

Seasonal variability in the effect of elevated CO₂ on ecosystem leaf area index in a scrub-oak ecosystem

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Abstract

We report effects of elevated atmospheric CO₂ concentration (C_a) on leaf area index (LAI) of a Florida scrub-oak ecosystem, which had regenerated after fire for between three and five years in open-top chambers (OTCs) and was yet to reach canopy closure. LAI was measured using four nondestructive methods, calibrated and tested in experiments performed in calibration plots near the OTCs. The four methods were: PAR transmission through the canopy, normalized difference vegetation index (NDVI), hemispherical photography, and allometric relationships between plant stem diameter and plant leaf area. Calibration experiments showed: (1) Leaf area index could be accurately determined from either PAR transmission through the canopy or hemispherical photography. For LAI determined from PAR transmission through the canopy, ecosystem light extinction coefficient (*k*) varied with season and was best described as a function of PAR transmission through the canopy. (2) A negative exponential function described the relationship between NDVI and LAI; (3) Allometric relationships overestimated LAI. Throughout the two years of this study, LAI was always higher in elevated C_a, rising from, 20% during winter, to 55% during summer. This seasonality was driven by a more rapid development of leaf area during the spring and a relatively greater loss of leaf area during the winter, in elevated C_a. For this scrub-oak ecosystem prior to canopy closure, increased leaf area was an indirect mechanism by which ecosystem C uptake and canopy N content were increased in elevated C_a. In addition, increased LAI decreased potential reductions in canopy transpiration from decreases in stomatal conductance in elevated C_a. These findings have important implications for biogeochemical cycles of C, N and H₂O in woody ecosystems regenerating from disturbance in elevated C_a.

Keywords: elevated CO₂, leaf area index, leaf expansion, leaf fall, open-top chambers, scrub-oak ecosystem

Received 9 January 2002; revised version received and accepted 22 March 2002

Introduction

Leaves provide the surface area for light interception and plant–atmosphere CO₂ and H₂O exchange, and are an important sink for plant nitrogen. Consequently, effects of growth in elevated atmospheric CO₂ concentrations (C_a) on ecosystem leaf area index (LAI) could result in

important feedbacks on ecosystem biogeochemical cycling of C, N and H₂O.

There is good reason to predict that ecosystem leaf area may change in elevated C_a. Changes in leaf and plant water status (Sasek & Strain, 1989), and effects of leaf sugar availability on cell division and expansion (Taylor *et al.*, 1994; Robertson & Leech, 1995; Masle, 2000; Ferris *et al.*, 2001), have been identified as mechanisms for increased leaf area in elevated C_a. However, the magnitude and direction of the effect of elevated C_a on leaf area has been found to vary both among and within plant species (Taylor *et al.*, 1994; Masle, 2000), and to depend on time of

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year (Ferris *et al.*, 1996). For plants grown predominantly in controlled environments, and for a maximum of one growing season, Pritchard *et al.*, (1999) found the stimulation of LAI in elevated C_a to be greater in crop species than for trees and wild herbaceous species. Conversely, Drake *et al.*, (1997) focused on the long-term response of field-grown crops to elevated C_a and found LAI at canopy closure to be unaffected by elevated C_a . Changes in leaf fall, either as a result of senescence or abscission may also change leaf area in elevated C_a . Studies have shown the rate of senescence to be increased (McConnaughay *et al.*, 1996), decreased (Curtis *et al.*, 1989; McConnaughay *et al.*, 1996; Li *et al.*, 2000) and unaffected by growth in elevated C_a (Gunderson *et al.*, 1993; McConnaughay *et al.*, 1996). For *Quercus myrtifolia*, an important species of the Florida scrub-oak ecosystem leaf abscission was significantly increased in elevated C_a throughout the year (Stiling *et al.*, 2002).

Ecosystem LAI changes with season, the result of the timing of leaf expansion and leaf fall of the constituent species. Because the effect of elevated C_a on leaf expansion and leaf fall can be species specific, growth in elevated C_a may change seasonal timing of increases and decreases in LAI. For complex ecosystems with many species, this may result in seasonal changes in the effect of elevated C_a on LAI. Currently, there is little data from which to draw conclusions about the effect of elevated C_a on LAI of woody ecosystems. For grasslands, the effect of elevated C_a on LAI is not clear. In a prairie ecosystem, elevated C_a increased maximum annual LAI during three dry years, but only in one of the three wet years (Owensby *et al.*, 1999). A common perception, that at canopy closure LAI will not be increased in elevated C_a , is supported by data from crop studies (Drake *et al.*, 1997). For woody ecosystems, any effects of elevated C_a on LAI at canopy closure may become apparent in time. However, it is likely that the effects of elevated C_a on LAI may be more pronounced prior to canopy closure. Data collected in studies on tree seedlings and saplings grown in isolation or controlled conditions, typically show leaf area to be increased in elevated C_a (Idso *et al.*, 1993; Ceulemans *et al.*, 1995; Heath & Kerstiens, 1997; Kellomäki & Wang, 1997; Tissue *et al.*, 1997). If these findings hold true for young trees in the field then, in ecosystems prone to disturbance, such as fire regenerated or managed ecosystems, the effects of elevated C_a on the establishment of LAI following disturbance may result in feedbacks on biogeochemical cycling of C, N and H_2O that could last until canopy closure.

There is good reason to assume that LAI of woody ecosystems may change in elevated C_a . In addition, in natural ecosystems species-specific effects on leaf area expansion and leaf fall may mean that changes in LAI will be seasonally dependent. To date, the lack of

appropriate experimental facilities have restricted detailed studies on LAI of natural woody ecosystems. Here, we report results of experiments designed to test two hypotheses.

- (1) Prior to canopy closure, elevated C_a will increase ecosystem LAI.
- (2) The magnitude of the increase will change with season.

This study was conducted in a Florida scrub-oak ecosystem, which had regenerated after fire in May, 1996 in both ambient and elevated C_a in open-top chambers (OTCs). The two-year study was performed prior to canopy closure, between three and five years into the fire regeneration cycle. Fundamental to this study, in which LAI was determined from regular measurements of PAR transmission through the canopy, were a series of experiments in February and June 2000, in which we determined a site-specific light extinction coefficient (k) and compared LAI determined from PAR transmission through the canopy, with LAI determined from three independent well established techniques, NDVI (Tucker, 1979; Gamon *et al.*, 1995), hemispherical photography (Evans & Coombe, 1959; Anderson, 1964; Neumann *et al.*, 1989) and allometric relationships (Ceulemans *et al.*, 1993; Delucia *et al.*, 2000).

Materials and methods

The site

The Smithsonian Environmental Research Center CO_2 project was on Merritt Island, Florida (28°38'N, 80°42'W) within NASA's Kennedy Space Center, in a scrub-oak, palmetto ecosystem (Schmalzer & Hinkle, 1992). Although 27 species of plants occur in this ecosystem, up to 85% of aboveground biomass can be composed of three rhizomatous evergreen scrub oak species, *Quercus myrtifolia* Willd., *Q. geminata* Small and *Q. chapmannii* Sargent (Schmalzer & Hinkle, 1992). The substrates were well drained Pomello (Arenic Haplahumod) and Poala (Spodic quartzipsamment) sands. Both were acidic and low in nutrients, most of which were locked up in the standing biomass and a soil organic layer approximately 30 cm in depth (Schmalzer & Hinkle, 1992). Sixteen open top chambers (OTC), each 2.5 m high with an octagonal surface area of 9.42 m², were established during spring, 1996 on a site that had been partially burnt in July of, 1995 and the burn was completed in January, 1996. Eight of the OTCs were maintained at current ambient C_a and eight at elevated C_a (current ambient + 350 $\mu\text{mol mol}^{-1} CO_2$) (Hymus *et al.*, 2002). In December 2000, the average number of shoots in

each OTC was 431 ± 45 and 462 ± 26 , and the average shoot height was 60 ± 5 cm and 69 ± 4 cm in the ambient and elevated OTCs, respectively.

Calibration plots

In February 2000, four 4 m² calibration plots (C1–C4) and in June 2000, two 4 m² calibration plots (C5–C6) were marked out. The plots were sited in close proximity to the OTCs, encompassing a range of LAI and enclosing vegetation of the same age and species composition as in the OTCs. The vegetation was low in stature. Four years after fire, the canopy height in these plots was between 50 and 60 cm. In each of these six calibration plots, we measured PAR transmission through the canopy, NDVI and stem diameter of the oaks, using methods described in detail below. After these measurements, the plots were defoliated, the area of a subsample of the leaves, c. 0.1 m² was measured using a leaf area meter (LI-3100, LI-COR, Lincoln, NB, USA), and all leaves, including those subsampled, and all woody biomass was dried to constant weight at 80 °C in a drying oven, and weighed. Leaf area of the calibration plots was determined by scaling from the leaf area and leaf dry weight of the subsample, to the leaf dry weight of the complete plot, this leaf area is subsequently termed 'True LAI'. From true LAI and measured PAR transmission through the canopy, we calculated *k* for each calibration plot using Beer's law. From the six calibration plots, we also developed a negative-exponential relationship between NDVI and LAI that could be used to determine LAI from NDVI measurements in the OTCs.

Determining LAI from PAR transmission through the canopy

Transmission of PAR through the canopy was measured in each calibration plot and in the OTCs in February and June 2000. These dates were chosen because they represented times of the year when LAI was at its annual minimum and maximum. Measurements were also made in the OTCs 10 times between May, 1999 and February 2001. All measurements were made on overcast days in diffuse light. For both the calibration plots and OTCs, PAR was measured 2.5 m above the canopy using a cosine corrected sensor with a gallium-arsenide photodiode (Pontailier, 1990). Measurements of PAR at ground level were made using a 35-cm long sensor containing 25 photodiodes having the same spectral response as the sensor used to measure above the canopy. Data from both sensors was scanned simultaneously using a CR 23X datalogger (Campbell Scientific, Logan, Utah, USA). For the calibration plots PAR was measured at 16 locations beneath the canopy. Within the OTCs PAR was

measured below the canopy in 18 fixed locations in every OTC which were revisited on every measurement date. The OTC structure decreased PAR incident above the canopy by 22% compared with PAR outside the OTC. All measurements made inside the OTCs were corrected for this effect by decreasing incident PAR measurements by 22% before calculating the interception ratio. Leaf area index was calculated by applying Beer's Law to the measurements of PAR transmission through the canopy, using a value of *k* that varied with PAR transmission, as determined in the calibration plots.

$$\text{LAI (m}^2 \text{ leaf area} \cdot \text{m}^{-2} \text{ ground area)} = -(1/k) \times \ln(I/I_0)$$

Where *I* was PAR measured below the canopy and *I*₀ was PAR measured above the canopy.

Determining LAI using NDVI

A sensor equipped with one gallium arsenide and one silicon photodiode, and appropriate long-pass filters, measured reflectance in the red (R), at 655 nm (640–665 nm), and near infrared (NIR), 830 nm (750–900 nm), parts of the solar spectrum (Pontailier & Genty, 1996). Twelve measurements were made 50 cm above the canopy in each OTC on three occasions in both February and June 2000 and in each calibration plot. The measurements made in the calibration plots were used to calibrate the method.

The sensor had a 60° field of view, consequently when positioned 50 cm above the canopy it viewed c. 0.5 m² of the canopy. The 12 measurements made inside the OTCs were made at regular positions above the canopy to avoid overlapping and seeing the chamber walls. In theory, 60% of the 9.42 m² surface area of the OTC was sampled.

$$\text{NDVI} = [(NIR - R)/(NIR + R)]$$

Measurements were made on clear days at a constant sun elevation of 40–45° (at solar noon in February and three and a half hours before or after noon in June). Reference measurements were made for incident light and showed a fairly stable R/NIR ratio, ranging from 1.52 in February to 1.67 in June. The higher ratio in June was due in part to the temperature sensitivity of the sensor, and increased water vapour in the atmosphere, which increased interception in the NIR range.

Determining LAI using hemispherical photography

Six photographs from beneath the canopy were shot at ground level in every OTC on overcast days in both February and June 2000. In the calibration plots, four photographs were taken during the same measurement periods. We used a digital camera (Nikon CoolPix 950, Nikon Corporation, Tokyo, Japan) fitted with a fisheye

adapter (Nikon FC-E8). The overall optical grouping had a 1.6-mm focal length, equivalent to 8 mm in a 35-mm SLR camera, and had a field of view of 183°. Images were 24-bit TIFF files (uncompressed) having a 1600 × 1200 pixel resolution. Pictures were analysed by converting data to a binary [1-bit] image according to a user-defined threshold, gap fraction determination and leaf area index calculation using 'Gap Light Analyser' software (Frazer *et al.*, 2000). Leaf area index was derived from gap fractions in five concentric rings covering zenith angles 0–75° (Welles & Norman, 1991).

Determining LAI using allometric relationships

Allometric relationships between plant stem diameter (SD) and plant leaf area (LA) were used to calculate leaf area for each individual oak within the six calibration plots. The relationships between SD and LA for *Q. myrtifolia* trees ($\ln(\text{LA}) = 1.798 + 2.648 (\ln(\text{SD}))$, $r^2 = 0.69$) and *Q. geminata* trees ($\ln(\text{LA}) = 2.419 + 2.088 (\ln(\text{SD}))$, $r^2 = 0.78$) were established in June, 1995 on three-year-old trees, during a pilot study performed at the same site (Day *et al.*, 1996; site description). In the absence of allometric relationships specific to *Q. chapmanii* we used the relationships for *Q. myrtifolia*. Leaf area was calculated from measurements of stem diameter made 1–2 cm aboveground level, using a digital caliper (NTD13-6, Mitotoyu Corp, Japan). For the six calibration plots, oaks accounted for between 81 and 93% of total aboveground biomass.

Leaf fall and leaf area expansion

In May, July, September and November, 1999 and February 2000, collections of leaf litter were made inside the OTCs within one week of measurements of LAI also inside the OTCs. From these measurements, leaf area expansion between each measurement date (L_{EXP} , $\text{m}^2 \text{m}^{-2}$ ground area), was calculated as the difference between the known change in LAI (ΔLAI) plus the known leaf fall (L_{FALL} , $\text{m}^2 \text{m}^{-2}$ ground area) between each measurement date.

Leaf fall ($\text{m}^2 \text{m}^{-2}$ ground area) was determined from the product of leaf fall (g m^{-2} ground area) and specific leaf area of green leaves (SLA, $\text{m}^2 \text{kg}^{-1}$). Leaf litter was collected in three trays (76 cm long, 5.25 cm wide and 3 cm deep) positioned beneath the canopy in each OTC. After collection, the litter was dried to constant weight at 80 °C and weighed. The SLA was determined for five green leaves from each of four *Q. myrtifolia* plants in each ambient and elevated OTC in June, September and November, 1999. Leaves were dried to constant weight at 80 °C in a drying oven, and weighed. For green leaves growing in ambient C_a , SLA was 5.35 ± 0.23 , 5.86 ± 0.31 , and 6.06 ± 0.28 in June, September and November, 1999,

respectively. For green leaves growing in elevated C_a , SLA was 4.81 ± 0.17 , 4.97 ± 0.18 and 5.21 ± 0.33 in June, September and November, 1999, respectively. In the OTCs 56% of the total oak aboveground biomass in December, 1999 was *Q. myrtifolia* (Dijkstra *et al.*, 2002). It was assumed that the absolute SLA values for *Q. myrtifolia* leaves were representative of the other species in the ecosystem. To determine whether individual leaf area was affected by growth in elevated C_a , we sampled 10 fully expanded upper canopy leaves of *Q. myrtifolia*, on six occasions in, 1998. The area of each leaf was measured using a leaf area meter (LI-3100).

Statistical analysis

Repeated measures analysis of variance was used to test for the effect of growth C_a on LAI. For LAI determined in February 2000 and June 2000, ANOVA tested for an effect of measurement technique on LAI measured, and for an effect of growth C_a on individual leaf area. Student's *t*-test was used to test for an effect of growth C_a on leaf expansion rate in the spring and leaf fall during the winter. An effect was described as statistically significant where $P < 0.05$. All analysis was performed using statistical software (Systat 7.0. SYSTAT, Evanstone, IL, USA).

Results

Calibration 1: determining k

Leaf area index values determined destructively in the six calibration plots were considered as 'true LAI' and ranged from 0.8 to 3.5 (Table 1). In the calibration plots, k

Table 1 Comparing techniques 1. Cross comparison of LAI measured in six calibration plots in February (C1–C4) and June (C5, C6), 2000. Leaf area index was determined nondestructively in 3 ways. (1) PAR transmission through the canopy (τ), (2) Hemispherical photography (HP) and (3) Allometric relationships (AR). For each of the six calibration plots, the percentage difference between true and measured LAI was calculated for each of the three techniques. These six values were then averaged to give a mean difference ($\% \pm 1 \text{ SE}$) from the true LAI (MD)

	True LAI	τ	HP	AR
C1	1.46	1.30	1.59 ± 0.15	2.99
C2	1.81	1.87	1.92 ± 0.14	2.27
C3	1.13	1.31	1.31 ± 0.07	2.62
C4	0.84	0.80	0.92 ± 0.04	2.62
C5	3.51	3.03	2.87 ± 0.39	4.05
C6	1.90	2.09	2.06 ± 0.11	2.79
MD	–	9.7 ± 2.0	11.0 ± 2.0	89.4 ± 30.8

For LAI determined from hemispherical photography 5% confidence limits are displayed.

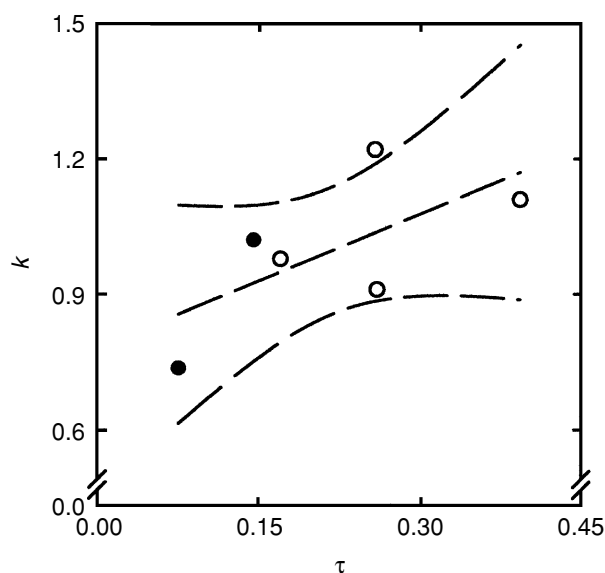


Fig. 1 Plot of k against PAR transmission through the canopy (τ) measured in four, 4 m^{-2} calibration plots in February (open circles) and two, 4 m^{-2} calibration plots in June 2000 (solid circles). The relationship between k and τ was described by the linear relationship $k = 0.9901 \tau + 0.7812$ ($t_5 = 1.77$; $P = 0.14$). The linear regression and 95% confidence intervals are shown (dashed lines).

was found to be a linear function of PAR transmission through the canopy and ranged from 0.73 to 1.22 (Fig. 1). The highest values of k were in February, when the ratio of woody biomass to leaf area was highest. This linear relationship was used to determine k whenever LAI was determined from PAR transmission through the canopy. By measuring in February and July, when LAI was at its annual minimum and maximum, respectively, values of k between 0.73 and 1.22 represented the annual range.

Calibration 2: NDVI

For the six calibration plots, the relationship of NDVI to LAI was described by a negative exponential function (Fig. 2). The slope of the relationship declined as LAI increased, evidence that the resolution of the NDVI measurements decreased as LAI increased. In addition, the relationship between NDVI and LAI approached saturation as LAI approached 3.5. At LAI less than 2.0, NDVI values were yet to saturate and the NDVI was most sensitive to small changes in LAI. This relationship between NDVI and LAI was used to determine LAI from NDVI in the OTCs in February and June 2000.

Calibration 3: comparing techniques February and June 2000

In the six calibration plots, LAI determined nondestructively, from PAR transmission through the canopy and

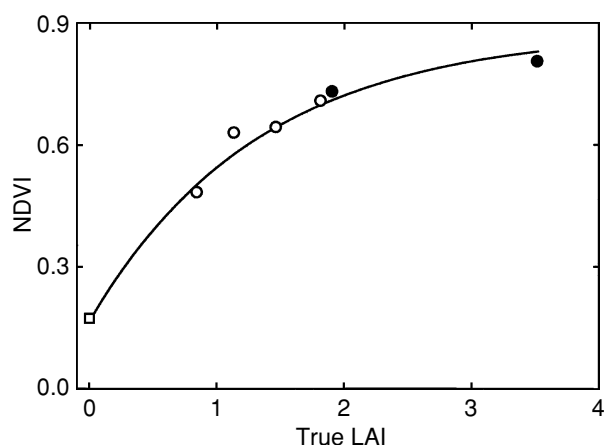


Fig. 2 Plot of true LAI, determined destructively, against NDVI measured in four, 4 m^{-2} calibration plots in February (open circles) and two, 4 m^{-2} calibration plots in June 2000 (solid circles). The value of NDVI at LAI of zero was measured over bare soil (open square). The relationship between LAI and NDVI was described by a negative exponential function as $\text{LAI} = -1.323 \ln((0.880 - \text{NDVI})/0.714)$ ($r^2 = 0.987$).

hemispherical photography, differed from true LAI by an average of $10 \pm 2\%$ and $11 \pm 2\%$, respectively (Fig. 3; Table 1). The accuracy of LAI determined from PAR transmission through the canopy was better for the calibration plots in February than June 2000 (Fig. 3; Table 1). For the six calibration plots, LAI determined from allometric relationships overestimated true LAI by $89 \pm 30\%$.

Effect of elevated CO₂ on LAI, leaf fall and leaf area expansion

Between May, 1999 and February 2001, LAI determined from the PAR transmission through the canopy ranged from 0.76 to 1.64 in the ambient OTCs, and from 1.06 to 2.34 in the elevated OTCs, the changes through time were statistically significant ($F_{9,63} = 21.4$; $P < 0.01$) (Fig. 4). The increase in LAI in elevated C_a was statistically significant ($F_{1,7} = 10.9$; $P = 0.01$) (Fig. 4a). However, the magnitude of the increase varied, ranging from a minimum of 22% in February 2000 to a maximum of 55% in June, 1999 (Fig. 4b). In February and June 2000, measurements of LAI made using NDVI and hemispherical photography were also made. In both February ($F_{2,40} = 0.18$; $P = 0.83$) and June 2000 ($F_{2,43} = 0.07$; $P = 0.94$) there was no difference between LAI measured using the three techniques (Table 2). The close agreement between LAI determined from NDVI and hemispherical photography, neither of which require knowledge of k , and LAI determined from PAR transmission through the canopy, which does require k , provides good evidence that growth in elevated C_a had not resulted in important changes in k .

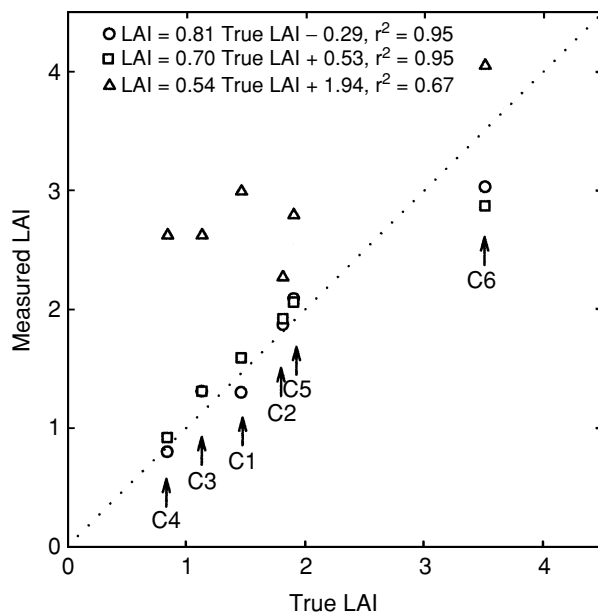


Fig. 3 Plot of true LAI, determined destructively, against measured LAI, determined using three different techniques: PAR transmission through the canopy (\circ); hemispherical photography (\square); and allometric relationships between stem diameter and leaf area (\triangle). True LAI was determined, and LAI measured using the three techniques, in four, 4 m^{-2} calibration plots in February (C1–C4) and two, 4 m^{-2} calibration plots in June 2000 (C5 and C6). The 1:1 relationship between true LAI and measured LAI is shown (dotted line).

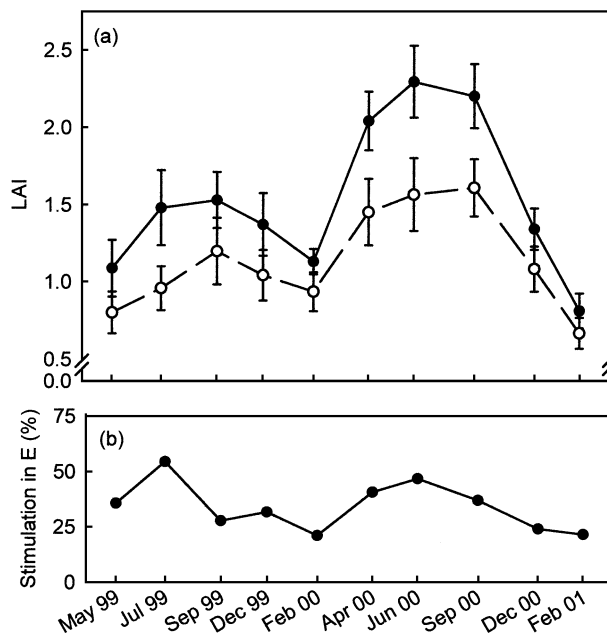


Fig. 4 Trends in ecosystem LAI between May 1999 and February 2001 for both ambient (\circ) and elevated (\bullet) OTCs (a), and the percentage increase of LAI in elevated C_a (b). Symbols shown in (a) are the mean values ($\pm 1\text{ SE}$) for 8 ambient and 8 elevated OTCs.

Table 2 Comparing techniques 2. Cross comparison of LAI measured in both ambient and elevated OTCs in February and June 2000. Leaf area index was determined from measurements of PAR transmission through the canopy (τ), NDVI and hemispherical photography. Values shown are the means ($\pm 1\text{ SE}$) for measurements made in eight ambient and eight elevated OTCs in both February and June 2000 using τ and NDVI. In February, photographs were made in six elevated OTCs and five ambient OTCs; in June, eight elevated OTCs and six ambient OTCs were photographed

	February 2000		June 2000	
	Ambient	Elevated	Ambient	Elevated
τ	1.13 (0.1)	1.16 (0.1)	1.56 (0.2)	2.29 (0.2)
NDVI	1.09 (0.1)	1.32 (0.1)	1.69 (0.2)	2.26 (0.2)
HP	1.11 (0.1)	1.21 (0.04)	1.78 (0.11)	2.17 (0.13)

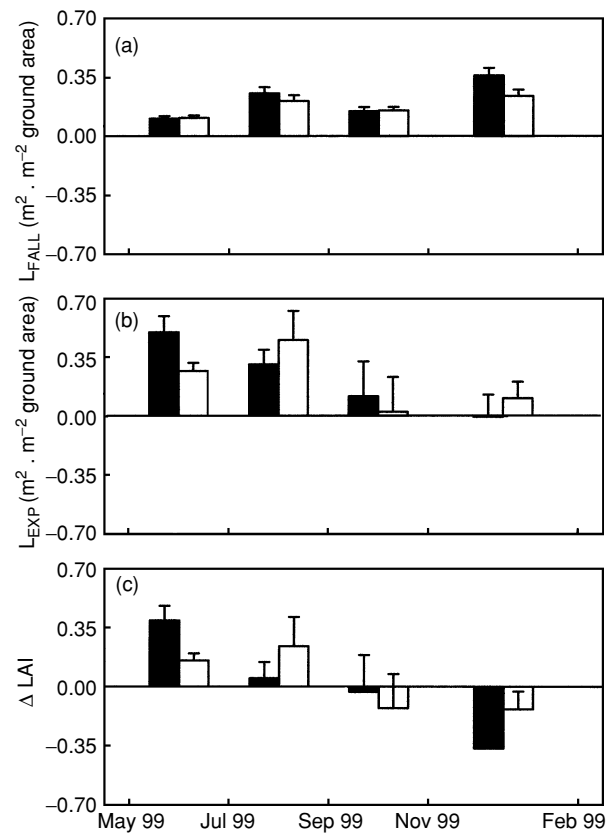


Fig. 5 Plots of leaf fall (L_{FALL} , $\text{m}^2 \cdot \text{m}^{-2}$ ground area) (a), leaf area expansion (L_{EXP} , $\text{m}^2 \cdot \text{m}^{-2}$ ground area) (b) and the change in LAI (ΔLAI) (c), between specific measurement dates between May 1999 and February 2000. Symbols shown are for either ambient (plain bars) or elevated (dark bars) OTCs. Bars shown are the mean ($\pm 1\text{ SE}$) for 8 replicate ambient and elevated OTCs.

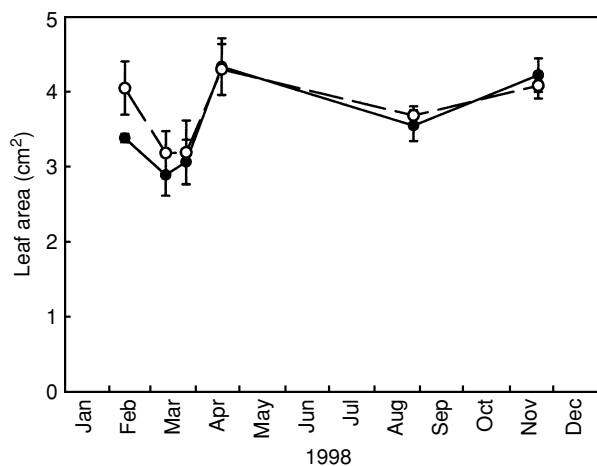


Fig. 6 Plot of leaf area (cm²), of sun leaves of *Q. myrtifolia* grown in either ambient (○) or elevated (●) OTCs. Symbols shown are the mean (\pm 1 SE.) for ≥ 6 replicate ambient and elevated OTCs.

The seasonal variability in the effect of elevated C_a on LAI was most likely the result of effects of elevated CO₂ on leaf area expansion and leaf fall. Between May and July, 1999 there was a large increase in the stimulation of LAI by elevated C_a , from 38 to 63%. During this time there was no difference in leaf fall between the two C_a treatments (Fig. 5a). Consequently, a statistically significant ($t_{14} = 2.14$; $P = 0.047$) increase in leaf area expansion in elevated C_a increased the stimulation of LAI (Figs 4b and 5b). Between July and September, 1999, leaf area expansion reached a peak in the ambient OTCs, resulting in a decline in relative effect of elevated C_a on LAI (Figs 4b and 5b). Between September and November, 1999 neither leaf fall, nor leaf area expansion, were effected by elevated C_a and the stimulation of LAI remained constant (Fig. 4b). Between November, 1999 and February 2000 there was a statistically significant ($t_{14} = 2.29$; $P = 0.038$) increase in leaf fall in elevated C_a , which reduced the stimulation of LAI by elevated C_a (Figs 5a and 4b). The decrease in the stimulation of LAI observed during both winters can only be explained by a rate of leaf fall that is greater relative to LAI during this time, in elevated C_a . Throughout, 1998, individual leaf area of *Q. myrtifolia* sun leaves was unaffected by elevated C_a ($F_{1,60} = 1.16$; $P = 0.29$) (Fig. 6).

Discussion

In this study, we have shown that elevated C_a increased LAI in a Florida scrub-oak ecosystem prior to canopy closure, and that the stimulation was seasonal. The stimulation of LAI in elevated C_a was highest during spring, when expansion of leaf area was faster in elevated C_a , and lowest in winter when leaf fall was greater,

relative to LAI, in elevated C_a (Figs 3, 4 and 5). These conclusions are based on a two-year study and supported by measurements made using three nondestructive techniques, PAR transmission through the canopy, NDVI and hemispherical photography (Fig. 3; Table 2). A fourth technique, the allometric relationship between stem diameter and leaf area, proved unsatisfactory, failing to accurately measure LAI in either of two seasons.

Central to this study were calibration experiments conducted in plots containing vegetation of the same age and species composition as in the OTCs. We found that k was dependent on PAR transmission through the canopy and changed with season, being highest in winter, when k reached 1.2 in one of the calibration plots. Similarly high values of k have been reported for a leafless oak forest (Baldocchi *et al.*, 1984). The seasonality in k was most likely due to leaf expansion in spring and partial leaf fall in winter, which resulted in the relatively larger impact of woody biomass on PAR transmission through the canopy in winter than summer (Fig. 1). By varying k according to the transmission of PAR through the canopy, seasonal changes in k were accounted for in the LAI measurements. We established a negative exponential relationship between NDVI and LAI in the calibration plots, which was used to determine LAI from NDVI measurements made in the OTCs (Fig. 2). We found that NDVI was best suited to measuring low LAI. As LAI increased above 2.0 the ability of NDVI to resolve small changes in LAI decreased, and ultimately NDVI values approached saturation at an LAI of 3.5. In this study LAI in the OTC's was always less than 3.0 giving confidence in the accuracy of our NDVI measurements. However, saturation of NDVI measurements, and loss of ability to resolve small changes in LAI as leaf area increases, will limit the utility of NDVI measurements as LAI increases to canopy closure.

In six calibration plots, LAI was measured to a high degree of accuracy from PAR transmission through the canopy and hemispherical photography (Fig. 3; Table 1). In contrast, allometric relationships generated on three-year-old trees in June, 1995, overestimated LAI of four-year-old trees in both June and February 2000. The magnitude of the error was a surprise. The scrub-oak ecosystem was evergreen and large seasonal changes in leaf area were not expected. However, the results show that in this evergreen system, seasonal leaf fall was sufficient to greatly decrease the accuracy with which leaf area in February could be predicted from allometry derived in June. More notable was the inability of allometric relationships generated for trees that were three years old, to predict the leaf area of four-year-old trees in the same season. This result showed that significant changes in allometry can occur within one year. These findings emphasize how sensitive allometric relationships

between stem diameter and leaf area may be to season and phenology, and how their use may be inappropriate for tracking seasonal changes in LAI.

Between May, 1999 and February 2001, elevated C_a significantly increased LAI (Fig. 4). Individual leaf area did not increase for *Q. myrtifolia* in elevated C_a (Fig. 6). If this holds for other species we can exclude the possibility that increased individual leaf area was the reason for increased LAI in elevated C_a . In addition, elevated C_a has had no effect on the number of plants in this ecosystem (Dijkstra *et al.*, 2002). In the absence of an effect of elevated C_a on individual leaf area or plant number, the increase in LAI could have been due to a change in the relationship between leaf area and stem diameter of individual trees in elevated C_a . However, for *Q. myrtifolia* and *Q. geminata*, which together can account for *c.* 70–80% of aboveground biomass at the site, the relationship between leaf area and stem diameter of individual trees was unchanged after three years growth in elevated C_a (data not shown). Consequently, in this study LAI was increased in elevated C_a because the trees were bigger (Dijkstra *et al.*, 2002). Importantly, the magnitude of the stimulation of LAI was variable, seemingly driven by effects of elevated C_a on the expansion of leaf area and leaf fall (Figs 4 and 5). Peak stimulation of LAI in elevated C_a occurred between March and May, after the first flush of the season in both, 1999 and, 2000. Between May and September, 1999, leaf fall was unaffected by elevated C_a and maximum annual LAI was attained by June, 1999 in elevated C_a , compared with September, 1999 in ambient C_a . We are unaware of other reports of this effect, although it is consistent with a study on sour orange trees in which spring branch growth was increased in elevated C_a (Idso *et al.*, 2000). During both winters, the stimulation of LAI in elevated C_a was at an annual minimum and this was consistent with higher leaf fall, relative to leaf area, in elevated C_a between November, 1999 and February, 2000 (Fig. 5).

Changes in LAI in elevated C_a will be a key feedback on the direct effects of elevated C_a on the biogeochemical cycling of C, N and H_2O within ecosystems. We can conclude that for this scrub-oak ecosystem, increased LAI has had important implications for the cycling of C, N and H_2O . For the three oak species that dominate aboveground biomass, photosynthesis per unit leaf area of sun leaves has always been higher in elevated C_a , regardless of acclimatory decreases in photosynthetic capacity (Li *et al.*, 1999; Li *et al.*, 2000; Ainsworth *et al.*, 2002; Hymus *et al.*, 2001; Hymus *et al.*, 2002). Conversely, respiration per unit leaf area of sun leaves of *Q. myrtifolia* and *Q. geminata* was typically unaffected by elevated C_a . Only for *Q. myrtifolia*, the leaf respiration increased during spring in elevated C_a (Hymus *et al.*, 2002). Consequently, increased leaf area has been a positive feedback on ecosystem C uptake in elevated C_a .

Decreases in foliar nitrogen concentrations of both sun and shade leaves of both *Q. myrtifolia* and *Q. geminata* have never been greater than 15% in elevated C_a (Li *et al.*, 2000) and have typically been much lower (Stiling *et al.*, 1999; Hymus *et al.*, 2002). Consequently, the stimulation of LAI between, 20 and 55% reported in this study has been a negative feedback, offsetting nitrogen savings at the leaf level and increasing canopy nitrogen content in elevated C_a . Increased leaf area has had complex effects on canopy transpiration, increasing both the evaporative surface and shading, at this site. These effects are in addition to direct effects of elevated C_a on stomatal conductance. Lodge *et al.*, (2001) reported decreases in stomatal conductance for *Q. myrtifolia* of *c.* 40%. If reflective of other species and indicative of the whole year, then a 40% decrease in stomatal conductance would be greater than the increase in LAI for most of the year, decreasing canopy transpiration in elevated C_a . Current research suggests that a 40% decrease in stomatal conductance may be a constraining limit, with actual decreases typically lower. Consequently, there are times of the year when increased leaf area increases canopy transpiration in elevated C_a (J. Li, unpublished data). In addition to effects on canopy transpiration, increased LAI has affected evapotranspiration, via increased shading of the soil surface. Throughout, 1998, maximum mid-day ecosystem evapotranspiration was on average 18% lower in elevated C_a (Hungate *et al.*, 2002).

To conclude, for the Florida scrub-oak ecosystem between three and five years into a fire-return cycle and prior to canopy closure, elevated C_a increased ecosystem LAI. Effects of elevated C_a on expansion of leaf area and increase of leaf fall were drivers of seasonal changes in the magnitude of the stimulation of LAI in elevated C_a . For ecosystems prone to regular disturbance, elevated C_a could stimulate LAI and have important consequences for biogeochemical cycling during regeneration from disturbance.

Acknowledgements

Technical assistance was provided by Mr David Johnson and Mr Tom Snead. Financial support for this study came from the Smithsonian Institution and the US Department of Energy. We also acknowledge the support and encouragement of NASA Kennedy Space Center and the Dynamac Corporation.

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