Genetics by Nutrient Availability Interactions on Short-term Carbon Pools and Fluxes in Young *Pinus taeda* Plantations

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ABSTRACT

The objective of this research was to determine how genetics and nutrient availability influence C cycling in intensively managed southern pine forests. This work consisted of a two year field and a complimentary one year greenhouse study each split into above- and belowground pools and fluxes. Both the greenhouse and field experiment showed differences between contrasting genotypes in gas exchange parameters and C partitioning patterns, but genetic by nutrient availability interactions were only observed in the field. In the field study, some genotypes were better able to tolerate nutrient limitations due to more favorable canopy architecture and lower N demand. Our results clearly show that contrasting ideotypes have the potential to respond differently to differences in nutrient availability in terms of biomass partitioning, leaf physiology, and leaf biochemistry (Chapter 3).

Both experiments showed short-term improvements to soil physical and chemical properties, which have been shown to correlate with higher site quality. In both the greenhouse and field experiment, we concluded that increased C loss by way of total soil CO₂ efflux \( F_S \) made up only a small percent total C incorporated as LR. Short-term results led us to conclude that combining LR treatments and planting of genotypes with low nutrient demand or high nutrient use efficiency may increase soil organic matter (SOM) while avoiding loss of stem volume from nutrient immobilization. Data from our field study showed a strong genotype by soil amendment interaction for \( F_S \) over all sampling dates with the relative importance of contributing factors (heterotrophic or root respiration) also changing (Chapter 5).

Overall, logging residue incorporation increased total system C gain per ha more than did fertilization alone, but there were differences between genotypes planted (Chapter 6). Data from the field experiment show that although LR incorporation did not decrease overall net primary productivity, it did decrease biomass partitioning to merchantable products (main stem) depending on genotype. These data underline the importance of matching appropriate genotypes to specific site conditions and silvicultural prescriptions.
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for foliar N content for *P. taeda*, and different letters indicate significant comparison-wise differences between nutrient manipulation treatments at the 0.05 alpha level (*n* = 12). Abbreviations: NoLR = no logging residue, NF = no fertilizer, LR = logging residue, F = fertilizer.

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Statistical analysis was performed using ANOVA with repeated measures in PROC MIXED using SAS version 9. Error bars equal ± 1 standard error of the mean ($P = 0.06, n = 6$).

**Figure 3.10** Clone by logging residue (LR; left sided) and CL by fertilizer (right side) interactions for maximum carboxylation capacity of Rubisco ($V_{C,\text{max}}$; panels A and B), maximum rate of RuBP regeneration ($J_{\text{max}}$; panels C and D), and foliar N levels. Variables were measured June 2006, October 2006, and March 2007. Photosynthetic parameters were estimated using $A/C_i$ curve fitting software developed by Sharkey et al. (2007) and adjusted to a common temperature of 25°C. Error bars represent ± 1 standard error of the mean.

**Figure 3.11** Linear relationship between maximum carboxylation capacity of Rubisco ($V_{C,\text{max}}$; top) and maximum RuBP regeneration mediated by electron transport ($J_{\text{max}}$; bottom) and foliar N (g N m$^{-2}$) between clones (CL). Clone 32 and 93 are represented by open (○) and closed (●) circles, respectively.

**Figure 3.12** Least squares means for $J_{\text{max}} / V_{C,\text{max}}$ ratio (panel A), apparent quantum yield ($Q_i$; panel B), light compensation point ($LCP$; panel C), and specific leaf area ($SLA$; panel D) for the clone by fertilizer interaction. Variables were estimated from $A/C_i$ and $A/PPFD$ response curves measured on three separate dates. $SLA$ was measured on needles used to generate $A/C_i$ curves.

**Figure 4.1** Average, minimum, and maximum daily greenhouse temperature monitored continuously over the course of the experiment.

**Figure 4.2** Mean soil pH by soil amendment treatment by sampling date. Bars represent average soil pH ($n = 12$) for three sampling dates. Error bars represent ± standard error from the mean.

**Figure 4.3** Mean soil nitrogen (N) and phosphorus (P) concentration for clone by fertilizer (F) treatments. Bars represent average N and P ($n = 36$) over three sampling dates. Error bars represent ± standard error from the mean. $P$-value represent significance of the clone by fertilizer interaction over all sampling dates.

**Figure 4.4** Least square mean soil CO$_2$ efflux rates ($F_{\text{Cont}}$) comparing effects of logging residue (LR; panel A) and fertilization (F; panel B) treatments. Daily average $F_{\text{Cont}}$ (7-9 measurement cycles per day) was measured continuously using the ACES starting April 2, 2006 thru May 18, 2007 and used to calculate an average monthly $F_{\text{Cont}}$. Monthly $F_{\text{Cont}}$ rates were transformed using their natural log to meet assumptions and analyzed using ANOVA with repeated measures in SAS version 9.0. Error bars
represent ± standard error of the mean and arrows indicate times of fertilization

**Figure 4.5** Non-linear relationship between soil temperature at 5 cm depth and total soil CO\(_2\) efflux \((F_{\text{Cont}})\) using data collected with the automated C efflux system (ACES) from April 2, 2006 thru May 18, 2007. Points represent average monthly \(F_{\text{Cont}}\) measured at an average monthly soil temperature. The \(r^2\) value was determined by linear regression between \(F_{\text{Cont}}\) and predicted value determined using the non-linear first order exponential equation.

**Figure 4.6** Least squares mean of soil CO\(_2\) efflux \((F_{\text{Point}})\) logging residue (LR) by fertilizer (F) by time three-way interaction (Panel A), percent \(F_{\text{Point}}\) treatment effect relative to control treatment (Panel B), and clone (CL) by time interaction (Panel C). Rates were transformed using their natural log to meet assumptions and analyzed using ANOVA with repeated measures in SAS version 9 (error bars represent ± standard error from the mean). Arrows indicate times of fertilization and dashed line represents control treatment.

**Figure 4.7** Least squares mean of index of heterotrophic respiration \((R_H)\) for the logging residue (LR) by fertilizer (F) by time three-way interaction (error bars represent ± one standard error of the mean, \(n = 12\); panel A). Percent \(R_H\) treatment effect relative to control containers measured on 10 sampling dates (Panel B). Arrows indicate times of fertilization and dotted line represents control treatment.

**Figure 4.8** Average microbial biomass C by soil amendment taken on two sampling dates February 2007 and July 2007. Each bar is an average of 12 samples and error bars represent ± one standard error from the mean.

**Figure 4.9** Least squares mean of percent decomposition by depth (Panel A) and soil amendment (Panel B). Two yellow pine, jointed dowel rods were buried vertically in the bulk soil of each container for one year (Appendix H). Subsamples were averaged and transformed using the arcsine of the square root prior to statistical analyses using ANOVA with depth as a split-plot treatment. Each bar is an untransformed average of 48 observations and error bars represent ± one standard error from the mean.

**Figure 4.10** Response surfaces for each soil amendment treatment representing the relationship between total soil CO\(_2\) efflux \((F_{\text{Point}})\), index of aboveground productivity and index of heterotrophic (microbial) respiration \((R_H)\). Index of aboveground productivity (AG index) was calculated by multiplying net photosynthesis per unit leaf area and stem volume (see chapter 2 for details). Both independent variables were measured concurrently on nine separate sampling dates. Each point on the response
curve represents an average of twelve experimental units. Multiple linear regression analyses were performed for each soil amendment treatment using the following model: 

\[ F_{\text{point}} = \beta_0 + \beta_1 \text{AG index} + \beta_2 \text{RH} \]

Coefficients of variation are reported below treatment labels on graphs.

**Figure 4.1** Cumulative monthly C loss in grams as total soil CO₂ efflux (\( F_{\text{Cont}} \); panel A) and as total C loss through leaching (Panel B) for logging residue (LR) treatment main effect. Daily average \( F_{\text{Cont}} \) (7-9 measurement cycles per day) was measured continuously using the ACES starting April 2, 2006 thru May 18, 2007 and used to calculate a cumulative monthly sum for \( F_{\text{Cont}} \). Monthly sums were transformed by their square root to meet the assumption of equal variance. Total C concentration in leachate (mg L⁻¹) was determined approximately monthly and multiplied by total volume of leachate collected. Each point is an average of 24 observations and error bars represent ± one standard error from the mean (\( n = 6 \)). Data were transformed by their natural log to meet assumption of equal variance.

**Figure 5.1** Mean soil bulk density between logging residue treatments for three soil depths. Five subsamples were taken to determine the plot average. Statistical tests were performed using analyses of variance with soil depth treated as a split-plot (\( n = 6 \)). Error bars represent ± standard error from the mean, significance at the 0.10, 0.05, and 0.001 alpha level is indicated by single (*), double (**), and triple asterisks (***) respectively.

**Figure 5.2** Mean percent volumetric soil water content by logging residue (LR) treatments. Data were collected over two years (error bars represent ± one standard error from the mean) using time domain reflectometry averaged over a depth of 13 cm. Inset graph shows mean (\( n = 48 \)) depth (cm) to reduced zone using rusty rod technique.

**Figure 5.3** Mean microbial biomass C content for clone (CL) and fertilizer treatments measured four times over the course of the experiment by chloroform fumigation extraction-procedure. Bars represent an average of six observations and error bars represent ± one standard error from the mean.

**Figure 5.4** Mean microbial biomass N content for clone (CL) by fertilizer (Panel A) and CL by logging residue (LR; Panel B) interactions measured four times over the course of the experiment by chloroform fumigation extraction-procedure. Bars represent an average of six observations and error bars represent ± one standard error from the mean.

**Figure 5.5** Mean total soil CO₂ efflux (\( F_S \)) for soil amendment by genotype interaction. Bars represent \( F_S \) rates measured over 13 sampling dates from January 2006 thru December 2007 and error bars indicate ± one standard error of the mean.
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Figure 6.1  Carbon budget for greenhouse experiment following the incorporation of logging residue (LR) relative to treatments not receiving LR incorporation ($n = 24$). *Pinus taeda* seedlings were planted in 170 L plastic containers and grown for one year in a greenhouse. Values contained in boxes represent the change in C (grams) for each pool or flux. Values in red indicate C pool or flux used to calculate total C balance. All values were measured at the end of one year................................................................. 213

Figure 6.2  Carbon budget for greenhouse experiment following fertilization with N and P relative to treatments not receiving nutrient additions ($n = 24$). *Pinus taeda* seedlings were planted in 170 L plastic containers and grown for one year in a greenhouse. Values contained in boxes represent the change in C (grams) for each pool or flux. Values in red indicate C pool or flux used to calculate total C balance. All values were measured at the end of one year...................................................................... 214

Figure 6.3  Relative C budget for field study two years following logging residue (LR) incorporation. Values in boxes represent changes in C content relative to control treatments (Mg C ha⁻¹) after two years. Values in red indicate pools and fluxes that were used to calculate total C budget.
Biomass values and logging residue values were calculated by multiplying by 0.5…………………………………………………………………………………………………………………… 215

**Figure 6.4** Relative C budget for field study two years following fertilization with N and P. Values in boxes represent changes in C content relative to control treatments (Mg C ha\(^{-1}\)) after two years. Values in red indicate pools and fluxes that were used to calculate total C budget. Biomass values were calculated by multiplying by 0.5………………………………………………………… 216

**Figure 6.5** Relative C budget for Cross carbon study two years following logging residue (LR) incorporation and fertilization with N and P. Values in boxes represent changes in C content relative to control treatments (Mg C ha\(^{-1}\)) after two years. Values in red indicate pools and fluxes that were used to calculate total C budget. Biomass values and logging residue values were calculated by multiplying by 0.5………………………………………………………… 217
Chapter 1
Context and Justification

Southern pine plantations in the southeastern United States are some of the most intensively managed and highly productive forested ecosystems in the world (Allen et al. 2005). Currently, southern pine plantations occupy more than 13 million ha and are forecast to increase 67% to 22 million hectares by the year 2040 (Wear and Greis 2002). Increasing future demands for forest products as well as growing concern over rising atmospheric CO₂ levels, has raised the question: can we manage these forests to maximize both forest production and C sequestration? Planted pine forests in the southeastern United States may create great opportunity for sequestering large amounts of C (Johnsen et al. 2001). Carbon storage in these ecosystems can be increased through two processes: i) by increasing the rate of C capture (i.e., increasing plant biomass and movement of C into forest soils), and ii) by decreasing the rate of C evolution from these soils back to the atmosphere. Manipulations of one or both of these processes by silvicultural practices can have a profound impact on whether these forests act as C sources or sinks. Over the last half century, intensive silviculture of pine plantations in the south has more than tripled productivity (forest yield) (Fox et al. 2004; Fox et al. 2007). This has been accomplished by improved methods of site preparation designed to maintain soil organic matter, fertilization, weed control, and the use of superior planting stock. Much work is still needed to satisfy the growing demand for forest products in a responsible way.

A typical harvesting operation in a southern pine stand can generate up to 50 Mg of logging debris ha⁻¹ (Allen et al. 2006), which historically has been gathered into large piles and burned or abandoned. These represent huge stores of organic C, which left exposed to the air will largely oxidize being released back into the atmosphere as CO₂. More recently, forest managers have begun spreading this logging debris back onto the site in an attempt to retain nutrients on site as well as minimize disturbance from trafficking. Furthermore, the idea has been proposed to incorporate this logging residue (LR) back to the soil (see review by Johnson and Curtis 2001). Not only would this provide nutrients to successive stands, but it may additionally increase soil C sequestration as it is likely some fraction will remain as
recalcitrant soil C. Agricultural studies have long shown that adding large amounts of C into the mineral soil will also impact nutrient cycling and decomposition rates of the material (Holland and Coleman 1987). Depending on the availability of organic C to decomposition and the ratio of C to N, there is both the potential to increase nutrient cycling, particularly N, P, and S, or cause short-term nutrient immobilization.

Adding nutrients leads to large increases in forest productivity and may help to overcome any immobilization caused by LR incorporation additions. Over the last three decades we have greatly increased our understanding of how to manipulate site nutrients to increase forest productivity. In fact, as of 2001, approximately 0.5 million hectares of planted pines have undergone some type of nutrient amendment (NCSFNC 2002). As Allen et al. (1990; 2005) stated, this is only a fraction of the forested stands that have the potential to respond to fertilization. As the use of chemical fertilizer increases in intensively managed pine stands it becomes necessary to understand how net ecosystem productivity will be impacted by such additions. For instance, it is well established in the literature that fertilization will increase aboveground productivity (Albaugh et al. 2004), but the mechanisms (i.e., increased photosynthetic capacity, change in C allocation, etc.) involved are not well understood. Additionally, there is growing evidence that fertilization, specifically N fertilization, suppresses the rate of OM decomposition (Fog 1988) either through reduced microbial activity (Homann et al. 2001; Blazier et al. 2005; Olsson et al. 2005), decreased microbial biomass (Bååth et al. 1981; Thirukkumaran and Parkinson 2000; Lee and Jose 2003), changes in microbial population composition (Lilleskov et al. 2002; Bittman et al. 2005), or some combination of these factors. Any of these outcomes could lead to increased net ecosystem productivity (NEP) (Maier et al. 2004; Sampson et al. 2006).

The use of superior genotypes has opened an exciting area of research that shows huge potential for increasing productivity of intensively managed pine stands. An estimated volume gain of 10 to 30% has been possible with selective breeding, and gains of 50% or more may be possible by combining the use of clones and intensive silviculture (Allen et al. 2005; Martin et al. 2005). Improvements in C allocation, photosynthetic capacity, drought and pest tolerance, and resource use efficiency are a few ways in which C capture (yield) can
be increased. Additionally, the use of clones in research aids in our ability to study these physiological processes by eliminating genetic variation and selecting clones that strongly express the trait we are interested in studying.

As previously mentioned, *P. taeda* is grown over a wide range and under diverse site conditions (i.e., climatic, moisture, soil type, topography, etc.) throughout the southeastern United States. Additional increases in yield may be realized by matching the most appropriate genotype and silvicultural prescription to specific site conditions. Given the large amount of clonal material being produced, an understanding of how specific clones will respond physiologically to changes in nutrition and site conditions will be necessary to match clones quickly to particular environments. Further, a physiological understanding of clonal growth responses may be useful for early, rapid screening of clonal material.

This research will utilize both a field and greenhouse study to determine what effects logging residue incorporation and fertilization have on the C capture and turnover in a *P. taeda* plantation. Additionally, we will study how these treatments, which impact nutrient availability, influence some of the physiological mechanisms of C capture and allocation in two superior *P. taeda* varieties believed to represent two distinct ideotypes (“crop” versus “competitor”). This research is a collaborative effort between Virginia Tech and the USDA Forest Service, MeadWestvaco, and North Carolina State University investigating how genotype and silvicultural interaction impact productivity and C sequestration by manipulating soil C and nutrients. Other researchers will focus on fine-root turnover, microbial responses, dissolved organic carbon (DOC), and C and N stabilization/destabilization.
Chapter 1

Section: Objectives

Objectives

General objectives:
The overall objective of this research is to monitor the short-term (1-3 years) effects of intensive silviculture on C pools and fluxes, involved in C capture, biomass partitioning, and C evolution back to the atmosphere, in young *Pinus taeda*. This research was broken into a greenhouse and field study, which complimented each other with respect to treatments and soil type, but offered widely different degrees of experimental control. Additionally, each study was broken into two experiments focusing on above and belowground C pools and fluxes. Specific questions involved in each aspect of this research combined to give a more complete picture of how silvicultural manipulations impact short-term physiological responses that will set the course for longer-term (rotation age) effects on site productivity (i.e., yield, NEP). Silvicultural treatments of interest are fertilization, incorporation of logging residue into the mineral soil, and planting of superior genotypes, which were chosen based on documented or predicted increases in yield as well as potential avenues for future C sequestration. Specific questions for each experiment were as follows:

**Short-term impacts of nutrient manipulations on leaf gas exchange and biomass partitioning in contrasting two-year-old *Pinus taeda* clones during seedling establishment (Chapter 2).**

1. What are the short-term physiological adjustments to leaf gas exchange that *P. taeda* make under varying nutrient availability, and are these adjustments stable across contrasting genotypes?

2. Is seedling growth in response to nutrient availability a function of photosynthetic efficiency ($A_{Sat}$) or mainly a result of changes in leaf area, and does this seem to be consistent across clones?
Interaction between contrasting *Pinus taeda* ideotypes and soil nutrient availability: effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology (Chapter 3).

1. How does biomass partitioning change with variations in nutrient availability between two contrasting ideotypes?

2. How does photosynthetic efficiency respond to changes in nutrient availability immediately following nutrient manipulation, and are there differences between contrasting genotypes?

3. Are there genetic by nutrient availability interaction with regard to photosynthetic capacity, leaf N, and leaf morphology?

Impacts of soil organic matter and nutrient manipulations on soil properties and their influence on belowground C cycling in a large pot study utilizing two contrasting *Pinus taeda* L. clones (Chapter 4).

1. What affect does adding LR alone and in combination with N and P fertilization have on total C loss from the soil by way of total soil CO$_2$ efflux from the soil surface ($F_S$) and leaching, and what percent of the added LR remained after one year?

2. What are the effects of N and P fertilization on microbial biomass, activity, and what is the influence on total C loss when both LR is present and absent?

3. What influence, if any, do contrasting *P. taeda* genotypes, which have been shown to differ in their fine- to coarse-root ratio, and their response to soil amendments (Chapter 2), have on belowground C cycling?
Interaction between contrasting *Pinus taeda* ideotypes and soil nutrient availability: effects of soil organic matter incorporation and fertilization on soil chemistry, microbial abundance, and soil respiration (Chapter 5).

1. Does LR incorporation improve factors associated with higher site quality such as: increased SOM, CEC, base cations, and decreased bulk density?

2. Does incorporating LR increase the biomass and activity of soil microbial populations resulting in immobilization of soil N?

3. Does the addition of N and P fertilization, which has shown to increase aboveground partitioning in both genotypes, decrease the biomass and activity of microbial populations?

4. Do genotypes shown to differ in biomass partitioning patterns have a significant influence on microbial biomass and CO$_2$ evolution from the soil surface ($F_s$)?
Literature Review

Changes made to soil organic matter (SOM) and nutrients can have diverse and long lasting impacts on site C pools and fluxes (Figure 1.1), and ultimately on the C balance of a site. Net ecosystem productivity (NEP) is the difference between two competing processes: 1. the capture of inorganic C in the form of CO$_2$ from the atmosphere and its fixation into biomass (gross primary productivity, GPP); and 2. the release of CO$_2$ back into the atmosphere as a result of decomposition of soil organic C by soil microbes (heterotrophic respiration, $R_H$) and whole-plant growth and maintenance respiration (ecosystem respiration). Active management of site nutrients and soil organic matter may dramatically impact one or both of these processes making our understanding of these changes crucial to our ability to manage, control, and predict changes to NEP on our intensively managed pine forests.

Impacts of SOM on soil properties

It has been well documented that leaving or removing logging debris on site during a harvesting operation can have profound effects on the soil physical (Powers et al. 2005), chemical (Ouro et al. 2001), and biological properties (Houghton et al. 1983; Aggangan et al. 1999; Li et al. 2004), which can impact the growth of successive stands (Tiarks et al. 2003; Merino et al. 2004). A typical logging operation in a southern pine plantation can produce anywhere from 5 to 50 Mg of C per hectare in the form of logging debris depending on age and extent of harvesting (Allen et al. 2006). This logging residue is in large part oxidized back into the atmosphere as CO$_2$ within the first couple years following harvest (Palviainen et al. 2004). Relative to the entire tree, these residues (e.g., needles, twigs, bark) contain a disproportionate amount of nutrients such as: nitrogen (N), phosphorus (P), and sulfur (S) (Ouro et al. 2001), which if left on-site can result in the slow release of nutrients back to the soil.

To slow the decomposition and loss of C from these organic matter stores some researchers have proposed incorporating this logging residue into the soil (Buford et al. 1998; Sanchez et al. 2000; Sanchez et al. 2001; Sanchez et al. 2003). Incorporating logging residue into the soil following harvesting could result in a number of different fates.
One fate could be the acceleration of microbial decomposition of the labile C pool leading to an increase in microbial populations (Aggangan et al. 1999; Ouro et al. 2001). Further, the sudden influx of logging residue with a high C:N ratio (≈ 700) could lead to the immobilization of essential nutrients such as N (Aggangan et al. 1999; Perez-Batallon et al. 2001), which may result in decreased tree growth. This is largely dependent on what is limiting microbial activity (i.e., C or some essential nutrient). Alternatively, there is the possibility that soil C storage could increase due to changes in soil nutrients, pH, or abiotic factors (moisture), which could slow microbial growth or cause changes in the microbial community. The breakdown of added LR could be slowed or prevented due to the physical protection of organic C compounds through adsorbing to soil particles. It is important to note that these are very complex processes which depend on soil type, the microbial community present, the quality of substrate being incorporated, and the environmental conditions.

Information gained from agricultural studies

Hypotheses of how incorporating residues into the mineral soil will respond are largely based on information gleaned from agriculture studies. Specifically, studies comparing no-tillage to conventional tillage which have dated back to the early 1900’s (see citations within Holland and Coleman 1987). Largely these studies have found increased decomposition when C rich residue was incorporated into mineral soil versus left on the soil surface (Holland and Coleman 1987; Coppens et al. 2007; Nicolardot et al. 2007). Coppens et al. (2007) hypothesized this was mostly due to more favorable moisture conditions when substrate was incorporated into mineral soil. Meanwhile, Holland and Coleman (1987) speculate that intimate contact between N rich mineral soil and a labile C source allow for more bacterial influence in decomposition. Similarly, Balota et al. (2004) found 45% increase in total C and an 83% increase in MBC in no-tillage versus conventional tillage in the upper 5 cm of soil 19 years after treatment initiation. Incorporation of pine sawdust into the mineral soil has been shown to effect soil pH, change microbial fungal composition, and increase the C:N two years following amendments (Kwasna et al. 2000).

Much of what we learned from agricultural studies can be used as a base for creating hypotheses in intensively managed forest stands, but it is important to note there are key
differences between them. Firstly, there are large differences in substrate quality (i.e., C:N and lignin content) between crop and forest residue (Table 1.1). High C:N ratios are likely to result in immobilization of N (Gok and Ottow 1988; Starbuck 1994) since soil microbes will likely be N limiting. Additionally, constituents of plant tissues that are resistant to decomposition such as lignin will also slow decomposition. There are a number of recent agricultural studies that have shown that additions with high C:N ratios and lignin contents decompose at slower rates than substrates with lower C:N and lignin contents (Coppens et al. 2007; Nicolardot et al. 2007). A second major difference between the two systems is time between rotations. While most agricultural crops are on yearly rotations (constant disturbance), intensively managed forests are on 20-25 year rotations, limiting the amount of disturbance to just one or a couple events. These differences between cropping systems further underline the need for studying the effects of LR incorporation in forested systems.

Soil physical and chemical properties

There are few studies, that we are aware of, that have looked at the effects of incorporating logging residue on soil physical and chemical properties in forested systems. One study on the Lower Coastal Plain region of NC by Sanchez et al. (2000) found increases in soil C and N 1.5 years following incorporation of logging slash into the soil profile. The authors found a 100% increase in soil C and decreased bulk density in treatments that were broadcast and bedded compared to treatments that were not bedded, although, decreased bulk density may be solely a function of bedding. The opposite was true in the high organic sites, but the effects were not statistically significant. A second study conducted by Sanchez et al (2003), tested the effects of leaving logging residue on the surface versus incorporation into the mineral soil on two sites with contrasting soil textures (sandy and clayey). The authors found increased soil C and N from 183% and 170%, respectively, in the low LR rates to as high as 265% and 220%, respectively, at the 2x LR rates 1.5 years following treatment initiation. Analyses of cost of broadcasting logging residue back onto the site showed that it is not currently economically feasible ($US 521 ha$\textsuperscript{-1} and $US 633 ha$\textsuperscript{-1} on the 'sandy' and 'clay' sites, respectively), but with advances in efficiency and productivity it may become feasible in the future.
One rationale for this research is that even if incorporation of LR does not directly increase long-term soil C, there are other indirect benefits to soil physical and chemical properties, which could lead to long-term increases in site quality. Soil organic matter is shown to increase CEC, as well as be a valuable store of nutrients, specifically, N, P, and S (Ouro et al. 2001; Sanchez et al. 2003). Logging residue incorporation has been shown to decrease bulk density and soil strength (Sanchez et al. 2001; Sanchez et al. 2003) as well as improve soil structure, aeration, and water holding capacity. Results from short-term studies have shown promise for increasing soil organic matter, but further inquiry is needed into the long-term impacts of logging residue incorporation.

**Soil biological properties**

Studies of the effects of OM manipulation on the soil biological properties of forested ecosystems are scarce, and of those studies we were unable to find any long-term studies. Total soil CO₂ efflux at the soil surface ($F_S$) is an in situ measure of both microbial (heterotrophic) respiration ($R_H$) and root respiration ($R_R$), which are the dominate emitters of CO₂ from soil. Therefore, manipulations (e.g., harvesting) of soil biotic properties (e.g., soil microbes and plant roots) and/or abiotic properties (e.g., temperature and moisture) may strongly influence $F_S$. For example, Perez-Batallon et al. (2001) found increased $F_S$ in plots which were harvested relative to the uncut check plot over the first year following treatment initiation in a *P. radiata* stand in NW Spain. Of the logging residue management techniques employed in the study, $F_S$ was consistently greatest in plots in which the logging residue was incorporated into the top 20 cm of mineral soil relative to treatments where logging residue was completely removed or left on the soil surface.

Microbial communities can be greatly influenced by LR additions to the soil increasing substrate and nutrients as well as effecting soil microclimate (temperature, moisture, and aeration). Perez-Batallon et al. (2001) observed an increase of 1.5 x in microbial biomass C ($MBC$) in plots where logging residue was incorporated. It is important to note that annual fluctuations in $MBC$ were mostly explained by temperature and moisture fluctuations. Likewise, Aggangan et al. (1999) found an increase in cumulative CO₂ respired following leaf litter incorporation in the soil in a 29 week laboratory incubation study. The authors
observed an increase in both MBC and microbial biomass N (MBN) with increasing leaf litter additions. The exception being at the higher leaf litter levels (20 and 30 Mg ha\(^{-1}\)) where MBC was reduced in treatments where leaf litter was incorporated. This corresponded with a decrease in N mineralization with higher levels of leaf litter in both treatments (soil surface and incorporated), but the response was much more pronounced when leaf litter was incorporated.

**Effects of nutrient additions on soil biological properties**

The application of fertilizers to forest soils has increased 800% since 1990 (NCSFNC 2002; Wear and Greis 2002). With pine plantations in the Southeastern United States projected to increase from 13 to 22 million hectares over the next 30 years fertilization on these, generally, degraded lands will likely follow that same trend (Wear and Greis 2002). Although, growing attention is being given to how this will impact belowground soil biological properties, there are still many questions which plague researchers. Can management of site nutrients lead to significant increases in the C holding capacity of managed southern pine plantations either through increased soil C, increased biomass production, or as observed by Leggett and Kelting (2006) both?

**Total soil CO\(_2\) efflux**

Total soil CO\(_2\) efflux can be used in conjunction with aboveground biomass estimates to estimate NEP of a stand. Therefore, understanding how fertilization will impact \(F_S\) is crucial to estimating NEP of managed southern pine plantations. The effects of fertilization on \(F_S\) have shown mixed results in forest ecosystems. In short-term studies, fertilization has resulted in decreases in \(F_S\) (Haynes and Gower 1995; Maier and Kress 2000; Butnor et al. 2003; Samuelson et al. 2004) or no change (Lai et al. 2002; Pangle and Seiler 2002; Lee and Jose 2003; Maier et al. 2004; Tyree et al. 2008). Longer-term (> 5 years) studies on the response of \(F_S\) to fertilization in forest ecosystems are less common, but have generally showed decreases following fertilization (Nohrstedt et al. 1989; Smolander et al. 1994; Mattson 1995; Bowden et al. 2004; Olsson et al. 2005). In contrast, Tyree et al. (2006) showed no change in \(F_S\) 24 years following fertilization in *P. taeda.*
Some have hypothesized this lack of response to fertilization by $F_S$ is a combination of increased $R_R$ and decreased $R_H$ offsetting each other (Lee and Jose 2003; Gough and Seiler 2004; Tyree et al. 2008). Many researchers have shown 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 2004), and a few have shown specific $R_R$ (Gough and Seiler 2004; Tyree et al. 2008) following nutrient additions in southern pines. To get a better understanding of what mechanisms are driving this response, or lack of response, it is useful to separate these two components. A review of the literature by Hanson et al. (2000) showed that $R_R$ contributes on average 46% of $F_S$ in forested ecosystems, but depending on climate, forest type, and methodology $R_R$ has been observed to contribute from 10 to 90% of $F_S$ (Raich and Schlesinger 1992; Andrews et al. 1999; Maier and Kress 2000; Widén and Majdi 2001; Pangle and Seiler 2002; Fahey et al. 2005). There are various techniques (trenching, girdling, stable isotopes, etc.) employed to separate the relative contribution of $R_R$ and $R_H$, but due to the intimate relationship between the two components (Bond-Lamberty et al. 2004) each method has its own pros and cons (see review by Hanson et al. 2000).

**Microbial activity and biomass**

An integral part of the terrestrial C cycle is the decomposition of site organic C and mineralization of essential nutrients by soil microbes. This process involves a diverse group of macro and microorganisms, which quickly consume labile C, and break down high molecular weight organic compounds such as humins and lignin into simpler, more labile forms that can be used to drive cellular respiration. As a consequence, inorganic C released back into the atmosphere can be used as a measure of microbial (heterotrophic) activity. Many researchers have observed changes in heterotrophic respiration ($R_H$) following fertilization. Of the literature we have examined, the majority has shown a decrease in $R_H$ following the application of fertilizer salts (Table 1.2). There are a few cases in which researchers observed an increase in $R_H$, but with the exception of Van Cleve and Moore (1978), these tended to be short-term studies (days) following the application of urea. A second estimate of microbial activity can be made by measuring decomposition of a substrate through loss of weight or strength. Fog (1988) in an extensive review of the literature found over 60 studies that reported no effect or a negative effect on decomposition of organic
matter following fertilization. Many different materials have been used with this measure, but they all use the same basic idea of measuring loss of material (i.e., weight, strength, nutrients) over a period of time (see review by Schmidt 2005). A second factor determining $R_H$ is the quantity of biomass present referred to as microbial biomass carbon ($MBC$). A quick literature search uncovered many studies which found changes to microbial biomass following the addition of nutrients. Of these studies reviewed, we found most observed decreases in microbial biomass, but some have shown increases or no change following fertilization (Table 1.3).

Although changes in microbial activity and biomass following nutrient amendments have been widely observed, the mechanisms driving this response are poorly understood. There have been many different hypotheses, but to date no one has been widely agreed upon. An early hypothesis stressed the importance of soil pH on $R_H$ rates. Support for this hypothesis came from studies where the addition of urea [(NH$_2$)$_2$CO] had been shown to increase soil pH accompanied by an increase in $R_H$ (Roberge 1976; Söderström et al. 1983; Thirukkumaran and Parkinson 2000). Further support comes from studies that showed ammonium nitrate (NH$_4$NO$_3$) and other ammonium salts to lower soil pH accompanied by a decrease in microbial respiration (Kowalenko et al. 1978; Thirukkumaran and Parkinson 2000; Bowden et al. 2004; Wallenstein et al. 2006). In contrast, others have found differences in respiration rates to be independent or weakly related to soil pH (Nohrstedt et al. 1989; Tyree et al. 2006).

Much emphasis has been given to whether soil microbes are limited by C or some other nutrient (often N and P). Management activities such as harvesting, fertilization, or LR additions can change this balance, which has been shown to impact microbial communities. For example, Bailey et al. (2002) found a high correlation between fungal activity and total soil C in a wide range of soils tested. The addition of fertilizer may directly affect microbial populations via pH or osmotic changes (Thirukkumaran and Parkinson 2000), or indirectly from longer-term changes to aboveground plant growth (Leckie et al. 2004), such as a reallocation of plant C from roots to shoots (Haynes and Gower 1995; Ryan et al. 1996).
This may cause a reduction in overall microbial biomass or activity, or simply a change in microbial population dominance.

The two most important soil microbes in C turnover are bacteria and fungi, and for that reason receive most attention from researchers. These two classes of organisms differ widely in their ability to acquire, store, and metabolize soil C (Bailey et al. 2002). For instance, Holland and Coleman (1987) in a review of the literature, reported that C assimilation efficiencies are significantly higher in fungi (40-73%) than bacteria (20-51%), meaning more C is retained as biomass and less is respired. Secondly, fungi are more resistant to decomposition because their cell walls are made up of polymers of melanin and chitin. In contrast, bacterial membranes are made up of easily decomposable phospholipids, giving fungi greater long-term storage of C as microbial biomass. Because of these differences it is not enough to simply measure overall microbial biomass, but some estimate of community composition should be made.

Fertilization has been shown to cause shifts in soil microbial composition (Bååth et al. 1978). Some studies have shown decreases in fungal components following fertilization (Wallander and Nylund 1992; Lilleskov et al. 2002; Nilsson and Wallander 2003; Bittman et al. 2005). For example, both Frey et al. (2004) and Wallenstein et al. (2006) found a decrease in fungal to bacterial activity ratios in both a hardwood and red pine stand following long-term chronic N additions on the Harvard forest. Based on just the few articles above it appears that changes to microbial composition may explain at least some of the decrease in $R_H$.

**Effects of nutrient amendments on C capture and allocation**

*Biomass and allocation*

A widely held belief among physiologists is that plants reroute, or reallocate, C from belowground to aboveground tissues following fertilization. This theory is intuitive and therefore easy for many to accept, but little attention is paid to physiological changes that occur at the leaf level. The presumption is that plants no longer have to compete for nutrients, therefore, allowing them to devote more of their resources to capturing light and converting CO$_2$ gas into biomass. Evidence comes from a highly cited study on 12-year-old
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*P. taeda* where the authors found an increase of 152% in stem volume increment and 101% in peak leaf area index in fertilized plots relative to unfertilized pots (Albaugh et al. 1998). The authors attribute this increase at least in part to differences in biomass partitioning between fertilized and unfertilized trees as they found a proportional decrease in fine-roots in fertilized plots, but an overall increase in total root biomass. The same trends in aboveground growth were observed 5 years later when the site was revisited in 2000 (Albaugh et al. 2004). Also, Samuelson (2000) found an increase in total root biomass and aboveground growth, and a decrease in fine-root biomass following fertilization in both *P. taeda* and *P. elliottii* pine seedlings. Others have also observed a shift in C allocation aboveground following fertilization in pines (Axelsson and Axelsson 1986; Green et al. 1994; Haynes and Gower 1995).

Contrary to Albaugh et al. (1998) findings of decreased fine-root (< 2mm) production in fertilized plots, King et al. (2002) found an increase of 108% in fine-root (0.5 – 1mm) growth in fertilized plots when measured on the same study. The authors attribute the discrepancy to differences in sampling method (coring vs. minirhizotrons) and what was considered fine-roots. Notably, the proportional change in fine-root production relative to overall growth was not reported in this article. Similarly, Pangle and Seiler (2002) found, using whole-tree harvest, an increase in fine-, medium-, and coarse-root biomass in two-year-old *P. taeda* seedlings that were fertilized.

Changes in C capture in response to fertilization are often a function of changing leaf area. Leaf area in *P. taeda* and other southern pines has been shown to increase following N fertilization on N deficient soils (Zhang et al. 1997a; Albaugh et al. 1998; King et al. 1999; Gough et al. 2004b; Will 2005). A widely cited study by Vose and Allen (1988) showed that leaf area index (*LAI*) responded to N fertilization on two of three stands sampled, but there was no response to P fertilization on any of the *P. taeda* stands. Maier et al. (2002) showed a 38% increase in leaf area in fertilized plots in 13-year-old *P. taeda*. This increase was attributed to significant increases in needle length, flush length, and number of needles per fascicle.
A change in C allocation and leaf area is one hypothesis for increased wood volume gain seen in *P. taeda* following N fertilization. Another hypothesis proposed by Gough et al. (2004b) and later supported by King et al. (2008) is that increases in $A_{sat}$ rates immediately following fertilization allow for increased photo-assimilates, which can be used to produce greater leaf areas for intercepting light. This greater leaf area and light interception can then lead to greater wood volume gains. These data come from studies which intensively monitored $A_{sat}$ and growth following fertilization in young *P. taeda*. Most studies supporting the hypothesis of increased shoot to root ratios mainly use differences in dry matter partitioning with little regard to physiological changes. Therefore, timing of fertilization and measurements is the main contributing factor hypothesized by both Gough et al. (2004b) and King et al. (2008) to explain the discrepancy between the two hypotheses.

**Leaf level physiology and biochemical properties**

Generally speaking, N fertilization results in increased foliar N ($[N]_f$) (Axelsson and Axelsson 1986; Green and Mitchell 1992; Mitchell and Hinckley 1993; Maier et al. 2002; Munger et al. 2003; Ripullone et al. 2003; Albaugh et al. 2004; Gough et al. 2004a; Gough et al. 2004b; Springer et al. 2005). The ease in measuring $[N]_f$ and its strong correlation with other physiological parameters such as: Rubisco activity, electron transport, and dark respiration to name a few is the reason for the large focus on this particular measure (Kellomaki and Wang 1997b; Kellomaki and Wang 1997a; Strand 1997; Samuelson 2000; Ripullone et al. 2003; Gough et al. 2004b; Manter et al. 2005). Foliar N concentration has shown a strong positive correlation with $A_{sat}$ (Green and Mitchell 1992; Mitchell and Hinckley 1993; Roberntz and Stockfors 1998; Schoettle and Smith 1999; Samuelson 2000; Ripullone et al. 2003; Gough et al. 2004b), although, some found a negative or very weak correlation between $A_{sat}$ and $[N]_f$ (Maier et al. 2002; Munger et al. 2003; Gough et al. 2004a; Warren et al. 2004; Springer et al. 2005; King et al. 2008). Further support comes from numerous studies listed in Table 1.4, which have shown increases in specific photosynthesis following fertilization. Of the studies examined, most observed an increase in $A_{sat}$ following fertilization; however, there were a number of studies that observed a weak increase or decrease in $A_{sat}$. Interestingly, most observed decreases in $A_{sat}$ tended to occur in studies that have undergone continuous fertilization. Lending support to the hypothesis posed by Gough
et al. (2004b), which suggests that increases in $A_{\text{Sat}}$ rates allow for increased photoassimilates, resulting in greater leaf area, and eventually decreased $A_{\text{Sat}}$ per unit leaf area.

Nitrogen is a major component in many of the macromolecules involved in photosynthesis (i.e., chlorophyll and Rubisco) making this element central to proper plant function. Studies have shown the response of specific molecules to N fertilization. For example, Chandler and Dale (1995) found increased chlorophyll and carotenoid concentrations in two-year-old N deficient, pot grown Sitka spruce (*Picea sitchensis*) that underwent different levels of N fertilization. Ripullone et al. (2003) found a positive correlation between $[\text{N}]_f$ and chlorophyll content in potted Douglas-fir and poplar seedlings. Tissue et al. (1993) also found increased chlorophyll content, and Rubisco content and activity in one-year-old potted *P. taeda* seedlings that had been fertilized with N and P. Bauer et al. (2004) found a 65 to 80% increase in protein and a 25 to 120% increase in chlorophyll content in red pines that underwent chronic N treatments. The authors found increased N as free amino acids in high N treated pines, but while glutamate, gaba, and leucine all increased by far the largest increase was observed in arginine. Warren et al. (2004) also found an increase in chlorophyll and Rubisco N after a N pulse in Douglas-fir seedlings, but as a percent of total N the authors found a decrease in Rubisco. Finally, Manter et al. (2005) found increased chlorophyll and Rubisco activity with N fertilization in Douglas-fir seedlings, but found a curvilinear trend in Rubisco activity. The authors observed a greater percent of inactivated Rubisco as $[\text{N}]_f$ increased and hypothesized that something else was limiting Rubisco function. Additionally, the authors observed an increase in Mn and no change in Mg$^{2+}$, which compete for binding sites. The authors hypothesized that an increase in the Mn:Mg$^{2+}$ ratio led to greater inactivation of Rubisco at higher $[\text{N}]_f$, although, they could not rule out the limitation of some other nutrient such as P. Similarly, based on calculated estimates from $A/C_i$ curves Ripullone et al. (2003) hypothesized the observed decrease in the percent of leaf N in active Rubisco could be due to more Rubisco existing in an inactive form thus serving as N storage.

Phosphorus may also play a critical role in how a plant responds to N additions. Warren and Adams (2002) demonstrated in a greenhouse and field study that the addition of P at varying
rates of N can result in changes in the Rubisco:Chl ratio. N appeared to increase Chl a and b content while showing only small increases in Rubisco content. In contrast, P fertilization showed a significant increase in Rubisco content while having no effect on Chl a and b. The authors further concluded that excess P tends to be stored as orthophosphate while N storage may be in the form of Rubisco.

**Questions answered and raised by clonal trials**

Many of the traits responsible for volume gain, disease resistance, C allocation patterns (Li et al. 1991b), LAI (McCready and Jokela 1996; McCrady and Jokela 1998), C capture (King et al. 2008), and N use efficiency (NUE) (Li et al. 1991a) have been shown to be genetically controlled. It has been well established that large increases in yield can be realized with the planting of superior seedling stock (Allen et al. 2005).

While increased yield is the primary benefit of the use of clones, there are others as well. For instance clones in research can be used to eliminate genetic variation in controlled studies. Additionally, clones known to differ in a particular trait of interest can be compared to gain better insight into certain plant processes. For example, Li et al. (1991a) showed that NUE is influenced by a number of different factors. Specifically, some genotypes are able to absorb N better and utilize N more efficiently at low fertility levels while other families are able to utilize N more efficiently at higher fertility levels. King et al. (2008) showed differences in physiological responses to fertilization in *P. taeda*, but even within full-sib families there was still significant variation in how the clones responded to fertilization. Another interesting finding from this study showed that high performing genotypes, in terms of C gain, accomplished this by different means (i.e., leaf area vs. C fixed per leaf area). Similarly, Koehn et al. (2003) found differences in leaf level physiology (i.e., photochemical quenching and yield of PSII) in full-siblings, open-pollinated, and self-pollinated slash pine seedlings. In contrast, Samuelson (2000) found little difference in physiological and growth traits among families in both *P. taeda* and *elliottii*. In summary, there is great potential to increase forest yield and advance our fundamental understanding of C cycling in managed pine systems by utilizing clones.
Table 1.1. Tissue chemistry of common crop and forest residues.

<table>
<thead>
<tr>
<th>Residue</th>
<th>C</th>
<th>N</th>
<th>C:N</th>
<th>Cellulose</th>
<th>Hemicellulose</th>
<th>Lignin</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Alfalfa¹ (Medicago sativa)</td>
<td>44.5</td>
<td>2.97</td>
<td>15</td>
<td>32.3</td>
<td>20.5</td>
<td>9.4²</td>
<td>(1)</td>
</tr>
<tr>
<td>Rye (Secale cereale)</td>
<td>--</td>
<td>--</td>
<td>16</td>
<td>21.2</td>
<td>24.9</td>
<td>1.4</td>
<td>(2)</td>
</tr>
<tr>
<td>Cuphea¹ (Cuphea spp.)</td>
<td>41.7</td>
<td>1.64</td>
<td>25</td>
<td>29.3</td>
<td>21.1</td>
<td>15.9²</td>
<td>(1)</td>
</tr>
<tr>
<td>Rape (Orobanche spp.)</td>
<td>--</td>
<td>--</td>
<td>29</td>
<td>33.2</td>
<td>14.4</td>
<td>6.7</td>
<td>(2)</td>
</tr>
<tr>
<td>Corn¹ (Maize spp.)</td>
<td>40.6</td>
<td>0.95</td>
<td>43</td>
<td>38.0</td>
<td>37.2</td>
<td>10.7²</td>
<td>(1)</td>
</tr>
<tr>
<td>Wheat straw (Triticum aestivum L.)</td>
<td>30.1</td>
<td>0.69</td>
<td>44</td>
<td>55.6</td>
<td>23.1</td>
<td>16.7</td>
<td>(3)</td>
</tr>
<tr>
<td>Switchgrass¹ (Panicum virgatum)</td>
<td>45.4</td>
<td>0.79</td>
<td>57</td>
<td>42.3</td>
<td>56.4</td>
<td>12.9²</td>
<td>(1)</td>
</tr>
<tr>
<td>Soybean¹ (Glycine spp.)</td>
<td>45.8</td>
<td>0.78</td>
<td>59</td>
<td>44.0</td>
<td>28.1</td>
<td>16.6²</td>
<td>(1)</td>
</tr>
<tr>
<td>Pines (Pinus spp.)</td>
<td>51.9</td>
<td>0.63</td>
<td>83</td>
<td>22.7-42.5</td>
<td>16.4-22.9</td>
<td>25.0-30.4</td>
<td>(4, 5)</td>
</tr>
<tr>
<td>red maple (Acer rubrum)</td>
<td>50.4</td>
<td>0.72</td>
<td>70</td>
<td>17.4</td>
<td>10.3</td>
<td>10.6</td>
<td>(4)</td>
</tr>
<tr>
<td>logging residue (incorporated on site)</td>
<td>--</td>
<td>--</td>
<td>700</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>(6)</td>
</tr>
<tr>
<td>Oak sawdust</td>
<td>50.0</td>
<td>0.1</td>
<td>--</td>
<td>70</td>
<td>--</td>
<td>27</td>
<td>(7)</td>
</tr>
</tbody>
</table>

¹ Average of leaves stems and roots (Johnson et al. 2007)
² (Coppens et al. 2007)
³ (Singh and Sharma 2002)
⁴ Values were taken on litter only (Delaney et al. 1996)
⁵ Values obtained from P. resinosa trees; age 21-25 (Berrocal et al. 2004)
⁶ Values obtained from Forest Service SRS.
⁷ Values obtained from (Starbuck 1994)
Table 1.2. Studies examining the effects of fertilization on soil microbial activity.

<table>
<thead>
<tr>
<th>Timber type</th>
<th>Age (yrs)</th>
<th>Type of Fertilizer</th>
<th>Freq. and/or last application</th>
<th>Parameter¹</th>
<th>Response¹</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picea spp.</td>
<td>--</td>
<td>Urea</td>
<td>13 days</td>
<td>Resp/Incub</td>
<td>Inc.</td>
<td>(Salonius 1972)</td>
</tr>
<tr>
<td>Picea mariana</td>
<td>75</td>
<td>NH₄NO₃ and NH₄SO₄, Urea</td>
<td>6 hours, 6 hours</td>
<td>Resp/Incub</td>
<td>Dec.</td>
<td>(Roberge 1976)</td>
</tr>
<tr>
<td>Field soil</td>
<td>--</td>
<td>NH₄NO₃</td>
<td>Annually for 3 years</td>
<td>Resp/NaOH</td>
<td>Dec.</td>
<td>(Kowalenko et al. 1978)</td>
</tr>
<tr>
<td>Populus tremuuloides</td>
<td>20</td>
<td>NH₄NO₃, TSP, KCl</td>
<td>Annually from 1969 thru 1975</td>
<td>Resp/Incub</td>
<td>Inc. N.S.</td>
<td>(Van Cleve and Moore 1978)</td>
</tr>
<tr>
<td>Pinus sylvestris</td>
<td>120</td>
<td>Urea and NH₄NO₃</td>
<td>5 years prior</td>
<td>Resp/Incub</td>
<td>Dec.</td>
<td>(Bååth et al. 1981)</td>
</tr>
<tr>
<td>Coniferous Forest</td>
<td>--</td>
<td>NH₄NO₃</td>
<td>3 months to 5 years</td>
<td>Resp/Incub</td>
<td>Dec.</td>
<td>(Söderström et al. 1983)</td>
</tr>
<tr>
<td>Field soil</td>
<td>--</td>
<td>Ca(H₂PO₄)₂·H₂O, Ca(OH)₂</td>
<td>16 weeks, 16 weeks</td>
<td>Resp/Incub/NaOH</td>
<td>Inc.</td>
<td>(Haynes and Swift 1988)</td>
</tr>
<tr>
<td>Pinus sylvestris</td>
<td>125</td>
<td>Urea and NH₄NO₃</td>
<td>10 years prior</td>
<td>Resp/Incub</td>
<td>Dec.</td>
<td>(Nohrstedt et al. 1989)</td>
</tr>
<tr>
<td>Mixed conifers</td>
<td>--</td>
<td>NH₄NO₃</td>
<td>5 months</td>
<td>Resp/Incub</td>
<td>Inc.</td>
<td>(Illmer and Schinner 1991)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>40-60</td>
<td>Urea, NH₄NO₃, NH₄SO₄, Rock and super phosphate</td>
<td>N – 2-5 years prior, P – 11-13 years prior</td>
<td>Resp/Incub</td>
<td>Dec.</td>
<td>(Smolander et al. 1994)</td>
</tr>
<tr>
<td>Pinus resinosa</td>
<td>31</td>
<td>NH₄⁺ and NO₃⁻</td>
<td>annually</td>
<td>Resp/Trench/SL</td>
<td>No resp.</td>
<td>(Haynes and Gower 1995)</td>
</tr>
<tr>
<td>Pinus contorta</td>
<td>--</td>
<td>Urea, NH₄NO₃, and TSP</td>
<td>120 days prior</td>
<td>Resp/Incub/IRGA</td>
<td>Dec.</td>
<td>(Thirukkumaran and Parkinson 2000)</td>
</tr>
<tr>
<td>Mixed deciduous</td>
<td>14-27</td>
<td>NH₄NO₃</td>
<td>every 4 weeks during growing seas.</td>
<td>Resp/NaOH</td>
<td>Dec.</td>
<td>(Fisk and Fahey 2001)</td>
</tr>
</tbody>
</table>
**Table 1.2 continued**

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Time</th>
<th>Method</th>
<th>Result</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinus sylvestris</td>
<td>NH₄Cl and C4 sucrose</td>
<td>9 days</td>
<td>Resp¹³C</td>
<td>Inc.</td>
<td>(Ekblad and Nordgren 2002)</td>
</tr>
<tr>
<td>Mixed Hardwoods</td>
<td>NH₄NO₃</td>
<td>Annually</td>
<td>Resp/Incub/IRGA</td>
<td>Dec.</td>
<td>(Bowden et al. 2004)</td>
</tr>
<tr>
<td>Pinus resinosa</td>
<td>NH₄NO₃</td>
<td>Annually</td>
<td>Resp/Incub/IRGA</td>
<td>Dec.</td>
<td>(Bowden et al. 2004)</td>
</tr>
<tr>
<td>Pinus taeda pot study</td>
<td>DAP</td>
<td>49 and 197 days prior</td>
<td>Resp/IRGA/Dist</td>
<td>Dec.</td>
<td>(Gough and Seiler 2004)</td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>Urea and DAP</td>
<td>&lt; 1 year</td>
<td>Dehydrogenase</td>
<td>Dec.</td>
<td>(Blazier et al. 2005)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>Optimal nutrition</td>
<td>every 2 days</td>
<td>Resp/IRGA/Gird</td>
<td>Dec.</td>
<td>(Olsson et al. 2005)</td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>DAP and NH₄NO₃</td>
<td>1 year</td>
<td>Resp/IRGA/Dist</td>
<td>Dec.</td>
<td>(Tyree et al. 2008)</td>
</tr>
</tbody>
</table>

¹ Abbreviations: Decreased (Dec.), Dehydrogenase activity (Dehyd), Increased (Inc.), No response (No resp.), Portable infrared gas analyzer (IRGA), Respiration (Resp), Soda-lime (SL), Soil incubation (Incub), Soil removed and measured in the field (Dist), Static absorption method (NaOH), Tree girdling (Gird), Trenching method (Trench).
Table 1.3. Studies examining the effects of fertilization on soil microbial biomass C.

<table>
<thead>
<tr>
<th>Timber type</th>
<th>Age (yrs)</th>
<th>Type of Fertilizer</th>
<th>Freq. and/or Method</th>
<th>Response</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed deciduous</td>
<td>--</td>
<td>Urea, Superphosphate</td>
<td>5 months prior</td>
<td>PC</td>
<td>N &lt; 5 months inc. bacteria returning to control levels P Decreased bacteria No response fungi to N or P (Kelly and Henderson 1978)</td>
</tr>
<tr>
<td><em>Pinus sylvestris</em></td>
<td>120</td>
<td>Urea and NH$_4$NO$_3$</td>
<td>5 years prior</td>
<td>ACO/FDA/MFM</td>
<td>Decrease in bacteria Decrease in fungi (Bååth et al. 1981)</td>
</tr>
<tr>
<td>Coniferous Forest</td>
<td>--</td>
<td>NH$_4$NO$_3$</td>
<td>3 months to 5 years</td>
<td>ACO/FDA/MFM</td>
<td>Decreased (Söderström et al. 1983)</td>
</tr>
<tr>
<td><em>Pinus sylvestris</em></td>
<td>125</td>
<td>Urea and NH$_4$NO$_3$</td>
<td>10 years prior</td>
<td>ATP/SIR</td>
<td>Decreased (Nohrstedt et al. 1989)</td>
</tr>
<tr>
<td>Mixed conifers</td>
<td>--</td>
<td>NH$_4$NO$_3$</td>
<td>5 months prior</td>
<td>SIR</td>
<td>Increased (Illmer and Schinner 1991)</td>
</tr>
<tr>
<td><em>Picea abies</em></td>
<td>40-60</td>
<td>Urea, NH$_4$NO$_3$ and NH$_4$SO$_4$</td>
<td>N – 2-5 years prior</td>
<td>CF/SIR/ERG</td>
<td>Decreased Increased No response to P (Smolander et al. 1994)</td>
</tr>
<tr>
<td><em>Pinus contorta</em></td>
<td>--</td>
<td>Urea, NH$_4$NO$_3$, and TSP</td>
<td>120 days prior</td>
<td>SIR</td>
<td>Decreased (Thirukkumaran and Parkinson 2000)</td>
</tr>
<tr>
<td><em>Pinus taeda</em></td>
<td>10</td>
<td>DAP</td>
<td>at establishment</td>
<td>SIR</td>
<td>No response (Bailey et al. 2002)</td>
</tr>
<tr>
<td>Psuedotsuga menziesii</td>
<td>20</td>
<td>Urea</td>
<td>3 years prior</td>
<td>SIR</td>
<td>No response (Bailey et al. 2002)</td>
</tr>
<tr>
<td>Mixed deciduous</td>
<td>14-27</td>
<td>NH$_4$NO$_3$</td>
<td>every 4 weeks during growing seas.</td>
<td>CF</td>
<td>Decreased (Fisk and Fahey 2001)</td>
</tr>
<tr>
<td><em>Pinus sylvestris</em></td>
<td>--</td>
<td>NH$_4$Cl and C4 sucrose</td>
<td>9 days</td>
<td>SIR</td>
<td>Increased (Ekblad and Nordgren 2002)</td>
</tr>
<tr>
<td><em>Picea glauca</em> Voss</td>
<td>110-150</td>
<td>Gaseous NH$_4^{+}$</td>
<td>Continuous inputs</td>
<td>Molecular tech. morphology Decreased Ectomycorrhizas</td>
<td>(Lilleskov et al. 2002)</td>
</tr>
<tr>
<td><em>Pinus taeda</em></td>
<td>7</td>
<td>N</td>
<td>Optimal</td>
<td>CF</td>
<td>Decreased (Lee and Jose 2003)</td>
</tr>
<tr>
<td>Specie</td>
<td>Treatment</td>
<td>Method</td>
<td>Result</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------------</td>
<td>-----------------</td>
<td>-------------------------------</td>
<td>------------------------------------</td>
<td></td>
</tr>
<tr>
<td><em>Thuja plicata</em></td>
<td>NPK 10 years prior</td>
<td>BIOLOG-PLFA</td>
<td>Increased</td>
<td>(Leckie et al. 2004)</td>
<td></td>
</tr>
<tr>
<td><em>Tsuga heterophylla</em></td>
<td>NPK 10 years prior</td>
<td>Direct counts</td>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Festuca arundinacea</em></td>
<td>NH₄NO₃ Annually</td>
<td>Direct counts</td>
<td>Decreased Fungal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pinus taeda</em></td>
<td>Optimal Continuous</td>
<td>CF</td>
<td>Increased</td>
<td>(Li et al. 2005)</td>
<td></td>
</tr>
<tr>
<td><em>Pinus contorta</em></td>
<td>Medium Urea 10 years prior</td>
<td>BIOLOG</td>
<td>No response</td>
<td>(Berch et al. 2006)</td>
<td></td>
</tr>
<tr>
<td><em>Pinus taeda</em></td>
<td>Urea and DAP &lt; 1 year</td>
<td>CF</td>
<td>Decreased</td>
<td>(Blazier et al. 2005)</td>
<td></td>
</tr>
<tr>
<td>Mixed hrdwds and con 80-120</td>
<td>(NH₄)₂SO₄, NH₄NO₃</td>
<td>CF/SIR</td>
<td>No response</td>
<td>(Wallenstein et al. 2006)</td>
<td></td>
</tr>
<tr>
<td>Mixed hrdwds and con 14</td>
<td>NH₄Cl</td>
<td>CF/SIR</td>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Picea rubens</em></td>
<td>NH₄Cl</td>
<td>CF/SIR</td>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Abbreviations: Acridine-orange stained smears to determine total bacterial number (ACO), ATP-synthase assay (ATP), Carbon substrate utilization method (BIOLOG), Chloroform fumigation (CF), Ergosterol content (ERG), FDA-active fungal length (FDA), Membrane-filter method to determine total fungal length (MFM), Plate count (PC), Phospholipid fatty acid profile (PLFA), and Substrate-induced respiration (SIR).
Table 1.4. Studies examining the effects of fertilization on leaf gas exchange.

<table>
<thead>
<tr>
<th>Timber type</th>
<th>Age (yrs)</th>
<th>Fertilizer / Freq. and/or last application</th>
<th>Cont / Fert Foliar N %</th>
<th>Parameter / Method</th>
<th>Response</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinus taeda</td>
<td>1</td>
<td>Complete Hoagland’s sol. with N ranging from 10-300mg l(^{-1}) N as NH(_4)NO(_3)</td>
<td>-- / --</td>
<td>(A_{\text{Sat}} / \text{IRGA})</td>
<td>Increased</td>
<td>(Green and Mitchell 1992)</td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>1</td>
<td>Low N, Low P, High N&amp;P twice weekly, mod. Hoaglands</td>
<td>-- / --</td>
<td>(A_{\text{Sat}} / \text{IRGA})</td>
<td>Increased</td>
<td>(Tissue et al. 1993)</td>
</tr>
<tr>
<td>Pinus elliottii</td>
<td>23</td>
<td>N, P, K, Ca, Mg, S, and Micros applied 3 times a year</td>
<td>0.75 / 1.16</td>
<td>(A_{\text{Sat}} / \text{IRGA})</td>
<td>Increased</td>
<td>(Teskey et al. 1994)</td>
</tr>
<tr>
<td>Picea sitchensis</td>
<td>2</td>
<td>Twice a week with 0, 7, 14, 28, 56, 112, 224 mg l(^{-1}) N (NO(_3) and NH(_4))</td>
<td>-- / --</td>
<td>(A_{\text{Sat}} / \text{IRGA})</td>
<td>Inc. with inc. N up to 28 Dec. with inc. N up to 56</td>
<td>(Chandler and Dale 1995)</td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>9</td>
<td>Optimal nutrition / Continuously</td>
<td>0.95 / 1.20</td>
<td>(A_{\text{Sat}} / \text{IRGA})</td>
<td>Increased</td>
<td>(Murthy et al. 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Murthy et al. 1997)</td>
</tr>
<tr>
<td>Picea rubens</td>
<td>Mature</td>
<td>Different levels of N fert. applied annually starting 1988</td>
<td>-- / --</td>
<td>(A_{\text{Sat}} / \text{IRGA})</td>
<td>Decreased; n.s.</td>
<td>(Schaberg et al. 1997)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>29</td>
<td>Optimal nutrient solution / Every day during growing season</td>
<td>-- / --</td>
<td>(O_2) exchange / (O_2)</td>
<td>Increased</td>
<td>(Strand 1997)</td>
</tr>
<tr>
<td>Pinus radiata</td>
<td>2</td>
<td>4 N treatments (100, 66, 33, 0%)</td>
<td>-- / --</td>
<td>(A_{\text{Sat}} / A/C_i)</td>
<td>Increased</td>
<td>(Walcroft et al. 1997)</td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>4</td>
<td>200 kg N ha(^{-1})</td>
<td>-- / --</td>
<td>(A_{\text{Sat}})</td>
<td>Slight increase; n.s.</td>
<td>(Zhang et al. 1997b)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>30</td>
<td>Optimal nutrient solution / Every day during growing season</td>
<td>-- / --</td>
<td>(A_{\text{Sat}} / \text{IRGA})</td>
<td>Increased; n.s.</td>
<td>(Roberntz and Stockfors 1998)</td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>1</td>
<td>Liquid fertilizer of 264 and 50</td>
<td>0.69 / 1.76</td>
<td>(A_{\text{Sat}} / \text{IRGA})</td>
<td>Increased</td>
<td>(Samuelson 2000)</td>
</tr>
<tr>
<td>Species</td>
<td>Treatment Details</td>
<td>Nitrogen (ppm)</td>
<td>Measure</td>
<td>Response</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------------------------------------------------------------------------------</td>
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<td>----------------------</td>
<td>-------------------</td>
<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Pinus elliottii</td>
<td>ppm N; weekly or bi-weekly</td>
<td>0.58 / 1.55</td>
<td>$R_D$</td>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinus taeda SETRES</td>
<td>Optimal nutrition / continuously</td>
<td>17 to 41%</td>
<td>$A_{Sat}$ / IRGA</td>
<td>No Change</td>
<td>(Maier et al. 2002)</td>
<td></td>
</tr>
<tr>
<td>Pinus taeda SETRES</td>
<td>Optimal nutrition / continuously</td>
<td>17 to 41%</td>
<td>$A_{Sat}$ / IRGA</td>
<td>No Change</td>
<td>(Maier et al. 2002)</td>
<td></td>
</tr>
<tr>
<td>Pinus pinaster</td>
<td>Complete nutrient with 2 levels</td>
<td></td>
<td>$A_{Max}$</td>
<td>No Change</td>
<td>(Warren and Adams 2002)</td>
<td></td>
</tr>
<tr>
<td>Pinus resinosa Harvard Forest</td>
<td>150 kg N ha$^{-1}$ year$^{-1}$ since 1988 chronic N additons</td>
<td>-- / --</td>
<td>$A_{Sat}$ / IRGA</td>
<td>Decreased</td>
<td>(Bauer et al. 2004)</td>
<td></td>
</tr>
<tr>
<td>Pinus taeda SETRES</td>
<td>Optimal nutrition / continuously</td>
<td>-- / --</td>
<td>$A_{Sat}$ / IRGA</td>
<td>No Change</td>
<td>(Gough et al. 2004a)</td>
<td></td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>DAP / single dose at start</td>
<td>0.76 / 1.7</td>
<td>$A_{Sat}$ / IRGA</td>
<td>Increased</td>
<td>(Gough et al. 2004b)</td>
<td></td>
</tr>
<tr>
<td>Pseudotsuga menziesii</td>
<td>Nutrient solution containing 250 ppm N as NH$_4$NO$_3$; cont.</td>
<td>0.86 / 1.79</td>
<td>$A_{Sat}$ / IRGA</td>
<td>Increased</td>
<td>(Warren et al. 2004)</td>
<td></td>
</tr>
<tr>
<td>Pinus taeda</td>
<td>DAP &amp; NH$_4$NO$_3$; single dose</td>
<td>1.49 / 1.67</td>
<td>$A_{Sat}$ / IRGA</td>
<td>Increased</td>
<td>(King et al. 2008)</td>
<td></td>
</tr>
<tr>
<td>Pinus radiata</td>
<td>N and P factorial</td>
<td>-- / --</td>
<td>$A_{Sat}$</td>
<td>Increased</td>
<td>(Bown et al. 2007)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: $C_i$ = internal CO$_2$ concentration, IRGA = infrared gas analyzer, O$_2$ = leaf-disc oxygen electrode, O$_2$ exchange = photosynthesis as measured by oxygen exchange, $A_{Sat}$ = net photosynthesis, $R_D$ = dark respiration.
Figure 1.1. Composite diagram depicting various C pools and fluxes in a typical managed *Pinus taeda* stand.
References


Chapter 1

Section: Reference Section


Chapter 1


Chapter 1

Section: Reference Section


Chapter 2

Short-term impacts of nutrient manipulations on leaf gas exchange and biomass partitioning in contrasting two-year-old Pinus taeda clones during seedling establishment.

Summary Differences in nutrient availability can influence the short-term capacity of conifer seedlings to collect and utilized light energy for photosynthesis, and the distribution of that photosynthate between plant tissues. With increased emphasis being placed on site-specific management there is a great need to evaluate how specific genotypes will vary across environments. We conducted a one year greenhouse experiment with a factorial combination of fertilization and high C:N logging residue (LR) incorporation applied to modify nutrient availability. The objectives were to assess the impact of nutrient manipulations on leaf gas exchange and biomass partitioning between two-year-old P. taeda clones, which represent two distinct ideotypes (“narrow crown” versus “broad crown”). Further, this experiment provides a detailed exploration of the short-term physiological adjustments to leaf gas exchange that P. taeda make under varying nutrient availability, and how these adjustments may differ between contrasting genotypes. Our data showed substantial differences between genotypes in both gas exchange parameters and biomass partitioning patterns. Additionally, fertilization with N and P did not result in an immediate and sustained increase in $A_{\text{Sat}}$ as we hypothesized, but did result in consistently lower $g_s$, $E$, and $C_i/C_a$ and improved WUE independently of genotype. When specific gas exchange data was scaled to the canopy level these data showed that both genotypes achieved similar canopy level CO$_2$ assimilation rates, but by different means. CL93 (“narrow crown” ideotype) produced greater leaf area with lower photosynthetic efficiency at low N concentrations while CL85 (“broad crown” ideotype) used the opposite strategy of lower leaf area, but greater efficiency. Although we did see a small effect of nutrient limitations in $A_{\text{Canopy}}$, $A_{\text{Sat}}$, and $TLA$, our foliar N concentration ([N]) indicated that our level of LR incorporation was not sufficient to cause [N] to decrease below critical limits. This work suggests that even within a single species, genotypes can express different mechanisms for capturing and partitioning C. From a practical standpoint, understanding these strategies could lead to better selection of clonal material to specific site resource availability optimizing productivity with the least amount of cost to the land owner.

Keywords: $A_{\text{Sat}}$, fertilization, genotype, leaf gas exchange, leaf morphology, loblolly pine
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbrev</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{\text{Canopy}}$</td>
<td>Canopy level net CO$_2$ assimilation</td>
<td>$\mu$mol CO$_2$ s$^{-1}$</td>
</tr>
<tr>
<td>$A_{\text{Sat}}$</td>
<td>Instantaneous specific net CO$_2$ assimilation under light and CO$_2$ saturation</td>
<td>$\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>$C_i/C_a$</td>
<td>Internal CO$_2$ to ambient CO$_2$ ratio</td>
<td>unitless</td>
</tr>
<tr>
<td>CSA</td>
<td>Canopy silhouette area</td>
<td>cm$^2$</td>
</tr>
<tr>
<td>$E$</td>
<td>Transpiration</td>
<td>mmol H$_2$O m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>$g_s$</td>
<td>Stomatal conductance</td>
<td>mol m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>LR</td>
<td>Logging residue</td>
<td>treatment</td>
</tr>
<tr>
<td>$[N]_a$</td>
<td>Nitrogen concentration per unit leaf area</td>
<td>g N m$^{-2}$ leaf</td>
</tr>
<tr>
<td>$[N]_f$</td>
<td>Foliar nitrogen concentration</td>
<td>unitless</td>
</tr>
<tr>
<td>$[N]_m$</td>
<td>Nitrogen concentration per unit leaf mass</td>
<td>mg N g$^{-1}$ leaf</td>
</tr>
<tr>
<td>$PNUE$</td>
<td>Instantaneous photosynthetic N use efficiency</td>
<td>$\mu$mol CO$_2$ g$^{-1}$ N s$^{-1}$</td>
</tr>
<tr>
<td>$SLA$</td>
<td>Specific leaf area</td>
<td>cm$^2$ g$^{-1}$</td>
</tr>
<tr>
<td>$TLA$</td>
<td>Total leaf area calculated from $SLA$ and leaf mass</td>
<td>m$^2$</td>
</tr>
<tr>
<td>$WUE$</td>
<td>Instantaneous water use efficient</td>
<td>$\mu$mol CO$_2$ mmol H$_2$O$^{-1}$</td>
</tr>
</tbody>
</table>
Introduction

Differences in nutrient availability can influence the short-term capacity of conifer seedlings to collect and utilize light energy for photosynthesis, and further, it can influence the distribution of that photosynthate between plant tissues. Nitrogen and phosphorus are usually the most limiting nutrients to plant growth and have been shown to exert a strong influence on leaf area (Zhang et al. 1997a; Albaugh et al. 1998; King et al. 1999), morphology (Niinemets et al. 2001; Maier et al. 2002; Will 2005), chemistry, as well as leaf level physiology in *Pinus* spp. (Tissue et al. 1993; Gough et al. 2004b; Samuelson et al. 2004; Bown et al. 2007; King et al. 2008). Nitrogen is a major component of most of the proteins and pigments involved in photosynthesis explaining why increases in net CO₂ assimilation under saturating light ($A_{Sat}$) have been commonly observed following N fertilization (Green and Mitchell 1992; Tissue et al. 1993; Samuelson 2000; Gough et al. 2004b). Increases in foliar N concentration ($[N]_f$) have been shown to correspond with increased chlorophyll content (Chandler and Dale 1995; Ripullone et al. 2003; Bauer et al. 2004; Chmura and Tjoelker 2008), Rubisco content, or both (Tissue et al. 1993; Warren et al. 2004; Manter et al. 2005) in conifer species.

Some studies have found no change or even a decrease in $A_{Sat}$ following fertilization (Zhang et al. 1997b; Maier et al. 2002; Warren and Adams 2002; Bauer et al. 2004; Gough et al. 2004a). In most of these instances measurements were taking after continuous or in some cases chronic N additions. One hypothesis proposed by Gough et al. (2004b) and later supported by King et al. (2008) is that increases in $A_{Sat}$ rates immediately following fertilization allow for increased photo-assimilates, which then can be used to produce greater leaf area for light interception, and lead to an eventual down regulation of $A_{Sat}$. Therefore, timing of fertilization and measurements is a contributing factor hypothesized by both Gough et al. (2004b) and King et al. (2008) that may help explain these discrepancies.

*Pinus taeda* is planted over a large geographic range within the southeastern United States, exposing it to a wide range of site conditions (e.g., climate and resource availability). Natural within species plasticity allows for tolerance of resource limitations while still achieving adequate growth, and has led to the widespread planting of *P. taeda* throughout the
southeast (Wear and Greis 2002). Extensive geographic range and site variation has allowed for large within species differences. Breeding programs have exploited this large genetic variation resulting in superior planting stock in terms of growth, disease resistance, and form. The widespread use of superior planting stock, and to a lesser extent clonal material, has been estimated to increase wood volume gains as much as 10-30% in southern pines. Further, it has been estimated that by combining clones and appropriate silvicultural prescriptions volume gains as high as 50% to over 60% could be achieved (Allen et al. 2005; Martin et al. 2005; McKeand et al. 2006).

With increased emphasis being placed on site-specific management there is much need to determine how specific genotypes will vary across environments (Fox 2000). There are conflicting opinions on importance of genetic by environment interactions (G x E). McKeand et al. (2006) in a summary of the current literature suggested that G x E are of little practical importance for open-pollinated, half-sib, and full-sib families, but more long-term studies are needed before the importance of G x E of clones are known. In contrast, Roth et al. (2007) found large G x E between full-sib genotypes when planted at different locations or managed with varying silvicultural prescriptions. Further, the authors concluded that matching the best genotype to site conditions may be necessary in the future to maximize productivity. Some studies have found significant family by fertilization interactions in stem growth (Li et al. 1991c), C allocation (Li et al. 1991b; Retzlaff et al. 2001), and nitrogen use efficiency (Li et al. 1991a) in *P. taeda*, but the effects of G x E have been less stable for leaf level gas exchange measurements. For example, researchers have found strong fertilizer effects on leaf photosynthesis and conductance, but no genotype by fertilizer interactions (Samuelson 2000; Bown et al. 2007; Chmura and Tjoelker 2008), while others have found differences in specific leaf photosynthesis between full-sib clones when fertilized (King et al. 2008).

The use of contrasting genotypes in physiological research has implications beyond matching specific genotypes to site conditions. For example, improved ability to detect treatment differences by eliminating genetic variability, or the use of contrasting clones combined with resource manipulations may allow for improved understanding of the mechanism involved in
C capture and partitioning. Additionally, their use in research may provide insight into the stability of these mechanisms within a single species under a range of resource availability. A greenhouse experiment with a factorial combination of fertilization and high C:N logging residue (LR) incorporation (applied to modify nutrient availability) was designed to assess the impact of nutrient manipulations on leaf gas exchange and biomass partitioning between two-year-old *P. taeda* clones representing two distinct ideotypes (“narrow crown” versus “broad crowns”). This experiment provides a detailed exploration of the short-term physiological adjustments to leaf gas exchange that *P. taeda* make under varying nutrient availability, and how these adjustments may differ between contrasting genotypes. Secondly, we ask if seedling growth response to nutrient availability is a function of increased photosynthetic efficiency (*A*<sub>sat</sub>) or mainly a result of changes in leaf area due to reallocation of C, and does this seem to be consistent across clones. From previous work, we hypothesize that specific net photosynthesis in both clones will increase immediately following fertilization, but to different degrees. We expected that one clone will invest more C to increasing leaf area and the other in photosynthetic machinery per unit leaf area leading to no overall difference in canopy level photosynthesis between genotypes. Further, we anticipated decreased leaf gas exchange in response to LR incorporation due to N limitations, but the degree of the decline will differ between clones possibly due to differences in biomass partitioning or photosynthetic N use efficiency (*PNUE*).

**Materials and Methods**

**Experimental design**

In April 2006, one-year-old *Pinus taeda* clones were planted in 170 L plastic containers (93cm x 53 cm x 50 cm) and grown in a greenhouse through July 2007. The greenhouse vents and climate settings were adjusted to provide plants with summer and winter conditions representative of the southeastern United States (Figure 2.1). The study design was a randomized complete block design replicated six times. Treatments were arranged in a full 2 by 2 by 2 factorial with two levels of logging residue (LR) incorporated into the soil (none, present), two levels of fertilization (none, present), and two clones (CL93, CL85). The forty-eight plastic containers were fitted with a single brass spigot for collecting water, and each was filled with approximately 0.17 m<sup>3</sup> of Eunola series (fine-loamy, siliceous, semiactive,
thermic Aquic Hapludults) soil two months prior to planting (Appendix A). Soil was collected in February 2006 to a depth of approximately one meter, which included the Ap, BE, and Bt horizons. Soil was collected from the Virginia Tech Tide Water Agricultural Research and Extension Center located in Holland, VA (Appendix B).

Treatments
Logging residue (LR; C:N = 128 ± 14; n = 4) was collected from residue piles near the logging deck of a *P. taeda* stand in South Carolina that had been harvested six months prior to collection. The residue consisted mainly of bark, needles, and small branches that remained following an onsite processing of merchantable timber. The LR was passed through a 5 cm x 10 cm screen and was mixed uniformly into the soil during pot filling at a rate of 4.92 kg LR o.d. container⁻¹ (equivalent to 25 Mg o.d. ha⁻¹). Fertilizer was applied on two separate dates. Due to slow initial growth of the clones, the first application was not applied until July 28, 2006. Fertilizer was in the form of diammonium phosphate (DAP) and ammonium nitrate (AN) at an equivalent rate of 200 kg N and 50 kg P ha⁻¹. The second fertilizer application took place on March 16, 2007 in the form of AN at a rate of 200 kg N ha⁻¹. Clonal seedlings representing “narrow crown” and “broad crown” ideotypes (CL93 and CL85, respectively) were donated by Mead Westvaco for use in this study and were chosen based on contrasting growth strategies (Phil Dougherty personal communication).

Repeated measurements
To test objective one, leaf gas exchange was measured seventeen times throughout the duration of the experiment. Measurements were taken at a greater frequency before and immediately following fertilization with the frequency decreasing to monthly by the end of the experiment. Specific net CO₂ assimilation under saturating light (*A*ₐₛₐ), stomatal conductance (*g*ₛ), transpiration (*E*), internal CO₂ to atmospheric CO₂ ratio (*C*ᵢ/*C*ₐ), and water use efficiency (*WUE*; μmol CO₂ mmol H₂O⁻¹) per unit leaf area were measured simultaneously using a Li-Cor 6400 portable open system infrared gas analyzer with a 2 x 3 cm cuvette and a blue–red LED light source (Li-Cor 6400, Lincoln, Nebraska). All measurements were made using the following chamber conditions: 1600 μmol m⁻² sec⁻¹ *PPFD*, 370 μmol mol⁻¹ reference CO₂ concentration, ambient chamber temperature and
humidity, and a flow rate of 300 μmol sec\(^{-1}\). Needles were excised from the upper portion of the seedling and placed into the cuvette. Data showed no significant \((P = 0.98; n = 8)\) differences between excised and attached needles over a two to three minute period after detachment. Following stabilization of the photosynthetic rate (approximately 2 min) measurements were logged three times at 10 second intervals, which were averaged to a single value. Needles were immediately removed from the cuvette and fascicle diameter measured to the nearest 0.01 mm using digital calipers and brought back to the lab for further analyses. All leaf gas exchange measurements were expressed on a leaf area basis using the following equation:

\[
LA = (n \times l \times d) + (\pi \times d \times l) \tag{2.1}
\]

where \(l\) is the length of the needle in the chamber, \(d\) is the diameter of the fascicle measured just above the sheath, and \(n\) the number of needles in the fascicle (Ginn et al. 1991).

Both seedling height (cm) and ground line diameter (mm) were measured approximately monthly from the time of planting through the end of the experiment. Aboveground stem volume was calculated using the following equation:

\[
\text{Stem volume (cm}^3\text{)} = \text{Height} \times (\text{basal dia.})^2. \tag{2.2}
\]

**Canopy silhouette area**

Canopy silhouette area (CSA) is defined as the total leaf and twig area contained within the tree canopy projected onto a plane (King et al. 2008). The aboveground portion of each seedling was cut above ground-line and transported to a staging area with a backdrop which provided good contrast with the tree. Two photographs were taken orthogonally to each other for each seedling using a Nikon D100 digital camera (Figure 2.2A). The color digital image was converted to a black and white image using SideLook 1.1 software (Nobis 2005) with the channel set to red. The programs automated threshold level was used as a first approximation (Figure 2.2B). In most instances the threshold level had to be adjusted. Adjustments were made by decreasing the threshold level to the point where the top of the
tree was visible (Figure 2.2C). Adobe® Photoshop® 6.0 (Adobe Systems Inc., San Jose, CA) was then used to clean up the black and white image and determine the number of pixels in the image (Figure 2.2D). A standard area reference in each photograph was used to convert from number of pixels in the image to the projected canopy area (cm²).

**Biomass partitioning, needle morphology and chemistry**

Following photographs of each canopy, seedlings were dissected into needles, branches, main stem, coarse- (> 2 mm) and fine-roots (< 2 mm). Samples were oven dried for at least two weeks at a temperature of 65 ± 5°C then weighed gravimetrically to the nearest 0.1 g. Needle morphology was determined by sampling five fascicles from most recent, fully elongated, current years flush (“new”), and five fascicles from the final, fully elongated, flush of previous season (“old”). Needle length from tip of needle to beginning of the fascicle sheath (mm), needle diameter (0.01 mm), number of needles per fascicle, and average oven dried (65 ± 5°C) weight of all five fascicles were measured for each seedling and needle age (480 needles). For each seedling by needle age combination total leaf area (TLA; m²) was calculated using equation 2.1 and specific leaf area (SLA; cm² g⁻¹) was calculated by dividing TLA by the weight. Using TLA, $A_{Sat}$ was scaled to a canopy level for the final measurement period only.

Needles from morphology measurements were then ground using a Wiley mill fitted with a number 20 screen and sent to USDA Forest Service Southern Research Station laboratory (Research Triangle Park, NC) for C and N determination using a Carlo-Erba elemental analyzer (Model NA-1500, Fison Instruments, Danvers, MA). To estimate instantaneous photosynthetic N use efficiency ($PNUE$) photosynthesis per unit N was calculated by dividing $A_{Sat}$ by the N concentration [$N$] of the needles for the final measurement period. Photosynthetic values from the late June 2007 sampling period were used to estimate $PNUE$ for new (current year flush) needles and $A_{Sat}$ values measured in late March 2007 were used to estimate $PNUE$ of old (last flush of previous year) needles. Due to different times of $A_{Sat}$ measurements, only relative treatment effects can be compared between $PNUE$ estimates of “old” and “new” needles.
Canopy level CO₂ assimilation

Objective two was tested by multiplying $A_{Sat}$ by estimates of $TLA$ to arrive at an estimate of instantaneous CO₂ assimilation at the canopy level ($A_{Canopy}$). Specific photosynthetic rates were scaled to the seedling level based on data collected from June 2007 sampling date only. This sampling date was chosen because temporally it was the closest sampling date to the time of destructive harvest. We recognize that canopy estimates of CO₂ assimilation are likely over estimates for a number of reasons, and therefore, should be interpreted with caution. First, $TLA$ does not account for differences in specific gas exchange rates due self shading or between “new” and “old” needles. Second, needle fascicles were included in the dry weight of the needles from the destructive harvest, but not when needles were sub-sampled for $SLA$ determination, which is an additional source of error leading to an overestimation of $TLA$. In the context of these cautions, we do believe this value gives a reasonable integration of differences in canopy area and $A_{Sat}$ rates.

Data analyses

Treatment differences over time were tested by analyses of variance with repeated measures (ANOVARM) using a MIXED model. Covariance structures were selected using AIC, AAIC, and BIC fit statistics included in the SAS output. Stomatal conductance and transpiration data for August 10, 2006, October 5, 2006, and October 16, 2006 were dropped due to unreasonably low internal CO₂ concentrations and $g_s$ values. Significant treatment by date interactions for repeated measurements, data from destructive harvest, needle morphology, and CSA were analyzed using a general linear model (GLM). For this experiment an alpha level of 0.10 was considered statistically significant. The relationship between $[N]_a$ and $A_{Sat}$ were modeled using linear regression. Comparison between $SCA$ and $TLA$ were modeled using nonlinear regression. Residuals and the normality curves were plotted for all analyses to confirm that the data meet assumptions of equal variance and normality for all parameters measured. When data were transformed to meet assumptions all values were expressed as untransformed averages and standard errors. All analyses were performed using the MIXED, GLM, REG, and NLIN procedures in SAS version 9 (SAS 2006).
Results

Total seedling biomass and C partitioning

The addition of fertilizer resulted in an 18% increase \((P = 0.07; n = 24)\) increase in total seedling biomass relative to unfertilized seedlings \((298 \pm 24 \text{ and } 252 \pm 18 \text{ g, respectively})\) by the end of the experiment. The average magnitude of the fertilizer response differed between genotypes with CL85 and CL93 increasing in total mass by 33 and 4%, respectively, but this difference was not statistically significant \((P = 0.15; n = 12)\). In contrast, there were no differences in total biomass or biomass partitioning between LR treatments either as a main effect or as an interaction between clones (Table 2.1).

The root to shoot ratio \((R:S)\) did not significantly \((P > 0.2)\) differ between treatment main effects, but we did observe a significant \((P = 0.10)\) LR by fertilizer interaction. The addition of both LR and fertilizer alone resulted in \(R:S\) slightly increasing from \(0.27 \pm 0.01\) to \(0.29 \pm 0.01\) and \(0.27 \pm 0.01\) to \(0.28 \pm 0.01\), respectively, but the addition of both LR and fertilizer together had no effect on \(R:S\). We observed large treatment main effects between the above and belowground tissues when analyzed individually. For example, the incorporation of LR led to a 30\% \((P = 0.004)\) increase in the stem to foliage ratio and a 16\% increase in the fine-to coarse-root ratio \((P = 0.006)\). However, LR did not significantly affect absolute biomass partitioning (Table 2.1). In contrast to LR additions, the addition of fertilizer showed a 20\% decrease \((P = 0.0002)\) in the fine- to coarse-root ratio relative to unfertilized seedlings, but this differences was due entirely to an increase in coarse-roots following fertilization (Figure 2.3A). The application of fertilizer resulted in 22\% more foliage \((P = 0.05)\) and 27\% more coarse-root \((P = 0.04)\) production among both clones (Figure 2.3A).

We found clonal differences in aboveground biomass partitioning. Clone 93 had 21\% more branches \((P = 0.05)\) than CL85 while CL85 had 23\% more stem biomass than CL93 \((P = 0.02; \text{Figure 2.3B})\). We also observed differences in proportional belowground partitioning between clones. For example, CL93 had 35\% greater F:C than CL85, but there was no difference in absolute belowground biomass partitioning \((P = 0.4)\). When we further explored the significant clone by LR interaction \((P = 0.07)\) we found that CL93 responded by increasing its F:C by 25\% while CL85 only increased its F:C by 9\%.
Stem volume, leaf morphology, and leaf area
We observed highly significant fertilizer by time ($P = 0.004$) and clone by time ($P < 0.0001$) interactions in calculated aboveground stem volume with the largest compounded treatment differences being expressed at the end of the experiment. Seedlings receiving fertilizer were 14% larger than control seedlings and CL85 seedlings were 41% larger than CL93 seedlings. When height and diameter were analyzed separately there were no significant ($P > 0.10$) differences between any of the treatments or their interactions for seedling height. In contrast, there was a highly significant LR by time ($P = 0.01$) interaction as well as a significant clone by fertilizer by time ($P = 0.03$) interaction in ground line diameter. Fertilizer increased ground line diameter to a greater degree in CL93 than it did in CL85. Finally, as a result of incorporating LR into the soil we observed a 5% increase in ground line diameter relative to treatments with no LR added.

Leaf morphology differed substantially between clones in both “old” and “new” needles for every needle parameter measured, and differed between fertilizer treatments for most parameters measured (Table 2.2 and 2.3). Clone 85 was greater than CL93 in most parameters measured except for the average number of needles per fascicle and SLA (Table 2.3). Overall, fertilizer resulted in decreased SLA in new needles relative to unfertilized seedlings as a main effect ($P = 0.04$), but there was a highly significant ($P = 0.03$) LR by clone by fertilizer interaction (Table 2.3). The addition of fertilizer by itself resulted in CL85 decreasing SLA while CL93 remained unresponsive. In contrast, when LR was applied without fertilization CL93 responded by increasing its SLA while CL85 remained unresponsive (Figure 2.4).

We observed good agreement between canopy silhouette area (CSA) and calculated total leaf area (TLA). When CSA was regressed with calculated TLA using a power function, 62% of the variation was explained (Figure 2.5). As TLA increased CSA increased at a diminishing rate likely due to overlapping of the needles. The various measures of foliage (i.e., weight, CSA, and TLA) were influenced by treatments differently. All three methods show an increase in foliage with fertilization, but the effect was statistically significant when
expressed on a weight basis \( (P = 0.05) \), and only significant \( (P = 0.10) \) when expressed as CSA and TLA. Similarly, both CSA and TLA showed 13 and 8% greater leaf area, respectively, in CL93 relative to CL85 \( (P = 0.06 \text{ and } P = 0.10, \text{ respectively}) \) while there was no statistical difference in foliage on a weight basis \( (P > 0.10) \).

**Leaf-level gas exchange**

We found a significant fertilizer by time \( (P = 0.09) \) interaction in \( A_{Sat} \). Following the first fertilizer application, there was a slight, but not significant increase in \( A_{Sat} \). Instantaneous CO\(_2\) assimilation rates in fertilized seedlings quickly returned to control levels, and on four sampling dates fertilized seedlings had significantly lower \( A_{Sat} \) values (Figure 2.6A). Following the second fertilizer application, \( A_{Sat} \) rates were lower in fertilized treatments until “new” needles were measured on the final sampling date in June 2007. We also found that fertilized seedlings had 5, 7, and 3% less \( (P < 0.05) \) \( g_s \), \( E \), and \( C_i/C_a \), respectively, and 5% greater \( (P < 0.01) \) WUE relative to unfertilized seedlings when averaged over the entire experiment irrespective of genotype (Figure 2.6B-D).

Contrary to our hypothesis there was no significant \( (P > 0.10) \) clone by fertilizer interaction over the entire study, but we did observe a highly significant difference between clones in many of parameters related to leaf gas exchange. Specifically, \( A_{Sat} \) was significantly \( (P = 0.01) \) greater in CL93 relative to CL85 through October of 2006, but this effect disappeared with both clones showing similar \( A_{Sat} \) rates by the end of the experiment (Figure 2.7A). With the exception of one sampling date in February, which was recorded as the lowest temperature for the year (Figure 2.1A), CL93 had significantly \( (P < 0.01) \) greater \( g_s \), \( E \), and \( C_i/C_a \) than CL85 (Figure 2.7B-D). The lack of clonal difference in \( A_{Sat} \) later in the experiment, and the increase in \( g_s \), led to significantly \( (P < 0.0001) \) greater WUE in CL85 relative CL93 when measured throughout the entire experiment. In fact, when averaged over all sampling dates we observed approximately a 20% greater instantaneous WUE in CL85 than CL93.
Chapter 2  Gas exchange and biomass partitioning in contrasting clones

Foliar N concentration and PNUE
Foliar N concentration and PNUE

Foliar samples collected at the end of the experiment showed that nutrient manipulations had a significant ($P < 0.05$) impact on $[N]_f$ in both “new” and “old” needles (Figure 2.8). Although the lowest foliar $[N]$ was observed in the LR only treatment, all treatments had foliar $[N]_m$ above the sufficiency limit (12 mg N g$^{-1}$ foliage) for P. taeda. “Old” needles had slightly greater foliar $[N]_m$, and substantially greater foliar $[N]_a$ than “new” needles. Overall, CL85 had 21 and 27% greater foliar $[N]_a$ than CL93 in “new” ($P = 0.0002$) and “old” ($P < 0.0001$) needles, respectively (Figure 2.9A and B). Fertilization resulted in increased foliar N ($P < 0.0001$) independently of clone, but in “old” needles the clonal response to fertilization differed in magnitude ($P = 0.06$) with CL85 increasing foliar $[N]_a$ 10% more than CL93 (Figure 2.9A and B).

The relationship between $A_{Sat}$ and $[N]_a$ was weak ($r^2 = 0.08$), but significant ($P < 0.10$), in “new” needles, and non-significant ($P > 0.10$) in “old” needles. We found in “new” needles differences in parameter estimates between CL93 and CL85 ($\beta_0 = 1.98$, $\beta_1 = 3.08$ and $\beta_0 = 3.77$, $\beta_1 = 0.72$, respectively) to not be statistically significant ($P > 0.10$). PNUE did not differ between clones when fertilizer was not applied in “new” needles, but when fertilizer was applied CL85 showed a decrease in PNUE while CL93 remained largely unchanged (Figure 2.9E). This was due to an increase in foliar $[N]_a$ with no accompanying change in $A_{Sat}$ (Figure 2.9A and C). In “old” needles we observed that CL93 had greater PNUE, relative to CL85, regardless of whether fertilizer was applied (Figure 2.9F). The individual components behaved similarly to that of fertilized “new” needles in that there was an increase in $[N]_a$ with no change in $A_{Sat}$.

Canopy level gas exchange
Canopy level CO$_2$ assimilation ($A_{Canopy}$) was increased ($P < 0.01$) by 29% in fertilized seedlings independent of genotype (Figure 2.10A). Analyses of the components making up $A_{Canopy}$ showed that CL93 responded to N and P fertilization by increasing $A_{Sat}$ while CL85 showed no change in $A_{Sat}$ (Figure 2.10B). In contrast, CL85 responded to fertilization by increasing TLA while CL93 showed no response (Figure 2.10C). Both clones showed two contrasting methods to increasing $A_{Canopy}$. There was no significant LR or LR by CL.
interactions in canopy level gas exchange or components making it up. However, $A_{\text{Canopy}}$ and $A_{\text{Sat}}$ did have a highly significant ($P < 0.01$ and $P = 0.02$, respectively) LR by fertilizer interaction and although $\text{TLA}$ was not significant ($P > 0.1$) the trend was the same (Figure 2.11A-C).

**Discussion**

*Differences in nutrient availability*

By the end of the experiment we found differences in biomass partitioning and leaf gas exchange between N availability treatments. Generally speaking, fertilization increased total seedling biomass (18%), foliar mass (22%), coarse-roots biomass (27%), and basal diameter (14%; Table 2.1; Figure 2.3A). Additionally, fertilization resulted in greater photosynthetic area when expressed as either $\text{TLA}$ or $\text{CSA}$ despite the contrasting effects of increased foliar biomass and decreased specific leaf area (Tables 2.1, 2.2, and 2.3). These findings are consistent with numerous studies that have thoroughly assessed the effects of fertilization on early growth in *P. taeda* (Zhang et al. 1997a; Albaugh et al. 1998; Samuelson 2000). In contrast to our hypothesis, the incorporation of LR did not result in reduced seedling biomass (Table 2.1), and in fact resulted in increased stem volume as a result of increased basal diameter. An analyses of $[N]_f$ showed that our level of fertilization and LR incorporation did result in changes in N availability, but those changes were weak and at no time resulted in foliar $[N]$ dropping below sufficiency limits (Figure 2.8). The research was aimed primarily at the effects of N fertilization, although, we recognize that P may also be a limiting factor in these soils.

In contrast to our hypothesis and others’ findings (Samuelson 2000; Gough et al. 2004b; King et al. 2008), we did not see a consistent increase in $A_{\text{Sat}}$ following fertilization with N and P. In fact, we observed a decrease on a number of sampling dates, but results were largely inconsistent (Figure 2.6A). One exception being on the final sampling date, which used the first fully elongated needles of the 2007 growing season, fertilized seedlings had slightly greater $A_{\text{Sat}}$ than control seedlings. This lack of consistent response has also been observed by others (Zhang et al. 1997b; Gough et al. 2004a; Chmura and Tjoelker 2008). We found a significant, but weak correlation between $[N]_a$ and $A_{\text{Sat}}$ in our study, which we
attributed to high $[N]_a$ found in all treatments. Similarly, Chmura and Tjoelker (2008) and King et al. (2008) found only a week correlation between $[N]_f$ and photosynthesis, which is likely a result of $[N]_f$ being maintained above the sufficiency limit of 12 mg g$^{-1}$.

Further, we observed decreased $g_s$, $E$, and $C_i/C_a$ following fertilization and throughout the entire experiment (Figure 2.6B-D). A decrease in $g_s$, $E$, and $C_i/C_a$ coincided in greater WUE, which may be a result of both increased stomatal control and changes in leaf morphology (decreased SLA) following fertilization (Green and Mitchell 1992; Samuelson 2000). Green and Mitchell (1992) concluded the increases in WUE with no response of $g_s$ following N fertilization was likely due to a decrease in non-stomatal limitations and not increased stomatal control. In our case we observed decreased $g_s$ and $C_i/C_a$ following fertilization and the increased WUE is likely due to increased stomatal control. Similar to our findings, Samuelson (2000) found that high N plants had significantly less $g_s$ in P. taeda seedlings, which with an observed increase in photosynthesis led to increased WUE.

**Clonal differences**

We did not observe differences in total biomass or stem height between genotypes, however, there were differences in the way that biomass was partitioned. For example, CL85 produced more stem biomass while CL93 produced more branch biomass (Figure 2.3B). This difference in biomass partitioning led to differences in canopy architecture between clones. Although, both clones produced the same amount of leaf biomass (Table 2.1) when leaf weight was converted to $T/L$ CL93 had 8% more leaf area than CL85. Similarly, an independent measure of projected canopy silhouette area (CSA) also showed a 13% greater photosynthetic area in CL93 relative to CL85. There are a couple of explanations for this, firstly, increased branch mass suggests CL93 had a more open canopy allowing more needles to participate in light interception. Although CL85 had greater stem biomass, an early partitioning of C to branches at the expense of diameter growth particularly in the first year of growth may give CL93 a more long-term advantage when it comes to capturing light per unit leaf area. Secondly, clonal differences in leaf morphology such as: number of needles per fascicle, needle weight, and leaf area led to greater leaf area per unit weight (SLA) in CL93 relative to CL85 irrespective of nutrient availability (Tables 2.2, 2.3 and Figure 2.4).
Clone by nutrient availability interactions

When all gas exchange data collected over the year were analyzed together we did not find a consistent clone by fertilizer interaction. However, data used on the final sampling date to scale values to the canopy level did show a strong clone by fertilizer interaction. In support of our hypothesis, fertilization significantly ($P < 0.01$) increased $A_{\text{Canopy}}$ in both genotypes by the end of the experiment, but the two genotypes achieved this in different ways (Figure 2.10). Clone 93 (“narrow crown” ideotype) was able to achieve similar $A_{\text{Canopy}}$ rates to CL85 (“broad crown” ideotype) by investing less in photosynthetic efficiency ($A_{\text{Sat}}$) and more in photosynthetic area at lower rates of N availability. This increase in $TLa$ was achieved by both increasing $SLA$ as well as partitioning more biomass to branches at the expense of diameter growth (stem volume), which led to a more open canopy allowing for more light penetration. King et al. (2008) found a similar response when they studied the effects of fertilization on eight different clones in the field. The authors found that some of clones responded by increasing leaf area while others increased photosynthetic efficiency. What was most surprising was that in some instances full-sib clones used contrasting strategies (King et al. 2008). In contrast, Chmura and Tjoelker (2008) found that in contrasting families of $P. \text{taeda}$, that photosynthetic efficiency was less important than full canopy light interception in predicting plant growth. Further support comes from data collected on “old” needles. In March 2007 the last fully elongated needles produced from the previous year were measured for $A_{\text{Sat}}, [N]_a$, and needle morphology. Regardless of N availability CL93 needles had greater $SLA$ relative to CL85 in “old” needles (Table 2.2 and 2.3) as well as maintained similar rates of $A_{\text{Sat}}$ at lower rates of $[N]_a$ leading to greater $PNUE$ in “old” needles (Figure 2.9B,D, and F). Similar plant biomass between clones indicates that both strategies were equally effective at capturing and assimilating CO$_2$ under optimal conditions. However under N limited conditions, the strategy for thinner leaves and more open canopy may be more advantageous for plant growth.

One aspect of this experiment was to change N availability by incorporating high C:N LR into the mineral soil. We were unable to achieve sufficient N immobilization with the level of LR we applied, but our data suggest that CL93 would have been better equipped to
tolerate N limitations due to greater PNUE in both “new” and “old” needles and its strategy for C assimilation. Despite weak treatment effects, at the level of LR we incorporated we found that CL93 had a higher fine- to coarse-root ratio and responded by increasing SLA relative to CL85 (Table 2.1 and Figures 2.4). We believe both of these actions would lead to increased resource acquisition (e.g., light and nutrients), but increased treatment intensity and more long-term evaluation is needed to determine the extent of this adjustment as well as if these effects will be present under field conditions. A component that we did not measure in this experiment that may have a strong influence is the amount of C lost through maintenance respiration and root exudates. Additionally, maintenance respiration (respiration measured in fully elongated needles) has been shown to differ between families for loblolly pine by some (Samuelson 2000) but not by others (King et al. 2008). Both of these potential losses of C need to be quantified to better judge these competing strategies.

Conclusion
Fertilization with N and P did not result in an immediate and sustained increase in $A_{\text{Sat}}$ as we hypothesized, but did result in consistently lower $g_s$, $E$, and $C_i/C_a$ and improved WUE independently of genotype. We found large differences between genotypes in both gas exchange parameters as well as in C partitioning. Clone 93 had similar seedling biomass to CL85, but invested more resources into producing branches and fine-roots at the expense of stem volume. Increased investment in branches and increased specific leaf area (cm$^2$ g$^{-1}$) led to more photosynthetic area in CL93 relative to CL85 despite similar foliage biomass. When final gas exchange rates were scaled to the canopy level by multiplying by the total leaf area (m$^2$) we found that both genotypes achieved similar canopy level CO$_2$ assimilation rates, but by different means. CL93 (“narrow crown” ideotype) produced greater leaf area with lower photosynthetic efficiency at low N rates while CL85 (“broad crown” ideotype) used the opposite strategy of lower leaf area, but greater efficiency. At higher rates of N availability, CL93 increased photosynthetic efficiency while CL85 increased leaf area. We believe the strategy that CL93 has shown would make it better able to compete in low N situations due to increased PNUE and fine- to coarse root ratio, but we were unable to verify this. Although we did see a small effect of nutrient limitations in $A_{\text{Canopy}}$, $A_{\text{Sat}}$, and TLA, our foliar $\left[N\right]$ indicated that our level of LR incorporation was not sufficient to cause $\left[N\right]$ below sufficiency.
limits. Our work shows that even within a single species different genotypes can express different mechanisms for capturing and partitioning C. Understanding these strategies could lead to better selection of clonal material to specific site resource availability to optimize productivity at the least amount of cost to the land owner. Based on current results, future research should focus differences in canopy architecture between genotypes at a young age and how it relates to wood volume at maturity.
Chapter 2  
Gas exchange and biomass partitioning in contrasting clones

Acknowledgments
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Table 2.1. Statistical summary of \(P\)-values for ANOVA of biomass partitioning between specific tissues of two-year-old *Pinus taeda* seedlings for logging residue (LR), clone (CL), and fertilizer (F) treatments main effects, two-way, and three-way interactions. Data were log transformed as needed to meet assumptions of normality and equal variance as indicated by plot of residuals and normality curves.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Main Effects</th>
<th>2-way Interaction</th>
<th>3-way Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LR(^1)</td>
<td>CL(^1)</td>
<td>F(^1)</td>
</tr>
<tr>
<td>Foliage</td>
<td>0.7361</td>
<td>0.9323</td>
<td><strong>0.0455</strong></td>
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<tr>
<td>Branches</td>
<td>0.6843</td>
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<td>0.1677</td>
</tr>
<tr>
<td>Stem(^4)</td>
<td>0.1959</td>
<td><strong>0.0203</strong></td>
<td>0.1367</td>
</tr>
<tr>
<td>Total above(^4)</td>
<td>0.8874</td>
<td>0.7446</td>
<td>0.1306</td>
</tr>
<tr>
<td>Coarse roots(^4)</td>
<td>0.8308</td>
<td>0.1286</td>
<td><strong>0.0396</strong></td>
</tr>
<tr>
<td>Fine roots</td>
<td>0.2635</td>
<td>0.3259</td>
<td>0.9935</td>
</tr>
<tr>
<td>Total below</td>
<td>0.8608</td>
<td>0.3854</td>
<td>0.1190</td>
</tr>
<tr>
<td>Fine:Coarse(^5)</td>
<td><strong>0.0057</strong></td>
<td>&lt;.0001</td>
<td><strong>0.0002</strong></td>
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<tr>
<td>Root:Shoot(^5)</td>
<td>0.6176</td>
<td>0.1964</td>
<td>0.9472</td>
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<tr>
<td>Stem:Leaf(^6)</td>
<td><strong>0.0041</strong></td>
<td>&lt;.0001</td>
<td><strong>0.0135</strong></td>
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<tr>
<td>Total biomass</td>
<td>0.8682</td>
<td>0.5240</td>
<td><strong>0.0664</strong></td>
</tr>
</tbody>
</table>

\(^1\) Sample size of 24  
\(^2\) Sample size of 12  
\(^3\) Sample size of 6  
\(^4\) Variable transformed by the natural log  
\(^5\) Variable transformed by the arcsin(square root)
Table 2.2. Statistical table of P-values for needle morphological parameters measured on 48 two-year-old *Pinus taeda* seedlings grown for one year in a greenhouse. Specific leaf area (SLA; cm\(^2\) g\(^{-1}\)), needle diameter (DIA; mm), needle length (LGNTH; mm), number of needles per fascicle (NDLS), dry weight (WT; mg), and calculated leaf area (LFAREA; cm\(^2\)) were measured on current year most recently elongated needles (New) and previous season last fully elongated needles (Old). Treatments were logging residue incorporation (LR), clone (CL), and fertilization (Fert).

*Variable was transformed by its natural log to meet assumptions of ANOVA*
Table 2.3. Least squares mean and (standard error) for leaf needle morphological parameters for treatment main effects in both “new” and “old” needles. Morphological parameters were measured on 48 two-year-old *Pinus taeda* seedlings grown for one year in a greenhouse. \( n = 24 \).

<table>
<thead>
<tr>
<th></th>
<th>Logging residue</th>
<th>Clone</th>
<th>Fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NOLR LR</td>
<td>CL85 CL93</td>
<td>NF F</td>
</tr>
<tr>
<td><strong>Specific leaf area</strong> (cm(^2) g(^{-1}))</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>New</td>
<td>209.8 (4.1)</td>
<td>216.2 (3.5)</td>
<td>205.3 (3.7)</td>
</tr>
<tr>
<td>Old</td>
<td>159.9 (2.9)</td>
<td>171.5 (6.7)</td>
<td>159.3 (2.9)</td>
</tr>
<tr>
<td><strong>Needle diameter</strong> (mm)</td>
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<tr>
<td>New</td>
<td>1.33 (0.02)</td>
<td>1.33 (0.02)</td>
<td>1.39 (0.02)</td>
</tr>
<tr>
<td>Old</td>
<td>1.31 (0.02)</td>
<td>1.28 (0.02)</td>
<td>1.34 (0.02)</td>
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<td><strong>Needle length</strong> (mm)</td>
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<tr>
<td>New</td>
<td>124 (4.3)</td>
<td>124 (4.8)</td>
<td>141 (3.5)</td>
</tr>
<tr>
<td>Old</td>
<td>125 (4.0)</td>
<td>130 (5.0)</td>
<td>134 (4.4)</td>
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<tr>
<td><strong>Number of needles</strong></td>
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<td>New</td>
<td>3.2 (0.05)</td>
<td>3.2 (0.05)</td>
<td>3.0 (0.01)</td>
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<tr>
<td>Old</td>
<td>3.3 (0.07)</td>
<td>3.2 (0.06)</td>
<td>3.1 (0.03)</td>
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<td><strong>Needle weight</strong> (mg)</td>
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</tr>
<tr>
<td>New</td>
<td>50.5 (2.8)</td>
<td>49.2 (2.8)</td>
<td>59.6 (2.4)</td>
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<tr>
<td>Old</td>
<td>66.4 (3.0)</td>
<td>64.4 (4.3)</td>
<td>72.3 (3.8)</td>
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<td><strong>Leaf area</strong> (cm(^2))</td>
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<tr>
<td>New</td>
<td>10.4 (0.47)</td>
<td>10.5 (0.53)</td>
<td>12.1 (0.44)</td>
</tr>
<tr>
<td>Old</td>
<td>10.5 (0.35)</td>
<td>10.6 (0.53)</td>
<td>11.3 (0.44)</td>
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Figure 2.1. Maximum daily photosynthetically active radiation (A), daily temperature (B), and daily relative humidity (C) of the greenhouse over the course of the experiment.
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Figure 2.8. Effect of nutrient manipulations on least squares means for Foliar N content of “new” and “old” needles expressed on a mass (Panel A and B) and area basis (Panel C and D). Dotted lines represent sufficiency limit for foliar N content for *P. taeda*, and different letters indicate significant comparison-wise differences between nutrient manipulation treatments at the 0.05 alpha level ($n = 12$). Abbreviations: NoLR = no logging residue, NF = no fertilizer, LR = logging residue, F = fertilizer.
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Figure 2.11. Least squares means for canopy level net CO₂ assimilation (A<sub>Canopy</sub>; panel A), specific net CO₂ assimilation (A<sub>Sat</sub>; panel B), and total leaf area (TLA; panel C) for each nutrient manipulation. TLA was calculated by multiplying total leaf weight and specific leaf area, which was averaged for “new” and “old” needles assuming both made up 50 percent of total leaf area. A<sub>Sat</sub> and TLA were measured on one occasion in late June 2007. Lower case letters indicate significant differences between treatments using p-diff option in SAS version 9.1 (alpha = 0.10; n = 12).
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Chapter 3

Interaction between contrasting Pinus taeda ideotypes and soil nutrient availability: effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology

Summary The combined effects of intensive management and planting of improved seedlings have led to large increases in productivity on intensively managed pine forests in the southeastern United States. To best match clones to particular site conditions an understanding of how specific clones respond in terms of biomass partitioning, leaf physiology, and biochemistry to changes in nutrition and site conditions will be necessary. This study was established to determine the response of biomass partitioning, net CO₂ assimilation ($A_{Sat}$), and photosynthetic capacity to logging residue (LR) incorporation and fertilization between two Pinus taeda clones that represent two distinct ideotypes. The “narrow crown” ideotype (CL93) has been shown to allocate more resources to stem growth while the “broad crown” ideotype (CL32) allocates more resources to leaf area. Under field conditions, we found consistent genetic by environment (varying nutrient regimes) interactions in biomass as well as leaf physiology. After inducing nutrient limitations by incorporation of LR we found a 25% loss in stem growth in CL32 while CL93 showed no negative response. We concluded this decrease was due to a combination of increased belowground maintenance costs associated with fine-root production, as well as a denser canopy in CL32 leading to reduced gross canopy CO₂ assimilation. In contrast, N and P fertilization resulted in a 21% greater increase in stem volume in CL93 relative to CL32. Fertilization increased $A_{Sat}$ temporarily in both clones, which eventually decreased below control levels by the end of the study. Although we found clone by fertilization interaction in leaf biochemistry, the greatest G x E interaction was found in leaf area, which appeared to have greater influence than photosynthetic efficiency on growth. This research demonstrates the importance of selecting appropriate clonal material and silvicultural prescription when implementing site-specific silviculture to maximize productivity in intensively managed southern pine forests.

Keywords: $A_{Sat}$, G x E interaction, $J_{max}$, loblolly pine, logging residue, photosynthesis, and $V_{C,max}$
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbrev</th>
<th>Description</th>
<th>Units</th>
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</thead>
<tbody>
<tr>
<td>$A_{\text{Canopy}}$</td>
<td>Canopy level photosynthesis</td>
<td>$\mu\text{mol CO}_2 \text{ s}^{-1}$</td>
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<tr>
<td>$A/C_i$</td>
<td>Net CO$_2$ assimilation-internal CO$_2$ partial pressures</td>
<td></td>
</tr>
<tr>
<td>$A/PPFD$</td>
<td>Net CO$_2$ assimilation-light response curve</td>
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<tr>
<td>$A_{\text{Sat}}$</td>
<td>Instantaneous net photosynthesis under saturating light and CO$_2$</td>
<td>$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>CSA</td>
<td>Canopy silhouette area</td>
<td>$\text{cm}^2$</td>
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<tr>
<td>$g_M$</td>
<td>Mesophyll conductance</td>
<td>$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$</td>
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<td>$J_{\text{max}}$</td>
<td>Maximum RuBP regeneration mediated by electron transport</td>
<td>$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$</td>
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<tr>
<td>$J_{\text{max}}/V_{\text{C,max}}$</td>
<td>Relative light to carboxylation efficiency</td>
<td>ratio</td>
</tr>
<tr>
<td>LA</td>
<td>Calculated leaf area</td>
<td>$\text{cm}^2$</td>
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<td>LCP</td>
<td>Light compensation point</td>
<td>$\mu\text{mol photons m}^{-2} \text{ s}^{-1}$</td>
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<tr>
<td>LR</td>
<td>Logging residue</td>
<td>Treatment</td>
</tr>
<tr>
<td>$[N]_a$</td>
<td>Nitrogen concentration per unit leaf area</td>
<td>$\text{g N m}^{-2} \text{ leaf}$</td>
</tr>
<tr>
<td>$[N]_f$</td>
<td>Foliar nitrogen concentration</td>
<td>unitless</td>
</tr>
<tr>
<td>$[N]_m$</td>
<td>Nitrogen concentration per unit leaf mass</td>
<td>$\text{mg N g}^{-1} \text{ leaf}$</td>
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<tr>
<td>PNUE</td>
<td>Instantaneous photosynthetic N use efficiency</td>
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<td>PPFD</td>
<td>Photosynthetic photon flux density</td>
<td>$\mu\text{mol photons m}^{-2} \text{ s}^{-1}$</td>
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<tr>
<td>Q</td>
<td>Apparent quantum photon yield</td>
<td>$\text{CO}_2 \text{ photons}^{-1}$</td>
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<tr>
<td>$R_L$</td>
<td>Leaf respiration estimated from $A/C_i$ curves</td>
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<tr>
<td>$R_M$</td>
<td>Mitochondrial respiration taken during the day</td>
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<td>SLA</td>
<td>Specific leaf area</td>
<td>$\text{cm}^2 \text{ g}^{-1}$</td>
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<tr>
<td>TPU</td>
<td>Triose phosphate utilization</td>
<td>$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$</td>
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<tr>
<td>$V_{\text{C,max}}$</td>
<td>Maximum carboxylation capacity</td>
<td>$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$</td>
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**Introduction**

Nitrogen and phosphorus deficiencies commonly limit growth in southern pine forests making fertilization a common management tool on nutrient poor sites (Fox et al. 2007). Increases in leaf area and total biomass following fertilization are well established in conifers (Vose and Allen 1988; Albaugh et al. 1998; Borders et al. 2004), but affects on leaf physiology are not. Studies utilizing young conifers have found increased photosynthetic efficiency following fertilization (Green and Mitchell 1992; Tissue et al. 1993; Murthy et al. 1996; Walcroft et al. 1997; Samuelson 2000; Gough et al. 2004b; Warren et al. 2004; Bown et al. 2007; King et al. 2008) while others have not (Maier et al. 2002; Warren and Adams 2002; Bauer et al. 2004). This inconsistency, in part, has led to the poor correlation some have found between specific photosynthesis under saturating light ($A_{Sat}$) and growth in conifers (Munger et al. 2003). When $A_{Sat}$ rates were scaled-up by amount of leaf area, others have found a strong correlation between CO$_2$ assimilation at the tree or stand level and plant growth (Kruger and Volin 2006; Chmura and Tjoelker 2008). Some have hypothesized that increased photosynthetic capacity following fertilization may only be a transient effect, but may be one mechanism for acquiring the photosynthesize necessary to increase leaf area (Gough et al. 2004a; Maier et al. 2008). Gough et al. (2004b) proposed a series physiological adjustments, or steps, which lead to increased growth in young *P. taeda* following fertilization. The authors theorized an early increase in photosynthetic efficiency led to greater photo-assimilates used to increase leaf area. The increased photosynthetic area later allowed for a down-regulation of photosynthetic efficiency while maintaining higher canopy level CO$_2$ assimilation.

Not surprisingly a seedling’s ability to acquire and utilize nutrients such as N and P efficiently is essential to maximizing growth. Nitrogen is a major component in all proteins and pigments (i.e., chlorophyll and Rubisco) involved in photosynthesis with a large proportion of foliar N being distributed between photosynthetic machinery associated with both light and dark reactions (Evans 1989). Researchers have found a strong positive correlation between foliar N concentration [$N_f$] and photosynthetic capacity such as maximum carboxylation capacity ($V_{C,max}$), maximum electron transport ($J_{max}$) (Kellomaki and Wang 1997; Strand 1997; Walcroft et al. 1997; Ripullone et al. 2003; Manter et al. 2005;
Bown et al. 2007), and not surprisingly, $A_{Sat}$ (Green and Mitchell 1992; Mitchell and Hinckley 1993; Roberntz and Stockfors 1998; Schoettle and Smith 1999; Samuelson 2000; Ripullone et al. 2003). Although, some have found poor correlations between $[N]_{f}$ and $A_{Sat}$ (Gough et al. 2004b; King et al. 2008; Chapter 2). Warren and Adams (2004) hypothesized that poor photosynthetic nitrogen use efficiency ($PNUE$; $CO_{2}$ assimilated per unit N) in conifers may be due to storage of excess N in the form of inactive Rubisco. The authors hypothesize that excess N as well as leaf anatomy of conifers allow conifers to cope with water and nutrient limitations better than their herbaceous counterparts. In fact, Warren et al. (2003) showed that as $[N]_{f}$ increased so did the amount of inactive Rubisco.

The combined effects of intensive management and planting of artificially selected, improved seedlings have led to large increases in productivity on intensively managed pine forests in the southeastern United States through improved growth, and pest resistance. Estimated volume gains of 10-30% have been made possible with selective breeding, and it is estimated that gains of 50% or more may be attained by combining the use of clones and intensive silviculture (Allen et al. 2005; Martin et al. 2005; McKeand et al. 2006). Large natural range and site conditions have led to large within species variation with the potential to respond very differently to silvicultural prescriptions. With a movement toward site-specific silviculture, a future challenge may be to match the most appropriate genotype and silvicultural prescription with specific site conditions (Fox 2000). Roth et al. (2007) has found large genetic by environment interactions ($G \times E$) in full-sib $P. \text{taeda}$ and $P. \text{elliottii}$ clones planted in different locations and undergoing different silvicultural treatments. In contrast, others contend that silvicultural effects are stable across open-pollinated, half-, and full-sib families (McKeand et al. 2006).

Research has shown genetic by environment interactions for C allocation, N use efficiency ($NUE$), and stem growth (Li et al. 1991a; Li et al. 1991b; Li et al. 1991c; Samuelson 2000; Retzlaff et al. 2001), but leaf physiological traits have been less consistent. Some have shown no $G \times E$ in leaf gas exchange (Samuelson 2000; Bown et al. 2007; Chmura and Tjoelker 2008). Koehn et al. (2003) found $G \times E$ in photochemical quenching and yield of PSII in full-sib slash pine seedlings. Additionally, King et al. (2008) showed differences in
$A_{\text{Sat}}$ responses to fertilization in *P. taeda* even among clones that shared the same parents. They found wide ranging differences between clones with some achieving superior growth by increasing photosynthetic efficiency while others achieved similar growth by simply increasing leaf area. Likewise, a greenhouse experiment using contrasting *P. taeda* clones, showed that when $A_{\text{Sat}}$ rates were scaled to the canopy level using estimates of total leaf area that contrasting clones showed the same increase in canopy level CO$_2$ assimilation ($A_{\text{Canopy}}$) following fertilization using two different mechanisms (Chapter 2). One clone achieved increased $A_{\text{Canopy}}$ by increasing leaf area while the other clone achieved the same $A_{\text{Canopy}}$ by increasing $A_{\text{Sat}}$. The authors caution that the final sampling date, which was used to scale $A_{\text{Sat}}$ to the canopy level, was the only date that $A_{\text{Sat}}$ rates were significantly greater in fertilized seedlings and are not representative of prior data, but warrant further investigation.

The objectives of this study were to investigate the interaction of nutrition and genetics on biomass partitioning and leaf physiology. This was achieved by manipulating site nutrient availability by incorporating high C:N (about 700) logging residue (LR) into the mineral soil or N and P fertilization. Two *P. taeda* clones selected for this study represented two distinct ideotypes ("narrow crown" versus "broad crown" ideotypes; see Martin et al. 2001) that have been shown to attain similar stem biomass, while maintaining large differences in leaf area (Figure 3.1). This experiment provided us the opportunity to ask three specific questions. First, how does biomass partitioning change with variations in nutrient availability between two contrasting ideotypes? We hypothesized that CL32 ("broad crown" ideotype) would show a decrease in growth with LR incorporation due to greater nutrient demand, and CL93 ("narrow crown" ideotype) would be less negatively affected by the incorporation of the LR and respond more positively to fertilization. Second, how does photosynthetic efficiency respond to changes in nutrient availability immediately following nutrient manipulation, and are there differences between contrasting genotypes? Finally, are there genetic by nutrient availability interaction with regard to photosynthetic capacity, leaf N, and leaf morphology? We hypothesized that manipulations to site nutrient availability would impact photosynthetic capacity, but to different degrees between clones. Specifically, the addition of N and P would lead to increased photosynthetic efficiency while the addition of high C:N ratio LR would lead to nutrient immobilization resulting in changes in $A_{\text{Sat}}$ and photosynthetic
capacity. Additionally, we hypothesized the clone that expressed less leaf area ("narrow crown" ideotype) would have greater $A_{\text{Sat}}$ and photosynthetic capacity than the clone that expressed more leaf area ("broad crown" ideotype).

**Materials and Methods**

*Site location, climate, and stand history*

The study site was located in Berkeley County, SC at an elevation of 24 m above mean sea level (Appendix C). Average annual temperature was 14.6°C and 17.4°C with an average daily maximum of 17.3°C and 25.2°C and an average daily minimum of 11.7°C and 11.2°C for the 2006 and 2007 year, respectively (Figure 3.2). Highest daily average temperature was 26.8°C and 32.5°C occurring in August 2006 and August 2007, respectively, and a low of -0.9°C and 0.4°C occurring in December 2006 and February 2007, respectively. Total precipitation was 90.2 cm in 2006 and 74.9 cm in 2007 spread evenly throughout the year, which was well below the average of 120 cm recorded between 1949 and 1973 (Long 1980). The dominant soil series is an Ocilla (loamy, siliceous, semiactive, thermic Aquic Arenic Paleudults). Harvest of the previous 21-year-old *P. taeda* stand took place in May 2004 and the site was sheared of residual material in July 2004. Logging residue treatments were applied in October 2004, and site preparation (bedding) took place in early November 2004. *Pinus taeda* clones were planted in January of 2005 and data for this study was collected between June 2006 and January 2008 (Appendix D).

*Study design and treatments*

The study design was a split-plot, randomized complete block design replicated three times with the whole-plot treatments arranged as a full 2 by 2 factorial, which was measured repeatedly. Each 0.18 ha plot (48 x 38 m) was planted with approximately 243 container grown, clonal *P. taeda* seedlings in nine rows at a 1.8 m spacing within rows and a 4.3 m spacing between row centers. Two levels of logging residue (LR) and two clones (CL32 and CL93) served as the whole-plot treatments. The two levels of LR were no LR incorporated (w/o LR) and LR incorporated into the mineral soil (LR) at a rate of 25 Mg o.d. wt. ha$^{-1}$, which was concentrated onto the beds (approximately 75 Mg o.d. wt. ha$^{-1}$; C:N = 700). Both LR treatments also incorporated the residual forest floor of approximately 25 Mg o.d. wt. ha$^{-1}$.
The two P. taeda clones chosen both exhibit superior height growth, but represent two distinct ideotypes. Clone 93 (“narrow crown” ideotype) has been shown to allocate more of its resources to stem growth while Clone 32 (“broad crown” ideotype) allocates more resources to leaf area (Figure 3.1).

Each plot was split into two 0.0013 ha measurement plots, located at opposite ends of the whole-plot, each consisted of six seedlings (4 measurement trees + 2 buffer trees) and served as the experimental unit (EU; Appendix C). Each split-plot received one of two fertilizer applications. No nutrient additions (NF) or N and P fertilization (F). During the 2006 growing season fertilizer was applied twice and totaled 209 kg N and 116 kg P ha\(^{-1}\) in the form of diammonium phosphate (DAP) and ammonium nitrate (AN). Roughly 1/3 was applied on April 6 and the remaining 2/3 applied on May 8, 2006. Fertilization for the 2007 growing season was applied on March 9, 2007 at a rate of 200 kg N ha\(^{-1}\) in the form of AN.

**Projected canopy area and biomass partitioning**

At the conclusion of the experiment a single tree, which most closely fit the mean tree height from each EU, was selected for biomass partitioning and projected canopy area determination. Canopy silhouette area (CSA) is defined as the total leaf and twig area contained within the tree canopy projected onto a plane (King et al. 2008). The aboveground portion of each seedling was cut 10 cm above ground-line and transported to a staging area where the sky was utilized as a backdrop that providing good contrast with the tree. Two photographs were taken orthogonally to each other for each tree using a 28-70 mm 1:2.8-4.0 D zoom lens (Sigma Corporation, Kanagawa, Japan) mounted on a D70 digital SLR (Nikon Corporation, Tokyo, Japan). The color digital image was converted to black and white using SideLook 1.1 software (Nobis 2005) with the channel set to red. Adobe® Photoshop® 6.0 (Adobe Systems Inc., San Jose, CA) was then used to clean up the black and white image and determine the number of pixels in the image. A standard reference in each photograph was used to convert from number of pixels in the image to the projected area (m\(^2\)). After photographing each tree the aboveground plant parts were wrapped in plastic and taken back to the lab. Belowground plant tissues were sampled by excavating a 1 x 1 x 0.5 meter volume around the main stem, which was immediately bagged and taken with the
aboveground tissues to the lab. At the lab each tree was dissected into needles, branches, main stem, tap root, and lateral roots. All samples were oven dried (> two weeks) at a temperature of 65 ± 5°C then weighed gravimetrically to the nearest gram.

**Instantaneous net photosynthesis**

Gas exchange measures were taken using an open-flow, infrared gas analyzer equipped with 2 x 3 cm cuvette with a blue–red LED light source (Li-Cor 6400, Lincoln, Nebraska). On 21 sampling dates between January 2006 and December 2008, $A_{sat}$ was measured on individual fascicles under saturating light between 1000 and 1600 hours. Measurements were taken at ambient temperature and relative humidity under the following settings: 1600 μmols m$^{-2}$ s$^{-1}$ photosynthetic photon flux density (PPFD), 370 μmol mol$^{-1}$ reference CO$_2$ concentration, and a flow rate of 300 μmol sec$^{-1}$. Most recently elongated needles were detached from the upper third, south facing side of each tree and immediately placed in the cuvette.

Preliminary tests using the same trees showed no difference in $A_{sat}$ between attached ($1.78 ± 0.12$) and detached ($1.78 ± 0.11$) needles ($P = 0.98; n = 8$). Once $A_{sat}$ stabilized (typically < 2 min), three measurements were taken at ten second intervals and averaged to a single rate.

Fascicle diameter was measured using digital calipers to the nearest 0.01 mm and leaf area ($LA; \text{cm}^2$) calculated using the following equation:

$$LA = (n \times l \times d) + (\pi \times d \times l)$$

[3.1]

where $l$ is the length (cm) of the leaf in the chamber, $d$ is the leaf diameter (cm) of the fascicle measured just above the sheath, and $n$ the number of needles in the fascicle (Ginn et al. 1991).

**Photosynthesis-CO$_2$ response curves**

Objective three was tested by calculating photosynthetic parameters derived from net CO$_2$ assimilation-internal CO$_2$ partial pressures ($A/C_i$) and light ($A/PPFD$) response curves constructed in the field on three sampling dates. June 2006, October 2006, and March 2007 were sampling dates chosen to represent three distinct phenological stages as well as investigate immediate adjustments in photosynthetic machinery between contrasting
genotypes immediately following nutrient manipulations. Net photosynthesis-CO$_2$ response curves ($A/C_i$) were constructed from field measurements of $A_{Sat}$ taken on attached needles over a range of external CO$_2$ partial pressures (370, 115, 150, 230, 300, 370, 570, 1000, 1500, and 1800 µmol mol$^{-1}$). $A/C_i$ curves were measured at 1600 µmol m$^{-2}$ s$^{-1}$ PPFD at a flow rate of 300 µmol s$^{-1}$ using an open-flow, infrared gas analyzer equipped with a blue–red LED light source (Li-Cor 6400). Leaf temperature was held to 25, 18, and 18°C for June 06, October 06, and March 07 sampling dates, respectively, which was near ambient temperature for that time of year. Relative humidity was held to a range of 60-70%, 40-50%, and 20-30% for June 06, October 06, and March 07 sampling dates, respectively. Needles and gas exchange rates were carefully monitored to insure the needles did not detach during measurements, which lasted about 45 minutes for each curve, and the IRGAs were matched between each partial pressure.

Nine pairs of $A_{Sat}$ and CO$_2$ partial pressures at the site of Rubisco were used to calculate in vivo maximum carboxylation rate ($V_{C,\text{max}}$), RuBP regeneration capacity ($J_{\text{max}}$) mediated by maximum electron transport rate, triose phosphate utilization ($TPU$), mesophyll conductance ($g_m$), and day time leaf respiration ($R_L$) using a freely available $A/C_i$ curve fitting utility version 1.1 (Sharkey et al. 2007; Appendix E). Briefly, photosynthetic parameters were estimated by assigning each $A_{Sat}$-[C$_i$] data pair to one of the three possible limitations to photosynthesis: Rubisco, RuBP regeneration, or $TPU$. A non-linear $A/C_i$ curve fitting utility written for Microsoft Excel was then used to fit the data by minimizing the sum of squares. Estimates of $g_m$ from both the Rubisco and RuBP regeneration curves were used to calculate CO$_2$ partial pressure at the site of Rubisco. For improved treatment comparisons, all parameters were scaled to a constant temperature of 25°C using scaling factors further explained by Sharkey et al. (2007).

**Net CO$_2$ assimilation-light response curves**

Net CO$_2$ assimilation-light response ($A/PPFD$) curves were measured by graphically comparing CO$_2$ assimilation within a range of light levels to determine light compensation point ($LCP$), mitochondrial respiration ($R_M$), and apparent quantum yield ($Q$). Measurements were taken at approximately the same date following the same procedure as CO$_2$ response
curves with the following changes. Reference CO₂ partial pressure was maintained at 370 μmol mol⁻¹ and measurements were made at the following light levels: 1800, 1400, 1000, 600, 400, 200, 100, 50, 20, and 0 μmol m⁻² s⁻¹ PPFD. Parameters for the A/PPFD curves were calculated by fitting response curves to a non-rectangular hyperbola using non-linear least squares regression (Hanson et al. 1987) using the following equation:

\[
A = A_{Sat} \times \left[ 1 - \left( 1 - \frac{R_M}{A_{Sat}} \right) \right]^{1 - \frac{PPFD}{LCP}} \tag{3.2}
\]

where \(A\) is net CO₂ assimilation at a given light level, \(A_{Sat}\) is light saturated net CO₂ assimilation, \(R_M\) is mitochondrial respiration taken during the day, \(PPFD\) is photosynthetic photon flux density, and \(LCP\) is light compensation point. Apparent quantum yield \((Q)\) was calculated using the first derivative of equation 3.2:

\[
Q = \left( \frac{A_{Sat}}{R_M} \right) \times \left( 1 - \frac{LCP}{A_{Sat}} \right) \times \ln \left( \frac{1 - LCP}{A_{Sat}} \right) \tag{3.3}
\]

**Foliar N and leaf morphology**

Foliar N and leaf morphology were measured on foliage samples used in \(A_{Sat}\) and \(A/C_i\) curves. Needle length (from tip to point where needles enter the fascicle), needles per fascicle, diameter, and photosynthetic \(LA\) (equation 3.1) were determined on fresh needles. Needles were then oven dried at 65°C for > 48 hours and weighed to the nearest mg. Specific needle area (SLA; cm² g⁻¹) was calculated as an estimate of needle dimensions and density. Following morphology measurements oven-dried needles were ground individually using a Wiley mill fitted with a number 20 screen then 15 ± 5 mg of powdered sample was weighed into an aluminum crucible. Foliar N was analyzed using a Carlo-Erba elemental analyzer (Model NA 1500; Fison Instruments, Danvers, MA) at the US Forest Service lab (Research Triangle Park, NC). Foliar \([N]\) was expressed on a leaf area (\([N]_a;\) g N m⁻²) and mass (\([N]_m;\) g m⁻²) basis. Photosynthetic N use efficiency (PNUE; μmol CO₂ g⁻¹ N s⁻¹) was also calculated using the following equation:

\[
PNUE = \frac{A_{Sat}}{[N]_a} \tag{3.4}
\]
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where $A_{\text{Sat}}$ is net CO$_2$ assimilation and $[N]_a$ is foliar N concentration expressed on an area basis.

Data analyses

Treatment differences for $A_{\text{Sat}}$, photosynthetic capacity, foliar $[N]$ and leaf morphology were determined using analysis of variance with repeated measures (ANOVA RM) using a MIXED model. Covariance structures were selected using AIC, AAIC, and BIC fit statistics included in the SAS output. When fit statistics were similar between covariance structures the appropriate structure was chosen subjectively by inspection of the correlation matrix. Treatment differences in biomass partitioning, CSA, and time interactions were analyzed using a general linear model (GLM). Relationship between $[N]_a$ and photosynthetic parameters were explored using linear regression. Residuals and the normality curves were plotted for all analyses to confirm that the data meet assumptions of equal variance and normality for all parameters measured. When data were transformed by their natural log to meet assumptions all values were expressed as untransformed least square means and standard errors. All analyses were performed using the MIXED, GLM, REG, and NLIN procedures in SAS version 9 (SAS 2006).

Results

Stem volume and biomass partitioning

A significant fertilizer and LR interaction ($P = 0.003$) was found for stem volume over the course of two growing seasons. At the end of two years stem volume increased by 63% when receiving fertilizer only, but in the presence of LR, fertilization only resulted in 28% increase in stem volume relative to controls (Figure 3.3A). We observed highly significant ($P \leq 0.01$) genotype by nutrient availability interactions over two growing seasons. When LR was added CL32 showed a 25% decrease in stem volume while CL93 showed no apparent decrease ($P = 0.01$; Figure 3.3B). The addition of fertilizer increased stem volume in both clones, but CL93 showed a 21% greater increase relative to CL32 (Figure 3.3C).

Estimates of biomass partitioning generally supported our stem volume data. We observed no significant clone main effect in biomass partitioning. There were significant LR and
fertilizer main effects and interactions in both total biomass and individual tissues; however, the largest differences were observed in the aboveground tissues (Table 3.1; Figure 3.4). We observed a number of genotype by treatment interactions in biomass partitioning. For example, we found significant genotype by LR interactions with stem biomass, lateral roots, and total belowground biomass decreasing by 36, 33, 30%, respectively, in CL32 with LR incorporation, while no differences were found in CL93 (Table 3.1). Fertilization resulted in increased leaf biomass in both clones ($P = 0.0004$), but the magnitude of the response was much greater in CL93 (Figure 3.5).

The effects of soil amendments on canopy silhouette area (CSA) were similar to our findings of leaf biomass with a significant LR by fertilizer interaction ($P = 0.08$). LR by itself had no effect on CSA. Fertilization in the absence of LR resulted in a 54% increase in CSA, but in the presence of LR only increased by 14% relative to control plots. In contrast with our leaf biomass findings, we found no CL by F interaction ($P > 0.10$). Both clones responded to fertilization ($P = 0.01$) by increasing CSA on average by 33%. There was a significant CL by LR interaction ($P = 0.006$). Clone 32 responded to the incorporation of LR by decreasing CSA by 25%, which brought it to the same level as CL93 with or without LR.

**Growth efficiency and canopy architecture**

Growth efficiency is defined as stem volume produced per unit CSA. We found significant CL by LR incorporation and CL by fertilization interactions. Regressing stem volume by CSA showed that the incorporation of LR resulted in a slope of zero in CL32, but had no significant ($P > 0.10$) affect on the slope of CL93 (one slope for both treatments; Figure 3.6A). Regardless of whether LR was added or not CL93 showed a significantly ($P = 0.08$) steeper slope than CL32, which is consistent with first year data from this site (Figure 3.1). Clone 32 showed an increase in the slope estimate when fertilized, but there was no significant fertilizer effect in CL93 (same slope used for both F and NF treatments). The slope estimates for CL93 and CL32, which underwent fertilization, were not significantly different, but the CL93 line was shifted to the left relative to CL32 indicating greater growth efficiency regardless of fertilization (Figure 3.6B).
Canopy silhouette area (CSA) was regressed by leaf biomass as an estimate of canopy density. The more shallow the slope estimates the more dense (greater leaf mass per unit canopy area) the canopy. Similar to our estimates of growth efficiency, we found that CL93 did not significantly change its slope with the addition of fertilizer (Figure 3.7A). In contrast, fertilized CL32 had significantly ($P = 0.08$) steeper slope than CL93 while the slope estimate for non-fertilized CL32 trees did not significantly differ from zero (Figure 3.7A). The addition of LR in CL32 resulted in a slope that did not differ from zero. When LR was not present the slope estimate for CL32 was steeper relative to CL93, but the differences was not significant ($P = 0.22$). The addition of LR had no effect on canopy architecture in CL93 (Figure 3.7B).

**Instantaneous leaf gas exchange and PNUE**

Net CO$_2$ assimilation ranged from 11.7 to 0.71 µmol CO$_2$ m$^{-2}$ s$^{-1}$. We found a weak ($r^2 = 0.02$), but highly significant ($P = 0.007$) relationship between $A_{\text{Sat}}$ and $[N]_a$. Fertilization significantly ($P = 0.05$) increased $[N]_a$ from 1.15 g N m$^{-2}$ in control plots to 1.32 g N m$^{-2}$ in fertilized plots. There was no significant difference between control and LR, but when LR was present fertilization only increased $[N]_a$ to 1.24 g N m$^{-2}$, which is 7% lower than the fertilizer effect when LR was not present.

When $A_{\text{Sat}}$ data from all 21 sampling dates were analyzed we found significant fertilizer by time ($P = 0.05$) and LR by CL by time ($P = 0.06$) interactions (Figure 3.8 and 3.9 top panels). To further explore these time interactions, data were divided into three discrete regions based on the year the needles were produced (2005, 2006, or 2007). Needles produced during the 2005 growing season (first year in the ground) showed no significant treatment effects. In 2006 needles, fertilization significantly ($P = 0.03$) increased $A_{\text{Sat}}$ relative to non-fertilized plots (F and NF were 7.24 and 6.98 µmol CO$_2$ m$^{-2}$ s$^{-1}$, respectively) through winter when rates did not differ between treatments (Figure 3.8 top panel). In contrast, needles produced in 2007 were significantly ($P = 0.02$) lower in fertilized plots relative to controls (5.59 and 5.98 µmol CO$_2$ m$^{-2}$ s$^{-1}$, respectively). A highly significant ($P < 0.0001$) fertilizer by time interaction showed that foliar N showed no difference between treatments until June 2006 and remained increased until October 2007. After October 2007 fertilized plots had lower
foliar $[N]_a$ than non-fertilized plots (Figure 3.8 middle panel). A significant ($P < 0.0001$) fertilizer by time interaction indicated that $PNUE$ decreased relative to non-fertilized plots starting in June 2006 and remained lower throughout the experiment with differences becoming smaller over the winter months (Figure 3.8 bottom panel).

There was no significant LR by CL interaction in $A_{Sat}$ observed in needles produced in 2006. Instead, we found weak LR ($P = 0.08$) and CL ($P = 0.07$) effects with LR increasing $A_{Sat}$ relative to plots w/o LR (7.24 and 6.97 µmol CO$_2$ m$^{-2}$ s$^{-1}$, respectively) and CL32 showing greater $A_{Sat}$ relative to CL93 (7.25 and 6.96 µmol CO$_2$ m$^{-2}$ s$^{-1}$, respectively). A highly significant LR by time ($P = 0.002$) and CL by time ($P = 0.002$) interaction showed that the magnitude of these responses varied over that time period with the largest differences being expressed between January and June of the 2007 growing season (Figure 3.9 top panel). Needles produced in 2007 showed a significant ($P = 0.06$) LR by CL interaction with CL32 increasing $A_{Sat}$ in the presence of LR from 5.59 to 6.04 µmol CO$_2$ m$^{-2}$ s$^{-1}$ and CL93 decreasing $A_{Sat}$ in the presence of LR from 6.03 to 5.48 µmol CO$_2$ m$^{-2}$ s$^{-1}$. We did not observe a significant LR by CL interaction in either foliar N or instantaneous photosynthetic N use efficiency. Both $[N]_a$ and $PNUE$ showed significant LR by time interaction ($P = 0.04$ and 0.003, respectively). LR resulted in lower $[N]_a$ and greater $PNUE$ throughout most of the experiment in both genotypes (Figure 3.9 middle and bottom panels).

**Photosynthetic capacity**

We estimated mesophyll conductance ($g_m$) for two of the three sampling dates that we measured $A/C_i$ curves. We did not find a significant treatment effect on $g_m$ for either date nor did we detect a difference in $R_{L}$. The incorporation of LR had a significant affect on both $V_{C,max}$ and $J_{max}$, but the effect was dependent on genotype ($P = 0.05$ and 0.06, respectively). LR resulted in a decrease in both photosynthetic parameters in CL32 while sharply increasing $V_{C,max}$ and $J_{max}$ in CL93 (Figure 3.10A and C). Notably, in the absence of any soil addition CL93 maintained $V_{C,max}$ and $J_{max}$ rates that were 19% and 17% lower than CL32, respectively. Finally, in contrast to $A_{Sat}$ data, the needles used to measure $A/C_i$ curves showed no significant ($P = 0.20$) difference in $[N]_a$ (Figure 3.10E).
The addition of fertilizer significantly ($P < 0.05$) increased $V_{C,\text{max}}, J_{\text{max}},$ and $[N]_a$ in both clones, but the magnitude of the response varied between clones ($P = 0.05, 0.10,$ and $0.07$, respectively). We observed only a small increase in all three parameters in CL32 and a large increase in CL93, although, following fertilization both $V_{C,\text{max}}$ and $J_{\text{max}}$ rates were the same in both genotypes (Figure 3.10B and D). Fertilizer resulted in higher $[N]_a$ in CL93 relative to CL32 in needles used to measure A/C$_i$ curves (Figure 3.10F). Triose phosphate utilization ($TPU$) was also significantly ($P = 0.02$) increased with fertilization to the same degree in both genotypes from $4.24$ to $4.77 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. We found $[N]_a$ to be a highly significant ($P \leq 0.01$) regressor for both $V_{C,\text{max}}$ and $J_{\text{max}}$ in CL93, but non-significant in CL32 (Figure 3.11). Despite the significance of $[N]_a$ in CL93 it explained only about 20% of the variation for both photosynthetic variables.

Significant CL by fertilization interactions were found in variables associated with light capture. Clone 32 responded to fertilization by increasing its $J_{\text{max}} / V_{C,\text{max}}$ ratio, apparent quantum efficiency ($Q$), and leaf area per unit mass (SLA), while decreasing its LCP. Clone 93 responded in an opposite fashion for all four variables by decreasing $J_{\text{max}} / V_{C,\text{max}}, Q,$ and SLA, and increasing LCP (Figure 3.12).

**Discussion**

In support of our hypothesis, we found response differences between clones when we induced nutrient limitations (see Chapter 5 for soils data) by incorporating LR into the mineral soil. Clone 32 showed a sharp decrease in stem volume and growth efficiency (stem growth per unit leaf area) with LR incorporation. Interestingly, we observed no short-term (two years) decrease in stem volume or growth efficiency in CL93 following the incorporation of LR. Our observed CL by LR interaction both highlights the importance of understanding the underlying mechanisms (e.g., biomass partitioning, photosynthetic efficiency) controlling these responses and the importance of selecting the proper genotype based on site conditions.

Our repeated stem growth data was largely consistent with destructive harvest data obtained at the end of the experiment (Table 3.1). In response to LR, CL32 decreased in total biomass
by 27% while CL93 showed only a 4% decrease, but notably, the CL by LR interaction in
total plant biomass was not statistically significant ($P = 0.14$) due to the high variation ($CV = 29\%$) and small sample size harvested ($n = 6$). The largest decreases in CL32 were observed in stem, lateral roots, and total belowground biomass. Similarly, Li et al. (1991a) showed that components (uptake and utilization efficiencies) of nitrogen use efficiency ($NUE$) (stem biomass produced per unit of N applied) in $P. \text{taeda}$ seedlings were moderately to highly dependent on genotype. Additionally, Samuelson (2000) found significant G x E interactions in root allocation under low, but not high, N availability in open-pollinated $P. \text{taeda}$ and $P. \text{elliottii}$ families.

The general decrease in biomass observed with LR incorporation in CL32 and not CL93 suggests that the mechanism(s) driving these differences are greater foliage and belowground maintenance cost, decreased gross canopy CO$_2$ assimilation, or a combination of both. Our $A_{Sat}$ data suggests photosynthetic efficiency of sun leaves was not contributing to this difference in growth since CL32 maintained either the same or greater $A_{Sat}$ relative to CL93 throughout the entire study (Figure 3.9). Further, we found that although photosynthetic capacity decreased in CL32 and increased in CL93 with the addition of LR, $V_{C,max}$ and $J_{max}$ rates in CL93 never exceeded CL32 rates (Figure 3.10A,C,and E). Despite these findings we postulate that the decrease in growth in CL32 with LR is largely a result of the amount of effective photosynthetic area, and there is some indirect evidence to support this. For example, the incorporation of LR did not impact leaf biomass in either clone, but decreased CSA by 25\% in CL32 while having no impact on CL93. From this we concluded that the incorporation of LR resulted in a denser canopy (shallower slope) in CL32, reducing light interception by interior leaves, and therefore, decreased gross CO$_2$ assimilation at the canopy level (Figure 3.7B). Since $A_{Sat}$, $A/PPFD$, and $A/C_i$ curve were always measured on needles receiving full sun, we would not be able to detect this difference, but a number of studies have shown large difference in photosynthetic capacity and $A_{Sat}$ between sun and shade leaves in $P. \text{taeda}$ (Maier et al. 2002; Gough et al. 2004a).

The majority of maintenance costs are associated with tissues which are most physiologically active such as foliage and fine-roots. We found no change in leaf dark respiration estimated
from light curves or leaf respiration \((R_L)\) estimated from \(A/C_i\) curves, but these data should be interpreted cautiously since they were measured on needles receiving full sun. We feel our method of root sampling was appropriate for exploring changes in biomass partitioning since fine-roots make up only a small fraction of total root biomass. Although notably, in terms of nutrient acquisition, belowground maintenance costs, and root turnover this method does not adequately estimate fine-roots. Data collected by a project collaborator between November 2005 and July 2006 using mini-rhizotrons showed that LR incorporation increased fine-root length in both clones, but CL32 maintained greater fine-root length regardless of whether LR was present or not (Seth Pritchard, Dept. of Biology, College of Charleston, personal communication). These findings contradict our findings of decreased lateral and tap roots in CL32, but not CL93 with the addition of LR. Logically, this would suggest that CL32 is better able to acquire nutrients than CL93 since fine-roots are the major source of nutrient uptake.

Foliar chemistry data collected during the winter of 2006 and 2007 show that the incorporation of LR did not decrease nutrient concentrations in 2006, and in 2007 led to increased foliar nutrient concentrations in both genotypes. Clone 32 maintained greater foliar nutrient concentrations than CL93, which would be consistent with increased fine-roots (Appendix F). Increased fine-roots may be responsible for the avoidance of nutrient stress, but the downside is that belowground maintenance costs may be substantially greater in CL32 relative to CL93 due to higher root turnover and increased physiological activity of fine- relative to coarse-roots despite the decrease in overall belowground biomass (Pregitzer et al. 1998). We roughly estimated total foliar N by multiplying average foliar \([N]\) taken from January 2006 and 2007 sampling periods (Appendix F) by foliar biomass data and found a highly significant \((P = 0.005)\) CL by fertilizer interaction. In plots not receiving N and P fertilization, CL32 maintained 22% greater total foliar N relative to CL93 \((34.4 \pm 3.8 \text{ and } 28.2 \pm 3.4 \text{ g N, respectively})\), which supports our hypothesis that CL93 has lower demand for N. However in fertilized plots, CL93 had 25% greater total foliar N relative to CL32 \((48.4 \pm 3.4 \text{ and } 38.6 \pm 3.1 \text{ g N, respectively})\) showing that CL93 is better able to respond to fertilization than CL32.
Consistent with most other reports our data showed a large increase in stem production, leaf area, and coarse-root biomass following the N and P fertilization in both clones (Axelsson and Axelsson 1986; Vose and Allen 1988; Albaugh et al. 1998; Allen and Lein 1998; King et al. 1999; Will 2005). The degree of response varied between clones leading to a significant genetic by fertilization interaction, which supported our hypothesis that CL93 (“narrow crown” ideotype) would be more responsive to fertilization than CL32. By the end of the experiment our calculated stem volume showed that CL93 increased stem volume 21% more than CL32 when fertilizer was added (Figure 3.3C). These findings are consistent with others who have found genotypic differences in growth and allocation to fertility in greenhouse studies using open pollinated families (Li et al. 1991c) and field trials using both open-pollinated (Retzlaff et al. 2001) and clonal material (King et al. 2008).

We did not observe a difference between genotypes in total tree biomass regardless of the soil amendment used, but there were differences in how that biomass was partitioned. Most notably, we observed a large CL by fertilizer interaction in leaf biomass. Clone 93 maintained much less leaf area relative to CL32 in non-fertilized plots, but with the addition of fertilizer CL93 responded by drastically increasing leaf biomass by 72% while CL32 increased leaf biomass very little (Figure 3.5). We were unable to see this same interaction when leaf quantity was expressed as CSA. Differences in canopy architecture may account for this discrepancy, which is supported by two related measurements that were sampled independently of each other. Firstly, the slope estimates of the relationship between CSA and leaf biomass (previously described as a measure of the canopy density) differed between genotypes. For example, CL93 when fertilized showed a denser canopy than CL32 when fertilized (Figure 3.7A). Secondly, needles that were used to construct A/Ci curves showed increased SLA in CL32 and decreased SLA in CL93 following fertilization due mainly to a larger increase in needle weight in CL93 relative to CL32 (Figure 3.12D).

Our $A_{Sat}$ and $[N]_a$ findings support the hypothesis proposed by Gough et al. (2004b). We observed an increase in $A_{Sat}$ following N and P fertilization, which disappeared with the onset of winter (Figure 3.8). At the end of the experiment the most recently fully elongated needles showed a decrease in $A_{Sat}$ and an increase in leaf biomass relative to non-fertilized
treatments (Figure 3.5). We found a weak relationship between photosynthesis and foliar N when estimated over the entire experiment. Foliar N was greater in fertilized trees except for the final two sampling dates where it was significantly less (Figure 3.8). In contrast to our hypothesis we did not find a clonal difference in $A_{\text{Sat}}$ over the experiment. Both clones responded positively to fertilization, however, we did find that fertilization with N and P resulted in differences in leaf biochemistry between clones. Similar to LR treatments CL93 showed the greatest response to fertilization by increasing $V_{C, \text{max}}$, $J_{\text{max}}$ rates, and $[N]_a$ to a greater extent than CL32 (Figure 3.10B, D, and F). As with LR treatments the increase in $V_{C, \text{max}}$ and $J_{\text{max}}$ with fertilization in CL93 simply elevated rates to the same level as CL32. The superior responsiveness of CL93 to fertilization is again echoed in the relationship between $V_{C, \text{max}}$, $J_{\text{max}}$ and foliar N. Again, the relationship between foliar N and photosynthetic parameters of CL32 was weak, but in CL93 the same relationship was highly significant.

Although fertilizer resulted in a temporary increase in $A_{\text{Sat}}$ in both clones there seemed to be a difference in how these two contrasting clones achieved this. For instance CL32 seemed to respond by increasing its ability to capture and utilize light energy while CL93 increased appeared to rely on increasing carboxylation efficiency. This can be seen by differences in the $J_{\text{max}}/V_{C, \text{max}}$ with fertilization (Figure 3.12A). This is further supported by the increase in quantum efficiency ($Q$; slope of CO$_2$ assimilation and PPFD), decrease in LCP, and increase in SLA (Figure 3.12B-D). In contrast CL93 showed an exact opposite trend. Low rates of $V_{C, \text{max}}$, $J_{\text{max}}$ in CL93 relative to CL32 in the absence of any nutrient additions lends further support that although CL93 may be less able to obtain nutrients (as mini-rhizotron fine-root data would suggest), but more tolerant of nutrient limitations and more efficient when N and P are in excess.
Conclusion
The focus of this experiment was to explore differences in stem growth, total biomass partitioning, and leaf physiology between two clones that were known to contrast in their growth efficiency and represent two different ideotypes. Our results have led us to largely reject the hypothesis that these two contrasting ideotypes do not differ in their response to nutrient manipulations in terms of growth, partitioning, and leaf gas exchange. In fact, under field conditions we found that CL93 was better able to tolerate nutrient limitations due to more favorable canopy architecture and perhaps due to lower belowground maintenance associated with fine-roots, which prevented a decrease in aboveground growth as was seen in CL32 with LR incorporation. In contrast, the ability of CL93 to increase leaf biomass with N and P fertilization allowed for greater gross CO$_2$ assimilation translating into greater stem volume. Our results clearly show that contrasting ideotypes have the potential to respond differently to differences in nutrient availability in terms of both partitioning and physiology. An implication of this research underlines the importance in clonal selection and ideotype development as well as silvicultural treatments when implementing site-specific silviculture to maximize productivity in intensively managed southern pine forests. Future research should focus on differences in belowground allocation (fine-root turnover, root respiration, and exudates) between these clones as well as the degree of mycorrhizal associations, which may provide insight into how one clone was able to maintain greater aboveground growth, similar foliar chemistry, and leaf area while possessing fewer fine-roots.
Chapter 3  

Genotype by nutrient interactions in C capture and partitioning

Acknowledgments
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Table 3.1. Statistical summary of $P$-values for logging residue (LR), clone (CL), and fertilizer (F) main effects and interactions for biomass partitioning. An average size tree was destructively harvested from each plot ($n = 24$) on January 2008. Statistical analyses was performed using the GLM procedure in SAS version 9 (SAS 2006).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Main Effects</th>
<th>2-way interactions</th>
<th>3-way interactions</th>
</tr>
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<td>LR CL F</td>
<td>CL<em>LR  F LR</em>F</td>
<td>CL<em>LR</em>F</td>
</tr>
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<td>n.s. 0.0060 0.0009</td>
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<tr>
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<td>n.s. 0.0227 n.s.</td>
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<td>0.0162 0.0942</td>
<td>n.s. 0.0125 n.s.</td>
</tr>
<tr>
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<td>0.0012 n.s.</td>
<td>n.s. 0.0018 n.s.</td>
</tr>
<tr>
<td>Lateral roots</td>
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<td>0.0022 0.0164</td>
<td>n.s. n.s. n.s.</td>
</tr>
<tr>
<td>Tap roots$^1$</td>
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<td>n.s. n.s. n.s.</td>
</tr>
<tr>
<td>Total belowground</td>
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<td>0.0098 0.0995</td>
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</tr>
<tr>
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<td>0.0009 n.s.</td>
<td>n.s. n.s. 0.0022</td>
</tr>
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<td>Root:Shoot</td>
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<td>n.s. n.s.</td>
<td>n.s. n.s. 0.1026</td>
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<td>Foliage:stem</td>
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<td>n.s. 0.0053</td>
<td>0.0625 n.s.</td>
</tr>
</tbody>
</table>

$^1$ Variable was log transformed to meet assumptions of ANOVA
Figure 3.1. Differences in growth efficiency between *P. taeda* clones following the end of the first growing season January 2006. Data collected from current study site by the USDA-Forest Service, Southern Research Station. Coefficient of determination ($r^2$) for Clone 93 and 32 are 0.92 and 0.72, respectively ($n = 18$).
Figure 3.2. Mean (solid line), minimum (dotted line), and maximum (dotted line) daily air temperature and total daily precipitation (bars) between January 2006 and January 2008 for the Cross study site located in Berkeley Co., SC.
Figure 3.3. Least squares mean stem volume as affected by logging residue (LR) by fertilizer (panel A), clone (CL) by LR (panel B), and CL by fertilizer interactions. Arrows indicate time of fertilization and error bars represent ± standard error from the mean and P-values represent treatment interactions with time. Aboveground volume was calculated using the following equation: Volume = ht × (basal dia)$^2$. 

(P = 0.003; n = 6)

(P = 0.01; n = 6)

(P = 0.003; n = 6)
Figure 3.4. Least squares mean of biomass partitioned among aboveground (AB) and belowground (BG) plant tissues. One three-year-old *P. taeda* was destructively harvested from each plot on January 2008. Numbers represent biomass for each tissue in grams (see Table 3.1 for list of *P*-values).
Figure 3.5. Clone by fertilizer two-way interaction in leaf biomass. Error bars represent ± 1 standard error of the mean.
Figure 3.6. Clone (CL) by logging residue (LR) interaction (panel A) and CL by fertilizer (F) interaction (panel B) for amount of stem volume produced per unit canopy area (CSA) in P. taeda clones. Panel A models: CL32 w/o LR (open circle, dashed line) volume = 1.04 CSA + 9414, $r^2 = 0.59$, $P = 0.08$; CL32 w/ LR (closed circle) slope not significantly different from zero $P > 0.1$; CL93 with and w/o LR (open and closed triangle, respectively, solid line) volume = 1.86 CSA - 5890, $r^2 = 0.87$, $P < 0.0001$. Panel B models: CL32, NF (open circle) slope not significantly different from zero, $P > 0.1$; CL32, F (closed circle, dashed line) volume = 1.24 CSA - 289, $r^2 = 0.63$, $P < 0.06$; CL93, NF and F (open and closed triangle, respectively, solid line) volume = 1.86 CSA - 5890, $r^2 = 0.87$, $P < 0.0001$. Linear regressions were performed using PROC REG in SAS version 9.
Figure 3.7. Linear relationship between canopy silhouette area (CSA) and leaf weight for clone (CL) by fertilizer (panel A) and CL by logging residue (LR; panel B) two-way interactions in *P. taeda* clones. Panel A model: CL32, NF (short dashed line) $CSA = 0.4 \times + 26363$, $r^2 = 0.00$, $P =$ n.s.; CL32, F (medium dashed line) $CSA = 14.2 \times - 4516$, $r^2 = 0.55$, $P = 0.09$; CL93, F and NF (solid line) $CSA = 6.7 \times + 9189$, $r^2 = 0.92$, $P < 0.0001$. Panel B model: CL32, w/o LR (medium dashed line) $CSA = 19.5 \times + 6936$, $r^2 = 0.58$, $P = 0.08$; CL32, LR (short dashed line) $CSA = 2.0 \times + 21701$, $r^2 = 0.05$, $P =$ n.s.; CL93, LR and w/o LR (solid line) $CSA = 6.7 \times + 9189$, $r^2 = 0.92$, $P < 0.0001$. 
Figure 3.8. Fertilizer by date interaction for net CO₂ assimilation under saturating light ($A_{Sat}$), foliar N per unit area ($N_a$), and instantaneous photosynthetic N use efficiency ($PNUE$). Arrows indicated time of fertilization and dotted lines represent date of transition from “old” to “new” fully elongated needles.
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References


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Chapter 4

Impacts of soil organic matter and nutrient manipulations on soil properties and their influence on belowground C cycling in a large pot study utilizing two contrasting Pinus taeda L. clones

Summary Intensively managed southern pine forests occupy an estimated 13 million hectares and may provide great opportunity for sequestering large amounts of C in both soil and plant biomass. We conducted a large lysimeter experiment using 170 liter closed plastic containers, which allowed for monitoring of the major C fluxes from the soil. Each container was planted with one of two Pinus taeda clones chosen based on differences in C allocation patterns. Additionally, each experimental unit underwent one of four soil amendments to modify soil organic matter (SOM) and nutrient availability. This was done by a factorial combination of fertilization with N and P, and high C:N logging residue (LR) incorporation. We measured how additions of LR and fertilizer modified soil chemical, physical, and biological properties and their influences on belowground C dynamics. Another objective was to determine if genotypes which were believed to differ in their C allocation patterns had a detectable effect on total soil CO₂ efflux (FS) and whether differences could be sufficient to impact net C exchange. We found that increased C loss by way of FS and through leaching made up approximately 7% of total C incorporated as LR. Our estimates showed that it would take a minimum of 15 years to fully decompose the material added, although, we feel decreases in decomposition rates with time from disturbance and more recalcitrant forms remaining make this a conservative estimate. Incorporation of LR increased FS as a result of increased microbial biomass and activity. Fertilization strongly decreased microbial activity and had a slight decrease in FS. Overall, we did not find consistent differences between genotypes, but work in older stands is still needed. Secondly, our data suggest that incorporation of LR over multiple rotations at a moderate rate after a stand has been harvested could increase SOM without decreasing plant growth, which could increase the ability of these stands to sequester C.

Keywords: Carbon sequestration, fertilization, logging residue incorporation, microbial biomass, soil respiration
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbrev</th>
<th>Description</th>
<th>Units</th>
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<tbody>
<tr>
<td>ACES</td>
<td>Automated Carbon Exchange System</td>
<td></td>
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<tr>
<td>CEC</td>
<td>Cation exchange capacity</td>
<td>meq 100 g⁻¹ soil</td>
</tr>
<tr>
<td>C:N</td>
<td>Carbon to nitrogen ratio</td>
<td>ratio</td>
</tr>
<tr>
<td>$F_{\text{Cont}}$</td>
<td>Continuous measure of total soil CO$_2$ efflux</td>
<td>µmol CO$_2$ m⁻² s⁻¹</td>
</tr>
<tr>
<td>$F_{\text{Point}}$</td>
<td>Point-in-time measure of total soil CO$_2$ efflux</td>
<td>µmol CO$_2$ m⁻² s⁻¹</td>
</tr>
<tr>
<td>$F_S$</td>
<td>Total soil CO$_2$ efflux</td>
<td>µmol CO$_2$ m⁻² s⁻¹</td>
</tr>
<tr>
<td>IRGA</td>
<td>Infrared gas analyzer</td>
<td></td>
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<tr>
<td>LR</td>
<td>Logging residue treatment</td>
<td>treatment variable</td>
</tr>
<tr>
<td>MBC</td>
<td>Microbial biomass carbon</td>
<td>mg C g⁻¹</td>
</tr>
<tr>
<td>MBN</td>
<td>Microbial biomass nitrogen</td>
<td>mg N g⁻¹</td>
</tr>
<tr>
<td>NUE</td>
<td>Nitrogen use efficiency</td>
<td>Growth per unit N</td>
</tr>
<tr>
<td>$R_{\text{H}}$</td>
<td>Index of heterotrophic (microbial) respiration</td>
<td>µmol CO$_2$ g⁻¹ s⁻¹</td>
</tr>
<tr>
<td>SM</td>
<td>Volumetric soil water content averaged over a 13 cm soil depth.</td>
<td>percent</td>
</tr>
<tr>
<td>SOM</td>
<td>Soil organic matter</td>
<td>unitless</td>
</tr>
<tr>
<td>TC</td>
<td>Soil temperature at 15 cm soil depth</td>
<td>degrees Celsius</td>
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</table>
Chapter 4  
*Genotype by nutrient effects on belowground C cycling*

**Introduction**

The combination of improved site resource management and planting of superior genotypes (Schultz 1997; Fox et al. 2004) has enabled intensively managed southern pine plantations to become some of the most productive forests in the world (Allen et al. 2005). Currently, managed southern pine forests occupy more the 13 million hectares and are forecast to increase to 22 million hectares by the year 2040 (Wear and Greis 2002). This may provide great opportunity to manage these forests to sequester large amounts of C in both soil and plant biomass (Johnsen et al. 2001). There has been a great deal of research into how standard silvicultural techniques such as fertilization (Leggett and Kelting 2006; Sampson et al. 2006), competition control (Shan et al. 2001; McFarlane 2006), and thinning (Ohashi et al. 1999; Tang et al. 2005; Selig et al. 2008) influence belowground C cycling and retention, but limited understanding of these processes still impede the ability to accurately model the effects of management activities on ecosystem C exchange in managed pine forests.

Decreases in site quality may be associated with intensive, short rotation harvests and the management of site resources. A typical logging operation in a southern pine plantation can produce anywhere from 5 to 50 Mg of C per hectare in the form of logging residue (LR) depending on age and extent of harvesting (Allen et al. 2006). Relative to the entire tree, these residues (e.g., needles, twigs, bark) contain a disproportionate amount of nutrients such as: nitrogen (N), phosphorus (P), sulfur (S), and calcium (Ca) (Ouro et al. 2001) as well as large quantities of organic C. Traditionally, management of LR has been limited to piling residue into windrows, which was left to decompose or burned. Retaining logging debris on site during and after harvesting has been shown to have profound effects on the soil physical (Powers et al. 2005), chemical (Ouro et al. 2001), and biological properties (Houghton et al. 1983; Aggangan et al. 1999; Li et al. 2004), which can impact the growth of successive stands (Tiarks et al. 2003; Merino et al. 2004). In addition to spreading this material back on to the site, the idea has been proposed to incorporate this LR back to the soil (see review by Buford et al. 1998; Johnson and Curtis 2001) in an attempt to both improve site quality by increasing soil organic matter and increase soil C sequestration.
Alternatively, incorporating large quantities of high C:N organic material into the mineral soil may result in short-term immobilization of nutrients leading to a loss of tree growth. A 29 week laboratory incubation study showed that the incorporation of *Eucalyptus globules* leaf litter into the mineral soil decreased N mineralization, but the there was poor correlation between microbial biomass N and N mineralization found in native soils (Aggangan et al. 1999). Ouro et al. (2001), Perez-Batallon et al. (2001), and LundmakThelin and Johansson (1997) found increased decomposition rates and nutrient release in coniferous stands when harvesting residue was incorporated into mineral soil relative to being left on the soil surface, which is likely a result of more favorable temperature and moisture conditions. Most work to date on the effects of LR incorporation into forest soils have focus primarily on effects to soil properties, but the effects on plant growth, and specifically, short-term seedling growth has been largely overlooked. It is important that we better understand how manipulating site resources through fertilization or the incorporation of organic material impacts plant growth. Recent research on clonal planting stock has shown that some genotypes respond differently to nutrient additions. Support for this comes from studies that have found significant genotype by fertilizer interactions in stem growth (Li et al. 1991c; King et al. 2008), C allocation (Li et al. 1991b; Retzlaff et al. 2001), and N use efficiency (Li et al. 1991a) in *P. taeda*. In fact, it has been estimated that combining selected genotypes and appropriate silvicultural prescriptions could result in volume gains as high as 50% to over 60% in *P. taeda* (Allen et al. 2005; Martin et al. 2005; McKeand et al. 2006).

Genotypes that vary in their belowground C allocation (e.g., root mass, specific root respiration, or exudates) could dramatically influence belowground C cycling. Total soil CO₂ efflux from the soil surface \( F_S \) is the second largest C flux in terrestrial systems (Raich and Schlesinger 1992) and a good *in situ* indicator of many interrelated belowground processes. This makes our understanding of this flux paramount to modeling the effects of forest management on ecosystem C cycling (King et al. 2004). The major contributors to \( F_S \) are heterotrophic (microbial, \( R_H \)) derived and root derived (including rhizosphere) respiration. Estimates of percent root derived respiration vary widely (10 – 90%) in forested ecosystems due to differences in climate, forest type, and sampling methodology, but a review by Hanson et al. (2000) showed that about half (average 46%) of \( F_S \) in forested
ecosystems is root derived. Ryan and Law (2005) in a recent review of the literature, further stressed importance of contributions of recently fixed photosynthate in driving soil respiration. In terms of modeling C cycling in intensively managed forests systems, large scale planting of genotypes that preferentially allocate resources aboveground may need to be accounted for in ecosystem C models. However, there are few studies comparing $F_S$ between clones. A single tree plot field study utilizing two-year-old $P. \text{taeda}$ clones found no clonal difference in $F_S$ measured at the base of each seedling (Tyree et al. 2008). Although there were no detectable clonal differences in specific root respiration, differences in belowground C allocation between clones was never assessed and may not have differed. In contrast, Kasurinen et al. (2004) found significant clone by CO$_2$ fertilization interaction in $F_S$ in pot grown, seven-year-old $Betula \text{pendula}$ Roth. trees grown in open-topped chambers. It is likely that as the trees grow and their roots occupy more of the site, that any clonal differences will be easier to detect, but notably; no studies on mature trees have been conducted.

A greenhouse experiment with a factorial combination of two contrasting $P. \text{taeda}$ clones, fertilization with N and P, and high C:N (approximately 120) logging residue (LR) incorporation was applied to modify nutrient availability. The overall goal of this experiment was to access the interaction between soil nutrient availability and genetics on soil physical, chemical, and biological properties, and how they influence belowground C cycling. Our primary objective was to measure the effects of adding LR alone and in combination with N and P fertilization on C loss from the soil by way of total soil CO$_2$ efflux from the soil surface ($F_S$) and leaching and determine what percent of the added LR remained after one year. Our second objective was to monitor the effects of N and P fertilization on microbial population, activity, and how that influences C loss from the system when both LR is present and absent. Thirdly, what influence does contrasting $P. \text{taeda}$ genotypes, which have been shown to differ in their fine- to coarse-root ratio, and response to soil amendments (Chapter 2), have on belowground C cycling.
Chapter 4  Genotype by nutrient effects on belowground C cycling

Materials and Methods

Experimental design
In April 2006, one-year-old P. taeda clones were planted in 170 L plastic containers (93 cm x 53 cm x 50 cm) and grown in a greenhouse through July 2007. The greenhouse vents and climate control were adjusted to provide plants with summer and winter conditions representative of the southeastern United States (Figure 4.1). The study design was a randomized complete block design replicated six times. Treatments were arranged in a full two by two by two factorial with two levels of logging residue (LR) incorporated into the soil (none, present), two levels of fertilization with N and P (none, present), and two clones (CL93, CL85). The forty-eight plastic containers were fitted with a single brass spigot for collecting water (Appendix A), and each was filled with approximately 0.17 m$^3$ of Eunola series (fine-loamy, siliceous, semi-active, thermic Aquic Hapludults) soil two months prior to planting. Soil was collected in February 2006 to a depth of approximately one meter, which included the Ap, BE, and Bt horizons. Soil was collected from the Virginia Tech Tide Water Agricultural Research and Extension Center located in Holland, VA (Appendix B).

Treatments
Six month old logging residue (LR) was collected from the logging deck of a harvested P. taeda stand in South Carolina (C:N = 128 ± 14; n = 4). The material consisted mainly of bark, needles, and small branches that remained following an onsite processing of merchantable timber. Residue that could pass through a 5 cm x 10 cm screen was incorporated with the soil during pot filling at a rate of 4.92 kg LR container$^{-1}$ (equivalent to 25 Mg o.d. ha$^{-1}$). Fertilizer was applied two times over the course of the experiment. Due to slow initial growth of the clones, the first application was on July 28, 2006 in the form of diammonium phosphate (DAP) and ammonium nitrate (AN) at an equivalent rate of 200 kg N and 50 kg P ha$^{-1}$. The second fertilization took place on March 16, 2007 and was applied in the form of AN, at a rate of 200 kg N ha$^{-1}$. Clonal seedlings (CL93 and CL85) were donated by Mead Westvaco for use in this study and were chosen based on contrasting growth strategies (Phil Dougherty personal communication). See chapter 2 for differences in C capture and biomass allocation between contrasting clones.
Chapter 4  Genotype by nutrient effects on belowground C cycling

\(C\) loss as soil \(CO_2\) efflux

To meet separate objectives for this experiment we employed two systems (automated system and portable manual system) for measuring total soil \(CO_2\) efflux emitting from the soil surface \((F_S)\). Continuous measures of total soil \(CO_2\) efflux \((F_{Cont})\) were made for 411 days starting April 2, 2006 through May 18, 2007 using the automated carbon exchange system (ACES). These data were used to investigate how soil amendment treatments differ in total C loss as \(CO_2\) efflux for the experiment. The ACES was developed at the USDA Forest Service, Southern Research Station Laboratory in Research Triangle Park (See Butnor et al. 2005 for detailed explanation). Briefly, the ACES system is an open-system infrared gas analyzer unit attached to 15 soil chambers that are measured sequentially. The chambers are constructed from 25 cm PVC pipe with a clear cover (dimensions are 491 cm\(^2\) by 10 cm height) and contain thermocouples to monitor both soil (5 cm depth) and air temperature. Every 160 minutes the ACES cycles through 15 measurement chambers and one null chamber giving approximately nine cycles per day. Every five days the ACES chambers were systematically rotated between two identical sets of three replications ensuring that five chambers were allocated to each replication (Appendix G). This approach ensured that each chambers spent equal time at all locations minimizing any bias caused by differences between chambers. Additionally, this allowed chambers to be cleaned and the soil surface scraped free of algae and any photosynthesizing vegetation. The null chamber was used to calibrate IRGA every 160 minutes to account for changes in temperature and pressure. Manual calibration of the IRGA took place once a month at which point soda lime was replaced and standard \(CO_2\) gas was used to make adjustments to the IRGA.

Raw data was downloaded from the system approximately every five days (during chamber relocation and cleaning) and checked for errors and machine malfunctions. Data validation involved ensuring chambers: 1) were equilibrating within 10 minutes, 2) were maintaining a slight (about 10%) positive pressure, 3) hosing was free of clogs and leaks, and 4) that thermocouples were operating. Following validation, data was prepared for analyses by deleting all but the data collected on the 10\(^{th}\) minute, and calculating a daily mean for each chamber. Seven cycles per 24 hour period was the minimum number to be considered a representative daily average, otherwise data for that day was deleted. Secondly, data
collected within 24 hours following relocation of chambers were dropped from the data set due to potential disturbance caused by relocating chambers, which resulted in approximately 20% of the data being deleted. Limitations by the number of chambers available resulted in an inability to test for genetic differences so only CL93 seedlings were used to test for differences between soil additions treatments. There were a total of 3907 useable daily averages (7-9 cycles per day) collected over 411 day period, which represented 79% of the total data collected. All missing dates (approximately 21%) were filled in by averaging the two closest daily values. Treatment effects were tested by averaging daily rates of $F_{\text{Cont}}$ for the month. Additionally, treatment effects on cumulative monthly C loss were tested by summing daily C loss for each month added to C loss from previous months over the experiment.

Manual point-in-time sampling of total soil CO$_2$ efflux ($F_{\text{Point}}$) was done using a Li-Cor 6200 infrared gas analyzer (Li-Cor Inc., Lincoln, Nebraska) with a dynamic closed soil chamber giving a total system volume of 6300 cm$^3$. These data were used to make more powerful comparisons between soil amendment treatments and investigate differences between genotypes. Soil respiration measurements were taken approximately every 1 to 1.5 months starting June 2006 thru June 2007 (11 sampling dates), but machine leaks forced us to remove the June and July 2006 sampling dates leaving nine dates over the course of the experiment. Further details concerning equipment design and methodology can be found in Selig et al. (2008) and Tyree et al. (2008). Twenty-four hours prior to $F_{\text{Point}}$ measurements the soil surface of each pot was lightly scraped to remove any algae, mold, or vegetation. Measurements were made in the same sequential blocking order starting between 1000 and 1600 hours and taking between 2 and 3 hours to complete. Carbon dioxide evolution was measured over a 30 second period and respiration rates calculated as $\mu$mol $\text{CO}_2$ m$^{-2}$ s$^{-1}$. Soil temperature and moisture were measured concurrently with $F_{\text{Point}}$ measurements. Soil temperature was measured to the nearest 0.1 °C at 15 cm depth using a Digi-sense temperature gauge (model no. 8528-20, Cole-Parmer Instrument Co., Niles, IL). Volumetric soil water content was averaged to a depth of 13 cm using a time domain reflectometer (Hydrosense 620 system, Campbell Scientific Inc., Logan, UT) to the nearest 1%.
Microbial biomass and activity

Microbial biomass C (MBC) and N (MBN) were estimated in February 2007 and June 2007 using chloroform fumigation-extraction procedure described by Jenkinson and Powlson (1976) and later modified by (Anderson and Domsch 1978). Six to eight random soil cores were taken to a depth of 20 cm using a 2.5 cm push tube. Soil samples were composited and passed through a 2 mm sieve and immediately stored at 4°C before analyses, which was always performed within 48 hours of collection. Two replicate 25 g fresh soil samples were weighed into 50 mL beakers and each placed into two vacuum desiccators. Half the samples were fumigated with ethanol-free chloroform (CHCl₃) for 24 hours while the other samples were left unfumigated to serve as controls. Each sample had 100 mL of 0.5 molar potassium sulfate (K₂SO₄) solution added and shaken on low for 2 hrs then allowed to settle for 4 hrs before being passed through Whatman #2 filter. A 20 mL subsample of filtered leachate was frozen and shipped to Analytical Services Laboratory at North Carolina State University, Raleigh, NC for total C and N using a TOC analyzer (TOC-5050 fitted with an autosampler model ASI-5000, Shimadzu Scientific Instruments, Columbia, Maryland). Microbial biomass C and N was calculated by subtracting complimentary fumigated from unfumigated samples divided by a constant of 0.35, which represents the extraction efficiency of the solution.

An index of heterotrophic respiration (Rₜ) was measured using a Li-Cor 6250 infrared gas analyzer (Li-Cor Inc., Lincoln, Nebraska) attached to a 0.25 L cuvette chamber, with a total system volume of 429 cm³. Soil samples were taken to a depth of 20 cm using a 2.5 cm push tube following Fₚₒᵢᵢₙ measurements using methods described by Gough and Seiler (2004) and Tyree et al. (2006; 2008). 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 the CO₂ concentration began to steadily rise (typically within one minute) Rₜ was measured over a 30 second period. Soil was oven-dried for 48 hours at 105°C and weighed gravimetrically to the nearest 0.01 g, and adjusted Rₜ rates were expressed as µmol CO₂ g⁻¹soil s⁻¹.
Two wooden dowel rods were buried in each container to a depth of 30 cm with the upper 10 cm remaining exposed to the atmosphere. Index of microbial decomposition was estimated using 0.64 cm (1/4 in) yellow pine dowels buried in the soil for approximately one year starting July 28, 2006. Loss of weight of a standard material has been used in previous studies (Neher et al. 2003; Jurgensen et al. 2006). Each dowel rod consisted of four 10 cm sections separated by silicone and held together with 1.5 cm long rubber hosing (3/8 inch outside diameter and ¼ inch inside diameter) to keep each section discrete from the others (Appendix H). Prior to assembly, all dowel rod sections were oven-dried to 65°C and weighed gravimetrically to the nearest 0.01 g. The ends were covered with silicone to prevent evaporation. At the end of the project (approximately one year) the dowels were removed rinsed with weak soap solution (shaken on low for 4 hours) followed by rinsing with DI water. Finally, wooden rods were oven-dried for 48 hours at a temperature of 65°C and weighed. Decomposition was determined by subtracting the final weight from the starting weight for each dowel and averaging the two subsamples for each EU.

**Total C loss in leachate**

Pots were watered consistently within reps to insure water was never limiting to plant growth. Approximately every month, each container was watered beyond pot capacity over the course of approximately 10 days. The addition of extra water over a 10 day period allowed time for water to penetrate the soil and prevented pooling of water on the soil surface. On the final day spigots were opened and allowed to drain into leachate catchments for 24 hours. Leachate was homogenized by stirring and a subsample passed through a Whatman #1 filter and poured into 20 ml scintillation vials, which was immediately frozen. Frozen samples were shipped to Analytical Services Laboratory at North Carolina State University, Raleigh, NC for total C and N using a TOC analyzer (TOC-5050 fitted with an autosampler model ASI-5000, Shimadzu Scientific Instruments, Columbia, Maryland). Remaining leachate was measured to the nearest ten milliliter to scale total C and N concentration to total C and N lost. In the event that drainage was not completed in 24 hours a fresh catchment was used to catch leachate for an additional 24 hour period.
**Soil chemical and physical properties**

Determination of soil chemical properties was performed on November 2006, February 2007, and June 2007 on fine-soil fraction (passed through 2 mm sieve). Samples collected in February 2007 and June 2007 for MBC determination were also used for soil chemical analyses. Approximately 15 g air dried soil was ground to a powder using a Micro-Mill® (Bel-Art Products, Pequannock, NJ) and 30 ± 5 mg of powdered soil weighed into 5 x 9 mm pressed tin capsules (Costech Analytical Technologies, Inc., Valencia, CA). Total soil C and N concentration were determined using a Carlo-Erba elemental analyzer (Model NA 1500; Fison Instruments, Danvers, MA) at the US Forest Service, Southern Research Station lab in Research Triangle Park, NC. The remaining soil sample was sent to the Virginia Tech Soil Testing Laboratory (Blacksburg, VA) and standard test run according to procedures described by Mullins and Heckendorn (2006). Briefly, soil pH was measured with a 1:1 (vol:vol) ratio of soil to distilled water. Cations were determined using a Mehlich 1 extracting solution and analyzed using an inductively coupled plasma atomic emission spectrometer (Thermo Elemental ICAP 61E). Soil physical properties were measured on one occasion at the conclusion of the experiment. One soil core was taken using a bulk density hammer to a depth of 10 cm at the base of each tree per container. Non-capillary porosity was determined by subtracting the weight of the soil core after 24 hours under 1/3 bar tension (field capacity) from the weight of the saturated soil core. Soil cores were then oven dried at 105°C and weight subtracted from saturated weight to estimate total porosity. Capillary porosity was determined by subtraction of non-capillary and total porosity. Total bulk density (including coarse fragments) was determined using the short core method (Grossman and Reinsch 2002). Cores were oven dried at 105°C and measured gravimetrically to the nearest 0.1 g. Average soil strength (KPa) from 0 to 10 cm depth was measured using Field Scout SC 900 soil compaction meter (Spectrum, Technologies, Inc., East-Plainfield, IL) and normalized to 10% soil moisture.

**Data analyses**

Treatment differences for $F_{\text{Point}}$, $F_{\text{Cont}}$, cumulative monthly C loss as $F_S$ and in leachate, $R_{\text{H}}$, and soil chemical properties were tested using analysis of variance with repeated measures (ANOVARM) using a MIXED model. Covariance structures were selected using AIC,
AAIC, and BIC fit statistics included in the SAS output. Treatment differences for soil physical properties, MBC, and index of decomposition were analyzed using a general linear model (GLM). Relationships between soil respiration ($F_{\text{Point}}$) and biotic and abiotic factors were explored using correlation and regression analyses for LR by fertilizer treatment combinations. Aboveground stem volume and specific photosynthesis under saturating light ($A_{\text{Sat}}$) data were collected concurrently with soils data and used to compare relationships between aboveground productivity and soil processes. See chapter 2 for explanation of methodology and treatment responses of aboveground variables. Correlations between soil chemical and biological properties and $R_H$ were explored for data collected on two sampling dates. For all analyses residuals and the normality curves were plotted to confirm that the data meet assumptions of equal variance and normality for all parameters measured. Values were expressed as untransformed least square means. All data preparation and analyses were performed using the CORR, GLM, MIXED, NLIN and REG procedures in SAS version 9 (SAS 2006).

Results

Soil physical and chemical properties

By the end of the experiment the incorporation of LR into the soil impacted soil physical properties by decreasing bulk density and increasing total porosity and soil strength (Table 4.1). The increase in porosity was due to greater capillary porosity, and had no apparent affect on non-capillary porosity. Our fertilization treatment resulted in a slight decrease in capillary porosity and an increase in non-capillary porosity, which is consistent with a decrease in the fine to coarse-root ratio (Chapter 2, Table 2.2), but did not have an affect any of the other soil physical properties measured. Finally, there were no differences in soil physical properties due to the presence of the clones. Our treatments had a significant effect on soil macro nutrients (Table 4.2). We observed an increase in soil N with LR additions over the first year following treatment initiation. LR incorporation appeared to decrease mineral soil P, but notably, no chemical analyses was performed on the logging residue (> 2 mm). On the final sampling date in June 2007 we found that fertilization with N and P resulted in a significant ($P < 0.05$) decrease in mineral soil K, Ca, and Mg levels. The incorporation of LR led to an immediate and sustained increase in soil C in the fine-soil
fraction (passed 2 mm sieved) throughout the experiment, which was further increased by the addition of fertilizer. We found that both LR and fertilization decreased soil pH and when LR and fertilizer were both applied and the effects appeared to be additive (Figure 4.2). Soil pH showed a consistent increase in the control treatments overtime. Finally, we found CL by fertilizer interactions for soil N and P ($P = 0.05$ and $0.09$, respectively). Soil N increased with fertilization in CL93, but not CL85, which may be a function of N uptake by the plants. In contrast, no substantial difference was observed in soil P (Figure 4.3).

**Total soil CO$_2$ efflux**

Results for $F_{\text{Cont}}$ using the ACES showed a highly significant ($P < 0.0001; n = 84$) increase with the addition of LR incorporated relative to plots not receiving LR additions ($2.62 \pm 0.13$ and $1.64 \pm 0.10$, respectively) over the entire experiment (Appendix I, Table I.1). Additionally, we observed a weak LR by time interaction ($P = 0.09; n = 6$) with treatment differences slightly diminishing with time (Figure 4.4A). The addition of N and P fertilization resulted in a highly significant ($P = 0.003$) fertilizer by time interaction (Figure 4.4B) with fertilizer reducing $F_{\text{Cont}}$; however, there was no significant ($P > 0.10$) interaction between LR and fertilizer treatments. Our pattern of $F_{\text{Cont}}$ over time showed a strong non-linear relationship ($r^2 = 0.78, n = 14$) with average soil temperature taken at 5 cm depth (Figure 4.5), which closely followed average air temperature (Figure 4.1).

Similar to $F_{\text{Cont}}$, soil CO$_2$ efflux data collected using point-in-time sampling ($F_{\text{Point}}$) showed an overall significant ($P < 0.0001$) increase with LR incorporation and significant ($P = 0.002$) decrease with fertilization as main effects (Appendix I, Table I.2). Two contrasting results appeared between the two methods employed for measuring $F_S$. First, we observed a significant LR by fertilizer by time three way interaction ($P = 0.04$) with $F_{\text{Point}}$. When LR was not present the application of fertilizer had little to no effect on $F_{\text{Point}}$. The incorporation of LR increased $F_{\text{Point}}$, but the effect was diminished when fertilizer was applied (Figure 4.6A and B). During the winter months all treatment effects disappeared, and reappeared following a rise in temperature. Second, our point-in-time sampling detected a significant CL by time interaction ($P = 0.05$). We observed no differences between clones throughout most of the study with the exception of the final two sampling dates where CL85 showed a
slight increase in $F_{\text{Point}}$ relative to CL93 (Figure 4.6C). Temperature explained 49% ($r^2 = 0.49; n = 9$) of the variation $F_{\text{Point}}$ between dates when fit using a first order exponential equation $y = a^{(bx)}$ where $y = F_{\text{Point}}$ and $x =$ soil temperature at 15 cm soil depth in degrees Celsius and parameter estimates for $a$ and $b$ are 0.25 ± 0.21 and 0.08 ± 0.03, respectively.

**Soil microbial respiration and biomass**
We observed a significant ($P = 0.006$) LR by fertilizer by time interaction for index of heterotrophic (microbial) respiration (Appendix I, Table I.3). With the exception of one sampling date in October 2006, the application of N and P fertilization decreased $R_H$ with increasing magnitude as the experiment progressed (Figure 4.7A and B). Overall, F treatments decreased $R_H$ by 28% relative to NF treatments (0.082 ± 0.005 and 0.11 ± 0.005 µmol CO$_2$ kg$^{-1}$ s$^{-1}$, respectively). In contrast, the incorporation of LR increased $R_H$ relative to control treatments with the difference decreasing over the course of the experiment. However, when fertilizer was applied in the presence of LR it led to an early increase in $R_H$ followed by a decrease in $R_H$ relative to control treatment with time. Correlation analyses conducted on data from February 2007 and June 2007 sampling dates showed a negative correlation between $R_H$ and N and P ($r = -0.26$ and -0.56, respectively), which further illustrates the negative impact of fertilization on $R_H$.

We observed differences in $MBC$ between nutrient amendments (Appendix I, Table I.4). For example, LR alone had the greatest $MBC$ on both sampling dates, but was slightly reduced when applied in combination with fertilizer (Figure 4.8). Across all data LR significantly ($P < 0.0001$) increased $MBC$ approximately 11% relative to treatments that did not receive LR (679 ± 26.4 and 514 ± 24.7 mg C kg$^{-1}$ soil, respectively; $n = 48$). Additionally, $MBC$ was slightly greater ($P = 0.05$) when sampled in February 2007 relative to July 2007. We did not observe a consistent effect of fertilizer on $MBC$ ($P = 0.23$), but overall F containers showed a decrease relative to NF treatments (579 ± 23.2 and 617 ± 32.5 mg C kg$^{-1}$ soil, respectively; $n = 48$). Comparison of $R_H$ and $MBC$ data from two sampling dates showed that these two measures were not significantly ($P = 0.35$) correlated.
We found a significant ($P < 0.0001$) difference in percent decomposition after one year between soil depth classes (Appendix I, Table I.5). Overall dowel rods buried in the 0-10 cm depth class decomposed at the fastest rate and the section of dowel rod left on the surface decomposed the slowest (Figure 4.9A). The significant LR by fertilizer interaction ($P = 0.003$) showed that when a C source (dowel road) was provided the application of fertilizer increases the rate of decomposition, but the rate was also affected by the presence or absence of LR (Figure 4.9B). When LR was present decomposition responded to fertilizer more than if LR was absent. Finally, application of N and P fertilization affected decomposition, but the response was dependent on soil depth ($P < 0.0001$). At the soil surface there was no difference in decomposition rates between F and NF treatments. The largest difference was found in the 0-10 cm depth where F treatments decomposed approximately 9% more than NF treatments, however, the deeper two depth classes both showed about 4.5% more decomposition in F treatments.

**Relationship between $F_{\text{Point}}$ and biotic and abiotic variables**

Temperature explained about half ($r^2 = 0.49; n = 9$) of the variation in $F_{\text{Point}}$ between dates. Similar to our ACES data (Figure 4.5), we also used a first order exponential equation to model the effects of temperature on $F_{\text{Point}}$ $y = a(\text{bx})$ where $y = F_{\text{Point}}$, $x =$ soil temperature ($^\circ\text{C}$) at 15 cm soil depth, and parameter estimates $a$ and $b$ are $0.25 \pm 0.21$ and $0.08 \pm 0.03$, respectively. Strong LR by fertilizer by time interaction prompted an investigation into factors driving these differences. As previously mentioned we found a relatively strong correlation between temperature and $F_{\text{Point}}$ for all soil amendment treatments, but not for soil moisture (Table 4.3). Stem volume and net photosynthesis collected concurrently in this study (Chapter 2) were used as indicators of aboveground productivity. The combination of these two variables into a single index of aboveground productivity correlated very positively with $F_{\text{Point}}$. Similarly, $R_H$ was used as an index of microbial activity and also showed a strong positive correlation with $F_{\text{Point}}$. These two variables explained between 80 and 94% of the temporal variation depending on which soil amendment treatment was used. When LR was incorporated alone $R_H$ was a good predictor of $F_{\text{Point}}$, but non-significant when fertilizer was added. Similarly, the addition of N and P fertilizer increased the influence of aboveground productivity and decreased the influence of $R_H$ on $F_{\text{Point}}$ (Table 4.3; Figure 4.10).
Chapter 4  Genotype by nutrient effects on belowground C cycling

Total C loss through $F_{\text{Cont}}$ and leaching

The two sources of C loss over the course of the experiment were $F_{\text{Cont}}$ and C loss by way of soil leaching. Soil CO$_2$ efflux from the soil surface dominated total C loss regardless of soil amendment or clone treatment. Relative to C loss through leaching, $F_{\text{Cont}}$ was more than two orders of magnitude greater. We observed a highly significant ($P < 0.0001$) increase in cumulative C loss as $F_{\text{Cont}}$ with the addition of LR (Figure 4.11A; Appendix I, Table I.6). Additionally, we observed a slight decrease in $F_{\text{Cont}}$ with the addition of fertilizer and a difference between genotypes, but these treatment main effects as well as interactions were not statistically significant ($P > 0.10$). Overall, C loss through leaching made up a minor portion of total C loss from the system, but was significantly ($P = 0.04$) increased by the addition of LR (Figure 4.11B). We observed no significant differences between other treatments main effects or interactions (Appendix I, Table I.7). The incorporation of LR resulted in an increase of 160 g of C loss through $F_{\text{Cont}}$ and leaching over treatments not receiving LR. Of the 2460 g of C that was incorporated in the form of LR approximately 93% of the C remained at the end of the experiment approximately one year later.

Discussion

Our soil amendment treatments had a positive affect on many soil physical and chemical properties, which are associated with increased site quality (Fisher and Binkley 2000). The incorporation of LR decreased bulk density and increased total soil porosity (Table 4.1) which is consistent with what Sanchez et al. (2000; 2003) found in the field following LR incorporation. Interestingly, we observed an increase in soil strength in plots with the addition of LR. We believe the increased soil strength was an artifact of the soil compaction meter coming into contact with a fragment of LR and not an accurate indicator of the resistance of soil to root penetration. The incorporation of LR immediately increased total C contained in the fine-soil fraction by 26% (Table 4.2), which was substantially less than that measured by Sanchez et al. (2000; 2003) in the field. We also measured increased total N, but found a decrease in soil P. In contrast, fertilization with N and P did not affect soil physical properties with the exception of increased non-capillary porosity, which was consistent with our observed decrease in the fine to coarse-root ratio in this experiment as
well as others (Chapter 2; Albaugh et al. 1998; Maier and Kress 2000). The addition of N and P did not impact soil C by itself, but when applied in combination with LR, soil C was increased relative to LR being applied alone. We attributed the decrease in K, Ca, or Mg following fertilization to NO$_3^-$ movement and possibly increased uptake as a result of improved growth (Chapter 2). Overall, the addition of both LR and fertilization decreased soil pH, but the increase in pH with time in control and LR treatments and not in the fertilizer treatments suggests that the negative effect of fertilizer additions on soil pH are being underestimated (Figure 4.2). Decreases in soil pH with the application of fertilizer salts have been widely observed accompanied by changes in microbial activity (Kowalenko et al. 1978, Thirukkumaran and Parkinson 2000, Bowden et al. 2004). One possible reason for the increase with time is that the water used for irrigation was slightly basic (pH = 7.43 ± 0.08; n = 4).

Although, the addition of LR has shown improvements to soil physical and chemical properties already mentioned, which in the long-term have been shown to improve site quality (Fisher and Binkley 2000), the next step was to determine if we created short-term nutrient limitations. Our seedling growth data suggest that our rates of LR incorporation did not result in N immobilization to a level that reduced growth, and in fact resulted in increased stem volume (Chapter 2). We did however observe a decrease in foliar N concentration with LR additions, but hypothesize that morphological and biochemical adjustments (e.g., SLA and inactive Rubisco content) in both clones were able to overcome these slight decreases. The incorporation of LR resulted in a slight decrease in N leaching (Data not shown), but did not have a corresponding increase in MBN, which would be an indication that N was being immobilized. This suggests that added N was either taken up by plants or volatilized, and based on the concentration of N remaining in the fine-soil fraction CL85 appears to be using more N than CL93 further leading us to conclude that N was being utilized by the plants and not, to a large extent, by soil microbial populations (Figure 4.3). In contrast, Aggangan et al. (1999) and Perez-Batallon et al. (2001) both found immobilization of N with incorporation of organic material in forest soils.
Incorporation of LR has commonly been shown to increase the rate of decomposition mainly by creating conditions (temperature and moisture) favoring increased microbial activity as well as putting C substrate in direct contact with soil biota. We hypothesized that incorporation of LR would increase decomposition as seen by others (LundmarkThelin and Johansson 1997; Ouro et al. 2001; Perez-Batallon et al. 2001), but that some fraction would remain as recalcitrant SOM. Overtime, successive rotations would result in a slow net gain of SOM, which could restore site quality as well as sequester C in both the soil and as increased forest products. To estimate total C loss we continuously monitored $F_{\text{Cont}}$ using the ACES and C loss through leaching by collecting all leachate and analyzing subsamples for total C. We found that the incorporation of LR increased C loss through both avenues, but C loss as leachate made up less than a percent of total C lost for the year (Figure 4.10). Subtracting excess C loss with LR treatments from control treatments showed that approximately 93% of added C still remained in the pot after one year. Further support comes from dowel rod decomposition data, in which there was approximately the same percent decomposition in the LR only treatments (Figure 4.9B). Assuming a constant rate of decomposition we calculated that it would take more than 15 years for all the incorporated LR to decompose, therefore, reaching a “net zero” point. This simple calculation makes a number of assumptions that would lead to an underestimation of the years before reaching the “net zero” point. Firstly, it assumes the increase in $F_S$ is due entirely to the decomposition of the added LR. Increases in aboveground productivity attributed to improvements to soil physical and chemical properties would likely contribute to C inputs and hence increased $F_S$. Secondly, we also assumed a constant rate of decomposition which is unlikely. We believe the rate of decomposition would diminish as we move further from the time of disturbance, and as more labile material would decomposed relatively early leaving more stable material with increased resistance to decay (Paul and Clark 1996).

The incorporation of LR into the mineral soil showed a substantial increase in $F_S$ (Figures 4.4A and 4.6A and B), which were consistent with our hypothesis as well as other studies that have shown increased $F_S$ with LR additions. For example, Aggangan et al (1999) observed increased $F_S$ with increasing rates of Eucalypt leaf litter incorporation during a 29 week incubation study. Additionally, Perez-Batallon et al. (2001) found increased $F_S$ in plots...
where LR was incorporated into the top 20 cm of mineral soil relative to treatments where LR was completely removed or left on the soil surface one year following harvest in a P. radiata stand in NW Spain. An observed interaction between LR and fertilization showed that although LR increased $F_S$, the increase was diminished with the addition of fertilizer relative to treatments not receiving any additions. Generally, fertilization with N and P resulted in a slight decrease in $F_S$ (Figure 4.4B and 4.6A and B). This has been shown in a number of short-term studies in conifers (Haynes and Gower 1995; Maier and Kress 2000; Butnor et al. 2003; Samuelson et al. 2004), however, others have found no decrease in $F_S$ (Lai et al. 2002; Pangle and Seiler 2002; Lee and Jose 2003; Maier et al. 2004; Tyree et al. 2008). Notably, fertilization resulted in a decrease in cumulative monthly C loss over the entire experiment, but the effect was found not to be statistically significant (Appendix I; Table I.6).

Investigation into the mechanisms driving our observed response of $F_S$ to soil amendments showed that drivers of $F_S$ were soil temperature, microbial activity and abundance, and aboveground productivity. Table 4.3 presents the correlations between $F_{Point}$ and a number of biotic and abiotic factors that have been shown in previous research to influence the rate of C loss from the soil surface. Not surprisingly soil temperature had a strong influence on $F_S$ and when modeled by itself explained approximately 50 to 75% of the temporal variation depending on which method was used to sample $F_S$. These findings are consistent with a large body of research showing increased $F_S$ with temperature (Lloyd and Taylor 1994; Boone et al. 1998; Davidson et al. 1998; Qi and Xu 2001), however due to soil moisture being maintained at moderate levels it did not have a strong influence on $F_S$ (Fang and Moncrieff 2001). We used growth and physiological data measured in this experiment to create an index of aboveground productivity. This is a composite, unitless variable used as an indicator of aboveground productivity with the assumption that the greater the aboveground growth and C capture the greater its allocation of C belowground. We found that both $R_H$ and aboveground productivity explained a large percentage of the temporal variation between soil amendment treatments (Figure 4.10). Our observed relationship between aboveground productivity and $F_{Point}$ is consistent with recent research showing a strong connection between recently fixed C and soil respiration (Ekblad and Högberg 2001;
Högberg et al. 2001; Irvine et al. 2005; Ryan and Law 2005). The relative contributions of each respiratory component changed depending on the soil amendment treatment. For example, fertilization increased “aboveground productivity”, which was highly correlated with an increase in $F_{\text{Point}}$ even though the overall effect of fertilization was to decrease $F_{\text{Point}}$ relative to control treatments. Likewise, the addition of LR resulted in greater correlation between $R_{\text{H}}$ and $F_{\text{Point}}$, although, “aboveground productivity” was still highly correlated (Table 4.3).

Index of heterotrophic (microbial) respiration ($R_{\text{H}}$), which is a measure of microbial activity, shows a similar interaction between LR and fertilization as did $F_{\text{Point}}$. We found that LR by itself increased $R_{\text{H}}$ relative to control treatments and fertilization by itself decreased $R_{\text{H}}$ relative to controls treatments. The combination of both LR and fertilization fell between these two extremes with $R_{\text{H}}$ rates decreasing with time relative to control treatments (Figures 4.7). Microbial respiration has been largely shown by others to decrease with fertilization (Bowden et al. 2004; Gough and Seiler 2004; Blazier et al. 2005; Tyree et al. 2008).

Similarly, microbial biomass C ($MBC$) increased with the addition of LR and decreased with fertilization (Figure 4.8), but notably, the effect of fertilization or its interaction with LR was not statistically significant (Appendix I, Table I.4). Our findings were consistent with others who found increased $MBC$ with the addition of organic material (Aggangan et al. 1999; Perez-Piqueres et al. 2006) and decreased $MBC$ with fertilization with N (Lee and Jose 2003; Blazier et al. 2005) and P (Kelly and Henderson 1978; Thirukkumararan and Parkinson 2000). Based on ours findings we hypothesize that the incorporation of a decomposable substrate into the mineral soil led to an increase in microbial biomass and activity which directly influenced $F_{\text{S}}$. Peculiarly, our index of decomposition responded to our soil amendments in an opposite fashion than would be expected based on $R_{\text{H}}$ and $MBC$ data. Decomposition of dowel rods increased with fertilization and decreased with the addition of LR (Figure 4.9B). Possible explanation for this finding include that adding N to a buried C source such as a wooden dowel rod increased decomposition at that point and not in the bulk soil. The addition of LR may have provided an alternate, more easily, decomposable source of C for soil microbes resulting in a dilution effect which decreased the rate of specific decomposition. If microbial population size was unable to increase due to limitation of some other nutrient, the added N would increase population mass and the addition of fertilizer and
LR would increase population size further corresponding to increased rates of decomposition, and this is what we observed. Poor agreement between decomposition and MBC could be attributed to sampling. MBC was only sampled on the fine-soil fraction and does not estimate the microbial population size associated with coarser material.

With the addition of both fertilization and LR our two methods of measuring $F_S$ arrived at the similar conclusion in terms of main effects. However, the point-in-time ($F_{\text{Point}}$) sampling detected a significant LR by fertilizer interaction where as the continuous monitoring ($F_{\text{Cont}}$) using the ACES did not (Figures 4.4 and 4.6A & B). The relationship between machines when compared directly was poor (Appendix J). Further investigation into machine differences pointed to two factors contributed to the increased apparent sensitivity of the point-in-time sampling in detecting a LR by fertilization interaction. First, with $F_{\text{Point}}$ we were able to measure all experimental units at one time ($n = 48$), which included both CL93 and CL85 in all six replications. Due to limited number of sampling chambers the ACES was only able to measure $F_{\text{Cont}}$ on CL93 for three of the six replications ($n = 12$) at any one time resulting in a loss of power in our analyses (see degrees of freedom Appendix I; table I.1 and I.2). To test this hypothesis we removed CL85 from the analyses and found the LR by fertilizer interaction to be non-significant ($P > 0.10$) for $F_{\text{Point}}$. Using both sampling methods clearly shows the trade-off between these two sampling methods and the need to select the correct approach specific to the question being asked. Continuous monitoring with an automated system allows for an account of diurnal variation and acquire a good daily averages $F_S$, however, this method poses limitations on accounting for spatial or treatment variability. Alternatively, point-in-time sampling allows for measuring more points within the same time period for more powerful comparison of relative treatment differences, but requires a knowledgeable operator.

We did not find an interaction between genotypes and soil amendments as we hypothesized. Overall, there was no differences in $F_{\text{Point}}$ between genotypes, although, there was a significant genotype by time interaction. Both clones showed similar rates of $F_{\text{Point}}$ throughout most of the experiment with differences between clones emerging at the end of the experiment (Figure 4.6C). Clone 85 showed increased rates of $F_{\text{Point}}$ relative to CL93 by
the end of the experiment, but it is unknown whether these differences would continue to be expressed or disappear as the seedlings matured. We speculated based on biomass partitioning data collected that CL93 would have greater $F_{\text{Point}}$, but this was not the case. Although CL85 had greater overall root mass, CL93 had greater fine-root mass and showed an increase in the fine- to coarse-root ratio at the end of the study. Both genotypes showed similar canopy level CO$_2$ assimilation and overall biomass when estimated at the end of the experiment, but the way that biomass was partitioned differed (Chapter 2). Differences in specific root respiration rates or root exudates between genotypes may help to explain our observed differences, but these data were not collected. *Pinus taeda* clones have been shown to differ in their growth and allocation patterns in response to nutrient additions (Li et al. 1991b; Retzlaff et al. 2001; King et al. 2008), but no studies that we are aware of have shown clonal differences in $F_S$ in conifer seedlings.

**Conclusion**

Our data lead us to conclude that the incorporation of LR into the mineral soil at similar rates used in this study would increase soil organic matter, site quality, and could lead to increased C sequestration on the site. We observed improved soil physical and chemical properties with LR incorporation without causing decreased plant growth. Increased C loss by way of $F_{\text{Cont}}$ and through leaching made up only a small percent total C incorporated as LR. The most conservative estimates showed that it would take 15 years to fully decompose, although, decreases in decomposition rates with time would substantially increase that amount of time. Microbial activity and aboveground productivity were the dominant biotic drivers of $F_{\text{Point}}$ and explained a great deal of the temporal variation between soil amendment treatments. When LR was incorporated microbial activity had increased influence over $F_{\text{Point}}$, whereas, application of fertilization resulted in greater explanatory power being given to aboveground productivity. These data support findings by others that have found a strong connection between recently fixed C and soil respiration. Heterotrophic respiration correspondingly increased with LR and decreased with fertilizer additions as did MBC. Finally, we did not find consistent differences between genotypes. For most of the experiment $F_{\text{Point}}$ did not differ between clones, but on the final two sampling dates a pattern emerged of one clone showing greater $F_{\text{Point}}$. If this pattern continues or becomes stronger
with increased occupation of soil by roots then this could have a significant influence on net C exchange, but further study on mature stands is needed.
Acknowledgments

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Table 4.1. Mean ± standard error for treatment main effects of soil physical properties. One soil core was taken per experimental unit to a depth of 10 cm using a hammer driven bulk density corer (n = 24).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bulk Density (g cm(^{-3}))</th>
<th>Total(^1)</th>
<th>Capillary(^2)</th>
<th>Non-capillary(^3)</th>
<th>Soil strength(^4) (KPa)</th>
<th>% Soil moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logging residue</td>
<td>1.39 ± 0.02</td>
<td>47.4 ± 0.57</td>
<td>26.4 ± 0.58</td>
<td>20.9 ± 0.69</td>
<td>1671 ± 112</td>
<td>9.8 ± 0.6</td>
</tr>
<tr>
<td>No logging residue</td>
<td>1.45 ± 0.01</td>
<td>45.5 ± 0.45</td>
<td>24.6 ± 0.50</td>
<td>20.9 ± 0.79</td>
<td>1111 ± 112</td>
<td>10.8 ± 0.7</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>n.s.</td>
<td>&lt; 0.001</td>
<td>0.09</td>
</tr>
<tr>
<td>Fertilization</td>
<td>1.41 ± 0.01</td>
<td>46.7 ± 0.48</td>
<td>24.9 ± 0.51</td>
<td>21.8 ± 0.83</td>
<td>1395 ± 147</td>
<td>10.5 ± 0.7</td>
</tr>
<tr>
<td>No fertilization</td>
<td>1.43 ± 0.02</td>
<td>46.3 ± 0.61</td>
<td>26.2 ± 0.59</td>
<td>20.0 ± 0.59</td>
<td>1387 ± 102</td>
<td>10.0 ± 0.6</td>
</tr>
<tr>
<td>P-value</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.08</td>
<td>0.06</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Clone 93</td>
<td>1.42 ± 0.02</td>
<td>46.5 ± 0.57</td>
<td>25.9 ± 0.58</td>
<td>20.6 ± 0.69</td>
<td>1380 ± 134</td>
<td>10.7 ± 0.8</td>
</tr>
<tr>
<td>Clone 85</td>
<td>1.42 ± 0.01</td>
<td>46.4 ± 0.52</td>
<td>25.1 ± 0.55</td>
<td>21.2 ± 0.79</td>
<td>1402 ± 118</td>
<td>9.8 ± 0.6</td>
</tr>
<tr>
<td>P-value</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

\(^1\)Determined by (2.65 – Bulk density) ÷ 2.65 \times 100

\(^2\)Determined by (wt soil at 1/3 bar – wt of oven-dried soil) ÷ volume of soil core \times 100

\(^3\)Determined by (Total porosity – capillary porosity)

\(^4\)Average soil strength from 0 to 10 cm soil depth and adjusted to 10 percent soil moisture (regression analysis).

\(^5\)Volumetric soil moisture averaged to a depth of 13 cm using time domain reflectometry.
Table 4.2. Average ± standard error soil macro nutrients measured three times during the experiment. Different letters indicate significant Fisher’s least square difference ($P \leq 0.05; n = 6$) between logging residue (LR) by fertilization (F) interaction within each sampling date. Containers were fertilized with N and P in July 2006 and again with N in March 2007.

<table>
<thead>
<tr>
<th>Soil amendment</th>
<th>C</th>
<th>N</th>
<th>C:N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>---------</td>
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<td>---------</td>
<td>----------</td>
<td>----------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>November 2006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td>3.17 ± 0.10 c</td>
<td>0.30 ± 0.01 c</td>
<td>10.4 ± 0.2 b</td>
<td>16.9 ± 0.5 a</td>
<td>38.6 ± 1.7 a</td>
<td>191 ± 4 ab</td>
<td>37.8 ± 1.3 a</td>
</tr>
<tr>
<td>Logging residue (LR)</td>
<td>4.35 ± 0.28 a</td>
<td>0.34 ± 0.02 ab</td>
<td>12.7 ± 0.3 a</td>
<td>16.5 ± 0.5 a</td>
<td>40.7 ± 0.8 a</td>
<td>197 ± 8 a</td>
<td>40.0 ± 0.7 a</td>
</tr>
<tr>
<td>Fertilization (F)</td>
<td>3.25 ± 0.09 c</td>
<td>0.31 ± 0.01 bc</td>
<td>10.4 ± 0.2 b</td>
<td>18.8 ± 0.6 b</td>
<td>37.0 ± 1.6 a</td>
<td>180 ± 6 bc</td>
<td>34.7 ± 1.0 b</td>
</tr>
<tr>
<td>LR + F</td>
<td>3.76 ± 0.12 b</td>
<td>0.35 ± 0.01 a</td>
<td>10.9 ± 0.3 b</td>
<td>19.0 ± 0.6 b</td>
<td>37.4 ± 1.1 a</td>
<td>168 ± 3 c</td>
<td>34.3 ± 0.9 b</td>
</tr>
</tbody>
</table>

| February 2007  |          |          |         |          |          |         |          |
| No addition    | 3.25 ± 0.16 bc | 0.29 ± 0.02 b | 11.6 ± 0.6 b | 20.3 ± 0.4 a | 56.9 ± 1.6 a | 239 ± 4 a | 51.1 ± 1.3 a |
| Logging residue (LR) | 3.79 ± 0.18 b | 0.27 ± 0.02 b | 14.6 ± 1.1 a | 18.5 ± 0.5 b | 58.0 ± 1.6 a | 228 ± 4 a | 50.7 ± 1.0 a |
| Fertilization (F) | 3.21 ± 0.10 c | 0.30 ± 0.02 ab | 11.3 ± 0.7 b | 25.6 ± 0.8 c | 58.0 ± 2.0 a | 240 ± 6 a | 53.6 ± 1.9 a |
| LR + F         | 4.50 ± 0.35 a | 0.33 ± 0.02 a | 13.5 ± 0.6 a | 23.8 ± 0.5 d | 56.7 ± 1.5 a | 227 ± 8 a | 50.0 ± 1.5 a |

| June 2007      |          |          |         |          |          |         |          |
| No addition    | 3.51 ± 0.12 b | 0.23 ± 0.01 b | 15.8 ± 0.9 a | 16.6 ± 0.4 b | 41.8 ± 1.5 a | 215 ± 7 a | 47.3 ± 1.5 a |
| Logging residue (LR) | 4.24 ± 0.22 a | 0.27 ± 0.02 a | 16.1 ± 0.9 a | 15.2 ± 0.3 c | 43.1 ± 0.7 a | 203 ± 4 a | 46.8 ± 1.1 a |
| Fertilization (F) | 3.54 ± 0.15 b | 0.26 ± 0.01 ab | 14.1 ± 0.8 a | 18.2 ± 0.4 a | 34.3 ± 1.1 b | 157 ± 3 b | 33.8 ± 0.7 b |
| LR + F         | 4.32 ± 0.14 a | 0.28 ± 0.02 a | 16.9 ± 1.9 a | 17.5 ± 0.3 ab | 35.4 ± 1.1 b | 161 ± 4 b | 34.6 ± 0.8 b |
Table 4.3. Correlations ($P$ – values) between total soil CO$_2$ efflux ($F_{\text{Point}}$) and biotic and abiotic variables for logging residue (LR) by fertilization (F) treatment combinations. Soil temperature and moisture, specific net photosynthesis ($A_{\text{Sat}}$), aboveground stem volume, index of aboveground productivity ($A_{\text{Sat}} \times$ stem vol.), and index of heterotrophic (microbial) respiration ($R_H$) data represents daily treatment averages (12 observations) measured on nine separate sampling dates from August 11, 2006 through June 19, 2007 ($n = 9$).

<table>
<thead>
<tr>
<th>Factors</th>
<th>None</th>
<th>LR only</th>
<th>F only</th>
<th>LR+F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil temperature at 15 cm soil depth</td>
<td>0.72 (0.03)</td>
<td>0.65 (0.06)</td>
<td>0.64 (0.07)</td>
<td>0.73 (0.01)</td>
</tr>
<tr>
<td>Percent volumetric soil moisture to 13 cm</td>
<td>0.09 (n.s.)</td>
<td>-0.03 (n.s.)</td>
<td>-0.03 (n.s.)</td>
<td>-0.06 (n.s.)</td>
</tr>
<tr>
<td>Specific net photosynthesis ($A_{\text{Sat}}$)</td>
<td>0.02 (n.s.)</td>
<td>-0.13 (n.s.)</td>
<td>-0.03 (n.s.)</td>
<td>-0.01 (n.s.)</td>
</tr>
<tr>
<td>Stem volume</td>
<td>0.73 (0.03)</td>
<td>0.72 (0.03)</td>
<td>0.82 (0.007)</td>
<td>0.79 (0.01)</td>
</tr>
<tr>
<td>Index of aboveground contribution</td>
<td>0.79 (0.01)</td>
<td>0.83 (0.005)</td>
<td>0.89 (0.001)</td>
<td>0.83 (0.006)</td>
</tr>
<tr>
<td>Index of heterotrophic respiration ($R_H$)</td>
<td>0.66 (0.05)</td>
<td>0.83 (0.006)</td>
<td>0.29 (n.s.)</td>
<td>0.23 (n.s.)</td>
</tr>
</tbody>
</table>
Figure 4.1. Average, minimum, and maximum daily greenhouse temperature monitored continuously over the course of the experiment.
Figure 4.2. Mean soil pH by soil amendment treatment by sampling date. Bars represent average soil pH ($n = 12$) for three sampling dates. Error bars represent ± standard error from the mean.
Figure 4.3. Mean soil nitrogen (N) and phosphorus (P) concentration for clone by fertilizer (F) treatments. Bars represent average N and P (n = 36) over three sampling dates. Error bars represent ± standard error from the mean. *P*-value represent significance of the clone by fertilizer interaction over all sampling dates.
Chapter 4  
Genotype by nutrient effects on belowground C cycling

Figure 4.4. Least square mean soil CO₂ efflux rates ($F_{\text{Cont}}$) comparing effects of logging residue (LR; panel A) and fertilization (F; panel B) treatments. Daily average $F_{\text{Cont}}$ (7-9 measurement cycles per day) was measured continuously using the ACES starting April 2, 2006 thru May 18, 2007 and used to calculate an average monthly $F_{\text{Cont}}$. Monthly $F_{\text{Cont}}$ rates were transformed using their natural log to meet assumptions and analyzed using ANOVA with repeated measures in SAS version 9.0. Error bars represent ± standard error of the mean and arrows indicate times of fertilization.
Figure 4.5. Non-linear relationship between soil temperature at 5 cm depth and total soil CO$_2$ efflux ($F_{\text{Cont}}$) using data collected with the automated C efflux system (ACES) from April 2, 2006 thru May 18, 2007. Points represent average monthly $F_{\text{Cont}}$ measured at an average monthly soil temperature. The $r^2$ value was determined by linear regression between $F_{\text{Cont}}$ and predicted value determined using the non-linear first order exponential equation.
Figure 4.6. Least squares mean of soil CO$_2$ efflux ($F_{Point}$) logging residue (LR) by fertilizer (F) by time three-way interaction (Panel A), percent $F_{Point}$ treatment effect relative to control treatment (Panel B), and clone (CL) by time interaction (Panel C). Rates were transformed using their natural log to meet assumptions and analyzed using ANOVA with repeated measures in SAS version 9 (error bars represent ± standard error from the mean). Arrows indicate times of fertilization and dashed line represent control treatment.
Figure 4.7. Least squares mean of index of heterotrophic respiration ($R_{H}$) for the logging residue (LR) by fertilizer (F) by time three-way interaction (error bars represent ± one standard error of the mean, $n = 12$; panel A). Percent $R_{H}$ treatment effect relative to control containers measured on 10 sampling dates (Panel B). Arrows indicate times of fertilization and dotted line represents control treatment.
Figure 4.8. Average microbial biomass C by soil amendment taken on two sampling dates February 2007 and July 2007. Each bar is an average of 12 samples and error bars represent ± one standard error from the mean.
Figure 4.9. Least squares mean of percent decomposition by depth (Panel A) and soil amendment (Panel B). Two yellow pine, jointed dowel rods were buried vertically in the bulk soil of each container for one year (Appendix H). Subsamples were averaged and transformed using the arcsine of the square root prior to statistical analyses using ANOVA with depth as a split-plot treatment. Each bar is an untransformed average of 48 observations and error bars represent ± one standard error from the mean.
Figure 4.10. Response surfaces for each soil amendment treatment representing the relationship between total soil CO₂ efflux (F<sub>Point</sub>), index of aboveground productivity and index of heterotrophic (microbial) respiration (R<sub>H</sub>). Index of aboveground productivity (AG index) was calculated by multiplying net photosynthesis per unit leaf area and stem volume (see chapter 2 for details). Both independent variables were measured concurrently on nine separate sampling dates. Each point on the response curve represents an average of twelve experimental units. Multiple linear regression analyses were performed for each soil amendment treatment using the following model:  

\[ F_{\text{Point}} = \beta_0 + \beta_1 \text{AG index} + \beta_2 R_H. \]

Coefficients of variation are reported below treatment labels on graphs.
Figure 4.11. Cumulative monthly C loss in grams as total soil CO$_2$ efflux ($F_{\text{Cont}}$; panel A) and as total C loss through leaching (Panel B) for logging residue (LR) treatment main effect. Daily average $F_{\text{Cont}}$ (7-9 measurement cycles per day) was measured continuously using the ACES starting April 2, 2006 thru May 18, 2007 and used to calculate a cumulative monthly sum for $F_{\text{Cont}}$. Monthly sums were transformed by their square root to meet the assumption of equal variance. Total C concentration in leachate (mg L$^{-1}$) was determined approximately monthly and multiplied by total volume of leachate collected. Each point is an average of 24 observations and error bars represent ± one standard error from the mean ($n = 6$). Data were transformed by their natural log to meet assumption of equal variance.
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Chapter 5

Interaction between contrasting *Pinus taeda* ideotypes and soil nutrient availability: effects of soil organic matter incorporation and fertilization on soil chemistry, microbial abundance, and soil respiration

**Summary** The long-term sustainability of intensively managed pine plantations should include a plan to manage soil organic matter (*SOM*) in order to preserve site quality. A combination of i) managing *SOM* through incorporating harvest residue (LR) into the mineral soil and ii) planting of high nutrient use efficiency genotypes to avoid nutrient immobilization has the potential to both improve soil quality and over time increase soil C sequestration. Our objectives with this research were to determine how LR incorporation, nutrient additions, and planting of contrasting *Pinus taeda* clones on the Lower Coastal Plain of South Carolina influences soil properties associated with site quality and total soil CO$_2$ efflux ($F_S$) two years following treatment initiation. We measured an immediate and sustained increase in soil properties: total C, CEC, pH, base saturation, and decreased bulk density, but the incorporation of LR resulted in decreased growth in one of the two genotypes. Genotypic differences in leaf area and fine-root length measured in an accompanying study led us to postulate that the main mechanism for avoiding N immobilization when LR was added for our two genotypes was a lower demand for N. Our data showed a strong genotype by soil amendment interaction in $F_S$ over all sampling dates. Individual components of $F_S$ (e.g., heterotrophic or root respiration) also changed between treatments. Microbial respiration data and known biomass partitioning data collected in an accompanying study led us to conclude that the amount of root biomass present was the main factor controlling $F_S$, which is further evidence supporting the strong link between gross primary productivity and soil respiration. Our results indicate that incorporated LR is a stable, and slow to decompose organic material making it likely that some percent of this material will remain at the end of the rotation. Overall, combining LR treatments and planting of genotypes with low nutrient demand or high efficiency may increase *SOM* while avoiding loss of *NPP* due to nutrient immobilization.

**Keywords:** Carbon sequestration, G x E interactions, intensive silviculture, loblolly pine, logging residue, total soil CO$_2$ efflux
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbrev</th>
<th>Description</th>
<th>Units</th>
</tr>
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<tbody>
<tr>
<td>CEC</td>
<td>Cation exchange capacity</td>
<td>meq 100 g(^{-1}) soil</td>
</tr>
<tr>
<td>C:N</td>
<td>Carbon to nitrogen ratio</td>
<td>ratio</td>
</tr>
<tr>
<td>(F_S)</td>
<td>Total soil CO(_2) efflux from the soil surface</td>
<td>(\mu)mol CO(_2) m(^{-2}) s(^{-1})</td>
</tr>
<tr>
<td>LR</td>
<td>Logging residue treatment</td>
<td>categorical variable</td>
</tr>
<tr>
<td>MBC</td>
<td>Microbial biomass carbon</td>
<td>mg C g(^{-1}) soil</td>
</tr>
<tr>
<td>MBN</td>
<td>Microbial biomass nitrogen</td>
<td>mg N g(^{-1}) soil</td>
</tr>
<tr>
<td>NUE</td>
<td>Nitrogen use efficiency</td>
<td>g biomass g(^{-1}) N</td>
</tr>
<tr>
<td>(R_H)</td>
<td>Index of heterotrophic (microbial) respiration</td>
<td>(\mu)mol CO(_2) g(^{-1}) s(^{-1})</td>
</tr>
<tr>
<td>SM</td>
<td>Volumetric soil water content averaged over a 13 cm soil depth.</td>
<td>percent</td>
</tr>
<tr>
<td>SOM</td>
<td>Soil organic matter</td>
<td>unitless</td>
</tr>
<tr>
<td>TC</td>
<td>Soil temperature at 15 cm soil depth</td>
<td>°C</td>
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**Introduction**

The combination of improved silviculture and use of superior planting stock has more than tripled volume production of intensively managed southern pine plantations over the last half century (Schultz 1997; Fox et al. 2004), and is estimated to result in future volume gains of up to 60% (Allen et al. 2005; Martin et al. 2005; McKeand et al. 2006). One concern is that increased productivity and shorter rotation lengths may put increased burden on the land requiring steps to insure long-term sustainability without decreasing site quality (Fox 2000).

Soil organic matter (SOM) is a principal indicator of site quality (Powers et al. 1990) as well as a possible sink for atmospheric C (Lal 2003), therefore, intensive silviculture should include some plan for managing SOM. Logging residue (LR) generated from forest harvests is a large source of C, which could potentially be used to manage SOM. Additionally, relative to the entire tree, LR contains a disproportionate amount of nutrients such as: N, P, and S (Ouro et al. 2001), which if left on-site in time will result in the slow release of nutrients back to the soil for use by successive stands.

Allen et al. (2006) estimated as much as 50 Mg of C ha⁻¹ in the form of LR (e.g., needles, twigs, bark) can be generated during a typical logging operation in a southern pine plantation. Removal of this material can have profound effects on the soil physical (Powers et al. 2005), chemical (Ouro et al. 2001), and biological properties (Li et al. 2004), which may have a dramatic impact on future forest productivity (Tiarks et al. 2003; Merino et al. 2004). However, LR left on the soil surface represents an obstacle for tree planting and site preparation and provides little opportunity to increase SOM. This in part has motivated some to investigate the effects of incorporating LR into the mineral soil to improve soil quality through the many benefits associated with increased SOM (Fisher and Binkley 2000) as well as increase soil C sequestration (Buford et al. 1998; Sanchez et al. 2000; Sanchez et al. 2001; Sanchez et al. 2003). The sudden influx of high C:N substrate has been shown to lead to the immobilization of essential nutrients such as N (Aggangan et al. 1999; Perez-Batallon et al. 2001), which can lead to decreased net primary productivity (NPP) until that organic material can be decomposed (Zimmerman et al. 1995). However, previous work showed that when LR was incorporated at a relatively low rate (equivalent to 25 Mg o.d. ha⁻¹) there was no detectable nutrient immobilization in large pots grown in a greenhouse over one year.
In contrast, an accompanying field experiment with three times the rate of LR incorporation (75 Mg o.d. ha\(^{-1}\)) showed a 25% decrease in stem volume in one *P. taeda* clone, but not in the other (Chapter 3). In addition, these genotypes responded differently to fertilization, which is consistent with other studies that have found fertilizer by genotype interactions in nutrient use efficiency (Li et al. 1991a) and biomass partitioning (Li et al. 1991b; Li et al. 1991c; Retzlaff et al. 2001; Chapter 3).

Total soil CO\(_2\) efflux from the soil surface \((F_S)\) is the second largest C flux in terrestrial systems (Raich and Schlesinger 1992) and is considered a good *in situ* indicator of many interrelated belowground processes (King et al. 2004). The major contributors to \(F_S\) are heterotrophic (microbial, \(R_H\)) derived and root derived (including rhizosphere) respiration from recently fixed CO\(_2\). Ryan and Law (2005) in a recent review of the literature, further stressed the importance of contributions of recently fixed photosynthate as a driver of soil respiration. The ability to allocate C can vary quite dramatically between genotypes within a specific species such as *P. taeda* (Chapter 4). Therefore, large scale planting of genotypes that differ in their belowground C allocation (e.g., root mass, specific root respiration, or exudates) by preferentially allocating resources aboveground may need to be accounted for in C models. Despite this, few studies to date have compared \(F_S\) between clones. A field study designed as a single tree plot utilizing two-year-old *P. taeda* clones found no clonal difference in \(F_S\) measured at the base of each seedling (Tyree et al. 2008). In contrast, Kasurinen et al. (2004) found significant clone by CO\(_2\) fertilization interaction in pot grown, seven-year-old *Betula pendula* Roth. trees grown in open-topped chambers. A more recent greenhouse study conducted over one year showed no difference in \(F_S\) for most of the experiment, but as *P. taeda* seedlings increased in size and occupied more of the soil, different clone effects on \(F_S\) emerged (Chapter 4).

Our objectives with this research were to determine how LR incorporation, nutrient additions, and planting of contrasting clones influence soil properties associated with site quality and \(F_S\) for two years following treatment initiation under field conditions. The incorporation of high C:N (about 700:1) logging residue (LR) into the mineral soil and fertilization with N and P as well as the combination of the two treatments provided us with
nutrient availability gradient, which ranged from nutrient immobilization to ample N and P (Table 5.1). Two *P. taeda* clones, each represented two distinct ideotypes ("narrow crown" versus "broad crown" ideotypes; see Martin et al. 2001), have been shown to differ in their biomass partitioning patterns, leaf physiology, and chemistry in response to nutrient availability (Chapter 3). This range of nutrient availability combined with planting of contrasting *P. taeda* genotypes was used to test the following hypotheses. First, that LR incorporation will improve factors associated with higher site quality such as: increased SOM, CEC, base cations, and decreased bulk density. Second, incorporating LR will increase the abundance and activity of soil microbial populations resulting in immobilization of soil N in the form of microbial biomass N. In addition, N and P fertilization, which has shown to increase aboveground partitioning in both genotypes, will decrease the abundance and activity of microbial populations. Finally, large differences in biomass partitioning that have been shown between genotypes in response to nutrient availability will have a significant influence on microbial populations and CO$_2$ evolution from the soil surface ($F_s$).

**Materials and Methods**

*Site location, climate, and stand history*

The study site was located in Berkeley County, SC at an elevation of 24 m above mean sea level (Appendix C). Average annual temperature was 14.6°C and 17.4°C with an average daily maximum of 17.3°C and 25.2°C and an average daily minimum of 11.7°C and 11.2°C for the 2006 and 2007 year, respectively. Highest daily average temperature was 26.8°C and 32.5°C occurring in August 2006 and August 2007, respectively, and a low of -0.9°C and 0.4°C occurring in December 2006 and February 2007, respectively. Total precipitation was 90.2 cm in 2006 and 74.9 cm in 2007 spread evenly throughout the year, which was well below the average of 120 cm recorded between 1949 and 1973 (Long 1980). The dominant soil series was an Ocilla (loamy, siliceous, semiactive, thermic Aquic Arenic Paleudults). Harvest of the previous 21-year-old *P. taeda* stand took place in May 2004 and the site was sheared of residual material in July 2004. Logging residue treatments were applied in October 2004, and site preparation (bedding) took place in early November 2004. *Pinus taeda* clones were planted in January of 2005 and data for this study were collected between June 2006 and January 2008 (Appendix D).
Study design and treatments

The study design was a split-plot, randomized complete block design replicated three times with the whole-plot treatments arranged as a full two by two factorial measured repeatedly. Each 0.18 ha plot (48 x 38 m) was planted with approximately 243 container grown, clonal P. taeda seedlings in nine rows at a 1.8 m spacing within rows and a 4.3 m spacing between row centers. Two levels of logging residue (LR) and two clones (CL32 and CL93) served as the whole-plot treatments. The two levels of LR were no LR incorporated (w/o LR) and LR incorporated into the mineral soil (LR) at a rate of 25 Mg o.d. wt. ha\(^{-1}\), which was concentrated onto the beds (approximately 75 Mg o.d. wt. ha\(^{-1}\)). Both LR treatments also incorporated the residual forest floor of approximately 25 Mg o.d. wt. ha\(^{-1}\). The two P. taeda clones chosen both exhibit superior height growth, but represent two distinct ideotypes. Clone 93 (“narrow crown” ideotype) has been shown to allocate more of its resources to stem growth while Clone 32 (“broad crown” ideotype) allocates more resources to leaf area and belowground tissues (Chapter 3).

Each plot was split into two 0.0013 ha measurement plots, located at opposite ends of the whole-plot, each consisted of six seedlings (4 measurement trees + 2 buffer trees) and served as the experimental unit (EU). Each split-plot received one of two fertilizer applications. No nutrient additions (NF) or N and P fertilization (F). During the 2006 growing season fertilizer was applied two times in the form of diammonium phosphate (DAP) and ammonium nitrate (AN) totaling 209 kg N and 116 kg P ha\(^{-1}\). Roughly 1/3 was applied on April 6 and the remaining 2/3 applied on May 8, 2006. Fertilizer application for the 2007 growing season was applied on March 9, 2007 at a rate equivalent to 200 kg N ha\(^{-1}\) in the form of AN.

Soil properties

Determination of soil chemical properties was performed on samples collected on February 2006 (pre-fertilization), June and December 2006, and July and December 2007 to a depth of 30 cm or to water saturated soil with a 2.5 cm push tube. Approximately 15 g air dried soil was ground to a powder using a Micro-Mill\(^{®}\) (Bel-Art Products, Pequannock, NJ) and 30 ± 5
mg of powdered soil weighed into 5 x 9 mm pressed tin capsules (Costech Analytical Technologies, Inc., Valencia, CA). Total soil C and N concentration were determined using a Carlo-Erba elemental analyzer (Model NA 1500; Fison Instruments, Danvers, MA) at the US Forest Service, Southern Research Station lab in Research Triangle Park, NC. The remaining soil sample was sent to the Virginia Tech Soil Testing Laboratory (Blacksburg, VA) and standard test run according to procedures described by Mullins and Heckendorn (2006). Briefly, soil pH was measured with a 1:1 (vol:vol) ratio of soil to distilled water. Cations were determined using a Mehlich 1 extracting solution and analyzed using an inductively coupled plasma atomic emission spectrometer (Thermo Elemental ICAP 61E).

**Total soil CO₂ efflux**

Manual point-in-time sampling of total soil CO₂ efflux ($F_S$) was performed using a Li-Cor 6200 portable infrared gas analyzer (Li-Cor Inc., Lincoln, Nebraska) with a dynamic closed soil chamber giving a total system volume of 6300 cm³ (Selig et al. 2008; Tyree et al. 2008). Soil respiration measurements were taken approximately every 1 to 1.5 months starting January 2006 thru December 2007 (16 sampling dates). A broken chamber hose on February 2006 and machine leaks on May and June 2006 forced us to remove all three sampling dates leaving 13 separate dates for the experiment. Measurements were taken at approximately the same location on each date and in the same sequential blocking order between 800 and 1600 hours and taking between 3 to 4 hours to complete. Two subsamples were taken per measurement plot. One measurement was taken at the base of the tree and the other taken between trees to better account for spatial variation on the planting bed. No measurements were taken between planting rows. Soil CO₂ evolution was measured over a 30 second period and efflux rates calculated as μmols CO₂ m⁻² s⁻¹. Soil temperature and moisture were measured concurrently with $F_S$ measurements. Soil temperature ($T_C$) was measured to the nearest 0.1 °C at 15 cm depth using a Digi-sense temperature gauge (model no. 8528-20, Cole-Parmer Instrument Co., Niles, IL). Percent volumetric soil water content ($SM$) was averaged to a depth of 13 cm using a time domain reflectometer (Hydrosense 620 system, Campbell Scientific Inc., Logan, UT) to the nearest 1%.
**Microbial biomass and activity**

Microbial biomass C (MBC) and N (MBN) were estimated in June and December 2006, and July and December 2007 using chloroform fumigation-extraction procedure described by Jenkinson and Powlson (1976) and later modified by (Anderson and Domsch 1978). Twenty-four random soil cores were taken to a depth of 20 cm, or depth to saturated soil, using a 2.5 cm push tube. Soil samples were composited and passed through a 2 mm sieve and stored at 4°C prior to analyses. Within 48 hours of collecting soil samples, two replicate 25 g fresh soil samples were weighed into 50 mL beakers and each placed into two separate vacuum desiccators. Half the samples were fumigated with ethanol-free chloroform (CHCl₃) for 24 hours while the other samples were left unfumigated to serve as controls. Following fumigation 100 mL of 0.5 molar solution of potassium sulfate (K₂SO₄) was added to each sample, shaken on low for 2 hrs, allowed to settle for 4 hrs, and then passed through a Whatman #2 filter. A 20 mL subsample of filtered leachate was frozen and shipped to Analytical Services Laboratory at North Carolina State University, Raleigh, NC for total C and N determination using a TOC analyzer (TOC-5050 fitted with an autosampler model ASI-5000, Shimadzu Scientific Instruments, Columbia, Maryland). Microbial biomass C and N was calculated by subtracting complimentary fumigated from unfumigated samples divided by a constant of 0.35, which represents the extraction efficiency of the solution.

An index of heterotrophic respiration ($R_{Hi}$) was measured using a Li-Cor 6250 infrared gas analyzer (Li-Cor Inc., Lincoln, Nebraska) attached to a 0.25 L cuvette chamber, with a total system volume of 429 cm³. Measurements were taken simultaneously with $F_S$ measurements using methods described by Gough and Seiler (2004) and Tyree et al. (2006; 2008). Two subsamples were measured per experimental unit. For each subsample, 12 soil cores were taken to a depth of 20 cm, or to the depth of water table, using a 2.5 cm push tube. Soil samples were composited, roots carefully removed by hand, and placed into an aluminum weigh boat (10 cm x 2 cm), which was immediately placed into the 0.25 L cuvette chamber. Once the CO₂ concentration began to steadily rise (typically within one minute), $R_{Hi}$ was measured over a 30 second period. Soil samples were transported back to the lab, oven-dried for 48 hours at 105°C, weighed gravimetrically to the nearest 0.01g. Index of heterotrophic respiration rates were expressed as µmol CO₂ kg⁻¹soil s⁻¹.
**Data analyses**

All subsamples were averaged prior to statistical analyses. Treatment differences for $F_S$, $R_H$, soil chemical properties, and $MBC$ were tested using analysis of variance with repeated measures (ANOVAR). Logging residue, clone, and fertilizer were treated as fixed effects and time as the repeated effect. Covariance structures were selected primarily using AIC, AAIC, and BIC fit statistics included in the SAS output. When fit statistics were similar between covariance structures (autoregressive versus compound symmetry) the appropriate structure was chosen subjectively by inspection of the correlation matrix. Relationships between $F_S$, soil biotic and abiotic properties as well as aboveground growth were explored using correlation and regression analyses. Correlations between soil chemical, $MBC$, and $R_H$ were explored for data collected on four sampling dates. For all analyses residuals and the normality curves were plotted to confirm that data meet assumptions of equal variance and normality. Data were transformed by their natural log to meet assumptions when appropriate. All values were expressed as untransformed least square means. Data preparation and analyses were performed using the CORR, MIXED, NLIN and REG procedures in SAS version 9.1 (SAS 2006).

**Results**

**Treatment effects on soil properties**

The incorporation of LR into the mineral soil decreased soil bulk density by 20, 8, and 11% at the 0-10, 10-20, and 20-30 cm soil depths, respectively, but was only statistically significant ($P = 0.008$) at the 0-10 cm soil depth (Figure 5.1). Overall, we observed a 15% increase ($P = 0.05$) in total soil C in the fine-soil fraction with LR relative to without LR treatments (Tables 5.1 and 5.2). Logging residue incorporation impacted soil chemistry by increasing base saturation, soil pH, and CEC, but the increase in CEC was not statistically significant. Base saturation increased ($P = 0.002$) 22% with LR from 11.9% to 14.5% due to a 26, 26, and 39% increase in Ca ($P = 0.02$), Mg ($P = 0.01$), and K ($P = 0.001$), respectively. LR increased soil pH slightly relative to treatments with out LR (4.0 and 3.9, respectively), but the most significant effect was the interaction between LR and fertilization. Fertilization decreased soil pH by 0.1 pH unit relative to control treatments when LR was not present, but
in the presence of LR fertilization had no effect on soil pH (Table 5.2). In contrast, we found no effect of LR on soil N and P as main effects, but did significantly \( (P = 0.0006) \) increase the C:N. We observed a 15%, 14%, and 14% decrease in fine-fraction (< 2mm) total soil C \((P = 0.02)\), total N \((P = 0.04)\), and P \((P = 0.10)\) in plots planted with CL93 relative to CL32 plots, respectively.

Logging residue, fertilizer, and genotype treatments influenced the soil environment in terms of temperature and moisture. For example, LR moderated soil water content by maintaining lower SM when the water table was high \((P < 0.0001)\). During periods when the water table was low differences between LR treatments diminished (Figure 5.2). Additionally, we observed an overall 14% decrease of SM in plots planted with CL32 relative to CL93 (14.6% ± 0.06 and 16.9% ± 0.07, respectively). Average soil temperature over the course of the entire experiment was significantly \((P = 0.04)\) greater in CL32 relative to CL93, but the difference in temperature was only 0.3°C. Finally, soil temperature slightly (no more than 0.4°C) increased with LR additions from 20.4 to 20.8°C when fertilizer was present, but did not differ when fertilizer was not present \((P = 0.01)\).

**Treatment interactions on MBC and MBN**

Microbial biomass data showed a significant CL by fertilizer interaction \((P = 0.02)\). The addition of fertilizer in CL32 increased MBC with time relative to non-fertilized treatments while the opposite trend was observed in CL93 (Figure 5.3). Overall, CL32 had 14% greater MBC present relative to plots planted with CL93 \((P = 0.06)\), but there was no significant interaction between LR and genotype or LR by itself. When all data were analyzed together, plots planted with CL32 had significantly \((P = 0.005)\) greater MBN than plots planted with CL93. The addition of fertilizer initially decreased MBN followed by an increase to control levels with time in CL32, but maintained consistently lower MBN with fertilization in CL93 relative to non-fertilized plots (Figure 5.4A). The addition of LR generally increased MBN over the course of the experiment \((P = 0.03)\), but the increase was greater in plots planted with CL32 relative to plots planted with CL93 for summer 2006 and winter 2007 sampling dates (Figure 5.4B).
Treatment effects on gas exchange
Total soil CO$_2$ efflux showed a significant ($P = 0.03$) soil amendment (LR and fertilization) by genetic interaction when all data were analyzed over the experiment. Clone 32 maintained approximately 38% and 36% greater $F_S$ in no soil amendment (None) and LR plus fertilizer (LR+F) treatments relative to CL93 (Figure 5.5). The addition of LR by itself resulted in decreased $F_S$ in CL32 and a slight increase in CL93. Fertilization with N and P resulted in a slight decrease in $F_S$ in both genotypes relative to control treatments with CL32 maintaining higher $F_S$ rates. Although time was a highly significant ($P < 0.0001$) variable, there were no statistically significant interactions between time and any of the other parameters. Differences in $F_S$ over time were largely attributed to soil temperature and percent volumetric soil water content. When daily average $F_S$ was regressed by both soil temperature and moister both variables were statistically significant ($P < 0.10$) and explained 66% ($r^2 = 0.66$, $n = 13$) of the temporal variation. Soil moisture modified the relationship between $F_S$ and $TC$ by decreasing $F_S$ at excessively high or low soil moistures (Figure 5.6).

Respiration by heterotrophic microbes ($R_H$) increased significantly ($P = 0.0006$, $n = 180$) with LR by approximately 18% relative to treatments not receiving LR (0.38 ± 0.02 and 0.32 ± 0.02 µmols CO$_2$ kg$^{-1}$ s$^{-1}$, respectively), and was dependent on genotypic (CL by LR interaction: $P = 0.04$, $n = 90$). The incorporation of LR increased $R_H$ in CL32, but appeared to have no effect on $R_H$ rates in CL93 (Figure 5.7). In contrast, fertilization with N and P resulted in an 11% decrease ($P = 0.02$, $n = 180$) in $R_H$ relative to NF treatments (0.33 ± 0.02 and 0.37 ± 0.02 µmols CO$_2$ kg$^{-1}$ s$^{-1}$, respectively). A slightly significant ($P = 0.09$, $n = 90$) CL by F interaction showed that $R_H$ decreased to a greater extent in CL93 relative to CL32 (19% and 3% decrease, respectively). As with our $F_S$ results, time was found to be a highly significant ($P < 0.0001$) term in our model, but in contrast, showed an interaction with both LR and CL. Both LR and CL32 showed increased $R_H$ relative to their contrasting treatments for most of the experiment. However, differences between LR treatments appeared to diminish during sampling dates with low $TC$ or high $SM$ (Figure 5.8A). Differences between genotypes diminished during winter measurements and on sampling dates with combined high $TC$ and low $SM$ such as in May and December 2007 (Figure 5.8B). Percent soil water
content explained 34\% of the temporal variation in daily average $R_H$ (Model: $R_H = 1.56(SM) + 0.11; P = 0.02; r^2 = 0.34$), but $TC$ was not a significant regressor.

A correlation analyses between $F_S$ and $R_H$ showed a weak correlation between these two variables in CL32 ($r = 0.40; P < 0.0001; n = 144$) and no correlation in CL93 ($r = 0.08; P = 0.34; n = 144$). However, when $F_S$ was adjusted to a constant $TC$ and $SM$ of 20\°C and 15\%, respectively, the correlation between $F_S$ and $R_H$ was considerably weaker in CL32 ($r = 0.28; P = 0.001; n = 144$) and still non-significant in CL93. Correlation analyses between $R_H$ and soil properties measured over four sampling dates showed its greatest correlation between $R_H$ and base saturation on three of the four sampling dates (Table 5.3). Soil CEC and pH showed no significant correlation with $R_H$.

Discussion

Effects LR on soil properties and nutrient availability

In support of our hypothesis the incorporation of LR led to an immediate and sustained increase in soil C over the first two years following treatment initiation. Additionally, this treatment improved soil properties that have been shown to be directly related to site quality such as CEC, pH, and base saturation (Table 5.1). These findings support other short-term studies which have shown decreased bulk density and increased SOM following LR incorporation (Sanchez et al. 2000; Sanchez et al. 2001; Sanchez et al. 2003; Chapter 4). Despite improvements to soil properties, LR incorporation resulted in the immobilization of N two years following treatment initiation. Supporting evidence for this claim comes from two independent data sources. First, treatments receiving LR showed a consistent increase in $MBN$ (Figure 5.4B). These data are consistent to N mineralization results observed on these same plots by a project collaborator (Tisdale 2008) as well as findings by others that observed N immobilization as increased $MBN$ following the incorporation of organic material (Gok and Ottow 1988; Aggangan et al. 1999; Perez-Batallon et al. 2001). Second, LR incorporation did result in decreased stem volume in one clone, but not the other (Chapter 3). Interestingly, both our $MBN$ and tree growth data show genotypic differences in the degree on N immobilization. For instance, CL32 (“broad crown” ideotype) experienced a 25\% decrease in stem volume while CL93 (“narrow crown” ideotype) showed no apparent
loss in stem volume (Chapter 3). Similarly, CL32 presented approximately 0.12 mg N g⁻¹, overall, greater MBN relative to CL93 (0.62 and 0.50 ± 0.03 mg N g⁻¹, respectively), although notably, both genotypes increased MBN following LR incorporation (Figure 5.4B). This increase in MBN corresponded to the 0.19 mg N g⁻¹, overall, greater total soil N found in CL32 plots relative to plots planted with CL93 (1.38 and 1.19 ± 0.06 mg N g⁻¹, respectively). This suggests that either CL93 is better able to extract N from the soil, or more likely, the greater total soil C associated with CL32 roots led to greater immobilization and less leaching of N from the system.

Genotypic differences in N use efficiency (NUE) have been observed in P. taeda (Li et al. 1991a). The authors showed that specifically, some families were better able to absorb N while others utilized N more efficiently. Data from our studies and a separate project collaborator suggests CL93 has less of a demand for N than CL32. Our biomass partitioning data showed greater growth efficiency (stem volume produced per crown area; less leaf are in CL93) in CL93 relative to CL32 regardless soil amendment treatment applied (Chapter 3, Figure 3.7). Fine-root data measured by a project collaborator using mini-rhizotrons showed greater fine-root length in CL32 relative to CL93 (Seth Pritchard, Dept. of Biology, College of Charleston, personal communication), which should favor better nutrient uptake in CL32. King et al. (2008) also found differences in crown area between full- and half-sib P. taeda clones under two soil fertility treatments. Similarly, Li et al. (1991b) and Samuelson (2000) both found genotypic differences in fine-root biomass in P. taeda families under low N availability. Greater leaf area and fine-root length, association with CL32, would likely increase the demand for N. These tissues have decreased turn-over time, are more physiologically active, and hence have greater N requirements relative to other plant tissues.

We roughly estimated total foliar N by multiplying average foliar [N] taken from January 2006 and 2007 sampling periods (Appendix F) by foliar biomass data (Chapter 3) and found a highly significant \( P = 0.005 \) CL by fertilizer interaction. In plots not receiving N and P fertilization, CL32 maintained 22% greater total foliar N relative to CL93 (34.4 ± 3.8 and 28.2 ± 3.4 g N, respectively), which supports our hypothesis that CL93 has lower demand for N. However in fertilized plots, CL93 had 25% greater total foliar N relative to CL32 (48.4 ± 3.9 g N).
3.4 and 38.6 ± 3.1 g N, respectively) showing that CL93 is better able to respond to fertilization than CL32.

**Genotype by soil amendment interaction on FS**

Another objective of this research was to determine if contrasting ideotypes planted under varying levels of nutrient availability would result in a detectable difference to $F_S$. Our data showed a strong genotype by soil amendment interaction over all sampling dates for $F_S$ and $R_H$. For example, when no soil amendments (control plots) were added $F_S$ was substantially greater in CL32 than CL93 (Figure 5.5). We attribute this increase simply to greater root respiration associated with increased fine-root length. The addition of LR by itself sharply decreased $F_S$ in CL32 and slightly increased in CL93. Examining just the $R_H$ data, which showed a large increase in CL32 and just a slight increase in CL93 (Figure 5.7), this would seem counter intuitive. The addition of LR was shown to not only reduce stem volume by 25%, but also reduce total belowground biomass by 30% in CL32 and not at all in CL93 (Chapter 3, table 3.1). Fertilization with N and P showed a decrease in $F_S$ in both clones relative to control treatments, but $F_S$ decreases to a much greater extent in CL32. This is a combination of two factors. One, a slight decrease in $R_H$ associated with a general decrease in MBC (Figure 5.3), which has been widely shown by others in *P. taeda* (Chapter 4; Lee and Jose 2003; Gough and Seiler 2004; Blazier et al. 2005; Tyree et al. 2008). Two, a decrease in root biomass associated with an increase in aboveground partitioning following fertilization with N and P, which has also been shown in this study and by others (Axelsson and Axelsson 1986; Green et al. 1994; Haynes and Gower 1995; Chapter 3). Unlike the LR alone treatment when both LR and fertilizer were applied we found that $F_S$ increased in CL32 and not in CL93. Again, LR resulted in significantly greater $R_H$ in CL32 only, although, the addition of fertilizer with the LR prevented a loss of growth in CL32.

Overall, we found poor correlation between $F_S$ and $R_H$ leading us to conclude that the main driving component of $F_S$ was root respiration or recently fixed C. Interestingly, the genetic by LR interaction we observed in $R_H$ as well as the lack of LR response in MBC would indicate that microorganisms are feeding mostly on plant derived C, which is being controlled by the plant and not decomposition of incorporated LR. Our results are consistent
with research showing a strong connection between recently fixed C and soil respiration (Ekblad and Högberg 2001; Högberg et al. 2001; Irvine et al. 2005; Ryan and Law 2005). A number of studies have shown that putting organic material belowground where more favorable moisture and temperature, and better proximity to soil microbes increases the rate of decomposition (LundmarkThelin and Johansson 1997; Ouro et al. 2001; Perez-Batallon et al. 2001; Chapter 4), thereby, potentially negating this as a method for increasing soil organic matter (SOM). We hypothesize that some fraction of the LR incorporated will remain throughout the rotations and that repeated incorporation of LR may increase overall SOM with time, but long-term (rotation length) studies are needed. Support for this comes from a greenhouse experiment where total C loss in the form of $F_S$ and total C within leachate was monitored for one year in large containers planted with a one year old $P. \text{taeda}$ seedling and a factorial combination of LR and fertilizer. Researchers found that increases in both $F_S$ and C loss as leachate amounted to only 7% (Chapter 4). The most conservative calculations showed that if the same rate of decomposition was maintained, which is unlikely, it would take at least 15 years to fully decompose. Notably the referenced study incorporated LR at a rate of 25 Mg C ha$^{-1}$ which is approximately less than 33% of the rate applied in the current study, and at that rate no N immobilization was detected.

**Future work**

The current work found weak correlations between $R_H$ and $MBC$ (Table 5.3). One reason for this is that our soil amendment treatments likely changed the composition of the microbial community, which may not be reflected in our $MBC$ data. Changes in microbial community composition have been widely reported following changes in available C and N (Bååth et al. 1978; Holland and Coleman 1987). For example, Holland and Coleman (1987) observed a decrease in the proportion of fungi when straw was incorporated into the mineral soil relative to being left on the soil surface. The authors speculate that fungi with their extensive hyphal network are able to access both N in the mineral soil and C on the surface, where as, bacteria require both C and N to be in more intimate contact. Additionally, studies have shown decreases in fungal components following fertilization (Wallander and Nylund 1992; Lilleskov et al. 2002; Nilsson and Wallander 2003; Bittman et al. 2005). For example, both Frey et al. (2004) and Wallenstein et al. (2006) found a decrease in fungal to bacterial
activity ratios in both a hardwood and red pine stand following long-term chronic N additions on the Harvard forest. This experiment did not attempt to characterize the microbial community following application of soil amendments, but recently, researchers have begun to more fully appreciate the complex relationship between plants and soil fungi and the role that root exudates play in regulating microbial community (Broeckling et al. 2008). A couple of studies have shown intra-species differences in microbial community composition in forest species such as *Populus angustifolia*, which is a species with high variation between genotypes (Schweitzer et al. 2008) and in *Betula pendula* (Kasurinen et al. 2005). *Pinus taeda* has a wide geographic range and large within species variation, making intra-specific differences in microbial community composition likely. Specifically, the two genotypes in our studies have been shown to vary in response to soil amendments with respect to biomass partitioning (Chapter 3), MBC, MBN, $R_H$, and $F_S$ (current study) making these clones good candidates for studying intra-species microbial community composition. Although we concluded that decreased demand was the main factor controlling the lack of decreased growth in CL93, it is possible that clones differ in their ability to interact with soil micro biota.

The scope of the current work dealt solely with the affects of LR on soil properties and factors controlling decomposition and eventual loss of belowground C. However, the authors recognize there are practical considerations such as economic feasibility and C cost associated with equipment that should be considered prior to implementation of such a treatment. A cost analyses, in terms of equipment hours associated the incorporation of LR, was performed by Sanchez et al. using two *P. taeda* plantations planted on different soil textures (2003). The authors found that with current technology it is not economically feasible ($US 521 \text{ ha}^{-1}$ and $US 633 \text{ ha}^{-1}$ on the 'sandy' and 'clay' sites, respectively), but the authors postulate that advances in efficiency and productivity may make it feasible in the future. An accounting of C costs associated with varying levels of intensive silviculture was performed by Markewitz (2006) illustrating the level of C accounting needed to access the viability of LR incorporation as a method for increasing soil C storage. Silvicultural treatments that manage SOM and the long-term sustainability of intensive pine silviculture are just in their infancy relative to what is known about other agriculture systems. However,
in addition to the previously mentioned economic considerations, future work is needed on the longer-term monitoring of tree growth, soil properties associated with site quality, and changes to SOM.

**Conclusion**

In support of our hypothesis, the incorporation of LR led to an immediate and sustained increase in soil C over the first two years following treatment initiation. We saw improvement in soil properties that have been shown to be directly related to site quality such as increased CEC, pH, base saturation, and decreased bulk density. Although these effects will likely lead to improved long-term site quality, in the short-term, the incorporation of LR resulted in N immobilization, but to different degrees between our contrasting genotypes. We found that both MBN and tree growth data show genotypic differences in the degree of N immobilization. Evidence from genotypic differences in biomass partitioning and fine-root length led us to conclude that the main driving factor between our two genotypes was that CL93 had less of a demand for N than CL32. In support of our hypothesis, our data showed a strong genotype by soil amendment interaction over all sampling dates with the factors (heterotrophic or root respiration) driving these differences also changing. Microbial respiration data and known biomass partitioning data collected in an accompanying study led us to conclude that the amount of root biomass present was the main factor controlling $F_S$. Our results indicate that our incorporated organic material is stable and slow to decompose, suggesting that some amount of this material will remain at the end of the rotation. Further, combining LR treatments and planting of genotypes with low nutrient demand or high efficiency may increase SOM while avoiding loss of NPP due to nutrient immobilization. This work underlines the importance of site-specific silviculture matched with appropriate genotypes that has been expressed by others (Fox 2000; Roth et al. 2007). Future work should focus on long-term effects of organic matter incorporation as well as the differences in microbial community between soil amendments and genotypes.
Acknowledgments

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Table 5.1. Mean ± std. err. soil chemistry for logging residue (LR) incorporation by fertilization (F) interactions.

<table>
<thead>
<tr>
<th>Nutrient addition</th>
<th>Total C %</th>
<th>Total N %</th>
<th>P ppm</th>
<th>C:N ratio</th>
<th>soil pH</th>
<th>CEC cmol kg⁻¹</th>
<th>Base Sat %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Samples collected winter 2005 (Pre-fertilization)</strong></td>
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<td></td>
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<td>5 ± 0.7</td>
<td>35 ± 2.8</td>
<td>4.04 ± 0.03</td>
<td>6.68 ± 0.62</td>
<td>12.18 ± 1.51</td>
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<td>5 ± 0.2</td>
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<td>7.55 ± 0.51</td>
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<td>3.99 ± 0.04</td>
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<tr>
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<td>14 ± 1.6</td>
<td>45 ± 3.0</td>
<td>4.13 ± 0.03</td>
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<td>15.38 ± 1.54</td>
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<tr>
<td><strong>Samples collected summer 2006</strong></td>
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<td>4 ± 0.2</td>
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<td>3.87 ± 0.05</td>
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<td>LR</td>
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<td>3.99 ± 0.05</td>
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<td><strong>Averaged over all sampling dates</strong></td>
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<td>3.88 ± 0.03</td>
<td>7.74 ± 0.25</td>
<td>11.78 ± 0.52</td>
</tr>
<tr>
<td>LR</td>
<td>5.15 ± 0.33</td>
<td>0.13 ± 0.01</td>
<td>5 ± 0.3</td>
<td>41 ± 1.8</td>
<td>4.02 ± 0.02</td>
<td>7.88 ± 0.28</td>
<td>14.77 ± 0.48</td>
</tr>
<tr>
<td>LR+F</td>
<td>5.34 ± 0.39</td>
<td>0.13 ± 0.01</td>
<td>12 ± 1.1</td>
<td>40 ± 1.6</td>
<td>4.06 ± 0.02</td>
<td>7.93 ± 0.28</td>
<td>14.25 ± 0.69</td>
</tr>
</tbody>
</table>
Table 5.2. *P*-value table for selected soil properties in the fine-soil fraction. All analyses on soil properties were performed with an autoregressive covariance structures using PROC MIXED in SAS version 9.1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total C(^1)</th>
<th>Total N(^1)</th>
<th>C:N(^1)</th>
<th>P(^2)</th>
<th>pH</th>
<th>Base Sat(^3)</th>
<th>CEC(^2)</th>
<th>SM(^3)</th>
<th>TC(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between-subject parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR</td>
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<td>n.s.</td>
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<td>n.s.</td>
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<td>n.s.</td>
<td>0.02</td>
<td>0.04</td>
</tr>
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<td>n.s.</td>
<td>0.03</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.09</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>F</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>&lt;0.0001</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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<td>n.s.</td>
<td>n.s.</td>
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<td>n.s.</td>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Within-subject parameters</strong></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIME</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<td>n.s.</td>
<td>n.s.</td>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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</tr>
<tr>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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<tr>
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<td>n.s.</td>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
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<td>n.s.</td>
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<tr>
<td>LRxCLxFxTIME</td>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

1 Determined on fine-soil fraction on 5 dates by the USFS SRS with Carlo-Erba elemental analyzer (Model NA 1500; Fison Instruments, Danvers, MA).
2 Determined on fine-soil fraction on 5 dates by Virginia Tech Soil Testing Laboratory (Blacksburg, VA) using Mehlich 1 extracting solution and analyzed using an inductively coupled plasma atomic emission spectrometer (Thermo Elemental ICAP 61E).
3 Volumetric soil water content (SM) and soil temperature (TC) Measured in situ on 16 sampling dates at a depth of 15 cm and averaged over 13 cm depth, respectively.
Table 5.3. Correlation between index of heterotrophic respiration and soil properties on four separate sampling dates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Statistic</th>
<th>Summer 06</th>
<th>Winter 06</th>
<th>Summer 07</th>
<th>Winter 07</th>
</tr>
</thead>
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<tr>
<td>MBC</td>
<td>Correlation</td>
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<td>--</td>
<td>0.42</td>
<td>0.46</td>
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<tr>
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<td>P-value</td>
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<td>n.s.</td>
<td>0.04</td>
<td>0.02</td>
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<td>--</td>
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</tr>
<tr>
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<td>--</td>
<td>0.34</td>
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<tr>
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<td>P-value</td>
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<td>n.s.</td>
<td>0.10</td>
</tr>
<tr>
<td>C:N</td>
<td>Correlation</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<tr>
<td></td>
<td>P-value</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>C:P</td>
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<td>0.37</td>
<td>--</td>
<td>--</td>
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<tr>
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<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>CEC</td>
<td>Correlation</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Base Sat.</td>
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<td>--</td>
<td>0.37</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.04</td>
<td>n.s.</td>
<td>0.08</td>
<td>0.05</td>
</tr>
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</table>
Figure 5.1. Mean soil bulk density between logging residue treatments for three soil depths. Five subsamples were taken to determine the plot average. Statistical tests were performed using analyses of variance with soil depth treated as a split-plot (n = 6). Error bars represent ± standard error from the mean, significance at the 0.10, 0.05, and 0.001 alpha level is indicated by single (*), double (**), and triple asterisks (***) respectively.
Figure 5.2. Mean percent volumetric soil water content by logging residue (LR) treatments. Dates were collected over two years (error bars represent ± one standard error from the mean) using time domain reflectometry averaged over a depth of 13 cm. Inset graph shows mean (n = 48) depth (cm) to reduced zone using rusty rod technique.
Figure 5.3. Mean microbial biomass C content for clone (CL) and fertilizer treatments measured four times over the course of the experiment by chloroform fumigation extraction-procedure. Bars represent an average of six observations and error bars represent ± one standard error from the mean.
Figure 5.4. Mean microbial biomass N content for clone (CL) by fertilizer (Panel A) and CL by logging residue (LR; Panel B) interactions measured four times over the course of the experiment by chloroform fumigation extraction-procedure. Bars represent an average of six observations and error bars represent ± one standard error from the mean.
Figure 5.5. Mean total soil CO$_2$ efflux ($F_S$) for soil amendment by genotype interaction. Bars represent $F_S$ rates measured over 13 sampling dates from January 2006 thru December 2007 and error bars indicate ± one standard error of the mean.
Figure 5.6. Relationship between total soil CO₂ efflux ($F_S$) and soil temperature ($TC$) taken at 15 cm soil depth and percent volumetric soil water content ($SM$) averaged over 13 cm soil depth. Points and drop lines represent the actual daily a means ($n = 24$) and the response grid was generated using the following model created based on the observed data. The relationship between $F_S$, $TC$, and $SM$ was modeled using the following second order exponential equation: $F_S = \beta_0 \times \exp(\beta_1 \times TC) \times SM^{\beta_2}$ where $\beta_0 = 1.62 \pm 0.95$, $P = \text{n.s.}$; $\beta_1 = 0.04 \pm 0.01$, $P = 0.004$; and $\beta_2 = 0.29 \pm 0.15$, $P = 0.08$) determined using Gauss-Newton non-linear procedure. A modified $r^2$ was calculated using linear regression between $F_S$ and the predicted value determine by the model. The inset shows a plot of the residuals.
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Figure 5.7. Mean index of heterotrophic (microbial) respiration ($R_H$) genetic by logging residue (LR) two way interaction. Bars represent an average of 90 $R_H$ rates measured 16 times from February 2006 thru December 2007 and error bars represent ± one standard error of the mean.
Figure 5.8. Mean logging residue (LR) by time (Panel A) and genotype (CL) by time (Panel B) interactions for index of heterotrophic (microbial) respiration ($R_H$). Inset represents average daily temperature in degrees Celsius ($T_C$) taken at 15 cm soil depth and volumetric soil water content ($S_M$) average over 13 cm soil depth. Error bars represent ± one standard error of the mean.
References


Long, B.M. 1980. Soil Survey of Berkeley County, South Carolina. United States Department of Agriculture, Soil Conservation Service and Forest Service in cooperation with South Carolina Land Resources Conservation Commission and South Carolina agricultural Experiment Station, pp. 18-19, 24-25, Map 30.


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Genotype by nutrient effects on belowground C in the field


Chapter 5

Genotype by nutrient effects on belowground C in the field


Tyree, M.C. 2008c. Chapter 4: Impacts of soil organic matter and nutrient manipulations on soil properties and their influence on belowground C cycling in a large pot study utilizing two contrasting Pinus taeda L. clones In Forestry. Virginia Tech, Blacksburg, p. 44.
Chapter 5  Genotype by nutrient effects on belowground C in the field


Chapter 6

Synthesis of Results
The underlying theme of this research was to determine how genetics and nutrient availability influence C cycling in intensively managed pine forests. This work consisted of a two year field study and a one year complementary greenhouse study each of which was split into above- and belowground pools and fluxes. In all experiments, we manipulated nutrient availability by a factorial combination of composted logging residue (LR) incorporation into the mineral soil and fertilization with N and P. Two contrasting Pinus taeda genotypes were selected for each study based on differences in biomass partitioning and hypothesized differences in nutrient use efficiency. In the greenhouse experiment clonal seedlings (CL85 and CL93) were selected and donated by Mead Westvaco based on differences in growth strategies (Phil Dougherty personal communication). The field study utilized CL93, which represented the “narrow crown” ideotype (low leaf area) and CL32, which represented the “broad crown” ideotype (high leaf area) (Figure 3.1). The aboveground experiments were reported in chapters 2 and 3, while the belowground experiments were discussed in chapters 4 and 5. Data from these four experiments were then synthesized and estimates of treatment effects on C cycling were made for the greenhouse study. Our microcosm (greenhouse) study was used to make inferences about how these treatments may have affected C cycling in the field.

Aboveground pools and fluxes
In the greenhouse study we found large differences between genotypes in gas exchange parameters (Figure 2.7) and C partitioning patterns (Figure 2.3). Early in the experiment (first 3 months) CL93 expressed greater specific CO₂ assimilation under saturating light ($A_{Sat}$), but differences decreased and slightly reversed by the end of one year. Additionally, CL93 maintained greater stomatal conductance ($g_s$), transpiration ($E$), and internal to ambient CO₂ concentration ratio ($C_i/C_a$) throughout the one year experiment. By the end of one year, CL93 and CL85 showed similar seedling biomass, but CL93 appeared to invest more resources into producing branches and fine-roots at the expense of stem volume relative to CL85. Increased investment in branches and increased specific leaf area (cm² g⁻¹) led to
more photosynthetic area in CL93 relative to CL85 despite similar foliar biomass. Interestingly, when final gas exchange rates were scaled to the canopy level we found that both genotypes achieved similar canopy level CO$_2$ assimilation rates, but by different means. Under low N availability, CL93 produced greater leaf area with lower photosynthetic efficiency while CL85 used the opposite strategy of lower leaf area, but greater photosynthetic efficiency. However, at higher rates of N availability CL93 increased photosynthetic efficiency while CL85 increased leaf area.

Consistent with the greenhouse study, we found genotype by nutrient interactions in biomass partitioning and leaf physiology in the field. However CL93, which was common to both studies, responded differently to fertilizer between studies. For example, CL93 maintained less leaf area than CL32 under control conditions, but sharply increased leaf biomass with N and P fertilization, which allowed for greater gross CO$_2$ assimilation and greater stem volume (Figures 3.5 and 3.3C). The second major difference between the two studies was that CL93 was better able to tolerate nutrient limitations brought about by LR incorporation due to more favorable canopy architecture and perhaps due to lower belowground maintenance associated with fine-roots. This prevented CL93 from experiencing a decrease in stem growth as was seen in CL32 following LR incorporation. Our results clearly show that contrasting ideotypes have the potential to respond differently to differences in nutrient availability in terms of both partitioning, leaf physiology, and leaf biochemistry (Chapter 3).

Belowground pools and fluxes
Both studies showed short-term improvements to soil physical and chemical properties as well as increased soil C due to LR incorporation. One discrepancy between our two experiments was the level of LR incorporation. The greenhouse experiment incorporated LR at a rate equivalent to 25 Mg o.d. ha$^{-1}$ while in the field study the same rate was concentrated onto the beds, which equaled roughly 75 Mg o.d. ha$^{-1}$. Second, the there were large differences in the quality of the added substrate. Residue C:N was 700 for the field study and approximately 120 for the greenhouse study. This large difference in substrate quality combined with higher soil pH and soil fertility in the greenhouse study relative to the field study likely contributed to differences between studies. We concluded this difference in
application rate was responsible for finding no decrease in growth in the greenhouse study while finding signs of nutrient immobilization in the field study. In both the greenhouse and field experiment, we concluded that increased C loss by way of $F_S$ made up only a small percent of total C incorporated as LR. The most conservative estimates showed that it would take 15 years to fully decompose, although, we feel decreases in decomposition rates with time would substantially increase that amount of time (Chapter 4). Data from a two year field study, also led us to conclude that incorporated LR was stable and slow to decompose, suggesting that some percent of this material will remain at the end of the rotation. Further, combining LR treatments and planting of genotypes with low nutrient demand or high efficiency may increase $SOM$ while avoiding loss of stem volume from nutrient immobilization.

Microbial activity and aboveground productivity were the dominant biotic drivers of $F_S$ and explained a great deal of the temporal variation between soil amendment treatments in the greenhouse study, but correlated very poorly in the field study. In the greenhouse study, when LR was incorporated microbial activity exerted increased influence over $F_S$, whereas, application of fertilization resulted in greater explanatory power given to aboveground productivity. These data support findings by others that a strong connection exists between recently fixed C and soil respiration (Irvine et al. 2005; Ryan and Law 2005). Heterotrophic respiration correspondingly increased with LR and decreased with fertilizer additions as did $MBC$.

**Genotypic differences in $F_S$**

In the greenhouse experiment, $F_S$ did not differ between clones for most of the year, but on the final two sampling dates a pattern emerged of one clone showing greater $F_S$. We concluded that if this pattern should continue or become stronger with increased occupation of soil by roots that there may be large differences in C cycling between genotypes, but further study on mature trees is needed. In support of our greenhouse study, data from our field study showed a strong genotype by soil amendment interaction on $F_S$ when analyzed over all sampling dates. Microbial respiration data and known biomass partitioning data
collected in an accompanying study (Chapter 4) led us to conclude that the amount of root biomass present was the main factor controlling genotype effects on $F_S$.

**Treatment effects on estimated total C budget**

Data from chapter two and chapter four were combined to approximate a one year C balance for the greenhouse experiment. In the greenhouse experiment we did not find a significant difference between planted genotypes in overall C pools and fluxes, but did find an obvious, large increase in total C with the incorporation of LR (Figure 6.1). Overall, the increase in loss of C as $F_S$ was a small percentage of the C added as LR. Further, we did not find a reduction in above or belowground biomass as a result of adding LR relative to controls in the greenhouse study. Total soil C contained in the fine-soil fraction was calculated by multiplying the estimated volume of soil in each pot by the bulk density of soil with and without LR present. We then used percent soil C values measured at the end of the experiment to estimate total soil C. We found a dramatic increase in C contained within the fine fraction. Visual inspection showed that a significant amount of coarse organic material remained after one year, which would lead us to conclude that a large portion of total C in the fine fraction was due to C being allocated belowground. Fertilization also resulted in an overall increase in total C contained within each experimental unit relative to non-fertilized treatments (Figure 6.2). Fertilizer resulted in a combination of increased biomass as well as decreased $F_S$ relative to controls. We hypothesized the decrease in $F_S$ was due to both a decrease in microbial biomass ($MBC$) and activity ($R_H$).

Data from chapters three and five were used to roughly estimate the C balance for the field study two years after treatment initiation. Total soil CO$_2$ efflux was estimated by taking an average from all 13 sampling dates to account for changes over time and with varying temperature and moistures. In the field, we found strong genotype by nutrient availability interactions for many of the C pools and fluxes leading us to separate clones for each soil manipulation treatment. We found that all soil manipulation treatments increased total C balance (Mg C ha$^{-1}$; Figures 6.3 and 6.4). Total C loss through leaching was not measured in the field, but data from the greenhouse experiment led us to conclude that it made up a very small fraction of the total C lost (Chapter 4). Logging residue incorporation increased total C
much more than did fertilizer alone also; CL32 had a larger increase in total C than CL93, which would be largely attributed to differences in $F_S$. These data also show that although LR incorporation did not decrease overall net primary productivity in CL32, it did decrease biomass partitioning to merchantable products (main stem). Notably, values contained in graphs are comparing treatment differences relative to control treatments and not comparing between genotypes.

**Contributions to Current Body of Knowledge and Implications**

This research has contributed to the current body of knowledge in a number of ways. The use of two contrasting genotypes has led to a greater fundamental understanding of the factors contributing to canopy level CO$_2$ assimilation in young conifer species such as: amount of leaf area, the orientation of that leaf area, needle morphology, and specific net photosynthesis. A one year greenhouse study showed differences in leaf physiology (Figure 2.7), needle morphology (Figure 2.4), and biomass partitioning (Table 2.1 and Figure 2.3) between two contrasting clones. Interestingly, when net canopy CO$_2$ assimilation was measured on one sampling date at the end of the experiment, data showed both clones achieved similar estimated canopy CO$_2$ assimilation, but utilized different strategies. One genotype invested more resources to developing leaf area while maintaining relatively low leaf efficiency, while the other investing more in leaf efficiency and less in leaf area. We speculate that this convergence could be an upper limit for *Pinus taeda* for CO$_2$ assimilation. Both clones were considered superior genotypes that likely share very similar genetics.

Similar findings have been observed in clonal field trials (King et al. 2008), but the current research focused on how these changes were achieved. For example, one genotype maintained greater specific leaf area and a more open canopy to achieve greater canopy area, while maintaining similar leaf biomass.

Changes in one or more factors contributing to canopy CO$_2$ assimilation following manipulation of nutrient availability can differ between genotypes. These genetic by nutrient availability interactions can have a significant influence on the growth partitioning of a particular genotype under specific site conditions. This was clearly shown in a field experiment conducted over two years during site establishment using contrasting genotypes.
(Chapter 3). We showed strong differences between genotypes in biomass partitioning and leaf physiology. Under natural site fertility conditions both genotypes achieved similar rates of growth, but differed dramatically in their response to nutrient limitations or surpluses (Figure 3.3B and C). Differences in biomass partitioning, leaf area (Figure 3.5), and leaf physiology (Figure 3.9 and 3.10) related to changes in N demand between genotypes. Under situations where soil N was limited (LR treatments), the low leaf area and N demand genotype excelled while high leaf area and N demand genotype declined in growth. These findings have a practical application as well. Implication of this research underline the importance in clonal selection and ideotype development (Nelson and Johnsen 2008) in addition to silvicultural treatments when implementing site-specific silviculture to maximize productivity in intensively managed southern pine forests. Additionally, development of genotypes with greater nutrient uptake or more efficient nutrient utilization would give forest managers more options, which is particularly relevant now with sharp increases in the price of fertilizers.

Our field data supports the hypothesis made by Gough and Seiler (2004) involving the series of adjustments to leaf physiology following fertilization with N and P to lead to increased leaf area and growth. We measured short-term increase in specific net photosynthesis under saturating light following fertilization, with rates returning to control levels, and eventually decreasing below control levels throughout the experiment (Figure 3.8A). This was observed in both genotypes in the field, but not the greenhouse experiment (Figure 2.6A). We observed increased growth and leaf area in both experiments, which suggests that the amount of leaf area is the dominant factor in controlling C assimilation. However, this does not rule out the importance of specific net photosynthesis as a key mechanism allowing for these adjustments to be made.

Another finding which advances our understanding of C cycling is the influence of genotypes on total soil CO₂ efflux ($F_5$). This has both fundamental as well as applied implications. From a more fundamental perspective intra-species variation in biomass allocation has been shown in the field to consistently influence $F_5$ (Figure 5.5). The greenhouse experiment showed no difference between genotypes in $F_5$ throughout most the experiment, but as the
tree grew and occupied more of the site, differences were observed (Figure 4.6C). We hypothesized these differences were a result of differences in root respiration, soil microbial community composition, or both. Both experiments failed to show differences in microbial biomass or heterotrophic respiration leading us to conclude that root respiration was the main factor, but further research is needed. From an applied viewpoint, differences in $F_S$ between clones could lead to large differences in C budgets. Total soil CO$_2$ efflux is the second largest C flux in the terrestrial biosphere making it a major factor in any C budget. Therefore, future C models may have to account for differences between genotypes.

Finally, one of our treatments included the incorporation of logging residue into the mineral soil. This has been hypothesized by some (Buford et al. 1998; Sanchez et al. 2000; Sanchez et al. 2001; Sanchez et al. 2003) as a way of increasing soil organic matter, which has been shown to be a principal indicator of site quality (Powers et al. 1990) due to its many benefits to soil chemical, physical, and biological properties (Fisher and Binkley 2000) as well as a potential sink for atmospheric C (Lal 2003). We hypothesized short-term nutrient immobilization as a result of adding high C:N organic material belowground, but found conflicting results between our two experiments. For instance, we did not detect any decrease in growth in the greenhouse experiment (Chapter 2), but did find a decrease in growth in the field experiment (Chapter 3). We concluded there were a couple differences between studies contributing to conflicting results. One was the amount of LR applied differed substantially between studies. In fact, we incorporated approximately 1/3 the amount of LR in the greenhouse study as in the field study. Second, residue quality differed between studies. Residue C:N ratio was about seven times higher in the field study. Finally, soil fertility was substantially higher in greenhouse soil, which was previously an agricultural soil that had been previously planted with soybeans.

We found increases in soil microbial biomass C and heterotrophic respiration in both studies as well as improvements to soil physical and chemical properties, which has also been observed by others (Sanchez et al. 2000; Sanchez et al. 2001; Sanchez et al. 2003). The incorporation of LR did increase $F_S$, but interestingly, did not lead to substantial amount of decomposition. For example, increased C loss by way of $F_{Cont}$ and through leaching made up
only an estimated 7% of total C incorporated as LR. Our most conservative estimates showed that it would take 15 years to fully decompose, although, we feel decreases in decomposition rates with time would substantially increase that amount of time. Results from our field study indicate that our incorporated organic material is stable and slow to decompose, suggesting that some percent of this material will remain at the end of the rotation. Further, combining LR treatments and planting of genotypes with low nutrient demand or high efficiency may increase SOM while avoiding loss of net primary productivity do to nutrient immobilization.

**Future Work**

Based on findings from current research we are able to make some recommendations for future work. Future work should fall into four broad categories each of which could be divided into multiple experiments.

1. Intra-species variation in microbial community composition
2. Effects of nutrient availability on leaf gas exchange of a single cohort of needles.
4. Economic and C budgets on the effectiveness of logging residue incorporation as a silvicultural treatments.

*Intra-species variation in microbial community composition*

Differences in belowground allocation between *P. taeda* genotypes can impact a number of factors that may influence microbial community composition such as: fine-root turnover, root respiration, and root exudates. Our results raise the question of how one clone was able to maintain similar aboveground growth and foliar chemistry while maintaining less leaf area and fewer fine-roots. We hypothesize that there is intra-species variation in microbial communities and specifically differences in mycorrhizal associations between these two clones. For instance, data from the current work found weak correlations between \( R_H \) and MBC (Table 5.3). One reason for this is that our soil amendment treatments likely changed the composition of the microbial community, which may not be reflected in our MBC data. Changes in microbial community composition have been widely reported following changes
in available C and N (Bååth et al. 1978; Holland and Coleman 1987). Additionally, studies have shown decreases in fungal components following fertilization (Wallander and Nylund 1992; Lilleskov et al. 2002; Nilsson and Wallander 2003; Bittman et al. 2005). This experiment did not attempt to characterize the microbial community composition following application of soil amendments, but recently, researchers have begun to more fully appreciate the complex relationship between plants and soil fungi and the role that root exudates play in regulating microbial community (Broeckling et al. 2008). Several studies have shown intra-species differences in microbial community composition in forest species such as Populus angustifolia, which is a species with high variation between genotypes (Schweitzer et al. 2008) and in Betula pendula (Kasurinen et al. 2005). Pinus taeda has an extensive geographic range and large within species variation, making intra-specific differences in microbial community composition likely. Specifically, the three genotypes in our studies have been shown to vary in response to soil amendments with respect to biomass partitioning (Chapter 3), MBC, MBN, RH, and FS (current study) making these clones good candidates for studying intra-species microbial community composition.

**Effects of nutrient availability on leaf gas exchange of a single cohort of needles**

The question of the effects on fertilization was not fully explored on a single cohort of needles. We propose a short pot experiment utilizing two or more genotypes that have been shown to represent a range of leaf areas such as clone 93 and clone 32 used in the field study (Chapter 3). Seedlings will be grown for one year in small pots half receive base rates of nutrients while the other half receives a base fertilizer rate plus N and P. The first emerging needles following fertilization will be measured repeatedly (four to five times) for net gas exchange, needle chemistry (N, P, carbohydrates, etc.), and needle morphology until fully elongated. The purpose will be to establish if fertilization increases net CO₂ assimilation and dark respiration, or simply advances physiological state of the plant as a percentage of elongation. There are a number of ways this question could be expanded. This project could be expanded to include the monitoring a multiple flushes over the year. Variables associated with changes in leaf biochemistry that could be explored are chlorophyll, Rubisco, and carbohydrate content. Variables related to needle morphology are specific needle area, weight, length, and cell wall thickness.
Long-term monitoring of incorporated logging residue decomposition

Silvicultural treatments that manage SOM and the long-term sustainability of intensive pine silviculture are just in their infancy relative to what is known about other agriculture systems. However, future work is needed on the longer-term monitoring of tree growth, soil properties associated with site quality, and changes to SOM. There are a number of agriculture experiments that have shown increased decomposition following the incorporation of organic material into the mineral soil. A major limiting factor in the rate of decomposition is the quality of substrate. Logging residue from forest harvest is high in lignin and phenolic compounds, which are resistant to decomposition. Data from current research showing the slow rate of decomposition in the first couple years following incorporation suggests that some percentage of logging residue will remain at the end of the rotation. This could mean that following a couple rotations there could be a significant increase in soil organic material in the long-term, but longer-term experiments are still needed. Short-term decreases in growth due to nutrient immobilization can be offset by planting genotypes with high nutrient use efficiency and in the longer-term those nutrients will be slowly released as the material eventually decomposes.

Analysis of Economic and C Cost

Finally, the scope of the current work dealt solely with the affects of LR on soil properties and factors controlling decomposition and eventual loss of belowground C. However, the authors recognize there are practical considerations such as economic feasibility and C cost associated with equipment that should be considered prior to implementation of such a treatment. A cost analysis, in terms of equipment hours associated with the incorporation of LR, was performed by Sanchez et al. using two P. taeda plantations planted on different soil textures (2003). The authors found that with current technology it is not economically feasible ($US 521 ha$−$1$ and $US 633 ha$−$1$ on the 'sandy' and 'clay' sites, respectively), but the authors postulate that advances in efficiency and productivity may make it feasible in the future. An accounting of C costs associated with varying levels of intensive silviculture was performed by Markewitz (2006) illustrating the level of C accounting needed to access the viability of LR incorporation as a method for increasing soil C storage. We recommend that
a similar accounting be made for the incorporation of logging residue. Results from longer-term monitoring of decomposition rates, and the interaction between logging residue incorporation and other silvicultural prescriptions will be needed prior to such an accounting.
Figure 6.1. Carbon budget for greenhouse experiment following the incorporation of logging residue (LR) relative to treatments not receiving LR incorporation \((n = 24)\). *Pinus taeda* seedlings were planted in 170 L plastic containers and grown for one year in a greenhouse. Values contained in boxes represent the change in C (grams) for each pool or flux. Values in red indicate C pool or flux used to calculate total C balance. All values were measured at the end of one year.
Figure 6.2. Carbon budget for greenhouse experiment following fertilization with N and P relative to treatments not receiving nutrient additions \((n = 24)\). *Pinus taeda* seedlings were planted in 170 L plastic containers and grown for one year in a greenhouse. Values contained in boxes represent the change in C (grams) for each pool or flux. Values in red indicate C pool or flux used to calculate total C balance. All values were measured at the end of one year.
Figure 6.3. Relative C budget for field study two years following logging residue (LR) incorporation. Values in boxes represent changes in C content relative to control treatments (Mg C ha\(^{-1}\)) after two years. Values in red indicate pools and fluxes that were used to calculate total C budget. Biomass values and logging residue values were calculated by multiplying by 0.5.
Figure 6.4. Relative C budget for field study two years following fertilization with N and P. Values in boxes represent changes in C content relative to control treatments (Mg C ha\(^{-1}\)) after two years. Values in red indicate pools and fluxes that were used to calculate total C budget. Biomass values were calculated by multiplying by 0.5.
Figure 6.5. Relative C budget for Cross carbon study two years following logging residue (LR) incorporation and fertilization with N and P. Values in boxes represent changes in C content relative to control treatments (Mg C ha\(^{-1}\)) after two years. Values in red indicate pools and fluxes that were used to calculate total C budget. Biomass values and logging residue values were calculated by multiplying by 0.5.
References


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Comprehensive literature cited


Comprehensive literature cited


Long, B.M. 1980. Soil Survey of Berkeley County, South Carolina. United States Department of Agriculture, Soil Conservation Service and Forest Service in cooperation with South Carolina Land Resources Conservation Commission and South Carolina agricultural Experiment Station, pp. 18-19 , 24-25, Map 30.


Comprehensive literature cited


Comprehensive literature cited


Comprehensive literature cited


Comprehensive literature cited


Comprehensive literature cited


Appendix A

Composite Image of Lysimeter

Figure A.1. Composite image of 170 L plastic pot fitted with a brass boiler spigot.
Appendices

Appendix B
Soil Chemical Analyses of Greenhouse Project

Table B.1. Soil chemical properties for Eunola series soil (fine-loamy, siliceous, semiactive, thermic Aquic Hapludults) Soil was collected in February 2006 to a depth of approximately one meter, which included the Ap, BE, and Bt horizons. Soil was collected from the Virginia Tech Tide Water Agricultural Research and Extension Center located in Holland, VA. Soil samples were taken from each pot within a replication (n = 8) prior to fertilization and combined. Composite samples were tested at the Virginia Cooperative Extension Soil Testing Laboratory.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Zn</th>
<th>Mn</th>
<th>Cu</th>
<th>Fe</th>
<th>B</th>
<th>CEC</th>
<th>Acidity</th>
<th>Base Sat.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep 1</td>
<td>5.68</td>
<td>15</td>
<td>59</td>
<td>205</td>
<td>44</td>
<td>1.9</td>
<td>4.0</td>
<td>0.7</td>
<td>14.8</td>
<td>0.1</td>
<td>1.6</td>
<td>7.2</td>
<td>92.8</td>
</tr>
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<td>Rep 2</td>
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<td>16</td>
<td>63</td>
<td>198</td>
<td>42</td>
<td>1.9</td>
<td>1.8</td>
<td>0.8</td>
<td>16.6</td>
<td>0.1</td>
<td>1.7</td>
<td>13.8</td>
<td>86.2</td>
</tr>
<tr>
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<td>14</td>
<td>63</td>
<td>190</td>
<td>42</td>
<td>1.9</td>
<td>1.4</td>
<td>0.8</td>
<td>14.8</td>
<td>0.1</td>
<td>1.5</td>
<td>4.0</td>
<td>96.0</td>
</tr>
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<td>Rep 4</td>
<td>5.64</td>
<td>15</td>
<td>58</td>
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<td>39</td>
<td>1.4</td>
<td>1.3</td>
<td>0.9</td>
<td>17.3</td>
<td>0.1</td>
<td>1.5</td>
<td>7.9</td>
<td>92.1</td>
</tr>
<tr>
<td>Rep 5</td>
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<td>15</td>
<td>52</td>
<td>187</td>
<td>38</td>
<td>1.2</td>
<td>1.1</td>
<td>0.8</td>
<td>15.9</td>
<td>0.1</td>
<td>1.4</td>
<td>4.2</td>
<td>95.8</td>
</tr>
<tr>
<td>Rep 6</td>
<td>5.65</td>
<td>15</td>
<td>54</td>
<td>192</td>
<td>40</td>
<td>1.2</td>
<td>1.3</td>
<td>0.8</td>
<td>13.7</td>
<td>0.1</td>
<td>1.7</td>
<td>14.3</td>
<td>85.7</td>
</tr>
<tr>
<td>Average</td>
<td>5.66</td>
<td>15.0</td>
<td>58.2</td>
<td>192.8</td>
<td>4.8</td>
<td>1.6</td>
<td>1.8</td>
<td>0.8</td>
<td>15.5</td>
<td>0.1</td>
<td>1.6</td>
<td>8.6</td>
<td>91.4</td>
</tr>
<tr>
<td>Coeff. Var.</td>
<td>1.3</td>
<td>4.2</td>
<td>7.8</td>
<td>3.9</td>
<td>5.5</td>
<td>22.4</td>
<td>60.2</td>
<td>7.9</td>
<td>8.6</td>
<td>0.0</td>
<td>7.7</td>
<td>52.9</td>
<td>5.0</td>
</tr>
</tbody>
</table>

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## Appendix D

### Stand History of Cross Carbon Study Site

Table D.1. Stand history for the Cross Carbon Study located outside of Summerville, SC. Data compiled by Steve Patterson.

<table>
<thead>
<tr>
<th>Date</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Previous stand</strong></td>
<td></td>
</tr>
<tr>
<td>1983</td>
<td>Site prep (shear, rake, and bed)</td>
</tr>
<tr>
<td>1984</td>
<td>Planted (superior loblolly pine)</td>
</tr>
<tr>
<td>1994 January</td>
<td>Prescribed burn</td>
</tr>
<tr>
<td>1996 January</td>
<td>Prescribed burn</td>
</tr>
<tr>
<td>1998 April</td>
<td>Application of fertilizer (200 and 25 lbs P/ ac as Urea and DAP)</td>
</tr>
<tr>
<td>2004 May</td>
<td>Stand harvested</td>
</tr>
<tr>
<td><strong>Current study</strong></td>
<td></td>
</tr>
<tr>
<td>2004 July</td>
<td>Forest floor removal (hand raked) and hand spread to 2X plots. Plots strip sheared on 14 foot centers.</td>
</tr>
<tr>
<td>2004 Aug – Nov</td>
<td>Debris material spread to 1X and 2X plots</td>
</tr>
<tr>
<td>2004 November</td>
<td>Plots double bedded</td>
</tr>
<tr>
<td>2005 January</td>
<td>Planting of container grown varietal loblolly pines</td>
</tr>
<tr>
<td>2005 March</td>
<td>Application of Permethrin 3.2 EC (4 oz/ gallon of water)</td>
</tr>
<tr>
<td>2005 April</td>
<td>Helicopter broadcast of Arsenal and Escort (4 and 0.5 oz/ 15 gallons, respectively)</td>
</tr>
<tr>
<td>2005 June</td>
<td>Spot spraying Oust (2 oz/ ac)</td>
</tr>
<tr>
<td>2005 July</td>
<td>Application of Waylay 3.2 AB (3.13% solution)</td>
</tr>
<tr>
<td>2005 August</td>
<td>Application of Permethrin (4 oz/ gallon of water) and 0.5% Arsenal, 5% glyphosate, and 0.25% Entry applied to beds</td>
</tr>
<tr>
<td>2005 September</td>
<td>0.5% Arsenal, 5% glyphosate, and 0.25% Entry applied to interbeds</td>
</tr>
<tr>
<td>2005 March</td>
<td>Oust and Escort applied to both beds and interbeds.</td>
</tr>
<tr>
<td>2006 April</td>
<td>Application of Waylay 3.2 (4.1 oz/ 3 gallons of water) and low level of fertilizer (59.75 kg N and 33.18 kg P ha⁻¹ DAP and NH₄NO₃)</td>
</tr>
<tr>
<td>2006 May</td>
<td>Application of high rate of fertilizer (149.38 kg N and 82.95 kg P ha⁻¹ DAP and NH₄NO₃)</td>
</tr>
<tr>
<td>2007 March</td>
<td>Application of fertilizer at a rate of 200 kg N ha⁻¹ in the form of NH₄NO₃.</td>
</tr>
<tr>
<td>2008 January</td>
<td>Destructive harvest of one tree per plot</td>
</tr>
</tbody>
</table>
Appendices

Appendix E

CO₂ Assimilation – CO₂ Partial Pressure Response Curves

Steps used to fit net photosynthesis – internal CO₂ partial pressure response curves (A/Cᵢ).
Carbon dioxide response curves were fitted using free online software developed by Sharkey et al. (2007), which can be downloaded from the following website: http://www.blackwellpublishing.com/plantsci/pcecalculation. The following is my interpretation of what is contained within the full article. For further explanation of equations and procedures see following article and references cited within (Sharkey et al. 2007).

Figure E.1. Net assimilation-CO₂ response curve showing three possible limitations of assimilation (A) at different levels of internal CO₂ concentrations. Graph adapted from A/Cᵢ curve fitting software (Sharkey et al. 2007).

For each A-Cᵢ pair (blue dot) one of three possible limitations to CO₂ assimilation (A) must be determined based on shape of curve. The three possible limitations are i.) Rubisco-limited state, ii.) RuBP regeneration-limited state, and iii.) triose phosphate use limited state (TPU). Rubisco-limited state is the portion of the curve that shows a linear relationship between A and internal CO₂ concentration (Cᵢ) shown by line 1 (red line in above figure). It is up to the user to determine the point at which the curve leaves this Rubisco-limited state and goes into
the RuBP regeneration-limited state \((x)\), typically \(< 200 \mu\text{mol mol}^{-1}\), but varies with each curve. The RuBP-regeneration-limited state, which typically occurs at a \(C_i\) between \(x\) and \(y\), is determined by the ability to reduce RuBP so it can be used in the carboxylation reaction. This portion of the curve is shown by line 2 (green line) in the figure above. The third limiting state is the \(TPU\) is the upper asymptote if one is present. Briefly, then the generation of products of the chloroplast out paces the ability of the leaf to use those products generally triose phosphates although glycine or serine use may also be included.

The following equations were used to estimate maximum carboxylation rate of Rubisco \((V_{C,\text{max}})\), maximum rate of RuBP regeneration \((J_{\text{max}})\), leaf respiration \((R_{L})\), triose phosphate use \((TPU)\), and mesophyll conductance \((g_m)\).

1. Rubisco limiting step (line 1)

\[
A = V_{C,\text{max}} \left[ \frac{C_c - \Gamma^*}{C_c + K_c (1 + O / K_o)} \right] - R_L
\]

Where \(A\) is net \(\text{CO}_2\) assimilation, \(K_c\) and \(K_o\) are the Michaelis constant of Rubisco for \(\text{CO}_2\) and \(\text{O}_2\), respectively, and \(O\) the partial pressure of \(\text{O}_2\) at the site of Rubisco. \(R_L\) is leaf respiration in the absence of photorespiration \((\Gamma^*)\), which are the \(y\)- and \(x\)-axis, respectively. \(C_c\) is the \(\text{CO}_2\) partial pressure within the chloroplast calculated using the following equation:

\[
C_c = C_i - A / g_m
\]

Where \(C_c\) is \(\text{CO}_2\) partial pressure at the site of Rubisco, \(C_i\) is internal \(\text{CO}_2\) partial pressure and \(g_m\) is mesophyll conductance, which can either be directly measured or calculated using a number of techniques explained in a review by Warren (2006). Maximum carboxylation rate of Rubisco is the slope of the linear portion of the curve (line 1; above figure).

2. RuBP regeneration-limiting step (line 2)
Appendices

\[ A = J_{\text{max}} \frac{C_c - \Gamma^*}{4C_c + 8\Gamma^*} - R_L \]  

[E.3]

Where \( J_{\text{max}} \) is the maximum rate of RuBP regeneration determined by the maximum rate of electron transport. \( \Gamma^* \) is photorespiration assuming 4 electrons per carboxylation and oxygenation.

3. **TPU-limiting step** (line 3)

\[ A = 3TPU - R_L \]  

[E.4]

4. Estimation of mesophyll conductance was calculated internally by the software, but briefly the technique uses a non-linear curve-fitting program using information from both the Rubisco-limited and RuBP regeneration-limited steps. This is done by fitting both equation 1 and 3 by substituting \((C_i - A/g_m)\) for \(C_c\) and minimizing the sum of squares. From this an estimation of \(g_m\) can be made from the data (Sharkey et al. 2007). As already mentioned this is just one of numerous techniques that can be used to estimate \(g_m\), which are discussed further by Warren (2006).

5. Temperature functions were used to adjust temperatures to 25°C causing the adjustment to be 1 at 25°C. The equations are shown below and the scaling parameters are found in the table E.2, which was copied from Sharkey et al. (2007) with appropriate credit cited within.

\[ Parameter = e^{\left(\frac{\Delta H}{R \cdot T}\right)} \]  

[E.5]

\[ Parameter = \frac{e^{\left(\frac{\Delta H}{R \cdot T}\right)}}{1 + e^{\left(\frac{\Delta S - \Delta H}{R \cdot T}\right)}} \]  

[E.6]

Where \( R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1} \) and \(0°C = 273.15\text{K}.\)
Table E.2. Table taken from Sharkey et al. (2007) showing the scaling functions for the temperature adjustment.

<table>
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<tr>
<th>Parameters used for fitting</th>
<th>25°C</th>
<th>c</th>
<th>ΔH_a</th>
<th>ΔH_d</th>
<th>ΔS</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_c (Pa)</td>
<td>27.238</td>
<td>35.9774</td>
<td>80.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K_o (kPa)</td>
<td>16.582</td>
<td>12.3772</td>
<td>23.72</td>
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<td></td>
</tr>
<tr>
<td>Γ_s (Pa)</td>
<td>3.743</td>
<td>11.187</td>
<td>24.46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Parameters used for scaling | | | | |
|-----------------------------|------|-------|------|------|-----|
| V_{C,max}                   | 1    | 26.355 | 65.33 |
| J_{max}                     | 1    | 17.71  | 43.9  |
| TPU                         | 1    | 21.46  | 53.1  | 201.8 | 0.65 |
| R_L                         | 1    | 18.7145 | 46.39 |
| g_m                         | 1    | 20.01  | 49.6  | 437.4 | 1.4  |


References


### Appendix F

**Foliar Chemistry of Cross Carbon Experiment**

Table F.1. Foliar nutrient analyses collected by USDA-US Forest Service, Southern Research Station. Least square means and pooled standard error are shown for each logging residue (LR) by clone (CL) interaction.

<table>
<thead>
<tr>
<th>Element</th>
<th>Critical(^1) value</th>
<th>Control</th>
<th>Control</th>
<th>LR</th>
<th>LR</th>
<th>Std. Err</th>
<th>P-values</th>
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<tr>
<td></td>
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<td></td>
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<td>TRT</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>CL</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TRT x CL</td>
</tr>
<tr>
<td>Collected January 2006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>1.2</td>
<td>1.66</td>
<td>1.57</td>
<td>1.59</td>
<td>1.53</td>
<td>0.06</td>
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<td>P (ppm)</td>
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<td>1351</td>
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<td>Mg (ppm)</td>
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<td>1251</td>
<td>1220</td>
<td>1198</td>
<td>45</td>
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<td>Ca (ppm)</td>
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<td>144</td>
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<tr>
<td>K (ppm)</td>
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<td>5480</td>
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<td>5334</td>
<td>166</td>
<td>n.s.</td>
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<tr>
<td>Collected January 2007</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>N (%)</td>
<td>1.2</td>
<td>1.23</td>
<td>1.20</td>
<td>1.27</td>
<td>1.35</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>P (ppm)</td>
<td>1200</td>
<td>1153</td>
<td>1013</td>
<td>1078</td>
<td>1142</td>
<td>57</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mg (ppm)</td>
<td>700</td>
<td>1225</td>
<td>1026</td>
<td>1174</td>
<td>1244</td>
<td>67</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ca (ppm)</td>
<td>1200</td>
<td>3772</td>
<td>3758</td>
<td>4883</td>
<td>5171</td>
<td>174</td>
<td>0.0004</td>
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<tr>
<td>K (ppm)</td>
<td>3500</td>
<td>5542</td>
<td>4614</td>
<td>6546</td>
<td>5449</td>
<td>639</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

\(^1\)Critical values for *P. taeda* taken from N.C. Cooperative Extension Service.
Appendices

Appendix G

Diagram of Treatment Layout of Greenhouse Study

Figure G.1. Diagram showing treatment layout of greenhouse study. Numbers to left of rows indicate replication numbers and codes within each box represent experimental units (EU). Top, middle, and bottom codes within each EU represent clone (85 and 93), logging residue (“cont” and “om”), and fertilization (“none” and “fert”) treatments, respectively.
Appendix H

Composite Drawing Wooden Dowel Rods

Figure H.1. Diagram and dimensions of wooden dowel rods that were installed in each pot for the greenhouse experiment. Company info where dowels were purchased: Bear Woods Supply Co. Inc. www.bearwood.com. Dowels were identified by Dr. Zink-Sharp as hard pine, but that is all the further she could identify them.
Appendices

Appendix I

Partial Analyses of Variance Tables for Belowground Greenhouse Study (CH4)

Table I.1. Average monthly total soil CO$_2$ efflux rate measured from April 2, 2006 thru May 18, 2007 using the automated C exchange system (ACES). Missing dates were filled in using the average value of the two closest dates. Data was log transformed to meet assumption of equal variance and the compound symmetry covariance matrix was selected based on fit statistics included in the SAS output.

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<td>6</td>
<td>94.53</td>
<td>&lt;.0001</td>
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<tr>
<td>F</td>
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<td>6</td>
<td>10.98</td>
<td>0.02</td>
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<td>CL$^1$</td>
<td>1</td>
<td>2</td>
<td>2.97</td>
<td>0.23</td>
</tr>
<tr>
<td>LR$^*$F</td>
<td>1</td>
<td>6</td>
<td>2.46</td>
<td>0.17</td>
</tr>
<tr>
<td>CL$^*$LR$^2$</td>
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<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>CL$^*$F$^2$</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>CL$^<em>$F$^</em>$LR$^2$</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<tr>
<td>TIME</td>
<td>13</td>
<td>104</td>
<td>119.48</td>
<td>&lt;.0001</td>
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<td>LR$^*$TIME</td>
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<td>104</td>
<td>1.63</td>
<td>0.09</td>
</tr>
<tr>
<td>F$^*$TIME</td>
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<td>104</td>
<td>2.65</td>
<td>0.003</td>
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<td>CL$^*$TIME$^1$</td>
<td>13</td>
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<td>0.15</td>
<td>1.00</td>
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<td>LR$^<em>$F$^</em>$TIME</td>
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<td>0.99</td>
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<td>LR$^<em>$CL$^</em>$TIME$^2$</td>
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<td>--</td>
<td>--</td>
<td>--</td>
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<tr>
<td>CL$^<em>$F$^</em>$TIME$^2$</td>
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<td>--</td>
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<td>--</td>
</tr>
<tr>
<td>LR$^<em>$F$^</em>$CL$^*$TIME$^2$</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

$^1$Analyses does not include any EU with LR or fertilizer additions.

$^2$Unable to perform analyses due to sampling limitations.
Table I.2. Point-in-time monitoring of total soil CO$_2$ efflux rate measured nine times from August 2006 thru June 2007 using a Li-Cor 6200 Infra-red gas analyzer. Data was log transformed to meet assumption of equal variance and the compound symmetry covariance matrix was selected based on fit statistics included in the SAS output.

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<th>Den df</th>
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<td>66.52</td>
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<td>35</td>
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<td>1</td>
<td>35</td>
<td>0.08</td>
<td>0.78</td>
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<td>1</td>
<td>35</td>
<td>1.22</td>
<td>0.28</td>
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<tr>
<td>CL*LR</td>
<td>1</td>
<td>35</td>
<td>0.32</td>
<td>0.57</td>
</tr>
<tr>
<td>CL*F</td>
<td>1</td>
<td>35</td>
<td>0.00</td>
<td>0.99</td>
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<tr>
<td>CL<em>F</em>LR</td>
<td>1</td>
<td>35</td>
<td>0.00</td>
<td>0.99</td>
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<td>TIME</td>
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<td>320</td>
<td>111.62</td>
<td>&lt;.0001</td>
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<td>320</td>
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<td>0.04</td>
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<tr>
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<td>320</td>
<td>1.99</td>
<td>0.05</td>
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<td>320</td>
<td>2.04</td>
<td>0.04</td>
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<td>320</td>
<td>1.28</td>
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<td>8</td>
<td>320</td>
<td>0.88</td>
<td>0.53</td>
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<td>8</td>
<td>320</td>
<td>0.22</td>
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Appendices

Table I.3. Index of heterotrophic respiration measured 10 times from July 2006 thru June 2007 using a Li-Cor 6250 Infra-red gas analyzer. Data was log transformed to meet assumption of equal variance and the compound symmetry covariance matrix was selected based on fit statistics included in the SAS output.

<table>
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<td>0.04</td>
<td>0.83</td>
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<td>0.64</td>
<td>0.43</td>
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<td>0.00</td>
<td>0.99</td>
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<td>1.73</td>
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Appendices

Table I.4. Microbial biomass C estimated from chloroform fumigation-extraction procedure. Soil samples were collected in the February 2007 and July 2007.

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<td>0.00</td>
<td>0.95</td>
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<td>0.05</td>
<td>0.82</td>
</tr>
<tr>
<td>F* TIME</td>
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<td>38</td>
<td>0.39</td>
<td>0.53</td>
</tr>
<tr>
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<td>0.49</td>
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<td>0.37</td>
<td>0.55</td>
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<td>LR<em>F</em>CL* TIME</td>
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<td>38</td>
<td>0.92</td>
<td>0.34</td>
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</table>
Table I.5. Index of decomposition of yellow pine dowel rods buried vertically from July 2006 thru July 2007. Two jointed dowel rods (Appendix H) were buried in each experimental unit and their percent decomposition averaged before statistical analyses. Data was log transformed by the arcsine of the square root to meet assumption of equal variance and normality and analyzed using ANOVA.

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Table I.6. Cumulative monthly C loss as CO$_2$ from the soil surface data collected from April 2, 2006 thru May 18, 2007 using the automated C exchange system (ACES). Data was transformed by its square root to meet assumption of equal variance and the autoregressive 1 covariance matrix was selected based on fit statistics included in the SAS output.

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<td>0.41</td>
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</tr>
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<td>CL*F$^2$</td>
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</tr>
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<td>--</td>
<td>--</td>
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<td>574.80</td>
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<td>8.85</td>
<td>&lt;.0001</td>
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<td>104</td>
<td>1.31</td>
<td>0.22</td>
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<td>0.63</td>
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<td>1.00</td>
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<td>--</td>
<td>--</td>
</tr>
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<tr>
<td>LR<em>F</em>CL*TIME$^2$</td>
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<td>--</td>
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</tr>
</tbody>
</table>

$^1$Analyses does not include any EU with LR or fertilizer additions.

$^2$Unable to perform analyses due to sampling limitations.
Table I.7. Cumulative monthly C loss (grams) through leachate measured on 12 occasions from June 2006 through June 2007. Data was transformed by its natural log to meet assumption of equal variance and the autoregressive 1 covariance matrix was selected based on fit statistics included in the SAS output.

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<td>0.35</td>
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<td>35</td>
<td>1.18</td>
<td>0.28</td>
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<tr>
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<td>0.00</td>
<td>0.98</td>
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Appendices

Appendix J

Direct Comparison between Point-In-Time and Automated Methods of Measuring Total Soil CO₂ Efflux

We made direct comparisons of paired $F_S$ rates by regressing Li-Cor 6200 with the ACES data sampled on eight separate dates. There was a weak relationship observed ($R^2 = 0.12$; slope = 0.30; n = 119 data pairs) between the two machines when all data pairs were used. Overall, the average $F_S$ rate measured using the ACES were significantly ($P < 0.0001$) greater than the average rate measured with the Li-Cor 6200 (1.43 ± 0.09 and 0.87 ± 0.07 μmol CO₂ m⁻² s⁻¹, respectively). Further regression analyses for each sampling date showed a large range in the relationship between these two methods. Approximately half the eight sampling dates showed a good agreement between methods while the other half showed a very weak relationship with the slope not being significantly different from zero.
Appendices

Figure J.1. Comparison of total soil CO$_2$ efflux ($F_S$) estimates using the Automated C Efflux System (ACES; open-system IRGA) and the Li-Cor 6200 (closed-system portable IRGA). Fifteen paired data points were taken on eight separate sampling dates from August 2006 through May 2007. The solid line represents the linear relationship between the paired data and dotted line represents the 1:1 relationship.