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Direct and indirect effects of elevated CO_2 on transpiration from *Quercus myrtifolia* in a scrub-oak ecosystem

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Abstract

Elevated atmospheric carbon dioxide (C_a) usually reduces stomatal conductance, but the effects on plant transpiration in the field are not well understood. Using constant-power sap flow gauges, we measured transpiration from *Quercus myrtifolia* Willd., the dominant species of the Florida scrub-oak ecosystem, which had been exposed *in situ* to elevated C_a (350 μmol mol^{-1} above ambient) in open-top chambers since May 1996. Elevated C_a reduced average transpiration per unit leaf area by 37%, 48% and 49% in March, May and October 2000, respectively. Temporarily reversing the C_a treatments showed that at least part of the reduction in transpiration was an immediate, reversible response to elevated C_a. However, there was also an apparent indirect effect of C_a on transpiration: when transpiration in all plants was measured under common C_a, transpiration in elevated C_a-grown plants was lower than that in plants grown in normal ambient C_a. Results from measurements of stomatal conductance (g_s), leaf area index (LAI), canopy light interception and correlation between light and g_s indicated that the direct, reversible C_a effect on transpiration was due to changes in g_s caused by C_a, and the indirect effect was caused mainly by greater self-shading resulting from enhanced LAI, not from stomatal acclimation. By reducing light penetration through the canopy, the enhanced self-shading at elevated C_a decreased stomatal conductance and transpiration of leaves at the middle and bottom of canopy. This self-shading mechanism is likely to be important in ecosystems where LAI increases in response to elevated C_a.

Keywords: carbon dioxide, LAI, open-top chamber, *Quercus myrtifolia*, sap flow, self-shading, stomatal conductance, transpiration

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Introduction

Doubling atmospheric carbon dioxide (C_a) reduces leaf stomatal conductance (g_s) in many species by 20–40% (Morison, 1987; Field *et al*., 1995; Drake *et al*., 1997), with somewhat smaller effects (11–21%) observed in trees (Curtis & Wang, 1998; Medlyn *et al*., 2001). If elevated C_a reduces plant transpiration and consequently evapotranspiration, the continuing rise in C_a may impact ecosystem, landscape and regional climate (Idso & Brazel, 1984; Field *et al*., 1995). An ongoing study on the responses of the Florida scrub-oak ecosystem to elevated C_a has been in progress since May 1996 at the Smithsonian CO_2 Site, Merritt Island National Wildlife Refuge, Florida, where 16, 9.45 m^2 plots within a scrub-oak community have been exposed to either ambient or elevated (350 μmol mol^{-1} above ambient) C_a within open-top chambers *in situ*. Previous studies in the system have examined effects of elevated C_a on leaf gas exchange and whole-system evapotranspiration during the first and second years of CO_2 exposure, finding that elevated C_a decreased stomatal conductance of *Quercus myrtifolia* Willd., the dominant species, by 40% (Lodge *et al*., 2001), and reduced mid-day evapotranspiration by 19%

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(Hungate *et al*., 2002). Presumably, the observed reduction in evapotranspiration is a direct consequence of the reduction in stomatal conductance. Here, we report the effects of elevated C_a on transpiration of *Q. myrtifolia* during the fourth year of CO_2 exposure, testing the hypothesis that elevated C_a decreases transpiration of *Q. myrtifolia*.

The reduction of transpiration measured on a leaf in a cuvette at elevated C_a is a result of decreased g_s (Field *et al*., 1995; Drake *et al*., 1997; Hsiao & Jackson, 1999), but for whole plants in the field, the effects of elevated C_a on transpiration are more complex. In Scots pine (*Pinus sylvestris* L.), elevated C_a increased transpiration at low levels of radiation or on cloudy days through increasing the sensitivity of stomatal conductance to low levels of light, but decreased it on clear days, especially in the afternoon through increasing the sensitivity of stomatal conductance to high vapor pressure deficit (VPD) (Kellomäki & Wang, 1998). Similar results were observed in sweetgum (*Liquidambar styraciflua* L.), where elevated C_a significantly decreased transpiration only at mean daily radiation levels above $400\,J\,m^{-2}\,s^{-1}$ and at vapor pressure deficits above 1.0 kPa (Wullschleger & Norby, 2001). On the contrary, a significant reduction of sap flow rate at elevated C_a occurred during cloudy days with low VPD, but not during clear days with high VPD in loblolly pine (*Pinus taeda* L.) (Ellsworth *et al*., 1995). Another aspect is the recently reported acclimation of transpiration to elevated C_a. Dugas *et al*. (2001) found that when the woody legume *Acacia farnesiana* (L.) plants grown for more than a year at $980\,\mu mol\,mol^{-1}$ C_a were exposed to $380\,\mu mol\,mol^{-1}$ C_a for 9 days, they transpired at half the rate of those that had been grown at $380\,\mu mol\,mol^{-1}$ C_a. Their results indicated that the reduction of transpiration by long-term CO_2 enrichment was greater than what would be predicted from transpiration of plants subject to short-term exposure to elevated C_a. Clearly, the mechanisms underlying the effects of elevated C_a on transpiration should be further examined.

When leaf areas index (LAI) increases at elevated C_a, as in the scrub-oak ecosystem (Hymus *et al*., 2002) as well as reported in some studies with forest species (Mauney *et al*., 1994; Heath & Kerstiens, 1997; Kellomäki & Wang, 1998), the light intercepted by the canopy per unit leaf area will decrease according to Beer's law (Nobel & Long, 1985). Leaves at the bottom or inside of the canopy may receive very low light, well below the saturating level for maximum stomatal opening. Under such low light, stomatal conductance decreases with decreasing photosynthetic photon flux density (PPFD) (Graham *et al*., 1982; Berryman *et al*., 1994; Will & Teskey, 1997). Thus, increased shading associated with increasing LAI under elevated C_a could possibly decrease g_s averaged over a whole plant, consequently reducing canopy transpiration per unit leaf area. The second objective of this study was to test the hypothesis that greater self-shading in plants grown under elevated C_a contributes to the reduction of transpiration.

Materials and methods

Experimental site

The study site was located on the Merritt Island National Wildlife Refuge, Cape Canaveral, Florida USA (28°38′N, 80°42′W). The subtropical climate is warm and humid. Annual precipitation averages 131 cm, with a dry period typically occurring between December and June. The mean daily maximum temperature is 22.3 °C for January and 33.3 °C for July, and the mean daily minimum temperature is 9.6 °C for January and 21.9 °C for July. Thunderstorms are common in the summer with frequent lightning strikes, which can cause wildfires.

The soil consists primarily of sand and sandy coquina deposited since the Pleistocene, and has an organic layer about 20 cm deep. The composition of aboveground biomass at the study sites was *Quercus myrtifolia* Willd. (76%), *Q. geminata* Small (15%), and *Q. chapmanii* Sarg (7%). Additional species included *Myrica serifera* L., *Lyonia ferruginea* (Walt.) Nutt, and *Galactia elliotti* Nuttall (Dijkstra *et al*., 2002).

Before the study, aboveground biomass was measured and the site was burned in January 1996. After burning, plots were assigned to blocks of three plots with similar preburn biomass characteristics. Sixteen open-top chambers (eight at ambient and eight at $350\,\mu mol\,mol^{-1}$ above ambient C_a) were erected over burned plots, with eight additional unchambered plots that served as chamber controls. The chambers were octagons, 3.6 m in diameter and 2.1 m in height having an area of $9.45\,m^2$. Pure CO_2 was added to the air stream blown into the elevated treatment chambers. Shoots of plants that had begun to grow after the site had been burned were removed before beginning treatment with elevated C_a on May 14, 1996. Elevated C_a ($350\,\mu mol\,mol^{-1}$ above ambient) treatments were monitored continuously, 24 h a day.

Sap-flow and transpiration

Constant-power stem flow gauges (Sakuratani, 1981; Baker & van Bavel, 1987) were used to measure transpiration of *Q. myrtifolia*. These gauges have been used to measure transpiration of *Q. virginiana* seedlings and other woody plants (Steinberg *et al*., 1989; Devitt *et al*., 1993). Previous research has shown this method to be accurate for woody plants with stem diameters similar to plants in this study (Heilman *et al*., 1989; Steinberg *et al*., 1989; Devitt *et al*., 1993; Dugas *et al*., 1993). The sap-flow

system was tested with plants outside the chambers and control plots before being used in chambers.

Sap-flow rates were measured three times in 2000 (Table 1) over periods of 14–18 days. During each measurement period, one plant from each of five ambient and elevated C_a treatments (Total number of samples = 10) were selected and gauged with constant-power stem flow gauges (Models SGA5, SGA10, Dynamax, Texas, USA). Gauge signals and heater voltage were sampled every 15 s and averaged for 15 min. A 15-min average of transpiration was derived from the sap flow rate calculated through stem heat balance (Dugas *et al.*, 1993). Average rates of transpiration throughout the day were also calculated for each plant, using the 9:00–16:00 h period to avoid effects of early-morning dew and to restrict analyses to daylight hours. The measurements were carried out on the same plants every time. Sap-flow rates were measured under growth conditions for most of the measurement period, but we also conducted C_a switching experiments to compare the short- and long-term effects of C_a on transpiration. On one day during each measurement period, C_a treatments were reversed at 12:00 h, such that transpiration was measured at 350 μmol mol^{-1} for plants grown at elevated C_a and at 700 μmol mol^{-1} for plants grown at ambient C_a. To evaluate possible indirect effects of elevated C_a, we also measured transpiration at ambient C_a of plants in all treatments by shutting off the CO_2 supply to the elevated C_a-treated plots.

Total leaf area of gauged plant

Total leaf area of each gauged plant was measured following the sap flow measurements. The method was adopted from traditional non-destructive estimation of LAI (Nobel & Long, 1985; Eschenbach & Kappen, 1996). Leaves were counted and about 3% of total leaves were randomly sampled from different canopy positions. The area of each individual leaf was measured with an area meter (LI-COR, Model 3100, Lincoln, Nebraska, USA) and averaged. Total plant leaf area is the product of total number of leaves by the average area per leaf.

Stomatal conductance and its light response curve

Stomatal conductance of sunlit leaves at the top of the canopy of gauged plants was measured *in situ* from 10:00 h to 14:30 h at 350 μmol mol^{-1} C_a first and then 700 μmol mol^{-1} C_a under 1200 μmol m^{-2} s^{-1} PPFD using the LI-COR 6400 photosynthesis system (LI-COR, Lincoln, Nebraska, USA). Stomatal conductance was recorded when a stable net photosynthetic rate was achieved following the change of CO_2 concentration, usually after about 10 min. The measurements were carried out on May 11 and October 31, when C_a was controlled to be 350 μmol mol^{-1} in both ambient and elevated C_a chambers. Stomatal conductance was not measured during the first period of sap flow measurements (February 29–March 16) due to a breakdown of our LI-COR 6400 photosynthesis system.

Light response curves of stomatal conductance were determined with the LI-COR 6400 photosynthesis system on November 1–3 during 9:00–12:00 h under growth C_a conditions in the field. Measurements were made at 1000 μmol m^{-2} s^{-1} PPFD, then 1500, 2000, 1000, 700, 400, 200, 100, 50 and 0 μmol m^{-2} s^{-1}. The readings for g_s following each step of changing PPFD were taken when photosynthetic rate and g_s were stable, typically after about 5 min.

Canopy leaf area index

Leaf area index (LAI) was determined non-destructively by applying Beer's Law to measurements of photosynthetic photon flux density (PPFD) interception by the canopy, using the equation:

$$\mathrm{LAI} = -\ln(I/I_o)/k, \quad (1)$$

where I was PPFD measured below the canopy, I_o was PPFD incident above the canopy and k was the extinction coefficient. A 40-cm long Sunfleck ceptometer containing 40 individual sensors (SF-40, Decagon, Pullman, WA, USA) was used to measure PPFD above and below the canopy in diffuse light conditions. The extinction coefficient was determined in six calibration plots, each 4 m^2 in area, of which four were harvested in February 2000 and

Table 1 Time and date (DOY-Day of year) of sap flow measurements in 2000

Date (DOY) of measurement	Under growth condition	Switched CO_2	All at ambient CO_2
Feb. 29–Mar. 16	Feb. 29–Mar. 10	12:00–18:00 h	9.15–15:00 h
(DOY 60–76)	(DOY 60–70)	Mar. 15 (DOY 75)	Mar. 16 (DOY 76)
Apr. 28–May 11	Apr. 28–May 5	12:00–18:00 h	8:00–16:00 h
(DOY 119–132)	(DOY 119–126)	May 8 (DOY 129)	May 11 (DOY 132)
Oct. 14–31	Oct. 14–23	12:00–18:00 h	8:00–16:00 h
(DOY 288–305)	(DOY 288–297)	Oct. 24 (DOY 298)	Oct. 31 (DOY 305)

two in July 2000. In the calibration plots, where I and I_o were measured, LAI was determined from destructive harvests and k was calculated from Eq. (1). The extinction coefficient was found to be a linear function of PPFD transmission through the canopy ($k = 0.9901\ I/I_o + 0.7812$) and ranged from 0.73 to 1.22. Measurements were made in February, April, September, and December, 2000. For each chamber, two measurements of I_o and 18 measurements of I were averaged to yield a single LAI value. This method was described in detail by Hymus *et al*. (2002).

Light interception by canopy of gauged plant

Intercepted PPFD was measured at top, middle and bottom of canopy of each gauged plant with Sunfleck ceptometer from 11:00 to 13:00 h on sunny July 6 and November 3, 2000, where a light stick (40 cm in length, with 40 PPFD sensors) was placed horizontally at four different compass directions (north–south, northeast–southwest, east–west, southeast–northwest) at each height. These measurements were done separately from LAI measurements.

Analysis of data

Results were statistically analyzed using SYSTAT 9.01 (SPSS Inc., Chicago, IL) using repeated measures analysis of variance (ANOVA). In all cases, growth C_a – the C_a treatment to which the plants were normally exposed – was considered the main effect, or between-subjects contrast. For comparing sap-flow rates under normal treatment conditions and under conditions when all plots were exposed to ambient C_a, time was the only repeated measure (within-subjects contrast). Hourly or daily averages were used for these statistical tests. Analyses of the C_a switching experiments included both time and measurement C_a as within-subject contrasts, where measurement C_a refers to the CO_2 concentration to which the plants were immediately exposed. Effects of growth C_a (between-subjects contrast) and measurement C_a (within-subjects contrast) on stomatal conductance were also assessed using repeated measures ANOVA. Leaf area index was analyzed with growth C_a as the main effect and time (month) as the repeated measure. Light interception was analyzed using a split-plot design, with growth C_a as the main effect and canopy position as the split-plot effect.

Results

Transpiration under growth conditions

The diurnal pattern of transpiration showed the expected relationship, tracking solar radiation throughout the day (Fig. 1, effect of time, $P < 0.001$ for all three measurement periods). Elevated C_a reduced transpiration per unit leaf area throughout the day under growth conditions in March (effect of C_a, $P < 0.001$), April ($P = 0.030$), and October ($P = 0.020$) 2000. Similarly, average transpiration between 9:00 and 16:00 h was consistently lower at elevated C_a than at ambient C_a during the three measurement periods (Fig. 2, effect of C_a, $P = 0.002$ for all periods combined). On average, elevated C_a reduced transpiration by 37% in February–March ($P < 0.001$), by 48% in April–May ($P = 0.012$) and by 49% in October ($P < 0.001$). Transpiration was highest in October, intermediate in February, and lowest in April (Fig. 2, effect of time, $P < 0.001$), likely reflecting the dry period that occurs in the Florida scrub-oak ecosystem between December and June (Mailander, 1990).

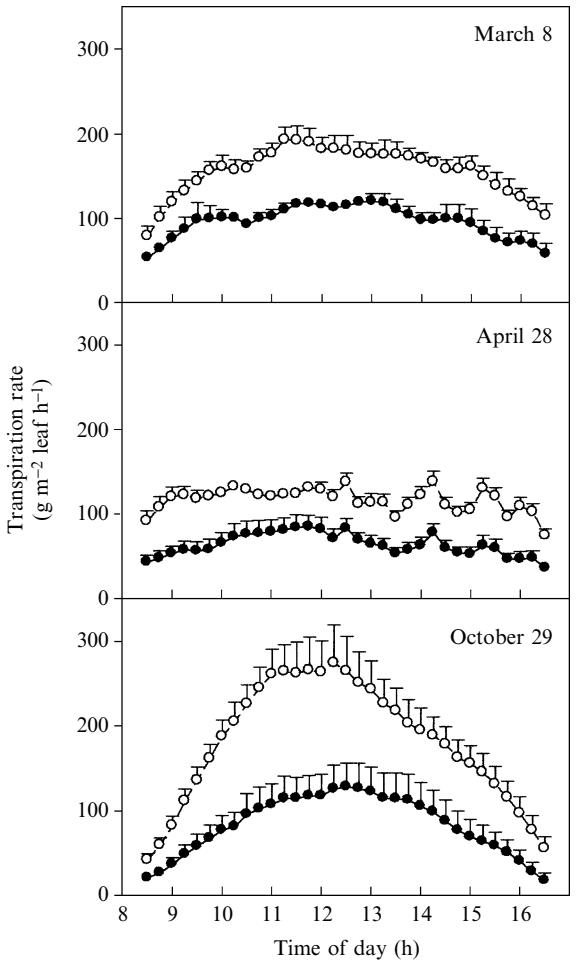

Fig. 1 Diurnal transpiration rate of *Q. myrtifolia*. The plants were growing and measured at ambient (--○--) and elevated (—●—) CO_2 in the field. Data are means + SE ($n = 5$).

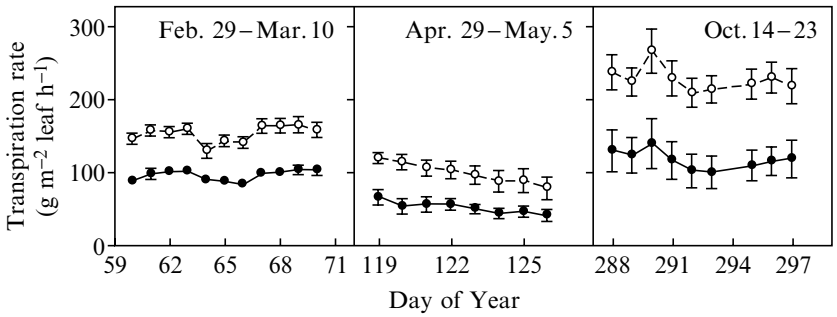

Fig. 2 Average transpiration rate of *Q. myrtifolia* between 9:00 and 16:00 h. The plants were growing and measured at ambient (--○--) and elevated (—●—) CO_2. Data are means ± SE ($n = 5$).

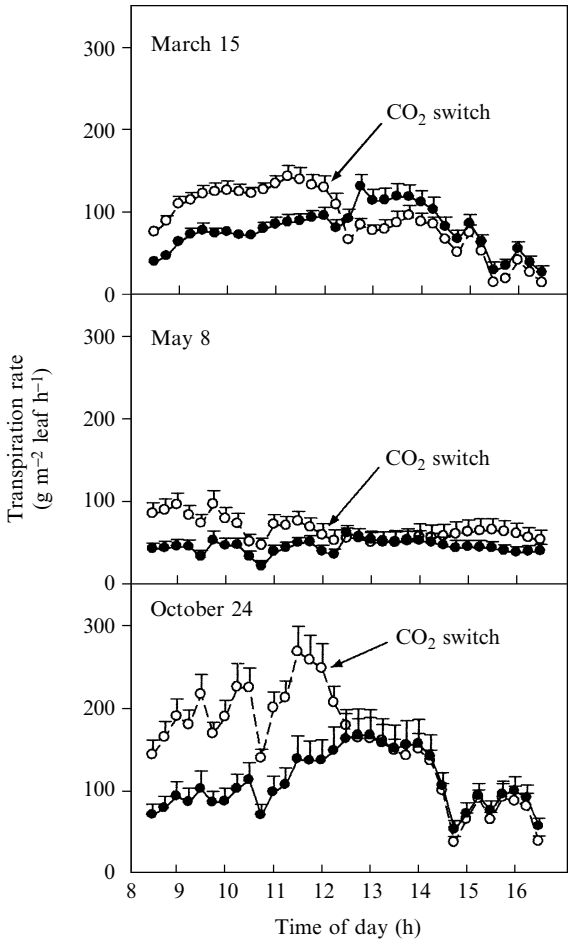

Fig. 3 Diurnal transpiration rate of *Q. myrtifolia* grown at ambient (--○--) and elevated (—●—) CO_2. The switch of CO_2 treatments at 12:00 h meant that CO_2 concentration temporarily became ambient for plants grown at elevated CO_2, and elevated for plants grown at ambient CO_2. Data are means + SE ($n = 5$).

Changes of transpiration rate following temporary CO_2 switch at midday

Reversing C_a treatments at midday caused an immediate change in transpiration rate in March, May, and October (Fig. 3, effect of measurement C_a, $P = 0.047$, 0.082 and 0.014, respectively). After the switch, transpiration decreased in ambient C_a-grown plants as C_a increased and *vice versa* in elevated C_a-grown plants, reflecting the physiological response of stomatal conductance to C_a. However, the effect of measurement C_a depended on growth C_a (interaction term, $P = 0.001$, 0.048, and < 0.001 for March, May, and October, respectively). For example, in March, although the C_a reversal caused transpiration in the ambient C_a-grown plants to be lower than that of the elevated C_a-grown plants, the difference observed (29%) was not as large as under growth conditions (40%). This interaction was even more pronounced in October: under growth C_a conditions, elevated C_a-grown plants had 50% lower transpiration rates than ambient-C_a grown plants, but after reversing C_a treatments, the rate of transpiration in the ambient-C_a grown plants was only 9% lower than, and statistically indistinguishable from, that of elevated C_a-grown plants (RMA, effect of measurement C_a under reversed conditions, $P = 0.599$). The interaction was similar in May: before the reversal, elevated C_a-grown plants transpired at rates 44% lower than ambient C_a-grown plants (RMA, effect of measurement C_a under growth conditions, $P = 0.006$), but after reversing C_a treatments, mean transpiration rates tended to be higher (25%) in ambient C_a-grown plants, the opposite of the expected pattern, though this effect was not significant (RMA, effect of measurement C_a under reversed conditions, $P = 0.814$). These switching experiments demonstrate that the rate of transpiration is sensitive to the C_a to which plants are immediately exposed, reflecting the reduction of stomatal conductance caused by elevated C_a. However, the interaction between growth and measurement C_a shows that this immediate physiological effect cannot fully explain the effects on transpiration of long-term growth under elevated C_a.

Stomatal conductance

Stomatal conductance of fully illuminated leaves was significantly lower when measured at elevated C_a compared to ambient C_a (Fig. 4), 39% lower in May and 31%

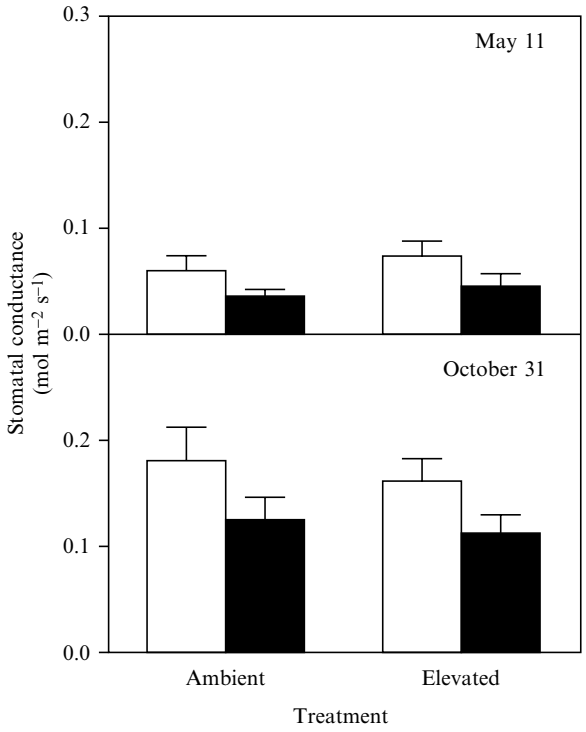

Fig. 4 Leaf stomatal conductance of *Q. myrtifolia* grown under different treatments and measured at ambient (□) and elevated (■) CO_2 under 1200 μmol m^{-2} s^{-1} photosynthetic photon flux density. Data are means + SE ($n = 5$).

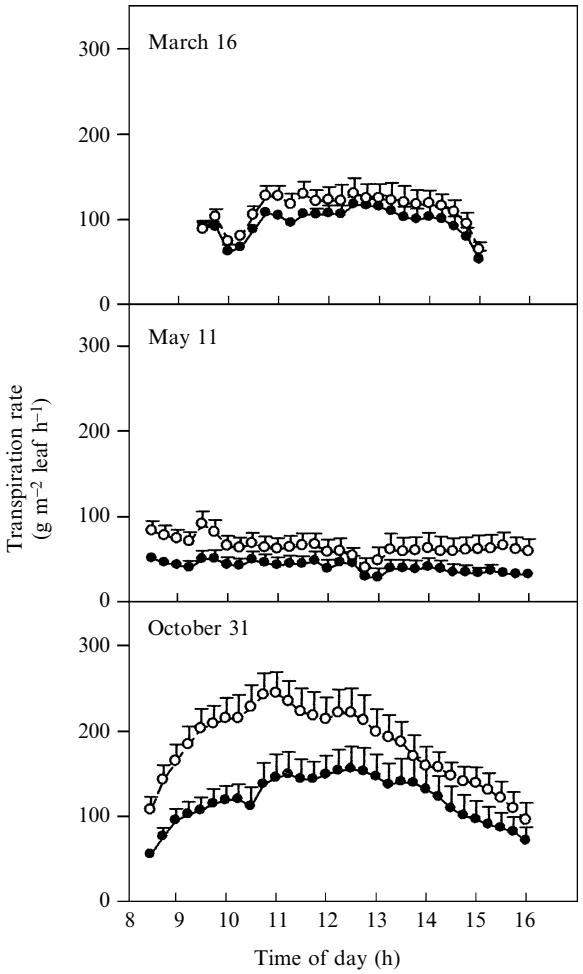

Fig. 5 Diurnal transpiration rate of *Q. myrtifolia* grown at ambient (--○--) or elevated (—●—) CO_2 and all measured at ambient CO_2 by temporarily shutting off pure CO_2 supply to elevated CO_2 chambers. Data are means + SE ($n = 5$).

lower in October (effect of measurement C_a, $P = 0.001$ and $P = 0.002$, respectively). By contrast, growth at elevated C_a had no effect on stomatal conductance when plants were measured at the same C_a (effect of growth C_a, $P = 0.541$ in May and $P = 0.587$ in October). When measured under growth C_a conditions, stomatal conductance was 22% and 27% lower in elevated C_a-grown plants. There was no interaction between growth and measurement C_a ($P < 0.6$ in both cases).

Transpiration measured at ambient CO_2 for all treatments

When pure CO_2 supply to elevated C_a chambers was temporarily shut off, and transpiration was measured under ambient C_a for plants from all the treatments, the rates of transpiration in elevated C_a-grown plants tended to be lower than rates from plants grown in ambient C_a (Fig. 5). Comparing hourly means for each measurement period revealed non-significant reductions of 13% in March (effect of growth C_a, $P = 0.383$), 33% in May ($P = 0.193$), and 32% in October ($P = 0.074$). When daily means were compared across all three measurement periods, the observed reduction in transpiration caused by growth in elevated C_a was significant ($P = 0.033$). Thus, the reduction of transpiration caused by growth under elevated C_a persisted even when plants were exposed to identical atmospheric CO_2 concentrations. The immediate (and reversible) reduction in stomatal conductance caused by short-term exposure to elevated C_a is insufficient to explain the long-term effects of elevated C_a on transpiration in this system.

Response of stomatal conductance to PPFD

Under growth conditions, g_s increased with PPFD before reaching its maximum at about 1000 μmol m^{-2} s^{-1} PPFD at both ambient and elevated C_a (Fig. 6). Elevated C_a reduced g_s at all PPFD levels ($P < 0.001$).

Leaf area index

LAI increased from February to April in all treatments because of emergence of new shoots around the end of March (Fig. 7, RMA, effect of time, $P < 0.001$). Elevated C_a significantly increased LAI ($P = 0.046$), with larger increases observed in April (41%) and September (37%) than in February (21%) and December (24%).

Canopy light interception of gauged plants

There was less light at the middle and bottom of canopy at elevated C_a than at ambient C_a (Figs 8 and 9). The integrated PPFD decreased rapidly from the top to the middle of the canopy for all treatments. The reduction was larger at elevated C_a than at ambient C_a. At the middle and bottom of the canopy, the integrated PPFD was 29% and 41% lower ($P = 0.023$), respectively, at elevated C_a than at ambient C_a in July. The absolute intercepted PPFD in December was presented in Fig. 9 in order to predict g_s at the middle and bottom of the canopy. The intercepted PPFD at the middle and bottom of the canopy was 428 ± 73 and $239 \pm 39\,\mu mol\,m^{-2}\,s^{-1}$, respectively, at ambient C_a and 299 ± 47 and $133 \pm 19\,\mu mol\,m^{-2}\,s^{-1}$ at elevated C_a.

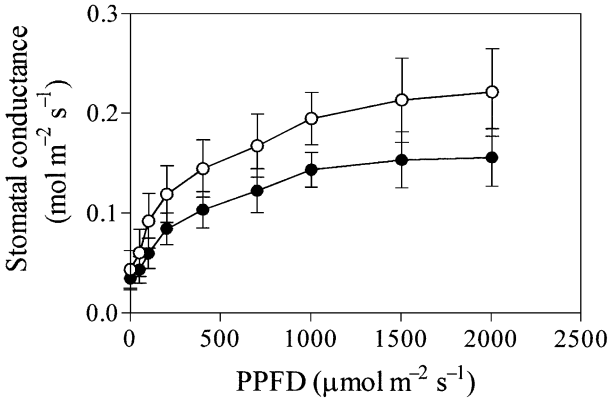

Fig. 6 Responses of leaf stomatal conductance of *Q. myrtifolia* to changing photosynthetic photon flux density (PPFD). Plants were growing and measured at ambient (--○--) and elevated CO_2 (—●—) in the field on November 1–3, 2000 between 9:00 and 12:00 h. Data are means ± SE ($n = 5$).

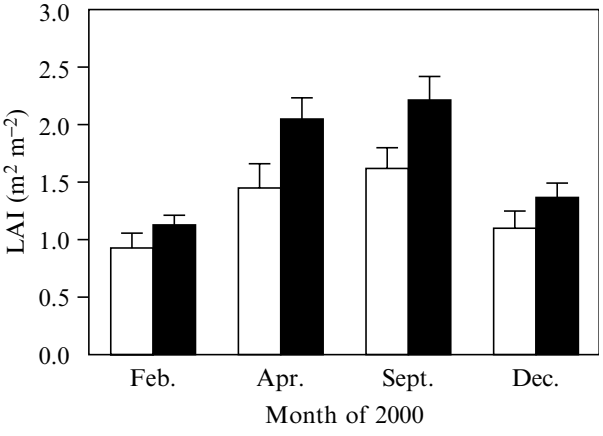

Fig. 7 Leaf area index (LAI) of the Florida scrub-oak ecosystem at ambient (open bars) and elevated CO_2 (fully filled bars). Data are means + SE ($n = 8$).

Discussion

The results presented here supported the hypothesis that elevated C_a reduced transpiration of *Q. myrtifolia*. This

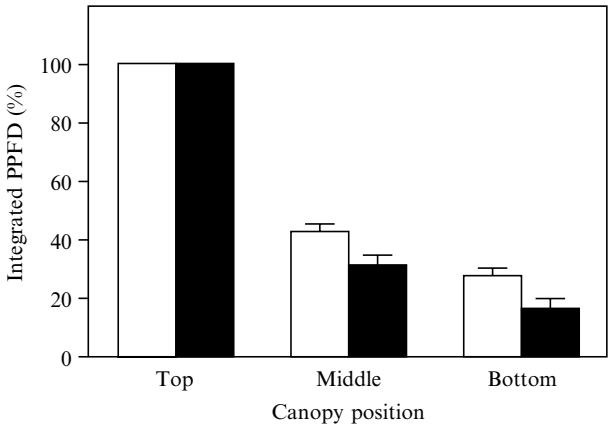

Fig. 8 Intercepted photosynthetic photon flux density (PPFD) at top, middle and bottom of canopy of *Q. myrtifolia* grown at ambient (open bars) and elevated CO_2 (fully filled bars) at 11:00–13:00 h on July 6, 2000. PPFD was expressed as percentage of that at top of canopy. Data are means + SE ($n = 5$).

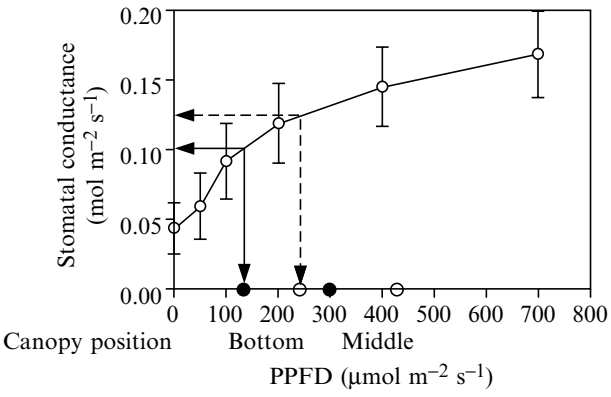

Fig. 9 PPFD at the middle and bottom of canopy of gauged plants measured on November 3 (○ for ambient CO_2 and ● for elevated CO_2) and light response curve of stomatal conductance at ambient CO_2. This graph illustrates how self-shading affects transpiration through decreasing stomatal conductance.

was evident from the transpiration diurnal courses (Fig. 1), the average between 9:00 and 16:00 h (Fig. 2) and the CO_2 switch at midday (Fig. 3). Elevated C_a reduced transpiration throughout the day and this effect was apparent throughout the year. These results are consistent with the reduction in evapotranspiration caused by elevated C_a observed earlier in this experiment (Hungate *et al*., 2002).

The observed reduction in transpiration is partly explained by reduced stomatal conductance. Stomatal conductance measured on sunlit leaves was 22–27% lower in plants grown under elevated C_a compared to ambient C_a-grown plants, a larger reduction than that reported from meta-analyses of tree species (11–21%, Curtis & Wang, 1998; Medlyn *et al*., 2001), though somewhat smaller than the 40% reduction reported from an earlier study of stomatal conductance of *Q. myrtifolia* in this ecosystem (Lodge *et al*., 2001). For some species, statistically significant effects of elevated C_a on stomatal conductance are not apparent (Bunce, 1992; Dixon *et al*., 1995; Atkinson *et al*., 1997; Ellsworth, 1999). The degree of stomatal control of canopy transpiration by stomatal conductance is given by the decoupling coefficient (Ω) (Jarvis & McNaughton, 1986; Martin, 1989), which is the ratio of the stomatal resistance to the total aerodynamic resistance (Gottschalck *et al*., 2001). Ω ranges from 0 to 1, indicating 100% to 0% stomatal control. The typical value of Ω for temperate forests is around 0.2 while those for crops and grassland generally range from 0.5 to 0.9 (Jarvis, 1985a, b; Meinzer & Grantz, 1989; Lee & Black, 1993; Gottschalck *et al*., 2001). For the Florida scrub-oak ecosystem, Ω was about 0.25 during this study (Sabina Dore, unpublished data). Preliminary results from energy balance measurements within the chambers showed that aerodynamic conductance was about 10 times larger than stomatal conductance (Oscar Monje, unpublished data). Thus, at this stage in canopy development, canopy transpiration of this ecosystem is mainly controlled by g_s. Since *Q. myrtifolia* is the dominant species (76% of aboveground biomass), the transpiration of this species should also be mainly controlled by g_s. Thus, decreases in g_s at elevated C_a could decrease transpiration.

The immediate responses of transpiration to short-term changes in C_a were qualitatively consistent with the effects of C_a on stomatal conductance (Fig. 3). Increasing C_a reduced transpiration, and decreasing C_a increased it. However, transpiration was significantly lower in plants grown at elevated C_a than that at ambient C_a when both of them were measured at the same ambient C_a (Fig. 5). This indicates an indirect effect of C_a on transpiration, or an acclimation of transpiration to elevated C_a as reported by Dugas *et al*. (2001) in *Acacia farnesiana*.

The acclimation of transpiration to elevated C_a in *Acacia farnesiana* was due to acclimation of stomata conductance (Dugas *et al*., 2001). For *Q. myrtifolia*, which had been exposed to elevated C_a for about 4 years, when measured at the same C_a, either ambient or elevated C_a, there was no difference in g_s between elevated C_a-grown plants and ambient C_a-grown plants (Fig. 4), indicating no acclimation of g_s to elevated C_a. A laboratory-based study by Lodge *et al*. (2001) found no reduction of stomatal density and only a minor stomatal acclimation in *Q. myrtifolia* (9–16% reduction in g_s when measured at 700 μmol mol^{-1} C_a) in 1997 and 1998. Because we observed no evidence for stomatal acclimation in our measurements, we explored other mechanisms for the indirect reduction in transpiration caused by growth in elevated C_a.

Elevated C_a increased LAI of the scrub-oak ecosystem by 21–41% (Fig. 7). Less light penetrated to the middle and bottom canopy layers in the elevated C_a-treated plots (Fig. 8). Based on the light response curves of g_s to PPFD, the stomatal conductance at the middle and bottom of the canopy would be lower in elevated C_a-grown plants than that in ambient C_a-grown plants when both of them were exposed to the same C_a (Fig. 9). Because of the lower g_s, the transpiration per unit leaf area would be reduced at elevated C_a. In addition, about 58% of the total leaf area of *Q. myrtifolia* is in the lower half of the canopy (J-H Li, unpublished data). Therefore, the increased shading resulting from enhanced LAI at elevated C_a is a simple and plausible explanation for the indirect reduction in transpiration caused by growth at elevated C_a. Consistent with this interpretation, when the C_a-enhancement of LAI increased from 21% in February to 41% in April, the indirect C_a effect increased from 13 to 33%. This indirect effect, caused by greater leaf area and increased self-shading, strongly influenced the treatment reversal experiments in May and October as well. Transpiration of the elevated C_a-grown plants increased when temporarily exposed to ambient C_a, but not to rates as high as those exhibited by ambient C_a-grown plants under growth conditions (Fig. 3). Increased LAI reduced light penetration through the canopy in the elevated C_a-treated plots, reducing the amount of energy available to drive transpiration at the middle and bottom of the canopy. This could reduce transpiration on a leaf area basis, independent of any direct effect of elevated C_a on stomatal conductance.

There are very few field-scale CO_2 enrichment experiments which have studied C_a effects on transpiration. Tognetti *et al*. (1999) found no difference in the mean transpiration per unit foliage area between plants of *Quercus pubescens* Willd. grown at a natural CO_2 spring and at a near-by control site. Elevated C_a reduced transpiration of Scots pine (*Pinus sylvestris* L.) by 15%

on the projected needle area based on sap-flow rate measurements (Kellomäki & Wang, 1998). For sweetgum (*Liquidambar styraciflua*) canopy transpiration per leaf area (transformed from stand transpiration, and LAI which was not affected by C_a in order to compare with this study) averaged 13% less in elevated compared with ambient C_a (Wullschleger & Norby, 2001). Compared with those studies, the C_a-induced reduction of transpiration from *Q. myrtifolia* (37–49%) reported here was much larger. One clear reason was the large reduction (22–27%) of stomatal conductance of *Q. myrtifolia* to elevated C_a. Another reason was the indirect effect that was caused by enhanced self-shading resulting from a 21–41% increase in LAI.

Sap-flow rate can be expressed on different bases: stem circumference (Hinckley *et al*., 1994), per plant or stem (Dugas *et al*., 1993; Ansley *et al*., 1994), per unit sapwood area (Köstner *et al*., 1998) and per unit leaf area (Meinzer *et al*., 1993; Kellomäki & Wang, 1998). In this study, transpiration was expressed per leaf area because of the clumped vegetation in the ecosystem and the method for measuring sap flow which does not require knowledge of the sapwood area. When scaling transpiration derived from sap flow rate, up from leaf to community level, the increase of LAI in elevated C_a will counteract the C_a effect on transpiration per unit leaf area. However, this negative feedback of increasing LAI on transpiration may be less than usually thought without consideration of the indirect C_a effect discussed here.

In conclusion, results from this field study indicate that elevated C_a decreased transpiration of *Q. myrtifolia* through a direct and an indirect effect. The reversible, direct effect was due to decreased stomatal conductance, a frequently observed response to elevated C_a (Morison, 1987). The indirect effect was apparently caused by higher self-shading resulting from enhanced LAI. The enhanced self-shading at elevated C_a reduces g_s at the middle and bottom of the canopy by reducing light availability. This self-shading mechanism is likely to be important in ecosystems where LAI increases in response to elevated C_a.

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