Seasonal CO$_2$ assimilation and stomatal limitations in a *Pinus taeda* canopy

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Summary   Net CO$_2$ assimilation (A_{net}) of canopy leaves is the principal process governing carbon storage from the atmosphere in forests, but it has rarely been measured over multiple seasons and multiple years. I measured midday A_{net} in the upper canopy of maturing loblolly pine (*Pinus taeda* L.) trees in the piedmont region of the southeastern USA on 146 sunny days over 36 months. Concurrent data for leaf conductance and photosynthetic CO$_2$ response curves (A_{net}–C_i curves) were used to estimate the relative importance of stomatal limitations to CO$_2$ assimilation in the field. In fully expanded current-year and 1-year-old needles, midday light-saturated A_{net} was constant over much of the growing season (5–6 µmol CO$_2$ m^{-2} s^{-1}), except during drought periods. During the winter season (November–March), midday A_{net} of overwintering needles varied in proportion to leaf temperature. Net CO$_2$ assimilation at light saturation occurred when daytime air temperatures exceeded 5–6 °C, as happened on more than 90% of the sunny winter days. In both age classes of foliage, winter carbon assimilation accounted for approximately 15% of the daily carbon assimilation on sunny days throughout the year, and was relatively insensitive to year-to-year differences in temperature during this season. However, strong stomatal limitations to A_{net} occurred as a result of water stress associated with freezing cycles in winter. During the growing season, drought-induced water stress produced the largest year-to-year differences in seasonal CO$_2$ assimilation on sunny days. Seasonal A_{net} was more drought sensitive in current-year needles than in 1-year-old needles. Relative stomatal limitations to daily integrated A_{net} were approximately 40% over the growing season, and summer drought rather than high temperatures had the largest impact on summer A_{net} and integrated annual CO$_2$ uptake in the upper crown. Despite significant stomatal limitations, a long duration of near-peak A_{net} in the upper crown, particularly in 1-year-old needles, conferred high seasonal and annual carbon gain.

Keywords: annual CO$_2$ assimilation, canopy carbon gain, climate variability, drought, evergreen photosynthesis, stomatal conductance, temperature.

Introduction

Evergreen trees have long-lived leaves with relatively low photosynthetic capacity but the ability to photosynthesize year-round (Reich et al. 1992, Larcher 1995). Few studies have measured net CO$_2$ assimilation (A_{net}) over the full life span of evergreen leaves, which can range from 2 to more than 20 years (Reich et al. 1995). Even studies examining a single annual cycle of A_{net} in evergreens are rare (Schulze et al. 1977, Troeng and Linder 1982, Matyssek 1986, Teskey et al. 1994), and such studies may not be representative because of year-to-year climate variability in temperate regions. In addition, there are few long-term studies of the physiological sensitivity of A_{net} to climatic conditions (Strain et al. 1976, Maier and Teskey 1992, Teskey et al. 1994). Knowledge of patterns in annual leaf A_{net} and its possible limitations is essential to assess the carbon sequestration capacity of temperate evergreen forests and the role of climate in year-to-year variability in carbon storage (Goulden et al. 1997).

In temperate conifers, net CO$_2$ assimilation during periods of favorable conditions for A_{net} outside the typical growing season may enhance tree carbon balance and hence competitiveness with deciduous species (Lassoie et al. 1985). However, because seasonal and year-to-year changes in water availability and temperature are likely to affect plant water relations, stomatal regulation of gas exchange in evergreen foliage must occur to minimize water stress throughout the year (Smith et al. 1984, Teskey et al. 1987). Although the magnitude of stomatal limitations to photosynthetic performance in forest trees has been analyzed for specific stresses such as drought (Kubiske and Abrams 1993), the long-term importance of such limitations to seasonal and annual carbon assimilation remains unclear.

To study the effects of climate conditions on annual carbon assimilation, daily and seasonal courses of photosynthesis were examined in different age classes of upper-canopy foliage in the coniferous species *Pinus taeda* L. (loblolly pine) over 36 months. Physiological controls on foliage A_{net} were also examined. Specific objectives were to determine (1) the amounts of seasonal and year-to-year variation in A_{net} and carbon gain in different age classes of needles in the upper canopy of loblolly pine, (2) the extent of stomatal limitations to A_{net}, and (3) the environmental factors causing large stomatal limitations. I hypothesized that 1-year-old needles have greater carbon gain than current-year needles, because of the longer

duration of net carbon gain. I also hypothesized that stomatal limitations during summer drought account for the large reductions in daily integrated carbon gain. *Pinus taeda* was selected for this study because it is grown commercially in areas with moderate winter climate where the period between frosts ranges from 200 to more than 300 days. In addition, loblolly pine is reputed to be sensitive to transitory low temperatures in some winters (Hocker 1956, Strain et al. 1976, Schulz 1997).

Methods

Study site

The study was conducted in the Blackwood Division of Duke Forest in Orange County, NC (35°58′ N, 79°5′ W) described by Ellsworth (1999) and Hendrey et al. (1999). The climate of the area is warm and humid with mild winters and an average frost-free season of 200 days. The 30-year mean January maximum and minimum temperatures are 9.2 and −3.5 °C, respectively, and mean July maximum and minimum temperatures are 31.5 and 18.3 °C, respectively. A 32-ha site was planted with a North Carolina piedmont provenance of loblolly pine in 1983 following clearing and burning the previous year. Several walk-up towers (Up-Right Corp., Selma, CA) were constructed in the stand to access upper-crown foliage of adjacent trees without obstructing upper crown branch growth. The loblolly pine overstory trees were approximately 11 m tall in the summer of 1995 and 13 m tall at the end of the study in 1998. Foliage located on the terminal shoot of major branches at the third whorl from the top of the tree was selected for gas exchange measurements to standardize light environment and location within the tree crown. Upper-crown, sun foliage was used because this foliage represents the physiological processes in the sunlit portion of the crown that dominate CO_2 uptake. To minimize within-crown shading, selected branches and foliage were south facing. Net CO_2 assimilation (A_{net}) and stomatal conductance to water vapor (g_s) of foliage were followed for 36 months at 1–3-week intervals. On all sampling dates, 3–7 trees were used for physiological measurements.

Major climatic conditions during the study, based on meteorological data from a National Weather Service site located 7 km from the study site, are summarized in Table 1. Site-specific air temperature measurements measured at the top of three towers at the site were begun in late 1996. For seasonal analyses, the cool season or winter is defined as the period when frosts are possible and extends from November to March in the central piedmont region. In all three study years, annual precipitation was at or above the 30-year mean of 1154 mm recorded for Chapel Hill, NC (National Weather Service, unpublished data), although in some months precipitation was below normal (Table 1). The 36-month measurement period included three extreme climate events: an unusual cold period in January–February, 1996 that included snow and freezing precipitation equivalent to more than 70 mm of water; a strong tropical storm (hurricane Fran) that passed over the site in September 1996; and a severe drought of an intensity that would normally occur in this region only once every 30 years (National Climatic Data Center, U.S. Dept. of Commerce, Asheville, NC) that occurred in August 1997 (Table 1; see Ellsworth 1999).

CO_2 assimilation measurements

On predominantly sunny days at 1–3 week intervals over 36 months, A_{net} was measured in single fascicles of upper-crown foliage receiving direct sunlight. Measurements on current-year needles were begun once needles were more than 50% fully elongated in late June. Light-saturated A_{net} (photon flux density > 1000 μmol m^{-2} s^{-1}) was measured in a leaf chamber attached to a portable infrared gas analyzer system for CO_2 and water vapor (CIRAS-1, PP-Systems, Hitchin, U.K.) in an open-flow configuration (see Ellsworth 1999). The leaf chamber was held at an angle of inclination close to that of the foliage, although shading from adjacent foliage was intentionally minimized. A Peltier-type cooling unit (Marlow Industries, Dallas, TX) was used to maintain foliage temperature close to ambient air temperature (within 1 °C) during measurements. Foliage temperatures during midday A_{net} measurements were within ± 8% (°C) of the maximum daily air temperature measured at the National Weather Service station nearby (7 km from study site). Other conditions inside the leaf chamber including atmospheric CO_2 concentration (C_a) and leaf–air water vapor pressure difference (ΔW) were maintained near ambient. The mean C_a for all measurements was 357 μmol mol^{-1}.

During the growing season, A_{net} measurements were generally made 1–2 h before solar noon, and during the cool season (November through March) measurements were made 1–2 h after solar noon. On 12 dates over all seasons, a full diurnal course of A_{net} measurements was made from dawn to dusk to confirm that A_{net} was maximal at the anticipated time of day. Periodic checks of A_{net} measured first and last on the same tree during the midday measurement cycle confirmed that A_{net} had not declined by more than 9% during this period, except during peak drought in August 1997 and 1998.

Measurements of the response of A_{net} to intercellular CO_2 concentration (C_i) (A_{net}–C_i curves) were conducted at different times of the year. These measurements were made at a leaf temperature (T_{leaf}) of 20–22 °C and leaf–air ΔW < 1.5 Pa kPa^{-1} in all months except July–September when measurements were instead made at 30 °C. During winter, A_{net}–C_i measurements were made on days when air temperature exceeded 15 °C to minimize immediate limitations of temperature on A_{net}. Daily maximum temperatures of at least 20 °C are possible on any day of the year in the central piedmont near Durham, NC (Figure 1, and unpublished data, National Climatic Data Center, U.S. Dept. of Commerce, Asheville, NC).

Both A_{net} and g_s are expressed on a surface area basis. Needle surface area was based on direct needle dimensional measurements, assuming that fascicles formed sectors of a cylinder. Needle dimensions were measured to the nearest 100 μm with an ocular micrometer or *in situ* to the nearest 200 μm with digital micrometers. In winter, needle surface area was estimated from previous data if dimensional mea-

Table 1. Summary of principal climatic factors during the study period based on meteorological data from Orange County, NC, 7 km from the study site. Climate normals are based on the 30-year mean (1960–1990) for Chapel Hill, NC (unpublished data from National Climatic Data Center, U.S. Dept. of Commerce, Asheville, NC).

Period of study	Mean daily maximum temperature (°C)	Departure from normal (°C)	Incidence of frost (% of days)	Longest period without rain (days)	Summer month with least precipitation (% below normal)
Nov. 1995–Mar. 1996	11.1	−2.0	61	–	–
May–Sept. 1996	28.6	−0.4	0	12	June (−54%)
Nov. 1996–Mar. 1997	13.7	+0.6	48	–	–
May–Sept. 1997	28.5	−0.5	0	35	Aug. (−82%)
Nov. 1997–Mar. 1998	13.1	0	43	–	–
May–Sept. 1998	30.3	+1.3	0	21	July (−43%)

surements for needles on the same tree had been made in the past 3–4 weeks.

Additional measurements

Xylem water potential (Ψ) was measured on needle fascicles with a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA). Predawn and midday Ψ of upper canopy foliage were measured in conjunction with days when A_{net} was measured. Needles were collected at predawn and sealed in a plastic bag for Ψ determinations within 30 min. In winter, these samples were allowed to thaw while sealed in the bag before measurement.

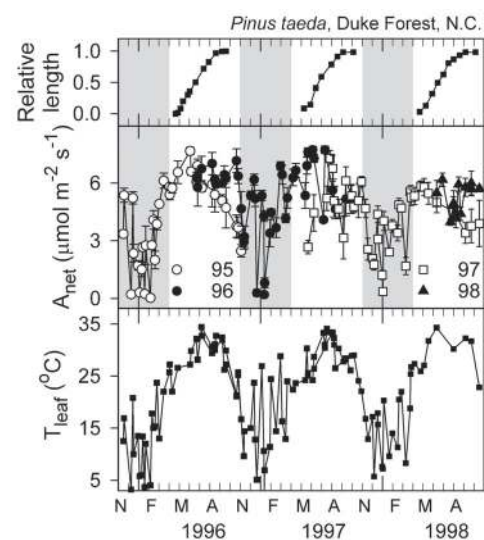Environmental parameters at the study site were monitored

Figure 1. Time course of seasonal pattern of current-year needle elongation (relative needle length), daily maximum net CO_2 assimilation (A_{net}) at light saturation in upper-crown foliage, and leaf temperature (T_{leaf}) at the time of measurement over 36 months (1995–98) in *Pinus taeda* at Duke Forest, NC. Different symbols represent different year classes of first-flush foliage, by year in which the foliage was produced (from 1995 to 1998). Data are from a total of 536 measurements on 146 days over a 36-month time span. Shaded areas represent the period outside the normal growing season (November to March) corresponding to the frost season. Months indicated are November (N), February (F), May (M), and August (A).

at the top of the crown on three towers beginning in late 1996. Photon flux density (PFD, measured as photosynthetically-active radiation in the 400–700 nm wavebands) and wind speed were measured above the canopy at the nearby free-air CO_2 enrichment (FACE) facility (Hendrey et al. 1999). Air temperature was measured with three shielded, wind-aspirated thermistors. Volumetric soil water content in the upper 30 cm horizon was measured by reflectometry techniques (Campbell Scientific, Logan, UT) and converted to soil matric potential (Ψ_m) by reference to a characteristic soil water release curve for the loamy clay soil (see Ellsworth 1999).

Modeling stomatal limitations and daily photosynthesis

Gas exchange variables were calculated according to Field et al. (1989). To determine seasonal variation in needle capacity for A_{net} and the relative limitation of stomata to photosynthesis (i.e., gas-phase limitation), I used the approach described by Farquhar and Sharkey (1982) as modified by Jones (1985).

Data from A_{net}–C_i curves were used to parameterize a leaf photosynthesis model to predict photosynthetic capacity at different times of the year based on Rubisco enzyme kinetics. From the initial linear slope of the A_{net}–C_i relationship, mesophyll carboxylation capacity was derived (Farquhar et al. 1980, Farquhar and Sharkey 1982). Field A_{net}–C_i curves were used to parameterize the biochemical model of C_3 photosynthesis described by Farquhar et al. (1980) and Farquhar and von Caemmerer (1982) with recent modifications (see Harley et al. 1992, Wullschleger 1993). The CO_2 demand in photosynthesis was calculated as the $V_{c,max}$ parameter of the Farquhar model as follows:

$$A_{net} = V_{c,max} \frac{C_i - \Gamma^*}{C_i + k_c \left(1 + \dfrac{O_i}{k_0}\right)} - R_d, \tag{1}$$

where $V_{c,max}$ is maximum Rubisco activity with saturating RuBP, R_d is non-photorespiratory CO_2 evolution in light, C_i and O_i are the intercellular CO_2 and O_2 concentrations, respectively, and k_c and k_o are the Michaelis coefficients of Rubisco for CO_2 and O_2. The CO_2 compensation point in the absence of photorespiration (Γ^*) was calculated according to Brooks and

Farquhar (1985). The temperature dependencies of k_c and k_0 are from Harley et al. (1992). The temperature dependence of $V_{c,max}$ and R_d at a given leaf temperature ($f(T_k)$) was calculated relative to their values at 25 °C ($T_k = 298$) as given by the simple Arrhenius function:

$$f(T_k) = f(298) \exp\left[\frac{(T_k - 298)H_a}{RT_k 298}\right], \qquad (2)$$

where $f(298)$ is the value of a given parameter at 25 °C, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), and H_a is the activation energy of the parameter (J mol^{-1}). In this treatment, H_a for k_c and k_o were considered to be the same as published for other C$_3$ species (Harley and Tenhunen 1991). However, the temperature dependence of $V_{c,max}$ used was 36 kJ mol^{-1}, lower than the values used by Harley et al. (1992), but in close agreement with field data for A_{net}–C_i curves of *P. taeda* measured at 10–30 °C in this study (data not shown) and for *Pinus radiata* L. (Walcroft et al. 1997). Equation 1 was solved for $V_{c,max}$ by least-squares linear regression as described by Wullschleger (1993). In the absence of detailed A_{net}–C_i curve information at different temperatures at all times of the year, I assumed that instantaneous limitations of temperature on $V_{c,max}$ were similar throughout the year and hence used a single H_a value to correct $V_{c,max}$ to the standard temperature.

Carbon dioxide supply to the mesophyll was calculated by Fick's Law as:

$$A_{net} = g_c(C_a - C_i), \qquad (3)$$

where g_c is stomatal conductance to CO$_2$ diffusion ($\approx g_s/1.6$), assuming that the CO$_2$ concentration at the site of carboxylation is close to the CO$_2$ concentration at the intercellular surfaces. The calculation was modified according to Field et al. (1989) to account for the diluting effect of water vapor on CO$_2$ concentration. Because stomatal limitations were calculated on a relative basis considering stomatal conductance to CO$_2$, gas and liquid-phase limitations by CO$_2$ diffusion from the cell wall to the site of carboxylation at the chloroplast are considered along with mesophyll limitations as the remaining limitations in this formulation. Relative stomatal limitation (l_g; Jones 1985) was calculated as:

$$l_g = \frac{r_g}{(r_g + r^*)}, \qquad (4)$$

where r_g is the reciprocal of g_c in Equation 1, and r^* is the slope of the supply function:

$$r^* = \frac{dC_i}{dA_{net}}, \qquad (5)$$

calculated from the carboxylation efficiency derived from $V_{c,max}$ from Equation 1 based on the measured A_{net}–C_i curve characteristics for the appropriate time of year. The supply function r^* represents residual limitations (i.e., non gas-phase)

to photosynthesis (Method V, Jones 1985). This approach has the advantage that effects of temperature and stress on the form of the A_{net}–C_i curve can be included. Given the initial linear portion of the A_{net}–C_i curve at a base temperature, the initial slope can be corrected according to the temperature-dependent kinetics described in the Arrhenius equations (see Equation 2). These equations produced a family of A_{net}–C_i curves as a function of temperature that were in good agreement with those measured at a range of controlled temperatures in the field (Ellsworth, unpublished data).

A simple empirical model was used to extrapolate midday A_{net} data and integrate A_{net} over the day for sunny days, for the purpose of estimating seasonal CO$_2$ assimilation over a year. The model was used to make predictions only for days for which there were specific midday A_{net} values. The model assumes that the form of the diurnal cycle of photosynthesis for a leaf at the top of the crown can be approximated by a sinusoidal function on days with no midday depression of photosynthesis. Irradiance is assumed to be close to the compensation point near sunrise and sunset, and daily maximum A_{net} is assumed to occur near midday. A modified sine-wave function is common for modeling diurnal cycles of light and temperature, and the form also approximates diurnal cycles in photosynthesis (Thornley and Johnson 1990). No *a priori* light response curve is needed with this approach, and the model assumes that any reductions in g_s in response to temperature and water stress will be reflected in maximum A_{net} on a given day. The model requires measurements of daily maximum A_{net} for parameterization (Figure 1), although a theoretical daily maximum A_{net} could also be predicted on the basis of leaf carboxylation capacity $V_{c,max}$ and the appropriate leaf C_i predicted by various empirical or theoretical models. From the appropriate daily maximum A_{net} from Figure 1, daily net CO$_2$ assimilation (A_{day}) was calculated as:

$$A_{day} = \int_{t_1}^{t_2} A_{max} \sin\left[\frac{\Pi(x - x_0)}{b}\right], \qquad (6)$$

where time t_1 to t_2 defines the period where $A_{net} > 0$ from 5° to 175° solar angle, and b is the day length calculated based on sun angle and equations in Campbell and Norman (1998). Numerical integration over the day was done by a trapezoidal approximation for 10-min intervals. The model fit was evaluated by comparing predicted A_{day} with actual A_{day} obtained from diurnal A_{net} measurements conducted in all seasons.

Results

During the 36-month study, over 540 upper canopy gas exchange measurements were made on 146 sunny days. Light-saturated daily maximum A_{net} varied seasonally from close to zero to 7 μmol CO$_2$ m^{-2} s^{-1} (Figure 1), but annual maximum A_{net} was constant from year to year for needles of a given age. Annual maximum A_{net} in the upper canopy (7.3 μmol m^{-2} needle surface s^{-1} or 70 nmol g^{-1} needle s^{-1}) occurred in late May–early June, concurrent with the emergence of new fo-

liage. For current-year foliage, annual maximum A_{net} on a surface area basis was similar to that for 1-year-old needles, but occurred in late July when foliage had reached 70–80% of full elongation (Figure 1). However, daily maximum A_{net} values close to 6 µmol m^{-2} s^{-1} were recorded at most times of the year under favorable conditions, even in January (Figure 1). Both temperature and daily maximum A_{net} were most variable in winter, suggesting that a single monthly measurement of A_{net} can be misleading when attempting to characterize seasonal A_{net} and winter carbon assimilation.

In winter, defined here as November–March, daily maximum A_{net} was proportional to T_{leaf} (Figure 2). There was little evidence of temperature preconditioning because A_{net} was a simple function of daily maximum temperature corresponding to measurement T_{leaf}. Thus, daily maximum A_{net} in overwintering foliage paralleled changes in daily maximum temperature (shaded areas in Figure 1). However, there was a tendency toward lower A_{net} for a given temperature on days when the ground surface had snow or ice than would be expected under other conditions (open symbols, Figure 2), suggesting that, on such days, cold soil or low daily temperatures have an effect on subsequent A_{net}. Persistent snow is rare in the central piedmont and was not observed in the four years before winter 1996. Light-saturated A_{net} at midday was close to zero on six occasions when T_{leaf} was less than 6 °C at the warmest part of the day, suggesting a lower temperature limit for net carbon gain of *P. taeda* foliage.

There were significant correlations between g_s and daily maximum A_{net} throughout the year (Figure 3). There was no difference in the A_{net}–g_s relationship between winter and the growing season ($P > 0.10$), or between different age classes of foliage ($P > 0.10$; Figure 3B). The similarity of A_{net}–g_s relationships between different seasons and foliage age classes indicates that a similar bulk leaf C_i is maintained over a wide variety of conditions with near-constant C_a, as implied by Equation 3. Across the 36-month study period, mean C_i was 220 µmol mol^{-1} with a coefficient of variation of 16% (data

Figure 3. Correlation between daily maximum net CO₂ assimilation (A_{net}) in upper-crown foliage of mature *Pinus taeda* with stomatal conductance (g_s) for foliage measured during the winter (A; data from December to March) and for different age classes measured during the summer (B; data from May to October). Current-year needles are indicated by open symbols, and 1-year-old needles are indicated by closed symbols. The least-squares regression line in A is $y = 0.46 + 0.061x$ ($r^2 = 0.82$, $P < 0.0001$), and the regression in B is $y = 2.03 + 0.053x$ ($r^2 = 0.67$, $P < 0.0001$).

not shown). Although g_s varied with A_{net} over the three years (Figures 1 and 4), the calculated relative stomatal limitations to A_{net} over the period were not constant (Figure 4). The largest relative stomatal limitations to A_{net} occurred in winter (shaded areas in Figure 4) and were associated with low temperatures (daily maximum $T_{air} < 10$ °C). Stomatal limitations were evident in the A_{net}–C_i curve data even at 10 °C, because A_{net} could be stimulated by the temporary addition of CO₂ to the leaf chamber (data not shown). Calculated stomatal limitations of more than 65% were associated with low temperatures but also occurred during a severe drought in late August 1997. Otherwise, limitations to A_{net} during the growing season were approximately evenly divided between stomatal and nonstomatal limitations with an assimilation-weighted mean l_g of 45% over the 3-year study. Assimilation-weighted mean l_g of current-year needles was higher during the drought year 1997 than during 1996 (56 versus 50% in 1996) (Figure 4). The relative stomatal limitations estimated by the approach of Farquhar and Sharkey (1982) over the 3 years were similar but slightly smaller (mean $l_g = 42\%$).

Because both drought and low temperatures were associated with major stomatal limitations to daily maximum A_{net} (Figure 4), relationships between g_s and environmental conditions were analyzed in more detail. During the coldest part of the year, g_s at maximum A_{net} was strongly correlated with the minimum temperature of the previous night when temperature
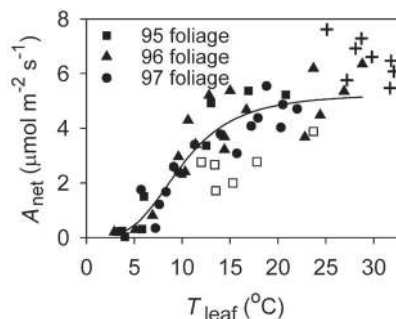

Figure 2. A composite of daily maximum A_{net} as a function of leaf temperature (T_{leaf}) for overwintering needles of *Pinus taeda* in three different years (closed symbols) based on data in Figure 1. All data are from December to March. Open symbols indicate overwintering foliage measured in January–February, 1996 coinciding with days when snow was present at the site. Plus symbols indicate foliage measured during May–June for comparison with winter measurements.
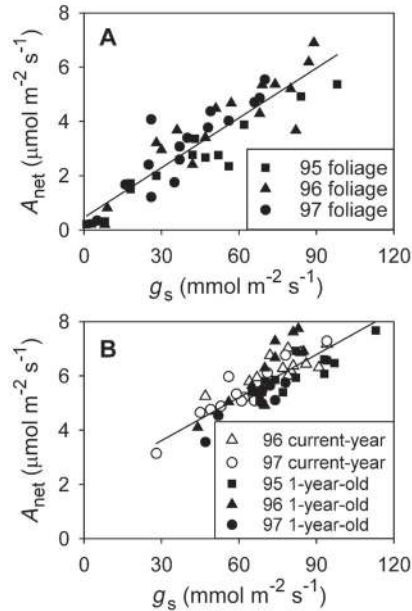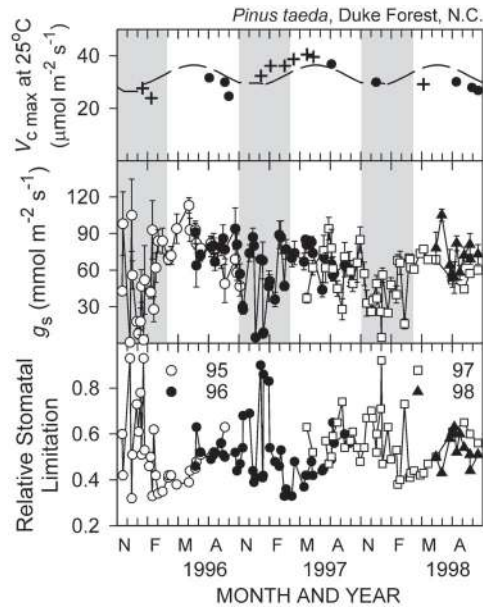
Figure 4. Time course of seasonal pattern of carboxylation capacity ($V_{c,max}$), stomatal conductance (g_s), and calculated relative stomatal limitations to CO_2 assimilation in upper-crown *Pinus taeda* needles over 36 months at Duke Forest, NC. Different year classes of needles are indicated by different symbols as in Figure 1, and months are designated as in Figure 1. The arbitrary dashed line drawn in the upper panel was used to estimate carboxylation capacity over the year for calculations of stomatal limitation (bottom panel).

Figure 5. Stomatal conductance (g_s; A, B) and predawn water potential (Ψ; C, D) during winter (left; panels A, C) and summer (right; panels B, D) in *Pinus taeda* as a function of environmental conditions. A. Stomatal conductance was significantly correlated with daily minimum temperature for temperatures < 5 °C in both years. A single regression line was fit to the data for nighttime T_{air} < 5 °C ($y = 65.7 – 4.9x$, $r^2 = 0.65$ for both years pooled). B. Stomatal conductance in current-year needles was significantly related to soil water potential (soil Ψ_m) during drought in 1997 ($\square$), but not during the less severe drought in 1998 ($\blacktriangle$). The curve shown is $y = 76.8 – 8.2x – 2.4x^2$ ($r^2 = 0.62$) and is fit to the 1997 data only. C. Relationship between predawn Ψ and nighttime minimum T_{air}. Data for 1996 are shown because predawn Ψ data were not collected in winter 1997. D. Declining predawn Ψ during the soil drying cycle (soil Ψ_m) in 1997. There was no significant relationship between predawn Ψ and soil Ψ_m in 1998.

mimima were sub-freezing or close to freezing ($r^2 = 0.68$, $P < 0.0001$; Figure 5). In 1996 and 1997, g_s declined more than fourfold as daily minimum temperature varied from 4 to –10 °C (Figure 5). The daily minimum temperature generally occurred at predawn. In winter, predawn Ψ was also strongly correlated with minimum overnight temperature ($r^2 = 0.88$, $P < 0.0001$), and low predawn Ψ (less than –1.5 MPa) was indicative of severe water stress on the coldest mornings. In current-year foliage in summer, g_s at maximum A_{net} varied significantly with soil Ψ_m over the drought cycle in 1997 ($r^2 = 0.60$, $P < 0.001$), although g_s was not correlated with soil Ψ_m in 1998 (Figure 5). Summertime predawn Ψ was related to soil Ψ_m during the early period in the dry-down cycle, but as soil Ψ_m reached –0.5 MPa, predawn Ψ declined no further.

A_{net} integrated over time

Diurnal patterns in A_{net} were sinusoidal in all seasons of the year (Figure 6), although during the drought in August 1997 the pattern was skewed, with higher A_{net} in morning hours. Net CO_2 assimilation at the top of the canopy was light saturated at all times except in early morning and late evening. Daily maximum A_{net} occurred at noon or slightly before noon in summer, and in mid-afternoon on cold days (maximum T_{air} < 15 °C; November). Midday depression of A_{net} was only observed on one occasion in summer (Figure 6). On the coldest diurnal measurement day (November 1997), positive A_{net} at light saturation was not measured until noon when T_{air} exceeded 5 °C,
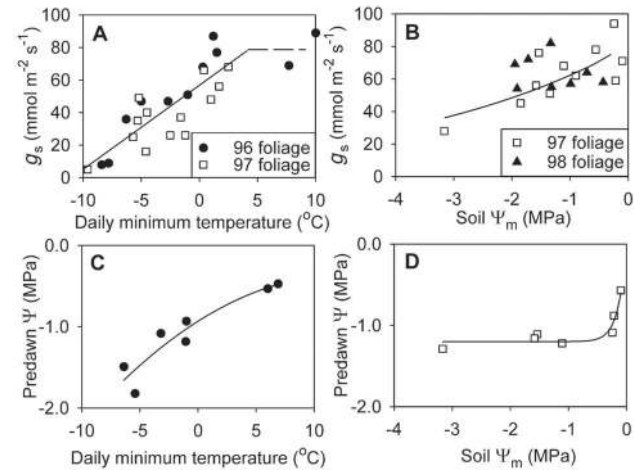
consistent with the seasonal sensitivity of A_{net} to temperature (Figure 2). The A_{day}, calculated by integrating the area under the diurnal A_{net} curves, was highly significantly related to maximum A_{net} (Figure 7A). There was a tendency for this relationship to depart from a zero intercept at low A_{net}, although a single cold day with low A_{net} was influential. Modeled A_{day} was closely related to A_{day} calculated from the diurnal courses (Figure 7B), suggesting that the model was a good approximation of the true A_{day} even on days when the diurnal pattern of A_{net} was skewed (Figure 6). During the drought, when midday depression of A_{net} occurred, A_{day} was overestimated by as much as 24%, indicating that the impact of drought on annual carbon gain may be underestimated, depending on the frequency of midday depression. The close relationship between predicted and measured A_{day} (Figure 7B) may have resulted from the close relationship between A_{net} and A_{day} (Figure 7A), although the relationship did not significantly depart from the 1:1 line as might be expected from the non-zero relationship in Figure 7A.

Predicted A_{day} for upper-canopy leaves on sunny days over the year had the expected parabolic pattern (Figure 8), indicating the importance of day length on seasonal carbon assimilation. Two age classes of foliage account for most carbon assimilation in *P. taeda*, because mean needle longevity at the top of the canopy is 19 months for first flush foliage in this region. The model of A_{day} over the year (Figure 8) predicts that,
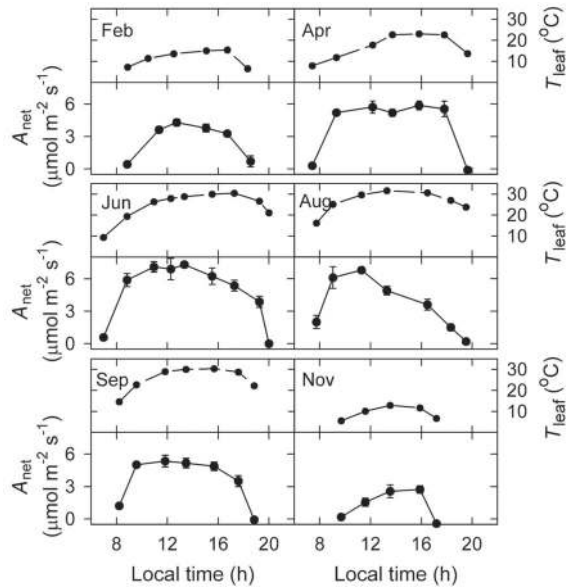
Figure 6. Typical diurnal courses of A_{net} in *Pinus taeda* foliage at the top of the canopy measured on sunny days at different times of year, shown with the measurement T_{leaf}. Diurnal courses of A_{net} in 1-year-old needles are for February (day of year (DOY) 32), April (DOY 98), and June (DOY 161), and diurnal courses in current-year needles are for August (DOY 220), September (DOY 273) and November (DOY 322). The measurements were made in direct sun and were light saturated (PFD > 800 µmol m^{-2} s^{-1}) at all times except for the first and last measurements in the diurnal cycle.

Figure 7. A. Daily integrated A_{net} (A_{day}) obtained from diurnal courses of A_{net} for upper-crown needles of *Pinus taeda* compared with maximum A_{net} for the same day. The solid line is the least-squares regression ($y = -77.0 + 43.0x$, $r^2 = 0.92$, $P < 0.0001$) and the dashed line is the regression forced through the intercept. B. Daily integrated A_{net} predicted by a simple model of the diurnal course of photosynthesis (predicted A_{day}) as a function of measured daily integrated A_{net} for data shown in A. The relationship shown is $y = 0.95x$, and the intercept term was not significant ($P > 0.10$).

on sunny days, current-year needles produce 51% of the annual carbon gain (Table 2), even though the effective presence of current-year needles is 60% of that of 1-year-old needles (e.g., late June–December versus January–October). However, this estimate is conservative because young expanding needles may exceed photosynthetic compensation before they reach 50% full expansion, as used here. Nevertheless, this phenological stage of photosynthetic compensation in *P. taeda* is consistent with data reported by Radoglou and Teskey (1997) and Higginbotham (1974).

There was considerable year-to-year variation in carbon gain in the upper-canopy foliage on sunny days that could be attributed to climate. Carbon gain integrated for current-year needles during sunny days in the growing season in 1997, which included a severe summer drought (Table 1; Ellsworth 1999), was 10% lower than in 1996, a wetter but cloudier year. Excluding the hurricane-induced rainfall on a single day in 1996, total rainfall for May–September measured 7 km from the study site was 33% lower in 1997 than in 1996. In over-wintering 1-year-old needles, carbon gain integrated over winter 1997 was 21% greater than in the previous, cooler winter (2 °C below the 30-year mean temperature, Table 1). However, photosynthesis during the winter (shaded areas in Figure 8) accounted for about 15% of the total estimated carbon gain for each foliage age class (Table 2). Thus, on an annual basis, the warm winter of 1997 (Table 1) yielded small increases in total carbon gain compared with the cold winter of 1996.
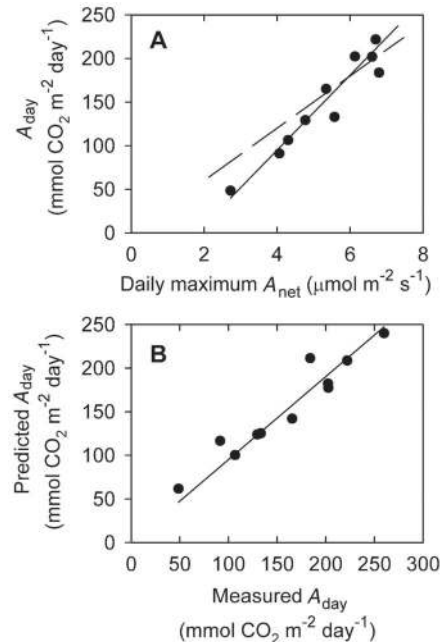
Discussion

The integration of A_{net} over daily and annual time scales provides a means of evaluating effects of climatic events and physiological limitations on long-term CO$_2$ exchange of forest canopies. An extended period of favorable climate should contribute to the annual carbon assimilation of *P. taeda* and enable this species to be one of the most productive conifers in North America (Teskey et al. 1987, Schulz 1997). In the studied *P. taeda* forest, A_{net} on sunny days was within 70% of the peak annual maximum A_{net} in the upper canopy in all months of the year except February (Figure 1). Thus, photosynthesis during frequent warm winter days as well as during the growing season contributed to annual carbon assimilation in the upper crown of *P. taeda*.

Annual carbon assimilation in over-wintering 1-year-old needles was substantial in the second growing season (Table 2), but A_{net} declined with time as current-year needles began to mature. Net assimilation was highest in 1-year-old needles in late spring when shoot growth was greatest (Figure 1), and carbon assimilation is important for supporting large growth sinks in developing shoots and needles. The 1-year needle age class also has 50% greater integrated carbon gain over the entire growing season than current-year needles, and comprises roughly half of the leaf area of mature *P. taeda* during the
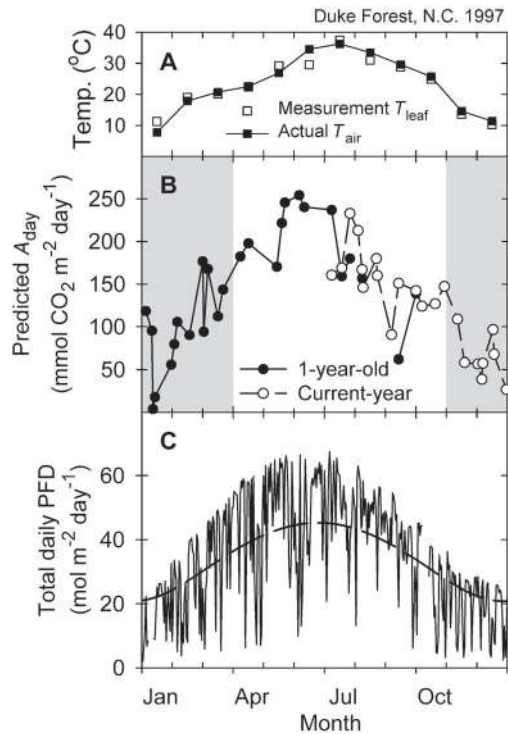
Figure 8. Seasonal course of temperature (Temp.; A), A_{day} for sunny days (B) and total PFD (daily sum; C) over the course of a year in Duke Forest, NC. A. Measurement T_{air} (□) is shown with the mean daily maximum T_{air} (■) for all sunny days throughout the year. B. Predicted A_{day} on sunny days for 1-year-old (●) and current-year (○) needles of *Pinus taeda* over 42 days of the year when needles were at least 80% fully expanded and non-senescent. Shaded areas are as in Figure 1. C. Total daily PFD (continuous line) over the year, and 70% of potential daily PFD assuming no clouds (broken line), which was used to define the sunny days when most photosynthesis occurred. Based on this definition, 222 out of the 354 days with full PFD records in 1997 were sunny.

Table 2. Predicted daily CO_2 assimilation (A_{day}) for sunny days integrated over the growing season and the winter for different needle age classes. The A_{day} integral for the specified period was discounted according to the proportion of sunny days (see Figure 8). Seasonal A_{day} in 1-year-old needles was evaluated during winter and over the growing season (frost-free period). The winter period was defined as January 1–March 31, and the growing season was from April 1 to October 31, corresponding to a 215-day season (unshaded area in Figure 8B). Current-year needle integrated A_{day} was evaluated from June 23 (50% maximum needle length) to October 31 (131-day season).

Needle age class	Period of study	Number of sunny days	Seasonal A_{day} integral (g C assimilated m^{-2} leaf)
1-year-old	Winter 1996	45	45
1-year-old	Growing season 1996	140	278
1-year-old	Winter 1997	44	55
1-year-old	Growing season 1997	155	305
Current-year	Growing season 1996	85	191
Current-year	Growing season 1997	94	173

rainy summer of 1996 and the summer drought of 1997, except that accounted for by the increased proportion of sunny days. In contrast to the 1-year-old needles, current-year needles were more sensitive to late summer drought, partly because these needles reach full expansion when drought is most common in this region. Ellsworth (1999) estimated that the 1997 summer drought reduced current-year A_{day} by 45% on sunny days in *P. taeda*, and carbon assimilation on sunny days integrated over the growing season was reduced by 10% compared with that in the wetter year of 1996 (Table 2). Although the estimated impacts of warming in winter and drought in summer are only applicable for sunny days, they provide an indication of how climatic conditions related to year-to-year changes in regional temperatures and precipitation (Graham 1995, Dai et al. 1998) may affect CO_2 assimilation in the upper crown.

The sum of A_{net} over time is a major determinant of carbon storage, but it is difficult to estimate for forest trees (Ford and Teskey 1991). The empirical approach used here to estimate annual leaf CO_2 assimilation does not represent total CO_2 assimilation because CO_2 assimilation on cloudy days was not considered. The proportion of sunny days is probably an important factor affecting annual CO_2 assimilation (Clark et al. 1999), and in this study a portion of the difference in growing season C gain between 1996 and 1997 could be attributed to the lack of consideration of A_{day} in cloudy conditions. Evidence from limited measurements during the growing season on uniformly cloudy days suggests that A_{day} is 30–40% of A_{day} for sunny days (Ellsworth, unpublished data). Also, the approach used here assumes that midday depression of A_{net} is infrequent or small, which is supported by the analysis of 12 diurnal courses of measurements (Figure 7B). Given the potential shortcomings of approaches for extrapolating leaf-scale measurements in time to estimate annual leaf carbon assimilation, seasonal variation in A_{net} under sunny conditions

growing season (Kinerson et al. 1974). Thus, over the period of winter to early summer, A_{net} may be more important in supporting growth of early wood and current-year needles than stored carbohydrates in *P. taeda*. This hypothesis is supported by a ^{14}C-labeling study on *Pinus sylvestris* L. showing that recently assimilated carbon from 1-year-old needles is allocated to bud growth in spring (Hansen et al. 1996). Thus, knowledge of year-to-year variation in seasonal integrated A_{net} and the sensitivity of A_{net} to adverse climate events may provide important mechanistic explanations for differing growth dynamics among years.

Year-to-year variability in A_{net} has rarely been quantified in long-term studies, although large inter-annual differences in CO_2 uptake by forest canopies have been observed (Goulden et al. 1996, Clark et al. 1999). Despite a 2.5 °C difference in daily maximum winter temperatures between 1996 and 1997, there was only a small difference in overall annual integrated carbon assimilation estimated for 1-year-old needles (Table 2). Similarly, there was little difference in seasonal integrated carbon assimilation estimated for 1-year-old needles between the

provides a means of examining mechanisms underlying year-to-year variability and climatic sensitivity of forest CO$_2$ exchange (Baldocchi et al. 1997, Goulden et al. 1997).

Winter carbon assimilation was estimated to contribute 15% of annual carbon gain in the upper crown (Figure 8, Table 2). Although the winter climate is relatively mild in the piedmont of North Carolina compared with northern temperate regions, there are several reasons why winter carbon assimilation is not more substantial in *P. taeda*. First, short day lengths limit the opportunities for daily carbon assimilation, even at this latitude. Second, it is possible that there is biochemical inactivation of photosynthetic enzymes or a decrease in enzyme pools caused by extended cold periods that precondition photosynthesis (cf. Strain et al. 1976). Depression of photosynthetic capabilities in winter and spring has been observed for northern temperate and boreal conifers, associated with long-term changes in the photosynthetic apparatus related to the prolonged influence of unfavorable conditions (Jurik et al. 1988, Hansen et al. 1996, Schwarz et al. 1997). However, little apparent physiological dormancy was observed in the present study even though the site is in the northern part of *P. taeda*'s range, because A_{net} was high whenever temperatures were favorable (Figure 1), and carboxylation capacity at a standard temperature was relatively constant throughout the year (Figure 4). Given the temperature dependence of A_{net} implied by Equation 2, it is not necessary to assume cold preconditioning beyond the immediate day of measurements to account for low winter A_{net}.

Understanding potential impacts of climate change on A_{net} and forest CO$_2$ uptake requires an understanding of temperature sensitivity under current conditions; however, data for multiple years and with better annual coverage than monthly measurements are lacking. Strain et al. (1976) concluded that *P. taeda* foliage showed strong seasonal changes in the temperature optimum of A_{net}, although their data included only a few days of winter measurements. In contrast, the maximum monthly A_{net} at light-saturation (Figure 1) as well as the seasonal A_{net}–C_i curve data corrected to a common temperature (Figure 4) showed relatively little seasonal variation. This suggests that temperature acclimation of the photosynthetic apparatus in *P. taeda* foliage throughout the year is small. However, A_{net} at light saturation in winter months closely followed the midday temperature from day to day (Figures 1 and 2). Understanding the effect of temperature on carbon gain as well as carbon losses is important for predicting effects of climate warming on forest species (Dewar et al. 1999).

In winter, large relative stomatal limitations to A_{net} were calculated and probably occurred as a result of cold-induced stomatal closure (Figures 4 and 5). Stomatal closure may restrict winter A_{net} in *P. taeda* (Teskey et al. 1987), although lower biochemical capacity for carboxylation in winter must also be considered (see $V_{c,max}$ in Figure 4A). In conifers, stomatal limitation at low temperatures is a result of freeze-induced water stress (Figure 5; Smith et al. 1984) or hydraulic limitations resulting from the high viscosity of water near 4 °C (Kramer 1940, Day et al. 1991), or both. No midday A_{net} was

observed on sunny days when maximum temperatures were below 5–6 °C (Figure 1) because of stomatal closure (Figures 4 and 5). However, such days were rare (5–7 days in winter 1997–98). Thus, stomatal closure (Teskey et al. 1987; Figure 5) associated with freeze-induced water stress and subsequent limitations to A_{net} (Figure 4) is a major factor reducing CO$_2$ assimilation in winter and hence the contribution of winter A_{net} to annual carbon assimilation. Efforts to predict climate-warming responses of *P. taeda* and temperature acclimation of needles must consider the importance of stomatal constraints on A_{net} in winter months. If climate warming is associated with increased variability in precipitation (Dai et al. 1998), stomatal responses will provide increasing limitations on A_{net} in summer as well (Figure 4, Figure 5C and 5D).

Drought induced limitations on A_{net} and seasonal carbon gain in *P. taeda* in both summer and winter (Figure 5), and in both cases these limitations were mediated by stomata (Figure 4). Relative stomatal limitations in *P. taeda* over the entire growing season were smaller than the stomatal limitations estimated by Kubiske and Abrams (1993) from instantaneous measurements for 19 hardwood species during a drought event. Despite a long season of near-peak A_{net} in the upper crown of the *P. taeda* trees and the capacity for peak rates of photosynthesis in most months of the year, stomata imposed significant physiological limitations on annual net CO$_2$ assimilation on sunny days throughout the year. The greatest stomatal limitations occurred when water supply was restricted because of low temperatures in winter or low soil water content in summer, both of which decrease xylem water potential. Thus, both stomatal behavior and environmental responses need to be considered when predicting seasonal and annual carbon assimilation and gross primary productivity in *P. taeda* forest canopies. Understanding the sources of limitations to canopy CO$_2$ assimilation capacity over the year in evergreen forest trees will improve assessments and models of how environmental variability affects annual carbon assimilation from the atmosphere.

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