

Canopy development of a model herbaceous community exposed to elevated atmospheric CO₂ and soil nutrients

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To test the prediction that elevated CO₂ increases the maximum leaf area index (LAI) through a stimulation of photosynthesis, we exposed model herbaceous communities to two levels of CO₂ crossed with two levels of soil fertility. Elevated CO₂ stimulated the initial rate of canopy development and increased cumulative LAI integrated over the growth period, but it had no effect on the maximum LAI. In contrast to CO₂, increased soil nutrient availability caused a substantial increase in maximum LAI. Elevated CO₂ caused a slight increase in leaf area and nitrogen allocated to upper canopy layers and may have stimulated leaf turnover deep in the canopy. Gas exchange measurements of intact communities

made near the time of maximum LAI indicated that soil nutrient availability, but not CO₂ enrichment, caused a substantial stimulation of net ecosystem carbon exchange. These data do not support our prediction of a higher maximum LAI by elevated CO₂ because the initial stimulation of LAI diminished by the end of the growth period. However, early in development, leaf area and carbon assimilation of communities may have been greatly enhanced. These results suggest that the rate of canopy development in annual communities may be accelerated with future increases in atmospheric CO₂ but that maximum LAI is set by soil fertility.

Introduction

The development and structure of a plant canopy are central factors determining net primary production (NPP). One attribute that determines the capacity of plant canopies to absorb light for photosynthesis is the leaf area index (LAI; m² leaf m⁻² ground surface). Changes in leaf orientation and position within a canopy influence the distribution of light and its absorption within canopies (Hirose and Werger 1987, Brown and Parker 1994). Leaf area index, however, determines total light absorption and is thus directly correlated with NPP across ecosystems (Hirose et al. 1997, Hirose and Bazzaz 1998).

In addition to directly stimulating photosynthesis (Pearcy and Björkman 1983) and the leaf area of individual plants (Gifford 1977, Long 1991, Gunderson and Wullschleger 1994, Taylor et al. 1994), exposure to elevated CO₂ may increase LAI in plant communities by increasing the retention of shade leaves, thereby increasing canopy light absorption (Long 1991, Eamus 1996). For rapidly growing tropical pioneer trees, shade leaves deep in the canopy are retained until their diurnal carbon balance approaches zero (Ackerly 1999). Elevated CO₂ suppresses photorespiration, thereby increasing light-limited photosynthesis (Pearcy and Björk-

man 1983, Long 1991, Singaas et al. 2000) and reducing the photosynthetic-light-compensation point (LCP; Long and Drake 1991). Together, these changes enable leaves to maintain positive photosynthesis rates at lower irradiance and may increase the longevity of shade leaves.

The response of LAI to elevated CO₂ may be strongly modified by nitrogen (N) status (Chu et al. 1996, Hirose et al. 1997). Canopy development is directly affected by nutrient availability. Nitrogen limitations, in particular, can counter the effect of elevated CO₂ on the retention of shade leaves by promoting the remobilization of N from shade to sun leaves (Bazzaz and Miao 1993, Ceulemans and Mousseau 1994, Chu et al. 1996, Hirose et al. 1996b, Poorter et al. 1996). A nutrient treatment was included in this study because of the strong, independent effect of N, in particular, and the potential interaction with CO₂ on canopy development (Chu et al. 1996, Hirose et al. 1996b, Hirose and Bazzaz 1998).

There have been relatively few attempts to quantify the effect of elevated CO₂ on LAI in species mixtures, and the results from these studies have been inconsistent. For example, Arnone and Körner (1993) found that monocultures of

Ricinus communis grown under ambient and twice-ambient CO₂ concentrations attained the same LAI after 21 days. Campbell et al. (1990) observed 5% lower LAI for a soybean canopy 37 days after planting for elevated (660 µl l⁻¹) compared to ambient (330 µl l⁻¹) CO₂ that increased to a 34% stimulation of LAI 54 days after planting. Young beech stands grown at elevated CO₂ had greater LAI after 4 months of treatment conditions, a stimulation that persisted through the following growing season (Overdieck 1993). The absence of an effect of elevated CO₂ on maximum LAI may result from nutrient limitations which reduce potential growth stimulation or enhanced respiration that counteracts the benefits achieved by higher photosynthesis.

Some of these studies (Campbell et al. 1990, Arnone and Körner 1993, Hirose et al. 1996b, Hirose et al. 1997) have relied on estimates of LAI obtained only once or twice during the growth period, potentially failing to detect how elevated CO₂ influences time-integrated leaf area development throughout the season. To test the prediction that maximum LAI will be greater under elevated atmospheric CO₂, we exposed assemblages of primarily annual species to a two-by-two factorial design of atmospheric CO₂ and soil nutrients. We calculated leaf area duration (LAD) from sequential estimates of LAI in model herbaceous communities grown under elevated CO₂ and soil fertilization. The species included genera with C₃ and C₄ photosynthesis and a range of growth forms that often co-occur in Illinois, USA.

Materials and methods

Plant material, growth conditions, and experimental design

We created model herbaceous communities by planting assemblages of 14 co-occurring species (Table 1), primarily annuals, in large plastic tubs. The species are typical of fallow agricultural fields in central Illinois (Garbutt et al. 1990), have rapid growth rates and are highly responsive to varying environmental conditions (Armesto and Pickett 1985). Seeds were sown in 21-l tubs (20 cm high × 34 cm

long × 31 cm wide) containing a quartz sand:loam soil (9:1 v/v) mixture. This soil mixture is well drained and nutrient-poor (0.48 mg N g⁻¹ soil; 0.02 mg P g⁻¹ soil). Based on a median density of 3 seeds cm⁻² for seedbanks in agricultural soil (Benoit et al. 1992, Cavers et al. 1992, Forcella et al. 1992), a total of 3 066 seeds, evenly divided among all species, was planted in each tub. Twenty-four tubs, hereafter referred to as communities, were placed in 4 growth chambers (1.46 m³; 1.37 m long × 0.91 m wide × 1.17 m high) inside a greenhouse room. The upper portion of each chamber was clear acetate. Incident irradiance was supplemented by a 1 000-W metal halide lamp above each chamber with a 14-h photoperiod.

A completely random split-plot experimental design was used, with two CO₂ levels and two soil nutrient levels. Atmospheric CO₂ concentration was maintained at 797 ± 50 µl l⁻¹ (mean ± SE) in treatment chambers and 370 ± 10 µl l⁻¹ in control chambers by daily manual adjustment of the frequency and duration of pure-CO₂ injection. Because of the difficulty of maintaining CO₂ concentrations during times with high respiration, on occasion the night-time CO₂ concentration in the chambers approached 1200 µl l⁻¹. Chamber temperatures were maintained at 27.4 ± 1.3°C for the 14-h light period and 24.7 ± 0.8°C for the 10-h dark period. The CO₂ concentration and temperature in each chamber were measured continuously with an infra-red gas analyzer (ADC LCA2, Analytical Development Company, Ltd., UK) and copper-constantan thermocouples, respectively, and recorded with a datalogger (CR21X, Campbell Scientific, Logan, UT, USA). Plants were watered to field capacity at 1- or 2-day intervals. Three communities in each chamber were randomly assigned to the fertilization treatment and were watered weekly with 1 l of commercial 20:20:20 N:P:K solution (nitrogen: 473 µl l⁻¹); the other communities were unfertilized throughout the experiment.

Six communities were positioned randomly in each growth chamber, and each group of 6 remained together throughout the experiment. The groups were systematically rotated among chambers each week to minimize potential chamber effects, and communities within each group were rotated each week to reduce neighbor effects.

Table 1. Growth form, leaf inclination, and photosynthetic pathway of plant species used to create experimental canopies. Abbreviations: H = herb, G = grass, W = wild, C = crop, A = annual, P = perennial, Ho = horizontal, D = diagonal, V = vertical, B = broadleaf, G = grass-like. *Sida spinosa* was assumed to be C₃ based on gross leaf morphology.

Species	Growth form	Leaf inclination		Photosynthetic pathway
<i>Abutilon theophrasti</i> Medic.	H W A	Ho	B	C ₃
<i>Amaranthus retroflexus</i> L.	H W A	Ho	B	C ₄
<i>Chenopodium album</i> L.	H W A	D	B	C ₃
<i>Datura stramonium</i> L.	H W A	D	B	C ₃
<i>Digitaria sanguinalis</i> (L.) Scop.	G W A	D	G	C ₄
<i>Kochia scoparia</i> (L.) Roth	H W A	D	G	C ₄
<i>Lepidium campestre</i> (L.) R. Br.	H W A	V	G	C ₃
<i>Polygonum pennsylvanicum</i> L.	H W A	D	B	C ₃
<i>Setaria faberi</i> Herrm.	G W A	V	G	C ₄
<i>Sida spinosa</i> L.	H W A	D	B	C ₃
<i>Solanum dulcamara</i> L.	H W A	Ho	B	C ₃
<i>Sorghum bicolor</i> (L.) Moench.	G C A	V	G	C ₄
<i>Sorghum halepense</i> (L.) Pers.	G W P	V	G	C ₄
<i>Xanthium pennsylvanicum</i> Wallr.	H W A	Ho	B	C ₃

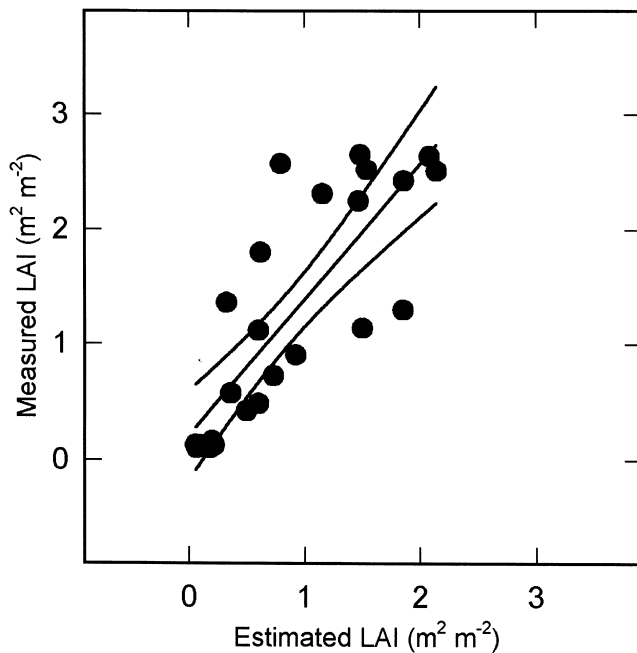


Fig. 1. Measured leaf area index (LAI; $\text{m}^2 \text{ leaf m}^{-2}$ ground surface) in relation to estimated LAI for 10-cm vertical canopy layers at the time of harvest for annual plant communities grown in a 2×2 factorial CO_2 and soil nutrient treatment regime. Data points were fit with a linear regression, and $\pm 95\%$ confidence intervals are shown. Parameters for the regression are $b_0 = 0.21$ and $b_1 = 1.18$ ($r^2 = 0.67$).

Canopy development

Leaf area index (LAI; $\text{m}^2 \text{ leaf m}^{-2}$ ground surface) and canopy height from the soil surface for each community were measured at approximately 5-day intervals for 6 weeks. They were measured in 16 locations in a 4×4 grid in each community. LAI, the cumulative leaf area from the top to the bottom of the canopy (Nobel et al. 1993), was estimated from measurements of light attenuation through the canopy using a derivation of the Beer-Lambert law: $\text{LAI} = [\ln(I_z/I_0)]/k$, where I_z and I_0 are the irradiance above the canopy and at the soil surface, respectively, and k is the extinction coefficient (Monsi and Saeki 1953, Werger and Hirose 1991, Nobel et al. 1993, Hirose et al. 1996a). We used an extinction coefficient of 0.5, which assumes a random azimuth orientation of foliage elements (Forseth and Norman 1993). We assumed constant k through the experiment due to the short duration of the experiment; however, k may be affected by various factors, including the mode of leaf arrangement, leaf size (Terashima and Hikosaka 1995), or the different timing of ontogenetic development among species in the communities.

We measured irradiance above the canopy and at the soil surface with two matched quantum sensors (LI-190SA; Li-Cor, Inc., Lincoln, NE, USA) used in conjunction with a datalogger (LI-1000; Li-Cor, Inc.). LAI was estimated on 8 days during the experiment. The outermost sampling locations were 5 cm from the edge of the tub and were entirely within the plant canopy. The quantum sensor received some irradiance from the side of the canopy, causing a slight

underestimate of LAI. Our 'optical' estimates of LAI were, however, highly correlated with a direct measurement of LAI made by a destructive harvest at the end of the experiment ($P < 0.001$; Fig. 1).

We calculated the mean LAI for each community per day. The vertical distribution of LAI at 5-cm intervals was measured 27 and 49 days from sowing.

Canopy CO_2 exchange

CO_2 exchange was measured for each community with a portable, closed-system gas analyzer (LI-6200; Li-Cor, Inc.) at the corresponding growth CO_2 concentration. Each community was placed in a clear plastic 140-l cuvette with a 15-cm diameter fan mounted inside for sufficient air mixing. Net ecosystem exchange (NEE) was measured 31–33 days from sowing (between 10.00 and 15.00 h, CST) on the entire plant-soil system (cuvette air temperature: $34.5 \pm 1.3^\circ\text{C}$). Sunlight was supplemented with a 1000-W metal halide lamp to provide $1040 \pm 109 \mu\text{mol m}^{-2} \text{ s}^{-1}$ during NEE measurements. Ecosystem respiration (R_{total}) was measured in the dark (between 20.30 and 24.00 h, CST) 38–40 days from sowing. Each community was placed in the cuvette and covered with black cloth (cuvette air temperature: $26.5 \pm 0.6^\circ\text{C}$). We assume a ratio of 0.60 to convert mitochondrial respiration in the day to night-time respiration (Kirschbaum and Farquhar 1984, Kromer 1995); the ratio may vary from 0.17 to 0.66 (Villar et al. 1994). To isolate the contribution of CO_2 from the soil ($R_{\text{soil}} = R_{\text{roots}} + R_{\text{microbes}}$) from R_{total} , CO_2 efflux was measured (between 09.00 and 18.00 h, CST) on each community immediately following harvest of the aboveground biomass 50–56 days from sowing (cuvette air temperature: $29.8 \pm 2.9^\circ\text{C}$; soil temperature: $23.3 \pm 2.7^\circ\text{C}$). Gross ecosystem exchange (GEE) was calculated from the sum of NEE and R_{total} ($\text{GEE} = \text{NEE} + R_{\text{total}}$).

Harvest

Aboveground biomass of each community was harvested and separated by species in 10-cm vertical layers beginning at the top of the canopy. Leaf area was measured by species, layer and community on fresh tissue with a video-area meter (Delta-T Area Meter, Decagon Devices, Inc., Pullman, WA, USA). Plant material (leaf, stem and reproductive mass) was dried at 70°C for at least 48 h and weighed. The collective root system of each community was washed and separated from the soil, dried and weighed. Leaf area duration (LAD; LAI integrated over time) was calculated between each measurement interval and summed over the growth period as $\text{LAD} = ([\text{LAI}_1 + \text{LAI}_2] \times [t_2 - t_1])/2$, where LAI_1 and LAI_2 are the LAIs at the initial time (t_1) and final time (t_2), respectively (expressed in days; Hunt 1990, Beadle 1993).

For each 10-cm layer (species were pooled by layer; total sample size = 202), total Kjeldahl N was extracted from ground plant tissue (20 mesh; Wiley Mill) by acid digestion, and N concentration was measured with an autoanalyzer (Traacs 800, Bran + Leubbe, Buffalo Grove, IL, USA). Nitrogen content was calculated following the harvest.

Data analysis

The changes in LAI and height across treatments were analyzed with an analysis of variance (PROC MIXED; SAS Institute, Inc., Cary, NC, USA) with repeated measures (Potvin et al. 1990) to test for effects of group, CO₂, nutrients and day. Group was included as a random effect in the analysis. The mean LAI and mean height for each community per measurement day were used for analysis.

Total leaf area, LAD, biomass, NEE, R_{total} and GEE, all of which were determined at one point in time during the experiment, were analyzed with an analysis of variance (PROC GLM; SAS Institute, Inc.). The model statement used in each case was: model y = (CO₂) (nutrients) (CO₂ × nutrients). Means were treated with a Bonferroni adjustment.

The Shannon-Wiener (S-W) diversity index (Barbour et al. 1987) was used to determine treatment effects on the species composition of communities; treatment differences were tested with an analysis of variance (Systat 5.2.1; Systat, Inc., SPSS, Evanston, IL, USA).

The vertical distribution of leaf area and N were standardized to account for differences in canopy height between fertilized and unfertilized communities and compared at each 5-cm (leaf area) or 10-cm (N) canopy interval with a paired *t*-test (Systat 5.2.1).

Regression models for LAI and N content of each treatment (Fig. 3) were compared using analysis of covariance (Systat 5.2.1). Significant differences were set at $\alpha = 0.05$.

Results

The initial increase in LAI was more rapid for communities grown under elevated than ambient CO₂, but there was no effect of growth CO₂ concentration ($F = 4.59$, $P = 0.96$) on maximum LAI (Fig. 2A). Fertilization increased LAI throughout the growth period ($F = 4536.60$, $P < 0.01$), and overall there was a significant interaction between CO₂ and nutrients ($F = 53.46$, $P < 0.05$) and CO₂ and day ($F = 227.58$, $P < 0.01$) on LAI. Elevated CO₂ caused a greater increase in the enhancement of LAI early in the experiment in fertilized compared to unfertilized communities, but there was no overall interaction of CO₂, nutrients, and day ($F = 1.55$, $P = 0.15$). Growth CO₂ concentration ($F = 4.65$, $P = 0.73$) had no effect on canopy height, but fertilization ($F = 924.51$, $P < 0.01$) increased canopy height (Fig. 2B). Although there was no significant interaction of CO₂ and nutrients ($F = 2.10$, $P = 0.29$), there was a significant interaction of CO₂ and day ($F = 2.31$, $P < 0.05$), nutrients and day ($F = 128.44$, $P < 0.01$), and CO₂, nutrients, and day ($F = 2.83$, $P = 0.01$) on height.

In both fertilized and unfertilized communities, growth in elevated CO₂ caused a slight shift in leaf area towards the top of the canopy ($P < 0.05$); this pattern was most evident in the unfertilized treatment (Fig. 3A). Although there tended to be more leaf area higher in the canopy under elevated CO₂, neither the CO₂ nor the nutrient level affected the N content (g m^{-2}) distribution among canopy layers (Fig. 3B). Elevated CO₂ increased the N concentration (%N)

in root mass and litter mass in unfertilized communities (Table 2). LAI was correlated with canopy N content (g m^{-2}) at the time of harvest ($r^2 = 0.74$; $F = 79.01$, $P < 0.001$; Fig. 4); in elevated CO₂, communities maintained a higher LAI for a given canopy N content across nutrient treatments, as indicated by different intercepts ($F = 6.81$, $P < 0.02$).

Fertilization, but not elevated CO₂, increased leaf area at the harvest (Table 2). Elevated CO₂ and fertilization enhanced LAD (LAI integrated over time), increasing the number of leaf-area days in elevated than in ambient CO₂. Total biomass was greater in elevated CO₂ and with fertilization, mainly through greater stem mass and a trend towards greater root mass.

The S-W diversity indices showed that the relative dominance of the 14 species in the communities was not different between CO₂ treatments ($F = 2.48$, $P = 0.13$), but it differed between nutrient treatments ($F = 45.12$, $P < 0.01$), with an interaction of CO₂ and nutrients ($F = 5.62$, $P < 0.05$). This

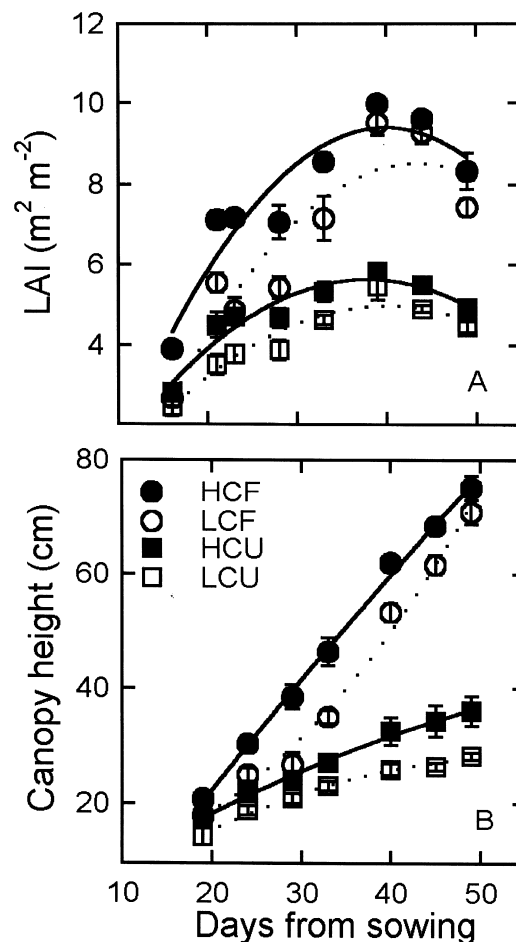


Fig. 2. (A) Estimated leaf area index (LAI; $\text{m}^2 \text{leaf m}^{-2}$ ground surface) for plant communities exposed to: high CO₂/fertilized (HCF, ●); low CO₂/fertilized (LCF, ○); high CO₂/unfertilized (HCU, ■); and low CO₂/unfertilized (LCU, □) treatments. Each point represents the mean of 6 communities (\pm SE). Error bars not shown are smaller than the symbols. There was an effect of fertilization on all measurements days. Data points were fit with a quadratic regression. (B) Height for canopies described above. Data points were fit with a quadratic regression.

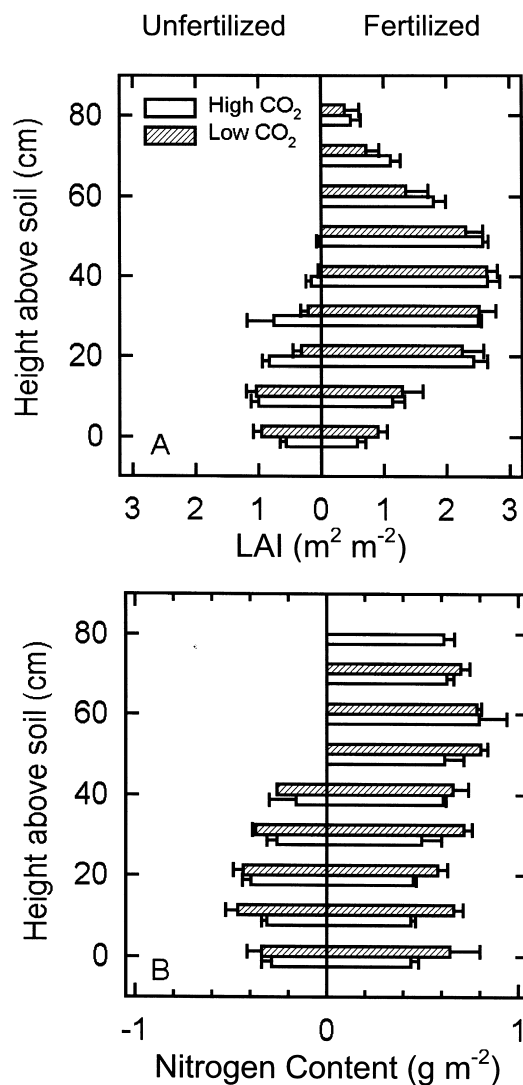


Fig. 3. (A) Vertical distribution of leaf area in 10-cm layers above the soil surface for communities exposed to: high CO₂/fertilized (HCF); low CO₂/fertilized (LCF); high CO₂/unfertilized (HCU); and low CO₂/unfertilized (LCU) treatments. High CO₂ communities are represented by open bars; low CO₂ communities are represented by striped bars. Each bar represents the layer mean for each of 6 communities (\pm SE). (B) Vertical distribution of leaf nitrogen content (g m^{-2}) in each 10-cm layer 27 days from sowing for communities described above.

index supports the observations that our fertilization treatment increased the relative dominance of certain species; ambient CO₂/fertilized communities had the lowest relative species evenness (mean S-W index \pm SE: 0.34 ± 0.05) and were primarily dominated by *Sorghum halepense*, whereas ambient CO₂/unfertilized communities had the highest relative species evenness (mean S-W index \pm SE: 1.18 ± 0.09).

There was no effect of elevated CO₂ on gross (GEE; $F = 0.02$, $P = 0.90$) or net (NEE; $F = 0.40$, $P = 0.53$) ecosystem exchange (Fig. 5A and B) measured near the end of the experiment, although fertilization significantly increased GEE ($F = 136.07$, $P < 0.001$) and NEE ($F = 75.27$, $P < 0.001$). A trend for lower NEE in elevated CO₂/fertilized communities was evident, and there was a significant CO₂ \times

nutrient interaction on NEE ($F = 5.22$, $P < 0.05$; Fig. 5B). In fertilized communities, there was a non-significant trend of higher total respiration (R_{total}) at elevated CO₂ (Fig. 5C). Pot (soil + root) respiration was unaffected by CO₂ ($F = 0.71$, $P = 0.41$), whereas leaf respiration (R_{total} pot respiration) was greater in elevated CO₂/fertilized communities (HCF: $4.42 \pm 1.54 \text{ mmol m}^{-2} \text{ s}^{-1}$; LCF: $1.66 \pm 1.04 \text{ mmol m}^{-2} \text{ s}^{-1}$; HCU: $0.89 \pm 0.68 \text{ mmol m}^{-2} \text{ s}^{-1}$; LCU: $0.34 \pm 0.97 \text{ mmol m}^{-2} \text{ s}^{-1}$; CO₂: $F = 3.29$, $P = 0.09$; fertilization: $F = 6.30$, $P = 0.02$; CO₂ \times fertilization: $F = 0.48$, $P = 0.55$).

Discussion

Elevated CO₂ caused faster canopy development without an increase in maximum LAI, resulting in greater LAD. A similar trend has been found for other experimental communities. Artificial tropical ecosystems (Körner and Arnone 1992), castor bean canopies (Arnone and Körner 1993), rice canopies (Ziska et al. 1996) and spruce canopies (Hättenschwiler and Körner 1996) showed no change in LAI, at least following short-term exposure to elevated CO₂. Ackerly et al. (1992) found *Abutilon* and *Amaranthus* had faster leaf initiation rates under elevated CO₂, and Campbell et al. (1990) found soybean canopies had faster LAI development that was not sustained throughout exposure to elevated CO₂. Despite faster initial growth of canopy leaf area in the present study, the timing of maximum LAI and of leaf senescence was not different between elevated and ambient CO₂ (Fig. 2), suggesting that canopy phenology was unaffected by CO₂ concentration. An accelerated rate of canopy development may be attributed to nutrient availability, higher turnover of carbon, greater net biomass accumulation or the rate of net carbon exchange.

Soil nutrient availability rather than atmospheric CO₂ concentration caused a large increase in maximum LAI. At a given N level, elevated CO₂ caused an additional small but statistically significant increase in LAI (Fig. 4), suggesting a higher efficiency of N use. Based on the responses of individual plants, greater photosynthetic NUE (carbon gain per unit leaf N; Lambers et al. 1998) is expected to increase canopy LAI through achieving maximal stand-level carbon gain (Hirose et al. 1996a). The hypothesis that leaf area development is controlled by N availability rather than CO₂ availability was proposed by Hirose et al. (1996b). The influence of N availability has been demonstrated in the leaf area growth of rice and understory plants to CO₂ elevation (Hättenschwiler and Körner 1996, Ziska et al. 1996). Davey et al. (1999) grew 3 grassland species in open-top chambers at 700 and 360 $\mu\text{l l}^{-1}$ CO₂ and found greater NUE at elevated compared to ambient CO₂. They related this to a reduction of leaf N content or to an enhancement of net photosynthesis. In the present work, the effect of CO₂ on the relationship between leaf area and leaf N was not strong enough to drive the community-level increases in LAI shown in Fig. 2. This may be due in part to the 3 outlying data points in the ambient CO₂/unfertilized treatment, which were derived from upper canopy layers that had very low leaf area. Hence, despite similar N content as the other data points in the same treatment, these layers had a very

small numerator in the calculation of LAI (m^2 leaf m^{-2} ground surface) and do not fall along the same line as the other layers.

We found no statistical evidence of greater maximum LAI or canopy height in elevated CO_2 . Rather, a higher concentration of CO_2 accelerated the rate at which the maximum LAI or height occurred. However, variation in the extinction coefficient of light, k , during the growth period, because of different growth rates among species (Terashima and Hikosaka 1995), could have concealed a difference in the maximum LAI attained.

Nutrient limitations and elevated CO_2 can drive a reallocation of N to upper canopy layers (Long and Drake 1991, Hirose et al. 1997). Typically, as total N availability increases, LAI is slightly greater in elevated CO_2 , resulting in lower leaf N concentrations (Hilbert et al. 1991). However, we found no shift in N distribution among canopy layers in elevated CO_2 communities (Fig. 3B). Our observation that elevated CO_2 did not alter the vertical distribution of N suggests that in multi-species canopies, the individual response of different species may obscure the pattern observed in simpler single-species canopies.

We expected that elevated CO_2 communities would be able to maintain more leaf area deep in the canopy through

a reduction of the LCP for deeply shaded leaves. Contrary to this expectation, we found a slight, significant increase in the distribution of leaf area toward upper canopy layers (Fig. 3A) for communities grown under elevated CO_2 . Faster leaf turnover in elevated CO_2 and an increase in the amount of N higher in the canopy resulted in faster senescence in 8 out of 10 Mediterranean herbaceous species grown in artificial communities (Navas et al. 1997). Faster development of upper canopy layers and possibly greater leaf turnover may have had a stronger influence on maximum LAI than the effects of CO_2 on photosynthetic properties including the light compensation point.

The early stimulation of LAI (Fig. 2A) during canopy development and presumably photosynthesis contributed to greater total biomass for elevated CO_2 communities at the time of harvest (Table 2). The 20–26% stimulation of biomass in the present study is within the range of stimulation in other multi-species communities (15–40%; Roy et al. 1996, Navas et al. 1997, Leadley et al. 1999, Norton et al. 1999) and slightly lower than that reported for primarily potted plants (Poorter et al. 1996).

There was no effect of elevated CO_2 on NEE at the community level when measured slightly after the middle of the growth period (Fig. 5B). This contradicts the common

Table 2. Leaf area, LAD, component biomass (leaf, stem and root mass), and nitrogen concentration for communities exposed to: high CO_2 /fertilized (HCF), low CO_2 /fertilized (LCF), high CO_2 /unfertilized (HCU) and low CO_2 /unfertilized (LCU). Each value is the mean (\pm SE) of six communities (LAD values presented are the cumulative leaf area duration for each treatment and day.) A significant effect of CO_2 , fertilization or interaction of CO_2 and fertilization represented by F-values and $P > F$ values.

	Treatment					
	HCF (mean \pm SE)	LCF (mean \pm SE)	HCU (mean \pm SE)	LCU		
Leaf area (m^2)	1.88 \pm 0.02	1.73 \pm 0.11	0.40 \pm 0.05	0.31 \pm 0.02		
Leaf area duration (days)	297 \pm 10	247 \pm 9	187 \pm 7	164 \pm 6		
Biomass (g)						
Total	205.5 \pm 10.6	162.6 \pm 12.7	86.8 \pm 7.8	72.3 \pm 7.5		
Leaf (L)	54.2 \pm 1.7	50.4 \pm 1.7	16.3 \pm 1.6	14.5 \pm 0.9		
Stem (S)	68.7 \pm 5.2	49.4 \pm 4.2	18.6 \pm 2.1	10.9 \pm 1.2		
Root (R)	82.6 \pm 7.1	62.7 \pm 8.3	52.0 \pm 8.2	46.9 \pm 7.1		
R:shoot ratio	0.68 \pm 0.05	0.62 \pm 0.06	1.59 \pm 0.35	1.85 \pm 0.27		
Nitrogen concentration (% N)						
Leaf	2.03 \pm 0.04	2.42 \pm 0.08	0.79 \pm 0.06	0.97 \pm 0.05		
Root	0.79 \pm 0.04	0.74 \pm 0.04	0.60 \pm 0.05	0.49 \pm 0.04		
Litter	1.72 \pm 0.09	2.05 \pm 0.15	0.70 \pm 0.03	0.61 \pm 0.08		
	Significance					
	CO_2		Fertilization		$\text{CO}_2 \times$ fertilization	
	F-value	$P > F$	F-value	$P > F$	F-value	$P > F$
Leaf area (m^2)	3.62	0.072	548.20	0.0001	0.15	0.700
Leaf area duration (days)	9.46	0.002	63.47	0.0001	1.44	0.231
Biomass (g)						
Total	8.46	0.009	112.22	0.0001	2.06	0.167
Leaf (L)	3.23	0.088	603.02	0.0001	0.37	0.575
Stem (S)	14.39	0.001	154.07	0.0001	2.65	0.119
Root (R)	2.61	0.122	9.06	0.007	0.93	0.346
R:Shoot ratio	1.07	0.312	70.48	0.0001	4.78	0.041
Nitrogen concentration (% N)						
Leaf	31.09	0.0001	388.02	<0.0001	2.28	0.133
Root	4.06	0.058	29.21	<0.0001	0.41	0.528
Litter	1.29	0.271	135.42	<0.0001	4.01	0.060

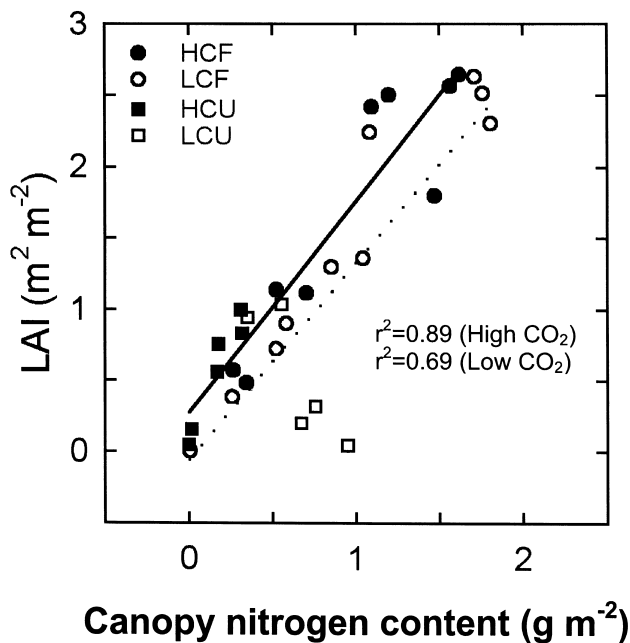


Fig. 4. Relationship of leaf area index ($\text{m}^2 \text{m}^{-2}$) and canopy nitrogen content per ground surface (g m^{-2}) at the time of harvest for communities exposed to: high CO_2 /fertilized (HCF); low CO_2 /fertilized (LCF); high CO_2 /unfertilized (HCU); and low CO_2 /unfertilized (LCU) treatments. Each point represents the mean of a given horizontal canopy layer averaged across 4–6 pots (because of variation in canopy height, and hence the number of layers harvested) in each 10-cm canopy layer.

result of increased NEE and net photosynthesis at the leaf-level (Campbell et al. 1990, Ziska et al. 1996; however, cf. Reekie and Bazzaz 1989). The timing of the NEE measurements (31–33 days from sowing) may have been too late to detect an effect of CO_2 , as the rate of canopy development appeared to decrease as the canopy approached maximum LAI (40–42 days from sowing; Fig. 2A).

Greater relative allocation of biomass to shoots with greater species diversity should have a positive feedback between CO_2 assimilation and aboveground biomass accumulation, although the effect is not likely to persist (Stocker et al. 1999). We found no effect of CO_2 on species diversity, NEE or GEE. However, small differences in instantaneous NEE could have large cumulative effects on plant biomass (Stocker et al. 1999). We found a tendency for increased NEE due in part to a small, offsetting effect of elevated CO_2 that increased total respiration for fertilized communities (Fig. 5C). The enhancement of total respiration may be related to greater belowground productivity, which accounts for over half of net primary productivity in grasslands (Stanton 1988, Stocker et al. 1999).

The results of community-wide carbon exchange in the present study differ from other studies, possibly because of the species composition of our communities, the timing of the measurements, or the lack of repeating the carbon exchange measurements. A tropical rainforest mesocosm had 71% greater NEE under elevated CO_2 (Lin et al. 1999); the enhancement occurred through a much larger increase in canopy carbon assimilation relative to a very small increase in night respiration. Lin et al. (1999) found in a desert

mesocosm that an enhancement of NEE occurred through a reduction in total respiration and an increase in canopy assimilation. An old-field grassland microcosm study had 50% greater net canopy photosynthesis during the first month and a 20% enhancement throughout the study (Roy et al. 1996). Communities of calcareous grassland species assemblages grown in elevated CO_2 had greater NEE over 2 years without a concomitant increase in aboveground biomass (Stocker et al. 1999). During the first year, they found lower NEE with lower species diversity. Leadley et al. (1999) found that plant biomass was increasingly greater during each year of a 4-year exposure to elevated CO_2 in a calcareous grassland community. They also found an in-

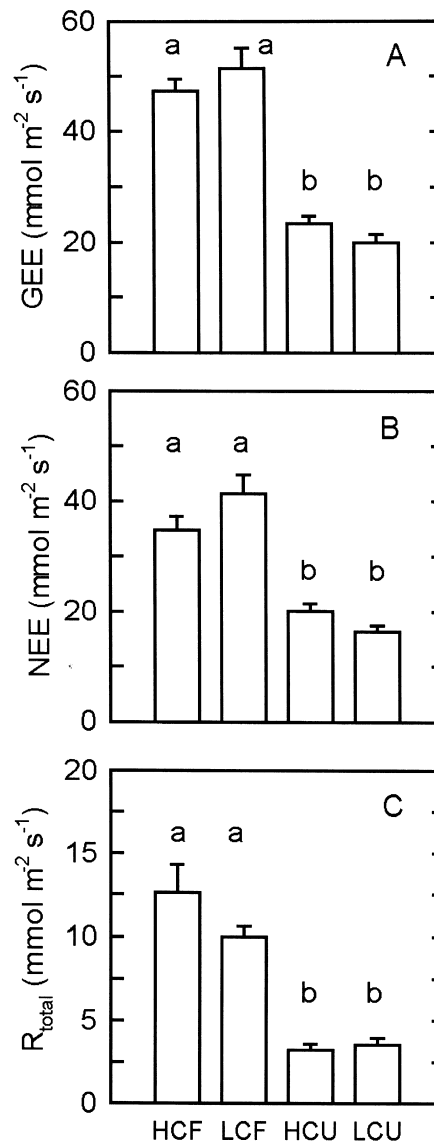


Fig. 5. Mean CO_2 exchange for communities exposed to: high CO_2 /fertilized (HCF); low CO_2 /fertilized (LCF); high CO_2 /unfertilized (HCU); and low CO_2 /unfertilized (LCU) treatments. Each bar represents the mean of 6 communities (\pm SE). (A) Gross ecosystem exchange ($\text{GEE} = \text{NEE} + R_{\text{total}}$); (B) net ecosystem exchange (NEE); and (C) total ecosystem respiration in the dark ($R_{\text{total}} = R_{\text{root}} + R_{\text{soil}} + R_{\text{leaf}}$). Different letters represent a significant difference between treatments within each plate.

crease in species diversity and interspecific variation with the potential to change community structure. Our measurements of NEE were made near the time of maximum LAI, and the initiation of senescence may have eliminated the potential effect of elevated CO₂ on NEE.

The biology of our experimentally assembled model communities may have modified the effect of CO₂ on canopy development. First, our communities became dominated by grasses (e.g., *Sorghum halepense* [L.] Pers. comprised 45–60% of total aboveground biomass in elevated and 10–20% in ambient CO₂ canopies); we may have overestimated LAI because grasses do not lose leaves in lower strata of the canopy (Werger and Hirose 1991). Second, numerous species of various functional types most likely had variable responses to elevated CO₂ that may have counteracted one another (e.g., Poorter et al. 1996, Schäppi and Körner 1996). Because our primary interest was in community level responses, we did not test for variation within or between species.

This study suggests that rapidly growing communities primarily comprised of annuals, especially those with plentiful nutrients, may utilize higher CO₂ concentrations initially upon exposure. However, as plants approached maximum size, respiration may dampen earlier increases in NEE or biomass. The productivity of annual plant communities in a future CO₂-enriched atmosphere ultimately may be slightly greater in elevated CO₂, supported by our finding of greater total biomass and the tendency for increased LAI at some points during the experiment. This study has provided support for the main effect of elevated CO₂ to be an acceleration of canopy development rather than stimulation of maximum LAI. Soil nutrient availability increased LAI, whereas CO₂ did not change for LAI in our communities. Care must be taken, however, for extrapolating these results to the field where greater rooting depth and differential access to water and nutrients may influence the effect of CO₂ on canopy development.

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