Seasonal Photosynthesis in Fertilized and Nonfertilized Loblolly Pine

Christopher M. Gough, John R. Seiler, Kurt H. Johnsen, and David Arthur Sampson

ABSTRACT. Net photosynthesis (P_n) of loblolly pine (*Pinus taeda* L.) foliage was monitored monthly in 14 yr old stands under near-ambient conditions over an entire year in upper and lower crowns and in both nonfertilized stands and stands receiving nutrient amendments for six consecutive years. Air temperature, humidity, vapor pressure deficit (VPD), photosynthetic photon flux density (PPFD), and plant water potential were monitored concurrently with P_n. Foliar nitrogen (N) concentration was also monitored. The effect of fertilization on P_n was inconsistent and generally not significant. Rates were consistently higher in the upper crown compared to the lower crown primarily due to variable light intensity. Multiple linear regression analysis shows that PPFD and VPD explain between 56% and 64% of the variability in foliar P_n, depending on the treatment. Little or no correlation between foliar N concentration and P_n was found, despite greater N concentrations in fertilized foliage, suggesting that fertilization does not enhance the photosynthetic capacity of loblolly pine foliage over the long term. Substantial amounts of carbon were fixed on measurement days during the winter season, even after freezing nights. Predicted light response curves indicate that foliar photosynthetic capacities are similar year-round, and gross primary productivity estimates (GPP) indicate that over 20% of the annual carbon fixation occurred during the nongrowing season. FOR. SCI. 50(1):1–9.

Key Words: Acclimation, empirical modeling, gas exchange, photosynthetic capacity, gross primary productivity.

FOLIAR NET PHOTOSYNTHESIS (P_n), or P_n per unit leaf area, in loblolly pine (*Pinus taeda* L.) is largely influenced by inherent site conditions and management-altering environmental conditions. Understanding the long-term effects of fertilization on loblolly pine gas exchange is critical since more hectares of forests are fertilized in the southeastern United States than in the rest of the world combined (NCSFNC 2002). Over 90% of the fertilized forest area in the southeast consists of managed loblolly pine plantations, with almost 470,000 ha of loblolly pine forest being fertilized in 2000 alone. Fertilization frequently results in increased productivity; however, the proposed mechanism(s) responsible for enhanced biomass production are not uniformly reported in the literature. Previous studies that investigated fertilization effects on pine foliar gas exchange provide mixed results. Enhanced stem wood production in fertilized stands may be the result of increased leaf area (Teskey et al. 1987, Vose and Allen 1988, Teskey et al.

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1994) or fertilization may be directly related to higher foliar P_n (Mitchell and Hinkley 1993, Murthy et al. 1996). Zhang et al. (1997) found that fertilization did not affect P_n per unit leaf area in loblolly pine grown on an infertile site in Oklahoma. Tang et al. (1999) similarly concluded that fertilization of loblolly pine on a well-drained site in Louisiana did not significantly impact foliar P_n. Neither of these studies examined gas exchange throughout an entire year however. Further, the individual studies did not examine fertilization effects on P_n over a range of environmental conditions typically encountered over the seasons by loblolly pine.

Traditionally, gas exchange studies have primarily focused on the growing season probably because measurable biological activity and growth is greatest, and because winter carbon fixation is often assumed to be negligible relative to the growing season. However, the contribution of carbon fixed during the nongrowing season to the yearly carbon pool remains uncertain. Sampson et al. (2001), using the process model BIOMASS, predicted that loblolly pine is capable of fixing and storing significant amounts of labile carbon during the nongrowing season. Verification of these results based on gas exchange data collected under realistic, ambient conditions is lacking. Previous studies examining winter carbon fixation only monitored photosynthesis under saturating light conditions (Murthy et al. 1997, Ellsworth 2000), providing limited information concerning carbon fixation rates over a realistic array of ambient environmental conditions. Murthy and coworkers measured light-saturated photosynthesis (A_{net}) in stands at the current study site (SETRES). However, they did not measure A_{net} over the entire year nor did they directly examine the effects of short-term and seasonal environmental dynamics on foliar carbon fixation capacity (i.e., the maximum possible rate of foliar carbon fixation given the immediate environment). Ellsworth measured midday A_{net} on sunny days in a loblolly pine stand located in the North Carolina Piedmont. He utilized a modeling approach to estimate total daily carbon assimilation (A_{day}) based on maximum photosynthesis (A_{max}) values, given the assumption that midday A_{net} rates for a particular day reflect physiological limitations imposed, in part, by current environmental stresses. In other words, Ellsworth assumed that water stress, for example, will result in lower than optimum maximum photosynthesis rates for the day. While this approach provides insight into seasonal carbon fixation based on variation in A_{net}, Ellworth's approach does not explore direct relationships between potentially complex and interacting environmental influences and carbon fixation. Our approach examines the importance of nongrowing season carbon fixation to the yearly carbon budget based on physiological observations under near-ambient conditions. Examining foliar P_n over a broad range of environmental conditions allowed us to investigate foliar carbon fixation capacities over a range of realistic environmental conditions.

Spatial and seasonal variation also influences foliar P_n. Physiological differences between sun and shade foliage are well documented. Foliage from the upper crown in loblolly pine generally exhibits higher P_n rates both due to higher photosynthetic capacity and greater light interception than lower crown foliage, suggesting that both environmental and physiological variation influence foliar P_n (Gravatt et al. 1997, Tang et al. 1999). Seasonal comparisons of intracanopy loblolly pine photosynthetic capacity have not been fully addressed, and therefore our understanding of seasonal crown acclimation remains poorly defined in loblolly pine.

In this study, foliar P_n rates were recorded on one day monthly in both fertilized and nonfertilized 14 yr old loblolly pine stands and in upper and lower crowns for an entire year. Mean monthly foliar P_n rates between fertilization treatments and crown positions were compared, and empirical models were developed to investigate differences in predicted response surfaces among foliage from fertilized and nonfertilized stands, upper and lower crown positions, and growing and nongrowing seasons. Further gross primary productivity (GPP) was estimated in order to compare carbon fixation among treatments and seasons. This study was designed to address the following major objectives: (1) to compare and quantify the potential rates of carbon fixation during the growing and non-growing seasons in loblolly pine grown in southern North Carolina, and (2) to determine if seasonal patterns in carbon fixation vary in fertilized and nonfertilized stands and in upper and lower crown foliage.

Methods

Study Site

All measurements were taken in Scotland County, North Carolina (35°N lat., 79°W long.) at the USDA Forest Service Southeastern Forest Tree Experiment and Education Site (SETRES). The stand consists of hand-planted loblolly pine (2×3 m spacing) established in 1985 (14 yr old at the beginning of the study). The site is flat, infertile, excessively drained, sandy, siliceous, and composed of thermic Psammentic Hapludult soil (Wakulla series). The average annual precipitation is 121 cm, but drought is common in the summer and early fall. The average summer temperature is 26°C, and the winter average is 9°C. The average annual temperature is 17°C. SETRES includes fertilized and nonfertilized treatment installations replicated four times. Interaction among belowground matter from adjacent plots is prevented by a 150 cm deep plastic liner that separates plots. Nonpine vegetation is controlled by mechanical and chemical (glyphosate) treatments such that no understory vegetation exists. Nutrient applications began in March 1992 and continued through March 1998. The cumulative amount of each nutrient (in kg ha^{-1}) added during that time is as follows: N (777), P (151), K (337), Ca (168), Mg (164), S (208), and B (3.9). In the fertilized plots, crown closure is common, and foliage is generally denser compared to control trees. Albaugh et al. (1998) found that total biomass accumulation at SETRES was 91% greater than control stands 4 yr after initial fertilization treatments began.

Measurements

Foliar P_n measurements were taken over a range of environmental conditions across an entire year in order to capture seasonal environmental variability. Since a major objective of our research was to compare seasonal photosynthetic trends and capacities for fertilized and nonfertilized foliage

in both the upper and lower crowns through empirical modeling, we required a dataset that captured the wide range of environmental conditions that occur during the growing and nongrowing seasons. Therefore, we measured foliar P_n within the growing and nongrowing seasons over a large range of light intensities, humidities, temperatures, vapor pressure deficits (VPDs), and water potentials.

Foliar P_n measurements were taken once monthly from April 1999 to March 2000 at SETRES using the LiCor 6400 Portable Photosynthesis System (LiCor, Lincoln, NE). Measurements were taken on cut current-year foliage (Ginn et al.1991) from the upper and lower third of crowns from a subsample of 2 individual trees per treatment/block combination for a total of 32 measurements per measurement period [2 treatments (control and fertilized) × 4 blocks × 2 crown positions × 2 subsamples)]. Multiple trees were sampled within a plot on each measurement day, and no attempt was made to resample branches. Gas exchange was measured in each block (containing both treatments) sequentially, and subsamples from each level within each treatment were chosen randomly for sampling. Blocks were always measured in the same order. Measurement periods outlined above were repeated a total of three times on the measurement day within a month in order to capture an abbreviated diurnal response to daily environmental changes. Rain on the December measurement day, however, prevented a morning measurement period. The three measurement periods were initiated at approximately 9 a.m., 11:30 a.m., and 1:30 p.m. on every monthly measurement day. Thus, a total of 96 measurements (three sampling sequences) were generally taken throughout the day. Ambient air temperature was monitored from an on-site temperature probe (Vaisala HMP35C, Helsinki, Finland).

Conditions in the LiCor's chamber during foliar P_n measurements represented the ambient environment of the fascicle prior to detachment. Shoots were cut using a pole pruner, and measurements were taken immediately on a detached fascicle. P_n of individual leaves was not recorded in the actual canopy on attached leaves since this would have greatly reduced our sample size by increasing the time required to collect one measurement. All measurements were taken at the ambient temperature, humidity, VPD, and CO_2 concentrations were held constant in the chamber at 350 ppm. The average photosynthetic photon flux density (PPFD) was estimated in the upper and lower crown using the LiCor's PPFD sensor. The PPFD for each crown level was determined by evaluating the average PPFD in full sunlight (for the upper third) and the average PPFD in the understory (for the lower third) prior to the measurement period. The PPFD levels for both control and fertilized stands were equally assigned for a given crown position despite slight differences in PPFD levels in the understory (due to leaf area differences). The PPFD was held constant in the cuvette throughout a measurement period using the LiCor's actinic light source. PPFD was reassessed and adjusted prior to each measurement period immediately before sampling allowing for diurnal light variation. Water potentials were determined for the branch from which the measurement fascicle was taken immediately after being cut using a field pressure chamber (PMS instrument Co., Corvallis, OR). All measurements were completed in one day. Needle diameter was immediately recorded and total foliar leaf area was later determined using the following equation (Ginn et al.1991):

$$LA_1 = (n * l * d) + (p * d * l)$$

where l = the length of the needle, d = fascicle diameter and n = number of needles in the fascicle. P_n values were adjusted to represent gas exchange on a per leaf area basis. Foliar N concentrations of measured needles were obtained from pooled samples collected from each block/fertilization/crown position combination during 8 of the 12 months using a Carlo-Erba elemental analyzer (Model NA 1500, Fison Instruments, Danvers, MA).

Statistical Analysis and Use of the Data

The foliar P_n data were analyzed as a time series with a split-block in which the whole plot is the fertilization treatment, and the split-block is the crown position (upper and lower thirds). The whole plot treatments were randomized across blocks while split-block treatments could not be randomly assigned since crown position is spatially fixed. Subsamples were averaged for analysis. Fertilization treatment and position impacts on gas exchange and foliar N percent were statistically assessed using analysis of variance.

The response of gas exchange to the environment was modeled using multiple linear regression since this approach provided the opportunity to determine the impact of individual environmental variables on foliar P_n. Gas exchange models were developed for all fertilization and crown position combinations based on the entire data set and seasonal data sets. Variables were ranked using the stepwise procedure in SAS (SAS Statistical Institute, Cary, NC). Potential explanatory variables assessed in the model selection procedure included PPFD, VPD, humidity, temperature, foliar N percent, and stem water potential. Stepwise procedures were executed with transformed and untransformed variables and residual plots were examined in order to minimize bias.

A common model including identical explanatory variables, but not necessarily the same coefficients, was developed to compare the relationship between foliar P_n and the environment among treatments. Model coefficients derived for all fertilization treatments and crown position combinations were developed for the entire year and for the growing (April–October) and nongrowing (November–March) seasons. Criteria for common model selection included common significant explanatory variables (transformed or untransformed) for all treatments, similar overall model R^2, and a simple overall model (five variables or less). Based on these criteria, the foliar P_n model we selected includes the following variables: PPFD, Ln(PPFD), and VPD. Parameter estimates for a given variable were compared statistically among treatments using indicator variables. Since we used a hierarchical approach to modeling (Montgomery et al. 2001), nontransformed PPFD remained in the

model despite being of little or no significance, and even though multicollinearity with Ln(PPFD) exists. In figures displaying predicted foliar P_n in response to PPFD, VPD was held constant. In order to compare the effect of light intensity on photosynthesis among treatments, average VPD for a given season were held constant when simulating light response curves. VPD values of 1.86 KPa and 1.00 KPa were assigned to growing season and nongrowing season, respectively, and represent the average VPD over those time periods.

Gross Primary Productivity Estimations

Pine gross carbon fixation—or gross primary productivity (GPP)—for the growing and nongrowing seasons by crown position and treatment was evaluated by combining outputs from a forest process model, in conjunction with meteorological data, as inputs into our empirical photosynthesis model. Specifically, we used the process model BIOMASS (Sampson et al. 2001) to estimate light (PPFD) interception by foliage within each crown level, and to output leaf area index (LAI), for the 14-yr-old loblolly pine stands at SETRES. Daily LAI was estimated using a statistical model developed at SETRES; this empirical model was based on 6 years of needle-count data, four destructive harvests, and monthly LI-COR LAI-2000 yr (Sampson et al., in press). Minor modification of the BIOMASS model was required to write as output average daily PAR absorbed by each crown position, and the associated LAI. The BIOMASS model was parameterized following Sampson et al. (2001) and updated to reflect stand structure and LAI's during our measurement years. An on-site weather station provided the meteorological data inputs to BIOMASS.

The LAI and light interception estimates derived using BIOMASS, and VPD data collected onsite, were inputs into our empirical models (daily time step) to estimate GPP for each fertilization treatment/crown position/season combination. Initially, daily PPFD for each fertilization treatment and crown position, and VPD values were input into empirical regression models with the appropriate coefficients resulting in an average carbon fixation rate for a given day by treatment, which was expressed in μmol C m^{-2} foliage s^{-1}. Using average carbon fixation rates for each day and time (s day^{-1}), total cumulative carbon fixation for a given day by treatment was calculated in terms of μmol C m^{-2} foliage day^{-1}. Next, LAI corresponding to the appropriate fertilizer treatment and day was used as a "multiplier" to scale up pine carbon fixation from the leaf level to the stand level in terms of land area. The multiplication of daily LAI by total cumulative daily carbon fixation rates provided daily GPP expressed as mol C m^{-2} land area day^{-1}. Finally, fixation rates were integrated over time (i.e., daily GPPs were grouped according to nongrowing and growing seasons), giving GPP in terms of mols C fixed per m^2 land area for each fertilization treatment/crown position/season.

Results

Effects of Fertilizer and Crown Position on Gas Exchange

Mean foliar P_n for a given measurement day within a month was assessed by treatment to examine the effects of fertilizer and crown position on gas exchange. A time series analysis indicated that foliar P_n rates differed between months among fertilization treatments and crown positions ($P < 0.01$). However, no time dependent interaction between fertilization treatment and crown position was found. Therefore, means of treatment main effects (i.e., fertilization treatment and crown position) for a given measurement day are presented independently (Figure 1). Fertilized stands had significantly higher foliar P_n rates only on the day of February measurements ($P = 0.09$) while mean rates were significantly greater in control stands on April ($P = 0.02$) and May ($P = 0.05$) measurement days. Mean monthly foliar P_n rates varied from approximately 1.5 μmol m^{-2} s^{-1} on November and December measurement days to around 3.5 μmol m^{-2} s^{-1} on the March measurement day for both fertilization treatments. Air temperature at SETRES varied seasonally, and sample dates were representative of this seasonal variation (Figure 2).

P_n rates of foliage sampled from the upper third of crowns were significantly greater than rates in the lower third of the crowns on all measurement days (Figure 1). This trend clearly parallels differences in ambient light levels between the upper and lower third of crowns, which were simulated in the measurement cuvette via the LiCor's actinic light source (Table 1). Mean monthly foliar P_n in the lower third of crowns varied from slightly less than 1 μmol m^{-2} s^{-1} in June to about 2.75 μmol m^{-2} s^{-1} in March; upper crown foliar P_n

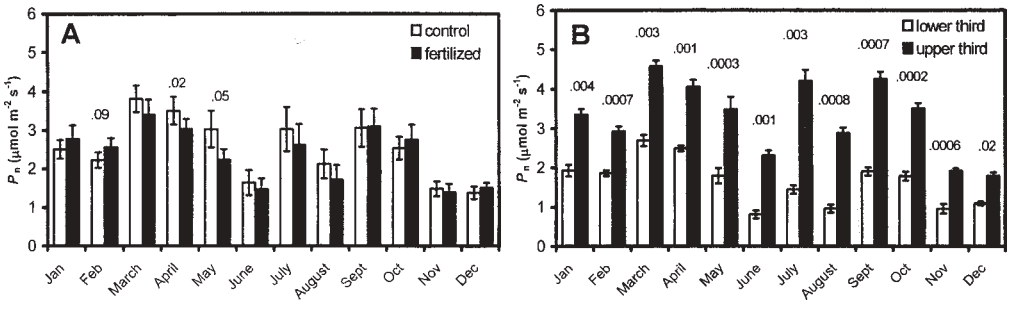

Figure 1. Mean monthly foliar P_n rates for control and fertilized stands (A) and upper third and lower third of crowns (B). Bars indicate the standard error. Numbers above bars are *P*-values indicating significance levels between fertilization treatments or crown positions within months. P-values are only given when $P < 0.1$. Each value is an average ($n = 16$) from three measurement periods throughout a single day, except for December when morning measurements were not used in the analysis because of rain.

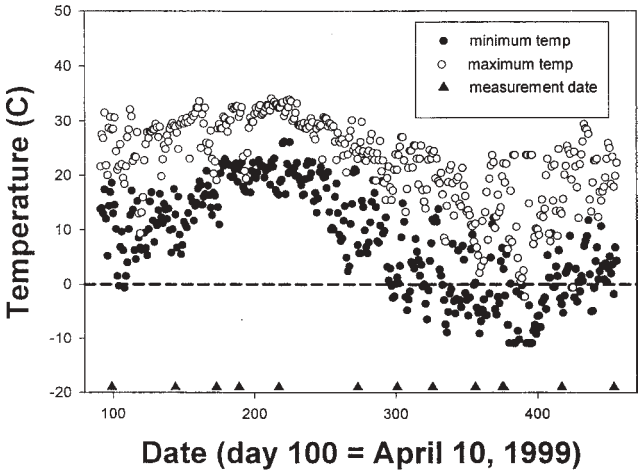

Figure 2. Daily minimum and maximum temperatures over the course of the study, and corresponding measurement dates at SETRES. Daily temperatures reported are high and low average hourly temperatures.

ranged from about 2 μmol m^{-2} s^{-1} in November and December to 4.5 μmol m^{-2} s^{-1} in March.

Monthly Foliar Nitrogen Concentrations

For 8 of the 12 months, foliar N concentration was determined for pooled measurement needles from a given block/fertilization treatment/crown position (Figure 3). Foliar N concentrations were significantly higher in fertilized foliage ($P < 0.1$) for all months sampled. Mean foliar N levels in control stands ranged from 1.0% to 1.2%, while foliage from fertilized stands ranged from 1.2% to over 1.%. Upper crown foliar N percents were only significantly greater than lower canopy needles for the months of January, July, and October ($P < 0.1$).

Common Model Parameters

Common significant parameters for empirical models developed to predict foliar P_n from all combinations of fertilization treatment/crown position/season include PPFD, Ln(PPFD), and VPD. Temperature, humidity, stem water potential, and foliar N concentration did not consistently correlate with foliar P_n or improve model R^2 values and therefore were not included in the common model. Since VPD is directly related to relative humidity and temperature, the latter variables may provide somewhat redundant information to the model resulting in statistical insignificance. Water potential was a significant variable in models only when VPD was removed. Since VPD was highly collinear with water potential and because VPD explained more variance in P_n than water potential, we chose VPD as an explanatory variable rather than water potential. PPFD and Ln(PPFD) generally accounted for 50% of the total variation among data within a fertilization treatment/crown position/season, and VPD explained approximately 5–10% of the variation in foliar P_n.

Common Model Analysis

Individual model coefficients for the model parameters were calculated for each fertilizer and crown position treatment combination in order to determine if simulated response surfaces differed among treatments. Statistical comparisons of parameter coefficients reveal that significant differences exist among coefficients for the four fertilization treatments and crown position combinations when models were developed based on data from the entire year, indicating that statistical differences exist among predicted response surfaces. Because we found statistical differences among model coefficients for the fertilizer and crown position treatments, we calculated individual coefficients (using the same common model) for the growing and nongrowing seasons for all fertilizer treatment/crown position combinations in order to examine seasonal differences in potential carbon fixation among treatments.

Model R^2 values generally improved when model coefficients were developed separately for both the growing and nongrowing seasons. R^2 values for models based on the entire year of data were 0.56 for the upper crown positions and 0.58 for the lower crown positions (for both treatments). R^2 values for both the growing season and nongrowing season models ranged from 0.58 to 0.64, with six of the eight models having R^2 values of at least 0.60 (Table 2).

Statistical comparisons of variable coefficients between the growing and nongrowing seasons confirm that environ-

Table 1. Mean PPFD in the lower and upper third of crowns, mean VPD, mean air temperature, mean relative humidity, and mean water potential for each month during measurements. Each value represents an average from the three measurement periods (morning, early afternoon, late afternoon) from a single day. Mean PPFD in the lower and upper third of crowns is an average of 8 samples. All other environmental variables represent an average from 16 samples.

Month	Mean lower crown PPFD	Mean upper crown	Mean VPD (kPa)	Mean air temperature (°C)	Mean relative humidity (%)	Mean water potential (Mpa)
	(μmol m^{-2} s^{-1})					
January	310	1154	1.2	18.1	45.8	–1.19
February	450	1200	0.88	12.8	42.4	–1.00
March	374	1093	1.6	19.2	28.2	–1.37
April	348	858	2.1	28.6	47.6	–1.27
May	471	1233	2.7	30.7	42.8	–1.47
June	74	283	1.0	23.7	62.7	–0.76
July	183	1183	1.8	33.0	66.4	–1.60
August	258	1066	3.2	34.3	47.9	–1.66
September	251	1383	1.4	21.2	48.0	–1.10
October	244	1166	1.3	17.9	37.5	–1.09
November	148	508	0.86	23.9	70.0	–0.74
December	66	151	0.61	12.3	53.7	–0.71

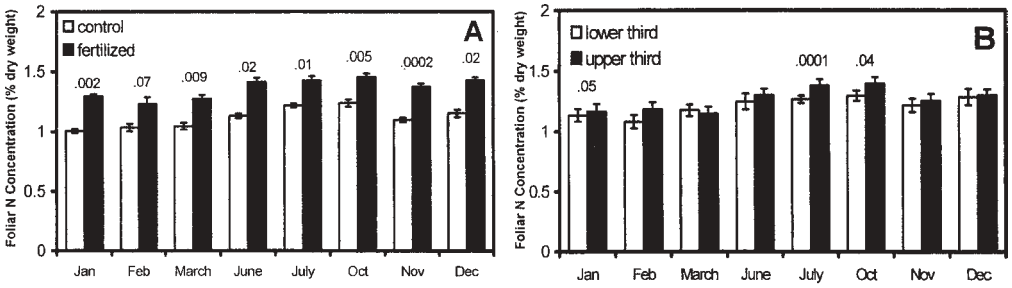

Figure 3. Mean monthly foliar N concentration expressed as percent of dry weight for control and fertilized stands (A) and upper third and lower third of crowns (B). Bars indicate standard error. Numbers above bars are *P*-values indicating significance levels between fertilization treatments or crown positions within months. *P*-values are only given when $P < 0.1$. Values are averages ($n = 8$) from pooled foliage in which foliar P_n rates were recorded.

mental influences on foliar P_n differ between seasons (Table 2). At least two (of four) parameter coefficients for the growing and nongrowing season models differed statistically for all fertilization treatment and crown position combinations ($P < 0.01$), indicating that separate models for the growing and nongrowing seasons are statistically appropriate. The predicted response to PPFD in the upper crown differs significantly between the growing and nongrowing seasons (Table 2, Figure 4). At low light levels (PPFD < 250 μmol $m^{-2}s^{-1}$) in both control and fertilized stands, foliage from the nongrowing season is more responsive to light as indicated by a lower predicted light compensation point (x-intercept) and quantum yield (initial slope). At PPFD levels greater than 250 μmol $m^{-2}s^{-1}$, growing season foliage has a higher predicted foliar P_n rate.

Seasonal Gross Primary Productivity

Although statistical differences exist among foliar P_n models for the growing and nongrowing seasons, the potential to maintain relatively high rates during the nongrowing season is apparent. For example, when light levels are saturating in the upper crown of control stands, predicted foliar P_n reaches a maximum of approximately 4.7 μmol $m^{-2}s^{-1}$ during the growing season and 3.3 μmol $m^{-2}s^{-1}$ during the nongrowing season. In order to examine cumulative seasonal carbon fixation, we estimated GPP for each treatment combination within both the growing and nongrowing seasons using our empirical models, meteorological data collected at SETRES, and LAIs and crown light levels generated using BIOMASS. Estimated GPP during the five months of the nongrowing season was a considerable fraction of yearly carbon uptake in both fertilized and nonfertilized treatments (Figure 5). The nongrowing season accounted for 20.5% of the total carbon fixed for the year in nonfertilized stands and 21.9% in fertilized stands. Thus, the GPP analysis and predicted light curves indicate that differences in carbon fixation between seasons are largely attributed to seasonal variability

Table 2. Foliar P_n prediction model parameter estimates, corresponding *P*-values, and model R^2 for the growing season (April–October) and nongrowing (November–March) season for all fertilization treatment and crown position combinations. Stars next to parameters indicate estimates differ significantly between growing and nongrowing season ($P < 0.1$).

	Control, lower crown			Control, upper crown		
Parameter	Estimate	*P*-value	Model R^2	Estimate	*P*-value	Model R^2
Growing season		$n = 240$			$n = 239$	
Intercept	–2.295	0.0001	0.58	–6.689	0.0001	0.64
PPFD	2.816×10^{-4}	0.4159		-9.561×10^{-4}	0.0003	
Ln(PPFD)*	0.8949	0.0001		1.876	0.0001	
VPD*	–0.3924	0.0001		–0.4976	0.0001	
Nongrowing season		n = 176			n = 176	
Intercept	–1.717	0.0001	0.64	–2.870	0.0005	0.63
PPFD	5.497×10^{-4}	0.2086		-3.303×10^{-4}	0.20	
Ln(PPFD)*	0.5466	0.0001		0.7980	0.0001	
VPD*	0.4673	0.0004		0.8098	0.0001	
	Fertilized, lower crown			Fertilized, upper crown		
Growing season		$n = 240$			$n = 239$	
Intercept*	–1.802	0.0001	0.59	–5.542	0.0001	0.60
PPFD*	7.237×10^{-4}	0.12		5.477×10^{-4}	0.042	
Ln(PPFD)*	0.7912	0.0001		1.653	0.0001	
VPD*	–0.5238	0.0001		–0.6983	0.0001	
Nongrowing season		$n = 175$			$n = 175$	
Intercept*	–0.4707	0.17	0.63	–2.048	0.025	0.62
PPFD*	0.001816	0.0001		3.670×10^{-4}	0.20	
Ln(PPFD)*	0.2684	0.004		0.7066	0.0003	
VPD*	0.2911	0.02		0.2356	0.20	

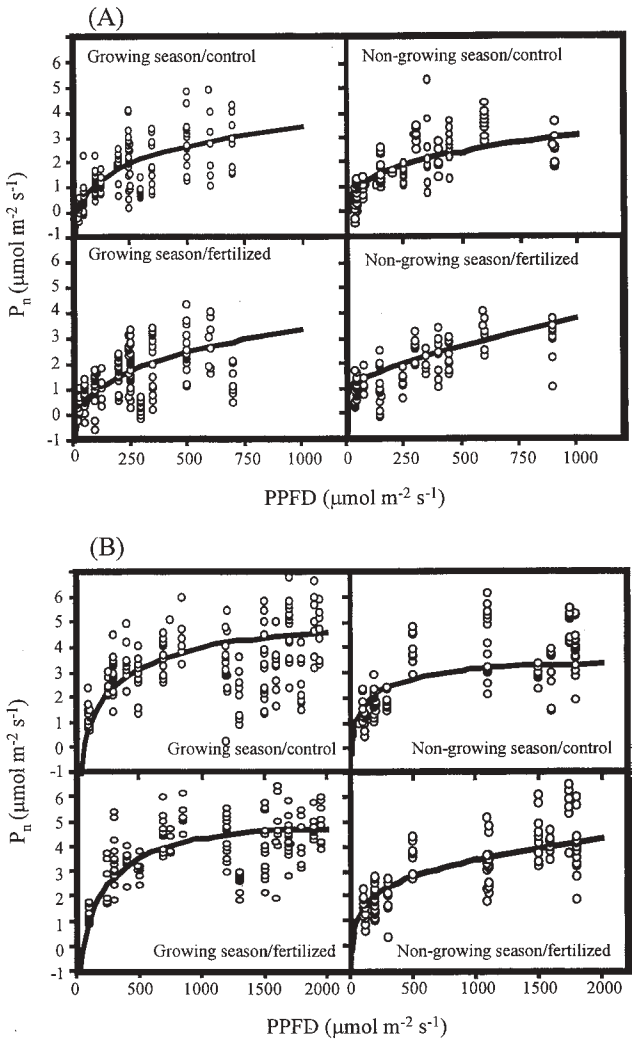

Figure 4. Predicted foliar P_n and actual data in relation to PPFD based on data for the growing (April—October) and nongrowing (November—March) seasons. (A) Lower crown, and (B) upper crown. Common foliar P_n models with the driving variables PPFD, Ln(PPFD), and VPD were developed using multiple linear regression for each fertilization and crown position combination. Average VPD for a given season was held constant when simulating light response curves. VPD values of 1.86 KPa and 1.00 KPa were assigned to growing season and non-growing season, respectively, and represent the average VPD over those time periods.

in the intensity of light reaching the crown and crown light interception. In other words, the altered light environment during the nongrowing season was largely responsible for the reduced rate of foliar carbon fixation since the physiological potential for the foliage to fix carbon was not greatly changed from the growing season to the nongrowing season. For the most part, carbon fixation was slightly higher in the upper crowns. GPP in nonfertilized stands for the year is an estimated 114.6 mol C m^{-2}, while the GPP estimation for fertilized stands is 188.7 mol C m^{-2}.

Discussion

Fertilization Effects

Previous studies provide inconsistent reports on the relationship between foliar N percent and foliar P_n rates. Mitchell and Hinckley (1993) found that, in Douglas-fir (*Pseudotsuga*

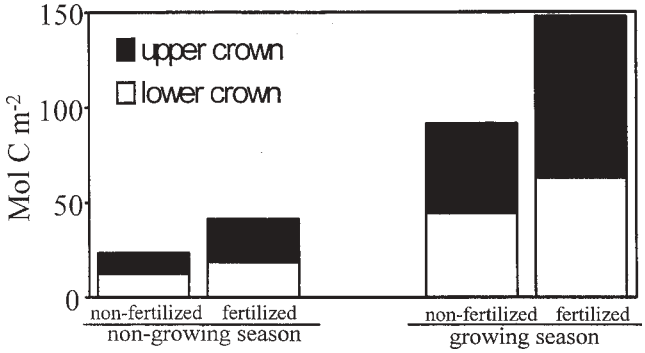

Figure 5. Estimated pine gross primary productivity SETRES at 14 yr of age for the upper and lower crowns of fertilized and nonfertilized stands for the growing and nongrowing seasons expressed in mols C per m^2 of land area. LAI and crown light interception values were estimated using the process model BIOMASS. Average monthly VPD was obtained from meteorological data collected on site. The empirical models presented in Table 2 were used to generate GPP for each fertilization treatment/crown position/season.

menziesii [Mirb.] Franco), photosynthetic rates and foliar N percents were positively correlated. In Scots pine (*Pinus sylvestris* L.) seedlings, foliar P_n was strongly correlated with foliar N percent only during the growing season (Vapaavuori 1995). Schoettle and Smith (1999) reported a weak relationship between foliar N and P_n in lodgepole pine (*Pinus contorta* Dougl. ex. Loud.), except in young leaves. However, no differences in P_n were found in mature slash pine (*Pinus elliottii* Engelm.) foliage that had been fertilized (Teskey et al. 1994). Zhang et al. (1997) reported that while N fertilization increased leaf N in loblolly pine, there was no increase in foliar P_n rates or quantum yield during the growing season. Results from our study indicate that there is little or no correlation between foliar N percent and foliar P_n rates in loblolly pine fertilized over the long term, particularly during the growing season. Despite the fact that foliar N was substantially greater in fertilized trees relative to nonfertilized trees (22%), foliar N percent was not a significant variable in our modeling analysis. Tang et al. (1999) found that fertilized loblolly pine in Louisiana had lower mean foliar P_n than nonfertilized trees from June through November, which is generally consistent with data presented in this study.

While we did not observe a consistent fertilization effect on photosynthesis after several years of nutrient additions, the relationship between fertilization and foliar photosynthetic efficiency has likely changed in the current stand. Previously published gas exchange data from the current study site suggest that photosynthetic capacities were initially enhanced in fertilized stands shortly after nutrient additions. Enhanced photosynthetic capacities may account for the greater leaf area and productivity currently observed in the fertilized stands. Murthy et al. (1997) reported that foliage from fertilized stands had higher photosynthetic capacities than control foliage during both the growing and nongrowing seasons 2 yr after fertilization began. Albaugh et al. (1998) reported that the rate of increase in leaf area index (LAI) was greater in fertilized stands relative to control stands from 1992 (initial fertilization) to 1996 (fourth year fertilization). Nine months after nutrient additions began, a

rapid increase in LAI was observed in fertilized stands. However, the enhanced rate of leaf area production in fertilized stands relative to control stands was reduced during measurements in 1995 and 1996. Thus, nutrient additions may have increased foliar P_n shortly after fertilization and provided the photoassimilates necessary to build additional leaf area in fertilized stands. Data from the current and previous studies conducted at SETRES imply that foliar P_n was adjusted downward after maximum increases in leaf areas were achieved in fertilized stands. Photosynthetic capacities have been shown to adjust to changing demands in carbohydrate sinks such as roots and fruits (Kramer and Kozlowski 1979), and also to changes in foliage growth (Boltz et al. 1986). Currently, the greater LAIs associated with fertilized stands at SETRES (Albaugh et al. 1998) are likely responsible for higher GPP in fertilized stands since photosynthetic capacities between fertilized and nonfertilized stands do not differ considerably.

Environmental Responses and Seasonal Variation

Seasonal changes in maximum foliar P_n in loblolly pine are well documented, and shifts in temperature optimums are known to occur throughout the year (Strain et al. 1976, Drew and Ledig 1981, Boltz et al. 1986, Teskey et al. 1986, Murthy et al. 1997). Our data indicate that temperature acclimation occurred between months. Since temperature is not a significant predictor of foliar P_n across time in our stands and because predicted photosynthetic capacity was not considerably impacted from the growing season to the nongrowing season despite considerable seasonal changes in temperature, shifts in temperature optimums must have occurred throughout the year. This is not to say that loblolly pine P_n does not display temperature sensitivity, especially when large shifts in temperature occur over a short period of time. However, a temperature influence was not detected in our stands across seasons since foliar P_n apparently adjusted to long-term changes in temperatures. In contrast to our findings, Ellsworth (2000), when measuring foliar P_n at saturating light levels from December through March, found a strong positive relationship between temperature and loblolly pine P_n, particularly between 5 and 15ºC. However, Ellsworth recorded foliar P_n under conditions of high light and low temperatures during the winter months, possibly resulting in photoinhibition. Seasonal changes in photoinhibition have been shown to occur in Scots pine (Ottander et al. 1995). Thus, measurement conditions may partially explain the apparent temperature sensitivity observed by Ellsworth. In addition, our site was approximately 120 km south of Ellsworth's and experiences warmer winter conditions; our coldest mean monthly cuvette temperatures were approximately 12ºC (December and February). Although we did not record P_n over the range of low temperatures that Ellsworth did, our measurement days were representative of seasonal temperatures at the study site (Figure 2). Thus, temperature acclimation along with higher winter temperatures, in part, allowed for the maintenance of near-growing season carbon fixation capacities throughout the winter in our stands.

Further, our data indicate little photosynthetic inhibition by subfreezing night temperatures. Below freezing temperatures, mostly at night, were common throughout our winter sampling period (Figure 2). Temperatures below freezing occurred on night or nights prior to sampling for January (–2.1º C), February (–3.7), and March (–1.8), and foliar P_n rates on these dates were not depressed relative to other dates. Thus, if freezing stress occurred, nonlethal freezing damage to photosynthetic apparatus was minor and undetectable in our foliar P_n rates. These findings are in contrast to reports by Teskey et al. (1987), who found significant reductions in foliar P_n rates following freezing nights. Thus, negative effects of temperature on P_n reported in some loblolly pine studies were not severe or even detectable in our stands. Based on our results, a single, uniform low temperature threshold cannot be assigned to all loblolly pine stands when quantifying carbon fixation. In process modeling situations, for example, a single temperature threshold for loblolly pine may be misleading since carbon fixation capacities are not uniformly compromised below a given temperature on all sites.

Our observed mean monthly foliar P_n rates, predicted foliar P_n rates, and GPP estimates indicate that loblolly pine may fix substantial amounts of carbon during the nongrowing season. Based on our findings, winter carbon fixation may be a crucial component of loblolly pine productivity in southern North Carolina and other locations in the southeastern United States. Sampson et al. 2001 suggested that winter P_n provides labile carbon stores that are later applied to growth during the summer months, when environmental stresses such as drought limit P_n. The relatively mild climate in the southeastern United States and the apparent elastic response to temperature may partially explain why P_n was not extensively reduced during the winter and reinforces the importance of winter P_n contributions to the loblolly pine annual carbon budget. Ellsworth (2000), in the same study discussed above, reported a somewhat lower contribution of winter daily carbon assimilation in a similar-aged loblolly pine stand located in the Duke University Forest located in North Carolina. While we estimated that over 20% of the year's total carbon assimilation occurs during the winter in control stands, Ellsworth reported a winter contribution of 15% on sunny days throughout the year. As discussed above, Ellsworth's carbon assimilation estimations may be highly influenced by the high sensitivity to low temperatures he observed. Also, our estimates may differ simply due to experimental design and analysis, and variability in climate and sites. Further, Ellsworth only measured photosynthesis under saturating light on sunny days. In another loblolly pine study conducted in the Duke Forest, Luo et al. (2001) estimated GPP using the process model MAESTRA, which was parameterized using eddy-flux and local meteorological data. MAESTRA was used to estimate annual carbon assimilation at the 14 yr old Duke site, which was determined to be 102 mol C $m^{-2}yr^{-2}$ in nonfertilized stands. The value calculated by Luo and his colleagues differs somewhat from our value of 114.6 mol C m^{-2}. However, our values may partly differ due to our different modeling approaches and the apparent sensitivity of MAESTRA to low temperatures, which resulted in modeled carbon fixation capacities that were re-

duced by half in the winter relative to the summer season. Again, we did not observe the temperature sensitivity that other investigators have reported.

Conclusions

Light intensity (PPFD) and VPD consistently explain a majority of the variability in photosynthesis across seasons and treatments. The effect of fertilization on foliar P_n appears to have changed over time after 6 yr of nutrient additions, and foliar N percent does not currently parallel foliar P_n rates. Our results also indicate that a significant amount of carbon (greater than 20% of the yearly total) is fixed by loblolly pine during the nongrowing season and therefore winter P_n should be a major component in mechanistic process models predicting yearly carbon uptake. Winter P_n was substantial despite low temperatures, which have been cited as limitations in winter P_n. The limited reduction in nongrowing season rates also implies that temperature acclimation effectively occurred throughout the year.

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