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Spatial and seasonal variability of photosynthetic parameters and their relationship to leaf nitrogen in a deciduous forest

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Summary We used gas exchange techniques to estimate maximum rate of carboxylation (V_{cmax}), a measure of photosynthetic capacity, in the understory and upper crown of a closed deciduous forest over two seasons. There was extensive variability in photosynthetic capacity as a result of vertical canopy position, species type, leaf age and drought. Photosynthetic capacity was greater in oaks than in maples and greater in the overstory than in the understory. Parameter V_{cmax} was maximal early in the season but declined slowly throughout most of the summer, and then more rapidly during senescence. There was also an apparent decline during drought in some trees. Variability in V_{cmax} as a result of species or vertical canopy gradients was described well by changes in leaf nitrogen per unit area (N_a). However, temporal changes in V_{cmax} were often poorly correlated with leaf nitrogen, especially in spring and summer and during drought. This poor correlation may be the result of a seasonally dependent fractional allocation of leaf nitrogen to Rubisco; however, we could not discount Rubisco inactivation, patchy stomatal closure or changes in mesophyll resistance. Consequently, when a single annual regression equation of V_{cmax} versus N_a was used for this site, there were substantial errors in the temporal patterns in V_{cmax} that will inevitably result in modeling errors.

Keywords: A/C_i curve, drought, gas exchange, leaf age, maple, oak.

Introduction

Photosynthetic capacity is closely linked to nitrogen through the nitrogen-rich carbon-fixing enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). A general relationship between leaf nitrogen and maximum assimilation often occurs across gradients in geography (Field and Mooney 1986, Reich et al. 1997), functional types (Field and Mooney 1986), species within functional types (Field and Mooney 1986, Harrington et al. 1989) and growth light environment (Field 1983, Niinemets and Tenhunen 1997). This discovery has been useful in confirming hypotheses about plant functioning and establishing relationships over broad gradients of leaf characteristics (Reich et al. 1997). A link to leaf nitrogen also provides the potential to estimate photosynthetic capacity at high temporal resolutions and large scales, especially if practical methods are developed to estimate nitrogen from remote sensing (Wessman 1990).

However, the utility of these relationships in describing all sources of variability at a single site, especially the temporal effects of leaf age and drought, is less certain. Typically, the relationship between nitrogen and maximum assimilation has been tested by comparing data across extreme gradients in leaf characteristics created by functional types or light gradients. Therefore, it is not always clear if more subtle variations, such as species, leaf age or drought converge on the same regressions or just cluster around it.

There is evidence indicating important variations in the relationship between leaf nitrogen and photosynthesis. Linear correlations do not always occur within species and the regression coefficients are often strongly dependent on species or treatment effects. For example, slopes between nitrogen and maximum assimilation rates can vary by a factor of 10 among species (Evans 1989). The relationship between maximum photosynthesis and leaf nitrogen can depend on whether nitrogen is increased by fertilization or by light gradients (DeJong et al. 1989, Reich et al. 1989, Walcroft et al. 1997) or whether drought is present (Reich et al. 1989, Abrams and Mostoller 1995). Several researchers have concluded that effects of leaf age can be described only by changes in leaf nitrogen (Field and Mooney 1983, Reich et al. 1991), but others have not. For example, there is evidence that nitrogen allocated to Rubisco and chlorophyll can vary with leaf age (Poorter and Evans 1998, Rey and Jarvis 1998).

A limitation of previous studies is that the relationship with leaf nitrogen has usually been linked to assimilation at optimal temperature and humidity, saturating light, adequate soil water content and ambient carbon dioxide concentrations, a parameter referred to as maximum assimilation. This parameter is not directly relevant in most current model applications. Furthermore, although maximum assimilation is expected to correlate strongly with photosynthetic capacity, confounding stomatal limitations can occur because of drought (Roupsard et al. 1996), leaf age effects (Radoglou 1996, Rey and Jarvis 1998), differences among forest genotypes (Radoglou 1996) and nitrogen fertilization (Reich et al. 1989).

To examine photosynthetic capacity independently of stomatal conductance, we characterized variability in the photosynthetic parameters of the model developed by Farquhar et al. (1980). We focused on maximum rates of carboxylation (V_{cmax}) of several understory and overstory trees in a deciduous broad-leaved forest in the southern USA. A large sample size and tower access to multiple mature trees in the upper portions of a closed canopy allowed comprehensive analysis of variability. Studies over short temporal scales have been conducted (Harley and Baldocchi 1995, Niinemets et al. 1998) and some general species differences have been identified (Wullschleger 1993). However, there are few data sets showing the full temporal and spatial range of parameter variability in a deciduous forest ecosystem and methods of deriving the parameters from nitrogen content or other leaf characteristics.

Our study centered on two aspects of photosynthetic capacity (V_{cmax}). First, we assessed the range of V_{cmax} and the factors affecting its spatial and temporal variability over two growing seasons, including growth light environment (i.e., differences between overstory and understory trees), species differences, leaf age effects and drought. We also tested the hypothesis that all variations in photosynthetic capacity caused by these factors are ultimately mediated by changes in leaf nitrogen. To test this hypothesis, we analyzed our data by an equation introduced by Niinemets and Tenhunen (1997) to describe the theoretical anatomical and biochemical interactions that may be sources of variation in V_{cmax}:

$$V_{cmax} = 6.25 V_{cr}\,\mathrm{LMA}\,N_m R_F, \qquad (1)$$

where 6.25 is the ratio of the weight of Rubisco to the weight of nitrogen in Rubisco; V_{cr} is the specific activity of Rubisco, which is assumed to be only a function of temperature (20.7 μmol CO_2 (g Rubisco)$^{-1}$ s^{-1} at 25 °C); LMA is the leaf mass per unit area (g m^{-2}); N_m (g g^{-1}) is the mass of nitrogen in the leaf per total mass of leaf; and R_F is the apparent fraction of that nitrogen allocated to Rubisco. We analyzed Equation 1 across differences in species, light environment, leaf age and soil water status to determine if nitrogen per unit leaf area (product of N_m and LMA) accounts for changes in V_{cmax} or if there are large variations in the apparent value of R_F.

Materials and methods

Site

Measurements were performed in a closed deciduous forest at Walker Branch watershed within the US Department of Energy reservation in Oak Ridge, TN, USA (35°58′ N, 84°17′ W, 365 m a.s.l.). The stand has been regenerating naturally since 1940 on abandoned pasture. Dominant species are chestnut oak (*Quercus prinus* L.), white oak (*Q. alba* L.) and red maple (*Acer rubrum* L.). Yellow poplar (*Liriodendron tulipifera* L.), sugar maple (*Acer saccarum* Marsh.), black gum (*Nyssa sylvatica* Marsh.), red oak (*Q. rubra* L.) and hickory (*Carya* sp.) are also present in smaller numbers. Maximum canopy height is about 26 m, and maximum leaf area index is about six. The understory consists of black gum, red maple, dogwood (*Cornus florida* L.), sassafras (*Sassafra albidum* (Nutt.) Nees), sourwood (*Oxydendrum arboreum* (L.) D.C.), yellow poplar, red oak, hickory, beech (*Fagus grandifolia* J.F. Ehrh.) and black cherry (*Prunus serotina* Ehrh.). Mean annual rainfall and temperature are 1372 mm and 13.9 °C. Rainfall is distributed fairly equally throughout the year, but with occasional summer droughts. The length of the growing season, defined here as the period when the forest was a net sink for carbon dioxide based on eddy covariance measurements, averages about 200 days, beginning on Day 111 and ending on Day 311 (Wilson and Baldocchi 2000). Details about other biological and environmental aspects of this site can be found in Johnson and Van Hook (1989).

Single-leaf gas exchange measurements were performed in two nearby regions in the watershed. The first region was on and around a meteorological tower. On this tower all measurements were on a white oak near the top of the canopy. All of the understory measurements were also performed in this region on saplings ranging from 1 to 5 m in height. The second region encompassed three access towers at the throughfall displacement experiment (TDE), described by Hanson et al. (1998). The TDE site consists of three 80 × 80 m treatments. Since July 1993, one treatment has received ambient precipitation, whereas the remaining two treatments have been subjected to a 33% reduction (dry treatment) or a 33% enhancement (wet treatment) of ambient precipitation. One tower is located on each of the three treatments, allowing access to a total of nine trees at heights exceeding 20 m. Although not all leaves were at the very top of the canopy, all were in the sunlit or primarily sunlit portion of the canopy. On each tree, we attempted to make measurements on the same branch or branches at similar heights throughout both years.

Experimental methods

Most of the gas exchange measurements were made with a steady-state system (LI-6400 Li-Cor, Inc., Lincoln, NE). About 5% of the measurements at ambient CO_2 dioxide were performed with a transient system (Li-Cor LI-6200), and cross comparisons with the LI-6400 showed that the two systems gave comparable data. Both analyzers were calibrated regularly with CO_2- and H_2O-free air and known standards. Measurements were performed during the 1997 and 1998 growing seasons on nearly 800 leaves of 65 trees encompassing 12 species.

During measurements, photosynthetically active radiation was at least 1100 μmol m^{-2} s^{-1}, which was above the 95% threshold for photosynthesis. Each leaf was initially measured at a reference ambient CO_2 concentration of 360 μmol mol^{-1}. The A/C_i response curves were produced on one-third of the leaves by increasing the reference CO_2 concentration from 50 to 1800 μmol mol^{-1} in about 11 steps; four of these steps were at, or less than, 200 μmol mol^{-1}. A full response curve took about 1 h to perform. Measurements were made in mid-morning and leaf temperatures were normally between 20 and 30 °C, with a few exceptions in early spring and autumn. The A/C_i curves on about 20 leaves were performed while leaf temperatures were manipulated over a 10 °C range to establish the temperature dependence of photosynthetic parameters. Because full A/C_i curves seriously constrained the replication needed for statistical inference, measurements on about two-thirds of the leaves were performed at ambient carbon dioxide concentration only.

About 70% of the measured leaves were harvested to determine area, dry weight, and nitrogen concentration. Leaf nitrogen was determined by gas chromotography (Carlo Erba NA1500 Analyzer, Carlo Erba Instruments, Rodano, Italy). Leaves were dried for 48 h at 65 °C, dry weights were obtained, then samples were ground and re-dried at 75 °C for several hours before being placed in the analyzer. Nitrogen concentrations were calibrated and checked against known standards.

Within each of the TDE treatments, soil water contents were periodically measured in the 0–300-mm depth, the portion of the soil profile containing at least 70% of all roots, with a time-domain reflectometer (Soil Moisture Equipment Corp., Santa Barbara, CA). In 1998, soil water content over the 300-mm profile was also measured hourly at the meteorological tower site with four water content reflectometers (Item CS615, Campbell Scientific Inc., Logan, UT) installed horizontally into the side of a subsequently backfilled soil pit. Soil water contents were converted to matric potentials (MPa) based on soil water release curves measured at the site (Hanson et al. 1998).

Calculation of photosynthetic parameters

In the model of Farquhar et al. (1980), photosynthesis is limited by either the maximum rate of carboxylation (V_{cmax}) or the maximum rate of electron transport (J_{max}), parameters that indicate internal biological limitations on photosynthetic capacity. Nonlinear regression methods (Draper and Smith 1966) were used to estimate V_{cmax}, J_{max} and dark respiration rate (R_d) from A/C_i curves. Both V_{cmax} and R_d were determined from the A/C_i curve when $C_i \leq 150$ μmol mol^{-1}, and the slope was quasi-linear, consistent with substrate limitations (Rubisco and CO_2) inhibiting photosynthesis (Wullschleger 1993). Values of Michaelis constants (K_c = 27.5 Pa and K_o = 42.0 kPa), activation energies ($H_a(K_c)$ = 80.5 kJ mol^{-1} and $H_a(K_o)$ = 14.5 kJ mol^{-1}), and the specificity for Rubisco (τ = 2321, $H_a(\tau)$ = –29.0 kJ mol^{-1}) were from Harley et al. (1992). Estimations of J_{max} followed the procedures outlined in Harley et al. (1992). Parameter J_{max} was estimated from the A/C_i curve when $C_i > 700$ μmol mol^{-1} with a fixed value of light energy conversion (α = 0.24) and the value of R_d obtained in the V_{cmax} regression.

The calculation of J_{max} required concentrations of CO_2 in excess of 1000 μmol mol^{-1}; however, under these conditions, there was normally not a unique relationship between C_i and assimilation, even after 1 h or more. Typically, stomatal conductance declined rapidly and often oscillated, a pattern consistent with patchy stomatal closure (Cardon et al. 1994, Mott 1995) and invalidating the interpretation of C_i (Laisk 1983). Because of this instability, measurements at CO_2 concentrations higher than ambient were not continued in 1998. Despite these problems, the slope of the relationship between J_{max} and V_{cmax} at 25 °C was 2.77, slightly above the mean found in other studies (Wullschleger 1993, Leuning 1997).

Extensive sampling was required to investigate all sources of variability in V_{cmax}, and several findings allowed us to make assumptions that substantially increased the quantity of data for analysis. After V_{cmax}, J_{max} and R_d were estimated, simulations of the Farquhar et al. (1980) model were performed with these parameter values over a full range of values of C_i, leaf temperature and irradiance. When the model was run with C_i values corresponding to measurements at reference CO_2 concentrations of 360 μmol mol^{-1} (ambient), the model estimates of assimilation were close to observed ambient values, even though the measurements at reference concentrations of 360 μmol mol^{-1} were not used to estimate model parameters. The model also estimated that, at ambient external CO_2 concentration, 95% of the leaves were substrate-limited, indicating that C_i was on the Rubisco-limiting portion of the curve, which is typical for trees at high and even moderate irradiances (Harley et al. 1986, Ni and Pallardy 1992). This finding allowed us to estimate V_{cmax} by a second method (one-point method), using only the measurements of assimilation rate and C_i at the ambient external CO_2 concentration (360 μmol mol^{-1}) and a value of R_d.

Sensitivity tests demonstrated that V_{cmax} was fairly insensitive to R_d over the range 0.10 to 0.90 μmol m^{-2} s^{-1} 25 °C. Based on the full A/C_i curve data, no clear relationship could be obtained between V_{cmax} and R_d as has sometimes been found in other studies (Field 1983, Niinemets and Tenhuenen 1997, Reich et al. 1998), so a constant value of R_d at 25 °C was assumed that was close to the mean (0.5 μmol m^{-2} s^{-1}). The error due to this assumption was probably minimal because sensitivity tests showed that inaccuracies in R_d of as much as 1.0 μmol m^{-2} s^{-1} resulted in V_{cmax} errors of about 3.5 μmol m^{-2} s^{-1}, or only about 10% of typical values of V_{cmax} at the top of the canopy. Because respiration was greater during the short period of leaf expansion in early spring than for the other periods, A/C_i curves were always performed to estimate R_d during this period. The temperature response of R_d was estimated from an Arrhenius function following Harley et al. (1986).

Although the loss of information associated with using the one-point method probably introduced errors in calculating V_{cmax}, there was a tight correlation between V_{cmax} estimated from the one-point method and V_{cmax} estimated from the A/C_i curve (R^2 = 0.97). The intercept was close to zero (0.13 μmol m^{-2} s^{-1}) and statistically insignificant, but there was a slight positive bias (slope of 1.08). The scatter around this regression line was much smaller than the variation in V_{cmax} caused by species, leaf age, drought or vertical position in the canopy. Because of the bias associated with comparing V_{cmax} calculated by the two methods, all of the data presented were obtained from measurements made at an external CO_2

concentration of 360 µmol mol^{-1}.

Based on the temperature response of the A/C_i curves, V_{cmax} was normalized to 25 °C. The activation energies (H_V = 52672 J mol^{-1} and H_d = 206083 J mol^{-1}) in Walcroft et al. (1997) provided the best fit to our data and were used for this normalization. The results were similar when the parameters given in Farquhar et al. (1980), but not in Harley et al. (1992), were used. Temperature dependence of J_{max} was not determined independently, but J_{max} was always estimated at 25 ± 1.2 °C.

Because LMA, N_m and V_{cmax} were obtained from measurements, R_F could be evaluated from Equation 1. Although R_F is a theoretical estimate for the fraction of leaf nitrogen that is invested in Rubisco, in practice R_F calculated from Equation 1 also represents all other factors that may limit carboxylation capacity (V_{cmax}). However, at high photon flux densities, Rubisco is often the most common limitation in the carboxylation process (Woodrow 1993) and very close correlations between assimilation and the amount and activity of Rubisco are frequently observed (Björkman 1981, Jacob et al. 1995, Rey and Jarvis 1998). Niinemets et al. (1998) found a good correlation between Rubisco activity determined from gas exchange (Equation 1) and enzyme extracts. In addition to the physical interpretation of R_F, R_F estimates also serve as an index of the stability of the relationship between V_{cmax} and leaf nitrogen.

Linear regressions were performed with V_{cmax} as a dependent variable against LMA and N_a (nitrogen per unit leaf area, the product of N_m and LMA). Mass-based regressions, V_{cmax} per unit leaf mass (V_{cmax_m}) against N_m, were also performed. Significant differences between slopes and intercepts were evaluated by analyses of covariance.

Results

Climate and soil water content

Climatic conditions differed in 1997 and 1998. In 1997, except very late in the growing season, precipitation was near or above normal and soil water was not limiting (Figure 1). In 1998, the spring and early summer were wet, but precipitation during July, August and September 1998 was 150 mm, or only about 36% of normal. Soil water deficits developed in midsummer, and by the first of September, soil water potentials were below –2.0 MPa on all but the wet TDE treatment site (Figure 1). Although the drought was not as severe at the wet TDE treatment site as at the other locations, differences in soil water between years was much greater than between TDE treatments. Therefore, the general effects of soil water on V_{cmax} were evaluated across years and not across TDE treatments.

Variability in V_{cmax}

Figures 2–5 show the mean seasonal patterns of V_{cmax}, N_a, LMA and R_F for each of the ten overstory trees in 1997 and 1998 for white oak (Figure 2), chestnut oak (Figure 3) red maple (Figure 4), and sugar maple and blackgum (Figure 5). Ambient photosynthetic rates were about one-fourth the value of V_{cmax}. Daily means for the understory trees are shown for all species in Figure 6.

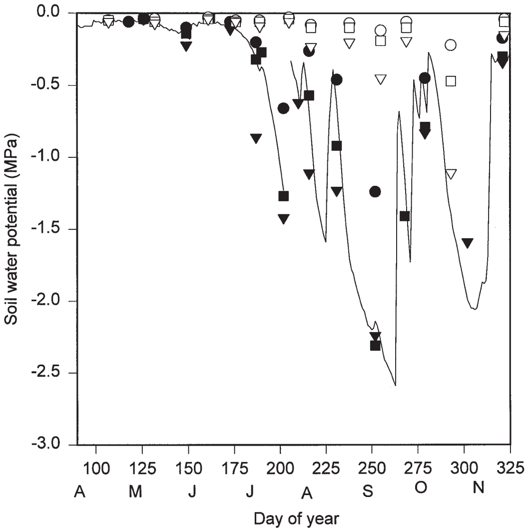

Figure 1. Soil water potential in the 0 to 300 mm horizon in the wet (○), ambient (□) and dry (▽) TDE (throughfall displacement experiment) treatments. The ambient treatment received ambient rainfall, the wet treatment received a 33% enhancement of ambient rainfall, and the dry treatment received a 33% reduction in ambient rainfall. Open symbols are for 1997 and closed symbols are for 1998. The solid line indicates soil water potential in 1998 at the meteorological tower.

Figures 2–6 show four factors causing variability in V_{cmax}. First, there were differences between species. Parameter V_{cmax} was generally greater in oaks than in maples (early season V_{cmax} averaging around 60 versus 40 µmol m^{-2} s^{-1}). Second, V_{cmax} was typically two to three times greater in the overstory (Figures 2a–5a) than in the understory (Figure 6a). Third, there were seasonal patterns in V_{cmax}. Maximum values of V_{cmax} typically occurred in May or June, just after the rapid spring increase in LMA had subsided, and then generally declined. A rapid decrease usually accompanied autumn senescence, but substantial reductions in V_{cmax} also occurred during the middle and late summer. As a result, for several months of the season most trees were operating well below their early season photosynthetic capacity. The summer decrease was generally less in white oak than in the other species.

The fourth factor that influenced V_{cmax} was soil water. Many of the lowest measured values of V_{cmax} in the overstory during the summer of 1998 occurred on Day 252, about 1 week before the peak drought. The drought-induced decrease in V_{cmax} occurred in addition to the leaf age effect, as indicated by the recovery of V_{cmax} to values similar to those in 1997 after modest rainfall and only partial recovery from soil water deficits (Figures 1–6). The V_{cmax} of white oak was less affected by drought than that of other species, and in some white oak trees the drought effect was limited to the most severe period of soil drying. The response of V_{cmax} to drought occurred sooner and was greater in the understory than in the overstory, except in understory oak and hickory, where the drought effects were minimal (data not shown).

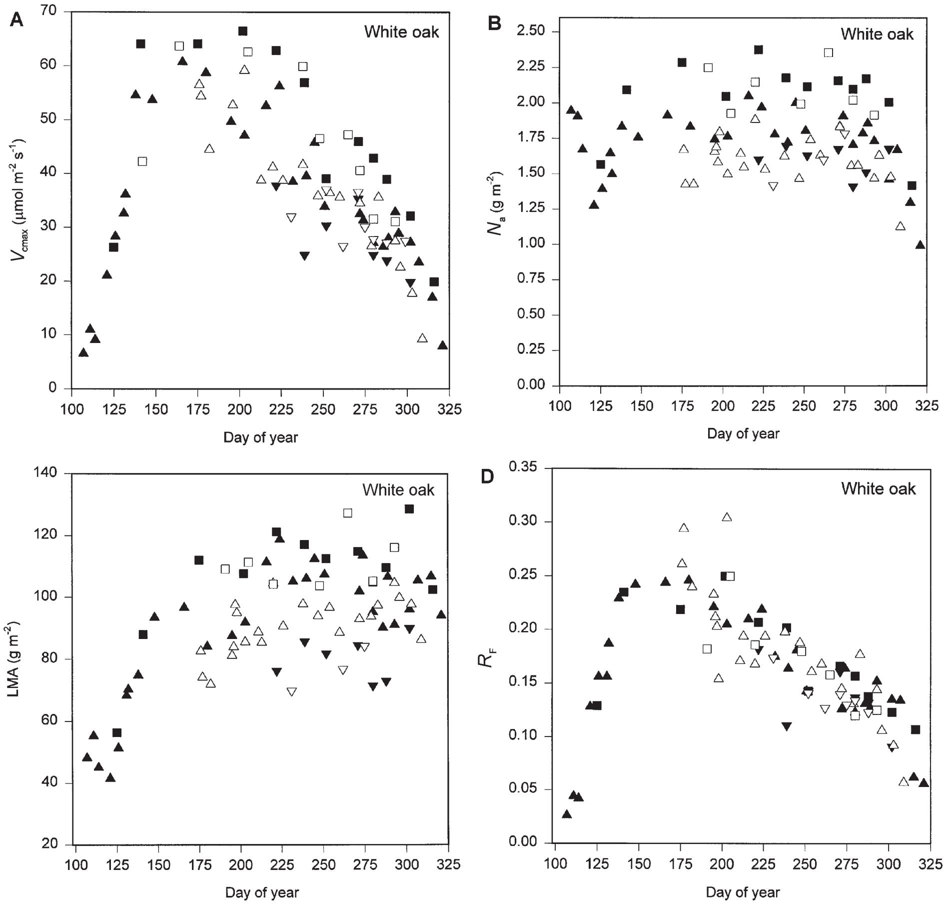

Figure 2. For white oak, (a) V_{cmax} at 25 °C, (b) nitrogen per unit leaf area (N_a), (c) leaf mass per unit area (LMA), and (d) apparent fraction of leaf nitrogen allocated to Rubisco (R_F, Equation 1) in 1997 (open symbols) and 1998 (closed symbols). Each symbol represents a daily mean for single trees at the meteorological tower (△) or in the ambient (□) or dry (▽) TDE treatment. Each point usually represents the mean value for two to six leaves on a single tree on one day. Often there were three or more replications in 1998, whereas there were frequently only two and sometimes only one in 1997, which increased the scatter in 1997.

Much of the variability in V_{cmax} caused by species and canopy position was explained by N_a. Oaks generally had larger N_a than maples (mid-season values of 1.8–2.0 versus 1.4 gm^{-2}) (Figures 2b–5b), and N_a was also substantially greater in the overstory than in the understory. Seasonal changes in N_a were usually small, except very early and late in the season (Figures 2b–5b). Although V_{cmax} was a function of leaf age and drought, N_a often was not. The effect of drought on V_{cmax} was not primarily mediated through decreases in leaf nitrogen. In sugar maple and blackgum, there was an apparent reduction in N_a coincident with and possibly attributable to drought, but in general N_a changed little during the drought period (Figures 2b–6b).

The values of R_F indicate that the relationship between V_{cmax} and N_a was strong when variability was driven by species type or canopy position; however, the relationship was weak when variability was caused by leaf age or drought. Scatter was greater in the understory data, but there was no large difference in mean values of R_F between overstory and understory species during unstressed periods (Figures 2d–6d). Except during drought, there were no large differences in R_F among species. However, R_F decreased with leaf age and during drought, suggesting that the relationship between V_{cmax} and N_a was seasonally dependent (see Equation 1). Because J_{max} decreased less with leaf age than V_{cmax}, the relationship between V_{cmax} and J_{max} was seasonally dependent (Figure 7), implying a possible seasonal dependence of nitrogen allocation within the leaf.

Regression equations

Regressions were performed to isolate the four factors (vertical gradients, species, leaf age, drought) affecting V_{cmax}. Mass-based regressions were also performed.

Vertical gradients The relationship between leaf nitrogen and V_{cmax} across vertical canopy gradients was evaluated from linear regressions based on the pooled understory and overstory data for July 1997 and 1998. Although this comparison also crossed species gradients and tree development gradients, similar results were found within species and from vertical gradients within a single white oak tree. Most of the variance in V_{cmax} was explained by N_a in both 1997 ($R^2 = 0.81$, intercept = –1.90 µmol m^{-2} s^{-1}, slope = 31.9 µmol g^{-1} s^{-1}, $n = 41$) and 1998 ($R^2 = 0.89$, intercept = –9.3, slope = 33.1, $n = 61$). There was a strong correlation between V_{cmax} and LMA in both years ($R^2 = 0.79$ in 1997 and 0.82 in 1998). Correlation on a mass basis (V_{cmax_m} versus N_m) was considerably lower ($R^2 = 0.06$ in 1997 and $R^2 = 0.35$ in 1998) than on an area basis. Most of the variance in J_{max} was also explained by N_a ($R^2 = 0.73$), and the ratio between J_{max} and V_{cmax} was similar for overstory (2.37) and understory (2.44) trees during this period.

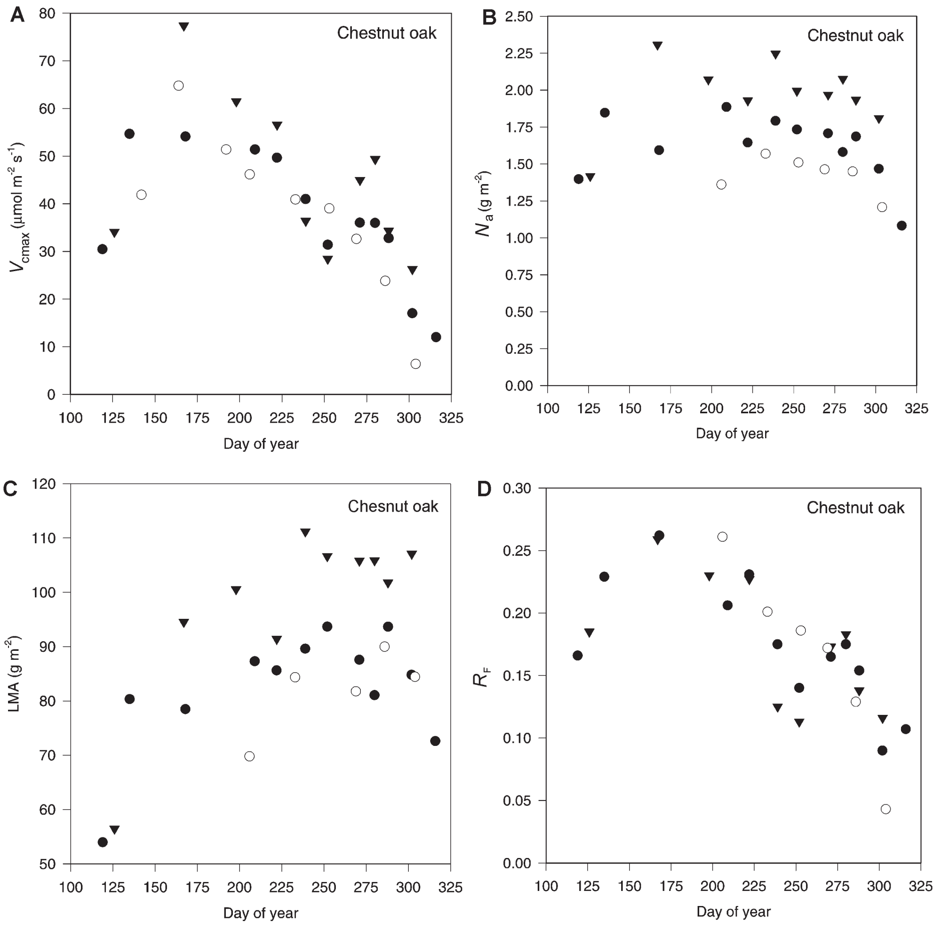

Figure 3. For chestnut oak, (a) V_{cmax} at 25 °C, (b) nitrogen per unit leaf area (N_a), (c) leaf mass per unit area (LMA), and (d) apparent fraction of leaf nitrogen allocated to Rubisco (R_F, Equation 1) in 1997 (open symbols) and 1998 (closed symbols). Each symbol represents a daily mean for single trees in the wet (○) or dry (▽) TDE treatment.

Species differences To test whether leaf nitrogen explained the differences in V_{cmax} among species, regressions were performed with data collected across the season from the 10 mature overstory trees. The species effect was isolated from leaf age effects by performing regressions on data that were grouped by day. Linear regressions were performed on four days in 1997 and nine days in 1998 (Table 1).

Figure 8 shows the relationship between N_a and V_{cmax} in the overstory on two days in 1998, one in late July and the other in early September. Variability in N_a and V_{cmax} on a given day in the overstory was mostly caused by species. Regressions for each of the 13 days across both seasons indicated that N_a explained between 60 and 80% of the variance in V_{cmax} ($P <$ 0.001 on all days except Day 205, 1997 when $P < 0.01$; Table 1). On all days, V_{cmax} was ranked by species in the same general manner as in Figure 8. Correlations between both V_{cmax} and LMA (Table 1) and V_{cmax} and N_m (data not shown) were always positive, suggesting that higher V_{cmax} in oak than in maple was a result of higher LMA and N_m. The correlation between V_{cmax} and LMA was usually greater than between V_{cmax} and N_m, implying that species differences in V_{cmax} are primarily a result of leaf thickness. Mass-based relationships were always inferior to the area-based relationships (Table 1). Although there was a strong correlation between N_a and V_{cmax} across species, the regression coefficients changed over the season (Figure 9 and Table 1), with V_{cmax} decreasing for a given value of N_a.

Seasonal patterns The temporal stability of relationships between N_a and V_{cmax} was examined in detail by sorting the overstory data into three seasons. These seasons did not match the calendar seasons but were roughly grouped as (1) spring, characterized by rapid increases in V_{cmax} during leaf expansion and development, (2) summer, characterized by full leaf expansion, relatively small changes in LMA and slowly or moderately decreasing V_{cmax} and (3) autumn, when there was a fairly rapid decrease in V_{cmax} during leaf senescence. The periods for these seasons were assigned slightly differently among the species. Maple and blackgum leaves developed and senesced sooner than oak leaves, and summer was defined between Days 140 to 280 (May 20–October 7), whereas in oak the summer was defined between Days 148 to 288 (May 28–October 15). All days before and after the summer boundary dates were considered spring and autumn, respectively. Summer data were collected over a longer period than for the other two seasons, and although V_{cmax} changed more slowly during summer than during spring and autumn, substantial decreases in V_{cmax} occurred throughout the summer period (Figures 2–6).

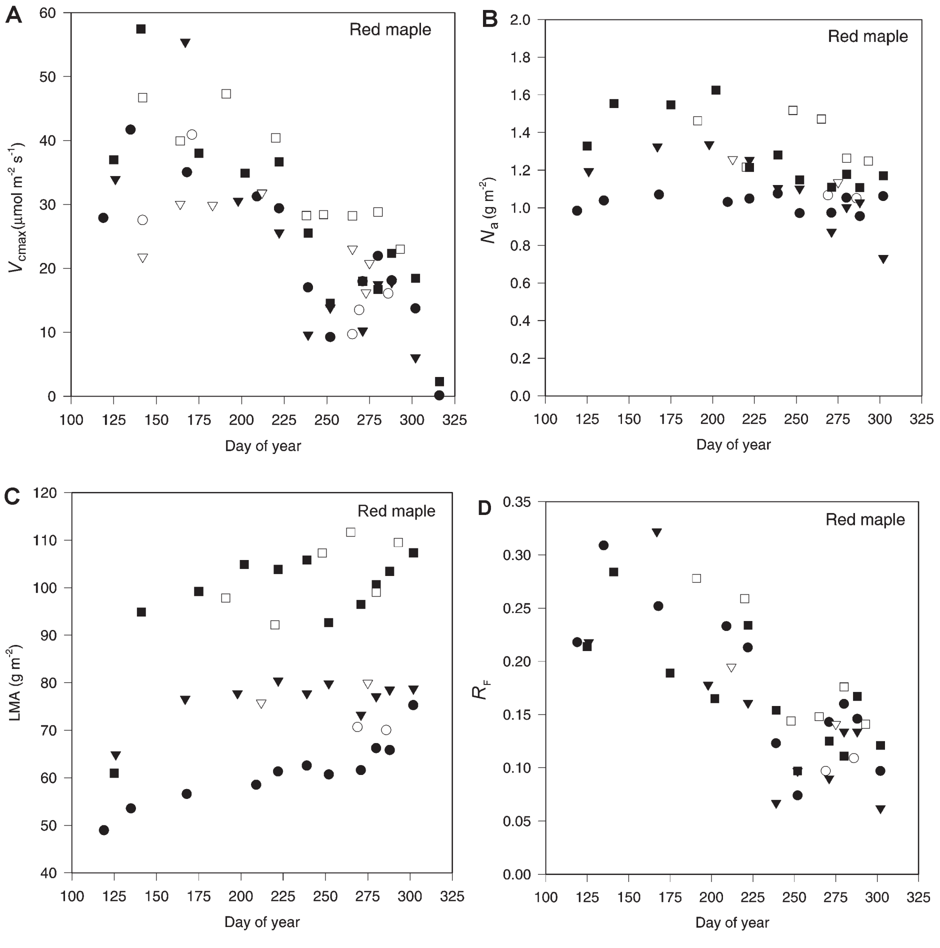

Figure 4. For red maple, (a) V_{cmax} at 25 °C, (b) nitrogen per unit leaf area (N_a), (c) leaf mass per unit area (LMA), and (d) apparent fraction of leaf nitrogen allocated to Rubisco (R_F, Equation 1) in 1997 (open symbols) and 1998 (closed symbols). Each symbol represents a daily mean for single trees in the in the ambient (□), wet (○) or dry (▽) TDE treatment.

For the combined spring overstory data (n = 45) in 1998, V_{cmax} was highly correlated with LMA ($R^2 = 0.73$, intercept = –18.6 µmol m^{-2} s^{-1}, slope = 0.73 µmol g^{-1} s^{-1}). There was a negative correlation between V_{cmax} and N_m ($R^2 = 0.55$) and there was no correlation between V_{cmax} and N_a ($R^2 = 0.08$).

All summer data for a given species in the overstory were pooled, and the same was done for the autumn data (Table 2). A second set of regressions was performed by pooling summer and autumn data by single trees. This second method provided a more rigorous test of chronological effects, because it is not confounded by tree-to-tree variation. Because the general tendencies were not substantially altered when based on individual tree data, only species data are shown in Table 2. The inclusion of 1997 data alone or in combination with the 1998 data did not change the general conclusions.

In both summer and autumn, changes in LMA were small, and LMA did not correlate with V_{cmax} for any species as it did in the spring (data not shown). In summer, the correlation between V_{cmax} and N_a was poor with none of the species exceeding an R^2 of 0.34 (Table 2), even though there were considerable reductions in V_{cmax} during this time (Figures 2a–5a). The mass-based regressions (V_{cmax_m} versus N_m) were slightly better than the area-based regressions during summer (Table 2).

In autumn, V_{cmax} was more strongly correlated with nitrogen on both an area and mass basis than during summer, especially in white oak (Table 2). The degree of correlation was similar whether the regressions were performed on an area or mass basis. The slopes of mass-based relationships were lower in autumn than in summer.

Drought Drought reduced V_{cmax} for several of the individual trees, usually with little or no change in N_a (Figures 2–6). A possible effect of drought on the overall relationship between N_a and V_{cmax} was observed (Figures 8 and 9; Table 1). For a given N_a, V_{cmax} was less on Day 252, 1998, near the period of most severe drought, than on most dates earlier and later in the same year, although the differences in slope were only statistically significant when compared with earlier in the year. However, on the two days when the soil was driest (Days 239 and 252, 1998), the intercepts were significantly less than zero (Table 1), which did not occur at any other time, suggesting that drought affected species with low N_a more than species with high N_a (cf. low N_a in drought-sensitive maples compared with high N_a in the more drought-tolerant oaks; Figures 2–5).

Annual regression

To test whether large errors occurred when a single relationship was used for the entire period of leaf maturity, we per-

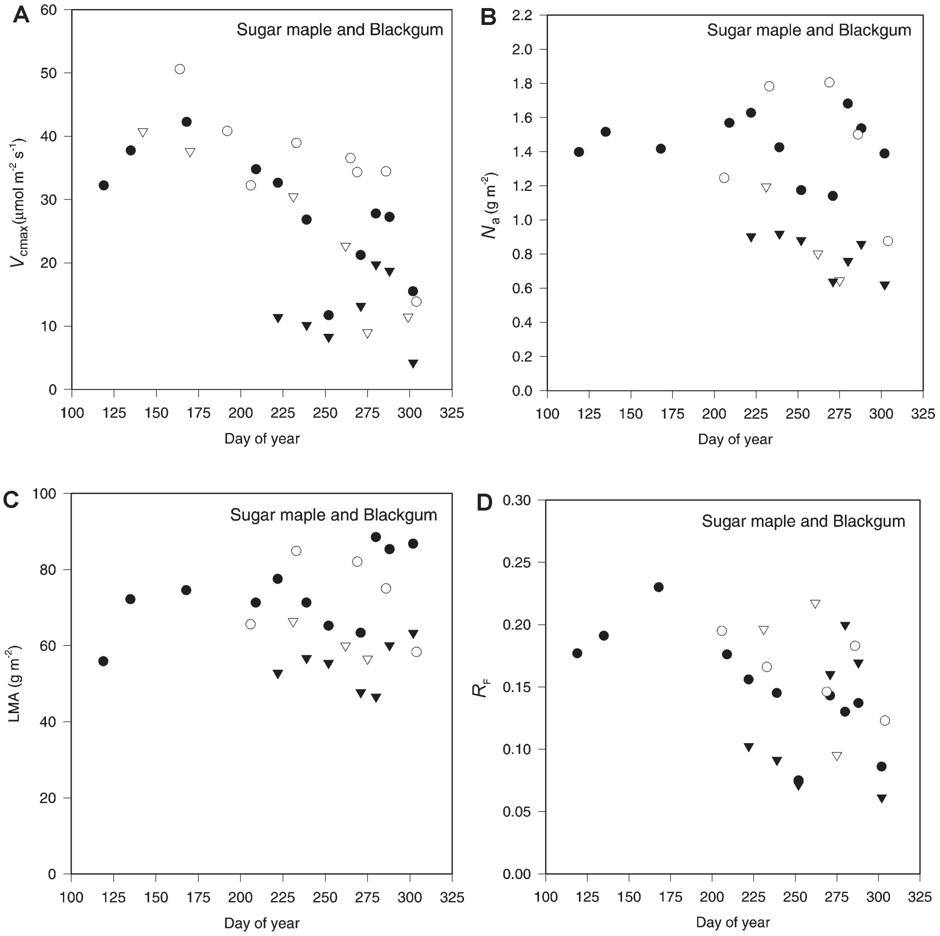

Figure 5. For blackgum and sugar maple, (a) V_{cmax} at 25 °C, (b) nitrogen per unit leaf area (N_a), (c) leaf mass per unit area (LMA), and (d) apparent fraction of leaf nitrogen allocated to Rubisco (R_F, Equation 1) in 1997 (open symbols) and 1998 (closed symbols). Each symbol represents a single blackgum (▽) in the dry TDE treatment and a single sugar maple (○) in the wet TDE treatment.

formed regressions based on all overstory data after Day 148 (Table 3). As expected from the seasonal dependence of the relationship between V_{cmax} and N_a (Table 1), lower correlations were obtained for data pooled across all dates than for data for single days.

During both years and for all trees, the single annual regressions underestimated V_{cmax} early in the year, often between 50 and 100%, and overestimated V_{cmax} later in the year (Figure 10). The drought in 1998 enhanced the overestimation of V_{cmax}. When mass-based regressions were applied in the same manner (data not shown), the overall trend was similar to that in Figure 10, but there was more scatter because mass-based regressions did not explain species differences well (Table 1).

Discussion

Photosynthetic capacity is a dynamic property in this temperate deciduous forest that varies considerably in both space and time. Species differences, light environment, leaf age and drought at a single location resulted in variability in V_{cmax} and photosynthesis that were comparable with those observed across broad vegetation types and geographical gradients (Peterson et al. 1999).

Variability caused by light environment

Parallel vertical gradients in light environment, maximum assimilation and leaf nitrogen per unit area (N_a) have been well established (e.g., Niinemets and Tenhunen 1997) and confirm hypotheses about optimal nitrogen allocation (Kull and Jarvis 1995). Often N_m is fairly constant with canopy height, whereas LMA and, therefore, N_a increase with height or with higher growth irradiance (Ellsworth and Reich 1993, Abrams and Mostoller 1995). Consistent with these studies, we found that most of the variations in N_a and V_{cmax} along vertical gradients were a result of thicker leaves (LMA) and not nitrogen concentration (N_m).

During unstressed periods, there was no consistent difference in R_F between understory and overstory trees. A change in R_F with height in the canopy would imply that the relationship between V_{cmax} and leaf nitrogen is dependent on growth irradiance. When R_F was calculated from Equation 1 and from enzyme extracts in other woody species, there was often no pattern with growth irradiance (Niinemets et al. 1998). In other cases where Rubisco has been measured directly, R_F sometimes (Evans 1989, Poorter and Evans 1998), but not always (Seemann et al. 1987) is a function of the growth irradiance.

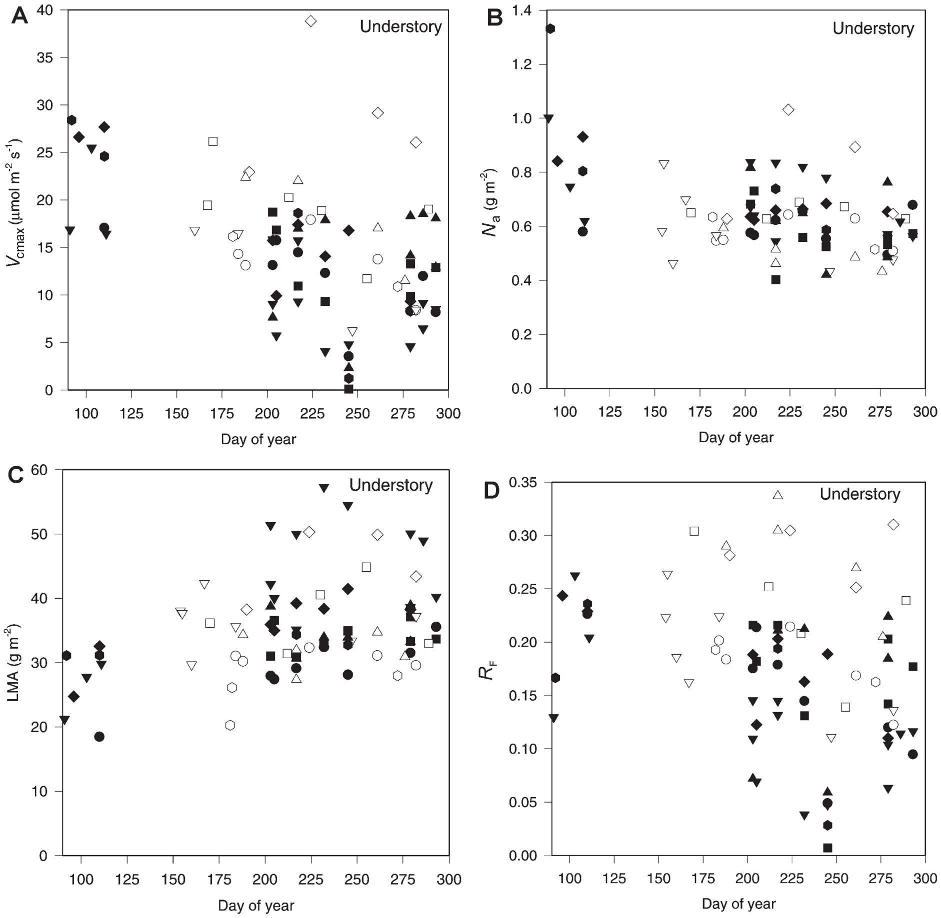

Figure 6. (a) Mean maximum rate of carboxylation (V_{cmax}), (b) nitrogen per unit leaf area (N_a), (c) leaf mass per unit area (LMA), and (d) apparent fraction of leaf nitrogen allocated to Rubisco (R_F, Equation 1) in 1997 (open symbols) and 1998 (closed symbols) for the understory species. Symbols are (○) beech, (□) sourwood, (▽) red maple, ( ) black cherry, (△) sassafrass and (◇) tulip poplar.

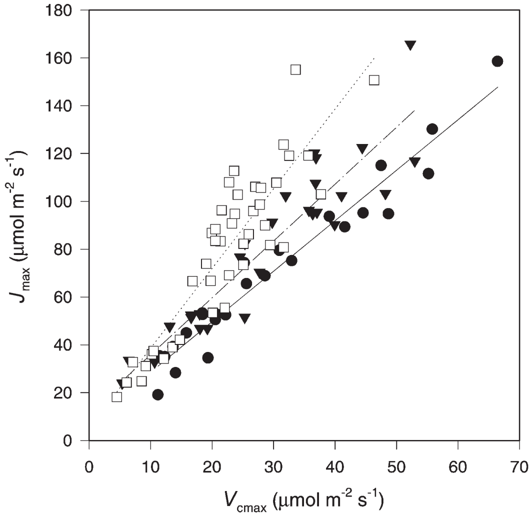

Figure 7. Relationship between J_{max} and V_{cmax} at 25 °C in 1997. Data were separated into three date groups: before Day 210 (●), between Days 210 and 255 (▼) and after Day 255 (□). Regression equations were: $J_{max} = 7.5 + 2.11\ V_{cmax}$ (solid line, $R^2 = 0.94$) before Day 210; $J_{max} = 12.1 + 2.38\ V_{cmax}$ (dash-dot line, $R^2 = 0.86$) between Days 210 and 255; $J_{max} = 5.4 + 3.33\ V_{cmax}$ (dash line, $R^2 = 0.81$) after Day 255. All slopes were significant ($P < 0.001$), and the slope on the last date group was significantly different from the two earlier date groups ($P < 0.01$). The intercepts for the first two date groups were significantly different from zero ($P < 0.05$) but not for the last group.

Variability caused by species type

A second source of variation in V_{cmax} was species type. Oaks had greater photosynthetic capacity and leaf nitrogen on a mass or area basis than maples. Leaf nitrogen was usually lower in red maple, especially on an area basis, than in other co-occurring species and this translated into lower photosynthetic capacity (cf. Reich et al. 1991, Abrams and Mostoller 1995, Sullivan et al. 1996, Abrams 1998). The strong correlation between LMA and V_{cmax} indicated that species with thicker leaves had greater photosynthetic capacity, consistent with previous studies (Abrams and Mostoller 1995, Niinemets et al. 1998).

Seasonal variability (leaf age)

A third and more complex source of variation in V_{cmax} was driven by changes in leaf age. The rapid increase in V_{cmax} during spring and decrease during autumn were similar to the patterns in maximum photosynthesis for deciduous forests in Wisconsin (Reich et al. 1991), Michigan (Jurik 1986) and Massachusetts (Bassow and Bazzaz 1998). At our forest, the duration of physiologically active leaves was longer and V_{cmax} decreased more over its duration than did maximum photosynthesis in these other studies. In Massachusetts, photosynthesis of oaks and maples was maximal or near maximal in middle and late August, much later than in our forest. At our site, large

Table 1. Linear regression coefficients for V_{cmax} versus N_a and LMA, and the massed-based coefficients (V_{cmax_m} versus N_m) for specified days in 1997 and 1998 based on all 10 overstory trees in the study. Significance of the slopes and non-zero intercepts were $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***). For the V_{cmax} versus N_a regression, significance differences in slopes ($P < 0.05$) are indicated by letters. Units: V_{cmax} (µmol m^{-2} s^{-1}), N_m (g g^{-1}), N_a (g m^{-2}), LMA (g m^{-2}) and V_{cmax_m} (µmol g^{-1} s^{-1}).

Day	Year	*n*	N_a			LMA			Mass		
			Intercept	Slope	R^2	Intercept	Slope	R^2	Intercept	Slope	R^2
171	1998	16	3.8	29.0***a	0.70	22.2	0.37***	0.26	–0.17	39.6**	0.48
203	1998	19	–11.2	34.3*** a	0.70	–12.5	0.68***	0.56	–0.03	29.1**	0.29
222	1998	20	–3.4	28.4*** ab	0.74	–8.0	0.57***	0.61	0.02	24.8**	0.30
239	1998	24	–15.2 *	28.6*** ab	0.73	–20.2*	0.57***	0.58	–0.19	29.4**	0.34
252	1998	25	–9.6 *	21.8*** bcd	0.79	–12.7*	0.42***	0.63	–0.22*	27.9***	0.43
271	1998	23	–5.9	23.7*** bc	0.77	–10.5	0.46***	0.54	–0.11	26.0***	0.58
280	1998	23	–3.1	22.1*** bcd	0.76	–0.8	0.36**	0.35	–0.11	26.5***	0.58
288	1998	24	4.7	15.8*** d	0.72	–4.0	0.36***	0.51	0.15*	10.0*	0.24
302	1998	20	–3.9	16.5*** d	0.69	–11.2	0.32***	0.50	–0.05	16.9***	0.48
205	1997	8	–14.5	40.6** a	0.68	–3.2	0.61*	0.67	0.28	15.6	0.05
225	1997	10	3.6	21.7*** bcd	0.76	15.7	0.27	0.20	0.39	4.0	0.01
265	1997	15	5.8	16.1*** bcd	0.63	7.4	0.27**	0.48	0.13	12.8*	0.21
282	1997	15	3.2	16.1*** bcd	0.63	–11.9	0.43***	0.60	–0.07	17.8**	0.37

reductions in V_{cmax} were evident 6 to 8 weeks before either visible evidence of senescence or the more rapid decrease in autumn. Mid-season reductions in photosynthesis have been observed in other trees near our deciduous forest (Edwards et al. 1994, Hanson et al. 1994), but not in a nearby forest in the Appalachian mountains (Sullivan et al. 1996).

Seasonal variability in V_{cmax}–N_a relationship

Unlike the variability driven by vertical gradients or species, the temporal relationship between V_{cmax} and leaf nitrogen was often poorly correlated, especially in spring and summer. In spring, V_{cmax} was well correlated with LMA. As observed by Reich et al. (1991), V_{cmax} was not correlated (area-basis) or was negatively correlated (mass-basis) with leaf nitrogen. The strong positive correlation with LMA, but not N_a or N_m, suggests that the leaves had adequate nitrogen but were not functionally developed (cf. Reich et al. 1991).

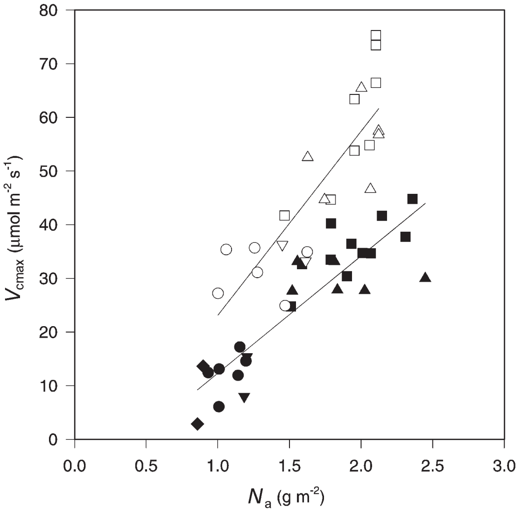

Figure 8. Relationships between N_a and V_{cmax} on Day 203 (open symbols) and Day 252 (closed symbols), 1998, for white oak (□), chestnut oak (△), red maple (○), sugar maple (▽) and blackgum (◇).

Because changes in V_{cmax} over the summer were greater than the decrease in N_a, it is unlikely that nitrogen translocation was responsible for the decrease in V_{cmax}. If V_{cmax} was determined exclusively by N_a, there would be no clear temporal pattern of R_F and a single regression against N_a would be suitable for the entire season; both of these conditions were violated. During the final period of senescence, N_a accounted for more of the variation in V_{cmax}, but R_F continued to decrease during autumn, suggesting that the relationship between V_{cmax} and N_a was not stable between summer and autumn.

One explanation is that the fraction of nitrogen allocated to Rubisco is a function of leaf age. During both years, R_F declined over the summer in all species, which may suggest a seasonal redistribution of leaf nitrogen. As inferred by the ratio of J_{max} to V_{cmax} in 1997, the allocation to electron transport may have been less seasonally dependent than R_F, leading to the possibility that allocation patterns change with leaf age.

The mid-season decline in apparent R_F contrasts with a sugar maple study by Niinemets and Tenhunen (1997). Several other studies have shown that the relationship between nitrogen and photosynthetic capacity is fairly constant across leaf age, implying no large temporal variations in R_F. A reduction in V_{cmax} with leaf age in chaparral shrub was explained by corresponding changes in leaf nitrogen (Field 1983, Field and Mooney 1983). A similar conclusion was reached with *Rhododendron* (Karlsson 1994). Reich et al. (1991) concluded

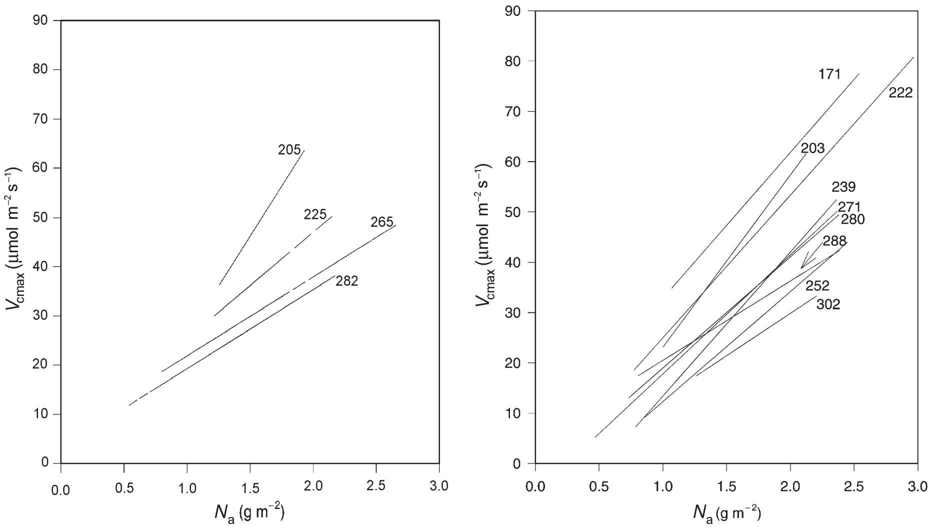

Figure 9. Summary of the regression equations between N_a and V_{cmax} for given dates across all overstory species in (a) 1997 and (b) 1998. The numbers beside the regressions refer to the day of year.

that, over the summer and autumn, nitrogen explained leaf age effects on maximum photosynthesis in oak and maples in Wisconsin. We also found that the correlations in our data were improved somewhat by including autumn data. However, the study of Reich et al. (1991) differs from our study in several respects. In our study, the growing season was longer and the mid-season decline in V_{cmax}, which was not explained by leaf nitrogen, was greater than the mid-season decline in maximum photosynthesis in the lower crown of open canopies reported by Reich et al. (1991). Nitrogen allocation patterns may differ for trees grown in open and closed environments. Also, ozone concentrations can be especially high in east Tennessee, and ozone has been shown to augment the seasonal decrease in maximum photosynthesis (Hanson et al. 1994).

Other studies present conclusions more similar to our findings. The fractional allocation of nitrogen to Rubisco in birch decreased with leaf age, and this decrease correlated with a down-regulation in photosynthesis and V_{cmax} (Rey and Jarvis 1998). Because the reduction of Rubisco was accompanied by an increase in starch accumulation, it was suggested that starch accumulation might create a feedback limitation to Rubisco synthesis. Another possibility is that V_{cmax} and Rubisco content remained constant, but appeared to decrease because of a seasonally dynamic additional resistance to carbon dioxide between the site of evaporation and the carboxylation site in the chloroplast (i.e., mesophyll resistance; Loreto et al. 1992, Roupsard et al. 1996). If mesophyll resistance increases as leaves age as a result of increased lignin concentrations, this increase would be interpreted as a decrease in V_{cmax} and R_F in our analysis, because we assumed that C_i was representative of the carbon dioxide concentration at the carboxylation sites.

Variability caused by drought

Drought stress in 1998 resulted in an apparent reduction in V_{cmax} in some trees without large changes in leaf nitrogen. Nitrogen-use efficiency (mol CO_2 assimilated per mol leaf N) has been observed to decrease in response to drought in other deciduous trees (Reich et al. 1989, Abrams and Mostoller 1995), but in those studies stomatal conductance declined, making it unclear whether there were changes in photosynthetic capacity. Consistent with our findings, overstory trees are often more drought tolerant than understory trees because of rooting depths (Dawson 1996), and several physiological adaptations enable white oak to be more drought tolerant than other co-occurring species (Abrams 1990, Epron and Dreyer 1993*a*).

Reports on the influence of drought on photosynthetic ca-

Table 2. Linear regression coefficients for V_{cmax} versus N_a (area based) and V_{cmax_m} versus N_m (mass based) for data pooled by species during the summer and autumn of 1998. Significance of the slopes and non-zero intercepts were $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***). Abbreviations: Qa (*Quercus alba*, white oak), Qp (*Quercus prinus*, chestnut oak) and Ar (*Acer rubrum*, red maple). Units: V_{cmax} (mmol m^{-2} s^{-1}), N_m (g g^{-1}), N_a (g m^{-2}), and V_{cmax_m} (mmol g^{-1} s^{-1}).

Species	Summer							Autumn						
	Area-based regression			Mass-based regression				Area-based regression			Mass-based regression			
	Intercept	Slope	R^2	Intercept	Slope	R^2	n	Intercept	Slope	R^2	Intercept	Slope	R^2	n
Qa	–8.2	27.4***	0.34	–0.18	32.3***	0.22	85	–13.6**	23.6***	0.65	–0.16**	25.0***	0.65	27
Qp	14.2	16.4*	0.12	–0.56**	51.8***	0.36	50	–4.7	21.1**	0.34	–0.18	28.5*	0.24	22
Ar	–13.9	32.2***	0.28	–0.32	41.7***	0.41	39	–7.9	23.9***	0.48	–0.12	25.8***	0.61	21

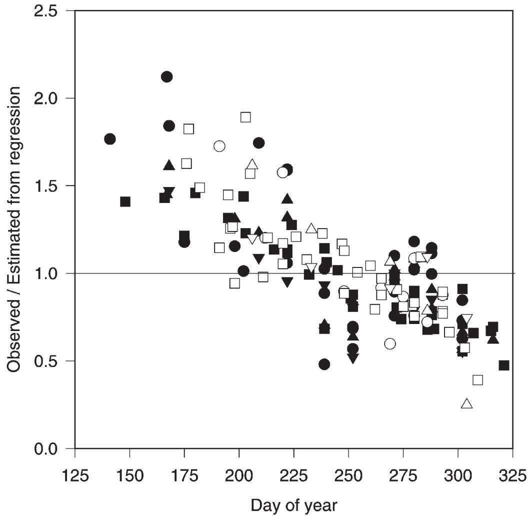

Figure 10. Ratio of observed to predicted V_{cmax} against day of year in 1997 (open symbols) and 1998 (closed symbols). Predicted V_{cmax} was obtained from the area-based regression equations (Table 3). Symbols: white oak (□), chestnut oak (△), red maple (○) and sugar maple (▽).

pacity are contradictory. When photosynthetic capacity was estimated from incubated tissues (Kaiser 1987), or from measurements at extremely high CO_2 concentrations (Epron and Dreyer 1993*b*) or from chlorophyll flourescence (Epron and Dreyer 1992), it was not affected by drought. However, assimilation rates frequently decline during drought even when C_i remains constant or increases (Pereria et al. 1986, Weber and Gates 1990, Ni and Pallardy 1992, Epron and Dreyer 1993*a*), and this decline has been interpreted as evidence of drought-induced reductions in photosynthetic capacity, as in our study. This interpretation is based on the assumption that gas exchange measurements provide the correct estimates of carbon dioxide concentration near the site of carboxylation; the existence of a mesophyll resistance or the occurrence of patchy stomatal closure could invalidate this assumption.

The assumption of a drought-induced reduction in V_{cmax} would be invalid if the mesosphyll resistance to carbon dioxide diffusion is altered by drought. Roupsard et al. (1996) used a combination of gas exchange and chlorophyll fluorescence techniques to show that estimated mesophyllic carbon dioxide concentration decreased with drought stress, in some cases even when C_i increased or remained constant. However, patchy stomatal closure cannot be eliminated as the cause, and although the presence of a significant mesophyll resistance is likely, no mechanism for an increase in this resistance with drought has been established (Roupsard et al. 1996).

Table 3. Coefficients for the area-based and mass-based regression of V_{cmax} on leaf nitrogen when overstory data during summer and autumn are pooled by year. All slopes were significantly different ($P <$ 0.001). Units: V_{cmax} (µmol m^{-2} s^{-1}), N_m (g g^{-1}), N_a (g m^{-2}) and V_{cmax_m} (µmol g^{-1} s^{-1}).

Year	*n*	Area-based regression			Mass-based regression		
		Intercept	Slope	R^2	Intercept	Slope	R^2
1997	126	1.2	20.1	0.28	–0.36	42.3	0.45
1998	252	–10.7	27.8	0.57	–0.26	35.9	0.48

Another difficulty is that the value of C_i calculated from gas exchange can be erroneous because of patchy stomatal closure (Laisk 1983, Epron and Dreyer 1993*b*, Mott 1995). Our interpretation that photosynthetic capacity, or V_{cmax}, was reduced during the peak period of drought would be invalid if patchy stomatal closure, which is known to be symptomatic of drought, was present. Instead, the apparent reductions in V_{cmax} would be an artifact caused by assuming uniform stomatal closure. However, actual reductions in V_{cmax} during drought have been reported. Drought-induced growth inhibition can result in sink limitation and feedback control on photosynthetic capacity (Kaiser 1987). Long-term water stress has been shown to inactivate Rubisco (Medrano et al. 1997). In our study, we assumed that Rubisco activity was constant, so any inactivation would appear as a decrease in R_F.

If either patchy closure or a drought-dependent mesophyll resistance is found to be common under natural conditions, these processes may require representation in leaf-level models (Buckley et al. 1999). However, diagnosing the necessary temporal and spatial parameterizations of these processes would be difficult, particularly for large-scale models. As a compromise, including a drought-dependent V_{cmax} parameterization may not be physiologically correct, but will accurately estimate fluxes in models that necessarily assume uniform stomatal conductance and no mesophyll resistance. Interpretation of model results would require the caveat that the simulated non-stomatal limitations may not represent actual reductions in photosynthetic capacity.

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