A PHYSIOLOGICAL AND MORPHOLOGICAL ANALYSIS OF THE EFFECTS OF NITROGEN SUPPLY ON THE RELATIVE GROWTH RATES OF NINE LOBLOLLY PINE (*Pinus taeda* L.) CLONES

A Thesis

by

COREY MICHAEL STOVER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2005

Major Subject: Forestry

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Approved as to style and content by:

Mark Tjoelker (Chair of Committee)	David Briske (Member)
Tom Byram (Member)	C.T. Smith (Head of Department)

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ABSTRACT

A Physiological and Morphological Analysis of the Effects of Nitrogen Supply on the Relative Growth Rates of Nine Loblolly Pine (*Pinus taeda* L.) Clones. (May 2005)
Corey Michael Stover, B.S., Texas A&M University
Chair of Advisory Committee: Dr. Mark Tjoelker

The influence of nitrogen supply on relationships of relative growth rate (RGR) to leaf physiology, structural and non-structural carbon partitioning, and nitrogen- and water-use efficiencies were examined in loblolly pine (*Pinus taeda* L.) clonal lines differing in growth potential. Nine 18-month-old loblolly pine clones were grown in a climate-controlled greenhouse for 20 weeks under two contrasting nitrogen (N) regimes (50 and 250 ppm) and a growth analysis was carried out. Higher nitrogen increased plant RGR and largely resulted in proportional shifts in biomass from roots and stems to needles. The RGR of plants receiving higher nitrogen was increased primarily through increased leaf area ratio (LAR), which was increased through higher leaf mass fraction (LMF) and not through changes in needle morphology. Although concentrations of needle glucose in plants receiving 250 ppm N were 22 percent higher than plants receiving lower N, total non-structural carbohydrate concentrations in needles of plants receiving 50 ppm N were nearly double that of clones receiving 250 pm N, primarily due to starch accumulation of the nitrogen-deficient plants. Plants receiving 250 ppm N also had 39 and 18 percent lower starch in the coarse and fine roots, respectively. Plants receiving higher nitrogen were also more water-use efficient, but had lower photosynthetic nitrogen-use efficiency. LAR, net assimilation rate (NAR), specific leaf area (SLA), and LMF were all positively correlated with RGR, but the main influence on RGR differences among clones was LAR. In addition, leaf-level rates of photosynthesis and respiration were positively correlated with RGR; however, faster-growing clones did not exhibit greater carbon economy at the leaf level. Both instantaneous water-use efficiency (A/E) and $\delta^{13}C$ were positively correlated with RGR and photosynthetic nitrogen-use efficiency was negatively correlated with RGR. The identification of physiological and

morphological traits underpinning differences in RGR among clones and how these traits are affected by nitrogen supply provides new information on trait correlations within species and parallels broader patterns observed among species.

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I. INTRODUCTION

According to the Food and Agricultural Organization, global demand for timber products is increasing at 1.5 to 2 percent per year, even as the land-area cover of the world's forests are decreasing (FAO 1999). As the land base for forestry decreases, a great deal of emphasis is being placed on increasing the productivity of existing forests, particularly industrial forest plantations, to meet the rising demand for forest products. Plantations of genetically improved trees have been recognized as being critical in providing reliable, ecologically sustainable, and economically affordable wood supplies (Retzlaff et al. 2001).

McKeand et al. (2003) recently stated that the productivity of southern United States plantations has local, regional, and global implications. In the past four decades, the southern United State's share of domestic timber production has increased from 41 to 58 percent and its share of world production has increased from 6.3 to 15.8 percent (McKeand et al. 2003). Foresters in the southern United States are responsible for more than 75 percent of the nation's tree planting, and more than 95 percent of the seedlings are genetically improved loblolly and slash pines (McKeand et al. 2003). Loblolly pine (*Pinus taeda* L.) is one of the most important tree species in the southern United States, with over 800 million seedlings planted annually (McKeand et al. 1999).

Loblolly pine (*Pinus taeda* L.) has a wide geographic distribution throughout the southeastern United States ranging from Texas in the west to southern New Jersey and central Florida in the east. Given a broad natural geographic distribution, it is common to find significant provenance and genotypic variation in growth (Bongarten and Teskey 1987). Provenance studies have shown differences in survival, growth, disease resistance, drought hardiness, and cold hardiness attributable to geographic source of the seed (Dorman 1976). For example, loblolly pine exhibits geographic variation in

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susceptibility to fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*). Some of the trait variation seen in loblolly pine is discontinuous, suggesting the species may consist of more than one ecotype. For example, loblolly pines from the geographically disjunct "Lost Pines" population in central Texas are more drought resistant than pines from elsewhere in the range. Al-Rabab'ah and Williams (2004) proposed that the "Lost Pines" population was part of the western refugium for *P. taeda* during Pleistocene glaciation. Clinal effects in growth also are exhibited by loblolly pine, with trees from coastal areas typically growing faster than those from inland sources (Dorman 1976).

Over the past few decades, tree improvement programs have exploited this genotypic variation to make significant gains in the productivity of loblolly pine plantations. Selection for fast-growing genotypes has increased yields on the order of 10 to 20 percent on forestlands in commercial production (Perry 1998). It is estimated that genetic gains from tree improvement programs within the southern United States vary, but in general, gains in volume production per unit area average 10 to 30 percent over unimproved planting stock (McKeand et al. 2003, Atwood et al. 2002, and Martin and Shiver 2002). Much of this effort has been expended to maximize productivity through genetic selection of trees that maximize the capture of carbon (C) and conversion into standing crop biomass (Retzlaff et al. 2001).

Although significant gains have been made through the deployment of elite loblolly pine genotypes, a large amount of intraspecific variation in growth is observed, even among progeny of crosses of elite loblolly parental genotypes. The quantification and control of intraspecific variation among elite loblolly pine genotypes is a necessary step to maximize gains in the productivity of forest plantations. The selection and use of clones of loblolly pine enables the control of intraspecific variation in elite loblolly pine deployments and has the potential to enhance uniformity in growth characteristics and other traits. Clonal forestry is becoming a practical reality with recent successes in mass propagation via rooted cuttings and somatic embryogenesis (McCall and Isik 2003). Clonal forestry consists of identifying individuals within crosses of elite parental genotypes that show superior growth and form and then mass-producing these

individuals through rooted cuttings or somatic embryogenesis. The selected mass-produced replications are genetically identical. Because clonal forestry is still in its infancy, few studies have been performed with clones to investigate how physiological and morphological traits of clones affect growth, especially in loblolly pine. Studies of how these traits respond to various environmental conditions are also lacking.

It is incompletely understood which growth traits are driving differences in growth potential among elite loblolly pine genotypes. Growth traits are phenotypic traits that plants exhibit when growing in specific environments and exhibit varying degrees of both genetic and environmental variation. Important morphological growth traits include biomass allocation patterns and the structure of plant parts (e.g. leaf morphology) that together determine key whole-plant processes such as leaf area development. Important physiological traits include leaf-level rates of photosynthesis and respiration, which underpin net carbon uptake. In addition, water-use efficiency and nutrient-use efficiency express the efficiency of carbon gain at leaf or plant level in relationship to water and nutrients, potentially influencing plant response to water and nutrient supply. Also, carbohydrate storage patterns reflect the relative balance between source and sink tissues. Ecophysiological investigations at the individual whole-tree level are an essential part of elucidating the growth traits and factors controlling the biological potential for tree improvement. Better understanding of the growth traits (or determinants) of inherently fast and slow-growing trees can provide valuable information to tree improvement programs by identifying physiological and morphological traits that can be exploited by tree breeders to potentially increase productivity through selective breeding. Due to the amount of growth trait variation within a species or even a family within a species, it is difficult to ascertain which traits or combinations of traits are underpinning differences in growth. Many studies have shown that there are broad interspecific patterns linking physiological and morphological traits and their effects on plant growth. Whether or not intraspecific differences in these traits follow these same broad patterns is not completely understood. Because clones are genetically identical, clones are useful and important tools for

identifying the traits underpinning intraspecific variation in growth rate among elite genotypes of loblolly pine. In addition, the use of genetically uniform individuals enables the quantification of the importance of phenotypic trait plasticity when grown in contrasting environments.

II. RELEVANT LITERATURE

Interspecific differences in relative growth rate (RGR) are of central importance in plant ecology because plant size and growth are important determinants of survival and reproduction in nature (Shipley 2002). When the external environment is constant, interspecific differences in RGR must be due to interspecific differences in plant physiology and morphology. It is therefore important to know how physiological and morphological differences contribute to variation in RGR (Shipley 2002).

The underlying physiological and morphological mechanisms controlling the growth rates of plants and plant responses to environmental conditions are incompletely understood. According to Lambers et al. (1998), leaf area ratio (LAR, the ratio of plant leaf area to plant dry mass) and whole-plant net assimilation rate (NAR, the rate of whole-plant dry mass gain per unit plant leaf area) are treated as driving variables of plant growth (see Appendix A). Researchers have commonly found that RGR is positively correlated with LAR, leaf mass fraction (LMF, the ratio of leaf dry mass to plant dry mass) and specific leaf area (SLA, the ratio of leaf area to leaf dry mass). However, this is only part of the equation. RGR also depends on NAR, which is dependent upon leaf-level carbon gain through photosynthesis and whole-plant carbon loss through respiration. Many studies have tried to link plant growth rate variation to physiological and morphological attributes. To date, these linkages remain incompletely understood.

Tilman (1988) hypothesized that "allocation to roots or stems comes at a cost – a decreased maximal rate of vegetative growth." Thus, increased allocation to below ground biomass equates to less above ground biomass and therefore lower growth rates. Poorter and Remkes (1990) carried out an experiment to test this hypothesis with 24 herbaceous C_3 species. Among species, they found a positive correlation with LMF and RGR, as a doubling in RGR was associated with a 22 percent increase in LMF. These results agree with Tilman's prediction that a larger investment in leaves is related to a higher growth rate, but LMF cannot fully explain the observed pattern quantitatively

(Poorter and Remkes 1990). They found a stronger positive correlation between LAR and RGR, as a doubling in RGR was associated with an 82 percent increase in LAR among species. These results suggest that LAR is a stronger determinant of RGR than LMF among species that differ widely in inherent growth rate. SLA is also an important determinant of growth and is directly related to whole-plant LAR, since the fraction of plant mass in leaves (LMF) multiplied by the SLA is, by definition, LAR.

Rates of photosynthesis and respiration are the key physiological traits associated with variation in NAR. Slower-growing species have lower rates of photosynthesis and use more of their carbon for respiration, mostly in their roots, whereas faster-growing species generally have higher rates of photosynthesis and use more of their newly fixed carbon for growth and lose less to respiration (Poorter et al. 1991, Tjoelker et al. 1998, 1999). Poorter et al. (1990) found that among fast- and slow-growing species, rates of net photosynthesis and respiration per unit shoot and respiration per unit root biomass were positively correlated with whole-plant RGR; however, photosynthesis per unit leaf area was not related to RGR due to the higher SLA of the faster-growing than slower-growing species. Thus, whole-plant growth is a function not only of allocation among plant parts, but also the specific rates of carbon gain and loss of plant parts.

Research on conifer seedlings has uncovered strong positive correlations with leaf mass-based rates of photosynthesis and RGR (Reich et al. 1998, Walters et al. 1993). In a study comparing the interspecific variation of RGR in nine boreal tree species, Reich et al. (1998) investigated whether differences in photosynthesis, respiration, and nitrogen uptake contribute to the differences in RGR. They found strong positive correlations between RGR of seedlings of the nine species and mass-based rates of light-saturated photosynthesis, nitrogen uptake rate, leaf dark respiration, and root respiration. Walters et al. (1993) also found a strong correlation with RGR and leaf mass-based photosynthesis and respiration rates for all plant organs in a study with three hardwood species. Walters et al. (1993) observed that RGR was positively correlated with both LAR and LMF and a stronger relationship existed between RGR and LAR in a high-light than low-light environment.

Investigations of differences in RGR among plant species have identified a number of underlying mechanisms responsible for growth differences. These studies have also revealed the magnitude of differences in RGR among species. In an extensive study on interspecific differences of RGR among 130 herbaceous annuals and perennials and tree seedlings, Grime and Hunt (1975) reported that RGR ranged more than twelve-fold from 31 mg g^{-1} day^{-1} to 386 mg g^{-1} day^{-1}. Although variation of RGR within plant species was estimated to be only 11 percent of the variation between species (Hunt 1984), the amount of intraspecific variation was still great.

In a study comparing intraspecific genetic variation in RGR of *Plantago major* L., Dijkstra and Lambers (1989) found that rates of leaf-level net photosynthesis and dark respiration on a leaf-area basis were 23 and 30 percent lower in the fast-growing line compared to the slow-growing line. NAR was also 11 percent higher in the slow-growing line. However, LMF was slightly higher and SLA was 30 percent higher in the fast than slow-growing line, resulting in increased LAR in the faster-growing line. The higher LAR of the faster-growing line more than offset the advantage of the higher NAR of the slow-growing line. Dijkstra and Lambers (1989) concluded that lower RGR of the slow grower was due to decreased SLA and reduced LAR, which was only partially compensated for by its higher leaf-level photosynthetic rate.

Genetic differences in net carbon gain in trees could be a function not only of differences in photosynthetic rate and total leaf area, but also of differences in respiration and immediate metabolic demands of leaf tissue, including partitioning to storage carbon (Yang et al. 2002). Yang et al. (2002) investigated diurnal and seasonal effects of fertilizer on leaf-level carbon acquisition and partitioning on recently assimilated carbon in fast- and slow-growing families of loblolly pine in a field experiment. Both of the slow-growing families exhibited higher leaf concentrations of total non-structural carbohydrates (TNC) and starch than the two faster-growing families. Optimal fertilization (maintaining foliar nutrient concentrations at levels described by Albaugh et al. 1998) decreased TNC and starch concentrations in all families during the growing season between the months of March and November.

Although some family and treatment differences were observed in the foliar concentrations of total non-structural carbohydrates (TNC) and starch, they concluded that growth differences among the families were not a function of differences in carbon acquisition or partitioning at the leaf level. Ongoing research at the same site suggests that growth differences may be closely associated with crown-level light interception and genetic differences in whole-tree carbon allocation, in particular, the carbon demands of the fine root system (Yang et al. 2002).

Nitrogen availability has been shown to have a positive relationship with RGR (Hirose 1988, Ingestadt 1979, van der Werf 1993b), but often correlations between RGR and other traits are unstable at varying nitrogen availabilities. In a study of the effects of N-supply on growth traits of inherently fast- and slow-growing monocotyledonous species, van der Werf et al. (1993b) reported that shoot respiration was negatively correlated with RGR and root respiration was positively correlated with RGR for plants receiving higher nitrogen, but both of these traits were not correlated to RGR in plants receiving lower nitrogen. Van der Werf et al. (1993a) reported that nitrogen productivity (g of plant biomass mol^{-1} plant N day^{-1}) and LAR were positively correlated with RGR under high N, but both were negatively correlated with RGR under lower N. Van der Werf et al. (1993b) found that photosynthetic nitrogen-use efficiency (PNUE) of faster-growing plant species was higher than the slower-growing species when given higher nitrogen, but PNUE was not significantly different among the species at lower levels of nitrogen supply. In addition, they found that nitrogen productivity of the faster-growing species decreased with decreasing nitrogen supply, whereas that of the slower-growing species remained the same as nitrogen supply decreased.

Nitrogen (N) availability is also one of the most important factors affecting growth partitioning in plants. In a seedling study investigating biomass partitioning for 23 open-pollinated loblolly pine families, low nitrogen availability resulted in significantly more biomass allocation to roots than in seedlings receiving a high N treatment (Bailian et al. 1991). Data from their study showed that compared to slow-

growing families, the faster-growing families had increased proportions of root biomass in low N and had the smallest standing crop of root biomass under the high N treatment.

Retzlaff et al. (2001) examined the genetic and fertilizer effect of whole-tree biomass allocation of fast and slow-growing loblolly pine families from each of two provenances – the Atlantic Coastal Plain and the Lost Pines in Texas. Optimal fertilization (maintaining foliar nutrient concentrations at levels described by Albaugh et al. 1998) increased aboveground and total tree biomass in all families in a field study. The faster-growing families had greater amounts of above- and below-ground biomass than the slower-growing families under both unfertilized and optimum fertilization treatments. Although differences in standing-crop biomass (roots, shoots, and foliage) among families and fertilizer treatments were observed, percent biomass partitioning to different tissues remained similar across families and fertilizer treatments. Under fertilization, both the fast- and slow-growing Atlantic Coastal Plain families as well as the fast-growing Lost Pines family had very similar above- and below-ground biomass and all were significantly higher than the slow-growing Lost Pines family. In all families, fertilization treatment resulted in increased total nonstructural carbohydrate (TNC) concentrations in roots, particularly starch concentrations in taproots. The Lost Pines families had higher levels of foliar starch than the Atlantic Coastal Plain families. The Lost Pines families also partitioned more TNC to needle tissues as starch on a percentage basis than the Atlantic Coastal Plain families. TNC analysis in this study suggests that genetic differences in aboveground growth are not the result of differences in carbon partitioning to the various TNC fractions (starch vs. soluble carbohydrates), but possibly the result of higher root system C demands of the slower growers.

Birk and Matson (1986) found that starch concentrations during the growing season were lower in current-year foliage of loblolly pine from high fertility sites than low fertility sites. Ludovici et al. (2002) also found that fertilization treatments (no fertilization versus optimal fertilization – as described by Albaugh et al. 1998) decreased growing season starch concentrations in all tissues (foliage, branch, bark, stem, and root) compared with unfertilized treatments, but that the absolute amount of starch was greater

in fertilized treatments than in unfertilized treatments due to increased biomass production (larger trees). In general, reductions in starch concentrations in response to increased nutrient supply are thought to reflect a reduction in carbon sink-limited growth (Chapin et al. 1990).

Within populations, intraspecific variation in physiological and morphological traits can lead to certain individuals exhibiting superior growth. In the practice of forestry, even small differences in intraspecific relative growth rate could compound through time, resulting in large differences in stand productivity (Atwood et al. 2002, Martin and Shiver 2002). Increased productivity of stands can be realized through exploitation of intraspecific variation in RGR among elite genotypes. This can be accomplished by selecting individuals exhibiting superior growth and mass multiplying these individuals through cloning.

Many questions concerning the growth strategies of trees have yet to be answered. It is not completely understood how variation in physiological and morphological traits affects tree growth. In order to explore these gaps, I used a continuum of nine slow to fast-growing loblolly pine clones with marked variation in initial size and growth potential. The use of genetically identical individuals of contrasting clones enabled a comparison of potential genetic differences in morphology and physiological traits and their correlations with variation in growth rates. Moreover, examination of growth trait variation among clones provided a test of the genetic underpinnings of broader patterns of growth trait correlations observed among taxa at family or species scales and provided new information on correlated trait variation in plants.

The objective of this study was to identify the physiological and morphological traits underpinning differences in relative growth rates of loblolly pine clones grown under contrasting nitrogen regimes. I hypothesized that inherently faster-growing clones attain higher relative growth rates by (1) exhibiting greater carbon economy at the leaf level than slower-growing clones by having a higher rates of net photosynthesis or ratio of net photosynthesis to leaf dark respiration, or by (2) exhibiting greater carbon gain at

the whole-plant level by partitioning more biomass to foliage mass or area. Alternatively, (3) faster-growing clones attain higher growth rates through a combination of altered leaf-level rates of carbon gain and whole-plant biomass allocation. I also hypothesized that nitrogen addition will increase relative growth rates by (1) increasing leaf area production and/or (2) increasing leaf-level net photosynthesis. Furthermore, nitrogen deficiency will cause accumulation of non-structural carbohydrates as nitrogen deficiency retards growth more than photosynthesis.

III. MATERIALS AND METHODS

Clonal plant material

A continuum consisting of nine loblolly pine clones ranging from relatively slow growers to fast growers under greenhouse conditions were examined in this study. The nine clones were selected from a large group of propagated clones on the basis of height relative to one another at an age of 12 months. Sixty ramets of each selected clone were obtained from the Temple Inland Forest Products Corporation nursery (Jasper, Texas, USA) in mid-June of 2003. Prior to selection for this study, the clones had been propagated by means of somatic embryogenesis (CellFor, Vancouver, BC, Canada) and grown until 2.5 cm tall prior to transplanting into 164 cm^2 Ray Leech conetainers® (Steuwe and Sons, Inc., Corvallis, Oregon, USA) containing 60 percent peat and 40 percent (by volume) coarse vermiculite. Each ramet was fertilized with one gram of 13-13-13 slow release Osmocote (Scotts Company, Inc., Marysville, Ohio, USA). The ramets were then moved into a greenhouse and spaced at a density of 540 per m^2.

The clones selected for this study originated from among progeny of six elite families generated via controlled pollination crosses (Table 1). The 12-month-old plants of the selected clones were transplanted individually into 11.4-liter square tree-pots® (Steuwe and Sons, Inc., Corvallis, Oregon, USA) with a 20 x 20 cm top and a depth of 38 cm. A mixture consisting of 50 percent peat and 50 percent coarse vermiculite (by volume) was used as the growing medium. The plants were grown in a greenhouse equipped with evaporative cooling pads and heated by passive trapping of long-wave radiation and supplemented with electric heaters. The plants were watered regularly to saturation with deionized water. 400 mL of a fertilizer solution (Peters 20-20-20 with micros, J.R. Peters, Inc., Allentown, PA, USA) was added once in mid-October. In November, the temperature in the ventilated greenhouse was maintained as close to the outside temperature as possible as part of an over-wintering treatment designed to initiate resting bud formation. The plants were exposed to the natural photoperiod. By mid-January, resting buds had satisfactorily developed in all of the plants.

Table 1. Family origin of clones of loblolly pine used in the study.

Family #	Controlled Pollination Cross (♀ x ♂)	Clone #
LP1	LSG-180 X LSG-008	7615, 7466, 7549
LP3	LSG-201 X LSG-104	7338
LP4	LSG-201 X LSG-062	7299
LP7	S4PT6 X LSG-008	7528
LP10	S4PT6 X 14-20	7378
LP11	LSG198 X 14-20	7924, 7544

On January 23, 2004, the plants were moved into a temperature-controlled greenhouse. Active growth was initiated by artificially extending the photoperiod to 14 hours of daylight (0600 to 2000 local standard time), using 1000-watt high-pressure sodium lamps in the morning and evening. During the 14-hour photoperiod, the lamps supplemented the natural sunlight when irradiance levels (photosynthetically active radiation (PAR) over the 400 to 700 nm waveband) fell below 700 watts per m^2. The average temperature of the greenhouse was maintained between 23 and 27 °C during the day and between 21 and 24 °C at night from January through March. The average temperature of the greenhouse was maintained between 24 and 29 °C during the day and between 21 and 24 °C at night from April through June. Greenhouse temperatures were maintained using evaporative cooling pads, gas-fired forced-fan heaters, and air circulation fans governed by a microprocessor-based control system.

Nitrogen treatment

Three weeks after transfer, when the plants were approximately 18-months old, nitrogen treatments were initiated. The treatment consisted of a modified half-strength Hoagland's solution containing all essential macro and micro-nutrients except nitrogen.

The nutrient solution was mixed just prior to each application and consisted of 1.0 mM $MgSO_4$, 1.25 mM K_2SO_4, 0.1 mM KH_2PO_4, 1.0 mM $CaSO_4$, 45.0 μM H_3BO_3, 9.1 μM $MnCl_2(4H_2O)$, 0.7 μM $ZnSO_4(7H_2O)$, 0.4 μM $CuSO_4(5H_2O)$, and 0.14 μM $NaMoO_4$. A 10 percent chelated iron solution containing 0.5 mL L^{-1} FeEDTA was also added. Two concentrations of nitrogen were added to the fertilizer solution. The concentrations consisted of a 50 ppm (0.625 mM) and 250 ppm (3.12 mM) total N solution of NH_4NO_3. Each week 500 ml of fertilizer solution was added to individual pots. Half of the plants of each clone received fertilizer with a 50 ppm nitrogen concentration and the remaining plants received fertilizer with a 250 ppm nitrogen concentration. In addition to the nutrient solution, pots were watered to capacity two to three times weekly with deionized water to ensure adequate water availability.

Experimental design

The experimental design was a randomized complete block design consisting of six blocks, arranged on greenhouse benches. Each block contained the nine clones and two fertilizer treatments (50 and 250 ppm N) in factorial combination with five replications (pots) of each clone and nitrogen treatment combination in each block for a total of 540 plants across the six blocks. The groups of 10 pots of each clone were randomly assigned a location within each block and nitrogen treatments were subsequently randomly assigned to each pot.

Growth analysis

The biomass growth data were collected in five destructive harvests at intervals of four to five weeks throughout the 20-week study. Destructive harvests took place on February 12, March 15, April 20, May 25, and June 28 of 2004. The first harvest was conducted upon initiation of the nitrogen treatments. At each harvest, one pot from each clone and nitrogen treatment combination was randomly selected from each of the six blocks. The plants were harvested and divided into separate tissues (needles, stems, and roots), oven dried (50 °C), and weighed to determine dry mass and dry mass fractions, including root mass fraction (RMF, g root g^{-1} plant), stem mass fraction (SMF, g stem g^{-1} plant), and

leaf mass fraction (LMF, g leaf g^{-1} plant). In addition to the destructive harvests, plant height was collected bi-weekly and diameter at the base of the stem was collected monthly throughout the course of the study. Relative growth rate (RGR) was calculated using a formula described by Hoffman and Poorter (2002):

$$RGR = \log_e(M_2) - \log_e(M_1)/(t_2 - t_1),$$

where M_1 and M_2 are the natural logarithm-transformed mean plant masses at time 1 (t_1) and time 2 (t_2) for each harvest interval. In addition, plots of natural log-transformed biomass against time were used to model plant growth throughout the experiment. The natural log-transformed plots were used to test whether nitrogen treatment significantly affected RGR of all clones combined and for individual clones and whether there were any clone x nitrogen treatment interactions. LAR was calculated by dividing total plant leaf area, determined for the first, third, and fifth harvests (see below) by total plant dry mass. Mean NAR was calculated on an interval basis by dividing increase in total plant dry mass between the first and third and third and fifth harvests by the mean of total leaf area between those two harvests.

Allometric analysis

Differences between N treatments and clones in dry mass allocation throughout the course of the experiment were examined using dry mass harvest data. Allometric relationships were examined, accounting for differences in plant size, by comparing the parameters in linear regression relationships of the form:

$$\log_e(y) = a + k \log_e(x),$$

where y is the dry mass of one plant part (roots, stems, or needles) and x is the total plant dry mass. The estimated parameters a and k are the y-intercept and slope of the allometric regression, respectively. Statistical tests of significant differences in intercepts and slopes were determined using analysis of covariance.

Needle morphological characteristics

A sub-sample of fresh needles from the first, third, and last harvests were collected from each harvested plant and frozen in sealed plastic bags prior to morphological analysis.

The samples were thawed at room temperature and digitally scanned at 400 dpi resolution with back-lighting using a calibrated flatbed optical scanner (STD 1600+, Epson, Long Beach, California, USA) and analyzed with WinSEEDLE analysis software (Version 2001a, Regent Instruments, Quebec City, Quebec, Canada) to determine total projected surface area, needle length, width, and volume. Subsequently, oven-dry mass (50 °C) of each sample was used to calculate specific leaf area (SLA, m^2 leaf area kg^{-1} leaf dry mass). Needles scanned from the first and third harvest were collected from the last fully elongated cohort on each of the plants and were formed during the previous growing season. Needles scanned from the last harvest were collected from the newest fully elongated cohort developed during the study.

Total non-structural carbohydrate analysis

Oven-dried needle and root tissues from the last harvest were pulverized in a sample mill (Tecator Cyclotec model 1093, Foss Analytical, AB, Sweden) and sub-sampled for analysis. Prior to grinding, root tissues were divided into two groups: (1) fine roots, consisting of roots less than 1 mm in diameter and (2) tap and coarse roots, consisting of the taproot and coarse roots greater than 1 mm in diameter. Only needles from the first fully elongated cohort developed during the study were sampled. Total non-structural carbohydrates (glucose and starch concentrations) analysis was conducted on dried and ground sub-samples using the method described by Oleksyn et al. (2000). Sugars were extracted from oven-dried tissue powder in methanol:chloroform:water (12:5:3 by volume), and the residue was used for starch determination. Extracted soluble sugars were determined colorimetrically with anthrone reagent at 625 nm. Starch in the insoluble material was converted to glucose with amyloglucosidase. Glucose obtained from the starch was measured with glucose oxidase by mixing the sample with glucose peroxidase/oxidase-*o*-dianisidine dihydrochloride reagent. Absorbance was measured at 540 nm after a 30-minute incubation at 37 ºC. Concentrations of soluble sugars and starch were expressed as parts per thousand of tissue dry mass. Soluble carbohydrate

concentrations were calculated from standard regression equations based on glucose and starch standard solutions.

Needle nitrogen concentration and carbon isotope composition

Analysis of nitrogen concentration and carbon isotope composition was conducted on dried and ground sub-samples of needle tissue collected from the first fully elongated cohort from the final harvest. Percent nitrogen concentration (N_f) and carbon isotope composition ($\delta^{13}C$) were determined using a continuous flow Isotope Ratio Mass Spectrometer (IRMS) at the University of California, Davis Stable Isotope Facility (Davis, California, USA).

Needle gas exchange

Gas exchange measurements were taken with a LI-COR 6400 infrared gas analyzer (LI-COR Corporation, Lincoln, Nebraska, USA) on the newest fully elongated needle cohort at the end of the study. Rates of light-saturated net photosynthesis were collected by placing two fascicles, which remained attached to the plant, inside the 2 x 3 cm standard cuvette. Rates of net CO_2 and water vapor exchange were determined at a photosynthetic photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1} (using the LI-COR red/blue LED light source), a controlled air temperature of 28 °C, and at ambient relative humidity (71.8 ± 4.6 percent). The reference CO_2 concentration was maintained at near ambient levels of 400 μmol mol^{-1}. Rates of photosynthesis were collected on a clear sunny day (June 21, 2004) between 1000 and 1600 h on six plants (one per block) of each clone and nitrogen treatment combination. Needle samples used for gas exchange measurements were frozen in sealed plastic bags prior to digital scanning and morphological analysis as described above. Light-saturated photosynthesis rates were expressed on a needle area (A_{area} μmol m^{-2} s^{-1}) and dry mass (A_{mass}, nmol g^{-1} s^{-1}) basis. Photosynthetic nitrogen-use efficiency (PNUE, μmol CO_2 g^{-1} N s^{-1}) was calculated by dividing A_{mass} by the nitrogen concentration of the leaf sample. Other calculated gas exchange parameters included

stomatal conductance (g_s, mmol m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}), and instantaneous water-use efficiency (A/E, mmol CO_2 mol^{-1} H_2O).

Rates of dark respiration were determined on approximately ten fascicles selected from the newest fully elongated needle cohort. The needle samples were detached and immediately sealed in an LI-COR conifer cuvette covered with an opaque cloth. Rates of net CO_2 efflux were determined at an air temperature of 28 °C, ambient relative humidity (49.2 ± 14.1 percent), and 400 µmol mol^{-1} CO_2 concentration. Rates of needle dark respiration were collected between 1000 and 1600 h on two consecutive days near the end of the study (June 22 and 23, 2004). In all, gas exchange measurements were collected on a total of 108 plants, six plants for each clone and N treatment combination. Rates were expressed on a needle area (R_{area}, µmol m^{-2} s^{-1}) and dry mass (R_{mass}, nmol g^{-1} s^{-1}) basis.

Data analysis

Analysis of variance (ANOVA) was used to analyze the effects of nitrogen treatment (1 degree of freedom), clone (8 degrees of freedom, and clone x nitrogen treatment interaction (8 degrees of freedom) on all measured growth and physiological traits. In addition, ANOVA was run separately in each nitrogen treatment to analyze clonal effects. Significant differences in means among treatments and clones were determined using Student's t test applied at $\alpha = 0.05$. In addition, a correlation and regression approach of examining traits in relationship to RGR among clones was used to test hypothesized trait relationships. All data were analyzed with statistical analysis software (Version 5, JMP, SAS Institute, Cary, North Carolina, USA).

IV. RESULTS

Growth

Relative growth rates of plants receiving 250 ppm N were significantly higher than plants receiving 50 ppm N ($P = 0.0001$, Table 2). The means of the interval-based RGR values are shown in Table 2. Averaged throughout the course of the study, plants receiving 250 and 50 ppm N had a mean RGR of 10.1 mg g^{-1} $day^{-1}$and 7.2 mg g^{-1} day^{-1}, respectively. Clones significantly differed in plant dry mass growth (Fig. 1) and RGR ($P = 0.027$).

The interval-based RGR of the clones varied during the course of the study (Fig. 2). The interaction effect of clone x N treatment was not statistically significant for RGR ($P = 0.15$), suggesting that RGR responded similarly to N treatment among the clones. Analyzing each clone separately, higher nitrogen significantly increased ($P < 0.05$) RGR

Table 2. Effects of nitrogen treatment on least squares means of interval-based relative growth rate (RGR) of plants combined throughout the study and RGR of roots and shoots at the final harvest interval among nine clones of loblolly pine.[1]

	RGR (mg g^{-1} day^{-1})		Root RGR (mg g^{-1} day^{-1})		Shoot RGR (mg g^{-1} day^{-1})	
Clone	250 ppm N	50 ppm N	250 ppm N	50 ppm N	250 ppm N	50 ppm N
7299	10.4	8.4	10.0	2.8	14.0	12.7
7338	11.2	6.5	6.6	7.0	14.7	6.4
7378	6.8	6.9	8.6	3.2	6.2	5.9
7466†	11.1	5.8	17.2	14.6	20.3	12.6
7528	11.8	7.6	9.2	7.8	6.8	5.8
7544*	8.7	5.5	10.5	5.0	12.5	4.7
7549†	12.3	8.1	7.9	3.9	12.0	1.2
7615†	8.9	6.3	13.7	4.1	12.6	5.6
7924*	10.0	9.5	13.1	10.0	8.6	13.1
SE	3.5	2.8	3.0	2.3	3.0	10.5
All Clones	10.1	7.2	10.8	6.5	12.0	7.6

[1]SE is the standard error for clonal means within each N treatment. Clones with identical symbols (*, †) are members of the same full-sib family.

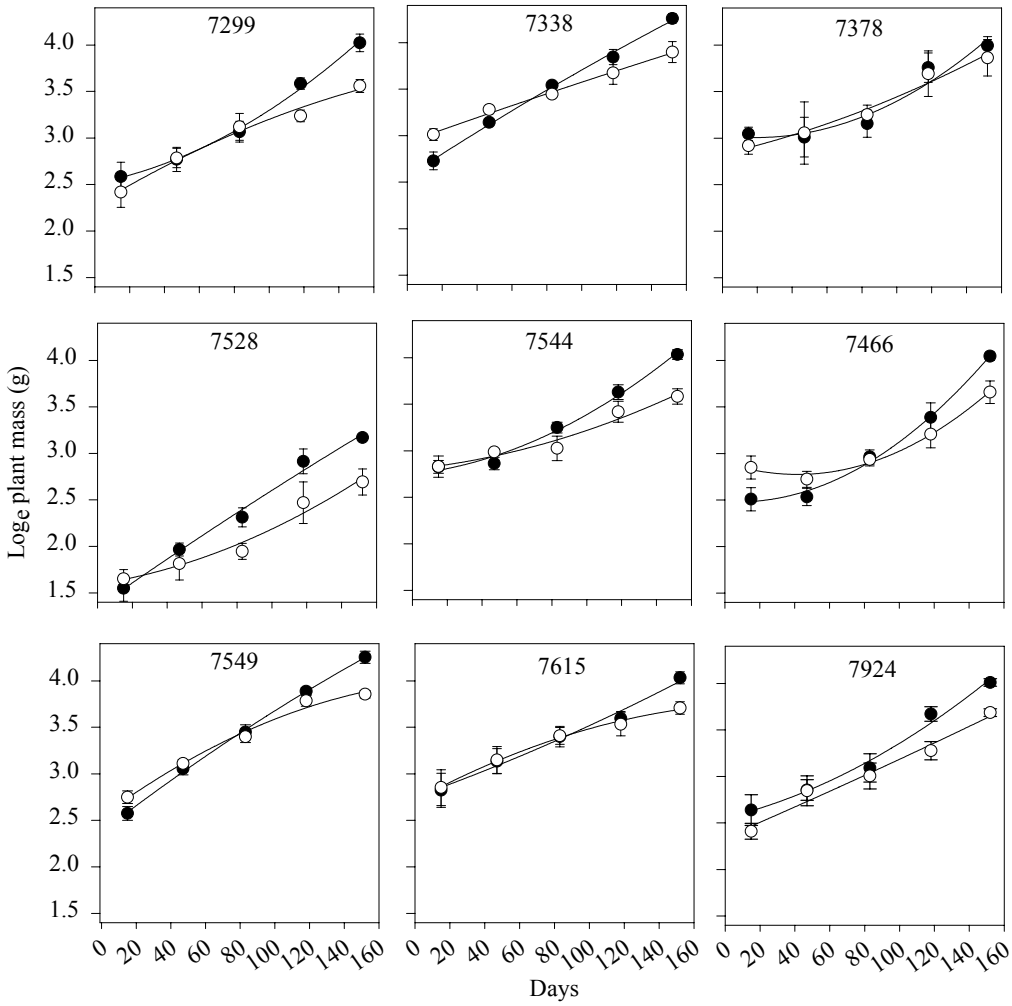

Figure 1. Time course of dry-mass growth of plants of nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)). Means of $\log_e$ transformed dry-mass and fitted second-order polynomials are shown. R^2 values ranged from 0.95 to 0.99. Each point is the average (± SE) of 6 plants during each harvest.

of all clones except 7378 ($P = 0.83$), 7615 ($P = 0.16$), and 7924 ($P = 0.20$). Higher N also increased the RGR of shoots ($P < 0.0001$) and roots ($P = 0.0018$) (Table 2). The clones differed in shoot RGR ($P = 0.017$), but not root RGR ($P = 0.12$) and there were no clone x N treatment interactions for either root or shoot RGR.

At the end of the study, clones receiving 250 ppm N had significantly higher ($P < 0.0001$) LAR ($cm^2\ g^{-1}$) and higher NAR ($P = 0.027$) than clones receiving 50 ppm N

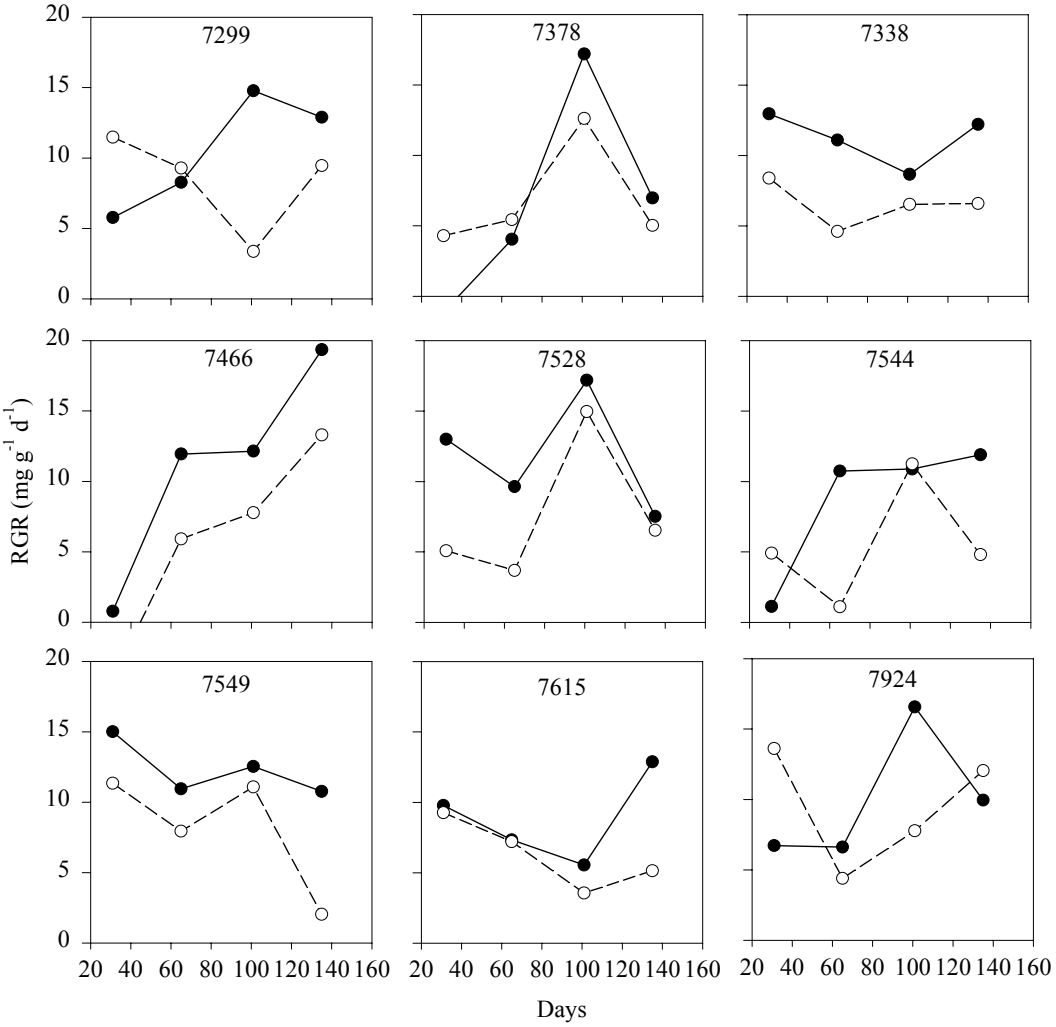

Figure 2. Time course of mean RGR of nine clones grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○). Mean values are shown for each harvest interval and plotted at the midpoint.

(Table 3). LAR was significantly different among the clones ($P = 0.0021$), but NAR was not ($P = 0.28$). The interaction effect of clone x N treatment was not statistically significant for LAR ($P = 0.15$) or NAR ($P = 0.89$).

At the end of the study mean total biomass was significantly increased by nitrogen treatment ($P < 0.0001$). Total plant biomass differed over three-fold among

Table 3. Effects of nitrogen treatment on least squares means of leaf area ratio (LAR) and net assimilation rate (NAR) among nine clones of loblolly pine at the end of the study.[1]

	LAR ($cm^2\ g^{-1}$)		NAR ($g\ m^{-2}\ day^{-1}$)	
Clone	250 ppm N	50 ppm N	250 ppm N	50 ppm N
7299	71.2 A	65.8 A	4.58 A	2.85 A
7338	68.4 AB	48.7 C	3.74 A	2.93 A
7378	60.3 BC	54.6 BC	4.29 A	4.61 A
7466†	60.8 BC	47.7 C	4.07 A	3.04 A
7528	54.4 C	51.5 C	5.94 A	4.63 A
7544*	63.7 ABC	54.0 BC	3.82 A	3.85 A
7549†	60.1 BC	53.0 BC	4.81 A	4.42 A
7615†	65.9 AB	51.5 C	3.07 A	2.38 A
7924*	63.0 ABC	62.3 AB	4.96 A	3.72 A
SE	3.4	3.6	1.08	1.07
All Clones	63.1	54.3	4.36	3.60

[1]LS mean values not sharing the same letter are significantly different at ($P < 0.05$) with Student's t. Clonal means are compared within each N treatment ($n = 6$). SE is the standard error for clonal means within each N treatment. Clones with identical symbols (*, †) are members of the same full-sib family.

clones ($P < 0.0001$) with means ranging from 23.8 g to 70.8 g in high N and 14.8 g to 49.3 g in the low N treatment. Dry mass of the roots, stems, and needles all were higher ($P < 0.0001$) in the 250 than 50 ppm N treatment (Table 4). The interaction effect of clone x N treatment was not statistically significant for total biomass, root, stem, or needle biomass at final harvest. Thus, clone and nitrogen treatment effects were independent.

Averaged throughout the course of the study, plants receiving 250 and 50 ppm N had a mean relative height growth rate (RHGR) of 4.50 mm cm^{-1} day^{-1} and 3.43 mm cm^{-1} day^{-1}, respectively. Clones significantly differed in RHGR ($P < 0.0001$, Table 5). The interaction effect of clone x N treatment was statistically significant for RHGR ($P < 0.0001$), suggesting that RHGR responded differently to N treatment among the clones. Consequently, a significant clone x N treatment interaction ($P = 0.017$) was found for final shoot height (Fig. 3, Table 5),

Table 4. Effects of nitrogen treatment on least squares means of final harvest dry mass of total plant, root, stem, and needles for nine clones of loblolly pine.[1]

	Total Biomass (g)				Root (g)				Stem (g)				Needle (g)			
Clone	250 ppm		50 ppm		250 ppm		50 ppm		250 ppm		50 ppm		250 ppm		50 ppm	
7299	55.9	B	35.1	B	15.1	C	10.2	C	10.4	BC	7.5	CD	30.3	BC	17.4	A
7338	70.8	A	49.3	A	19.5	AB	17.6	A	14.4	A	11.3	A	36.9	A	20.5	A
7378	54.3	B	47.6	AB	17.6	ABC	14.9	AB	10.1	BC	10.1	ABC	26.6	C	22.7	A
7466†	57.1	B	28.8	AB	16.6	BB	13.0	BC	12.6	AB	9.1	ABCD	27.9	C	16.7	A
7528	23.8	C	14.8	C	7.7	D	5.4	D	3.6	D	2.1	E	12.5	C	7.2	B
7544*	56.6	B	36.0	B	17.6	ABC	13.5	BC	9.1	C	6.9	D	29.7	C	15.5	A
7549†	70.7	A	47.2	AB	20.3	A	15.1	AB	14.2	A	10.2	AB	35.8	AB	22.0	A
7615†	56.4	B	40.8	AB	15.1	C	13.0	BC	11.0	BC	9.9	ABC	30.4	BC	17.8	A
7924*	55.2	B	39.9	AB	17.6	ABC	12.6	BC	9.8	C	8.3	BCD	27.8	C	19.0	A
SE	3.69		4.7		1.3		1.3		0.9		0.9		2.6		2.7	
All Clones	55.9		38.8		16.7		12.8		10.6		8.4		28.7		17.6	

[1]Mean values not sharing the same letter are significantly different at ($P < 0.05$) with Student's t ($n = 6$). Clonal means are compared within each N treatment. SE is the standard error for clonal means within each N treatment. Clones with identical symbols (*, †) are members of the same full-sib family.

indicating that the response of height growth to N treatment differed among clones. Comparing N treatment means of each clone separately at final harvest, the height of three clones (7924, 7378, and 7528) did not significantly differ between N treatments and the height of clone 7615 was significantly higher ($P < 0.05$) in the lower N treatment. In the five remaining clones, the final height of plants receiving 250 ppm N was significantly higher ($P < 0.001$) than plants receiving 50 ppm N. At final harvest, the height of clones receiving 250 ppm N differed over three-fold with means ranging from 19.8 to 61.6 cm. The height of clones receiving 50 ppm N differed more than two fold with means ranging from17.8 to 49.1 cm.

At the end of the study, the average diameter of the plants receiving 250 ppm N was 10.2 mm, which was on average 7.3 percent higher ($P < 0.0001$) than the plants receiving 50 ppm N (average diameter of 9.5 mm, Table 5). The final diameters of the clones were significantly different ($P < 0.0001$). At final harvest, the diameter of plants

Table 5. Effects of nitrogen treatment on least squares means of shoot height and diameter at final harvest and mean relative height growth rate (RHGR) for nine clones of loblolly pine.[1]

	Height (cm)				Diameter (mm)				RHGR (mm cm^{-1} day^{-1})	
Clone	250 ppm N		50 ppm N		250 ppm N		50 ppm N		250 ppm N	50 ppm N
7299	48.7	B	40.3	BC	9.8	CD	9.0	C	4.9	2.9
7338	56.8	A	46.8	AB	11.3	A	11.4	A	5.3	3.7
7378	42.6	BC	42.0	BC	10.0	CD	10.1	B	3.7	3.0
7466†	46.0	B	38.7	CD	11.2	AB	9.8	BC	5.0	3.9
7528	19.8	D	17.8	E	8.4	E	7.2	D	4.4	3.9
7544*	36.7	C	32.8	D	10.4	BCD	9.4	BC	5.2	4.5
7549†	61.6	A	49.0	A	10.5	BC	9.8	BC	3.0	2.3
7615†	44.7	B	49.1	A	10.4	BCD	9.7	BC	3.8	2.7
7924*	42.8	BC	43.6	ABC	9.6	D	9.1	BC	5.3	3.9
SE	2.4		2.4		0.3		0.4		2.4	2.2
All Clones	44.4		40		10.2		9.5		4.5	3.4

[1]Mean values not sharing the same letter are significantly different at ($P < 0.05$) with Student's t. Clonal means are compared within each N treatment. SE is the standard error for clonal means within each N treatment. Clones with identical symbols (*, †) are members of the same full-sib family.

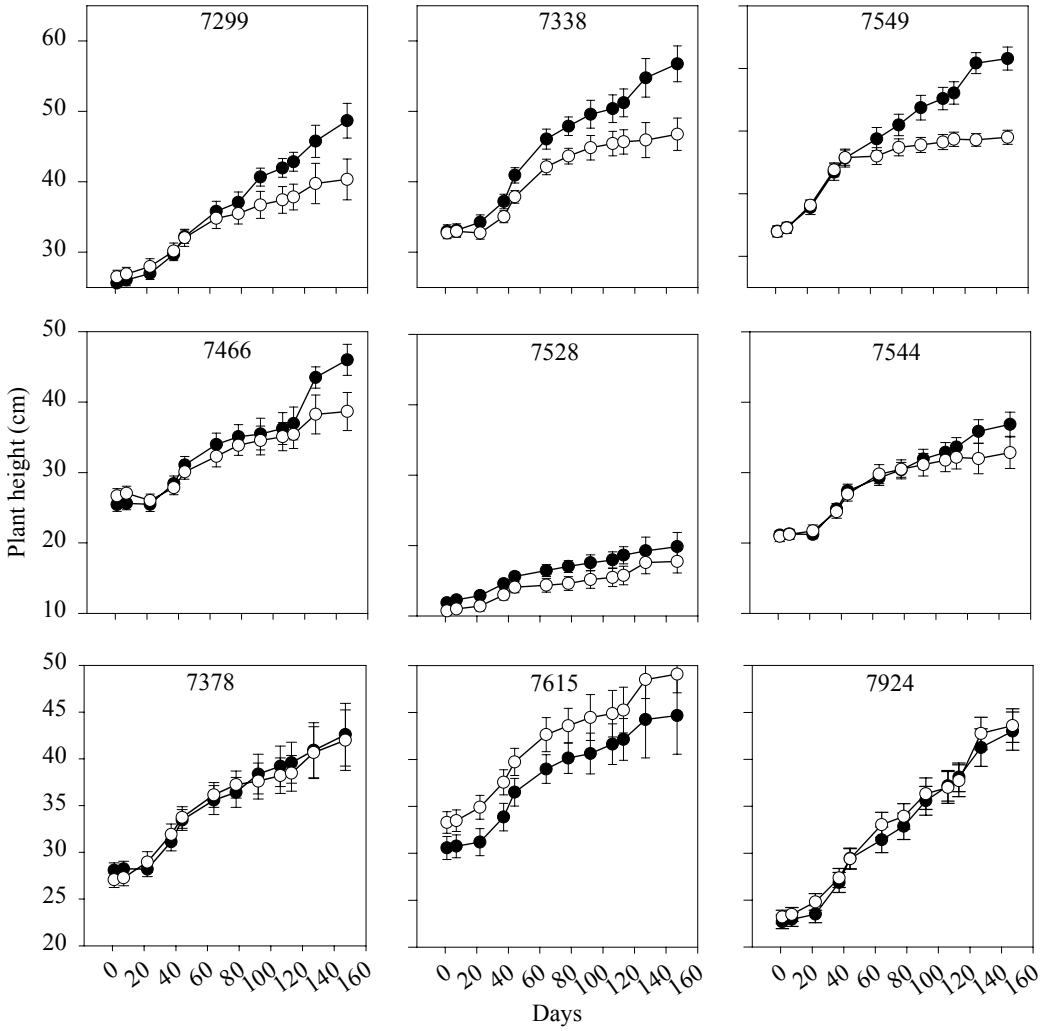

Figure 3. Time course of height growth of nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)) (n = 30 at day 0 and n = 6 at day 152). Mean (± SE) are shown. Note the different y-axis scales.

receiving 250 ppm N and 50 ppm N ranged from 8.4 mm to 11.3 mm and 7.2 mm to 11.4 mm, respectively. The interaction effect of clone x N treatment was not statistically significant for diameter (P = 0.42). Thus, clone and nitrogen treatment effects were independent.

Biomass allocation and partitioning

Differences in allocation patterns among the clones and between the N treatments were identified based on allometric parameters in regression equations relating root, stem, and needle dry mass to total plant biomass growth over the course of the 20-week study. Comparisons made between N treatments for individual clones revealed that some clones showed significant responses to nitrogen treatment through shifts in allometry and others did not (Table 6). Intercepts of allometric regressions were significantly lower in the higher N treatment for clones 7338, 7466, and 7615 for roots, for clones 7299, 7338, 7544, and 7615 for stems, and significantly higher in the higher N treatment for clones 7299, 7338, 7466, 7544, and 7615 for needles. Slopes of allometric regressions were significantly lower in the higher N treatment for clone 7615 for roots, clones 7544, 7615, and 7924 for stems, and significantly higher for clones 7544 and 7615 for needles. Significant differences in intercepts of allometric regressions signify that biomass allocation to plant parts is different between N treatments at a common plant mass. Significant differences in slopes of allometric regressions signify that N treatment is affecting allocation as time progresses. Although differences in slopes and intercepts were not all significant, plants grown under 250 compared to 50 ppm N had largely decreased slopes of root and stem allometric regressions and increased the slopes of the needle allometric regressions, indicating higher N shifted biomass allocation away from roots and stems towards needles (Table 6). Comparing common slopes models, higher N by in large resulted in reductions in the intercepts of root and stem allometric regressions and increased intercepts of needle allometric regressions, indicating that at a common plant size, plants receiving 250 ppm N allocated more biomass to foliage and less to roots and stems. Overall, the slopes of the root allometric regressions were below 1.0 indicating that as the plants grew in size, proportionally less biomass was allocated to roots. The slopes of the needle allometric regressions were all above 1.0 indicating that as the plants grew in size, proportionally greater amounts of biomass were allocated to needles. The slopes of the stem allometric regressions ranged from 0.69 to 1.11 in the 50

ppm N treatment and from 0.74 to 1.07 in the 250 ppm N treatment indicating that as the plants grew in size, some clones allocated more to stems and some less.

Clones differed in dry mass allocation to roots in each of the N treatments. In the 250 ppm N treatment, clones 7466 and 7378 had significantly higher allocation

Table 6. Allometric coefficients of dry mass allocation to roots, stems, and needles of clones grown in 50 and 250 ppm N. Significance levels due to N treatments for differences in the intercepts and slopes of allometric regressions are shown for roots, stems, and needles for individual clones. Values for slopes and intercepts ± SE are shown. $P > F$ for equality of intercepts and homogeneity of slopes were tested using analysis of covariance.[1]

	Intercept (adjusted least squares mean)			Slope		
Clone	250 ppm	50 ppm	($P > F$)	250 ppm	50 ppm	($P > F$)
			Root			
7299	2.07 ± 0.027	2.11 ± 0.027	0.268	0.68 ± 0.041	0.74 ± 0.058	0.402
7338	2.46 ± 0.019	2.54 ± 0.026	0.003	0.68 ± 0.030	0.74 ± 0.059	0.349
7378	2.29 ± 0.028	2.34 ± 0.033	0.206	0.81 ± 0.056	0.83 ± 0.043	0.805
7466†	1.96 ± 0.026	2.05 ± 0.026	0.013	0.83 ± 0.039	0.84 ± 0.064	0.900
7528	1.26 ± 0.033	1.29 ± 0.043	0.514	0.72 ± 0.053	0.82 ± 0.057	0.182
7544*	2.33 ± 0.025	2.39 ± 0.031	0.120	0.65 ± 0.051	0.77 ± 0.064	0.156
7549†	2.32 ± 0.023	2.35 ± 0.022	0.419	0.80 ± 0.032	0.69 ± 0.056	0.105
7615†	2.23 ± 0.035	2.32 ± 0.028	0.086	0.73 ± 0.067	0.98 ± 0.082	0.022
7924*	2.15 ± 0.026	2.17 ± 0.022	0.585	0.72 ± 0.033	0.74 ± 0.056	0.782
			Stem			
7299	1.50 ± 0.025	1.58 ± 0.025	0.017	0.94 ± 0.037	0.98 ± 0.056	0.584
7338	1.90 ± 0.026	2.00 ± 0.026	0.008	1.07 ± 0.058	1.01 ± 0.051	0.506
7378	1.73 ± 0.033	1.76 ± 0.033	0.641	0.92 ± 0.061	0.87 ± 0.056	0.533
7466†	1.62 ± 0.026	1.60 ± 0.026	0.627	0.91 ± 0.031	0.98 ± 0.077	0.342
7528	0.52 ± 0.043	0.43 ± 0.043	0.136	0.74 ± 0.055	0.69 ± 0.091	0.614
7544*	1.53 ± 0.030	1.63 ± 0.031	0.054	0.88 ± 0.052	1.11 ± 0.090	0.029
7549†	1.87 ± 0.022	1.89 ± 0.022	0.494	0.86 ± 0.034	0.95 ± 0.052	0.169
7615†	1.80 ± 0.028	1.89 ± 0.028	0.039	0.89 ± 0.047	1.08 ± 0.071	0.025
7924*	1.48 ± 0.022	1.52 ± 0.022	0.265	0.90 ± 0.031	1.06 ± 0.048	0.005
			Needle			
7299	2.20 ± 0.026	2.12 ± 0.026	0.041	1.31 ± 0.035	1.28 ± 0.063	0.677
7338	2.58 ± 0.024	2.46 ± 0.024	0.001	1.24 ± 0.038	1.27 ± 0.074	0.701
7378	2.37 ± 0.030	2.31 ± 0.030	0.169	1.24 ± 0.062	1.24 ± 0.043	0.921
7466†	2.17 ± 0.023	2.10 ± 0.023	0.039	1.20 ± 0.036	1.17 ± 0.055	0.617
7528	1.21 ± 0.057	1.28 ± 0.058	0.336	1.50 ± 0.091	1.38 ± 0.103	0.387
7544*	2.33 ± 0.031	2.24 ± 0.031	0.079	1.40 ± 0.056	1.22 ± 0.088	0.085
7549†	2.54 ± 0.023	2.51 ± 0.023	0.336	1.25 ± 0.031	1.32 ± 0.059	0.230
7615†	2.48 ± 0.035	2.36 ± 0.035	0.024	1.25 ± 0.063	1.02 ± 0.085	0.034
7924*	2.22 ± 0.028	2.18 ± 0.028	0.389	1.31 ± 0.036	1.23 ± 0.064	0.292

[1]Clones with identical symbols (*, †) are members of the same full-sib family.

coefficients than the other clones, signifying that these clones allocated more biomass to roots than the other clones. By comparison, clone 7544 allocated less biomass to roots than the other clones. In addition, there were significant differences in the stem dry mass allometric regressions for clones 7338 and 7528, which exhibited the highest and lowest stem dry mass allocation among the clones. Also, there were significant differences in needle dry mass allometric regressions. Clones 7528 and 7544 had greater slopes and 7466 had a lower slope than the other clones, indicating comparatively higher and lower biomass allocation to needles among the clones. In the 50 ppm N treatment, clones 7615 and 7549 exhibited the highest and lowest root dry mass allocation among the clones. Clones 7544 and 7615 had significantly higher allocation coefficients indicating that these clones allocated more biomass to stems than the other clones. By comparison, clones 7466 and 7378 had significantly lower allocation coefficients, signifying that these clones allocated less biomass to stems than the other clones as time progressed. In addition, there were significant differences in the needle dry mass allometric regressions for clones 7528 and 7615, which exhibited the highest and lowest needle dry mass allocation among the clones.

At the end of the study, higher N significantly increased ($P < 0.0001$) biomass partitioning to needles by 6.4 percent and significantly decreased ($P < 0.0001$) biomass partitioning to roots and stems by 2.8 percent and 3.5 percent, respectively (Table 7). Biomass partitioning differed among clones for roots ($P < 0.0001$), for stems ($P < 0.0001$) and for needles ($P = 0.02$) (Table 7). At final harvest, the fraction of biomass that plants receiving 250 ppm N partitioned to roots, stems, and needles was 0.297, 0.187, and 0.516, respectively. The fraction of biomass that plants receiving 50 ppm N partitioned to roots, stems, and needles was 0.336, 0.212, and 0.452, respectively. There was also a significant clone x N treatment interaction for RMF ($P = 0.04$), but no significant interactions for partitioning to stems and needles. Root biomass partitioning at final harvest did not differ between nitrogen treatments in two of the nine clones (7378 and 7924), while the other clones showed varying degrees of increased partitioning to roots in the 50 than 250 ppm N treatment.

Needle traits

The mean length and dry mass of individual needles differed between nitrogen treatments ($P < 0.0001$) at the final harvest (Table 8). There were no nitrogen treatment effects on specific leaf area (SLA) ($m^2\ kg^{-1}$) at the end of the study ($P = 0.31$). Clones receiving 250 ppm N had 22.4 percent longer and 18.8 percent heavier needles than clones receiving 50 ppm N. Needle length, individual needle mass, and SLA differed among the clones (needle length $P = 0.0003$, needle mass $P = 0.0066$, SLA $P < 0.0001$, Table 8). There were no clone x N treatment interaction effects for needle length, individual needle mass, or SLA.

In contrast to individual needle mass, length, and SLA, there were significant clone x N treatment effects for percent needle nitrogen (N_f) ($P = 0.019$, Table 8). Comparing clonal means within each nitrogen treatment at final harvest, needle nitrogen concentrations among the clones differed in the 250 ppm N treatment ($P < 0.0001$), but not under the 50 ppm treatment ($P = 0.16$). The differences in N_f between N treatments were greater for clones 7544 and 7528. At final harvest, clones receiving 250 ppm N and 50 ppm N had a mean N_f of 0.76 percent and 0.45 percent, respectively in the fully elongated cohort of needles developed during the study. However, mean needle nitrogen concentrations among clones ranged widely from 0.63 to 1.11 percent in the 250 ppm and from 0.35 to 0.51 percent in the 50 ppm N treatment.

Table 7. Biomass partitioning on a fractional basis to roots (RMF), stems (SMF), and leaves (LMF) of nine clones of loblolly pine receiving 250 ppm N and 50 ppm N at the final harvest.[1]

	RMF		SMF		LMF	
Clone	250 ppm N	50 ppm N	250 ppm N	50 ppm N	250 ppm N	50 ppm N
7299	0.271 D	0.289 D	0.188 BC	0.216 ABC	0.541 A	0.496 A
7338	0.275 D	0.359 ABC	0.203 AB	0.230 AB	0.522 AB	0.412 C
7378	0.326 A	0.326 BCD	0.186 BCD	0.223 ABC	0.488 B	0.451 ABC
7466†	0.292 BCD	0.340 ABC	0.220 A	0.232 AB	0.488 B	0.428 BC
7528	0.322 AB	0.374 AB	0.152 E	0.146 D	0.525 AB	0.480 AB
7544*	0.313 ABC	0.378 A	0.162 DE	0.193 C	0.525 AB	0.429 BC
7549†	0.288 CD	0.319 CD	0.203 AB	0.217 ABC	0.509 AB	0.465 ABC
7615†	0.269 D	0.321 CD	0.193 BC	0.245 A	0.539 A	0.434 BC
7924*	0.320 ABC	0.317 CD	0.177 CDE	0.210 BC	0.504 AB	0.474 AB
SE	0.012	0.017	0.009	0.011	0.015	0.020
All Clones	0.297	0.336	0.187	0.212	0.516	0.452

[1]Mean values not sharing the same letter are significantly different at ($P < 0.05$) with Student's t ($n = 6$). Clonal means are compared within each N treatment. SE is the standard error for clonal means within each N treatment. Clones with identical symbols (*, †) are members of the same full-sib family.

Table 8. Effects of nitrogen treatment and clone on least squares means of needle length (mm), individual needle mass (mg), SLA (m^2 kg^{-1}), and percent needle nitrogen (N_f) at the final harvest among nine clones of loblolly pine.[1]

	Needle length (mm)		Individual needle mass (mg)		SLA (m^2 kg^{-1})		N_f (%)	
Clone	250 ppm N	50 ppm N	250 ppm N	50 ppm N	250 ppm N	50 ppm N	250 ppm N	50 ppm N
7299	165.6 BC	145.2 B	25.0 C	22.3 BC	13.2 A	13.2 A	0.67 D	0.43 ABC
7338	166.2 BC	136.4 BC	25.2 C	24.4 BC	13.1 A	11.8 BCDE	0.65 D	0.40 BC
7378	164.5 BC	139.3 BC	28.4 ABC	25.9 B	12.3 AB	12.2 ABCD	0.78 BCD	0.51 ABC
7466†	152.8 C	134.2 BC	25.4 BC	26.0 AB	12.4 AB	11.2 DE	0.87 B	0.55 AB
7528	151.7 C	124.5 BC	29.8 ABC	23.2 BC	10.3 C	10.7 E	1.11 A	0.56 AB
7544*	181.4 AB	114.8 C	32.6 AB	18.8 C	12.1 AB	12.6 ABC	0.82 BC	0.35 BC
7549†	185.9 AB	170.7 A	32.6 AB	32.5 A	11.8 B	11.4 CDE	0.69 CD	0.43 ABC
7615†	191.0 A	146.0 AB	35.3 A	26.1 AB	12.2 AB	11.9 BCDE	0.63 D	0.42 ABC
7924*	167.3 ABC	135.9 BC	27.8 ABC	21.0 BC	12.5 AB	13.1 AB	0.65 D	0.45 ABC
SE	8.3	8.8	2.7	2.3	0.4	0.5	0.05	0.06
All Clones	169.6	138.6	29.1	24.5	12.2	12.0	0.76	0.45

[1]Mean values not sharing the same letter are significantly different at (P <0.05) with Student's t (n = 6). Clonal means are compared within each N treatment. SE is the standard error for clonal means within each N treatment. Clones with identical symbols (*, †) are members of the same full-sib family.

Gas exchange

Overall, light-saturated rates of net photosynthesis increased at the higher nitrogen treatment on both area and mass bases (A_{area}: $P = 0.013$, A_{mass}: $P = 0.0008$, Table 9), although a significant clone x N treatment interaction was found for A_{area} ($P = 0.016$). Mean A_{area} ranged from 3.3 to 6.7 and from 3.0 to 5.4 μmol CO_2 m^{-2} s^{-1} for clones receiving 250 and 50 ppm N, respectively. Mass-based means ranged from 52.4 to 91.2 and from 45.9 to 74.4 nmol CO_2 $g^{-1}s^{-1}$ for clones receiving 250 ppm N and 50 ppm N, respectively. On average, rates of net photosynthesis differed between the N treatments proportionally more on a mass-basis (23 percent) than area-basis (16 percent). Comparing clones, clones 7378 and 7528 had greater increases in A_{area} in the 250 ppm N compared to 50 ppm N treatment. In addition, clone 7466 had higher A_{area} in the 50 ppm

Table 9. Mean light-saturated rates of net photosynthesis in needles expressed on an area (A_{area}), mass, (A_{mass}) and nitrogen basis (PNUE) in nine clones of loblolly pine.[1] Rates were determined at the end of the study.

	A_{area}		A_{mass}		PNUE	
	(μmol CO_2 m^{-2} s^{-1})		(nmol CO_2 g^{-1} s^{-1})		(μmol CO_2 g^{-1} N s^{-1})	
Clone	250 ppm N	50 ppm N	250 ppm N	50 ppm N	250 ppm N	50 ppm N
7299	3.7 CD	3.4 B	63.3 B	60.6 AB	9.75 ABC	14.13 A
7338	3.3 CD	3.1 B	56.7 B	45.9 B	8.89 ABCD	11.47 A
7378	5.2 B	3.4 B	88.8 A	52.7 B	11.54 A	11.56 A
7466†	4.2 BCD	5.4 A	68.2 B	74.4 A	6.83 D	13.53 A
7528	6.7 A	4.4 AB	91.2 A	65.6 AB	8.30 BCD	12.02 A
7544*	4.0 CD	3.3 B	61.9 B	51.1 B	6.89 D	14.59 A
7549†	3.4 CD	3.2 B	52.4 B	47.0 B	7.49 CD	11.12 A
7615†	4.3 BC	3.9 B	68.4 B	57.4 AB	10.90 AB	13.94 A
7924*	4.2 BCD	3.0 B	69.6 B	49.3 B	11.17 A	11.57 A
SE	0.4	0.5	6.1	7.2	1.00	1.26
All Clones	4.3	3.7	69.1	56.0	9.08	12.66

[1]Mean values not sharing the same letter are significantly different at ($P < 0.05$) with Student's t ($n = 6$). Clonal means are compared within each N treatment. SE is the standard error for clonal means within each N treatment. Clones with identical symbols (*, †) are members of the same full-sib family.

N treatment than in the 250 ppm N treatment, which is opposite to the response of the other clones.

Overall, photosynthetic nitrogen-use-efficiency (PNUE, μmol CO_2 g^{-1} N s^{-1}) was greater for plants receiving lower N ($P < 0.0001$), although a significant clone x N treatment interaction was found ($P = 0.015$). Comparing clonal means within each N treatment at final harvest, clones differed in PNUE in the 250 ppm treatment ($P = 0.04$), but not the 50 ppm N treatment ($P = 0.33$). Clones 7466 and 7544 had greater increases in PNUE in the 250 compared to 50 ppm N treatment than the other clones. There were no differences in PNUE between N treatments for clones 7378 and 7924. Mean PNUE for clones receiving 250 ppm N and 50 ppm N was 9.08 and 12.66 μmol CO_2 g^{-1} N s^{-1}, respectively.

Needle dark respiration rates were generally greater in the higher nitrogen treatment on both area and mass bases ($P < 0.0001$), although a significant clone x N treatment interaction was found for R_{area} ($P = 0.0086$, Table 10). Comparing clonal

Table 10. Nitrogen effects on R_{area}, R_{mass}, and A/R (ratio of photosynthesis to respiration) at the final harvest among nine clones of loblolly pine.[1]

	R_{area} μmol CO_2 m^{-2} s^{-1}				R_{mass} nmol CO_2 g^{-1} s^{-1}				A/R (area-based)			
Clone	250 ppm N		50 ppm N		250 ppm N		50 ppm N		250 ppm N		50 ppm N	
7299	0.58	B	0.41	E	7.5	BC	5.3	B	6.8	B	9.5	A
7338	0.62	B	0.47	DE	7.9	B	5.5	B	5.4	B	7.3	ABCD
7378	0.80	A	0.63	AB	9.9	A	7.6	A	6.5	B	5.5	D
7466†	0.51	B	0.61	ABC	6.4	C	6.7	AB	10.8	A	9.2	AB
7528	0.90	A	0.68	A	9.3	A	7.2	A	7.5	AB	6.4	BCD
7544*	0.62	B	0.43	DE	7.4	BC	5.4	B	6.5	B	8.5	ABC
7549†	0.58	B	0.55	BCD	6.9	BC	6.2	AB	5.7	B	6.1	CD
7615†	0.59	B	0.46	DE	7.1	BC	5.5	B	7.4	AB	8.4	ABC
7924*	0.63	B	0.50	CDE	7.9	B	6.5	AB	6.8	B	6.0	CD
SE	0.04		0.04		0.5		0.6		1.1		1.0	
All Clones	0.65		0.53		7.8		6.2		6.7		7.4	

[1]Mean values not sharing the same letter are significantly different at ($P < 0.05$) with Student's t ($n = 6$). Clonal means are compared within each N treatment. SE is the standard error for clonal means within each N treatment. Clones with identical symbols (*, †) are members of the same family.

means within each N treatment at final harvest, R_{area} and R_{mass} were significantly different among clones (R_{area} 250 ppm N: $P < 0.0001$; R_{area} 50 ppm N: $P = 0.0003$; R_{mass} 250 ppm N: $P < 0.0001$; R_{mass} 50 ppm N: $P = 0.05$).

There were no significant N treatment effects on A/R ratio whether expressed on a needle area or dry mass basis (area: $P = 0.15$, mass: $P = 0.33$); however, comparing clonal means within each N treatment, there were significant differences among clones on a needle area basis in both N treatments and only in the 50 ppm N treatment on a dry mass basis (area 250 ppm N: $P < 0.05$, area 50 ppm N: $P < 0.05$ mass 250 ppm N: $P = 0.13$ mass 50 ppm N: $P < 0.05$). There were no clone x N treatment interaction effects for A/R on an area or mass basis ($P > 0.40$).

Stomatal conductance and water-use efficiency

There was no significant clone x N treatment interaction for stomatal conductance (g_s), mmol H_2O m^{-2} s^{-1}, $P = 0.22$, Table 11). At final harvest, mean g_s of the clones was lower in the 250 than 50 ppm N treatment ($P < 0.0001$). The mean g_s for clones receiving 250 ppm and 50 ppm N was 41.1 and 60.3 mmol H_2O m^{-2} s^{-1}, respectively. Clones differed in mean g_s in both N treatments ($P < 0.0001$), ranging nearly four-fold from 19.8 to 86.9 mmol H_2O m^{-2} s^{-1} in the 250 ppm N treatment and two-fold from 42.7 to 91.2 mmol H_2O m^{-2} s^{-1} in the 50 ppm treatment (Table 11).

Although instantaneous water-use efficiency (A/E, mmol CO_2 mol^{-1} H_2O) of the clones was higher in the 250 than 50 ppm N treatment ($P < 0.0001$), a significant clone x nitrogen treatment interaction was found ($P = 0.075$). Overall, plants receiving 250 ppm N had a mean A/E of 7.88 and plants receiving 50 ppm N had a mean A/E of 4.09 (Table 11). Comparing clonal means within each N treatment at final harvest, clones differed in A/E in the 250 ppm N treatment ($P = 0.0039$), but not in the 50 ppm N treatment ($P = 0.61$). N treatment did not affect A/E for clones 7378 and 7528 as much as the other clones. Clone 7544 showed a greater response to N treatment than the other clones.

As a measure of water-use efficiency, A/E provides only a snapshot of the water-use efficiency of a plant at one particular point in time. Long-term measures of water-

Table 11. Effects of nitrogen treatment on stomatal conductance ($g_{s,}$) instantaneous water-use-efficiency (A/E), $\delta^{13}C$ (‰), and internal CO_2:ambient CO_2 ratio (C_i/C_a) among nine clones of loblolly pine.[1]

	g_s (mmol H_2O m^{-2} s^{-1})		A/E (mmol CO_2 mol^{-1} H_2O)		$\delta^{13}C$ (‰)		Ci/Ca ratio	
Clone	250 ppm N	50 ppm	250 ppm N	50 ppm N	250 ppm N	50 ppm N	250 ppm N	50 ppm N
7299	33.8 B	42.7 C	7.84 AB	4.71 A	-29.29 ABC	-30.30 A	0.418 B	0.638 B
7338	21.8 B	48.0 BC	9.66 A	4.34 A	-29.24 AB	-30.60 AB	0.335 B	0.694 AB
7378	73.7 A	70.6 ABC	5.38 C	3.95 A	-30.43 EF	-30.97 AB	0.629 A	0.705 AB
7466†	35.1 B	68.4 ABC	8.17 AB	4.92 A	-29.56 ABC	-30.28 A	0.425 B	0.643 AB
7528	86.9 A	91.2 A	5.55 BC	3.84 A	-30.98 F	-31.90 C	0.638 A	0.749 AB
7544*	29.9 B	75.4 AB	8.49 A	2.85 A	-29.93 CDE	-31.13 BC	0.372 B	0.780 A
7549†	19.8 B	53.6 BC	9.70 A	4.28 A	-29.15 A	-30.59 AB	0.315 B	0.683 AB
7615†	38.9 B	48.2 BC	8.00 AB	3.45 A	-29.85 BCD	-31.15 BC	0.416 B	0.762 AB
7924*	27.1 B	44.7 BC	8.53 A	4.51 A	-30.06 DE	-30.22 A	0.395 B	0.687 AB
SE	8.1	10.8	0.84	0.73	0.23	0.29	0.059	0.048
All Clones	41.1	60.3	7.88	4.09	-29.83	-30.79	0.439	0.705

[1]Mean values not sharing the same letter are significantly different at ($P < 0.05$) with Student's t ($n = 6$). SE is the standard error for clonal means within each N treatment. Clones with identical symbols (*, †) are members of the same full-sib family.

use efficiency can be inferred from measures of the carbon isotope composition of plant tissues ($\delta^{13}C$). Clone and nitrogen treatment effects on $\delta^{13}C$ were independent ($P = 0.26$). Nitrogen treatment had a significant effect on $\delta^{13}C$ ($P < 0.0001$). Clones receiving 250 ppm N had a mean $\delta^{13}C$ of –29.82 ‰ and clones receiving 50 ppm N had a mean $\delta^{13}C$ of –30.79 ‰, reflecting greater water-use efficiency in the 250 than 50 ppm N treatment. Comparing clonal means within each N treatment at final harvest, there were also significant clonal differences in $\delta^{13}C$ in the 250 ppm N treatment ($P < 0.0001$) and in the 50 ppm N treatment ($P = 0.0027$). The $\delta^{13}C$ of clones receiving 250 ppm N ranged from –30.98 ‰ to –29.12 ‰ and clones receiving 50 ppm N had $\delta^{13}C$ values ranging from –31.90 ‰ to –30.22 ‰ (Table 11).

Although the Ci/Ca ratio of the clones was higher in the 50 than 250 ppm N treatment ($P < 0.0001$, Table 11), a significant clone x nitrogen treatment interaction was found ($P = 0.031$). Comparing clonal means within each N treatment at final harvest, there were significant clonal differences in Ci/Ca ratio in the 250 ppm N treatment ($P = 0.002$), but not in the 50 ppm N treatment ($P = 0.40$).

Non-structural carbohydrate concentrations

Clone and nitrogen treatment effects on needle carbohydrate concentrations were independent. Concentrations of glucose, starch, and TNC in the fully elongated cohort of needles collected at the final harvest were significantly affected by nitrogen treatment. Clones receiving 250 ppm N had 22 percent higher needle glucose ($P < 0.0001$), less than half the needle starch ($P = 0.075$), and 43 percent less needle TNC ($P = 0.038$) than clones receiving 50 ppm N (Table 12). Comparing clonal means within each N treatment at final harvest, needle glucose, starch, and TNC concentrations were significantly different in the 250 ppm N treatment ($P < 0.05$) and in the 50 ppm N treatment ($P < 0.05$). Clones receiving 250 ppm N had mean needle glucose concentrations ranging from 28.5 to 45.5 mg g^{-1}, needle starch concentrations ranging from 29.6 to 90.3 mg g^{-1}, and TNC concentrations ranging from 73.4 to 118.8 mg g^{-1} (Table 12). Clones receiving 50 ppm N had needle glucose concentrations ranging from 24.3 to 43.1 mg g^{-1}, needle

Table 12. Effects of nitrogen and clone on least squares means of concentrations of glucose, starch, and total non-structural carbohydrates (TNC) in needles, tap and coarse roots, and fine roots of nine clones of loblolly pine.[1]

	Glucose (mg g^{-1})				Starch (mg g^{-1})				TNC (mg g^{-1})			
Clone	250 ppm N		50 ppm N		250 ppm N		50 ppm N		250 ppm N		50 ppm N	
					Needle							
7299	32.6	CD	27.3	CD	50.9	B	94.7	B	83.4	B	121.9	B
7338	28.5	D	24.5	D	90.3	A	138.4	AB	118.8	A	162.9	AB
7378	32.4	CD	29.0	CD	47.5	B	110.9	AB	79.8	B	139.9	B
7466†	43.9	A	35.9	B	30.0	B	119.5	AB	73.9	B	155.4	AB
7528	45.5	A	43.1	A	54.0	B	179.6	A	99.5	AB	222.6	A
7544*	43.8	A	30.3	BCD	29.6	B	118.1	AB	73.4	B	148.4	B
7549†	32.1	CD	24.3	D	56.5	B	148.4	AB	88.7	AB	172.7	AB
7615†	43.4	AB	33.5	BC	45.0	B	119.5	AB	88.4	AB	152.9	AB
7924*	36.9	BC	30.0	BCD	48.6	B	84.8	B	85.5	B	114.9	B
SE	2.32		2.19		11.74		25.61		11.26		24.79	
All Clones	37.7		30.9		50.4		123.8		88.1		154.6	
					Tap + coarse root							
7299	16.7	B	19.3	A	46.8	CD	61.6	D	63.5	C	80.9	C
7338	16.0	B	22.2	A	60.5	BCD	102.3	ABC	76.5	BC	124.5	AB
7378	24.1	B	17.6	A	83.0	AB	129.1	A	107.1	A	146.6	A
7466†	35.9	A	22.6	A	39.3	D	68.9	CD	75.2	BC	91.5	BC
7528	22.6	B	21.3	A	72.3	ABC	126.2	A	94.9	AB	147.5	A
7544*	23.0	B	20.3	A	59.2	BCD	108.4	AB	82.3	BC	128.7	AB
7549†	22.3	B	15.8	A	88.3	A	119.7	A	110.6	A	135.5	A
7615†	16.2	B	21.8	A	63.5	BCD	123.5	A	79.7	BC	145.3	A
7924*	23.0	B	20.6	A	40.6	D	72.7	BCD	63.6	C	93.3	BC
SE	3.92		3.22		8.95		13.35		8.45		13.38	
All Clones	22.2		20.2		61.5		101.4		83.7		121.5	
					Fine root							
7299	34.0	AB	32.5	A	6.6	C	10.4	BCD	40.6	BC	42.9	B
7338	33.8	AB	36.9	A	7.9	BC	12.8	BCD	41.7	BC	49.7	AB
7378	26.2	B	34.8	A	8.3	BC	8.8	CD	34.5	C	43.5	B
7466†	31.3	AB	32.7	A	10.0	BC	16.9	B	41.3	BC	49.6	AB
7528	41.1	A	32.1	A	22.4	A	26.4	A	63.5	A	58.4	A
7544*	39.2	A	33.3	A	10.2	BC	13.2	BCD	49.4	B	46.5	B
7549†	33.6	AB	34.8	A	13.8	B	14.8	BC	47.4	BC	49.6	AB
7615†	35.6	AB	32.9	A	12.5	BC	10.7	BCD	48.0	BC	43.6	B
7924*	36.4	AB	32.0	A	7.5	BC	7.1	D	44.0	BC	39.1	B
SE	3.82		3.25		2.39		2.48		4.85		3.89	
All Clones	34.6		33.5		11.1		13.5		45.7		47.0	

[1]Mean values not sharing the same letter are significantly different at ($P < 0.05$) with Student's t ($n = 6$). SE is the standard error for clonal means within each N treatment. Clones with identical symbols (*, †) are members of the same full-sib family.

starch concentrations ranging from 84.8 to 179.6 mg g^{-1}, and needle TNC concentrations ranging from 114.9 to 222.6 mg g^{-1}.

Clone and nitrogen treatment effects on tap and coarse root carbohydrate concentrations were independent. Concentrations of starch and TNC (but not glucose) in the tap and coarse roots collected at the final harvest were significantly affected by nitrogen treatment (glucose: $P = 0.22$; starch: $P < 0.0001$; TNC: $P < 0.0001$). Clones receiving 250 ppm N had 39 percent lower starch and 31 percent lower TNC in tap and coarse roots than clones receiving 50 ppm N. Comparing clonal means within each N treatment at final harvest, tap and coarse root glucose, starch, and TNC concentrations were significantly different in the 250 ppm N treatment ($P < 0.01$), but only tap and coarse root starch and TNC concentrations (not glucose) in the 50 ppm N treatment were significantly differed among clones (starch and TNC: $P < 0.01$; glucose: $P = 0.85$).

Clone and nitrogen treatment effects on fine root carbohydrate concentrations were independent. At final harvest, nitrogen treatment had a significant effect on concentrations of starch in the fine roots (starch: $P = 0.037$), but not on concentrations of glucose or TNC (glucose: $P = 0.52$; TNC: $P = 0.52$). Clones receiving 250 ppm N had 18 percent less fine root starch than clones receiving 50 ppm N. Comparing clonal means within each N treatment at final harvest, fine root glucose, starch, and TNC concentrations were significantly different in the 250 ppm N treatment ($P < 0.05$), but only fine root starch and TNC concentrations (not glucose) in the 50 ppm N treatment were significantly different (starch and TNC: $P < 0.05$; glucose: $P = 0.88$).

Growth trait correlations

Mean RGR was positively correlated with mean LAR and mean NAR (Fig. 4). Pooling all clones and N treatments together, mean LAR was more highly correlated ($r = 0.73$, $P < 0.0001$) with mean RGR than was NAR ($r = 0.29$, $P = 0.022$). Also, RGR was positively correlated with LMF ($r = 0.73$ $P = 0.0003$) and SLA ($r = 0.61$ $P = 0.0071$) (Fig. 4) and negatively correlated with RMF ($r = -0.64$ $P = 0.0046$, not shown) at final harvest. Overall, LAR was positively correlated with LMF ($r = 0.84$ $P < 0.0001$, Fig. 5).

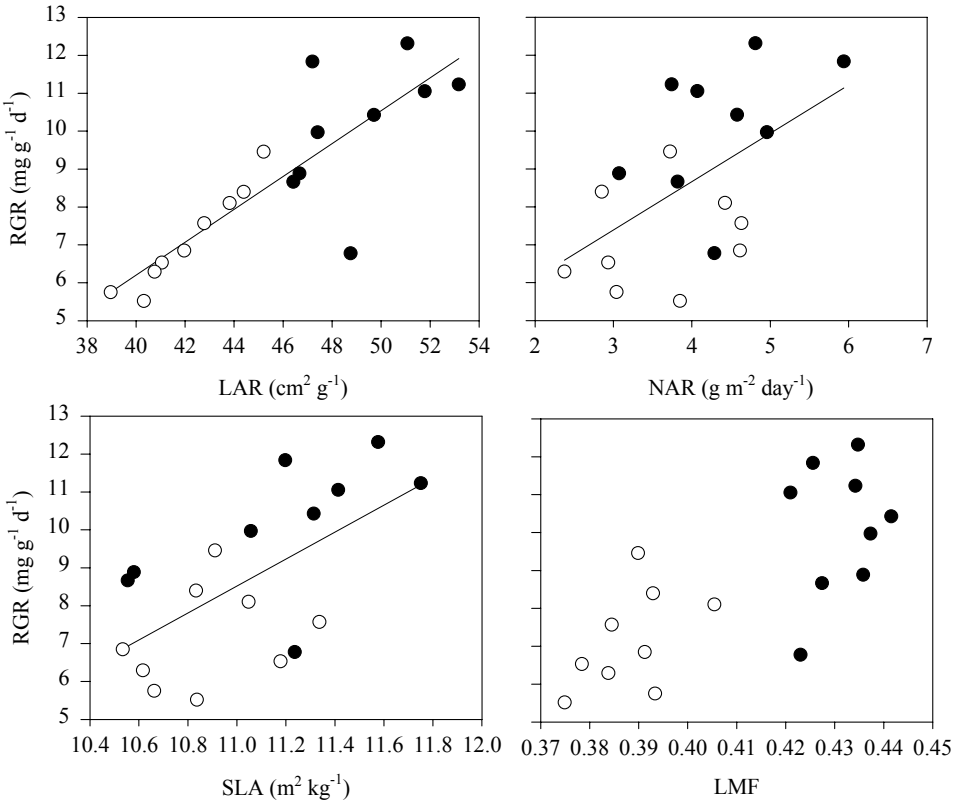

Figure 4. Relationships of RGR (mg g^{-1} day^{-1}) with leaf area ratio (LAR, cm^2 g^{-1}), whole-plant net assimilation rate (NAR, g m^{-2} day^{-1}), specific leaf area (SLA, m^2 kg^{-1}), and leaf mass fraction (LMF, g leaf g^{-1} plant) for nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)).

However, LAR was more highly correlated with SLA in the 250 ppm N treatment (r = 0.89 P = 0.0012) than in the 50 ppm N treatment (r = 0.33 P = 0.39, Fig. 5).

Leaf-level rates of net CO_2 exchange and whole-plant RGR at the last harvest interval were positively correlated among clones and N treatments (Fig. 6). The correlations of needle photosynthesis and respiration rates with RGR were stronger on a mass basis (A_{mass}, r = 0.57, P = 0.014; R_{mass}, r = 0.61, P = 0.0066) than area basis (A_{area}, r = 0.44, P = 0.068; R_{area}, r = 0.51, P = 0.03; not shown). N_f was positively correlated with RGR (r= 0.76 P = 0.0003, Fig. 7) as well as LAR (r = 0.64, P = 0.0041), and NAR

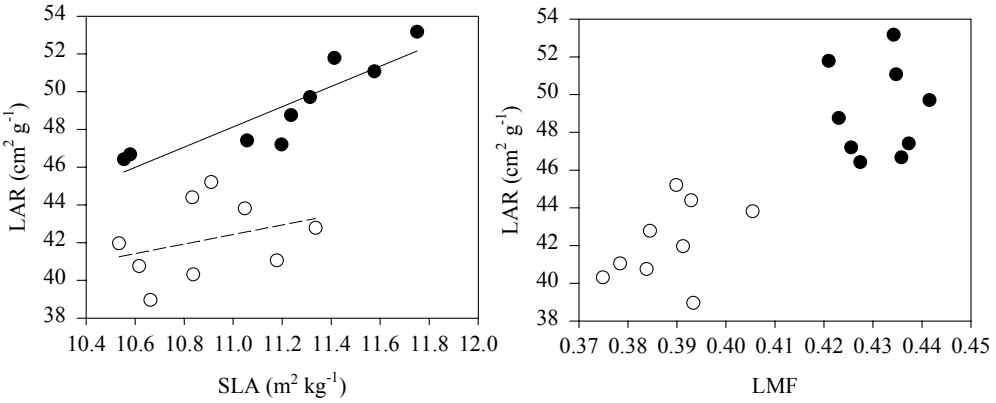

Figure 5. Relationships of LAR (cm^2 g^{-1}) with SLA (m^2 kg^{-1}),and LMF (g leaf g^{-1} plant) for nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)).

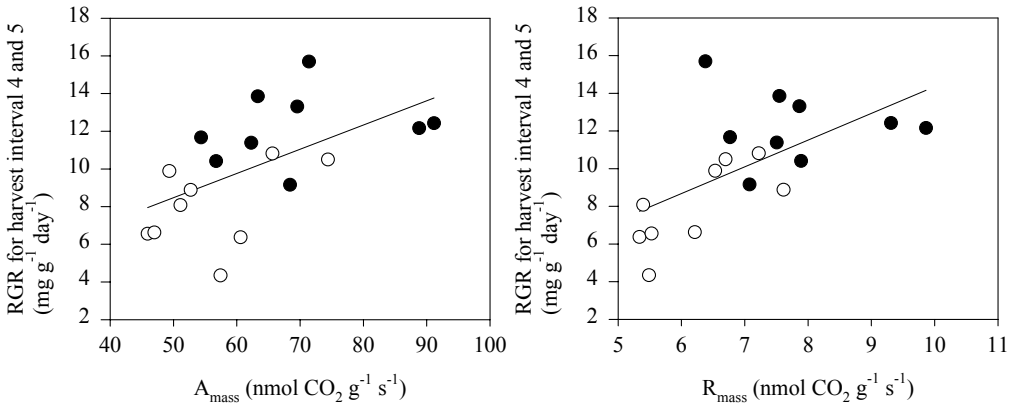

Figure 6. Relationships of RGR (mg g^{-1} day^{-1}) with A_{mass} (nmol CO_2 g^{-1} s^{-1}) and R_{mass} (nmol CO_2 g^{-1} s^{-1}), for nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)).

(r = 0.60, P = 0.0079) (not shown). Mean RGR was negatively correlated with PNUE (r = -0.79 P < 0.0001, Fig. 7).

Shoot RGR was negatively correlated with needle total nonstructural carbohydrates (TNC) (r = -0.58 P = 0.011, Fig. 8) and root RGR was negatively correlated with tap and coarse root TNC (r = -0.63 P = 0.0051). Therefore, carbohydrate

accumulation was associated with declining RGR of both roots and shoots among clones and N treatments.

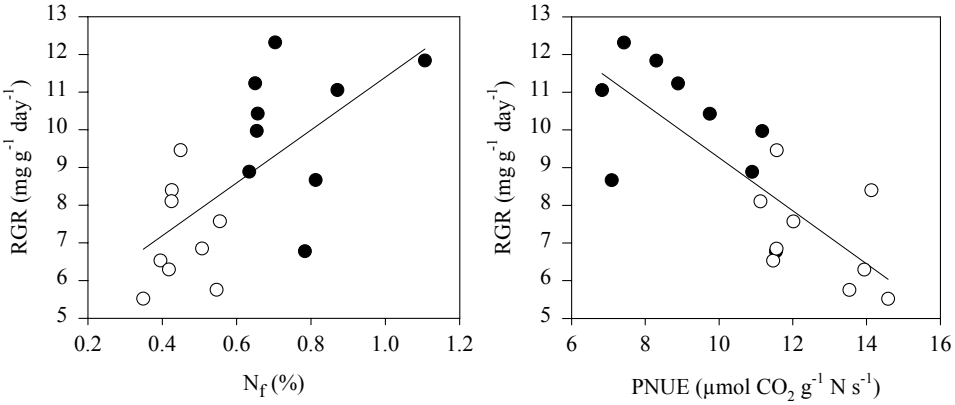

Figure 7. Relationships of RGR (mg g^{-1} day^{-1}) with N_f (%) and PNUE (µmol CO_2 g^{-1} N s^{-1}) for nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)).

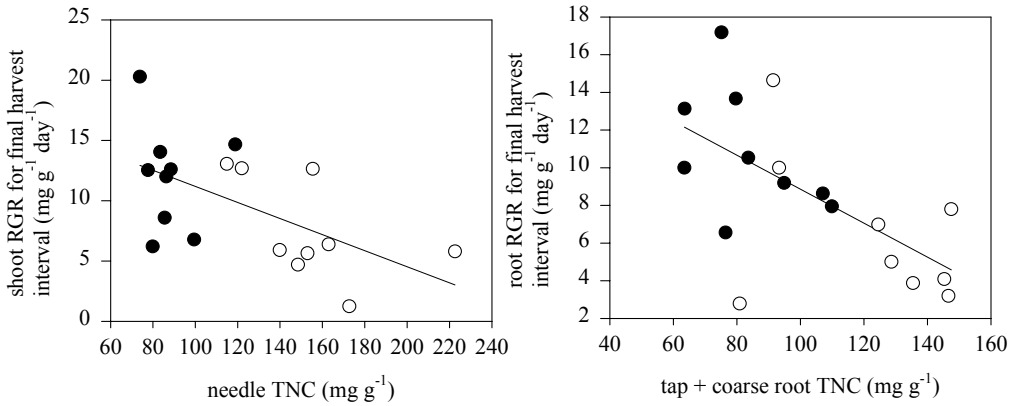

Figure 8. Relationships of shoot RGR (mg g^{-1}) and needle TNC (mg g^{-1}) and root RGR (mg g^{-1}) and tap and coarse root TNC (mg g^{-1}) for nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)).

Gas exchange trait correlations

Among clones and N treatments A_{mass} was positively correlated with needle nitrogen (r = 0.77 P = 0.0002, Fig. 9). Likewise, R_{mass} was positively correlated with needle nitrogen (r = 0.74, P = 0.0004, Fig. 9), suggesting that leaf-level rates of net CO_2 exchange were associated with N concentration.

A_{mass} was significantly negatively correlated with needle starch (r = -0.48 P = 0.045, Fig. 10), suggesting that starch accumulation was inhibiting photosynthesis. Needle glucose concentrations were positively correlated with A_{mass} (r = 0.65 P = 0.0032, Fig. 10), suggesting that plants with higher rates of photosynthesis have short-term accumulation of soluble sugars that are readily available to satisfy immediate metabolic demands.

Whole-plant RGR was correlated with water-use efficiency based on needle gas exchange measures near the end of the study (A/E, r = 0.76 P = 0.0003, Fig. 11) as well as long-term $\delta^{13}C$ (r = 0.89 P < 0.0001, Fig. 11). Consequently, mean needle $\delta^{13}C$ among clones and N treatment combinations was positively correlated with total seedling dry mass, height, and diameter at final harvest (Fig. 12). Furthermore, differences in water-use efficiency among clones and N treatments were associated with

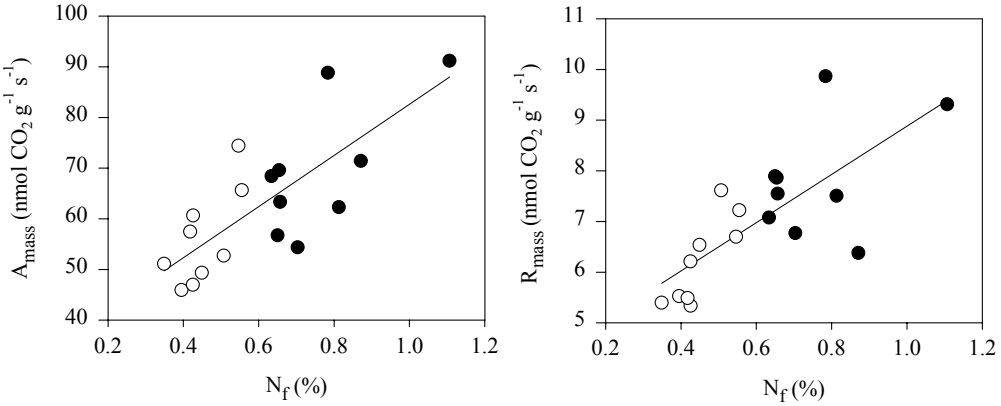

Figure 9. Relationships of A_{mass} (nmol CO_2 g^{-1} s^{-1}) and R_{mass} (nmol CO_2 g^{-1} s^{-1}) and N_f (%) for nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)).

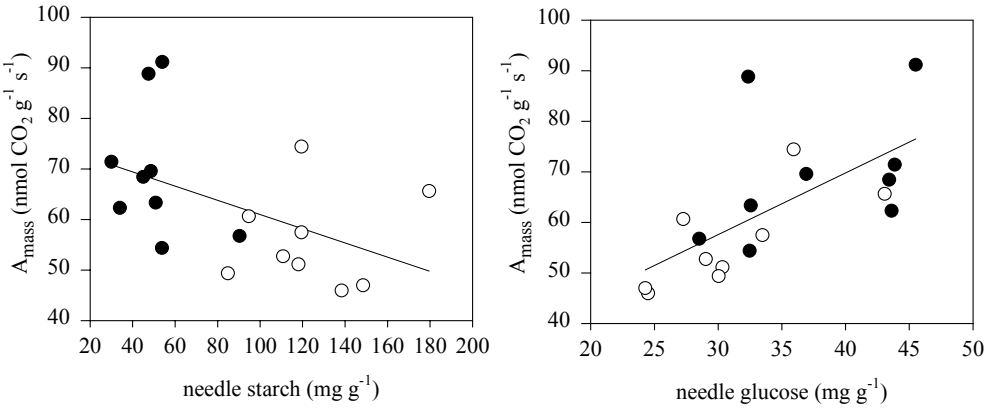

Figure 10. Relationships of A_{mass} (nmol CO_2 g^{-1} s^{-1}) and needle starch (mg g^{-1}) and needle glucose for nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)).

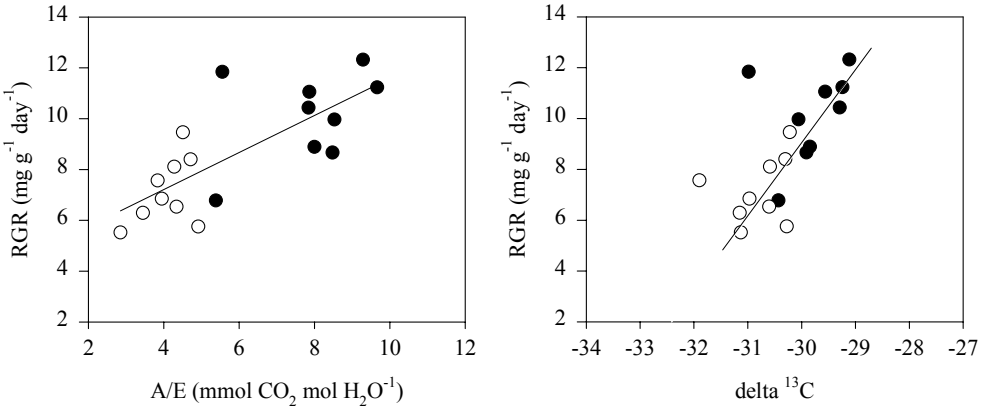

Figure 11. Relationship of RGR (mg g^{-1} day^{-1}) and A/E (mmol CO_2 mol^{-1} H_2O) and $\delta^{13}C$ (delta ^{13}C, ‰) for nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)). The relationship in the right panel excludes the two data points for clone 7528.

differences in biomass partitioning to roots. Mean root mass fraction at final harvest among clones and N treatments was positively correlated with g_s (r = 0.69 P = 0.0014), and negatively correlated with A/E (r = -0.71 P = 0.0009) and $\delta^{13}C$ (r = -0.79 P < 0.0001, Fig. 13). There were no correlations between PNUE and growth traits,

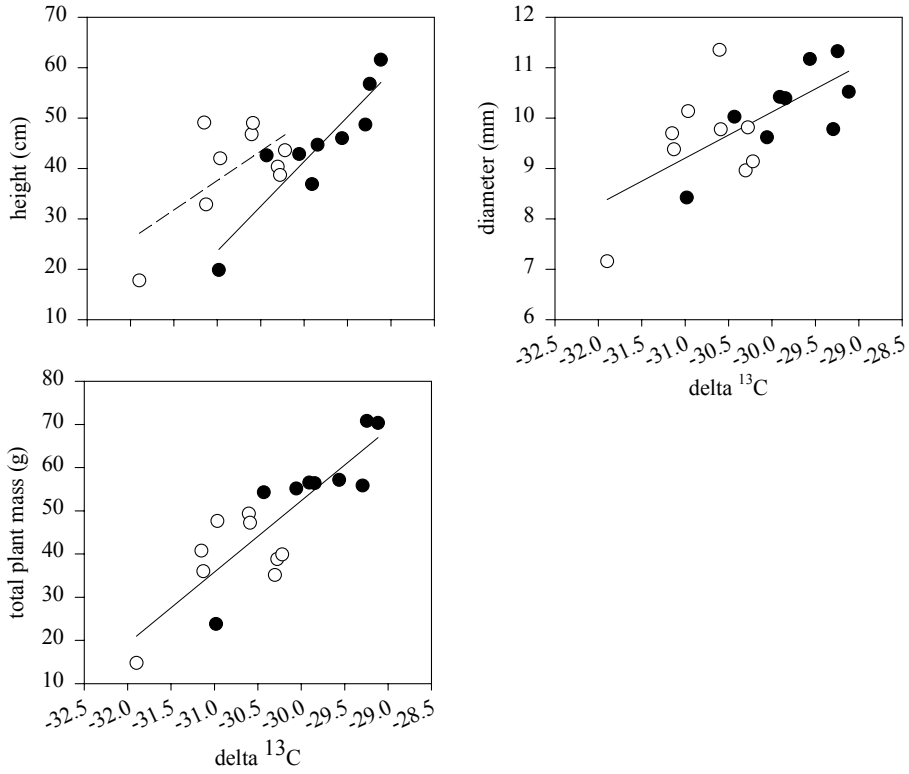

Figure 12. Relationships of $\delta^{13}C$ (delta ^{13}C, ‰) and height (cm), diameter (mm), and total plant mass (g) for nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)).

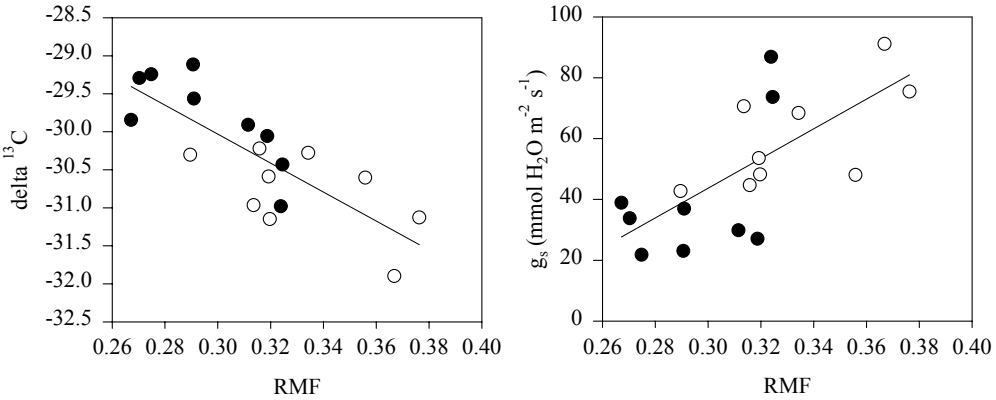

Figure 13. Relationships of $\delta^{13}C$ and g_s (mmol H_2O m^{-2} s^{-1}) and root mass fraction (RMF) of nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)).

suggesting no relationships between leaf-level nitrogen-use efficiency and plant growth traits. Furthermore, there was also a strong negative correlation between A/E and PNUE (r = -0.76 P = 0.0003, Fig. 14) suggesting there is a trade-off between water- and nitrogen-use efficiency.

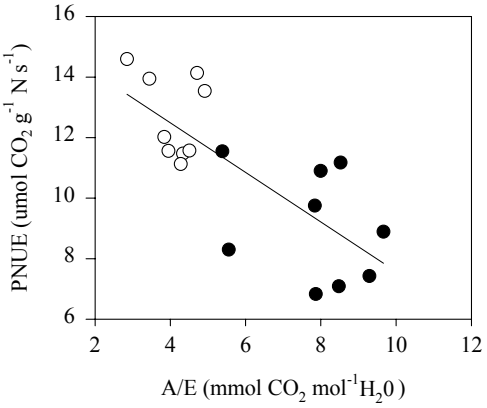

Figure 14. Relationship of PNUE (µmol CO_2 g^{-1} N s^{-1}) and A/E (mmol CO_2 mol^{-1} H_2O) of nine clones of loblolly pine grown under two nitrogen treatments (250 ppm (●) and 50 ppm (○)).

V. DISCUSSION

Determinants of RGR

The difference in RGR among the clones was largely associated with differences in biomass partitioning. RGR can be divided into two components: leaf area ratio (LAR), which is the amount of leaf area per unit total plant mass, and net assimilation rate (NAR), which is the rate of increase of plant mass per unit leaf area. LAR can be subdivided into SLA, which is the amount of leaf area per unit leaf mass and LMF, which is the fraction of total plant biomass allocated to leaves. Increased leaf area allows a plant to intercept greater amounts of photosynthetically active radiation, which in turn can provide greater amounts of fixed carbon to be used for growth and maintenance. RGR was better correlated with LAR than with NAR, suggesting that LAR was a stronger determinant of clonal growth rate variation.

RGR was positively correlated with LMF and negatively correlated with RMF indicating that clones that allocated a greater proportion of their biomass to needles and a lower proportion of their biomass to roots had higher RGR. Because clones that allocated and partitioned more biomass to needles and less to roots had higher RGR, differences in biomass allocation and partitioning are a plausible explanation of growth differences among the clones under the conditions of this study.

Although LAR appears to be the strongest determinant in clonal variation in RGR, it appears that the LAR of the plants in the two nitrogen treatments is responding oppositely. LAR of higher N plants is correlated with SLA differences more so than LMF. In contrast, the LAR of low N plants is more strongly correlated with LMF than SLA differences. SLA and LMF are the underlying components of LAR and RGR was significantly positively correlated with both SLA and LMF. The regression relationship between LAR and RGR appears to be stronger for the plants receiving 50 ppm N and the regression relationship between LMF and RGR appears mostly driven by differences in LMF between N treatments. This suggests that it is the combined effects of differences in SLA and LMF, particularly at lower N that are strongly correlated with RGR

differences. Optimizing leaf display by increasing SLA and increasing LMF at the expense of roots and stems will result in increased LAR.

Increased LAR could have allowed the clones to gain more carbon at the whole plant level by having proportionally more photosynthesizing needle surface area relative to total respiring plant biomass. Overall, decreased losses of carbon as respiration could have been achieved by having lower proportions of stem and root mass, which depend upon carbon from photosynthesis for growth and maintenance. Greater net whole-plant carbon gain will result in increased RGR. RGR of the clones was not related to carbon economy at the leaf level, but was positively correlated with LMF, SLA, and LAR. Since greater partitioning to needles (carbon source) and decreased partitioning to roots and stems (carbon sinks) will lead to an overall more net positive carbon gain (all other things being equal), this suggests that biomass partitioning to needles at the expense of roots is driving clonal and nitrogen treatment differences in RGR, hence LAR, not NAR, appeared more important as a determinant of RGR. The finding that LAR rather than NAR had the greater influence on RGR is consistent with some literature (Poorter and Remkes 1990, Saverimuttu and Westoby 1996), but contradicts Shipley (2002). My hypothesis that faster-growing trees would exhibit greater carbon gain at the whole-plant level through increased biomass partitioning to foliage mass and leaf area was supported by the positive correlations between RGR and LMF and LAR.

Effect of nitrogen supply on growth

At the end of the study mean total height and biomass was significantly increased by nitrogen treatment in agreement with previously reported increases in mean total biomass for N-fertilized loblolly pine (Bailian et al. 1991, Griffin et al. 1995, Retzlaff et al. 2001). While nitrogen fertilization significantly increased the total biomass of all clones, N fertilization significantly affected the allometric relationships between total dry mass and other plant tissues of roughly half the clones, although all clones exhibited similar trends. Higher nitrogen supply largely shifted biomass allocation away from roots and stems towards needles. Bailian et al. (1991) reported that nitrogen fertilization

had significant effects on the intercepts of root, stem, and needle allometric regressions, but not slopes. Bailian et al. (1991) found that loblolly pine seedlings favored biomass allocation to needles or both needles and stems and away from roots with nitrogen fertilization. In a field study on five-year-old loblolly pine, Retzlaf et al. (2001), found that supplying optimal fertilization had no effect on the allometric relationships between plant dry mass and various above- and below-ground tissues. Although higher nitrogen resulted in shifts in biomass allocation across most clones in the present study, there were no differences in the responsiveness of faster-growing compared to slower-growing clones to nitrogen fertilization in terms of dry mass growth.

Overall, increased nitrogen supply appeared to increase RGR through higher LAR. Nitrogen supply increased LAR by increasing LMF. At the final harvest, the higher N treatment increased LAR and LMF on average 16.2 percent, and 12.8 percent, respectively, over the lower N treatment, while SLA was did not differ between N treatments. Although SLA did not differ between N treatments, SLA was highly correlated with LAR in the higher N treatment ($r = 0.89$ $P = 0.0012$), suggesting that clonal differences in SLA influenced LAR more so under higher than lower N supply. Increasing LMF accounted for most of the observed increase in LAR in the higher N treatment, but increased SLA within the higher N treatment further increased LAR. Also, ANCOVA common slopes models showed that the intercepts of the allometric regressions of leaf mass allocation of higher N plants were higher than those of the lower N plants. This suggests that the partitioning patterns for LMF also held at a common plant size and not just at the final harvest among plants of vastly differing sizes. Because enhancing leaf area display by increasing SLA appears to be secondary to increasing the percentage of foliage biomass in response to increased N supply, this suggests that LMF is more important than SLA in increasing LAR under nitrogen fertilization. In conclusion, clones receiving 250 ppm N achieved higher RGR than clones receiving 50 ppm N through increased LAR. The increase in LAR was largely caused by a shift in biomass allocation from roots to shoots.

Gas exchange trait correlations

NAR is the balance of daily carbon gain through photosynthesis in the leaves and carbon use in the respiration of the entire plant, including leaves, stems, and roots. A/R ratios collected near the end of the study showed no correlation to mean plant RGR, implying that clones with higher RGR do not have a greater carbon economy (or carbon-use efficiency) at the leaf level, at least at the time when gas exchange data were collected. Rates of respiration were not collected on roots and stems, precluding a determination of whole-plant carbon economy. Other studies suggest that RGR differences among species may, in part, be linked to reductions in root respiratory costs related to nutrient uptake (Poorter et al. 1991). Although A/R ratios were not correlated with mean RGR, rates of A and R were positively correlated with mean RGR for the last harvest interval, suggesting that faster-growing clones have higher rates of gas-exchange. Other studies of broad interspecific variation among tree species have reported a positive correlation between RGR and photosynthesis and respiration (Reich et al. 1998, Tjoelker et al. 1998, 1999, Walters et al. 1993). Although the faster-growing clones had higher rates of gas exchange, my hypothesis that the faster-growers would exhibit greater carbon economy at the leaf level than the slower-growers was not supported by the gas exchange data. Higher N also increased rates of A and R and the relationship of RGR to A and R was partly linked to these increases. There were no differences in the responsiveness of rates of A and R of faster-growing compared to slower-growing clones to nitrogen fertilization.

Carbohydrate and growth relationships

Because rates of A were positively correlated with needle glucose concentrations and plants receiving 250 ppm N had rates of A_{area} and A_{mass} that were 16 percent and 23 percent higher than plants receiving 50 ppm N, elevated levels of needle glucose in the plants receiving higher N may be a result of short-term accumulation due to higher rates of A. Since rates of R_{area} and R_{mass} in plants receiving 250 ppm N were 23 percent and 26 percent higher than plants receiving 50 ppm N, the increased needle glucose

concentrations may be a function of the increased C sink strength of the needles and perhaps greater maintenance requirements.

Penning de Vries (1975) reported that respiration was roughly proportional to tissue protein content. The decrease in respiration in the needles of the plants receiving 50 ppm N could be linked to decreased protein content brought on by N deficiency. Nitrogen deficiency should cause a decrease in the protein content in the needles of N-deficient plants leading to lower respiration rates. Declines in respiration due to nitrogen deficiency have been previously reported (Brady 1973, Ryle et al. 1969).

According to Chapin (1980), nutrient stress has a greater effect on growth than on photosynthesis so that TNC concentrations rise above levels immediately needed for growth. In this study, the plants receiving 50 ppm N had a mean needle nitrogen concentration of nearly only one-third the reported critical level of 1.2 percent for loblolly pine (Allen 1987, Colbert and Allen 1996). Starch accumulation in the needles of nitrogen stressed plants indicates that growth is retarded more than photosynthesis (Wardlaw 1990). Lawlor et al. (1987) reported that inadequate nitrogen also prevents growth of sink/storage organs so these organs may accumulate carbohydrates as well. TNC concentrations in the needles of clones receiving 50 ppm N were nearly twice as high as the clones receiving 250 ppm N supporting my hypothesis that nitrogen deficient plants would accumulate non-structural carbohydrates. Presumably, the stress caused by nitrogen deficiency in the clones receiving 50 ppm N apparently resulted in the accumulation of starch as carbon gains exceeded the carbon demands for growth.

The accumulation of starch under low nutrient conditions may, in turn, result in reduced photosynthetic rates (Selga et al. 1983). In this study, the accumulation of carbohydrates under N stress indicates that photosynthesis may not be limiting growth, but the limited growth of the N-stressed clones may be interfering with photosynthesis. Carbohydrate accumulation in photosynthetic cells may slow rates of photosynthesis through "feedback inhibition." Starch accumulation in the chloroplasts decreases cytosolic P_i, which in turn results in decreased formation of ATP. A drop in supply of ATP causes a decline in the activity of the Calvin cycle. Less RuBP is regenerated

resulting in decreased carboxylating activity of Rubisco and photosynthesis (Lambers et al. 1998). Carbohydrate accumulation in photosynthetic cells may also slow rates of particular enzyme reactions in the photosynthetic carbon reduction cycle (PCR) (Lawlor and Keys 1993). Accumulation of carbohydrates in photosynthetic cells may increase the production of inhibitors that bind to Rubisco, namely carboxyarabinitol 1-phosphate (CA1P), which is derived from carbohydrate metabolism and linked to carbohydrate status (Lawlor and Keys 1993).

The TNC concentrations in the roots of plants receiving 250 ppm N were lower than those grown at 50 ppm, owing to differences in starch concentrations. Concentrations of glucose in the tap + coarse and fine roots were not significantly different between N treatments. Although it might be expected that concentrations of glucose in the tap, coarse, and fine roots would differ between N treatments because glucose is in a form that is more readily available for use in N assimilation (or other functions), this was not the case. Tap roots are important storage organs and the accumulation of starch in the roots of clones receiving lower N may result from reduced growth elsewhere in the plants. Because plants receiving lower N experienced shifts in biomass partitioning away from shoots and toward roots, the sink strength of the roots may have been greater than that of the needles. According to Chapin (1980), TNC reserves are allocated to roots for root growth at the expense of shoot growth in response to reduced nutrient status at low nutrient availabilities. Because clones receiving 50 ppm N had a 13 percent higher root mass fraction than clones receiving 250 ppm N and TNC concentrations in the roots were positively correlated with root mass fraction, the greater accumulation of starch reserves in the roots of clones receiving 50 ppm N was likely a function of the shift in growth of shoots to root growth, increasing the potential for N acquisition. In both N treatments, the RGR of the roots were lower than the RGR of the shoots. This is expected because shoots are usually the dominant carbon sink. Also, the root RGR in the 50 ppm N treatment was 47 percent lower than the shoot RGR, while the root RGR in the 250 ppm N treatment was only 43 percent lower than the shoot RGR. This is interesting because the RMF of the plants receiving 50 ppm N was

significantly higher than plants receiving more N and it would be expected that the ratio of root RGR to shoot RGR of the plants receiving 50 ppm N would be higher than that of the plants receiving higher N. This finding may relate back to the notion that growth is more retarded than photosynthesis under nutrient deficiency. RGR of plant organs was also negatively related to TNC concentrations and it appears that TNC accumulation was the result of these organs becoming sink limited.

Water-use and nitrogen-use efficiency

Higher rates of A combined with lower g_s resulted in the clones receiving 250 ppm N to have higher instantaneous water-use efficiency (A/E). The combination of high A and lower g_s also resulted in decreased Ci/Ca ratios of the plants receiving higher N, leading to reduced discrimination of ^{13}C during photosynthesis and therefore less negative $\delta^{13}C$ values. Significantly less negative $\delta^{13}C$ values also suggested a higher integrated water-use efficiency for clones receiving 250 ppm N. One explanation of the decreased Ci/Ca ratios of plants receiving higher N was that it was possibly caused by stomatal limitations brought on by mild water stress. According to Farquar et al. (1989), discrimination against ^{13}C decreases when stomatal conductance is reduced in relation to the capacity for CO_2 fixation. This was most likely the cause of lower internal concentration of CO_2 in clones receiving 250 ppm N. Less negative $\delta^{13}C$ is likely a consequence of higher photosynthetic capacity given the positive correlation with N_f (Prasalova et al. 2003). Clones receiving 250 ppm N had higher A at almost any given value for $\delta^{13}C$ than clones receiving 50 ppm N, and clones receiving higher N also had a significantly lower mean Ci/Ca ratio. The positive relationship between $\delta^{13}C$ and growth suggests that the faster-growing plants among clones and N treatments had greater water-use efficiency. The strong positive relationship between A/E and RGR supports this postulation. The finding that $\delta^{13}C$ was significantly correlated with short-term A/E measures is consistent with other studies (Craig and Zhang 2001, Olivas-Garcia et al. 2000).

There is also a trade-off between water-use efficiency and photosynthetic nitrogen-use efficiency (Livingston et al. 1999, Reich et al. 1989, Wang et al. 1998). This relationship was mostly the result of stomatal limitations to photosynthesis in the plants receiving higher N. Photosynthesis does not decline as much as transpiration with decreasing Ci, resulting in increasing water-use efficiency (A/E) with decreasing stomatal conductance. Although photosynthesis does not decline as much as transpiration under decreasing Ci, less photosynthetic capacity is used leading to a reduced photosynthetic nitrogen-use efficiency (PNUE) (Lambers et al. 1998). The trade-off between water- and nitrogen-use efficiency reflects the ability of loblolly pine to maximize resource-use efficiency during periods when water availability may become limited.

The balance between water uptake and water loss via transpiration at the whole-plant level can be modified by plant biomass partitioning patterns. In fact, RMF was positively correlated with stomatal conductance and negatively correlated with A/E and $\Delta^{13}C$, suggesting that clones partitioning less biomass to roots were more water-use efficient, although they likely exhibited lower whole-plant transpiration rates, and may in fact have been water-limited. A high root:shoot ratio increases the ability of plants to gain water by increasing the absorptive root surfaces relative to transpiring leaf area. Shifts in biomass allocation among clones and between N treatments likely caused the increase in stomatal conductance of clones with high a root:shoot ratio, because the balance shifted in favor of water acquisition. The significantly higher root:shoot ratio of clones receiving 50 than 250 ppm N is a likely cause of the lower water-use efficiency observed in these plants. Conversely, the reduced root:shoot ratio in plants grown under 250 compared to 50 ppm N, in part, resulted in a less favorable root to shoot balance in terms of water limitations to leaf-level gas exchange.

Field implications

The results of this study provide useful information for selecting loblolly pine genotypes that exhibit fast growth during early stages of development. Morphological traits

associated with leaf area development appear to be the key determinants of early growth rate differences among loblolly pine clones and early selection of genotypes with the greatest leaf area ratio could maximize gains during the initial development of stands. In this regard, dry mass allocation to leaf area was a key trait, although under higher nitrogen supply, genotype differences in specific leaf area emerged as a strong correlate of growth rate differences. Although early growth of plants in greenhouse studies do not always correlate to growth differences observed in field plantings, studies such as this might provide useful information on physiological and morphological traits that correlate to mature field performance. Early growth rate differences varied nearly two-fold among this limited set of clones, suggesting that potentially large selection gains are possible. Field trials will be necessary to separate genetic from environmental effects and to determine whether the faster-growing genotypes use the same growth strategies in both the laboratory and field.

The response of the clones to variation in nitrogen supply provides information on the potential responses of loblolly pine clones to environmental conditions typically present in the western gulf coast region. Differences in biomass allocation in response to nitrogen-supply were linked to water- and nitrogen-use efficiency and may affect early performance of clones in the field. Forest soils in the western gulf region are typically nutrient-limited and very dry during the summer months. Decreased nitrogen supply increased biomass allocation to roots and decreased water-use efficiency, but increased nitrogen-use efficiency. Increased root mass fraction may be helpful to newly planted clones by increasing their ability to acquire water and nutrients, but this comes at a cost – less efficient use of water. On the other hand, plants that received less nitrogen had greater photosynthetic nitrogen-use efficiency. Because there was a trade-off between water- and nitrogen-use efficiency, it appears that early selection for both of these traits cannot be simultaneous. Although early selection for both water- and nitrogen-use efficiency cannot be simultaneous, both WUE and PNUE might be maximized by matching nitrogen supply to the early demands of loblolly pine trees.

VI. SUMMARY

LAR appeared to be the key determinant of differences in relative growth rates among the clones of loblolly pine and faster-growing clones generally had greater LAR than slower-growers. Increased growth and growth rates of plants receiving higher nitrogen was also realized through increased LAR, which was increased by increasing LMF. SLA was a more important determinant of LAR differences in plants receiving higher N. LMF was a more important determinant of LAR differences in plants receiving lower nitrogen. In addition, faster-growing clones were more water-use efficient and likely exhibited lower whole-plant transpiration. Plants receiving lower nitrogen also exhibited lower water-use efficiency, which likely resulted from the significantly higher root mass fraction of the nitrogen-stressed plants. Moreover, nitrogen stress affected growth more than photosynthesis, resulting in the accumulation of non-structural carbohydrates.

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APPENDIX A

Appendix A. Definitions and units for gas-exchange rates, growth analysis, and tissue morphology terms used in this paper.

Term	Definition	Units
A_{area}	Net photosynthetic rate (area basis)	μmol CO_2 m^{-2} s^{-1}
A_{mass}	Net photosynthetic rate (mass basis)	nmol CO_2 g^{-1} s^{-1}
A/E	Instantaneous water-use efficiency (photosynthesis/transpiration)	mmol CO_2 mol^{-1} H_2O
A/R	Ratio of photosynthesis to respiration	
Ci/Ca	Ratio of internal CO_2 concentration to atmospheric CO_2 concentration	
g_s	Stomatal conductance	mmol H_2O m^{-2} s^{-1}
LAR	Leaf area ratio (total leaf area/total plant mass)	cm^2 g^{-1}
LMF	Leaf mass fraction	g leaf g^{-1} plant
NAR	Net assimilation rate	g m^{-2} day^{-1}
N_f	Foliar nitrogen concentration	%
PNUE	Photosynthetic nitrogen-use efficiency	μmol CO_2 g^{-1} N s^{-1}
R_{area}	Leaf respiration rate (area basis)	μmol CO_2 m^{-2} s^{-1}
RGR	Relative growth rate	mg g^{-1} day^{-1}
RHGR	Relative height growth rate	mm cm^{-1} day^{-1}
R_{mass}	Leaf respiration rate (mass basis)	nmol CO_2 g^{-1} s^{-1}
RMF	Root mass fraction	g root g^{-1} plant
SMF	Stem mass fraction	g stem g^{-1} plant
SLA	Specific leaf area	m^2 kg^{-1}
TNC	Total non-structural carbohydrate concentration (glucose + starch)	mg g^{-1}
$\delta^{13}C$	carbon isotope ratio (delta ^{13}C)	‰

VITA

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