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Effect of elevated carbon dioxide and water stress on gas exchange and water use efficiency in corn

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ABSTRACT

CO_2 has been predicted to increase in the future, and thus leading to possible changes in precipitation patterns. The objectives of this study were to investigate water use and canopy level photosynthesis of corn plants, and to quantify water use efficiency in corn plants under two different CO_2 levels combined with four different water stress levels. Corn plants were planted in sunlit plant growth chambers and a day/night temperature of (28/18 °C) was applied. From 21 days after emergence (DAE), the eight treatments including two levels of carbon dioxide concentrations (400 and 800 μmol mol^{-1}) and four levels of water stress (well-watered control, "mild", "moderate", and "severe" water stress) treatments at each CO_2 level were imposed. Height, number of leaves, leaf lengths, and growth stages of corn plants were monitored from nine plants twice a week. Corn plants were separately collected, dried, and analyzed for the biomass accumulation at 21 and 60 DAE. Soil water contents were monitored by a time domain reflectometry (TDR) system (15 probes per chamber). The "breaking points" (changes from high to low rates of soil water uptake) were observed in the bottom of soil depth for the water stressed conditions, and the "breaking points" under ambient CO_2 appeared 6–9 days earlier than under elevated CO_2. Although approximately 20–49% less water was applied for the elevated CO_2 treatments than for ambient CO_2 from 21 DAE, higher soil water contents were recorded under elevated CO_2 than under ambient CO_2. However, corn growth variables such as height, leaf area, and biomass accumulation were not significantly different in CO_2 or water stressed treatments. This result may be explained by considering that significant differences in canopy level gross photosynthesis among the water stress treatments was observed only toward the end of the experiment. The higher soil water contents observed under elevated CO_2 resulted mainly from less water use than under ambient CO_2. WUE (above ground biomass per water use since 21 DAE) at the final harvest was consistently higher and varied with a smaller range under elevated CO_2 than under ambient CO_2. This study suggests that less water will be required for corn under high-CO_2 environment in the future than at present.

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1. Introduction

Fossil fuel combustion and land use change are contributing to increased atmospheric CO_2 concentrations (Keeling and Whorf, 2001) at an unprecedented rate. This increase in atmospheric CO_2 concentrations may contribute to changes both in precipitation and in evapotranspiration (Kruijt et al., 2008; Long et al., 2004; Schneider, 2001). Consequently, the risks of flooding and drought may increase in many areas due to the changes (Bates et al., 2008).

It is generally recognized that elevated CO_2 concentrations increase crop photosynthesis and yield for many crops. For C_3 plants at elevated atmospheric CO_2, growth and yield will increase by reducing photorespiration and enhancing photosynthetic CO_2 exchange rates (CER), while the photosynthetic mechanism for C_4 plants at elevated atmospheric CO_2 still remains uncertain (Vu and Allen, 2009; Leakey et al., 2006). Some C_4 plants respond to increased CO_2 (Ziska and Bunce, 1997; LeCain and Morgan, 1998; Wand et al., 2001) and some do not (Morison and Gifford, 1984b; Wilsey et al., 1994; Ward et al., 1999; Wand et al., 2001). Unlike C_3 plants, little direct effects of increase in atmospheric CO_2 on C_4 photosynthesis are theoretically expected (Vu and Allen, 2009).

There have been many studies on the interaction of CO_2 and water on plant growth. Under elevated CO_2, less water is used to produce each unit of dry matter by reducing stomatal conductance (Morison, 1993). For many C_4 plants, the reduction in crop water use under elevated CO_2 does occur even though there is not an increase in photosynthesis (Leakey et al., 2006; Long et al., 2006).

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Loomis and Lafitte (1987) reported that corn growth rates were very little affected by large changes in the supplies of CO_2 and water. Clark et al. (1999) found that there was a strong interaction between CO_2 and water on net photosynthesis in temperate pasture species (C_3 and C_4). However, Surano and Shinn (1984) found that elevated CO_2 increased WUE independent of water supply.

It has been reported that elevated CO_2 may have the potential to enhance plant water use efficiency (WUE) in C_3 or C_4 plants (Rogers et al., 1983; Amthor, 1995; Kimball et al., 2002). This increase in WUE at elevated CO_2 is largely due to decreases in stomatal conductance and transpiration (Ghannoum et al., 2001; Prior et al., 2010). In C_3 plants, increased photosynthesis as well as reduced transpiration contributes to determination of increased WUE, whereas decreased transpiration contributes in C_4 plants (Rogers and Dahlman, 1993). Prior et al. (2010) reported that elevated CO_2 significantly increases WUE, and concluded that soil moisture can be better conserved at elevated CO_2 during reproductive growth.

The objectives of this study were (1) to investigate water uptake and canopy level photosynthesis of corn plants grown under ambient (400 μmol mol^{-1}) and elevated (800 μmol mol^{-1}) CO_2 combined with four different water stress levels, and (2) to quantify water use efficiency in corn plants under those treatments.

2. Materials and methods

2.1. SPAR chambers

Corn plants were grown in naturally sunlit soil–plant–atmosphere-research (SPAR) chambers at the Henry A. Wallace Agricultural Research Center, Beltsville, MD in which temperature, humidity, and carbon dioxide concentrations were precisely controlled. Transparent chamber tops (2.2 m long by 1.4 m wide by 2.5 m high) were constructed of 0.0127 m thick Plexiglas. These chamber tops are mounted to steel soil bins (2.0 m long by 0.5 m wide by 1.0 m deep). The physical and environmental description and operation SPAR chambers have been previously described (Fleisher et al., 2008; Timlin et al., 2007; Baker et al., 2004; Kim et al., 2004). Table 1 summarizes the statistics for CO_2 and temperature control in the growth chamber during growth period. CO_2 and temperature in the SPAR units were maintained within 20 μmol mol^{-1} of the set points (400 and 800 μmol mol^{-1} for ambient and elevated CO_2 levels, respectively) and 1 °C of the day/night set points (28/18 °C), respectively. Opening the chamber doors to measurements mainly resulted in the difference of CO_2 level, but the doors were open less than 1 h.

The soil bins (2.0 m long by 0.5 m wide by 1.0 m deep), where the chamber tops are mounted, were filled with a mixture of 75% coarse sand and 25% vermiculate (Grace Construction Products, Cambridge, MA, USA). Soil water contents were monitored by a time domain reflectometry (TDR) system. Fifteen TDR probes per chamber were installed at five different depths (0 cm, 15 cm, 30 cm, 50 cm and 75 cm from the soil surface) in three rows and monitored hourly. For the soil surface, 15 cm-long TDR waveguides (three rod) were vertically installed to better estimate infiltrated water in the surface soil as suggested by Timlin et al. (2007). From the second depth, 30 cm-long TDR waveguides were horizontally installed. Total water volumes in the soil bins for each hourly measurement were obtained by multiplying water contents by soil volume. Water use per day was assumed to be the difference between the 08:00 and 22:00 h water contents to minimize the variations of water content during night time when irrigation was applied (Timlin et al., 2007). More detailed information on the TDR system and soil characteristics can be found in Timlin et al. (2007).

Table 1
Statistics for CO_2 and temperature control in the growth chamber. A_SVR: ambient CO_2 and "severe" water stress, A_MOD: ambient CO_2 and "moderate" water stress, A_MLD: ambient CO_2 and "mild" water stress, A_CTR: ambient CO_2 and control, E_SVR: elevated CO_2 and "severe" water stress, E_MOD: elevated CO_2 and "moderate" water stress, E_MLD: elevated CO_2 and "mild" water stress, and E_CTR: elevated CO_2 and control.

Treatment	Period	CO_2 (μmol m^{-2} s^{-1})		Temperature (°C)	
		Mean	Standard error	Mean	Standard error
A_SVR	Day	400.8	0.7	27.826	0.003
	Night	N/A[a]	N/A	18.024	0.001
E_SVR	Day	794.8	0.2	27.919	0.003
	Night	N/A	N/A	18.017	0.003
A_MOD	Day	392.4	0.7	27.794	0.004
	Night	N/A	N/A	17.952	0.001
E_MOD	Day	795.0	0.2	28.007	0.002
	Night	N/A	N/A	18.098	0.001
A_MLD	Day	402.7	0.5	27.944	0.002
	Night	N/A	N/A	18.118	0.001
E_MLD	Day	794.4	0.1	27.950	0.003
	Night	N/A	N/A	18.128	0.002
A_CTR	Day	393.8	0.6	27.939	0.004
	Night	N/A	N/A	17.948	0.002
E_CTR	Day	793.8	0.1	27.663	0.002
	Night	N/A	N/A	17.784	0.002

[a] Not applicable. CO_2 was not controlled at night.

2.2. Plant culture

Three corn seeds (*Zea mays* L., Pioneer brand hybrid corn, 33M15) per location were planted in 9 rows with 5 plants in each row with 20 cm row spacing on 20/07/2009 and corn plants uniformly emerged on 24/07/2009. Corn plants were thinned out at 6 days after emergence (DAE) so that only single corn plant remained at each location. The plants were fertigated nightly at 23:00 h with full-strength Hoagland's nutrient solution (Hewitt, 1952). This night fertigation minimizes the redistribution of irrigated water during the daylit period. Four different water stress levels ("control", and "severe", "moderate", and "mild" water stress levels) combined with ambient (400 μmol mol^{-1}) and elevated CO_2 (800 μmol mol^{-1}) were applied to the corn plants at 21 days after emergence (DAE). These treatments were denoted with letters "A"/"E" for ambient/elevated CO_2, and "CTR", "MLD", "MOD", and "SVR" for "control", "mild", "moderate", and "severe", respectively. For example, A_SVR stands for ambient CO_2 and "severe" of the water stress level, and E_CTR represents elevated CO_2 and "control" of the water stress level (i.e. well-watered conditions). The irrigation amounts were estimated considering the average of water use and water amount in soil bins for previous two days. Time release fertilizer (Osmocote 14-14-14, The Scotts Company, OH, USA) was applied in each chamber at a rate of 134 g m^{-2} to avoid possible nitrogen deficiency in corn plants due to decreases of fertigation for the targeting water stress levels.

2.3. Measurements

Nine plants were selected to monitor growth development of corn plants in three center rows per chamber and three center plants per the center row. Plant height, number of leaves, leaf lengths, and growth stages of corn plants were measured twice a week for early growth stages or once a week after tassel emergence. Means were separated by the Proc Mixed lsmeans macro as described by Saxton (1998).

Plant height was measured from soil surface to the base of a youngest, fully expanded leaf. Corn plants were separately collected, dried, and analyzed for leaf, stem, and ear dry weights at 21 and 60 DAE. Individual laminar area was measured with a leaf area meter (LI-COR, LI-3000, Lincoln, NE, USA). The harvested plant parts were dried at 70 °C at least 7 days prior to measurements of dry weight. These two destructive harvests were used to develop relationships between leaf areas and leaf lengths. Leaf lengths were fitted to power functions similar forms to that of Zhao et al. (2003) to estimate individual leaf area. The fitted curves are:

For "not fully expanded leaves" or "fully expanded leaves" higher than leaf number 11 or equal to

$$A = 0.394\,L^{1.608}(R^2 = 0.948^{***}) \qquad (1)$$

For "fully expanded leaves" lower than leaf number 10 or equal to

$$A = 0.0065\,L^{2.435}(R^2 = 0.925^{***}) \qquad (2)$$

where A is leaf area in cm^2 and L is leaf length in cm. More than 900 leaves were used for the development of the relationships at the two destructive harvests.

2.4. Data analysis

The carbon exchange rate (CER) represents net photosynthesis (P_n), when corn plants are growing in the chambers. Dark respiration, R_D at night-time temperature was estimated as the mean CER at night time (from 01:00 to 04:00 h) when there is no significant light, and R_D at daytime temperature was estimated using relationships from Reddy et al. (1991). These R_D values were used to estimate gross photosynthesis, P_G, as in Eq. (3). This method to estimate P_G and R_D has been used to relate seasonal carbon assimilation to dry matter (van Iersel and Kang, 2002; Reddy et al., 1989; Dutton et al., 1988; Fleisher et al., 2008).

$$P_G = P_N + R_D \qquad (3)$$

Here P_G is the gross instantaneous photosynthetic rate in $\mu mol\,CO_2\,m^{-2}\,s^{-1}$, P_N is the net instantaneous photosynthetic rate in $\mu mol\,CO_2\,m^{-2}\,s^{-1}$, and R_D is the dark respiration in $\mu mol\,CO_2\,m^{-2}\,s^{-1}$. Canopy gas exchange data were averaged at 15-min intervals. To interpolate measurements of P_G and to analyze light–response curves, a maximum function (P_{MAX}) of similar form to that of Constable and Rawson (1980) and Milroy and Bange (2003) was fitted to the relationship between P_G and incident PAR. The equation is given as

$$P_G = P_{MAX}(1 - \exp[-a \times I]) \qquad (4)$$

where I is light intensity (PPFD) in μmol Quanta $m^{-2}\,s^{-1}$, P_{MAX} is the asymptotic rate of gross carbon assimilation in $\mu mol\,CO_2\,m^{-2}\,s^{-1}$ at light saturation, a is a coefficient with units of $\mu mol\,photons^{-1}\,m^2\,s$. The NLIN procedure in SAS statistical software (The SAS system for Windows, 9.2, SAS Institute Inc., Cary, NC, USA) was used to fit the parameters, a and P_{MAX} in Eq. (4).

Daily water use efficiency (WUE) and WUE at the final harvest time were calculated. Leaf level water use efficiency can be calculated as (e.g. Prior et al., 2010):

$$WUE_L = \frac{P_N}{T_r} \qquad (5)$$

where WUE_L is the leaf level water use efficiency in $\mu mol\,CO_2\,mmol^{-1}$ H_2O, P_N is the photosynthesis rate in $\mu mol\,CO_2\,m^{-2}\,s^{-1}$, and T_r is the transpiration rate in $mmol\,H_2O\,m^{-2}\,s^{-1}$. For this study, daily WUE is defined as a similar form of that of Prior et al. (2010), dividing daily canopy P_G by daily canopy water use. Because crop canopy was closed in all chambers by the time water stress levels were imposed and the differences of evaporation from the soil surface among chambers can be considered negligible, water use per day estimated as the difference between the 08:00 and 22:00 h water contents (Timlin et al., 2007) can be assumed as water uptake per day (i.e. transpiration). Another WUE ($g\,L^{-1}$) at the final harvest time (60 DAE) was calculated as above ground biomass ($g\,plant^{-1}$) divided by water use ($L\,plant^{-1}$) since 21 DAE.

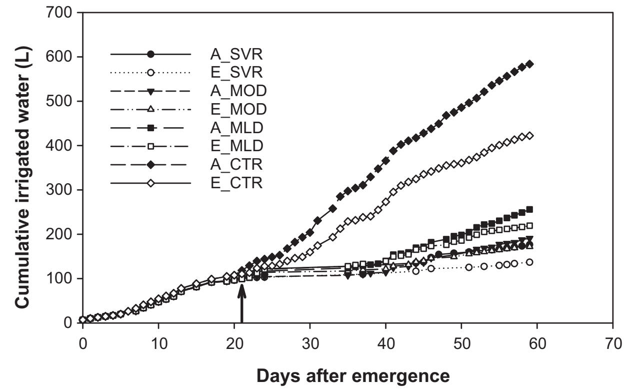

Fig. 1. Cumulative irrigated water amounts. The vertical arrow along the *x*-axis indicates initiation of the water stress treatment (21 days after emergence). A_SVR: ambient CO_2 and "severe" water stress, A_MOD: ambient CO_2 and "moderate" water stress, A_MLD: ambient CO_2 and "mild" water stress, A_CTR: ambient CO_2 and control, E_SVR: elevated CO_2 and "severe" water stress, E_MOD: elevated CO_2 and "moderate" water stress, E_MLD: elevated CO_2 and "mild" water stress, and E_CTR: elevated CO_2 and control.

3. Results and discussion

3.1. Soil water uptake

A total 140–580 L (14.0–58.0 cm cm^{-2}) of water was supplied to the different treatments during the growth period (Fig. 1). Approximately 40–480 L (4.0–48.0 cm cm^{-2}) of water was applied as irrigation from the time the water treatments were imposed (21 DAE). From 21 DAE, approximately 20 ("moderate") to 49% ("severe") less water was applied to the elevated CO_2 treatments than for the ambient CO_2. Higher soil water contents were recorded under elevated CO_2 than under ambient CO_2 (Fig. 2). More irrigation water was applied to each ambient CO_2 water stress treatment compared to the corresponding elevated treatment. These results suggest that water demand for corn plants will be lower in the future under the predicted higher CO_2 concentrations.

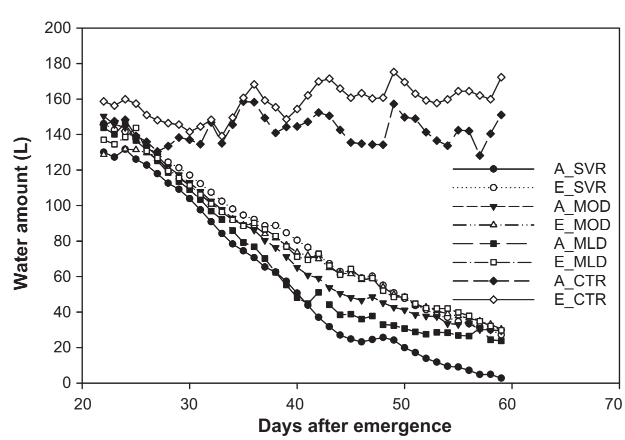

Fig. 2. Daily water amount in the soil bins. A_SVR: ambient CO_2 and "severe" water stress, A_MOD: ambient CO_2 and "moderate" water stress, A_MLD: ambient CO_2 and "mild" water stress, A_CTR: ambient CO_2 and control, E_SVR: elevated CO_2 and "severe" water stress, E_MOD: elevated CO_2 and "moderate" water stress, E_MLD: elevated CO_2 and "mild" water stress, and E_CTR: elevated CO_2 and control.

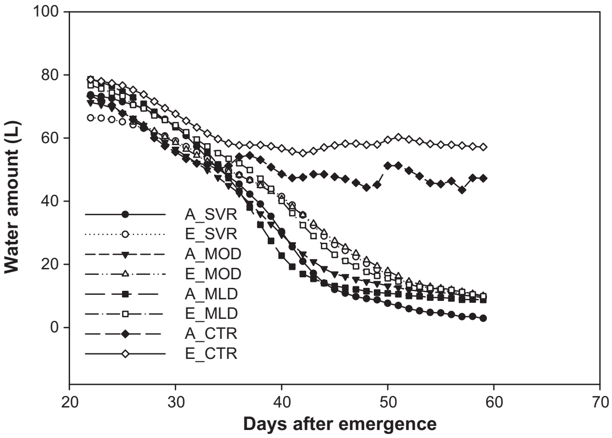

Fig. 3. Temporal changes in soil water amounts between 0.625 and 0.85 m. A_SVR: ambient CO_2 and "severe" water stress, A_MOD: ambient CO_2 and "moderate" water stress, A_MLD: ambient CO_2 and "mild" water stress, A_CTR: ambient CO_2 and control, E_SVR: elevated CO_2 and "severe" water stress, E_MOD: elevated CO_2 and "moderate" water stress, E_MLD: elevated CO_2 and "mild" water stress, and E_CTR: elevated CO_2 and control.

Daily water amounts in the soil bins are shown in Fig. 2. At harvest, the soil bins under well-watered conditions retained approximately 140–160 L (14.0–16.0 cm cm^{-2}) of water, while less than 40 L (4.0 cm cm^{-2}) of water remained in the soil bins under the "severe" water stress level. Overall, higher soil water contents under elevated CO_2 were observed at both well-watered and water stressed conditions. This result is in agreement with that of Nelson et al. (2004). They reported that soil moisture throughout the soil profile (the soil surface to 105 cm deep) under elevated CO_2 (720 μL L^{-1}) was higher than under ambient CO_2 (360 μL L^{-1}) at a study site mixed with C_3 and C_4 grasses.

Water use from the time water stress treatments were imposed (21 DAE) is shown in Table 2. Consistently lower water use (35% for the well-watered conditions and 13–20% for the water stressed conditions) was observed under elevated CO_2 than under ambient CO_2. Water use from 21 DAE under the well-watered conditions was about 18.9 L $plant^{-1}$ for the ambient CO_2 treatment (A_CTR), and 12.3 L $plant^{-1}$ for the elevated CO_2 treatment (E_CTR). Under the water stressed conditions, water use from 21 DAE was 8.5 L $plant^{-1}$ and 6.8 L $plant^{-1}$, for A_SVR and E_SVR, respectively. The water use data showed that the ambient well watered CO_2 treatment (A_CTR) used more water; about 1.54 times greater, than the corresponding elevated CO_2 treatment (E_CTR). However, for water stressed levels ("mild", "moderate", and "severe"), about 1.15–1.25 times greater water use by corn plants was observed under the ambient CO_2 treatments than under the corresponding elevated CO_2 treatments. Water use relative to "control" was 45 (A_SVR) to 77% (E_MLD). These results are similar to those of van Vuuren et al. (1997) who reported about 1.25 times greater water was used by spring wheat under the ambient CO_2 treatments (350 μmol mol^{-1}) than under the elevated CO_2 treatments (700 μmol mol^{-1}).

Apparent "breaking points" (changes from high to low rates of soil water uptake) were observed in the bottom depth (between 0.625 and 0.85 m from the soil surface) for the water stressed conditions (Fig. 3). Changes in slope of water uptake rates over time indicate a decrease in water availability. Similar phenomenon was observed in a field study by Starr and Paltineanu (1998). However, the "breaking points" (i.e. slope changes) in Fig. 3 were more evident than those in the study by Starr and Paltineanu (1998). The breaking points under ambient CO_2 were between approximately 42 and 47 DAE, and those under elevated CO_2 were between approximately 51 and 53 DAE. This suggests that it took longer

Table 2
Leaf area, dry matter, and height at the final destructive harvest A_SVR: ambient CO_2 and "severe" water stress, A_MOD: ambient CO_2 and "moderate" water stress, A_MLD: ambient CO_2 and "mild" water stress, A_CTR: ambient CO_2 and control, E_SVR: elevated CO_2 and "severe" water stress, E_MOD: elevated CO_2 and "moderate" water stress, E_MLD: elevated CO_2 and "mild" water stress, and E_CTR: elevated CO_2 and control.

Treatment	Height (m $plant^{-1}$)		Leaf area (m^2 $plant^{-1}$)		Leaf biomass (g $plant^{-1}$)		Specific leaf area (m^2 g^{-1})	Above ground biomass (g $plant^{-1}$)		Water use (L $plant^{-1}$)[a] (%)	WUE[b] (g L^{-1})
	Mean	Standard error	Mean	Standard error	Mean	Standard error		Mean	Standard error		
A_SVR	2.19 b[†]	0.06	0.37 b	0.03	17.12 cd	1.15	0.022	59.3 d	4.7	8.5 (45)	7.0
E_SVR	2.37 a	0.06	0.48 ab	0.02	19.33 bcd	1.09	0.025	70.5 bc	4.8	6.8 (55)	10.4
A_MOD	2.47 a	0.08	0.42 ab	0.04	16.72 d	2.55	0.025	64.1 cd	9.8	9.0 (48)	7.1
E_MOD	2.44 a	0.02	0.43 ab	0.02	20.06 abcd	1.16	0.021	80.0 b	4.8	7.8 (64)	10.2
A_MLD	2.43 a	0.04	0.47 ab	0.03	22.28 abc	1.56	0.021	71.4 bc	3.8	11.6 (61)	6.2
E_MLD	2.49 a	0.02	0.47 ab	0.02	20.29 abcd	0.83	0.023	74.1 bc	1.7	9.5 (77)	7.8
A_CTR	2.51 a	0.03	0.54 a	0.05	24.84 a	3.72	0.022	107.7 a	4.5	18.9 (100)	5.7
E_CTR	2.49 a	0.01	0.53 a	0.02	23.72 ab	1.86	0.022	97.7 a	3.4	12.3 (100)	8.0

[a] Water use was estimated as sum of the difference of soil water amount between 20 DAE and 59 DAE and cumulative irrigated water during the period. Water use relative to "control" is shown within parentheses in the water use column.
[b] Water use efficiency: defined as above ground biomass (g $plant^{-1}$) divided by water use (L $plant^{-1}$) since 21 days after emergence.
[†] Values followed by the same lowercase letter indicate no significant different by the *F* test and the Proc Mixed lsmeans macro described by alpha = 0.05.

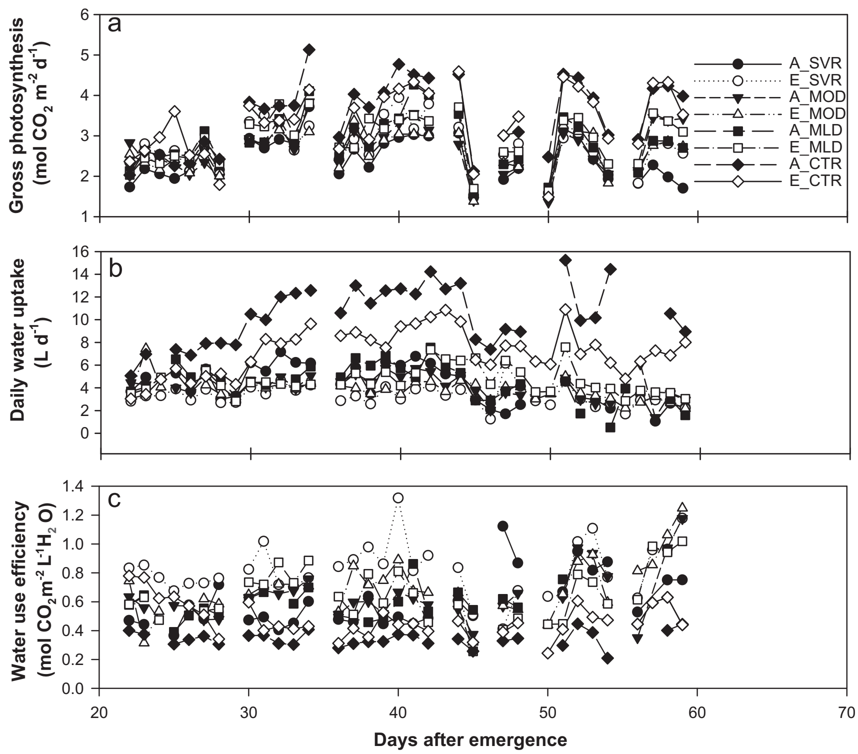

Fig. 4. Gas exchange and water uptake since water stress treatments were initiated (21 days after emergence): (a) gross photosynthesis, (b) daily water uptake, and (c) water use efficiency. A_SVR: ambient CO_2 and "severe" water stress, A_MOD: ambient CO_2 and "moderate" water stress, A_MLD: ambient CO_2 and "mild" water stress, A_CTR: ambient CO_2 and control, E_SVR: elevated CO_2 and "severe" water stress, E_MOD: elevated CO_2 and "moderate" water stress, E_MLD: elevated CO_2 and "mild" water stress and E_CTR: elevated CO_2 and control.

for the easily available water to become depleted for the elevated CO_2 treatments than for the ambient even when less irrigation was applied to the elevated CO_2 treatments.

3.2. Gas exchange rates and water use efficiency

The canopy level gross photosynthesis (P_G) is shown in Fig. 4a. No significant differences among CO_2 and water stress treatments were observed at the beginning of the water stress treatments. However, there were significances in P_G among the water stress treatments within CO_2 treatments at the end of the experiment, but no significance differences between the CO_2 treatments. This lack of differences in P_G due to CO_2 may be explained by considering the CO_2 concentrating mechanism in C_4 leaves that C_4 photosynthesis is nearly saturated in current atmospheric conditions (Cure, 1986; Ghannoum et al., 2000, 2001). Little direct response to elevated CO_2 concentrations has theoretically been expected based on the CO_2 concentrating mechanism in C_4 leaves.

Water use on a daily basis is shown in Fig. 4b. For the well-watered conditions, corn plants used less water under the elevated CO_2 (E_CTR) than under the ambient (A_CTR). However, unlike water use amounts from 21 DAE (Table 2), there were no apparent trends in the daily data for the water stressed conditions. Daily water uptake under well-watered conditions largely varied with between 5.1 and 15.2 L (0.5–1.52 cm cm^{-2}) day^{-1} for A_CTR, and with the range between 3 and 10.9 L (0.3–1.09 cm cm^{-2}) day^{-1} for E_CTR. However, under water stressed conditions, the ranges of daily water uptake [2–7.5 L (0.2–0.75 cm cm^{-2}) day^{-1}] were smaller than under well-watered conditions. Daily water uptake under the well-watered conditions increased at 30 DAE and were maintained until 45 DAE (Fig. 4b).

The daily WUE under well-watered conditions ranged from 0.2 to 0.7 mol CO_2 m^{-2} L^{-1} H_2O, and that under water stressed conditions ranged from 0.3 to 1.3 mol CO_2 m^{-2} L^{-1} H_2O (Fig. 4c). Consistently higher daily WUE under the elevated CO_2 and well-watered conditions (E_CTR) than under ambient CO_2 and well-watered conditions (A_CTR) was observed during the experiment. This result supports previous studies demonstrating that elevated CO_2 concentrations contribute to significant increases in WUE (Ghannoum et al., 2001; Morison and Gifford, 1984a,b; Bremer et al., 1996; Samarakoon and Gifford, 1996; Owensby et al., 1997).

Table 2 summarizes height, leaf area, above ground biomass, and WUE at the final destructive harvest (60 DAE). The height of corn plants ranged from 2.19 to 2.51 m. The height was not significantly different in the CO_2 or irrigation treatments except for A_SVR. The leaf area and the above ground biomass of corn plants varied with the range of 0.37–0.54 m^2 $plant^{-1}$ and of 59.3–107.7 g $plant^{-1}$, respectively. There were no significant CO_2 effects for leaf area or above ground biomass. Significances in P_G among the water stress treatments started to be observed at the end of the experiment (Fig. 4a). This result implies that 60 DAE may not be enough to show the effects of the water stress treatments on the biomass. Biomass and leaf area for the well-watered treatments were significantly greater than those of the stressed treatments. There were no significant differences between irrigation treatments within a CO_2 level. These results are in agreement with those of Samarakoon and Gifford (1996) and Surano and Shinn (1984). Samarakoon and Gifford (1996) reported that elevated CO_2 did not significantly affect dry matter or plant height in corn, and Surano and Shinn (1984) found that corn growth and yield were not largely affected by elevated CO_2.

Leaf areas under well-watered conditions (A_CTR and E_CTR) at the final destructive harvest were not statistically different from those under water stressed conditions (Table 2). However, leaf areas estimated with Eqs. (1) and (2) were significant in water stress treatments (Fig. 5). Leaf areas were slightly overestimated (slope: 1.03 and R^2: 0.86) using Eqs. (1) and (2). This overestimation and

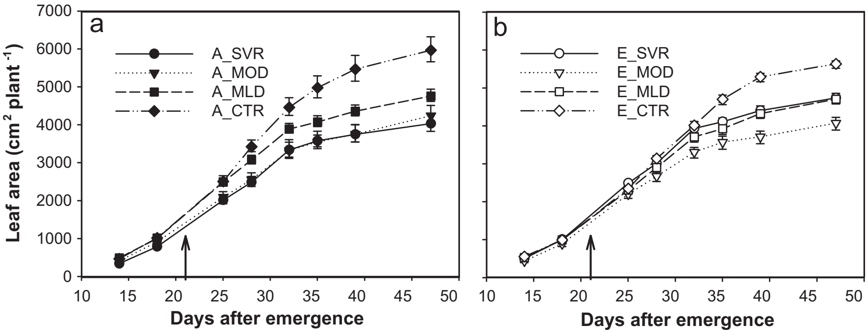

Fig. 5. Leaf area during growth period. The vertical arrow along the x-axis indicates initiation of the water stress treatment (21 days after emergence): (a) ambient CO_2 and (b) elevated CO_2. A_SVR: ambient CO_2 and "severe" water stress, A_MOD: ambient CO_2 and "moderate" water stress, A_MLD: ambient CO_2 and "mild" water stress, A_CTR: ambient CO_2 and control, E_SVR: elevated CO_2 and "severe" water stress, E_MOD: elevated CO_2 and "moderate" water stress, E_MLD: elevated CO_2 and "mild" water stress, and E_CTR: elevated CO_2 and control. Each data point is the mean ± standard error of nine plants.

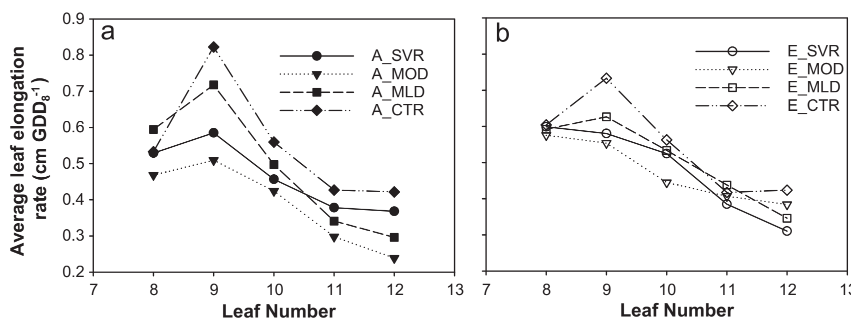

Fig. 6. Leaf elongation rates from leaf numbers 8–12: (a) ambient CO_2 and (b) elevated CO_2. A_SVR: ambient CO_2 and "severe" water stress, A_MOD: ambient CO_2 and "moderate" water stress, A_MLD: ambient CO_2 and "mild" water stress, A_CTR: ambient CO_2 and control, E_SVR: elevated CO_2 and "severe" water stress, E_MOD: elevated CO_2 and "moderate" water stress, E_MLD: elevated CO_2 and "mild" water stress, and E_CTR: elevated CO_2 and control.

the nature of power functions used for the estimation of leaf areas may have led to this discrepancy.

Average leaf elongation rates defined as slopes between 10 and 90% of the maximum leaf length are illustrated in Fig. 6. Average leaf elongation rates of leaf number 8–12 for A_MOD were lower than those for A_SVR. The range of the average leaf elongation rates for elevated CO_2 was smaller than those for ambient CO_2. This smaller range of leaf elongation rates may lead to smaller range of the leaf biomass for elevated CO_2 than for ambient CO_2. Further investigation on an interaction water use and biomass accumulation is recommended to explain this phenomenon. However, specific leaf areas (SLAs) defined as the ratio of leaf area to leaf dry mass were consistent with the range of 0.021–0.025 $m^2\,g^{-1}$. These results indicate that leaf thickness among the treatments did not vary greatly.

Water use efficiency (defined as above ground biomass per water use since 21 DAE) under the elevated CO_2 conditions was consistently higher than under ambient CO_2. The range of WUE under ambient CO_2 (5.7–7.0 $g\,L^{-1}$) was less than that under elevated CO_2 (7.8–10.4 $g\,L^{-1}$). Similar results were reported in a study on potato by Fleisher et al. (2008). Since the above ground biomasses of corn plants were not significantly different in the CO_2 treatment for each water stress treatment, the higher WUE indicates that reduced water use under the elevated CO_2 condition leads to increases in WUE.

4. Conclusions

Corn plants were grown under ambient (400 $\mu mol\,mol^{-1}$) and elevated (800 $\mu mol\,mol^{-1}$) CO_2 combined with four different irrigation treatments, to investigate water use and canopy level photosynthesis and to quantify water use efficiency. Fifteen TDR probes per chamber were used to monitor hourly soil water contents. Both at well-watered and at water stressed conditions, higher water contents maintained under the elevated CO_2 conditions than under the ambient CO_2, even though 20–49% less water was irrigated for the elevated CO_2 conditions since 21 DAE than for the ambient CO_2 conditions. Approximately 13–20% and 35% less water was used under the elevated CO_2 conditions than under the ambient CO_2 conditions, for the water stressed conditions and for the well-watered conditions, respectively. These results suggest that under increased CO_2 concentrations as generally predicted in the future, less water will be required for corn plants than at present.

At the end of the experiment, significant differences in canopy gross photosynthesis between well watered and water stressed treatments within a CO_2 treatment were observed, while no significant differences between the CO_2 treatments were observed. Daily WUE was defined as daily gross photosynthesis divided by daily water use. Approximately 50% less differences in magnitude of daily WUE was observed under the well-watered condition than under the water stressed conditions. However, daily WUE under the elevated CO_2 treatment were mainly higher than under the ambient CO_2 treatment. The "breaking points" (changes from high to low rates of soil water uptake) were observed in the bottom of soil bins (between 0.625 and 0.85 m from the soil surface) for water stressed conditions, and the "breaking points" under ambient CO_2 appeared 6–9 days earlier than under elevated CO_2. This result suggests that it took longer for the easily available water to become depleted for the elevated CO_2 treatments than for the ambient.

This study does not show evidence that elevated CO_2 treatment has a strong effect on plant height, leaf area, or above ground biomass. No significance differences were observed among the water stressed conditions, either. However, above ground biomass of corn plants for this study was significantly different between the well-watered condition and the water stressed conditions. WUE (above ground biomass per water use since 21 DAE) at the final harvest was consistently higher under the elevated CO_2 conditions than under the ambient CO_2 conditions, and WUE under elevated CO_2 varied with a smaller range than under ambient CO_2. Since no significance in the CO_2 treatment was observed, this higher WUE indicates that less water was used under the elevated CO_2 condition to produce similar biomass as that in the ambient CO_2 treatment. This study suggests that less water will be required under high-CO_2 environment in the future than at present.

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