



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

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ABSTRACT

We conducted a 1-year greenhouse experiment to assess the impact of nutrient manipulations on seedling growth, biomass partitioning, and leaf gas exchange between two fast growing *Pinus taeda* clones that differed in growth efficiency. After 1 year we observed significant treatment and treatment by clone effects on growth, biomass partitioning, and gas exchange parameters. Fertilization increased total seedling biomass 18% primarily through an increase in foliage and coarse-roots. Clones did not differ in total seedling biomass, however, clone 85 produced more stem than clone 93 leading to 37% greater stem:leaf, while clone 93 maintained more branch biomass. The logging residue treatment increased stem:leaf by 30%, but had no effect on total biomass or partitioning. Differences in leaf morphology resulted in significantly greater canopy leaf area in clone 93 than clone 85. Increased foliar N concentration from fertilization had only minor effects on specific photosynthesis under saturating light (A_{Sat}), but lowered stomatal conductance (g_s), transpiration (E), and internal to external CO_2 concentration ratio (C_i/C_a) as well as improved water use efficiency (WUE) independently of genotype. When gas exchange data was scaled to the canopy level both genotypes achieved similar canopy level CO_2 assimilation rates, but our data suggests they did this by different means. Although we did see a small effect of nutrient limitations in total canopy photosynthesis under saturating light (A_{Canopy}), A_{Sat} , and total leaf area (TLA), our foliar N concentration ($[N]$) indicated that our level of logging residue incorporation did not cause $[N]$ to decrease below sufficiency limits. From a practical standpoint, a better understanding of strategies for capturing and partition C may lead to better selection of clonal material, thereby, optimizing productivity.

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1. Introduction

Differences in nutrient availability can influence the short-term capacity of conifer seedlings to collect and utilize light energy from photosynthesis as well as the distribution of that photosynthate between plant tissues. Nitrogen and phosphorus are usually the most limiting nutrients to plant growth and have been shown to exert a strong influence on leaf area (Zhang et al., 1997a; Albaugh et al., 1998; King et al., 1999), leaf morphology (Niinemets et al., 2001; Maier et al., 2002; Will, 2005) and chemistry, as well as leaf-level physiology in *Pinus* spp. (Tissue et al., 1993; Gough et al., 2004b; Samuelson et al., 2004; Bown et al., 2007; King et al., 2008). Nitrogen is a major component of most of the proteins and

pigments involved in photosynthesis, therefore, it is not surprising that increases in specific net CO_2 assimilation under saturating light (A_{Sat} ; Table 1) have been commonly observed following N fertilization (Green and Mitchell, 1992; Tissue et al., 1993; Murthy et al., 1996, 1997; Samuelson, 2000; Gough et al., 2004b). Increases in foliar N concentration ($[N]_f$) have been shown to correspond with increased chlorophyll content (Chandler and Dale, 1995; Ripullone et al., 2003; Bauer et al., 2004; Chmura and Tjoelker, 2008), Rubisco content, or both (Tissue et al., 1993; Warren et al., 2004; Manter et al., 2005) in conifer species.

However, some studies have found no change or even a decrease in A_{Sat} following fertilization in both young (Zhang et al., 1997b; Samuelson et al., 2001; Warren and Adams, 2002) and mature trees (Tang et al., 1999; Maier et al., 2002; Bauer et al., 2004; Gough et al., 2004a). In most of these instances measurements were taken after continuous or in some cases chronic N additions. One hypothesis proposed by Gough et al. (2004b) and

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Table 1
List of abbreviations.

Abbreviation	Description	Units
A_{canopy}	Canopy level net CO ₂ assimilation	$\mu\text{mol CO}_2 \text{ s}^{-1}$
A_{sat}	Instantaneous specific net CO ₂ assimilation under light and CO ₂ saturation	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
C_i/C_a	Internal CO ₂ to ambient CO ₂ ratio	Unitless
CSA	Canopy silhouette area	cm ²
E	Transpiration	mmol H ₂ O m ⁻² s ⁻¹
g_s	Stomatal conductance	mol m ⁻² s ⁻¹
LR	Logging residue	Treatment
$[N]_a$	Nitrogen concentration per unit leaf area	g N m ⁻² leaf
$[N]_f$	Foliar nitrogen concentration	Unitless
$[N]_m$	Nitrogen concentration per unit leaf mass	mg N g ⁻¹ leaf
$PNUE$	Instantaneous photosynthetic N use efficiency	$\mu\text{mol CO}_2 \text{ g}^{-1} \text{ N s}^{-1}$
SLA	Specific leaf area	cm ² g ⁻¹
TLA	Total leaf area calculated from SLA and leaf mass	m ²
WUE	Instantaneous water use efficiency	$\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$

later supported by King et al. (2008) is that increases in A_{sat} rates immediately following fertilization allow for increased photo-assimilates, which then can be used to produce greater leaf area for light interception, and lead to an eventual down regulation of A_{sat} . Therefore, timing of fertilization and measurements is a contributing factor hypothesized by both Gough et al. (2004b) and King et al. (2008) that may help explain these discrepancies.

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

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

photosynthesis between full-sib clones when fertilized (King et al., 2008).

The use of contrasting genotypes in physiological research has implications beyond matching specific genotypes to site conditions. For example, improved ability to detect treatment differences by eliminating genetic variability, or the use of contrasting clones combined with resource manipulations may allow for improved understanding of the mechanism involved in C capture and partitioning. Additionally, their use in research may provide insight into the stability of these mechanisms within *P. taeda* under a range of resource availability. We conducted a greenhouse experiment with a factorial combination of fertilization and high C:N logging residue (LR) incorporation (applied to modify nutrient availability) to assess the impact of nutrient manipulations on leaf gas exchange and biomass partitioning between 2-year-old *P. taeda* clones believed to maintain different growth efficiencies (stem produced per unit leaf area). We ask if seedling growth response to nutrient availability is a function of increased A_{sat} or a result of changes in leaf area due to reallocation of C, and is this response consistent across clones. We hypothesize that A_{sat} in both clones will increase immediately following fertilization, but to different degrees. We expected that one clone would invest more C to increasing leaf area and the other in photosynthetic machinery per unit leaf area leading to no overall difference in canopy level net CO₂ assimilation (A_{canopy}) between genotypes. Similarly, we anticipated clones to respond differently to LR incorporation due to differences in biomass partitioning and photosynthetic N use efficiency ($PNUE$). We hypothesize that the clone that maintains more leaf area will show greater declines in overall productivity (growth) relative to the clone which maintains less leaf area.

2. Materials and methods

2.1. Experimental design

In April 2006, 1-year-old *P. taeda* clones were planted in 170-L plastic containers (93 cm × 53 cm × 50 cm) and grown in a greenhouse through July 2007. The greenhouse vents and climate settings were adjusted to provide a summer and winter temperature regime representative of the southeastern United States. The study design was a randomized complete block design replicated six times. Treatments were arranged in a full 2 by 2 by 2 factorial with two levels of LR incorporated into the soil (none, present), two levels of fertilization (none, present), and two clones (CL93, CL85). Forty-eight plastic containers were fitted with a single brass spigot for collecting water, and each was filled with approximately 0.17 m³ of Eunola series (fine-loamy, siliceous, semiactive, thermic Aquic Hapludults) soil 2 months prior to planting. Soil was collected from the Virginia Tech Tidewater Agricultural Research and Extension Center located in Holland, VA on February 2006 to a depth of approximately one meter, which included a mixture of the Ap, BE, and Bt horizons.

2.2. Treatments

Logging residue (LR; C:N = 128 ± 14; n = 4) was collected from residue piles near the logging deck of a *P. taeda* stand in South Carolina that had been harvested 6 months prior to collection. The residue consisted mainly of bark, needles, and small branches that remained following an onsite processing of merchantable timber. The LR was passed through a 5 cm × 10 cm screen and was mixed uniformly into the soil during pot filling at a rate of 4.92 kg LR o.d. container⁻¹ (equivalent to 25 Mg o.d. ha⁻¹). Fertilizer was applied on two separate dates. Due to slow initial growth of the clones, the first application was not applied until July 28, 2006. Fertilizer was in the form of diammonium phosphate (DAP) and ammonium nitrate (AN)

at an equivalent rate of 200 kg N and 50 kg P ha⁻¹. The second fertilizer application took place on March 16, 2007 in the form of AN at a rate of 200 kg N ha⁻¹. Clonal seedlings were donated by Mead Westvaco for use in this study and were chosen to represent contrasting biomass partitioning patterns (Phil Dougherty personal communication).

2.3. Repeated measurements

Leaf gas exchange was measured 17 times during the experiment. Measurements were taken at a greater frequency before and immediately following fertilization with the frequency decreasing to monthly by the end of the experiment. Specific net CO₂ assimilation under saturating light (A_{Sat} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s ; $\text{mol m}^{-2} \text{ s}^{-1}$), transpiration (E ; $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), internal CO₂ to atmospheric CO₂ ratio (C_i/C_a), and water use efficiency (WUE ; $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) per unit leaf area were measured simultaneously using a Li-Cor 6400 portable open system infrared gas analyzer with a 2 cm × 3 cm cuvette and a blue–red LED light source (Li-Cor 6400, Lincoln, Nebraska). All measurements were made using the following chamber conditions: 1600 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD, 370 $\mu\text{mol mol}^{-1}$ reference CO₂ concentration, ambient chamber temperature and humidity, and a flow rate of 300 $\mu\text{mol s}^{-1}$. Needles were excised from the upper portion of the seedling and placed into the cuvette. Data showed no significant ($P = 0.98$; $n = 8$) differences between excised and attached needles over a 2–3-min period after detachment (data not shown). Following stabilization of the photosynthetic rate (approximately 2 min) measurements were logged three times at 10-s intervals, which were averaged to a single value. Needles were immediately removed from the cuvette and fascicle diameter measured to the nearest 0.01 mm using digital calipers and brought back to the lab for chemical analyses. All leaf gas exchange measurements were expressed on a leaf area basis using an equation developed by Ginn et al. (1991).

Both seedling height (cm) and ground line diameter (mm) were measured approximately monthly from the time of planting through the end of the experiment. Aboveground stem volume was calculated by multiplying aboveground seedling height by ground line diameter squared.

2.4. Canopy silhouette area

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

2.5. Biomass partitioning, needle morphology and chemistry

Seedlings were dissected into needles, branches, main stem, coarse- (>2 mm) and fine-roots (<2 mm). Samples were oven dried for 2 weeks at a temperature of 65 ± 5 °C then weighed

gravimetrically to the nearest 0.1 g. Needle morphology was determined by sub-sampling five fascicles from most recent, fully elongated, current years flush (“new”), and five fascicles from the final, fully elongated, flush of previous season (“old”). Needle length from tip of needle to beginning of the fascicle sheath (mm), needle diameter (0.01 mm), number of needles per fascicle, and average oven dried (65 ± 5 °C) weight of all five fascicles were measured for each seedling and needle age (480 needles). Specific leaf area (SLA ; $\text{cm}^2 \text{ g}^{-1}$) was calculated from the sub-sample and used to estimate total leaf area (TLA ; m^2) based on the total foliar biomass of each seedling.

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

2.6. Data analyses

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

3. Results

3.1. Total seedling biomass and biomass partitioning

The addition of fertilizer resulted in an 18% increase ($P = 0.07$; $n = 24$) in total seedling biomass relative to unfertilized seedlings (298 ± 24 and 252 ± 18 g, respectively) by the end of the experiment. Additionally, fertilizer affected how biomass was partitioned within both aboveground and belowground tissues. For example, fertilizer additions resulted in a 20% decrease ($P = 0.0002$) in the fine- to coarse-root ratio relative to unfertilized seedlings, but this difference was due mainly to a 27% increase ($P = 0.04$) in coarse-roots (Fig. 1A). Also, the application of fertilizer resulted in 22% more foliage ($P = 0.05$) among both clones (Fig. 1A).

In contrast, the incorporation of LR had no significant ($P > 0.10$) effect on total seedling biomass or any of the individual components (Table 2). However, LR did lead to a 30% ($P = 0.004$) increase in the stem to leaf ratio (stem:leaf) and a 16% increase in the fine- to coarse-root ratio (F:C; $P = 0.006$).

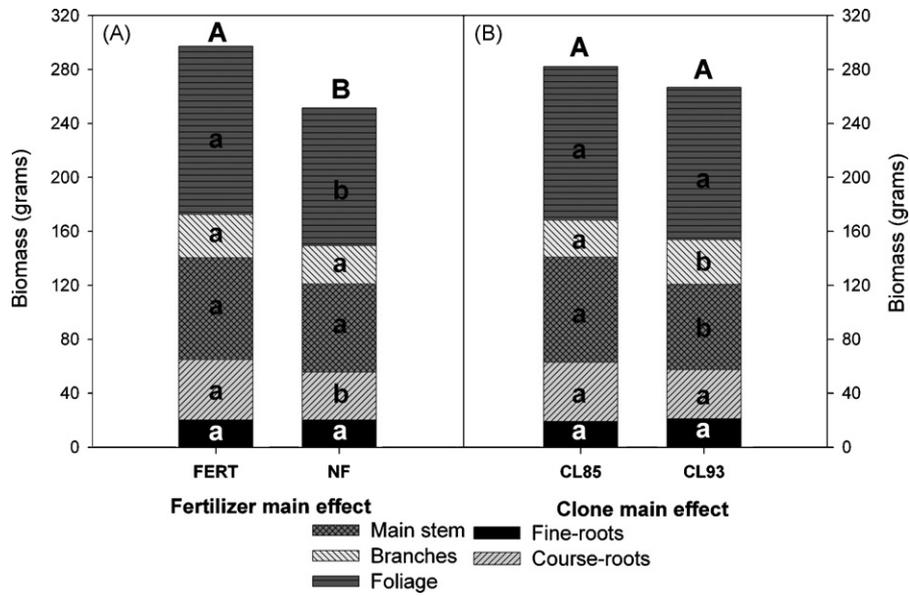


Fig. 1. Biomass partitioning between plant tissues for fertilizer (A) and clone (B) main effects for *Pinus taeda* seedlings at the end of 1 year. Different lowercase letters indicate significant ($P \leq 0.10$) treatment differences within individual tissues and capital letters indicate treatment differences in total biomass (see Table 2 for list of P -values; $n = 24$).

Table 2
Statistical summary of P -values for ANOVA of biomass partitioning between specific tissues of 2-year-old *Pinus taeda* seedlings for logging residue (LR), clone (CL), and fertilizer (F) treatments main effects, two-way, and three-way interactions. Data were log transformed as needed to meet assumptions of normality and equal variance as indicated by plot of residuals and normality curves.

Variable	Main effects			Two-way interaction		Three-way Interaction	
	LR ^a	CL ^a	F ^a	LR × CL ^b	LR × F ^b	CL × F ^b	LR × CL × F ^c
Foliage	0.74	0.93	0.05	0.72	0.50	0.19	0.30
Branches	0.68	0.05	0.17	0.95	0.34	0.10	0.31
Stem ^d	0.20	0.02	0.14	0.89	0.63	0.55	0.56
Total above ^d	0.89	0.74	0.13	0.74	0.62	0.55	0.42
Coarse-roots ^d	0.83	0.13	0.04	0.69	0.92	0.33	0.25
Fine-roots	0.26	0.33	0.99	0.92	0.79	0.12	0.25
Total below	0.86	0.39	0.12	0.58	0.92	0.11	0.28
Fine:coarse ^e	0.006	<0.0001	0.0002	0.07	0.99	0.42	0.45
Root:shoot ^e	0.62	0.20	0.95	0.13	0.10	0.13	0.24
Stem:leaf ^e	0.004	<0.0001	0.01	0.10	0.18	0.25	0.98
Total biomass	0.87	0.52	0.07	0.89	0.56	0.15	0.34

^a Sample size of 24.
^b Sample size of 12.
^c Sample size of 6.
^d Variable transformed by the natural log.
^e Variable transformed by the arcsin (square root).

Table 3
Statistical table of P -values for needle morphological parameters measured on 48 2-year-old *Pinus taeda* seedlings grown for 1 year in a greenhouse. Specific leaf area (SLA; $\text{cm}^2 \text{g}^{-1}$), needle diameter (DIA; mm), needle length (LGNTN; mm), number of needles per fascicle (NDLS), dry weight (WT; mg), and calculated leaf area (LFAREA; cm^2) were measured on current year most recently elongated needles (new) and previous season last fully elongated needles (old). Treatments were logging residue incorporation (LR), clone (CL), and fertilization (Fert).

Source	SLA		DIA		LGNTN		NDLS		WT		LFAREA	
	New	Old	New	Old	New	Old	New	Old	New ^a	Old	New	Old ^a
Block	0.14	0.07	0.15	0.003	0.04	0.002	0.19	0.005	0.10	0.001	0.08	0.002
LR	0.16	0.09	0.82	0.15	0.87	0.33	0.75	0.59	0.56	0.59	0.88	0.99
CL	0.001	0.06	0.0001	0.0001	0.0001	0.009	0.0001	0.0001	0.0001	0.001	0.0001	0.005
LR × CL	1.00	0.15	0.81	0.47	0.74	0.94	0.53	0.42	0.90	0.59	0.79	1.00
Fert	0.04	0.69	0.02	0.002	0.08	0.26	0.75	0.005	0.007	0.009	0.03	0.02
LR × Fert	0.28	0.61	0.20	0.13	0.04	0.99	0.75	0.42	0.28	0.68	0.05	0.52
CL × Fert	0.87	0.18	0.28	0.65	0.29	0.33	0.53	0.01	0.32	0.30	0.22	0.92
LR × CL × Fert	0.03	0.26	0.61	0.39	0.41	0.27	1.00	0.59	0.69	0.04	0.41	0.14

^a Variable was transformed by its natural log to meet assumptions of ANOVA.

Table 4

Arithmetic mean and (standard error) for leaf needle morphological parameters for treatment main effects in both “new” and “old” needles. Morphological parameters were measured on 48 2-year-old *Pinus taeda* seedlings grown for 1 year in a greenhouse ($n = 24$).

	Logging residue		Clone		Fertilization	
	NOLR	LR	CL85	CL93	NF	F
Specific leaf area ($\text{cm}^2 \text{g}^{-1}$)						
New	209.8 (4.1)	216.2 (3.5)	205.3 (3.7)	220.7 (3.4)	217.9 (3.8)	208.1 (3.7)
Old	159.9 (2.9)	171.5 (6.7)	159.3 (2.9)	172.1 (6.6)	167.0 (3.3)	164.4 (6.7)
Needle diameter (mm)						
New	1.33 (0.02)	1.33 (0.02)	1.39 (0.02)	1.27 (0.01)	1.30 (0.02)	1.36 (0.02)
Old	1.31 (0.02)	1.28 (0.02)	1.34 (0.02)	1.25 (0.02)	1.26 (0.02)	1.33 (0.02)
Needle length (mm)						
New	124 (4.3)	124 (4.8)	141 (3.5)	107 (2.2)	121 (4.3)	127 (4.7)
Old	125 (4.0)	130 (5.0)	134 (4.4)	120 (4.1)	124 (3.6)	130 (5.2)
Number of needles						
New	3.2 (0.05)	3.2 (0.05)	3.0 (0.01)	3.3 (0.05)	3.2 (0.04)	3.2 (0.05)
Old	3.3 (0.07)	3.2 (0.06)	3.1 (0.03)	3.4 (0.07)	3.1 (0.04)	3.3 (0.08)
Needle weight (mg)						
New	50.5 (2.8)	49.2 (2.8)	59.6 (2.4)	40.1 (1.3)	46.3 (2.7)	53.4 (2.8)
Old	66.4 (3.0)	64.4 (4.3)	72.3 (3.8)	58.4 (3.1)	60.2 (3.0)	70.5 (4.1)
Leaf area (cm^2)						
New	10.4 (0.47)	10.5 (0.53)	12.1 (0.44)	8.8 (0.25)	9.9 (0.46)	11.0 (0.51)
Old	10.5 (0.35)	10.6 (0.53)	11.3 (0.44)	9.8 (0.39)	9.9 (0.36)	11.2 (0.47)

Finally, the root to shoot ratio (R:S) did not significantly ($P > 0.2$) differ between treatment main effects, but we did observe a significant ($P = 0.10$) LR by fertilizer interaction. The addition of either LR and fertilizer alone resulted in R:S slightly increasing from 0.27 ± 0.01 to 0.29 ± 0.01 and 0.27 ± 0.01 to 0.28 ± 0.01 , respectively, but the addition of both LR and fertilizer together had no effect on R:S.

We found clonal differences in aboveground biomass partitioning. Clone 93 had 21% more branches ($P = 0.05$) than CL85 while CL85 had 23% more stem biomass ($P = 0.02$; Fig. 1B). In addition, CL85 had a 37% greater stem:leaf relative to CL93 (0.77 ± 0.10 and 0.57 ± 0.02 , respectively). We also observed differences in proportional belowground partitioning between clones. For example, CL93 had 35% greater F:C than CL85, but there was no difference in absolute belowground biomass partitioning ($P = 0.4$). When we further explored the significant clone by LR interaction ($P = 0.07$) we found that CL93 responded by increasing its F:C by 25% while CL85 only increased its F:C by 9%.

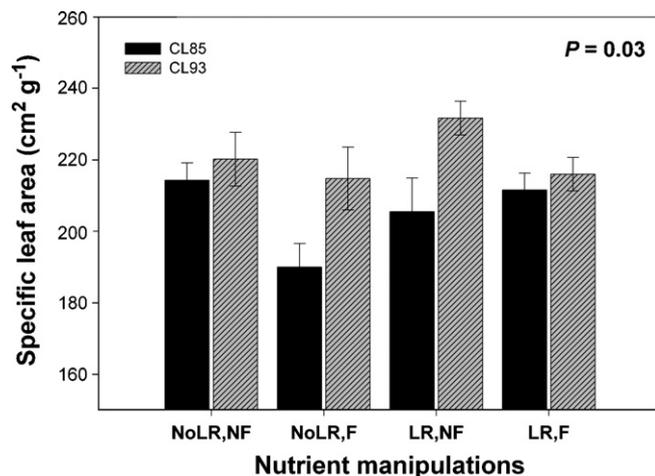


Fig. 2. Impact of logging residue (LR) and fertilization (F) on specific leaf area (SLA) between two clones (CL). SLA was calculated for current year needles collected from 2-year-old pot grown *Pinus taeda* grown in a greenhouse for 1 year (error bars represent ± 1 standard error; $n = 6$). The P -value reported in the upper right corner refers to the three-way clone by fertilizer by LR interaction.

3.2. Stem volume, leaf morphology, and leaf area

Significant fertilizer by time ($P = 0.004$) and clone by time ($P < 0.0001$) interactions were observed in aboveground stem volume with the largest compounded treatment differences being expressed at the end of the experiment. Seedlings receiving fertilizer were 14% larger than control seedlings and CL85 seedlings were 41% larger than CL93 seedlings. When height and diameter were analyzed separately there were no significant ($P > 0.10$) differences between any of the treatments or their interactions for seedling height. In contrast, there was a highly significant LR by time ($P = 0.01$) interaction as well as a significant clone by fertilizer by time ($P = 0.03$) interaction in ground line diameter. Fertilizer increased ground line diameter to a greater degree in CL93 than it did in CL85. Finally, as a result of incorporating LR into the soil we observed a 5% increase in ground line diameter relative to treatments with no LR added, but we observed no difference in stem volume or leaf area.

Leaf morphology differed substantially between clones in both “old” and “new” needles for every needle parameter measured,

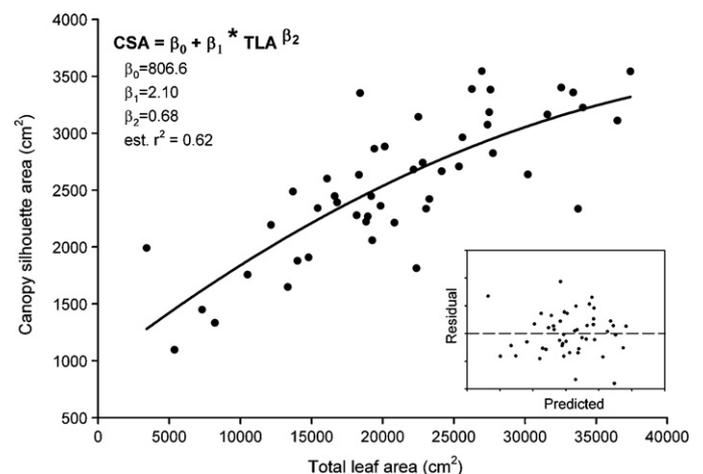


Fig. 3. Canopy silhouette area plotted against total leaf area using a three parameter, nonlinear power function with diminishing returns ($r^2 = 0.62$; $n = 48$). The inset is a plot of the residuals.

and differed between fertilizer treatments for most parameters measured (Tables 3 and 4). Clone 85 needles were larger in weight than CL93 resulting in lower *SLA* (Table 4). Overall, fertilizer resulted in decreased *SLA* in new needles relative to unfertilized seedlings as a main effect ($P = 0.04$), but there was a highly significant ($P = 0.03$) LR by clone by fertilizer interaction (Table 4). The addition of fertilizer by itself resulted in CL85 decreasing *SLA* while CL93 remained unresponsive. In contrast, when LR was applied without fertilization CL93 responded by increasing its *SLA* while CL85 remained unresponsive (Fig. 2).

We observed good agreement between *CSA* and *TLA*. When *CSA* was regressed with *TLA* using a power function, 62% of the variation was explained (Fig. 3). As *TLA* increased *CSA* increased at a diminishing rate likely due to overlapping of the needles. The various estimates of foliage (i.e., weight, *CSA*, and *TLA*) were influenced by treatments differently. All three measures showed an increase in foliage with fertilization. For example, fertilization increased foliage on a weight basis ($P = 0.05$) and when expressed as *CSA* and *TLA* ($P = 0.10$). Both *CSA* and *TLA* showed 13 and 8%

greater area, respectively, in CL93 relative to CL85 ($P = 0.06$ and 0.10, respectively) while there was no statistical difference in foliage on a weight basis ($P > 0.10$).

3.3. Leaf-level gas exchange

We found a significant fertilizer by time ($P = 0.09$) interaction in A_{Sat} . On four sampling dates fertilized seedlings had significantly lower A_{Sat} values (Fig. 4A). Following the second fertilizer application, A_{Sat} rates were lower in fertilized treatments until “new” needles were measured on the final sampling date in June 2007. We also found that fertilized seedlings had 5, 7, and 3% less ($P < 0.05$) g_s , E , and C_i/C_a , respectively, and 5% greater ($P < 0.01$) WUE relative to unfertilized seedlings when averaged over the entire experiment irrespective of genotype (Fig. 4B–D). In contrast, we found no difference in gas exchange parameters due to LR incorporation.

There was no significant ($P > 0.10$) clone by fertilizer interaction over the entire study in A_{Sat} , but we did observe a highly significant difference between clones in many of parameters

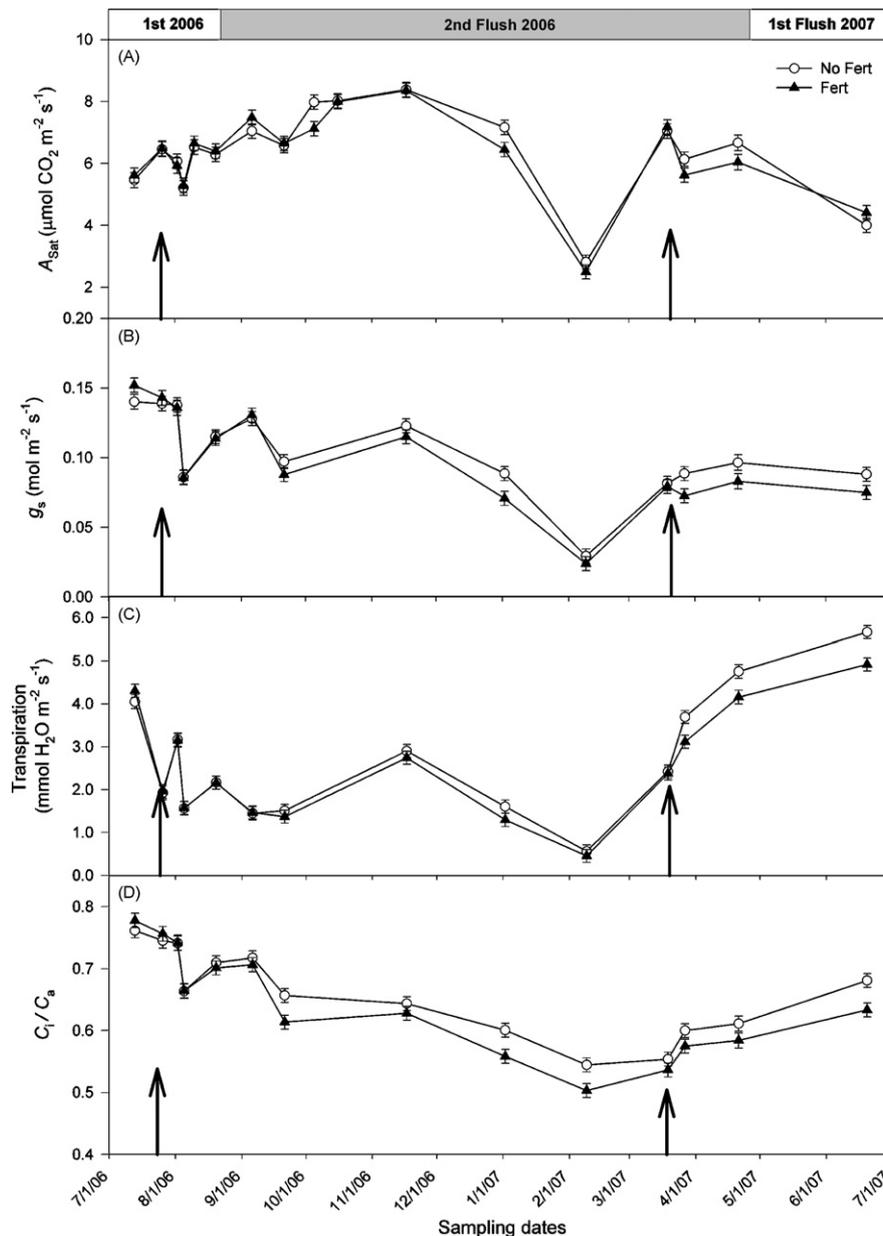


Fig. 4. Plot of fertilizer by time interaction for net photosynthesis (A, A_{Sat}), stomatal conductance (B, g_s), transpiration (C), and ratio of internal to ambient CO_2 concentration (D). Bar across top of graph indicates the age of needles measured. Arrows indicate times of fertilization.

related to leaf gas exchange. Specifically, A_{Sat} was significantly ($P = 0.01$) greater in CL93 relative to CL85 through October of 2006, but this effect disappeared with both clones showing similar A_{Sat} rates by the end of the experiment (Fig. 5A). With the exception of one sampling date in February, which was recorded as the lowest temperature for the year, CL93 had significantly ($P < 0.01$) greater g_s , E , and C_i/C_a than CL85 (Fig. 5B–D). The lack of clonal difference in A_{Sat} later in the experiment, and the increase in g_s , led to significantly ($P < 0.0001$) greater WUE in CL85 relative to CL93 when measured throughout the entire experiment. When averaged over all sampling dates we observed approximately a 20% greater instantaneous WUE in CL85 than CL93.

3.4. Foliar N concentration and PNUE

Foliage samples collected at the end of the experiment showed that nutrient manipulations had a significant ($P < 0.05$) impact on $[N]_f$ in both “new” and “old” needles (Fig. 6). “Old” needles had

slightly greater foliar $[N]_f$ than “new” needles when expressed on a weight $[N]_m$ or area $[N]_a$ basis. Overall, CL85 had 21 and 27% greater foliar $[N]_a$ than CL93 in “new” ($P = 0.0002$) and “old” ($P < 0.0001$) needles, respectively (Fig. 7A and B). Fertilization resulted in increased foliar N ($P < 0.0001$) independently of clone, but in “old” needles the clonal response to fertilization differed in magnitude ($P = 0.06$) with CL85 increasing foliar $[N]_a$ 10% more than CL93 (Fig. 7A and B). Although the lowest foliar $[N]$ was observed in the LR only treatment, all treatments had foliar $[N]_m$ above the sufficiency limit (12 mg N g^{-1} foliage) for *P. taeda*.

Photosynthetic N use efficiency did not differ between clones when fertilizer was not applied in “new” needles, but when fertilizer was applied CL85 showed a decrease in $PNUE$ while CL93 remained largely unchanged (Fig. 7E). This was due to an increase in foliar $[N]_a$ with no accompanying change in A_{Sat} (Fig. 7A and C). In “old” needles we observed that CL93 had greater $PNUE$, relative to CL85, regardless of whether fertilizer was applied (Fig. 7F). The individual components behaved similarly to that of fertilized

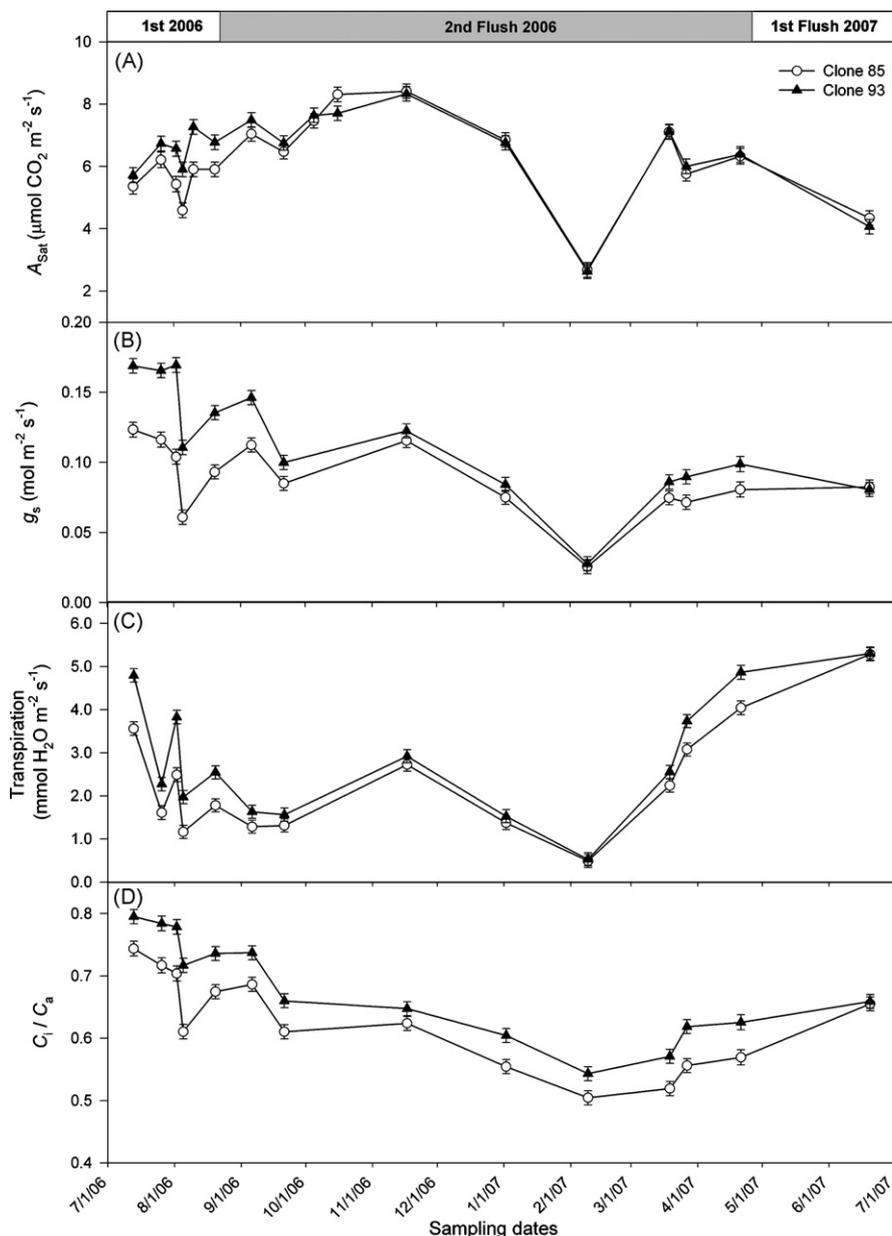


Fig. 5. Plot of genotype by time interaction for net photosynthesis (A, A_{Sat}), stomatal conductance (B, g_s), transpiration (C), and ratio of internal to ambient CO_2 concentration (D). Bar across top of graph indicates the age of needles measured.

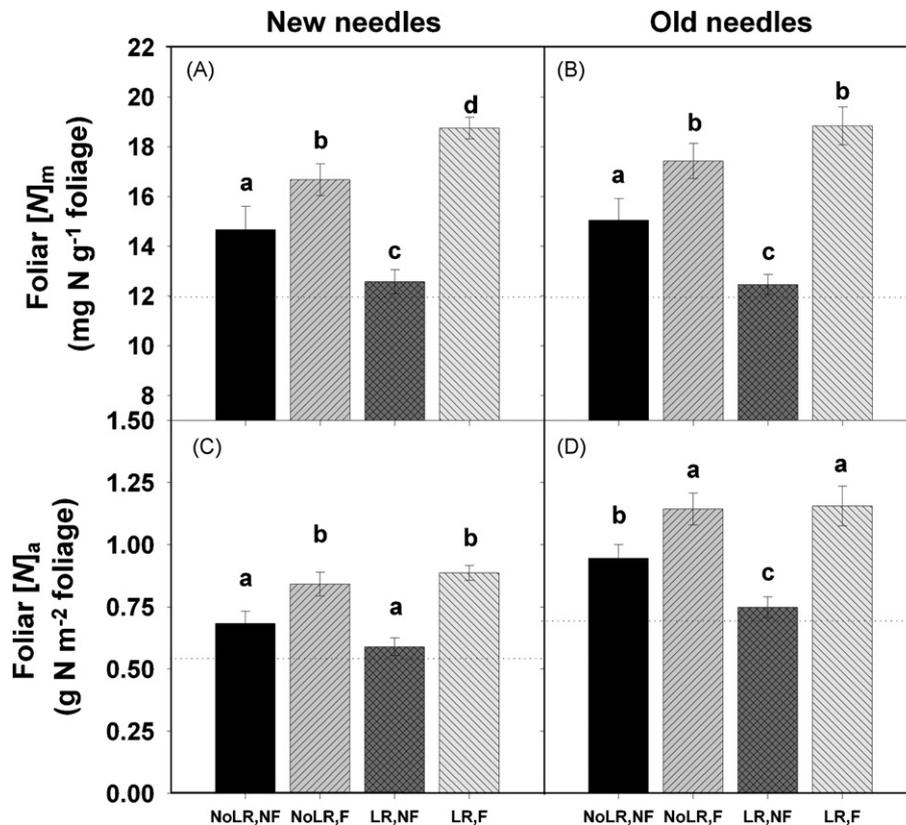


Fig. 6. Effect of nutrient manipulations on arithmetic means for foliar N content of “new” and “old” needles expressed on a mass (panels A and B) and area basis (panels C and D). Dotted lines represent sufficiency limit for foliar N content for *P. taeda*, and different letters indicate significant comparison-wise differences between nutrient manipulation treatments at the 0.05 alpha level ($n = 12$). Abbreviations: NoLR = no logging residue, NF = no fertilizer, LR = logging residue, and F = fertilizer.

“new” needles in that there was an increase in $[N]_a$ with no change in A_{sat} .

4. Discussion

4.1. Differences in nutrient availability

By the end of the experiment we found differences in biomass partitioning and leaf gas exchange between N availability treatments. Fertilization increased total seedling biomass (18%), foliar mass (22%), coarse-root biomass (27%), and basal diameter (14%; Table 2 and Fig. 1A). Additionally, fertilization resulted in greater photosynthetic area when expressed as either TLA or CSA despite the contrasting effects of increased foliar biomass and decreased specific leaf area (Tables 2–4). These findings are consistent with numerous studies that have thoroughly assessed the effects of fertilization on early growth in *P. taeda* (Zhang et al., 1997a; Albaugh et al., 1998; Samuelson, 2000). In contrast to our hypothesis, the incorporation of LR did not result in reduced seedling biomass (Table 2), and in fact resulted in increased stem volume as a result of increased basal diameter. An analyses of $[N]_f$ showed that our level of fertilization and LR incorporation did result in changes in N availability, but those changes were weak and at no time resulted in foliar [N] dropping below sufficiency limits (Fig. 6).

In contrast to our hypothesis and others' findings (Samuelson, 2000; Gough et al., 2004b; King et al., 2008), we did not see a consistent increase in A_{sat} following fertilization with N and P. In fact, we observed a decrease on a number of sampling dates, but results were largely inconsistent (Fig. 4A). One exception being on the final sampling date, which used the first fully elongated needles of the 2007 growing season, fertilized seedlings had

slightly greater A_{sat} than control seedlings. This lack of consistent response has also been observed by others (Zhang et al., 1997b; Gough et al., 2004a; Chmura and Tjoelker, 2008).

We observed a consistent decrease in g_s , E , and C_i/C_a following fertilization throughout the experiment (Fig. 4B–D). Decreased g_s , E , and C_i/C_a coincided in greater WUE, which may have been a result of both increased stomatal control and changes in leaf morphology (decreased SLA) following fertilization (Green and Mitchell, 1992; Samuelson, 2000). Green and Mitchell (1992) concluded the increases in WUE with no response of g_s following N fertilization was likely due to a decrease in non-stomatal limitations and not increased stomatal control. In our case we observed decreased g_s and C_i/C_a following fertilization and the increased WUE is likely due to increased stomatal control. Similar to our findings, Samuelson (2000) and Munger et al. (2003) both found that high N plants had significantly less g_s in *P. taeda* seedlings, which with an observed increase in photosynthesis as observed by Samuelson (2000), led to increased WUE.

4.2. Clonal differences

We did not observe differences in total biomass or stem height between genotypes, however, there were differences in the way biomass was partitioned. For example, CL85 produced more stem biomass while CL93 produced more branch biomass (Fig. 1B). This difference in partitioning led to differences in canopy architecture between clones. Although, both clones produced the same amount of leaf biomass (Table 2) when leaf weight was converted to TLA CL93 had 8% more leaf area than CL85. Similarly, an independent measure of projected CSA also showed a 13% greater photosynthetic area in CL93 relative to CL85. There are a couple of explanations for this, first, increased branch mass suggests CL93

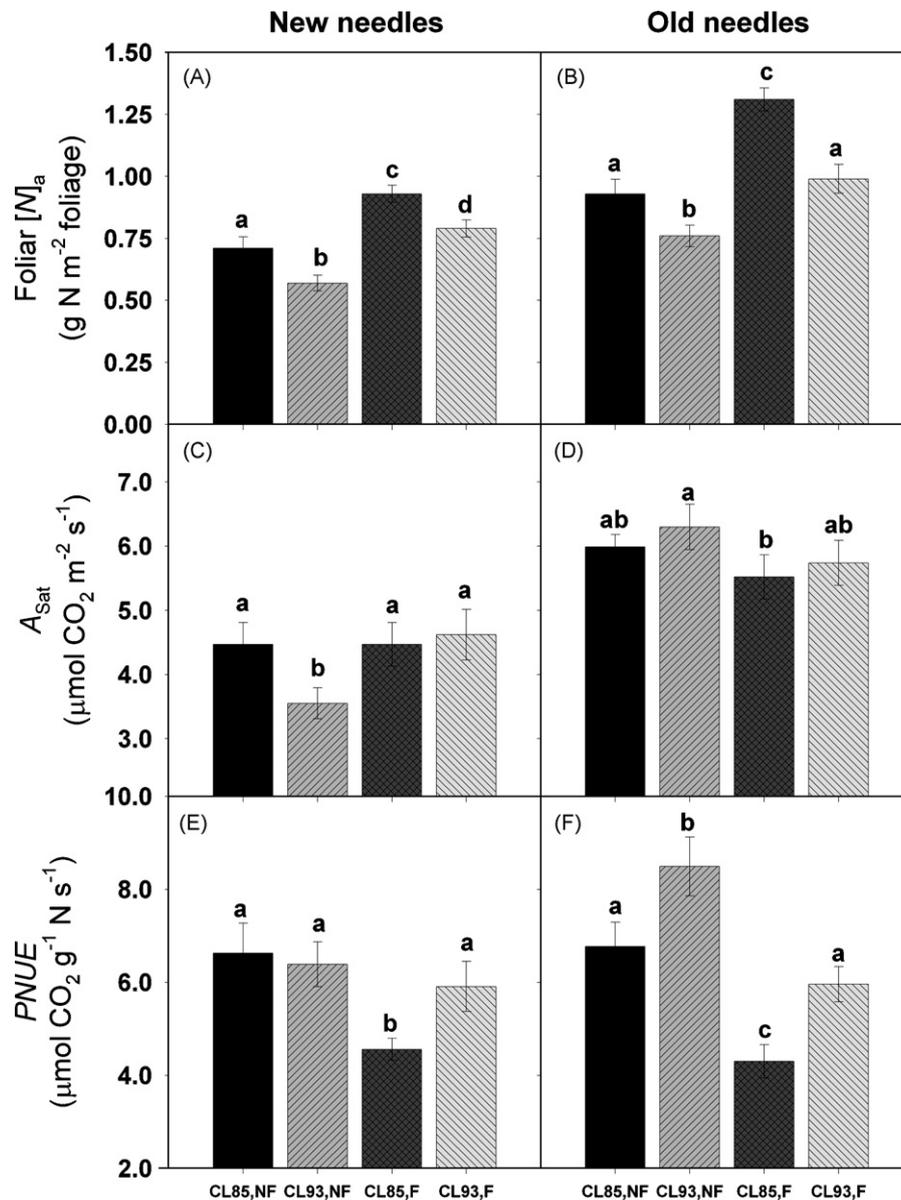


Fig. 7. Foliar N content on an area basis (A and B), net CO₂ assimilation (C and D), and photosynthetic N use efficiency (E and F) for current year (“new”) and previous year needles (“old”) for clone by fertilizer two-way interaction. A_{Sat} measured in late June 2007 and late March 2007 were used to calculate PNUE for “new” and “old” needles, respectively. Error bars represent ± 1 standard error from the mean ($n = 12$). Lower case letters indicate significant differences between treatments using *p*-diff option in SAS version 9.1 ($\alpha = 0.10$; $n = 12$).

had a more open canopy allowing more needles to participate in light interception. Although CL85 had greater stem biomass, an early partitioning of C to branches at the expense of diameter growth particularly in the 1st year of growth may give CL93 a more long-term advantage when it comes to capturing light per unit leaf area. Second, clonal differences in leaf morphology: number of needles per fascicle, needle weight, and leaf area led to greater leaf area per unit weight (SLA) in CL93 relative to CL85 irrespective of nutrient availability (Tables 3 and 4 and Fig. 2).

4.3. Canopy level CO₂ assimilation

Instantaneous CO₂ assimilation at the canopy level (A_{Canopy}) was calculated by multiplying A_{Sat} by estimates of TLA. Specific photosynthetic rates (A_{Sat}) measured in June 2007 were used to scale to the whole-tree level because temporally it was the closest sampling date to the time of destructive harvest, and also represented the first fully elongated flush of the current year’s growth. However,

these results need to be viewed with caution since this sampling date was one of the few in which fertilizer had a positive effect on A_{Sat} .

In support of our hypothesis, we found that A_{Canopy} increased ($P < 0.01$) 29% in fertilized seedlings in both genotypes (Fig. 8A), but both clones showed two contrasting methods to increasing A_{Canopy} . For example, an analysis of the components making up A_{Canopy} showed that CL93 responded to N and P fertilization by increasing A_{Sat} while CL85 showed no change in A_{Sat} (Fig. 8B). In contrast, CL85 responded to fertilization by increasing TLA while CL93 showed no response (Fig. 8C). This increase in TLA was achieved by both increasing SLA as well as partitioning more biomass to branches at the expense of diameter growth (stem volume), which led to a more open canopy allowing for more light penetration.

Similar to our findings, King et al. (2008) found a $G \times E$ interaction on the effects of fertilization in eight different clones in the field. The authors observed that some clones responded by increasing leaf area while others increased A_{Sat} . What was most surprising was that in some instances full-sib clones used opposite

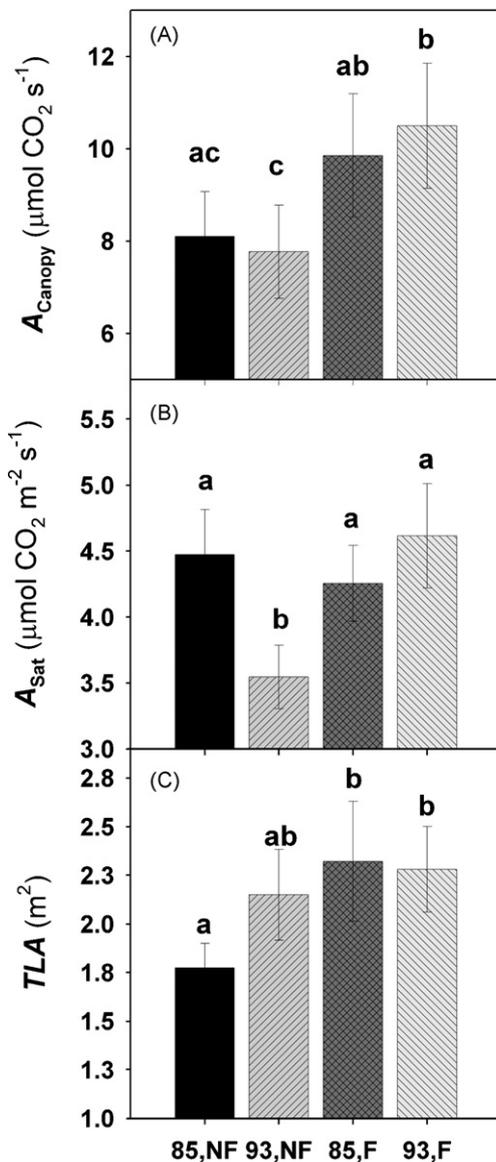


Fig. 8. Least squares means for canopy level net CO_2 assimilation (A_{Canopy} ; panel A), specific net CO_2 assimilation (A_{Sat} ; panel B), and total leaf area (TLA ; panel C) for clone by fertilizer interactions. TLA was calculated by multiplying total leaf weight and specific leaf area, which was averaged for “new” and “old” needles assuming both made up 50% of total leaf area. A_{Sat} and TLA were measured on one occasion in late June 2007. Lower case letters indicate significant differences between treatments using p -diff option in SAS version 9.1 ($\alpha = 0.10$; $n = 12$).

strategies (King et al., 2008). In contrast, Chmura and Tjoelker (2008) found that in contrasting families of *P. taeda*, that A_{Sat} was less important than full canopy light interception in predicting plant growth. Further support comes from data collected on “old” needles. In March 2007 the last fully elongated needles produced from the previous year were measured for A_{Sat} , $[N]_a$, and needle morphology. Regardless of N availability CL93 needles had greater SLA relative to CL85 in “old” needles (Tables 3 and 4) as well as maintained similar rates of A_{Sat} at lower rates of $[N]_a$ leading to greater $PNUE$ in “old” needles (Fig. 7B, D, and F). Similar plant biomass between clones indicates that both strategies were equally effective at capturing and assimilating CO_2 under optimal conditions. However under N limited conditions, the strategy for thinner fascicle diameter needles and more open canopy may be more advantageous for plant growth.

Our estimates of A_{Canopy} represent upper limits for a number of reasons. First, TLA does not account for differences in specific gas

exchange rates due self-shading or between “new” and “old” needles. Second, needle fascicles were included in the dry weight of the needles from the destructive harvest, but not when needles were sub-sampled for SLA determination. Both sources of error would lead to an overestimation of TLA . In the context of these cautions, we do believe this value gives a reasonable integration of differences in canopy area and A_{Sat} rates.

No significant LR or LR by CL interaction was observed in A_{Canopy} or its corresponding components. However, A_{Canopy} and A_{Sat} did have a highly significant ($P < 0.01$ and $P = 0.02$, respectively) LR by fertilizer interaction and although TLA was not significant ($P > 0.1$) the trend was the same (Fig. 9). Our lack of LR response could be due to our inability to achieve sufficient N immobilization with the level of LR applied. However, from our data we inferred that CL93 might have been better equipped to tolerate N limitations due impart to greater $PNUE$ in both “new” and “old” needles. Despite weak treatment effects, at the level of LR we incorporated we found

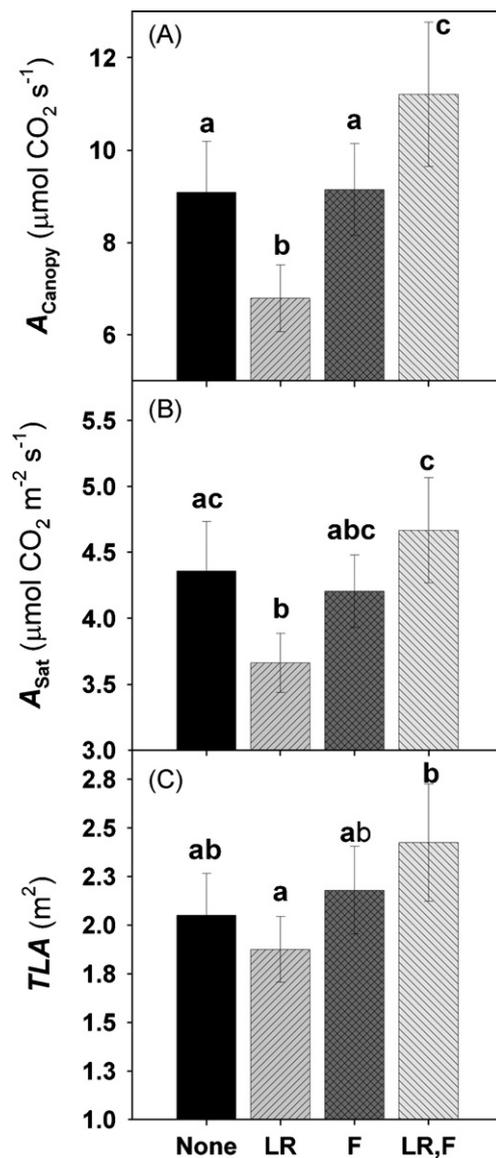


Fig. 9. Least squares means for canopy level net CO_2 assimilation (A_{Canopy} ; panel A), specific net CO_2 assimilation (A_{Sat} ; panel B), and total leaf area (TLA ; panel C) for each nutrient manipulation. TLA was calculated by multiplying total leaf weight and specific leaf area, which was averaged for “new” and “old” needles assuming both made up 50% of total leaf area. A_{Sat} and TLA were measured on one occasion in late June 2007. Lower case letters indicate significant differences between treatments using p -diff option in SAS version 9.1 ($\alpha = 0.10$; $n = 12$).

that CL93 had a higher fine- to coarse-root ratio and responded by increasing *SLA* relative to CL85 (Table 2 and Fig. 2). We believe both of these responses would lead to increased resource acquisition (e.g., light and nutrients), but increased treatment intensity and more long-term evaluation is needed to determine the extent of these adjustment as well as if these effects will be present under field conditions. Finally, a component that we did not measure in this experiment that may have a strong influence is the amount of C lost through maintenance respiration and root exudates. Additionally, maintenance respiration (respiration measured in fully elongated needles) has been shown to differ between families for loblolly pine by some (Samuelson, 2000) but not by others (King et al., 2008). Both of these potential losses of C need to be quantified to better judge these competing strategies.

5. Conclusion

Fertilization with N and P did not result in an immediate and sustained increase in A_{Sat} as we hypothesized, but did result in consistently lower g_s , E , and C_i/C_a and improved *WUE* independently of genotype. We found large differences between genotypes in both gas exchange parameters as well as in biomass partitioning. Clone 93 had similar seedling biomass to CL85, but invested more resources into producing branches and fine-roots at the expense of stem volume. Increased investment in branches and increased specific leaf area ($\text{cm}^2 \text{g}^{-1}$) led to more photosynthetic area in CL93 relative to CL85 despite similar foliage biomass. When final gas exchange rates were scaled to the canopy level by multiplying by the total leaf area (m^2) we found that both genotypes achieved similar canopy level CO_2 assimilation rates, but appeared to do so by different means. CL93 produced greater leaf area with lower A_{Sat} at low N rates while CL85 used the opposite strategy of lower leaf area, but greater A_{Sat} . At higher rates of N availability, CL93 increased photosynthetic efficiency while CL85 increased leaf area. We believe the strategy that CL93 has shown would make it better able to compete in low N situations due to increased *PNUE* and fine- to coarse-root ratio, but we were unable to verify this. Although we did see a small effect of nutrient limitations in A_{Canopy} , A_{Sat} , and *TLA*, our foliar [N] indicated that our level of LR incorporation did not cause [N] to drop below sufficiency limits. Based on current results, future research should focus on differences in canopy architecture between genotypes at a young age and how it relates to wood volume at maturity. Finally, our work suggests that within *P. taeda* genotypes may express different mechanisms for capturing and partitioning C, which should be accounted for when matching clonal material to specific site conditions.

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