LONG-TERM RESPONSE OF PHOTOSYNTHESIS TO ELEVATED CARBON DIOXIDE IN A FLORIDA SCRUB-OAK ECOSYSTEM

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Abstract. The response of photosynthesis was analyzed during canopy closure in a Florida scrub-oak ecosystem exposed to elevated $[CO_2]$ (704 μmol CO_2/mol air; concentration of CO_2). The species were measured on six occasions, covering different seasons, during the third and fourth year of exposure to elevated $[CO_2]$. The entire regrowth cycle of this community has been under elevated $[CO_2]$, providing a rare opportunity to assess the differential responses of species during the critical phase of canopy closure. Measurements were taken in order to determine both season-specific and species-specific differences in the response of photosynthesis to elevated $[CO_2]$. Photosynthesis was measured with an open-gas exchange system, and in vivo rates of Rubisco carboxylation ($V_{c,max}$) and electron transport (J_{max}) were derived to assess changes in the photosynthetic capacity in the codominant, evergreen oak species. *Quercus myrtifolia* did not show any change in photosynthetic capacity with prolonged exposure to elevated $[CO_2]$ during any season, and as a result the increase in photosynthesis due to the increased supply of CO_2 was sustained at 72%. The codominant, *Q. geminata*, showed a loss of photosynthetic capacity with growth at elevated $[CO_2]$, such that during most measurement periods light-saturated photosynthesis in leaves grown and measured at elevated $[CO_2]$ was no higher than in leaves grown and measured at ambient CO_2. A third oak, *Q. chapmanii*, showed a response similar to that of *Q. myrtifolia*. This suggests that at the critical phase of canopy closure in a woody community, elevation of $[CO_2]$ causes a species-dependent and time-dependent change in the capacity of the codominants to acquire carbon and energy.

Key words: canopy closure; climate change; elevated CO_2; Florida scrub oak; intergeneric variation; interspecific variation; photosynthesis; Quercus; *Rubisco.*

INTRODUCTION

Despite a plethora of studies of the effects of elevated $[CO_2]$ (concentration) on photosynthesis of trees, very few have considered effects on mature dominant tree species at canopy closure in natural stands. We are not aware of any other experimental studies that have considered multiple species at this stage of development in a natural forest system during different seasons. How elevated $[CO_2]$ affects photosynthesis and whether codominants are affected differentially is a critical step in understanding whether the function and structure of these ecosystems will be affected by elevated $[CO_2]$ in the long term. Recent meta-analyses and reviews of the response of trees to rising $[CO_2]$ have reported significant increases in photosynthesis in trees grown under elevated $[CO_2]$ (Ceulemans and Mousseau 1994, Gunderson and Wullschleger 1994, Drake et al. 1997, Curtis and Wang 1998, Medlyn et al. 1999, Norby et al. 1999). However, the majority of the studies are performed with young trees and seedlings that are physiologically very

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different from trees at and beyond canopy closure (Ward and Strain 1999). The loss of photosynthetic capacity under elevated $[CO_2]$ is commonly linked to an inability of the plant to utilize additional photosynthate (Drake et al. 1997). This is less likely to occur in rapidly growing seedlings or saplings than in closed canopy stands. Therefore, there can be little certainty that the findings of elevated $[CO_2]$ effects obtained with these juvenile stages of trees will have relevance to natural closed canopies (Norby et al. 1999, Lee and Jarvis 1995). If trees that develop at elevated $[CO_2]$ have stimulated photosynthesis sustained over time and integrated over the entire canopy, then those forests show the potential to assimilate, but not necessarily sequester, increasing amounts of atmospheric CO_2. If, on the other hand, down-regulation of photosynthesis limits increases in leaf area and biomass, carbon assimilation may not change from current levels.

As any forest matures, canopy closure increases competition for light and space between individuals and species. In forest ecosystems, competition and abiotic factors can equally limit productivity (Barton 1993). It is unknown whether a physiological advantage gained at elevated $[CO_2]$ for an individual or species growing in isolation will be amplified or dimin-
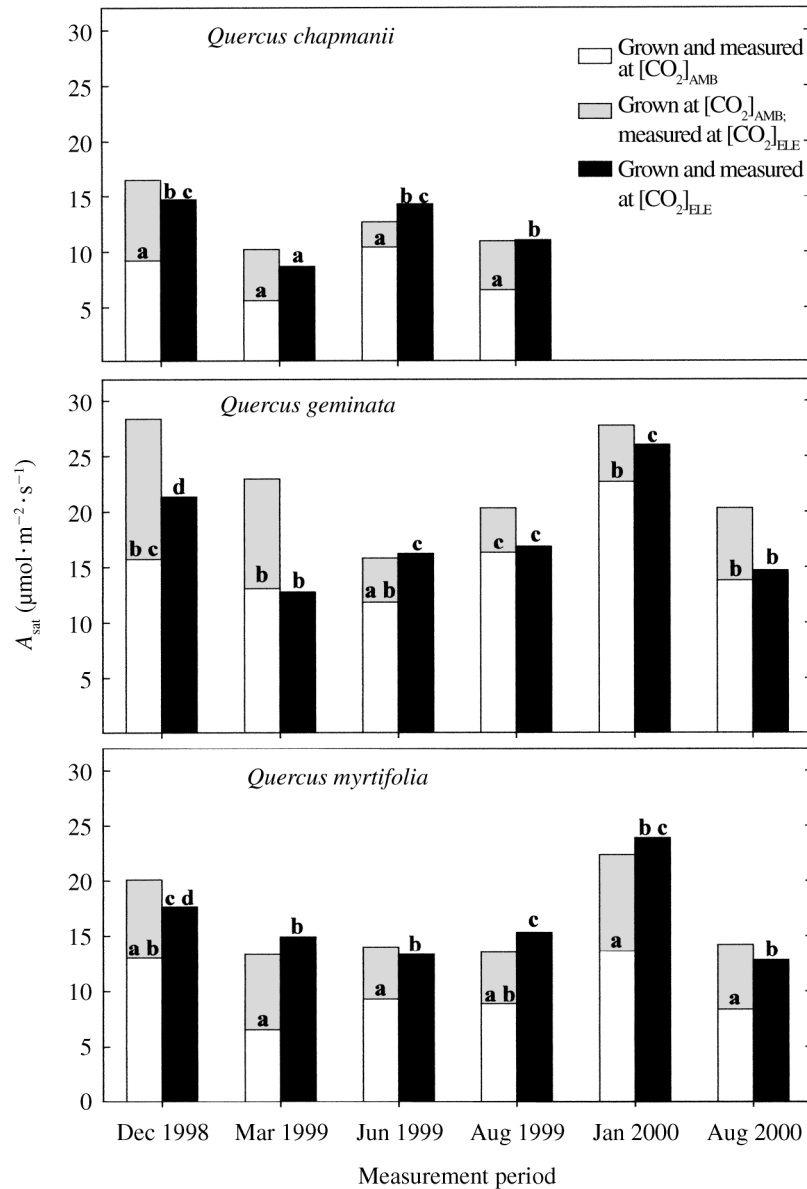
FIG. 1. The immediate response (white vs. gray bars) and long-term response (white vs. black bars) of photosynthesis to elevated [CO_2]. Preplanned comparisons of light-saturated rate of CO_2 uptake (A_{sat}) at growth [CO_2] were determined within each measurement period by least-square means (SAS "Proc Mixed," SAS Institute, Cary, North Carolina). Statistically significant differences ($P < 0.05$) within each measurement period only are indicated by lowercase letters. The results of the statistical analysis of A_{sat} measured at growth [CO_2] are shown in Table 3.

ished under conditions of competition. Current evidence shows that trees grown under different degrees of competition have contrasting responses to elevated [CO_2]. For example, birch showed opposing responses to growth at elevated [CO_2] depending upon seedling density, and therefore the degree of competition between individuals (Bazzaz et al. 1995). In addition to competition among individuals, competition among species is also important. Analysis of one species within a mixed community ignores the potential of natural selection for species that take maximum advantage of

elevated [CO_2]. If competition does amplify a physiological advantage gained by one species, then the pressure for natural selection will increase. In this case, the long-term response of a community will not be the mean response of the current species, but that of the species with the largest response to elevated [CO_2]. Studies of mixed-grassland species have predicted that species composition in natural plant communities is likely to change at elevated [CO_2] (Jongen and Jones 1998, Lüscher et al. 1998); however, similar studies have not been performed in forest ecosystems. Cur-

TABLE 1.   Dates of photosynthetic measurements and replicate numbers of A/c_i curves.

Measure-ment period	Measurement dates	Daily temperature (°C)			Precipita-tion† (mm)	Number of A/c_i curves‡		
		Mean	Maxi-mum	Mini-mum		Q. chap-manii	Q. gemi-nata	Q. myrti-folia
Dec 1998	9–15 Dec	18.0	20.7	10.7	9.1	8	8	8
Mar 1999	20 Mar–5 Apr	20.0	31.9	9.8	25.0	10	9	12
Jun 1999	24 May–24 Jun	25.0	35.5	17.8	119.9	9	64	64
Aug 1999	18–25 Aug	28.1	39.3	21.2	190.0	13	16	16
Jan 2000	21 Dec–15 Jan	14.9	24.4	7.1	62.6	0	60	59
Aug 2000	5–24 Aug	27.4	37.5	20.4	7.9	0	56	56

† Total precipitation for the entire calendar month in which measurements were taken.
‡ Number of curves taken per species in each measurement period.

rently the response of codominants to rising [CO_2] dur-ing canopy closure is largely unknown. Moreover, the few studies which examine co-occurring trees have, for logistical reasons, failed to follow the photosynthetic response through to maturity (Norby et al. 1999).

Another limitation to understanding future carbon exchange of forests is determining if and how time or season will modify responses to elevated [CO_2]. Leaf age, leaf developmental stage, weather, and cumulative exposure to [CO_2] are factors that alter results of ex-periments that analyze the effects of elevated [CO_2] on trees (Bazzaz et al. 1995, Lee and Jarvis 1995, Lewis et al. 1996, Turnbull et al. 1998, Jach and Ceulemans 2000, Li et al. 2000). It is therefore critical to make measurements over an entire growing season in order

to characterize changes in photosynthesis associated with elevated [CO_2] and accurately predict the long-term responses of forest systems to rising [CO_2] (Turn-bull et al. 1998).

This study, performed in a Florida scrub-oak com-munity, analyzes the long-term consequences of ele-vated [CO_2] on photosynthesis of subtropical oak spe-cies. The majority of global change studies of forests have been carried out in temperate ecosystems, while tropical and subtropical ecosystems are vastly under-represented. It is currently unknown how the different climate and growth cycle of a subtropical system will modify the photosynthetic response to elevated [CO_2]. Because the scrub-oak community has a short life cy-cle, burning every ~10 yr, (Schmalzer and Hinkle 1992) it is possible to examine the response to elevated [CO_2] over the entire burn cycle of the ecosystem. A second advantage of the scrub-oak community is its low stature, which facilitates the enclosure of numerous individuals inside an open-top chamber. Additionally, the scrub-oak community accommodates sixteen cham-bers, eight at a current [CO_2] level and eight at an elevated [CO_2] level, which provide high statistical power for the experiment.

This study tests the hypothesis that there is signifi-cant interspecific and seasonal variation in three scrub-oak species in the ability of photosynthesis to respond to rising [CO_2] at canopy closure. Photosynthetic ac-climation, a change in photosynthetic capacity with growth in elevated [CO_2], was evaluated by measuring the response of light-saturated photosynthesis to chang-es in intercellular CO_2 concentration in the three dom-inant Florida scrub-oak species. Acclimation is defined as a phenomenon whereby living organisms adjust to the present environmental conditions, where adjust-ments are on the time scale of less than one generation (Calow 1998). Acclimation of photosynthesis is gen-erally said to occur when plants grown in elevated [CO_2] have lower photosynthetic rates than plants grown in ambient [CO_2], when compared at a common [CO_2] (Long 1991). Acclimation to elevated [CO_2] most commonly involves a decrease in photosynthetic
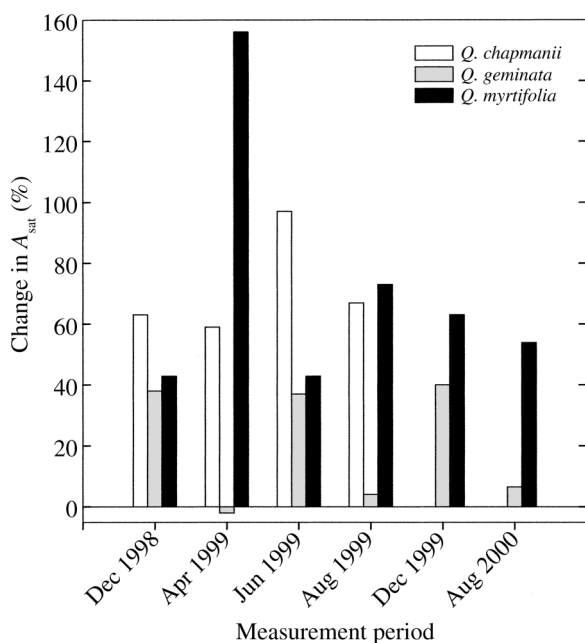

FIG. 2.   Interspecific variation in percentage change of net photosynthetic rates in response to [CO_2] enrichment for three Quercus species. Percentage change in photosynthetic rate, measured at growth [CO_2], due to elevated [CO_2] [((A_{satELE} − A_{satAMB})/A_{satAMB}) × 100] is shown.

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TABLE 2. [CO$_2$], RubP-saturated carboxylation, and electron transport values.

Species and parameter	[CO$_2$] (µmol CO$_2$/mol air)	Measurement period (mean ± 1 SE [n])		
		Dec 1998	Mar 1999	Jun 1999
Q. chapmanii				
$V_{c,max}$	379	53.4 ± 5.7 (3)	30.4 ± 6.6 (4)	49.9 ± 5.2 (4)
	704	45.5 ± 5.7 (4)	23.9 ± 5.2 (4)	50.1 ± 6.6 (4)
J_{max}	379	130.6 ± 9.5 (3)	74.0 ± 10.9 (4)	86.4 ± 8.5 (4)
	704	114.24 ± 9.5 (4)	66.88 ± 8.5 (4)	110.7 ± 10.9 (4)
Q. geminata				
$V_{c,max}$	379	86.6 ± 5.7 (4)*	66.6 ± 3.8 (4)*	62.7 ± 4.1 (8)
	704	65.0 ± 6.6 (4)*	29.9 ± 4.8 (4)*	63.9 ± 4.1 (8)
J_{max}	379	220.3 ± 9.5 (4)*	156.9 ± 6.3 (4)*	105.1 ± 6.8 (8)
	704	168.6 ± 10.9 (4)*	96.9 ± 8.0 (4)*	104.6 ± 6.8 (8)
Q. myrtifolia				
$V_{c,max}$	379	65.8 ± 6.6 (4)	36.7 ± 5.2 (6)	55.4 ± 4.1 (8)
	704	53.7 ± 5.7 (4)	44.6 ± 5.2 (6)	49.6 ± 4.1 (8)
J_{max}	379	149.2 ± 10.9 (4)	96.3 ± 8.5 (6)	97.2 ± 6.8 (8)
	704	125.5 ± 9.5 (4)	107.5 ± 8.5 (6)	93.1 ± 6.8 (8)

Notes: Definitions are as follows: $V_{c,max}$, maximum rate of Ribulose-1,5-bisphosphate saturated carboxylation in vivo; J_{max}, maximum in vivo rate of electron transport contributing to RubP regeneration. All values except [CO$_2$] (concentration) are given as means ± 1 SE, with sample size per species per measurement period, i.e., number of replicate chambers, in parentheses. Parameters are calculated from A/c_i response curves.

* Significant difference within each species and measurement period only ($P < 0.05$).

capacity resulting from a decrease in the maximum rate of Rubisco carboxylation ($V_{c,max}$) and/or a decrease in the maximum rate of electron transport contributing to Ribulose-1,5-bisphosphate (RubP) regeneration (J_{max}).

This study extends earlier work in the Florida scrub-oak ecosystem conducted after three months of exposure to elevated CO$_2$. Earlier findings showed acclimation of photosynthesis in *Q. myrtifolia*, but not *Q. geminata*, at a very early stage of regrowth in August 1996 (Li et al. 1999). More recent findings have also shown that growth at elevated [CO$_2$] resulted in increased leaf-area index (G. J. Hymus, *unpublished data*), increased belowground biomass (Day et al. 1996), species-specific increases in aboveground biomass (Dijkstra et al. 2002), and no change in respiration (Hymus et al. 2002). With no decline in leaf area and no variation in respiration with growth at elevated [CO$_2$], photosynthesis is the critical process that will then determine whether the ability to acquire carbon and energy will change under elevated [CO$_2$]. This current study evaluates the long-term and seasonal response of the system as canopy closure commences, during the third and fourth years of exposure to elevated [CO$_2$].

METHODS

Experimental site and CO$_2$ fumigation

This experiment was conducted on the Merritt Island Wildlife Refuge at Cape Canaveral, on the east coast of central Florida, USA (28°38′ N, 80°42′ W). The vegetation at the site is Florida scrub, a pyrogenic, xeromorphic shrub community dominated by perennial evergreen oaks (Myers 1990). *Quercus myrtifolia* Willd., *Quercus geminata* Small, and *Quercus chapmanii* Sarg. dominate the biomass at the experimental site. These perennial evergreen oak species remain

green until winter (~February) when they begin to senesce (Li et al. 2000). By early spring (late March to April), one-year-old leaves have fully senesced and the current year's leaves have flushed. Although the majority of leaves live one year, i.e., from April until early February of the following year, a few leaves form later and similarly senesce at a different time (P. Stiling, *personal communication*).

The experimental site was burned in August 1995 and again in December 1995 through January 1996 to simulate the natural fire cycle. The chamber sites were established in May 1996 when all regrowth was cut to the ground prior to treatment with CO$_2$. Sixteen octagonal open-top chambers, 3.5 m in diameter and 3.7 m high, constructed with PVC framework and covered with square panels of mylar (Melinex 071, Courtaulds Performance Films, Virginia, USA) were used to enclose areas of vegetation. The chambers were divided equally among two [CO$_2$] (concentration) treatments: eight chambers were continuously elevated to a day- and nighttime [CO$_2$] of 704 µmol CO$_2$/mol air ([CO$_2$]$_{ELE}$) and eight remained at the current ambient [CO$_2$], 379 µmol CO$_2$/mol air ([CO$_2$]$_{AMB}$). These are the mean [CO$_2$] levels from the continuous measurements conducted since treatment was imposed. Elevated [CO$_2$] was monitored by an infrared gas analyzer (Licor 6262, LICOR, Lincoln, Nebraska, USA) with pure CO$_2$ added to the air stream and blown into chambers at a constant rate.

Photosynthetic gas exchange

The specific dates of photosynthetic measurements and replicate number of A/c_i curves analyzed are shown in Table 1. Leaves that flushed in the spring of 1998 were measured during the December 1998 and March 1999 measurement period. June 1999, August 1999,

Table 2. Extended.

Measurement period (mean ± 1 SE [n])		
Aug 1999	Jan 2000	Aug 2000
51.5 ± 4.1 (6)	...	...
46.5 ± 4.4 (6)	...	...
82.7 ± 6.8 (6)	...	...
85.5 ± 7.3 (6)	...	...
98.4 ± 4.1 (8)*	86.3 ± 4.1 (8)*	93.2 ± 4.1 (8)*
69.3 ± 4.1 (8)*	66.7 ± 4.1 (8)*	62.5 ± 4.1 (8)*
132.3 ± 6.8 (8)	180.8 ± 6.8 (8)*	149.3 ± 6.8 (8)*
116.2 ± 6.8 (8)	171.8 ± 6.8 (8)*	119.3 ± 6.8 (8)*
58.4 ± 4.1 (8)	74.5 ± 4.1 (8)	72.0 ± 4.1 (8)
62.1 ± 4.1 (8)	68.1 ± 4.1 (8)	62.1 ± 4.1 (8)
101.7 ± 6.8 (8)	154.9 ± 6.8 (8)	112.6 ± 6.8 (8)
117.9 ± 6.8 (8)	156.4 ± 6.8 (8)	110.6 ± 6.8 (8)

and January 2000 measurements were taken on leaves that flushed in the spring of 1999, and August 2000 measurements were taken on leaves that flushed in the spring of 2000. Therefore, measurements of three annual flushes of leaves are discussed in this study.

Leaf CO_2 assimilation rate (A) and stomatal conductance to water (g_s) were determined in response to changes in the intercellular CO_2 concentration (c_i) with a portable, steady-state gas-exchange system, incorporating an infrared gas analyzer (LI-6400, Li-Cor, Lincoln, Nebraska, USA). Photosynthesis was initially induced at the growth [CO₂]. The reference CO_2 concentration was reduced stepwise to a lower concentration of 50 μmol CO_2/mol air and then increased stepwise to an upper limit of 1400 μmol CO_2/mol air. Ten or eleven points were measured in each A/c_i curve. Each stepwise measurement was completed within one or two minutes in order to minimize alteration of the activation state of Rubisco. Leaf temperature was maintained at 25°C with a vapor-pressure deficit in the air of the cuvette of 1.7 ± 0.2 kPa and a photon flux density of 1250 μmol·m⁻²·s⁻¹. Following gas exchange, leaf areas were measured with a leaf area meter (LI-3100, Li-Cor, Lincoln, Nebraska, USA) calibrated with a metal disc of known area.

Gas analysis was conducted on cut leaves sampled predawn and kept at low light prior to measurement. Leaves were sampled from the uppermost canopy and then removed by cutting the petiole underwater. It should be noted that cutting the leaves predawn and analyzing them in the controlled environment of a field laboratory avoided photoinhibition, water stress, or triose-phosphate utilization limitation that might develop over the diurnal course. This method also ensured that differences reflected long-term acclimation rather than short-term diurnal effects. This procedure measured the effect of growth at elevated [CO₂] on potential photosynthetic rates at the dates of measurement. As such, it will show if there is any intrinsic basis for differences between leaf photosynthetic rates in the field.

Calculation of $V_{c,max}$, J_{max}, and c_i/c_a

Photosynthetic parameters were calculated by fitting the equations of Farquhar et al. (1980) and by maximum likelihood regression (Sigmaplot, Jandel Scientific, Erkrath, Germany) according to the method of McMurtrie and Wang (1993). $V_{c,max}$ and J_{max} were calculated from different phases of the A/c_i response plot. $V_{c,max}$ was determined from points below the inflexion of the A/c_i plot and J_{max} was determined from values determined to be above the inflexion. Atmospheric [CO₂] is denoted by the variable c_a. The ratio of internal to atmospheric [CO₂] (c_i/c_a) was calculated at the growth c_a.

CHN measurements

Following leaf gas-exchange measurements in June 1999 and January 2000, one leaf section per chamber was cut and dried to constant mass at 80°C. Total leaf N was determined by thermal conductivity detection following combustion and chemical reduction of leaf material (CE440, Exeter Analytical, N. Chelmsford, Massachusetts, USA). The analyzer was standardized with acetanilide (Exeter Analytical, N. Chelmsford, Massachusetts, USA). Photosynthetic nitrogen-use efficiency (PNUE), the ratio of A_{sat} at the growth [CO₂] to leaf N content (mmol CO_2·mol·N⁻¹·s⁻¹), was then calculated. (A_{sat} denotes light-saturated leaf CO_2 assimilation rate.)

Statistical analysis

For the overall comparison of $V_{c,max}$, J_{max}, and A_{sat} between treatments, a mixed model analysis of variance (ANOVA) was used with time of year (measurement period), species, and treatment as fixed effects and chamber as the random effect. There was no correlation between measurement periods, therefore, a repeated-measures analysis was not required. Because *Q. chapmanii* was not present in all chambers, sample size was not consistent for each species during each time period; therefore, a mixed model ANOVA was used. The total number of A/c_i curves per species and measurement period is given in Table 1. Up to four A/c_i curves per species per chamber (subsamples) were averaged to give a replicate chamber value for $V_{c,max}$, J_{max}, and A_{sat}. All statistics were performed on chamber means. Preplanned pairwise comparisons of estimated means within measurement periods were obtained using the paired differences of means (PDIFF) option on the least squares means (LSMEANS) statement (SAS Institute, Cary, North Carolina, USA; Steel et al. 1997).

Specific leaf area, leaf nitrogen content, and photosynthetic nitrogen use efficiency of *Q. myrtifolia* and *Q. geminata* were determined for one plant per chamber during two measurement periods. A three-way ANOVA was performed with measurement period, species, treatment, and the interaction terms included in the model. Means were separated as described above. All

statistical analyses were performed with SAS software (SAS Institute, Cary, North Carolina, USA) at an alpha level of 0.05.

RESULTS

Light-saturated photosynthesis

Within each species, a seasonal pattern of photosynthesis was evident (Fig. 1). A_{sat} of *Q. chapmanii* grown and measured at $[CO_2]_{AMB}$ was higher in December 1998 and June 1999 than in March 1999 and August 1999. *Q. chapmanii* grown and measured at $[CO_2]_{ELE}$ showed the same trends. *Q. geminata* and *Q. myrtifolia* had similar seasonal patterns of photosynthesis, with the highest absolute rates occuring in the winter (December 1998 and January 2000 measurement periods; Fig. 1). Comparisons of A_{sat} between the species grown at $[CO_2]_{AMB}$ revealed that *Q. geminata* had higher rates of photosynthesis than the codominants, *Q. chapmanii* and *Q. myrtifolia* (Fig. 1, white bars). This trend was observed in each measurement period except June 1999. However, when species were grown and measured at $[CO_2]_{ELE}$, *Q. geminata* did not have higher rates of photosynthesis than *Q. chapmanii* and *Q. myrtifolia*, during most measurement periods (Fig. 1, black bars).

An immediate stimulation of A_{sat}, common to all species and measurement periods, was observed when leaves grown at $[CO_2]_{AMB}$ were measured at $[CO_2]_{ELE}$ (Fig. 1, white vs. gray bars). Growth at $[CO_2]_{ELE}$ (the long-term response of photosynthesis to CO_2) resulted in species- and time-specific patterns of stimulation (Fig. 1, white vs. black bars). When *Q. myrtifolia* grown and measured at $[CO_2]_{ELE}$ were compared with *Q. myrtifolia* grown and measured at $[CO_2]_{AMB}$, a significant increase in A_{sat} was observed in every measurement period. The relative degree of stimulation varied between measurement periods from 37% in June 1999 to >150% in April 1999 (Fig. 2). *Q. chapmanii* also showed sustained increases in photosynthesis with growth at $[CO_2]_{ELE}$, although the 57% stimulation of A_{sat} during March 1999 was not significant, possibly due to the limited sample size during that measurement period. In contrast to *Q. myrtifolia* and *Q. chapmanii*, *Q. geminata* grown at $[CO_2]_{ELE}$ did not show a significant stimulation of photosynthesis during every measurement period (Fig. 2). In March 1999, August 1999, and August 2000, there was no significant difference between *Q. geminata* grown and measured at $[CO_2]_{AMB}$ and *Q. geminata* grown and measured at $[CO_2]_{ELE}$ (Fig. 1).

The A/c_i response indicated that *Q. geminata* grown at $[CO_2]_{ELE}$ had significant in vivo reductions in both Rubisco activity ($V_{c,max}$) and the capacity to regenerate Ribulose-1,5-bisphosphate (RubP) by electron transport (J_{max}), when compared to *Q. geminata* grown and measured at $[CO_2]_{AMB}$ (Table 2). This decrease in photosynthetic capacity in *Q. geminata* grown at $[CO_2]_{ELE}$ accounted for the loss of stimulation of A_{sat} seen in March 1999, August 1999, and August 2000 (Fig. 2). Within *Q. myrtifolia* and *Q. chapmanii*, there was no significant $[CO_2]$ effect on $V_{c,max}$ or J_{max} within any measurement period. Therefore, within those species, $[CO_2]$ did not affect photosynthetic capacity, and as described above, A_{sat} was stimulated during every measurement period.

Leaf nitrogen and photosynthesis

Photosynthetic nitrogen-use efficiency (PNUE) was highest for *Q. myrtifolia* and *Q. geminata* grown at $[CO_2]_{ELE}$ in January 2000 (Fig. 3A, see also Table 4). During January 2000, growth at $[CO_2]_{ELE}$ stimulated PNUE 125% for *Q. myrtifolia* and 50% for *Q. geminata*. The increase in PNUE in *Q. myrtifolia* was caused by a 63% stimulation of A_{sat} and a 37% decrease in leaf N content (Figs. 2, 3B). However, the stimulation in PNUE in *Q. geminata* was caused only by the 40% increase in A_{sat}, as leaf N content was not significantly different between plants grown $[CO_2]_{AMB}$ and $[CO_2]_{ELE}$. The ratio $V_{c,max}/J_{max}$, which indicated the allocation of N to Rubisco capacity relative to electron transport capacity, varied between measurement periods (Table 3). For both ambient and elevated $[CO_2]$ treatments, the $V_{c,max}:J_{max}$ ratio was higher in summer (0.60) than in winter (0.41). Therefore, for both *Q. geminata* and *Q. myrtifolia*, stimulation of PNUE with growth at $[CO_2]_{ELE}$ in January 2000 corresponded to a time period when N was favorably allocated towards electron transport capacity rather than to Rubisco capacity.

Leaf N content, measured on the basis of area, was significantly higher in January 2000 than June 1999, for both *Q. geminata* and *Q. myrtifolia* (Fig. 3B, Table 4). However, within *Q. geminata*, elevated $[CO_2]$ caused a significant decrease in leaf N during June 1999 and not during January 2000. Within *Q. myrtifolia*, leaf N was significantly affected by $[CO_2]$ in January 2000, but not in June 1999. Specific leaf area was not changed with growth at $[CO_2]_{ELE}$ in *Q. myrtifolia*, but was significantly lower in *Q. geminata* grown at $[CO_2]_{ELE}$ and measured in June 1999 (Fig. 3C, Table 4).

DISCUSSION

Lee and Jarvis (1995) concluded that a major limitation in understanding forest responses to atmospheric change is that the majority of work has been done with seedlings, saplings, and juvenile plants during short time periods. Such plants may respond very differently as they mature. This long-term study was performed during canopy closure in a Florida scrub-oak system, which has regrown entirely under elevated $[CO_2]$. The seasonal pattern of photosynthesis in this subtropical system is clearly different from temperate systems, as the absolute rates of photosynthesis were highest in the winter measurement periods (December 1998, January 2000). Leaf N content was significantly lower in older leaves measured in January 2000 than young leaves measured in June 1999, a trend consistent with other
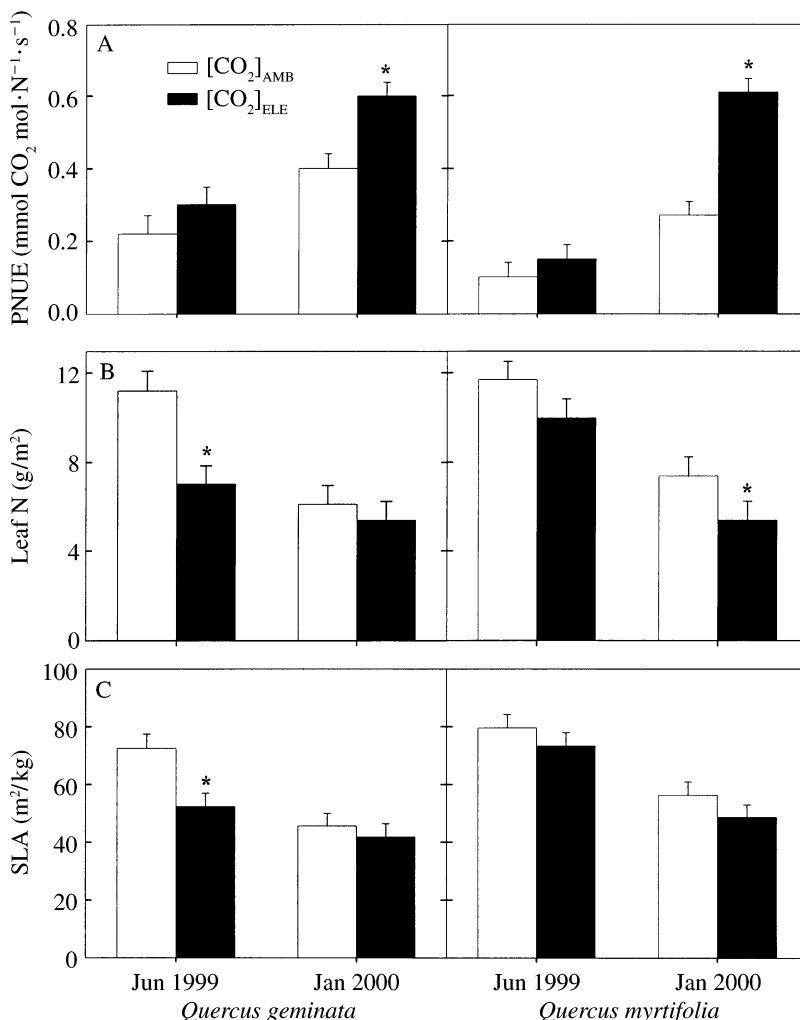
FIG. 3. Photosynthetic nitrogen-use efficiency (PNUE), leaf nitrogen measured on an area basis (Leaf N), and specific leaf area (SLA) of *Q. geminata* and *Q. myrtifolia* measured in June 1999 and January 2000. Means + 1 SE are shown. Preplanned comparisons of the [CO$_2$] effect were determined within each species and measurement period (SAS "Proc Mixed," PDIFF option). An asterisk indicates significantly different ($P < 0.05$) means within a species and measurement period.

reports of these species (Li et al. 2000). Contrary to the idea that the results from studies performed on seedlings are unlikely to translate to mature trees (Lee and Jarvis 1995), the Florida scrub-oak system maintained the stimulation of photosynthesis initially reported for the first three months of the experiment (Li et al. 1999). After three years of exposure to elevated [CO$_2$], there was a mean increase of 53% in A_{sat} for all three oak species. There were, however, significant interspecific differences in the photosynthetic response of codominant oak species grown at [CO$_2$]$_{ELE}$, reinforcing the idea that tree species differ in their response to elevated [CO$_2$] (Lee and Jarvis 1995, Saxe et al. 1998, Norby et al. 1999).

As predicted by the initial hypothesis, growth at elevated [CO$_2$] resulted in significant species- and season-dependent differences in the photosynthetic re-

sponse of *Quercus* codominants in a mature canopy. Rates of photosynthesis equalled or exceeded rates measured for these plants in situ (Hymus et al. 2001) suggesting that our method of sampling did not damage photosynthetic capacity. *Q. myrtifolia* and *Q. chapmanii* grown at [CO$_2$]$_{ELE}$ showed large, consistent increases in A_{sat} throughout the year. This contrasted to *Q. geminata*, which in March 1999, August 1999, and August 2000 showed no stimulation of A_{sat} with growth at [CO$_2$]$_{ELE}$. Analysis of the A/c_i response revealed decreases in $V_{c,max}$ and J_{max} for *Q. geminata* trees grown at [CO$_2$]$_{ELE}$, but no change in $V_{c,max}$ or J_{max} for *Q. myrtifolia* or *Q. chapmanii* (Table 2). The decreases in $V_{c,max}$ and J_{max} in *Q. geminata* were absent in young leaves measured in June 1999, but occurred in all other measurement periods. Similar seasonal patterns of down-regulation of photosynthesis, absent in young leaves,

TABLE 3. Analysis of variance of photosynthetic and gas-exchange parameters measured on three *Quercus* species grown at ambient $[CO_2]$ (379 μmol CO_2/mol air) and elevated $[CO_2]$ (704 μmol CO_2/mol air).

Sources of variation	df	A_{sat} F	A_{sat} P	$V_{c,max}$ F	$V_{c,max}$ P	J_{max} F	J_{max} P	$V_{c,max}/J_{max}$ F	$V_{c,max}/J_{max}$ P
Month	4	53.7	<**0.01**	31.0	<**0.01**	59.6	<**0.01**	7.6	**0.01**
Species	2	56.4	<**0.01**	51.9	<**0.01**	70.1	<**0.01**	1.5	0.24
Month × species	7	2.97	<**0.01**	2.6	**0.01**	4.9	<**0.01**	0.76	0.47
$[CO_2]$	1	107.5	<**0.01**	18.1	<**0.01**	4.4	**0.04**	48.2	<**0.01**
Month × $[CO_2]$	4	2.16	0.06	2.0	0.07	4.2	<**0.01**	3.2	<**0.01**
Species × $[CO_2]$	2	9.03	<**0.01**	15.1	<**0.01**	13.5	<**0.01**	3.3	<**0.01**
Month × species × $[CO_2]$	7	1.88	0.07	3.0	<**0.01**	2.2	**0.03**	1.07	0.39
Error	31								

but developing over the growing season, have been reported for *Pinus sylvestris*, *Betula pendula*, *Pinus taeda*, and *Castanea sativa* (El Kohen and Mousseau 1994, Tissue et al. 1996, Rey and Jarvis 1998, Jach and Ceulemans 2000).

Interestingly, growth measurements of these Florida scrub-oak species correlate with the photosynthetic results. *Q. myrtifolia* and *Q. chapmanii* maintained significant increases in biomass with growth in $[CO_2]_{ELE}$ even after three years of CO_2 treatment (Dijkstra et al. 2002). Elevated $[CO_2]$ had no effect on biomass of *Q. geminata*, the species that had significant down-regulation of photosynthesis during most measurement periods. These reported differences support the idea that interspecific variation and seasonal variation in photosynthesis are critical considerations for accurate modeling of ecosystem responses to rising $[CO_2]$ (Tjoelker et al. 1998). If the response of only *Q. myrtifolia* were incorporated into an ecosystem model of the Florida scrub-oak system, then photosynthesis would be overestimated. Also, if only *Q. geminata* were included, net photosynthesis would be largely underestimated. Similarly, if a model was based on only one measurement period, then the estimate of ecosystem photosynthesis in elevated $[CO_2]$ would not be accurate.

While it has been predicted that in the long term, forests will fail to respond to elevated $[CO_2]$ as other resources, such as nitrogen supply, become limiting to

growth (McGuire et al. 1995), this has not yet been the case in the Florida scrub-oak ecosystem. Both *Q. myrtifolia* and *Q. geminata* showed seasonal increases in PNUE with growth at $[CO_2]_{ELE}$, even after three years of regrowth. Sustained increases in A_{sat} exhibited by *Q. myrtifolia* and *Q. chapmanii* have translated to increased growth in these species, and there is no suggestion that this trend is changing. However, seasonal patterns of down-regulation of photosynthesis in *Q. geminata* may be responsible for the lack of growth response in that species. If *Q. myrtifolia* and *Q. chapmanii* continue to have sustained increases in photosynthesis, translating to sustained increases in growth and biomass, they have the potential to compete better against *Q. geminata* for light and other resources. This indicates that elevated $[CO_2]$ has the potential to alter the fitness of these species, and the future species composition of this ecosytem may be different in an atmosphere with elevated CO_2 concentration.

TABLE 4. Analysis of variance of photosynthetic nitrogen use efficiency, leaf N concentration (measured on an area basis), and specific leaf area of *Q. geminata* and *Q. myrtifolia*.

Sources of variation	Photosynthetic nitrogen use efficiency (PNUE) F	Photosynthetic nitrogen use efficiency (PNUE) P	Leaf nitrogen concentration† F	Leaf nitrogen concentration† P	Specific leaf area F	Specific leaf area P
$[CO_2]$	29.8	<**0.01**	7.6	**0.02**	6.1	**0.03**
Species	11.1	<**0.01**	5.2	**0.03**	14.3	<**0.01**
$[CO_2]$ × species	0.5	0.47	0.3	0.56	0.7	0.41
Month	71.8	<**0.01**	56.4	<**0.01**	50.9	<**0.01**
$[CO_2]$ × month	9.8	<**0.01**	2.3	0.14	1.5	0.23
Species × month	2.32	0.13	1.3	0.27	0.8	0.39
$[CO_2]$ × species × month	2.69	0.11	3.4	0.07	2.3	0.14

Note: There is one degree of freedom for each interaction; error df is 7.
† Measured on the basis of area.

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