Canopy radiation- and water-use efficiencies as affected by elevated [CO$_2$]

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Abstract

This study used an environmentally controlled plant growth facility, EcoCELLs, to measure canopy gas exchanges directly and to examine the effects of elevated [CO$_2$] on canopy radiation- and water-use efficiencies. Sunflowers (*Helianthus annus* var. Mammoth) were grown at ambient (399 µmol mol^{-1}) and elevated [CO$_2$] (746 µmol mol^{-1}) for 53 days in EcoCELLs. Whole canopy carbon- and water-fluxes were measured continuously during the period of the experiment. The results indicated that elevated [CO$_2$] enhanced daily total canopy carbon- and water-fluxes by 53% and 11%, respectively, on a ground-area basis, resulting in a 54% increase in radiation-use efficiency (RUE) based on intercepted photosynthetic active radiation and a 26% increase in water-use efficiency (WUE) by the end of the experiment. Canopy carbon- and water-fluxes at both CO$_2$ treatments varied with canopy development. They were small at 22 days after planting (DAP) and gradually increased to the maxima at 46 DAP. When canopy carbon- and water-fluxes were expressed on a leaf-area basis, no effect of CO$_2$ was found for canopy water-flux while elevated [CO$_2$] still enhanced canopy carbon-flux by 29%, on average. Night-time canopy carbon-flux was 32% higher at elevated than at ambient [CO$_2$]. In addition, RUE and WUE displayed strong diurnal variations, high at noon and low in the morning or afternoon for WUE but opposite for RUE. This study provided direct evidence that plant canopy may consume more, instead of less, water but utilize both water and radiation more efficiently at elevated than at ambient [CO$_2$], at least during the exponential growth period as illustrated in this experiment.

Keywords: canopy development, carbon flux, elevated [CO$_2$], global change, radiation-use efficiency, sunflower, water flux, water-use efficiency.

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Introduction

Numerous studies in the past decades have led to a general conclusion that elevated [CO$_2$] enhances photosynthesis, decreases transpiration, and increases radiation- and water-use efficiencies (RUE and WUE) at the leaf level (Kimball & Idso 1983; Cure & Acock 1986; Lawlor & Mitchell 1991; Polley *et al.* 1993; Drake *et al.* 1997; Farquhar 1997; Murray 1997). For example, by averaging over many greenhouse and growth chamber studies, Kimball *et al.* (1993) reported that plant growth

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and yield have typically increased more than 30% and stomatal conductance decreased about 37% with a doubling of [CO$_2$]. A synthesis of experimental data from 38 studies by the statistical meta-analysis suggests that leaf photosynthesis increased by 50% (Curtis 1996). Jackson *et al.* (1994) found that elevated [CO$_2$] decreased leaf stomatal conductance, reduced transpiration by 50%, increased mid-day photosynthetic rates by 70%, and approximately doubled WUE compared to that at ambient [CO$_2$]. Despite advances in our knowledge of CO$_2$ effects on leaf-level physiology, the understanding of CO$_2$-induced changes in carbon- and water-fluxes at

the ecosystem level is greatly limited. Indeed, the changes in ecosystem carbon- and water-fluxes in various climatic scenarios are more relevant to future agricultural productivity and ecosystem functions than leaf-level changes. Thus, it is imperative to develop predictive understanding of ecosystem carbon- and water-fluxes as affected by rising atmospheric [CO_2].

Canopy carbon- and water-fluxes at elevated [CO_2] have usually been inferred using indirect methods or scaled up from leaf-level measurements using models. For example, Field *et al.* (1997) and Ham *et al.* (1995) found that soil moisture content at elevated [CO_2] increased in comparison to that at ambient [CO_2], leading to the conclusion that canopy transpiration at elevated [CO_2] must be reduced. Models have also been used to scale leaf-level results to predict canopy carbon- and water-fluxes with consideration of canopy structure (e.g. Wang & Jarvis 1990; Sellers 1992; Norman 1993; Amthor 1994; Leuning *et al.* 1995; Wang & Polglase 1995; Dewar 1997). For example, Baldocchi & Harley (1995) used the canopy photosynthesis and evaporation model for the temperate broadleaf forest and indicated that an increase of [CO_2] from 350 to 600 μmol mol^{-1} may increase canopy photosynthesis by 45% and reduce canopy stomatal conductance by 16%.

Although modelling is a powerful tool, it may or may not incorporate factors that regulate canopy transpiration. Those factors include: (i) canopy conductance; (ii) leaf temperature; (iii) feedbacks from improved plant water status via enhanced leaf area production; (iv) plant physiological feedback control of stomatal conductance with respect to optimizing the balance between carbon gain vs. water loss; (v) prolonged availability of soil moisture and thus less temporal restriction of transpiration; (vi) contributions of soil evaporation and understorey evapotranspiration to canopy-scale water balance; and (vii) planetary boundary layer conductance (Mooney *et al.* 1999; Amthor 1999). With such unknown feedback between leaf-level physiology and canopy processes, the direct measurement of canopy fluxes becomes an indispensable approach.

Several experimental techniques such as lysimetry, soil water balance, energy balance and sap flow methods have been developed to address plant water-use (e.g. Dugas *et al.* 1994; Kimball *et al.* 1994; Hunsaker *et al.* 1996; Senock *et al.* 1996). However, canopy carbon-flux has not been well estimated until very recently. Using the eddy-covariance technique or mesocosms, whole-ecosystem carbon- and water-fluxes can be quantified at the same time. The eddy-covariance technique allows continuous monitoring of carbon- and water-fluxes of plant canopy in the field with high time-resolution (e.g. Wofsy *et al.* 1993; Rochette *et al.* 1996; Arneth *et al.* 1998; Grace *et al.* 1998). However, this technique has limited capabilities in

studying the mechanisms of ecosystem-level responses and cannot be applied to elevated [CO_2] plots. Enclosure measurements with growth chambers and mesocosms have been used at small scales (e.g. Acock *et al.* 1985; Drake *et al.* 1989; Caporn & Wood 1990; Griffin *et al.* 1996). While enclosure measurements may result in modification of physical properties and possible damage to biological structures, these experiments have the potential to make accurate measurements and to contribute to our mechanistic understanding of canopy responses to elevated [CO_2] by controlling other environmental conditions.

This study used a unique plant growth facility, EcoCELLs, to quantify the carbon- and water-fluxes of sunflower canopies at ambient and elevated [CO_2]. As a model laboratory mesocosm, EcoCELLs is large enough for sunflower plants to develop a natural canopy (2.85$\times$3.9 m^2) similar to that in the field. Simultaneously, EcoCELLs offers the possibility to control and manipulate the major environmental factors, which may not be possible in field experimental studies. EcoCELLs studies have been successfully used for addressing leaf-to-canopy scaling issues (Griffin *et al.* 1996), for balancing ecosystem carbon budget (Cheng *et al.* 2000), for examining leaf acclimation with a canopy (Sims *et al.* 1999), and for investigating canopy physiology (Luo *et al.* 2000). This study was designed to examine the effect of elevated [CO_2] on canopy carbon- and water-fluxes, radiation- and water-use efficiencies, and focused particularly on water flux and water-use efficiency. The covariance between canopy carbon- and water-fluxes at both ambient and elevated [CO_2] treatments was also explored.

Materials and methods

Plant material, experimental facility and precision test

Seeds of sunflower (*Helianthus annus* var. Mammoth) were planted in a plant growth facility EcoCELLs (Ecologically Controlled Enclosed Lysimeter Laboratory) at Desert Research Institute, Reno, NV, USA. Technical detail is described in Griffin *et al.* (1996). Briefly, EcoCELLs comprises several EcoCELL units which are environmentally controlled, naturally lit, open-flow, mass-balance systems that function at the mesocosm scale. Gas flux measurements at the whole-system level can be made with a high degree of accuracy similar to that of a well-designed leaf-level gas exchange system. The dimensions of each EcoCELL were 7.3 $\times$ 5.5 $\times$ 4.5 m (L $\times$ W $\times$ D), providing a total volume of 183.5 m^3. There were three 6.7 m^3 pots positioned side-by-side in each EcoCELL so that sunflowers developed a continuous canopy, which measured 2.85 $\times$ 3.9 m^2. The

pots were filled, in layers starting from the bottom, with 1 m washed river bed pebbles, 0.4 m washed river sand and 0.4 m of a 1:1 (v:v) mixture of washed river sand and top soil from the tallgrass prairie (Konza Prairie Long-term Ecological Site, Manhattan, KS, USA).

The measurement and control systems of each EcoCELL were kept completely separate whenever possible. For example, the relative humidity of the EcoCELL was controlled by STEAFA (Stefa control system Inc., San Diego, CA) while the measurement of water vapour flux used an infrared gas analyser (IRGA) monitored by software RTMS (Campbell Scientific Inc., Logan, UT). Three IRGAs were dedicated to the monitoring system: two were run continuously in differential mode to record the flux of carbon and water across each EcoCELL, and the third ran in absolute mode, and sequentially sampled a standard gas as it entered and exited each EcoCELL. All three IRGAs sampled at 5-s intervals and recorded as 60-s averages.

Prior to the experiment, all equipment in the whole gas exchange system was calibrated either by the manufacturer or by DRI laboratory personnel. In addition, we checked and quantified the accuracy of system-level measurements five times by injecting a known amount of CO_2 gas through a calibrated mass-flow meter. Results showed that more than 95% of 96 data points over a 24-h period varied within $\pm 0.5 \, \mu mol \, m^{-2} s^{-1}$ in both EcoCELLs. This variation is very small compared to the magnitude of canopy CO_2 exchange, which ranged from $5 \, \mu mol \, m^{-2} s^{-1}$ in the early stage of canopy development to $50 \, \mu mol \, m^{-2} s^{-1}$ toward the end of the experiment. It is a common practice in biophysical studies that measurements are made with no additional or less replications if instruments have high accuracy. For example, canopy flux measurements made by the eddy-covariance technique were generally not replicated (e.g. Wofsy et al. 1993; Arneth et al. 1998; Grace et al. 1998). In this study, canopy gas exchange measurements were made with a high accuracy and with no replication of treatments at the ecosystem scale.

During the experiment, CO_2 concentration was set to $399 \pm 13 \, \mu mol \, mol^{-1}$ (mean $\pm$ SD) in one EcoCELL for ambient $[CO_2]$ treatment and $746 \pm 14 \, \mu mol \, mol^{-1}$ in another for elevated $[CO_2]$ treatment. Each EcoCELL contained 108 plants planted in rows with a space of 0.33 m between plants. Water supply was controlled by whole-system weighing lysimeter data and plants were watered with the drip irrigation system to maintain soil water content within the range 60–90% field water holding capacity. Air temperature, relative humidity and CO_2 concentration were controlled automatically by computer. Daytime air temperature was controlled at $28 \pm 0.5 \, °C$ and night-time at $13 \pm 0.5 \, °C$. Daytime relative humidity was controlled at $30 \pm 5\%$ and night-time at

$60 \pm 5\%$. The chambers received sunlight. Photosynthetic active radiation (PAR) in the EcoCELLs was approximately 85% of that incident on the greenhouse and averaged $32 \pm 6 \, mol \, m^{-2} d^{-1}$ with a mean maximum instantaneous PAR of $1545 \pm 107 \, \mu mol \, m^{-2} s^{-1}$ over the course of the experiment. Most of the days during the experimental period (from 7 July to 28 August 1997) were cloudless.

Gas exchange measurements

Canopy carbon- and water-fluxes (plant + soil) in the EcoCELLs were measured continuously using a Li-Cor 6262 gas exchange system at an interval of 15 min during the experimental period. Carbon- and water-flux calculations were made as open system differential measurements as described by Field et al. (1991) and expressed on the ground surface area basis.

Light levels in each of the EcoCELLs were monitored with a quantum sensor mounted parallel to the surface of the pots, in the centre of the middle pot, which was well above the plant canopy. Because the canopy in the EcoCELLs had a cubic shape and did not form an infinite canopy surface area, as in the natural field, incident irradiance on the canopy was adjusted from the measured light levels by considering direct solar radiation on the edges. The correction is described in detail by Luo et al. (2000).

Belowground respiration was measured at 12.00 hours using a portable CO_2 analyser (Model LI-6200, Li-Cor Comp.) connected to a soil respiration chamber LI-100. Nine plastic rings were inserted 0.05 m into the soil at each EcoCELL randomly. Measurements were made four times during the experiment. Daytime canopy respiration (i.e. plant and soil respiration) was measured by shading the EcoCELL with black polyethylene plastic sheets for four hours in the afternoon on 25 August, three days before harvesting.

Canopy development and biomass measurement

Leaf areas were calculated from measurements of leaf length and width using allometric relationships developed from a subset of similar leaves. Leaf area of all leaves on the six randomly selected plants in each chamber were measured four times during canopy development and used to calculate total leaf area index (LAI) for the canopy. Measured LAI was linearly interpolated to estimate daily LAI values during the experiment.

Shoot biomass was measured in the final harvest. Root biomass was measured by hand washing soil blocks measuring $0.30 \times 0.30 \times 0.40 \, m$ ($L \times W \times D$) from each EcoCELL with nine replicates. The sampling depth of

0.40 m was adequate because virtually no roots were found below the top soil layer in this experiment.

Data analysis

Radiation-use efficiency (RUE) was defined as a ratio of canopy photosynthesis to intercepted PAR by canopy. Intercepted PAR (IPAR) was estimated using IPAR = PAR $(1 - e^{(-LAI*k)})$ (Campbell & Norman 1998), where k is the canopy extinction coefficient (equalling 0.97 for sunflower canopy; Monteith 1973), LAI is canopy leaf area index, and PAR is the measured photosynthetic active radiation. Water-use efficiency (WUE) was defined as a ratio of canopy photosynthesis to canopy evapo-transpiration (ET).

Daily gross canopy carbon-fluxes were estimated by integrating 24-h measurements of net canopy carbon-flux plus ecosystem dark respiration. Dark respiration was estimated from night-time ecosystem respiration corrected for the temperature difference between day and night with $Q_{10} = 1.5$. Daily water fluxes and IPAR were calculated by integrating 24-h measurements. Night-time canopy carbon- and water-fluxes were estimated by averaging night-time measurements from 00.00 to 03.45 hours and from 22.00 to 24.00 hours. Daily RUE and WUE were calculated by using the corresponding daily canopy carbon-flux, water flux, and IPAR.

In order to show the diurnal variations, we calculated RUE and WUE from measurements of canopy carbon- and water-fluxes at 15 min intervals. In order to condense data without loss of information on canopy development effects, we averaged the corresponding 15-min values over every 8 days from 22 DAP to the end of experiment (53 DAP). Within each of the four 8-day periods, the change of LAI was relatively small.

Relationships between canopy fluxes and IPAR were analysed with a rectangular hyperbolic equation (Ruimy *et al.* 1995; Luo *et al.* 2000)

$$F_c = \frac{F_{max}\alpha I}{F_{max} + \alpha I} - F_0$$

where F_c is the canopy carbon or water-fluxes, F_{max} is the maximum canopy carbon or water-flux, α is canopy quantum yield, I is IPAR, and F_0 is canopy carbon or water-flux when $I = 0$. The statistical analyses were carried out with the SAS package (SAS Institute Inc., Cary, NC).

Results

Canopy development at two CO_2 treatments

During the experimental period, sunflower plants were in vegetative phase. No flower or bud was observed at either ambient or elevated $[CO_2]$. Canopy leaf area index (LAI) increased nearly linearly from 0.6 at 32 days after planting (DAP) to final observations of 4.5 and 5.0 at ambient and elevated $[CO_2]$, respectively (Fig. 1). The nominal probability of the difference in LAI between elevated and ambient $[CO_2]$ was probably due to random error. The slight increase in leaf area at elevated $[CO_2]$ was a result of the increased expansion of individual leaves in the centre of the canopy (Sims *et al.* 1999). The total number of leaves was not affected by elevated $[CO_2]$. The harvested total biomass (shoot + root) was 57.5 g plant^{-1} at elevated $[CO_2]$ which was 22% higher than that at ambient $[CO_2]$ (47.1 g plant^{-1}).

Canopy carbon- and water-fluxes during canopy development

During the first 21 DAP, both daily total canopy carbon- and water-fluxes were low (data not shown) as a consequence of the small leaf area index. Canopy LAI was less than 1 until 33 days after planting. Canopy carbon-flux was very small at 22 DAP, gradually increased to 1.1 and 1.7 mol m^{-2} day^{-1} at 46 DAP at ambient and elevated $[CO_2]$, respectively, and then levelled off until the end of experiment (Fig. 2a). Canopy carbon-flux was higher by 44% during the experimental period at elevated $[CO_2]$ than that at ambient $[CO_2]$. At the end of experiment, elevated $[CO_2]$ enhanced canopy carbon-flux by 53%.

Daily canopy water-flux showed a similar pattern to canopy carbon-flux during canopy development (Fig. 2b). It increased from 100 mol m^{-2} day^{-1} at 22 DAP
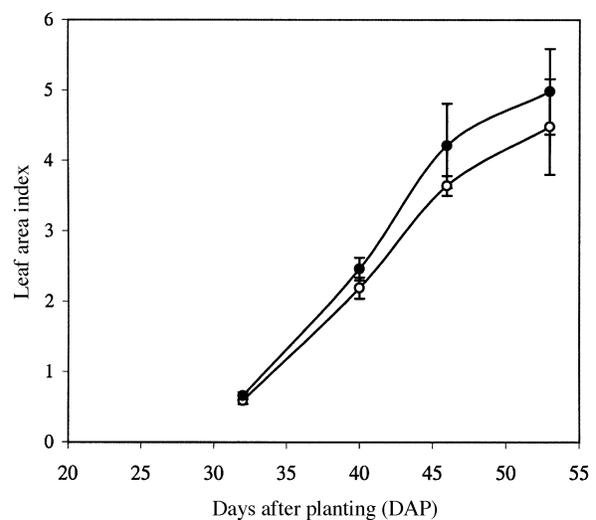

Fig. 1 Canopy leaf-area index during canopy development at ambient $[CO_2]$ (○, mean ± 1 SE) and elevated $[CO_2]$ (●, mean ± 1 SE).
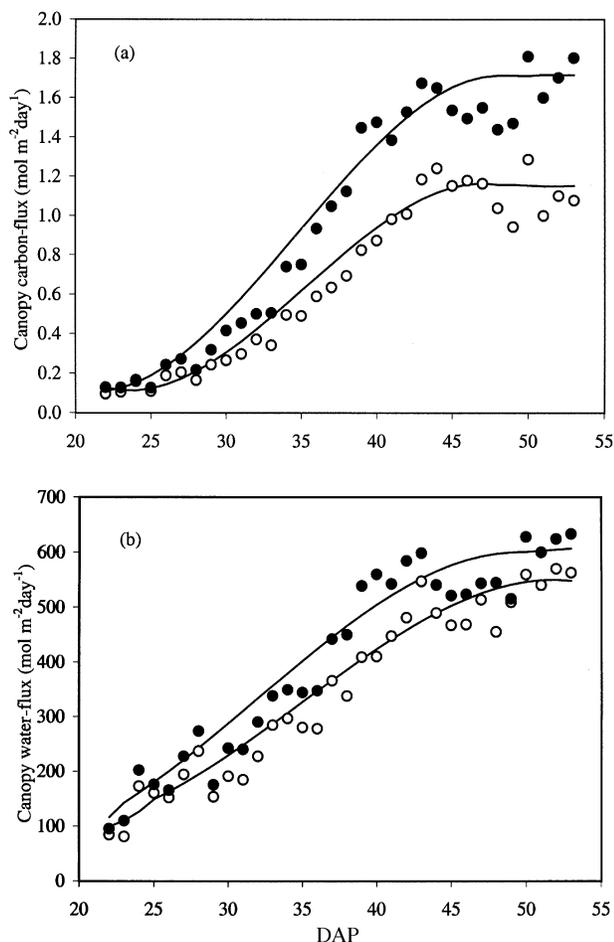
Fig. 2 Daily canopy carbon (a) and water (b) fluxes at ambient [CO_2] (○) and elevated [CO_2] (●) during canopy development.

Fig. 3 Daily canopy carbon (a) and water (b) fluxes based on a leaf area basis at ambient [CO_2] (○) and elevated [CO_2] (●) during canopy development.

to 550 and 600 mol m^{-2} day^{-1} at ambient and elevated [CO_2], respectively, at the end of experiment. In contrast to most other studies, we found an 18% increase in canopy water-flux at elevated [CO_2] in comparison to that at ambient [CO_2] during the experimental period and an 11% increase at the end of experiment. When canopy water-flux was expressed as per unit leaf area, no effect of elevated [CO_2] was found, especially at the late stage of canopy development (Fig. 3b). Canopy carbon-flux based on per unit leaf area was still consistently higher at elevated [CO_2] than at ambient [CO_2] (Fig. 3a). On average, canopy carbon-flux was enhanced by 29% at elevated [CO_2].

Night-time canopy carbon-flux (i.e. plant and soil respiration) also changed during canopy development (Fig. 4). In the early stage, night-time canopy carbon-flux had no statistical difference between ambient and elevated CO_2 treatments. But after 35 DAP, night-time canopy carbon-flux at elevated [CO_2] was considerably more negative than that at ambient [CO_2]. At the end of
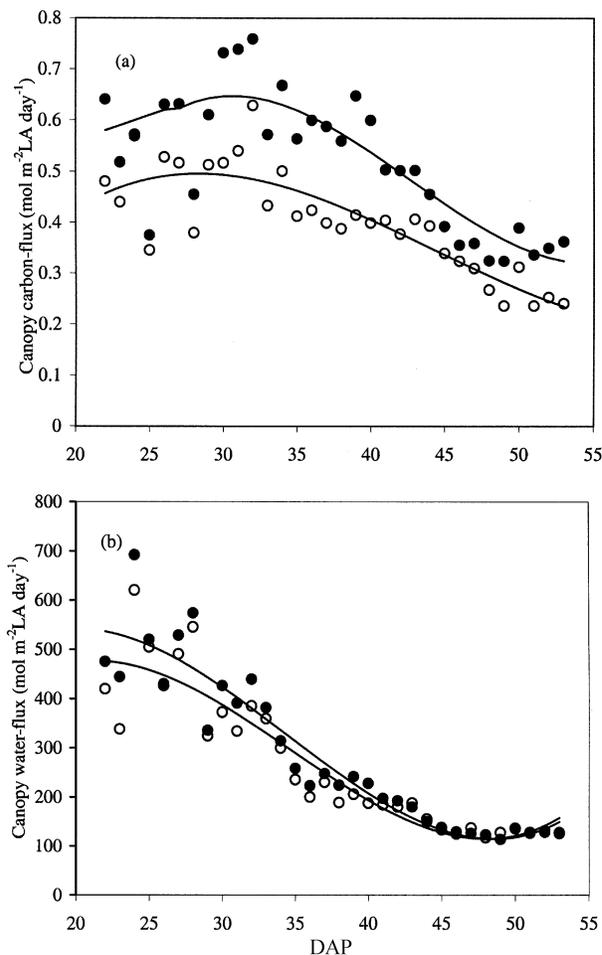
the experiment, elevated [CO_2] enhanced night-time carbon-flux by 32%. Night-time water-flux showed a different pattern compared to night-time canopy carbon-flux. The values of night-time canopy water-flux were small (~1 mmol m^{-2} s^{-1}) and did not show a correlative change with canopy development (data not shown). The reason for these results may be that as stomata closed at night, the night-time canopy water-flux was mainly from soil evaporation which was shown to be less affected by elevated [CO_2] compared to canopy night-time respiration.

Daytime measurements of belowground respiration showed very similar trends with night-time carbon-flux (Table 1). It was enhanced by 41% at elevated [CO_2] at 47 DAP. By shading the entirety of both EcoCELLs with black polyethylene plastic sheets at 50 DAP, we measured daytime canopy respiration. The values were −6.5 and −7.7 μmol m^{-2} s^{-1} at ambient and elevated [CO_2], respectively. These values were comparable with
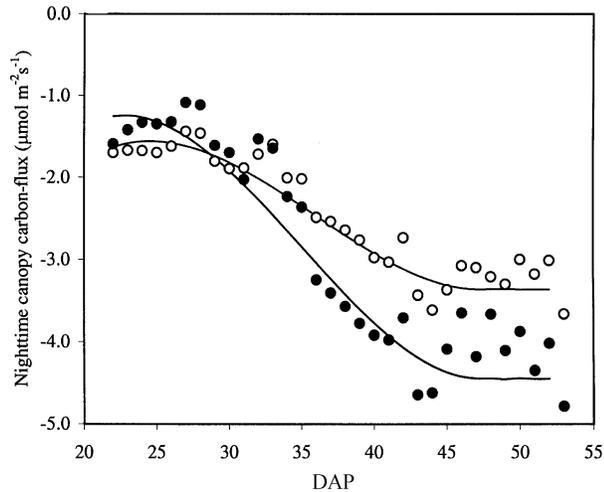
Fig. 4 Night-time canopy carbon-fluxes at ambient (○) and elevated [CO_2] (●). Each circle represents the daily mean of measurements recorded between 20.00 and 03.45 hours.

Table 1 Belowground respiration measured at noon with LI-6200 at 19, 30, 39 and 47 days after planting (DAP) and whole canopy respiration measured at 50 DAP by shading the whole EcoCELLs for 4 h in the afternoon

Days after planting	Respiration (μmol m^{-2}s^{-1})	
	Ambient [CO_2]	Elevated [CO_2]
19	−2.60	−2.22
30	−2.53	−2.68
39	−3.73	−5.19
47	−4.34	−6.11
50	−6.51	−7.70

the values converted from the night-time respiration measurements corrected with temperature differences between daytime and night-time with $Q_{10} = 1.5$.

Radiation- and water-use efficiencies during canopy development

Radiation-use efficiency (RUE) calculated from daily canopy carbon-flux and intercepted PAR during canopy development closely reflected variation in canopy carbon-fluxes (Fig. 5a). RUE gradually increased to 0.027 μmol CO_2 μmol^{-1} photon at ambient [CO_2] and 0.043 μmol CO_2 μmol^{-1} photon at elevated [CO_2] by the end of the experiment. Plants at elevated [CO_2] had a 45% higher RUE than those at ambient [CO_2] during the experimental period. Water-use efficiency (WUE) showed a quadratic increase (Fig. 5b). Although water loss was higher at elevated than at ambient [CO_2], WUE was still enhanced 22% by elevated [CO_2] during the
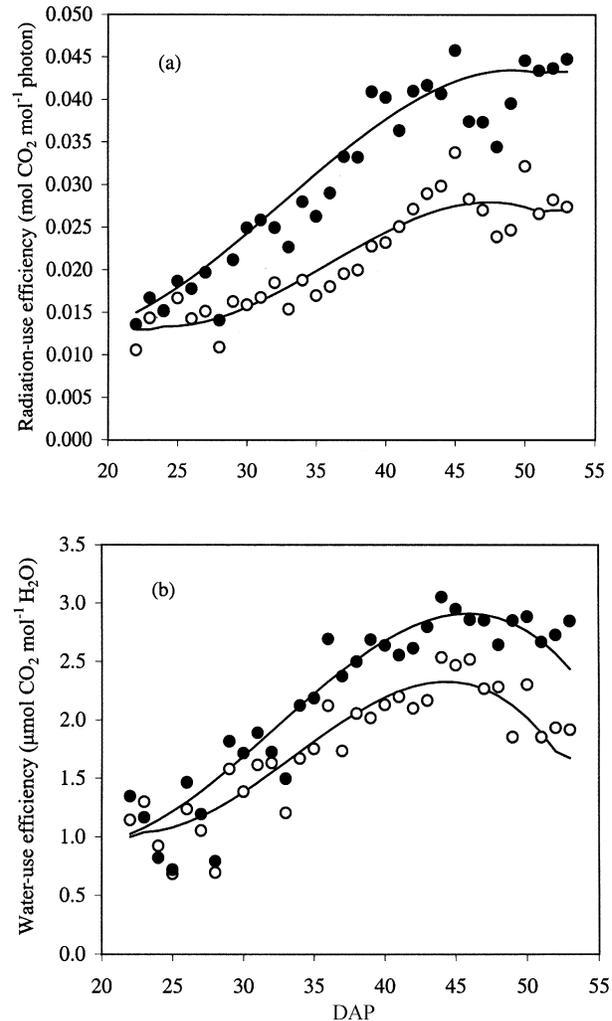

Fig. 5 Canopy radiation-use efficiency (a) and water-use efficiency (b) at ambient [CO_2] (○) and elevated [CO_2] (●) during canopy development.

experimental period due to the increased canopy carbon-flux. Water-use efficiency increased to the maximum value of 2.9 μmol CO_2 mol^{-1} H_2O at elevated [CO_2] at 46 DAP, which was 26% higher than that at ambient [CO_2]. It decreased until the end of the experiment at both ambient and elevated [CO_2] as a result of the increased canopy water-use and relatively stable canopy carbon-flux.

Diurnal variations in canopy carbon- and water-fluxes

Diurnal variations of canopy carbon- and water-fluxes at a 15-min interval displayed a similar pattern during the four time-periods (Fig. 6). The pattern was low at night, increased in the morning, reached the peak at noon, and decreased in the afternoon. The difference between canopy carbon-fluxes was small in the early morning
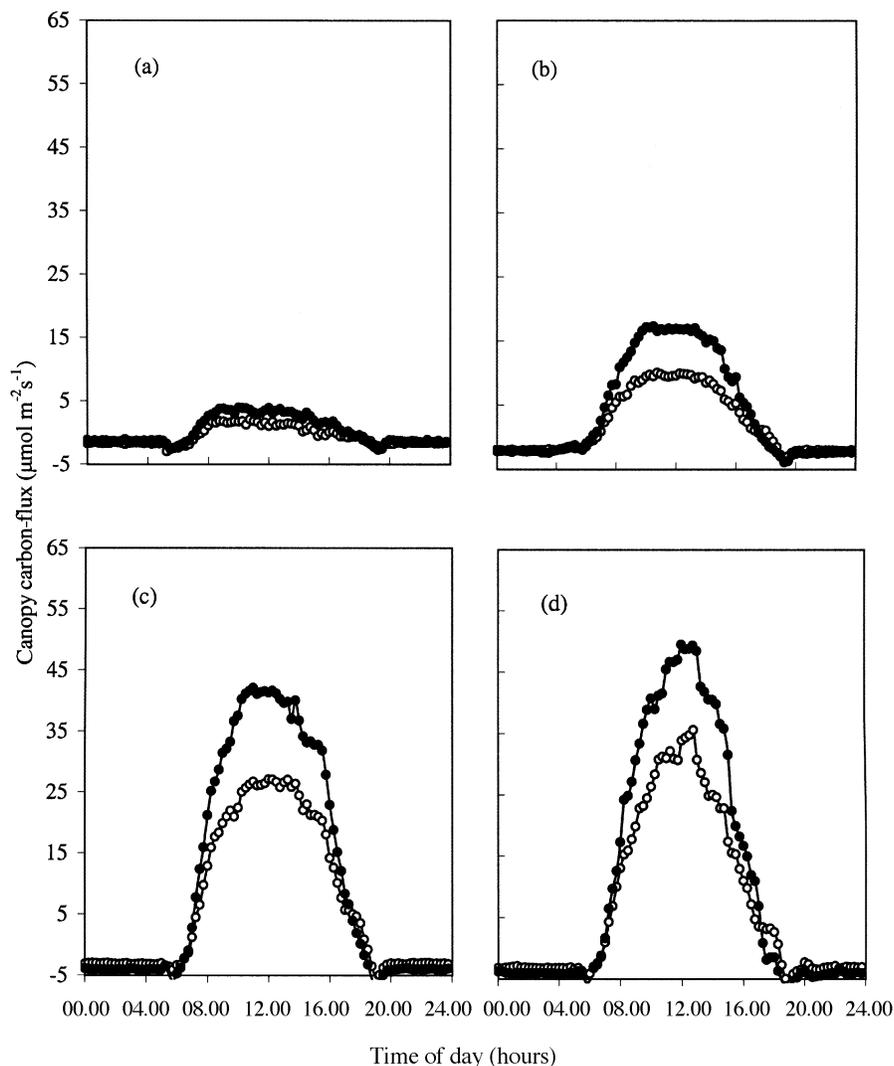
Fig. 6 Diurnal courses of averaged instantaneous canopy carbon-fluxes at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles). Each circle represents the mean of 8-day measurements. (a): 22–29 DAP; (b) 30–37 DAP; (c) 38–45 DAP; (d) 46–53 DAP.

and late afternoon, became large and reached maximum at noon between ambient and elevated [CO_2]. The peak canopy carbon-flux was only 3.5 and 4.0 μmol m^{-2} s^{-1} at ambient and elevated [CO_2], respectively, at 22–29 DAP. It increased to 50 μmol m^{-2}s^{-1} at 12.00 hours at elevated [CO_2] at 46–53 DAP, 38% higher than that at ambient [CO_2]. At night, canopy carbon-flux was consistently more negative at elevated [CO_2] than at ambient [CO_2], indicating increased ecosystem respiration.

The diurnal change in canopy water-flux was similar to that in canopy carbon-flux (Fig. 7). Elevated [CO_2] enhanced canopy water-flux during daytime for all four time-periods. The difference of canopy water-fluxes was larger at noon than the rest of the day. The maximum canopy water-flux at elevated [CO_2] reached nearly 3.5 and 4.1 mmol / m^{2}s^{-1} during 22–29 DAP at ambient and elevated [CO2], respectively. At 46–53 DAP, it was 15 mmol / m^{2}s^{-1}, 17% higher than that at ambient

[CO_2]. In contrast to the night-time canopy carbon-flux, night-time canopy water-flux was consistently higher at elevated than at ambient [CO_2], although the difference between ambient and elevated [CO_2] was rather small.

Diurnal variation in radiation- and water-use efficiencies

Instantaneous RUE and WUE during daytime (from 08.00 to 16.45 hours) were calculated by averaging 15-min measurements of canopy carbon, water-fluxes, and IPAR. We excluded data from 17.00 to 07.45 hours because it was less meaningful to study RUE and WUE at night and because the variability in RUE and WUE was large when the light was low. While the general pattern of the diurnal change in RUE or WUE was similar for the four time-periods, the values increased gradually as canopy developed. Elevated [CO_2] enhanced both RUE
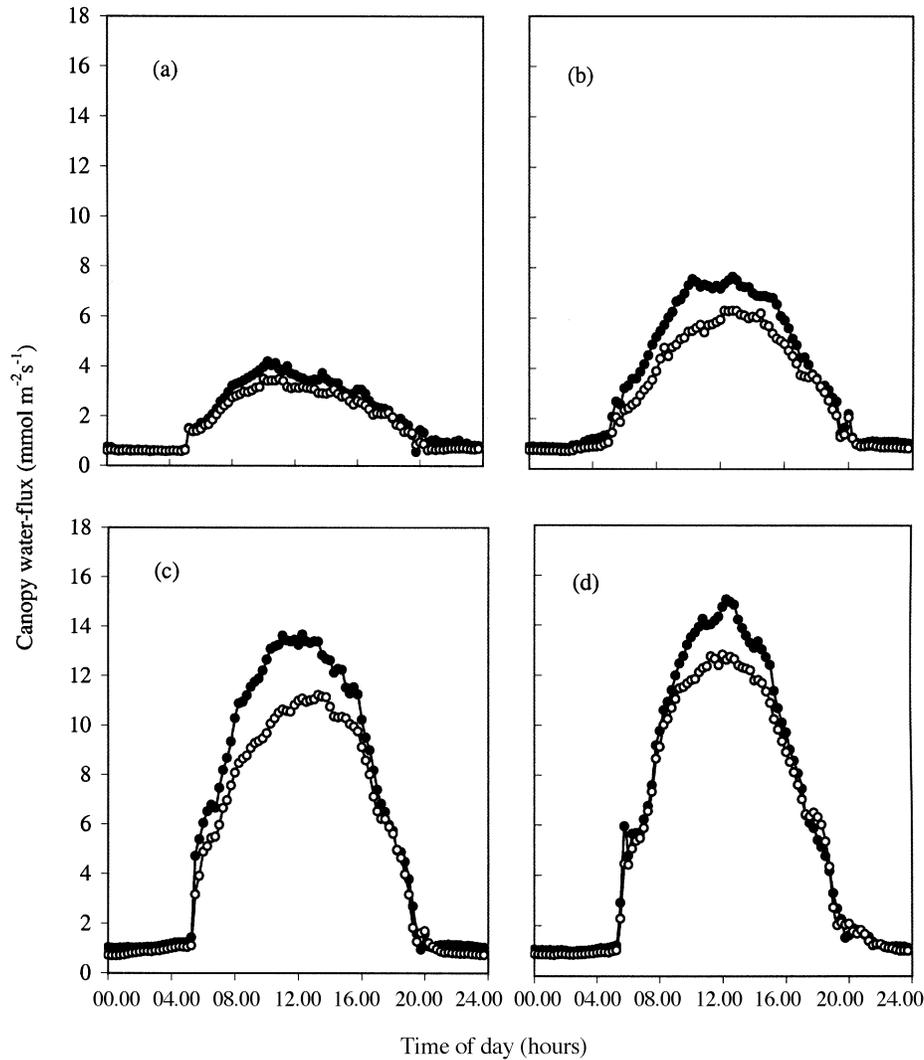
Fig. 7 Diurnal courses of averaged instantaneous canopy water-fluxes at ambient [CO_2] (○) and elevated [CO_2] (●). Each circle represents the mean of 8-day measurements. (a): 22–29 DAP; (b) 30–37 DAP; (c) 38–45 DAP; (d) 46–53 DAP.

and WUE during a day. While RUE reached the minimum value at 12.00 hours (noon), WUE was maximal (Figs 8 and 9). The enhancement of RUE by elevated [CO_2] was relatively constant during the day, whereas the enhancement of WUE by elevated [CO_2] was greater at noon than in the morning and afternoon. For example, at 38–45 DAP, plants at elevated [CO_2] had a RUE of 0.046 $\mu mol\ CO_2\ \mu mol^{-1}$ photon in the morning, decreased to 0.026 $\mu mol\ CO_2\ \mu mol^{-1}$ photon at noon, then increased to 0.045 $\mu mol\ CO_2\ \mu mol^{-1}$ photon again in the afternoon (Fig. 8c). Elevated [CO_2] enhanced RUE by 53% at noon than that at ambient [CO_2]. WUE increased from 2.2 $\mu mol\ CO_2\ mol^{-1}\ H_2O$ in the morning to 3.2 $\mu mol\ CO_2\ mol^{-1}\ H_2O$ at noon, then decreased to 1.5 $\mu mol\ CO_2\ mol^{-1}\ H_2O$ in the afternoon at 38–45 DAP (Fig. 9c). The maximum WUE was enhanced by 26% at noon. WUE at other time periods showed a similar pattern.

Responses of canopy carbon- and water-fluxes to IPAR

The responses of canopy carbon- and water-fluxes to IPAR showed typical curvilinear patterns (Figs 10 and 11). A rectangular hyperbolic equation was fitted for canopy carbon- and water-fluxes (Table 2). Estimated maximum photosynthetic capacity changed from 5 $\mu mol\ m^{-2}s^{-1}$ at 22–29 DAP to 72 $\mu mol\ m^{-2}s^{-1}$ at 46–53 DAP at ambient [CO_2] and from 8 to 107 $\mu mol\ m^{-2}s^{-1}$ at elevated [CO_2]. Elevated [CO_2] enhanced photosynthetic capacity by 61%. Canopy quantum yield was estimated from 0.022 to 0.051 $\mu mol\ CO_2\ \mu mol^{-1}$ photon at ambient [CO_2] and 0.034–0.068 $\mu mol\ CO_2\ \mu mol^{-1}$ photon at elevated [CO_2]. The reason behind canopy water-flux response to IPAR may be the same reason behind canopy carbon-flux, as light induced stomatal opening and closure. The estimated values for maximum canopy water-flux were 2.8
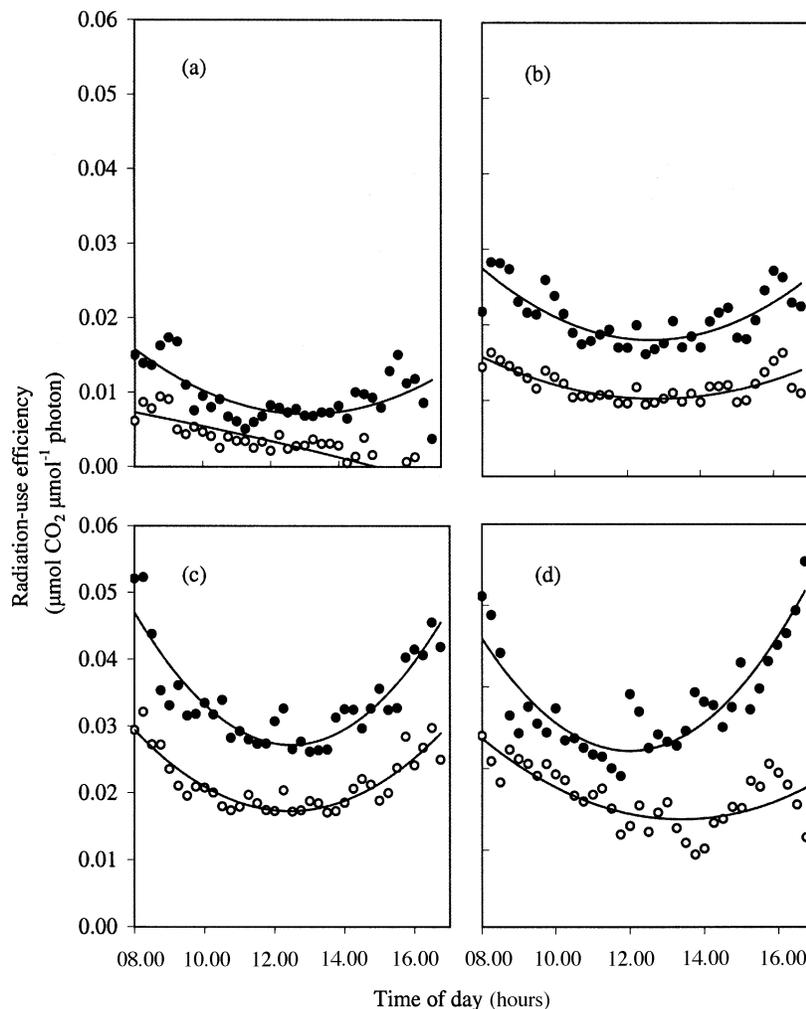
Fig. 8 Diurnal courses of canopy radiation-use efficiency at ambient $[CO_2]$ ($\circ$) and elevated $[CO_2]$ ($\bullet$). (a): 22–29 DAP; (b) 30–37 DAP; (c) 38–45 DAP; (d) 46–53 DAP.

and $3.17\,\text{mmol}\,\text{m}^{-2}\,\text{s}^{-1}$ at 22–29 DAP at ambient and elevated $[CO_2]$, respectively. These values increased to 12.6 and $14.6\,\text{mmol}\,\text{m}^{-2}\,\text{s}^{-1}$ at 46–53 DAP. Maximum canopy water-flux was enhanced by 17% at elevated $[CO_2]$.

The relationship between daytime (from 08.00 to 16.45 hours) canopy carbon- and water-fluxes was described by a linear regression at both ambient and elevated $[CO_2]$ (Fig. 12). The slope of the line at elevated $[CO_2]$ was larger than that at ambient $[CO_2]$ except at the early stage, which indicated that plants grown at elevated $[CO_2]$ gained more carbon per unit water loss than plants grown at ambient $[CO_2]$.

Discussion

This study used a unique facility and continuous, whole canopy measurements to quantify ecosystem-level carbon- and water-fluxes as affected by rising atmospheric $[CO_2]$. Our study has demonstrated that elevated $[CO_2]$

enhanced canopy water-flux consistently throughout the experiment per ground-area unit. By the end of the experiment, the ecosystem water loss was 11% higher at elevated than at ambient $[CO_2]$. This is consistent with several results from other studies. Chaudhuri *et al*. (1990) grew winter wheat in CO_2-enriched greenhouses for 3 years and found that although evapotranspiration (ET) increased by 16% at elevated $[CO_2]$ ($825\,\mu\text{mol}\,\text{mol}^{-1}$) in one year, there was little effect of CO_2 on ET for the other two years. Kimball *et al*. (1994) reported a 13% increase in ET of cotton in the CO_2 enriched plots ($550\,\mu\text{mol}\,\text{mol}^{-1}$) compared with that under ambient conditions ($370\,\mu\text{mol}\,\text{mol}^{-1}$) in a free-air CO_2 enrichment (FACE) experiment. Samarakoon & Gifford (1995) compared cotton, wheat and maize using temperature- and relative humidity-controlled glasshouses, and found that water use per pot of cotton increased as a consequence of a large increase in leaf area and small change in conductance at elevated $[CO_2]$, while maize had very little leaf-area response and resulted in significant water conservation. Fredeen *et al*.
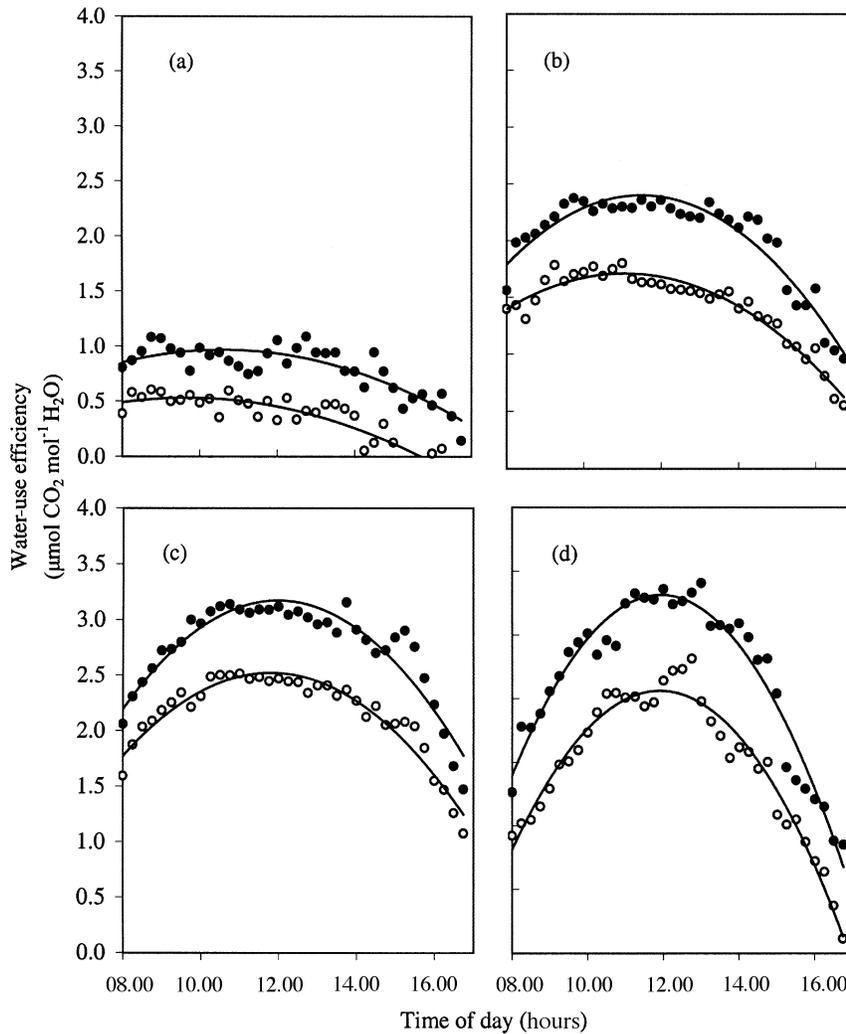
Fig. 9 Diurnal courses of canopy water-use efficiency at ambient [CO$_2$] (○) and elevated [CO$_2$] (●). (a): 22–29 DAP; (b) 30–37 DAP; (c) 38–45 DAP; (d) 46–53 DAP.

(1998) found that water fluxes were enhanced by elevated [CO$_2$] for *Avena* but reduced for another two species *Planto* and *Lasthenia* in comparison to that at ambient [CO$_2$]. Wheeler *et al.* (1999) found that canopy water-use by potato increased as [CO$_2$] increased from 400 to 1000 and 10,000 µmol mol^{-1} in a growth chamber. By applying a soil–vegetation–atmosphere model to corn and soybean, Carlson & Bunce (1996) found that a doubling of [CO$_2$] could lead to a small seasonal increase in transpiration for these crops.

However, numerous studies indicate that canopy water-fluxes are virtually unchanged at elevated [CO$_2$]. For example, several years of studies on cotton in an FACE site (AZ, USA) revealed, in general, that ET was unaffected at elevated [CO$_2$], and the effect of elevated [CO$_2$] was too small to be detected (Dugas *et al.* 1994; Hileman *et al.* 1994; Hunsaker *et al.* 1994; Kimball *et al.* 1994). In addition, Jones *et al.* (1985a) grew plants at controlled-environment chambers and found that tran-

spiration rates were essentially equivalent at ambient and elevated [CO$_2$]. Centritto *et al.* (1999) found that water loss did not differ in either well watered or droughted cherry seedlings between elevated and ambient [CO$_2$]. In a FACE experiment at Duke forest, Ellsworth (1999) did not find evidence of water savings in elevated [CO$_2$] plots compared to ambient plots under drought and nondrought conditions.

Decrease of ET was also observed in several field experiments. Jones *et al.* (1985b) reported that soybean canopies at 660 µmol mol^{-1} [CO$_2$] in sunlit, controlled environmental chambers transpired about 10% less over the whole season than those at 330 µmol mol^{-1}. Evapotranspiration was reduced by 17–22% in the C3 and 28–29% in the C4 community in a wetland ecosystem (Drake *et al.* 1997). Ham *et al.* (1995) measured whole-chamber water vapour fluxes and showed that elevated [CO$_2$] reduced ET by 22% compared to that at ambient [CO$_2$]. Fredeen & Field (1995) found a lower ecosystem
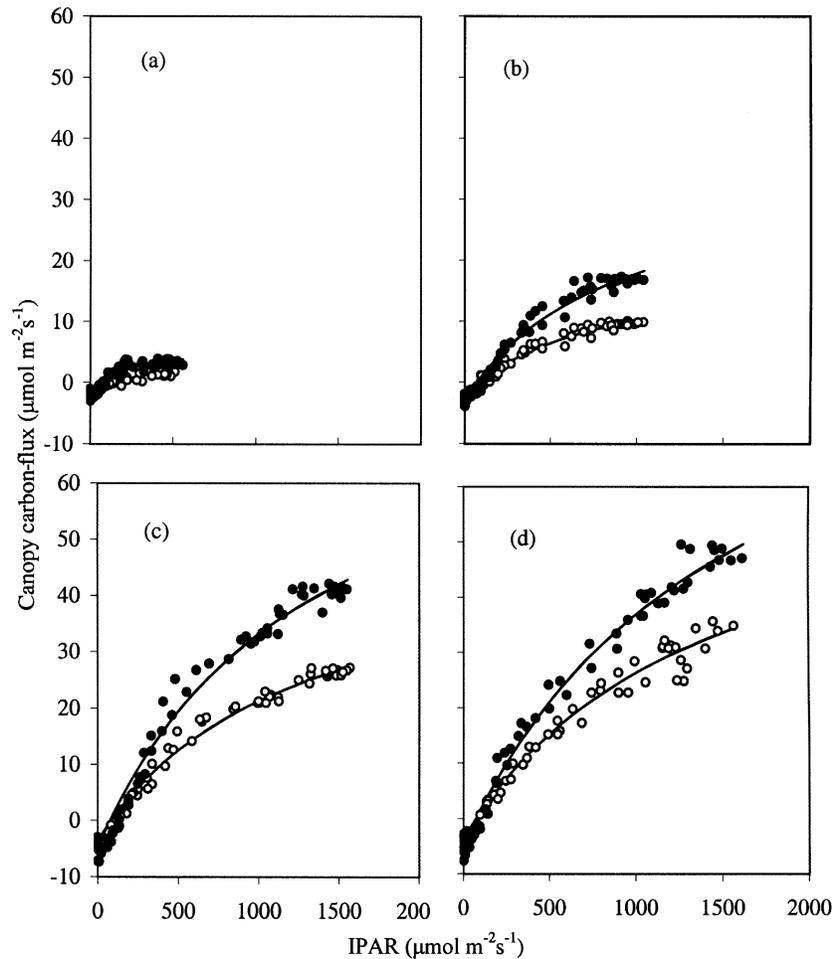
Fig. 10 Variation of canopy carbon-fluxes with photosynthetic active radiation (PAR) at ambient $[CO_2]$ (○) and elevated $[CO_2]$ (●). The relationships were fitted by rectangle hyperbolic equations. Their parameter values were listed in Table 2.

ET at elevated $[CO_2]$ throughout most of the experiment. In the same FACE site in Arizona using wheat, FACE reduced seasonal ET by 4.5% to 11% in well-watered wheat plots (Kimball *et al*. 1995; Hunsaker *et al*. 1996; Pinter *et al*. 1996; Kimball *et al*. 1999).

The variable responses of canopy ET to elevated $[CO_2]$ possibly result from multiple mechanisms and factors. In addition to leaf stomatal conductance, factors that influence canopy ET include canopy leaf-area, canopy temperature, irradiance, wind speed, leaf and canopy conductance, vapour pressure deficit (VPD) above canopy, and vegetation structure (McNaughton & Jarvis 1983; Morison & Gifford 1984; Jarvis & McNaughton 1986; Baldocchi 1994; Morecroft & Roberts 1999). Gifford (1988) hypothesized that adjustment in both stomatal conductance and leaf-area development for plants grown in drying soil is regulated genetically by cues other than by elevated $[CO_2]$. Martin *et al*. (1989) analysed variations of ET using Penman–Monteith models and found that ET differed from the control by about −20 to 40%, depending on ecosystem and on

climate and plant input used. Idso & Idso (1993) found that high temperature caused by increasing $[CO_2]$ influenced plant transpiration. Bunce (1998) reported that air-to-leaf water pressure difference was responsible to the variations of stomatal conductance in wheat and barley. Although our study was not designed to identify mechanisms causing discrepancy between leaf- and canopy-level ET, results do help to exclude several possible mechanisms. Because this study was conducted in an environmentally controlled mesocosm, factors such as temperature, relative humidity and VPD, which may be altered by elevated $[CO_2]$ in the field, were unlikely to cause an increase in canopy ET. There was a slight increase of canopy leaf area index at elevated $[CO_2]$. When canopy water-flux was expressed on the leaf area bases, no effect of elevated $[CO_2]$ was found on canopy water losses, especially at the late stage of canopy development (Fig. 5b). In other words, the 11% increase of canopy water-flux at elevated $[CO_2]$ resulted mainly from the increased canopy leaf-area. Variable responses of canopy water-fluxes to elevated $[CO_2]$ indicated that
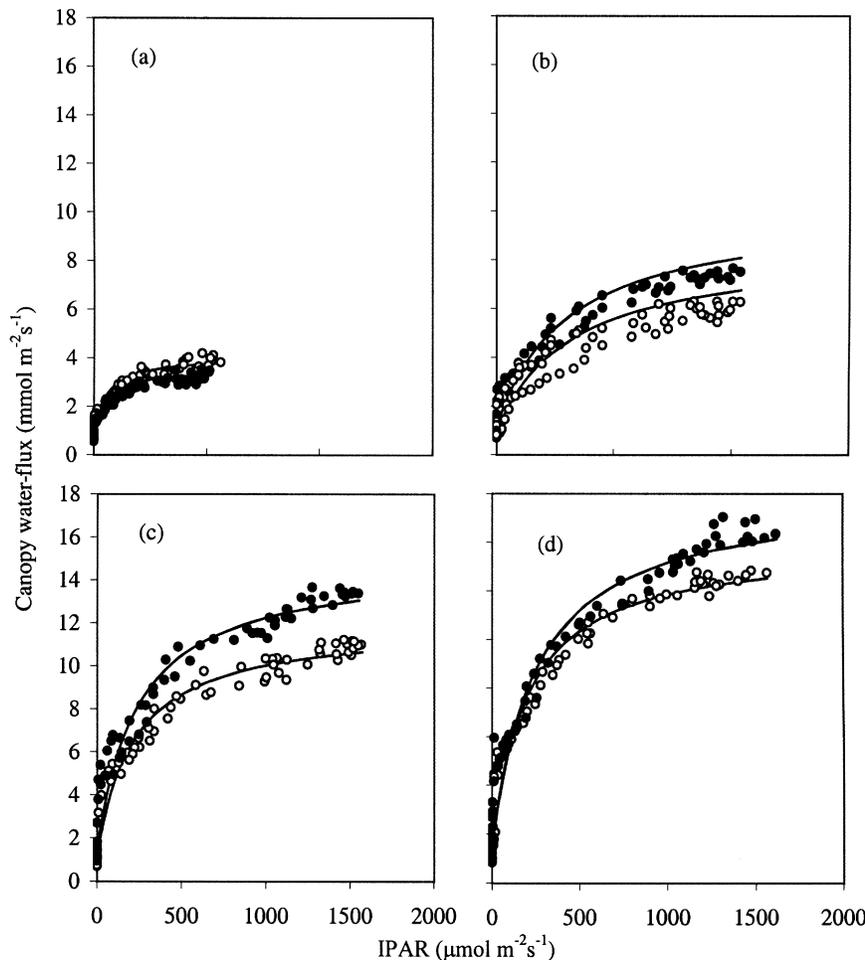
Fig. 11 Variation of canopy water-fluxes with photosynthetic active radiation (PAR) at ambient $[CO_2]$ (○) and elevated $[CO_2]$ (●). The relationships were fitted by rectangle hyperbolic equations. Their parameter values were listed in Table 2.

feedback between leaf-level physiology and canopy-level processes is one of several important issues deserving careful studies in the future.

In spite of diverse responses of canopy water-fluxes, water-use efficiency (WUE) at elevated $[CO_2]$ is consistently increased in comparison to that at ambient $[CO_2]$. We found in this study that WUE was 22% higher at elevated than at ambient $[CO_2]$. Similarly, Reddy *et al.* (1995) found a doubling of $[CO_2]$ improved WUE by an average of 50% using a growth chamber. Water-use efficiency was enhanced by 20% at elevated $[CO_2]$ microcosms in comparison to that at ambient $[CO_2]$ with serpentine soils (Field *et al.* 1997). Samarakoon *et al.* (1995) found that WUE of two wheat cultivars grown in the Canberra sunlit phytotron was increased by 60% and 78%, respectively, for the well-watered treatment. In the FACE experiment on cotton, WUE was found to be improved 28% to 39% for well-watered plots when $[CO_2]$ was elevated from ambient to 550 μmol mol⁻¹ (Mauney *et al.* 1994). The increase in WUE was caused mainly by a greater increase in canopy carbon-flux, with either a

decrease in canopy ET, or no change of ET at elevated $[CO_2]$. In some cases, as demonstrated in this study, canopy WUE was still higher at elevated $[CO_2]$ even though ET was also enhanced, because elevated $[CO_2]$ stimulated more canopy carbon fixation than water transpiration.

Rising atmospheric $[CO_2]$ enhances canopy carbon-flux and canopy radiation-use efficiency (RUE) across almost all studies. For example, Hendrey *et al.* (1993) reported that canopy-level photosynthesis of cotton in the FACE experiment was enhanced at elevated $[CO_2]$ (550 ppm) by 18–35% compared to that at ambient $[CO_2]$. Ryle *et al.* (1992) showed that whole-plant net photosynthesis rates of ryegrass were 33% higher at elevated than ambient $[CO_2]$. Elevated $[CO_2]$ increased daily canopy photosynthesis of *Abutilon* and *Ambrosia* by 30–50% (Hirose *et al.* 1997; model result), by 54% in a rice stand (Allen *et al.* 1989), and by 40–80% in a salt marsh community (Drake & Leadley 1991) relative to their corresponding values at ambient $[CO_2]$. RUE for soybean canopy was found to be 40% higher in 800 μmol mol⁻¹
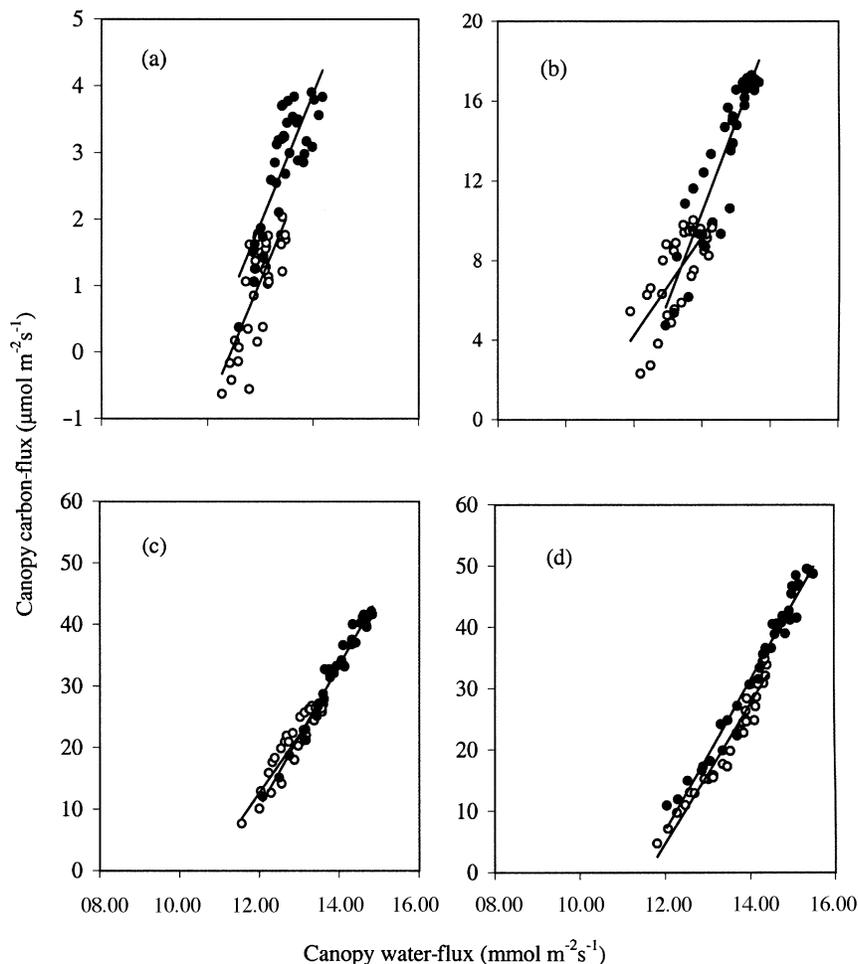
Fig. 12 Relationship between canopy carbon-flux and canopy water-flux time at ambient $[CO_2]$ ($\circ$) and elevated $[CO_2]$ ($\bullet$). Linear equations are fitted. (a) 22–29 DAP.

$F_{CO2,amb} = -4.68 + 1.92\,F_{H2O,\,amb}$, $r^2 = 0.59$; $F_{CO2,ele} = -3.90 + 1.94\,F_{H2O,ele}$, $r^2 = 0.68$; (b) 30–37 DAP. $F_{CO2,amb} = -5.80 + 2.51\,F_{H2O,amb}$, $r^2 = 0.58$; $F_{CO2,ele} = -16.77 + 4.55\,F_{H2O,ele}$, $r^2 = 0.85$; (c) 38–45. $F_{CO2,amb} = -24.72 + 4.67\,F_{H2O,amb}$, $r^2 = 0.88$; $F_{CO2,ele} = -35.60 + 5.74\,F_{H2O,ele}$, $r^2 = 0.97$; (d) 46–53 DAP. $F_{CO2,amb} = -40.73 + 5.68\,F_{H2O,amb}$, $r^2 = 0.94$; $F_{CO2,ele} = -42.06 + 6.11\,F_{H2O,ele}$, $r^2 = 0.97$. $F_{CO2,\,amb}$, $F_{CO2,ele}$, $F_{H2O,amb}$ and $F_{H2O,ele}$ represent canopy carbon-flux at ambient and elevated $[CO_2]$ and canopy water-fluxes at ambient and elevated $[CO_2]$, respectively. $r^{2'}$ determination coefficient.

than in 330 $\mu mol\ mol^{-1}$ CO_2 treatments (Acock *et al.* 1985). Using the enclosed rainforest in Biosphere 2, Lin *et al.* (1998) found that the whole-ecosystem RUE was 0.022–0.032 $mol\ CO_2\ mol^{-1}$ photon at high $[CO_2]$, which was, on average, 100% higher than that at low $[CO_2]$. These results, together with ours, have revealed that plants grown at elevated $[CO_2]$ have a higher RUE than those grown at ambient $[CO_2]$.

Radiation-use efficiency is influenced by many factors such as PAR availability, temperature, vapour pressure deficit (VPD), nitrogen supply and plant species (e.g. Bartelink *et al.* 1997; Mariscal *et al.* 2000). In the present experiment, temperature and VPD were controlled while nitrogen was adequately supplied. Thus, these factors are unlikely to be the major causes of RUE change at elevated $[CO_2]$. Change of canopy leaf area, especially the change of leaf distribution and canopy structure, may determine the quantity of radiation intercepted by the canopy and become one of the major causes of increased RUE at elevated $[CO_2]$. Sinclair & Horie (1989) showed that leaf RUE theoretically depends on maximum leaf photosyn-

thetic rate. The higher values of canopy quantum yield and canopy photosynthetic capacity revealed in this study may have contributed to the higher canopy carbon-flux and RUE at elevated $[CO_2]$, which resulted in a 22% higher harvested biomass at the end of the experiment. The increase of canopy RUE during the experimental period may be explained by the gradual increase of photosynthesis rate for leaves at the top of the canopy measured at both ambient and elevated $[CO_2]$ (Sims *et al.* 1999). That leaf-scattered light was captured and utilized efficiently at lower intensity by shaded leaves may also attribute to the changes of RUE during canopy development. Similar patterns of RUE change were observed during the early growth seasons in a young olive orchard (Mariscal *et al.* 2000; Fig. 6).

How elevated $[CO_2]$ affects whole-ecosystem (combined plant and soil) respiration is a critical issue in understanding ecosystem carbon processes because it reflects how fast the additional fixed carbon is cycled through the ecosystems. Poorter *et al.* (1992) analysed the effects of elevated $[CO_2]$ on dark respiration rate for a

Table 2 Response of canopy gas fluxes (*F*) to intercepted photosynthetic active radiation (IPAR) at ambient and elevated [CO_2]. Values are estimates $\pm$ standard errors.

DAP	CO_2 treatment	F_{max}	α	F_0	R^2
Canopy carbon-flux (μmol m^{-2}s^{-1})					
22–29	A	5.02 ± 0.47	0.0220 ± 0.0032	-1.81 ± 0.07	0.89
	E	7.62 ± 0.55	0.0342 ± 0.0041	-1.61 ± 0.09	0.92
30–37	A	21.40 ± 0.95	0.0292 ± 0.0015	-2.25 ± 0.08	0.99
	E	39.25 ± 2.38	0.0434 ± 0.0024	-2.77 ± 0.15	0.98
38–45	A	54.63 ± 2.14	0.0456 ± 0.0019	-3.56 ± 0.16	0.99
	E	87.65 ± 4.44	0.0674 ± 0.0033	-4.85 ± 0.28	0.99
46–53	A	72.23 ± 4.42	0.0511 ± 0.0026	-3.62 ± 0.23	0.98
	E	106.94 ± 6.09	0.0680 ± 0.0031	-4.74 ± 0.28	0.99
Canopy water-flux (mmol m^{-2}s^{-1})					
22–29	A	2.82 ± 0.08	0.0688 ± 0.0081	0.68 ± 0.04	0.95
	E	3.17 ± 0.11	0.0556 ± 0.0070	0.90 ± 0.05	0.94
30–37	A	7.59 ± 0.36	0.0235 ± 0.0024	0.95 ± 0.08	0.95
	E	8.96 ± 0.29	0.0297 ± 0.0021	1.13 ± 0.07	0.97
38–45	A	10.84 ± 0.23	0.0501 ± 0.0037	1.11 ± 0.09	0.98
	E	13.45 ± 0.31	0.0557 ± 0.0042	1.40 ± 0.11	0.98
46–53	A	12.62 ± 0.26	0.0636 ± 0.0043	1.31 ± 0.10	0.98
	E	14.57 ± 0.34	0.0570 ± 0.0042	1.54 ± 0.11	0.98

DAP, days after planting; F_{max}, maximum canopy carbon or water-flux; α, canopy quantum yield; F_0, canopy carbon or water-flux when IPAR $= 0$; R^2, determinant coefficient; A, ambient; E, elevated

wide range of plant species and found that leaf respiration was, on average, slightly higher for plants grown at high [CO_2] (16%) than those at ambient [CO_2]. Luo *et al.* (1996) found that soil-surface respiration in the sandstone grassland in California was 42% higher at elevated than ambient [CO_2]. Soil-surface respiration in the Duke Forest at elevated [CO_2] exhibited no difference in the first 10 months after CO_2 fumigation, but increased by 33% in the second growing season and by 45% in the third growing season in comparison to that at ambient [CO_2] (J.A. Andrews and W.B. Schlesinger, pers. comm.). In the present study, ecosystem respiration at elevated [CO_2] was progressively higher in magnitude than that at ambient [CO_2] after 35 DAP. By the end of the experiment, ecosystem respiration was 32% higher at elevated than that at ambient [CO_2]. This enhancement was probably a result of both enhanced aboveground and belowground respiration. Root/rhizosphere respiration as a portion of total ecosystem respiration was higher at elevated [CO_2] (Cheng *et al.* 2000). Root-to-shoot ratio was also higher at elevated [CO_2], suggesting that plants grown at elevated [CO_2] allocated more photosynthate to belowground components than did plants at ambient [CO_2].

Responses of canopy carbon-flux to radiation have been reported in the literature either as a linear (Wall *et al.* 1990; Baldocchi 1994; Soegaard & Thorgeirsson 1998) or nonlinear relationship (Jones *et al.* 1985a; Drake

& Leadley 1991; Rochette *et al.* 1996; Lin *et al.* 1998). In spite of the fact that the linear relationship between net primary productivity and absorbed photosynthetic active radiation (APAR) is conveniently useful in remote sensing for quantification of large-scale productivity, numerous recent studies have suggested a nonlinear relationship between photosynthesis and radiation. For example, a rectangular hyperbolic relationship between photosynthesis and PAR can be well applied to almost all of the 122 datasets in a review study (Ruimy *et al.* 1995). In a mesocosm study, Lin *et al.* (1998) found that the response of net ecosystem exchange of carbon to PAR was nonlinear at both a low and a high [CO_2] phase. Our results have supported the nonlinear relationship. Canopy carbon- and water-fluxes, on the one hand, and PAR, on the other, were well described by a hyperbolic equation similar to leaf level (Figs 10 and 11, also see Luo *et al.* 2000).

This study also demonstrated a positive correlation between daytime canopy carbon-flux and water-flux at both ambient and elevated [CO_2] (Fig. 12). Such a correlation has also been shown in other leaf- and canopy-level studies. Grace *et al.* (1998) showed that canopy CO_2 assimilation rate was linearly correlated with canopy stomatal conductance of a C4 pasture. Cox *et al.* (1998) revealed a linear relationship between canopy photosynthesis and canopy conductance using a modelling approach. In a field experiment, when ET was

normalized by vapour pressure deficit, the relationship between canopy photosynthesis and ET was linear (Rochette *et al*. 1996). This linearity may be interpreted largely in terms of a pathway across the air boundary layer and stomata shared by the CO_2 assimilation and transpiration process. Another important fact is that canopy photosynthesis and evapotranspiration also have in common the reliance upon radiation absorption as the energy source to drive the process (Amthor 1999). Further, changes in leaf area and display affect the energy supply for the two processes in a nearly identical manner. Leaf area change enhanced by elevated $[CO_2]$ has the same impact on canopy carbon- and water-fluxes.

In summary, elevated $[CO_2]$ enhanced canopy carbon- and water-fluxes, radiation- and water-use efficiencies during canopy development. The diurnal change of RUE and WUE was also enhanced by elevated $[CO_2]$. Sunflower plants grown at elevated $[CO_2]$ consumed more, instead of less, water to gain more carbon than those grown at ambient $[CO_2]$ as a consequence of the slightly increased leaf area, at least during the exponential growth period as illustrated in this experiment. This study also confirmed that the effect of elevated $[CO_2]$ was smaller on canopy water-flux than that on canopy carbon-flux. Comparison of this study with other studies reported in the literature suggests that feedback between leaf-level physiology and canopy-level processes is complex and that leaf-level results of water use at elevated $[CO_2]$ may not be easily extrapolated to predict of canopy water-flux. There is no sufficient evidence from canopy water studies to conclude that reductions of ET and plant water requirements would occur in the future high-CO_2 world.

Acknowledgements

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