

Evapotranspiration and water use efficiency in a Chesapeake Bay wetland under carbon dioxide enrichment

JIAHONG LI*, JOHN E. ERICKSON*¹, GARY PERESTA* and BERT G. DRAKE*

*Smithsonian Environmental Research Center, PO Box 28, Edgewater, MD 21037, USA

Abstract

Wetlands evapotranspire more water than other ecosystems, including agricultural, forest and grassland ecosystems. However, the effects of elevated atmospheric carbon dioxide (CO₂) concentration (C_a) on wetland evapotranspiration (ET) are largely unknown. Here, we present data on 12 years of measurements of ET, net ecosystem CO₂ exchange (NEE), and ecosystem water use efficiency (EWUE, i.e. NEE/ET) at 13:00–15:00 hours in July and August for a *Scirpus olneyi* (C3 sedge) community and a *Spartina patens* (C4 grass) community exposed to ambient and elevated (ambient + 340 μmol mol⁻¹) C_a in a Chesapeake Bay wetland. Although a decrease in stomatal conductance at elevated C_a in the *S. olneyi* community was counteracted by an increase in leaf area index (LAI) to some extent, ET was still reduced by 19% on average over 12 years. In the *S. patens* community, LAI was not affected by elevated C_a and the reduction of ET was 34%, larger than in the *S. olneyi* community. For both communities, the relative reduction in ET by elevated C_a was directly proportional to precipitation due to a larger reduction in stomatal conductance in the control plants as precipitation decreased. NEE was stimulated about 36% at elevated C_a in the *S. olneyi* community but was not significantly affected by elevated C_a in *S. patens* community. A negative correlation between salinity and precipitation observed in the field indicated that precipitation affected ET through altered salinity and interacted with growth C_a. This proposed mechanism was supported by a greenhouse study that showed a greater C_a effect on ET in controlled low salinity conditions compared with high salinity. In spite of the differences between the two communities in their responses to elevated C_a, EWUE was increased about 83% by elevated C_a in both the *S. olneyi* and *S. patens* communities. These findings suggest that rising C_a could have significant impacts on the hydrologic cycles of coastal wetlands.

Keywords: Chesapeake Bay wetland, elevated CO₂, evapotranspiration, precipitation, salinity, sea level, stomatal conductance, water use efficiency

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Introduction

The largest flow of material in the biosphere is the movement of water through the hydrologic cycle (Chahine, 1992). About 75% of the annual precipitation of the coterminous United States is evapotranspired (Walter *et al.*, 2004). Evapotranspiration (ET) is thus intricately related to droughts, floods, wild fires, and

other natural disasters. Moreover, since the latent heat flux associated with ET consumes much of the water and energy fluxes that are available on the surface of the earth, it influences both climatology and hydrology at local, regional, and global scales (Karl & Trenberth, 2003; Huntington, 2006). Therefore, understanding the effects of climate change on ET is critical, but has to date received comparatively little attention compared with carbon cycling.

One of the most consistent responses of plants to growth in elevated atmospheric carbon dioxide (CO₂) concentration (C_a) is the lowering of leaf stomatal conductance (g_s). In various reviews, the reduction was

Correspondence: Jiahong Li, tel. +1 321 861 3223, fax +1 321 861 2954, e-mail: lij@si.edu

¹Present address: University of Florida, PO Box 110500, Gainesville, FL 32611, USA.

found to be about 22% at 550 $\mu\text{mol mol}^{-1}$ C_a (Ainsworth & Rogers, 2007) and to be 20–40% at 700 $\mu\text{mol mol}^{-1}$ C_a (Drake *et al.*, 1997; Medlyn *et al.*, 2001). Whether or not the reduction in g_s will cause a reduction in canopy transpiration and ET has been a major question for scientists trying to understand regional responses to rising C_a and climate change. Field experiments have produced a variety of outcomes, ranging from 0% to 23% reduction of ET at elevated C_a (Kimball *et al.*, 1994; Bernacchi *et al.*, 2007; Polley *et al.*, 2007). Although wetlands evapotranspire more water than arable land, forest or grasslands (Bullock & Acreman, 2003), the effects of elevated C_a on wetland ET are largely unknown.

A long-term experiment on the effects of elevated C_a on a brackish tidal wetland ecosystem has been conducted since May 1987 on the Rhode River subestuary of the Chesapeake Bay and includes a *Scirpus olneyi* (C3) community and a *Spartina patens* (C4) community. It is the longest running experiment on natural ecosystem responses to elevated C_a in the world to date. The objectives of this study were: (i) to investigate the effects of elevated C_a on ET from the *S. olneyi* and *S. patens* communities; (ii) to examine the control of ET by leaf area index (LAI), precipitation, salinity, and mean sea level (MSL) at ambient and elevated C_a; and (iii) to determine how elevated C_a altered ecosystem water use efficiency (EWUE) in the *S. olneyi* and *S. patens* communities.

Materials and methods

Experimental site

The site for this study is located on the Rhode River, a subestuary of the Chesapeake Bay (38°51'N, 76°32'W). It is in the high marsh (40–60 cm above mean low water), which is flooded approximately 2% of the time and is representative of most brackish high marshes along Mid-Atlantic North America (Jordan & Correll, 1991). The soil contains approximately 80% organic matter to 4.5 m depth and the bulk density of the surface 30 cm is 0.12 g d.w. cm⁻³ (Marsh *et al.*, 2005). Dominant vegetation in the high marsh includes the C3 sedge *S. olneyi* (A.) Gray [a.k.a. *Schoenoplectus americanus* (Pers.) Volk. Ex Schinz & R. Keller], the C4 grass *S. patens* (Aiton) Muhl., and the C4 grass *Distichlis spicata* (L.) Greene. Within the high marsh, *S. patens* tends to occur at relatively high elevations, *S. olneyi* at relatively low elevations, and *D. spicata* occurs throughout the marsh (Arp *et al.*, 1993; Erickson *et al.*, 2007). A *S. olneyi* community and a *S. patens* community have been exposed to elevated CO₂ concentrations (ambient + 340 $\mu\text{mol mol}^{-1}$) using open-top chambers (0.8 m in dia-

meter, 1 m tall) since 1987 (Drake *et al.*, 1989). In order to distribute CO₂ enriched air uniformly throughout the plant canopy and ensure strong coupling between the plant canopy and the atmosphere, there are two blowers on each chamber: one to introduce air into the chamber, and the second to draw air through a perforated skirt and to recirculate it into the chamber (Drake *et al.*, 1989). Twenty open-top chambers were employed for the two communities, five ambient and five elevated C_a chambers for each community, respectively.

ET, net ecosystem CO₂ exchange (NEE), and EWUE

The open-top chambers were used as gas exchange cuvettes to measure ET and NEE (Arp, 1991; Leadley & Drake, 1993). When the measurements were conducted, a transparent plastic top with a restricted exit tube was placed over the chamber opening to prevent the incursion of external air. ET and NEE were determined by multiplying the flow rate of air through the open-top chambers by the difference in water vapor and CO₂ concentrations between air coming into and leaving the chambers, respectively. The water vapor concentrations were determined using DEW-10 dewpoint hygrometers (General Eastern Instruments Inc., Watertown, MA, USA) from 1989 to 1991 and infrared gas analyzers for 1992–1996 (Binos, Inficon Inc., Hanau, Germany) and 2001–2004 (Li-6262; LI-COR, Lincoln, NE, USA). The CO₂ measurements, gas circuit, and other related information were described in detail by Leadley & Drake (1993). Flow rate of air passing through the chambers was measured using a hot wire anemometer (1440M, Kurz Institute Inc., Monterey, CA, USA). The approach of using chambers for ecosystem gas exchange measurements has been validated by an eddy covariance technique for NEE in a Florida scrub-oak ecosystem (Dore *et al.*, 2003). As reported in the scrub-oak ecosystem (Hungate *et al.*, 2002), evaporation of water condensed on the chamber walls often caused a spike in ET during the morning hours. Therefore, the analysis focused on data collected after the spike had subsided. In addition, we used data with photosynthetic photon flux density (PPFD) over 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which is the saturating PPFD for photosynthesis of *S. olneyi*, in order to reduce the variability in ET and NEE due to variable incident light. Thus, the average ET at 13:00–15:00 hours was chosen in this analysis. This period also had the least variation in air temperature throughout the study. In July and August of 1989–1996 and 2000–2004, ET and NEE were measured in the *S. olneyi* and *S. patens* communities for 1 week every month. ET and NEE data were not available for the *S. patens* community in 1991 and ET data were not available in 1997–2000 for both *S. olneyi* and *S. patens*.

communities due to an instrument malfunction. The average ET measured in July and August were used for each year in order to compare with LAI at the peak of the growing season, which occurred during the time between the last week of July and first week in August (Curtis *et al.*, 1989). EWUE is defined here as the ratio of NEE to ET.

LAI

Each year, at the peak of the growing season, photosynthetically active shoots (green tissue) of *S. olneyi* in each chamber were counted and measured for length and width at 1/2 the shoot length. Leaf area for each shoot was estimated as a product of shoot length and width at 1/2 the shoot length. Because of high shoot densities and small leaves in the *S. patens* community, LAI was determined by shoot biomass and a regression between leaf area (LA, cm²) and shoot biomass (SB, g in dry weight) established from a destructive harvest in 1989 (at ambient C_a: LA = 0.0266 SB + 0.491, R² = 0.85** (p ≤ 0.01); at elevated C_a: LA = 0.0238 SB + 4.752, R² = 0.79**). These relationships held when compared with data from a destructive harvest in 2008. The shoot biomass of the *S. patens* grasses was estimated by subsampling five randomly selected 25 cm² quadrats in each chamber at the same time when the annual survey was conducted in the *S. olneyi* community. No shoot biomass data were available for the *S. patens* community in 1995. Further details regarding the measurements on the LAI and biomass of the *S. olneyi* and *S. patens* communities were reported by Curtis *et al.* (1989).

Salinity, MSL, and meteorological measurements

Salinity of the marsh was measured by refractometry every 2 weeks at the closest point from the chambers where open water was available (about 200 m). As described by Rasse *et al.* (2005), no *a priori* information as to the period of time when salinity of the marsh influences *S. olneyi* and *S. patens* communities is available, so we used the March–August period of each year. March was chosen as a starting point because, for most years, data had not been collected in January and February. August was chosen as an end period because ET and NEE were measured in August of each year. Influence of the salinity of the previous year was ruled out by a multiple correlation study.

MSL data measured as mean water level relative to a NOAA/NOS tidal gauge were obtained from the US Naval Academy in nearby Annapolis, MD (station 8575512). Mean hourly water level data were averaged each year for the months of March to August to arrive at a growing season mean.

PPFD was measured using Eppley PSP radiometers with filter domes for 400 and 715 nm (The Eppley Laboratory Inc., Newport, RI, USA), the difference being PPFD. Precipitation was recorded at the Weather Station of Smithsonian Environmental Research Center, which is <1 km from the site.

Stomatal conductance

Stomatal conductance of *S. olneyi* was measured on August 20–24, 2001 and August 19–25, 2003 *in situ* from 10:00 to 14:00 hours under growth conditions at 1000 μmol m⁻² s⁻¹ PPFD, which is the saturating light for the photosynthesis, using the LI-COR 6400 photosynthesis system (LI-COR). The measurements were done at three ambient and three elevated CO₂ chambers because the rest of chambers were being used for other measurements at that time. In each chamber, five plants were randomly chosen and used for the measurements.

Greenhouse experiment

Dormant rhizomes of *S. olneyi* were collected in early spring (March 2005) from Kirkpatrick marsh, a subestuary of the Chesapeake Bay. Rhizomes were stored on cool damp trays until shoots and roots began to emerge. Following emergence, viable plants were separated from rhizomes and potted into PVC pots (10 cm in diameter and 16 cm in length) containing a 60:40 root-zone mix of peat obtained from the rhizosphere of the marsh and a commercially available peat (Pioneer Peat Inc., Grand Forks, ND, USA). Four shoots were planted in each of 72 pots, and along with 12 plant-free soil evaporation control pots, were randomly assigned to one of six growth chambers at the Smithsonian Environmental Research Center (Edgewater, MD, USA) in early April, 2005. The chambers were 1.5 m wide × 0.9 m deep × 1.5 m high and were covered with a clear polyester film. Chambers were equipped with blowers that drew ambient air through the chambers. Within a chamber, plastic tubing with an inlet port above the canopy drew air samples that were pumped to an Inficon Binos infrared gas analyzer (Oerlikon Corp., Pfäffikon, Switzerland), which measured C_a in the chambers every 15 min throughout the experiment.

A multifactorial treatment structure with a split-plot experimental design was used, whereby the six individual growth chambers served as whole-plots and CO₂ treatments were applied at the whole-plot level. After the germinants were potted, atmospheric CO₂ was elevated to an average of 765 ± 5 μmol mol⁻¹ in three of the chambers by adding pure CO₂ to the ambient air stream, while the other three were ventilated with ambient air (average C_a of 406 ± 3 μmol mol⁻¹). A

shielded copper–constantan thermocouple was used to measure air temperature within the chambers, which averaged (24 h) from 21.5 to 33.6 °C with a mean of 28.3 ± 0.3 °C during the entire study period. Mid-day (1000–1500) PPFD measured by Li-Cor quantum sensors (Li-Cor) averaged between 300 μmol m⁻² s⁻¹ on an extremely overcast day to about 1000 μmol m⁻² s⁻¹ on clear days.

Salinity was manipulated for each of the pots within each chamber to create four salinity treatments with three replications each at the subplot level. The pots containing the plants were placed into larger PVC pots (15 cm in diameter and 17 cm in length) to maintain the water level (subirrigation) and salinity treatments. After the plants were established (approximately 2 weeks), pots were salinized with Instant Ocean (Aquarium Systems, Mentor, OH, USA), a synthetic sea salt mixture, at 2, 6, 12, or 20 ppt (g L⁻¹ Instant Ocean in distilled water) to a water level about 6 cm below the sediment surface. These values encompass the range of salinity levels commonly encountered by *S. olneyi* in the marsh (Broome *et al.*, 1995; Erickson *et al.*, 2007). To maintain salinity concentrations, pots were flushed bi-weekly when solutions were changed and water levels were maintained with distilled water in the interim. Plants were grown for 100 days and harvested at the end of the study.

Following initiation of the salinity treatments water use by each of the pots in each chamber were measured gravimetrically over a fixed time interval. Plant canopy transpiration was estimated by subtracting evaporation from plantless control pots in each chamber. The data were expressed in a basis of the surface area of the pot. ET was negligible at night, so data are presented over the period of daylight hours (sunrise to sunset). Data presented here are from the measurements on June 12, 2005 (around peak biomass).

Analysis of data

Results of ET, NEE, EWUE, LAI (Fig. 1), and stomatal conductance (Fig. 6) were analyzed with SYSTAT 11 software package (Systat Software Inc., CA, USA, 2004). One-way repeated measures analysis of variance (ANOVA) was used for analyzing each species separately, where C_a was considered the between-subjects contrast and the year was the repeated measure (within-subjects contrast). Two-way repeated measures ANOVA was used for analyzing the two species together, where the species and C_a were considered the between-subjects contrasts and the year was the repeated measure (within-subjects contrast). Two-way ANOVA was used to analyze stomatal conductance measured at different C_a and in different years (Fig. 6). Parameters from regressions

in Figs 2–5 were analyzed using dummy variable regression analysis with error terms generated by SIGMAPLOT 8.0 to determine whether the coefficients for different regression lines were significantly different (Powell *et al.*, 2005). Dummy variables are variables that take values of only 0 or 1. In a linear regression model, they are used to define subsets of observations that have different slopes and/or intercepts to test for equality between sets of coefficients (Gujarati, 1970).

For the greenhouse experiment, significant treatment effects and their interactions were analyzed using the mixed-model procedure of the SAS system (Littell *et al.*, 1998). The experimental treatment design was a multi-factorial design with two levels of CO₂ (ambient, elevated) by four levels of salinity (2, 6, 12, and 20 ppt). The experimental design was a split-plot design. CO₂ treatment was randomly assigned to the whole-plot experimental units (chamber) and salinity was randomly assigned at the subplot level to each pot. CO₂ treatment and salinity were treated as fixed effects and chamber within CO₂ treatment was treated as a random effect. Degrees of freedom were determined using the Satterthwaite approximation. All treatment effects were considered significant at *P* < 0.05. No significant chamber effect on presented data was identified in the statistical analyses, so data were pooled across chambers to present treatment means and standard errors in the figure.

Results

ET

Elevated C_a reduced ET by 19% (*P* = 0.007) on average over the 12 years in the *S. olneyi* community (Fig. 1). The reduction varied with the year, ranging from 9% to 31%. In the *S. patens* community, the reduction in ET at elevated C_a was 34% (*P* = 0.013) on average, ranging from 26% to 42%. At ambient C_a, the difference in ET between the *S. olneyi* and *S. patens* communities was not statistically significant (*P* = 0.268). At elevated C_a, ET in the *S. olneyi* community was 45% higher than that in the *S. patens* community (*P* = 0.007).

NEE

Elevated C_a increased NEE by 36% (*P* = 0.005) on average over the 12 years in the *S. olneyi* community (Fig. 1). The stimulation varied with the year, ranging from 9% to 73% (*P* < 0.001). In the *S. patens* community, the stimulation of 13% in NEE on average over the years by elevated C_a was not statistically significant (*P* = 0.428). At ambient C_a, the difference in NEE between the *S. olneyi* and *S. patens* communities was not

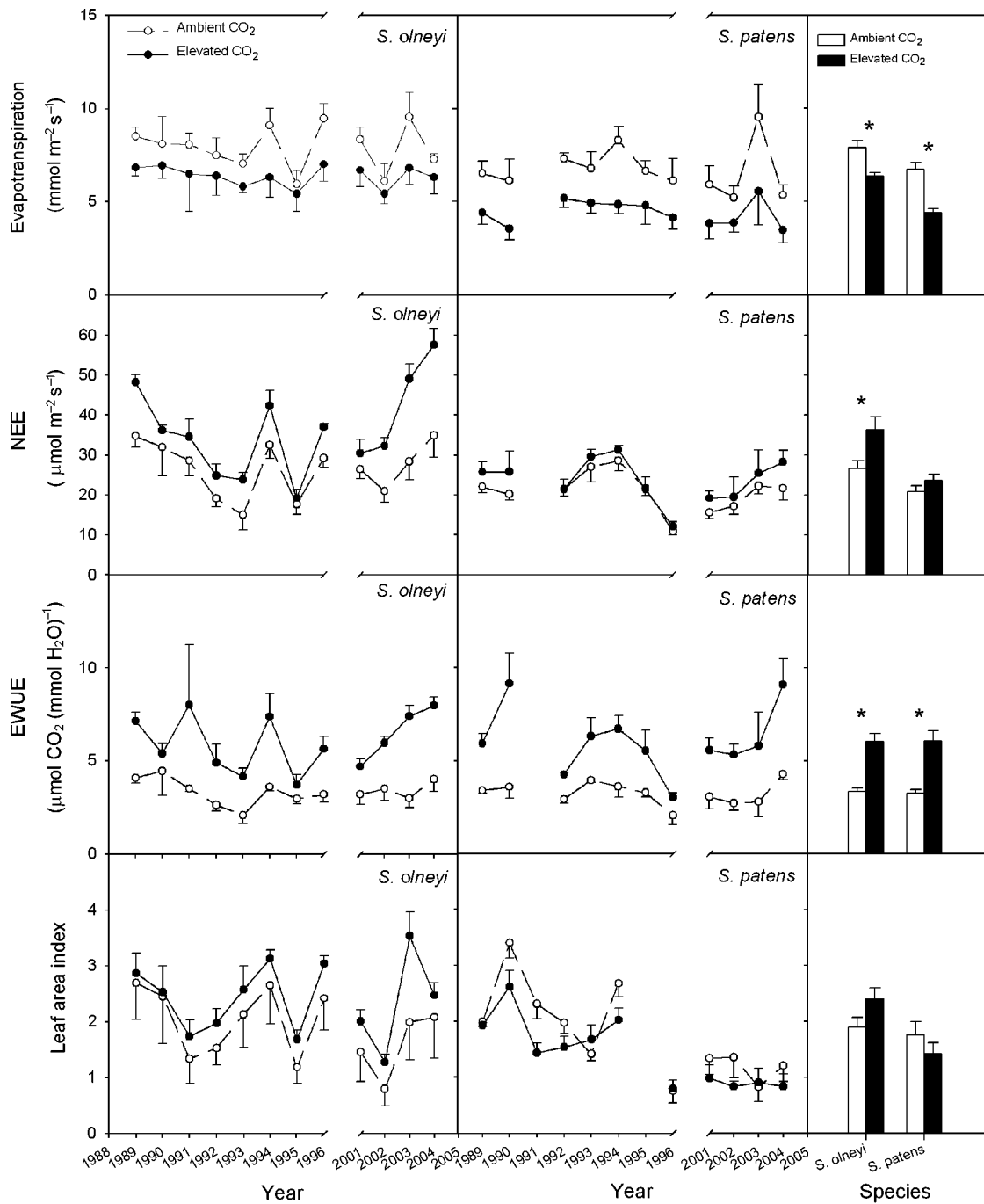


Fig. 1 The eight panels on the left: average evapotranspiration, net ecosystem CO₂ exchange (NEE), and ecosystem water use efficiency (EWUE) at 13:00–15:00 hours and leaf area index measured in July and August in *Scirpus olneyi* and *Spartina patens* communities at ambient (open circles and dashed lines) and elevated (filled circles and solid lines) CO₂. Data are means plus or minus standard errors (*n* = 5). Both the means of evapotranspiration and NEE represent 1–7 sunny days of measurements. The four panels on the right: average evapotranspiration, NEE, EWUE, and leaf area index measured over the years in *S. olneyi* and *S. patens* communities at ambient (open bars) and elevated (filled bars) CO₂. Data are means plus standard errors (*n* = 10–12). *Indicate significant CO₂ effect (*P* ≤ 0.05) between treatments from one-way repeated measures analysis of variance.

statistically significant ($P = 0.105$). At elevated C_a , NEE in the *S. olneyi* community was 55% higher than that in the *S. patens* community ($P = 0.001$).

EWUE

EWUE increased at elevated C_a by 82% ($P < 0.001$) and 84% ($P = 0.001$) on average over the 12 years in the *S. olneyi* and *S. patens* communities, respectively (Fig. 1). The EWUE of *S. olneyi* was not statistically different from that of *S. patens* at ambient ($P = 0.174$) or elevated ($P = 0.473$) C_a . EWUE varied significantly from year to year in the *S. olneyi* community ($P < 0.001$) but not in the *S. patens* community ($P = 0.178$).

LAI, precipitation, salinity, and MSL

On average over the 12 years, *S. olneyi* had 27% higher LAI at elevated C_a compared with ambient C_a , but the difference was not statistically significant ($P = 0.393$) due to the large variation among the chambers (Fig. 1). The LAI varied among the years ($P < 0.001$). The *S. patens* community had 19% lower LAI at elevated C_a than at ambient C_a on average over the years, but the difference was not statistically significant ($P = 0.069$). Similar to *S. olneyi*, *S. patens* had varied LAI from year to year ($P < 0.001$). At ambient C_a , the *S. olneyi* community had a similar LAI with the *S. patens* community on average over the 12 years. At elevated C_a , the 70% higher LAI in the *S. olneyi* community compared with *S. patens* was statistically significant ($P = 0.002$).

Precipitation and salinity varied a great deal with the year but showed no trend (Fig. 2). They were negatively correlated with each other (salinity = -0.0897 precipitation + 13.726, $R^2 = 0.581^{**}$, $n = 12$). MSL increased from 1989 to 2004 at an average rate of 0.35 cm yr^{-1} (Fig. 2, MSL = $0.3463 \text{ year} - 528.12$, $R^2 = 0.361^*$, $n = 12$).

In the *S. olneyi* community, LAI was positively correlated with precipitation (at ambient C_a : LAI = 0.024 precipitation + 0.217, $R^2 = 0.50^*$; at elevated C_a : LAI = 0.031 precipitation + 0.279, $R^2 = 0.67^{**}$) but negatively with salinity (at ambient C_a : LAI = -0.256 salinity + 3.81, $R^2 = 0.78^{**}$; at elevated C_a : LAI = -0.299 salinity + 4.64, $R^2 = 0.88^{**}$) at ambient and elevated C_a (Fig. 3). The slopes of these linear regression lines for precipitation and salinity were not different between the CO₂ treatments (Dummy variable regression analysis, $P = 0.39$ for precipitation and $P = 0.448$ for salinity). In the *S. patens* community, no correlation at the 5% level of significance was found between LAI and precipitation or salinity (Fig. 3). LAI was not correlated with MSL in the *S. olneyi* community at ambient or elevated C_a but was correlated with MSL in the *S. patens* community (at ambient C_a : LAI = -0.200 MSL + 34.33,

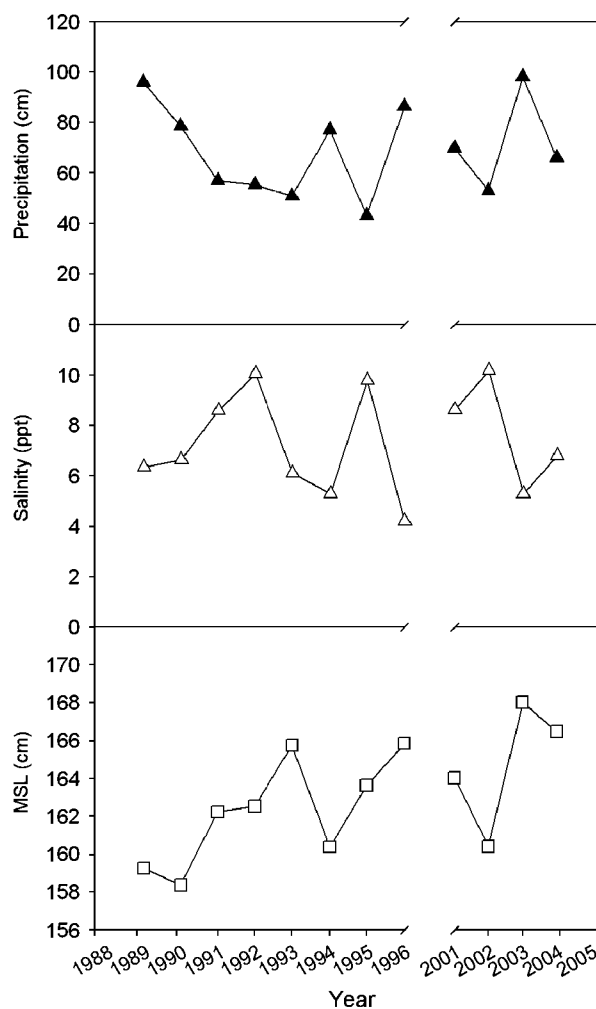


Fig. 2 The accumulated precipitation, mean marsh salinity and mean sea level (MSL) from March to August at the experimental site.

$R^2 = 0.65^{**}$; at elevated C_a : LAI = -0.132 MSL + 22.9, $R^2 = 0.45^{**}$, Fig. 3).

Correlations of ET with LAI, precipitation, salinity, and MSL

ET was linearly correlated with LAI at ambient and elevated C_a in the *S. olneyi* community but not in the *S. patens* community (*S. olneyi* at ambient C_a : ET = 1.28 LAI + 5.50, $R^2 = 0.44^*$; *S. olneyi* at elevated C_a : ET = 0.51 LAI + 5.14, $R^2 = 0.40^*$; *S. patens* at ambient or elevated C_a : no correlation at the 5% level of significance) (Fig. 4). The slopes of the linear regression lines in the *S. olneyi* community were not significantly affected by the CO₂ treatments (Dummy variable regression analysis, CO₂ effect on the slope, $P = 0.116$).

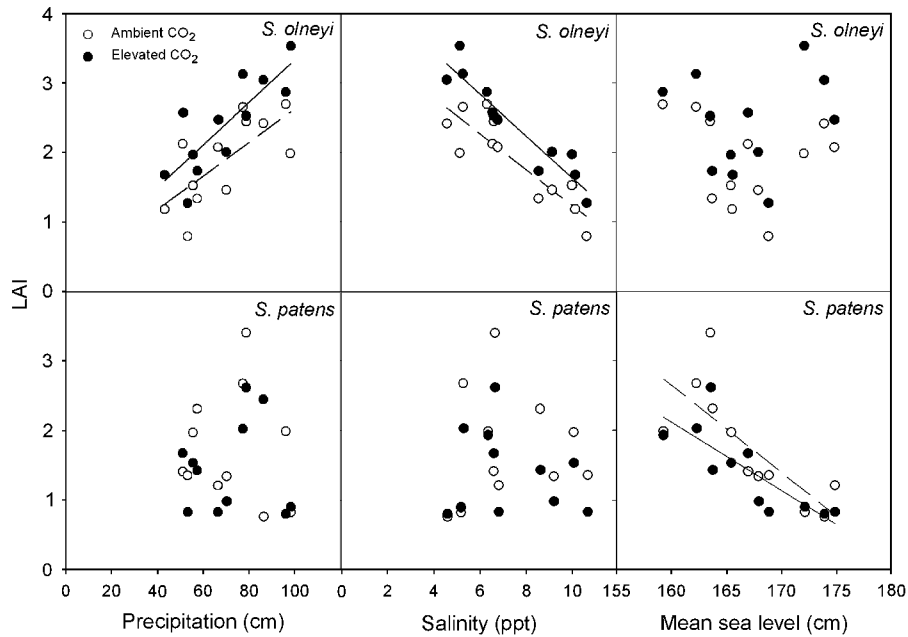


Fig. 3 Correlations of leaf area index (LAI) with precipitation, salinity and mean sea level in *Scirpus olneyi* and *Spartina patens* communities at ambient (open circles) and elevated (filled circles) CO₂. Data are as described in Figs 1 and 2.

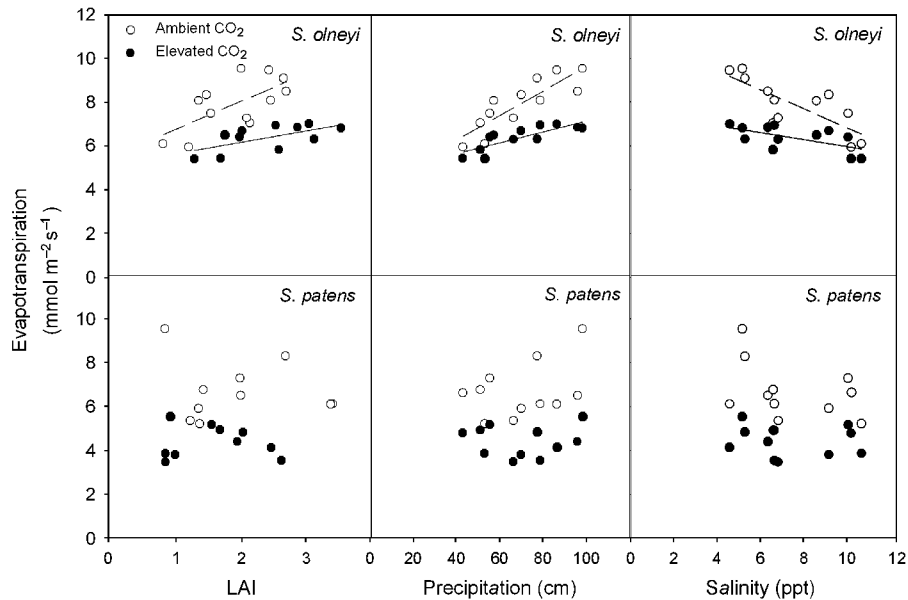


Fig. 4 Correlations of evapotranspiration with leaf area index (LAI), precipitation, and salinity in *Scirpus olneyi* and *Spartina patens* communities at ambient (open circles) and elevated (filled circles) CO₂. Data are as described in Figs 1 and 2.

In the *S. olneyi* community, ET increased with increasing precipitation at both ambient and elevated C_a (Fig. 4). The linear correlation between ET and precipitation was significant at both ambient and elevated C_a (*S. olneyi* at ambient C_a: ET = 0.056 precipitation + 4.01, R² = 0.74**; *S. olneyi* at elevated C_a: ET = 0.025 precipitation + 4.65, R² = 0.66**; *S. patens* at ambient or elevated

C_a: no correlation at the 5% level of significance). The slope of the regression line was 127% larger at ambient than at elevated C_a (Dummy variable regression analysis, P = 0.017). In the *S. patens* community, no correlation at the 5% level of significance between ET and precipitation was found at ambient or elevated C_a. In contrast, rising salinity decreased ET at both ambient and

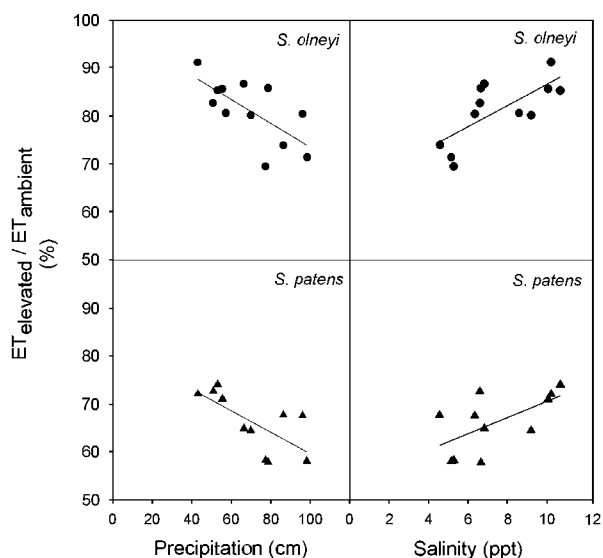


Fig. 5 Correlations of evapotranspiration ratio (elevated/ambient CO₂ treatments) with precipitation and salinity in *Scirpus olneyi* and *Spartina patens* communities. Data are as described in Fig. 1.

elevated C_a in the *S. olneyi* community. ET was negatively correlated with salinity (at ambient C_a : $ET = -0.43\text{salinity} + 11.18$, $R^2 = 0.61^{**}$; at elevated C_a : $ET = -0.17\text{salinity} + 7.67$, $R^2 = 0.40^*$) (Fig. 4). Elevated C_a decreased the slope between ET and salinity by 60% (Dummy variable regression analysis, CO₂ effect on the slope, $P = 0.043$). No correlation at the 5% level of significance between ET and MSL was found at ambient or elevated C_a .

Controls of precipitation and salinity on the CO₂ effects on ET

The reduction of ET at elevated C_a was greater at higher precipitation and lower salinity (Fig. 5). The ratio of ET at elevated C_a to ET at ambient C_a ($ET_{\text{elevated}}/ET_{\text{ambient}}$) was negatively correlated with the total precipitation in both the *S. olneyi* and *S. patens* communities (*S. olneyi*: $ET_{\text{elevated}}/ET_{\text{ambient}} = -0.0025$ precipitation + 0.98, $R^2 = 0.47^*$; *S. patens*: $ET_{\text{elevated}}/ET_{\text{ambient}} = -0.0022$ precipitation + 0.82, $R^2 = 0.47^*$) but positively with salinity (*S. olneyi*: $ET_{\text{elevated}}/ET_{\text{ambient}} = 0.022$ salinity + 0.65, $R^2 = 0.51^*$; *S. patens*: $ET_{\text{elevated}}/ET_{\text{ambient}} = 0.0169$ salinity + 0.54, $R^2 = 0.38^*$).

Stomatal conductance

Precipitation was 70% less in August 2001 than in August 2003 but salinity 64% higher (Fig. 6). Compared with 2003, stomatal conductance in 2001 was reduced

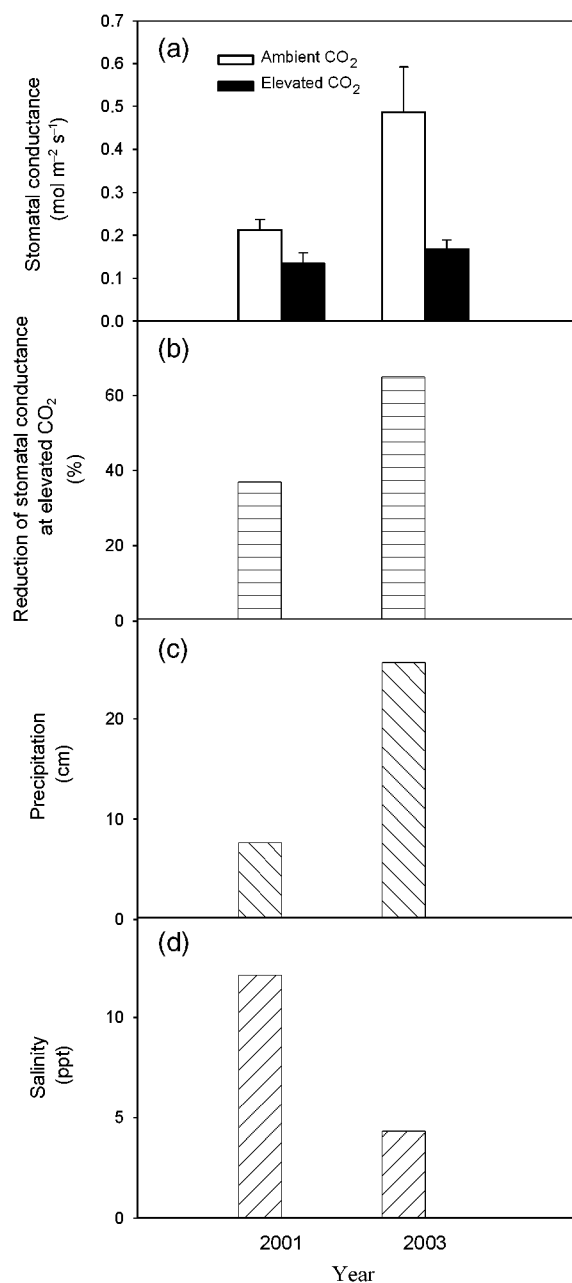


Fig. 6 Stomatal conductance of *Scirpus olneyi* at ambient (open bars) and elevated (filled bars) CO₂, the percentage of reduction in stomatal conductance at elevated CO₂ (partially filled bars with horizontal lines), the total precipitation (partially filled bars with both forward and back slashes), and salinity (partially filled bars with forward slashes) in August 2001 and 2003. Stomatal conductance values are means plus standard errors from ambient and elevated CO₂ ($n = 3$).

by 56% at ambient C_a ($P = 0.033$), but the 20% reduction at elevated C_a was not statistically significant ($P = 0.122$). Elevated C_a reduced stomatal conductance by 37% and 65% in the August of 2001 and 2003,

respectively ($P = 0.008$), indicating a lower CO_2 effect under higher salinity.

CO₂ effect on canopy transpiration under different salinity

In the greenhouse experiment, canopy transpiration decreased with an increase in salinity at both ambient and elevated C_a ($P < 0.001$) (Fig. 7). Elevated C_a reduced the canopy transpiration overall ($P = 0.015$). The extent of reduction depended on salinity, decreasing with increasing salinity as indicated by a significant interactive effect on canopy transpiration between CO_2 and salinity ($P < 0.001$). The ratio of canopy transpiration (CT) at elevated C_a to that at ambient C_a ($\text{CT}_{\text{elevated}}/\text{CT}_{\text{ambient}}$) was correlated with salinity ($\text{CT}_{\text{elevated}}/\text{CT}_{\text{ambient}} = 1.25 \text{ salinity} + 67.95$, $R^2 = 0.93^*$).

Discussion

CO₂ effect on ET

The 19% reduction on average over the 12 years in the *S. olneyi* community is in the range of reported CO_2 effects in other communities (0–23%; Kimball *et al.*, 1994; Bernacchi *et al.*, 2007; Polley *et al.*, 2007). The 34% reduction in the *S. patens* community is larger compared with the reported CO_2 effects in many other

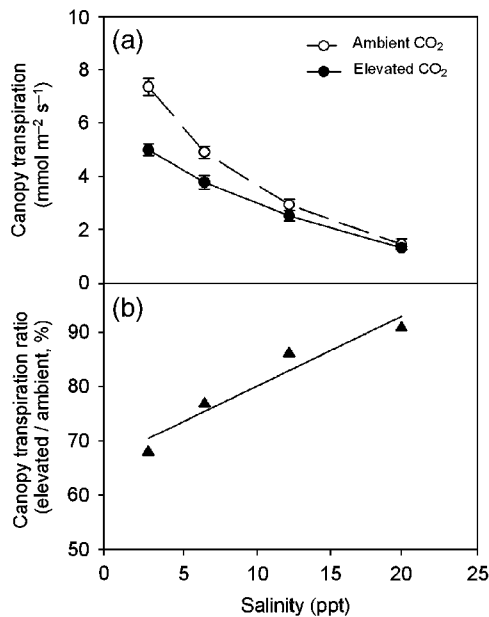


Fig. 7 Responses of *Scirpus olneyi* canopy transpiration to salinity of potted plants grown in ambient (open circles) and elevated (filled circles) CO_2 in the greenhouse. Data are means plus and minus standard errors ($n = 9$).

communities. However, very large reduction of transpiration in *S. patens* has been seen before: it was reduced by 41% when C_a was raised from 340 to 580 $\mu\text{mol mol}^{-1}$ (Rozema *et al.*, 1991). In addition, LAI was not significantly affected by elevated C_a in *S. patens* (Fig. 1), and consequently could not have counteracted the reduction in ET resulting from decreased stomatal conductance.

The reduction of ET in elevated C_a in the wetland communities demonstrated in this study suggests that elevated C_a could have a strong impact on functioning of wetlands through altered ET. For example, reduced ET could increase carbon uptake by mitigating the effects of drought on carbon uptake (Rasse *et al.*, 2005) and could also facilitate ground water recharge to counteract salinity intrusion in coastal areas caused by rising sea level from global warming, a serious problem in the United States (Stevenson *et al.*, 1988; Day *et al.*, 2000). On the other hand, reduced ET may weaken the role of wetlands in reducing floods (Bullock & Acreman, 2003).

Controls of LAI and stomatal conductance on ET

LAI was strongly responsive to salinity in the *S. olneyi* community, but not MSL, whereas LAI in the *S. patens* community was not responsive to salinity but was strongly responsive to sea level (Fig. 3). This interesting finding is consistent with the distribution of these two species in the marsh (i.e. *S. olneyi* at lower and *S. patens* at higher elevation). Previous research has shown *S. olneyi* growth to be more affected by salinity than flooding, due to its well-developed aerenchyma, and *S. patens* growth to be more affected by flooding, given its relatively poor aerenchyma, and less affected by salinity, due to the fact that it contains glands that secrete salt (Broome *et al.*, 1995).

ET was linearly correlated with LAI at ambient and elevated C_a in the *S. olneyi* community, indicating a control of LAI over ET (Fig. 2). On average, the *S. olneyi* community had about 27% more LA at elevated C_a than at ambient C_a (Fig. 1). The CO_2 effect on increasing ET through enhancing LAI was overridden by a decline in stomatal conductance, 37% and 65% in 2001 and 2003 (Fig. 6). The reduction of stomatal conductance in *S. olneyi* was larger than the commonly reported 20–40% at 700 $\mu\text{mol mol}^{-1}$ CO_2 (Morison, 1987; Drake *et al.*, 1997; Medlyn *et al.*, 2001), but reductions up to 60% have been reported in other field studies (Hsiao & Jackson, 1999).

In the *S. patens* community, no correlation between ET and LAI was found and this may be due to several factors. First, LAI decreased over the experimental period, co-occurring with the widely observed marsh dieback in *Spartina* along the eastern seaboard and the

Gulf of Mexico (Alber *et al.*, 2008). Although there is currently no single mechanism that explains this die-back, water logging from rising sea level is considered one of the main factors (Alber *et al.*, 2008; Gedan *et al.*, 2009). This is consistent with the findings that *S. patens* is more sensitive to flooding compared with *S. olneyi* as discussed above (Broome *et al.*, 1995). In addition, the decline in LAI was correlated with rising sea level over the experimental period (Fig. 3), suggesting that rising sea level could be responsible for the decline in LAI. Second, the shoot density in the *S. patens* community was about 10 times higher than that in the *S. olneyi* community (Curtis *et al.*, 1989), resulting in a dense mat at the ground level. Since stem is a major part of the shoots (~ 58% of shoot biomass), the ground was fully covered by shoots even when LAI was below 1. Because of such a dense canopy, leaves within plant canopies are exposed to lower levels of light and consequently have lower stomatal conductance (Li *et al.*, 2003). Finally, a larger LAI may also shade the soil and reduce evaporation from the soil surface. The combination of these factors may cause ET to change very little as LAI increases. For example, for tall grass prairie vegetation, doubling LAI from 1 to 2 increased ET by just 12% at ambient C_a (Polley *et al.*, 2007). This deserves further study.

Since NEE was not affected by elevated C_a in *S. Patens* (Fig. 1), measurements of gas exchange at the leaf level have been primarily focused on *S. olneyi* and not on *S. patens*. Stomatal conductance of *S. patens* has not been measured. It has been generally recognized that stomata in C₄ species and C₃ species have similar responses to elevated C_a (Leakey *et al.*, 2004; Ainsworth & Rogers, 2007).

Controls of precipitation and salinity on the CO₂ effect on ET

The percentage of reduction of ET at elevated C_a was positively correlated with precipitation in both the *S. olneyi* and *S. patens* communities (Fig. 5) suggesting that water savings in wetlands from reduced ET with rising C_a would be larger under wet conditions. In *S. olneyi*, the larger reduction of ET at a higher precipitation was unlikely related to LAI because the slopes in the regressions between LAI and precipitation were the same at ambient and elevated C_a (Fig. 3). Salinity is most likely the causative factor resulting in relatively larger effects on plants exposed to the ambient treatment than on those exposed to elevated C_a treatments (Figs 6 and 7). Unlike many coastal wetland plant communities that are directly influenced by seawater and have little fresh water inflow (e.g. Morris *et al.*, 1990), Chesapeake Bay has 150 tributaries that drain over 16 million ha, covering much of the northeastern United States (White,

1989). This fresh water inflow has a large dilution effect on the salinity environment of plants growing in the marsh at our study site. As a result, the data from the field are consistent with the mechanism that precipitation affected the response of ET to elevated C_a through altering salinity, as salinity was negatively correlated with precipitation at the experimental site. However, the data from the field could not explicitly distinguish the effects of salinity from precipitation because of their correlation. In the greenhouse experiment with salinity as the sole variable, the reduction of canopy transpiration at elevated C_a was much larger at a lower salinity level (Fig. 7). These results confirmed the findings from the field observations and supported the hypothesis that relations among elevated C_a effects on ET and precipitation were mediated through the effects of precipitation on marsh salinity.

A similar mechanism may also apply to *S. patens* according to a laboratory study on the transpiration of *S. patens* under different C_a and salinity levels (Rozema *et al.*, 1991), where transpiration of *S. patens* at 580 μmol mol⁻¹ C_a was reduced by 41% at a salinity of 10 mM NaCl, but had no reduction at a salinity of 250 mM NaCl compared with ambient C_a. Because salinity was negatively correlated with precipitation at the study site, a larger reduction in transpiration in *S. patens* in the field was likely to occur when there was more precipitation.

The increase in intercellular CO₂ associated with elevated C_a commonly results in reduced stomatal conductance due to both stomatal closure (Drake *et al.*, 1997) and decreased stomatal densities over longer time scales (Bettarini *et al.*, 1998). Similarly, reduced stomatal conductance is common in response to salinity (Hussain *et al.*, 2004), a mechanism to decrease plant transpiration and concomitantly reduce ion buildup in plant tissues, which can injure cells and disrupt metabolic processes (Munns, 2002, 2005). In the present study, we observed relatively large reductions in stomatal conductance and transpiration in response to high salinity at ambient C_a or in response to elevated C_a at low salinity, however the effects of these factors (i.e. high salinity and elevated C_a) on stomatal conductance and transpiration were not additive in that the difference in ambient and elevated C_a was not as great at high salinity as it was at low salinity. Thus, the elevated C_a effect on stomatal closure was at least partly obscured by the salinity stress, which we interpreted as reduced stomatal sensitivity to elevated C_a as salinity increased.

CO₂ effect on EWUE

Elevated C_a had the same effect on EWUE for *S. olneyi* and *S. patens* in spite of the significantly different effects

on their NEE and ET (Fig. 1). These results are consistent with the widely held conclusion that plant or ecosystem water and CO₂ exchanges are tightly coupled (Cowan & Farquhar, 1977; Hsiao & Jackson, 1999; Hetherington & Woodward, 2003). In this study, elevated C_a increased NEE in *S. olneyi* but not in *S. patens*. At the same time, elevated C_a induced more water savings in *S. patens* than in *S. olneyi*. These results suggest that elevated C_a could give C3 species a competitive advantage in the carbon cycle; while at the same time could bring an advantage to C4 species in the hydrologic cycle.

Conclusions

Much of our research at the long-term elevated CO₂ experimental site on the Chesapeake Bay wetland as well as that of many other field experiments has focused on the carbon cycle. This study demonstrated that elevated C_a can have a very significant impact on ecosystem hydrologic cycle through reducing ET. As it is known that the hydrology of an ecosystem is determined by many biotic and abiotic factors, the mechanisms underlying the impact of rising atmospheric C_a on ecosystem hydrology are complex as demonstrated in the current study. For the salt marsh studied here, the effect of elevated C_a on ET was determined by species functional group (C3 vs. C4 photosynthetic pathway) as well as environmental conditions (precipitation, salinity and sea level). In the *S. olneyi* community, the decline in ET from decreased stomatal conductance at elevated C_a was counteracted to some extent by an increase in LAI, resulting in an average 19% reduction of ET over the 12 years included in this report. In the *S. patens* community, LAI was not significantly affected by elevated C_a and the average reduction of ET by 34% at elevated C_a was much greater. For both communities, the magnitude of the relative reduction in ET depended on precipitation, increasing with more precipitation due to a larger relative effect of precipitation on stomatal conductance in the control plants. The negative correlation between salinity and precipitation observed in the field and the results from an experiment on canopy transpiration with salinity as the sole variable in a greenhouse indicated that precipitation affects the response of ET to elevated C_a through altered salinity. In spite of the differences between the two communities in responses to elevated C_a, their EWUE had the same response to elevated C_a, increasing about 83% on average. While the findings from this study should be applicable to other coastal salt marsh ecosystems, elevated C_a may have much different effects on ET in nonsaline wetland ecosystems.

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