Growth and Physiological Responses to Fertilizer Application in Clonal Loblolly Pine

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

Doctor of Philosophy
In
Forestry

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June 16, 2010
Blacksburg Virginia

Keywords: allometry, carbon allocation, photosynthesis, respiration, plantation forestry

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ABSTRACT

More than 20 million clonal loblolly pines have been planted throughout the southeastern United States. Fertilizer has been applied to more than 6.5 million hectares of plantations to alleviate deficiencies of nitrogen and phosphorus that limit growth. Because cloning loblolly pine in large numbers has only become possible in the last decade, it is unknown how clones may respond differently to fertilizer application. Growth, growth efficiency, and biomass partitioning responses to fertilizer application were investigated among 25 clones planted in the Virginia Piedmont. Closely related clones varied in their fertilizer stem volume responses, but not enough to be statistically significant ($p = 0.11$). Clones varied in growth efficiency and partitioning to individual tissues, but clone-by-fertilizer interactions were not observed. Clonal variability was observed in root morphology, and maximum rooting depth showed a significant clone-by-fertilizer interaction. Clones with rapid growth rates can be selected with a range of other desirable traits.

Short-term (i.e. weeks) responses to fertilization are often inconsistent with long-term (i.e. years) responses, but are critical to understanding growth responses. We investigated carbon allocation in two full-sibling clones of loblolly pine under two levels of fertilizer application over four months in a greenhouse. Using monthly harvests of some trees and ecophysiological measurements throughout, we determined carbon allocation on a monthly scale. In response to fertilizer application, both clones reduced allocation belowground and increased allocation to foliage to some extent, increasing whole-canopy photosynthetic capacity. However, these changes in allocation were ephemeral. By the end of the experiment, root-shoot ratios were no longer significantly affected by fertilizer application. Clones had allocation patterns distinct from one another, with one allocating more belowground and the other allocating more to stem mass. While their overall growth responses to fertilizer application were similar, the physiological mechanisms that resulted in these responses were different between clones.

Results of the two studies indicate that while fertilizer responses may not need to be included when testing clones for deployment, knowledge of the fertilizer responses of widely-deployed clones would offer forest managers opportunities to apply clone-specific precision-silvicultural systems to optimize growth rates and manage for a range of products.

Acknowledgements

Many people were critical to the efforts that culminated in this manuscript, but none more so than John Seiler and Tom Fox. John motivated me by example with his intense level of energy directed towards research, his passion for teaching, and his constant drive to seek sound explanations for any unexplained observation. Tom inspired in me the importance of clearly communicating complex results, critically and skeptically thinking about the real-world significance of data, and tackling complex problems both thoughtfully and with a dedicated work ethic. Both provided me with countless opportunities and funding to attend conferences, workshops, and field tours that broadened my experiences and connections. I am thankful to have had the opportunity to work for both of them.

The members of my dissertation committee, Chris Maier, Mike Aust, and Amy Brunner have provided insightful thoughts and criticisms on my research at various stages in this process. This manuscript and my own understanding of the topics herein are better thanks to their efforts, both in the classroom and in numerous meetings with each. I'd like to particularly thank Chris Maier for making several trips to and from Blacksburg for preliminary exams and data meetings.

In my three years at Virginia Tech John Peterson, Tal Roberts, Dave Mitchem, Kathie Hollandsworth, Sue Snow, and Tracey Sherman all provided critical logistical support.

The enormous undertaking that was the Reynolds clone-by-fertilizer biomass harvest would not have been possible without the help of countless individuals. First and foremost, John Peterson spent many days working underground, whether during the root harvest in the field or the month of needle picking in a basement in Blacksburg. I'd still be processing samples were it not for his dedication and bull-headed work ethic. The Reynolds Homestead staff, including Kyle Peer, Ben Templeton, Clay Sawyers, and Debbie Bird was instrumental throughout both the above and belowground harvests. Dan Evans, Joe Secoges, Tyler Wright, and Jake Carter also spent days in the field moving trees and digging roots.

The voluntary efforts of friends and fellow graduate students accounted for more than a third of the 240 hand-numbing hours spent processing needle samples from the Reynolds harvest, which

included time spent working late into Friday nights and on weekends. The following people all helped with needle picking or unloading some of the 1000 grocery bags full of samples into the Latham drying oven: Tyler Wright, Bonnie Stovall, Danielle Gift, John Seiler, James Freeman, Jess Walker, Scott Bates, Claudia Cotton, Joe Secoges, Alecia Peduzzi, Matt Brinkman, Jesse Reed, Rebecca Reed, Terry Vogler, Logan Park, Sara Murrill, Katie Hoover, Salli Dymond, Kathie Hollandsworth, and Richard Oderwald. Thanks to John James for providing much needed storage for all the samples. Matthew Seiler spent many hours in the head shed processing the root samples we collected. Colleen Carlson offered some much needed advice on statistical methods, and previous work on this trial conducted by Colleen, Mike Tyree and Nathan King proved extremely useful in interpreting some of our results.

Soil used in the greenhouse experiment was donated by the USDA Forest Service from the SETRES experiment. Thanks to Kurt Johnsen for allowing us to collect soil, and to Pete Anderson for his assistance in doing so. John Peterson, Bonnie Stovall, Kelly Merkl, and Matthew Seiler all put in significant efforts in sample processing for the greenhouse experiment. Chris Maier and Karen Sarsony kindly offered their assistance with the foliar N analyses.

Clonal material planted in these experiments was generously donated by the Forest Biology Research Cooperative and by Phil Dougherty and Jeff Wright at ArborGen. Funding for the work described on the following pages was provided by the NSF Center for Advanced Forestry Systems, the Forest Nutrition Cooperative, and the Virginia Tech Cunningham Doctoral Scholarship.

None of this would have been possible without the loving support of my wife, Bonnie. Bonnie spent innumerable hours picking needles, listening to me talk about projects, dealing with the mud and pine needles I tracked everywhere for months, and keeping me sane. Her ability to do all this while undertaking her own graduate work was all the more impressive.

Last, I'd like to thank my parents, John and Therese Stovall. Early on they inspired in me a love of the woods and analytical thought that has since determined the course of my career. Their continued support has been instrumental since long before I entered graduate school.

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1. The ecophysiology of *Pinus taeda* plantations: Responses of different genotypes to fertilizer application over varying temporal scales

1.1. Justification

Clonal plantations of eucalypts are currently common in South America. Improvements in somatic embryogenesis made for *Pinus taeda* (L.) in the last two decades will likely make this same trend a reality in North America in the foreseeable future. Clones will be deployed over large areas that encompass a gradient of site resources, and it may be possible to optimize growth by selecting individual clones adapted to specific site conditions. Silvicultural systems will likely be required to vary by genotype depending on different clonal growth or competition strategies and nutrient use efficiencies. We need to better understand how individual genotypes are utilizing site resources to better manage plantations for improved growth. Furthermore, genotypes may modify the soil environment differently, which could alter our ability to accurately predict changes in soil carbon (C) dynamics, and thus sustainability and carbon sequestration.

Clonal screening is the process by which individual genotypes are selected for large scale propagation and deployment. Screening is currently a time and money intensive process that does not fully account for the range of silvicultural tools available to modern managers, such as fertilizer application. Opportunities exist to determine whether a suite of observed morphological and ecophysiological processes and traits follow common patterns that allow for categorization of clones, or whether they vary independently and thus require development of more detailed information for each clone. Doing so may improve the efficiency of clonal screening programs so that they may better predict a range of responses of individual clones to specific silvicultural treatments. Screening methods must involve rapid assessments of a large number of trees to be financially attractive to companies producing clones. Crown ideotypes are one potential methodology that may simplify this process by grouping clones based upon easily identifiable traits, such as crown width or branch morphology.

1.2. Literature Review

1.2.1. Plantation Forestry: More Wood From Less Land

Plantation forests produce a disproportionate quantity of timber from only a small fraction of the world's forests (Paquette and Messier 2009). Only 4% of global forests are allocated to plantation forestry, although in the United States this figure is slightly higher at 5.6% (FAO 2007). Despite comprising such a small fraction of total forest cover, these forests are highly productive (5-40 m^3 yr^{-1} useable wood) compared to natural forests (< 3 m^3 yr^{-1} useable wood), and thus account for approximately one third of the world's industrial wood production (Sedjo and Botkin 1997; Paquette and Messier 2009). The United States produces more than 25% of the world's industrial roundwood on an annual basis (FAO 2007), of which approximately 60% originates from the Southeast (Wear and Greiss 2002; Adams, Haynes et al. 2006). *Pinus taeda* (L.) and less commonly *Pinus elliottii* (Engelm.) plantations are widespread across the Southeast, currently covering more than 13 million hectares, or approximately 75% of the 17 million total hectares of plantation forestland in the United States (Wear and Greiss 2002; FAO 2007). Plantation forestry is becoming increasingly common in the Southeast, as *P. taeda* plantations are expected to increase by 67% to approximately 22 million hectares by 2040 (Wear and Greiss 2002). Due in large part to the productivity and extent of these plantations, the southeastern United States alone produces more timber (approximately 16% of global supply) than any other country in the world (Wear and Greiss 2002).

Such high productivity is achieved through intensive silvicultural practices including site preparation, competition control, and fertilizer application, which combined are estimated to have increased productivity per land area by approximately 40% over natural stands (Fox, Jokela et al. 2007). Since the early 1990's millions of hectares of plantations have been fertilized, at an annual rate of 0.5 million hectares as of 2004 (Fox, Allen et al. 2007). Fertilizer application increases growth rates primarily by ameliorating deficiencies of nitrogen (N) and phosphorus (P), which allows greater developed leaf area to fix more carbon (C) (Vose and Allen 1988; Albaugh, Allen et al. 1998; Fox, Allen et al. 2007). Fertilizer growth responses vary across sites, but average 25% in response to mid-rotation application of N and P (Fox, Allen et al. 2007). Both P and potassium (K) fertilizers have been shown to improve growth rates on some sites for

multiple rotations after a single application (Fox 2000), while N fertilizer requires more frequent additions, with mean growth responses lasting approximately eight years (Fox, Allen et al. 2007). Micronutrient deficiencies (e.g. manganese, boron) are also sometimes observed in plantations, particularly after application of macronutrient fertilizers (i.e. N, P, K), although these deficiencies are not as well-understood, are less frequently diagnosed, and are less likely to be amended through fertilizer application compared to macronutrient deficiencies (Stone 1990; Jokela, Mcfee et al. 1991; Albaugh, Allen et al. 2007).

The first applied forest tree improvement program began in the southeastern United States in 1951 (Zobel and Talbert 1984). Today, of the more than 1 billion seedlings planted annually in the United States, 75% are planted in the Southeast covering more than 800,000 hectares; 95% of those seedlings are genetically improved *P. taeda* or *P. elliottii* (McKeand, Mullin et al. 2003). Several breeding cycles have been completed for *P. taeda*, resulting in seedlings with improved growth rates, disease resistance, stem form, and value to the landowner (McKeand, Abt et al. 2006; McKeand, Jokela et al. 2006). Improved stem volumes per land area for open-pollinated seedlings have been observed, and are estimated at 7-12% for first-cycle selections, 13-21% for second-cycle selections, and approximately 35% for third-cycle selections (Li, McKeand et al. 1999; Byram, Gwaze et al. 2003). Planting and tree-improvement combined currently account for gains of approximately 33% in stand volumes versus natural stands averaged across the existing plantation land-base (Fox, Jokela et al. 2007).

While open-pollinated seedlings still represent the vast majority of those planted in the Southeast, a number of methods for the production of elite genotypes have been developed in the last two decades (McKeand, Mullin et al. 2003). Elite genotypes produced from known crosses (i.e. full-sib families, mass-control-pollinated seedlings, and clonal seedlings) are now being deployed throughout the Southeast (McKeand, Mullin et al. 2003). A process known as somatic embryogenesis is utilized to produce millions to billions of genetically identical clonal seedlings from a single seed (Li and Huang 1996; Merkle and Dean 2000; Pullman and Johnson 2002). More than 20 million clonal *P. taeda* seedlings have been planted as of 2010, and production and planting of clones is only expected to increase as production capabilities of the companies

producing clonal seedlings increases (McKeand, Zobel et al. 2007; Bettinger, Clutter et al. 2009; Whetten and Kellison 2010).

Deployment of high stem volume clones may increase productivity by 100% over unimproved seed, with commensurate gains in stand value (McKeand, Mullin et al. 2003; Dougherty and Wright 2009; Whetten and Kellison 2010). There are currently many opportunities for research on clonal plantations, as many of the silvicultural and biological implications of their widespread deployment across the Southeast have not yet been fully ascertained (Lambeth and McCullough 1997; Bettinger, Clutter et al. 2009). Precision-silvicultural systems tailored to the traits of individual clones planted in block plots offer the greatest potential opportunity to maximize gains from clonal plantations (Allen, Fox et al. 2005; Dougherty and Wright 2009). Identifying suites of clonal traits, or crop tree ideotypes, that are respond consistently to silvicultural inputs could greatly increase the capabilities of land managers to appropriately manage clonal plantations (Martin, Johnsen et al. 2001; Nelson and Johnsen 2008).

1.2.2. Genetic Variation of Carbon Physiology and Genotype-by-Environment Interactions

P. taeda is characterized by high within-species genetic diversity (Lambeth and McCullough 1997), resulting in wide ranges in growth rates and the timing and magnitude of different ecophysiological processes among individuals within the breeding population. For example, open-pollinated families of *P. taeda* have shown variation in height growth (Paul, Foster et al. 1997; Xiao, Jokela et al. 2003), fine root allocation under N limitation (Samuelson 2000), foliar nutrient content (Xiao, Jokela et al. 2003), leaf area and light interception (McCrady and Jokela 1998; Xiao, Jokela et al. 2003), growth response to fertilizer application and weed control (Roth, Jokela et al. 2007), the efficiency of nitrogen use in producing stem volume (Li, Mckeand et al. 1991), biomass partitioning and depth of rooting (Barnes 2002). Clones also tend to show considerable variability. Clonal variability in a number of different tree species has been shown for survival (Bitoki 2008), growth and phenology (Paul, Foster et al. 1997; Emhart, Martin et al. 2006), soil CO_2 efflux (Kasurinen, Kokko-Gonzales et al. 2004), light-saturated net-photosynthetic rates (King, Seiler et al. 2008), crown structure and radiation interception (Emhart, Martin et al. 2007), biomass partitioning (Scarascia-Mugnozza, Ceulemans et al. 1997), and partitioning of gross primary production (Bown, Watt et al. 2009). As many of these

processes affect fertilizer growth response, this suggests the possibility that clone-by-fertilizer interactions may be widespread.

However, genotype-by-environment interactions are not problematic in open-pollinated *P. taeda*: high-performing families surpass low-performing families across a range of sites (McKeand, Jokela et al. 2006; Roth, Jokela et al. 2007). Further, research suggests that across a large number of clones, clone-by-site interactions may not be any more common than G x E interactions in open-pollinated families (McKeand, Jokela et al. 2006). By contrast, some studies among dissimilar sites have observed notable clone-by-site interactions that were more prevalent than interactions observed among half-sib families (Isik, Li et al. 2003; McKeand, Jokela et al. 2006). However, it remains uncertain the extent to which clone-by-silviculture interactions may play a role in clonal plantations comprised of a small number of individual genotypes.

Even if interactions are not prevalent among a large number of clones, if they do occur within the small proportion of high-performing outliers that are selected for wide-spread clonal deployment, opportunities to optimize silvicultural regimes could be forgone. The ability to predict potential site-specific interactions between widespread clonal genotypes and fertilizer application may be increasingly necessary as we deploy less diverse genetic sources (i.e. clones) over a broader land base. Some evidence does exist among small groups of clones for the presence of clone-by-fertilizer interactions in *P. taeda*. Interactions have been observed for growth, stem form defects, and a range of physiological processes including biomass partitioning and leaf-level gas exchange (King, Seiler et al. 2008; Espinoza 2009; Tyree, Seiler et al. 2009a; Tyree, Seiler et al. 2009b). However it should be noted that these are all single site studies, and both genotypic variance and genotype-by-environment interactions tend to be overestimated based on single site studies (White, Adams et al. 2007).

1.2.3. Biomass Partitioning and Allometric Responses to Fertilizer Application

Biomass partitioning is the distribution of mass among different plant organs (e.g. stem, foliage, roots). Allometry is similar in definition, but refers more narrowly to relative growth and is most often used to reference comparisons of the biomass of various plant organs against one another

(e.g. root-shoot ratio) (Ledig, Bormann et al. 1970). Some allometric relationships, particularly the stem-foliar mass or stem volume to foliar mass ratios, have been used to infer growth efficiency (Colbert, Jokela et al. 1990; Will, Munger et al. 2002; Burkes, Will et al. 2003; Samuelson, Johnsen et al. 2004a). Others, like root-shoot ratios, are used to assess how plants exploit different resource environments (Green, Mitchell et al. 1994; Griffin, Winner et al. 1995; Gedroc, McConnaughay et al. 1996; Gautam, Mead et al. 2003; Albaugh, Allen et al. 2006). Fertilizer application has been shown in numerous studies to result in changes in biomass partitioning (Axelsson and Axelsson 1986; Ingestad and Agren 1991; Gebauer, Reynolds et al. 1996; Albaugh, Allen et al. 1998; King, Albaugh et al. 1999; Albaugh, Allen et al. 2006). From an operational standpoint, the absolute magnitude of these changes is all that is relevant for C sequestration belowground, and the growth of various plant biomass components. However, in order to determine the exact physiological cause of these allometric shifts further analysis is required.

Allometric relationships change over time in tree species as a function of tree size (Ledig, Bormann et al. 1970). Tree size is governed by growth, which is a function of both age and site quality. Thus trees of the same age may have allometric differences that are due to site quality effects on growth rates. However, treatments, such as fertilizer application, may also directly affect allometry in addition to altering growth rates. To discriminate between these causes of observed fertilizer effects on allometry, it is necessary to determine whether 1) changes were due to treatment effects on growth rates only, 2) changes were due to treatment effects directly on allometry, or 3) changes were due to treatment effects on both allometry and growth rates. This is accomplished by performing an allometric analysis (Ledig, Bormann et al. 1970). An allometric analysis utilizes the linear regression of the natural log transformed biomass of one plant component against another for a sample population including a range of tree sizes, and then tests whether the regression coefficients are different. Regressions are conventionally reported in the form:

$$[1] \qquad \ln(y) = a + k \ln(x)$$

where ‘ln(y)’ is the natural log of one biomass component, ‘ln(x)’ is the natural log of another biomass component, ‘a’ is the intercept, and ‘k’ is the slope. Based on allometric analyses, it has been determined that fertilizer effects on biomass partitioning are due almost entirely to more rapid growth after fertilizer application, and not to changes in allometry when trees are compared on a similar size basis (Colbert, Jokela et al. 1990; King, Albaugh et al. 1999; Jokela and Martin 2000; Coyle and Coleman 2005).

Figure 1-1 illustrates the possible interpretations that may be derived from an allometric analysis. The figure depicts two treatments: W and Z. From an operational standpoint, the allometric relationship observed in the field is really the only consideration that is important. However, from an ecophysiological standpoint, the information contained in the figure below allows us to determine if the differences in allometry are a treatment effect (e.g. clones are different) or if the differences occurred because treatment comparisons were made on trees of different sizes that would be expected to have distinct allometric relationships with respect to components X and Y due to differences in development.

Assume treatments W and Z in Figure 1-1 represent two different clones. In the upper-left panel allometric differences are only the effect of clones growing at different rates. Their allometry is the same because both clones fall along the same regression line (i.e. the regression coefficients are not significantly different between clones). In the upper-right panel, clone Z has a significantly greater slope ‘k’, indicating that over time, it will allocate more to component Y at the expense of component X relative to clone W. In the lower-left panel, clone Z has a greater intercept ‘a’, indicating that clone Z always allocates more to component Y versus X than clone W does if they are compared on a similar size basis. In the lower-right panel is the most complex case, where both slope ‘k’ and intercept ‘a’ are different between clones W and Z. In this case, clone Z initially allocates more to component X at the expense of component Y when compared to clone W, but when the clones become larger, this trend reverses itself. Similar interpretations can be made if W is considered a control treatment and Z a fertilizer treatment. Thus, the upper-left panel would indicate fertilizer does not affect allometry, only growth rates, while the other three panels would indicate that fertilizer does directly affect the allometric relationship between components X and Y.

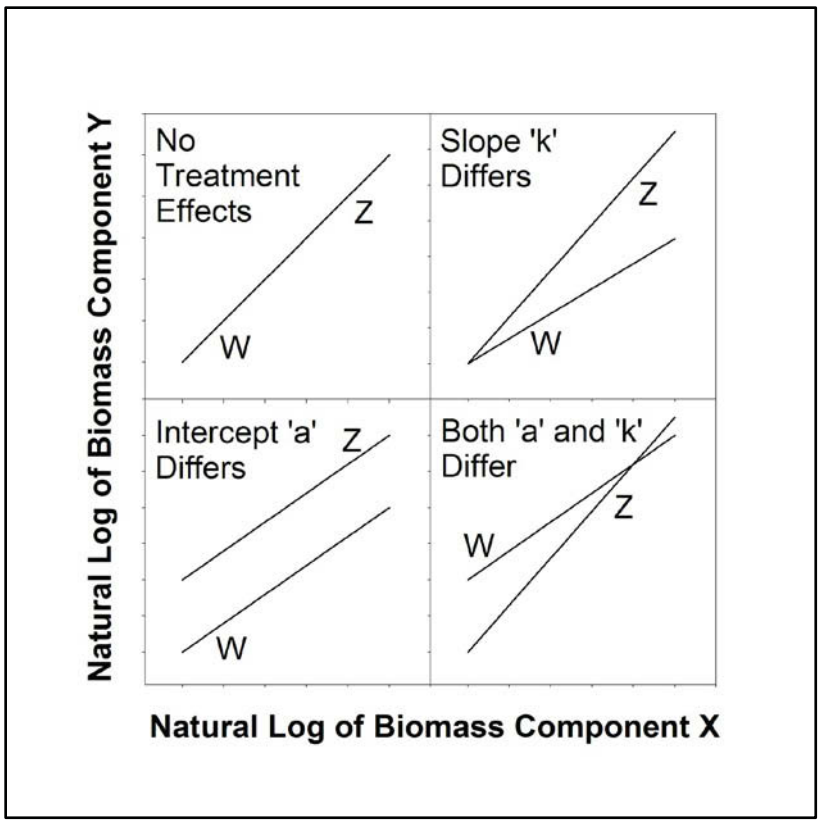

Figure 1-1. Hypothetical example of the allometric analyses utilized in Chapters 3 and 4. Two biomass components (X and Y) are regressed against one another for two different treatments (W and Z). The four possible outcomes are illustrated. In the upper-left panel, treatments W and Z have no affect on allometry, indicating that any observed allometric differences are due to growth effects. The other three panels all show allometric shifts due to treatments based on significant differences in one or both of the allometric regression coefficients 'a' and 'k'.

1.2.4. Carbon Allocation and Respiratory Flux Responses to Fertilizer Application

Carbon partitioned to biomass represents less than 50% of the total C fixed by a tree through gross primary productivity (GPP), but should not be used to infer either GPP or how GPP is partitioned among plant tissues (Ryan, Hubbard et al. 1996; Litton, Raich et al. 2007). The remaining 50% or more of GPP is partitioned to respiratory C fluxes (Kinerson, Ralston et al. 1977; Ryan 1991a; Ryan 1991b). It has been shown that while N fertilizer application increases respiratory C fluxes, this is a result of fertilized trees being larger and having higher N concentrations, and not a result of a changes in C use efficiency or the percentage of GPP partitioned to respiratory C fluxes, which remain relatively constant (Ryan, Hubbard et al. 1996; Maier, Zarnoch et al. 1998; Maier 2001; Maier, Albaugh et al. 2004; Wertin and Teskey 2008).

While the majority of available data shows no effect of fertilizer application on the partitioning of GPP, several studies have shown increased relative partitioning to aboveground net primary productivity (NPP) and aboveground respiration as a result of fertilizer application (Giardina, Ryan et al. 2003; Bown, Watt et al. 2009). Overall, whether a forest is a net source or sink of C is largely determined by respiratory C fluxes including soil CO_2 efflux (Valentini, Matteucci et al. 2000). Through fertilizer application's effect on the absolute magnitude of these fluxes, silviculture may determine if a *P. taeda* plantation is a net source or sink of atmospheric C (Lai, Katul et al. 2002; Maier, Albaugh et al. 2004).

As with considerations of aboveground or total biomass partitioning, biomass allocated to root tissues does not represent the total belowground C flux. Inclusion of root respiration, fine root turnover, and root exudation all increase belowground C allocation estimates beyond what is often assessed in published studies of biomass partitioning (Cheng, Coleman et al. 1993; Kuzyakov 2006b). Belowground C allocation may also be underestimated by 'black-box' modeling approaches that do not account for within-tree C fluxes, such as the movement of root-respiration-derived C up the stem in the transpiration stream (Aubrey and Teskey 2009). Reported values for total belowground C allocation as a percentage of GPP range from 40 to 73% for various tree species (Grayston, Vaughan et al. 1997). Further, fertilizer application has been shown to result in lower overall partitioning of GPP belowground across a large number of studies (Haynes and Gower 1995; Ryan, Hubbard et al. 1996; Giardina and Ryan 2002; Giardina, Ryan et al. 2003; Giardina, Binkley et al. 2004).

1.2.5. Soil CO_2 Efflux, Its Sources, and Its Response to Fertilizer Application

Soil CO_2 efflux is the single largest respiratory C flux in forested ecosystems (Lavigne and Ryan 1997), and includes both heterotrophic and autotrophic belowground respiratory components (Hogberg, Nordgren et al. 2001; Kuzyakov 2006b). Heterotrophic organisms such as bacteria and fungi metabolize root exudate compounds, dead fine roots, sloughed off root cells, leaf litter inputs, and soil organic matter. This is the heterotrophic component of soil CO_2 efflux (R_H). Root respiration and the respiration of symbiotic mycorrhiza comprise the autotrophic component of soil CO_2 efflux (R_A). Although mycorrhiza are not autotrophic, they are typically included in R_A due to methodological limitations and their intimate symbiosis with the roots of

many species (Hogberg, Buchmann et al. 2006; Kuzyakov 2006b; Kuzyakov 2006a). Efflux varies spatially and temporally both within pine plantations (Fang, Moncrieff et al. 1998; Lin, Rygiewicz et al. 2001; Gough and Seiler 2004b) and regionally (Gough, Seiler et al. 2005) in response to temperature, moisture, and soil factors (Maier and Kress 2000; Fang and Moncrieff 2001; Qi and Xu 2001; Pangle and Seiler 2002; Dilustro, Collins et al. 2005; Palmroth, Maier et al. 2005).

Total soil CO_2 efflux decreases after fertilizer application in mid-rotation (i.e. 11-17 years) *P. taeda* plantations (Maier and Kress 2000; Butnor, Johnsen et al. 2003). Similar results have been observed in younger (i.e. six-year-old) plantations (Samuelson, Johnsen et al. 2004b). Reduced efflux increases C sequestration, and can reduce the time necessary for a stand to become a net C sink (Maier and Kress 2000). However, fertilizer application may ultimately increase efflux by rotation age (e.g. 33 years), even if fertilizer is only applied within 10 years of planting (Tyree, Seiler et al. 2006). While overall efflux is often reduced by fertilizer application, detailed partitioning studies reveal that this is the result of R_H declining while R_A increases one year after fertilizer application in clones of *P. taeda* (Tyree, Seiler et al. 2008). The same trends were observed in pot-grown seedlings (Gough and Seiler 2004a). However, R_H and R_A both declined in fertilized versus unfertilized plots in a boreal forest (Olsson, Linder et al. 2005). These differences are likely due to relative root growth response to fertilizer application.

Changes in biomass partitioning likely cause increased R_A due to fertilizer application. Increases in R_A correlate with increased fine root production in fertilized stands (Lee and Jose 2003; Gough and Seiler 2004a). However, the plant physiological mechanisms responsible for fertilizer application induced reduction in R_H are not well-understood. Reductions in R_H with fertilizer application are proximately caused by reductions in microbial biomass (Smolander, Kurka et al. 1994). But plants and nutrient availability both ultimately play a critical role in constraining and structuring microbial communities (Smolander, Kurka et al. 1994; Marschner, Yang et al. 2001; Thirukkumaran and Parkinson 2002; Leckie, Prescott et al. 2004; Marschner, Crowley et al. 2004; Lagomarsino, Moscatelli et al. 2006; Cleveland, Nemergut et al. 2007). Root exudation is a likely physiological link between observed plant and microbial responses to fertilizer application. Root exudate quantity and chemical composition structure soil microbial

communities (Marschner, Crowley et al. 2004). Increased root exudates in experimental systems increase R_H (Landi, Valori et al. 2006). Thus, likely reductions in root exudation associated with fertilizer application could limit R_H in *P. taeda* plantations. Different C substrates found in exudates (e.g. glucose vs. oxalic acid) have been observed to increase R_H to different extents (Landi, Valori et al. 2006). Thus, potential changes in root exudate chemistry with fertilizer application could also reduce R_H.

1.2.6. Root Exudate Fertilizer Responses

Root exudates are compounds released from roots, either passively or actively, which increase nutrient availability, modify soil pH, reduce metal toxicity, and enable symbiotic relationships with mycorrhiza that can greatly increase effective rooting density (Grayston, Vaughan et al. 1997; Jones 1998; Dakora and Phillips 2002; Jones, Hodge et al. 2004). Root exudates are comprised of low molecular weight organic acids, amino acids, lipids, proteins, carbohydrates, and phenolic compounds, among others (Nguyen 2003; Sandnes, Eldhuset et al. 2005). Plants control the extent and spatial distribution of microbial and mycorrhizal activity near the root by constraining root exudation and reabsorbing exudates (Jones, Hodge et al. 2004; Sauer, Kuzyakov et al. 2006). Estimates of the proportion of GPP allocated to root exudation in forests are uncertain due to difficulties in measuring in-situ rates, but are thought to range up to 10% (Grayston, Vaughan et al. 1997; Phillips, Bernhardt et al. 2009). Estimates for *P. taeda* are few, but one detailed in-situ study has found that exudation comprised 1-2% of GPP in a 24-year-old plantation (Phillips, Erlitz et al. 2008) . Root exudates of *P. taeda* are highly labile, or readily available to bacterial and fungal metabolism, and have been shown to vary across soil types (Sanchez and Bursey 2002). While we are unaware of any research on differences among clones in root exudation quality or quantity, inter-specific variation of both has been observed (Smith 1976; Grayston, Vaughan et al. 1997).

Changes in root exudate quantity or chemical composition with N fertilizer application have only very recently begun to be quantified in *P. taeda* (Phillips, Erlitz et al. 2008; Phillips, Bernhardt et al. 2009). The limited number of studies done have shown conflicting results, with either no response of exudation across an N availability gradient (Phillips, Erlitz et al. 2008), or a relatively large response to N (Phillips, Bernhardt et al. 2009). Results from other systems

indicate that conflicting results are common with regards to N availability's effects on exudation, with reports of increased exudation under both higher (Henry, Nguyen et al. 2005) and lower (Haase, Neumann et al. 2007) N availability in annual crops. In forest ecosystems, root exudation decreased after N fertilizer application in a northern hardwood stand (Phillips and Fahey 2007) and after N and P fertilizer application in a humid tropical forests (Giardina, Binkley et al. 2004). Results for P gradients are more consistent, with studies typically showing inverse relationships between exudation rate and P availability. For instance, studies have shown that P deficiency caused increased root exudation for woody (Ratnayake, Leonard et al. 1978) and herbaceous annual crops (Egle, Romer et al. 2003). Low molecular weight organic acids present in root exudates have been shown to increase P availability by releasing tightly bound P from clay surfaces (Fox, Comerford et al. 1990; Strom 1997). Thus, reductions in exudates would be expected to occur once P becomes more readily available immediately after fertilizer application. In *P. taeda* plantations dissolved organic C chemical composition has been shown to differ in fertilized versus control plots (Sanchez, Leggett et al. 2005), although this may have been primarily due to changes in litter layer chemistry (Michalzik, Kalbitz et al. 2001; Sanchez 2004).

1.2.7. Leaf Area and Leaf-Level Photosynthetic Responses to Fertilizer Application

Low leaf area, rather than water availability, most commonly limits long-term growth in *P. taeda* across its natural range (Sampson and Allen 1999). Nutrient deficiencies in turn, particularly N and P, result in lower than optimal leaf area (Fox, Allen et al. 2007). In the long term, increased growth rates observed after fertilizer application with N and P correlate with increases in leaf area (Vose and Allen 1988; Dalla-Tea and Jokela 1991; Albaugh, Allen et al. 1998; Jokela and Martin 2000), but not always with changes in photosynthetic rate (Samuelson 1998). Photosynthetic rates of fertilized stands are often comparable to unfertilized stands once leaf area is significantly greater (Teskey, Gholz et al. 1994), even if increased leaf area results in greater shading lower in the canopy (Zhang, Hennessey et al. 1997; Niinemets, Ellsworth et al. 2001). Thus, in the long-term increased photosynthetic fixation of C through increased leaf area is a consistent mechanism of fertilizer growth response.

For increased leaf area to develop in the long-term, more C must be allocated to new leaf growth immediately following fertilizer application. Observations on a single *P. taeda* family indicated that photosynthetic rates increase immediately after fertilizer application, but decline back to pre-fertilizer application levels once leaf area increases (Gough, Seiler et al. 2004). Foliar N content positively correlates to variation in photosynthetic rates in newly developed fascicles (Strand 1997; Maier, Palmroth et al. 2008) and the amount of C fixed aboveground per unit of leaf area (Martin and Jokela 2004). Shortly after fertilizer application existing foliage appears to store N, but does not show increased photosynthetic rates in response (Maier, Palmroth et al. 2008). Thus, short-term increases in photosynthetic rate in newly developing foliage allow greater C fixation. Greater C fixation results in increased allocation to new leaf area. Once new leaves develop, N is mobilized within the plant and reallocated between new and old foliage, diluting N concentrations and reducing photosynthetic rates to pre-fertilizer application levels (Gough, Seiler et al. 2004; Adegbidi, Jokela et al. 2005). However the increased leaf area has created the capacity for greater C fixation, even at pre-fertilizer application photosynthetic rates.

1.2.8. Temporal-Scale Considerations for Ecophysiological Responses to Fertilizer Application

Consideration of the temporal scales of ecophysiological responses to fertilizer application is necessary to fully understand how responses lead to altered morphology and increased growth following fertilizer application (Gough, Seiler et al. 2004; King, Seiler et al. 2008; Maier, Palmroth et al. 2008; Tyree, Seiler et al. 2009a). Using the example described in the section above, increased leaf area, and thus the increased ability to fix C, has been demonstrated as one of the primary morphological mechanisms responsible for fertilizer stem growth response in *P. taeda*. Changes in morphology operate over longer temporal scales (e.g. growing seasons up to rotation length), as production of greater foliar area and greater crown size requires one or more growing seasons following fertilizer application to occur. But before any changes in morphology can occur, rates of ecophysiological processes constrained by existing morphology must respond in the short-term (e.g. days to weeks) following fertilizer application. C fluxes or allocation must change in response to fertilizer application to allow for greater C allocation to new leaf area development. A generalized model of the ultimate short-term physiological responses to

fertilizer application that cause later morphological responses that proximately drive fertilizer growth-response in the long-term is depicted in Figure 1-2.

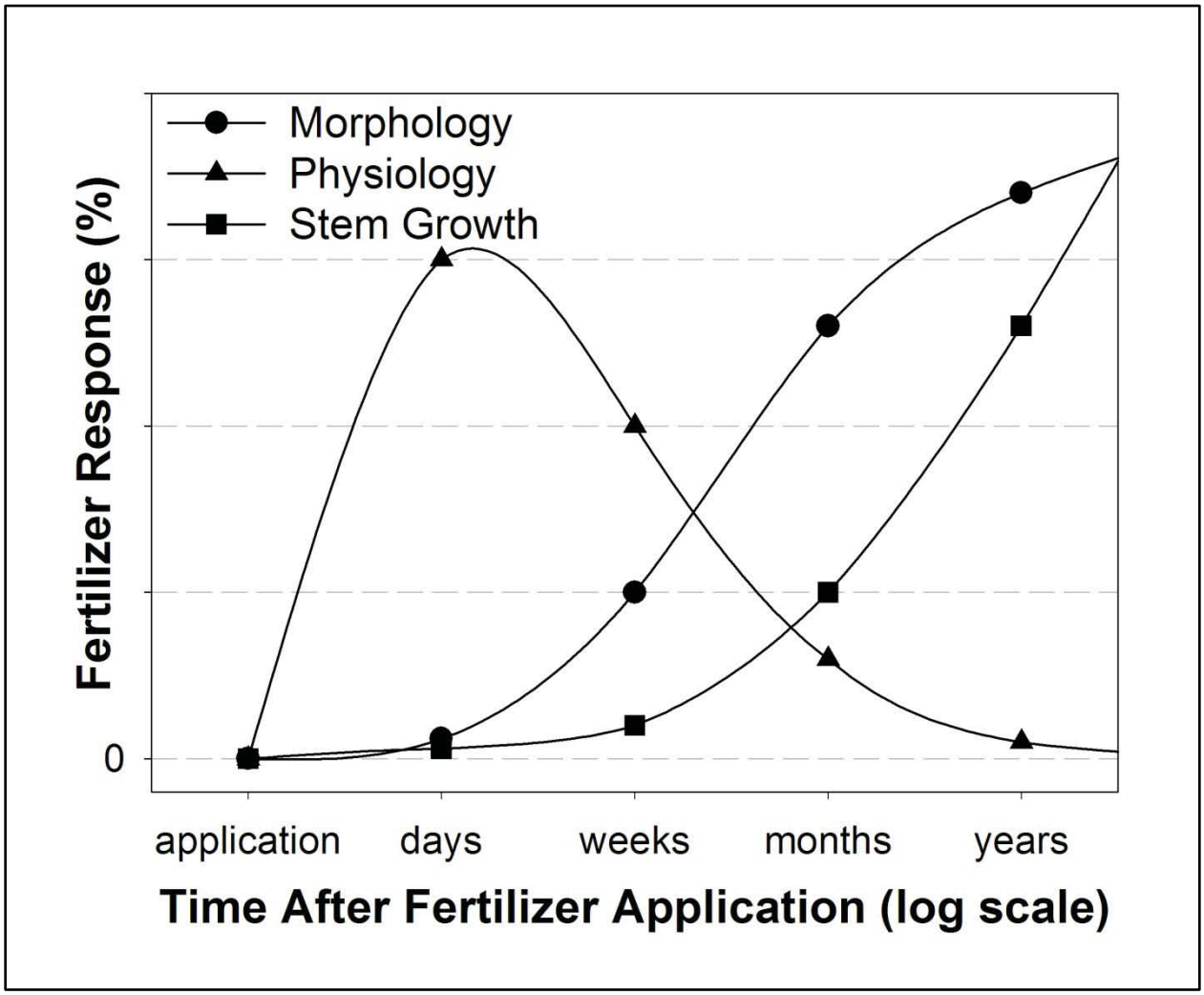

Figure 1-2. Generalized model of fertilizer response showing that short-term changes in ecophysiological processes are the ultimate causes of fertilizer stem growth response, while changes in morphology (e.g. increased foliage) that are associated with fertilizer response are proximate causes.

1.2.9. Crown Ideotypes as a Tool for Clonal Precision Silviculture

Originally defined by Donald (1968), ideotypes are simplified models of plants described with the intent of increasing yields when defined traits are selected for in a cultivar. Ideotypes may be as simple as a single characteristic, such as ground-line diameter (Britt, Mitchell et al. 1991), or may involve a complex list of many traits (Dickmann 1985; Martin, Johnsen et al. 2001; Nelson and Johnsen 2008). Inclusion of ecophysiological variables in ideotype development and clonal

screening may be common practice in the near future, as *P. taeda* is one of the best studied forest tree species in the world with regard to ecophysiology (Martin, Dougherty et al. 2005). The development of ideotypes for clones of *P. taeda* offers a simplified process for making silvicultural recommendations for individual clones. Rather than screening each clone for its response to silvicultural treatments individually (i.e. planting density, fertilizer application, weed control, etc.), clones could be classified into a small number of ideotypes that have been previously tested for response to silviculture. The efficacy of this system requires ideotypes to 1) be quantifiable, 2) be stable across sites, and 3) respond consistently to silvicultural manipulations.

Figure 1-3. A comparison of contrasting crown ideotypes between six-year-old fertilized ramets of clone H2 (panel A) and clone C2 (panel B). There was less than a 3% difference in stem volumes between these individuals, despite the 110% difference in foliar mass. The square being held in each photo for scale is 25 x 36 cm. These clones are described in greater detail in Chapters 2 and 3.

Crown variables will be an important component to any *P. taeda* ideotype (See Figure 1-3), as they constrain radiation interception, and thus ultimately carbon gain (Chmura, Rahman et al. 2007; Emhart, Martin et al. 2007; Nelson and Johnsen 2008). Crown ideotypes have been previously evaluated in terms of how specific crown geometry (e.g. shape, surface area) affects interception and penetration of solar radiation (Chen, Ceulemans et al. 1994). Chen et al., in a purely theoretical context, described how crown height-to-width ratio was a critical property in evaluation crown surface area, light interception, and critical solar elevation for maximum light interception (1994). Other mechanistic approaches to crown ideotypes have maximized models of crown N distribution, light extinction coefficients, and LAI to determine the ideal crown form for carbon gain (Wu 1993). While these ideotypes improve our understanding of crown level physiology, they are not simply or rapidly measured in the field with current technology, and thus are not practically applicable in the context of current clonal screening programs prior to deployment (Nelson and Johnsen 2008). To be useful from a tree-breeding (i.e. clonal screening) standpoint, ideotypes developed for southern pines should be constrained to one or few rapidly assessable characteristics that are highly correlated to rotation-length stem volume, or some other economically relevant trait (Martin, Johnsen et al. 2001). However, more complex ideotypes may be applicable to clones that have already been widely deployed in order to develop genotype-specific precision-silvicultural systems.

1.3. Objectives, Experiments, and Brief Chapter Descriptions

Preliminary results in early 2007 indicated substantial variability among clones in their stem volume growth response at a field trial in the Virginia Piedmont described in detail in Chapters 2 and 3. This unexpected observation, coupled with some of the variability in physiological processes between clones in response to fertilizer application described above, informed the following research objectives. The objectives of Chapters 2 and 3 are

1. To quantify the range of growth responses to fertilizer application in different genotypes of *P. taeda* (Chapter 2).

2. To quantify the extent of stem form defect responses to fertilizer application in different genotypes of *P. taeda* (Chapter 2).
3. To quantify the extent of allometric and growth efficiency responses to fertilizer application in different genotypes of *P. taeda* (Chapter 3).

Specific hypotheses are described in the relevant chapters. One of the advantages of the field trial described in chapters 2 and 3 was that clone-by-fertilizer interactions were tested for a greater number of clones (21 in chapter 2, 10 in chapter 3), than the small number of previous reports in the literature, allowing for greater inference to be drawn from conclusions.

Based on the previously described model of short-term ecophysiological responses to fertilizer application driving long-term growth responses, and previous research (Gough, Seiler et al. 2004; King, Seiler et al. 2008; Tyree, Seiler et al. 2009a), we tested the following series of alternative but not necessarily exclusive hypotheses in a four month greenhouse experiment (Chapters 4, 5, and 6).

1. Increased N and P availability reduces C allocation to roots, increasing aboveground C allocation for new leaves (Chapter 4).
2. Increased N and P availability reduces C allocation to respiratory fluxes, increasing aboveground C allocation for new leaves (Chapter 5).
3. Increased N and P availability reduces C allocation to root exudates, increasing aboveground C allocation for new leaves (Chapter 5).
4. Increased N availability increases photosynthetic rates, thus fixing more C for allocation to new leaves (Chapter 6).
5. Fertilizer application results in some combination of these mechanisms (Chapter 7).
6. Clones respond differently, which may explain differences in growth.

While each of these mechanisms has been observed independently, we are aware of no current studies that have simultaneously explored as comprehensive a suite of C allocation pathways

leading to long-term growth response following fertilizer application in clones of *P. taeda*. These hypotheses were tested in two full-sib clones with contrasting crown ideotypes, as some evidence shows that at least for mechanism 4 response of C allocation to fertilizer application varies among clones (King, Seiler et al. 2008). A whole-plant C allocation method similar to that reported in Chapter 7 has been successfully applied to *Pinus radiata* (D. Don) among four clones with factorial N and P additions (Bown, Watt et al. 2009).

Variability in belowground C allocation and root exudate quantity and quality between clones could also affect total soil C sequestration, soil CO_2 efflux rates, how soil CO_2 efflux is partitioned between autotrophic and heterotrophic components, and rhizosphere C forms. Differences in clonal influence on soil C processes could have implications for future efforts to quantify C sequestration in clonal plantations for carbon markets. Chapter 5 further sought to test whether observed reductions in the heterotrophic component of soil CO_2 efflux were affected by changes in root exudate quantity and chemical composition in response to fertilizer application.

1.4. Literature Cited

Adams, D. M., R. W. Haynes and A. J. Daigneault (2006). Estimated timber harvest by U.S. region and ownership, 1950-2002, USDA Forest Service General Technical Report PNW-GTR-659, Pacific Northwest Rearch Station, Portland, OR.

Adegbidi, H. G., E. J. Jokela and N. B. Comerford (2005). "Factors influencing production efficiency of intensively managed loblolly pine plantations in a 1-to 4-year-old chronosequence." Forest Ecology and Management **218**(1-3): 245-258.

Albaugh, T. J., H. L. Allen, P. M. Dougherty, L. W. Kress and J. S. King (1998). "Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions." Forest Science **44**(2): 317-328.

Albaugh, T. J., H. L. Allen and T. R. Fox (2007). "Historical patterns of forest fertilization in the southeastern United States from 1969 to 2004." Southern Journal of Applied Forestry **31**(3): 129-137.

Albaugh, T. J., H. L. Allen and L. W. Kress (2006). "Root and stem partitioning of *Pinus taeda*." Trees-Structure and Function **20**(2): 176-185.

Allen, H. L., T. R. Fox and R. G. Campbell (2005). "What is ahead for intensive pine plantation silviculture in the south?" Southern Journal of Applied Forestry **29**(2): 62-69.

Aubrey, D. P. and R. O. Teskey (2009). "Root-derived CO_2 efflux via xylem stream rivals soil CO_2 efflux." New Phytologist **184**(1): 35-40.

Axelsson, E. and B. Axelsson (1986). "Changes in carbon allocation patterns in spruce and pine trees following irrigation and fertilization." Tree Physiology **2**: 189-204.

Barnes, A. D. (2002). "Effects of phenology, water availability and seed source on loblolly pine biomass partitioning and transpiration." Tree Physiology **22**(10): 733-740.

Bettinger, P., M. Clutter, J. Siry, M. Kane and J. Pait (2009). "Broad implications of southern United States pine clonal forestry on planning and management of forests." International Forestry Review **11**(3): 331-345.

Bitoki, O. (2008). "Comparing early survival and growth of varietal and open-pollinated loblolly pine seedlings." VDOF Forest Research Review **April 2008**: 4-5.

Bown, H. E., M. S. Watt, P. W. Clinton, E. G. Mason and D. Whitehead (2009). "The influence of N and P supply and genotype on carbon flux and partitioning in potted *Pinus radiata* plants." Tree Physiology **29**(7): 857-868.

Britt, J. R., R. J. Mitchell, B. R. Zutter, D. B. South, D. H. Gjerstad and J. F. Dickson (1991). "The influence of herbaceous weed-control and seedling diameter on 6 years of loblolly-pine growth - A classical growth analysis approach." Forest Science **37**(2): 655-668.

Burkes, E. C., R. E. Will, G. A. Barron-Gafford, R. O. Teskey and B. Shiver (2003). "Biomass partitioning and growth efficiency of intensively managed *Pinus taeda* and *Pinus elliottii* stands of different planting densities." Forest Science **49**(2): 224-234.

Butnor, J. R., K. H. Johnsen, R. Oren and G. G. Katul (2003). "Reduction of forest floor respiration by fertilization on both carbon dioxide-enriched and reference 17-year-old loblolly pine stands." Global Change Biology **9**(6): 849-861.

Byram, T. D., D. P. Gwaze, L. G. Miller and E. M. Raley (2003). "Fifty-first progress report of the cooperative forest tree improvement program." Texas Forest Service Circular **407**: 22.

Chen, S. G., R. Ceulemans and I. Impens (1994). "Is there a light regime determined tree ideotype." Journal of Theoretical Biology **169**(2): 153-161.

Cheng, W. X., D. C. Coleman, C. R. Carroll and C. A. Hoffman (1993). "In-situ measurement of root respiration and soluble C-concentrations in the rhizosphere." Soil Biology & Biochemistry **25**(9): 1189-1196.

Chmura, D. J., M. S. Rahman and M. G. Tjoelker (2007). "Crown structure and biomass allocation patterns modulate aboveground productivity in young loblolly pine and slash pine." Forest Ecology and Management **243**(2-3): 219-230.

Cleveland, C. C., D. R. Nemergut, S. K. Schmidt and A. R. Townsend (2007). "Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition." Biogeochemistry **82**(3): 229-240.

Colbert, S. R., E. J. Jokela and D. G. Neary (1990). "Effects of annual fertilization and sustained weed-control on dry-matter partitioning, leaf-area, and growth efficiency of juvenile loblolly and slash pine." Forest Science **36**(4): 995-1014.

Coyle, D. R. and M. D. Coleman (2005). "Forest production responses to irrigation and fertilization are not explained by shifts in allocation." Forest Ecology and Management **208**(1-3): 137-152.

Dakora, F. D. and D. A. Phillips (2002). "Root exudates as mediators of mineral acquisition in low-nutrient environments." Plant and Soil **245**(1): 35-47.

Dalla-Tea, F. and E. J. Jokela (1991). "Needlefall, canopy light interception, and productivity of young intensively managed slash and loblolly-pine stands." Forest Science **37**(5): 1298-1313.

Dickmann, D. I. (1985). The ideotype concept applied to forest trees. Attributes of trees as crop plants. M. G. R. Cannell and J. E. Jackson. Huntington, England, Institute of Terrestrial Ecology**:** 89-101.

Dilustro, J. J., B. Collins, L. Duncan and C. Crawford (2005). "Moisture and soil texture effects on soil CO_2 efflux components in southeastern mixed pine forests." Forest Ecology and Management **204**(1): 85-95.

Donald, C. M. (1968). "The breeding of crop ideotypes." Euphytica **17**: 385-403.

Dougherty, D. and J. Wright (2009). "Improved returns on forestlands: a financial analysis of mass control pollinated and varietal seedlings." Tree Farmer **January / February 2009**: 42-46.

Egle, K., W. Romer and H. Keller (2003). "Exudation of low molecular weight organic acids by *Lupinus albus* L., *Lupinus angustifolius* L. and *Lupinus luteus* L. as affected by phosphorus supply." Agronomie **23**(5-6): 511-518.

Emhart, V. I., T. A. Martin, T. L. White and D. A. Huber (2006). "Genetic variation in basal area increment phenology and its correlation with growth rate in loblolly and slash pine families and clones." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **36**(4): 961-971.

Emhart, V. I., T. A. Martin, T. L. White and D. A. Huber (2007). "Clonal variation in crown structure, absorbed photosynthetically active radiation and growth of loblolly pine and slash pine." Tree Physiology **27**(3): 421-430.

Espinoza, J. A. (2009). Genetic and nutritional effects on stem sinuosity in loblolly pine. Forestry. Raleigh, NC, North Carolina State University. **Ph.D. Dissertation:** 102 p.

Fang, C. and J. B. Moncrieff (2001). "The dependence of soil CO_2 efflux on temperature." Soil Biology & Biochemistry **33**(2): 155-165.

Fang, C., J. B. Moncrieff, H. L. Gholz and K. L. Clark (1998). "Soil CO_2 efflux and its spatial variation in a Florida slash pine plantation." Plant and Soil **205**(2): 135-146.

FAO (2007). State of the world's forests 2007. Rome, Italy, Food and Agricultural Organization of the United Nations**:** 144 p.

Fox, T. R. (2000). "Sustained productivity in intensively managed forest plantations." Forest Ecology and Management **138**(1-3): 187-202.

Fox, T. R., H. L. Allen, T. J. Albaugh, R. Rubilar and C. A. Carlson (2007). "Tree nutrition and forest fertilization of pine plantations in the southern United States." Southern Journal of Applied Forestry **31**(1): 5-11.

Fox, T. R., N. B. Comerford and W. W. Mcfee (1990). "Phosphorus and aluminum release from a spodic horizon mediated by organic-acids." Soil Science Society of America Journal **54**(6): 1763-1767.

Fox, T. R., E. J. Jokela and H. L. Allen (2007). "The development of pine plantation silviculture in the southern United States." Journal of Forestry **105**: 337-347.

Gautam, M. N. K., D. J. Mead, P. W. Clinton and S. X. Chang (2003). "Biomass and morphology of *Pinus radiata* coarse root components in a sub-humid temperate silvopastoral system." Forest Ecology and Management **177**(1-3): 387-397.

Gebauer, R. L. E., J. F. Reynolds and B. R. Strain (1996). "Allometric relations and growth in *Pinus taeda*: The effect of elevated CO_2 and changing N availability." New Phytologist **134**(1): 85-93.

Gedroc, J. J., K. D. M. McConnaughay and J. S. Coleman (1996). "Plasticity in root shoot partitioning: Optimal, ontogenetic, or both?" Functional Ecology **10**(1): 44-50.

Giardina, C. P., D. Binkley, M. G. Ryan, J. H. Fownes and R. S. Senock (2004). "Belowground carbon cycling in a humid tropical forest decreases with fertilization." Oecologia **139**(4): 545-550.

Giardina, C. P. and M. G. Ryan (2002). "Total belowground carbon allocation in a fast-growing *Eucalyptus* plantation estimated using a carbon balance approach." Ecosystems **5**(5): 487-499.

Giardina, C. P., M. G. Ryan, D. Binkley and J. H. Fownes (2003). "Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest." Global Change Biology **9**(10): 1438-1450.

Gough, C. M. and J. R. Seiler (2004a). "Belowground carbon dynamics in loblolly pine (*Pinus taeda*) immediately following diammonium phosphate fertilization." Tree Physiology **24**(7): 845-851.

Gough, C. M. and J. R. Seiler (2004b). "The influence of environmental, soil carbon, root, and stand characteristics on soil CO_2 efflux in loblolly pine (*Pinus taeda* L.) plantations located on the South Carolina Coastal Plain." Forest Ecology and Management **191**(1-3): 353-363.

Gough, C. M., J. R. Seiler and C. A. Maier (2004). "Short-term effects of fertilization on loblolly pine (*Pinus taeda* L.) physiology." Plant Cell and Environment **27**(7): 876-886.

Gough, C. M., J. R. Seiler, P. E. Wiseman and C. A. Maier (2005). "Soil CO_2 efflux in loblolly pine (*Pinus taeda* L.) plantations on the Virginia Piedmont and South Carolina Coastal Plain over a rotation-length chronosequence." Biogeochemistry **73**(1): 127-147.

Grayston, S. J., D. Vaughan and D. Jones (1997). "Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability." Applied Soil Ecology **5**(1): 29-56.

Green, T. H., R. J. Mitchell and D. H. Gjerstad (1994). "Effects of nitrogen on the response of loblolly-pine to drought. 2. Biomass allocation and C-N balance." New Phytologist **128**(1): 145-152.

Griffin, K. L., W. E. Winner and B. R. Strain (1995). "Growth and dry-matter partitioning in loblolly and ponderosa pine-seedlings in response to carbon and nitrogen availability." New Phytologist **129**(4): 547-556.

Haase, S., G. Neumann, A. Kania, Y. Kuzyakov, V. Romheld and E. Kandeler (2007). "Elevation of atmospheric CO_2 and N-nutritional status modify nodulation, nodule-carbon supply, and root exudation of *Phaseolus vulgaris* L." Soil Biology & Biochemistry **39**(9): 2208-2221.

Haynes, B. E. and S. T. Gower (1995). "Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin." Tree Physiology **15**(5): 317-325.

Henry, F., C. Nguyen, E. Paterson, A. Sim and C. Robin (2005). "How does nitrogen availability alter rhizodeposition in *Lolium multiflorum* Lam. during vegetative growth?" Plant and Soil **269**(1-2): 181-191.

Hogberg, P., N. Buchmann and D. J. Read (2006). "Comments on Yakov Kuzyakov's review 'Sources of CO_2 efflux from soil and review of partitioning methods' [Soil Biology & Biochemistry 38, 425-448]." Soil Biology & Biochemistry **38**(9): 2997-2998.

Hogberg, P., A. Nordgren, N. Buchmann, A. F. S. Taylor, A. Ekblad, M. N. Hogberg, G. Nyberg, M. Ottosson-Lofvenius and D. J. Read (2001). "Large-scale forest girdling shows that current photosynthesis drives soil respiration." Nature **411**(6839): 789-792.

Ingestad, T. and G. I. Agren (1991). "The influence of plant nutrition on biomass allocation." Ecological Applications **1**(2): 168-174.

Isik, F., B. L. Li and J. Frampton (2003). "Estimates of additive, dominance and epistatic genetic variances from a clonally replicated test of loblolly pine." Forest Science **49**(1): 77-88.

Jokela, E. J. and T. A. Martin (2000). "Effects of ontogeny and soil nutrient supply on production, allocation, and leaf area efficiency in loblolly and slash pine stands." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **30**(10): 1511-1524.

Jokela, E. J., W. W. Mcfee and E. L. Stone (1991). "Micronutrient deficiency in slash pine - Response and persistence of added manganese." Soil Science Society of America Journal **55**(2): 492-496.

Jones, D. L. (1998). "Organic acids in the rhizosphere - a critical review." Plant and Soil **205**(1): 25-44.

Jones, D. L., A. Hodge and Y. Kuzyakov (2004). "Plant and mycorrhizal regulation of rhizodeposition." New Phytologist **163**(3): 459-480.

Kasurinen, A., P. Kokko-Gonzales, J. Riikonen, E. Vapaavuori and T. Holopainen (2004). "Soil CO_2 efflux of two silver birch clones exposed to elevated CO_2 and O_3 levels during three growing seasons." Global Change Biology **10**(10): 1654-1665.

Kinerson, R. S., C. W. Ralston and C. G. Wells (1977). "Carbon cycling in a loblolly pine plantation." Oecologia **29**(1): 1-10.

King, J. S., T. J. Albaugh, H. L. Allen and L. W. Kress (1999). "Stand-level allometry in *Pinus taeda* as affected by irrigation and fertilization." Tree Physiology **19**(12): 769-778.

King, N. T., J. R. Seiler, T. R. Fox and K. H. Johnsen (2008). "Post-fertilization physiology and growth performance of loblolly pine clones." Tree Physiology **28**(5): 703-711.

Kuzyakov, Y. (2006a). "Response to the comments by Peter Hogberg, Nina Buchmann and David J. Read on the review 'Sources of CO_2 efflux from soil and review of partitioning methods' (Soil Biology & Biochemistry 38, 425-448)." Soil Biology & Biochemistry **38**(9): 2999-3000.

Kuzyakov, Y. (2006b). "Sources of CO_2 efflux from soil and review of partitioning methods." Soil Biology & Biochemistry **38**(3): 425-448.

Lagomarsino, A., M. C. Moscatelli, P. De Angelis and S. Grego (2006). "Labile substrates quality as the main driving force of microbial mineralization activity in a poplar

plantation soil under elevated CO_2 and nitrogen fertilization." Science of the Total Environment **372**(1): 256-265.

Lai, C. T., G. Katul, J. Butnor, M. Siqueira, D. Ellsworth, C. Maier, K. Johnsen, S. Mckeand and R. Oren (2002). "Modelling the limits on the response of net carbon exchange to fertilization in a South-Eastern pine forest." Plant Cell and Environment **25**(9): 1095-1119.

Lambeth, C. C. and R. B. McCullough (1997). "Genetic diversity in managed loblolly pine forests in the southeastern United States: Perspective of the private industrial forest land owner." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **27**(3): 409-414.

Landi, L., F. Valori, J. Ascher, G. Renella, L. Falchini and P. Nannipieri (2006). "Root exudate effects on the bacterial communities, CO_2 evolution, nitrogen transformations and ATP content of rhizosphere and bulk soils." Soil Biology & Biochemistry **38**(3): 509-516.

Lavigne, M. B. and M. G. Ryan (1997). "Growth and maintenance respiration rates of aspen, black spruce and jack pine stems at northern and southern BOREAS sites." Tree Physiology **17**(8-9): 543-551.

Leckie, S. E., C. E. Prescott and S. J. Grayston (2004). "Forest floor microbial community response to tree species and fertilization of regenerating coniferous forests." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **34**(7): 1426-1435.

Ledig, F. T., F. H. Bormann and K. F. Wenger (1970). "The distribution of dry matter growth between shoots and roots in loblolly pine." Botanical Gazette **131**(4): 349-359.

Lee, K. H. and S. Jose (2003). "Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient." Forest Ecology and Management **185**(3): 263-273.

Li, B., S. McKeand and R. Weir (1999). "Tree improvement and sustainable forestry - impact of two cycles of loblolly pine breeding in the U.S.A." Forest Genetics **6**(4): 229-234.

Li, B., S. E. Mckeand and H. L. Allen (1991). "Genetic-variation in nitrogen use efficiency of loblolly-pine seedlings." Forest Science **37**(2): 613-626.

Li, X. Y. and F. H. Huang (1996). "Induction of somatic embryogenesis in loblolly pine (*Pinus taeda* L)." In Vitro Cellular & Developmental Biology-Plant **32**(3): 129-135.

Lin, G. H., P. T. Rygiewicz, J. R. Ehleringer, M. G. Johnson and D. T. Tingey (2001). "Time-dependent responses of soil CO_2 efflux components to elevated atmospheric [CO_2] and temperature in experimental forest mesocosms." Plant and Soil **229**(2): 259-270.

Litton, C. M., J. W. Raich and M. G. Ryan (2007). "Carbon allocation in forest ecosystems." Global Change Biology **13**(10): 2089-2109.

Maier, C. A. (2001). "Stem growth and respiration in loblolly pine plantations differing in soil resource availability." Tree Physiology **21**(16): 1183-1193.

Maier, C. A., T. J. Albaugh, H. L. Allen and P. M. Dougherty (2004). "Respiratory carbon use and carbon storage in mid-rotation loblolly pine (*Pinus taeda* L.) plantations: The effect of site resources on the stand carbon balance." Global Change Biology **10**(8): 1335-1350.

Maier, C. A. and L. W. Kress (2000). "Soil CO_2 evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **30**(3): 347-359.

Maier, C. A., S. Palmroth and E. Ward (2008). "Short-term effects of fertilization on photosynthesis and leaf morphology of field-grown loblolly pine following long-term exposure to elevated CO_2 concentration." Tree Physiology **28**(4): 597-606.

Maier, C. A., S. J. Zarnoch and P. M. Dougherty (1998). "Effects of temperature and tissue nitrogen on dormant season stem and branch maintenance respiration in a young loblolly pine (*Pinus taeda*) plantation." Tree Physiology **18**(1): 11-20.

Marschner, P., D. Crowley and C. H. Yang (2004). "Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type." Plant and Soil **261**(1-2): 199-208.

Marschner, P., C. H. Yang, R. Lieberei and D. E. Crowley (2001). "Soil and plant specific effects on bacterial community composition in the rhizosphere." Soil Biology & Biochemistry **33**(11): 1437-1445.

Martin, T. A., P. M. Dougherty and S. E. McKeand (2005). "Strategies and case studies for incorporating ecophysiology into southern pine tree improvement programs." Southern Journal of Applied Forestry **29**(2): 70-79.

Martin, T. A., K. H. Johnsen and T. L. White (2001). "Ideotype development in southern pines: Rationale and strategies for overcoming scale-related obstacles." Forest Science **47**(1): 21-28.

Martin, T. A. and E. J. Jokela (2004). "Developmental patterns and nutrition impact radiation use efficiency components in southern pine stands." Ecological Applications **14**(6): 1839-1854.

McCrady, R. L. and E. J. Jokela (1998). "Canopy dynamics, light interception, and radiation use efficiency of selected loblolly pine families." Forest Science **44**(1): 64-72.

McKeand, S., T. Mullin, T. Byram and T. White (2003). "Deployment of genetically improved loblolly and slash pines in the South." Journal of Forestry **101**(3): 32-37.

McKeand, S., B. Zobel, T. Byram and D. Huber (2007). Southern pine tree improvement - A living success story. Proceedings of the 29th Southern Forest Tree Improvement Conference. T. Byram and M. L. Rust. Galveston, TX**:** 3-6.

McKeand, S. E., R. C. Abt, H. L. Allen, B. L. Li and G. P. Catts (2006). "What are the best loblolly pine genotypes worth to landowners?" Journal of Forestry **104**(7): 352-358.

McKeand, S. E., E. J. Jokela, D. A. Huber, T. D. Byram, H. L. Allen, B. L. Li and T. J. Mullin (2006). "Performance of improved genotypes of loblolly pine across different soils, climates, and silvicultural inputs." Forest Ecology and Management **227**(1-2): 178-184.

Merkle, S. A. and J. F. D. Dean (2000). "Forest tree biotechnology." Current Opinion in Biotechnology **11**(3): 298-302.

Michalzik, B., K. Kalbitz, J. H. Park, S. Solinger and E. Matzner (2001). "Fluxes and concentrations of dissolved organic carbon and nitrogen - a synthesis for temperate forests." Biogeochemistry **52**(2): 173-205.

Nelson, C. D. and K. H. Johnsen (2008). "Genomic and physiological approaches to advancing forest tree improvement." Tree Physiology **28**: 1135-1143.

Nguyen, C. (2003). "Rhizodeposition of organic C by plants: Mechanisms and controls." Agronomie **23**(5-6): 375-396.

Niinemets, U., D. S. Ellsworth, A. Lukjanova and M. Tobias (2001). "Site fertility and the morphological and photosynthetic acclimation of *Pinus sylvestris* needles to light." Tree Physiology **21**(17): 1231-1244.

Olsson, P., S. Linder, R. Giesler and P. Hogberg (2005). "Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration." Global Change Biology **11**(10): 1745-1753.

Palmroth, S., C. A. Maier, H. R. McCarthy, A. C. Oishi, H. S. Kim, K. H. Johnsen, G. G. Katul and R. Oren (2005). "Contrasting responses to drought of forest floor CO_2 efflux in a loblolly pine plantation and a nearby oak-hickory forest." Global Change Biology **11**(3): 421-434.

Pangle, R. E. and J. Seiler (2002). "Influence of seedling roots, environmental factors and soil characteristics on soil CO_2 efflux rates in a 2-year-old loblolly pine (*Pinus taeda* L.) plantation in the Virginia Piedmont." Environmental Pollution **116**: S85-S96.

Paquette, A. and C. Messier (2009). "The role of plantations in managing the world's forests in the Anthropocene." Frontiers in Ecology and the Environment **8**(1): 27-34.

Paul, A. D., G. S. Foster, T. Caldwell and J. McRae (1997). "Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine." Forest Science **43**(1): 87-98.

Phillips, R. P., E. S. Bernhardt and W. H. Schlesinger (2009). "Elevated CO_2 increases root exudation from loblolly pine (*Pinus taeda*) seedlings as an N-mediated response." Tree Physiology: tpp083.

Phillips, R. P., Y. Erlitz, R. Bier and E. S. Bernhardt (2008). "New approach for capturing soluble root exudates in forest soils." Functional Ecology **22**(6): 990-999.

Phillips, R. P. and T. J. Fahey (2007). "Fertilization effects on fineroot biomass, rhizosphere microbes and respiratory fluxes in hardwood forest soils." New Phytologist **176**(3): 655-664.

Pullman, G. S. and S. Johnson (2002). "Somatic embryogenesis in loblolly pine (*Pinus taeda* L.): improving culture initiation rates." Annals of Forest Science **59**(5-6): 663-668.

Qi, Y. and M. Xu (2001). "Separating the effects of moisture and temperature on soil CO_2 efflux in a coniferous forest in the Sierra Nevada mountains." Plant and Soil **237**(1): 15-23.

Ratnayake, M., R. T. Leonard and J. A. Menge (1978). "Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation." New Phytologist **81**: 543-552.

Roth, B. E., E. J. Jokela, T. A. Martin, D. A. Huber and T. L. White (2007). "Genotype x environment interactions in selected loblolly and slash pine plantations in the southeastern United States." Forest Ecology and Management **238**(1-3): 175-188.

Ryan, M. G. (1991a). "Effects of climate change on plant respiration." Ecological Applications **1**(2): 157-167.

Ryan, M. G. (1991b). "A simple method for estimating gross carbon budgets for vegetation in forest ecosystems." Tree Physiology **9**(1-2): 255-266.

Ryan, M. G., R. M. Hubbard, S. Pongracic, R. J. Raison and R. E. McMurtrie (1996). "Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status." Tree Physiology **16**(3): 333-343.

Sampson, D. A. and H. L. Allen (1999). "Regional influences of soil available water-holding capacity and climate, and leaf area index on simulated loblolly pine productivity." Forest Ecology and Management **124**(1): 1-12.

Samuelson, L. J. (1998). "Influence of intensive culture on leaf net photosynthesis and growth of sweetgum and loblolly pine seedlings." Forest Science **44**(2): 308-316.

Samuelson, L. J. (2000). "Effects of nitrogen on leaf physiology and growth of different families of loblolly and slash pine." New Forests **19**(1): 95-107.

Samuelson, L. J., K. Johnsen and T. Stokes (2004a). "Production, allocation, and stemwood growth efficiency of *Pinus taeda* L. stands in response to 6 years of intensive management." Forest Ecology and Management **192**(1): 59-70.

Samuelson, L. J., K. Johnsen, T. Stokes and W. L. Lu (2004b). "Intensive management modifies soil CO_2 efflux in 6-year-old *Pinus taeda* L. stands." Forest Ecology and Management **200**(1-3): 335-345.

Sanchez, F. G. (2004). "Irrigation, fertilization and initial substrate quality effects on decomposing loblolly pine litter chemistry." Plant and Soil **270**(1-2): 113-122.

Sanchez, F. G. and M. M. Bursey (2002). "Transient nature of rhizosphere carbon elucidated by supercritical freon-22 extraction and C-13 NMR analysis." Forest Ecology and Management **169**(3): 177-185.

Sanchez, F. G., Z. H. Leggett and S. Sankar (2005). "Analyzing water soluble soil organics as trifluoroacetyl derivatives by liquid state proton nuclear magnetic resonance." Communications in Soil Science and Plant Analysis **36**(19-20): 2793-2805.

Sandnes, A., T. D. Eldhuset and G. Wollebaek (2005). "Organic acids in root exudates and soil solution of Norway spruce and silver birch." Soil Biology & Biochemistry **37**(2): 259-269.

Sauer, D., Y. Kuzyakov and K. Stahr (2006). "Spatial distribution of root exudates of five plant species as assessed by C-14 labeling." Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde **169**(3): 360-362.

Scarascia-Mugnozza, G. E., R. Ceulemans, P. E. Heilman, J. G. Isebrands, R. F. Stettler and T. M. Hinckley (1997). "Production physiology and morphology of *Populus* species and their hybrids grown under short rotation. 2. Biomass components and harvest index of hybrid and parental species clones." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **27**(3): 285-294.

Sedjo, R. A. and D. Botkin (1997). "Using forest plantations to spare natural forests." Environment **39**(10): 14-30.

Smith, W. H. (1976). "Character and significance of forest tree root exudates." Ecology **57**(2): 324-331.

Smolander, A., A. Kurka, V. Kitunen and E. Malkonen (1994). "Microbial biomass C and N, and respiratory activity in soil of repeatedly limed and N-fertilized and P-fertilized Norway spruce stands." Soil Biology & Biochemistry **26**(8): 957-962.

Stone, E. L. (1990). "Boron deficiency and excess in forest trees: A review." Forest Ecology and Management **37**(1-3): 49-75.

Strand, M. (1997). "Effect of mineral nutrient content on oxygen exchange and chlorophyll *a* fluorescence in needles of Norway spruce." Tree Physiology **17**(4): 221-230.

Strom, L. (1997). "Root exudation of organic acids: Importance to nutrient availability and the calcifuge and calcicole behaviour of plants." Oikos **80**(3): 459-466.

Teskey, R. O., H. L. Gholz and W. P. Cropper (1994). "Influence of climate and fertilization on net photosynthesis of mature slash pine." Tree Physiology **14**(11): 1215-1227.

Thirukkumaran, C. M. and D. Parkinson (2002). "Microbial activity, nutrient dynamics and litter decomposition in a Canadian Rocky Mountain pine forest as affected by N and P fertilizers." Forest Ecology and Management **159**(3): 187-201.

Tyree, M. C., J. R. Seiler, W. M. Aust, D. A. Sampson and T. R. Fox (2006). "Long-term effects of site preparation and fertilization on total soil CO_2 efflux and heterotrophic respiration in a 33-year-old *Pinus taeda* L. plantation on the wet flats of the Virginia Lower Coastal Plain." Forest Ecology and Management **234**(1-3): 363-369.

Tyree, M. C., J. R. Seiler and T. R. Fox (2008). "The effects of fertilization on soil respiration in 2-year-old *Pinus taeda* L. clones." Forest Science **54**(1): 21-30.

Tyree, M. C., J. R. Seiler and C. A. Maier (2009a). "Short-term impacts of nutrient manipulations on leaf gas exchange and biomass partitioning in contrasting 2-year-old *Pinus taeda* clones during seedling establishment." Forest Ecology and Management **257**(8): 1847-1858.

Tyree, M. C., J. R. Seiler, C. A. Maier and K. H. Johnsen (2009b). "*Pinus taeda* clones and soil nutrient availability: Effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology." Tree Physiology **29**(9): 1117-1131.

Valentini, R., G. Matteucci, A. J. Dolman, E. D. Schulze, C. Rebmann, E. J. Moors, A. Granier, P. Gross, N. O. Jensen, K. Pilegaard, A. Lindroth, A. Grelle, C. Bernhofer, T. Grunwald, M. Aubinet, R. Ceulemans, A. S. Kowalski, T. Vesala, U. Rannik, P. Berbigier, D. Loustau, J. Guomundsson, H. Thorgeirsson, A. Ibrom, K. Morgenstern, R. Clement, J. Moncrieff, L. Montagnani, S. Minerbi and P. G. Jarvis (2000). "Respiration as the main determinant of carbon balance in European forests." Nature **404**(6780): 861-865.

Vose, J. M. and H. L. Allen (1988). "Leaf-area, stemwood growth, and nutrition relationships in loblolly-pine." Forest Science **34**(3): 547-563.

Wear, D. and J. Greiss (2002). Southern forest resource assessment, USDA Forest Service General Technical Report SRS-53, Southern Rearch Station, Asheville, NC.

Wertin, T. M. and R. O. Teskey (2008). "Close coupling of whole-plant respiration to net photosynthesis and carbohydrates." Tree Physiology **28**(12): 1831-1840.

Whetten, R. W. and R. Kellison (2010). "Research gap analysis for application of biotechnology to sustaining US forests." Journal of Forestry **108**: 193-201.

White, T. L., W. T. Adams and D. B. Neale (2007). Forest Genetics. Cambridge, MA, CABI Publishing.

Will, R. E., G. T. Munger, Y. J. Zhang and B. E. Borders (2002). "Effects of annual fertilization and complete competition control on current annual increment, foliar development, and growth efficiency of different aged *Pinus taeda* stands." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **32**(10): 1728-1740.

Wu, R. L. (1993). "Simulated optimal structure of a photosynthetic system - Implication for the breeding of forest crop ideotype." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **23**(8): 1631-1638.

Xiao, Y., E. J. Jokela and T. L. White (2003). "Growth and leaf nutrient responses of loblolly and slash pine families to intensive silvicultural management." Forest Ecology and Management **183**(1-3): 281-295.

Zhang, S. S., T. C. Hennessey and R. A. Heinemann (1997). "Acclimation of loblolly pine (*Pinus taeda*) foliage to light intensity as related to leaf nitrogen availability." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **27**(7): 1032-1040.

Zobel, B. and J. Talbert (1984). Applied forest tree improvement. New York, NY, John Wiley & Sons.

2. Growth and stem quality fertilizer response of loblolly pine clones in the Virginia Piedmont

2.1. Introduction

Clonal *Pinus taeda* (L.) plantations are currently limited in their deployment throughout the southeastern United States (Bettinger, Clutter et al. 2009). However, increased production capabilities and the development of silvicultural systems specific to clonal plantations may result in a greater land area allocated to clonal plantations in the near future (Wright and Dougherty 2006). One current barrier to the widespread deployment of clonal plantations is the higher cost per seedling versus mass control pollinated or improved open pollinated seedlings (Wright and Dougherty 2007). In order to offset higher costs at planting, it has been argued that it is necessary to manage clonal plantations with a high level of silvicultural inputs to maximize growth rates and improve product class distribution at harvest (Dougherty 2007; Wright and Dougherty 2007). Fertilizer application is among the most commonly prescribed management activities currently in *P. taeda* plantations (Fox, Allen et al. 2007), and likely will be a prescription for many clonal plantations in the future. The objective of this chapter was to determine the range of stem growth, stem form, and two generalized growth efficiency metrics response to fertilizer application in a large number of clones. We further sought to ascertain if clone-by-fertilizer interactions for any of these traits occurred, and to determine if their occurrence might be of concern or represent unique opportunities for the management of clonal plantations and the selection of clones for deployment.

Clones of *P. taeda* have been previously shown to vary significantly in their growth rates (Paul, Foster et al. 1997; King, Seiler et al. 2008). Additionally, variable growth responses to fertilizer application have been observed among a small number of clones (King, Seiler et al. 2008; Espinoza 2009; Tyree, Seiler et al. 2009b). However, we are aware of no information currently available in the literature on clonal fertilizer growth response involving more than eight clones of *P. taeda*. While information on clone-by-fertilizer interactions is currently based on small samples of clones, reports of clone-by-site interactions are inconsistent among trials planted with large numbers (e.g. > 100) of clones (Paul, Foster et al. 1997; Baltunis, Huber et al. 2007). It

remains uncertain to what extent genotype-by-environment interactions may play a role in operational clonal plantations, although it is important to note that even if such interactions occur at a low rate, they may remain an issue if they randomly occur in any of the relatively small number of high-performing clones that are selected and deployed over a wide geographic area. While potential genotype-by-environment or genotype-by-silviculture interactions clearly represent a challenge for the management of clonal plantations, they may also present a range of management opportunities. For instance, if highly responsive genotypes are identified prior to planting, appropriate silvicultural systems may be implemented to take full advantage of their potential growth responses to treatments (Roth, Jokela et al. 2007).

Stem form defects such as forks, ramicorns, or sinuosity have previously been observed in *P. taeda* plantations, and have generally been associated with greater levels of silvicultural inputs, particularly fertilizer application, in some genotypes (McKeand, Jokela et al. 2006; Espinoza 2009). Forking is a dichotomous branching of the main leader, ramicorns are excessively steeply angled large branches, and sinuosity is a repeated deviation from vertical in the main leader. All are significant form problems that may reduce wood quality and yield if present at harvest. As with growth, we are aware of no published reports on the effects of fertilizer application on stem form defects in a sample of more than six clones. More experiments involving a greater number of clones are required to determine the possible extent and severity of stem form responses to fertilizer application. Branch morphology, including branch diameter, angle, and number, also affects stem quality by determining the size and number of knots. Branch diameter and number have both been shown to increase with fertilizer application in *P. taeda*, although not to an extent that would negatively impact stem quality (Albaugh, Allen et al. 2006). In a study of two contrasting clones of *P. taeda* allocation to branch biomass varied between clones in response to fertilizer application (Tyree, Seiler et al. 2009a). It is uncertain to what extent branch morphology may vary among a larger number of clones, and how common clone-by-fertilizer interactions may be for branch morphology.

Crown morphology, or the size and spatial distribution of foliage and branches throughout the canopy, is an important determinant of growth efficiency. Crown morphology and growth efficiency, or the amount of stem volume produced per unit foliage, have been shown to vary

among different *Pinus* species (Xiao, Jokela et al. 2003). Among open-pollinated families of *P. taeda* genotypic variability in crown morphology has been observed and correlated to growth (McCrady and Jokela 1996; Chmura, Rahman et al. 2007). A trial with 300 clones of *P. taeda* similarly found differences in crown morphology that were related to growth (Emhart, Martin et al. 2007). Differences in clonal crown morphology offer the opportunity to select individual clones based both on different growth efficiency and different crown-based ideotypes (Martin, Johnsen et al. 2001; Emhart, Martin et al. 2007; Nelson and Johnsen 2008). For instance, narrow-crown clones could be planted at a low density to maximize growth rates and production of high-quality sawtimber without problems associated with large branches or ramicorns. Further information on potential clone-by-fertilizer interactions in crown morphology is required before such clone-specific silvicultural systems can be successfully applied.

Numerous studies have examined the effects of fertilizer application on crown morphology without accounting for genotypic variability. It is widely accepted that fertilizer application results in an increase in leaf-area at the whole crown scale (Vose and Allen 1988; Dalla-Tea and Jokela 1991; Albaugh, Allen et al. 1998). Increased leaf area in response to fertilizer application has been shown to result in greater allocation to stem mass (Colbert, Jokela et al. 1990; Samuelson, Johnsen et al. 2004). However, a number of studies among open-pollinated trees have found little effect of fertilizer application on crown morphology other than increased leaf area (Xiao, Jokela et al. 2003; Yu, Chambers et al. 2003; Chmura, Rahman et al. 2007). Clone-by-fertilizer interactions for crown morphology have been observed in a field trial with a pair of clones with contrasting ideotypes (Tyree, Seiler et al. 2009b). However, studies examining clone-by-fertilizer interactions among a greater number of clones will be required to better elucidate the extent of these interactions and the range of silvicultural possibilities they may present.

2.2. Materials and Methods

2.2.1. Site, Study, and Plant Material Descriptions

Our study was installed at the Reynolds Homestead Forestry Research Center on a typical site in the upper Piedmont of Patrick County, Virginia, USA (Latitude: 36° 40’ N, Longitude: 80° 10’

W). The site is 320 to 340 m in elevation with topography consisting of gradually sloping hills. Average annual precipitation is 1,300 mm and mean annual temperature is 12.8° C. Soils located at the site include a Fairview sandy clay loam (fine, kaolinitic, mesic Typic Kanhapludults) and a French loam (fine-loamy over sandy or sandy-skeletal, mixed, active, mesic Fluvaquentic Dystrudepts). Soils generally have a truncated Ap horizon leading directly into a clayey B horizon as a result of extensive erosion due to poor agricultural practices over the last several centuries.

At this site a split-plot experimental design was installed and replicated four times by block. The whole-plot treatment was control versus fertilizer application. Fertilizer was hand-banded at a rate of 224 kg ha^{-1} of diammonium phosphate and 184 kg ha^{-1} of ammonium nitrate per each application, equivalent to 103 kg ha^{-1} of elemental N and 45 kg ha^{-1} of elemental P per application. Fertilizer was applied on May 4, 2004, May 4, 2006, and July 16, 2008. The sub-plot treatment consisted of 25 clones in single-tree plots, with a single ramet of each clone per plot. Clonal material was donated by the Forest Biology Research Cooperative (Gainsville, Florida, USA). All clones were rooted cuttings from crosses of the Loblolly Pine Lower Gulf Elite Breeding Population that includes Atlantic Coastal Plain and Florida provenances. Clones are labeled by letter according to each cross, and are numbered by genotype within each cross (i.e. B1 and B2 are full-sib to one another but not to A). The site was cleared of competing vegetation with glyphosate (Round Up®), ripped, and the planting rows were shallowly cultivated. Seedlings were hand planted at 3.0-by-2.5 m spacing in May 2003. A border row of open-pollinated seedlings was planted around each plot. Complete weed control was maintained for the first two years. After the first two years the rows were mowed at least twice per year to control competing vegetation. Prior to root growth between plots, we dug trenches and lined them with plastic between the plots to ensure fertilizer did not contaminate control plots. Further descriptions of this trial are available in King et al. (2008) and Tyree et al. (2008).

2.2.2. Data Collection

Stem height was measured each year in the dormant season. Prior to trees reaching 1.37 m ground-line diameter was measured with calipers. Following the third growing season dbh was measured annually with calipers until the trial was harvested after the sixth growing season. A

simple biomass index was calculated for the first year by multiplying height by the square of ground-line diameter. This biomass index was used as a covariate in analyses of stem volumes from years three through six. Stem volume was calculated using empirical equations from Burkhart (1977) for outside the bark volume of plantation grown trees.

Stem form was assessed following the third, fifth, and sixth growing seasons. Each tree was given a binary score for the presence or absence of stem forking and ramicorns. Only forks that were present for at least two previous growing seasons were scored. The number of ramicorns was not recorded, only their presence or absence. Sinuosity was scored based on a subjective categorical system where a score of one indicated no sinuosity, two indicated sinuosity was evident but not severe, three indicated sinuosity was severe and might still be present at the end of a 20 year rotation, and four indicated that sinuosity was so severe that it would either severely limit growth, or would still be present at the end of a 20 year rotation.

A number of crown metrics were quantified on the same dates stem form was assessed. For a single representative whorl of branches nearest to 1.37 m above ground-line, crown width of an inter-row branch was measured, the number of primary branches was counted, and the angle and basal diameter of the average branch in each whorl was scored on a categorical basis. Crown volume was then calculated as a cone based on crown width and total tree height, since all measurements on this trial were prior to crown closure, and live-crown ratios were all approximately equal to unity. After calibrating the measurer's eye with a protractor on the first several trees, branch angle was visually assessed and assigned to one of four categories numbered one to four for branch angles from horizontal of 0° to 15°, 15° to 30°, 30° to 45°, and greater than 45°, respectively. Branch basal diameter was similarly assigned to one of four categories numbered one through four for branch diameters of 0 to 0.5 cm, 0.5 to 1 cm, 1 to 2 cm, and greater than 2 cm, respectively. The number of flushes in the previous growing season was also tallied.

The winter of 2005-06 was relatively severe, and included several unusual periods of prolonged subfreezing temperatures, and several precipitation events that resulted in the accumulation of up to 1 cm of ice. These events offered the opportunity to examine the range of response among

different genotypes to cold damage. Damage hypothesized to be the result of this unusual cold was observed on the leaders of a number of ramets. Cold damage was scored for all ramets in March 2006 according to a subjective categorical score. A score of 0 was given to trees with no observable damage, 0.5 to trees where only the tips of the needles of the top whorl were minimally affected, 1 to trees where the terminal buds of the top whorl were minimally affected, 2 to trees with the terminal buds of the top two or three whorls and the leader were markedly affected, and 3 to trees with severe dieback of the top. Only one tree was included in the most severe category, so categories two and three were combined for further analysis.

Two different methods were employed in order to assess growth efficiency, or unit stem produced per unit foliage. First, we divided stem volume by crown volume to create a growth efficiency ideotype metric (GEI). Assuming that crown volume may be used as an inexact surrogate for leaf area, the GEI can be interpreted as the volume of wood produced per unit foliage. We recognize that this approach is simplistic, and ignores differences in photosynthetic rates, specific leaf area, and foliar display. The second method involved the harvest of a single representative branch from the north side of each tree on January 4, 2006, after the third growing season. Foliage and branches were separated, oven dried, and weighed. Foliar mass was then expressed on a branch mass basis to account for differences in harvested branch size and to better represent crown development (Xiao, Jokela et al. 2003). These data were then compared with growth in the fourth growing season as another indicator of growth efficiency, where the mass of foliage from a single branch was a surrogate for the total foliar mass of the tree.

2.2.3. Statistical Analysis

Prior to analysis all variables were checked for assumptions of normality and heteroscedasticity. Where assumptions were violated variables were power transformed. All means and standard errors presented are of untransformed data. Analyses were performed in SAS software version 9.2 (SAS Institute Inc., Cary, North Carolina, USA). All analyses included appropriate error structures for a split-plot experimental design (block and fertilizer-by-block interactions). Repeated measures data was analyzed in PROC MIXED with either an unstructured, compound symmetry, or first-order autoregressive covariance structure selected utilizing information criteria in the output. Some models failed to converge, despite dependent variables having been

transformed to normal and checked for outliers. These variables (height, dbh, stem volume) were analyzed in PROC GLM with all appropriate time and time-by-treatment interactions included in the model. First-year covariates of height, ground-line diameter, and biomass index were included in these models. Variables measured at only one date were analyzed in PROC MIXED. Categorical, count, and binary data (sinuosity, ramicorns, forks, cold damage, branch number, branch diameter, branch angle, flush number) were analyzed with PROC LOGISTIC. Problems arose with the analysis of forking, ramicorn, and cold damage data due to the quasi-complete separation (QCS) of data points. QCS occurs when one or more independent variables perfectly predict the dependent variable. QCS in this data resulted from the large number of clones that had dramatically different values for each of these variables (e.g. some clones had no incidence, others high incidence). As a result, no statistics are presented for inference for these three independent variables, only mean and frequency data with interpretations of meaningful differences left to the reader. Regressions described are simple linear models implemented in PROC REG.

2.3. Results

2.3.1. Survival

Survival after six growing seasons in this trial was 88% (175 of 200 ramets) across all 25 clones. Most mortality occurred in the first growing season after planting, accounting for 16 of the eventual 25 deaths. Survival was greater than or equal to 75% in 21 of the 25 clones. With the exception of survival data, analyses in the remainder of this chapter are based only on these 21 clones. Of the four clones that were excluded from further analyses due to excessive mortality reducing our ability to make statistical inferences, three had survival rates of 63%, while the fourth had a survival rate of only 50%. Mortality in these four clones accounted for 13 of 25 total tree deaths in this trial. All mortality in these four clones occurred in either the first or second growing seasons. For the remaining 21 clones on which analyses were performed, survival after the first growing season was 97% (163 of 168 ramets), which declined to 93% (156 of 168 ramets) by the end of the sixth growing season. No incidence of insect or disease damage was observed in this trial, and mortality was not attributed to any specific cause.

2.3.2. Stem Growth

Clones showed a dramatic range of stem growth over time. Height, dbh, and stem volume were all significantly influenced by the clonal main effect and the clone-by-year interaction ($p < 0.01$; Table 2-1). By the end of the sixth growing season, the smallest clone, clone E, had a mean dbh of 9.2 cm, a mean height of 5.4 m, and a mean stem volume of 11,453 cm^3 (Figure 2-1). By contrast, the largest clone based on stem volume, clone I2, had a mean dbh of 13.0 cm, a mean height of 7.3 m, and a mean stem volume of 13,723 cm^3, differences of 41.2%, 35.5%, and 19.8%, respectively, compared to clone E. When the full-sib pairs in the trial were compared, some grew at similar rates. For instance, clones C1 and C2 showed less than 1% difference in dbh and stem volume, and only a 12.4% difference in height. However, other pairs of full-sib clones grew at dramatically different rates from one another. For example, after six growing seasons clone I2 had 30.0% greater mean dbh and 11.8% greater mean stem volume than clone I3 while showing a less than 1% difference in height. Trends among different clones presented in Figure 2-1 reflect that clonal differences in stem volume and height increase in absolute magnitude over time.

Averaged across all clones, fertilizer had a small but statistically significant impact on stem growth by the end of the sixth growing season. Height and stem volume showed significant fertilizer-by-time interactions, and dbh showed a significant fertilizer main effect ($p < 0.05$; Table 2-1). After the sixth growing season, trees in fertilizer plots had a mean dbh 6.1% greater than controls, a mean height 1.4% greater, and a mean stem volume 2.5% greater (Figure 2-2). While these differences were small in magnitude averaged across all clones, fertilizer growth response varied among clones for height ($p < 0.10$; Figure 2-3). Although the clone-by-fertilizer interaction was not statistically significant for stem volume ($p = 0.11$), data are presented in Figure 2-3. A small number of clones displayed greater growth responses to fertilizer application versus the mean of all clones in this trial. For example, clone D had 12.2% greater stem volume in fertilizer plots, and clone F2 had 14.2% greater volume. By contrast, other clones like clones F1 or I3 showed almost no fertilizer growth response. Still others, like clones A and K actually had reduced mean stem volume in fertilizer plots, although the reductions in stem volume were small compared to the magnitude of variability observed. While some full-sib

pairs of clones showed similar fertilizer growth responses (e.g. clones C1 and C2), others were markedly different in their response to fertilizer application (e.g. clones F1 and F2).

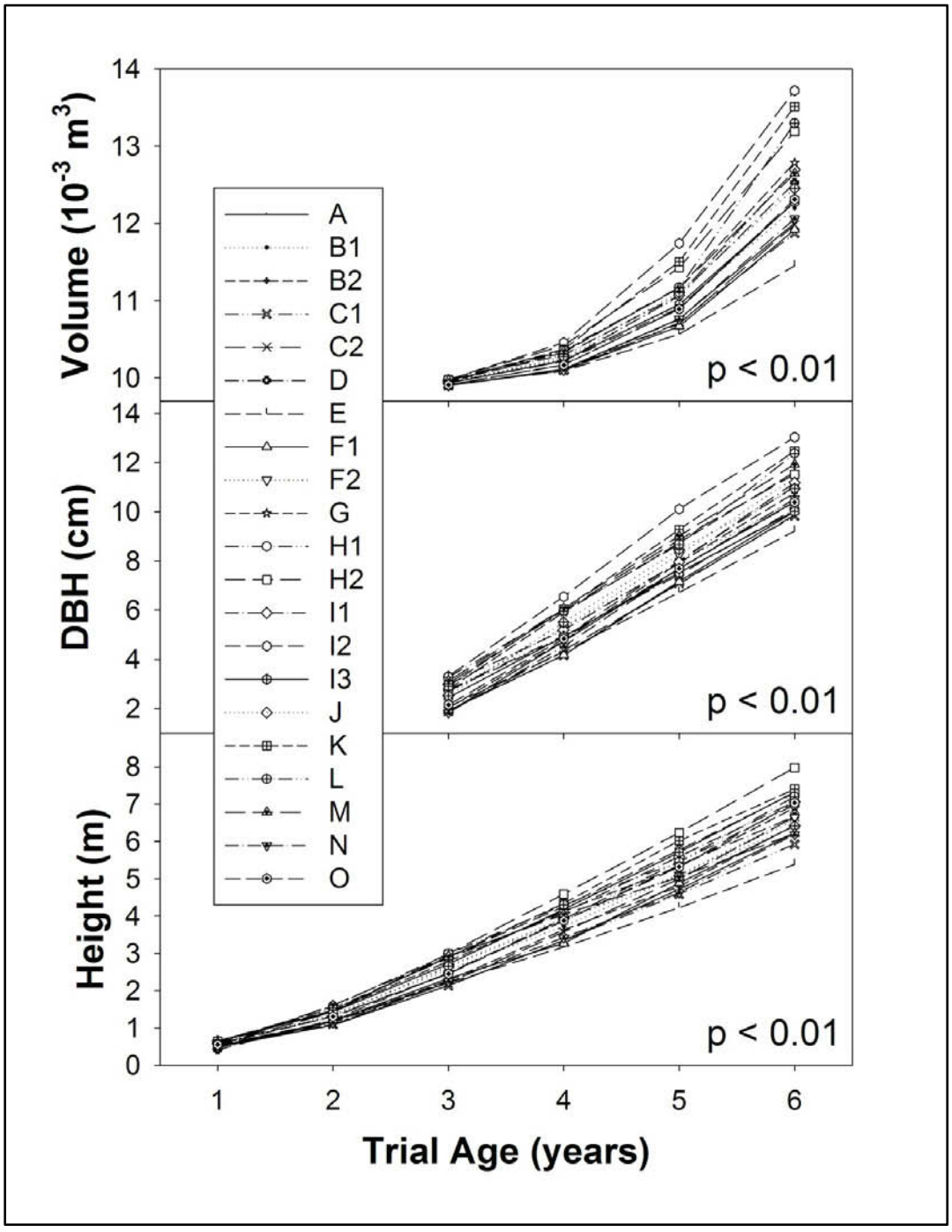

Figure 2-1. Mean stem growth of 21 clones of *P. taeda* replicated four times in the Virginia Piedmont. Full-sib clones are named with the same letter (e.g. B1, B2). Stem volume was calculated based on the empirical formula found in Burkhart (1977).

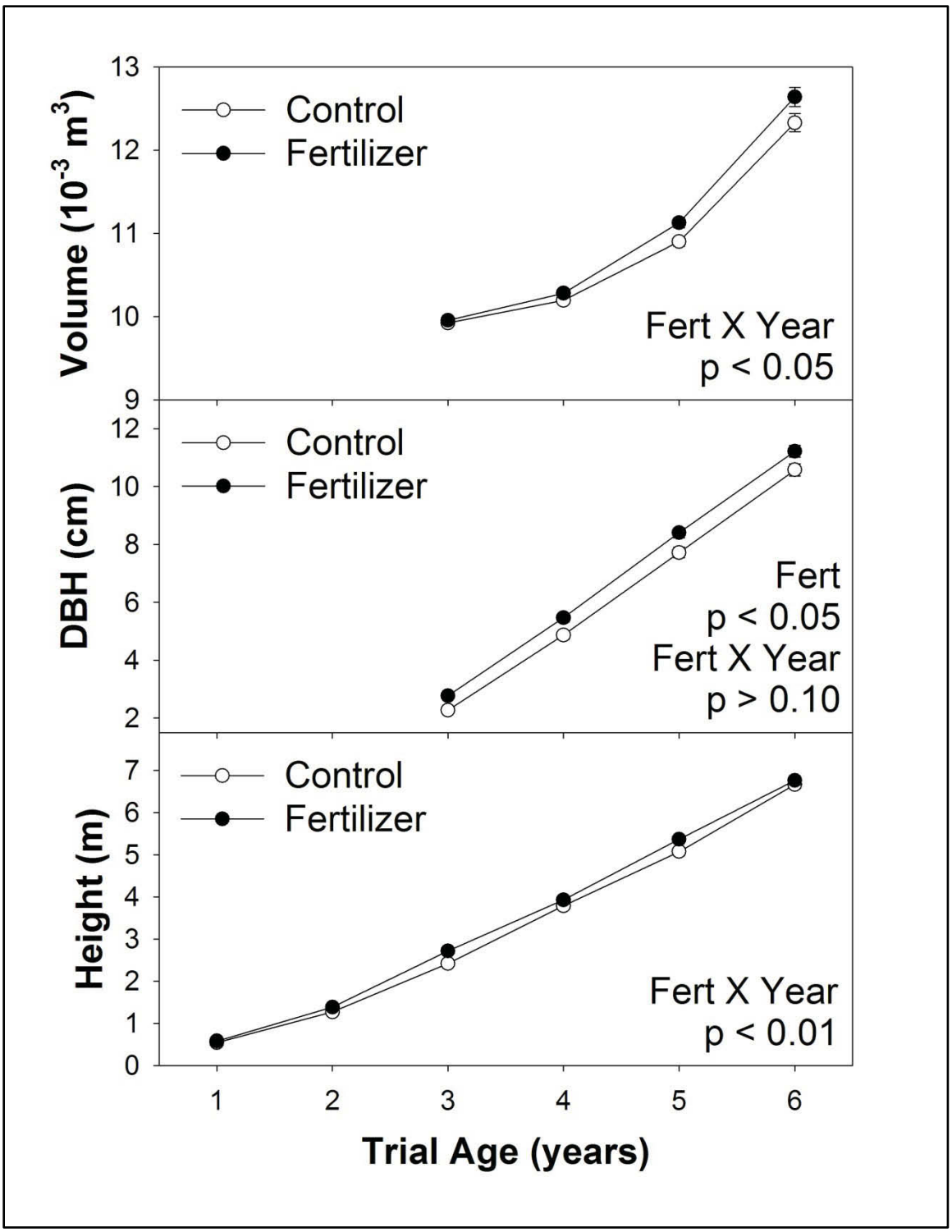

Figure 2-2. Stem growth of fertilized and unfertilized ramets averaged across 21 clones of *P. taeda* replicated four times in the Virginia Piedmont. Stem volume was calculated based on the empirical formula found in Burkhart (1977). Standard error bars are shown.

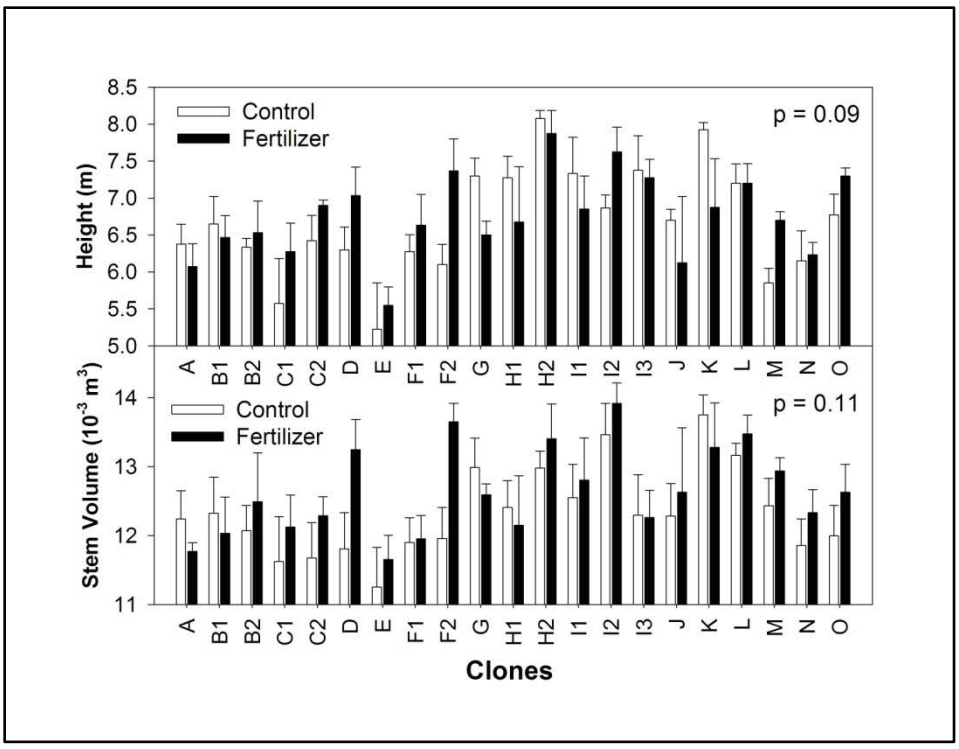

Figure 2-3. Clone-by-fertilizer interactions at age six for stem growth of 21 clones of *P. taeda* replicated four times in the Virginia Piedmont. Stem volume was calculated based on the empirical formula found in Burkhart (1977). Standard error bars are shown.

Table 2-1. P-values for variables measured on a six-year-old *P. taeda* trial replicated four times in the Virginia Piedmont. Crown volume, crown width, and growth efficiency ideotype were analyzed with repeated measures, while foliage per branch mass was only assessed after year 3. Significant p-values are shown in bold face.

Variable	Clone	Fertilizer	C x F	Year	C x Y	F x Y	C x F x Y
Height	**0.00**	**0.08**	**0.09**	**0.00**	**0.00**	**0.00**	0.10
dbh	**0.00**	**0.02**	0.23	**0.00**	**0.00**	0.88	1.00
Stem volume	**0.00**	**0.02**	0.11	**0.00**	**0.00**	**0.05**	0.89
Sinuosity score	**0.00**	0.11	0.99	0.25	1.00	0.49	1.00
Branch number	**0.00**	0.97	0.10	0.71	0.68	**0.02**	0.76
Branch diameter score	**0.00**	0.97	0.53	0.97	0.40	0.99	0.59
Branch angle score	**0.00**	0.99	**0.02**	**0.02**	0.46	0.92	0.80
Flush number	**0.00**	0.14	0.13	**0.00**	**0.00**	0.66	**0.09**
Crown volume	**0.03**	**0.05**	0.18	---	---	---	---
Crown width	**0.04**	**0.04**	**0.06**	---	---	---	---
Growth efficiency ideotype	**0.01**	**0.07**	0.21	---	---	---	---
Foliage per branch mass	**0.00**	0.27	**0.00**	---	---	---	---

2.3.3. Stem Form

By the end of the sixth growing season, some degree of stem sinuosity affected 25% of the surviving ramets in the trial. However, only 6% of surviving ramets were categorized in the worst sinuosity category, indicating that sinuosity was so severe that it would likely still be present at the end of a 20 year rotation. Neither height growth the year before quantifying sinuosity, nor height growth the year after were significantly correlated with the severity of sinuosity observed ($p > 0.10$). However, there were differences among clones in the frequency and severity of sinuosity observed ($p < 0.01$; Figure 2-4). For example, at age six 75% of the ramets of clone I2 were affected with sinuosity of a mean score of 3, indicating severe sinuosity that may still be observed at the end of a 20 year rotation. However, clone I3, full-sib to clone I2, showed no observable sinuosity in any ramet at age six.

Overall the incidence of forking observed in this trial, only 7% at age six, was very low. Only 5% of ramets in control plots (4 ramets) and 9% of ramets in fertilizer plots (7 ramets) were forked after six growing season. Only one clone had more than one forked ramet. Clone J had two forked ramets, one in a fertilizer plot and the other in a control plot. Ramicorns were observed at a much greater frequency, affecting 24% of all ramets after the sixth growing season. Ramicorns were nearly twice as common in fertilizer plots versus controls; while only 18% of control ramets had at least one ramicorn, 30% of ramets in fertilizer plots did. Ramicorn incidence also varied among clones (Figure 2-5). For example, clone H2 had no incidence of ramicorns at age six, while clone H1, full-sib to H2, had at least one ramicorn present on five of eight ramets. Fertilizer response in terms of ramets also varied among clones. For example, in clone A ramicorn incidence increased from 25% in control plots to 75% in fertilizer plots. Other clones showed little effect of fertilizer, such as clone I2 which had incidence rates of 33% and 25% in control and fertilizer plots, respectively. Still other clones showed no ramicorns in fertilized plots despite a high incidence in control plots, such as clone F2 (Figure 2-5). Again, no statistics are available for forking or ramicorn data due to issues with quasi-complete separation of data points among such a large number of clones.

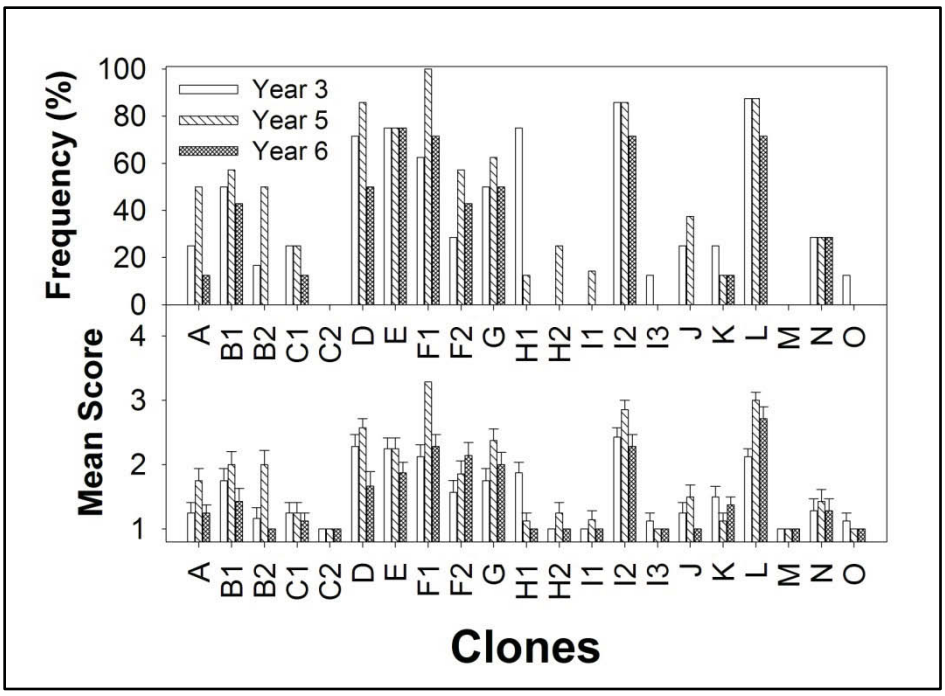

Figure 2-4. Sinuosity over three different years of 21 clones of *P. taeda* replicated four times in the Virginia Piedmont. Standard error bars are shown. Sinuosity increases in severity as the score increases. Specific detail on scoring may be found in the methods section.

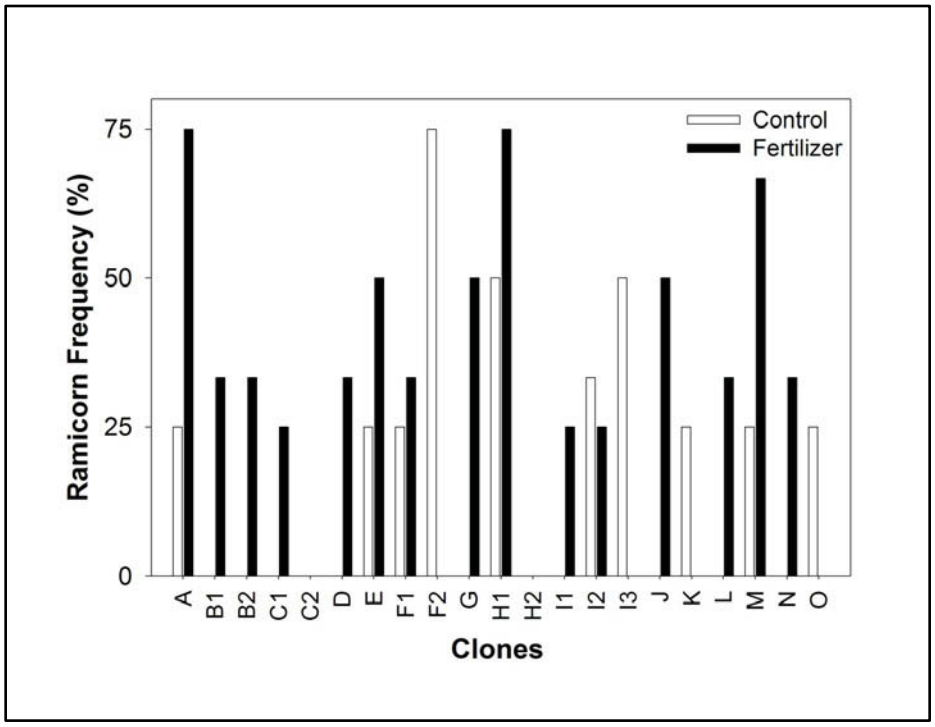

Figure 2-5. Ramicorn frequency at age six in fertilized and unfertilized ramets of 21 clones of *P. taeda* replicated four times in the Virginia Piedmont.

2.3.4. Cold Damage and Height Growth

Cold damage affected 18% of ramets in the winter of 2005-2006, after the third growing season. Table 2-2 displays the relationship between cold damage score and height growth in both the previous and subsequent growing seasons. Cold damage preferentially affected ramets that had displayed greater height growth in the previous growing season. Ramets affected by cold damage had grown an average of 1.44 m in the previous growing season, compared to mean height growth of only 1.20 m for unaffected ramets. Data were of insufficient temporal resolution to determine whether these ramets grew more rapidly or whether they continued to produce flushes later in the growing season. While ramets in the least severe cold damage category (score = 0.5) again showed the greatest height gains the following growing season, ramets that received the most severe cold damage showed significantly reduced height growth in 2006 ($p < 0.10$; Table 2-2). Cold damage appears to have been more severe for some clones compared to others (Figure 2-6). While some clones showed no cold damage in any ramet (e.g. clones B1, B2, H1, and H2). Other clones showed a high incidence of relatively minor cold damage (e.g. clones G, I1), while still others showed a high incidence of relatively major cold damage (e.g. clone M). This was despite all clones having been derived from Florida or Atlantic Coastal Plain provenances, two or more USDA plant hardiness zones away from this trial's location in the Virginia Piedmont. As with forking and ramicorn data, issues with quasi-complete separation of these data resulted in spurious statistical results, so no statistics are presented for inference.

Table 2-2. Cold damage data from the 2005-06 winter measured on a four-year-old *P. taeda* trial replicated four times in the Virginia Piedmont. Means are shown with one standard error in parentheses. Letters denote significantly different means based on Tukey's HSD test with $\alpha = 0.10$. Cold damage increases in severity as the score increases. Specific detail on scoring may be found in the methods section.

Cold damage score	N	Height growth previous growing season (m)	Height growth next growing season (m)
0	131	1.20 (0.03) A	1.29 (0.03) AB
0.5	15	1.44 (0.07) AB	1.42 (0.07) A
1	6	1.45 (0.08) B	1.17 (0.13) AB
2	8	1.44 (0.07) AB	1.03 (0.12) B

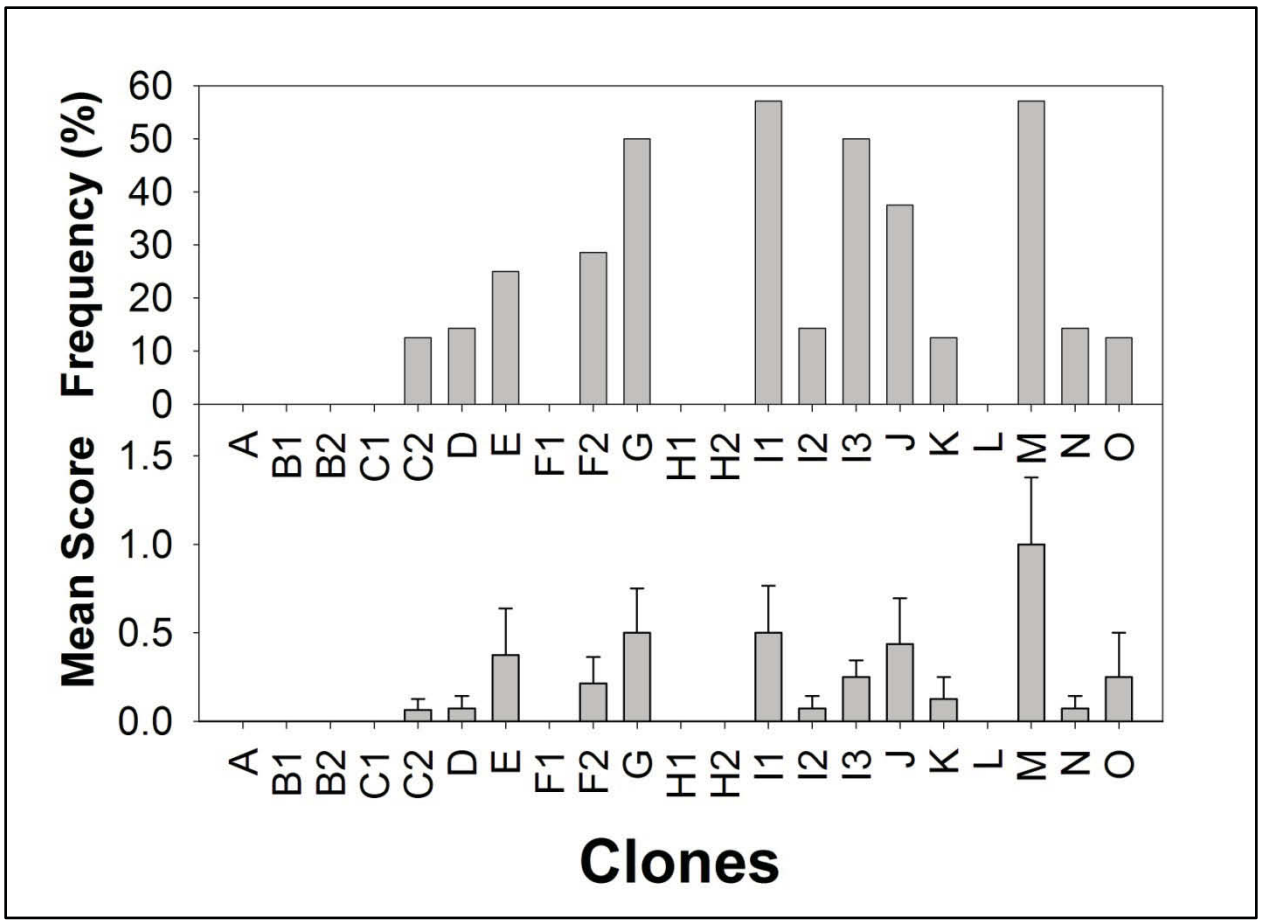

Figure 2-6. Cold damage from the 2005-06 winter of 21 clones of *P. taeda* replicated four times in the Virginia Piedmont. Standard error bars are shown. Cold damage increases in severity as the score increases. Specific detail on scoring may be found in the methods section.

2.3.5. Crown Morphology and Growth Efficiency

Clones varied in their branch morphology, and in the response of branch morphology to fertilizer application. Branch number and branch diameter varied among clones, but not in response to fertilizer application (Table 2-1; Figure 2-7). Branch number ranged from a mean of 2.6 branches per whorl in clone N to a mean of 4.0 in clone F2. Branch diameter score ranged from a category mean of 2.1 in clone H1 up to 3.8 in clone B2. While branch number and diameter did not vary in their fertilizer response among clones ($p > 0.10$), branch angle and crown width both showed significant clone-by-fertilizer interactions ($p < 0.10$; Figure 2-7). Some clones did not respond to fertilizer application in terms of branch angle (e.g. clones E and I3), while others

responded by reducing branch angle (e.g. clones F2 and K). Crown widths varied among clones, ranging from 119 cm in clone H1 to 166 cm in clone G. While crown width generally increased with fertilizer application, the magnitude of increase varied among clones. Additionally, there were several notable exceptions where fertilizer application resulted in reduced crown width (e.g. clone I2). The number of flushes per year also significantly varied among clones, ranging in the sixth growing season from a low mean of 2.0 in clone K to a high mean of 3.8 in clone B2. Flush number across the three growing seasons assessed showed a small but significant negative correlation to height growth ($p < 0.01$, $R^2 = 0.21$, data not shown), with fewer flushes corresponding to greater height growth.

There was a strong positive correlation between stem volume and crown volume across all data from years three, five, and six ($p < 0.01$, $R^2 = 0.79$; Figure 2-8). Despite this correlation, there was significant clonal variability in the stem volume per crown volume ratio, which we are interpreting as a growth efficiency ideotype ($p < 0.01$; Figure 2-9). The ratio ranged from a low of 2.1×10^{-3} in clone B2 to a high of 3.8×10^{-3} in clone O. While some full-sib pairs had similar ratios (e.g. clones B1 and B2), others contrasted in their ratios (e.g. clones H1 and H2). A positive correlation was observed between the foliar mass from a single representative branch harvested after the third growing season and stem volume increment in the fourth growing season ($p < 0.10$, $R^2 = 0.29$; Figure 2-10). When foliar mass was standardized to the mass of the branch from which it was harvested, a significant clone-by-fertilizer interaction emerged ($p < 0.01$; Figure 2-11). While a number of clones showed little response to fertilizer application (e.g. clones I2 and K), others showed increases (e.g. clones B2 and H1) while still others showed decreases (e.g. clones C2 and O). As with other clonal comparisons, some full-sib pairs showed similar fertilizer responses (e.g. clones F1 and F2) while others showed dissimilar responses (e.g. clones I1 and I2).

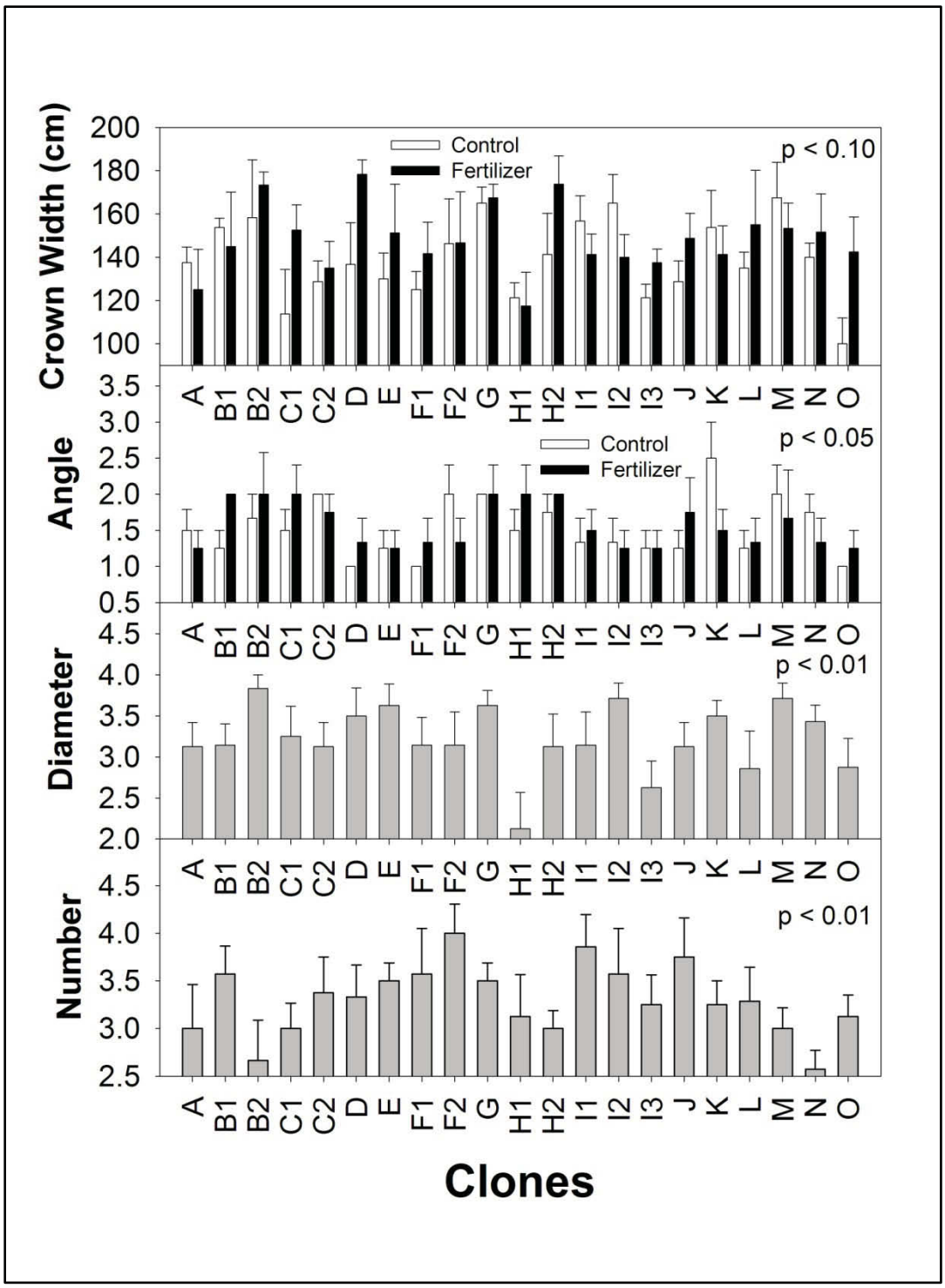

Figure 2-7. Branch morphology metrics at age six of 21 clones of *P. taeda* replicated four times in the Virginia Piedmont. The top two panels show clone-by-fertilizer interactions, while the bottom two depict only the clonal effect. Standard error bars are shown. Branch diameter increases in size and branch angle becomes increasingly vertical as scores increase. Specific detail on scoring may be found in the methods section.

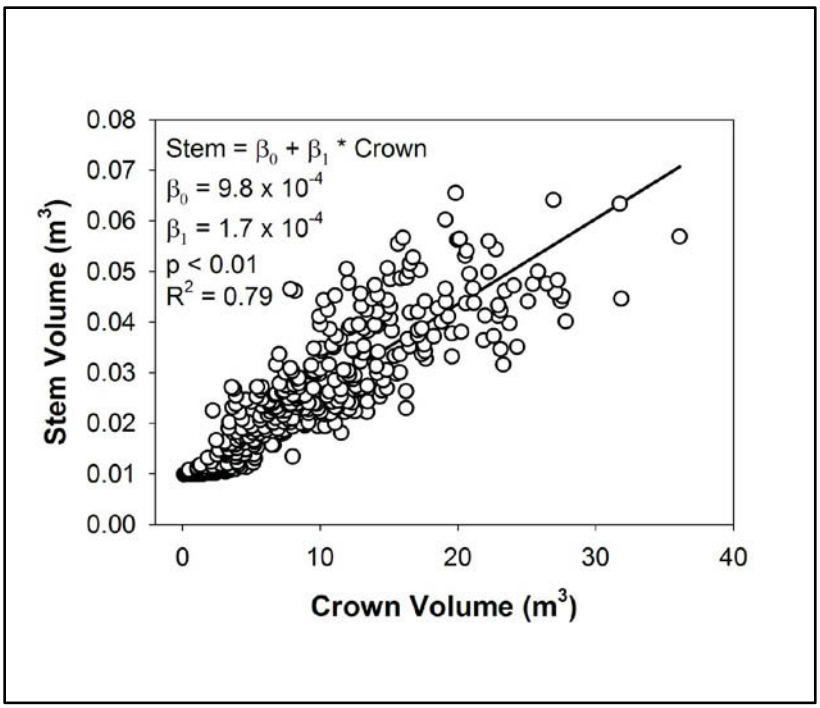

Figure 2-8. Simple linear regression of crown volume to stem volume with data from three different years from 21 clones of fertilized and unfertilized ramets of *P. taeda* replicated four times in the Virginia Piedmont. N = 468, regression statistics are shown.

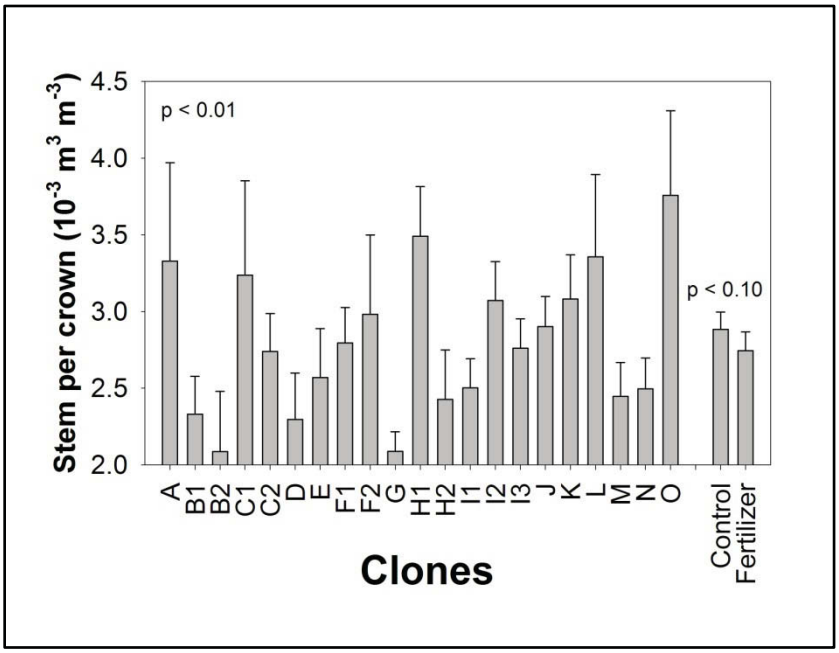

Figure 2-9. Clone and fertilizer main effects at age six for stem volume per crown volume from fertilized and unfertilized ramets of 21 clones of *P. taeda* replicated four times in the Virginia Piedmont. Stem volume per crown volume can be interpreted as an approximation of growth efficiency, or stem produced per unit foliage. Standard error bars are shown.

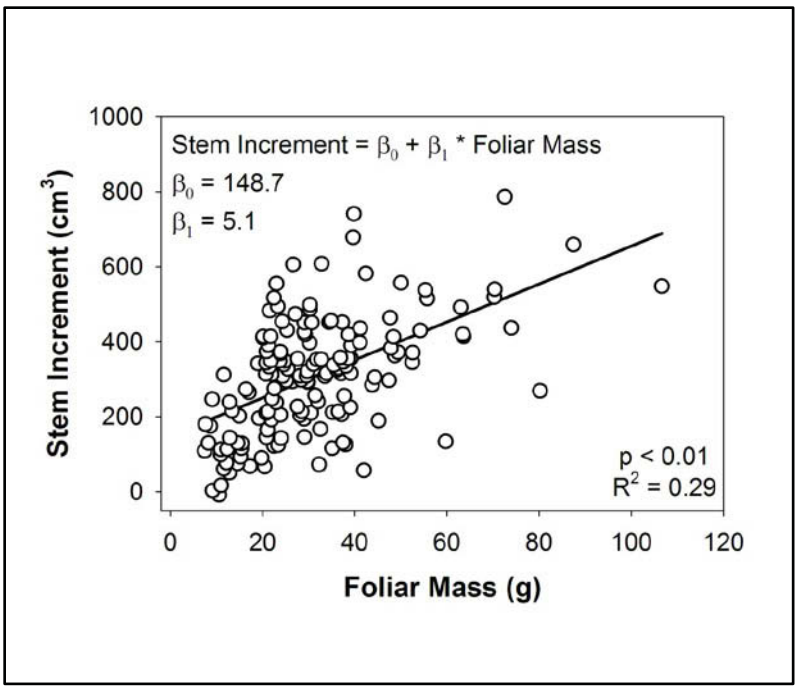

Figure 2-10. Simple linear regression of foliar mass per branch mass from a single representative branch harvested in January 2006 to stem volume increment the following growing season from 21 clones of fertilized and unfertilized ramets of *P. taeda* replicated four times in the Virginia Piedmont. N = 159, regression statistics are shown.

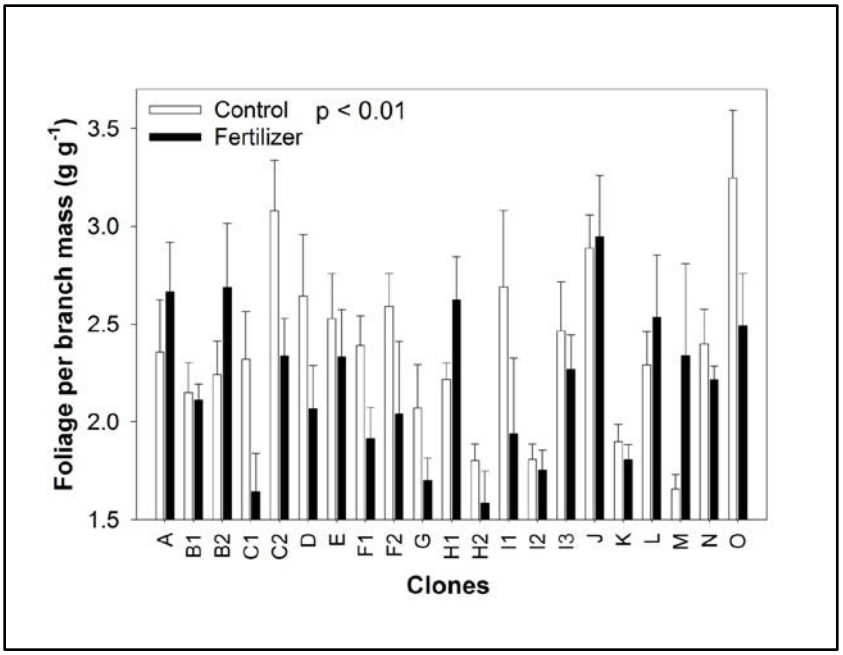

Figure 2-11. Clone-by-fertilizer interactions for foliar mass per branch mass from a single representative branch harvested in January 2006 from fertilized and unfertilized ramets of 21 clones of *P. taeda* replicated four times in the Virginia Piedmont. Standard error bars are shown.

2.4. Discussion

2.4.1. Growth, Growth Efficiency, and Implications for Clonal Testing

The range of growth rates observed in this trial among different clones was consistent with previous reports in the literature (Paul, Foster et al. 1997). The 19.8% difference in stem volume observed between the best and worst performing clones would likely increase by rotation age if growth trends observed by age six continued. The nearly continuous distribution of different clones within this range reflects the high degree of within-species genetic diversity in *P. taeda* breeding populations and the complex polygenic nature of growth (Williams, Hamrick et al. 1995; Kaya, Sewell et al. 1999). While the clone-by-time interaction was significant, it appears that this was largely the result of scale-effect rather than rank-shift interactions. For example, between the fifth and sixth years, no clone changed stem volume rank by more than three places out of 21 clones. This data supports that clonal selection at any time within years three to six will likely yield similar results in terms of identifying the top performing clones based on growth. However, whether these clones remain top performers at full rotation age is a distinct question not addressed by these data, although other evidence suggests that early selection based on growth data is likely predictive at rotation age (Lambeth 1980; Foster 1986; McKeand 1988).

The fertilizer growth response observed in this trial, while statistically significant, was small in magnitude (< 15% for all clones). The average growth response for *P. taeda* plantations in the southeast is 25% when fertilizer is applied midrotation (Fox, Jokela et al. 2007). It is possible that this site had sufficient nutrient availability for a newly initiated stand prior to crown closure, based on the model of stand nutrition over the length of a rotation described in Fox et al. (2007). However, prior to crown closure at approximately age 8 to 12, many plantations are not limited primarily by nutrients due to the small size of the trees relative to the large nutrient pool available from decomposing slash from the previous rotation (Switzer and Nelson 1972; Piatek and Allen 1999). While there was no slash on our site at planting, this experiment may still not have yet developed nutrient deficiencies substantial enough to result in a large fertilizer growth response. Further evidence to support this hypothesis includes relatively high foliar nitrogen content at age two as was reported in King et al. (2008) in eight of these clones. Levels were generally greater than 1.2%, the established critical limit for nitrogen limitation in *P. taeda*

(Comerford and Fisher 1984). While foliar nitrogen content is not as strong a predictor of fertilizer growth response as leaf area index (Vose and Allen 1988; Albaugh, Allen et al. 1998), we do not have leaf area index data for this trial.

The lack of a significant clone-by-fertilizer interaction for stem volume indicates that widespread clonal screening for these interactions may be unnecessary. However, we should note that a small number of clones did show substantial growth responses to fertilizer application compared to the trial average. While the 12 to 14% stem volume responses observed in these highly responsive clones is considerably less than average midrotation fertilizer growth responses in *P. taeda* as discussed above, a similar growth increase in a high performing clone may represent a substantial increase in both yield and value at harvest if single genotype stands are deployed. It is also possible that fertilizer response in these highly responsive genotypes might have been much more substantial on a site with lower native nutrition. While our data suggests that widespread clonal screening for fertilizer response is likely unnecessary, there do appear to be opportunities to realize substantial growth increases with appropriate nutrient management in a small number of genotypes, while other genotypes may be less sensitive to nutrient additions and require lower intensity, and thus lower cost, inputs. One potential solution suggested by our results is to test the fertilizer response of clones produced in large numbers as they are deployed on a site with minimal native nutrition over the first several years after planting to establish midrotation fertilizer recommendations for those same genotypes in operational plantations. This approach would likely provide adequate information for the management of deployed clones in a timely fashion, while eliminating the expense of testing clones that have not been operationally deployed for responsiveness to fertilizer application.

Clonal variability in growth efficiency or crown metrics that affect growth efficiency has been previously reported among clones of *P. taeda* (Emhart, Martin et al. 2007; Tyree, Seiler et al. 2009b). These results are consistent with the clonal main effect we observed in the stem volume per crown volume ratio. The clone-by-fertilizer interaction observed for foliage mass per branch mass indicated differences in foliar display in response to fertilizer application similar to previous reports (Tang, Chambers et al. 1999; Maier, Palmroth et al. 2008). However, our results point toward foliar display response to fertilizer application varying among genotypes,

demonstrating that results reported for two clones in Tyree et al. (2009b) are likely applicable across a greater population of clones. The fact that foliar mass per branch mass only accounted for 29% of the variability in stem growth in the following growing season indicates that photosynthetic rates, foliar morphology, and other traits that we did not assess likely varied among clones in response to fertilizer application, as has previously been reported for this and other studies (King, Seiler et al. 2008; Tyree, Seiler et al. 2009b). Variability in branch metrics among clones and in response to fertilizer application have both been previously observed, and are another source of fertilizer and clonal variability in crown and foliar display (Maier, Johnsen et al. 2002; Albaugh, Allen et al. 2006; Tyree, Seiler et al. 2009a). The extent of variation in crown traits and growth efficiency offers numerous opportunities for ideotype-based clonal selection, as has been previously discussed in Emhart et al. (2007).

2.4.2. Stem Form Defects

Stem form defects were found only at relatively low incidence rates in this trial, although their incidence did vary significantly among clones in some cases. While sinuosity was not severe in this trial when averaged across clones, we did find that some clones displayed a high incidence of severe sinuosity, indicating that clonal screening for severe cases of sinuosity may be advisable. Similar to our findings, sinuosity has previously been shown to vary among both open-pollinated families and clones in the *Pinaceae* family (Schermann, Adams et al. 1997; Espinoza 2009). Also consistent with our results, sinuosity in *Pseudotsuga menziesii* (Mirb.) Franco showed little correlation between leader height growth and sinuosity class (Gartner and Johnson 2006). Due to the extremely low incidence of forking in this trial, we can make no inference on treatment effects, although previous work has shown both genotypic variability in forking frequency and increased incidence forking as a result of fertilizer application (Schermann, Adams et al. 1997; McKeand, Jokela et al. 2006; Espinoza 2009). Ramicorns were a more common defect present in this trial. While ramicorn incidence almost doubled with fertilizer application, clones did vary widely in their ramicorn fertilizer response. Past studies have found conflicting results with regard to genetic variation in ramicorns, with some studies finding little variability among open-pollinated families (Codesido and Fernandez-Lopez 2008), while others observed different family means (Schermann, Adams et al. 1997). As with some of

our genotypes, *P. taeda* has previously shown worsening of stem form defects with fertilizer application in some provenances (Espinoza 2009).

2.4.3. Cold Damage and Survival

While we did find differences among genotypes in the frequency and severity of cold damage occurrence, overall incidence was low in this trial. There was no mortality that could be directly attributed to cold damage, and only eight ramets were assigned the worst cold damage score that was correlated with significantly reduced growth in the subsequent growing season. Our results are consistent with the literature in terms of genotypic variability in cold damage, which has previously been shown to vary among different open-pollinated families of *P. taeda* (Kolb, Steiner et al. 1985). Our ability to infer that cold damage will not be a problem for genotypes deployed far from their recommended hardiness zone (see Schmidtling 2001) is very limited based on this dataset from a single site over a single severe winter in only these 25 genotypes. However, the fact that some genotypes of Atlantic Coastal Plain and Florida provenances had no incidence of cold damage is promising for specific clones with regards to moving seed sources large distances from their origin. While inference space based on these data is similarly limited for survival, mortality was higher in four genotypes than the rest. The fact that all of this mortality occurred prior to the third growing season in these clones suggests that the seedlings may have had varying levels of fitness across clones, but we cannot attribute this mortality to a more specific cause. Genotype-by-environment interactions for survival across a number of sites are already implicitly considered in clonal testing, since dead trees cannot be selected for favorable traits, so little change in current practices is suggested by these data.

2.5. Conclusion: Implications for Clone-Specific Silvicultural Systems

Differences in clonal traits offer a number of different opportunities for precision silvicultural systems targeted to specific clones planted in single-genotype blocks. For instance, clones with rapid stem volume growth rates and narrow crowns might be planted at a wide spacing if they do not have problems with ramicorns, allowing for the production of a high percentage of sawtimber without necessitating intermediate treatments beyond fertilizer application and possibly pruning. Even if pruning becomes necessary, selecting clones with a lower number of

smaller diameter branches may reduce effort and thus costs involved with pruning operations. Clones with lower foliage per branch mass ratios may allow more light to reach the understory through their more diffuse crowns, and thus be more appropriate for inter-row planting with biomass crops such as switch grass. Alternatively, clones with high foliage per branch mass ratios may create a more heavily shaded understory, possibly reducing requirements for competition control earlier in the rotation. These examples highlight but a few of the possibilities for clone-specific silvicultural systems that depend on the specific traits of individual clones that are selected for deployment over large acreages. These results also suggest that companies practicing clonal forestry could select a number of similarly performing clones in order to eliminate unnecessary complications that might arise from multiple clone-specific silvicultural regimes. Further work to optimize the management of individual genotypes produced in large numbers for specific product classes may greatly increase the value of clonal plantations in the coming decades.

Our results also indicate that clone-by-fertilizer interactions for a number of traits, such as height growth, branch traits, and foliage per branch mass may offer specific silvicultural opportunities in the management of clonal plantations. Genotypes with growth rates that are known to be more responsive to fertilizer application can be managed with maximal nutrient inputs in order to take full advantage of their responsiveness. By contrast, genotypes that are less responsive to fertilizer application may be fertilized at lower rates, thus reducing costs without substantially reducing potential growth. Minimizing fertilizer rates in these genotypes may also reduce the incidence of stem quality defects that have been associated with greater nutrient additions in other studies (e.g. Espinoza 2009). While clone-by-fertilizer interactions can create management opportunities, lacking information on the fertilizer response of specific genotypes may make it difficult to optimize the management of clonal plantations. Fertilizer applied to unresponsive genotypes could be an unnecessary expense, while on the other end of the spectrum insufficient fertilizer application for clones that are highly responsive could result in yields substantially below what might be economically achieved. While further research is necessary on fertilizer interactions with a greater number of clones on a greater number of sites, the results from this

trial suggest that opportunities do exist for the design and application of clone-specific silvicultural systems for widely deployed *P. taeda* genotypes.

2.6. Literature Cited

Albaugh, T. J., H. L. Allen, P. M. Dougherty, L. W. Kress and J. S. King (1998). "Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions." Forest Science **44**(2): 317-328.

Albaugh, T. J., H. L. Allen and T. R. Fox (2006). "Individual tree crown and stand development in *Pinus taeda* under different fertilization and irrigation regimes." Forest Ecology and Management **234**(1-3): 10-23.

Baltunis, B. S., D. A. Huber, T. L. White, B. Goldfarb and H. E. Stelzer (2007). "Genetic analysis of early field growth of loblolly pine clones and seedlings from the same full-sib families." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **37**(1): 195-205.

Bettinger, P., M. Clutter, J. Siry, M. Kane and J. Pait (2009). "Broad implications of southern United States pine clonal forestry on planning and management of forests." International Forestry Review **11**(3): 331-345.

Burkhart, H. E. (1977). "Cubic-foot volume of loblolly pine to any merchantable top limit." Southern Journal of Applied Forestry **1**: 7-9.

Chmura, D. J., M. S. Rahman and M. G. Tjoelker (2007). "Crown structure and biomass allocation patterns modulate aboveground productivity in young loblolly pine and slash pine." Forest Ecology and Management **243**(2-3): 219-230.

Codesido, V. and J. Fernandez-Lopez (2008). "Juvenile genetic parameter estimates for vigour, stem form, branching habit and survival in three radiata pine (*Pinus radiata* D.Don) progeny tests in Galicia, NW Spain." European Journal of Forest Research **127**(4): 315-325.

Colbert, S. R., E. J. Jokela and D. G. Neary (1990). "Effects of annual fertilization and sustained weed-control on dry-matter partitioning, leaf-area, and growth efficiency of juvenile loblolly and slash pine." Forest Science **36**(4): 995-1014.

Comerford, N. B. and R. F. Fisher (1984). "Using foliar analysis to classify nitrogen-deficient sites." Soil Science Society of America Journal **48**: 910-913.

Dalla-Tea, F. and E. J. Jokela (1991). "Needlefall, canopy light interception, and productivity of young intensively managed slash and loblolly-pine stands." Forest Science **37**(5): 1298-1313.

Dougherty, D. (2007). "Improved returns on forestlands: A financial analysis of MCP and varietal seedlings on private land in the southeastern United States." Forest Landowner **66**: 15-18.

Emhart, V. I., T. A. Martin, T. L. White and D. A. Huber (2007). "Clonal variation in crown structure, absorbed photosynthetically active radiation and growth of loblolly pine and slash pine." Tree Physiology **27**(3): 421-430.

Espinoza, J. A. (2009). Genetic and nutritional effects on stem sinuosity in loblolly pine. Forestry. Raleigh, NC, North Carolina State University. **Ph.D. Dissertation:** 102 p.

Foster, G. S. (1986). "Trends in genetic parameters with stand development and their influence on early selection for volume growth in loblolly pine." Forest Science **32**: 944-959.

Fox, T. R., H. L. Allen, T. J. Albaugh, R. Rubilar and C. A. Carlson (2007). "Tree nutrition and forest fertilization of pine plantations in the southern United States." Southern Journal of Applied Forestry **31**(1): 5-11.

Fox, T. R., E. J. Jokela and H. L. Allen (2007). "The development of pine plantation silviculture in the southern United States." Journal of Forestry **105**: 337-347.

Gartner, B. L. and G. R. Johnson (2006). "Is long primary growth associated with stem sinuosity in Douglas-fir?" Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **36**(9): 2351-2356.

Kaya, Z., M. M. Sewell and D. B. Neale (1999). "Identification of quantitative trait loci influencing annual height- and diameter-increment growth in loblolly pine (*Pinus taeda* L.)." Theoretical and Applied Genetics **98**(3): 586-592.

King, N. T., J. R. Seiler, T. R. Fox and K. H. Johnsen (2008). "Post-fertilization physiology and growth performance of loblolly pine clones." Tree Physiology **28**(5): 703-711.

Kolb, T. E., K. C. Steiner and H. F. Barbour (1985). "Seasonal and genetic variations in loblolly-pine cold tolerance." Forest Science **31**(4): 926-932.

Lambeth, C. C. (1980). "Juvenile-mature correlations in *Pinaceae* and implications for early selection." Forest Science **26**(4): 571-580.

Maier, C. A., K. H. Johnsen, J. Butnor, L. W. Kress and P. H. Anderson (2002). "Branch growth and gas exchange in 13-year-old loblolly pine (*Pinus taeda*) trees in response to elevated carbon dioxide concentration and fertilization." Tree Physiology **22**(15-16): 1093-1106.

Maier, C. A., S. Palmroth and E. Ward (2008). "Short-term effects of fertilization on photosynthesis and leaf morphology of field-grown loblolly pine following long-term exposure to elevated CO_2 concentration." Tree Physiology **28**(4): 597-606.

Martin, T. A., K. H. Johnsen and T. L. White (2001). "Ideotype development in southern pines: Rationale and strategies for overcoming scale-related obstacles." Forest Science **47**(1): 21-28.

McCrady, R. L. and E. J. Jokela (1996). "Growth phenology and crown structure of selected loblolly pine families planted at two spacings." Forest Science **42**(1): 46-57.

McKeand, S. E. (1988). "Optimum age for family selection for growth in genetic tests of loblolly pine." Forest Science **34**: 400-411.

McKeand, S. E., E. J. Jokela, D. A. Huber, T. D. Byram, H. L. Allen, B. L. Li and T. J. Mullin (2006). "Performance of improved genotypes of loblolly pine across different soils, climates, and silvicultural inputs." Forest Ecology and Management **227**(1-2): 178-184.

Nelson, C. D. and K. H. Johnsen (2008). "Genomic and physiological approaches to advancing forest tree improvement." Tree Physiology **28**: 1135-1143.

Paul, A. D., G. S. Foster, T. Caldwell and J. McRae (1997). "Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine." Forest Science **43**(1): 87-98.

Piatek, K. B. and H. L. Allen (1999). "Nitrogen mineralization in a pine plantation fifteen years after harvesting and site preparation." Soil Science Society of America Journal **63**(4): 990-998.

Roth, B. E., E. J. Jokela, T. A. Martin, D. A. Huber and T. L. White (2007). "Genotype x environment interactions in selected loblolly and slash pine plantations in the southeastern United States." Forest Ecology and Management **238**(1-3): 175-188.

Samuelson, L. J., K. Johnsen and T. Stokes (2004). "Production, allocation, and stemwood growth efficiency of *Pinus taeda* L. stands in response to 6 years of intensive management." Forest Ecology and Management **192**(1): 59-70.

Schermann, N., W. T. Adams, S. N. Aitken and J. C. Bastien (1997). "Genetic parameters of stem form traits in a 9-year-old coastal Douglas fir progeny test in Washington." Silvae Genetica **46**(2-3): 166-170.

Schmidtling, R. C. (2001). Southern pine seed sources, USDA Forest Service General Technical Report SRS-44, Southern Rearch Station, Asheville, NC.

Switzer, G. L. and L. E. Nelson (1972). "Nutrient accumulation and cycling in loblolly pine (*Pinus taeda* L.) plantation ecosystems: The first twenty years." Soil Science Society of America Journal **36**(1): 143-147.

Tang, Z. M., J. L. Chambers, S. Guddanti, S. F. Yu and J. P. Barnett (1999). "Seasonal shoot and needle growth of loblolly pine responds to thinning, fertilization, and crown position." Forest Ecology and Management **120**(1-3): 117-130.

Tyree, M. C., J. R. Seiler and T. R. Fox (2008). "The effects of fertilization on soil respiration in 2-year-old *Pinus taeda* L. clones." Forest Science **54**(1): 21-30.

Tyree, M. C., J. R. Seiler and C. A. Maier (2009a). "Short-term impacts of nutrient manipulations on leaf gas exchange and biomass partitioning in contrasting 2-year-old *Pinus taeda* clones during seedling establishment." Forest Ecology and Management **257**(8): 1847-1858.

Tyree, M. C., J. R. Seiler, C. A. Maier and K. H. Johnsen (2009b). "*Pinus taeda* clones and soil nutrient availability: Effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology." Tree Physiology **29**(9): 1117-1131.

Vose, J. M. and H. L. Allen (1988). "Leaf-area, stemwood growth, and nutrition relationships in loblolly-pine." Forest Science **34**(3): 547-563.

Williams, C. G., J. L. Hamrick and P. O. Lewis (1995). "Multiple-population versus hierarchical conifer breeding programs - a comparison of genetic diversity levels." Theoretical and Applied Genetics **90**(3-4): 584-594.

Wright, J. and D. Dougherty (2007). "Silviculture for your varietal loblolly pine plantation." Forest Landowner **66**: 26-29.

Wright, J. and P. Dougherty (2006). "Varietal forestry." Forest Landowner **65**(5): 3-4.

Xiao, Y., E. J. Jokela and T. L. White (2003). "Species differences in crown structure and growth performance of juvenile loblolly and slash pine." Forest Ecology and Management **174**(1-3): 295-313.

Yu, S. F., J. L. Chambers, Z. M. Tang and J. P. Barnett (2003). "Crown characteristics of juvenile loblolly pine 6 years after application of thinning and fertilization." Forest Ecology and Management **180**(1-3): 345-352.

3. Allometry and growth efficiency varies among six-year-old *Pinus taeda* (L.) clones in the Virginia Piedmont

3.1. Introduction

Clonal plantation forestry has become a practical reality in the southeastern United States due to technological advances in somatic embryogenesis for *Pinus taeda* (L.) (Stasolla and Yeung 2003; Bettinger, Clutter et al. 2009; Whetten and Kellison 2010). Clones are being deployed now primarily to improve growth rates, with gains in stem volume growth of up to 100% predicted (McKeand, Mullin et al. 2003; Whetten and Kellison 2010). Clonal seedlings cost considerably more than either open pollinated or mass control pollinated seedlings, thus necessitating relatively intensive silvicultural inputs to maximize productivity and justify higher initial costs at planting (Dougherty 2007). Fertilizer is already applied to approximately 650,000 ha annually as of 2004, and typically yields a growth response of 25% when applied to mid-rotation stands (Fox, Allen et al. 2007). It is likely that fertilizer application will be a common cultural input for the majority of clonal plantations in the future.

Clonal tests have revealed a wide range of stem volume growth rates (Paul, Foster et al. 1997; Coyle, Coleman et al. 2006). While considerable work has been done to understand the ecophysiological differences between clones leading to dissimilar growth rates (e.g. King, Seiler et al. 2008; Bown, Watt et al. 2009; Tyree, Seiler et al. 2009a), uncertainty remains regarding what specific physiological process or processes are most responsible for regulating stem volume production in different clonal genotypes. Further, the possibility of clone-by-fertilizer interactions and the ecophysiological mechanisms underlying such potential interactions requires further investigation. Most studies of genotype-by-environment interactions involving clonal material have been performed in young stands (i.e. < 5 years), some of which found interactions (Paul, Foster et al. 1997), and others did not (Baltunis, Huber et al. 2007). Results from single-site studies have shown clone-by-fertilizer interactions for growth (King, Seiler et al. 2008; Tyree, Seiler et al. 2009a; Tyree, Seiler et al. 2009b). While leaf-level gas exchange does not consistently explain these differences in clonal fertilizer growth response (King, Seiler et al. 2008), canopy-level CO_2 assimilation coupled with clonal differences in biomass partitioning,

the amount of biomass allocated to various plant organs, was shown to be consistent with different clonal fertilizer-growth responses in a greenhouse experiment with two *P. taeda* genotypes (Tyree, Seiler et al. 2009a). However, it remains uncertain if differences in biomass partitioning will remain a consistent explanation of fertilizer growth response among a larger population of clones. The primary objective of this chapter was to determine whether clonal differences in biomass partitioning and allometry are consistent with observed differences in clonal growth rates and potential clone-by-fertilizer interactions.

Biomass partitioning in trees is an important variable in determining stem volume growth efficiency (Albaugh, Allen et al. 1998), ecosystem carbon balance (Maier, Albaugh et al. 2004), and belowground carbon sequestration in stumps and coarse roots (Van Lear, Kapeluck et al. 2000; Miller, Allen et al. 2006). Partitioning varies with availability of nutrients, typically resulting in greater allocation to woody perennial tissues and decreased root-shoot ratios with fertilizer application (Ingestad and Agren 1991; King, Albaugh et al. 1999). However, biomass partitioning also changes with stand development and growth, requiring a direct comparison of plants of similar size to determine whether treatment effects are altering partitioning directly, or are merely altering growth rates (Ledig, Bormann et al. 1970). When growth is accounted for in analyses of biomass partitioning, allometric relationships typically show minimal or no effect of fertilizer application compared to treatment effects on growth rates (King, Albaugh et al. 1999; Coyle and Coleman 2005). While biomass partitioning has been shown to vary among both open-pollinated seedlings of different provenance and different clonal genotypes (Barnes 2002; Bown, Watt et al. 2009; Tyree, Seiler et al. 2009a; Tyree, Seiler et al. 2009b), studies determining whether these effects are due to genotypic variability in allometry versus genotypic differences in growth rate are lacking.

In addition to biomass partitioning to roots, root morphology may also vary between clones and in response to fertilizer application. A study of *Pinus radiata* (D. Don) found significant differences between clonal and open-pollinated genotypes not only for root-shoot ratio, but also for distribution of root mass among lateral and vertical coarse roots (Gautam, Mead et al. 2003). In open-pollinated bare-root seedlings of *P. taeda* the vertical distribution of root biomass and

maximum rooting depth have been shown to vary by provenance (Barnes 2002). Given these results and the overall phenotypic plasticity of *P. taeda* (Samuelson, Johnsen et al. 2004), it seems likely clonal differences in coarse root morphology will be observed. A secondary objective of this chapter was to assess genotypic variability in coarse root morphology and maximum depth of rooting in clones of *P. taeda* and determine whether these traits differ among clones in response to fertilizer application.

Biomass partitioning to foliage and patterns of foliar display have previously been correlated to growth in *P. taeda* (Chmura, Rahman et al. 2007; Emhart, Martin et al. 2007; Tyree, Seiler et al. 2009b). Clonal variability has been observed in crown size (Tyree, Seiler et al. 2009b), and crown size has shown a strong genetic correlation with growth in clonal *P. taeda* (Emhart, Martin et al. 2007). Additionally, the relationship between foliar mass and crown size was shown to vary in response to a nutrient availability gradient among two clones of *P. taeda* in a single-site field trial (Tyree, Seiler et al. 2009b). Stem-foliage mass ratios can be considered as an index of growth efficiency (i.e. unit wood produced per unit photosynthetic tissue). This ratio has been shown to vary between clones, and has shown significant clone-by-nutrient-availability interactions (Tyree, Seiler et al. 2009a; Tyree, Seiler et al. 2009b). Considering differences in crown size and display along with differences in biomass partitioning may improve our ability to determine the physiological basis of variable clonal growth rates. The final objective of this chapter was to determine whether a clone-by-fertilizer interaction for crown traits such as that observed in Tyree et al. (2009b) would be observed among a greater number of clones.

3.2. Materials and Methods

3.2.1. Site and Study Descriptions

Our study was located at the Reynolds Homestead Forestry Research Center (36° 40’ N, 80° 10’ W) in the upper Piedmont of Patrick County, Virginia, USA. Annual precipitation for the site is 1,308 mm, while mean annual maximum and minimum temperatures are 18.5° C and 7.0° C, respectively. Average July high temperature is 29.3° C, and average January low temperature is -4.0° C. Topography consists of gently sloping hills ranging in elevation from 320 to 340 m.

Past intensive agricultural land use has resulted in a truncated Ap horizon, with clayey B horizons mixed with the A. Mapped soil series include a Fairview sandy clay loam (fine, kaolinitic, mesic Typic Kanhapludults) and a French loam (fine-loamy over sandy or sandy-skeletal, mixed, active, mesic Fluvaquentic Dystrudepts). Site and study design descriptions can also be found in Tyree et al. (2008) and King et al. (2008).

A split-plot experimental design was installed in May 2003, with the whole plots being two levels of fertilizer application (with or without) and the split-plot factor being 25 clones. Whole plot treatments were blocked and replicated four times. One ramet (experimental unit) of each clone was planted in each plot. Ramets were rooted cuttings planted at a 3.0-by-2.5 m spacing. Clonal material donated by the Forest Biology Research Cooperative (Gainsville, Florida, USA) was from the Loblolly Pine Lower Gulf Elite Breeding Population, which includes both Atlantic Coastal and Florida provenances. Site preparation prior to planting included application of glyphosphate (Round Up®) for weed control. The site was subsequently ripped and the planting rows were shallowly cultivated. A border row of open pollinated seedlings was planted around each plot. After planting complete weed control was maintained for the first two years. Prior to root growth between plots trenches were dug and lined with plastic along intersecting plot boundaries to contain the fertilizer treatment. Fertilizer was applied by hand-banded application on May 4, 2004, May 4, 2006, and July 16, 2008. Each application consisted of 224 kg ha^{-1} of diammonium phosphate and 184 kg ha^{-1} of ammonium nitrate, yielding 103 kg ha^{-1} of elemental N and 45 kg ha^{-1} of elemental P.

3.2.2. Destructive Harvest

Aboveground biomass for the trial was harvested after six complete growing seasons in February, 2009. Fresh weights were obtained for all surviving ramets immediately after harvest. Ten clones (75 ramets total) were selected from the full trial for belowground destructive harvest. These ten clones included four full-sib pairs and two other clones. Clones were selected so that they included five of the best performing clones based on fresh weight and five of the poorest performing clones. Each of these ten clones had at least three of four ramets surviving in each fertilized and unfertilized plots. After ramets were felled with a chainsaw 10 cm above

ground line, they were partitioned into stem, branch, and foliar components, which were each oven-dried at 65° C for > 15 days and weighed.

In the field immediately prior to dissection, each ramet of the ten selected clones was photographed against a cloudless blue sky for determination of the crown silhouette area (CSA), an index of displayed leaf area (see King, Seiler et al. 2008). Ramets were photographed twice from right angles about the stem and values were averaged to yield CSA. A Nikon D70 digital SLR camera with an AF-S DX Zoom-Nikkor 18-70mm f/3.5-4.5G IF-ED lens was used for all photographs (Nikon Inc, Melville, New York, USA). Underexposure of 1.0 eV was automatically set to maximize contrast between canopy and sky. Crown images were then thresholded in SideLook v.1.1 (Nobis and Hunziker 2005) and post-processed for analysis in Adobe PhotoShop 6.0 (Adobe Systems Inc., San Jose, California, USA). Pixels containing canopy elements were counted and scaled to area based on comparison with a photographed object of known area, resulting in CSA in units of m^2.

The root systems of the ten clones selected for belowground harvest were excavated from a one meter diameter by one meter deep cylindrical pit. No roots below one meter were sampled, and if a stump ended before one meter, maximum rooting depth was measured and recorded. A Supersonic X-LT air knife (Supersonic Inc, Allison Park, Pennsylvania, USA) connected to 0.5 l s^{-1}, 850 kPa, diesel air compressor was employed to loosen the bulk soil and remove soil from roots. All roots within the sampled volume known to be from that specific ramet (i.e. those still attached to the taproot) were collected. Soils were not sieved, and no attempt was made to sample all fine roots. Fine root cores or pits were not attempted since a clonal effect could not be determined with certainty in this trial due to the single-tree clonal plots.

Roots were further pressure washed of soil, and separated into taproot and lateral root components. Root systems observed were characterized by a large, single primary tap root with numerous large lateral and sinker roots emanating from the primary taproot. Taproots were considered any growing downward within 45° of vertical, and thus included both the primary taproot and all sinker roots, while lateral roots were considered those growing within 45° of

horizontal. Lateral roots were further subdivided into coarse (> 15 mm), medium (5 to 15 mm) and fine (< 5 mm) fractions. All roots emanating from the primary taproot were excised as close to the main stump as possible, categorized as either lateral (< 45° from horizontal) or sinker (> 45° from horizontal) roots, and measured for diameter along two axes at the point of excision. For each stump root number, average diameter, and standard deviation of diameter were calculated for both sinker and lateral root fractions. All roots were then oven-dried at 65° C for > 15 days and weighed by fraction.

3.2.3. Data Analysis

All analyses were performed with SAS software version 9.2 (SAS Institute, Cary, North Carolina, USA). Model assumptions were checked and data was power or natural log transformed as necessary, although all reported means and standard errors are untransformed. All variables were analyzed with PROC MIXED with block and block-by-fertilizer modeled as random effects. The Kenward-Roger method for calculating denominator degrees of freedom was applied (Littell, Milliken et al. 2006). Multiple comparisons utilized a Tukey HSD procedure implemented in PROC GLM at an $\alpha = 0.10$ significance level. Since many ramets rooted to the full sample depth, we were unable to adequately transform root depth data to normal. The non-parametric Friedman's Chi Square test was utilized for analysis of this variable and was implemented in PROC FREQ. Regression of CSA to foliar mass was done with PROC REG.

Allometric relationships were examined by regressing natural log transformed biomass components against one another. This technique is necessary to determine whether shifts in allometry are attributable to treatment effects or whether they occur principally as a result of treatments influencing size. We observed a relatively large range in tree masses as a result of fertilizer treatments and block effects (mean = 26.2 kg, standard deviation = 11.4 kg, N = 75). The range of natural log transformed whole tree biomass observed was 2.79 across all trees and averaged 1.34 within each clone. This technique has previously been applied to data obtained from a single destructive harvest (Colbert, Jokela et al. 1990), and is typically applied to a population with a range of natural log transformed whole tree biomass of 2 to 5 (Gebauer,

Reynolds et al. 1996; King, Albaugh et al. 1999; Adegbidi, Jokela et al. 2002; Barnes 2002; Coyle and Coleman 2005). These regression equations were of the form:

$$[1] \qquad \ln(y) = a + k \ln(x)$$

where 'ln(x)' was the natural logarithm of one biomass component, 'ln(y)' was the natural logarithm of another biomass component, and 'a' and 'k' were the regression coefficients (Ledig, Bormann et al. 1970).

Previous biomass partitioning studies employing this technique test the slope, 'k' (e.g. Coyle and Coleman 2005) or test 'a' and 'k' independently using ANCOVA (e.g. Colbert, Jokela et al. 1990; Jokela and Martin 2000). However, we utilized a procedure known as conditional error (Swindel 1970) to simultaneously test both regression coefficients. Conditional error calculates an F statistic by comparing the sum of squares of errors from a combined model using data from multiple sample populations and the reduced models for each sample population separately, as given by the equation:

$$[2] \qquad F = [f \times (SSE^* - SSE)] / [(f^* - f) \times SSE]$$

where 'F' is the test statistic, 'f*' is the error degrees of freedom of the combined model, 'f' is the summed error degrees of freedom from the reduced models, 'SSE*' is the sum of squares of errors of the combined model, and 'SSE' is the total of all sums of squares of errors of the reduced models. A significant p-value indicates that the reduced models have significantly different matrices of regression coefficients, and should thus not be combined into a single model. At least one pair of reduced models has a different slope, intercept, or both a different slope and intercept. This procedure was employed to test both clone and fertilizer main effects. Clone-by-fertilizer interactions for allometric equations were not examined; this would have involved comparing regressions based on only three or four data points, and would not have yielded robust results.

3.3. Results

3.3.1. Biomass Partitioning

Significant clone-by-fertilizer interactions were observed for aboveground, belowground, and total tree mass ($p < 0.10$; Table 3-1). Clones showed variability in their mean mass and did not all respond similarly to fertilizer application, sometimes even between clones from the same full-sib crosses (Figure 3-1). Some clones showed significant increases in some biomass components with fertilizer application. For example Clone C2 increased in total mass by 33%, belowground mass by 32%, and aboveground mass by 33% with fertilizer application ($p < 0.10$; Figure 3-1). By contrast, clone C1, from the same full-sib cross showed no significant increases in any of these three biomass components in the fertilizer plots ($p > 0.10$). Additionally, other clones actually showed significantly decreased biomass with fertilizer application. Clone K had 13% less total mass and 17% less aboveground mass in fertilized plots ($p < 0.10$), with no difference in belowground biomass ($p > 0.10$). Across all clones there was substantial variability in biomass, with clone K having the greatest average total dry mass at 35.4 kg, 77% greater than clone I3, the least massive clone at 20.0 kg ($p < 0.10$). Full-sib pairs of clones also varied in the magnitude of the differences between them. For example, clone I2 had significantly greater total, aboveground, and belowground biomass than clone I3 in control plots ($p < 0.10$; Figure 3-1). By contrast clones C2 and C3 showed no significant differences in any of these three biomass components in either fertilized or control plots ($p > 0.10$).

Averaged across the clones, fertilizer application increased foliar, branch, and taproot biomass by 18%, 27%, and 18% respectively ($p < 0.10$), but had no effect on stem or lateral root mass ($p > 0.10$; Table 3-1; Figure 3-2). There were highly significant clonal effects for each of these five components ($p < 0.01$) except for taproot biomass, which was nonetheless significant ($p < 0.10$). The greatest clonal differences observed for foliar and branch mass were actually between full-sib clones I2 and I3. Clone I2 had 149% greater foliar mass and 234% greater branch mass than clone I3 ($p < 0.10$; Figure 3-2). By contrast other full-sib pairs, such as clones C1 and C2, showed no differences for foliar or branch biomass ($p > 0.10$). Stem mass also varied considerably among clones, with clone K having the greatest stem dry mass of 16.7 kg, 93%

greater than the clone with the least stem mass, clone C2, with an average mass of only 8.7 kg ($p < 0.10$). Taproot and lateral root biomass followed similar trends. Taproot dry mass ranged from 2.7 kg in clone C2 to 5.0 kg in clone K, a difference of 84% ($p < 0.10$). Again, some full-sib pairs had different taproot mass (e.g. clones I2 and I3), while other full-sib pairs showed no difference in taproot mass (e.g. clones C1 and C2). Attached lateral root dry mass within the sampled soil volume ranged from 0.6 kg in clone I3 to 1.7 kg in clone K, a difference of 184% ($p < 0.10$). Lateral root dry mass also varied between clones from the same full-sib crosses (Figure 3-2).

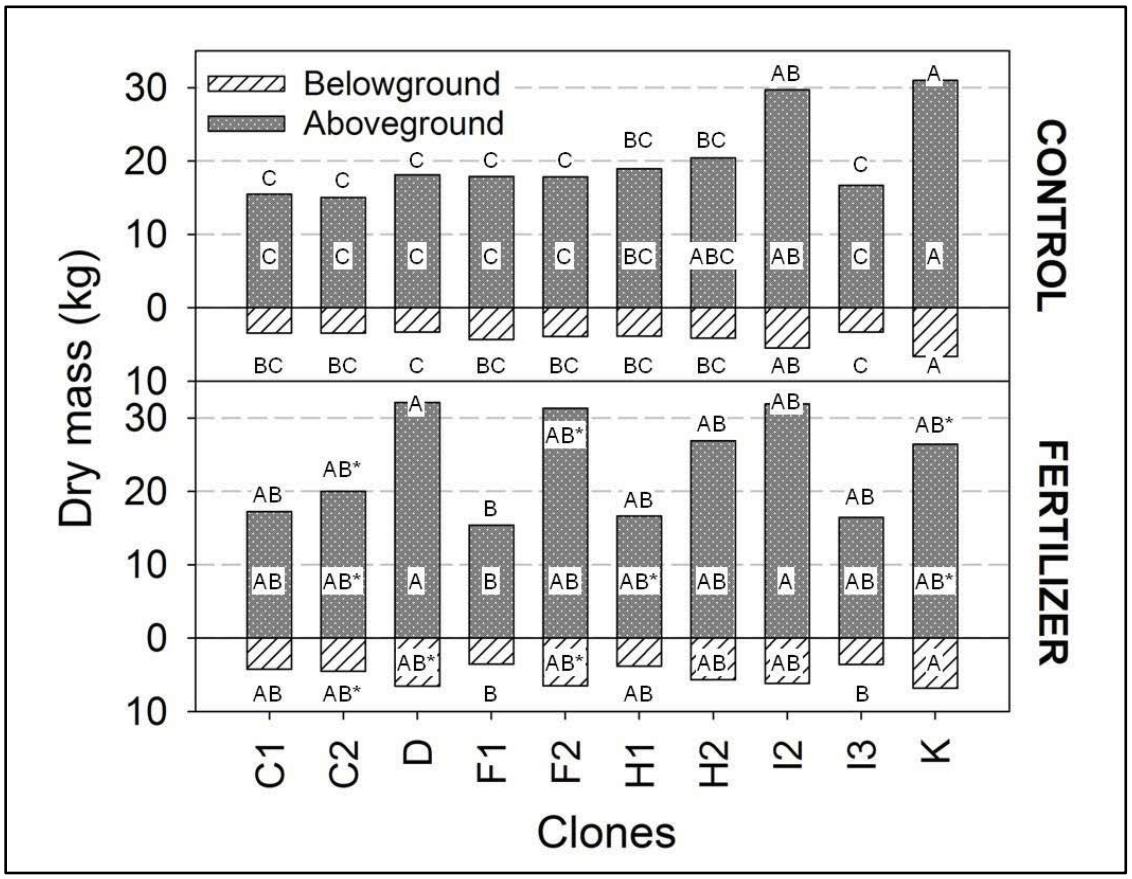

Figure 3-1. Clone-by-fertilizer interactions for aboveground ($p = 0.06$), belowground ($p = 0.09$), and total tree ($p = 0.06$) biomass from the destructive harvest of ten clones under two fertilizer regimes (operational and control). Letters show significantly different clonal means for the control (top) and fertilizer (bottom) treatments separately based on Tukey's HSD at an $\alpha = 0.10$ significance level. The upper letter indicates total biomass, the middle aboveground biomass, and the lower belowground biomass. Asterisks denote significant fertilizer effects for biomass components of individual clones based on a t-test for that clone only at an $\alpha = 0.10$ significance level. Full-sib clonal pairs are denoted with the same letter in the clonal ID.

Table 3-1. P-values for main effects and interaction effect for all variables from the destructive harvest of a six-year-old clonal trial with ten clones and two fertilizer levels (operational and control). Significant ($p < 0.10$) values are shown in bold. Analysis was done with SAS software version 9.2 using mixed models for all variables except for rooting depth. Rooting depth was analyzed with the non-parametric Friedman's Chi-Square test. Each treatment group was replicated three or four times, depending on mortality; 75 trees were harvested in total.

Variable	Clone	Fertilizer	C X F
Total mass	**0.0001**	**0.0136**	**0.0559**
Aboveground mass	**0.0001**	**0.0236**	**0.0577**
Belowground mass	**0.0001**	**0.0760**	**0.0870**
Lateral root mass	**0.0001**	0.1463	0.1192
Tap root mass	**0.0557**	**0.0886**	0.6396
Stem mass	**0.0001**	0.2431	0.1285
Branch mass	**0.0001**	**0.0975**	0.1962
Foliar mass	**0.0001**	**0.0319**	0.1746
Lateral root fraction	**0.0015**	0.3541	0.8081
Tap root fraction	**0.0050**	0.7033	0.1282
Stem fraction	**0.0001**	0.2457	0.3594
Branch fraction	**0.0001**	**0.0602**	0.3815
Foliar fraction	**0.0001**	0.5940	0.4453
Root-shoot ratio	**0.0446**	0.1986	0.5447
Stem-foliage ratio	**0.0001**	0.6847	0.5402
Branch-foliage ratio	**0.0001**	0.2242	**0.0984**
Taproot-stem ratio	**0.0002**	0.3046	**0.0509**
Number lateral roots	**0.0174**	0.4554	0.5244
Average lateral root diameter	0.1465	0.2223	0.3989
Standard deviation lateral root diameter	0.5165	0.7469	0.6424
Number tap roots	**0.0625**	**0.0240**	0.6073
Average tap root diameter	**0.0001**	0.8228	0.3257
Standard deviation tap root diameter	**0.0731**	0.8138	0.3772
Fine lateral root mass	**0.0002**	**0.0990**	**0.0381**
Medium lateral root mass	**0.0001**	**0.0978**	0.3823
Coarse lateral root mass	**0.0001**	0.3188	0.1337
Rooting depth	**0.0188**	**0.0881**	**0.0379**
Crown Silhouette Area	**0.0001**	0.1054	0.2699
Crown Silhouette Area per foliar mass	**0.0004**	0.3676	0.7309

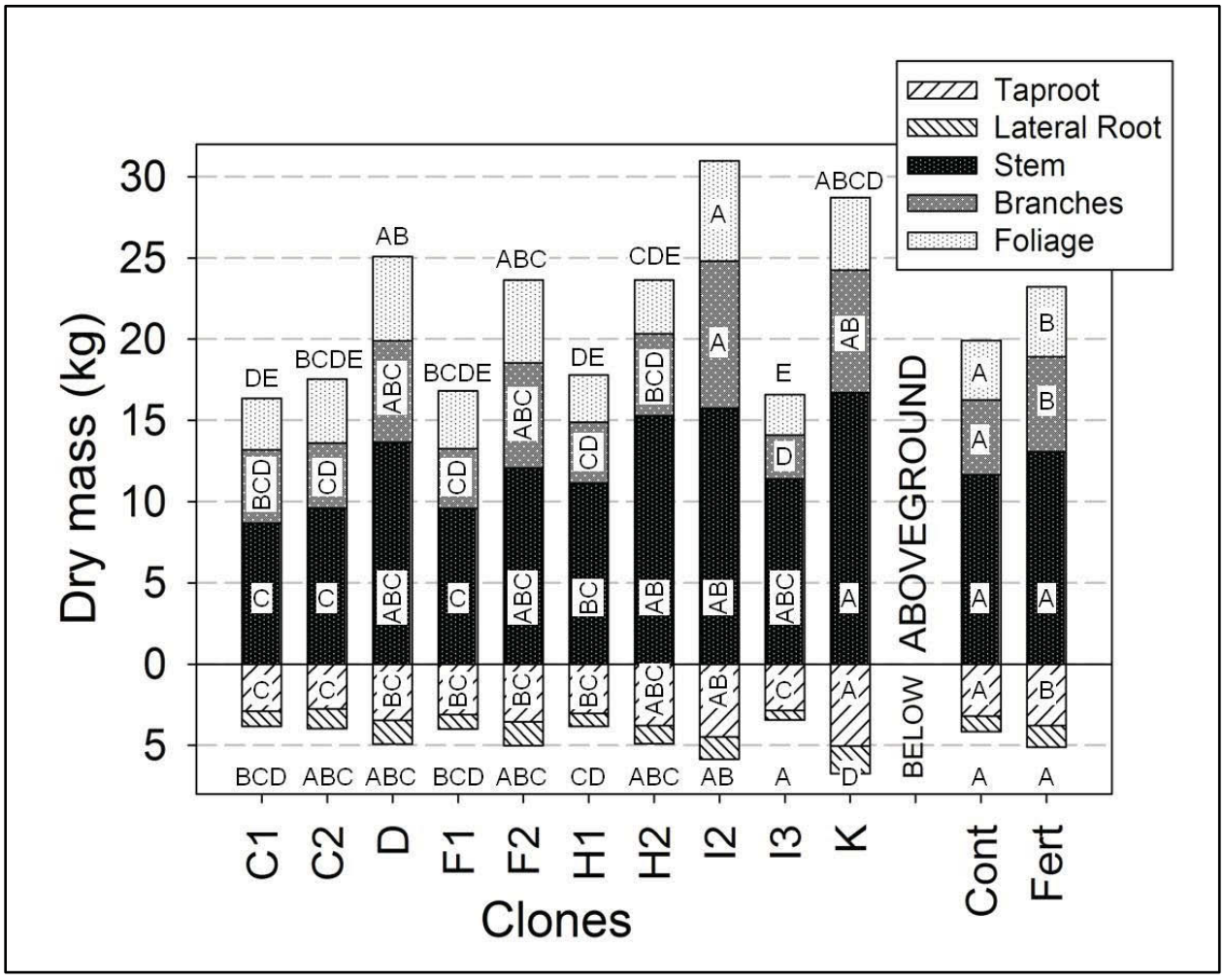

Figure 3-2. Clonal and fertilizer main effects for each of five absolute biomass components from the destructive harvest of ten clones under two fertilizer regimes (operational and control). Letters show significantly different treatment effects for each of the five biomass components based on Tukey's HSD at an α = 0.10 significance level. Full-sib clonal pairs are denoted with the same letter in the clonal ID.

3.3.2. Allometry

Fertilizer application only significantly changed relative biomass partitioning to branch mass, which increased from 17.8% to 19.3% of tree total mass ($p < 0.10$; Figure 3-3). None of the other four biomass components assessed differed between the fertilizer and control treatment when considered as a fraction of total tree mass ($p > 0.10$). However, foliar, branch, stem, taproot, and lateral root fractions all differed substantially among the clones ($p < 0.01$; Figure 3-3). A comparison of the clones with the greatest and least relative partitioning for each of these components revealed differences of 6.3%, 11.0%, 15.0%, 2.5%, and 3.3%, respectively ($p <$

0.10). As with comparisons of full-sib pairs of clones for absolute biomass components, some pairs displayed similar relative partitioning to one another (e.g. clones C2 and C3), while others displayed dissimilar patterns (e.g. clones I2 and I3).

Any biomass partitioning changes in response to fertilizer application were the result of changes in growth rates, not direct effects on allometry ($p > 0.10$; Table 3-2). A non-significant p-value indicates that compared groups did not differ in regression coefficients, and thus any changes in allometry are growth-induced (i.e. they are on the same line). By contrast, allometry did vary between clones even after accounting for growth for all but the lateral root fraction ($p < 0.10$; Table 3-2). The magnitude of the allometric differences observed in this trial between clones was substantial. In order to illustrate the extent of variability in allometry among clones, clone-specific allometric equations (Table 3-2) were used to calculate the average biomass partitioning for each clone at the average tree mass for all trees (26.2 kg).

When clones were directly compared at the same size (i.e. after considering growth effects), clone I3 had the greatest allocation to stem of 14.4 kg dry mass, or 54.9% of its total dry mass. By contrast clone F2 had the least allocation to stem of 10.6 kg dry mass, only 40.7% of its total dry mass. Thus, for trees of equal size, clone I3 had 16.9% greater stem dry mass than the average of all clones, while clone F2 had 13.4% less stem dry mass. The clone-specific regressions utilized for this comparison all had an $R^2 > 0.82$ for the natural logs of stem versus total tree mass (Table 3-2). Even for clones that did not have dissimilar slopes of their allometric regressions, differences in intercepts sometimes resulted in substantial differences in allometry. For example, clones F2 and I3 each had $k = 0.89$. However, the difference in their intercepts (clone F2: $a = 0.22$; clone I3: $a = 0.52$) resulted in a difference in allocation to stem mass of approximately 14% of total tree biomass between these clones across the range of tree masses observed in this trial (e.g. a 3.7 kg difference in stem mass at a tree mass of 26.2 kg). While the above comparisons emphasize the difference between clones, there were also pairs of clones with nearly identical allometry and growth rates observed in this trial. For instance, clones H2 and I3 showed less than a 0.3 kg difference in stem mass (< 1% of total tree mass) across the range of tree masses observed.

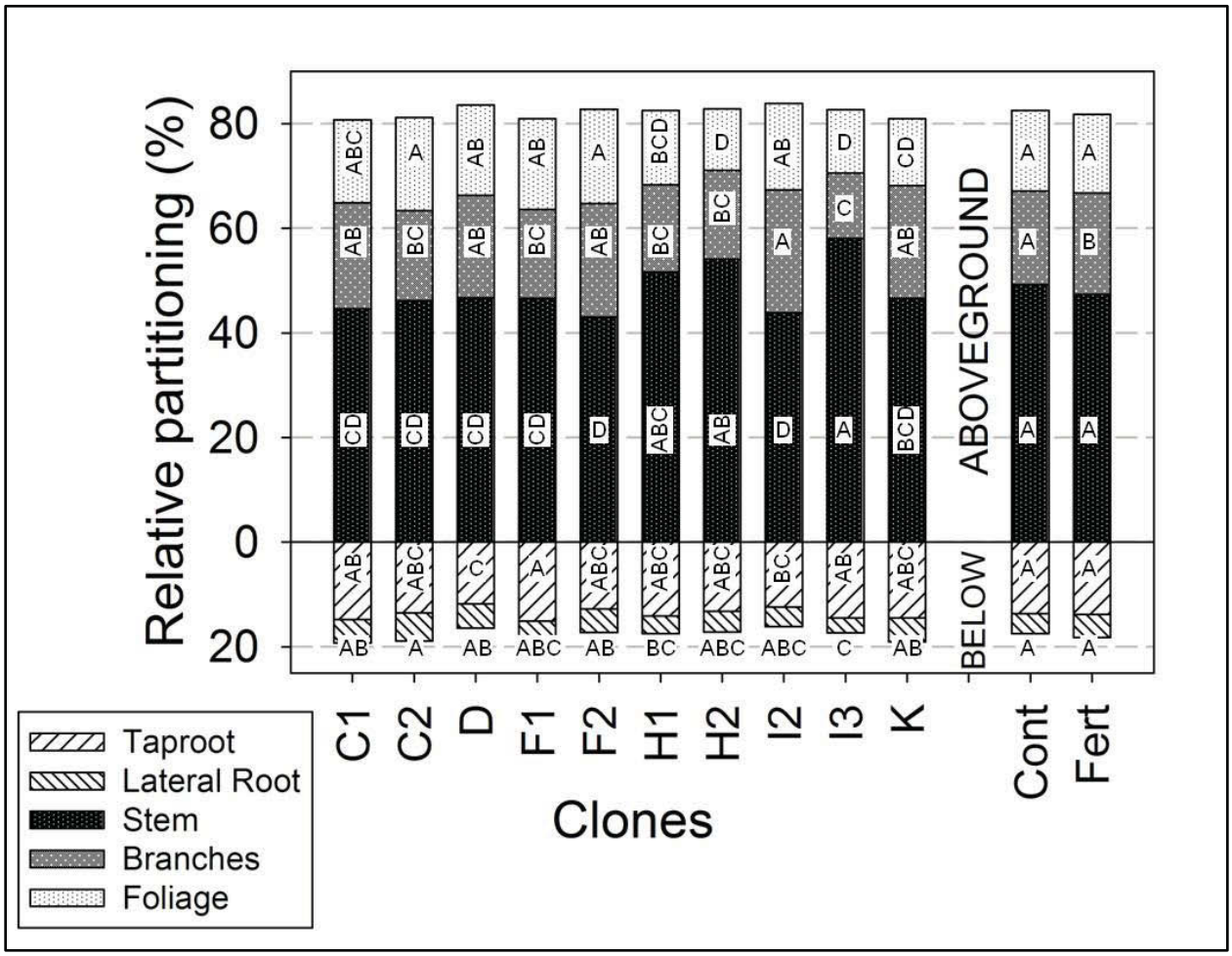

Figure 3-3. Clonal and fertilizer main effects for each of five relative biomass components from the destructive harvest of ten clones under two fertilizer regimes (operational and control). Letters show significantly different treatment effects for each of the five biomass components based on Tukey's HSD at an $\alpha = 0.10$ significance level. Full-sib clonal pairs are denoted with the same letter in the clonal ID.

Table 3-2. Allometric analysis of five biomass components from the destructive harvest of ten clones under two different fertilizer regimes (operational and control). Regressions were of the form ln(y) = a + k ln(x), where x = total mass and y = the biomass component. Main effects were tested using conditional error, which tests for significant differences between regression lines for all coefficients simultaneously. Regression coefficients and the coefficient of determination are shown for the regression of all data (N = 75), regressions of each individual clone (6 ≤ N ≤ 8), and the regression of each fertilizer treatment (37 ≤ N ≤ 38). Full-sib clonal pairs are designated with the same letter.

	Foliar vs. Total Mass				Branch vs. Total Mass				Stem vs. Total Mass				Lateral Root vs. Total Mass				Tap Root vs. Total Mass			
	Clone	**Fert**			**Clone**	**Fert**			**Clone**	**Fert**			**Clone**	**Fert**			**Clone**	**Fert**		
Num DF	18	2			18	2			18	2			18	2			18	2		
Den DF	55	71			55	71			55	71			55	71			55	71		
F	3.5	0.3			4.3	0.1			3.6	0.7			1.3	0.9			1.6	1.3		
P-Values	**<0.01**	0.71			**<0.01**	0.90			**<0.01**	0.52			0.21	0.40			**0.09**	0.29		
	a	**k**	**R²**		**a**	**k**	**R²**		**a**	**k**	**R²**		**a**	**k**	**R²**		**a**	**k**	**R²**	
All	-1.39	0.95	0.85		-5.04	1.33	0.92		0.18	0.91	0.92		-5.33	1.21	0.76		-0.90	0.89	0.92	
Clone C1	-1.25	0.94	0.93		-4.19	1.26	0.96		0.15	0.90	0.98		-4.21	1.11	0.90		-1.95	1.00	0.96	
Clone C2	-3.18	1.15	0.97		-5.73	1.40	0.98		0.72	0.85	0.98		-5.06	1.21	0.91		0.61	0.73	0.94	
Clone D	-2.03	1.03	0.99		-5.06	1.33	0.99		0.48	0.88	0.96		-5.84	1.26	0.69		-0.69	0.86	0.84	
Clone F1	-0.60	0.88	0.90		-6.03	1.43	0.97		0.37	0.88	0.97		-7.98	1.47	0.73		-1.10	0.92	0.90	
Clone F2	-0.73	0.90	0.93		-3.27	1.17	0.90		0.22	0.89	0.88		-9.24	1.60	0.92		-0.57	0.85	0.97	
Clone H1	0.89	0.71	0.87		-2.84	1.10	0.91		-1.07	1.04	0.97		-6.60	1.32	0.85		-1.74	0.98	0.98	
Clone H2	-0.81	0.87	0.83		-6.34	1.45	0.93		0.61	0.88	0.90		-2.82	0.95	0.39		-2.43	1.04	0.87	
Clone I2	-3.69	1.18	0.94		-8.16	1.64	0.90		2.71	0.66	0.83		-5.18	1.18	0.69		0.95	0.71	0.85	
Clone I3	-3.05	1.10	0.96		-7.37	1.54	0.98		0.52	0.89	0.99		-5.26	1.17	0.78		-1.00	0.90	0.97	
Clone K	-0.85	0.88	0.90		0.55	0.80	0.70		-2.75	1.19	0.91		-6.03	1.28	0.50		0.43	0.77	0.79	
Fertilizer	-1.89	1.00	0.84		-5.21	1.35	0.89		0.47	0.88	0.89		-6.01	1.28	0.74		-0.35	0.84	0.89	
Control	-1.11	0.92	0.85		-4.89	1.31	0.93		-0.08	0.94	0.94		-4.65	1.13	0.77		-1.16	0.92	0.94	

Root-shoot and stem-foliage ratios also varied among the ten clones ($p < 0.05$; Figure 3-4). When growth was also considered neither of these allometric relationships was significantly affected by the fertilizer treatment ($p > 0.10$). By contrast branch-foliage and stem-taproot ratios both showed significant clone-by-fertilizer interactions ($p < 0.10$; Figure 3-4). Differences between clones for all four of these ratios could be attributed to differences in allometry as well as differences in growth rate ($p < 0.10$; Table 3-3). Root-shoot ratio clonal means ranged from 0.19 to 0.24, a difference of 25.1%. Three of the clones had slopes greater than unity for root-shoot regressions, indicating that they were allocating proportionally more to root biomass as trees grew larger. The remaining seven clones with slopes less than unity were reducing allocation to roots as trees grew larger. While we were unable to run regressions to determine what role allometric differences played for the clone-by-fertilizer interaction observed for branch-foliage and stem-taproot ratios, there were clonal differences in the allometric regressions for both these ratios ($p < 0.05$). For both ratios clones varied in their allometry as growth proceeded, but most clones allocated more to branch versus foliage and more to taproot versus stem as they grew larger (Table 3-3).

Stem-foliage ratio (i.e. growth efficiency) clonal means ranged from 2.5 to 4.9, a difference of 97.9%. Eight of the clones had slopes less than unity, indicating a greater allocation to foliage versus stem as growth continued. However, much of the allometric variability was attributable to differences between clones even after considering their variation in growth rates. When clones were compared as described above at the average tree size for the trial (26.2 kg) to account for different growth rates, stem-foliage ratios ranged from 2.3 in clone C2 to 4.6 in clone H2. This indicates that clone H2 was able to produce twice as much stem mass per unit foliar mass as clone C2. More efficient growth, however, did not necessarily correlate to greater growth rates at either the stem or tree scale. While clone I3 had the second greatest growth efficiency, it was the least massive overall clone in the trial and ranked sixth in stem mass. Clone F2 ranked ninth in growth efficiency, but was fourth in total mass and fifth in stem mass.

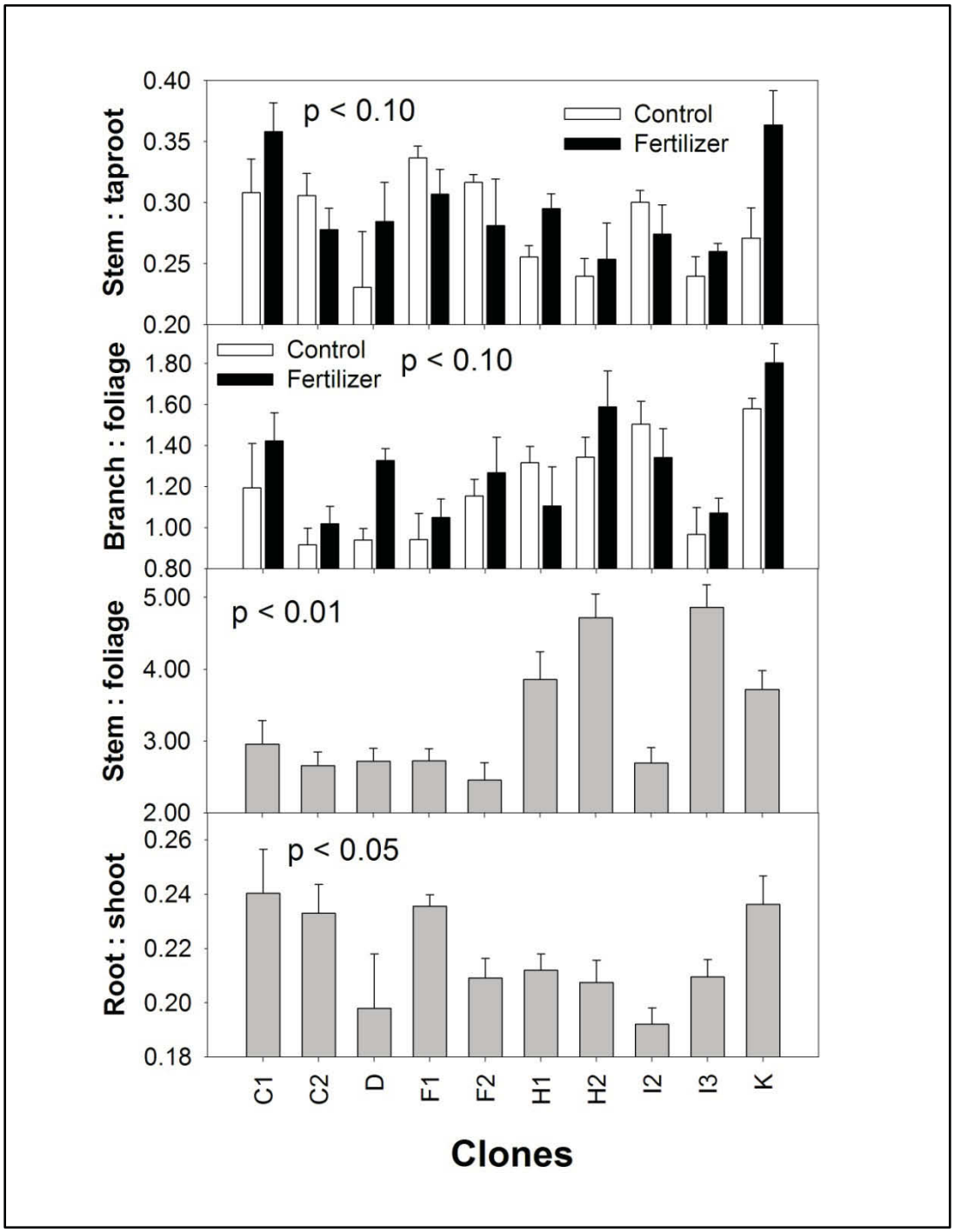

Figure 3-4. Four different allometric relationships are shown from the destructive harvest of ten clones under two fertilizer regimes (operational and control). Error bars are one standard error, for the lower two panels only showing a clonal effect ($6 \leq N \leq 8$), while for the upper two panels showing the interaction ($3 \leq N \leq 4$). Full-sib clonal pairs are denoted with the same letter in the clonal ID.

Table 3-3. Allometric analysis of four allometric relationships from the destructive harvest of ten clones under two different fertilizer regimes (operational and control). Regressions were of the form ln(y) = a + k ln(x), where x = the second component listed and y = the first component listed. Main effects were tested using conditional error, which tests for significant differences between regression lines for all coefficients simultaneously. Regression coefficients and the coefficient of determination are shown for the regression of all data (N = 75), regressions of each individual clone (6 ≤ N ≤ 8), and the regression of each fertilizer treatment (37 ≤ N ≤ 38). Full-sib clonal pairs are designated with the same letter.

	Root Mass vs. Shoot Mass			Stem Mass vs. Foliar Mass			Branch Mass vs. Foliar Mass			Stem Mass vs. Taproot Mass		
	Clone	**Fert**		**Clone**	**Fert**		**Clone**	**Fert**		**Clone**	**Fert**	
Num DF	18	2		18	2		18	2		18	2	
Den DF	55	71		55	71		55	71		55	71	
F	1.7	1.1		4.2	0.5		5.7	1.0		2.0	1.4	
P-Values	**0.07**	0.35		**<0.01**	0.60		**<0.01**	0.37		**0.02**	0.26	
	a	**k**	**R^2**	**a**	**k**	**R^2**	**a**	**k**	**R^2**	**a**	**k**	**R^2**
All	1.72	0.98	0.92	3.28	0.74	0.65	-2.20	1.29	0.91	-0.42	0.91	0.86
Clone C1	2.02	0.93	0.94	2.10	0.87	0.85	-2.06	1.29	0.95	-2.01	1.10	0.96
Clone C2	0.36	1.13	0.95	3.36	0.70	0.91	-1.61	1.19	0.97	0.17	0.85	0.92
Clone D	3.01	0.84	0.78	2.37	0.84	0.93	-2.29	1.28	0.97	-0.13	0.87	0.69
Clone F1	1.89	0.95	0.99	2.12	0.86	0.80	-4.15	1.51	0.93	-1.53	1.04	0.94
Clone F2	2.26	0.92	0.98	2.25	0.84	0.67	-2.29	1.29	0.97	0.07	0.86	0.90
Clone H1	2.11	0.93	0.98	-0.30	1.20	0.75	-3.66	1.49	0.95	-0.56	0.92	0.97
Clone H2	2.35	0.91	0.88	3.82	0.72	0.54	-3.75	1.51	0.92	-1.69	1.03	0.73
Clone I2	-0.58	1.26	0.96	5.48	0.48	0.64	-2.85	1.37	0.93	0.46	0.82	0.60
Clone I3	1.28	1.04	0.97	3.38	0.76	0.91	-2.89	1.38	0.99	-1.39	1.00	0.95
Clone K	1.91	0.95	0.82	0.35	1.11	0.69	0.92	0.95	0.86	3.09	0.56	0.65
Fertilizer	1.50	1.00	0.92	3.90	0.67	0.61	-1.66	1.23	0.88	0.01	0.87	0.82
Control	1.72	0.98	0.92	2.86	0.79	0.67	-2.50	1.33	0.93	-0.53	0.92	0.88

3.3.3. Root Morphology

In addition to clonal differences in partitioning to belowground biomass, we observed distinct differences in the morphology of lateral and sinker roots emerging from the primary taproot. The number of sinker roots emanating from each taproot, the average diameter of these sinker roots, and the standard deviation of their diameters all differed among clones ($p < 0.10$; Figure 3-5). While the number of lateral roots emanating from each taproot significantly varied among clones ($p < 0.05$), their diameter and the standard deviation of their diameters were not different

among clones ($p > 0.10$). From this data we can infer that some clones (e.g. clone K) had root systems that were comprised of a small number of relatively large diameter sinker roots, while other clones (e.g. clone I3) had a greater number of relatively small diameter sinker roots.

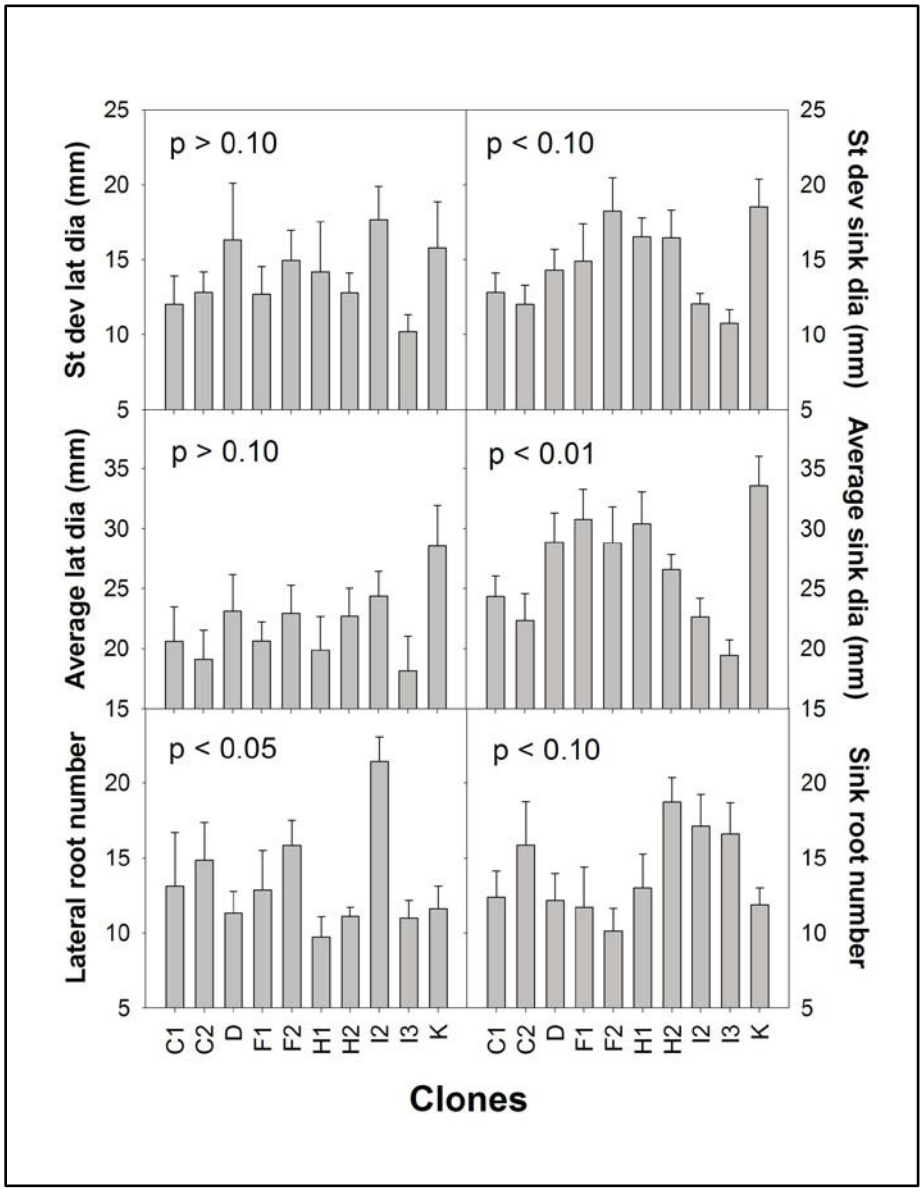

Figure 3-5. Root morphological variables measured for all roots emanating from the tap root from the destructive harvest of ten clones under two fertilizer regimes (operational and control). Lateral roots, which were < 45° from horizontal, are shown in the left panels, while sinker roots, which were < 45° from vertical, are shown in the right panels. Error bars are one standard error, sample size was ($6 \leq N \leq 8$). Full-sib clonal pairs are denoted with the same letter in the clonal ID.

Clones also varied in the mass of lateral roots observed in each size class from the sampled soil volume. Coarse (> 15 mm) and medium (5 to 15 mm) lateral root mass both showed significant clonal effects ($p < 0.01$; Figure 3-6). The medium size fraction also showed a significant fertilizer effect, with a greater mass of roots found in fertilized plots versus controls ($p < 0.10$; Figure 3-6). The fine fraction of lateral roots (< 5 mm) varied among clones depending on their fertilizer status ($p < 0.01$; Figure 3-6). Generally, most clones responded to fertilizer application with an increase in root mass in the smallest size fraction, but some (e.g. clones C2 and H2) did so to a much greater extent than others (e.g. clones C1 and H1). Again, we must emphasize that this data only accurately reflects the dynamics of the coarser root fraction, and is not representative of fine root dynamics that we were unable to adequately assess in this trial.

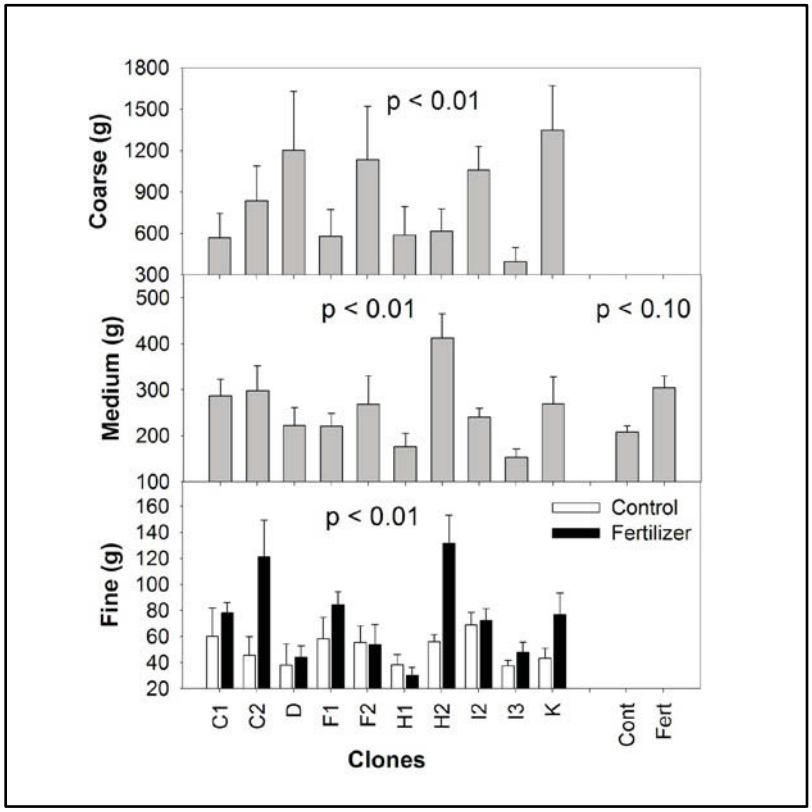

Figure 3-6. Mass of coarse (>15 mm), medium (5 to 15 mm), and fine (<5 mm) lateral roots from the destructive harvest of ten clones under two fertilizer regimes (operational and control). Error bars are one standard error, for the upper two panels only showing a clonal effect ($6 \leq N \leq 8$), for the center panel showing a fertilizer effect ($37 \leq N \leq 38$), while for the lower panel showing the interaction ($3 \leq N \leq 4$). Full-sib clonal pairs are denoted with the same letter in the clonal ID.

There was a clone-by-fertilizer interaction for rooting depth observed in this trial ($p < 0.05$). Some clones, such as F2, always rooted below our maximum sampling depth (1 m) in both control and fertilizer plots (Figure 3-7). Other clones, such as H2, did not root to the maximum sampling depth in any of the control or fertilizer plots. Of the remaining clones, most showed reduced rooting depth in response to fertilizer application, although there were a number of notable exceptions that showed greater rooting depth with fertilizer application (i.e. clones C1 and D).

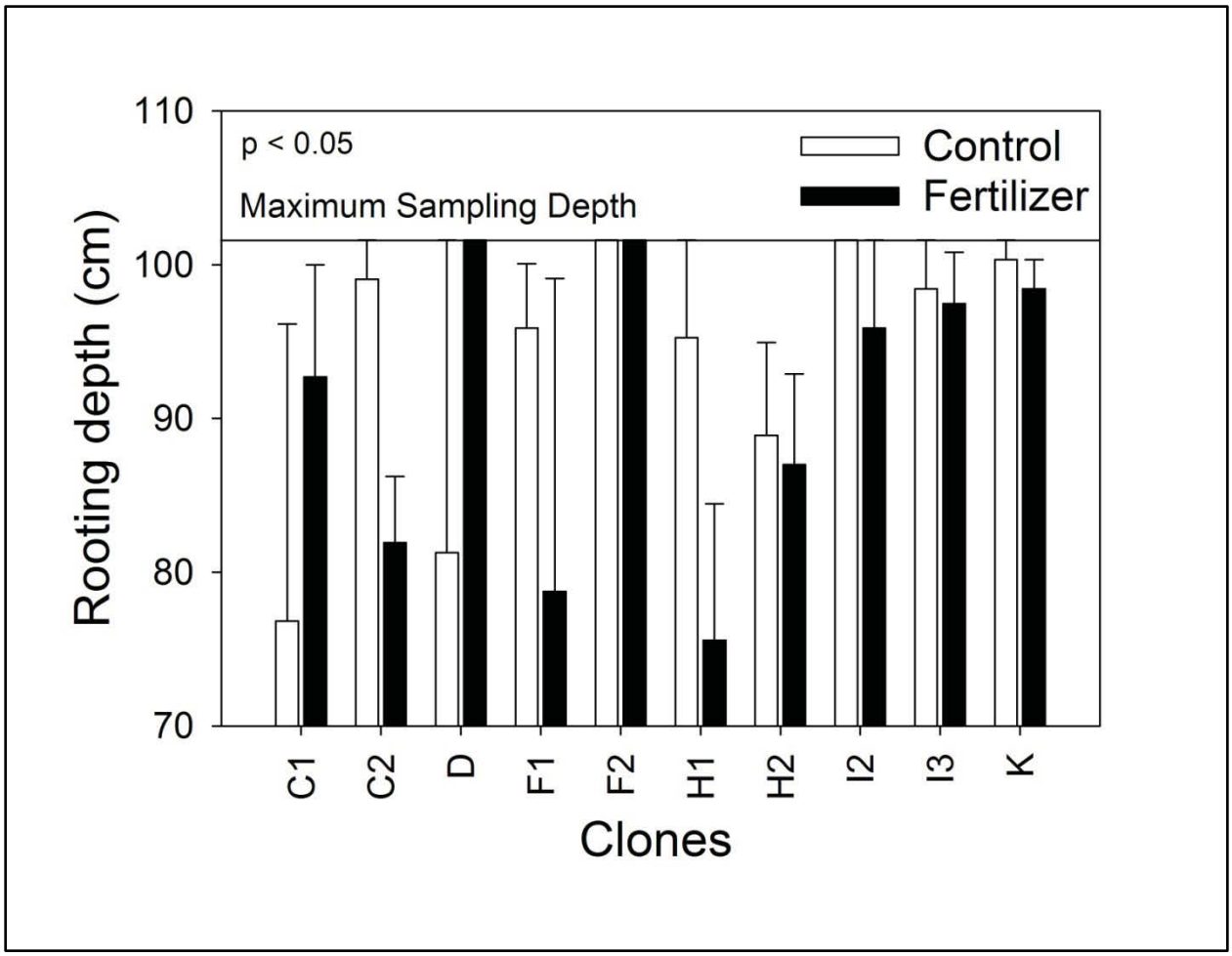

Figure 3-7. Maximum depth of rooting from the destructive harvest of ten clones under two fertilizer regimes (operational and control). Note that no attempt was made to sample roots below 101.6 cm despite the fact that many ramets exceeded this depth. Error bars are one standard error, sample size was ($3 \leq N \leq 4$). Full-sib clonal pairs are denoted with the same letter in the clonal ID.

3.3.4. Crown Size and Display

CSA and CSA per foliar mass both varied significantly among clones ($p < 0.01$; Figure 3-8), but did not differ among fertilizer treatments ($p > 0.10$). The greatest difference in CSA was observed between a full-sib pair. Clone I2 had the greatest CSA of 6.9 m^2, 104% greater than clone I3 with only 3.4 m^2. This pair of clones also showed among the greatest differences in CSA per foliar mass, with clone I3 displaying more crown area per foliar mass at 1.5 $m^2\ kg^{-1}$ versus clone I2 at 1.2 $m^2\ kg^{-1}$, a difference of 26%. Thus, clone I3 can be characterized as having a smaller but more efficiently displayed crown relative to clone I2, although it must be noted that much of this difference is likely due to the marked differences in total tree mass between these two clones (20.0 kg for clone I3 versus 36.8 kg for clone I2). By contrast, other full-sib pairs like clones H1 and H2 showed little difference in either CSA or CSA per foliar mass (Figure 3-8). Overall, foliar mass and CSA were highly correlated across all trees ($R^2 = 0.87$, $p < 0.01$; Figure 3-9). Based on data from this trial, CSA is an accurate index of foliar mass that is far less labor-intensive than direct measurement. CSA was also assessed by King et al. (2008) at age two in this trial on seven of the same clones we quantified at age six. At age two, King et al. observed a clone-by-fertilizer interaction that was the result of clone F2 more than doubling in CSA in response to fertilizer application ($p < 0.10$). When the year-two and year-six ranks of the common seven clones were compared, the only clone to change rank by more than two places was clone I3, which was third largest at year two, but had dropped to the smallest among the seven by year six. Clone F2 had the greatest CSA of the group of seven in both years.

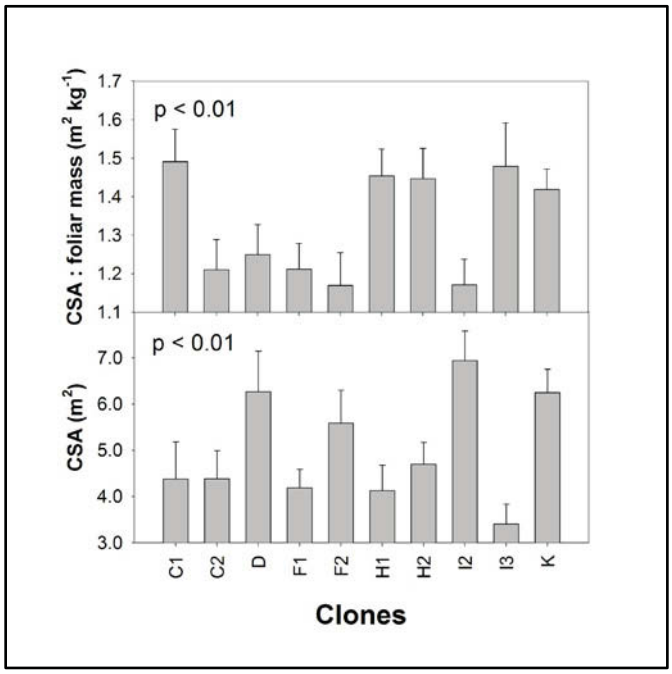

Figure 3-8. Crown silhouette area (lower panel) and crown silhouette area per foliar mass (upper panel) from the destructive harvest of ten clones under two fertilizer regimes (operational and control). Error bars are one standard error, sample size was ($6 \leq N \leq 8$). Full-sib clonal pairs are denoted with the same letter in the clonal ID.

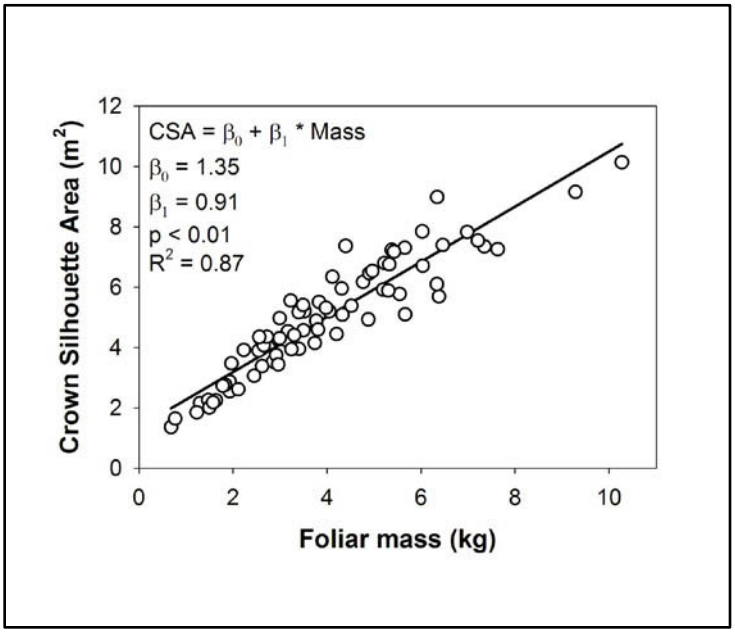

Figure 3-9. Pooled simple linear regression of foliar mass to crown silhouette area for all data from the destructive harvest of ten clones under two fertilizer regimes (operational and control). Sample size was 75, regression and statistics are shown.

3.4. Discussion

3.4.1. Biomass Partitioning, Allometry, and Stem Growth Efficiency Vary Among Clones

We observed substantial variability among clones in the absolute magnitude of biomass allocated to each of the five components we assessed: lateral roots, taproot, stem, branches, and foliage. While overall the most massive clones tended to have the greatest stem mass, there was significant clonal variability in relative allocation to the stem component, indicating fundamental genotypic differences in efficiency of stem production. For instance, clone H2 had significantly less foliar mass than clones D or I2, despite having a stem mass that was not significantly different. King et al. (2008) assessed photosynthetic rates on this trial during the second growing season for seven of the ten clones presented in this chapter (although not for clones D or I2), and found that clone H2 consistently had among the highest rates of any of those seven clones. Higher photosynthetic rates are consistent with clone H2 developing similar stem mass despite than clones D or I2 despite significantly lower foliar mass. Whatever the physiological cause of its variability, stem growth efficiency is an important consideration for clonal plantations since higher-valued products, primarily saw-timber, are the primary management objective given higher initial costs (Dougherty 2007). The variability observed among clones in this trial suggests that stem growth efficiency is a trait that should be considered along with other important physiological characteristics for the development of crop ideotypes for clonal selection (see Nelson and Johnsen 2008).

Any differences in biomass partitioning we observed between fertilizer and control treatments appeared to be primarily attributable to treatment effects on growth rates, as has previously been established in the literature (King, Albaugh et al. 1999; Coyle and Coleman 2005). However, the high degree of variability among clones was attributable primarily to changes directly in allometry even after accounting for growth. Clones varied in biomass partitioning when compared at similar sizes. Our results also emphasize the importance of statistically assessing the intercept of allometric regressions as well as the slope, as a number of previous examples from the literature have done (Colbert, Jokela et al. 1990; Adegbidi, Jokela et al. 2002). While the intercept may not be subject to any meaningful interpretation at the y-axis, those clones with

greater intercepts but similar slopes (e.g. clone I3 versus F2) will always have greater partitioning to the assessed biomass component at any size. Analysis of only the slope of allometric relationships, 'k', may result in simplistic interpretations leading to the spurious conclusion that no difference in partitioning between treatments exists.

Despite these differences in allometry, no clear trends emerged between biomass partitioning and either total tree or stem growth. For instance, the most massive clone, I2, had the least allocation to stem while the similarly sized second most massive clone, F2, had the fourth greatest allocation to stem. That clone H2 had the greatest growth efficiency ratio (stem-foliage), yet was ranked fifth in stem mass is indicative of the lack of correlation between growth efficiency and actual growth. Clonal mass, whether for the whole tree or only the stem fraction varied independently from allocation to stem and foliar components and to the stem-foliage growth efficiency ratio ($p > 0.10$; $R^2 < 0.01$ for all). From this we conclude that other physiological processes besides biomass partitioning are responsible for differences in growth observed between clones. It is likely from our results and previous results in the literature that there are multiple different combinations of physiological processes and morphology that can result in similar growth between clones with dissimilar allometry, or dissimilar growth between clones with similar allometry (King, Seiler et al. 2008; Tyree, Seiler et al. 2009a; Tyree, Seiler et al. 2009b). While assessment of biomass partitioning for determining harvest index or belowground C sequestration in coarse roots remains important, it appears to be a predictive variable in and of itself for neither growth nor long-term fertilizer growth response.

Regardless of whether the clone-by-fertilizer interactions for total, aboveground, and belowground biomass were attributable to fertilizer effects on partitioning or to variation in growth, from the perspective of selecting individual clones for operational deployment this interaction may require further attention. Fertilizer applied to nonresponsive clones may be an unnecessary expense. Conversely, not applying fertilizer to highly responsive clones would represent a lost opportunity to maximize the growth potential of expensive planting stock. Our results are somewhat inconsistent with the literature on genotype-by-environment interactions in *P. taeda*. Genotype-by-environment interactions for growth rates are not commonly observed in

P. taeda, even in clones (McKeand, Jokela et al. 2006; Roth, Jokela et al. 2007). Additionally, biomass partitioning has previously shown little effect attributable to G x E interactions between fast and slow growing open-pollinated families (Retzlaff, Handest et al. 2001). However, we are not currently aware of any other studies examining biomass partitioning in more than two clones of *P. taeda* in response to fertilizer application. The fertilizer-by-clone interactions we observed in this trial indicate that further research on biomass partitioning is needed across a greater number of sites and clones to determine whether the extent of the interaction we observed in this trial warrants attention in the future screening of clonal material.

The range of biomass partitioning patterns observed among these clones and that partitioning varied independently from growth represents an opportunity to select clones with rapid stem growth rates and a range of other favorable characteristics. Precision-silvicultural systems could benefit from the consideration of biomass partitioning patterns along with other traits in developing crop ideotypes for *P. taeda* (Nelson and Johnsen 2008). For example, a clone with low allocation to branch mass and high allocation to taproot mass could maximize saw-timber value by reducing knots while simultaneously increasing C sequestered in taproots in subsequent rotations. Clones with lower allocation to foliar mass may transmit more light to the understory, and thus be better suited to silvicultural systems focused on inter-planting rows with biomass energy crops. While clonal variability in partitioning such as that observed in this trial will require greater effort and expense in screening clones for production, it does represent an opportunity for producing a number of different ideotypes appropriate to different management objectives.

3.4.2. Root Morphology Shows Genotypic Variability

Phenotypic plasticity was defined by Valladares et al. (2006) as "the capacity of a given genotype to render different phenotypic values for a given trait under different environmental conditions." Whether implicit or explicit, this is the interpretation of phenotypic plasticity prevalent throughout the literature (Bradshaw 2006). However, many studies assessing phenotypic plasticity do so not with clones, but with a population containing a range of genotypes (Valladares, Chico et al. 2002; Sanchez-Gomez, Valladares et al. 2006). It has long

been assumed based on such studies that *P. taeda* is a species with high phenotypic plasticity (e.g. Samuelson, Johnsen et al. 2004). The results of our study, which showed distinctly different root morphology among closely related clones of *P. taeda* on a small, relatively uniform site, draw into question the true source of the phenotypic plasticity observed in many studies examining populations rather than clones. It appears at least possible that a substantial component of what is identified as phenotypic plasticity may in fact be genotypic variability even among closely related individuals of the same species. Phenotypic variability between tree species, including *P. taeda*, has been found to be far greater than their plasticity in response to environmental gradients (Coleman 2007). Based on our results the same conclusions may be as apt within a species as between species.

The range of coarse root morphologies observed among our clones may influence their abilities to exploit the soil environment across a range of resource availability. Our discussion here is limited to coarse root morphology, as we did not assess fine roots in this trial and have no information on how fine root dynamics may have varied among clones. The ability of some clones to exploit the soil volume to a greater depth than others may have implications for water relations, as deeper rooted clones will have access to deeper water sources. *P. taeda* has been shown to access water from different depths in the profile at different times of year, although no differences were found in one study among families differing in their drought tolerance (Retzlaff, Blaisdell et al. 2001). Drought treatments have been shown to increase rooting at greater depths, particularly in the fall (Barnes 2002). Given the genotypic differences we observed in rooting depth, it seems likely that clones that tend to root deeper may be better able to acclimate to drought conditions. Since we lack fine root data, it is difficult to draw conclusions based on coarse root morphology that are relevant to nutrition. However, clones that root to greater depth should be able to exploit a greater soil volume. Soil depth has been found to be a key variable of site quality in other forested ecosystems (Fisher and Binkley 2000), although this effect of depth cannot be directly attributed to nutrition as it is confounded by greater water availability.

3.4.3. Crown Size and Display Vary Among Clones

In the second growing season a significant clone-by-fertilizer interaction was observed for CSA in this trial for eight clones, seven of which are presented in this chapter (King, Seiler et al. 2008). This contrasts with our six-year data showing only a significant clonal effect on CSA. However, the range of relative differences among clones in CSA remained approximately equal between year two and year six, with the larger clones having approximately twice the CSA of the smaller clones. The strong relationship we observed between foliar mass and CSA is consistent with previous results employing this technique (Tyree, Seiler et al. 2009b). CSA has also shown relatively strong correlations with stem volume and leaf area (Tyree, Seiler et al. 2009a; Tyree, Seiler et al. 2009b). Despite the correlation with foliar mass, we did observe significant differences among clones in CSA per foliar mass, indicating differences in either foliar display or specific leaf area among clones, although these differences were not assessed further in this trial. Genotypic differences in crown architecture and specific leaf area that are correlated to growth have both previously been observed for *P. taeda* (McCrady and Jokela 1998; Chmura and Tjoelker 2008; Tyree, Seiler et al. 2009b). However, there appears to be no clear effect of the CSA to foliar mass ratio on growth in this trial. Among the five largest clones, three had among the lowest ratios (clones D, F2, and I2) while two had among the highest ratios (clones H2 and K).

3.5. Conclusion

Clones show variability in biomass partitioning and allometry. Allometric differences were a combination of differences in partitioning at similar sizes and differences in growth rates among clones. Allometry varied independently from stem and whole tree growth rates, indicating that partitioning is likely not a principle cause of dissimilar clonal growth rates without consideration of other physiological processes. While screening clones for their biomass partitioning is likely a difficult and expensive task, different clonal partitioning patterns offer unique opportunities for precision-silvicultural systems being employed now and those that are developed in the future. Based on clonal differences in coarse root morphology, it is uncertain to what extent genotypic effects cause what is commonly believed to be the highly phenotypically plastic nature of *P.*

taeda. Differences in root depth exist between clones, which leads to the hypothesis that different clones may be adapted to better exploit a given resource environment than others. While crown size did differ among clones, more comprehensive assessments of canopy physiology are required for any correlation with growth rates to emerge. Conclusions based on clone-by-fertilizer interactions observed for total tree, above, and belowground biomass require further research on more sites and a greater number of clones to be validated. Awareness of potential clone-by-fertilizer interactions in biomass partitioning is likely necessary in future studies examining clonal variability in the ecophysiology of *P. taeda*.

3.6. Literature Cited

Adegbidi, H. G., E. J. Jokela, N. B. Comerford and N. F. Barros (2002). "Biomass development for intensively managed loblolly pine plantations growing on Spodosols in the southeastern USA." Forest Ecology and Management **167**(1-3): 91-102.

Albaugh, T. J., H. L. Allen, P. M. Dougherty, L. W. Kress and J. S. King (1998). "Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions." Forest Science **44**(2): 317-328.

Baltunis, B. S., D. A. Huber, T. L. White, B. Goldfarb and H. E. Stelzer (2007). "Genetic analysis of early field growth of loblolly pine clones and seedlings from the same full-sib families." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **37**(1): 195-205.

Barnes, A. D. (2002). "Effects of phenology, water availability and seed source on loblolly pine biomass partitioning and transpiration." Tree Physiology **22**(10): 733-740.

Bettinger, P., M. Clutter, J. Siry, M. Kane and J. Pait (2009). "Broad implications of southern United States pine clonal forestry on planning and management of forests." International Forestry Review **11**(3): 331-345.

Bown, H. E., M. S. Watt, P. W. Clinton, E. G. Mason and D. Whitehead (2009). "The influence of N and P supply and genotype on carbon flux and partitioning in potted *Pinus radiata* plants." Tree Physiology **29**(7): 857-868.

Bradshaw, A. D. (2006). "Unravelling phenotypic plasticity - why should we bother?" New Phytologist **170**(4): 644-648.

Chmura, D. J., M. S. Rahman and M. G. Tjoelker (2007). "Crown structure and biomass allocation patterns modulate aboveground productivity in young loblolly pine and slash pine." Forest Ecology and Management **243**(2-3): 219-230.

Chmura, D. J. and M. G. Tjoelker (2008). "Leaf traits in relation to crown development, light interception and growth of elite families of loblolly and slash pine." Tree Physiology **28**(5): 729-742.

Colbert, S. R., E. J. Jokela and D. G. Neary (1990). "Effects of annual fertilization and sustained weed-control on dry-matter partitioning, leaf-area, and growth efficiency of juvenile loblolly and slash pine." Forest Science **36**(4): 995-1014.

Coleman, M. (2007). "Spatial and temporal patterns of root distribution in developing stands of four woody crop species grown with drip irrigation and fertilization." Plant and Soil **299**(1-2): 195-213.

Coyle, D. R. and M. D. Coleman (2005). "Forest production responses to irrigation and fertilization are not explained by shifts in allocation." Forest Ecology and Management **208**(1-3): 137-152.

Coyle, D. R., M. D. Coleman, J. A. Durant and L. A. Newman (2006). "Survival and growth of 31 *Populus* clones in South Carolina." Biomass & Bioenergy **30**(8-9): 750-758.

Dougherty, D. (2007). "Improved returns on forestlands: A financial analysis of MCP and varietal seedlings on private land in the southeastern United States." Forest Landowner **66**: 15-18.

Emhart, V. I., T. A. Martin, T. L. White and D. A. Huber (2007). "Clonal variation in crown structure, absorbed photosynthetically active radiation and growth of loblolly pine and slash pine." Tree Physiology **27**(3): 421-430.

Fisher, R. F. and D. Binkley (2000). Ecology and management of forest soils. New York, NY, John Wiley & Sons, Inc.

Fox, T. R., H. L. Allen, T. J. Albaugh, R. Rubilar and C. A. Carlson (2007). "Tree nutrition and forest fertilization of pine plantations in the southern United States." Southern Journal of Applied Forestry **31**(1): 5-11.

Gautam, M. N. K., D. J. Mead, P. W. Clinton and S. X. Chang (2003). "Biomass and morphology of *Pinus radiata* coarse root components in a sub-humid temperate silvopastoral system." Forest Ecology and Management **177**(1-3): 387-397.

Gebauer, R. L. E., J. F. Reynolds and B. R. Strain (1996). "Allometric relations and growth in *Pinus taeda*: The effect of elevated CO_2 and changing N availability." New Phytologist **134**(1): 85-93.

Ingestad, T. and G. I. Agren (1991). "The influence of plant nutrition on biomass allocation." Ecological Applications **1**(2): 168-174.

Jokela, E. J. and T. A. Martin (2000). "Effects of ontogeny and soil nutrient supply on production, allocation, and leaf area efficiency in loblolly and slash pine stands." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **30**(10): 1511-1524.

King, J. S., T. J. Albaugh, H. L. Allen and L. W. Kress (1999). "Stand-level allometry in *Pinus taeda* as affected by irrigation and fertilization." Tree Physiology **19**(12): 769-778.

King, N. T., J. R. Seiler, T. R. Fox and K. H. Johnsen (2008). "Post-fertilization physiology and growth performance of loblolly pine clones." Tree Physiology **28**(5): 703-711.

Ledig, F. T., F. H. Bormann and K. F. Wenger (1970). "The distribution of dry matter growth between shoots and roots in loblolly pine." Botanical Gazette **131**(4): 349-359.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger and O. Schabenberger (2006). SAS for mixed models, second edition. Cary, NC, SAS Institute Inc.

Maier, C. A., T. J. Albaugh, H. L. Allen and P. M. Dougherty (2004). "Respiratory carbon use and carbon storage in mid-rotation loblolly pine (*Pinus taeda* L.) plantations: The effect of site resources on the stand carbon balance." Global Change Biology **10**(8): 1335-1350.

McCrady, R. L. and E. J. Jokela (1998). "Canopy dynamics, light interception, and radiation use efficiency of selected loblolly pine families." Forest Science **44**(1): 64-72.

McKeand, S., T. Mullin, T. Byram and T. White (2003). "Deployment of genetically improved loblolly and slash pines in the South." Journal of Forestry **101**(3): 32-37.

McKeand, S. E., E. J. Jokela, D. A. Huber, T. D. Byram, H. L. Allen, B. L. Li and T. J. Mullin (2006). "Performance of improved genotypes of loblolly pine across different soils, climates, and silvicultural inputs." Forest Ecology and Management **227**(1-2): 178-184.

Miller, A. T., H. L. Allen and C. A. Maier (2006). "Quantifying the coarse-root biomass of intensively managed loblolly pine plantations." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **36**(1): 12-22.

Nelson, C. D. and K. H. Johnsen (2008). "Genomic and physiological approaches to advancing forest tree improvement." Tree Physiology **28**: 1135-1143.

Nobis, M. and U. Hunziker (2005). "Automatic thresholding for hemispherical canopy-photographs based on edge detection." Agricultural and Forest Meteorology **128**: 243-250.

Paul, A. D., G. S. Foster, T. Caldwell and J. McRae (1997). "Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine." Forest Science **43**(1): 87-98.

Retzlaff, W. A., G. K. Blaisdell and M. A. Topa (2001). "Seasonal changes in water source of four families of loblolly pine (*Pinus taeda* L.)." Trees-Structure and Function **15**(3): 154-162.

Retzlaff, W. A., J. A. Handest, D. M. O'Malley, S. E. McKeand and M. A. Topa (2001). "Whole-tree biomass and carbon allocation of juvenile trees of loblolly pine (*Pinus taeda*): Influence of genetics and fertilization." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **31**(6): 960-970.

Roth, B. E., E. J. Jokela, T. A. Martin, D. A. Huber and T. L. White (2007). "Genotype x environment interactions in selected loblolly and slash pine plantations in the southeastern United States." Forest Ecology and Management **238**(1-3): 175-188.

Samuelson, L. J., K. Johnsen and T. Stokes (2004). "Production, allocation, and stemwood growth efficiency of *Pinus taeda* L. stands in response to 6 years of intensive management." Forest Ecology and Management **192**(1): 59-70.

Sanchez-Gomez, D., F. Valladares and M. A. Zavala (2006). "Functional traits and plasticity in response to light in seedlings of four Iberian forest tree species." Tree Physiology **26**(11): 1425-1433.

Stasolla, C. and E. C. Yeung (2003). "Recent advances in conifer somatic embryogenesis: Improving somatic embryo quality." Plant Cell Tissue and Organ Culture **74**(1): 15-35.

Swindel, B. F. (1970). Some applications of the principles of conditional error, USDA Forest Service Research Paper SE-59, Southern Rearch Station, Asheville, NC.

Tyree, M. C., J. R. Seiler and T. R. Fox (2008). "The effects of fertilization on soil respiration in 2-year-old *Pinus taeda* L. clones." Forest Science **54**(1): 21-30.

Tyree, M. C., J. R. Seiler and C. A. Maier (2009a). "Short-term impacts of nutrient manipulations on leaf gas exchange and biomass partitioning in contrasting 2-year-old *Pinus taeda* clones during seedling establishment." Forest Ecology and Management **257**(8): 1847-1858.

Tyree, M. C., J. R. Seiler, C. A. Maier and K. H. Johnsen (2009b). "*Pinus taeda* clones and soil nutrient availability: Effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology." Tree Physiology **29**(9): 1117-1131.

Valladares, F., J. M. Chico, I. Aranda, L. Balaguer, P. Dizengremel, E. Manrique and E. Dreyer (2002). "The greater seedling high-light tolerance of *Quercus robur* over *Fagus sylvatica* is linked to a greater physiological plasticity." Trees-Structure and Function **16**(6): 395-403.

Valladares, F., D. Sanchez-Gomez and M. A. Zavala (2006). "Quantitative estimation of phenotypic plasticity: Bridging the gap between the evolutionary concept and its ecological applications." Journal of Ecology **94**(6): 1103-1116.

Van Lear, D. H., P. R. Kapeluck and W. D. Carroll (2000). "Productivity of loblolly pine as affected by decomposing root systems." Forest Ecology and Management **138**(1-3): 435-443.

Whetten, R. W. and R. Kellison (2010). "Research gap analysis for application of biotechnology to sustaining US forests." Journal of Forestry **108**: 193-201.

4. Short-term changes in biomass partitioning of two full-sib clones of *Pinus taeda* (L.) under differing fertilizer regimes over four months

4.1. Introduction

Clonal plantations are now becoming common in the southeastern United States as improvements in somatic embryogenesis make possible the mass production of *Pinus taeda* (L.) clones (Stelzer and Goldfarb 1997; Whetten and Kellison 2010). Fertilizer application is also a common practice in plantations, with average growth increases across the Southeast of approximately 25% to mid-rotation additions of nitrogen (N) and phosphorus (P) (Fox, Jokela et al. 2007). In order to maximize growth potential in clonal plantations to justify more expensive genetic material at planting, fertilization and other intensive silvicultural practices will need to be applied (Dougherty 2007). However, differences in growth strategy among clones may require different silvicultural prescriptions for different clones (Roth, Jokela et al. 2007; Tyree, Seiler et al. 2009b).

Biomass partitioning changes naturally as trees grow larger. Thus fertilizer application may alter partitioning through its effect on growth rates, but it may also cause allometric shifts as a direct response to a resource-availability gradient. Some studies of open-pollinated *P. taeda* have shown a resource-availability effect of small magnitude directly on allometry (King, Albaugh et al. 1999; Jokela and Martin 2000), while others have not (Ledig, Bormann et al. 1970; Samuelson, Johnsen et al. 2004; Coyle and Coleman 2005; Coyle, Coleman et al. 2008). Results are mixed when the effect of genetics on biomass partitioning is considered. In one study different families or provenances did not appear to differ in partitioning patterns under similar environments (Retzlaff, Handest et al. 2001). However in another, families with the greatest stem mass and height displayed the greatest allocation to root biomass under low N environments, but the least under high N (Li, Allen et al. 1991). This would seem to indicate that high performing families had greater plasticity in allometry that allowed them to better

acclimate to their environment. The shift in root-shoot ratios was consistent with the observed fertilizer growth response in the study by Li et al. (1991).

However, all these results are based upon averages of many individual genotypes, and thus may not be expected to show similar outcomes to studies of a small number of individual genotypes replicated over larger areas, as would be found in a clonal plantation. Field observations among different clones reveal significantly different growth rates (Paul, Foster et al. 1997; Baltunis, Huber et al. 2007; Bitoki 2008). Additionally, pairs of clones display different biomass partitioning patterns across a range of resource availabilities, indicating that partitioning may be at least partially responsible for observed variability in growth responses to treatments (Tyree, Seiler et al. 2009a; Tyree, Seiler et al. 2009b). Much more information on biomass partitioning in a large number of clones is needed to determine whether allometric variation between individual genotypes is of sufficient magnitude to require individual consideration of each genotype, or of genotypes with similar allometry.

The vast majority of information available on biomass partitioning in both open-pollinated and clonal *P. taeda* pertains to longer-term responses to varying resource availability. Results are typically derived from either 1) a single harvest (e.g. Colbert, Jokela et al. 1990; Retzlaff, Handest et al. 2001), or 2) multiple annual harvests (e.g. Samuelson, Johnsen et al. 2004; Coyle and Coleman 2005). While this information is important to assess effects of treatments on stand development and ecosystem level processes that occur over years, these studies are not designed to address how trees respond to fertilizer application in the short-term. While several studies have incorporated multiple harvests over a single growing season across a N gradient, they have done so to compare only total tree growth response (Griffin, Winner et al. 1995) or to ensure a range of seedling sizes appropriate for allometric analyses (Gebauer, Reynolds et al. 1996). Neither of these studies examined the effect of N availability in altering a time series of biomass partitioning. Green et al. (1994) did find significant changes in root-shoot ratio over a ten day period in response to a combination of drought and fertilizer treatments. While this time series was not long enough for any observable growth response to treatments, it does reveal that biomass partitioning can vary in seedlings on the scale of days.

Despite the lack of information on short-term variability in biomass partitioning across a resource-availability gradient, short-term shifts in partitioning in the days, weeks, or months immediately following fertilizer application may explain long-term growth responses. Ephemeral reductions in root allocation could allow greater C allocation to foliar biomass, leading to a long-term growth response to fertilizer application even if partitioning to roots later increased. Other physiological processes, such as photosynthesis and root respiration, have been shown to respond differently in the short-term to fertilizer application than they do in the long-term, indicating that information on short-term changes in physiology are necessary to improve our understanding of longer-term fertilizer growth response. (Gough and Seiler 2004; Gough, Seiler et al. 2004). Since short-term differences in photosynthetic rates between clones do not appear to consistently correlate with growth responses (King, Seiler et al. 2008), short-term shifts in allometry may be an important mechanism for long-term fertilizer growth responses. We hypothesized that 1) biomass partitioning would show a short-term increase in foliar allocation at the expense of root allocation with fertilizer application as a mechanism of short-term fertilizer growth response and that 2) this ephemeral response would vary in magnitude, even between full-sib clones. To address these questions multiple harvests over four months following fertilizer application were performed in two clones produced from a single full-sib cross.

4.2. Materials and Methods

4.2.1. Study Description

This experiment was installed in a greenhouse at Virginia Tech in Blacksburg, Virginia, USA (37.24°N 80.43°W). Trees were potted on April 30, 2009 in homogenized A-horizon soil collected from the USDA Forest Service's Southeast Tree Research and Education Site (SETRES). The soil was a Wakulla series (siliceous, thermic Psammentic Hapludult) that was chosen because its low inherent fertility enabled us to manipulate nutrient availability. Coarse roots and organic matter were removed from the soil by first passing it through a 1-cm sieve. Sufficiently large pots (15 x 15 x 38 cm, 8,550 cm^3) were used to attempt to prevent excessive root binding for the duration of the four month experiment. Watering was sufficient in quantity and frequency to prevent visual signs of drought stress while still limiting leaching. Temperature

was set to a nighttime minimum of 18° C and averaged 25.3° C throughout the experiment. Relative humidity was at atmospheric ambient. Day length averaged 12.9 hours over the duration of the experiment and was extended during the last 30 days (September 15 to October 15) with artificial sodium lighting turned on daily for approximately three hours pre-dawn.

4.2.2. Experimental Design

The experiment was a randomized complete block design with a two-by-two-by-four factorial structure replicated eight times (128 trees total). Treatments were clone (GE034 and GE769 provided by ArborGen LLC (Summerville, South Carolina, USA), fertilizer application (fertilized vs control) and tree-for-time substitution (harvests day 30, 61, 91, and 121). Clones were produced from the same-full sib cross by somatic embryogenesis. These two clones were the first selected by ArborGen in 2005, and are both fast-growing elite selections (Bitoki 2008). Clone 34 is characterized by greater growth rates and a narrower crown with a greater number of smaller branches versus clone 769 (Bitoki 2008). The clonal seedlings were grown in containers containing a mixture of peat and vermiculite. They were planted with this root-bound soil media left intact in order to prevent substantial root death and potential tree mortality. Survival rate until planned harvest date was 100%. Fertilizer (N and P) was applied at an operational rate (DAP and ammonium nitrate at 225 kg N per hectare, 56 kg elemental P per hectare) on June 16, 2009. The four tree-for-time substitution destructive harvests were conducted monthly on July 16, August 16, September 15, and October 15, 2009, allowing for four months of data collection. At the beginning of the experiment, when fertilizer was applied, and prior to each destructive harvest heights and basal diameters of all trees were measured to ensure that there was no significant pre-existing growth difference between harvest groups and that the tree-for-time substitution assumption was valid. The eight blocks consisted of two greenhouse benches with four blocks per bench and 16 trees per block.

4.2.3. Data Collection

On each harvest date pots were carefully overturned to remove the entire tree intact. Roots were washed with tap-water, and any remaining plug material was carefully removed by hand. The entire tree was hand dissected into components (foliage, branches, stem, taproot, coarse roots > 2

mm diameter, and fine roots < 2 mm diameter). Fine root and coarse root data were later combined into a lateral root category due to a low incidence of coarse roots found in the first two harvests (most trees had none). These components were oven-dried in paper bags at 65° C for > 10 days and weighed to determine biomass partitioning. Results are expressed on both an absolute (g per component) and relative (% of total tree mass) basis in order to examine actual fertilizer and clonal effects versus changes in partitioning normalized to tree size.

4.2.4. Statistics

All analyses were run with SAS software version 9.2 (SAS Institute, Cary, North Carolina, USA). Normality was checked and variables were natural log transformed as necessary, although all reported means and standard errors are untransformed. Q-Q plots were used to examine all outliers. Absolute biomass components, relative biomass components, and total tree mass were analyzed using ANOVA implemented in PROC MIXED with block as a random effect. The Kenward-Roger method for calculating denominator degrees of freedom was used (Littell, Milliken et al. 2006). All two-way and three-way effect-by-time interactions were included in the model. If data violated assumptions of homogenous variance, variance was modeled separately for each treatment combination using the "group" option in the "repeated" statement of PROC MIXED.

Natural log transformed regressions of biomass components versus total tree mass were utilized to determine if differences in observed partitioning were due to treatment effects directly on allometry or on growth rates. Regression equations were in the form

$$[1] \qquad \ln(y) = a + k \ln(x)$$

where 'ln(x)' is the natural logarithm of total tree biomass, 'ln(y)' is the natural logarithm of the biomass component being analyzed, and 'a' and 'k' are regression coefficients (Ledig, Bormann et al. 1970). Sample populations with significantly different values of 'k' have different allocation patterns after accounting for changes in allometry due to growth rate. For instance, a population with a greater value of 'k' for root-shoot ratio will partition more biomass belowground as they grow larger versus a population with a lower value of 'k'. Even if 'k' does

not significantly differ among populations, significant differences in 'a' may also indicate distinct patterns of biomass partitioning. For example, a population with a greater value of 'a' despite similar 'k' will always allocate more to the given tissue when compared at any similar overall tree size with a population with a lower value of 'a'.

While most studies employing allometric regressions only test the slope, 'k' (Coyle and Coleman 2005) or test 'a' and 'k' independently using ANCOVA (e.g. Colbert, Jokela et al. 1990; Jokela and Martin 2000), we employed the technique of conditional error (Swindel 1970) to simultaneously test the full matrix of regression coefficients. Conditional error calculates an F statistic by comparing the sum of squares of errors from a combined model using data from multiple sample populations and the reduced models for each sample population separately. A significant p-value indicates that the reduced models have significantly different matrices of regression coefficients, and should thus not be combined into a single model. We utilized this procedure to run pair-wise comparisons of all clone-by-fertilizer treatment combinations with a Bonferroni correction for a family-wise error rate of $\alpha = 0.05$.

4.3. Results

4.3.1. Fertilizer Response in All Biomass Components

Over the course of the experiment fertilizer application increased absolute growth of each of the five biomass components and of total tree biomass, as indicated by fertilizer-by-harvest-date interactions ($p < 0.01$; Table 4-1) and results depicted in the upper panels of Figure 4-1. At the day 30 harvest fertilized trees had 10% lower total tree mass, although this difference was not significant ($p > 0.10$) for any component but lateral roots ($p < 0.01$). However, by the day 121 harvest, fertilized trees showed greater average mass versus unfertilized trees of 53% for total tree mass, 52% for foliar mass, 99% for branch mass, 41% for stem mass, 77% for taproot mass, and 44% for lateral root mass ($p < 0.01$; Figure 4-1). The only clone-by-fertilizer interaction based on absolute mass data was observed for the branch component ($p < 0.10$). While clone 34 showed a 132% increase in branch mass due to fertilizer application, clone 769 only displayed an

80% increase. The lack of other significant interactions in terms of absolute mass indicates that aside from the branch component, both clones responded similarly to fertilizer application.

The above results are based on the absolute magnitude of biomass. To examine normalized changes in partitioning we also examined partitioning relative to tree size. When biomass partitioning was considered from a relative, rather than an absolute perspective there were fewer effects attributable to fertilizer application. Lateral root fraction averaged 3.2% less partitioning to total tree mass in fertilized ramets across all dates ($p < 0.01$), reaching a low of 5.6% by the day 91 harvest ($p < 0.01$; Figure 4-1 lower panel). The tap root fraction did not vary among fertilizer treatments across all dates or on any individual date ($p > 0.10$). While stem and branch fractions were not different in the fertilizer treatment across all dates ($p > 0.10$), they were affected on the fourth harvest individually, with 1.7% less allocation to stem and 1.3% more allocation to branches in control versus fertilizer treatments ($p < 0.10$). Foliar fraction showed a clone-by-fertilizer-by-harvest interaction, which is discussed in detail below.

Table 4-1. ANOVA results for both absolute and relative components of tree biomass, as well as total tree biomass and root-shoot ratio. P-values are presented, values less than 0.10 are shown in bold, N = 8.

Variable	Clone	Fertilizer	C x F	Harvest Date	C x HD	F x HD	C x F x HD
total tree mass	**0.0001**	**0.0001**	0.6525	**0.0001**	0.5680	**0.0001**	0.5886
lateral root mass	**0.0176**	0.3049	0.4356	**0.0001**	0.2156	**0.0001**	0.5778
tap root mass	**0.0001**	**0.0001**	0.6836	**0.0001**	0.2497	**0.0001**	0.3263
stem mass	**0.0005**	**0.0001**	0.8638	**0.0001**	0.3718	**0.0005**	0.4650
branch mass	**0.0001**	**0.0008**	**0.0747**	**0.0001**	**0.0308**	**0.0008**	0.4726
foliar mass	0.8915	**0.0001**	0.4864	**0.0001**	0.8712	**0.0001**	0.7643
lateral root fraction	0.2232	**0.0001**	0.2106	**0.0099**	**0.0516**	**0.0480**	0.2025
tap root fraction	**0.0001**	0.1336	0.8587	**0.0026**	0.4671	0.4623	0.5914
stem fraction	0.7601	0.9993	0.5928	0.2470	**0.0159**	0.1406	0.2297
branch fraction	**0.0001**	0.1510	**0.0143**	**0.0070**	**0.0280**	0.1774	0.6486
foliar fraction	**0.0001**	**0.0013**	0.7724	**0.0001**	0.1059	0.1139	**0.0715**
root-shoot ratio	**0.0001**	**0.0001**	0.1118	**0.0001**	0.1378	**0.0145**	0.1512
Stem-foliage ratio	**0.0001**	0.1380	0.5878	**0.0019**	0.1305	0.8006	0.1865

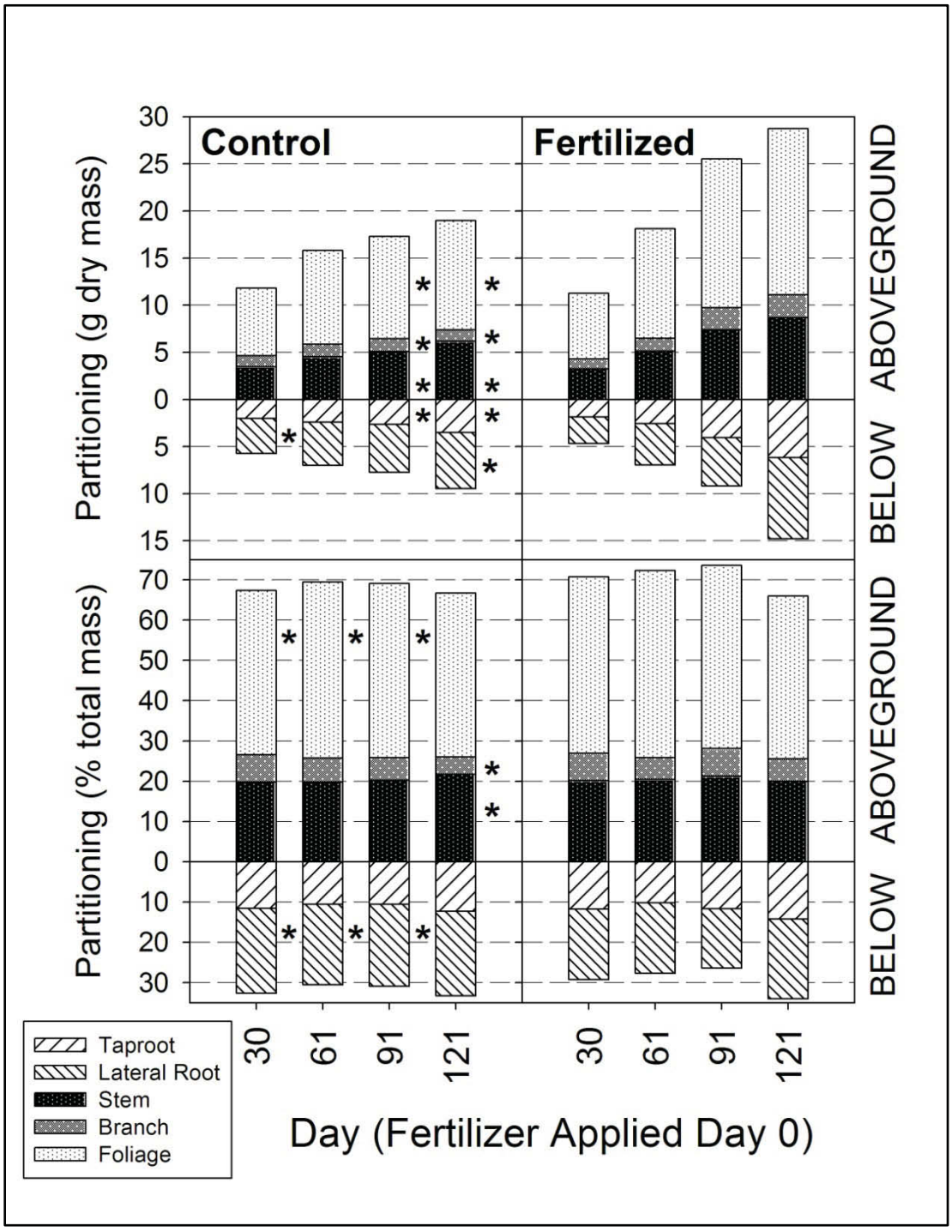

Figure 4-1. Fertilizer effects for absolute (upper panels) and relative (lower panels) biomass partitioning from four destructive harvests. Aboveground biomass is shown above the axis, belowground biomass below the axis. Control time-series is shown in the left panels, fertilized in the right panels. An asterisk to the right of a biomass component indicates a significant difference between control and fertilizer treatments ($p < 0.10$). N=16, Day 0 = June 16, 2009.

4.3.2. Ephemeral Shifts in Partitioning Vary Between Clones

When absolute mass was considered, the full-sib clones were different from one another in every biomass component considered with the exception of foliar mass ($p < 0.05$; Figure 4-2). Clone 769 had greater mass of most components at almost all dates (Figure 4-2, upper panel). For example, for the day 121 harvest, clone 769 had 15% greater total tree mass, 40% greater branch mass, 68% greater taproot mass, and 27% greater lateral root mass than clone 34 ($p < 0.05$). While at the first harvest date clone 769 had 23.8% greater stem mass ($p < 0.05$), by day 121 the more rapid stem growth of clone 34 brought it within 5% of the stem mass of clone 769, a difference that was no longer significant ($p > 0.10$). No observed clonal differences in absolute foliar mass across dates or on any individual date were observed ($p > 0.10$; Figure 4-2).

We did observe several differences between these full-sib clones in relative biomass partitioning response to fertilizer application. While the clone-by-fertilizer-by-harvest interaction for root-shoot ratio was not significant ($p > 0.10$), an interaction was observed at the day 30 harvest ($p < 0.01$; Figure 4-3). While clone 769 had less than a 3% mean difference between fertilizer and control ramets, clone 34 showed a 41% mean difference, with less allocation to roots in fertilized versus unfertilized ramets. Figure 4-3 shows significantly lower root-shoot ratios in fertilized trees averaged across both clones versus controls for the second and third harvests ($p < 0.10$). However, reduced partitioning to belowground tissues was only an ephemeral response to fertilizer application, with no significant differences between fertilized and unfertilized root-shoot ratios by the day 121 harvest ($p > 0.10$), although the clones continued to display different root-shoot ratios ($p < 0.01$).

Clonal differences in root-shoot ratios were not the result of uniform responses in both tap and lateral root fractions. For instance, while clone 769 had greater lateral root biomass at the first and final harvests ($p < 0.05$), there were no differences in relative partitioning to lateral roots between clones ($p > 0.10$; Figure 4-2 lower panel). Conversely there was no difference in absolute lateral root mass at the third harvest ($p > 0.10$), although clone 34 partitioning 2.2% more of its relative biomass to lateral roots than clone 769 ($p < 0.05$). Despite asynchronous patterns of absolute versus relative partition to lateral root biomass, across all four harvest dates

clone 769 allocated an average of 5.8% more of its total biomass to taproots ($p < 0.01$), which also resulted in greater tap root mass from an absolute basis on all four dates ($p < 0.01$).

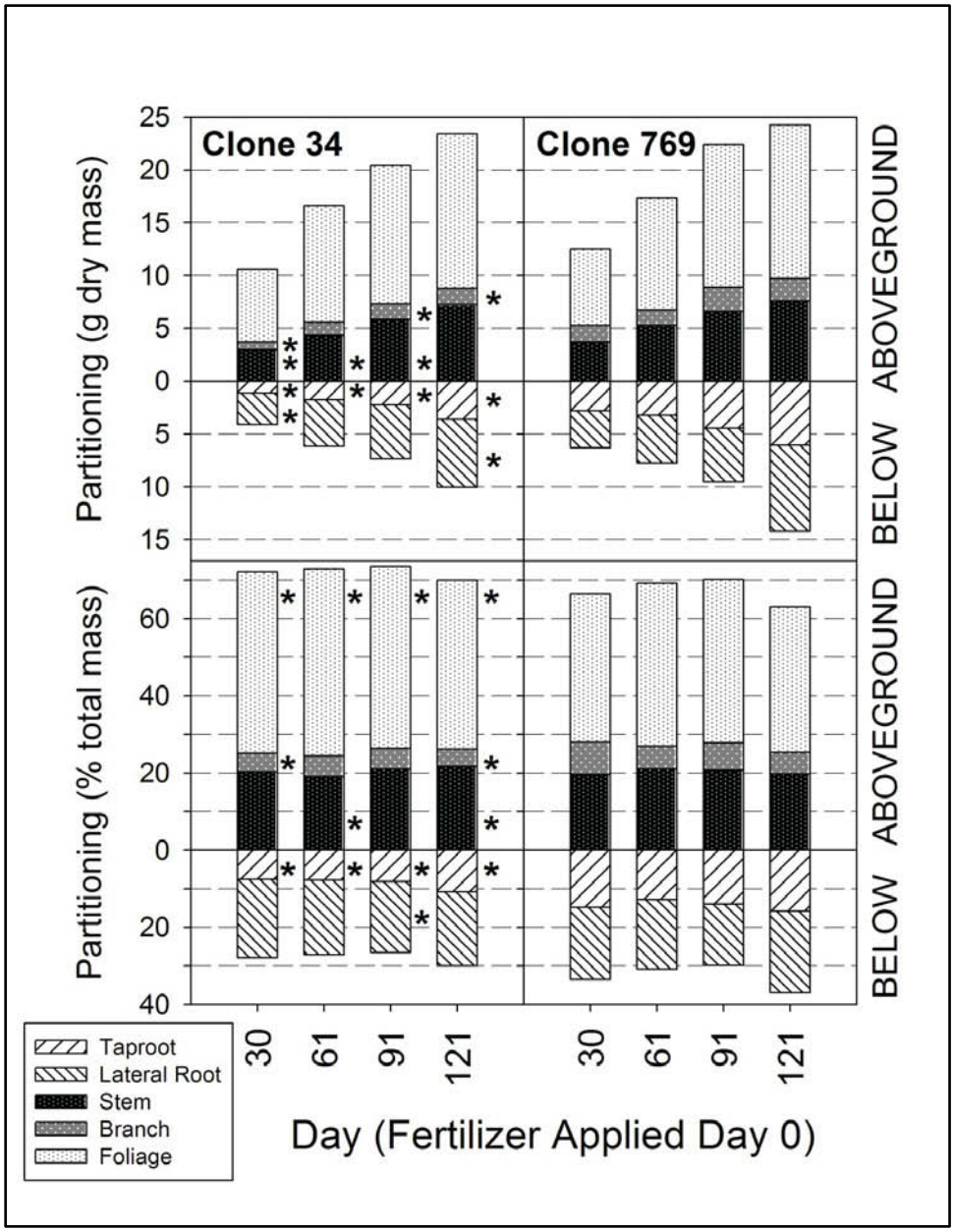

Figure 4-2. Clonal effects for absolute (upper panels) and relative (lower panels) biomass partitioning from four destructive harvests. Aboveground biomass is shown above the axis, belowground biomass below the axis. Clone 34 time-series is shown in the left panels, Clone 769 in the right panels. An asterisk to the right of a biomass component indicates a significant difference between clones ($p < 0.10$). N=16, Day 0 = June 16, 2009.

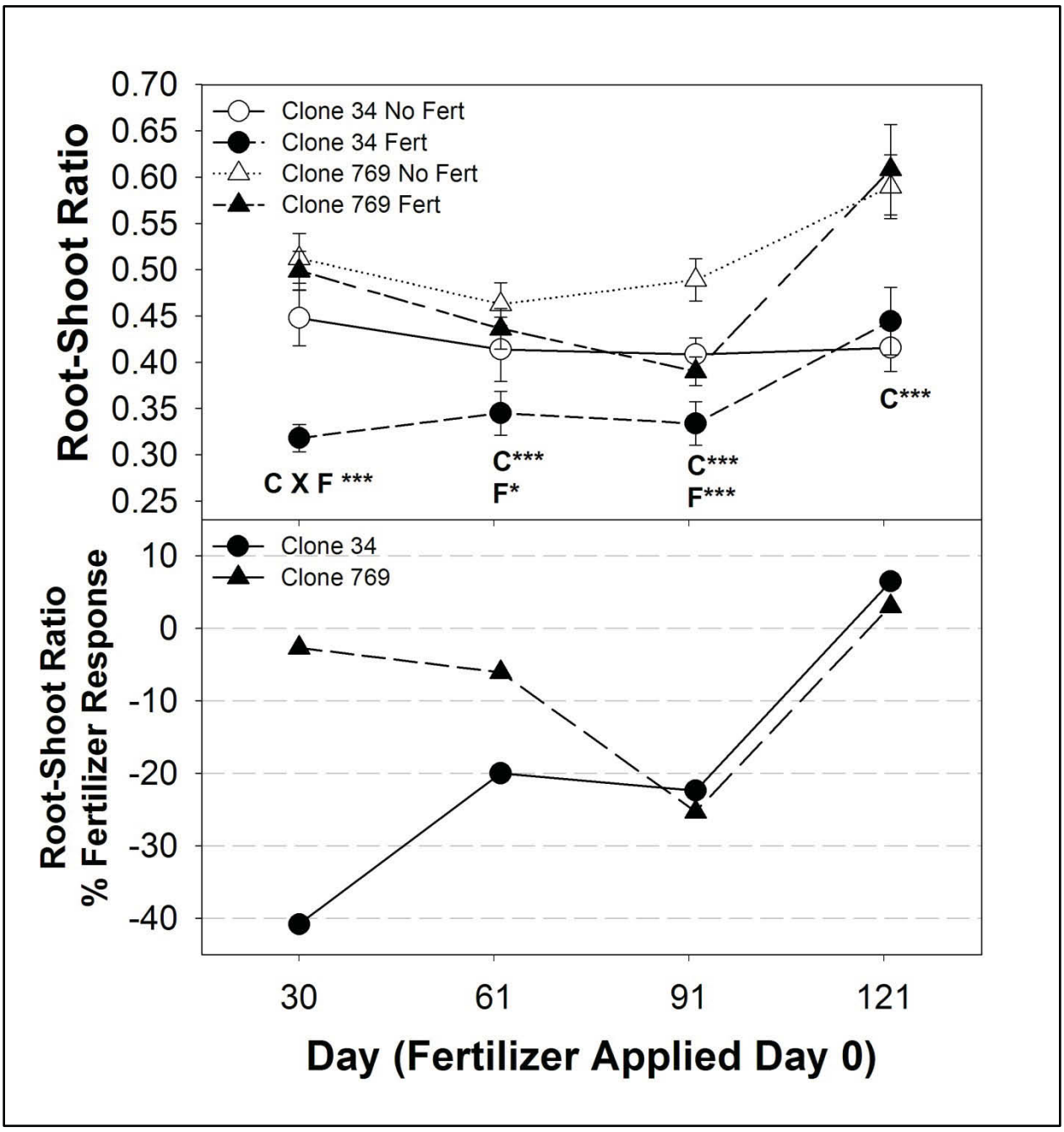

Figure 4-3. Root-shoot ratio for two full-sib clones under two fertilizer treatments from each of the four destructive harvests is shown in the top panel, while the percent fertilizer response is shown in the lower panel. Significant effects are noted, with C indicating clone, F indicating fertilizer, and C X F indicating the clone-by-fertilizer interaction (* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$). Standard errors are shown on the top panel, N = 8. Day 0 = June 16, 2009.

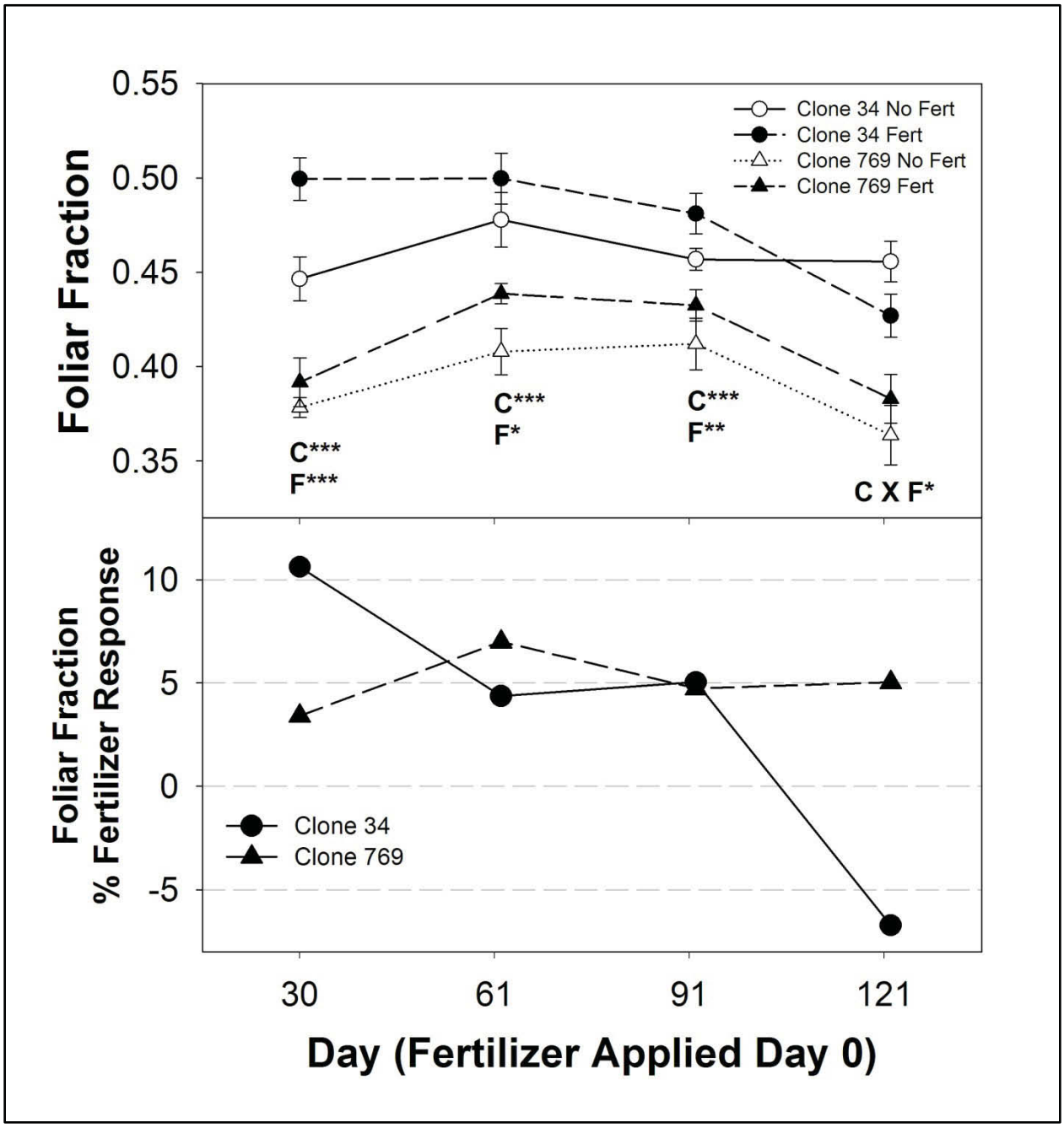

Figure 4-4. Foliar mass fraction for two full-sib clones under two fertilizer treatments from each of the four destructive harvests is shown in the top panel, while the percent fertilizer response is shown in the lower panel. Significant effects are noted, with C indicating clone, F indicating fertilizer, and C X F indicating the clone-by-fertilizer interaction (* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$). Standard errors are shown on the top panel, N = 8. Day 0 = June 16, 2009.

There were also aboveground differences between these full-sib clones in relative biomass partitioning. Branch fraction showed a clone-by-fertilizer interaction across all dates, with clone 34 partitioning 1.2% more of its total biomass to branches in fertilized ramets while clone 769 partitioned 0.4% less ($p < 0.05$). Relative stem partitioning varied between the clones over time ($p < 0.05$), with individually significant differences of 2.3% less total mass partitioned to stem by clone 34 at day 61, but 1.6% more at day 121 ($p < 0.10$). A clone-by-fertilizer-by-harvest interaction for foliar fraction was observed, and is depicted in Figure 4-4 ($p < 0.10$). While clone 769 maintains a relatively steady difference of approximately 5% between fertilizer and control treatments, the fertilizer response of clone 34 declines from a 10.6% difference at day 30 to a -6.7% difference at day 121. Thus, while clone 769 does not appear to demonstrate an ephemeral shift in foliar fraction in response to fertilizer application, clone 34 does. When the stem mass to foliar mass ratio is compared, clone 34 has 16.4% less stem mass per foliar mass averaged across all dates ($p < 0.01$; Figure 4-5). However, clone 34 increased in total mass by 228% between days 30 and 121, compared to a 205% increase for clone 769, which indicated that while clone 34 may have had lower growth efficiency with respect to stem produced per unit of foliage, it still grew at a more rapid rate. The lower root-shoot ratios of clone 34 (e.g. less partitioning belowground) and greater foliar fraction at all dates ($p < 0.01$), appeared to compensate for reduced growth efficiency, resulting in a lack of any difference between clones in absolute stem mass by the final harvest ($p > 0.10$).

4.3.3. Allometry Varies Between Clones

Allometric analyses comparing the natural log transformed data from one biomass component against another indicated there were no significant differences in regression coefficients among treatment combinations for either the stem or lateral root components ($p > 0.10$; Figure 4-6; Table 4-2). A non-significant p-value indicates that allometric differences are only attributable to changes expected due to growth, while a significant p-value indicates that allometry is shifting in response to treatments even after accounting for changes due to growth. Thus, we infer that any differences observed in partitioning to stem or lateral root components were attributable to changes in partitioning that occur over the course of development, and did not represent an

allometric response to fertilizer application or an allometric difference between clones. For the tap root component, results indicated that while the within clone allometric response to fertilizer application was only due to treatment effects on growth ($p > 0.10$), these clones produced from the same full-sib cross did have different allometry even after accounting for tree size ($p < 0.05$; Figure 4-6; Table 4-2). When either total root biomass or the root-shoot ratios were considered, a more complex pattern emerged. While changes in partitioning in response to fertilizer application in clone 769 were only attributable to growth ($p > 0.10$), clone 34 shifted its allometry in response to fertilizer application even after accounting for ontogeny ($p < 0.05$; Figure 4-6; Figure 4-7; Table 4-2). An identical trend was observed for the branch component with one notable exception. When unfertilized, there was no significant trend between natural log transformations of total tree mass and branch mass in clone 769 ($p > 0.10$, $R^2 < 0.05$), indicating very poor ontogenetic control over branch allocation for this clone under conditions of nutrient deficiency. Fertilizer responses in biomass partitioning to foliar mass and stem-foliage ratios were not attributable to changes in allometry within clones ($p > 0.10$; Figure 4-6; Figure 4-7; Table 4-2). While there were significant differences in allometry among clone 34 when fertilized and clone 769 when unfertilized, these comparison are not inferentially useful.

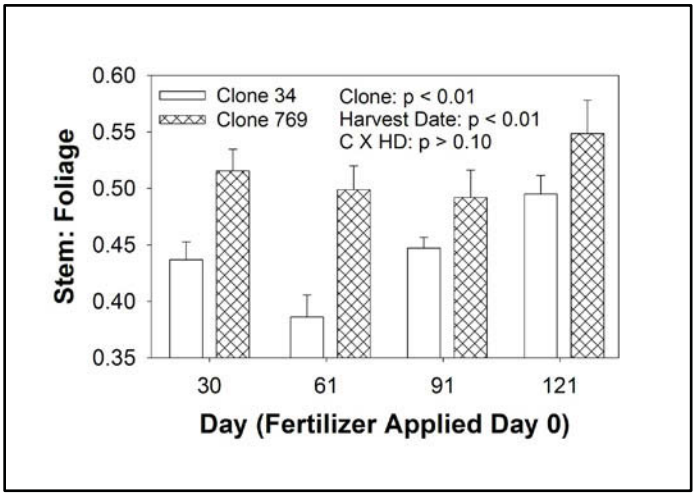

Figure 4-5. Clonal effect on the stem-foliage ratio for four destructive harvests. A lower bar indicates lower growth efficiency, or unit stem produced per unit foliage. Standard errors are shown, N = 16. Day 0 = June 16, 2009.

Table 4-2. Regression analysis of natural log transformed biomass components versus one another. The p-values presented are derived from a conditional error analysis testing whether the vector of regression coefficients was equal for each clone-by-fertilizer treatment combination. If not significant, only the overall regression R^2 is presented (N = 128). If significant all four clone-by-fertilizer group R^2 values are shown (N = 32). Allometric coefficient 'k' and intercept 'a' are shown for each regression. Letters shown in the 'group' rows indicate significantly different regressions based on pair-wise conditional error tests with a Bonferroni adjustment to the p-value for a family-wise error rate of 0.05.

				Clone 34		Clone 769	
Variable	**P-Value**		**All Data**	**No Fert**	**Fert**	**No Fert**	**Fert**
Lateral Root	> 0.10	**R^2**	0.65	---	---	---	---
vs		***a***	-1.48	---	---	---	---
Total Mass		***k***	0.94	---	---	---	---
		group	---	---	---	---	---
Tap Root	< 0.01	**R^2**	---	0.69	0.86	0.49	0.74
vs		***a***	---	-3.00	-3.42	-2.91	-2.10
Total Mass		***k***	---	1.15	1.29	1.28	1.05
		group	---	A	A	B	B
Total Root	< 0.01	**R^2**	---	0.85	0.93	0.85	0.89
vs		***a***	---	-1.40	-1.72	-1.54	-1.16
Total Mass		***k***	---	1.06	1.11	1.14	1.00
		group	---	A	B	A	A
Stem	> 0.10	**R^2**	0.90	---	---	---	---
vs		***a***	-1.72	---	---	---	---
Total Mass		***k***	1.04	---	---	---	---
		group	---	---	---	---	---
Branches	< 0.01	**R^2**	---	0.44	0.77	n.s.	0.48
vs		***a***	---	-2.72	-3.35	n.s.	-3.14
Total Mass		***k***	---	0.85	1.13	n.s.	1.09
		group	---	A	B		AB
Foliage	< 0.01	**R^2**	---	0.94	0.97	0.82	0.95
vs		***a***	---	-0.64	-0.39	-0.74	-0.85
Total Mass		***k***	---	0.95	0.89	0.94	0.99
		group	---	AC	A	B	BC
Root	<0.01	**R^2**	---	0.70	0.85	0.64	0.76
vs		***a***	---	-0.76	-1.35	-0.76	-0.52
Shoot		***k***	---	0.96	1.11	1.03	0.92
		group	---	A	B	C	AC
Stem	<0.01	**R^2**	---	0.80	0.86	0.43	0.88
vs		***a***	---	-0.88	-1.24	-0.16	-0.59
Foliage		***k***	---	1.03	1.16	0.78	0.95
		group	---	AB	A	B	AB

Note: n.s. indicates that the regression was not significant (i.e. p > 0.10).

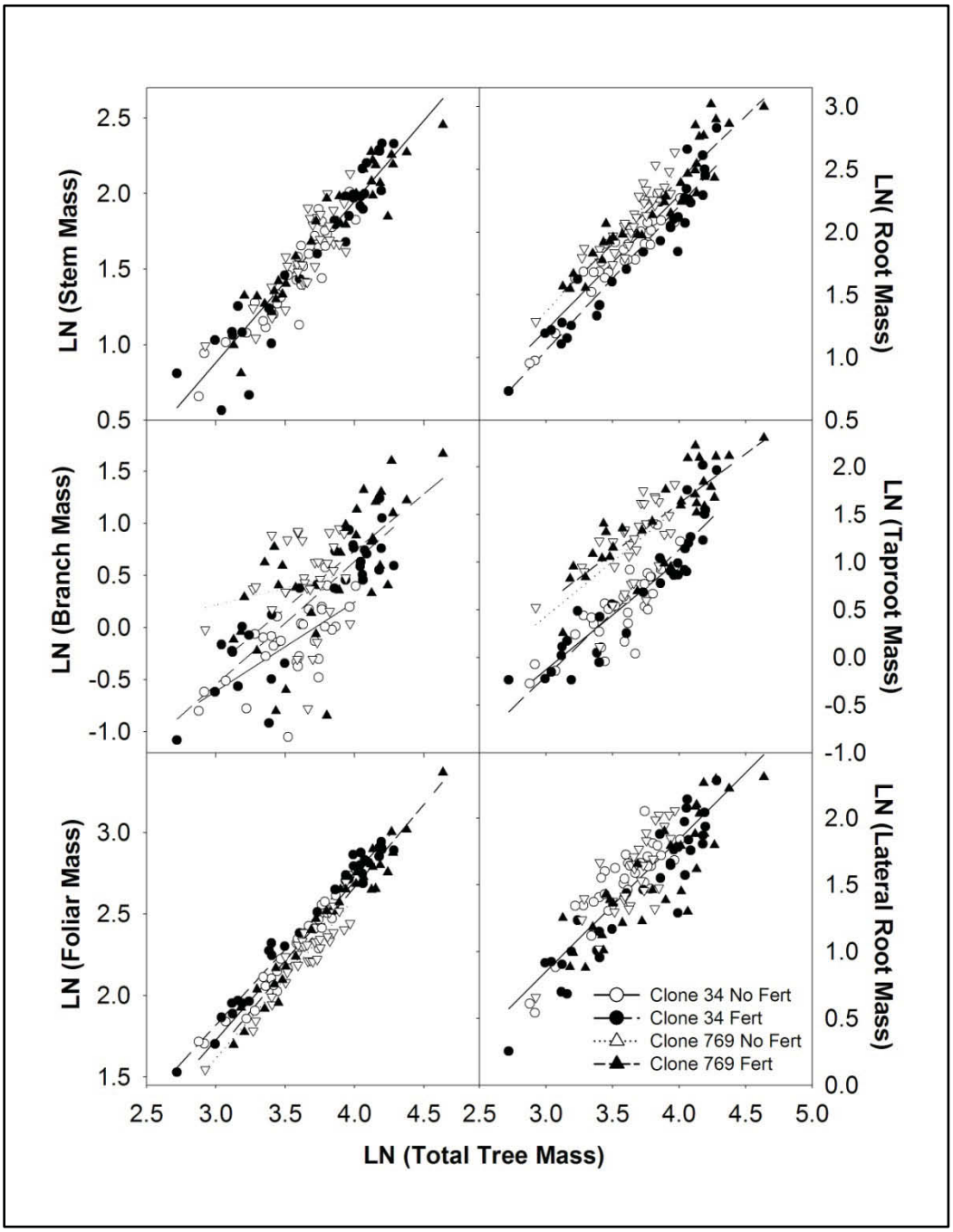

Figure 4-6. Natural log transformations of biomass components (y-axis) regressed against total tree mass for trees from all four destructive harvests. Of the six panels, two components (stem and lateral root) did not show significant differences between the vectors of regression coefficients, so only one regression for all (N=128) data points is shown. Each of the remaining four panels showed significant ($p < 0.05$) differences between regression coefficients, indicating differences in allometry due to treatments when development was considered. For each of these four regressions N = 32. All relevant statistics are presented in Table 4-2.

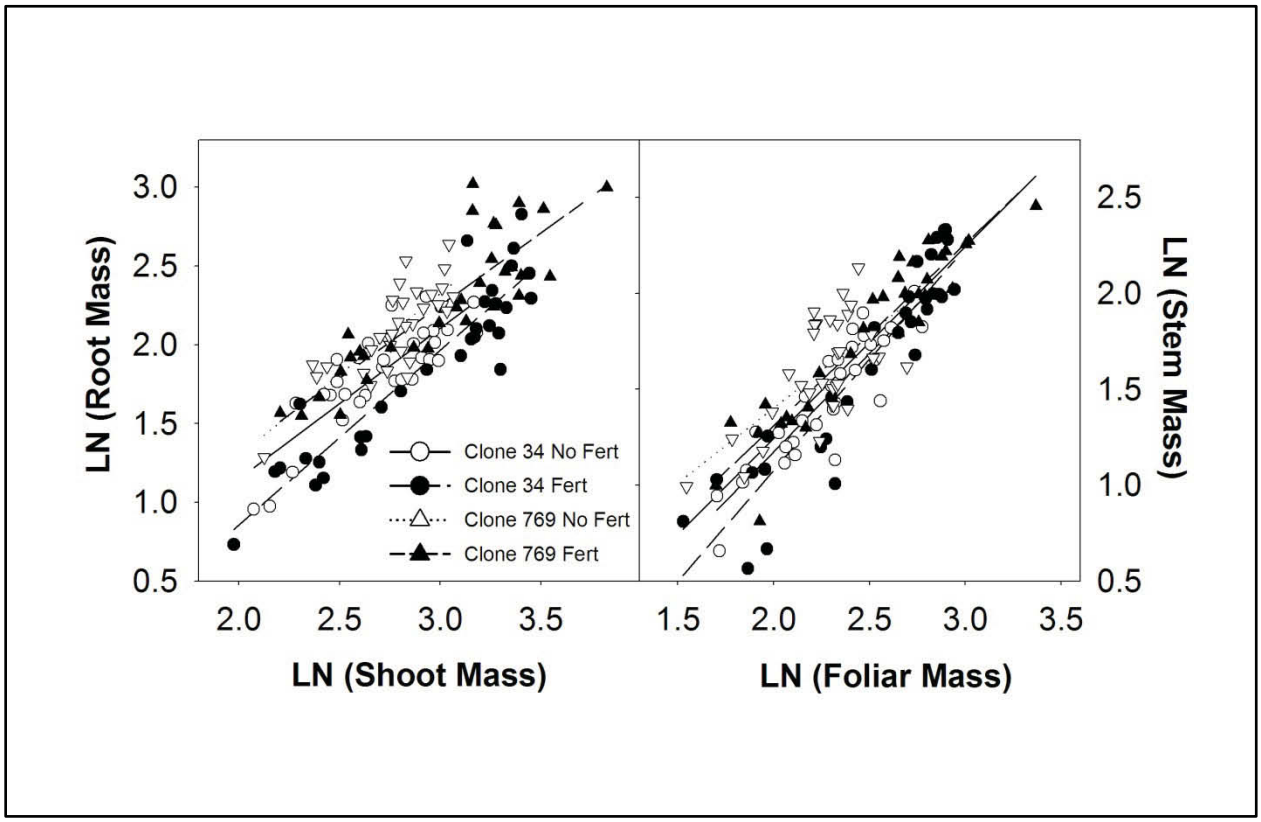

Figure 4-7. Natural log transformations of root versus shoot mass (left panel) and shoot versus foliar mass (right panel) for two full-sib clones under two levels of fertilizer from four destructive harvests. Both panels showed significant ($p < 0.05$) differences between regression coefficients, indicating differences in allometry due to treatments when development was considered. For each of these regressions N = 32. All relevant statistics are presented in Table 4-2.

4.4. Discussion

4.4.1. Ephemeral Changes in Partitioning with Fertilizer Application

Our first hypothesis, that fertilizer application would result in a short-term change in biomass partitioning, was supported by the data observed in this experiment. Figure 4-3 clearly shows reduced root-shoot ratios (i.e. less partitioning to roots) at some time in the four months post-fertilizer application in both of these clones from the same full-sib cross. This reduced partitioning to roots was consistently of a large magnitude for the first three harvest dates in clone 34, while it increased in magnitude over the same time period in clone 769. However, this

shift in partitioning was ephemeral. By day 121 both clones showed no significant difference between fertilized and unfertilized root-shoot ratios. A single harvest conducted during the dormant season would have concluded that fertilizer application did not affect root-shoot ratios in either clone, completely missing short-term reductions to belowground biomass partitioning.

Figure 4-4 shows significantly increased allocation to the foliar fraction in both clones during at least one harvest. By the final harvest foliar fraction in fertilized trees is either not significantly different than controls (clone 769) or shows a reversed trend from the previous three harvests (clone 34). This again indicates an ephemeral shift in partitioning that may explain a portion of the fertilizer growth response that would be missed based on a dormant season only harvest. Short-term changes in partitioning to both root-shoot ratios and foliar mass are theoretically consistent with the observed 53% total tree fertilizer growth response. A reduction in root partitioning in response to increased nutrient availability allowed an increase in foliar partitioning, thus increasing the photosynthetic capacity of the fertilized trees, allowing for long-term growth response to fertilizer application.

Based on this data we infer that short-term changes in biomass partitioning, whether due to changes in allometry or growth rate, are real and are a plausible mechanism of short-term growth response to fertilizer application. While several studies have shown that allometry does not change across a resource gradient in *P. taeda*, these studies are all focused on long-term responses to fertilizer treatments (Ledig, Bormann et al. 1970; Coyle, Coleman et al. 2008). Reduced below-ground biomass allocation in the weeks and months following fertilizer application allows carbon to be allocated to the more rapid development of a greater foliar biomass in fertilized trees. Once fertilized trees have attained a greater photosynthetic capacity, root-shoot ratios return to levels similar to those of unfertilized controls. However, by this time the canopy-level photosynthetic capacity of fertilized trees has increased, even if no change in photosynthetic rates occurs. This then results in a whole tree growth response to fertilizer application, as we observed, with all plant tissues having a greater mass versus unfertilized controls. Short-term changes in partitioning are consistent with long-term growth responses to

fertilizer application, even when considered independently of other potentially contributing physiological responses to fertilizer application (e.g. photosynthetic and dark respiration rates).

4.4.2. Implications for Episodic Nutrient Deficiencies and Seasonal Dieback

Biomass partitioning, particularly root-shoot ratios, have been shown to vary throughout the year in a number of species (Cannell and Willett 1976). Typically growth shifts aboveground in the late spring to early summer as shoots and new foliage elongate. Root growth is minimal during this period. Later in the growing season, in the late summer to early fall, aboveground growth ceases, allowing C allocation to shift belowground as roots grow throughout the fall and into the winter, depending upon soil temperature and water relations (Edwards, Friend et al. 1992; Iivonen, Rikala et al. 2001; Barnes 2002). Shoot and foliar yellowing or dieback has previously been observed towards the end of the growing season in heavily managed plantations after the application of macronutrients (Martin and Blakeslee 1998; South, Brown et al. 2002). While there is not yet definitive data ascribing a specific cause to this phenomenon, it has been hypothesized that it may be the result of micronutrient, particularly boron, deficiencies that have been shown to occur in *P. taeda* with application of high rates of N and P fertilizers (Stone 1990; South, Brown et al. 2002; Blazier and Hennessey 2008). While our experiment was not intended to address this hypothesis, our results support this hypothetical framework. The ephemeral reductions we observed in root-shoot ratios would limit the ability of the fertilized tree to fully exploit the soil volume for micronutrients in the growing season following fertilizer application. By the time root growth again commenced towards the end of the growing season, a short-term micronutrient deficiency could have occurred, resulting in the observed dieback symptoms. Our results support both the occurrence of a micronutrient induced dieback and why dieback is not as frequently observed after the growing season during which macronutrients are applied.

In *P. taeda* seedlings, episodic growth phases shift between aboveground and belowground tissues on a monthly to bi-monthly basis to a lesser extent than larger seasonal growth phases, resulting in varying root-shoot ratios within a single growing season (Drew and Ledig 1980). This variability does not change our conclusions on ephemeral fertilizer partitioning responses, since they are based on simultaneous comparison of fertilized ramets with controls throughout

the growing season. The difference in root-shoot ratios observed in this study between fertilized and unfertilized ramets indicates that resource availability over the short-term, through either its effects on allometry or development rate, may be at least as large a source of variability in root-shoot ratios as seasonal fluctuations in some clones (Figure 4-3, Figure 4-4). It should be noted that the fertilizer responses in this experiment may be of greater magnitude than average since we used an extremely infertile soil (150 mg kg^{-1} total N, < 2 mg kg^{-1} Mehlich 1 extractable P) in order to increase our ability to detect treatment differences.

4.4.3. Varying Clonal Partitioning Strategies

Our second hypothesis that short-term shifts in partitioning would vary in magnitude between clones was also supported by the data. Clones had similar total growth responses to fertilizer application, with no significant clone-by-fertilizer interactions in either stem mass or total tree mass observed. Clone 769 did have significantly greater whole tree mass in both fertilized and control treatments, and both clones showed several different partitioning responses to fertilizer application. Clone 34 showed a greater magnitude of reduction in the root-shoot ratios over the first two harvest dates versus clone 769 (Figure 4-3) despite the fact that they are full-sib to one another. The different partitioning patterns observed between the two clones appear to be theoretically consistent with the differences observed in growth rates. Greater partitioning to foliage and less partitioning to roots in clone 34 are consistent with its greater overall growth rate. While it is less efficient from a stem mass per foliar mass production basis, clone 34 may still outperform clone 769 in the long-term due to its lesser belowground allocation. This is supported by results from the literature comparing growth of these two clones in a field trial (Bitoki 2008). However, based on the previous discussion it is also possible that reduced belowground allocation could lead to a greater probability of micronutrient deficiencies in clone 34 versus clone 769.

Results of this study show that not only did allometry vary substantially between a full-sib pair of clones, but so did the cause of changes in allometry. Allometric analyses of the total root fraction show that while clone 34 responded to fertilizer application by changing its allometry, clone 769 only showed growth rate effects (Table 4-2). We are unable to determine based on the

time-scale of this experiment whether the shift in allometry was a short-term or long-term response to fertilizer application. Clones of other species have shown markedly different patterns of biomass partitioning within a single growing season (Scarascia-Mugnozza, Ceulemans et al. 1997) and significant differences in allometric coefficients for some biomass components across a resource gradient in the long-term (Coyle and Coleman 2005). While it is possible based on the literature that the allometric shifts we observed may be longer-term differences between these clones, we cannot conclude so without further experiments.

The vast majority of previous studies of allometric shifts in response to a resource-availability gradient have been based on genotypic averages (e.g. open-pollinated trees). It is possible that the mixed results found in the literature are to some extent due to the randomly selected genotypes specific to each study. Studies finding positive results for allometric shifts may have had more genotypes like clone 34, while those finding no allometric shifts may have had more genotypes like clone 769. While we have no direct evidence of this, it is possible given the relatively small number of replications in most studies due to the large investments of time and labor that collecting biomass partitioning data requires. Of the studies on biomass partitioning in *P. taeda* we examined, five had five or fewer replications per treatment per harvest date (Li, Allen et al. 1991; Green, Mitchell et al. 1994; Retzlaff, Handest et al. 2001; Samuelson, Johnsen et al. 2004; Coyle, Coleman et al. 2008), five had ten or fewer (Colbert, Jokela et al. 1990; Griffin, Winner et al. 1995; Gebauer, Reynolds et al. 1996; Jokela and Martin 2000; Adegbidi, Jokela et al. 2002), and only two had more than ten (Gough and Seiler 2004; Albaugh, Allen et al. 2006). If genotypes are highly variable in direct allometric shift versus growth rate effects as the source of fertilizer partitioning changes in biomass partitioning, relatively small samples sizes taken from random genotypes could randomly determine the outcome of each individual allometric analyses. As we've demonstrated, even full-sib clones may differ markedly in allometry, indicating that even constraining experiments to full-sib families would still likely result in a range of genotype-specific partitioning strategies.

Regressions across a large number of studies, such as the one shown in Coyle (2008), indicate that changes in the rate of development are the predominant mode of partitioning change found

in *P. taeda* across all genotypes. But, the fact that these two full-sib clones behaved so differently when fertilized in terms of allometric shifts versus growth rate effects raises the question of how variable individual genotypes may be. Of the studies we are aware of examining biomass partitioning in *P. taeda* clones, none have yet attempted to ascribe changes in partitioning to their fundamental causes, changes allometry or changes associated with growth (Tyree, Seiler et al. 2009a; Tyree, Seiler et al. 2009b). Further studies with a greater number of clones will be necessary in order to assess how variable *P. taeda* is in its mode of fertilizer response to partitioning.

4.5. Conclusion

Short-term changes in biomass partitioning in response to fertilizer application occurred in two full-sib clones of *P. taeda*. Ephemeral reductions in allocation to roots and increases in allocation to foliage in the several months following fertilizer application are consistent with a theory of long-term fertilizer-growth response caused by short-term physiological changes. The magnitude of changes in partitioning differed between the two full-sib clones, and was consistent with each clone's observed whole-tree growth response to fertilizer. Allometric analysis revealed that in response to fertilizer application one clone only altered growth rates, causing corresponding changes with respect to belowground biomass partitioning, while the other shifted its allometry. The variable modes of partitioning response to fertilizer application indicate that different clones may have fundamentally different physiological capacities to respond to fertilizer application. These results emphasize the importance of understanding how different clones respond to fertilizer application in order to optimize management and accurately model carbon dynamics in clonal plantations.

4.6. Literature Cited

Adegbidi, H. G., E. J. Jokela, N. B. Comerford and N. F. Barros (2002). "Biomass development for intensively managed loblolly pine plantations growing on Spodosols in the southeastern USA." Forest Ecology and Management **167**(1-3): 91-102.

Albaugh, T. J., H. L. Allen and L. W. Kress (2006). "Root and stem partitioning of *Pinus taeda*." Trees-Structure and Function **20**(2): 176-185.

Baltunis, B. S., D. A. Huber, T. L. White, B. Goldfarb and H. E. Stelzer (2007). "Genetic gain from selection for rooting ability and early growth in vegetatively propagated clones of loblolly pine." Tree Genetics & Genomes **3**(3): 227-238.

Barnes, A. D. (2002). "Effects of phenology, water availability and seed source on loblolly pine biomass partitioning and transpiration." Tree Physiology **22**(10): 733-740.

Bitoki, O. (2008). "Comparing early survival and growth of varietal and open-pollinated loblolly pine seedlings." VDOF Forest Research Review **April 2008**: 4-5.

Blazier, M. A. and T. C. Hennessey (2008). "Seasonal soil and foliage nutrient dynamics of a juvenile loblolly pine plantation: Impacts of fertilizer formulation and vegetation suppression." Forest Ecology and Management **255**(8-9): 3404-3415.

Cannell, M. G. R. and S. C. Willett (1976). "Shoot growth phenology, dry matter distribution and root: shoot ratios of provenances of *Populus trichocarpa*, *Picea sitchensis* and *Pinus contorta* growing in Scotland." Silvae Genetica **25**(2): 49-59.

Colbert, S. R., E. J. Jokela and D. G. Neary (1990). "Effects of annual fertilization and sustained weed-control on dry-matter partitioning, leaf-area, and growth efficiency of juvenile loblolly and slash pine." Forest Science **36**(4): 995-1014.

Coyle, D. R. and M. D. Coleman (2005). "Forest production responses to irrigation and fertilization are not explained by shifts in allocation." Forest Ecology and Management **208**(1-3): 137-152.

Coyle, D. R., M. D. Coleman and D. P. Aubrey (2008). "Above- and below-ground biomass accumulation, production, and distribution of sweetgum and loblolly pine grown with irrigation and fertilization." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **38**: 1335-1348.

Dougherty, D. (2007). "Improved returns on forestlands: A financial analysis of MCP and varietal seedlings on private land in the southeastern United States." Forest Landowner **66**: 15-18.

Drew, A. P. and F. T. Ledig (1980). "Episodic growth and relative shoot - root balance in loblolly-pine seedlings." Annals of Botany **45**(2): 143-148.

Edwards, G. S., A. L. Friend, E. G. Oneill and P. T. Tomlinson (1992). "Seasonal patterns of biomass accumulation and carbon allocation in *Pinus taeda* seedlings exposed to ozone, acidic precipitation, and reduced soil Mg." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **22**(5): 640-646.

Fox, T. R., E. J. Jokela and H. L. Allen (2007). "The development of pine plantation silviculture in the southern United States." Journal of Forestry **105**: 337-347.

Gebauer, R. L. E., J. F. Reynolds and B. R. Strain (1996). "Allometric relations and growth in *Pinus taeda*: The effect of elevated CO_2 and changing N availability." New Phytologist **134**(1): 85-93.

Gough, C. M. and J. R. Seiler (2004). "Belowground carbon dynamics in loblolly pine (*Pinus taeda*) immediately following diammonium phosphate fertilization." Tree Physiology **24**(7): 845-851.

Gough, C. M., J. R. Seiler and C. A. Maier (2004). "Short-term effects of fertilization on loblolly pine (*Pinus taeda* L.) physiology." Plant Cell and Environment **27**(7): 876-886.

Green, T. H., R. J. Mitchell and D. H. Gjerstad (1994). "Effects of nitrogen on the response of loblolly-pine to drought. 2. Biomass allocation and C-N balance." New Phytologist **128**(1): 145-152.

Griffin, K. L., W. E. Winner and B. R. Strain (1995). "Growth and dry-matter partitioning in loblolly and ponderosa pine-seedlings in response to carbon and nitrogen availability." New Phytologist **129**(4): 547-556.

Iivonen, S., R. Rikala and E. Vapaavuori (2001). "Seasonal root growth of Scots pine seedlings in relation to shoot phenology, carbohydrate status, and nutrient supply." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **31**(9): 1569-1578.

Jokela, E. J. and T. A. Martin (2000). "Effects of ontogeny and soil nutrient supply on production, allocation, and leaf area efficiency in loblolly and slash pine stands."

Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **30**(10): 1511-1524.

King, J. S., T. J. Albaugh, H. L. Allen and L. W. Kress (1999). "Stand-level allometry in *Pinus taeda* as affected by irrigation and fertilization." Tree Physiology **19**(12): 769-778.

King, N. T., J. R. Seiler, T. R. Fox and K. H. Johnsen (2008). "Post-fertilization physiology and growth performance of loblolly pine clones." Tree Physiology **28**(5): 703-711.

Ledig, F. T., F. H. Bormann and K. F. Wenger (1970). "The distribution of dry matter growth between shoots and roots in loblolly pine." Botanical Gazette **131**(4): 349-359.

Li, B. L., H. L. Allen and S. E. McKeand (1991). "Nitrogen and family effects on biomass allocation of loblolly-pine seedlings." Forest Science **37**(1): 271-283.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger and O. Schabenberger (2006). SAS for mixed models, second edition. Cary, NC, SAS Institute Inc.

Martin, T. A. and G. Blakeslee (1998). Needle and shoot blight of loblolly pine. Forest Biology Research Cooperative 2nd Annual Report. University of Florida. School of Forest Resources and Conservation**:** 4-11.

Paul, A. D., G. S. Foster, T. Caldwell and J. McRae (1997). "Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine." Forest Science **43**(1): 87-98.

Retzlaff, W. A., J. A. Handest, D. M. O'Malley, S. E. McKeand and M. A. Topa (2001). "Whole-tree biomass and carbon allocation of juvenile trees of loblolly pine (*Pinus taeda*): Influence of genetics and fertilization." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **31**(6): 960-970.

Roth, B. E., E. J. Jokela, T. A. Martin, D. A. Huber and T. L. White (2007). "Genotype x environment interactions in selected loblolly and slash pine plantations in the southeastern United States." Forest Ecology and Management **238**(1-3): 175-188.

Samuelson, L. J., K. Johnsen and T. Stokes (2004). "Production, allocation, and stemwood growth efficiency of *Pinus taeda* L. stands in response to 6 years of intensive management." Forest Ecology and Management **192**(1): 59-70.

Scarascia-Mugnozza, G. E., R. Ceulemans, P. E. Heilman, J. G. Isebrands, R. F. Stettler and T. M. Hinckley (1997). "Production physiology and morphology of *Populus* species and their hybrids grown under short rotation. 2. Biomass components and harvest index of hybrid and parental species clones." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **27**(3): 285-294.

South, D. B., P. Brown, P. M. Dougherty, S. Olykan, B. Runion, A. Singh and M. Skinner (2002). Tip-dieback in young loblolly pine plantations. Proceedings of the eleventh biennial southern silvicultural research conference. K. W. Outcalt, General Technical Report SRS–48. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station**:** 574-578.

Stelzer, H. E. and B. Goldfarb (1997). "Implementing clonal forestry in the southeastern United States: SRIEG satellite workshop summary remarks." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **27**(3): 442-446.

Stone, E. L. (1990). "Boron deficiency and excess in forest trees: A review." Forest Ecology and Management **37**(1-3): 49-75.

Swindel, B. F. (1970). Some applications of the principles of conditional error, USDA Forest Service Research Paper SE-59, Southern Rearch Station, Asheville, NC.

Tyree, M. C., J. R. Seiler and C. A. Maier (2009a). "Short-term impacts of nutrient manipulations on leaf gas exchange and biomass partitioning in contrasting 2-year-old *Pinus taeda* clones during seedling establishment." Forest Ecology and Management **257**(8): 1847-1858.

Tyree, M. C., J. R. Seiler, C. A. Maier and K. H. Johnsen (2009b). "*Pinus taeda* clones and soil nutrient availability: Effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology." Tree Physiology **29**(9): 1117-1131.

Whetten, R. W. and R. Kellison (2010). "Research gap analysis for application of biotechnology to sustaining US forests." Journal of Forestry **108**: 193-201.

5. Respiratory C fluxes and root exudation in two clones of *Pinus taeda* (L.) under varying fertilizer regimes

5.1. Introduction

Pinus taeda (L.) plantations span some 13 million hectares across the southeastern United States (Wear and Greiss 2002), and are responsible for production of a disproportionate amount of timber on a national basis (Adams, Haynes et al. 2006). These plantations are often fertilized with N and P, with over 6 million hectares already fertilized over the last several decades (Albaugh, Allen et al. 2007). Increasingly clonal material is being planted in these plantations in order to increase productivity (Bettinger, Clutter et al. 2009). An understanding of the ecophysiology of different clones under fertilizer regimes similar to those found in operational plantations is necessary both to understand varying observed clonal growth responses to fertilizer application (King, Seiler et al. 2008), and to better understand the carbon (C) cycling of this rapidly expanding intensively managed forest ecosystem. The purpose of this chapter is to examine how respiratory carbon fluxes and root exudation change in the short-term between contrasting yet closely related clones in response to an operational fertilizer application, and to determine if these changes in ecophysiology are consistent with a theory of short-term growth response to fertilizer application.

Ecosystem respiration, or C emitted by aboveground biomass respiration and soil CO_2 efflux, is one of the primary determinants in whether forest ecosystems are net sources or sinks of C relative to the atmosphere (Valentini, Matteucci et al. 2000). Numerous studies have shown that nutrient availability affects various respiratory fluxes in forest ecosystems, including foliar, woody, and root biomass respiration, soil microbial respiration, and total soil CO_2 efflux (Ryan, Hubbard et al. 1996; Vose and Ryan 2002; Phillips and Fahey 2007). Fertilizer and irrigation treatments have been shown to affect whether mid-rotation *P. taeda* plantations on a poor quality site may be net sources or sinks for C, in part due to treatment effects on ecosystem respiration (Maier, Albaugh et al. 2004). Intra-specific variability in various respiration rates has also been observed in the literature. Lower leaf respiration rates were found in faster growing families of *P. taeda* and *PInus elliottii* (Englm.) (Samuelson 2000). In another study no difference was

observed in foliar respiration among clones of *P. taeda*, although only a relatively small foliar sample (5 fascicles) was measured (King, Seiler et al. 2008). Clonal variability in respiratory C fluxes could play a significant role in determining differences that have been observed in clonal growth rates (e.g. Paul, Foster et al. 1997).

Soil CO_2 efflux (F_S) is the largest respiratory CO_2 flux in forested ecosystems, and is in fact greater in magnitude on average than net primary productivity (Raich and Schlesinger 1992). A number of studies have shown that in *P. taeda* plantations, fertilizer application typically results in reduced F_S rates (Maier and Kress 2000; Butnor, Johnsen et al. 2003; Giardina, Binkley et al. 2004; Samuelson, Johnsen et al. 2004). However, other studies have shown that F_S increased in either the short-term (< 1 year) or long-term (> 20 year) following fertilizer application (Gough and Seiler 2004; Tyree, Seiler et al. 2006). Increases in F_S such as these are often attributed to greater root biomass, and thus greater root respiration in fertilized treatments versus controls. However, the heterotrophic component of soil CO_2 efflux (F_H) consistently shows a decline with fertilizer application that has not yet been linked to a specific causal mechanism (Gough and Seiler 2004; Olsson, Linder et al. 2005; Tyree, Seiler et al. 2008). Research in the field on young (e.g. one-year-old) clones has shown no variability in F_S, or its autotrophic or heterotrophic components due to genotype (Tyree, Seiler et al. 2008). This last study hypothesized that clonal effects may not have manifested yet in one-year-old trees, and that any treatment effects would have been difficult to detect in single-tree plots. However, pot-based studies have shown that F_S response to fertilizer application may occur within days (Gough and Seiler 2004). Thus, a pot-based study involving clones would be ideal to detect any short-term changes in belowground respiratory C fluxes following fertilizer application.

The first objective of this chapter was to determine whether differences in total ecosystem respiration, aboveground biomass respiration, and F_S would be consistent with observed differences clonal growth rates. Clones with lower respiratory C fluxes may allocate more C to net primary production, allowing for more rapid short-term growth. Further, we hypothesized that different clonal growth responses to fertilizer application would be consistent with changes in respiratory C fluxes across a nutrient availability gradient. Reduced respiratory C fluxes in

response to fertilizer application may be a short-term mechanism by which trees can allocate more C to canopy development, the widely recognized long-term mechanism of fertilizer growth response (Vose and Allen 1988; Albaugh, Allen et al. 1998; Fox, Allen et al. 2007).

Root exudates are composed of numerous organic and inorganic compounds that are either passively or actively transferred from living roots to the rhizosphere (Nguyen 2003). Among a range of other functions, root exudates are known to increase nutrient availability in the rhizosphere, either through ligand exchange reactions with organic acids (e.g. P) or by increasing rates of microbial mineralization by providing a labile C source (e.g. N) (Hinsinger 2001; Dakora and Phillips 2002; Landi, Valori et al. 2006). Estimates of the percentage of GPP allocated to root exudation range from less than 1% up to 10% (Grayston, Vaughan et al. 1997; Phillips, Erlitz et al. 2008; Phillips, Bernhardt et al. 2009). While the carbon flux that can be attributed to exudation is of a much lower magnitude than respiratory C fluxes, root exudates remain disproportionately important in governing plant-soil interactions through their role in mediating the biological and chemical nature of the rhizosphere. Differences in root exudation may thus play a critical ecophysiological role in linking plant growth response to fertilizer additions and genotypic variability in fertilizer growth response.

Fertilizer application has been shown to alter both quantity and quality of root exudates. Reduced exudation quantity and reduced total extractable rhizosphere C have been observed with the addition of N fertilizers (Henry, Nguyen et al. 2005; Lagomarsino, Moscatelli et al. 2006). Other studies have shown both quantitative and qualitative changes in root exudation in response to a P gradient (Ratnayake, Leonard et al. 1978; Egle, Romer et al. 2003). Generally, exudation rates are greatest under more nutrient deficient conditions, and thus are reduced by fertilizer application. Studies have also found quantitative and qualitative differences in root exudation between different species and between different cultivars of the same species (Egle, Romer et al. 2003). The chemical composition of exudates varies among tree species, even those in the same family or genera (Grayston, Vaughan et al. 1997). However, data pertaining to intra-specific variability of exudate quantity and quality in tree species are lacking in the literature. The second objective of this study was to determine if root exudation quantity and quality varies

between clones in response to fertilizer application. Further, we hypothesized that any differences would be consistent with differences in clonal growth responses to fertilizer application, either through the role of exudation as a C sink or through altering nutrient uptake efficiencies between clones.

Root exudation affects CO_2 efflux from the rhizosphere. Inputs of root exudate organic acids into simulated rhizospheres have been linked to changes in microbial N immobilization and mineralization processes, community structure, and microbial activity (Landi, Valori et al. 2006). Up to 36% of microbial biomass C may be root derived, a major source of which could be exudates (Werth and Kuzyakov 2008). Numerous studies have showed rhizosphere priming effects, or increases in soil organic matter turnover as a result of labile root exudate inputs into soil (Kuzyakov 2002; Bol, Moering et al. 2003). Each of these processes mediated by root exudate quantity and quality plays a role in the magnitude of F_H from rhizosphere soils. While there is much tangential evidence to support the hypothesis that F_H may be regulated by root exudate inputs into the rhizosphere, we are not aware of any studies that have yet examined causal links between root exudation and F_H across a resource availability gradient. The final objective of this study was to determine if reduced root exudation as a result of fertilizer application may be the causal mechanism responsible for reductions in F_H routinely observed with fertilizer application (Gough and Seiler 2004; Olsson, Linder et al. 2005; Tyree, Seiler et al. 2008).

5.2. Materials and Methods

5.2.1. Study Description and Experimental Design

Ramets of two clones were potted on April 30, 2009 in a coarse, nutrient and organic matter deficient soil in a greenhouse at Virginia Tech, Blacksburg, Virginia, USA. The two contrasting clones, GE34 and GE769, were a full-sib pair originally produced by ArborGen in 2005 (Arborgen LLC, Summerville, South Carolina, USA) (Bitoki 2008). Clone 769 has slower growth rates and a wider crown with a lesser number of larger branches compared to clone 34 (Bitoki 2008). Ramets were potted in 15-by-15-by-38 cm deep pots (8,550 cm^3) that were

sufficiently large to minimize substantial root-binding through the four months of this experiment. Trees were not root-bound even by the final day 121 harvest. Ramets were planted in their original plugs containing previously fertilized media in order to minimize root mortality and turnover. The soil utilized in this study was the sieved (1-cm mesh) A horizon of a Wakulla series (siliceous, thermic Psammentic Hapludult) obtained from the USDA Forest Service's Southeastern Tree Research and Education Site (SETRES). Trees were watered daily to minimize drought stress while also avoiding excessive leaching from the bottom of the pots. Nighttime minimum temperature was set to 18° C in the greenhouse, and while the vents were set to open during the day at 25° C daytime temperatures did exceed this frequently.

The ramets were randomly assigned to fertilizer and control treatments, and fertilizer was applied to the selected ramets on June 16, 2009. This date will be referred to as day 0 throughout the remainder of this chapter. Fertilizer was applied at an operational rate with DAP and ammonium nitrate at 225 kg N per hectare and 56 kg elemental P per hectare. Control trees received no fertilizer. Following fertilizer application, ramets from each treatment combination were harvested monthly on July 16, August 16, September 15, and October 15, 2009 (30, 61, 91, and 121 days after fertilizer application). Thus the experiment was a two-by-two-by-four factorial randomized complete block design replicated eight times (128 trees total), with treatments consisting of clone, fertilizer, and sequential harvest, respectively. Some measurements were only made on the final harvest group (day 121 harvest) throughout the experiment. These variables are described below, and may be considered a two-by-two randomized complete block design with repeated measures. Other variables were measured on each tree at harvest and thus reflect a tree-for-time-substitution assumption.

5.2.2. Biomass and Stem Growth Measurements

At each of the four destructive harvests the entire tree was partitioned into components. Fine roots were considered those < 2 mm diameter, with coarse roots being any root > 2 mm diameter. All biomass components were oven-dried at 65° C for > 10 days, and weighed. Throughout the experiment ground-line diameter and total height were measured weekly on the final harvest group. A stem volume index was calculated for each tree with the formula height × (basal

diameter)^2. Prior to each destructive harvest heights and basal diameters of all trees were measured to ensure that no significant growth differences existed between harvest groups, and that tree-for-time-substitution assumptions were valid.

5.2.3. Soil CO_2 Efflux and Respiratory C Flux Measurements

Total soil CO_2 efflux (F_S) was assessed in the morning between 10:00 and 12:00 EDT using a small dynamic closed (231 cm^3 volume, 55 cm^2 area) cuvette with no fan. A LI-6200 infrared gas analyzer (IRGA) was used for all respiratory C flux measurements (LiCor Biosciences Inc., Lincoln, Nebraska, USA). The IRGA was zeroed daily immediately prior to the first F_S measurement and a blank reading on a sealed cuvette with no soil was taken to ensure the apparatus was operating correctly. Soil temperature (thermocouple) and volumetric moisture content (TDR) were measured concurrently with efflux for use as covariates in statistical analyses. These measurements were made on 22 separate dates. Procedures were based on those described in Gough and Seiler (2004).

The heterotrophic component of soil CO_2 efflux (F_H) was measured on all four destructive harvest groups one or two days prior to harvest. One soil sample (mean 19.9 g, standard error 0.1 g) was taken with a push-tube from the top 17 cm of soil near the edge of each pot to minimize damage to the root system. Roots were removed from the soil by hand, and the soil was then placed in an aluminum boat in a 0.25 L cuvette equipped with a small fan for quantification of F_H. Soil was then weighed fresh within the hour, oven dried at 65° C for > 24 hours and weighed again dry so that gravimetric moisture content could be calculated and used as a covariate. Rates were expressed on a soil dry mass basis.

The autotrophic component of soil CO_2 efflux (F_A) was measured at days 61, 91, and 121 after fertilizer application on the harvested trees. Autotrophic respiration was not measured for the first destructive harvest. Between 13:00 and 16:00 on each harvest date, each pot was carefully overturned to remove the entire tree intact. Roots were then washed with tap-water, and trees were transported to the lab, a process taking no longer than 30 minutes for each block. A small sample (mean 0.34 g, standard error 0.02 g) of fine roots (< 2 mm) were carefully excised from

harvested trees immediately after their transport back to the lab, soaked in deionized water, and then F_A was determined in sequential tree order by blocks in a 0.25 L cuvette equipped with a small fan. Roots were then oven dried at 65° C for > 24 hours and weighed so that respiration rates could be expressed on a mass basis. Both F_H and F_A are intended for use only as treatment indices, and do not represent rates that would be observed in intact plant-soil systems (Hanson, Edwards et al. 2000; Tyree, Seiler et al. 2008). Care was taken to obtain roots with a similar diameter distribution in all treatments for both F_A and exudate measurements.

Dark respiration rates were measured at night between 23:00 and 5:00 EDT within two days prior to each destructive harvest. Two distinct measurements were made: 1) total aboveground dark respiration (R_{AG}) and 2) total ecosystem respiration rate (R_{ECO}), which included aboveground biomass, belowground biomass, and the entire potted soil mass. For R_{AG} a large inverted trash-can (volume = 120,000 cm^3) was used as a cuvette. An incision was made along the radius of the lid, so that it could be sealed with weather-stripping around the base of the stem of the seedling being measured without damaging the seedling. A small fan was installed in the cuvette to mix the air volume inside. Due to technical difficulties with the cuvette, aboveground respiration was only measured for harvests 30 and 91 days after fertilizer application. For R_{ECO} measurements, the cuvette used was one large trashcan inverted atop another (total volume = 240,000 cm^3). The entire seedling while still potted was placed in the bottom trashcan, the top trashcan was sealed on top of it, and air was mixed with a small fan. Ambient temperature inside the cuvette was measured with a thermocouple during each measurement so that respiration rates could be standardized to 20° C assuming a Q_{10} of 2.0 (Ryan 1991). Both rates were expressed on a plant mass basis in order to account for differences in tree size.

5.2.4. Root Exudation and Soil Chemical Analysis

Root specific exudation rates for a subsample of fine roots (mean 0.78 g, standard error 0.03 g) were determined as per Egle et al. (2003) in the lab beginning no more than 30 minutes after the harvest of each tree. Exudation was assessed while the tree was completely intact, save for a small sample of separate roots that had been excised for determination of F_A. Briefly, this process involved immersing a portion of the washed root system in 75 ml of aerated deionized

water for one hour to allow the roots to equilibrate, then repeating the process with 75 ml of fresh, aerated deionized water for one more hour. After removing roots from the solution, this subsample of fine roots was excised and refrigerated for later morphological analysis. Root morphology (length, surface area) was determined by scanning the root sample taken for exudate measurements and processing images with WinRhizo 5.0A software (Regent Instruments Inc., Quebec, Quebec, Canada).

The second solution was immediately filtered through a number 2 Whatman qualitative filter, and then frozen at -20° C. Details of the procedure (time of soak, volume of soak solution, adequate concentrations of exudates obtained) were determined in a pilot study conducted prior to the first harvest using 12 ramets of clone 769. Total organic carbon (TOC) was later determined on an Elementar LiquiTOC Analyzer (Elementar Americas, Inc., Mt. Laurel, New Jersey, USA). Further analyses of exudate composition were performed by ion chromatography with an AS17C column at a 1 ml/min flow rate through a 25 µl sample loop at 149 mA current. An EG40 Eluent Generator was utilized with an IP25 Isocratic pump, a CD25 Conductivity detector, and a LC25 Chromatography oven (Dionex Corporation, Sunnyvale, California, USA). Anions assessed included acetate, chloride, citrate, formate, lactate, nitrate, oxalate, phosphate, sulfate, and tartrate. All runs were checked for accuracy with standards that were prepared daily.

All soils were sieved through a 2-mm mesh and air dried. Soil C and N were determined on an Elementar CNS Analyzer (Elementar Americas, Inc., Mt. Laurel, New Jersey, USA). Other soil nutrients were analyzed by the Virginia Tech Soils Testing Laboratory using a Mehlich I procedure and a Thermo Elemental ICAP 61E (Thermo Scientific, Walthman, Massachusetts, USA) (Mullins and Heckendorn 2009).

5.2.5. Statistical Analysis

All analyses were performed in SAS software v. 9.2 (SAS Institute Inc., Cary, North Carolina, USA). All variables were transformed as appropriate to meet assumptions of normality, but all reported means and standard errors are untransformed. Repeated measures analyses utilized covariance structures appropriate for unevenly spaced data (unstructured, compound symmetry,

spatial power, spatial Gaussian, and spatial spherical) that were selected by minimizing AIC_c values (Littell, Milliken et al. 2006). For data from the four harvest dates, all harvest interactions were included in analyses. Block was modeled as a random effect, and PROC MIXED was implemented using the Kenward-Roger method for calculating denominator degrees of freedom (Littell, Milliken et al. 2006). To meet assumptions of homogenous variance, variance was modeled separately for each treatment combination using the "group" option in the "repeated" statement as necessary.

Root exudate qualitative data for oxalate, citrate, phosphate, and lactate were corrected by changing all non-detect values to the value of half the detection limit for that compound (Smith 1991). As it was not possible to transform these data to normal, the non-parametric Friedman's Chi-Square Test was implemented using PROC FREQ. No analysis was performed for the fertilizer effect on soil P data, since only two of 64 control samples were above the detection limit. Statistical values reported for soil P data pertain only to the fertilized treatment.

5.3. Results

5.3.1. Stem Growth Response to Treatments

Both clonal and fertilizer main effects were statistically significant in the repeated measures analysis for stem volume (Table 5-1). These differences appear to be driven by height growth for the clonal effect and basal diameter growth for the fertilizer effect (Figure 5-1). By day 121 fertilized trees showed a 53% increase in volume over controls and a 25% increase in basal diameter ($p < 0.05$). While clone 769 had 50% greater volume and 10% greater height at day 0 ($p < 0.10$), by day 121 clone 34 had 3% greater volume and was 5% taller, although these day 121 differences between clones were no longer statistically significant ($p < 0.10$). This indicates that clone 34 is the faster growing of these two clones, since it had less stem volume at day 0 but was not significantly different from clone 769 by day 121. Both clones responded similarly to fertilizer application as is evidenced by lack of significant clone-by-fertilizer interactions for any of the stem growth metrics ($p > 0.10$).

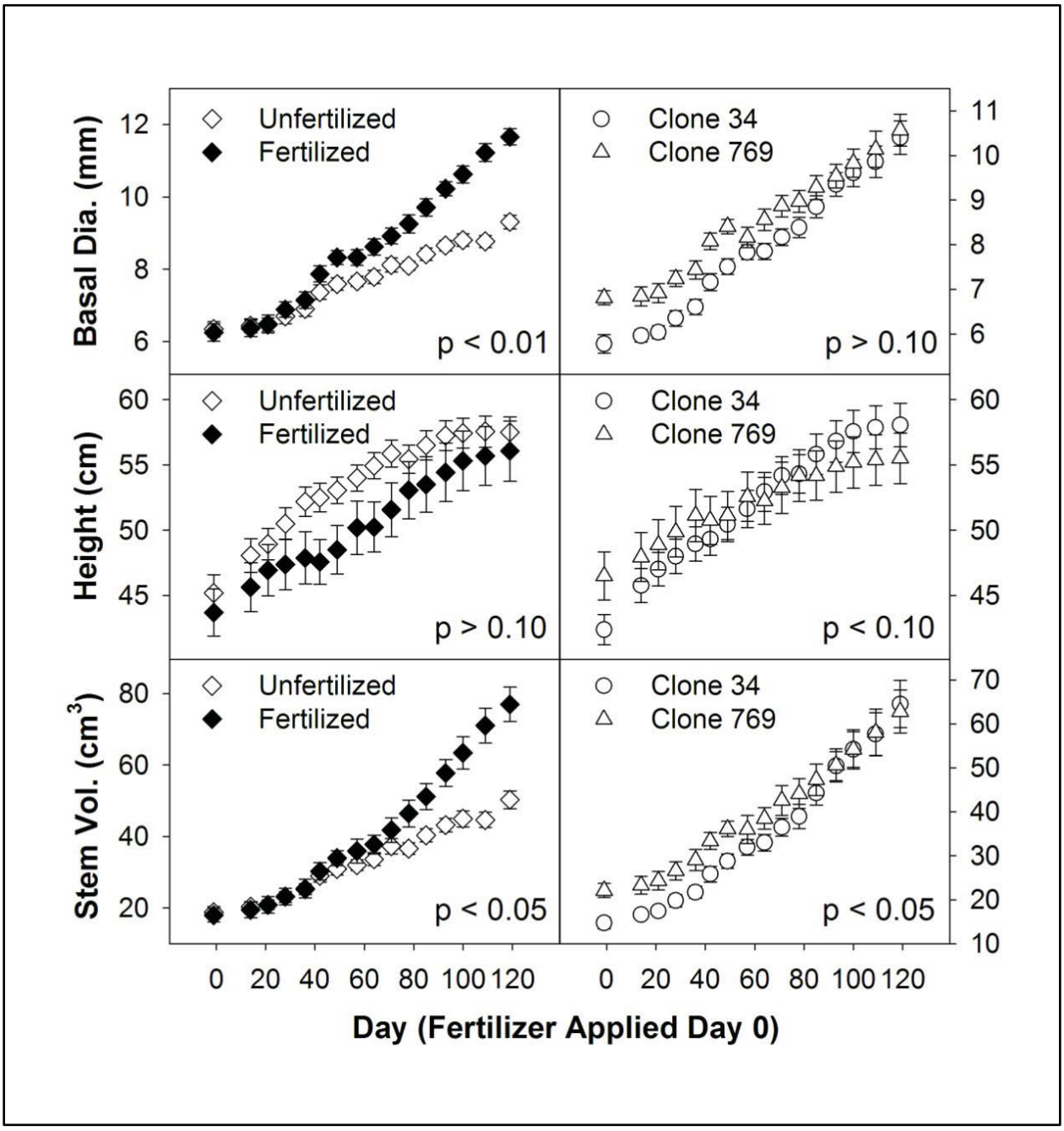

Figure 5-1. Fertilizer and clonal effects for stem dimensions of the final harvest group. Standard error bars are shown, N = 16. Fertilizer was applied day 0 = June 16, 2009.

Table 5-1. P-values for stem metrics, respiratory C fluxes, root exudation quantity, and soils data. When harvest effects are not shown, data were collected only on the final harvest group trees over time and were analyzed using repeated measures. When harvest effects are shown, data were collected from each of the four harvest groups at the corresponding monthly destructive harvest following fertilizer application. P-values < 0.10 are indicated in boldface.

Variable	Clone	Fertilizer	C x F	Harvest Date	C x HD	F x HD	C x F x HD
tree height	**0.0661**	0.9439	0.7599	---	---	---	---
basal diameter	0.9363	**0.0001**	0.6374	---	---	---	---
stem volume	**0.0207**	**0.0132**	0.7427	---	---	---	---
F_S	0.6381	**0.0001**	**0.0002**	---	---	---	---
F_A per root mass	0.7201	**0.0024**	0.1332	**0.0023**	0.8547	0.4251	0.7429
Fine root mass	**0.0152**	0.4468	**0.0973**	**0.0001**	**0.0674**	**0.0001**	0.3088
F_A per tree	0.4636	**0.0038**	**0.0876**	0.3119	0.8411	0.4933	0.6314
F_H	0.5154	**0.0001**	0.1984	**0.0635**	0.6867	0.1269	0.3962
R_{ECO}	**0.0014**	0.4210	0.7487	**0.0001**	0.2450	0.7945	0.2553
R_{AG}	0.1186	**0.0990**	0.6042	**0.0001**	0.2034	0.8625	0.7875
root exudation per mass	**0.0189**	0.2436	0.4513	**0.0001**	0.8019	0.2690	0.1830
root exudation per length	0.1411	0.9904	0.2692	**0.0001**	0.4072	0.3580	**0.0848**
root exudation per area	0.1607	0.5376	0.3202	**0.0001**	0.5123	**0.0401**	**0.0564**
soil pH	**0.0778**	**0.0001**	0.3228	**0.0002**	0.3131	0.1347	0.6248
soil N	0.4411	**0.0001**	0.7230	**0.0001**	0.8696	**0.0125**	0.8391
soil K	0.5439	**0.0009**	0.4738	**0.0135**	0.7579	**0.0154**	0.9764
soil Ca	**0.0419**	0.1984	0.2708	**0.0001**	**0.0944**	**0.0209**	0.6776
soil Mg	0.1001	**0.0398**	0.4273	**0.0001**	0.1115	**0.0007**	0.6331

5.3.2. Respiratory C Fluxes and Soil CO_2 Efflux

R_{ECO} was greater in clone 34 than clone 769 by 32.4% averaged across all dates ($p < 0.01$; Figure 5-2). This effect was only significant when tested on individual dates for the 30 and 121 harvests, where clone 34 had 35.5 and 53.1% greater flux rates, respectively ($p < 0.10$). No fertilizer or clone-by-fertilizer effects were observed ($p > 0.10$). By contrast, no significant clonal differences were found in R_{AG} ($p > 0.10$). R_{AG} only varied among fertilizer treatments when averaged across both measurement dates ($p < 0.10$; Figure 5-2), although this effect was not individually significant on either date ($p > 0.10$). R_{AG} was 14.7% higher across both dates in fertilized ramets. A number of factors could result in this disparity between R_{ECO} and R_{AG} fluxes among treatments. F_S may be sufficiently large to mask any signal from R_{AG} as both are

components of R_{ECO}. However, it is not possible to determine what proportion of R_{ECO} was attributable to R_{AG} due to chamber effects. R_{ECO} and R_{AG} measurements should not be directly compared on the same scale, but rather should only be considered as treatment indices (Norman, Kucharik et al. 1997).

F_S response to fertilizer application differed significantly between the two clones over time ($p < 0.01$; Table 5-1; Figure 5-3). In the 10 days immediately following fertilizer application efflux rates spiked in fertilized ramets of both clones, reaching a peak on day 2 at approximately 140% the level of unfertilized ramets. Following day 10, F_S was not significantly affected by fertilizer application in either clone until day 36. From day 36 to day 78 clone 34 consistently and significantly ($p < 0.05$) had less than 60% of the F_S rates in fertilized ramets compared to controls. During this period unfertilized ramets of clone 34 also consistently had the highest efflux rates of any treatment combination, while fertilized ramets consistently had the lowest rates. However, during this same period clone 769 showed no significant F_S response to fertilizer application. While the treatment effect diminished in clone 34 following day 78, fertilized ramets still had less than 80% of the rates observed in controls. Clone 769 continued to show no significant treatment effects between days 78 and 120 ($p > 0.10$).

Despite the different clonal responses found in F_S rates, neither F_A nor F_H showed any significant clonal effects across all dates (Table 5-1). F_H was significantly depressed on days 30, 60, and 90 following fertilizer application ($p < 0.05$; Figure 5-4). However, on day 120 there was a significant clone-by-fertilizer interaction, with clone 34 showing a 20% increase while clone 769 showed a 46% decrease in fertilized ramets ($p < 0.05$). While F_H is only intended as a treatment index, since approximately the same soil mass was contained in each pot these rates can be inferentially scaled to the whole plot level for more direct comparison with F_S data. The lack of consistency between the distinctly different clonal F_S fertilizer responses and F_H would seem to indicate that F_H alone is not driving F_S in this nutrient-deficient coarse sand.

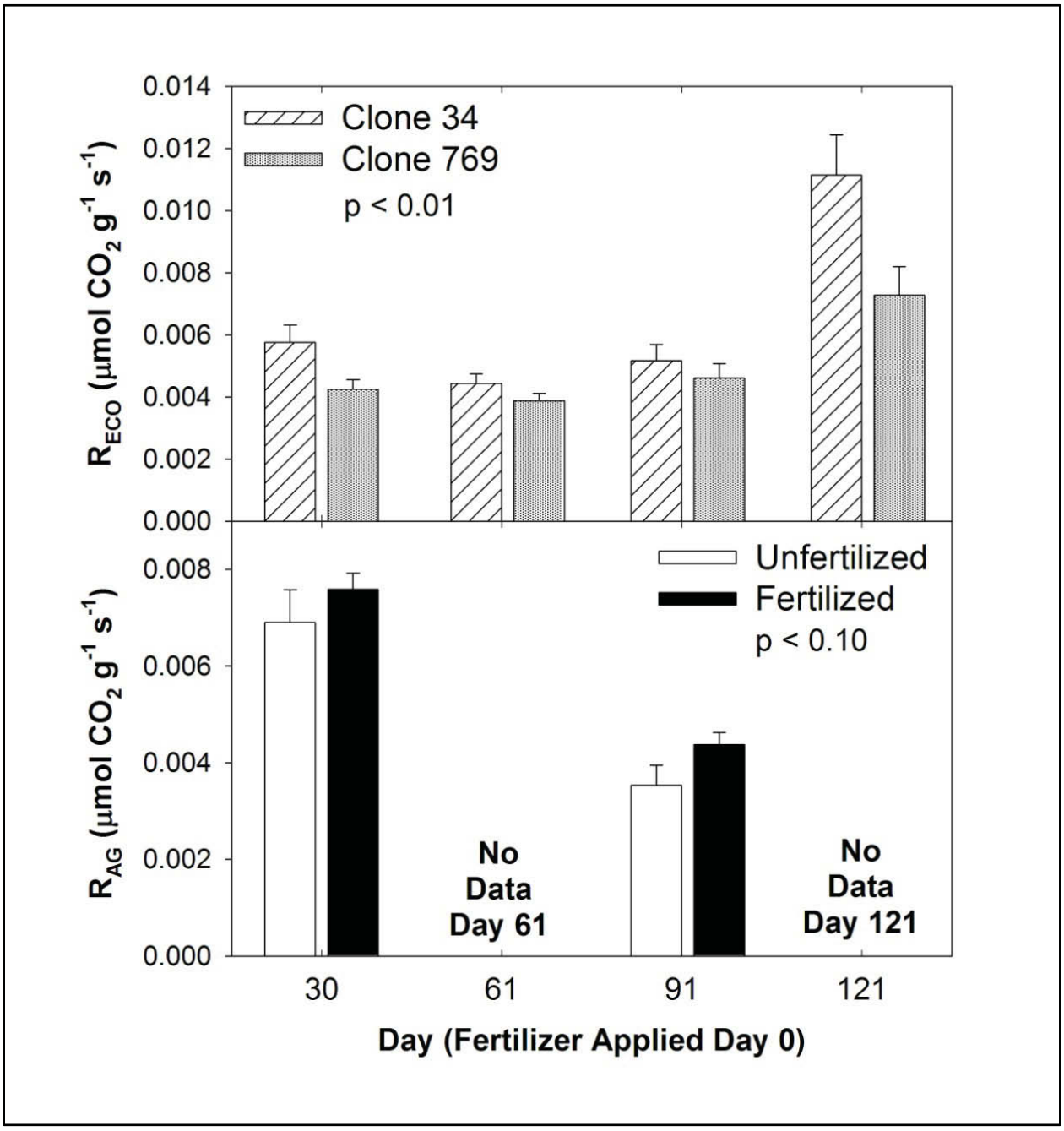

Figure 5-2. Clone and fertilizer effects for total ecosystem dark respiration and aboveground plant dark respiration, respectively, measured prior to each of four destructive harvests. Data are not available for R_{AG} for days 61 or 121. Measurements were made with a LICOR 6200 IRGA. Respiration is expressed on a plant biomass basis for both metrics. Standard error bars are shown, N=16. Fertilizer was applied day 0 = June 16, 2009.

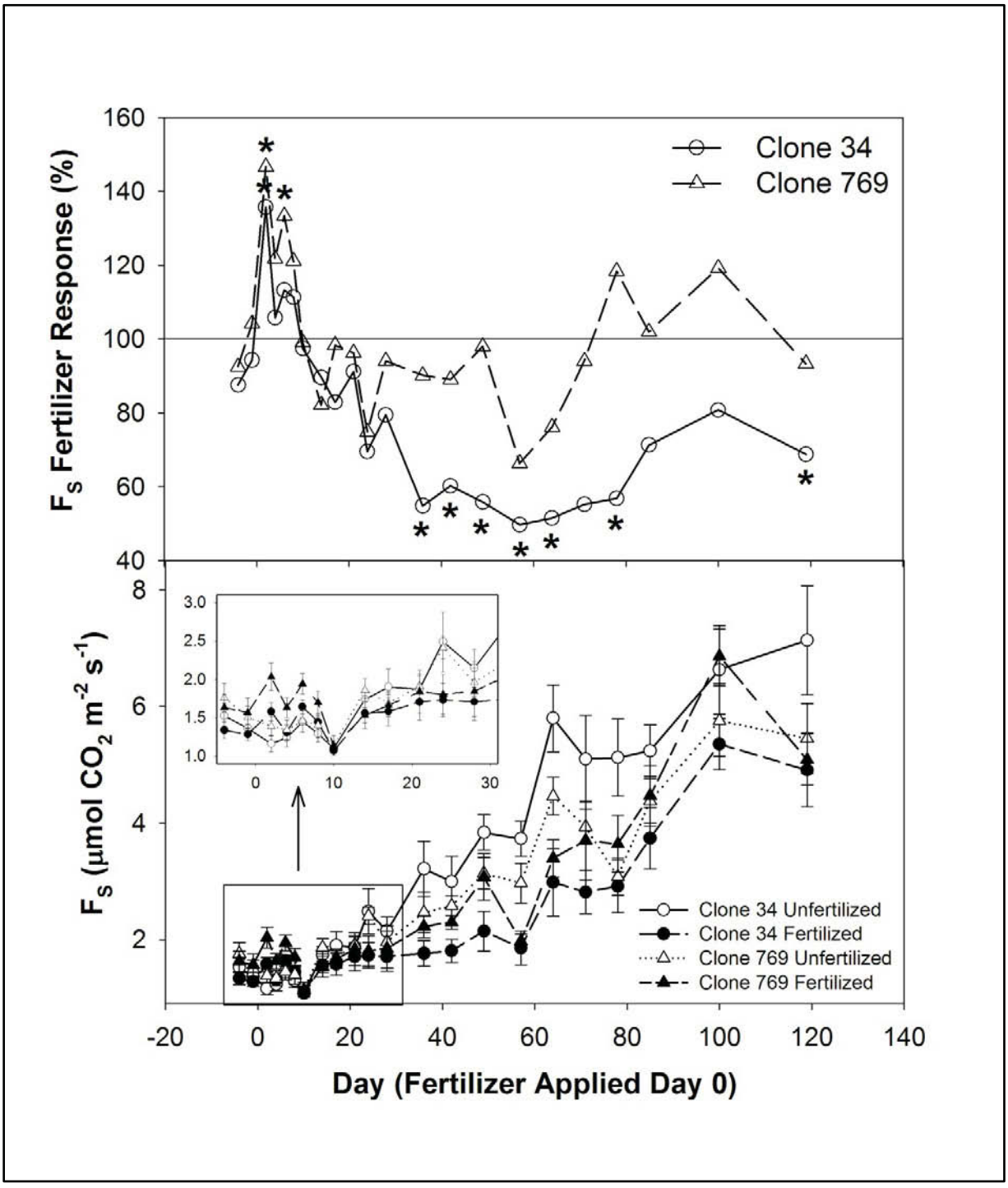

Figure 5-3. Clone-by-fertilizer interaction for soil CO_2 efflux from harvest 4 trees (bottom panel). The fertilizer effect for each clone is shown in the top panel, with significant differences for each clone ($p < 0.10$) denoted by an asterisks. Measurements were made using a LICOR 6200 with a 231 cm^3 cuvette. Standard errors are shown, N = 8. Fertilizer was applied day 0 = June 16, 2009.

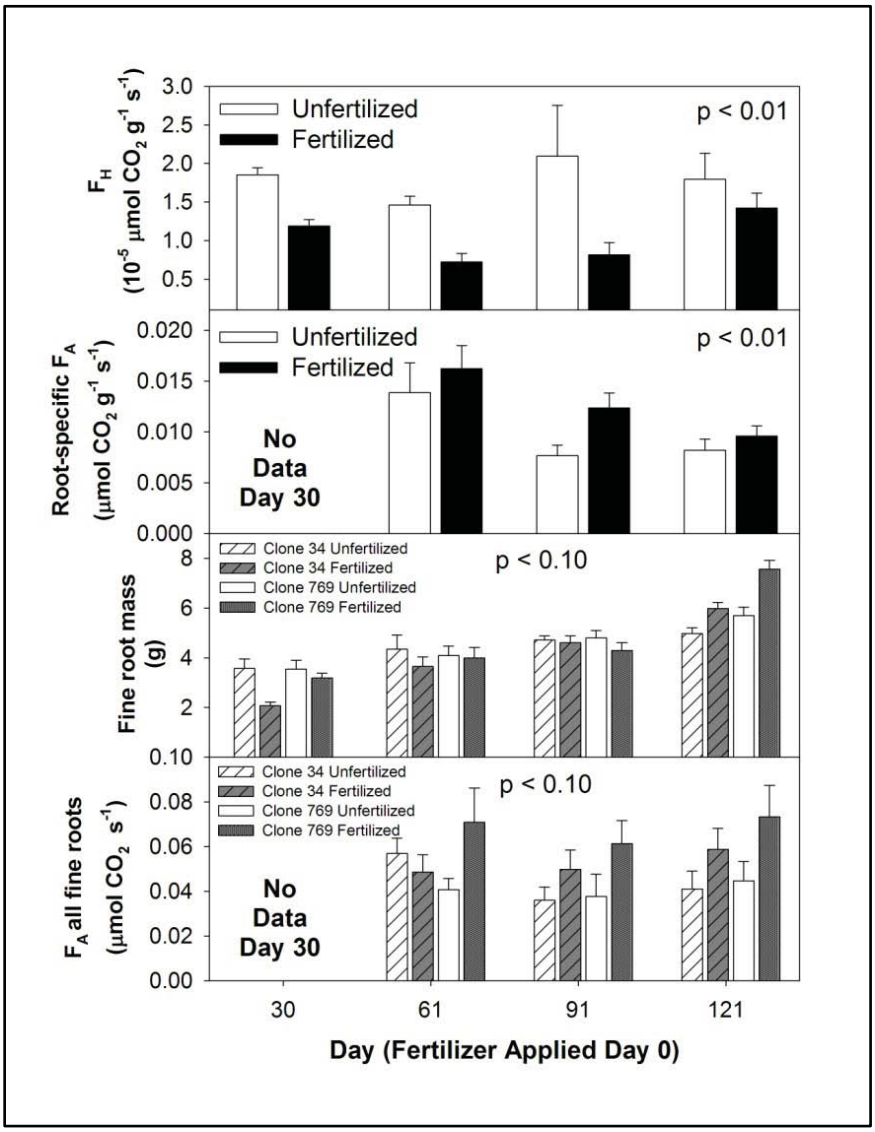

Figure 5-4. Fertilizer effects for heterotrophic and autotrophic components of soil CO_2 efflux in the top two panels from four destructive harvest dates. Measurements were made using a LICOR 6200 and are standardized to soil mass and root mass, respectively. The bottom two panels show clone-by-fertilizer interactions for fine root mass and F_A scaled to all fine roots. Standard errors are shown, N = 16 for the upper two panels, N = 8 for the lower two panels. Fertilizer was applied day 0 = June 16, 2009.

Across all dates root-specific F_A rates were 29% higher in the fertilizer treatment (Table 5-1). The only individual date for which this effect was significant was day 60 ($p < 0.01$; Figure 5-4). As with F_H, there were no significant clonal effects for root-specific F_A. However, there was a significant clone-by-fertilizer interaction in fine root biomass across harvest dates ($p < 0.10$; Figure 5-4). When this was taken into account by scaling root-specific F_A rates up to the tree

level by multiplying by total fine root biomass, a significant clone-by-fertilizer interaction emerged for F_A ($p < 0.10$; Figure 5-4). Based on the data shown in Figure 5-4, it would appear that the clonal differences in F_S fertilizer response are consistent with the combined effects of fertilizer on F_A scaled to all fine roots and F_H. Fertilized ramets of clone 769 had total fine root biomass F_A rates that were the greatest of any other treatment combination. This substantial increase coupled with reduced F_H rates in fertilized ramets is consistent with the lack of fertilizer effect observed for F_S for clone 769. In clone 34, reduced fine root mass in fertilized ramets at days 30 and 60 lead to reduced F_A when scaled to all fine roots, despite increased root-specific F_A (Figure 5-4). While we lack day 30 F_A data, it appears likely that the reduction in F_A found in clone 34, coupled with the reduced F_H observed across both clones combined to cause the substantial and significant reductions observed in F_S for fertilized ramets of clone 34 between days 36 and 78.

5.3.3. Root Exudate Quantity and Quality and Soil Nutrients

Root exudate TOC varied between clones depending on fertilizer status and time since fertilizer application (Table 5-1). Figure 5-5 depicts exudate TOC expressed on a root-length basis. Unfertilized ramets of clone 34 had substantially elevated exudation rates (63% greater) on day 30 ($p < 0.05$). Rates were still higher by day 60, but not significantly so versus the other three treatment combinations ($p > 0.10$). At days 90 and 120 there were no significant differences among the four treatment combinations. Similar results were observed whether data were expressed on a root-mass or root-surface-area basis (Table 5-1). This pattern of exudation was inconsistent with the observed reductions in F_H rates across both clones, and thus did not support our hypothesis that reduced root exudation in fertilized ramets would be correlated with reduced F_H. Across all ramets F_H was not correlated to root exudate TOC ($R^2 < 0.01$, $p > 0.10$).

Root exudate anion composition varied markedly among treatment groups. Of the anions we assessed through ion chromatography, formate was observed in no samples, acetate in only one, and tartrate in only eight of 128. No data analysis was preformed and no further data are shown for these compounds. Phosphate and citrate were each observed in 22% of samples, oxalate in 38%, lactate in 92%, and sulfate in 100% (Figure 5-6). While we cannot determine whether the

phosphate and sulfate we observed originated from root exudates or from the minimal amount of rhizosphere soil we were unable to wash from the roots, both anions have been previously collected in tree root exudates (Nguyen, Nakabayashi et al. 2003; Qin, Hirano et al. 2007). Citrate and lactate showed no significant differences among treatment combinations ($p > 0.10$), although they did vary in concentration across treatment dates (Table 5-2, Figure 5-6). Sulfate showed a significant clone-by-fertilizer interaction. While sulfate was found at increased concentrations in fertilized ramets of both clones at days 61 and 91, the magnitude of this increase differed between clones. Oxalate showed a significant clone-by-fertilizer interaction across all dates ($p < 0.01$) and was present in exudates sampled from a greater number of the control ramets of both clones at greater levels than the fertilized ramets (Figure 5-6). Phosphate was observed in 87.5% of control ramets for both clones at day 30, but only in 50% or fewer of fertilized ramets (Figure 5-6). After day 30, phosphate declined dramatically, and was only observed in five of 32 ramets at day 60, two of 32 at day 90, and none at day 120. At day 30 phosphate concentrations showed a strong clone-by-fertilizer interaction ($p < 0.01$), with clone 34 having much greater levels in control ramets than any of the other treatment combinations.

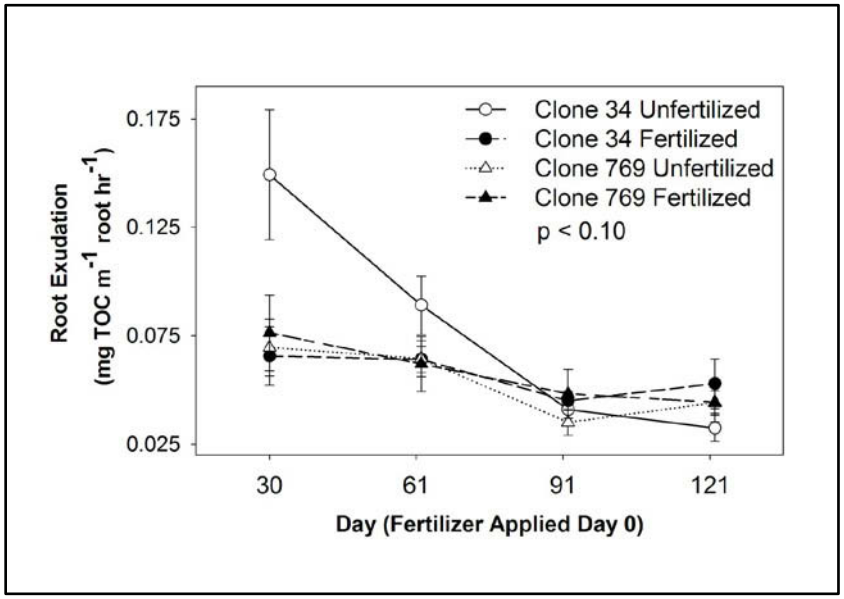

Figure 5-5. Total organic carbon in root exudates analyzed with an Elementar LiquiTOC and expressed on a root length basis for each of four destructive harvests. Root exudates were collected per Egle et al. (2003). Standard error bars are shown, N=8. Fertilizer was applied day 0 = June 16, 2009.

Fertilizer treatments significantly affected soil nutrient concentrations. Soil P was below the detection limit (2.0 ppm) of the Virginia Tech Soil Testing Laboratory Mehlich I procedure for all but two of 64 unfertilized soil samples, and was substantially greater than the detection limit in all 64 fertilized soils (Table 5-3) (Mullins and Heckendorn 2009). P uptake varied between clones in fertilized ramets, with clone 769 showing significantly higher soil P levels at day 30 while clone 34 had significantly greater soil P at days 91 and 121 ($p < 0.05$). Soil N was significantly elevated in fertilized soils at 30, 60, and 90 days post-fertilizer application ($p < 0.01$), but had returned to control levels by day 120 ($p > 0.10$). No significant clonal differences were observed for N. Fertilizer application resulted in significantly greater pH across all dates and for each individual date ($p < 0.01$; Table 5-1, Table 5-3). Additionally there was a small but significant clonal effect at day 30, with clone 769 having slightly greater pH ($p < 0.10$). Base cations showed a trend consistent with pH, with lower concentrations observed for K, Mg, and Ca in fertilized ramets at 120 days after fertilizer application ($p < 0.05$). K was also found at significantly lower concentrations in fertilized ramets at day 60. At day 30 only, clone 769 showed significantly greater concentrations of both Mg and Ca ($p < 0.05$).

Table 5-2. P-values for the Friedman's Chi Square non-parametric test for rhizosphere anion composition data. While phosphate and sulfate observed may have originated from root exudation, they may also have been sourced from rhizosphere soil. P-values < 0.10 are indicated in boldface.

Variable	Clone	Fertilizer	C x F	Harvest Date	Detection Limit (ppm)
lactate	0.1015	0.8997	0.3621	**0.0028**	0.02086
oxalate	**0.0630**	**0.0002**	**0.0008**	**0.0001**	0.00360
citrate	0.6831	0.4142	0.8271	**0.0002**	0.00851
phosphate	0.8273	**0.0030**	**0.0003**	**0.0001**	0.00430
sulfate	0.1336	**0.0455**	**0.0251**	**0.0012**	0.01156

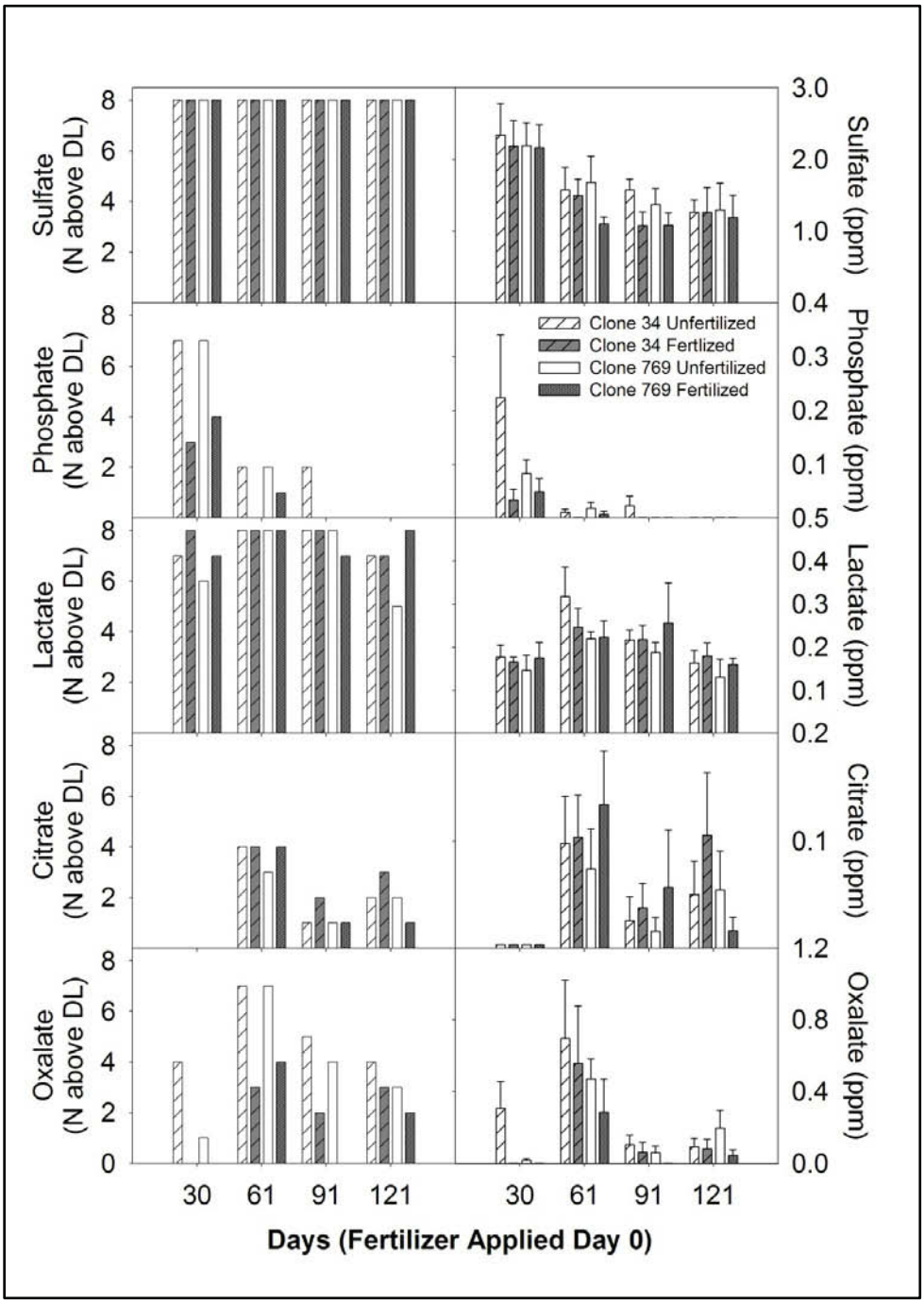

Figure 5-6. Root exudate and rhizosphere anion composition analyzed with a Dionex ion chromatograph for each of four destructive harvests. Left panels show number of samples above the detection limit, while the right panels show the mean of each treatment combination. Values below the detection limit were corrected to half the detection limit and are included in data shown in the right panel. Root exudates were collected per Egle et al. (2003). Standard error bars are shown in the right panels, N=8. Fertilizer was applied day 0 = June 16, 2009.

Table 5-3. Means and standard errors (in parentheses) for soils data from each of four destructive harvest dates. N and P fertilizer was applied at an operational rate on day 0 = June 16, 2009 to a Wakulla sand.

Variable	Day	34 Unfertilized	769 Unfertilized	34 Fertilized	769 Fertilized
pH	30	4.90 (0.04)	5.03 (0.03)	4.56 (0.07)	4.67 (0.07)
	61	4.89 (0.05)	4.92 (0.04)	4.48 (0.07)	4.56 (0.09)
	91	4.84 (0.05)	4.89 (0.05)	4.61 (0.06)	4.58 (0.08)
	121	5.05 (0.04)	5.19 (0.12)	4.70 (0.05)	4.61 (0.07)
N	30	0.0148 (0.0010)	0.0149 (0.0007)	0.0193 (0.0011)	0.0182 (0.0011)
(%)	61	0.0152 (0.0012)	0.0142 (0.0006)	0.0185 (0.0010)	0.0179 (0.0012)
	91	0.0130 (0.0006)	0.0134 (0.0006)	0.0150 (0.0007)	0.0149 (0.0009)
	121	0.0141 (0.0011)	0.0137 (0.0008)	0.0144 (0.0007)	0.0145 (0.0008)
P	30	2.00 (0.00)	2.00 (0.00)	9.63 (1.43)	13.38 (1.45)
(ppm)	61	2.00 (0.00)	2.00 (0.00)	10.00 (1.38)	10.63 (1.99)
	91	2.00 (0.00)	3.00 (1.00)	8.88 (0.72)	5.75 (0.62)
	121	2.00 (0.00)	2.13 (0.13)	8.50 (0.53)	6.88 (0.85)
K	30	5.25 (0.25)	5.25 (0.25)	5.25 (0.31)	6.00 (0.78)
(ppm)	61	5.25 (0.31)	4.75 (0.25)	4.38 (0.42)	4.25 (0.25)
	91	4.75 (0.31)	5.13 (0.58)	4.63 (0.60)	5.00 (0.73)
	121	5.88 (0.30)	6.38 (0.63)	4.25 (0.25)	4.50 (0.27)
Ca	30	41.25 (1.41)	43.63 (0.86)	41.13 (1.44)	48.63 (3.50)
(ppm)	61	44.00 (1.38)	42.50 (1.49)	43.25 (1.80)	45.00 (1.60)
	91	46.50 (1.69)	49.38 (4.08)	45.13 (1.51)	46.75 (3.23)
	121	53.13 (1.69)	56.13 (2.83)	46.25 (1.21)	47.13 (1.55)
Mg	30	11.00 (0.50)	11.75 (0.37)	10.88 (0.48)	12.50 (0.46)
(ppm)	61	12.13 (0.44)	11.63 (0.50)	12.13 (0.48)	12.50 (0.57)
	91	12.88 (0.55)	13.50 (0.85)	12.50 (0.42)	12.38 (0.42)
	121	15.63 (0.53)	15.75 (0.49)	13.00 (0.46)	13.25 (0.41)

5.4. Discussion

5.4.1. Respiratory C Fluxes

Neither R_{ECO} nor R_{AG} responses to fertilizer application in either clone was consistent with our hypothesis of short-term fertilizer growth response via reduced respiratory C fluxes. R_{AG} showed slight but significant increases with fertilizer application, while R_{ECO} showed no effect. Thus, fertilized ramets did not have more C to allocate to greater leaf area as we hypothesized

due to reduced respiratory C fluxes. Additionally, while the two clones showed different growth rates, there were no observed clonal differences in R_{AG}. R_{ECO} was greater in clone 34, the faster growing clone, at days 30 and 121, which again is contrary to our hypothesis of short-term growth response. We cannot attribute these clonal effects to a more specific cause, as trends in F_S do not appear to explain the clonal difference in R_{ECO}. While fertilized ramets of clone 34 consistently had the lowest F_S rates of any treatment combination on almost all dates, they displayed R_{ECO} rates 19.7% higher than unfertilized ramets of clone 769 and 21.6% higher than unfertilized ramets. Unfertilized ramets of clone 34 consistently had both the highest F_S and R_{ECO} rates.

Increased R_{AG} due to fertilizer application has been previously observed in the literature. In one study with *Pinus radiata* (D. Don) clones, it was found that R_{AG} increased in fertilized ramets, yet remained a constant fraction of gross primary production, indicating that the observed fertilizer effect was primarily due to greater overall productivity resulting from fertilizer application (Bown, Watt et al. 2009). This is consistent with the findings of Wertin and Teskey (2008), who observed a tight relationship between net photosynthetic rates and foliar dark respiration rates. Maier et al. (2004) observed both increased R_{ECO} and R_{AG} due to fertilizer treatments in a midrotation *P. taeda* plantation, consistent with our results in this study. They also found that R_{AG} comprised a greater fraction of R_{ECO} in fertilized plots, which is consistent with our observed increase in R_{AG} in fertilized ramets coupled with reduced F_S due to fertilizer application, at least in clone 34. Bown et al. (2009) did observe a significant clonal effect for R_{AG}, similar to what we observed in R_{ECO} but not R_{AG}. Based on the results of this study and our own it appears that while clonal differences in respiratory C fluxes may not bear a direct causal link to short-term fertilizer growth responses, higher respiratory rates are consistently observed in faster, not slower, growing clones. This is consistent with the 'rising tide lifts all boats' hypothesis of Litton et al. (2007)

5.4.2. Soil CO_2 Efflux and Root Exudation

Clones 34 and 769 responded differently to fertilizer application with regard to F_S despite being closely related (full-sib) clones. While clone 769 showed almost no response at all, clone 34

generally had the highest rates when unfertilized, and the lowest when fertilized. The clonal differences we observed in F_S fertilizer response were surprising, given that no previous studies we are aware of have shown an effect of genotype on F_S fertilizer response. Several studies with clonal *Pinus* species have shown no effect of clone or clone-by-fertilizer interactions on F_S (Tyree, Seiler et al. 2008; Bown, Watt et al. 2009). A study of *Betula pendula* (Roth) clones did show clonal differences in F_S response to elevated CO_2 and O_3 that were not entirely consistent with observed root or aboveground biomass responses to treatments.

Clone 34, when unfertilized, showed both the highest F_S rates and the highest root exudate TOC rates. However, these two trends did not appear to be completely synchronous: exudation peaked in the day 30 and 60 harvests, while F_S rates did not become elevated in the unfertilized ramets versus the fertilized ramets until day 36. F_S rates remained elevated through much of the duration of the experiment, while exudation rates dropped to similar levels to the other treatment combinations at days 90 and 120. Previous research in artificial rhizospheres has shown that root exudates can alter rhizosphere microbial communities and diurnal patterns of F_S (Landi, Valori et al. 2006). This is one possible operating mechanism that explains our observed temporal asynchrony between exudation and F_S in clone 34. It is also possible that these observations are consistent with a rhizosphere priming effect, whereby addition of a labile C source alters the microbial community, increasing its capacity for later breakdown of soil organic C (Kuzyakov and Bol 2006; Cleveland, Nemergut et al. 2007). Whatever the cause, the F_S and exudate responses of these two clones to fertilizer application remain markedly different. Clone 769, by contrast to clone 34, showed little response in terms of exudate quantity or F_S rates to fertilizer application.

Our hypothesis of reduced root exudation as a possible mechanism of short-term fertilizer growth response does not seem to be supported by the data. While we did observe this trend in clone 34, we did not observe any differences in root exudate TOC in clone 769, which also showed a substantial fertilizer growth response. Other short-term ecophysiological responses of clones that are inconsistent with their growth responses to fertilizer application have previously been observed for net photosynthetic rates in *P. taeda* (King, Seiler et al. 2008). Conclusions

derived from these types of observations can be categorized as either 1) the observed ecophysiological trait is not the primary mechanism of short-term fertilizer growth response or 2) the short-term mechanism of fertilizer growth response is genotype specific, and a generalized theory of response is not feasible for clonal plantation forestry. Without assessing the full C budget of a larger number of contrasting clones over time, it is not possible to distinguish between these two competing hypotheses. This remains an avenue of research worth further pursuit.

Our hypothesis that reduced root exudates with fertilizer application could explain observed reductions in F_H were not supported by the data. Despite a clear difference between clones in root exudation, there was no significant clonal effect observed for F_H. Additionally, the observed reduction in F_H with fertilizer application was based on a sample from the edge of the pot, which was primarily bulk, not rhizosphere, soil. Root exudates are often rapidly consumed in the rhizosphere and have a lesser effect on bulk soil (Landi, Valori et al. 2006). Our results are consistent with reduced F_H with fertilizer application, which has been observed in numerous studies (Gough and Seiler 2004; Olsson, Linder et al. 2005; Tyree, Seiler et al. 2008), being a direct effect of fertilizer on bulk soil microbial communities without any significant plant mediation involved in this process. Direct effects of fertilizer application on bulk soil microbial communities have been reported previously for laboratory experiments involving fertilizer application in systems lacking growing plants (Thirukkumaran and Parkinson 2000). Root exudation and F_H do not appear to be closely linked in fertilized *P. taeda* plantations.

5.4.3. Root Exudate Chemistry

In soils containing Al oxides that bind tightly with P, oxalate has been shown to release potentially large quantities of inorganic P through ligand exchange reactions (Fox, Comerford et al. 1990; Fox and Comerford 1992). The higher concentrations of oxalate observed in the root exudates of unfertilized ramets of primarily clone 34 at days 30 and 61 appear to be consistent with higher levels of phosphate also found in these exudates. Although roots were washed, it was impossible to remove all rhizosphere soil in the short (< 30 min) time-frame prior to exudate collection from sampled trees. It is likely that greater oxalate concentrations in these exudates

had increased labile phosphate in the rhizosphere soil, which we also detected with our sampling protocol. Considering that phosphate was observed above the detection limit in more than twice as many unfertilized ramets as fertilized ramets at both days 30 and 61 and at much higher concentrations despite phosphate having been directly added to the fertilized ramets, it seems highly likely that the observed phosphate was released from the soil as a result of root exudate ligand exchange reactions. However, we should note that a number of previous studies have observed phosphate in root exudates (Pellet, Grunes et al. 1995; Pellet, Papernik et al. 1996; Nguyen, Nakabayashi et al. 2003), although observed releases of phosphate have typically been in low pH soils (< 4.5) under conditions of Al toxicity.

While sulfate is not typically observed in studies of root exudation, one previous study did find that sulfate was released in root exudates of *Populus tremula* (L.) (Qin, Hirano et al. 2007). This study also speculated that sulfate was released through similar channels as malate and formate as they found a high correlation among the release of these three anions. While we did observe an increase of sulfate we did not observe formate concentrations above the detection limit in any samples, and did not assess malate. It is possible that the sulfate we observed originated from the rhizosphere soil, and not from root exudation. We did not observe any significant treatment effects for either citrate or lactate, despite the previously observed role of citrate release in P uptake in some species (Grayston, Vaughan et al. 1997).

5.5. Conclusion

Respiratory C fluxes, F_S, and root exudation did not change in either of these full-sib clones consistent with a hypothesis of short-term fertilizer growth response due to reduced respiratory C fluxes. We observed greater R_{ECO} and reduced F_S when fertilized as well as greater total exudation of higher TOC and oxalate concentration when unfertilized in clone 34 relative to clone 769. The clones showed different patterns of F_A scaled to all fine roots that explained their different fertilizer F_S responses. Exudation was not consistent with observed reductions in F_H, indicating that F_H response to fertilizer application is probably a direct effect of fertilizer on soil microbial communities and is not the result of plant-mediated processes. Overall, our results

show that different clones may display unique responses to fertilizer treatments that affect their plant-soil interactions. Respiratory C fluxes may also vary between clones. The range of C cycling processes we observed between these two clones implies that genotype-specific belowground C allocation may allow some clones to better exploit a given resource environment than others. At an ecosystem scale, large clonal plantations comprised of a small number of genotypes may interact with the soil in dramatically dissimilar patterns, potentially resulting in overall changes to stand-scale C budgets. Much more research on this topic will be necessary to scale results to the field.

5.6. Literature Cited

Adams, D. M., R. W. Haynes and A. J. Daigneault (2006). Estimated timber harvest by U.S. region and ownership, 1950-2002, USDA Forest Service General Technical Report PNW-GTR-659, Pacific Northwest Rearch Station, Portland, OR.

Albaugh, T. J., H. L. Allen, P. M. Dougherty, L. W. Kress and J. S. King (1998). "Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions." Forest Science **44**(2): 317-328.

Albaugh, T. J., H. L. Allen and T. R. Fox (2007). "Historical patterns of forest fertilization in the southeastern United States from 1969 to 2004." Southern Journal of Applied Forestry **31**(3): 129-137.

Bettinger, P., M. Clutter, J. Siry, M. Kane and J. Pait (2009). "Broad implications of southern United States pine clonal forestry on planning and management of forests." International Forestry Review **11**(3): 331-345.

Bitoki, O. (2008). "Comparing early survival and growth of varietal and open-pollinated loblolly pine seedlings." VDOF Forest Research Review **April 2008**: 4-5.

Bol, R., J. Moering, Y. Kuzyakov and W. Amelung (2003). "Quantification of priming and CO_2 respiration sources following slurry-C incorporation into two grassland soils with different C content." Rapid Communications in Mass Spectrometry **17**(23): 2585-2590.

Bown, H. E., M. S. Watt, P. W. Clinton, E. G. Mason and D. Whitehead (2009). "The influence of N and P supply and genotype on carbon flux and partitioning in potted *Pinus radiata* plants." Tree Physiology **29**(7): 857-868.

Butnor, J. R., K. H. Johnsen, R. Oren and G. G. Katul (2003). "Reduction of forest floor respiration by fertilization on both carbon dioxide-enriched and reference 17-year-old loblolly pine stands." Global Change Biology **9**(6): 849-861.

Cleveland, C. C., D. R. Nemergut, S. K. Schmidt and A. R. Townsend (2007). "Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition." Biogeochemistry **82**(3): 229-240.

Dakora, F. D. and D. A. Phillips (2002). "Root exudates as mediators of mineral acquisition in low-nutrient environments." Plant and Soil **245**(1): 35-47.

Egle, K., W. Romer and H. Keller (2003). "Exudation of low molecular weight organic acids by *Lupinus albus* L., *Lupinus angustifolius* L. and *Lupinus luteus* L. as affected by phosphorus supply." Agronomie **23**(5-6): 511-518.

Fox, T. R., H. L. Allen, T. J. Albaugh, R. Rubilar and C. A. Carlson (2007). "Tree nutrition and forest fertilization of pine plantations in the southern United States." Southern Journal of Applied Forestry **31**(1): 5-11.

Fox, T. R. and N. B. Comerford (1992). "Influence of oxalate loading on phosphorus and aluminum solubility in spodosols." Soil Science Society of America Journal **56**(1): 290-294.

Fox, T. R., N. B. Comerford and W. W. Mcfee (1990). "Kinetics of phosphorus release from spodosols - effects of oxalate and formate." Soil Science Society of America Journal **54**(5): 1441-1447.

Giardina, C. P., D. Binkley, M. G. Ryan, J. H. Fownes and R. S. Senock (2004). "Belowground carbon cycling in a humid tropical forest decreases with fertilization." Oecologia **139**(4): 545-550.

Gough, C. M. and J. R. Seiler (2004). "Belowground carbon dynamics in loblolly pine (*Pinus taeda*) immediately following diammonium phosphate fertilization." Tree Physiology **24**(7): 845-851.

Grayston, S. J., D. Vaughan and D. Jones (1997). "Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability." Applied Soil Ecology **5**(1): 29-56.

Hanson, P. J., N. T. Edwards, C. T. Garten and J. A. Andrews (2000). "Separating root and soil microbial contributions to soil respiration: A review of methods and observations." Biogeochemistry **48**(1): 115-146.

Henry, F., C. Nguyen, E. Paterson, A. Sim and C. Robin (2005). "How does nitrogen availability alter rhizodeposition in *Lolium multiflorum* Lam. during vegetative growth?" Plant and Soil **269**(1-2): 181-191.

Hinsinger, P. (2001). "Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review." Plant and Soil **237**(2): 173-195.

King, N. T., J. R. Seiler, T. R. Fox and K. H. Johnsen (2008). "Post-fertilization physiology and growth performance of loblolly pine clones." Tree Physiology **28**(5): 703-711.

Kuzyakov, Y. (2002). "Review: Factors affecting rhizosphere priming effects." Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde **165**(4): 382-396.

Kuzyakov, Y. and R. Bol (2006). "Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar." Soil Biology & Biochemistry **38**(4): 747-758.

Lagomarsino, A., M. C. Moscatelli, P. De Angelis and S. Grego (2006). "Labile substrates quality as the main driving force of microbial mineralization activity in a poplar plantation soil under elevated CO_2 and nitrogen fertilization." Science of the Total Environment **372**(1): 256-265.

Landi, L., F. Valori, J. Ascher, G. Renella, L. Falchini and P. Nannipieri (2006). "Root exudate effects on the bacterial communities, CO_2 evolution, nitrogen transformations and ATP content of rhizosphere and bulk soils." Soil Biology & Biochemistry **38**(3): 509-516.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger and O. Schabenberger (2006). SAS for mixed models, second edition. Cary, NC, SAS Institute Inc.

Litton, C. M., J. W. Raich and M. G. Ryan (2007). "Carbon allocation in forest ecosystems." Global Change Biology **13**(10): 2089-2109.

Maier, C. A., T. J. Albaugh, H. L. Allen and P. M. Dougherty (2004). "Respiratory carbon use and carbon storage in mid-rotation loblolly pine (*Pinus taeda* L.) plantations: The effect of site resources on the stand carbon balance." Global Change Biology **10**(8): 1335-1350.

Maier, C. A. and L. W. Kress (2000). "Soil CO_2 evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **30**(3): 347-359.

Mullins, G. L. and S. E. Heckendorn (2009). Laboratory procedures: Virginia Tech soil testing laboratory. Blacksburg, VA, Publication 452-881, Virginia Cooperative Extension.

Nguyen, C. (2003). "Rhizodeposition of organic C by plants: Mechanisms and controls." Agronomie **23**(5-6): 375-396.

Nguyen, N. T., K. Nakabayashi, J. Thompson and K. Fujita (2003). "Role of exudation of organic acids and phosphate in aluminum tolerance of four tropical woody species." Tree Physiology **23**(15): 1041-1050.

Norman, J. M., C. J. Kucharik, S. T. Gower, D. D. Baldocchi, P. M. Crill, M. Rayment, K. Savage and R. G. Striegl (1997). "A comparison of six methods for measuring soil-surface carbon dioxide fluxes." Journal of Geophysical Research-Atmospheres **102**(D24): 28771-28777.

Olsson, P., S. Linder, R. Giesler and P. Hogberg (2005). "Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration." Global Change Biology **11**(10): 1745-1753.

Paul, A. D., G. S. Foster, T. Caldwell and J. McRae (1997). "Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine." Forest Science **43**(1): 87-98.

Pellet, D. M., D. L. Grunes and L. V. Kochian (1995). "Organic-acid exudation as an aluminum-tolerance mechanism in maize (*Zea mays* L)." Planta **196**(4): 788-795.

Pellet, D. M., L. A. Papernik and L. V. Kochian (1996). "Multiple aluminum-resistance mechanisms in wheat - Roles of root apical phosphate and malate exudation." Plant Physiology **112**(2): 591-597.

Phillips, R. P., E. S. Bernhardt and W. H. Schlesinger (2009). "Elevated CO_2 increases root exudation from loblolly pine (*Pinus taeda*) seedlings as an N-mediated response." Tree Physiology: tpp083.

Phillips, R. P., Y. Erlitz, R. Bier and E. S. Bernhardt (2008). "New approach for capturing soluble root exudates in forest soils." Functional Ecology **22**(6): 990-999.

Phillips, R. P. and T. J. Fahey (2007). "Fertilization effects on fineroot biomass, rhizosphere microbes and respiratory fluxes in hardwood forest soils." New Phytologist **176**(3): 655-664.

Qin, R. J., Y. Hirano and I. Brunner (2007). "Exudation of organic acid anions from poplar roots after exposure to Al, Cu and Zn." Tree Physiology **27**(2): 313-320.

Raich, J. W. and W. H. Schlesinger (1992). "The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate." Tellus Series B-Chemical and Physical Meteorology **44**(2): 81-99.

Ratnayake, M., R. T. Leonard and J. A. Menge (1978). "Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation." New Phytologist **81**: 543-552.

Ryan, M. G. (1991). "Effects of climate change on plant respiration." Ecological Applications **1**(2): 157-167.

Ryan, M. G., R. M. Hubbard, S. Pongracic, R. J. Raison and R. E. McMurtrie (1996). "Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status." Tree Physiology **16**(3): 333-343.

Samuelson, L. J. (2000). "Effects of nitrogen on leaf physiology and growth of different families of loblolly and slash pine." New Forests **19**(1): 95-107.

Samuelson, L. J., K. Johnsen, T. Stokes and W. L. Lu (2004). "Intensive management modifies soil CO_2 efflux in 6-year-old *Pinus taeda* L. stands." Forest Ecology and Management **200**(1-3): 335-345.

Smith, R. L. (1991). EPA Region 3 guidance on handling chemical concentration data near the detection limit in risk assessments. US EPA Region 3 HSCD: Risk Assessment, Technical Guidance Manual. Philadelphia, PA, United States Environmental Protection Agency.

Thirukkumaran, C. M. and D. Parkinson (2000). "Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers." Soil Biology & Biochemistry **32**(1): 59-66.

Tyree, M. C., J. R. Seiler, W. M. Aust, D. A. Sampson and T. R. Fox (2006). "Long-term effects of site preparation and fertilization on total soil CO_2 efflux and heterotrophic respiration in a 33-year-old *Pinus taeda* L. plantation on the wet flats of the Virginia Lower Coastal Plain." Forest Ecology and Management **234**(1-3): 363-369.

Tyree, M. C., J. R. Seiler and T. R. Fox (2008). "The effects of fertilization on soil respiration in 2-year-old *Pinus taeda* L. clones." Forest Science **54**(1): 21-30.

Valentini, R., G. Matteucci, A. J. Dolman, E. D. Schulze, C. Rebmann, E. J. Moors, A. Granier, P. Gross, N. O. Jensen, K. Pilegaard, A. Lindroth, A. Grelle, C. Bernhofer, T. Grunwald, M. Aubinet, R. Ceulemans, A. S. Kowalski, T. Vesala, U. Rannik, P. Berbigier, D. Loustau, J. Guomundsson, H. Thorgeirsson, A. Ibrom, K. Morgenstern, R. Clement, J. Moncrieff, L. Montagnani, S. Minerbi and P. G. Jarvis (2000). "Respiration as the main determinant of carbon balance in European forests." Nature **404**(6780): 861-865.

Vose, J. M. and H. L. Allen (1988). "Leaf-area, stemwood growth, and nutrition relationships in loblolly-pine." Forest Science **34**(3): 547-563.

Vose, J. M. and M. G. Ryan (2002). "Seasonal respiration of foliage, fine roots, and woody tissues in relation to growth, tissue N, and photosynthesis." Global Change Biology **8**(2): 182-193.

Wear, D. and J. Greiss (2002). Southern forest resource assessment, USDA Forest Service General Technical Report SRS-53, Southern Rearch Station, Asheville, NC.

Werth, M. and Y. Kuzyakov (2008). "Root-derived carbon in soil respiration and microbial biomass determined by C-14 and C-13." Soil Biology & Biochemistry **40**(3): 625-637.

Wertin, T. M. and R. O. Teskey (2008). "Close coupling of whole-plant respiration to net photosynthesis and carbohydrates." Tree Physiology **28**(12): 1831-1840.

6. Short-term effects of fertilizer application on crown architecture and photosynthesis in two full-sib *Pinus taeda* clones

6.1. Introduction

As demand for wood products increases while the available land base for forestry declines over the coming century, timber harvested from plantations will become increasingly important (Paquette and Messier 2009). To meet the demand for wood products silvicultural practices maximizing productivity of plantations, including use of clonal material and optimal application of fertilizer, will be necessary (Fox, Jokela et al. 2007). Deployment of clonal *Pinus taeda* (L.) is becoming increasingly common throughout the southeastern United States as somatic embryogenesis processes improve (Bettinger, Clutter et al. 2009; Whetten and Kellison 2010). Forest fertilization, primarily to alleviate deficiencies of nitrogen and phosphorous, is already a widespread practice in *P. taeda* plantations (Fox, Allen et al. 2007). Clones show a wide range of stem volume growth rates and often respond dissimilarly to fertilizer application, sometimes even if closely related genotypes are compared (Paul, Foster et al. 1997; King, Seiler et al. 2008; Tyree, Seiler et al. 2009a; Tyree, Seiler et al. 2009b). In order to maximize productivity of clonal plantations we require a better understanding of the ecophysiological mechanisms constraining stem volume production and the range of variability of these mechanisms among widely deployed clonal genotypes.

Clonal variation in stem dimension growth rates has been observed in numerous field trials (Isik, Goldfarb et al. 2005; King, Seiler et al. 2008; Tyree, Seiler et al. 2009b). Clones also have been shown to vary in stem dimension response to fertilizer application when relatively few (i.e. < 10) clones are compared (King, Seiler et al. 2008; Tyree, Seiler et al. 2009a). However, studies involving larger numbers of clones (i.e. > 500) typically find little evidence of genotype-by-environment interactions over multiple field sites (Paul, Foster et al. 1997; Baltunis, Huber et al. 2007). Since the practice of clonal forestry is still relatively novel in the southeastern United States, the ideal number of clones planted per area or by individual landowners has not yet been widely established (Lambeth and McCullough 1997). It is possible that potential economic gains from planting a relatively small number of outstandingly high performing genotypes (i.e.

phenotypic outliers) may outweigh the risks entailed by such low genetic diversity for some land-owners (McKeand, Mullin et al. 2003). In situations such as this it is critical to have a priori knowledge of the responses of each genotype to silvicultural prescriptions in order to prevent the mismanagement of high-initial-cost genetic material.

Crown attributes have been shown repeatedly to vary with both genetics and cultural treatments. Dramatic increases in leaf area and mass with fertilizer application are widely observed in *P. taeda* (Vose and Allen 1988; Dalla-Tea and Jokela 1991; Albaugh, Allen et al. 1998; Jokela, Dougherty et al. 2004), and are likely the predominant cause of long-term growth response to fertilizer application (Fox, Allen et al. 2007). Fertilized trees typically develop larger crowns with greater foliar mass per branch (Gillespie, Allen et al. 1994). Genotypic variability in vertical distribution of leaf area has been correlated to growth, with faster growing families having more leaf area in the middle third of the crown (McCrady and Jokela 1996). Crown structure and leaf area distribution have also been shown to be mechanisms of interspecific differences in light interception and tree growth within different members of the *Pinus* genus (Chmura and Tjoelker 2008). Clones of *P. taeda* have been shown to vary considerably in crown size and leaf area, both traits that were strongly genetically correlated to growth (Emhart, Martin et al. 2007).

While increased leaf-level photosynthetic rates are often observed following fertilizer application (Green and Mitchell 1992; Maier, Palmroth et al. 2008), leaf-level photosynthetic rates do not explain variation in stem growth (McGarvey, Martin et al. 2004), because they do not always increase after fertilizer application (Samuelson 1998; Munger, Will et al. 2003). Variability in leaf-level photosynthetic rates due to fascicle elongation (Radoglou and Teskey 1997), seasonal trends (Gough, Seiler et al. 2004a), timing of measurements after fertilizer application (Gough, Seiler et al. 2004b), age of foliage measured (McGarvey, Martin et al. 2004), and whether measured foliage developed before or after fertilizer application (Maier, Palmroth et al. 2008) all complicate broad generalizations of photosynthetic rate response to fertilizer application. Although there are many technical difficulties, photosynthetic rates must be scaled to a canopy level for any sizeable correlation with growth to emerge (McGarvey, Martin et al. 2004; Chmura

and Tjoelker 2008). Further integration of crown traits with photosynthetic rates can yield even more detailed conclusions. A comparison of two-clones with contrasting crown ideotypes revealed similar canopy-level photosynthetic rates that were arrived at by different combinations of leaf mass, SLA, and leaf-level photosynthetic rates (Tyree, Seiler et al. 2009a).

A study of open-pollinated seedlings showed that leaf-level photosynthetic rates increase after fertilizer application for several months due to elevated foliar nitrogen concentration ($[N]_f$) (Gough, Seiler et al. 2004b). $[N]_f$ subsequently drops as N is reallocated to newly developing foliage, and photosynthetic rates return to pre-fertilizer application levels. This short-term response to fertilizer application explains one physiological mechanism that leads to long-term fertilizer growth response. However, when this framework has been applied to clones, short-term leaf-level photosynthetic response to fertilizer application varies among genotypes and does not consistently explain growth responses (King, Seiler et al. 2008). In this study our objective was to determine whether short-term changes in leaf-level photosynthetic rates, when coupled with various metrics of crown dynamics, were consistent with a theory of short-term fertilizer physiological responses explaining differences among clones in long-term fertilizer growth response. We hypothesized that by considering a whole suite of crown metrics we would be able to find short-term responses to fertilizer application that would explain differences in whole-tree carbon gain associated with clonal differences in stem-volume growth response. The suite of crown metrics we examined included branch number, branch mass, foliar mass, SLA, crown silhouette area, two novel indices of foliar display density, $[N]_f$, leaf-level photosynthetic rates, and canopy-level photosynthetic rates.

6.2. Materials and Methods

6.2.1. Study Description

Clones GE034 and GE769 (ArborGen LLC, Summerville, South Carolina, USA) were planted in this study. These two clones, produced from a single full-sib cross, were among the first selected by ArborGen in 2005 (Bitoki 2008), and have contrasting crown ideotypes. Clone 34 is the faster growing of the two, has a narrower crown than clone 769, and allocates less to branches

(Bitoki 2008). Trees were removed from the cooler (4° C) where they had been for two months and were potted on April 30, 2009 in their plugs. Plug media was comprised of a mixture of peat moss and vermiculite and contained an undisclosed quantity of fertilizer. They were left in plugs to minimize reductions in growth rates or survival due to excessive root mortality that would have resulted from removing the trees from the densely rooted plugs. Trees were potted in homogenized A-horizon soil from the USDA Forest Service's Southeastern Tree Research and Education Site (SETRES). The soil is a Wakulla series (siliceous, thermic Psammentic Hapludult), and was selected to minimize native nutrition to allow for as complete nutrient control as is possible in a natural soil. Soil was sieved through a 1-cm mesh to remove any coarse roots, was homogenized, and was placed into 15-by-15-by-38 cm pots (8,550 cm^3) that were sufficiently large to limit extensive root binding during this four month experiment.

Trees were in the greenhouse in Blacksburg, Virginia, USA, (37.24°N 80.43°W) from April 30 to October 15, 2009. Watering was conducted daily in an attempt to prevent drought stress while also minimizing leaching from the bottom of the pots. Nighttime minimum temperature was set to 18° C in the greenhouse, and while the vents were set to open during the day at 25° C daytime temperatures did exceed this frequently. Relative humidity was allowed to fluctuate with the ambient air. High-pressure sodium lights were turned on daily for several hours pre-dawn from September 15 to October 15 to augment photoperiod, which averaged 12.9 hours throughout the experiment. Environmental conditions were recorded by a single HOBO datalogger (Onset Computer Corp., Bourne, Massachusetts, USA) placed in the center of the experiment. Greenhouse environmental data are shown in Figure 6-1.

6.2.2. Experimental Design

This experiment was a two-by-two-by-four factorial randomized complete block design replicated eight times on two greenhouse benches for a total of 128 trees. Treatments were the two clones, fertilizer application (none versus fertilized), and four sequential destructive harvests. Trees randomly assigned to the fertilizer treatment were fertilized at an operational rate with diammonium phosphate and ammonium nitrate at 225 kg N per hectare and 56 kg elemental

P per hectare. Control trees received no fertilizer. Fertilizer was applied on June 16, 2009, which is referred to as day 0 throughout the rest of the chapter.

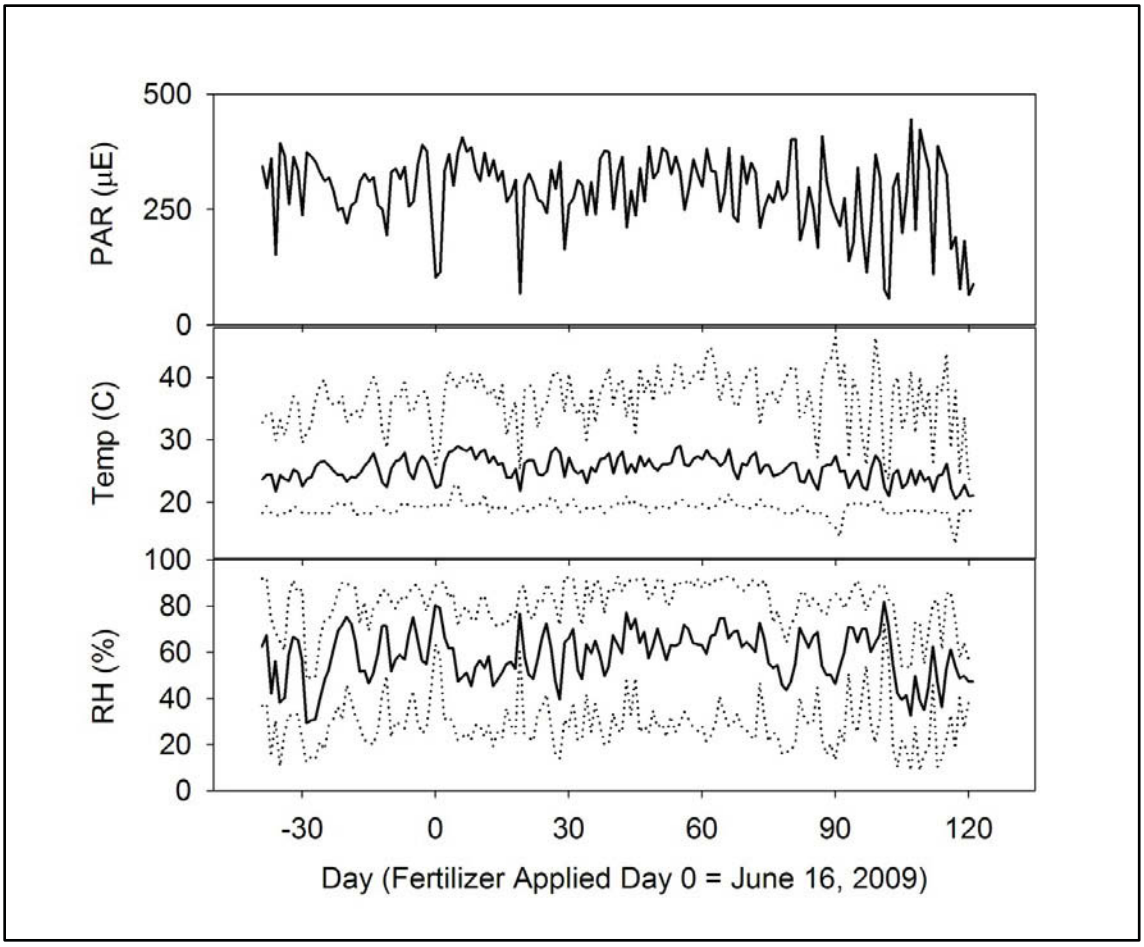

Figure 6-1. Ambient conditions in the greenhouse during the duration of the experiment from a single HOBO data station placed between both greenhouse benches. Solid lines indicate daily means, while dotted lines indicate maximum and minimum hourly averages for each day for relative humidity and temperature. PAR values represent daytime means excluding one hour post-dawn and pre-dusk.

6.2.3. Measurements

Thirty-two trees were randomly assigned to each of the four destructive harvest dates, which took place monthly on July 16, August 16, September 15, and October 15, 2009 (30, 61, 91, and 121 days after fertilizer application). The 32 trees selected for the final harvest at day 121 were used for detailed physiological measurements throughout the experiment. Trees from remaining three harvest groups were used to quantify changes in crown morphology and size over time.

Table 6-1 details which variables were measured on all trees at harvest, and which were made over time on the day 121 harvest group. The following paragraphs detail procedures followed at each of the four destructive harvests. This is then followed by procedures for the physiological measurements made on the day 121 harvest group over the full duration of the experiment.

Table 6-1. Description of variables measured. Measurements made on the trees during monthly destructive harvests following fertilizer application are indicated by "all", while measurements made throughout the experiment on the final harvest group only are indicated by "H4".

Variable	Units	Description	Trees
tree height	cm	Total tree height	H4
basal diameter	mm	Basal caliper	H4
stem volume	cm^3	$dia^2 \times ht$	H4
foliar mass	g	Oven dry mass all foliage	All
foliar mass per branch	g	Oven dry mass foliage per branch	All
average branch mass	g	Oven dry mass branches / branch number	All
total branch mass	g	Oven dry mass all branches	All
primary branches	#	Number of branches originating from stem	All
secondary branches	#	Number of branches originating from branches	All
SLA	$cm^2\ g^{-1}$	Single sided leaf area per mass	All
leaf area per tree	cm^2	SLA × foliar mass	All
CSA	m^2	Canopy Silhouette Area	All
CDDI	$m^2\ m^{-2}$	Crown Density Display Index	All
FDDI	$m^2\ m^{-2}$	Foliar Density Display Index	All
canopy A_{SAT}	$\mu mol\ CO_2\ s^{-1}$	A_{SAT} scaled to canopy using SLA and foliar mass	H4
A_{SAT} (First Flush)	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	Light saturated photosynthetic rate	H4
A_{SAT} (Second Flush)	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	Light saturated photosynthetic rate	H4
$[N]_f$ (First Flush)	%	CN analyzer results from A_{SAT} foliage	H4
$[N]_f$ (Second Flush)	%	CN analyzer results from A_{SAT} foliage	H4
PNUE	$\mu mol\ CO_2\ g^{-1}\ N\ s^{-1}$	Photosynthetic nitrogen use efficiency	H4

On the date of each destructive harvest, each tree was transported from the greenhouse to the lab and cut at the root collar. Prior to further dissection of each tree, crown architecture was assessed on each tree by taking a number of crown silhouette area (CSA) photos to determine the displayed crown area (see King, Seiler et al. 2008). The entire crown was photographed twice from right angles about the stem and these values were averaged to yield CSA. All photos were

taken with a Nikon D70 digital SLR with an AF-S DX Zoom-Nikkor 18-70mm f/3.5-4.5G IF-ED lens against a uniform white background (Nikon Inc, Melville, New York, USA). Images were underexposed by 1.0 eV in order to maximize contrast between canopy and background. All canopy photographs were thresholded in SideLook v.1.1 (Nobis and Hunziker 2005) and further post-processed and analyzed in Adobe PhotoShop 6.0 (Adobe Systems Inc., San Jose, California, USA). Canopy pixels were automatically counted and compared to an object of known area in each photograph to calculate CSA in units of m^2. One representative branch was then selected, excised, and photographed individually to yield a branch silhouette area (BSA), which was used in calculating a foliar density display index described below.

After being photographed, each tree was carefully dissected into components. All foliage was excised from each tree, and branches were then removed separately. The number of primary (originating from stem) and secondary (originating from primary branch) branches were tallied. Very few secondary branches were observed in this trial, so all data presented is for primary branches only. Branch and foliar samples were oven-dried at 65° C for > 10 days and weighed. These data were used to calculate average mass per branch (total branch mass divided by primary branch number) and foliar mass per branch (total foliar mass divided by primary branch number).

In order to calculate specific leaf area (SLA, cm^2 / g), foliage from the single representative branch that had already been photographed was excised and processed separately from foliage from the rest of the crown. Single-sided leaf area was determined on this subsample only on a LI-3100 scanner (LI-COR Biosciences, Lincoln, Nebraska, USA). The subsample was then oven-dried and weighed as described above. SLA was calculated by dividing scanned leaf area by subsample foliar mass. Leaf area from the subsample was then scaled to the whole canopy by multiplying SLA by the foliar biomass of the entire tree.

We created two indices to determine the amount of foliar and branch overlap for each tree. Foliar density display index (FDDI) and crown density display index (CDDI) were calculated based on the following equations:

[1] FDDI = BSA / (Single-sided leaf area from the representative branch subsample)

[2] CDDI = CSA / (Single-sided leaf area scaled to the whole tree)

These indices thus correspond to the displayed leaf area per the actual leaf area. Indices are inversely related to foliar overlap or foliar density; decreasing values of these indices represent less displayed foliage (i.e. more overlap or greater density) per unit actual leaf area. Because FDDI is based only on data from a single representative branch, it only accounts for foliar overlap. However, CDDI is scaled to the entire tree, and thus incorporates both foliar and branch-scale overlaps.

Physiological measurements were made on the 32 trees from the day 121 harvest group throughout the four month duration of the experiment at a high frequency immediately before and after application of the fertilizer treatment, and at a sufficient frequency there-after to account for temporal variability. Light saturated net photosynthetic rate (A_{SAT}) was measured between 13:00 and 16:00 EDT on one fascicle from a uniform canopy position on each tree with an LI-6400 infrared gas analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA). Prior to day 28 all measurements were made on foliage from the preexisting growth flush. On day 28 measurements were made on both first flush and second flush fascicles. After this date only second flush fascicles were measured. Block temperature was held at 30°C, flow rate at 300 μmol s^{-1}, reference CO_2 at 380 ppm, and photosynthetic photon flux density at 1600 μmol m^{-2} s^{-1}. A 2-by-3 cm leaf cuvette was used, with foliar area later adjusted based on measured fascicle diameters and needles per fascicle as per Ginn et al. (1991). All appropriate steps were taken to ensure the LI-6400 yielded accurate data: fresh soda lime and desiccant, IRGA zeroing, mixer and light calibrations each measurement day, and matching reference and sample cells immediately prior to each measurement.

Fascicles on which A_{SAT} was measured were then immediately oven-dried at 65° C for > 10 days. After drying they were ground through a Wiley mill with a number 20 screen and analyzed for foliar N concentration ($[N]_f$) by the USDA-Forest Service Southern Research Station laboratory (Research Triangle Park, North Carolina, USA) with a Carlo-Erba elemental analyzer (Model NA-1500, Fison Instruments, Danvers, Massachusetts, USA). Photosynthetic nitrogen use efficiency (PNUE) was calculated by converting $[N]_f$ from a mass fraction to N concentration per unit leaf area by multiplying by SLA, and then multiplying this value by (A_{SAT}) to yield units of μmol CO_2 g^{-1} N s^{-1}. PNUE, like A_{SAT}, is the flux from the single fascicle on which photosynthetic measurements were made. PNUE was not scaled to the canopy-level, but was calculated on the same four dates as canopy-level A_{SAT} for the reasons described below.

Leaf-level A_{SAT} data was scaled to the entire canopy to assess crown-level effects of treatments on gas exchange. A regression on all 128 trees of stem volume at harvest versus foliar mass at harvest was performed (foliar mass = 0.16 × stem volume + 4.22; $p < 0.0001$, $R^2 = 0.73$). Using this regression we calculated foliar mass for the 32 trees on which A_{SAT} was measured at dates prior to their actual destructive harvest. Foliar mass then multiplied by SLA to yield whole-tree leaf area on these dates, and A_{SAT} was then scaled to the whole canopy. Because SLA could only be measured at harvest dates, and varied with treatments over time, canopy A_{SAT} was only calculated for the four measurement dates most proximate to harvest dates (days 28, 70, 86, and 119). Only second flush data was used in calculating canopy A_{SAT}.

6.2.4. Statistics

All analyses were run with SAS software version 9.2 (SAS Institute, Cary, North Carolina, USA). Normality was checked and variables were transformed as necessary, although all reported means and standard errors are untransformed. Q-Q plots were used to examine all outliers. If data violated assumptions of homogenous variance, variance was modeled separately for each treatment combination using the "group" option in the "repeated" statement.

Measurements made over time on only the final harvest group were analyzed using repeated measures ANOVA implemented in PROC MIXED with block as a random effect. The

Kenward-Roger method for calculating denominator degrees of freedom was implemented (Littell, Milliken et al. 2006). The following covariance structures appropriate to repeated measures data with unequal spacing were modeled for each analysis: unstructured, compound symmetry, spatial Gaussian, spatial power, and spatial spherical (Littell, Milliken et al. 2006). The best covariance structure was selected by minimizing AIC_C. Harvest-date-only variables were analyzed using ANOVA implemented in PROC MIXED with block as a random effect. All two-way and three-way effect-by-harvest-date interactions were included in the model. Regression results presented were implemented in PROC REG.

6.3. Results

6.3.1. Stem Growth Response

There were no significant clone-by-fertilizer interactions in stem dimensions, indicating that both clones responded similarly to fertilizer application ($p > 0.10$; Table 6-2). While clone 769 had 50% greater volume at fertilizer application ($p < 0.05$), by day 121 there was no difference between clones ($p > 0.10$). Basal diameter and stem volume index both increased over time with fertilizer application ($p < 0.05$; Figure 6-2). Overall fertilized trees increased in volume by 53% over controls by day 121 ($p < 0.05$). Tree height showed no difference among fertilized trees and controls in the repeated measures analysis ($p > 0.10$). However, both tree height and stem volume varied between clones ($p < 0.10$). There was a delay of approximately 40 days in the fertilizer growth response we observed. Trees were planted in plugs that had received an undisclosed dose of fertilizer both in the media mix and later during seedling development (personal communication, Dr. Phil Dougherty, ArborGen). Despite this delay, the day 121 data shows that the fertilizer treatment applied was effective in producing a stem volume growth response.

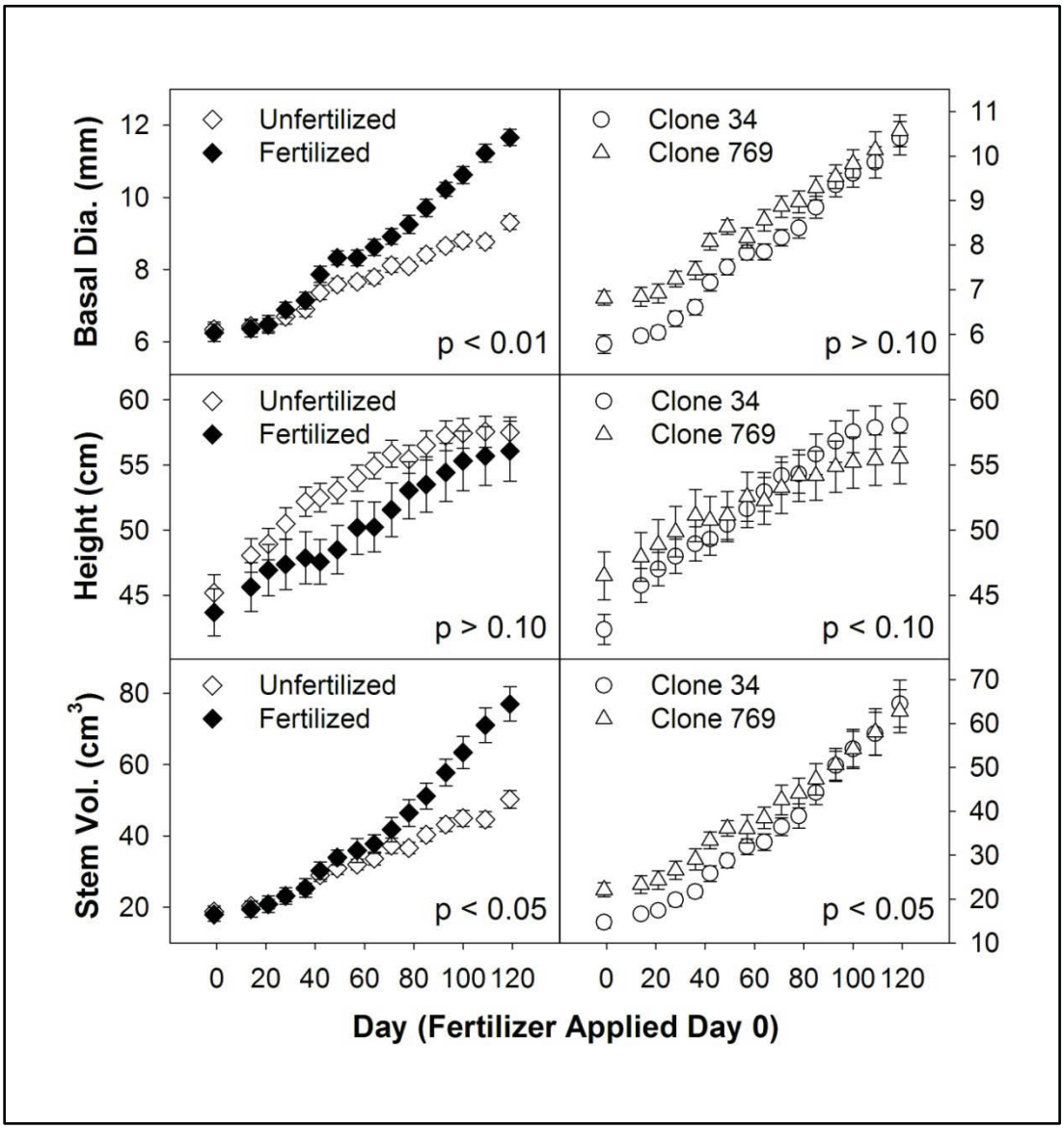

Figure 6-2. Fertilizer and clonal effects for stem dimensions of the final harvest group. Standard error bars are shown, N = 16. P-values include dimensions at planting as a covariate. Fertilizer was applied day 0 = June 16, 2009.

6.3.2. Branch Architecture

The two clones differed markedly in their branch architectures (Figure 6-3). Clone 769 had fewer but more massive branches with more foliar mass per branch versus clone 34 ($p < 0.01$; Table 6-2). By day 121 clone 769 had 34% fewer primary branches per tree and 47% greater foliar mass per branch ($p < 0.05$). There were significant clone-by-fertilizer interactions with

regard to average branch mass and total mass of all branches ($p < 0.10$). Clone 769 always had more massive branches and more total branch mass than clone 34, but fertilizer application reduced the difference between clones. When unfertilized, clone 769 had 138% greater average branch mass than clone 34 compared to a 122% increase when fertilized. For total mass of all branches clone 769 showed a 67% increase when unfertilized but only a 29% increase when fertilized versus clone 34.

Table 6-2. P-values for crown variables and gas exchange data. When harvest date effects are not shown, data were collected only on the final harvest group trees over time and were analyzed using repeated measures. When harvest date effects are shown, data were collected from each of the four harvest groups at the corresponding monthly destructive harvest following fertilizer application. P-values < 0.10 are indicated in boldface.

Variable	Clone	Fertilizer	C x F	Harvest Date	C x HD	F x HD	C x F x HD
tree height	**0.0661**	0.9439	0.7599	---	---	---	---
basal diameter	0.9363	**0.0001**	0.6374	---	---	---	---
stem volume	**0.0207**	**0.0132**	0.7427	---	---	---	---
foliar mass	0.8915	**0.0001**	0.4864	**0.0001**	0.8712	**0.0001**	0.7643
foliar mass per branch	**0.0001**	**0.0042**	0.6516	**0.0001**	0.1129	0.6790	0.3894
average branch mass	**0.0001**	**0.0069**	**0.0651**	**0.0087**	0.6356	0.1275	0.2392
total branch mass	**0.0001**	**0.0008**	**0.0747**	**0.0001**	**0.0308**	**0.0008**	0.4726
primary branches	**0.0001**	0.1732	0.6953	**0.0322**	**0.0309**	**0.0209**	0.7262
secondary branches	0.9671	0.9834	0.9821	1.0000	1.0000	1.0000	1.0000
SLA	**0.0943**	**0.0066**	**0.0098**	**0.0003**	0.7157	**0.0008**	**0.0003**
leaf area per tree	0.4453	**0.0001**	**0.0189**	**0.0001**	0.4267	**0.0001**	**0.0020**
CSA	0.3519	**0.0001**	0.5333	**0.0001**	0.4977	**0.0007**	0.4118
CDDI	0.1087	**0.0686**	**0.0272**	**0.0001**	0.4691	**0.0066**	**0.0383**
FDDI	0.9602	0.2626	0.2599	**0.0001**	0.4973	**0.0659**	0.2236
canopy A_{SAT}	0.7481	**0.0001**	0.2641	**0.0001**	0.5658	**0.0001**	0.5733
A_{SAT} (First Flush)	0.9639	**0.0001**	**0.0924**	---	---	---	---
A_{SAT} (Second Flush)	0.7717	0.3155	0.3911	---	---	---	---
$[N]_f$ (First Flush)	**0.0153**	**0.0001**	0.6897	---	---	---	---
$[N]_f$ (Second Flush)	0.6880	**0.0001**	0.5482	---	---	---	---
PNUE	**0.0062**	**0.0001**	**0.0074**	**0.0001**	0.6107	**0.0414**	0.8639

Branch metrics also showed substantial responses to fertilizer application. Overall, the canopies of fertilized trees were characterized by a greater number of more massive branches with more leaf area per branch (Figure 6-3). The number of primary branches and the total mass of all branches increased with fertilizer application over time, showing 30% and 99% increases respectively averaged across both clones by day 121 ($p < 0.05$). Mass per branch and foliar mass per branch increased with fertilizer application by 31% and 15% averaged over both clones by day 121 ($p < 0.01$).

6.3.3. Leaf Area, Canopy Density, Crown Silhouette Area, and Foliar Mass

Both total tree leaf area and SLA showed significant clone-by-fertilizer-by-harvest interactions ($p < 0.01$; Table 6-2; Figure 6-4). Overall, fertilized trees generally had greater total leaf area and SLA. Total leaf area fertilizer response continued to increase in magnitude over the final three harvest dates, with a 49% increase observed by day 121. While fertilizer was the main cause of differences in total leaf area, the clones did respond differently. When fertilized, clone 769 showed a consistently greater increase in leaf area over time versus unfertilized ramets across all four harvest dates. In contrast, clone 34 showed a substantial 210% increase in leaf area in fertilized ramets between days 30 and 61, but thereafter showed a relatively consistent difference versus unfertilized ramets. The interaction in SLA appears to be largely the effect of very different trends in the first harvest versus the last three harvests (Figure 6-4). For the last three harvests, both clones have similarly greater SLA when fertilized.

Display density indices showed that both foliar and total canopy display density increased as the growing season progressed, since density increases as these indices decrease (Figure 6-5). FDDI showed only a fertilizer-by-harvest interaction ($p < 0.10$; Table 6-2). Fertilized trees had a lower density of foliar display per branch at the first harvest, higher densities at the second two harvests, with no observed difference between fertilizer treatments by the final harvest ($p < 0.10$). As the growing season progressed, the negative trends observed across all trees indicated an increase in foliar display density on a representative branch, or greater foliar overlap. At the whole canopy scale similar trends in density were observed, but with a significant clone-by-fertilizer-by-harvest interaction ($p < 0.05$; Figure 6-5). Clone 769 showed a consistent increase

in display density with fertilizer application of a greater magnitude than clone 34 across the first three harvests. Clone 34 showed greater canopy display density when unfertilized at day 30, followed by greater densities when fertilized at the second and third harvest dates. By day 121, there was no difference among treatments ($p > 0.10$); all ramets had relatively densely displayed crowns.

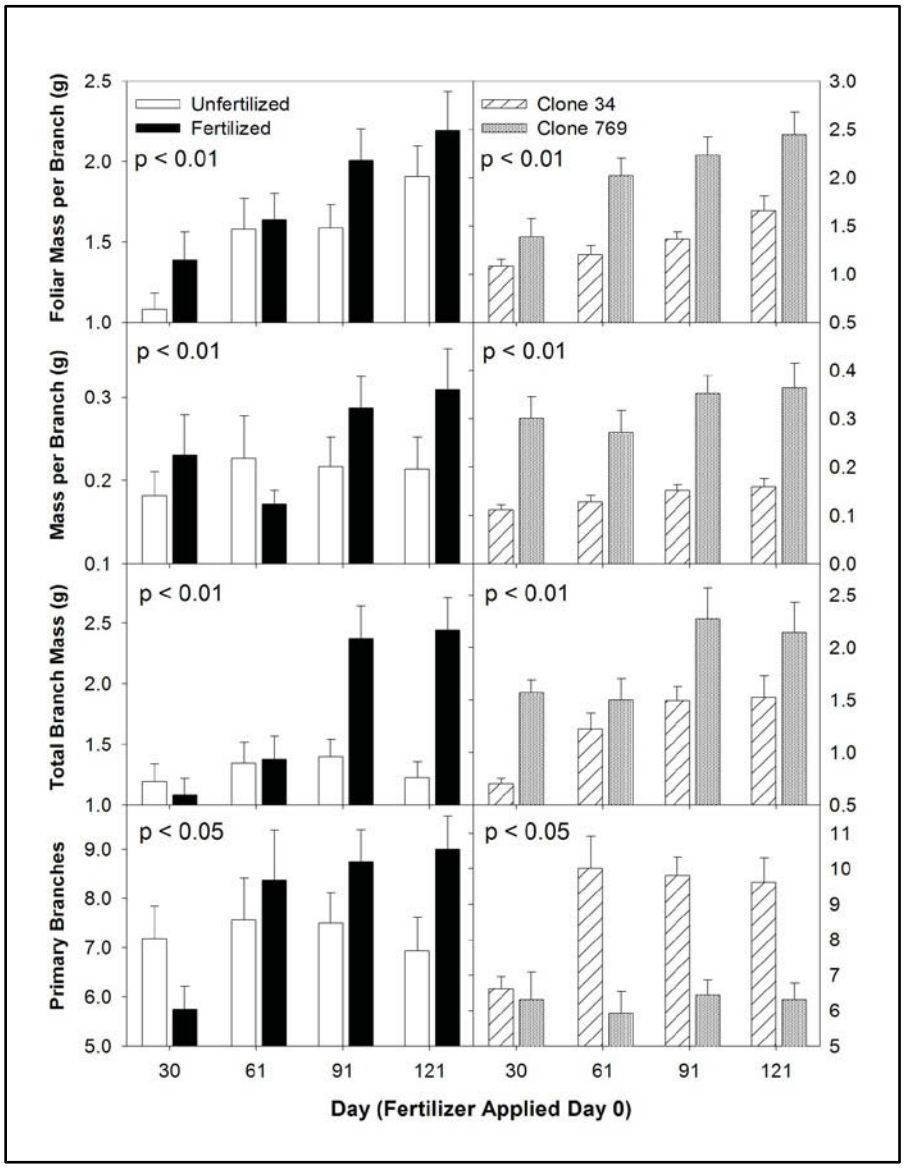

Figure 6-3. Clonal and fertilizer effects for foliar mass per branch, mass per branch, total branch mass, and primary branch number from four monthly post-fertilizer application destructive harvests. Standard errors are shown, N =16. Fertilizer was applied day 0 = June 16, 2009.

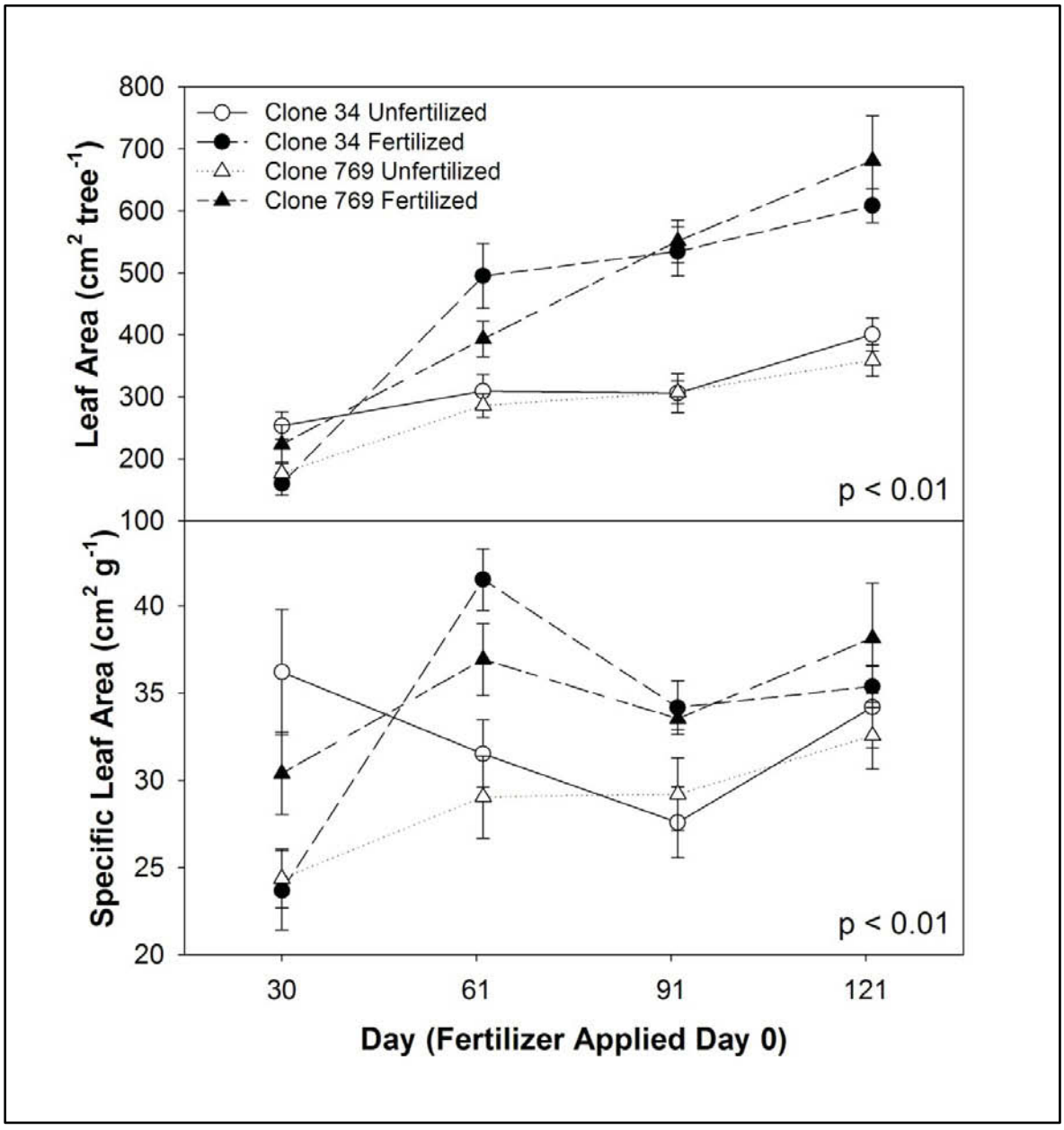

Figure 6-4. Clone-by-fertilizer interactions for leaf area per tree (top panel) and specific leaf area (bottom panel) from four monthly post-fertilizer application destructive harvests. Leaf area was determined on a subsample of foliage using a LICOR 3100 scanner. Specific leaf area is calculated based on sub-sampling a single representative branch, while leaf area per tree scales this data to the tree using total foliar mass. Standard errors are shown, N = 8. Fertilizer was applied day 0 = June 16, 2009.

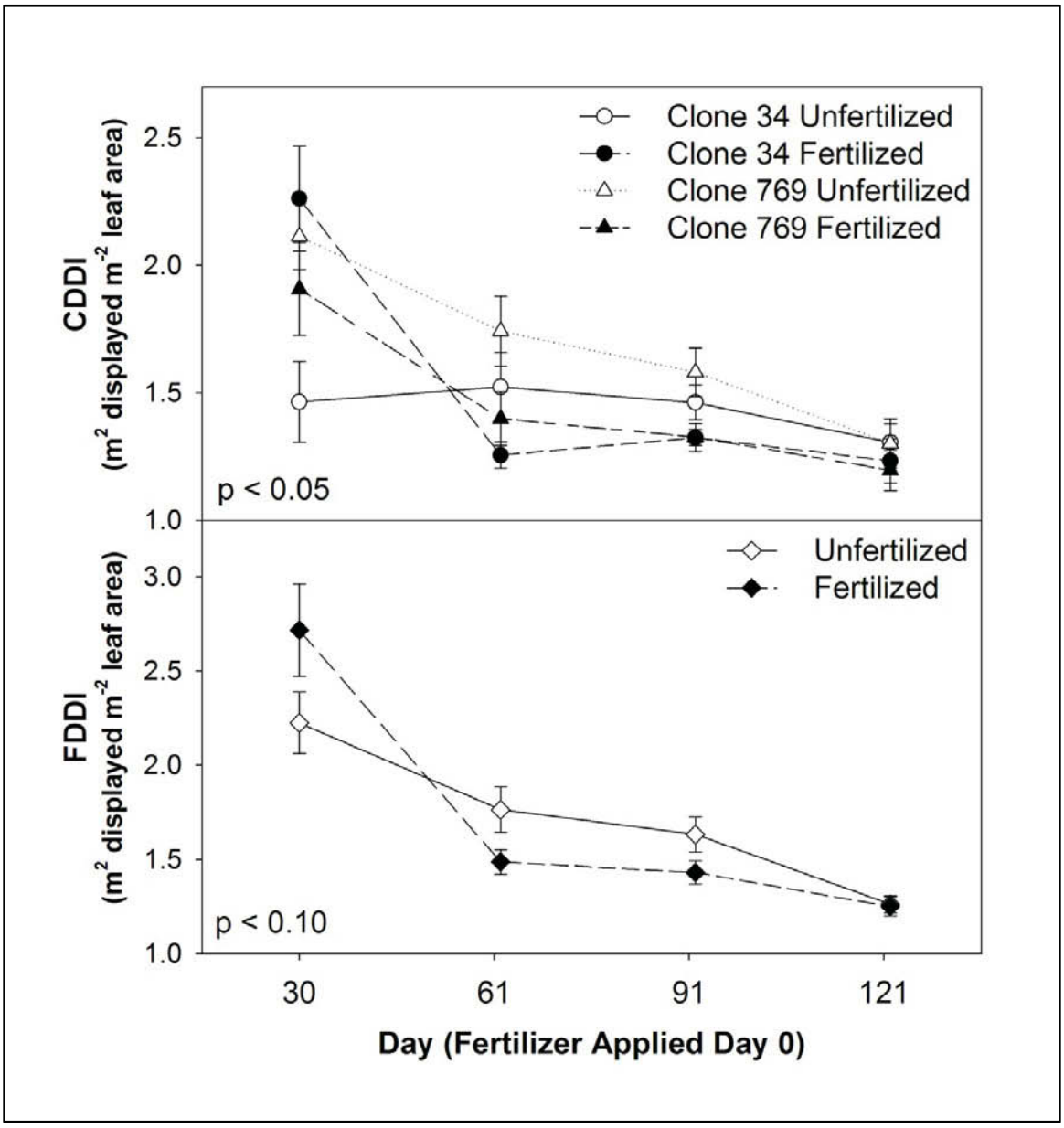

Figure 6-5. Clone-by-fertilizer interaction for Crown Display Density Index (CDDI) is shown in the top panel for data from four monthly post-fertilizer application destructive harvests. Only the fertilizer effect is shown in the bottom panel for the Foliar Display Density Index (FDDI). These indices are calculated by dividing photographed silhouette area by scanned foliar area for the whole canopy and a single representative branch, respectively. While FDDI incorporates only the relative amount of overlap of foliage on a single branch, CDDI also incorporates overlap of branches at a whole canopy scale. Trees with more overlapping, or a greater density of displayed foliage, have lower index values, while trees with less overlapping foliage have higher index values. Standard errors are shown N=8 for CDDI and N=16 for FDDI. Fertilizer was applied day 0 = June 16, 2009.

Foliar and canopy display density indices were highly correlated with one another (Figure 6-6; $p < 0.0001$, $R^2 = 0.72$). This may have been an artifact of the indices themselves, as the denominators of both were based on scanned leaf area of the representative branch subsample. Data from the scanned foliage of the representative branch subsample were used as the denominator of FDDI, while for the denominator of CDDI these branch-level data were scaled to the canopy level using total foliar biomass. The denominators were significantly correlated ($p < 0.0001$, $R^2 = 0.60$). However, the high correlation between these indices may also reflect that the predominant source of canopy-level overlap was foliar overlap at the branch scale rather than branch overlap at the crown scale. If branch overlap was a substantial source of canopy density, we would have expected a lower, not a higher, correlation between FDDI and CDDI compared to the autocorrelation between their denominators.

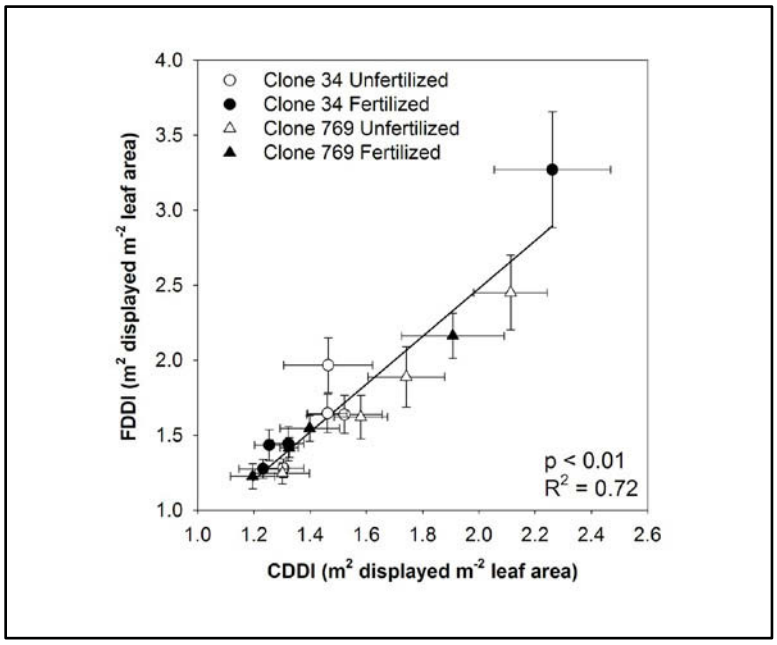

Figure 6-6. Regression of mean Crown Density Display Index (CDDI) versus Foliar Density Display Index (FDDI) by clone-by-fertilizer-by-harvest-date treatment groupings. These indices are calculated by dividing photographed silhouette area by scanned foliar area for the whole canopy and a single representative branch, respectively. While FDDI incorporates only the relative amount of overlap of foliage on a single branch, CDDI also incorporates overlap of branches at a whole canopy scale. Trees with more overlapping, or a greater density of displayed foliage, have lower index values, while trees with less overlapping foliage have higher index values. Standard errors are shown, N=8.

Despite the observed differences in stem volume, branch architecture, leaf area, and canopy density between clones 34 and 769, they showed no differences in either CSA or total foliar mass ($p > 0.10$; Table 6-2). While their overall crown architectures were very different, they had similar foliar masses that were displayed over a similar total crown cross-sectional area. However, there were significant fertilizer-by-harvest interactions for both these variables. While there were no differences in foliar mass or CSA at day 30 between fertilizer treatments ($p > 0.10$), by day 121 fertilized trees showed 52% greater foliar mass and 58% greater CSA ($p < 0.01$; Figure 6-7).

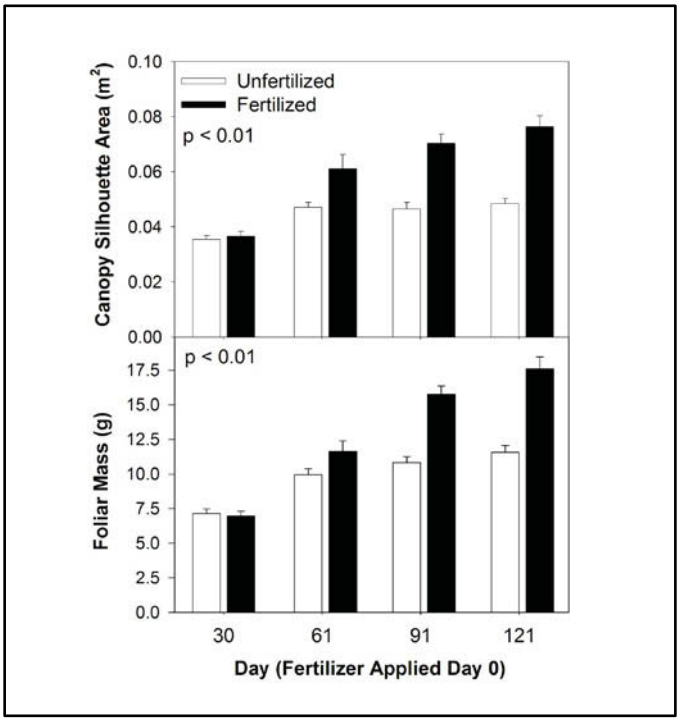

Figure 6-7. Fertilizer effect for Crown Silhouette Area (CSA) and foliar dry mass from four monthly post-fertilizer application destructive harvests. CSA is a metric of canopy display synthesizing amount of foliage with degree of overlap. Standard errors are shown, N =16. Fertilizer was applied day 0 = June 16, 2009.

6.3.4. Leaf-Level Physiology

There was an unusual overall decline in mean leaf-level A_{SAT} between days 2 and 42 (Figure 6-8; Figure 6-9). Mean A_{SAT} dropped from a four-day-prefertilizer application high of 4.3 to a day 28 low of 1.1 μmol CO_2 m^{-2} s^{-1} before eventually rebounding to levels similar to those prior to fertilizer application by day 42. All trees were watered daily each morning in an attempt to

prevent drought stress, however measurements were taken at mid-afternoon during this period of decline (13:00 to 15:00 EDT). Examination of greenhouse environmental conditions revealed that mean leaf-level A_{SAT} was negatively correlated to vapor pressure deficit in the three hours preceding each measurement period across all dates (Figure 6-9; $p = 0.02$, $R^2 = 0.36$). Four of the five dates with unusually low A_{SAT} occurred on dates with the five highest VPD's (Figure 6-9). Temperature and PAR were not significantly correlated with mean A_{SAT} when considered 3, 2, or 1 hour prior to measurements, or during the actual measurement period ($p > 0.10$). The day 2 to day 42 decline in A_{SAT} appears to be due at least partially to higher VPD prior to those particular measurements.

Leaf-level A_{SAT} was not different between treatments 1 and 4 days prior to fertilizer application ($p > 0.10$; Figure 6-8). Immediately after fertilizer application (day 2), rates dropped in fertilized trees compared to controls for first flush foliage ($p < 0.10$). This reduction was consistent throughout all measurements on the first flush, which ended day 28. During this period a clone-by-fertilizer interaction was also observed in the repeated measures analysis of the first flush measurements ($p < 0.10$; Table 6-2). After fertilizer application, clone 34 averaged a 23% decline in A_{SAT}, while clone 769 averaged a 41% decline. While no differences in A_{SAT} were observed in the repeated measures analysis of the second flush ($p > 0.10$), days 86 and 100 individually showed significantly greater rates in fertilized trees versus controls ($p < 0.01$).

When scaled to the whole canopy on the measurement dates nearest the four destructive harvests, A_{SAT} showed a fertilizer-by-time interaction ($p < 0.01$; Table 6-2; Figure 6-8). At day 28 fertilized trees had lower rates, but at days 70, 86, and 119 rates were higher in fertilized trees. This appears to be a combined effect of 1) higher leaf-level A_{SAT} rates (Figure 6-8), 2) greater leaf area (Figure 6-4) and 3) greater SLA (Figure 6-4) in fertilized versus unfertilized ramets. While leaf-level A_{SAT} rates were not different between fertilized and control treatments on day 119 ($p > 0.10$), canopy-level A_{SAT} was 60% greater in fertilized trees ($p < 0.01$).

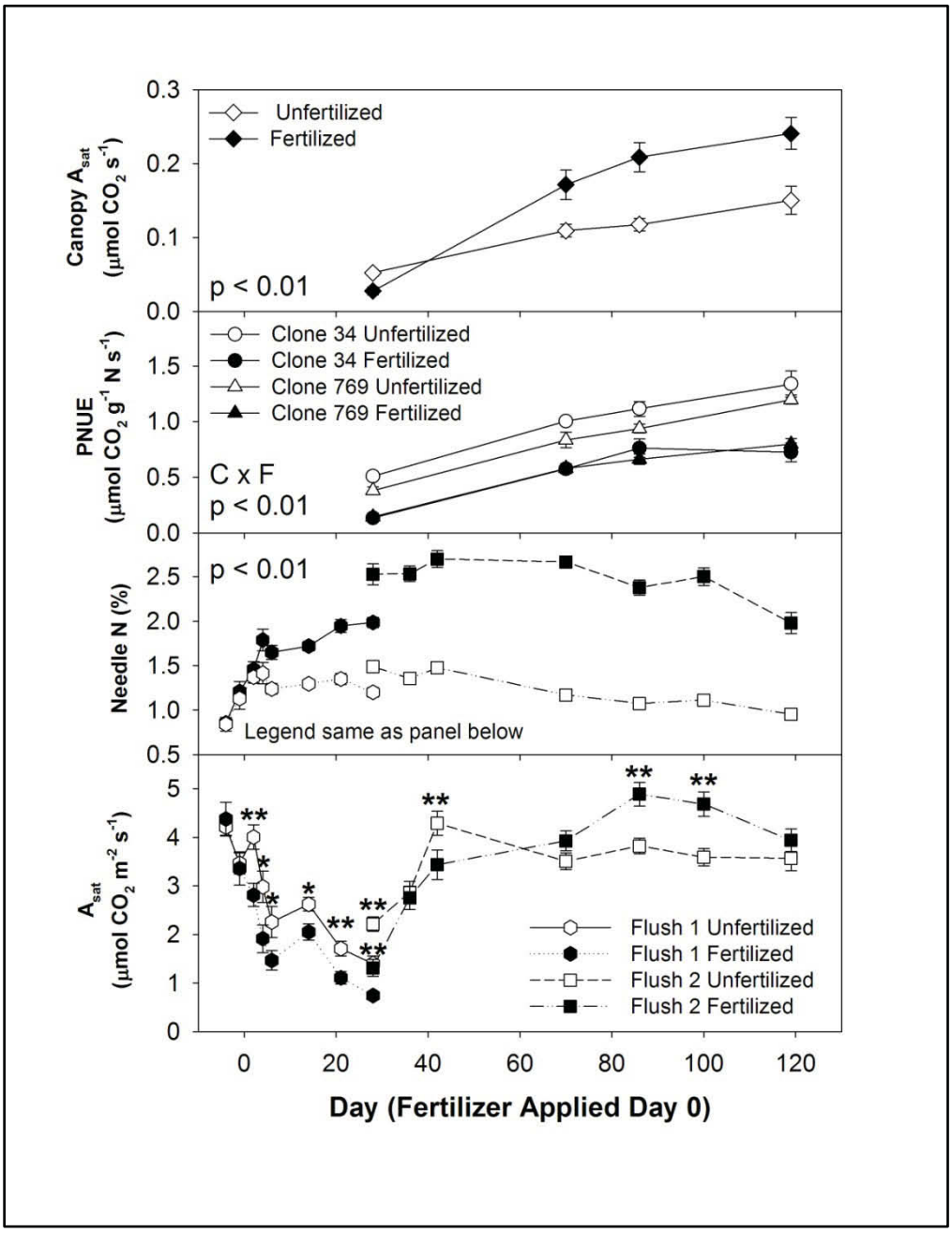

Figure 6-8. Canopy-level photosynthetic rate, photosynthetic nitrogen use efficiency (PNUE), foliar nitrogen concentration, and leaf-level light-saturated net photosynthetic rates from the final harvest group. Only the fertilizer effect is shown for all but PNUE, where the clone-by-fertilizer interaction is shown. All data are from gas exchange measurements on a single fascicle per date using a LICOR 6400. PNUE and canopy-level photosynthetic rate data are only shown for the four measurement dates most proximate to the four destructive harvests due to temporal variability in specific leaf area. Asterisks denote individually significant dates (* p < 0.10, ** p < 0.01). Standard errors are shown, N = 16 for all but PNUE, where N = 8. Fertilizer was applied day 0 = June 16, 2009.

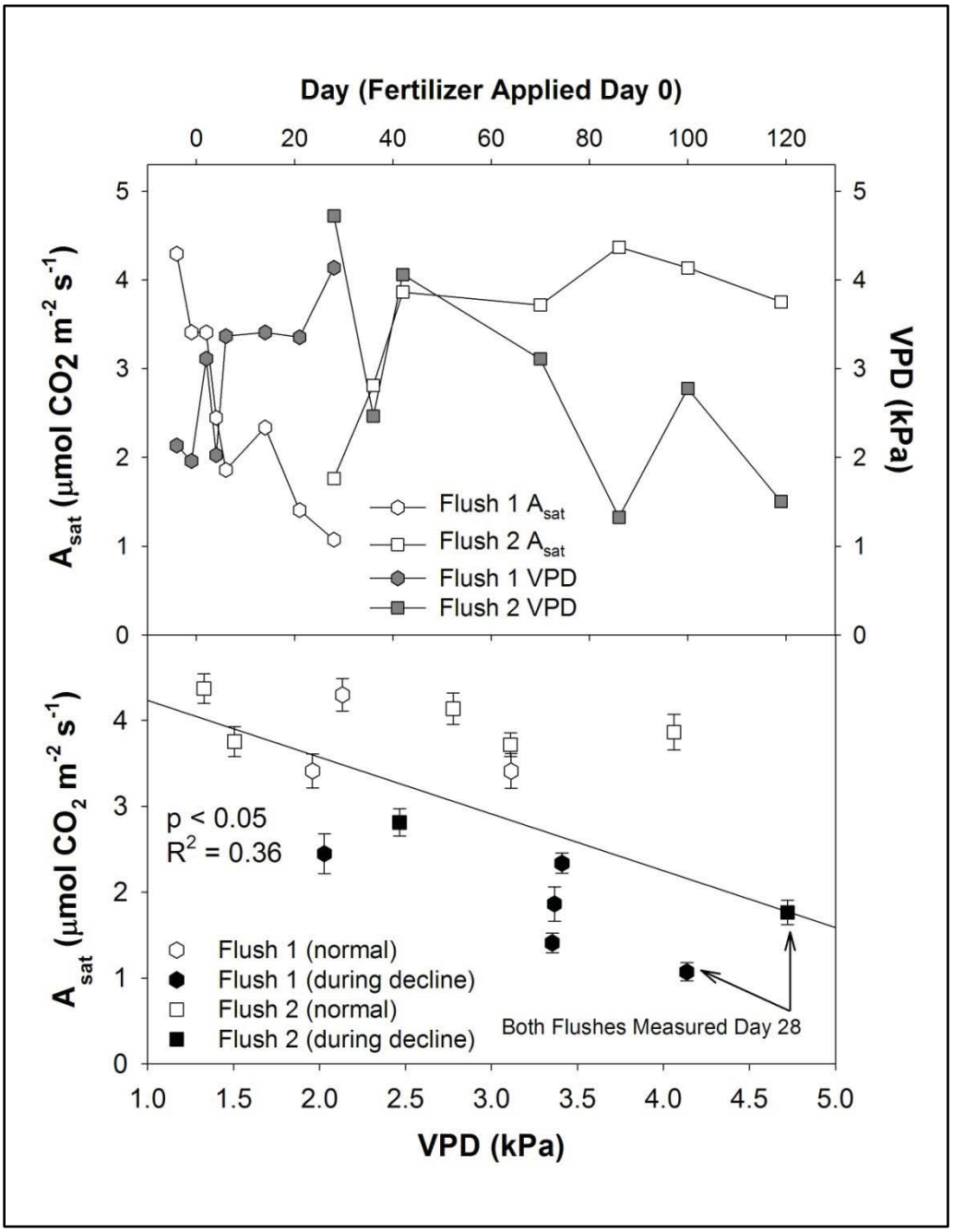

Figure 6-9. Leaf-level light-saturated net photosynthetic (A_{SAT}) rates are plotted against vapor pressure deficit (VPD) averaged for the three hours prior to each measurement period in the lower plot. Symbols shown in black represent days 4 to 36, which had below-normal A_{SAT} rates for *P. taeda*. Data presented are means of all measured trees from the final harvest group on each of 14 measurement dates. Standard errors are shown, N = 32. The upper plot shows the time trend of mean A_{SAT} and VPD. VPD was measured at a single HOBO data station in the center of the experiment. First and second flush measurements are distinguished in both plots.

Foliar nitrogen content was not correlated to leaf-level A_{SAT} in this experiment ($p > 0.10$, $R^2 < 0.01$). Fertilizer application did result in greater $[N]_f$ in both the first and second flushes ($p < 0.01$; Table 6-2; Figure 6-8). Clone 34 had 15% greater $[N]_f$ in the first flush than clone 769 (1.5% vs. 1.3%; $p < 0.05$). There was no clonal difference in $[N]_f$ in the second flush ($p > 0.10$). PNUE was lower in fertilized trees, largely as a result of increased $[N]_f$, but showed a clone-by-fertilizer interaction with clone 34 having a greater magnitude of reduction than clone 769 at all dates ($p < 0.01$; Figure 6-8). When unfertilized, clone 34 had slightly greater PNUE across all dates than other treatment combinations.

6.4. Discussion

6.4.1. Foliar Response to Fertilizer Inconsistent with Stem Growth Response

Fertilized ramets showed numerous physiological and morphological responses to fertilizer application that are consistent with many long-term observations of fertilizer application in *P. taeda*. However, we did not observe canopy physiological or morphological responses in this trial that were consistent with the theory of short-term fertilizer response posited in Gough (2004b). The reduced leaf-level A_{SAT} rates found in the fertilized treatment until day 70 are inconsistent with the substantial stem-volume growth response observed beginning at approximately day 40. While the fertilized ramets did have increased leaf area, foliar mass, and CSA by the day 61 harvest, they did not increase foliar allocation by increasing carbon gain via elevated A_{SAT} rates consistent with greater observed $[N]_f$. Even when scaled to the whole-canopy level, prior to day 70 A_{SAT} rates were lower in fertilized trees. We hypothesize based on these results that changes in biomass partitioning or other carbon fluxes not assessed in this chapter must be responsible for the increased foliar allocation observed, and that these processes are the physiological mechanisms of short-term growth response to fertilizer application that were operating in these two clones in this trial.

This conclusion is supported by the results of King et al. (2008). King et al. also found photosynthetic responses to fertilizer application inconsistent with fertilizer growth responses in a two-year-old field trial with four pairs of clones produced through full-sib crosses. Some

clones increased A_{SAT} and showed a growth response to fertilizer, while others with increased A_{SAT} but showed no growth response. Some with no change in A_{SAT} with fertilizer application showed a fertilizer growth response, as did some clones that even had negative A_{SAT} responses. These patterns were inconsistent among full-sib pairs of clones. While we did not observe clonal differences in A_{SAT} between our clones, it is possible that they were both in the group described by King et al. that showed negative A_{SAT} but positive growth responses to fertilizer application.

6.4.2. Foliar Display Inconsistent with Clonal Stem Growth Response

While there were marked differences in these two full-sib clones in their crown architecture, the lack of differentiation observed between clones in photosynthetic rates (at the leaf or canopy-level), total foliar mass, CSA, or total leaf area indicates that the clones were performing similarly with regard to whole-tree carbon gain. We failed to support our hypothesis that differences observed in stem volume growth between these clones were consistent with differences in canopy morphology or physiology. The clonal differences in growth rates observed must be due to differences in other ecophysiological processes, potentially biomass partitioning or respiratory carbon fluxes that were not assessed in this chapter. The reduction in leaf-level A_{SAT} rates in fertilized ramets through day 42 post-fertilizer application further indicates that the short-term mechanism of fertilizer growth response in both of these clones was not directly attributable to canopy physiology.

The branch data coupled with the CDDI index showed that clone 769 generally had a less dense crown with less foliar and branch overlap. The display of foliage on a lesser number of substantially larger branches should result in less self-shading and greater PAR interception, which at similar photosynthetic rates and foliar mass would result in more carbon gain. Greater PAR interception resulting in greater growth has been observed among different species of the *Pinus* genus (Chmura, Rahman et al. 2007). While branch data supported the validity of the CDDI index, the clonal growth trend observed in this study is contrary to what we would expect based on this hypothesis. The clone with favorable traits allowing greater PAR interception, clone 769, had lower growth rates. This lends further support to our conclusion that canopy morphology is not the primary mechanism of short-term fertilizer growth response in these two

clones. It should be noted that while CDDI and FDDI values theoretically should be less than unity (displayed foliar area divided by scanned single-sided foliar area), the CSA index on which displayed area was based 1) is an index and is only intended to compare treatment effects and 2) includes woody biomass area as well as foliar area, and thus should consistently overestimate actual displayed foliar area. While values greater than unity are not intuitively meaningful, these indices remain useful for treatment comparisons over time.

6.4.3. Implications and Caveats of Clonal Carbon Gain

The similarity in carbon gain of these two full-sib clones despite dramatically different crown architecture may have a number of useful implications for attempts to select crop ideotypes based in part or whole on crown metrics (Chen, Ceulemans et al. 1994; Nelson and Johnsen 2008). First, differences in crown morphology cannot necessarily be assumed to correlate to differences in crown physiology between clones. While it may appear depending upon the metrics selected that one clone is displaying its foliage in a more efficient manner, at a canopy scale there may be no substantial difference in photosynthetic capacity. Second, if two clones with dramatically different crown architecture can have similar photosynthetic capacities, the clone with more favorable branch characteristics for the intended product class could be selected without necessarily reducing growth potential. Our results highlight the value of including crown traits when developing crop tree ideotypes for clonal deployment. However, because growth was not correlated to crown variables in this study, independent assessment of growth would be necessary. These conclusions are based on results similar to those found for two other clones deployed in another greenhouse experiment (Tyree, Seiler et al. 2009a).

It is somewhat surprising given the differences in crown morphology between these two clones that no difference in overall carbon gain was observed. While the clones had distinct patterns of branching and foliar display per branch, this did not apparently affect the overall efficiency of canopy-level carbon gain. However, it is important to note that the photosynthetic rates we quantified were light-saturated rates from uniform canopy positions on all trees, and thus did not incorporate any measure of intercepted PAR at different positions within the canopy. While branch data and CDDI do not show trends consistent with those hypothesized between foliar

display and growth, a more detailed assessment of PAR interception throughout the crown may have revealed differences in actual carbon gain between these genotypes that would be independent of their light-saturated photosynthetic capacity and consistent with differences in clonal growth rates. Other studies have shown that genotypic differences in crown morphology are consistent with differences in stem growth rates (Dalla-Tea and Jokela 1991; Xiao, Jokela et al. 2003; Chmura, Rahman et al. 2007; Emhart, Martin et al. 2007), a correlation that may be directly attributed to PAR interception (McCrady and Jokela 1998). Future assessment of clonal differences in short-term growth response to fertilizer application should incorporate some metric of PAR interception rather than merely comparing light-saturated photosynthetic rates scaled to the canopy-level.

6.4.4. Foliar Nitrogen, Photosynthesis, and Nitrogen Use Efficiency

The drop in A_{SAT} we observed between days 2 and 42 was to rates typically below those reported for *P. taeda*, which are in the 3 to 10 μmol CO_2 m^2 s^{-1} range (Samuelson 1998; Gough, Seiler et al. 2004a; Maier, Palmroth et al. 2008). While pre-measurement period VPD explains a substantial portion of the variance associated with this decline, consistent with the literature for conifers (Grieu, Guehl et al. 1988; LeBude, Goldfarb et al. 2005), it does not explain the decline on all dates, or the rates observed within a normal range on several other dates with high VPD. It is possible that rates were declining in the older foliage (flush 1), and we then switched to the new foliage (flush 2) before the needles had fully matured. *P. taeda* in the field typically does not reach full needle elongation until late-summer (Tang, Chambers et al. 1999), so it is possible that switching to the second flush foliage on day 28 (July 14) preceded their full elongation when A_{SAT} would be maximized and construction respiration would have approached zero. Previous work on *P. taeda* has shown that after fertilizer application, $[N]_f$ increases in existing foliage but does not result in an increase in A_{SAT} (Maier, Palmroth et al. 2008). By contrast, both $[N]_f$ and A_{SAT} increase in newly developed current year foliage (Maier, Palmroth et al. 2008). This may partially explain while A_{SAT} decreased while $[N]_f$ increased in first flush foliage of fertilized ramets. While all these hypotheses are consistent with our data, we cannot definitively attribute the decline observed to any one cause.

Although we did not observe clonal differences in the ability to take advantage of varying resource environments based on stem volume growth, differences in $[N]_f$ and PNUE point to varying capacities to exploit available nutrients. Clone 34 had the greatest PNUE and higher stem volume growth rates when unfertilized, signifying that it may outperform clone 769 on poorer sites or where silvicultural inputs are less intensive. That clone 34 was also the fastest growing of the two may be related to its greater efficiency in N metabolism, although we did not directly assess this correlation in this study. After fertilizer application similar PNUE and $[N]_f$ levels in both clones revealed that they were similarly capable of increasing carbon gain through their uptake of available N and P. However, these physiological differences are far less important from a clonal selection context than the differences in growth rates and branch characteristics we observed. Indeed, the overall lack of clone-by-fertilizer interactions found with respect to canopy metrics in this study supports the selection of clonal material with little regard to potential rank-change G x E interactions, as has been found in previous studies (McKeand, Jokela et al. 2006; Roth, Jokela et al. 2007).

The lack of any relationship observed between A_{SAT} and $[N]_f$ measured on the same foliage in this study is somewhat inconsistent with the majority of results in the literature. This may have been in part due to the substantial decline in A_{SAT} rates between days 4 and 42 we observed. Many studies have found relatively strong relationships between A_{SAT} and $[N]_f$ when expressed either per foliar mass or area (Green and Mitchell 1992; Gough, Seiler et al. 2004b; Crous, Walters et al. 2008; Maier, Palmroth et al. 2008). It is possible that we did not observe any relationship because even our control treatment had $[N]_f$ of 1.23% averaged across all dates, which is generally viewed as being at or above sufficiency levels for *P. taeda* (Comerford and Fisher 1984). Only a very weak relationship between A_{SAT} and $[N]_f$ was observed in King (2008), where $[N]_f$ levels were also above sufficiency in the vast majority of measured ramets. Several studies have found that increased $[N]_f$ leads to increased A_{SAT} following fertilizer application (Samuelson 2000; Gough, Seiler et al. 2004b). However we observed the opposite trend, consistently reduced A_{SAT} and consistently higher $[N]_f$ following fertilizer application.

6.5. Conclusion

Based on the results of this study increased photosynthetic rates following fertilizer application are not consistently the primary mode of short-term fertilizer growth response in all genotypes of *P. taeda*. The two full-sib clones in this study differed markedly in their branch architecture, foliar display, and nitrogen use efficiency. However, they did not differ in whole-canopy traits such as foliar area or foliar mass. When coupled with the lack of clonal difference in photosynthetic rates, we conclude that carbon gain from a whole-canopy perspective was not significantly different between clones. Despite similar carbon gain, clones displayed different stem volume growth rates. To explain discrepancies between crown physiologies and stem volume growth rates we hypothesize that the clones were likely varying in physiological processes not assessed in this chapter such as biomass partitioning or respiratory carbon fluxes. These results emphasize that physiological responses to fertilizer application observed across a genotypic average of *P. taeda* may not necessarily apply equally to all genotypes. A better understanding of the physiology of individual clones that will be deployed over large acreages may be necessary in order to optimally manage them. Additionally, these results imply that crop ideotypes for *P. taeda* clones should assess crown traits and stem growth as independent variables, since all clones do not display strong correlations between crown morphology or physiology and growth rates.

6.6. Literature Cited

Albaugh, T. J., H. L. Allen, P. M. Dougherty, L. W. Kress and J. S. King (1998). "Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions." Forest Science **44**(2): 317-328.

Baltunis, B. S., D. A. Huber, T. L. White, B. Goldfarb and H. E. Stelzer (2007). "Genetic analysis of early field growth of loblolly pine clones and seedlings from the same full-sib families." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **37**(1): 195-205.

Bettinger, P., M. Clutter, J. Siry, M. Kane and J. Pait (2009). "Broad implications of southern United States pine clonal forestry on planning and management of forests." International Forestry Review **11**(3): 331-345.

Bitoki, O. (2008). "Comparing early survival and growth of varietal and open-pollinated loblolly pine seedlings." VDOF Forest Research Review **April 2008**: 4-5.

Chen, S. G., R. Ceulemans and I. Impens (1994). "Is there a light regime determined tree ideotype." Journal of Theoretical Biology **169**(2): 153-161.

Chmura, D. J., M. S. Rahman and M. G. Tjoelker (2007). "Crown structure and biomass allocation patterns modulate aboveground productivity in young loblolly pine and slash pine." Forest Ecology and Management **243**(2-3): 219-230.

Chmura, D. J. and M. G. Tjoelker (2008). "Leaf traits in relation to crown development, light interception and growth of elite families of loblolly and slash pine." Tree Physiology **28**(5): 729-742.

Comerford, N. B. and R. F. Fisher (1984). "Using foliar analysis to classify nitrogen-deficient sites." Soil Science Society of America Journal **48**: 910-913.

Crous, K. Y., M. B. Walters and D. S. Ellsworth (2008). "Elevated CO_2 concentration affects leaf photosynthesis-nitrogen relationships in *Pinus taeda* over nine years in FACE." Tree Physiology **28**(4): 607-614.

Dalla-Tea, F. and E. J. Jokela (1991). "Needlefall, canopy light interception, and productivity of young intensively managed slash and loblolly-pine stands." Forest Science **37**(5): 1298-1313.

Emhart, V. I., T. A. Martin, T. L. White and D. A. Huber (2007). "Clonal variation in crown structure, absorbed photosynthetically active radiation and growth of loblolly pine and slash pine." Tree Physiology **27**(3): 421-430.

Fox, T. R., H. L. Allen, T. J. Albaugh, R. Rubilar and C. A. Carlson (2007). "Tree nutrition and forest fertilization of pine plantations in the southern United States." Southern Journal of Applied Forestry **31**(1): 5-11.

Fox, T. R., E. J. Jokela and H. L. Allen (2007). "The development of pine plantation silviculture in the southern United States." Journal of Forestry **105**: 337-347.

Gillespie, A. R., H. L. Allen and J. M. Vose (1994). "Amount and vertical-distribution of foliage of young loblolly-pine trees as affected by canopy position and silvicultural treatment." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **24**(7): 1337-1344.

Ginn, S. E., J. R. Seiler, B. H. Cazell and R. E. Kreh (1991). "Physiological and growth-responses of 8-year-old loblolly-pine stands to thinning." Forest Science **37**(4): 1030-1040.

Gough, C. M., J. R. Seiler, K. H. Johnsen and D. A. Sampson (2004a). "Seasonal photosynthesis in fertilized and nonfertilized loblolly pine." Forest Science **50**(1): 1-9.

Gough, C. M., J. R. Seiler and C. A. Maier (2004b). "Short-term effects of fertilization on loblolly pine (*Pinus taeda* L.) physiology." Plant Cell and Environment **27**(7): 876-886.

Green, T. H. and R. J. Mitchell (1992). "Effects of nitrogen on the response of loblolly-pine to water-stress. 1. Photosynthesis and stomatal conductance." New Phytologist **122**(4): 627-633.

Grieu, P., J. M. Guehl and G. Aussenac (1988). "The effects of soil and atmospheric drought on photosynthesis and stomatal control of gas-exchange in 3 coniferous species." Physiologia Plantarum **73**(1): 97-104.

Isik, F., B. Goldfarb, A. LeBude, B. L. Li and S. McKeand (2005). "Predicted genetic gains and testing efficiency from two loblolly pine clonal trials." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **35**(7): 1754-1766.

Jokela, E. J., P. M. Dougherty and T. A. Martin (2004). "Production dynamics of intensively managed loblolly pine stands in the southern United States: A synthesis of seven long-term experiments." Forest Ecology and Management **192**(1): 117-130.

King, N. T., J. R. Seiler, T. R. Fox and K. H. Johnsen (2008). "Post-fertilization physiology and growth performance of loblolly pine clones." Tree Physiology **28**(5): 703-711.

Lambeth, C. C. and R. B. McCullough (1997). "Genetic diversity in managed loblolly pine forests in the southeastern United States: Perspective of the private industrial forest land owner." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **27**(3): 409-414.

LeBude, A. V., B. Goldfarb, F. A. Blazich, J. Frampton and F. C. Wise (2005). "Mist level influences vapor pressure deficit and gas exchange during rooting of juvenile stem cuttings of loblolly pine." Hortscience **40**(5): 1448-1456.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger and O. Schabenberger (2006). SAS for mixed models, second edition. Cary, NC, SAS Institute Inc.

Maier, C. A., S. Palmroth and E. Ward (2008). "Short-term effects of fertilization on photosynthesis and leaf morphology of field-grown loblolly pine following long-term exposure to elevated CO_2 concentration." Tree Physiology **28**(4): 597-606.

McCrady, R. L. and E. J. Jokela (1996). "Growth phenology and crown structure of selected loblolly pine families planted at two spacings." Forest Science **42**(1): 46-57.

McCrady, R. L. and E. J. Jokela (1998). "Canopy dynamics, light interception, and radiation use efficiency of selected loblolly pine families." Forest Science **44**(1): 64-72.

McGarvey, R. C., T. A. Martin and T. L. White (2004). "Integrating within-crown variation in net photosynthesis in loblolly and slash pine families." Tree Physiology **24**(11): 1209-1220.

McKeand, S., T. Mullin, T. Byram and T. White (2003). "Deployment of genetically improved loblolly and slash pines in the South." Journal of Forestry **101**(3): 32-37.

McKeand, S. E., E. J. Jokela, D. A. Huber, T. D. Byram, H. L. Allen, B. L. Li and T. J. Mullin (2006). "Performance of improved genotypes of loblolly pine across different soils, climates, and silvicultural inputs." Forest Ecology and Management **227**(1-2): 178-184.

Munger, G. T., R. E. Will and B. E. Borders (2003). "Effects of competition control and annual nitrogen fertilization on gas exchange of different-aged *Pinus taeda*." Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **33**(6): 1076-1083.

Nelson, C. D. and K. H. Johnsen (2008). "Genomic and physiological approaches to advancing forest tree improvement." Tree Physiology **28**: 1135-1143.

Nobis, M. and U. Hunziker (2005). "Automatic thresholding for hemispherical canopy-photographs based on edge detection." Agricultural and Forest Meteorology **128**: 243-250.

Paquette, A. and C. Messier (2009). "The role of plantations in managing the world's forests in the Anthropocene." Frontiers in Ecology and the Environment **8**(1): 27-34.

Paul, A. D., G. S. Foster, T. Caldwell and J. McRae (1997). "Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine." Forest Science **43**(1): 87-98.

Radoglou, K. and R. O. Teskey (1997). "Changes in rates of photosynthesis and respiration during needle development of loblolly pine." Tree Physiology **17**(7): 485-488.

Roth, B. E., E. J. Jokela, T. A. Martin, D. A. Huber and T. L. White (2007). "Genotype x environment interactions in selected loblolly and slash pine plantations in the southeastern United States." Forest Ecology and Management **238**(1-3): 175-188.

Samuelson, L. J. (1998). "Influence of intensive culture on leaf net photosynthesis and growth of sweetgum and loblolly pine seedlings." Forest Science **44**(2): 308-316.

Samuelson, L. J. (2000). "Effects of nitrogen on leaf physiology and growth of different families of loblolly and slash pine." New Forests **19**(1): 95-107.

Tang, Z. M., J. L. Chambers, S. Guddanti, S. F. Yu and J. P. Barnett (1999). "Seasonal shoot and needle growth of loblolly pine responds to thinning, fertilization, and crown position." Forest Ecology and Management **120**(1-3): 117-130.

Tyree, M. C., J. R. Seiler and C. A. Maier (2009a). "Short-term impacts of nutrient manipulations on leaf gas exchange and biomass partitioning in contrasting 2-year-old *Pinus taeda* clones during seedling establishment." Forest Ecology and Management **257**(8): 1847-1858.

Tyree, M. C., J. R. Seiler, C. A. Maier and K. H. Johnsen (2009b). "*Pinus taeda* clones and soil nutrient availability: Effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology." Tree Physiology **29**(9): 1117-1131.

Vose, J. M. and H. L. Allen (1988). "Leaf-area, stemwood growth, and nutrition relationships in loblolly-pine." Forest Science **34**(3): 547-563.

Whetten, R. W. and R. Kellison (2010). "Research gap analysis for application of biotechnology to sustaining US forests." Journal of Forestry **108**: 193-201.

Xiao, Y., E. J. Jokela and T. L. White (2003). "Species differences in crown structure and growth performance of juvenile loblolly and slash pine." Forest Ecology and Management **174**(1-3): 295-313.

7. Synthesis of experimental results: Clonal variability in ecophysiology and modeling short-term changes in carbon budget response to fertilizer application

7.1. Chapter Synopses

7.1.1. Chapter 2: Growth and Stem Form Defects in the Reynold's Homestead Trial

Variation in nutrient use efficiency that exists among clones could affect growth responses to fertilizer application. Our research objective in this chapter was to determine the range of growth and stem form quality responses due to fertilizer application in clones of *Pinus taeda* (L.). A split-plot experimental design was used, with the whole plots being two levels of fertilizer application (fertilizer versus control) and the split plot factor being 25 clones. Whole plot treatments were blocked and replicated four times. Six years after planting and five years after fertilizer application, a repeated measures analysis showed fertilizer-by-time and clone-by-time interactions affected stem volume ($p < 0.10$). Clone-by-fertilizer interactions were observed for tree height, branch traits, and a metric of foliar display. While these interactions were primarily due to scale-effect phenomena rather than rank shifts, the magnitude of fertilizer responses observed in a small number of genotypes suggests that while testing clones for fertilizer responses may be unnecessary prior to deployment, knowledge of fertilizer responses in widely deployed genotypes, if developed by mid-rotation, may better optimize management of single-clone blocks. Our results further indicated that a range of possibilities exist for the design and application of clone-specific precision silvicultural systems.

7.1.2. Chapter 3: Allometry and Growth Efficiency in the Reynold's Homestead Trial

Differences in aboveground and belowground carbon allocation between individual genotypes could have significant implications for productivity and carbon sequestration and cycling of clonal plantations. We assessed biomass partitioning, allometry, coarse root morphology, and crown size of ten *P. taeda* clones at age six in the Virginia Piedmont in both control and fertilizer plots. Clonal effects were observed for biomass partitioning to foliar, branch, stem, taproot, and lateral root fractions; allometric relationships including root-shoot, stem-foliage, branch-foliage,

and stem-taproot ratios; root morphology, and crown size. Clonal differences in biomass partitioning were the result of both differences in allometry and differences in growth rates. Growth efficiency (stem-foliage ratio) showed a two-fold difference among the two most disparate clones even after accounting for differences in growth rate. Clone-by-fertilizer interactions were observed for total, above, and belowground biomass, and for rooting depth. Differences in partitioning and allometry were not clearly tied to tree or stem growth rates, indicating that there may be opportunities to select and deploy clones with rapid stem volume growth rates that have biomass partitioning patterns tailored to various precision-silviculture applications.

7.1.3. Chapter 4: Ephemeral Shifts in Allometry in a Four Month Greenhouse Experiment

Short-term shifts in allometry of *P. taeda* clones in response to fertilizer application may be a mechanism of long-term fertilizer growth response. Reduced belowground C allocation and increased allocation to foliage following fertilizer application would result in a whole-tree growth response. Our research objective was to quantify differences in biomass partitioning due to fertilizer application in contrasting clones of *P. taeda* produced from the same full-sib cross. A two (clone) by two (fertilizer) by four (sequential harvest) factorial randomized complete block design was replicated eight times (128 trees total) in a greenhouse for four months. Thirty-two trees, eight per each fertilizer-clone combination, were destructively harvested 30, 61, 91, and 121 days after fertilizer application. Each tree was separated into component tissues (foliage, branch, stem, taproot, lateral roots), oven-dried (65° C), and weighed. Both clones responded to fertilizer with a short-term reduction in root-shoot ratio and increase in relative allocation to foliar biomass, as hypothesized. However, these changes were ephemeral, returning to control levels by the end of the experiment. Further, allometric analysis revealed that fertilizer responses in below-ground partitioning were due to treatment effects on allometry for one clone but were only attributable to more rapid growth rates in the other. Ephemeral changes in biomass partitioning in response to fertilizer application were consistent with the theory of short-term physiological response to increased nutrient availability fueling long-term fertilizer growth responses posited in Chapter 1.

7.1.4. Chapter 5: Respiratory C Fluxes and Root Exudation in a Four Month Greenhouse Experiment

We investigated whether changes in respiratory C fluxes, soil CO_2 efflux, or root exudate quantity or quality explained differences in growth rates between contrasting yet closely related clones of *P. taeda*. A factorial design with two clones, fertilized and control treatments, and four sequential harvests was installed in a greenhouse for 121 days. Results show that while the clones did differ in growth rates, the C fluxes assessed in this chapter were not consistent with increased C available for stem growth in the short-term following fertilizer application due to reduced C fluxes belowground or to respiration. Changes in root exudation were not consistent with reduced heterotrophic soil CO_2 efflux, which does not appear to be a plant-mediated process. However, the two clones did show significant differences in respiratory C fluxes, soil CO_2 efflux, and root exudation quantity and quality in response to fertilizer application. These results indicated that if single genotypes are deployed over large land areas in plantations, dramatic differences between clonal plant-soil interactions may require consideration in ecosystem C budgets. Further, the range of belowground fluxes observed implies that genotype-specific C allocation may make some clones better able to exploit a given resource environment than others.

7.1.5. Chapter 6: Canopy Dynamics and Foliar Gas-Exchange in a Four Month Greenhouse Experiment

Increased photosynthetic rates immediately following fertilizer application have been established as a short-term mechanism of fertilizer growth response across a genotypic average, but this observation does not appear to apply consistently among individual clones. In order to better assess how two full-sib clones respond to fertilizer application in the short-term, an integrated suite of crown metrics were measured over four months. Metrics included light-saturated net-photosynthetic rates (A_{SAT}), foliar nitrogen content($[N]_f$), photosynthetic nitrogen use efficiency (PNUE), foliar mass, foliar area, branch architecture, specific leaf area (SLA), crown silhouette area (CSA), and two indices of foliar area displayed per actual leaf area. Measurements were made on a two-by-two factorial randomized complete block design replicated eight times in a

greenhouse, with two full-sib clones and two levels of nutrient availability. While the two clones in this study differed markedly in their branch architecture, foliar display, SLA, and PNUE, overall they showed little difference in foliar mass, foliar area, CSA, A_{SAT}, or $[N]_f$. Canopy-level responses were not consistent with the theory of short-term increased carbon gain with fertilizer application leading to greater leaf-area and increased stem volume growth, and further did not explain differences between clones in stem volume growth. It appears that, at least in these two clones, the mechanism of short-term fertilizer growth response was most likely changes in biomass partitioning described in Chapter 4.

7.2. Modeling the Carbon Budget of the Four-Month Greenhouse Experiment

7.2.1. Partitioning Gross Primary Productivity

Quantifying C allocated to biomass and comparing individual fluxes does not accurately assess the full partitioning of gross primary productivity (GPP) to various plant organs and processes (Litton, Raich et al. 2007). Modeling of GPP partitioning integrated over time is possible by scaling measurements of biomass, aboveground respiratory C fluxes, and soil CO_2 efflux (Ryan 1991b; Ryan 1991a; Ryan, Hubbard et al. 1996; Giardina and Ryan 2002; Giardina, Ryan et al. 2003). In order to develop a more comprehensive representation of the C budget in the four month greenhouse experiment, we applied this modeling approach as adapted to container-based seedlings by Bown et al. (2009).

The model is shown in equations 1 through 7, and each variable is described in Table 7-1. Equation 1 partitions GPP into aboveground net primary productivity (ANPP), aboveground plant respiration (APR), and total belowground C flux (TBCF).

$$[1] \quad GPP = ANPP + APR + TBCF$$

Partitioning to ANPP is the sum of litter-fall production, mortality, and changes in woody and foliar biomass C storage (Equation 2). Because litter-fall and mortality were not observed in our four month greenhouse study, we set these fluxes equal to zero, yielding Equation 3 for ANPP.

[2] $ANPP = F_A + F_W + \Delta C_F + \Delta C_w$

[3] $ANPP = \Delta C_F + \Delta C_w$

Partitioning to TBCF is the sum of soil CO_2 efflux, C lost through erosion or leaching, changes in soil C, changes in root biomass C, and changes in litter layer C less new litterfall that was previously quantified as part of ANPP (Equation 4). We were again able to eliminate some of these variables, resulting in the Equation 5. We assumed that there was no erosion or leaching, and we observed no litter layer or litter-fall in this study. Analysis of soil data between days 30 and 121 showed no significant changes in soil C, so this flux was also set equal to zero.

[4] $TBCF = F_S + F_E + \Delta C_S + \Delta C_R + \Delta C_L - F_A$

[5] $TBCF = F_S + \Delta C_R$

Two further terms are defined in Equation 6 and Equation 7. Net primary productivity (NPP) is the sum of ANPP and the change in root biomass C, or total aboveground and belowground change in biomass C. Carbon use efficiency (CUE) is defined as the proportion of GPP partitioned to NPP, or biomass.

[6] $NPP = ANPP + \Delta C_R$

[7] $CUE = NPP / GPP$

In the greenhouse study various C fluxes were quantified with a variety of different cuvettes of different sizes and shapes. As a result, the individual fluxes measured represent an accurate comparison of treatments, but likely did not reflect the magnitude of the absolute fluxes (Norman, Kucharik et al. 1997). Further, we did not apply multiple measurement techniques to each flux to determine the accuracy of our methods in assessing the actual rates. Thus, while this modeling approach likely did not reflect the absolute magnitude of GPP partitioned to each component assessed, it remained an accurate treatment index that allowed us to compare the effects of fertilizer application on the C budget of both clones (Bown, Watt et al. 2009).

Table 7-1. Description of all variables utilized in the C budget modeling of the greenhouse clone-by-fertilizer-by-sequential-harvest study. Variables assumed to equal zero in this simplified greenhouse pot study are noted in the description.

Acronym		Variable	Description
GPP		Gross Primary Productivity	All C fixed through photosynthesis
ANPP		Aboveground Net Primary Productivity	C stored in aboveground biomass
	F_A	Sum of litterfall C production	Assumed equal to 0 (no litterfall)
	F_W	Sum of mortality C production	Assumed equal to 0 (no mortality)
	ΔC_F	C content change of live foliage	C stored in foliar biomass
	ΔC_w	C content change of aboveground woody tissue	C stored in branch and stem biomass
APR		Aboveground Plant Respiration	Sum of construction and maintenance
TBCF		Total Belowground Carbon Flux	All C allocated belowground
	F_S	Sum of soil respiration	C lost from the soil surface
	F_E	C lost from system through erosion or leaching	Assumed to equal 0 (no leaching)
	ΔC_S	C content change of soil	Assumed equal to 0 (no change)
	ΔC_R	C content change of root biomass	C stored in tap and lateral root biomass
	ΔC_L	C content change of litter layer	Assumed to equal 0 (no litter layer)
NPP		Net Primary Productivity	All C stored in biomass
CUE		Carbon Use Efficiency	Portion of GPP allocated to NPP

7.2.2. Applying a Gross Primary Productivity Partitioning Model to Greenhouse Data

Experimental design and measurements are described in detail in Chapters 4, 5, and 6. The following methods section will not describe procedures that have been detailed elsewhere, but the corresponding chapters can be referenced where appropriate. The model was applied to only the day 121 harvest group of trees and represents data integrated over the duration of the experiment (i.e. days 0 through 121). Thus, all values reflect the change in biomass C pools or the integrated total of respiratory C fluxes from the time of fertilizer application through the final destructive harvest four months later. Data from all trees was utilized in order to estimate parameters for the day 121 harvest group trees.

ANPP was calculated as the change in aboveground biomass from day 0 to day 121, assuming that 50% of biomass was carbon. Aboveground biomass was the sum of foliar, branch, and stem mass. Treatment specific non-linear regressions on all trees were applied to determine the relationship between stem dimensions and aboveground biomass. Regressions were of the form

shown in Equation 8, and were estimated using PROC NLIN in SAS software version 9.2. (SAS Institute Inc., Cary, North Carolina, USA). Coefficients and statistics for each regression are shown in Table 7-2. Regressions were then applied to stem dimension measurements taken on each tree from the day 121 harvest group on day 0. Difference between actual aboveground biomass from the day 121 harvest, and estimated aboveground biomass at day 0 was then calculated for each tree.

[8] $$\text{Aboveground biomass} = a\,(\text{basal diameter})^{b}\,(\text{height})^{c}$$

Table 7-2. Non-linear regressions of above and belowground biomass based on stem dimensions at harvest for all 128 trees from the greenhouse clone-by-fertilizer-by-sequential-harvest study. Equations were of the form: biomass = a (basal diameter)^{b} (height)^{c}. Regressions were implemented in PROC NLIN in SAS software version 9.2.

Aboveground Biomass

Treatments		Coefficients			Statistics			
Clone	Fert	a	b	c	F	p-value	R^2	N
34	0	0.2920	1.1369	0.3848	476.51	<0.0001	0.980	32
34	1	0.1231	1.2144	0.5794	622.51	<0.0001	0.985	32
769	0	0.4868	1.2454	0.1919	856.21	<0.0001	0.989	32
769	1	0.0362	1.6936	0.6130	575.99	< 0.0001	0.983	32

Belowground Biomass

Treatments		Coefficients			Statistics			
Clone	Fert	a	b	c	F	p-value	R^2	N
34	0	0.3013	1.1540	0.1640	307.11	<0.0001	0.969	32
34	1	0.0434	2.1348	0.0833	254.35	<0.0001	0.963	32
769	0	0.0734	1.2373	0.5320	355.50	<0.0001	0.974	32
769	1	0.1332	2.2059	-0.1686	295.06	<0.0001	0.968	32

Modeling efforts found in the literature typically calculate APR by including maintenance respiration rates separately for foliage and wood, and then assume construction respiration as a uniform fraction of biomass (Ryan 1991b; Maier, Albaugh et al. 2004; Bown, Watt et al. 2009). This was unnecessary in our experiment, as we directly measured APR by placing the entire aboveground portion of the tree in a cuvette. The flux we measured included maintenance respiration of both foliage and wood as well as construction respiration due to elongating shoots and fascicles or secondary woody growth.

APR was measured on days 30 and 91 on those respective harvest groups, and was converted to 20° C using ambient air temperature measured concurrently by assuming a Q_{10} of 2.0 (Ryan 1991b). APR was expressed per plant mass based on harvest data to account for variability in tree size. The average mass-specific APR rates for each treatment group were calculated, and the day 30 rates were applied to days 0 through 59 (inclusive), while the day 91 rates were applied to days 60 through 121. Rates were back-corrected to the temperature measured at the single data logger in the center of the experiment at two minute intervals, again assuming a Q_{10} of 2.0. Total daily mean mass-specific APR CO_2 flux was calculated for each treatment group based on this data. Stem dimension measurements made weekly throughout the trial were linearly interpolated for each tree in the final harvest group between measurement dates at a daily resolution. The regression in Equation [8] was then applied to calculate estimated daily aboveground biomass for each tree in the day 121 harvest group on each day. Mass was then multiplied by the daily mean mass-specific APR CO_2 flux for the corresponding treatment group. Daily CO_2 yields attributable to APR for each tree were summed from days 0 to 121, and converted from a CO_2 basis to a C basis to give APR used in the model.

TBCF was calculated as shown in Equation [5]. Change in root biomass C (ΔC_R) was calculated in the same manner as ANPP was calculated above. The non-linear regression relating stem dimensions to belowground mass is shown in Equation [9], and coefficients and statistics are shown in Table 7-2.

[9] $$\text{Belowground biomass} = a\,(\text{basal diameter})^{b}\,(\text{height})^{c}$$

F_S had been measured on all trees from the day 121 harvest group throughout the trial. F_S data was scaled to the soil surface area of each pot and was converted to C mass basis. Rates were then scaled up to a daily flux. Daily fluxes were linearly interpolated between measurement dates for each tree, and all daily values were summed for each tree to yield the integrated F_S flux over 121 days. Using midmorning rates to reflect daily fluxes required the assumption that midmorning rates represented the average daily rate, which was unlikely. In order to adjust for the close coupling of photosynthetic and respiratory fluxes (i.e. respiration also declines at night)

observed in similar sized trees in other studies (Wertin and Teskey 2008), daily fluxes were multiplied by 0.5. This further reflected that rates likely declined at night due to lower temperatures, and likely declined later in the day due to lower soil moisture availability, as watering was done each morning (Fang and Moncrieff 2001; Qi and Xu 2001; Dilustro, Collins et al. 2005). The magnitude of this adjustment was arbitrary, but it does not alter the validity of modeling efforts as a treatment index.

Various ratios (e.g. CUE) were calculated from the fluxes and biomass pools described above. Data was transformed as necessary to meet statistical assumptions, although all reported values are untransformed. All variables were analyzed in PROC MIXED with block as a random effect, and comparisons were made in PROC GLM with Tukey's HSD test at a significance level of $\alpha = 0.10$.

7.2.3. Model Results and Discussion

Fertilizer application increased GPP and resulted in corresponding increases in the absolute magnitudes of NPP, ANPP, and ΔC_R (Figure 7-1; Table 7-3; $p < 0.01$). A clone-by-fertilizer interaction occurred for APR, whereby both clones showed increases with fertilizer application, but clone 34 increased more ($p < 0.05$). Partitioning to TBCF and F_S also showed clone-by-fertilizer interactions ($p < 0.01$). TBCF was not different between fertilizer treatments in clone 34 due to a reduction in F_S coupled with an increase in ΔC_R as a result of fertilizer application. By contrast clone 769 showed a significant increase in TBCF with fertilizer application that was the result of increased ΔC_R but no significant F_S response to fertilizer application.

When considered on the basis of percentage of GPP partitioned, rather than on terms of absolute fluxes and pools, similar trends emerged. Fertilizer application resulted in increased CUE in both clones (Table 7-3; $p < 0.01$). Overall clone 769 had slightly greater CUE ($p < 0.10$). Greater proportional allocation to ANPP was observed in fertilized ramets of both clones ($p < 0.01$), although the clones were not different in this regard ($p > 0.10$). For APR clone 34 showed no effect of fertilizer application, while clone 769 decreased partitioning from 50.9% to 41.5% of GPP ($p < 0.10$). Conversely, for TBCF clone 769 showed no effect of fertilizer application,

while clone 34 decreased partitioning from 36.2% to 27.3% of GPP ($p < 0.10$). These results contrasted with those based on absolute fluxes, and were driven by both clones reducing partitioning to F_S as a portion of TBCF when fertilized, but clone 34 doing so to a greater extent than clone 769 ($p < 0.10$).

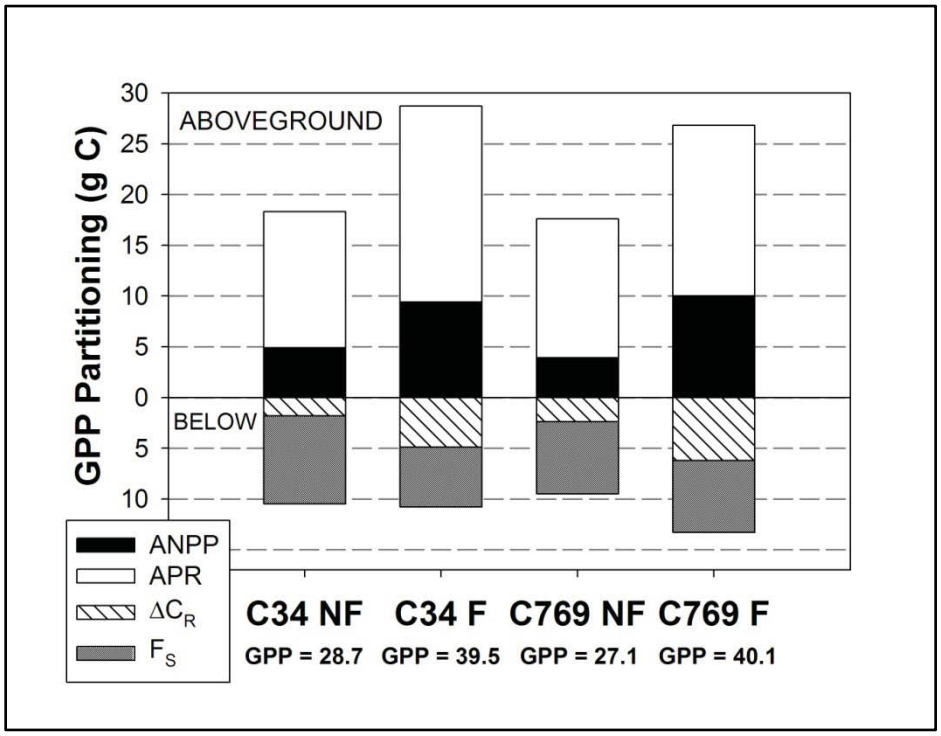

Figure 7-1. Carbon budget for two clones (C34 and C769) under two fertilizer regimes (F = fertilizer, NF = no fertilizer) over 121 days. GPP = gross primary productivity, ANPP = aboveground net primary productivity, APR = aboveground plant respiration, F_S = soil CO_2 efflux, and ΔC_R = C accumulation in roots, or belowground net primary productivity. Statistics are shown in Table 7-3.

Interpretation of these results indicated that while both clones showed remarkably similar absolute values of GPP, and GPP responses to fertilizer application, they did so by partitioning GPP in different ways in response to fertilizer application. Clone 769 partitioned less of GPP belowground in controls but more in the fertilizer treatment, indicating that plant-soil interactions could be affected by clone-specific GPP partitioning patterns in response to fertilizer application. Differences CUE showed that the clones were similar without fertilizer application, but with fertilizer application clone 769 had greater growth efficiency. Thus while the clones may perform similarly on a nutrient deficient site, with fertilizer application clone 769 would

likely be the best performer based on this data. This conclusion pertains to total biomass, not only to stem mass or volume as has been discussed in previous chapters. As with the conclusions presented in Chapter 4, 5, and 6, a range of clonal responses to fertilizer application suggests opportunities for clone-specific silvicultural systems, and ideotype based clonal selection.

Table 7-3. Treatment means and statistics for C allocation in a four month greenhouse experiment with two clones under two fertilizer treatments. Means are shown with standard errors in parentheses. Different letters denote significant differences ($p < 0.10$) based on Tukey's HSD test. P-values are shown in the rightmost three columns. Acronyms are defined in Table 7-1.

Variable	C34 Control	C34 Fert	C769 Control	C769 Fert	Overall Mean	Clone	Fert	C X F
GPP (g C)	28.7 (1.4) A	39.5 (1.5) B	27.1 (0.9) A	40.1 (2.7) B	33.9 (1.4)	0.60	**<0.01**	0.51
NPP (g C)	6.7 (0.6) A	14.2 (0.5) B	6.3 (0.4) A	16.2 (1.0) B	10.8 (0.9)	0.48	**<0.01**	0.19
ANPP (g C)	4.9 (0.5) A	9.4 (0.5) B	3.9 (0.3) A	10.0 (0.9) B	7.0 (0.6)	0.72	**<0.01**	0.16
APR (g C)	13.4 (0.5) A	19.3 (0.9) B	13.7 (0.3) AB	16.8 (1.6) BC	15.8 (0.6)	0.27	**< 0.01**	**0.05**
TBCF (g C)	10.4 (0.6) A	10.8 (0.7) A	9.5 (0.5) A	13.3 (0.5) B	11.0 (0.4)	0.18	**<0.01**	**<0.01**
F_S (g C)	8.7 (0.6) A	5.9 (0.5) B	7.1 (0.4) AB	7.1 (0.4) AB	7.2 (0.3)	0.68	**<0.01**	**<0.01**
ΔC_R (g C)	1.8 (0.2) A	4.9 (0.4) B	2.4 (0.3) A	6.2 (0.4) C	3.8 (0.4)	**< 0.01**	**< 0.01**	0.85
CUE (%)	22.9 (1.4) A	36.2 (1.0) B	23.0 (1.0) A	40.6 (1.4) C	30.7 (1.5)	**0.09**	**<0.01**	0.11
ANPP/GPP (%)	16.8 (1.0) A	23.8 (1.1) B	14.2 (1.0) A	24.7 (1.2) B	19.9 (1.0)	0.42	**<0.01**	0.10
APR / GPP (%)	47.0 (1.5) A	48.9 (0.9) A	50.9 (1.1) A	41.5 (1.3) B	47.1 (0.9)	0.15	**<0.01**	**<0.01**
TBCF / GPP (%)	36.2 (0.8) A	27.3 (1.4) B	35.0 (1.1) A	33.7 (1.7) A	33.1 (0.9)	**0.02**	**<0.01**	**<0.01**
F_S / TBCF (%)	83.2 (1.9) A	54.6 (2.5) B	74.9 (1.9) C	53.2 (2.2) B	66.5 (2.5)	**0.03**	**<0.01**	0.13

Limited inferences may be drawn from comparisons of our results with ecosystem-level studies in older stands due to differences in processes between tree ages and between single-tree and stand scales. Nonetheless, previous research in older stands has found that fertilizer amendment typically does not result in large changes in CUE, contrary to our results (Lai, Katul et al. 2002; Giardina, Ryan et al. 2003; Maier, Albaugh et al. 2004). While we found that even control treatments represented a net C sink (GPP was positive), results from a 12-year-old stand with the same soil indicate control treatments may not be an atmospheric C sink, with GPP values of approximately zero (Maier, Albaugh et al. 2004). The proportion of GPP partitioned to respiration has also been found to vary little across treatments, again conflicting with our results (Litton, Raich et al. 2007). However, in the one study did show an effect on APR / GPP, an

increase was observed (Giardina, Ryan et al. 2003), contrary to the reduction observed in our study for clone 769. However, when our results are compared with the only study we are aware of comparing GPP partitioning among clonal seedlings under different levels of fertilizer application (Bown, Watt et al. 2009), our results were surprisingly consistent. Bown et al. observed clone-by-fertilizer interactions for CUE and APR / GPP, fertilizer effects for F_S / TBCF and ANPP / GPP, and clonal effects for ANPP / GPP and TBCF / GPP. This further supports that while pot-based seedling studies may produce similar results, these results should not be inferentially scaled to the ecosystem level for older plantations.

The model results do provide greater context for individual flux and biomass results presented in Chapters 4, 5, and 6. ANPP and ΔC_R were both increased by fertilizer but, only ΔC_R differed between clones. These results are the same as those presented in Chapter 4, as expected since they are based on the same data, and are indicative that the regression of stem dimensions to biomass applied in this chapter performed adequately, which the regression statistics suggested. Results presented in Chapter 5 for aboveground mass-specific respiration (R_{AG}) differed from results presented in this chapter when scaled to tree size and integrated over time. R_{AG} showed only a weak fertilizer effect in Chapter 5 when expressed on a mass-specific basis, but when scaled to tree size, APR showed a significant clone-by-fertilizer interaction. This most likely resulted from treatment effects on aboveground biomass. Trends in F_S were relatively consistent between Chapter 5 and the results presented in this chapter. In both chapters clone 34 had the highest flux in control ramets and the lowest flux with fertilizer application, while clone 769 had an intermediate flux that did not appear to show a fertilizer response. The lack of a clonal effect observed for leaf or canopy-level A_{SAT} rates or foliar mass or area in Chapter 6 was entirely consistent with the lack of difference between clones in GPP, reinforcing both conclusions since these represented two completely distinct methodologies for assessing whole-tree C gain.

7.3. Synthesis of Experiments and Conclusion

Differences among clones in growth, crown form, and allometry may represent opportunities for clone-specific silvicultural systems to be applied to optimize growth rates while simultaneously

selecting clones with traits chosen for specific products. Companies practicing clonal forestry have the opportunity to select clones that perform similarly across a variety of traits, and thus simplify their management systems for clonal plantations. Alternately they may select clones adapted to different site conditions or silvicultural systems, as results of numerous studies including those presented in this manuscript suggest some clones may be better suited to specific silvicultural systems than others.

For example, Table 7-4 shows that clone K from the Reynolds Homestead trial would be ideal for maximizing biomass (above or belowground) and stem volume production. Clone K had deep roots, a large crown, a high growth efficiency, and without fertilizer application showed no incidence of sinuosity or cold damage. It may be ideal for deployment with minimal or no application of fertilizer on certain sites. However, when fertilizer is applied, clone I2 may be advantageous over clone K in terms of stem volume production, although it may be disadvantageous by comparison with regard to other traits (e.g. less root mass). Essentially, for whatever range of traits are preferred for a particular management objective (e.g. maximizing growth, biomass, stocking, or belowground C sequestration) a clone with appropriate traits can be selected and deployed.

Table 7-4. Comparison of the ten clones described in Chapter 3 based on different traits. The best three clones are listed for each trait based on overall ranking (all ramets), unfertilized ranking, and fertilized ranking. All clones with no incidence of sinuosity or cold damage are listed for those traits.

	Best Performing Clones		
Trait	Overall	No Fertilizer	Fertilizer
Largest Total Mass	I2, K, D	K, I2, H2	D, I2, F2
Largest Root Mass	K, I2, F2	K, I2, F2	K, D, F2
Largest Stem Mass	K, I2, H2	K, I2, H2	D, I2, H2
Largest Stem Volume	I2, K, H2	K, I2, H2	I2, F2, H2
Lowest Branch Number	H2, C1, H1	I3, H2, C1	H1, H2, C1
Largest Crown Volume	H2, D, K	K, I2, H2	H2, D, F2
Smallest Crown Volume	H1, F1, C1	C1, F1, H1	H1, C2, I3
Highest Growth Efficiency	H1, C1, K	C1, H1, F1	H1, F1, I2
Deepest Rooting	F2, K, I2	F2, I2, K	F2, D, K
No Sinuosity	C2, H1, H2, I3	C1, C2, H1, H2, I3, K	C2, H1, H2, I3
No Cold Damage	C1, F1, H1, H2	C1, C2, D, F1, H1, H2, I2, K	C1, F1, H1, H2

Based on the results of the Reynolds Homestead clone-by-fertilizer trial, and the greenhouse short-term ecophysiology study, clones respond differently to fertilizer application in their stem volume growth, allometry, growth efficiency, and the magnitude and timing of whole-tree C allocation and fluxes. Short-term changes in allometry appear to be one mode by which trees acclimate to fertilizer application immediately after changes in soil nutrient availability. Short-term changes in C allocation lead to long-term fertilizer growth responses through changes in morphology. Further, clones may differ in their allometric responses, indicating some may be fundamentally more responsive to fertilizer application based on their ecophysiology. Variability among clones in C allocation belowground and to respiratory fluxes and root exudation suggests that clones differ in plant-soil interactions that may affect C sequestration and soil organic matter dynamics. Consideration of clone-by-silviculture interactions in future clonal plantations may prevent problems from occurring and offer unique opportunities for forest managers.

7.4. Literature Cited

Bown, H. E., M. S. Watt, P. W. Clinton, E. G. Mason and D. Whitehead (2009). "The influence of N and P supply and genotype on carbon flux and partitioning in potted *Pinus radiata* plants." Tree Physiology **29**(7): 857-868.

Dilustro, J. J., B. Collins, L. Duncan and C. Crawford (2005). "Moisture and soil texture effects on soil CO_2 efflux components in southeastern mixed pine forests." Forest Ecology and Management **204**(1): 85-95.

Fang, C. and J. B. Moncrieff (2001). "The dependence of soil CO_2 efflux on temperature." Soil Biology & Biochemistry **33**(2): 155-165.

Giardina, C. P. and M. G. Ryan (2002). "Total belowground carbon allocation in a fast-growing *Eucalyptus* plantation estimated using a carbon balance approach." Ecosystems **5**(5): 487-499.

Giardina, C. P., M. G. Ryan, D. Binkley and J. H. Fownes (2003). "Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest." Global Change Biology **9**(10): 1438-1450.

Lai, C. T., G. Katul, J. Butnor, M. Siqueira, D. Ellsworth, C. Maier, K. Johnsen, S. Mckeand and R. Oren (2002). "Modelling the limits on the response of net carbon exchange to fertilization in a South-Eastern pine forest." Plant Cell and Environment **25**(9): 1095-1119.

Litton, C. M., J. W. Raich and M. G. Ryan (2007). "Carbon allocation in forest ecosystems." Global Change Biology **13**(10): 2089-2109.

Maier, C. A., T. J. Albaugh, H. L. Allen and P. M. Dougherty (2004). "Respiratory carbon use and carbon storage in mid-rotation loblolly pine (*Pinus taeda* L.) plantations: The effect of site resources on the stand carbon balance." Global Change Biology **10**(8): 1335-1350.

Norman, J. M., C. J. Kucharik, S. T. Gower, D. D. Baldocchi, P. M. Crill, M. Rayment, K. Savage and R. G. Striegl (1997). "A comparison of six methods for measuring soil-surface carbon dioxide fluxes." Journal of Geophysical Research-Atmospheres **102**(D24): 28771-28777.

Qi, Y. and M. Xu (2001). "Separating the effects of moisture and temperature on soil CO_2 efflux in a coniferous forest in the Sierra Nevada mountains." Plant and Soil **237**(1): 15-23.

Ryan, M. G. (1991a). "Effects of climate change on plant respiration." Ecological Applications **1**(2): 157-167.

Ryan, M. G. (1991b). "A simple method for estimating gross carbon budgets for vegetation in forest ecosystems." Tree Physiology **9**(1-2): 255-266.

Ryan, M. G., R. M. Hubbard, S. Pongracic, R. J. Raison and R. E. McMurtrie (1996). "Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status." Tree Physiology **16**(3): 333-343.

Wertin, T. M. and R. O. Teskey (2008). "Close coupling of whole-plant respiration to net photosynthesis and carbohydrates." Tree Physiology **28**(12): 1831-1840.

"You're still here? It's over. Go home. Go."

-Ferris Bueller's Day Off (1986)