**Tree Physiology** 29, 1117–1131
*doi:10.1093/treephys/tpp050*

# *Pinus taeda* clones and soil nutrient availability: effects of soil organic matter incorporation and fertilization on biomass partitioning and leaf physiology

MICHAEL C. TYREE,[1,2] JOHN R. SEILER,[3] CHRIS A. MAIER[4] and KURT H. JOHNSEN[4]

[1] *School of Forestry, Louisiana Tech University, P.O. Box 10138, Ruston, LA 71272, USA*

[2] *Corresponding author (mtyree@latech.edu)*

[3] *Department of Forest Resources and Environmental Conservation, Virginia Tech, 228 Cheatham Hall, Blacksburg, VA 24061, USA*

[4] *USDA-Forest Service, Southern Research Station, Research Triangle Park, NC 27709, USA*

Received October 29, 2008; accepted June 11, 2009; published online July 16, 2009

**Summary** The combined effects of intensive management and planting of improved seedlings have led to large increases in productivity on intensively managed pine forests in the southeastern United States. To best match clones to particular site conditions, an understanding of how specific clones respond to changes in nutrition in terms of biomass partitioning, leaf physiology and biochemistry will be necessary. This study measured the response of biomass partitioning, light-saturated net photosynthesis ($A_{Sat}$) and photosynthetic capacity to a range in soil fertility and fertilization between two contrasting *Pinus taeda* L. clone ideotypes: a 'narrow crown' clone (NC) that allocates more resources to stem growth and a 'broad crown' clone (BC) that allocates more resources to leaf area (LA). Under field conditions, we found consistent clone by environment (i.e., varying nutrient regimes) interactions in biomass as well as leaf physiology. Nutrient limitations induced by logging residue incorporation resulted in a 25% loss in stem growth in BC, while NC showed no response. We postulated that the decrease in BC was due to the differences in canopy architecture leading to a reduced canopy $CO_2$ assimilation, as well as to increased belowground maintenance costs associated with fine-root production. In contrast, N and P additions resulted in a 21% greater increase in stem volume in NC relative to BC. Fertilization increased $A_{Sat}$ temporarily in both clones, but $A_{Sat}$ eventually decreased below control levels by the end of the study. Although we found a clone by fertilization interaction in leaf physiology, the greatest genotype by environment interaction was found in the LA that appeared to have a greater influence than $A_{Sat}$ on growth. This research demonstrates the potential importance of selecting appropriate clonal material and silvicultural prescription when implementing site-specific silviculture to maximize productivity in intensively managed southern pine forests.

*Keywords: $A_{Sat}$, $G \times E$ interaction, $J_{max}$, loblolly pine, logging residue, photosynthesis, $V_{C,max}$.*

## Introduction

The combination of intensive management and planting of genetically improved seedlings has led to large increases in productivity on intensively managed pine forests in the southeastern United States. Estimated volume gains of 10–30% are possible with selective breeding and gains of 50% or more may be attained using the combination of clones and intensive silviculture (Allen et al. 2005, Martin et al. 2005, McKeand et al. 2006). Roth et al. (2007) found large genotype by environment interactions ($G \times E$) in full-sib *Pinus taeda* L. and *Pinus elliottii* Engelm. clones planted in different locations and undergoing different silvicultural treatments. In contrast, others contend that silvicultural effects are stable across broad numbers of open-pollinated, half- and full-sib families and clones (McKeand et al. 2006). As forest managers move toward site-specific silviculture, an understanding of how clones respond to changes in site resource availability is necessary for matching the most appropriate planting stock to site conditions and for maximizing productivity in intensively managed southern pine forests (Fox 2000).

Past research had demonstrated genotype by environment interactions for C allocation, N use efficiency (NUE; stem biomass per unit N) and stem growth (Li et al. 1991*a*, 1991*b*, 1991*c*, Samuelson 2000, Retzlaff et al. 2001), but leaf physiological traits have proven to be less

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consistent. For example, some studies show no G × E in leaf gas exchange (Samuelson 2000, Bown et al. 2007, Chmura and Tjoelker 2008), while Koehn et al. (2003) observed G × E in photochemical quenching and yield of PSII in full-sib *P. elliottii* seedlings. Additionally, King et al. (2008) showed different responses in net photosynthesis under saturating light ($A_{Sat}$) to fertilization in *P. taeda* among clones that shared the same parents. They found wide ranging differences between clones with some increasing growth and $A_{Sat}$, while others achieved similar growth responses by simply increasing leaf area (LA) with little or no increase in $A_{Sat}$. Likewise, in a greenhouse experiment using contrasting *P. taeda* clones, one clone achieved an increased canopy level $CO_2$ assimilation ($A_{Canopy}$) following fertilization by increasing LA, while the other clone achieved the same $A_{Canopy}$ by increasing $A_{Sat}$ (Tyree et al. 2009).

Nitrogen and phosphorus fertilization to increase growth on nutrient poor sites is a common management tool in the southern United States pine forests (Fox et al. 2007). Increases in LA and total biomass following fertilization are well established in conifers (Vose and Allen 1988, Albaugh et al. 1998, Borders et al. 2004), but effects on leaf physiology are not. Studies using young conifers have found increased photosynthesis on a LA basis following fertilization (Green and Mitchell 1992, Tissue et al. 1993, Murthy et al. 1996, Walcroft et al. 1997, Samuelson 2000, Gough et al. 2004*b*, Warren et al. 2004, Bown et al. 2007, King et al. 2008); however, others have not (Maier et al. 2002, Warren and Adams 2002, Bauer et al. 2004). This inconsistency, in part, has led to the poor correlation between $A_{Sat}$ and growth in conifers (Munger et al. 2003). However, there is a strong correlation between $A_{Canopy}$ at the seedling, tree or stand level and plant growth (Kruger and Volin 2006, Chmura and Tjoelker 2008). Increased photosynthetic capacity following fertilization may only be a transient effect, disappearing with increased LA (Gough et al. 2004*a*, Maier et al. 2008). Gough et al. (2004*b*) proposed a series of physiological adjustments, or steps, that lead to an increased growth in young *P. taeda* following fertilization. The authors theorized an early increase in $A_{Sat}$ that led to greater photo-assimilates which were used to increase LA. The increased photosynthetic area later facilitated a down-regulation of $A_{Sat}$, while maintaining higher $A_{Canopy}$.

Nitrogen is a major component in all proteins and pigments (i.e., chlorophyll and Rubisco) involved in photosynthesis with a large proportion of foliar N distributed between photosynthetic machinery associated with both light and dark reactions (Evans 1989). The photosynthetic parameters maximum carboxylation capacity ($V_{C,max}$), maximum electron transport ($J_{max}$) (Kellomaki and Wang 1997, Strand 1997, Walcroft et al. 1997, Ripullone et al. 2003, Manter et al. 2005, Bown et al. 2007) and $A_{Sat}$ (Green and Mitchell 1992, Mitchell and Hinckley 1993, Roberntz and Stockfors 1998, Schoettle and Smith 1999, Samuelson 2000, Ripullone et al. 2003) are strongly correlated with foliar N concentration ($[N]_f$), although some have found poor correlations between $[N]_f$ and $A_{Sat}$ (Will et al. 2001, Gough et al. 2004*b*, King et al. 2008) in conifers. Warren et al. (2003) showed that inactive Rubisco was positively correlated with $[N]_f$ in *Pinus sylvestris* L. and hypothesized that poor photosynthetic nitrogen use efficiency (PNUE; $CO_2$ assimilated per unit N) in conifers may be due to the storage of excess N in inactive Rubisco and amino acids (Warren and Adams 2004).

The objective of this study was to investigate the interaction between nutrient availability and genetics on biomass partitioning and leaf gas exchange. Site nutrient availability was manipulated by incorporating high C:N (about 700) logging residue (LR) into the mineral soil, fertilization with N and P (F) or both (LR + F). Previous work found that the incorporation of LR decreased N mineralization rates (Tisdale 2008) and increased microbial biomass C and N (Tyree 2008) after 2 years. Two *P. taeda* clones, belonging to two distinct ideotypes ('narrow crown' versus 'broad crown'; see Martin et al. 2001), were selected for this study. Both clones attained a similar stem biomass, while maintaining large differences in LA (Figure 1). This experiment provided an opportunity to ask two specific questions. First, are changes in stem volume, biomass partitioning, $A_{Sat}$ and photosynthetic capacity (i.e., $V_{C,max}$ and $J_{max}$) in response to LR incorporation (nutrient limitations) different between clones? We hypothesized that the greater nutrient demand associated with the 'broad crown' clone (BC) would result in decreased stem growth and increased root to shoot ratio in treatments receiving LR. In contrast, the 'narrow crown' clone (NC) would be less negatively affected by the incorporation of LR. We also hypothesized that LR incorporation would result in decreased $A_{Sat}$ and photosynthetic capacity in both clones, but to a greater extent in BC. Second, are changes in stem volume, biomass partitioning, $A_{Sat}$ and photosynthetic capacity in response

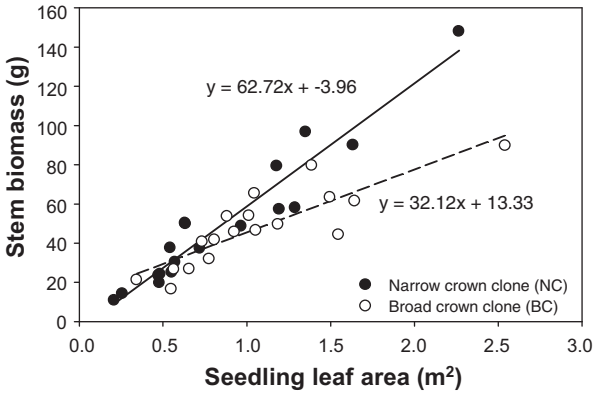


Figure 1. Differences in growth efficiency between *P. taeda* clones following the end of the first growing season January 2006. Data were collected from the current study site by the USDA-Forest Service, Southern Research Station. Coefficient of determination ($r^2$) for NC and BC is 0.92 and 0.72, respectively ($n$ = 18).

to N and P fertilization different between clones? We hypothesized that the NC would be less able to respond to N and P additions in terms of increased stem volume and biomass partitioning relative to BC. In addition, we hypothesized that fertilization would lead to an immediate increase in $A_{Sat}$ in both clones followed by an eventual decrease in $A_{Sat}$ at or below control levels with increasing LA.

## Materials and methods

### *Site location, climate and stand history*

The study site was located in Berkeley County, SC (33°16′49.94″ N and 80°10′9.46″ W) at an elevation of 24 m above mean sea level. Average annual temperature was 14.6 and 17.4 °C with an average daily maximum of 17.3 and 25.2 °C, and an average daily minimum of 11.7 and 11.2 °C for 2006 and 2007, respectively (Figure 2). Highest daily average temperature was 26.8 and 32.5 °C occurring in August 2006 and August 2007, respectively, and a low daily average of −0.9 and 0.4 °C occurring in December 2006 and February 2007, respectively. Total precipitation was 90.2 cm in 2006 and 74.9 cm in 2007 spread evenly throughout the year, which was well below the average of 120 cm recorded between 1949 and 1973 (Long 1980). The dominant soil series is Ocilla (loamy, siliceous, semiactive, thermic Aquic Arenic Paleudults). Harvest of the previous 21-year-old *P. taeda* plantation took place in May 2004 and the site was sheared of residual material in July 2004. The LR treatments were applied in October 2004, and site preparation (bedding) took place in early November 2004. *Pinus taeda* clones were planted in January 2005, and the data for this study were collected between January 2006 and January 2008.

### *Study design and treatments*

The study design was a split-plot, randomized complete block design replicated three times with the whole-plot treatments arranged as a full 2 × 2 factorial. Two levels of LR and two clones (BC and NC) served as the whole-plot treatments and fertilization constituted the split-plot treatment. Each 0.18 ha plot (48 × 38 m) was planted with ∼ 243 container grown, clonal *P. taeda* seedlings in nine rows at a 1.8 m spacing within rows and at a 4.3 m spacing between row centers. Within each main plot, two 0.0013 ha split plots were located at opposite ends. Each split plot consisted of six seedlings from one row (four measurement trees + two buffer trees) and served as the experimental unit (EU).

The two levels of LR were no LR incorporated (w/o LR) and LR incorporated during bedding into the mineral soil (LR) at a rate of 25 Mg o.d. wt. $ha^{-1}$, which was concentrated onto the beds (∼ 75 Mg o.d. wt. $ha^{-1}$; C:N = 700). Both LR treatments also incorporated the residual forest floor of ∼ 25 Mg o.d. wt. $ha^{-1}$. The two *P. taeda* clones chosen both exhibit a superior height growth, but represent two distinct ideotypes. The NC has been shown to allocate more of its resources to stem growth, while the BC allocates more resources to LA (Figure 1). Each split plot received one of the two fertilizer applications: no nutrient additions (NF) or N and P fertilization (F). During the 2006 growing season, F was applied twice and totaled 209 kg N and 116 kg P $ha^{-1}$ in the form of diammonium phosphate and ammonium nitrate (AN). Roughly one-third was applied on 6 April 2000 and the remaining two-thirds applied on 8 May 2006. Fertilization for the 2007 growing season was applied on 9 March 2007 at a rate of 200 kg N $ha^{-1}$ in the form of AN.

### *Stem volume, projected canopy area and biomass partitioning*

Tree height was initially measured to the nearest centimeter using a meter stick and later to the nearest 20th of an inch using a Philadelphia rod (Model 903043; Crain Enterprises, Mound, IL). Basal diameter was measured at ∼ 2–5 cm above the ground line to the nearest millimeter using digital calipers. Aboveground stem volume was calculated for all 16 measurement dates by multiplying aboveground tree height by ground line diameter squared.

At the conclusion of the experiment, a single tree, which most closely fits the mean tree height from each EU, was selected to estimate biomass partitioning and canopy silhouette area (CSA). CSA is defined as the total leaf and twig area contained within the tree canopy projected onto a plane (King et al. 2008). The aboveground portion of each seedling was cut 10 cm above the ground line and

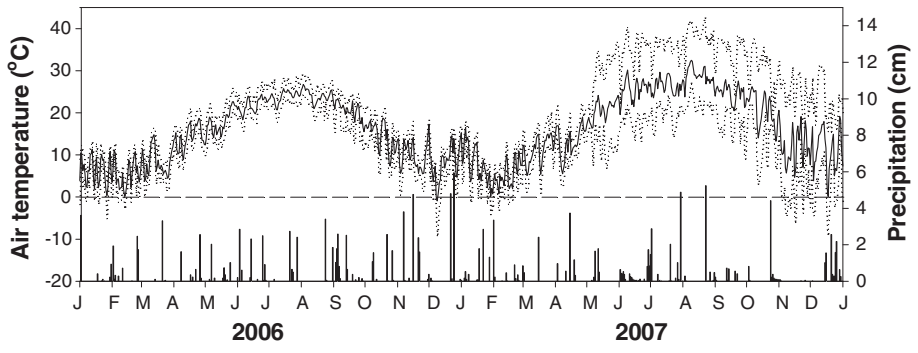


Figure 2. Mean (solid line), minimum (dotted line) and maximum (dotted line) daily air temperature and total daily precipitation (bars) between January 2006 and January 2008 for the cross-study site located in the Berkeley County, SC.

transported to a staging area where the sky was used as a high contrast backdrop. Two photographs were taken orthogonally to each other for each tree using a 28–70 mm 1:2.8–4.0 D zoom lens (Sigma Corp., Kanagawa, Japan) mounted on a D70 digital SLR (Nikon Corp., Tokyo, Japan). The color digital image was converted to black and white using SideLook 1.1 software (Nobis 2005) with the channel set to red. Adobe® Photoshop® 6.0 (Adobe Systems Inc., San Jose, CA) was then used to clean up the black and white image, and to determine the number of pixels in the image. A standard known area reference in each photograph was used to convert from the number of pixels in the image to the projected area ($m^2$).

After photographing each tree, the aboveground plant parts were wrapped in plastic, taken back to the laboratory and dissected into needles, branches and main stem. Belowground plant tissues were sampled by excavating a $1.0 \times 1.0 \times 0.5$ m volume around the main stem. Roots were handpicked from the soil, bagged and taken with the aboveground tissues to the laboratory. In the laboratory, the root system was separated into tap root and lateral roots and washed. All the separated plant parts were oven dried ($>$ 2 weeks) at a temperature of 65 $\pm$ 5 °C and then weighed gravimetrically to the nearest gram.

### *Instantaneous net photosynthesis*

Gas exchange was sampled on two trees per EU using an open-flow, infrared gas analyzer (IRGA) equipped with a $2 \times 3$ cm cuvette with a blue–red LED light source (Li-Cor 6400, Lincoln, NE). On 21 sampling dates between January 2006 and December 2008, $A_{Sat}$ was measured on individual fascicles under saturating light between 10:00 and 16:00 h. Measurements were taken at ambient temperature and relative humidity under the following settings: 1600 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density (PPFD), 370 μmol $mol^{-1}$ reference $CO_2$ concentration and a flow rate of 300 μmol $s^{-1}$. Most recently elongated needles were detached from the upper third, south facing side of each tree and immediately placed in the cuvette. Preliminary tests using the same trees showed no difference in $A_{Sat}$ between attached (1.78 $\pm$ 0.12) and detached (1.78 $\pm$ 0.11) needles ($P = 0.98$; $n = 8$). Once $A_{Sat}$ stabilized (typically $<$ 2 min), three measurements were taken at 10 s intervals and averaged to a single rate. Fascicle diameter was measured using digital calipers to the nearest 0.01 mm and the LA ($cm^2$) calculated (Ginn et al. 1991).

### *Photosynthesis–$CO_2$ response curves*

Photosynthetic parameters were derived from net $CO_2$ assimilation–internal $CO_2$ partial pressures ($A/C_i$) and light ($A$/PPFD) response curves constructed in the field on three sampling dates. June 2006, October 2006 and March 2007 were the sampling dates chosen to represent three distinct phenological stages as well as to investigate immediate adjustments in photosynthetic machinery between contrasting genotypes immediately following nutrient manipulations. Net photosynthesis–$CO_2$ response curves ($A/C_i$) were constructed from field measurements of $A_{Sat}$ taken on attached needles over a range of external $CO_2$ partial pressures (370, 115, 150, 230, 300, 370, 570, 1000, 1500 and 1800 μmol $mol^{-1}$). $A/C_i$ curves were measured at 1600 μmol $m^{-2}$ $s^{-1}$ PPFD at a flow rate of 300 μmol $s^{-1}$ using an open-flow, IRGA equipped with a blue–red LED light source (Li-Cor 6400). Leaf temperature was held to 25, 18 and 18 °C for June 2006, October 2006 and March 2007 sampling dates, respectively, which was near ambient temperature for that time of year. Relative humidity was held to a range of 60–70%, 40–50% and 20–30% for June 2006, October 2006 and March 2007 sampling dates, respectively. Needles and gas exchange rates were carefully monitored to insure the needles did not detach during measurements, which lasted about 45 min for each curve, and the IRGA was matched between each partial pressure.

Nine pairs of $A_{Sat}$ and $CO_2$ partial pressures were used to calculate in vivo maximum carboxylation rate ($V_{C,max}$), RuBP regeneration capacity ($J_{max}$) mediated by maximum electron transport rate, triose phosphate utilization (TPU), mesophyll conductance ($g_m$) and day time leaf respiration ($R_L$) using a freely available $A/C_i$ curve fitting utility Version 1.1 (Sharkey et al. 2007). Briefly, photosynthetic parameters were estimated by assigning each $A_{Sat}$–[$C_i$] data pair to one of the three possible limitations to photosynthesis: Rubisco, RuBP regeneration or TPU. A non-linear $A/C_i$ curve fitting utility was then used to fit the data by minimizing the sum of squares. Estimates of $g_m$ from both the Rubisco and RuBP regeneration curves were used to calculate $CO_2$ partial pressure within the chloroplast. For improved treatment comparisons, all parameters were scaled to a constant temperature of 25 °C using scaling factors further explained by Sharkey et al. (2007).

### *Net $CO_2$ assimilation–light response curves*

Net $CO_2$ assimilation–light response ($A$/PPFD) curves were measured by graphically comparing $CO_2$ assimilation within a range of light levels to determine light compensation point (LCP), mitochondrial respiration ($R_M$) and apparent quantum yield ($Q$). Measurements were taken at approximately the same date following the same procedure as $CO_2$ response curves with the following changes. Reference $CO_2$ partial pressure was maintained at 370 μmol $mol^{-1}$ and measurements were made at the following light levels: 1800, 1400, 1000, 600, 400, 200, 100, 50, 20 and 0 μmol $m^{-2}$ $s^{-1}$ PPFD. Parameters for the $A$/PPFD curves were calculated by fitting response curves to a non-rectangular hyperbola using non-linear least-squares regression (Hanson et al. 1987) using the following equation:

$$A = A_{Sat} \times \left\{ [1 - (1 - R_M/A_{Sat})]^{(1-PPFD/LCP)} \right\}, \qquad (1)$$

where $A$ is net $CO_2$ assimilation at a given light level, $A_{Sat}$ is light-saturated net $CO_2$ assimilation, $R_M$ is

mitochondrial respiration taken during the day, PPFD is photosynthetic photon flux density and LCP is light compensation point. Apparent quantum yield ($Q$) was calculated using the first derivative of Eq. (1)

$$Q = (A_{Sat}/R_M) \times (1 - LCP/A_{Sat}) \times \ln(1 - LCP/A_{Sat}). \quad (2)$$

*Foliar N and leaf morphology*

Foliar N and leaf morphology were measured on foliage samples used in $A_{Sat}$ and $A/C_i$ curves. Needle length (from tip to point where needles enter the fascicle), needles per fascicle, diameter and photosynthetic LA were determined on fresh needles. Needles were then oven dried at 65 °C for more than 48 h and weighed to the nearest milligram. Specific needle area (SLA; $cm^2\ g^{-1}$) was calculated as an estimate of needle dimensions and density. Following morphology measurements, oven-dried needles were ground individually using a Wiley mill (Model 3; Arthur H. Thomas Co., Philadelphia, PA) fitted with a number 20 screen, and then 15 ± 5 mg of powdered sample was weighed into an aluminum crucible. Foliar N was analyzed using a Carlo-Erba elemental analyzer (Model NA 1500; Fison Instruments, Danvers, MA). Foliar [N] was expressed on a LA ($[N]_a$; $g\ N\ m^{-2}$) and mass ($[N]_m$; $g\ g^{-1}$) basis. The PNUE ($\mu mol\ CO_2\ g^{-1}\ N\ s^{-1}$) was also calculated by dividing $A_{Sat}$ by $[N]_a$.

*Data analyses*

For $A_{Sat}$ measurements, subsamples were averaged to the plot level before statistical analysis. Treatment differences for $A_{Sat}$, photosynthetic capacity, foliar [N] and leaf morphology were determined using analysis of variance with repeated measures (ANOVARM) using a MIXED model approach (SAS 1999). LR, clone (CL), fertilizer and interaction terms were analyzed as fixed effects and time was treated as a random effect. LR, CL and LR × CL were analyzed using Rep × LR × CL as an error term. F, F × LR, F × CL and F × LR × CL were tested using Rep × LR × CL × F as an error term. All terms including time were tested using Rep × LR × CL × F × time. Covariance structures were selected by comparing Akaike's Information Criterion and Schwarz's Bayesian Criterion fit statistics across covariance structures, which are included in the default SAS output (Littell et al. 2006). Significant time interactions were analyzed for individual sampling dates, in addition to differences in biomass partitioning and CSA, using a general linear model. Rep, LR, CL and LR × CL were analyzed using Rep × LR × CL as an error term and F, F × LR, F × CL and F × LR × CL were tested using Rep × LR × CL × F as an error term. When significant treatment differences were observed in leaf physiology data, post hoc tests of differences between least-squares means were performed for all pair-wise comparisons using the P-diff option in the LSMEANS statement in SAS. Growth efficiency (the slope of the relationship between stem volume and CSA), canopy density (relationship between leaf biomass and CSA) and the relationship between $[N]_a$ and photosynthetic capacity were explored using linear regression. Indicator (dummy) variables were used to test for significant differences between slope estimates. When data did not meet assumptions of normality and equal variance, as indicated by viewing plots of residuals and normality curves, response variables were transformed by their natural log. All values were expressed as untransformed least-squares means and standard errors. Due to the low replication of this experimental design, an alpha level of 0.10 was considered statistically significant. All analyses were performed using the MIXED, GLM, REG and NLIN procedures in SAS Version 9 (SAS 2006).

## Results

*Stem volume and biomass partitioning*

At the end of two growing seasons, stem volume differed between fertilizer and LR treatment combinations ($P = 0.003$). The application of fertilizer (F) by itself increased the stem volume by 63%, whereas, the application of fertilizer and LR (LR + F) suppressed the F response resulting in only a 28% increase in stem volume relative to control treatments (Figure 3A). Nutrient manipulation treatments impacted stem volume differently between clones. For example, the incorporation of LR resulted in a 25% decrease in stem volume in BC, while no decrease was observed in NC ($P = 0.01$; Figure 3B). In addition, the application of F resulted in a 21% greater increase in stem volume in NC relative to BC ($P = 0.003$; Figure 3C).

Estimates of biomass partitioning generally supported our stem volume data with our largest nutrient manipulation effects being observed in the aboveground tissues (Table 1; Figure 4). Other than the ratio of foliage to stem mass, we observed no differences in biomass partitioning between clones as a main effect; however, we did observe a number of interactions between clone and nutrient manipulation treatments (Table 1). For example, LR incorporation decreased stem, lateral root and total belowground biomass by 36, 33 and 30%, respectively, in BC, but showed no response in NC (Table 1). Fertilization resulted in an increased foliar biomass in both clones ($P = 0.0004$), but the magnitude of the response was 60% greater in NC relative to BC (Figure 5).

The effects of soil amendments on CSA were consistent with our findings of foliar biomass with a significant interaction between LR and F treatments ($P = 0.08$). LR by itself had no effect on CSA relative to control (None) treatments (24,891 and 24,768 $cm^2$, respectively; $P = 0.97$). Fertilization in the absence of LR resulted in

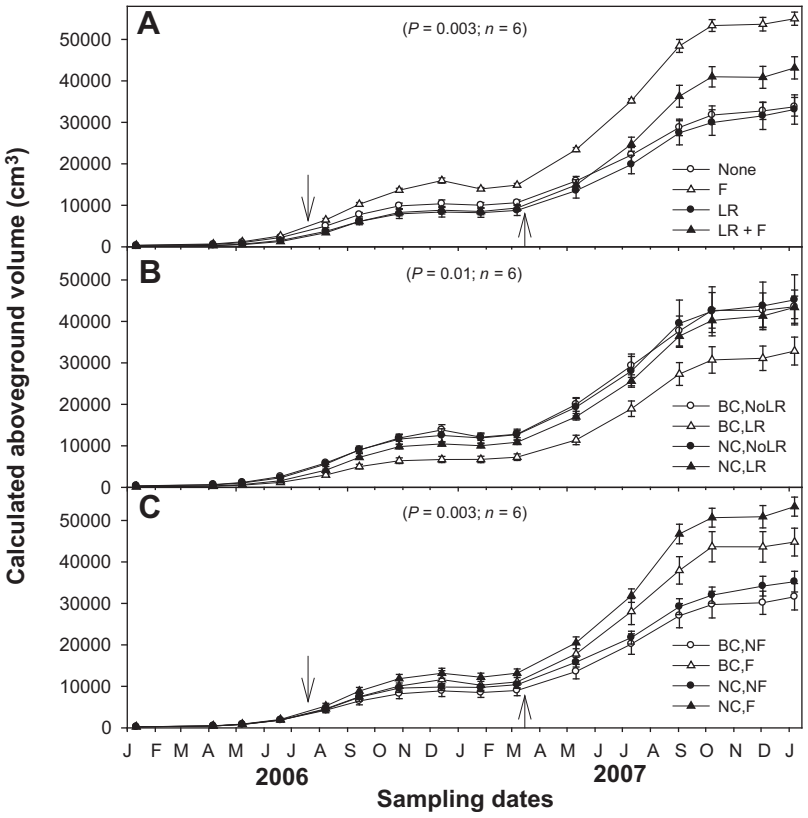


Figure 3. Least-squares means stem volume as affected by LR by fertilizer (panel A), clone (CL) by LR (panel B) and CL by fertilizer interactions. Arrows indicate time of fertilization and error bars represent ± standard error from the mean and *P* values represent treatment interactions with time. Aboveground volume was calculated using the following equation: volume = height × (basal diameter)$^2$.

Table 1. Statistical summary of *P* values for LR, clone (CL) and fertilizer (F) main effects and interactions for biomass partitioning. An average size tree was destructively harvested from each plot (24 trees in total) on January 2008. Statistical analyses were performed using the GLM procedure in SAS Version 9 (SAS 2006).

| Parameter | Main effects | | | Two-way interactions | | | Three-way interactions |
|---|---|---|---|---|---|---|---|
| | LR | CL | F | CL × LR | CL × F | LR × F | CL × LR × F |
| Foliage | n.s. | n.s. | 0.0004 | n.s. | 0.0060 | 0.0009 | n.s. |
| Branches | 0.1096 | n.s. | 0.0043 | n.s. | n.s. | 0.0227 | n.s. |
| Stem | 0.0857 | n.s. | 0.0162 | 0.0942 | n.s. | 0.0125 | n.s. |
| Total aboveground | 0.1064 | n.s. | 0.0012 | n.s. | n.s. | 0.0018 | n.s. |
| Lateral roots | 0.1075 | n.s. | 0.0022 | 0.0164 | n.s. | n.s. | n.s. |
| Tap roots[1] | n.s. | n.s. | 0.0952 | n.s. | n.s. | n.s. | n.s. |
| Total belowground | 0.0483 | n.s. | 0.0098 | 0.0995 | n.s. | 0.0807 | n.s. |
| Total biomass | 0.0695 | n.s. | 0.0009 | n.s. | n.s. | 0.0022 | n.s. |
| Root:shoot | n.s. | n.s. | n.s. | n.s. | n.s. | 0.1026 | n.s. |
| Foliage:stem | 0.0123 | 0.0346 | n.s. | 0.0053 | 0.0625 | n.s. | n.s. |

[1]Variable was transformed by its natural log to meet assumptions of ANOVA.

a 54% increase in CSA relative to controls (38,071 and 24,768 cm$^2$, respectively; $P = 0.005$), but in the presence of LR (LR + F) only increased by 14% relative to control plots (28,223 and 24,768 cm$^2$, respectively; $P = 0.35$). However, unlike our foliar biomass data, we found no interaction between clone and F ($P > 0.10$), instead, both clones responded to F ($P = 0.01$) by increasing CSA on average by 33%. In contrast, we did observe an interaction between clone and LR ($P = 0.006$). We found that BC responded to LR by decreasing CSA by 25%, which brought it to the same level as NC with or without LR.

### *Growth efficiency and canopy architecture*

We defined growth efficiency as the slope of the linear relationship between stem volume produced and CSA. We found a greater response in growth efficiency to F in

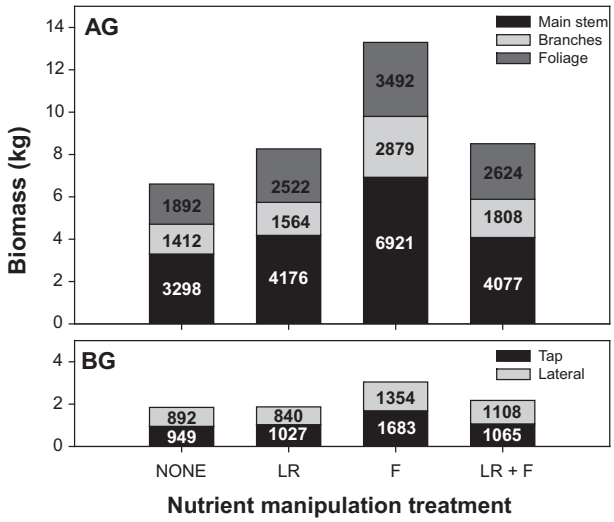


Figure 4. Least-squares means of biomass partitioned among aboveground (AG) and belowground (BG) plant tissues. One 3-year-old *P. taeda* was destructively harvested from each plot on January 2008. Numbers represent biomass for each tissue in grams (see Table 1 for list of *P* values).

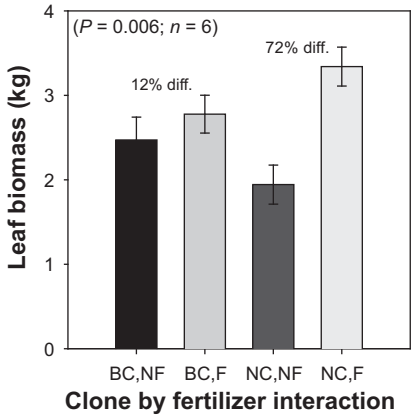


Figure 5. Clone by fertilizer two-way interaction in leaf biomass. Error bars represent ± 1 standard error of the mean.

BC relative to NC (Figure 6A). For example, F increased growth efficiency in BC by 80% ($m$ = 0.69 and 1.24 for NF and F, respectively), whereas we observed a much smaller increase (24%) between F treatments in NC ($m$ = 1.57 and 1.95 for NF and F, respectively). In BC, growth efficiency (slope estimate) ranged from not being significantly different from zero in NF treatments ($P$ = 0.41) to being significant in F treatments ($P$ = 0.06), although a comparison of slope estimates showed no difference between F treatments in either clones ($P$ > 0.10). Finally, we observed a greater growth efficiency regardless of F treatment in NC relative to BC as indicated by a shift to the left in the regression line of NC relative to BC (Figure 6A). We also observed a much greater response to LR incorporation in BC, but in the opposite direction of its response to F (Figure 6B). For instance, the incorporation of LR decreased growth efficiency by 60% in BC ($m$ = 1.04 and 0.42 in NoLR and LR, respectively) and had no effect in NC ($m$ = 1.87 and 1.73 in NoLR and LR, respectively). Regardless of whether LR was added or not, NC showed a greater growth efficiency than BC as indicated by a significantly ($P$ = 0.08) steeper slope estimate.

Canopy density was estimated by regressing leaf biomass by CSA. This estimate of canopy architecture was interpreted as a smaller (shallow) slope estimate representing a denser canopy (greater foliar mass per unit canopy area). In contrast to our biomass partitioning and growth efficiency data, we found that BC responded to F by decreasing ($P$ = 0.08) canopy density, whereas F had no effect ($P$ > 0.10) in NC (Figure 7A). Further, in F plots BC had a significantly ($P$ = 0.08) lower canopy density than NC, which would allow for a greater light penetration. Consistent with our biomass partitioning and growth efficiency data, we found that BC responded to LR treatments by increasing canopy density (shallower slope) to a greater degree than NC; however, there was no significant ($P$ > 0.10) difference in our slope estimates (Figure 7B).

### *Instantaneous leaf gas exchange and PNUE*

Net $CO_2$ assimilation under saturating light ($A_{Sat}$) ranged from 11.7 to 0.71 μmol $CO_2$ $m^{-2}$ $s^{-1}$ and was weakly related to $[N]_a$ ($r^2$ = 0.02, $P$ = 0.007). Our nutrient manipulation treatments affected $[N]_a$ ($P$ = 0.05) with F increasing $[N]_a$ from 1.15 ± 0.03 to 1.32 ± 0.03 g N $m^{-2}$. There was no difference between control (none) and LR treatments (1.15 ± 0.03 and 1.16 ± 0.03 g N $m^{-2}$, respectively), but when both F and LR were added (LR + F) $[N]_a$ increased to 1.24 ± 0.03 g N $m^{-2}$, which was 7% lower than the addition of F alone.

We found significant F by time interaction in $A_{Sat}$ ($P$ = 0.05) (Figure 8 top panel). To further explore this time interaction, data were divided into three discrete regions based on the year the needles were produced (2005, 2006 or 2007). Needles produced during the 2005 growing season (first year in the ground) showed no significant treatment effects. In 2006 needles, F significantly ($P$ = 0.03) increased $A_{Sat}$ relative to NF plots (F and NF were 7.24 and 6.98 μmol $CO_2$ $m^{-2}$ $s^{-1}$, respectively) during the growing season, although rates did not differ between treatments in the winter. In contrast, needles produced in 2007 were significantly ($P$ = 0.02) lower in F plots relative to controls (5.59 and 5.98 μmol $CO_2$ $m^{-2}$ $s^{-1}$, respectively). There were significant F by time interactions in $[N]_a$ and PNUE ($P$ < 0.0001 and $P$ = 0.0003, respectively). There were no differences in $[N]_a$ between F treatments until June 2006, from June 2006 through October 2007 F plots had greater $[N]_a$ and after October 2007 the trend reversed with F plots having a lower $[N]_a$ relative to NF plots (Figure 8 middle panel). Instantaneous PNUE decreased in F plots relative to NF plots starting in June

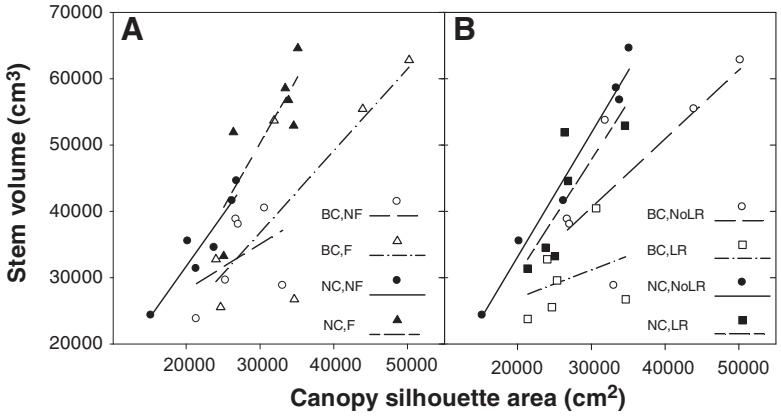


Figure 6. Interaction between clone (CL) and fertilizer treatments (F; panel A), and CL and LR treatments (panel B) for the amount of stem volume produced per unit CSA in *P. taeda* clones. Panel A: BC,NF (○, dashed line) volume = 0.69 CSA + 14432, $r^2$ = 0.17, $P$ = 0.41; BC,F (△, dash-dot line) volume = 1.24 CSA − 289, $r^2$ = 0.63, $P$ = 0.06; NC,NF (●, solid line) volume = 1.57 CSA + 379, $r^2$ = 0.88, $P$ = 0.006; and NC,F (▲, short-long-short dash) volume = 1.95 CSA − 8363, $r^2$ = 0.66, $P$ = 0.05. Panel B: BC,NoLR (○, dashed line) volume = 1.04 CSA + 9413, $r^2$ = 0.58, $P$ = 0.08; BC,LR (□, dash-dot line) volume = 0.42 CSA + 18514, $r^2$ = 0.12, $P$ = 0.51; NC,NoLR (●, solid line) volume = 1.87 CSA − 4281, $r^2$ = 0.98, $P$ = 0.0002; and NC,LR (■, short-long-short dash) volume = 1.73 CSA − 4261, $r^2$ = 0.65, $P$ = 0.05. Linear regressions were performed using PROC REG in SAS Version 9.

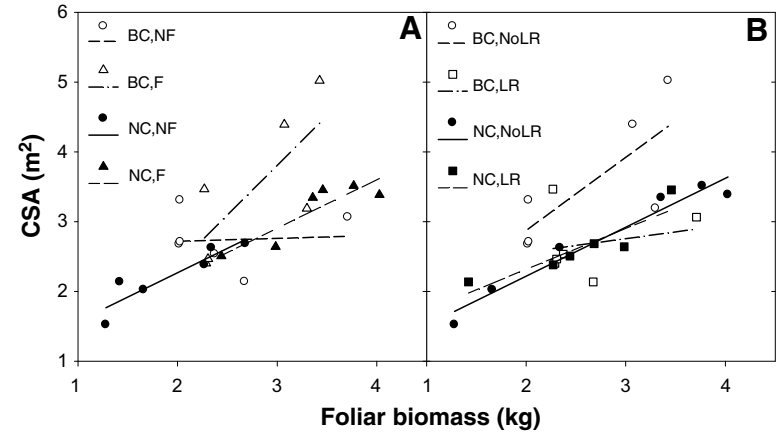


Figure 7. Linear relationship between CSA and foliar biomass for clone (CL) by fertilizer (panel A) and CL by LR (panel B) two-way interactions in *P. taeda* clones. Panel A: BC,NF (○, dashed line) CSA = 0.41 leaf + 2.6, $r^2$ = 0.00, $P$ = 0.90; BC,F (△, dash-dot line) CSA = 14.2 leaf − 0.5, $r^2$ = 0.55, $P$ = 0.09; NC,NF (●, solid line) CSA = 7.0 leaf + 0.9, $r^2$ = 0.83, $P$ = 0.01; and NC,F (▲, short-long-short dash) CSA = 7.0 leaf + 0.8, $r^2$ = 0.78, $P$ = 0.02. Panel B: BC,NoLR (○, dashed line) CSA = 10.5 leaf + 0.8, $r^2$ = 0.58, $P$ = 0.08; BC,LR (□, dash-dot line) CSA = 2.0 leaf + 2.2, $r^2$ = 0.05, $P$ = 0.67; NC,NoLR (●, solid line) CSA = 7.0 leaf + 0.8, $r^2$ = 0.95, $P$ = 0.0009; and NC,LR (■, short-long-short dash) CSA = 5.9 leaf + 1.1, $r^2$ = 0.82, $P$ = 0.01. Linear regressions were performed using PROC REG in SAS Version 9.

2006, and remained lower throughout the experiment with differences becoming smaller over the winter months (Figure 8 bottom panel).

There was no significant LR by CL interaction in $A_{Sat}$ observed in needles produced in 2006. Instead, we found weak LR ($P$ = 0.08) and CL ($P$ = 0.07) effects with LR increasing $A_{Sat}$ relative to plots without LR (7.24 and 6.97 μmol $CO_2$ $m^{-2}$ $s^{-1}$, respectively) and BC showing a greater $A_{Sat}$ relative to NC (7.25 and 6.96 μmol $CO_2$ $m^{-2}$ $s^{-1}$, respectively). A highly significant LR by time ($P$ = 0.002) and CL by time ($P$ = 0.002) interaction showed that the magnitude of these responses varied over that time period with the largest differences being expressed between January and June of the 2007 growing season (Figure 9 top panel). Needles produced in 2007 showed a significant ($P$ = 0.06) LR by CL interaction with BC increasing $A_{Sat}$ in the presence of LR from 5.59 to 6.04 μmol $CO_2$ $m^{-2}$ $s^{-1}$ and NC decreasing $A_{Sat}$ in the presence of LR from 6.03 to 5.48 μmol $CO_2$ $m^{-2}$ $s^{-1}$. We did not observe a significant LR by CL interaction in either $[N]_a$ or PNUE. Both $[N]_a$ and PNUE showed a significant LR by time interaction ($P$ = 0.04 and 0.003, respectively). LR resulted in lower $[N]_a$ and

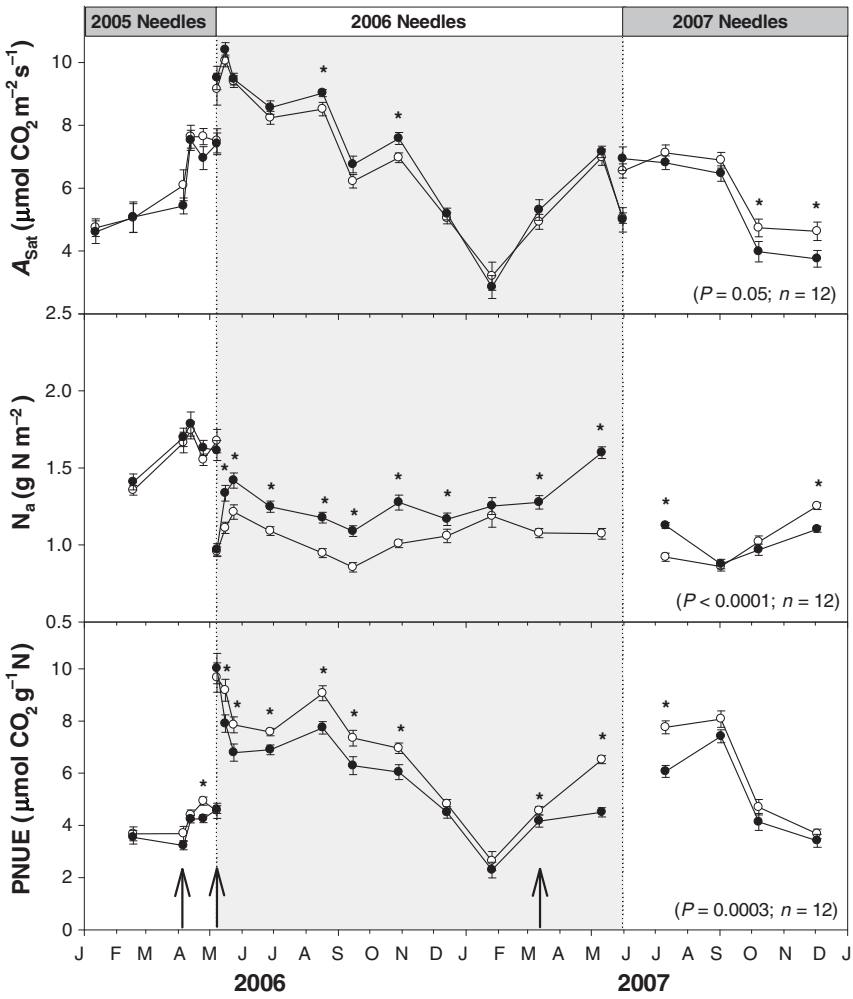


Figure 8. Fertilizer by date interaction for net $CO_2$ assimilation under saturating light ($A_{Sat}$), foliar N per unit area ($[N]_a$) and instantaneous PNUE. Arrows indicate the time of fertilization and dotted lines represent the date of transition from 'old' to 'new' fully elongated needles. Statistical analysis was performed using ANOVA with repeated measures in PROC MIXED and significant ($P < 0.10$) treatment differences on individual sampling are indicated using single asterisks and were analyzed using PROC GLM in SAS (error bars equal ± 1 standard error of the mean, $n = 6$).

greater PNUE throughout most of the experiment in both genotypes (Figure 9 middle and bottom panels).

*Photosynthetic capacity*

We estimated mesophyll conductance ($g_m$) for two of the three sampling dates that we measured using $A/C_i$ curves. We did not find any significant effect in our treatments on $g_m$ for either date nor did we detect any difference in $R_L$. The incorporation of LR had a significant effect on both $V_{C,max}$ and $J_{max}$, but the effect was dependent on genotype ($P = 0.05$ and 0.06, respectively). LR resulted in a decrease in both photosynthetic parameters in BC, while sharply increasing $V_{C,max}$ and $J_{max}$ in NC (Figure 10A and C). Notably, in the absence of any soil addition NC maintained $V_{C,max}$ and $J_{max}$ rates that were 19% and 17% lower than BC, respectively. Finally, in contrast to $A_{Sat}$ data, the needles used to measure $A/C_i$ curves showed no significant ($P = 0.20$) difference in $[N]_a$ (Figure 10E).

The addition of F significantly ($P < 0.05$) increased $V_{C,max}$, $J_{max}$ and $[N]_a$ in both clones, but the magnitude of the response varied between clones ($P = 0.05$, 0.10 and 0.07, respectively). We observed only a small increase in all three parameters in BC and a large increase in NC (Figure 10B and D). However, following F, both $V_{C,max}$ and $J_{max}$ rates were similar in both clones, indicating that NC maintained lower photosynthetic capacity in NF treatments relative to BC. Fertilizer resulted in a greater $[N]_a$ in NC relative to BC in needles used to measure $A/C_i$ curves (Figure 10F). TPU was also significantly ($P = 0.02$) increased with F to the same degree in both genotypes from 4.24 to 4.77 μmol $CO_2$ $m^{-2}$ $s^{-1}$. We found $[N]_a$ to be a highly significant ($P \leq 0.01$) regressor for both $V_{C,max}$ and $J_{max}$ in NC, but non-significant in BC, although $[N]_a$ only explained about 20% of the variation in both $V_{C,max}$ and $J_{max}$. Despite these differences, we found no significant differences in parameter estimates between clones.

Significant interactions between CL and F were found in variables associated with light capture. BC responded to F by increasing its $J_{max}/V_{C,max}$ ratio, apparent quantum efficiency ($Q$) and leaf area per unit mass (SLA), while decreasing its LCP. NC responded in an opposite fashion for all

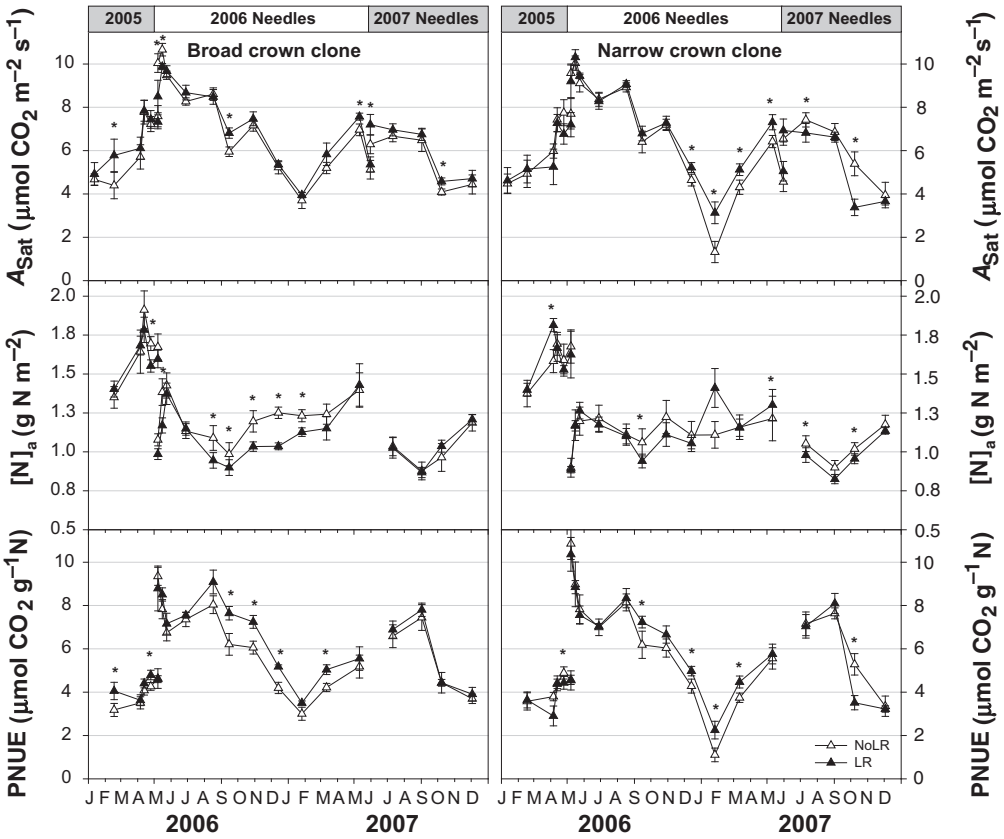


Figure 9. Light-saturated net $CO_2$ assimilation ($A_{Sat}$) clone by LR by time three-way interaction. Open (△) and closed (▲) triangles represent NoLR and LR, respectively. BC (left side) and NC (right side) were separated to assist in viewing interaction. Statistical analysis was performed using ANOVA with repeated measures in PROC MIXED and significant ($P < 0.10$) treatment differences on individual sampling are indicated using single asterisks and were analyzed using PROC GLM in SAS (error bars equal ± 1 standard error of the mean, $n = 6$).

four variables by decreasing $J_{max}/V_{C,max}$, $Q$ and SLA, and by increasing LCP (Figure 11).

## Discussion

### *Interaction between clone and low nutrient availability*

We showed that the two clones differed in stem volume and biomass partitioning in response to changes in soil nutrient availability. These findings are consistent with Li et al. (1991*a*), who found that NUE (stem biomass produced per unit of N applied) in *P. taeda* seedlings was moderately to highly dependent on genotype. The incorporation of LR decreased stem volume and growth efficiency (slope of the relationship between stem growth CSA), as well as changed canopy architecture and biomass partitioning in BC, but had no effect on NC (Figures 3B, 6B and 7B; Table 1). The incorporation of LR resulted in decreased lateral root and total belowground biomass (~ 33% and 30%, respectively) in BC, but had no effect on NC. Nutrition by family interactions in belowground partitioning has also been observed by Samuelson (2000) in a young *P. taeda* pot study and in woody roots (> 5 mm) of 5-year-old *P. taeda* grown in the field (Retzlaff et al. 2001), but notably, both these studies were adding F not inducing nutrient limitations.

Our observed interaction between LR and clones highlights both the importance of selecting the proper genotype based on site conditions and the importance of understanding the underlying mechanisms (e.g., biomass partitioning, canopy architecture and $A_{Sat}$) controlling these responses. Our data suggest that net photosynthesis of sun leaves was not contributing to this difference in growth as BC maintained either the same or greater $A_{Sat}$ relative to NC throughout the entire study (Figure 9). Further, we found that although photosynthetic capacity decreased in BC and increased in NC with the addition of LR, $V_{C,max}$ and $J_{max}$ rates in NC never exceeded BC rates (Figure 10A, C and E). We estimated the canopy level N use by multiplying average foliar $[N]_m$, from needles used to measure $A_{Sat}$ over all sampling dates ($n > 20$), by leaf biomass determined by destructive harvest. LR incorporation decreased $[N]_m$ in BC by < 3% and increased NC by 4% ($P = 0.02$). However,

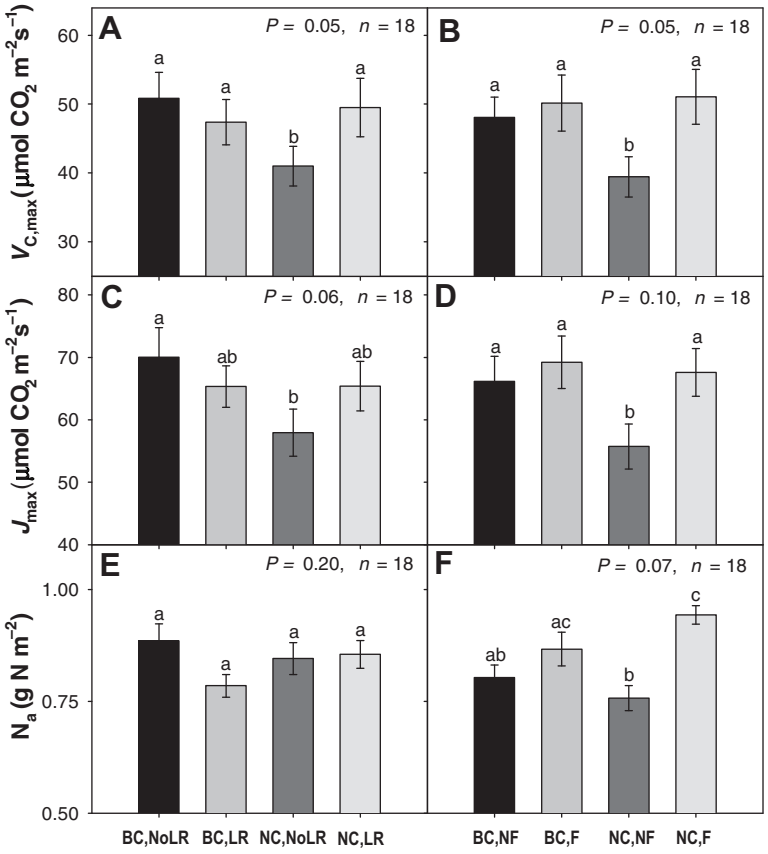


Figure 10. Interaction between clone and LR (left side), and CL and fertilizer (right side) for maximum carboxylation capacity of Rubisco ($V_{C,max}$; panels A and B), maximum rate of RuBP regeneration ($J_{max}$; panels C and D) and $[N]_f$. Variables were measured in June 2006, October 2006 and March 2007. Photosynthetic parameters were estimated using the $A/C_i$ curve fitting software developed by Sharkey et al. (2007) and adjusted to a common temperature of 25 °C. *P* values show significance of interaction term, lowercase letters represent all pair-wise comparisons between least-squares means and error bars represent ± 1 standard error of the mean.

at the canopy level there were no differences observed between clones ($P = 0.88$), which suggest that there was little difference in the N uptake under nutrient stress between clones.

We hypothesize that the decrease in growth in BC with LR was largely a result of the changes in the amount of effective photosynthetic area. The incorporation of LR did not impact foliar biomass in either clone, but decreased CSA by 25% in BC, while having no impact on NC. From this we inferred that the incorporation of LR resulted in a denser canopy in BC, reducing light interception by interior leaves, and therefore, decreased gross $CO_2$ assimilation at the canopy level (Figure 7B). Since $A_{Sat}$, $A$/PPFD and $A/C_i$ curve were always measured on needles receiving full sun, we were not able to detect this difference. A number of studies have shown a large difference in the photosynthetic capacity and $A_{Sat}$ between sun and shade leaves in *P. taeda* (Zhang et al. 1997, Maier et al. 2002, Gough et al. 2004*a*) as well as a wide variety of other plants (Meir et al. 2002). We found no change in leaf dark respiration estimated from light curves or leaf respiration ($R_L$) estimated from $A/C_i$ curves, but these data should be interpreted cautiously as they were also measured on needles receiving full sun.

Differences in belowground C allocation between clones may have also contributed to growth differences with LR incorporation. Litton et al. (2007) cautioned against using biomass as a sole estimate of C allocation. Biomass alone ignores differences in retention times (Coleman et al. 2000) and specific respiration rates between fine and coarse roots (Ryan et al. 1996, Pregitzer et al. 1998) as well as C lost as root exudates. In light of this, two independent sources suggest that BC allocated more C belowground relative to NC. First, data collected by a project collaborator between November 2005 and July 2006 using mini-rhizotrons showed that LR increased fine-root length in both clones, but BC maintained greater fine-root length, regardless of whether LR was present or not (Seth Pritchard, Department of Biology, College of Charleston, personal communication). However, increased fine-root production in BC may have been at the expense of coarser laterals and tap roots as we found a decrease in these roots with the incorporation of LR in BC. Second, in an accompanying study using these same plots soil heterotrophic respiration was found to increase ~ 45% with LR incorporation in BC and not in NC ($P = 0.07$, $n = 84$; Tyree 2008). This increase in soil heterotrophic respiration also suggests greater belowground C allocation in BC relative to NC with LR incorporation.

### *Interaction between clone and N and P fertilization*

The addition of N and P resulted in substantial increases in stem production, LA and coarse-root biomass in both

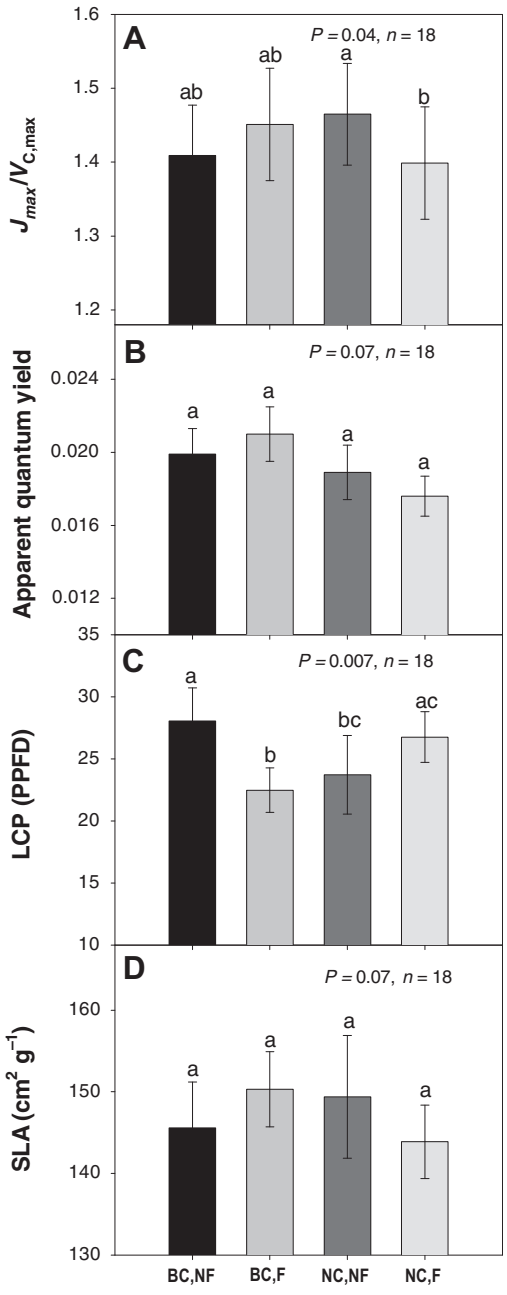


Figure 11. Least-squares means for $J_{max}/V_{C,max}$ ratio (panel A), apparent quantum yield ($Q$; panel B), LCP (panel C) and SLA (panel D) for the clone by fertilizer interaction. Variables were estimated from $A/C_i$ and $A$/PPFD response curves measured on three separate dates. SLA was measured on needles used to generate $A/C_i$ curves. $P$ values show significance of interaction term, lowercase letters represent all pair-wise comparisons between least-squares means and error bars represent ± 1 standard error of the mean.

clones. Although these increases are consistent with other reports (Axelsson and Axelsson 1986, Vose and Allen 1988, Albaugh et al. 1998, Allen and Lein 1998, King et al. 1999, Will 2005), we found that BC and NC responded to F to different degrees. In contrast to our hypothesis, BC was not more responsive to F than NC, in fact, by the end of the experiment NC increased leaf biomass 60% more than BC when F was added (Figure 5). The increase in foliage biomass facilitated a 21% greater increase in stem volume in NC relative to BC following F (Figure 3C). The increases in growth and leaf biomass are consistent with others who found genotype by fertility interactions in growth and allocation in greenhouse studies using open-pollinated families (Li et al. 1991*c*) and field trials using both open-pollinated (Retzlaff et al. 2001) and clonal material (King et al. 2008). Interestingly, unlike our leaf biomass data, there was no clone by F interaction on CSA. This led to NC expressing a denser canopy (relationship between CSA and foliar biomass) than BC with F (Figure 7A). This increase in canopy density was achieved by both a dramatic increase in leaf biomass and a decrease in SLA in NC (Figure 11D).

As hypothesized, we observed a short increase in $A_{Sat}$ following N and P fertilization with an eventual decrease in $A_{Sat}$ (Figure 8). This down-regulation in $A_{Sat}$ was accompanied by greater CSA and foliar biomass relative to NF treatments (Figure 5), which are consistent with those of Gough et al. (2004*b*) who also found a transient effect of F on $A_{Sat}$ in a pot study conducted on *P. taeda*. In contrast, others have not found the same increase in $A_{Sat}$ immediately following F (Zhang et al. 1997, Tyree et al. 2009). We did not find consistent differences between clones in $A_{Sat}$ over the experiment; however, N and P additions did result in differences in leaf biochemistry between clones. For example, NC showed a greater response to F by increasing $V_{C,max}$, $J_{max}$ rates and $[N]_a$ to a greater extent than BC (Figure 10B, D and F). However, the increase in $V_{C,max}$ and $J_{max}$ with F in NC simply elevated rates to the same level as BC. These data along with our growth and partitioning data indicated that although F resulted in a temporary increase in $A_{Sat}$ in both clones, there was a difference in how this was achieved. The BC clone responded by increasing its ability to capture and use light energy, while NC appeared to rely on an increase in carboxylation efficiency. This can be seen by differences in the $J_{max}/V_{C,max}$ with F (Figure 11A) and further supported by a decrease in LCP (Figure 11C).

The NC clone maintained a lower demand for N in NF treatments, but responded to F to a greater extent than the BC clone. In plots not receiving N and P fertilization, BC maintained 30% greater total canopy N (foliar $[N]_m \times$ total leaf biomass) relative to NC (43.1 and 33.2 g N, respectively); however in F plots, NC had 21% greater total foliar N relative to BC (63.1 and 52.2 g N, respectively). This ability to increase total canopy N contradicts the hypothesis that BC would be better able to respond to F. However, it is unknown whether increased total canopy N is due to a greater uptake or a preferential allocation of N to foliage rather than fine roots in NC.

## Conclusion

Our results showed that these two contrasting clones differed in their response to nutrient manipulations in terms of growth, partitioning and leaf gas exchange. Under field conditions, we found that NC was better able to tolerate nutrient limitations relative to BC. This was achieved by maintaining less leaf biomass, more favorable canopy architecture and perhaps lower belowground maintenance associated with fine roots. We conclude that these differences prevented a decrease in aboveground growth as was seen in BC with LR incorporation. In contrast, the ability of NC to increase leaf biomass with N and P fertilization allowed for a greater gross $CO_2$ assimilation translating into greater stem volume. Our results clearly show that contrasting clones have the potential to respond differently to differences in nutrient availability in terms of both partitioning and physiology. This research underlines the benefits of clonal selection and possibly ideotype development (Nelson and Johnsen 2008) as well as silvicultural treatments when implementing site-specific silviculture to maximize productivity on intensively managed southern pine forests. Future research should focus on differences in belowground allocation (fine-root turnover, root respiration and exudates) and foliar display efficiency between these clones, which may provide an insight into how one clone was able to maintain greater aboveground growth, similar foliar chemistry and LA while possessing fewer fine roots.

### Acknowledgments

The authors thank the USDA-Forest Service, Agenda 2020 for funding this research and MeadWestvaco for their monumental effort in preparing, maintaining and providing access to the study site. Specifically, thanks to Steve Patterson, Phil Dougherty, Daniel Mcinnis and Karen Sarsony for their technical support. Special thanks to John Peterson, Pete Anderson, Ben Templeton, Sarah Seiler and Isaiah Miller for their tireless efforts in the field and laboratory. Finally, thanks to Seth Pritchard for use of mini-rhizotron data, Erik Nilsen for use of his Li-Cor 6400 and Thomas Fox, Erik Nilsen, Lee Daniels, Chris Gough and John Peterson and anonymous reviewers for their thoughtful suggestions to improve this manuscript.

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