

# Short-term effects of fertilization on loblolly pine (*Pinus taeda* L.) physiology

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## ABSTRACT

**Fertilization commonly increases biomass production in loblolly pine (*Pinus taeda* L.). However, the sequence of short-term physiological adjustments allowing for the establishment of leaf area and enhanced growth is not well understood. The effects of fertilization on photosynthetic parameters, root respiration, and growth for over 200 d following the application of diammonium phosphate were intensively investigated in an effort to establish a relative sequence of events associated with improved growth. Root respiration, foliar nitrogen concentration  $[N]_f$ , and light-saturated net photosynthesis ( $A_{sat}$ ) temporarily increased following fertilization.  $A_{sat}$  was correlated positively with  $[N]_f$  when non-fertilized and fertilized treatments were pooled ( $R^2 = 0.47$ ). Increased photosynthetic capacity following fertilization was due to both improved photochemical efficiency and capacity and enhanced carboxylation capacity of Rubisco. Positive effects of fertilization on growth were observed shortly after  $A_{sat}$  increased. Fertilized seedlings had 36.5% more leaf area and 36.5% greater total dry weight biomass at 211 d following fertilization. It is concluded that fertilization temporarily increased photosynthetic capacity, which resulted in a pool of photo-assimilate used to build leaf area. The N from fertilizer initially invested in photosynthetic structures and enzymes probably re-translocated to newly developing foliage, explaining the reduction in  $[N]_f$  and  $A_{sat}$  that was observed after peak levels were achieved following fertilization.**

*Key-words:* carboxylation capacity; chlorophyll fluorescence; leaf area; nitrogen; photochemical efficiency; photosynthesis; root respiration.

## INTRODUCTION

Fertilization is commonly applied in forest management to enhance productivity. However, the initial physiological changes permitting increased biomass productivity following fertilization are not well characterized. Fertilization generally increases leaf area in pine, which enhances over-

all productivity by increasing whole-plant carbon (C) fixation (Teskey *et al.* 1987; Vose & Allen 1988; Teskey, Gholz & Cropper 1994; Albaugh *et al.* 1998). Vose & Allen (1988) reported an increase in leaf area index (LAI) of up to 60% with N fertilization in loblolly pine (*Pinus taeda* L.). Albaugh *et al.* (1998) reported a LAI increase of 101% in nutrient-poor loblolly pine stands amended with optimum levels of fertilizer. The establishment of greater leaf area requires that one of the following must occur: (1) fertilization may have a direct effect on photosynthetic capacity (i.e. maximum net photosynthesis per unit leaf area) resulting in more C being fixed per unit leaf area with the 'surplus' photo-assimilate being subsequently used to build additional leaf area; (2) biomass allocation may shift from root to leaf components, thereby reducing C sink tissue and increasing source tissue; or (3) increased leaf area is the result of both mechanisms. While any of the above mechanisms could account for increased biomass productivity in response to N fertilization, the literature does not clearly identify a single mechanism.

Previous studies that investigated fertilization effects on Pinaceae leaf-level net assimilation ( $A$ ) provide mixed results. Fertilization has directly improved  $A$  in some cases (Mitchell & Hinckley 1993; Murthy *et al.* 1996). Other studies report that fertilization had little or no impact on  $A$  (Zhang, Hennessey & Heinemann 1997; Tang *et al.* 1999; Samuelson *et al.* 2001; Maier *et al.* 2002; Gough *et al.* 2004). Similarly, previous studies report conflicting results concerning the relationship between foliar N status and  $A$ . Some reports indicate that foliar N in Pinaceae species is positively correlated with  $A$  (Mitchell & Hinckley 1993; Vapaavuori *et al.* 1995; Murthy *et al.* 1996), whereas others report a weak or non-existent relationship between foliar N and  $A$  (Teskey *et al.* 1994; Zhang *et al.* 1997; Schoettle & Smith 1999; Maier *et al.* 2002; Gough *et al.* 2004). Generally, the literature suggests that any increase in  $A$  following fertilization is brief, providing the necessary photo-assimilate for enhanced leaf area. Once greater leaf area is established following nutrient amendments,  $A$  may return to pre-fertilization levels. The preceding hypothesis could explain why the literature as a whole provides conflicting reports concerning the effects of fertilizer on  $A$ , since measurement timing following fertilization may be critical to the detection of changes in photosynthetic capacity. This hypothesis has never been tested in a single study that intensively

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monitors changes in photosynthetic capacity immediately following fertilization.

Shifts in plant biomass partitioning and allocation are also common following fertilization. King *et al.* (1999) reported a 6% increase in biomass partitioning to perennial tissues including coarse roots and branches after 4 years of fertilization in a nutrient-poor 12-year-old loblolly pine stand. In the same stands, Albaugh *et al.* (1998) reported 8 and 22% of total biomass was allocated to fine roots in fertilized and non-fertilized stands, respectively. Other investigators reported both an absolute and relative decrease in root biomass production in fertilized pine (Axelsson & Axelsson 1986; Griffin, Winner & Strain 1995; Haynes & Gower 1995; Gebauer, Reynolds & Strain 1996).

We present results from a study in which seedlings were fertilized and intensively monitored for short-term physiological changes associated with productivity shifts. Specifically, our objectives included determining the short-term photosynthetic, root respiratory and biomass allocation responses to nutrient additions. We hypothesized that root respiration ( $R_r$ ) would increase following fertilization due to increasing metabolic demands. Shortly after fertilization, we expected to observe an increase in photosynthetic capacity, which would parallel enhanced foliar nitrogen concentration  $[N]_f$ . We predicted that any changes in photosynthetic capacity would reflect adjustments in photochemical and/or carboxylation capacity monitored using chlorophyll fluorescence and  $A/C_i$  derived parameters. Further, we anticipated that photosynthetic capacity would eventually return to non-fertilized levels following the establishment of greater leaf area. We also expected a return in  $[N]_f$  levels to non-fertilized levels over time since N is 'diluted' during the production of greater leaf area. Finally, we hypothesized that a final harvest of seedlings would show that leaf area was in fact greater in fertilized plants and shifts in relative biomass allocation would favour above-ground tissues rather than root biomass.

## METHODS

### Study design

An infertile (sandy, siliceous, thermic Psammentic Hapludult; Wakulla series) (Abrahamson, Dougherty & Zarnoch 1998) was collected to a depth of 0.2 m from the Forest Service's South-eastern Tree Research and Education Site (SETRES) located in Scotland County, North Carolina (35°N lat., 79°W long.) in the Sandhills region. This soil was chosen for the experiment because of its relatively low fertility and the gains in loblolly pine productivity following fertilization of the soil (Albaugh *et al.* 1998). The soil was sieved through a 6.4-mm screen to remove coarse woody debris and coarse roots, and homogenized by manually mixing the soil. Forty containerized 1-0 half-sibling (MeadWestvaco Corp., Summerville, SC, USA, family: WV-3) loblolly pine seedlings were planted in 29 L containers (dimensions: 355.6 mm × 292.1 mm × 279.4 mm, Custom™ 2800C, Hummert International, Earth City, Missouri, USA) in an effort

to minimize physical restrictions on root growth. The total soil volume of each pot was approximately 0.025 m<sup>3</sup>. All pots initially received a low-level dose of fertilizer (15 kg ha<sup>-1</sup> of elemental N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O) in order to prevent or alleviate nutrient deficiencies prior to actual fertilization treatment application. Potted seedlings were randomly chosen and paired. A single pair of pots placed next to each other on a greenhouse bench served as a block. The study was arranged as a randomized complete block design with fertilized and non-fertilized treatments.

### Growth conditions

The seedlings, which were 1 year old at the time of potting, were grown for approximately 1 year in a greenhouse prior to the fertilizer application outlined below. Plants were grown under a 16-h photoperiod for the duration of the study. The greenhouse thermostat was set for 25 °C daytime and 18 °C night-time temperatures; however, the actual greenhouse temperatures were not recorded. Temperature was regulated in the summer using a cooling pad. Seedlings were watered frequently and evenly in order to prevent water stress. Excessive watering was avoided in efforts to prevent leaching of nutrients from soil.

### Nutrient additions and prior measurements

Within each block (two seedlings), seedlings were assigned randomly to either the fertilized or non-fertilized treatment. Seedling height and ground-line diameter were determined, and foliage samples were collected from each seedling for  $[N]_f$  analysis prior to fertilizer treatment application. In addition,  $A$ , discussed below in detail, was measured prior to fertilization to ensure that both treatments had statistically identical physiological and biomass parameters prior to fertilizer application. On the day of the fertilizer application, but prior to fertilization, seedling heights were 0.85 and 0.88 m, the ground-line diameters were 13.9 and 14.2 mm for the control and fertilized plants, respectively, and differences were not significant ( $P > 0.05$ ). Each seedling assigned to the fertilization treatment received 1.85 g (0.39 g N and 0.43 g P) of diammonium phosphate (DAP), which is the recommended operational level for seedling fertilization of loblolly pine (Jokela & Long 1999). The equivalent rate is 280 kg DAP ha<sup>-1</sup> or 59.4 kg ha<sup>-1</sup> of elemental N and 65.8 kg ha<sup>-1</sup> of elemental P.

### Growth measurements

Seedling growth was monitored over the course of the study by tracking changes in ground-line diameter and height. Seedling growth generally was monitored every week or every other week.

### Root respiration

The value of  $R_r$  was measured 49 and 197 d after fertilization using a LiCor 6200 closed dynamic system with a LiCor

6250 infrared gas analyser (IRGA) and a 0.25-L cuvette chamber (LiCor Inc., Lincoln, NE, USA). For each pot, approximately 10 000 mm<sup>2</sup> of fine roots (< 2 mm diameter) close to the soil surface were excavated gently and loose soil was shaken free. The roots were placed on a moist paper towel positioned on the cuvette to prevent them from rapidly drying. Respiration was measured over a 30-s sampling period. Roots were scanned electronically, digitized, and surface area was determined using WINRHIZO 5.0 A software (Regent Instruments Inc., Quebec, Canada).

### Photosynthesis measurements

Net photosynthesis under saturating light levels ( $A_{\text{sat}}$ ) was measured on young, fully expanded foliage using a LiCor 6400 portable photosynthesis system (LiCor Inc.). Leaf  $A_{\text{sat}}$  was measured within 1 min following the detachment of a fascicle, which is common practice in loblolly pine studies since *in situ* photosynthesis rates are maintained for several minutes following detachment (Ginn *et al.* 1991). We monitored  $A_{\text{sat}}$  prior to and following fertilization over a 190-day period. Measurements initially were taken several days a week and later continued at less frequent intervals until physiological changes resulting from fertilization stabilized. The value of  $A_{\text{sat}}$  was measured in the same sequential blocking order during every measurement period. Chamber conditions were maintained at 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density (PPFD), 370  $\mu\text{mol mol}^{-1}$  [CO<sub>2</sub>], 25 °C temperature, and >55% humidity. The PPFD level chosen represents a photosynthetically active saturating light intensity in loblolly pine (Teskey *et al.* 1987). The other values, although somewhat arbitrary, served to minimize the confounding effects of environmental variation during measurements.  $A_{\text{sat}}$  was expressed on a per unit leaf area basis using the following equation (Ginn *et al.* 1991):

$$LA_1 = (n \times l \times d) + (\pi \times d \times l)$$

where  $l$  is the length of the needle,  $d$  is the fascicle diameter and  $n$  is the number of needles in the fascicle.

### Foliar nitrogen concentration

Following  $A_{\text{sat}}$  measurements, each detached fascicle was pooled by treatment into groups of four (i.e. four blocks were combined) on each measurement date since one needle was not of sufficient mass for [N]<sub>f</sub> analysis. Needles were oven-dried at 65 °C for 48 h and ground in a Wiley mill (Model 3; Arthur H. Thomas Co., Philadelphia, PA, USA). Samples were analysed for [N]<sub>f</sub> by the USDA Forest Service Southern Research Station laboratory (Research Triangle Park, NC, USA) using a Carlo-Erba elemental analyser (Model NA 1500; Fison Instruments, Danvers, MA, USA).

### Chlorophyll fluorescence measurements

We used chlorophyll fluorescence to examine the impact fertilization has on the short-term efficiency of the photosynthetic machinery and the light reactions since photo-

chemical efficiency may vary depending on plant nutrient status (e.g. Kellomaki & Wang 1997). Chlorophyll fluorescence parameters and  $A_{\text{sat}}$  were measured simultaneously on young, fully expanded needles attached to the stem. Measurements were taken 22 and 204 d following fertilization using the LiCor 6400–40 fluorescence attachment (LiCor Inc.). The LiCor fluorescence attachment allows for concurrent monitoring of gas exchange from the same tissue subjected to fluorescence. Chamber CO<sub>2</sub> concentration, temperature, and relative humidity parameters were controlled as described above. Attached needles were dark-adapted for 20 min prior to the measurement of dark-adapted or minimum fluorescence ( $F_o$ ), which was measured using a weak measurement beam (< 1  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Maximum fluorescence of both dark-adapted ( $F_m$ ) and light-adapted ( $F_m'$ ) was determined on attached foliage following a red light saturating pulse >7000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and centred at a wavelength of 630 nm. Steady-state fluorescence of light-adapted leaves ( $F_s$ ) was determined following the application of 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of continuous actinic light. The minimal fluorescence of a light-adapted leaf ( $F_o'$ ) was determined after a brief 1-s period of darkness.  $F_s$ ,  $F_o'$ ,  $F_m'$  and  $A_{\text{sat}}$  can take several minutes to reach equilibrium due to the lagging activity of photochemical and non-photochemical reactions following initial exposure to actinic light after dark acclimation (van Kooten & Snel 1990). Therefore, we measured  $F_s$  and  $F_m'$  sequentially five times at 3-min intervals under continuous actinic light levels of 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to obtain steady-state measurements. Using the parameters  $F_o$ ,  $F_o'$ ,  $F_m$ ,  $F_s$  and  $F_m'$ , the following photochemical and non-photochemical parameters were calculated:

- 1 Maximum photochemical efficiency of photosystem II (PSII) ( $F_v/F_m$ ) =  $(F_m - F_o)/F_m$
- 2 Quantum efficiency of PSII ( $\Phi_{\text{PSII}}$ ) =  $\Delta F/F_m' = (F_m' - F_s)/F_m'$
- 3 Photochemical quenching ( $q_p$ ) =  $(F_m' - F_s)/(F_m' - F_o')$
- 4 Non-photochemical quenching ( $q_N$ ) =  $(F_m - F_m')/(F_m - F_o')$

(Butler 1978 [ $F_v/F_m$ ], Genty, Briantais & Baker 1989 [ $\Phi_{\text{PSII}}$ ], van Kooten & Snel 1990 [ $q_p$ ,  $q_N$ ]).  $F_v/F_m$  is the fraction of photons used for photochemistry by dark-adapted leaves and  $\Phi_{\text{PSII}}$  represents the fraction of absorbed photons that are used for photochemistry by light-adapted leaves. Photochemical quenching is the conversion of light energy to biochemical energy. In the photochemical quenching of light energy, light is principally used to drive the biochemical reactions of photosynthesis and photorespiration. Non-photochemical quenching occurs when excess light energy is converted to heat energy through processes such as xanthophyll epoxidation (Malkin & Niyogi 2000).

### A/C<sub>i</sub> curves

In other efforts to monitor changes in the photosynthetic machinery following fertilization, we measured the response of  $A$  to internal CO<sub>2</sub> partial pressures ( $A/C_i$

response curves) *in situ* with a LiCor 6400 portable photosynthesis system. Young, fully expanded needles were selected from shoots adjacent to those measured for  $A_{\text{sat}}$ . Chamber conditions were maintained at  $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD,  $25^\circ\text{C}$  and  $>55\%$  relative humidity. The attached foliage was sealed into the leaf chamber and allowed to equilibrate to chamber conditions and at an initial  $[\text{CO}_2]$  of  $370 \mu\text{mol mol}^{-1}$ . Measurements of  $A$ ,  $C_i$  and stomatal conductance to water vapour were made at nine steps of external  $[\text{CO}_2]$  (70, 150, 230, 290, 370, 570, 890, 1500 and  $1900 \mu\text{mol mol}^{-1}$ ) ensuring that at each step chamber  $[\text{CO}_2]$  and  $A$  had stabilized before recording the measurement. Subsets of seedlings from eight blocks were measured three times, once before fertilization and then at 22 and 204 d after fertilization, coinciding with chlorophyll fluorescence measurements. Leaf area, mass and  $[\text{N}]_f$  were measured for each leaf sampled. The photosynthetic response curve parameters  $V_{\text{c,max}}$  (maximum carboxylation rate of Rubisco) and  $J_{\text{max}}$  (RuBP regeneration capacity mediated by maximum electron transport rate) were calculated with the Farquhar biochemical model of photosynthesis (Farquhar, von Caemmerer & Berry 1980) using the approach described in Crous & Ellsworth (2004).

### Partitioning and post-harvest measurements

Seedlings were harvested 211 d after fertilization and dry weight biomass of the roots, shoots, and foliage was determined for the two treatments. The roots were washed thoroughly in an effort to remove soil following harvest. Differences in dry matter partitioning between treatments were examined using an allometric relationship between roots and shoots where shoot weight =  $a(\text{root weight})^k$  (Ledig, Bormann & Wenger 1970). Parameter coefficients were compared statistically in order to determine whether partitioning differed between fertilized and control plants. Projected leaf area of fresh foliage was determined using a LI-3100 Area Meter (LiCor Inc.). Following leaf area measurements, biomass components were oven-dried at  $65^\circ\text{C}$  for 1 week and weighed to determine the biomass for each treatment.

### Statistical analysis

Variables measured repeatedly over time were analysed using repeated measures analyses and variables measured only at harvest were analysed as a randomized complete block design (with 20 replications per treatment). Repeated measures analyses were used to examine the effect of time, fertilization and time  $\times$  fertilization on all variables measured on two or more dates over the study. All statistical analyses were performed using the PROC GLM procedure in SAS (SAS Institute, Cary, NC, USA). Variables compared between fertilization treatments include  $A_{\text{sat}}$ ,  $R_r$ ,  $[\text{N}]_f$ , chlorophyll fluorescence-derived parameters,  $A/C_i$ -derived parameters, tree height growth, stem diameter growth, and root, shoot and foliage biomass. A significance level of  $\alpha = 0.05$  was applied to all analyses.

## RESULTS

### Foliar nitrogen concentration

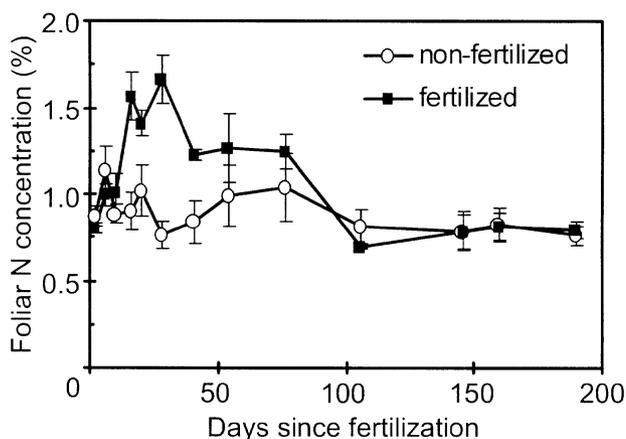
$[\text{N}]_f$  was significantly greater in the fertilized plants compared with the controls beginning 16 d following the treatment application.  $[\text{N}]_f$  in the fertilized treatment remained elevated over the control for approximately 50 d ( $P < 0.05$ ; Fig. 1). The peak difference in  $[\text{N}]_f$  between treatments was observed 28 d following fertilization, when foliage from the fertilized treatment contained almost 1.7% N and controls contained 0.76% N.

### Growth measurements

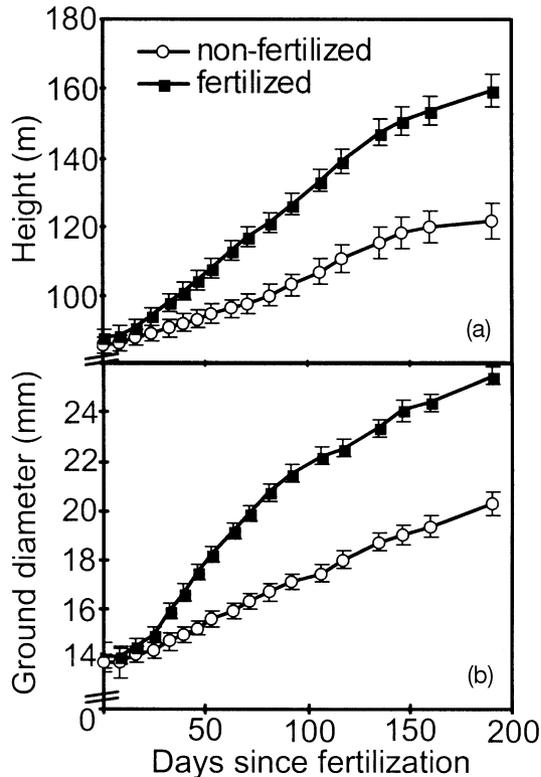
Fertilized seedling heights and ground diameters were significantly greater than the controls beginning 32 d following fertilization and continuing for the duration of the study ( $P < 0.05$ ; Fig. 2). The trajectory of growth and the fact that we observed a time-fertilizer treatment interaction indicates that growth rates were consistently greater for the fertilized seedlings in comparison with the controls. Mean heights and ground diameters of seedlings increased by 44.3 and 46.4%, respectively, in the control treatment over the course of the study. In comparison, mean seedling heights and diameters grew by 82.4 and 79.6% in the fertilized treatment over the study period.

### Root respiration

The  $R_r$  of fertilized plants was significantly elevated 49 d after fertilization ( $P < 0.05$ ; Fig. 3), averaging 32% higher than the non-fertilized plants. No significant difference between treatments was apparent at 197 d following fertilization.



**Figure 1.** Foliar nitrogen concentration  $[\text{N}]_f$  expressed as a percentage of dry weight tissue biomass in fertilized and non-fertilized loblolly pine seedlings following treatment application. Fertilized seedlings received  $1.85 \text{ g diammonium phosphate per pot}$ , which is an equivalent to  $280 \text{ kg ha}^{-1}$ .  $[\text{N}]_f$  was determined for foliage sampled for  $A_{\text{sat}}$ . Needles collected from four blocks were pooled for  $[\text{N}]_f$  analysis ( $n = 5$  replications/treatment).



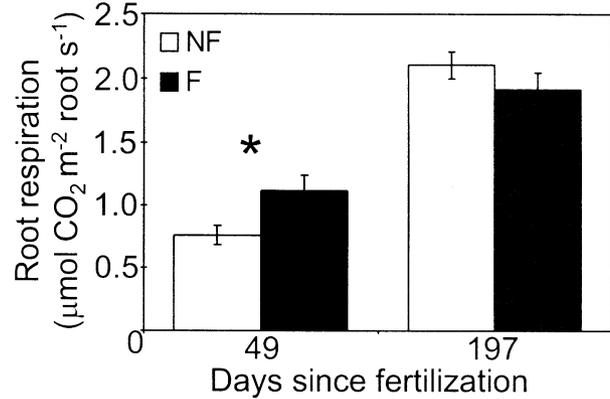
**Figure 2.** Height (a) and ground diameter (b) growth in non-fertilized and fertilized loblolly pine seedlings following treatment application. Fertilized seedlings received 1.85 g diammonium phosphate per pot, which is equivalent to 280 kg ha<sup>-1</sup> ( $n = 20$  replications/treatment).

### Photosynthesis measurements

The value of  $A_{\text{sat}}$  was greater in fertilized treatments in comparison with the controls beginning 8 d after fertilization ( $P < 0.05$ ; Fig. 4) and  $A_{\text{sat}}$  generally remained elevated in the fertilized foliage until the last two measurement dates. However, the difference in  $A_{\text{sat}}$  between the control and fertilized foliage was reduced over the last 100 d of the study, indicating a gradual return of  $A_{\text{sat}}$  in the fertilized foliage to control levels. Mean  $A_{\text{sat}}$  on a single measurement date ranged from approximately 1.5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to nearly 6  $\mu\text{mol m}^{-2} \text{s}^{-1}$  from foliage in the fertilized treatment. Both the time effect and the time-fertilizer treatment interaction were significant ( $P < 0.05$ ).

### Light-saturated photosynthesis and foliar nitrogen percentage

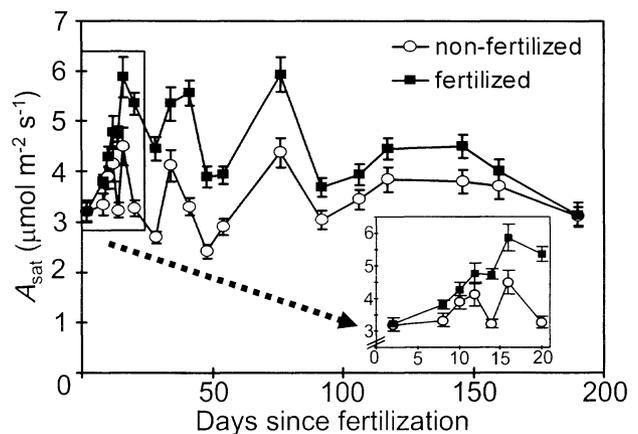
The relationship between  $A_{\text{sat}}$  and  $[\text{N}]_f$  was examined using linear regression. Mean  $A_{\text{sat}}$  values for a measurement date were regressed against corresponding mean  $[\text{N}]_f$  from each treatment for the analysis. A significant relationship between  $A_{\text{sat}}$  and  $[\text{N}]_f$  was observed ( $P < 0.05$ ) with  $[\text{N}]_f$  explaining 47% of the variance in  $A_{\text{sat}}$  when data from non-fertilized and fertilized treatments were pooled (Fig. 5).



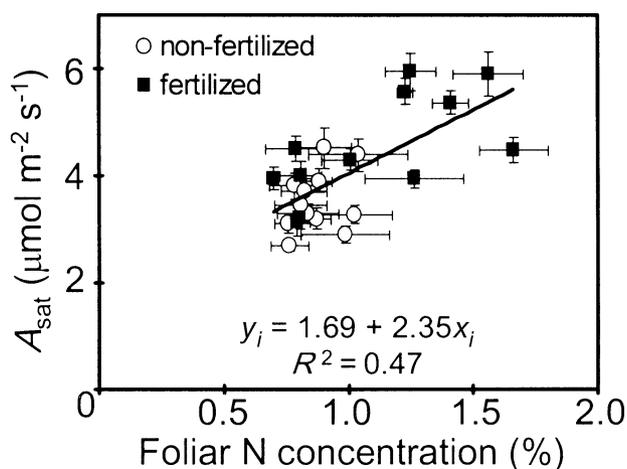
**Figure 3.** Fine root respiration ( $R_r$ ) in non-fertilized and fertilized loblolly pine observed on two measurement dates. Star above bars indicates significance between treatment means on the corresponding measurement date ( $\alpha = 0.05$ ). NF, non-fertilized; F, fertilized ( $n = 20$  replications/treatment).

### Chlorophyll fluorescence measurements

Chlorophyll fluorescence was used to examine the competency of the photosynthetic machinery associated with the electron transport chain. Fluorescence-derived parameters indicate an increase in electron transport efficiency 22 d following fertilization ( $P < 0.05$ ; Table 1). Specifically,  $F_v/F_m$ ,  $\Phi_{\text{PSII}}$ , and  $q_p$  were significantly greater in foliage from the fertilized treatment and coincided with elevated  $[\text{N}]_f$ . The values of  $A_{\text{sat}}$  measured concurrently with fluorescence measurements was also greater in the fertilized treatment on day 22 ( $P < 0.05$ ) but  $q_N$  was not significantly different between treatments 22 d following fertilization. Fluorescence-derived parameters and simultaneously measured



**Figure 4.** Light-saturated net photosynthesis ( $A_{\text{sat}}$ ) in non-fertilized and fertilized loblolly pine seedlings following treatment application.  $A_{\text{sat}}$  was measured on young, fully expanded needles immediately following detachment. Fertilized seedlings received 1.85 g diammonium phosphate per pot, which is an equivalent to 280 kg ha<sup>-1</sup>. The inset figure highlights the first 20 d of measurement following fertilization ( $n = 20$  replications/treatment).



**Figure 5.** Relationship between foliar nitrogen concentration  $[N]_f$  and light-saturated photosynthesis ( $A_{sat}$ ) in loblolly pine seedlings. Fertilized seedlings received 1.85 g diammonium phosphate per pot, which is an equivalent to 280 kg ha<sup>-1</sup>. Regression is based on pooled non-fertilized and fertilized treatment observations ( $n = 24$  pooled observations). Foliar nitrogen concentration was measured on a subset of samples for which  $A_{sat}$  was measured.

$A_{sat}$  were not significantly different between treatments 204 d following fertilization.

### A/C<sub>i</sub> curves

Foliage from fertilized seedlings exhibited greater  $J_{max}$  and  $V_{c,max}$ , and reduced electron transport capacity ( $J_{max}/V_{c,max}$ ) in comparison with foliage from non-fertilized seedlings 22 d following fertilization ( $P < 0.05$ ; Table 2). Enhanced electron transport and carboxylation rates coincided with

the temporary increase in  $[N]_f$  observed in the fertilized treatment.  $J_{max}$ ,  $V_{c,max}$ , and  $J_{max}/V_{c,max}$  did not differ between treatments prior to fertilization. Differences in  $J_{max}$ ,  $V_{c,max}$ , and  $J_{max}/V_{c,max}$  between treatments were not detected at 204 d following fertilization. The transient increase in the A/C<sub>i</sub>-derived parameters  $J_{max}$ ,  $V_{c,max}$ , and  $J_{max}/V_{c,max}$  in foliage from fertilized seedlings corresponded to enhanced electron transport efficiency determined using chlorophyll fluorescence and improved  $A_{sat}$ . There was a good correlation between  $[N]_f$ , expressed on a per unit area basis, and  $V_{c,max}$  and  $J_{max}$  when data from pre- and post-fertilization measurements were combined (Fig. 6). There was no fertilization effect on leaf mass per unit area (LMA, Table 2).

### Biomass and partitioning

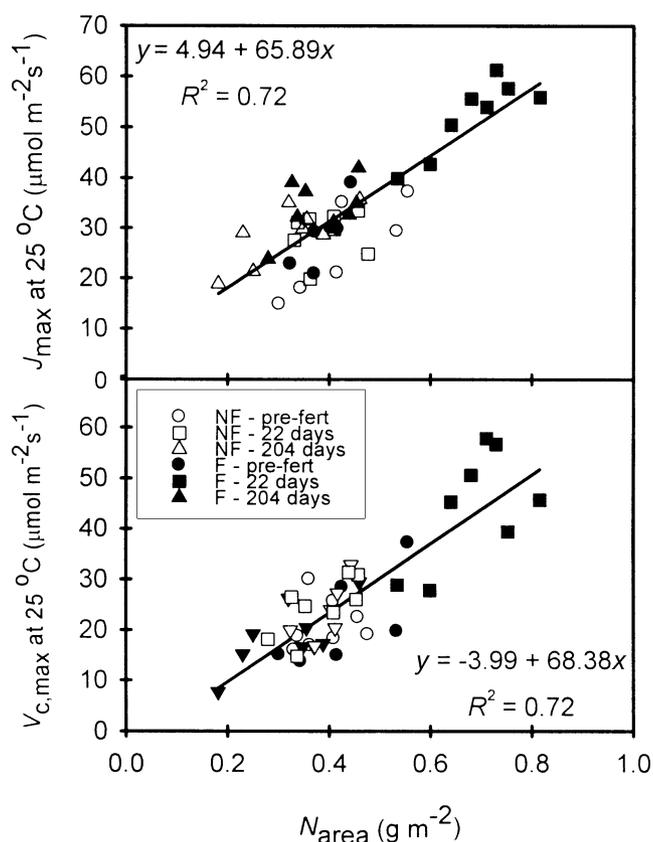
Foliage, stem, and root dry weights at harvest (211 d following fertilization) were determined for the non-fertilized and fertilized treatments. All tissues from fertilized plants had greater biomass than the controls ( $P < 0.05$ ). There was no significant difference in partitioning based on our analysis of the allometric relationship between roots and shoots for each treatment. Coefficients for the allometric relationship between roots and shoots where shoot weight =  $a(\text{root weight})^k$  were not significantly different between treatments ( $P > 0.05$ ). However, we observed a non-significant relationship between root and shoot dry weight biomass in both treatments. The coefficient  $k$  in the above function was not significant when estimated for each treatment individually ( $P > 0.05$ ), which prevented us from making meaningful statistical comparisons of root and shoot partitioning between treatments.

Projected leaf area was 36.5% greater in fertilized seedlings compared with the controls at harvest ( $P < 0.05$ ; Table 3). Control seedlings had an average projected leaf

Variable	Treatment	Days since fertilization			
		22		204	
		Mean	P-value	Mean	P-value
$F_v/F_m$	NF	0.756	0.0021	0.790	0.2638
	F	0.800		0.777	
$\Phi_{PSII}$	NF	0.058	<0.0001	0.051	0.8601
	F	0.107		0.054	
$q_P$	NF	0.204	0.0009	0.244	0.7128
	F	0.313		0.273	
$q_N$	NF	0.911	0.1966	0.931	0.9502
	F	0.901		0.932	
$A_{sat}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	NF	2.789	0.0365	3.191	0.6683
	F	3.937		2.404	

**Table 1.** Chlorophyll fluorescence-derived parameters and light-saturated net photosynthesis ( $A_{sat}$ ) in non-fertilized (NF) and fertilized (F) treatments measured 22 and 204 d following fertilization

Mean maximum photochemical efficiency of PSII ( $F_v/F_m$ ), quantum efficiency of PSII ( $\Phi_{PSII}$ ), photochemical quenching ( $q_P$ ), non-photochemical quenching ( $q_N$ ) were monitored simultaneously with  $A_{sat}$  on the young, fully expanded and attached loblolly pine foliage.  $P$ -values were obtained from pairwise comparisons of mean values for non-fertilized and fertilized treatments ( $n = 14$  replications/treatment).



**Figure 6.** Correlation between foliar nitrogen content and estimated  $J_{\max}$  and  $V_{c,\max}$  based on gas exchange analyses. Data are shown for pre-fertilization, 22 and 204 d after fertilization for both non-fertilized (NF) and fertilized (F) treatments. Data from both treatments and the three dates were pooled for regression analysis. Data points are individual tree observations ( $n = 44$  observations for  $J_{\max}$  versus N;  $n = 46$  observations for  $V_{c,\max}$  versus N).

area of 0.346 m<sup>2</sup> at harvest, whereas the fertilized seedlings averaged 0.545 m<sup>2</sup> leaf area.

## DISCUSSION

### Short-term physiological changes associated with fertilization

Our results suggest that a series of physiological events leads to enhanced leaf area and growth following DAP fertilization of loblolly pine seedlings. The value of  $R_t$  was elevated 49 d after fertilization relative to the controls, which may be due to increased nutrient uptake and assimilation following fertilization (Vessey & Layzell 1987; Ryan 1991; Zogg *et al.* 1996; Griffin *et al.* 1997; Lu *et al.* 1998). Within 16 d following fertilization, we observed greater  $[N]_f$  in the fertilized treatment, which coincided with increased  $A_{\text{sat}}$ .  $[N]_f$  was correlated with  $A_{\text{sat}}$  ( $R^2 = 0.47$ ) suggesting that N directly impacted photosynthetic capacity. Our findings are consistent with some previous reports that investigated the effects of foliar N and/or N fertilization on  $A_{\text{sat}}$  of Pinaceae species (e.g. Mitchell & Hinckley 1993; Vapaavuori *et al.* 1995; Schoettle & Smith 1999), but not others (e.g. Teskey *et al.* 1994; Zhang *et al.* 1997). Increased  $A_{\text{sat}}$  was due to greater photosynthetic electron transport capacity and efficiency and enhanced carboxylation capacity of foliage from fertilized plants. Changes in both the photosynthetic apparatus and carboxylation capacity have been positively related to N fertilization and foliar N in Pinaceae species (Kellomaki & Wang 1997; Strand 1997; Wang & Kellomaki 1997; Cheng, Fuchigama & Breen 2000; Lavigne, Little & Major 2001; Livonen, Rikala & Vapaavuori 2001).

Less than 4 weeks following the initial rise in photosynthetic capacity, fertilized seedlings had greater ground diameters and heights, demonstrating a short lag between

**Table 2.** Maximum carboxylation rate of Rubisco ( $V_{c,\max}$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), maximum electron transport rate ( $J_{\max}$ ,  $\mu\text{mol RuBP m}^{-2} \text{ s}^{-1}$ ), electron transport capacity ( $J_{\max}/V_{c,\max}$ ), foliar nitrogen concentration  $[N]_f$ , and leaf mass per unit area (LMA,  $\text{g m}^{-2}$ ) of sampled leaves prior to fertilization and 22 and 204 d following fertilization

Variable	Treatment	Days after fertilization					
		Pre-fertilization		22		204	
		Mean	<i>P</i> -value	Mean	<i>P</i> -value	Mean	<i>P</i> -value
$V_{c,\max}$	NF	21.4	0.7530	21.1	0.0001	18.8	0.1188
	F	22.4		44.1		24.1	
$J_{\max}$	NF	25.8	0.5441	29.5	<0.0001	27.5	0.0936
	F	28.1		52.0		32.4	
$J_{\max}/V_{c,\max}$	NF	1.24	0.5848	1.43	0.0029	1.58	0.3189
	F	1.29		1.22		1.38	
$[N]_f$	NF	0.925	0.5712	0.796	<0.0001	0.618	0.0571
	F	0.819		1.40		0.751	
LMA	NF	48.4	0.8947	49.8	0.4875	51.7	0.7007
	F	48.1		48.7		50.8	

$V_{c,\max}$ , and  $J_{\max}$  were calculated from  $A/C_i$  curves performed on young, fully expanded and attached loblolly pine foliage. *P*-values were obtained from pairwise comparisons of mean values for non-fertilized (NF) and fertilized (F) treatments ( $n = 8$  replications/treatment).

**Table 3.** Projected leaf area (LA) of fresh foliage along with actual biomass and percentage of total biomass allocated to foliage, stem and roots 211 d following diammonium phosphate fertilization application of non-fertilized (NF) and fertilized (F) loblolly pine seedlings

	LA (m <sup>2</sup> )	Foliage		Stem		Root		Total (g)	
		Actual (g)	Percentage						
NF	0.346	63.1	34.9	63.7	35.2	54.1	29.9	180	100
F	0.545	99.9	35.1	110	38.8	74.2	26.1	284	100
<i>P</i>	<0.0001	<0.0001		<0.0001		0.0155		<0.0001	

*P*-values are associated with treatment mean comparisons of LA and absolute foliage, stem, root, and total dry weight biomass. Trends in dry weight partitioning (or relative allocation) of roots and shoots were compared between treatments using a regression approach. As the relationship between root and shoot biomass for treatments individually was not significant ( $P < 0.05$ ), meaningful statistical comparisons between treatments were not possible and are not presented ( $n = 20$  replications/treatment).

enhanced photosynthetic capacity and improved growth. Further, projected leaf area was 36.5% greater in fertilized seedlings relative to the controls at the end of the study, implying that enhanced photosynthetic capacity allowed for the production of greater leaf area. Enhanced leaf area production following fertilization is consistent with stand-level loblolly pine studies (Vose & Allen 1988; Albaugh *et al.* 1998). Over time, the relative difference in  $A_{\text{sat}}$  between the fertilized and non-fertilized treatments narrowed, which corresponded to the return of  $[\text{N}]_{\text{f}}$  in the fertilized seedlings to non-fertilized levels. Finally,  $A_{\text{sat}}$  returned to non-fertilized levels in the fertilized seedlings, which coincided with the return of electron transport and carboxylation capacities to non-fertilized levels.

Although we realize that the exact timing and sequence observed in this study may vary in a field setting, combined evidence from previous studies suggest that the series of changes leading to greater productivity following fertilization is similar. Results from multiple studies conducted at the USDA Forest Service's South-eastern Tree Research and Education Site (SETRES) in North Carolina suggest that fertilization had an effect on both photosynthetic capacity and biomass allocation, which resulted in the establishment of greater leaf area and enhanced growth. Since 1992 (stand age of 6 years), fertilized stands at SETRES received nutrient amendments considered optimal for loblolly pine (Allen 1987). Murthy *et al.* (1996) reported that fertilized stands had higher  $[\text{N}]_{\text{f}}$  and greater photosynthetic capacities than foliage from non-fertilized stands following 1 and 2 years of nutrient additions, which coincided with the establishment of greater leaf area in fertilized stands (Albaugh *et al.* 1998). Investigators also observed shifts in biomass allocation with fertilization, including an initial reduction in relative root biomass (Albaugh *et al.* 1998; King *et al.* 1999). Ultimately, these changes increased total biomass by 99% in fertilized stands after the fourth year of nutrient additions at SETRES (Albaugh *et al.* 1998).  $A$  was generally the same in fertilized and non-fertilized stands during the eighth year (stand age 14 years) of fertilization at SETRES. Thus, following the establishment of greater leaf area in fertilized stands,  $A$

returned to pre-fertilization levels, which is consistent with the current study.

### Photosynthesis and foliar nitrogen concentration

Our findings are consistent with the general trend in the literature that indicates  $[\text{N}]_{\text{f}}$  and  $A$  are positively correlated in Pinaceae species (e.g. Teskey *et al.* 1994; Murthy *et al.* 1996; Meir *et al.* 2002). The large range in  $[\text{N}]_{\text{f}}$  that was observed in the fertilized treatment over the course of the study may in part explain why we detected a relationship between  $[\text{N}]_{\text{f}}$  and  $A_{\text{sat}}$ . The  $[\text{N}]_{\text{f}}$  in both treatments combined varied considerably over the course of the study, ranging from less than 0.8% to nearly 1.6%. The lowest  $[\text{N}]_{\text{f}}$ -values we recorded were well below the reported critical level of 1.2% (Allen 1987; Colbert & Allen 1996). Thus, photosynthetic capacity of foliage from fertilized seedlings may have been especially impacted by N additions in our study due to low  $[\text{N}]_{\text{f}}$  prior to fertilization. Although we found a significant relationship between  $A_{\text{sat}}$  and  $[\text{N}]_{\text{f}}$  when data from both treatments were combined, we observed no significant relationship between  $A_{\text{sat}}$  and  $[\text{N}]_{\text{f}}$  within a range of 1.23 to 1.66%, suggesting that there was no effective change in photosynthetic capacity above the suggested critical N level of 1.2% in loblolly pine. A lack of  $[\text{N}]_{\text{f}}$  observations below the critical level may have precluded some previous researchers from documenting a positive relationship between  $A$  and N in loblolly pine, even after fertilization. Zhang *et al.* (1997), Samuelson *et al.* (2001) and Gough *et al.* (2004) failed to observe a relationship between  $A$  and  $[\text{N}]_{\text{f}}$  in loblolly pine, but their observations ranged from 1.13 to 1.8%  $[\text{N}]_{\text{f}}$ . Murthy *et al.* (1996) reported elevated  $A_{\text{sat}}$  1 year following initial applications of optimum nutrient additions in loblolly pine, which corresponded to  $[\text{N}]_{\text{f}}$  ranging from less than 0.7% to greater than 1.3%. Five years later in the same stands, Maier *et al.* (2002) found a mixed relationship between  $A_{\text{sat}}$  and N when  $[\text{N}]_{\text{f}}$  ranged from about 0.7% to nearly 1.5%, indicating that N is not the exclusive driver of photosynthetic capacity below the reported critical level. Reports cited above suggest that the

variable relationship between foliar N and  $A$  may also be due to differences in genetics, foliage age, crown position and seasonal dynamics.

The present results suggest that measurement timing following fertilization may be important in efforts to detect the effect of N on  $A$  since the rise and subsequent return of  $[N]_f$  and  $A_{sat}$  to non-fertilized levels following fertilization occurred within a time frame of just over 100 d. Other investigators have found similar short-term responses to fertilization (Foyer *et al.* 1994; Livonen *et al.* 2001). Thus, the pulse of N concentrated in the foliage may have re-translocated to developing foliage or other organs. Zhang & Allen (1996) found that fertilized 13-year-old loblolly pine re-translocated 75% of its foliar N, which corresponded to simultaneous reductions of N in old foliage and increases in developing foliage. The redistribution or re-translocation of N to developing foliage probably occurred in the current study since we observed greater leaf area in the fertilized seedlings at harvest and a concurrent return of  $[N]_f$  to control levels. However, it is interesting that  $[N]_f$  levels eventually returned to pre-fertilization levels in the fertilized seedlings, rather than stabilizing at the suggested critical  $[N]_f$  of 1.2%. The transient nature of foliar N accumulation and subsequent dilution of N in developing photosynthetic tissues may explain why some studies fail to link fertilization with changes in foliar N or  $A$ , but do report increased leaf area production following fertilization. For example, Tang *et al.* (1999) found no change in  $A$  5-years following fertilization applications in a loblolly pine stand that had already established greater leaf area than the controls.

The photosynthetic efficiency and capacity of the electron transport chain and the carboxylation capacity of Rubisco were improved temporarily by fertilization and paralleled changes in  $[N]_f$ . The rise in  $[N]_f$  coincided with improved maximum photochemical efficiency ( $F_v/F_m$ ), quantum efficiency of PSII ( $\Phi_{PSII}$ ) and maximum rate of electron transport ( $J_{max}$ ), which allowed for a temporary improvement in photochemical quenching ( $q_P$ ) following fertilization. Higher photosynthetic capacity also coincided with a temporary enhancement in the carboxylation capacity of Rubisco ( $V_{c,max}$ ). However, although both photochemistry and carboxylation capacity were temporarily improved in fertilized plants, electron transport capacity ( $J_{max}/V_{c,max}$ ) decreased temporarily, suggesting that N resources were allocated preferentially to Rubisco rather than to proteins involved in electron transport. It is important to note that we observed no differences between treatments in leaf mass per unit area (LMA), indicating that improved photosynthetic capacity was due to improvements in photochemistry rather than changes in mass. Improved photosynthetic capacity in relation to  $[N]_f$  of Pinaceae species is frequently due to more efficient and enhanced capacity of electron transport (Kellomaki & Wang 1997; Strand 1997; Wang & Kellomaki 1997; Cheng *et al.* 2000; Lavigne *et al.* 2001; Livonen *et al.* 2001) and/or greater carboxylation capacity (Kellomaki & Wang 1997; Meir *et al.* 2002). Our results suggest that N provided from fertilizer was

initially used in part to build and maintain proteins associated with both electron transport and carboxylation (i.e. Rubisco). The return of photosynthetic capacity in fertilized seedlings to non-fertilized levels indicates that diminished production and greater turnover of photosynthetic proteins and enzymes probably contributed to the mobile N pool. The contribution of N from protein turnover to the mobile N pool is well documented (Sprengli 2000).

### Biomass partitioning and fertilization

Although enhanced biomass production in fertilized seedlings was clearly related to increased photosynthetic capacity, we cannot rule out that temporary shifts in biomass allocation may have also contributed to the building of additional leaf area. Shifts in biomass partitioning immediately following fertilization may have contributed to leaf area production in fertilized seedlings. We can draw limited conclusions from our results as we did not observe a strong relationship between root and shoot dry weight biomass within treatments and because we did not measure changes in partitioning over time. However, other studies suggest that shifts in biomass partitioning account for some, but not all changes in leaf area and productivity associated with loblolly pine fertilization (Vose & Allen 1988; Albaugh *et al.* 1998; King *et al.* 1999). Complicating the issue is the observation that changes in allocation associated with fertilization are inconsistent across ages in loblolly pine (Griffin *et al.* 1995; Gebauer *et al.* 1996; King *et al.* 1999). King *et al.* (1999) reported enhanced leaf area production despite an increase in below-ground biomass allocation in 7- and 9-year-old loblolly pine trees continuously fertilized for 2 and 4 years, respectively, which is in contrast to findings from seedling studies (Griffin *et al.* 1995; Gebauer *et al.* 1996). The increase in below-ground biomass allocation observed by King and coworkers implies that the establishment of greater leaf area preceding nutrient additions must have been due to C gain from enhanced photosynthetic capacity rather than reallocation of resources from roots to foliage.

### Summary of physiological sequence following fertilization

Our results and collective findings from other studies discussed above suggest the following sequence of physiological events occurs following fertilization in loblolly pine: (1)  $R_r$  temporarily increases following fertilization due to increased nutrient uptake and assimilation demands. (2)  $A_{sat}$  improves as N accumulates in foliage and contributes to larger pools of proteins associated with electron transport and carboxylation. (3) Greater C fixation per unit leaf area generates the photo-assimilate necessary to build additional leaf area and improve growth. (4) N from fertilizer that was initially incorporated into photosynthetic proteins is re-translocated to developing foliage, decreasing the pool of photosynthetic proteins per unit leaf area and effectively reducing  $A_{sat}$ . (5) Improved growth continues in fertilized

plants despite the return of photosynthetic capacity per unit leaf area to control levels due to enhanced photosynthetic surface area. Allocation shifts from the roots to foliage may also occur concurrently with enhanced photosynthetic capacity, but we were unable to detect a change in partitioning in our study.

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