next two years. Michael earned a B.S. in forest biology from The Pennsylvania State University in December 2002. He plans to complete his M.S. at Virginia Polytechnic Institute and State University in forestry under Dr. John Seiler in 2005. Starting fall 2005 he plans to continue work under Dr. John Seiler toward his Ph.D. studying gas exchange in loblolly pine.