

Effects of elevated atmospheric CO₂ on net ecosystem CO₂ exchange of a scrub–oak ecosystem

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Abstract

We report the results of a 2-year study of effects of the elevated (current ambient plus 350 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) atmospheric CO₂ concentration (C_a) on net ecosystem CO₂ exchange (NEE) of a scrub–oak ecosystem. The measurements were made in open-top chambers (OTCs) modified to function as open gas-exchange systems. The OTCs enclosed samples of the ecosystem (ca. 10 m² surface area) that had regenerated after a fire, 5 years before, in either current ambient or elevated C_a . Throughout the study, elevated C_a increased maximum NEE (NEE_{max}) and the apparent quantum yield of the NEE (ϕ_{NEE}) during the photoperiod. The magnitude of the stimulation of NEE_{max} expressed per unit ground area, was seasonal, rising from 50% in the winter to 180% in the summer. The key to this stimulation was effects of elevated C_a , and their interaction with the seasonal changes in the environment, on ecosystem leaf area index, photosynthesis and respiration. The separation of these factors was difficult. When expressed per unit leaf area the stimulation of the NEE_{max} ranged from 7% to 60%, with the increase being dependent on increasing soil water content (W_{soil}). At night, the CO₂ effluxes from the ecosystem (NEE_{night}) were on an average 39% higher in elevated C_a . However, the increase varied between 6% and 64%, and had no clear seasonality. The partitioning of NEE_{night} into its belowground (R_{below}) and aboveground (R_{above}) components was carried out in the winter only. A 35% and 27% stimulation of NEE_{night} in December 1999 and 2000, respectively, was largely due to a 26% and 28% stimulation of R_{below} in the respective periods, because R_{below} constituted ca. 87% of NEE_{night} . The 37% and 42% stimulation of R_{above} in December 1999 and 2000, respectively, was less than the 65% and 80% stimulation of the aboveground biomass by elevated C_a at these times. An increase in the relative amount of the aboveground biomass in woody tissue, combined with a decrease in the specific rate of stem respiration of the dominant species *Quercus myrtifolia* in elevated C_a , was responsible for this effect. Throughout this study, elevated C_a had a greater effect on carbon uptake than on carbon loss, in terms of both the absolute flux and relative stimulation. Consequently, for this scrub–oak ecosystem carbon sequestration was greater in the elevated C_a during this 2-year study period.

Keywords: elevated CO₂, NEE , open-top chambers, respiration, scrub–oak ecosystem

Received 16 January 2003; revised version received and accepted 9 May 2003

Introduction

Major research initiatives into the biological effects of global climate change are currently focused on increasing the understanding of the environmental controls on

ecosystem carbon cycling, particularly those factors affected by increasing atmospheric CO₂ concentration (C_a). It is likely that increasing C_a will result in an increase in the net exchange of CO₂ between the biosphere and the atmosphere (NEE), through a stimulation of photosynthesis, increased water and nitrogen use efficiency and growth. (Recent reviews. Leaf physiology: Drake *et al.*, 1997; Jarvis *et al.*, 1999; Growth and phenology: Norby *et al.*, 1999; Pritchard *et al.*, 1999; Water relations: Wullschleger *et al.*, 2002; Soil

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processes: Zak *et al.*, 2000.). The results from field experiments have reported that a step increase in C_a to the elevated levels predicted within the next hundred years results in increased carbon sequestration in aboveground (DeLucia *et al.*, 1999; Norby *et al.*, 2001; Oren *et al.*, 2001; Dijkstra *et al.*, 2002) and belowground biomass (DeLucia *et al.*, 1999; Schlesinger & Lichter, 2001; Dilustro *et al.*, 2002). A net increase in ecosystem carbon uptake in elevated C_a would be expected to underlie these changes in biomass accumulation.

Currently, the number of studies that have measured *NEE* at both current ambient and elevated C_a is limited, both with respect to the ecosystems in which they have been made and also the duration of the data sets generated. This is unfortunate for two key reasons. First, regular *NEE* measurements are ideally suited to the establishment of causative relationships between ecosystem carbon cycling, biotic and abiotic factors and their interaction with elevated C_a. Second, long-term measurements of *NEE* at elevated C_a will yield data sets against which ecosystem models, which are typically parameterized using leaf level data (McMurtrie & Wang, 1993; Medlyn & Dewar, 1996; Rasse *et al.*, 2001), can be tested.

Enclosure methods remain the only way that *NEE* measurements can currently be made at elevated C_a. Consequently, field experiments have been limited to low stature ecosystems. Studies in a C₃ grassland (Stocker *et al.*, 1999; Niklaus *et al.*, 2000) and a marsh (Drake *et al.*, 1996) demonstrated the potential for increased *NEE* during the photoperiod in elevated C_a. In contrast, the stimulation of *NEE* in elevated C_a was transient in both the Arctic tundra (Oechel *et al.*, 1994) and a C₃ alpine grassland (Diemer & Körner, 1998), while for a C₄ grassland the stimulation of *NEE* in elevated C_a was only observed late in a 34-day measurement period, due to delayed senescence (Ham *et al.*, 1995). With the exception of Drake *et al.*, 1996, who observed a decrease in marsh ecosystem respiration in elevated C_a, effects on ecosystem respiration have not been typically observed (Diemer, 1994; Ham *et al.*, 1995; Stocker *et al.*, 1997). Currently, field studies on woody ecosystems are missing from the literature.

Here, we report *NEE* measurements made during a 2-year period between July 1999 and 2001 at current ambient and elevated C_a in a fire-dependent scrub-oak ecosystem of central Florida. The key findings at the site have been a sustained stimulation of photosynthesis for the main species (Li *et al.*, 1999, 2000; Hymus *et al.*, 2001; Ainsworth *et al.*, 2002; Hymus *et al.*, 2002a), little effect of elevated C_a on leaf or soil respiration (Johnson *et al.*, 2001; Hymus *et al.*, 2002a) and a strong stimulation of aboveground biomass and ecosystem leaf area index (LAI) by elevated C_a (Dijkstra *et al.*, 2002; Hymus *et al.*, 2002b). These findings provide the basis for testing the

hypothesis that elevated C_a will increase carbon uptake during the photoperiod, primarily due to the increased photosynthesis and leaf area, and increase carbon loss at night, due to increased biomass.

Materials and methods

Site description

The open-top chamber (OTC) project was sited in the fire-dependent, scrub-oak ecosystem of coastal central Florida on Merritt Island (28°38'N, 80°42'W), within NASA's Kennedy Space Center. The substrates were well-drained Pomello (Arenic Haplhumod) and Poala Sands (Spodic quartzipsamment). 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 (Schmaltzer & Hinkle, 1992). The experimental site was representative of a fire-maintained scrub-oak palmetto community (Breininger & Schmalzer, 1990). The shrub layer was comprised of rhizomatous sclerophyllous evergreen oaks, which resprouted from belowground after fire (Breininger & Schmalzer, 1990). Three oak species, *Quercus myrtifolia*, *Quercus geminata* and *Quercus chapmanii*, typically comprise up to 85–90% of the aboveground biomass in this ecosystem (Schmaltzer & Hinkle, 1992). The climate was subtropical, warm and humid, with an average annual precipitation of 131 cm masking high year-to-year variability. The mean maximum and minimum temperatures in July, the hottest month, were 33.3 °C and 21.8 °C, respectively, and 22.3 °C and 9.5 °C in January, the coldest month (Schmaltzer & Hinkle, 1992). The combination of frequent fires, seasonal water stress and nutrient poor soil conditions maintains the composition and structure of the ecosystem.

Sixteen OTCs, 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 then completely burnt in a second fire in January 1996. Eight of the OTCs were maintained at the current ambient C_a and eight at elevated C_a (current ambient + 350 μmol CO₂ mol⁻¹). Here, we present the data of 2 years collected between June 1999, 3 years into the fire regeneration cycle, and July 2001, 5 years into the regeneration cycle. Within the context of a natural fire regeneration cycle that maybe between 7 and 40 years in length, the data presented here come from an early stage of ecosystem regeneration after fire.

Measurement of *NEE*

With the addition of custom-designed Lexan lids (Commercial Plastics, Orlando, FL, USA), the OTCs

functioned as an open gas-exchange system. Lids were added and *NEE* measured monthly for periods of 5–10 days. Air was blown through the OTCs at a rate of ca. $27 \text{ m}^3 \text{ min}^{-1}$, entering through four circular ducts each of 20.3 cm diameter, total surface area 0.123 m^2 , and exiting through exhaust ports in the lids with a total exit surface area of 0.096 m^2 . The difference in the entrance and exit area increased the chamber air pressure, which provided protection against leaks of outside air into the OTCs. However, the increased pressure also suppressed soil CO_2 efflux (R_{soil} , see the artifacts below). The ventilation rate was sufficient such that the chamber air was replaced 1.5 times a minute. An IRGA (LI 6262; LI-COR, Lincoln, NEB, USA) operated in the differential mode, measuring the difference between a reference CO_2 mol fraction, from one of four chamber inlet ducts, and a sample CO_2 mol fraction inside the chambers, at the canopy height. Midway through each OTC sampling, a switching valve reversed the cells of the IRGA through which the two air streams flowed. A differential was measured in each configuration with the final differential used being the mean of these two measurements. This protocol accounted for the problems associated with the contamination or aging of the IRGA cells. A second IRGA continuously measured the reference CO_2 mol fraction (chamber inlet) and fed this value into the differential analyzer, which then used an internal algorithm to correct the measured differential for changes in the reference C_a . Both the reference and sample air streams were drawn from the OTCs at a flow rate of 5 L min^{-1} . After exhausting, the sample was fed to the IRGAs at a rate of 1 L min^{-1} after being individually mixed in flask volumes of 1.9 L. All 16 OTCs were sampled once every 26 min until August 2000, then afterwards once every 15 min. The molar flow rate of air through each chamber was determined at the end of each measurement period from the dilution of a known CO_2 flux injected into each blower, by calculating the flow rate required to give the measured C_a (Garcia *et al.*, 1990). We calculated the *NEE* per m^2 of ground area (s) from the difference between reference and sample C_a (ΔC_a), and the flow rate of air through the chamber (f) (mol s^{-1}).

$$NEE (\mu\text{mol m}^{-2} \text{ ground area s}^{-1}) = (\Delta C_a \times f) / s. \quad (1)$$

Experimental artifacts

Leaks of air into the OTC, and the suppression of R_{soil} due to increases in OTC air pressure are artifacts of measurement in chambers (Fang & Moncrieff, 1998; Lund *et al.*, 1999; Niklaus *et al.*, 2000). Throughout the duration of this study, experiments were carried out

from which we developed protocols to account for both these artifacts in this system. Leaks of outside air into the OTCs were investigated using N_2O as an inert tracer, and found to occur only when wind speed exceeded 5 m s^{-1} . By excluding any data collected when the wind speeds exceeded 4 m s^{-1} , we minimized the possibility of the contaminated data entering our analysis. At night, the data collected at low wind speeds ($< 2 \text{ m s}^{-1}$) were also excluded. This was because at night, a wind speed greater than 2 m s^{-1} was necessary to mix air in and around the canopy to provide a stable reference C_a of the air drawn into the OTCs. In the most extreme cases, the requirement of wind speeds greater than 2 m s^{-1} resulted in the exclusion of 80% of the night data collected in any given measurement period. Turbulent atmospheric conditions during the day resulted in good mixing of the air around the canopy, even at wind speeds below 2 m s^{-1} , and exclusion of the data was not required.

Accounting for the suppression of R_{soil} by increases in the air pressure inside the OTC was essentially a modeling exercise performed in two steps. First, we parameterized the model of Hanson *et al.* (1993) using concurrent measurements of R_{soil} , soil temperature (T_{soil}) and soil water content (W_{soil}) collected at the site throughout the study period (Dore *et al.*, 2003). The linear relationship between the modeled and measured fluxes was highly significant ($t_{1,15} = 7.6$; $P < 0.01$; $r^2 = 0.80$; Dore *et al.*, 2003). Second, on nine occasions between March 2000 and July 2001, we measured the R_{soil} in all the OTCs both before and after the addition of the lids. The percentage suppression of the R_{soil} (g), due to pressurization of the OTC after the addition of the lids, varied from 32% to 63% on these occasions and was found to be significantly dependent on increases in W_{soil} from 1.9% to 6.3% ($g = 0.43 \times \ln(W_{\text{soil}}) - 0.24$; $P < 0.01$; $r^2 = 0.84$; D. P. Johnson, unpublished results). From a modeled estimate of the R_{soil} (mR_{soil}) and an estimate of the percentage of R_{soil} that was suppressed (g), we could correct each measurement in each chamber for the suppressed R_{soil} (z). This procedure resulted in the *NEE* being

$$NEE = NEE(1) + z, \quad (2)$$

where *NEE* (1) is the *NEE* calculated as described in Eqn (1), and

$$z = mR_{\text{soil}} \times g.$$

The confidence in the effectiveness of these protocols to account for the experimental artifacts was provided by Dore *et al.* (2003), who showed that at night, when the OTC has a negligible effect on the microclimate, the *NEE*_{night} measured inside the OTCs was not different

from the *NEE* measured in the same stand using an eddy covariance system.

Derivation of *NEE* parameters

For each measurement period, data were divided into those collected during the photoperiod (PPFD > 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$; wind speed < 4 m s^{-1}) and those collected at night (PPFD < 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$; wind speed > 2 < 4 m s^{-1}). For the data collected in each OTC during the photoperiod, the response of *NEE* to PPFD was plotted and a non-rectangular hyperbola was fitted (Long & Hällgren, 1993). From each curve fit, *NEE* at the maximum PPFD measured during the measurement period (*NEE*_{max}), and the apparent quantum yield of *NEE* (ϕ_{NEE}), were obtained. Ecosystem respiration (*NEE*_{night}) was calculated as the average of all the individual *NEE* measurements made at night in each measurement period. The parameters *NEE*_{max}, ϕ_{NEE} and *NEE*_{night} were all determined for individual OTCs during each measurement period. In this study *NEE*_{max} per unit leaf area was also expressed. This was possible in 11 periods when the *NEE* measurements coincided with measurements of LAI. Previously published data revealed LAI to be higher in elevated *C_a*, rising from 20% higher in the winter to a maximum of 55% higher during the summer months. In ambient *C_a*, LAI increased from 0.8 to 1.2 from 0.9 to 1.6 and from 0.7 to 1.7 in 1999, 2000 and 2001, respectively. In elevated *C_a*, LAI increased from 1.1 to 1.5 from 1.1 to 2.3 and from 0.8 to 2.3 in 1999, 2000 and 2001, respectively. The ecosystem LAI was determined from measurements of light penetration through the canopy using Beer's law, and is described in detail in Hymus *et al.* (2002b).

Partitioning of Ecosystem Respiration

Partitioning of *NEE*_{night} into its above- (*R*_{above}) and below (*R*_{below})-ground components was carried out by subtracting *R*_{above} from *NEE*_{night} in December 1999 and 2000 only. Aboveground respiration (*R*_{above}) was estimated at the same temperature as *NEE*_{night} by applying the temperature dependencies of leaf and stem respiration, determined for both *Q. myrtifolia* and *Q. geminata* in December 2000, to the biomass measurements of the aboveground stem biomass determined from the allometric relationships (Dijkstra *et al.*, 2002) and leaf area (Hymus *et al.*, 2002b) made in both December 1999 and 2000. The aboveground stem dry weight (SDW) of *Q. myrtifolia* and *Q. geminata* grown in the ambient and elevated *C_a* was determined from the measurements of stem basal diameter (SBD) using the following allometric relationships: $\ln(\text{SDW}) = -3.005 + 3.231(\ln(\text{SBD}))$ ($r^2 = 0.91$) and $\ln(\text{SDW}) = -2.913 + 3.196(\ln(\text{SBD}))$ ($r^2 =$

0.95) for *Q. myrtifolia* and *Q. chapmanii* growing in the elevated and ambient *C_a*, respectively. And $\ln(\text{SDW}) = -3.059 + 3.154(\ln(\text{SBD}))$ ($r^2 = 0.92$) and $\ln(\text{SDW}) = -2.962 + 3.094(\ln(\text{SBD}))$ ($r^2 = 0.93$) for *Q. geminata* growing in the elevated and ambient *C_a*, respectively. The stem biomass was 303 and 572 g C m^{-2} in the ambient and elevated treatments, respectively, in December 1999, and 402 and 727 g C m^{-2} in the ambient and elevated treatments, respectively, in December 2000. The temperature dependence of the leaf (*R*_{leaf}) and stem (*R*_{stem}) respiration for both *Q. myrtifolia* and *Q. geminata* was measured using the experimental setup described in detail in Hymus *et al.* (2002a). The selection of the current year foliage was as described in Hymus *et al.* (2002a). The samples of current year woody stem tissue (at least 1.5 g), of ca. nine months age, were removed by a razor within an hour of sunrise from both *Q. myrtifolia* and *Q. geminata* in each OTC. For each species in each OTC, a sample consisted of stem sections of up to 15 cm in length taken from at least five plants. Both the leaf and stem respiration rates were measured at 16 °C, 20 °C, 23 °C, 26 °C and 30 °C at their respective growth *C_a*. The measurements were made at least 30 min after each step change in the temperature of the air flowing through the cuvettes. This time was found to be sufficient for respiration rates to stabilize at the new temperature.

Micrometeorological measurements

Continuous measurements of *T*_{soil} (°C) measured at 10 cm depth, *W*_{soil} (%vol) measured between 0 and 15 cm depth and wind speed (m s^{-1}) and PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$) both measured at a height of 3 m were recorded with a datalogger (CR7, Campbell Scientific, Logan, UT, USA). The soil temperature and *W*_{soil} were measured in each plot using copper-constantan thermocouples (Omega Engineering, Stanford, CT, USA) and water content reflectometers (CS615, Campbell Scientific, Logan, UT, USA), respectively. The wind speed was measured with a cup-anemometer (R. M. Young Wind Sentry Set, Campbell Scientific, Logan, UT, USA) and PPFD using a quantum sensor (LI 190, LICOR, Lincoln, NB, USA).

Statistical analysis

The non-rectangular hyperbola, from which *NEE*_{max} and ϕ_{NEE} were derived, was fitted to plots of PPFD and *NEE* using graphical software (Sigma plot 2001, SPSS, Chicago, IL, USA). Repeated-measures analysis of variance was used to test for an effect of *C_a* and time on *NEE*_{max}, ϕ_{NEE} and *NEE*_{night}. Two-way ANOVA was used to test (i) the effect of *C_a* and temperature on leaf

and stem respiration of *Q. myrtifolia* and *Q. geminata* in December 2000, and (ii) the effect of the C_a and year, on R_{above} , R_{below} and total ecosystem respiration in December 1999 and 2000. For all the analyses, individual OTCs were used as replicates. For all the repeated measures and two-way ANOVAs carried out in this study, none of the interactions between the factors were significant ($P > 0.05$). Consequently, the results of the interactions have not been reported in the text. All analysis was carried out using statistical software (Systat 7.0, Systat, Evanstone, IL, USA).

Results

Throughout this study, the summer months were characteristically warm and wet, the winter months were cooler and dryer (Fig. 1). The trends in T_{air} exhibited patterns typical of the geographic location, ranging from 19 °C in January 2001 to 35 °C in May 2001 during the photoperiod, and from 8 °C in December 2001 to 26 °C in August 2000 during the night (Fig. 1b). The monthly rainfall totals followed general long-term precipitation patterns. However, the specific months often differed substantially from long-term averages. In particular, July 1999 and August 2000 were very dry for summer months, while September 1999 and March 2001 were much wetter than average (Fig. 1c).

Photoperiod

The seasonal changes in NEE_{max} (expressed per m^2 ground area) in the elevated C_a treatment tracked those in ambient C_a . In both treatments, NEE_{max} rose sharply from winter minima during the spring, to reach summer maxima. This transition reflected increases in the temperature, rainfall and leaf area. The decline in NEE_{max} during the autumn and winter months reflected decreases in both the temperature and rainfall, in combination with decreased and senescing leaf area (Figs 1 and 2). The changes in NEE_{max} over time were statistically significant ($F_{25,75} = 11.6$; $P < 0.01$). Throughout the measurement period, NEE_{max} was significantly higher in elevated C_a ($F_{1,3} = 30.3$; $P = 0.01$). However, there was a marked seasonality to the degree of stimulation (Fig. 2b). During the spring, the stimulation of NEE_{max} increased, reaching a maximum during the summer months of ca. 180%. The stimulation then declined during the autumn and winter to a minimum of ca. 50%. When expressed per unit leaf area, minimizing the effect of the increased LAI in elevated C_a , the stimulation of NEE_{max} was lower but still variable, and ranged from ca. 7% to ca. 60% (Fig. 2b). This variation was not dependent on the variation in T_{air} ($P = 0.78$; $r^2 = 0.01$) or T_{soil} ($P = 0.56$; $r^2 = 0.04$), but on

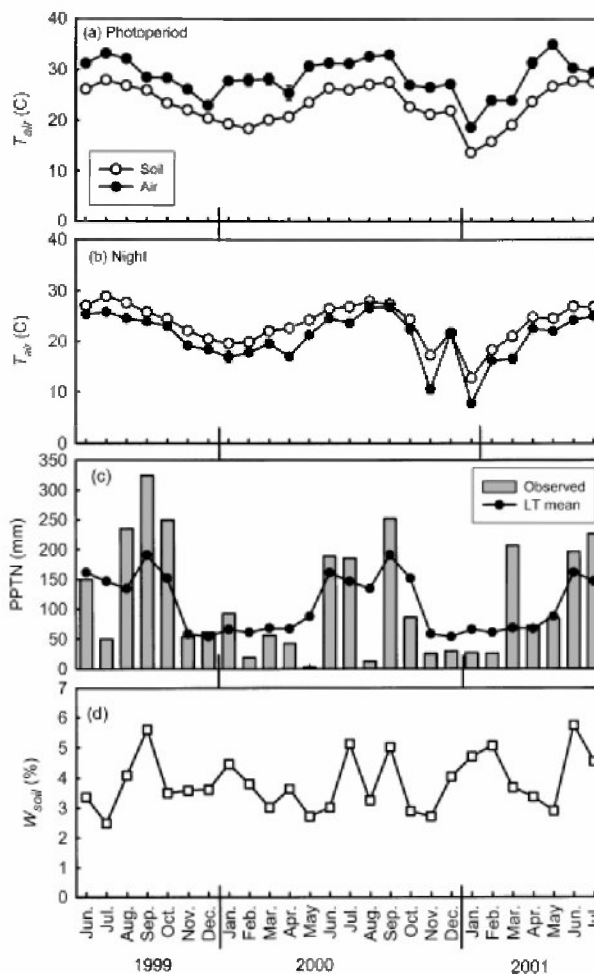


Fig. 1 Climate data. Monthly averages of T_{air} (solid circles) and T_{soil} (open circles) measured during the photoperiod (a), and at night (b). Monthly precipitation totals (PPTN) measured at the site (solid bars) and long-term mean monthly precipitation totals averaged between 1878 and 1956 (LT mean, circles) (c), and W_{soil} (d) during measurement periods in each month between June 1999 and July 2001, are also shown. For T_{air} , T_{soil} and W_{soil} symbols are the mean (± 1 SE) of measurements made in ≥ 10 OTCs. Because T_{air} , T_{soil} and W_{soil} were unaffected by the C_a , the data shown are averaged for the two treatments. The precipitation was measured at one location within the site.

W_{soil} ($P = 0.01$; $r^2 = 0.54$), with the stimulation of NEE_{max} in elevated C_a increasing as W_{soil} increased (Fig. 3). Importantly, the changes in W_{soil} were independent of changes in T_{air} ($P = 0.31$; $r^2 = 0.04$), minimizing the possibility that the dependence of the stimulation of NEE_{max} in elevated C_a on W_{soil} , was actually due to increases in T_{air} .

At low light, elevated C_a significantly stimulated ϕ_{NEE} ($F_{1,3} = 31.3$; $P = 0.01$; Fig. 4). While ϕ_{NEE} was significantly dependent on time of the year ($F_{25,75} = 6.2$;

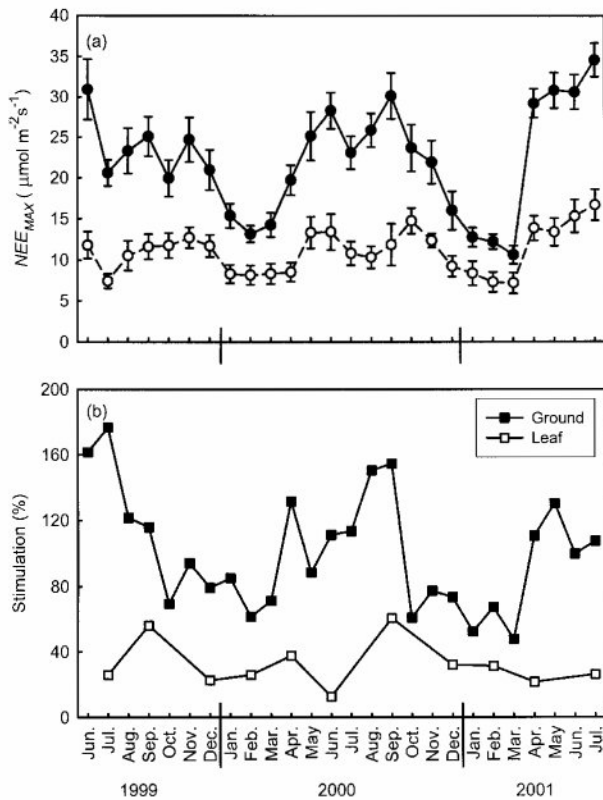


Fig. 2 Trends in NEE_{max} , measured monthly in both ambient (open circles) and elevated (closed circles) C_a , between June 1999 and July 2001; each data point is the mean (± 1 SE) of measurements made in ≥ 6 OTCs (a). For each month the percentage stimulation of NEE_{max} in elevated C_a is also shown, expressed both per m² ground area (solid squares) and per m² leaf area (open squares) (b).

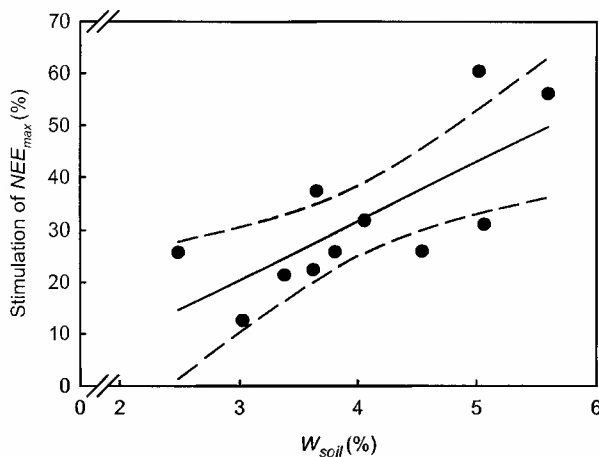


Fig. 3 Plot of the percentage stimulation of NEE_{max} expressed per m² leaf area, in elevated C_a against W_{soil} measured between 0 and 15 cm. A linear regression and 95% confidence intervals are shown.

$P < 0.01$) and the greatest stimulation of the ϕ_{NEE} in elevated C_a was during the summer, a clear seasonality to the effect was not obvious.

Night

Both the C_a treatments exhibited the same significant changes in NEE_{night} throughout the study ($F_{25,125} = 28.9$; $P < 0.01$). The increases from the winter minima to summer maxima followed increases in T_{soil} (Figs 5 and 6). Night respiration was significantly higher in elevated C_a , averaging 39% over the study period, and varying between 6% and 64% in the elevated C_a (Fig. 5) ($F_{1,5} = 16.7$; $P = 0.01$). However, there was no clear seasonality to the effect.

The partitioning of NEE_{night} into its above- and belowground components was attempted in the winter only. In December 1999 and 2000, R_{below} accounted for 86% and 89% of the ecosystem respiration, respectively (Table 1). The R_{below} was 26% and 28% higher in elevated C_a in December 1999 and 2000, respectively; neither effect was significant when testing at $P < 0.05$, but both were significant at $P < 0.1$. During both the months, R_{above} was significantly higher in elevated C_a , by 35% ($t_{1,14} = 2.25$; $P = 0.02$) and 42% ($t_{1,14} = 1.94$; $P = 0.04$) in 1999 and 2000, respectively (Table 1). The partitioning of NEE_{night} between R_{above} and R_{below} was achieved by scaling up leaf and stem respiration measurements to the ecosystem using biomass inventories. The R_{leaf} and R_{stem} of *Q. geminata* and *Q. myrtifolia* were always significantly dependent on T_{air} ($P < 0.01$). The temperature dependence of R_{leaf} and R_{stem} of *Q. geminata* and R_{leaf} of *Q. myrtifolia* was unaffected by growth in elevated C_a (Fig. 7a, b and d).

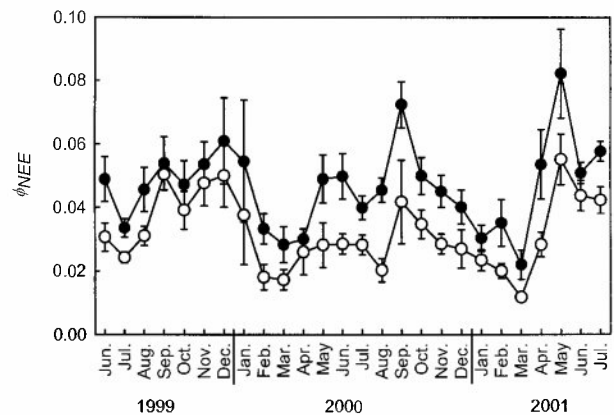


Fig. 4 Trends in ϕ_{NEE} , measured monthly in both ambient (open circles) and elevated (closed circles) C_a , between June 1999 and July 2001; each data point is the mean (± 1 SE) of measurements made in ≥ 6 OTCs.

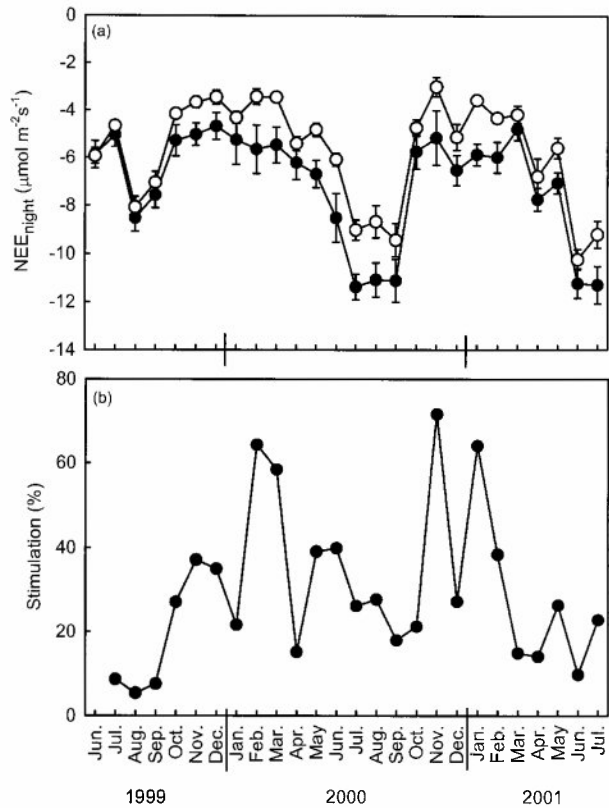


Fig. 5 Trends in NEE_{night} measured monthly in both ambient (open circles) and elevated (closed circles) C_a , between June 1999 and July 2001; each data point is the mean (± 1 SE) of measurements made in ≥ 6 OTCs (a). For each month the percentage stimulation of NEE_{night} in elevated C_a is also shown (b).

However, R_{stem} of *Q. myrtifolia* was significantly lower in elevated C_a at all temperatures ($F_{1,61} = 14.4$; $P < 0.01$; Fig. 7c). For both *Q. geminata* and *Q. myrtifolia*, R_{leaf} was higher than the R_{stem} .

Discussion

This study explored the effect of elevated C_a on NEE of a scrub-oak ecosystem. The study was performed over a 25-month period, beginning 3 years into a fire-

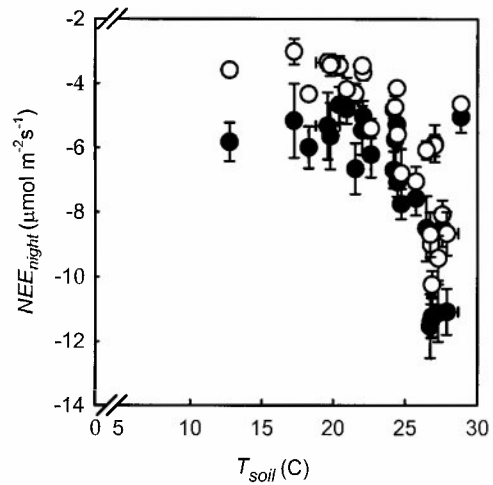


Fig. 6 Plot of the dependence of NEE_{night} on T_{soil} for measurements made in both ambient (open circles) and elevated (closed circles) C_a . Symbols shown are the averages of measurements made during the measurements periods in each month. Each symbol is the mean (± 1 SE) of measurements made in ≥ 6 OTCs.

regeneration cycle. We tested the hypothesis that elevated C_a would stimulate ecosystem carbon uptake during the photoperiod, and carbon loss at night. This hypothesis was confirmed. However, at night the increase in aboveground respiration was less than the increase in the aboveground biomass.

Photoperiod

During the photoperiod maximum and light-limited NEE , both expressed per unit ground area, were significantly higher in elevated C_a (Figs 2a and 4). For NEE_{max} there was a clear seasonality to this stimulation, rising to a summer maximum from a winter minimum. For ϕ_{NEE} any seasonality was less obvious; however, the largest effect of the elevated C_a was also observed in the summer months. The increase in the effect of the elevated C_a on NEE_{max} during the spring and summer months was consistent with increases in LAI during

Table 1 Partitioning ecosystem respiration

	R_{below} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)			R_{above} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)			NEE_{night} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
	Ambient	Elevated	S (%)	Ambient	Elevated	S (%)	Ambient	Elevated	S (%)
Dec. 1999	2.98 \pm 0.22	3.81 \pm 0.50	28*	0.48 \pm 0.06	0.65 \pm 0.04	37**	3.46 \pm 0.26	4.53 \pm 0.49	31**
Dec. 2000	4.59 \pm 0.49	5.78 \pm 0.61	26*	0.55 \pm 0.07	0.78 \pm 0.09	42**	5.13 \pm 0.55	6.53 \pm 0.63	27**

The partitioning was achieved by subtracting R_{above} from NEE_{night} with the residual being R_{below} in December 1999 and 2000. The stimulation of each component in elevated C_a is also shown (S). All the data are expressed per m^2 ground area. Statistical significance of the differences between means is shown (** $P < 0.05$; * $P < 0.1$).

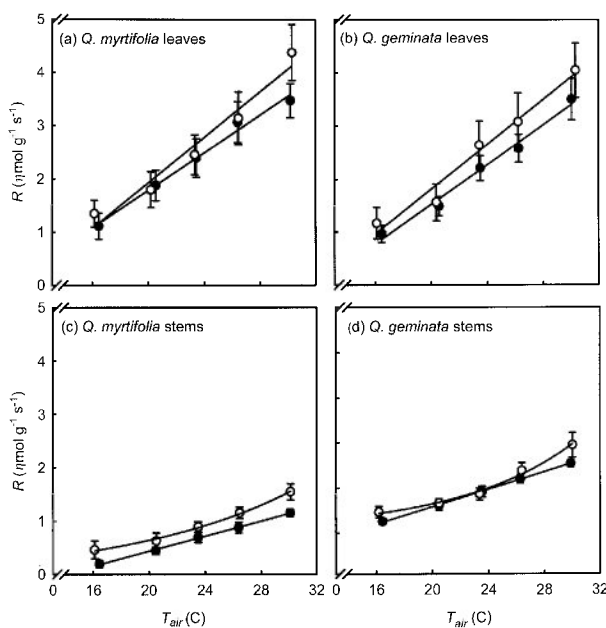


Fig. 7 Plots of the temperature dependence of R_{leaf} and R_{stem} for *Q. myrtifolia* (a, c) and *Q. geminata* (b, d). Measurements were made for material grown and measured at either the ambient (open circles) or elevated (closed circles) C_a . Symbols shown are the mean (± 1 SE, $n = 8$).

this period in elevated C_a (Hymus *et al.*, 2002b). During the winter months, the leaf-fall rates increase in elevated C_a , relative to those in the ambient C_a , and the stimulation of the LAI declines (Stiling *et al.*, 2002; Hymus *et al.*, 2002b). However, for both NEE_{max} and ϕ_{NEE} , when expressed per unit ground area, the changes in the leaf area would not have been solely responsible for the observed seasonality to the stimulation. The following three key factors should in theory have affected the stimulation of NEE in elevated C_a .

(1) *Higher summer temperatures.* Long (1991) provided a physiological basis for increasing stimulation of both light-saturated and light-limited photosynthesis as leaf temperature increases in elevated C_a . This is because elevated C_a will inhibit the oxygenation of Rubisco, which becomes progressively favored relative to carboxylation at higher temperatures, due to a relatively greater decrease in both the solubility of CO₂ and specificity of Rubisco for CO₂ (Jordan & Ogren, 1984). Long (1991) extended this analysis to a hypothetical canopy with an LAI of three (a value being approached at this site), to show that at 35 °C, a temperature frequently experienced at this site, the canopy photosynthesis would double with an increase in C_a from 350 to 650 $\mu\text{mol mol}^{-1}$.

(2) *Leaf photosynthetic capacity.* Between June 1999 and August 2000, Ainsworth *et al.* (2002) showed that stimulation of light-saturated photosynthesis of sun leaves of the three oak species in elevated C_a displayed seasonal and inter-annual variability. For *Q. geminata*, which exhibited varying degrees of acclimatory decreases in $V_{c,\text{max}}$ in elevated C_a , the stimulation of A_{sat} ranged from a maximum of ca. 40% in December 1999 to a complete loss of stimulation in August 1999. For *Q. myrtifolia*, which did not acclimate to the elevated C_a , the stimulation of A_{sat} ranged from a maximum of ca. 150% for young leaves in the spring, to ca. 40% for mature leaves in June 1999. For senescing *Q. myrtifolia* leaves in 1998 the stimulation of A_{sat} in the elevated C_a was 97% (Li *et al.*, 2000).

(3) *Respiratory fluxes.* Because NEE during the photoperiod is the difference between carbon uptake by photosynthesis and carbon loss by both autotrophic and heterotrophic respiration, an effect of elevated C_a on any component of ecosystem respiration would have affected the stimulation of the NEE in elevated C_a during the photoperiod. While no statistically significant effects of elevated C_a on soil respiration have yet been shown at this site (Johnson *et al.*, 2001; Table 1), this should not hide the fact that on occasions soil respiration is higher in elevated C_a (Table 1). Similarly, elevated C_a was shown to have no direct effect on leaf respiration of two oak species in this study; however, the indirect effects of long-term growth in elevated C_a have been observed. For *Q. myrtifolia*, leaf respiration was increased during the spring (Hymus *et al.*, 2002a), while in this study the stem respiration was decreased during the winter (Fig. 7).

Separating the relative influence of the factors described above on increasing NEE in elevated C_a is not easy. A start was to express NEE_{max} on a unit leaf area basis, thereby accounting for the increase in LAI in the elevated C_a . When expressed per unit leaf area the stimulation of the NEE_{max} ranged from 7% to 60%. Contrary to expectations, the stimulation of NEE_{max} , expressed per unit leaf area, was not temperature dependent, but dependent on increases in W_{soil} (Fig. 3). This finding is novel and it suggests that as the water availability increases, the ecosystem carbon uptake may be increasingly stimulated in elevated C_a . The fact that changes in W_{soil} were found to be independent of changes in temperature strengthens the finding. However, we are unable to discount the possibility that the periods of increased W_{soil} coincided with other factors that would have increased the stimulation of NEE in elevated C_a , for example little or no acclimation of

photosynthetic capacity to elevated C_a . Alternatively, it is possible that the small range in what are very low W_{soil} values, cannot really be thought of as representing a transition from low to high W_{soil} . This study has highlighted how difficult it is to separate the many processes responsible for changing NEE in elevated C_a , which was one of the stated aims of this study. It is expected that future modeling studies at this site will provide insights into the relative importance of the multiple mechanisms by which NEE is increased in elevated C_a .

Throughout this study, the stimulation of NEE_{max} expressed per unit leaf area, averaged 29%. This was lower than the average 69% and 32% stimulation of A_{sat} for sun leaves of *Q. myrtifolia* and *Q. geminata*, respectively, determined from multiple studies carried out at this site (Li *et al.*, 2000; Hymus *et al.*, 2001, 2002a; Ainsworth *et al.*, 2002). Given that the stimulation of soil, leaf and woody tissue respiration is smaller than the stimulation of A_{sat} in elevated C_a , at this site, we would have expected the stimulation of the leaf area-based NEE_{max} to be greater than the stimulation of sun leaf A_{sat} . Clearly, there is a significant factor decreasing the effect of the elevated C_a on NEE_{max} . Increased shade in the denser elevated C_a canopy is most likely this factor. While photosynthesis of the light-limited leaves within the canopy will still be stimulated by elevated C_a , due to the competitive suppression of photorespiration, the stimulation will be much less than that of the sun leaves (Long & Drake, 1991; Osborne *et al.*, 1997). The combination of this physiological response with the fact that light penetrating the denser canopy will be greatly reduced in elevated C_a is important. It has been shown that through the combination of these two factors, photosynthesis of *Q. myrtifolia* leaves within the canopy was only 6% higher in elevated C_a compared with a 34% stimulation of the sun leaf photosynthesis (J. H. Li unpublished results).

Night

In this system, changes in NEE_{night} tracked changes in temperature in both the C_a treatments (Figs 1, 5 and 6). Over the study period, elevated C_a significantly increased NEE_{night} . This increase averaged 39%, but ranged from 6% to 64%. While the highest stimulation was during the winter months, this effect was far from consistent.

In this study we made two attempts to partition NEE_{night} . During December 1999 and 2000, the increase in NEE_{night} in elevated C_a was driven by increases in R_{below} which accounted for up to ca. 87% of NEE_{night} (Table 1). A high within-treatment variability meant

that the increase in R_{below} in elevated C_a in both December 1999 and 2000 was not statistically significant ($P = 0.06$ and 0.08). These two isolated measurement periods, in which R_{below} was increased in the elevated C_a , need to be placed within the context of long-term data sets in which there has been no statistically significant effect of elevated C_a on either the soil [CO_2] (Johnson *et al.*, 2001) or R_{soil} (unpublished results), even though there are times (also winter months) when the R_{soil} is higher in elevated C_a . It is conceivable that during the winter months when the respiratory fluxes from the soil are low, the increases in the R_{soil} that are small in absolute terms will yield large relative stimulations.

Aboveground respiration was significantly increased by 35% and 42% in December 1999 and December 2000. However, R_{above} accounted for only ca. 13% of NEE_{night} . The stimulation in R_{above} in elevated C_a was less than the increase in aboveground biomass of 66% and 79% in December 1999 and 2000, respectively, reported in Dijkstra *et al.* (2002). In the absence of any direct effect of elevated C_a on mitochondrial respiration (Jahnke, 2001; Hymus *et al.*, 2002a), two indirect effects were likely occurring. First, growth in elevated C_a decreased stem respiration indirectly in *Q. myrtifolia* (Fig. 7), this may well have been due to a decrease in the ratio of respiring to non-respiring tissue in the larger stems in elevated C_a . This finding remains to be tested on stems of increasing age. Second, the ratio of leaf biomass to woody biomass was decreasing in elevated C_a . In December 1999 and 2000, the woody biomass was increased by 89% and 80%, respectively, in elevated C_a , while leaf biomass, which had higher specific respiration rates than the woody biomass (Fig. 7), was increased by only 40% and 22% in December 1999 and 2000, respectively.

In this study, we partitioned NEE_{night} by subtracting R_{above} from NEE_{night} to yield R_{below} . An alternative was to have subtracted R_{soil} , modeled from soil chamber measurements, from NEE_{night} to yield R_{above} . We chose the former method because of the comprehensive nature of our biomass sampling, in which the woody biomass of every oak in each chamber is determined. This removed the problems of trying to sample representatively on a smaller scale, as had to be carried out using the soil chamber to measure R_{soil} . Also, given the large proportion of the NEE_{night} that was derived from the soil, even the small relative errors in measuring this flux would lead to large partitioning errors.

As far as we know, this study constitutes the largest data set showing the effects of the elevated C_a on NEE measured *in situ*, and is the first to be carried out in a woody ecosystem. In the context of previous field studies (Diemer, 1994; Oechel *et al.*, 1994; Ham *et al.*,

1995; Drake *et al.*, 1996; Stocker *et al.*, 1997; Diemer & Körner, 1998; Stocker *et al.*, 1999; Niklaus *et al.*, 2000), our finding of a sustained stimulation of both light-limited and maximum *NEE* during the photoperiod, which was still evident after 6 years regeneration in the elevated C_a is novel (in the marsh ecosystem of the Chesapeake Bay, an increased ecosystem carbon uptake in the elevated C_a has been observed for 16 years, B. G. Drake, unpublished results). As expected, this effect was due to the numerous interactions between elevated C_a, climate, ecosystem processes and physiology. The key factor among those responsible for the seasonal variability in the effect was increases in ecosystem leaf area in the elevated C_a and changes in *W*_{soil}. Also novel was the sustained increase in ecosystem respiration in elevated C_a, and the smaller stimulation than would have been expected from the increase in the above-ground biomass. Given that the night-time flux, and its stimulation by elevated C_a, was smaller than CO₂ uptake during the photoperiod and its stimulation by elevated C_a, we conclude that during this two-year period more carbon was sequestered at elevated C_a in this ecosystem. Based on these findings, elevated C_a would be expected to speed up the rate of postfire biomass accumulation, which would be expected to shorten the fire return cycle in this fire-dependent ecosystem.

Acknowledgements

Dr Jiahong Li is thanked for his scientific input to this paper during many discussions and the permission to mention unpublished data. The authors also gratefully acknowledge the continuing support of this project by the US Department of Energy and The Kennedy Space Center.

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