Initial response of evapotranspiration from tallgrass prairie vegetation to CO_2 at subambient to elevated concentrations

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Summary

1. Effects of CO_2 enrichment on leaf transpiration are well-documented, but our understanding of how CO_2 interacts with other variables to regulate evapotranspiration from plant communities is more limited.

2. A series of weighing lysimeters in which tallgrass prairie species had been planted were exposed to a subambient to elevated gradient in CO_2 in a field chamber. Lysimeters with intact monoliths of three soil types were represented along the CO_2 gradient. We used regression analysis to determine how CO_2 effects on evapotranspiration per unit of soil surface area (ET_{soil}) and per unit of leaf area (ET_{la}) depended on variation in leaf area index (LAI) and diurnal changes in environmental variables during the initial 6 weeks of CO_2 treatment.

3. CO_2 enrichment reduced ET_{soil} and ET_{la}, and together with air temperature and LAI accounted for most of the variance in daily values of evapotranspiration explained by multiple regression models. The CO_2 effect on ET_{soil} did not depend on values of other variables, but CO_2 enrichment reduced ET_{la} most at relatively low air temperatures and low LAI for all soils combined. Higher temperatures countered the CO_2 effect by increasing ET_{la} more at elevated than subambient CO_2. Higher LAI countered the CO_2 effect by decreasing ET_{la} more at subambient than elevated concentrations. Plant (LAI) and environmental effects on ET_{la} differed among soils, possibly because plant growth patterns and physiology differed among soils.

4. Our results imply that the CO_2 effect on evapotranspiration per unit of leaf area will vary with seasonal change in temperature and LAI, independent of seasonal shifts in leaf age and physiological activity.

Key-words: C_3–C_4 grassland, leaf area index, lysimeter, soil type, temperature

Introduction

Atmospheric carbon dioxide (CO_2) concentration has increased by > 35% since Industrialization to the current level near 380 μmol mol^{-1} (Keeling & Whorf 2005) and may reach twice the pre-Industrial concentration by mid-century (IPCC 2001). Increasing CO_2 usually reduces stomatal conductance to water vapour (Morison 1987; Field, Jackson & Mooney 1995). For leaves and isolated plants, partial stomatal closure often leads to slower transpiration rates, an increase in the water use efficiency of CO_2 fixation, and improved leaf and plant water status. Leaf-level impacts of CO_2 enrichment are well-documented, but field studies of CO_2 effects on rates of water loss from plant–soil systems and on ecosystem water

balance are relatively few. At these expanded spatial and temporal scales, daily or seasonal changes in environmental conditions and feedbacks of CO_2 enrichment on soil and atmospheric water content and canopy development may confound projections based on physiological responses of sunlit leaves. No field manipulation of CO_2 fully incorporates canopy and higher-scale regulation of water loss (Field *et al.* 1995). But, these experiments can provide insight into how CO_2 effects on evapotranspiration rates (ET) and ecosystem water balance vary with local environmental conditions and with feedback effects of CO_2 enrichment on soil water content and plant growth.

Viewed simply, ET per unit of soil surface area (ET_{soil}) is the product of leaf area index (LAI) and ET per unit of leaf area (ET_{la}). Leaf area and ET_{la} are regulated by different factors that operate on different temporal scales. The ET_{la} varies with

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environmental variables like temperature, air vapour pressure deficit, and light that fluctuate on diurnal and seasonal time-scales, whereas LAI in relatively undisturbed ecosystems is regulated primarily by factors like water and nitrogen availability that vary over longer time periods. Because LAI and ET_{la} are regulated differently, the two factors often are treated as independent contributors to water loss in CO_2 experiments. But, ET_{la} also may depend on LAI. Water loss becomes less sensitive to an increase in leaf area as LAI approaches values of about 4 (Schulze *et al.* 1994), implying that ET_{la} declines as LAI increases. To the extent greater LAI reduces ET_{la}, it also may lessen the amount by which CO_2 enrichment reduces water loss per unit of leaf area. Wullschleger *et al.* (2002) showed that the CO_2 effect on stomatal conductance varied with canopy position, but to our knowledge no published study has addressed the question of whether LAI affects how water loss responds to CO_2 by changing the CO_2 response of ET_{la}.

We modified a field chamber used to regulate CO_2 along a subambient to elevated gradient (Johnson, Polley & Whitis 2000) in order to facilitate measurements of ET and of water drainage below the rooting zone. With these improvements, CO_2 effects on the water balance of different soil–plant systems can be studied under realistic field conditions. We briefly describe modifications to the CO_2 facility, and then demonstrate the utility of the improved facility for measuring the response of ET to a continuous gradient in CO_2 over subambient to elevated concentrations.

Canopy LAI differed among the experimental units we studied prior to CO_2 exposure, but variation in LAI was relatively evenly distributed along CO_2 gradients. We used this initial variation in leaf area together with day-to-day changes in environmental factors during the period of CO_2 exposure to determine how LAI and environmental factors influence responses of ET_{soil} and ET_{la} to CO_2. We were concerned primarily with the immediate, physiological effect of CO_2 enrichment on ET_{la}, so we restricted our analysis to the 6 weeks after CO_2 treatment was first imposed when indirect effects of CO_2 on water loss as mediated by changes in species composition or soil water content should have been minimal. In order to maximize the range of CO_2 treatments considered, we operated the system with an expanded CO_2 gradient for the first 6 weeks (200–550 µmol mol^{-1}, as compared to the long-term gradient of 250–500 µmol mol^{-1}).

Materials and methods

EXPERIMENTAL FACILITY

We used elongated field chambers designed after the system described by Johnson *et al.* (2000) to expose perennial C_3–C_4 vegetation to a continuous gradient in CO_2 spanning pre-Industrial to elevated concentrations. The CO_2 facility is located in central Texas, USA (31°05′ N, 97°20′ W) and consists of two transparent, tunnel-shaped chambers aligned parallel along a north–south axis. Each chamber is divided into 10 consecutive compartments each 1·2 m wide × 1·5 m tall × 5 m long. Aerial growth of chambered vegetation is enclosed in a transparent polyethylene film which transmits > 90% of incident irradiance with minimal effects on spectral quality. Chambers

depend upon photosynthesis during daylight and upon respiration at night to create CO_2 gradients. Air introduced into chambers during daylight is progressively depleted of CO_2 by photosynthesizing plants as it is moved by blowers toward the air outlet of the system. The direction of air flow is reversed at night, and respiration by the enclosed ecosystem progressively increases the CO_2 concentration of chamber air. Desired CO_2 concentration gradients are maintained by automatically varying the rate of air flow through chambers in response to changes to photosynthetic (daylight) or respiration rates (night). During daylight, pure CO_2 is injected into air introduced into the south end of one chamber (elevated chamber; compartments 1–10) to elevate CO_2 concentration. The CO_2 level is depleted to 370 µmol mol^{-1}. Ambient air is introduced into the south end of the second chamber (subambient chamber; compartments 11–20) to initiate an ambient to subambient gradient in CO_2 (370–200 µmol mol^{-1}). Night-time CO_2 concentrations are regulated at about 170 µmol mol^{-1} above daytime values along each chamber. Ambient air is introduced into the north end of the subambient chamber at night. Pure CO_2 is injected into air blown from the north of the superambient chamber at night to increase the initial CO_2 concentration by 170 µmol mol^{-1}. Air temperature and vapour pressure deficit are regulated near ambient values by cooling and dehumidifying air at 5-m intervals along chambers.

The CO_2 concentration and dew point of air are measured each 20 min at air entry and exit points of each 5-m compartment along chambers. Air temperatures at the southern and northern extremes of each compartment are measured every 15 s with fine-wire (0·5 mm) thermocouples. The temperature, CO_2 concentration, and dew point temperature of air at each sampling location were averaged over each daytime period of CO_2 regulation. Incident photosynthetically active radiation (PAR; 400–700 nm) is measured with silicon photo-diodes mounted atop blower housings.

SOIL MONOLITHS

In order to measure water balance, we installed containerized monoliths of soil vegetated with C_3–C_4 species characteristic of tallgrass prairie along chambers. Each of the ten 5-m long compartments of each chamber was constructed above a steel container that is 1·2 m wide × 1·6 m deep × 5·0 m long and that was buried to 1·5 m depth in soil. Into each of these outer containers we installed four steel boxes (each 1 m wide and long × 1·5 m deep) containing intact soil monoliths.

We installed monoliths of three soil types along chambers. Two of the three soil types are common to the Blackland Prairie region of central Texas, USA and include soils from the Austin series (Udorthentic Haplustolls, a mollisol) and Houston Black series (Udic Haplusterts, a vertisol). The Austin soil contains 35%–40% clay in the upper 40 cm of the profile, underlain by chalks and marls. The Houston Black soil is deeper, and contains 45%–55% clay to > 1·5 m depth. To expand the range of soil types considered, we also included a sandy loam alfisol from the Bastrop series (Udic Paleus-talfs). The Bastrop soil contains 60%–73% sand in the upper 50 cm of the profile and 48% sand from 50 to 150 cm depth. Two mono-liths each of a Houston Black soil and of an Austin series soil were placed into 12 of the total of 20, 5-m long steel containers along chambers, including odd-numbered compartments along each chamber and one additional compartment in each chamber (compartments 2 and 20). Two monoliths of a sandy Bastrop soil were placed into each of the remaining eight (even-numbered) of the 5-m containers. The Bastrop soil in these eight containers was paired with two monoliths of either the Houston soil (four containers) or Austin soil

(four containers). Monoliths of the appropriate soil were randomly assigned to 5-m containers. Positions of monoliths within each 5-m container were randomly assigned.

Hydraulic pressure was used to press open-ended boxes (1×1 m base $\times 1·5$ m height) constructed of 8 mm thick steel into soil. Boxes were placed on the soil surface with an open end facing downward. Steel I-beams, each 4·25 meters long, then were placed parallel to and on opposite sides of the box. The I-beams were secured at each end to 30-cm diameter helical anchors (Atlas Systems, Inc., Rockwall, TX, USA) drilled to 3 m depth in soil. Hydraulic cylinders then were attached at one end to an I-beam and at the other end to a steel 'header' mounted atop boxes. Hydraulic pressure was used to push boxes into soil. Soil adjacent to one side of each buried box then was excavated to a depth of about 1·7 m. A hydraulically powered blade of 6 mm thick steel (1 m long $\times 0·2$ m wide) was used to separate the encased monolith from soil beneath. A custom-built cart was attached to the exposed face of the steel box. The encased monolith then was pushed over onto the cart and removed up the sloped face of the excavation using a tractor.

In order to facilitate drainage from monoliths, we removed 5 cm of soil from the base of each monolith and dispersed filaments separated from the upper 50 cm of a 70-cm long fibreglass wick (3 cm diameter) across the soil surface. Wicks then were covered with soil that had been removed. The intact 20 cm portion of the wick was passed through a hole in the centre of a 1×1 m steel plate that was welded to the base of each steel box and into a reservoir created from 5 cm diameter PVC pipe. The reservoir was attached to the steel box with a 5 cm length of 5 cm diameter steel pipe.

VEGETATION

We used a non-residual herbicide (glyphosate [*N*-(phoshonomethyl)glycine], formulated as Roundup) to kill vegetation present when monoliths were collected, then transplanted perennial forb and grass species characteristic of tallgrass prairie in central Texas, USA into all monoliths. Eight plants of each of five grass species [*Bouteloua curtipendula* (side-oats grama), *Panicum obtusum* (vinemesquite), *Schizachyrium scoparium* (little bluestem), *Sorghastrum nutans* (Indian grass) and *Tridens albescens* (white tridens)] and three forb species [*Salvia azurea* (pitcher sage), *Solidago canadensis* (goldenrod) and the legume *Desmanthus illinoensis* (Illinois bundleflower)] were transplanted into each monolith (total density of 64 plants per m^2) during May 2003. Planting locations of species within each monolith were randomized according to a Latin Square design. Transplants were watered during the initial 2 months to promote establishment, but received only rainfall thereafter. Plants that emerged from the seed bank were removed. The grass *P. obtusum* proved to be highly aggressive. Stolons from plants in a given monolith sometimes rooted in adjacent monoliths, so the grass was removed in 2004 by cutting each plant beneath the crown.

Above-ground production in each monolith was measured each year prior to CO₂ treatment (2003–2005) by clipping vegetation to 5 cm height at the end of the growing season in early December. Plant material removed in December was returned to the monolith from which it was removed in January before the next growing season. By returning harvested material, we sought to accommodate indirect effects of CO₂ on element (i.e. C, N) cycling that may be mediated by differences in the quantity or quality of above-ground production.

In this study, 60 of the 80 monoliths including all monoliths within odd-numbered of the 5-m long compartments along each chamber and all monoliths of the Bastrop soil (located in 8 of 10 even-numbered compartments), were mounted on balances or scales

(Model DSL3636–10, Avery Weigh-Tronix, Farimont, MN, USA; total of 60 monoliths). Remaining monoliths functioned as non-weighing lysimeters. Each balance consists of a steel platform ($0·9 \times 0·9$ m) mounted above 4 load cells and has a capacity of 4500 kg and precision of 450 g.

Each monolith was equipped with a dedicated surface irrigation system, and with a system for collecting and quantifying the volume of water that drained through soil into collection reservoirs located beneath steel boxes encasing monoliths. Drainage water was removed from collection reservoirs using a vacuum pump. Acrylic pipe was installed to a depth of 1·2 m in each monolith for neutron probe measurements of soil water content.

ET MEASUREMENTS

CO₂ control was initiated in June 2006. Mean rates of ET_{soil} and ET_{la} were calculated daily from 7 June through 19 July 2006 for each monolith mounted on a scale using daily changes in scale readings and measurements of LAI. The LAI in each monolith was measured in early June, late June and early August using a SunScan canopy analysis system (Delta-T Devices Ltd., Burwell, Cambridge, UK). LAI on intervening days was calculated assuming a linear relationship between LAI and days between measurement dates. In order to minimize disturbances to plants during ET measurements, we did not destructively measure leaf area but note that above-ground biomass harvested from monoliths at the end of the prior growing season was strongly and linearly correlated with LAI calculated from end-of-season measurements with the SunScan system ($r^2 = 0·68$, $P < 0·0001$, $n = 80$).

In late May prior to initiating CO₂ control, we irrigated each monolith to increase soil water content to near field capacity and to equalize soil water content among monoliths of each soil type. During each of the following 2 months (June and July), we added water equivalent to the historical mean of rainfall for the 2-month period (total of 150 mm; 89 years mean) to each monolith (108 mm and 42 mm in June and July, respectively). Water was added in approximately equal weekly increments.

STATISTICAL ANALYSES

We used stepwise regression (forward selection of variables) to determine effects of LAI and environmental variables (predictor variables) on ET_{soil} and ET_{la} during daylight hours. Mean daytime rates of ET_{soil} and ET_{la} were modelled as a linear function of soil water deficit from field capacity (deficit), LAI, and of daytime means of photosynthetically active radiation (PAR), air temperature (T), air vapour pressure deficit (vpd) and CO₂ concentration, and of all possible two- and three-parameter cross-product interaction terms with predictor variables (interaction regression model; Neter *et al.* 1996). Daytime means of T, vpd and CO₂ concentration for each monolith were calculated assuming each changed linearly with distance between measurement locations at extremes of each 5-m compartment in chambers. Soil water deficit was calculated each day using the difference between the measured weight of each monolith and the weight of each monolith measured prior to the experiment when soil was near field capacity. Prior to analysis, we excluded data from days on which scales malfunctioned and data from days on which monoliths were irrigated or chambers were uncovered for maintenance or vegetation studies. A total of 1174 observations from 50 monoliths (including 19 Austin, 15 Bastrop and 16 Houston monoliths) were analysed.

Table 1. Means and ranges of plant and environmental variables used with CO_2 concentration as independent variables in stepwise regression to model daytime means of ET rates from monoliths with tallgrass prairie vegetation. Values of each variable for each monolith were averaged over each daylight period of CO_2 control. Table entries represent means and ranges of daily averages calculated considering all days of ET measurements and all monoliths or CO_2 treatments ($n = 1174$)

Variable	Mean	Minimum	Maximum
Photosynthetically active radiation ($\mu mol\ m^{-2}\ s^{-1}$)	1213	840	1700
Temperature (°C)	31	21	41
Vapour pressure deficit (kPa)	3·0	0·9	6·7
Soil water deficit (mm)	63	0	188
Leaf area index	1·5	0·4	4·7

Results

ENVIRONMENTAL VARIABLES

The regressions used to model water loss have utility as prognostic tools for CO_2 effects only if means of independent variables did not change systematically with CO_2 concentration and independent variables exhibited similar variability at different CO_2 levels. There was no relationship between means of LAI or environmental variables and CO_2 concentration when monoliths of all soil types were considered (r^2 of linear regressions on CO_2 concentration ranged from 0·058 for temperature to 0·00021 for vpd). Soil water deficit was slightly greater on average in Bastrop than Austin or Houston soils (means across days and CO_2 treatments = 67·3, 60·2 and 60·3 mm, respectively), and LAI was smaller on average in Austin than Bastrop or Houston soils (means across days and CO_2 treatments = 1·1, 1·8 and 1·8, respectively). Variation in other variables was similar among soil types. No water drained through soil into collection reservoirs located beneath monoliths during June and July 2006.

Environmental variables and LAI varied considerably among days and monoliths (CO_2 treatments) during the 6 weeks of ET measurements (Table 1), but most observations fell within a moderate range. Air temperature ranged between 26 °C and 36 °C for 80% of day–CO_2 observations. LAI varied between 0·5 and 3·0 for 94% of observations, and soil water deficit ranged between 0 and 120 mm for 92% of days and CO_2 treatments.

Variation in LAI and in environmental variables during the experiment was relatively evenly distributed across CO_2 treatments, although the primary source of variation differed among variables. Day-to-day change in ET_{la} of each monolith was highly correlated with variation in PAR. At the current CO_2 concentration, for example, ET_{la} increased linearly as the daytime mean of PAR increased (see Supplementary Fig. S1). We assumed that on a given day each monolith was exposed to the same level of PAR. During the period of measurements, therefore, each monolith was exposed to the full range of variation in PAR. Temperature and vpd were positively correlated with PAR ($r = 0.22$ for each correlation). Each monolith thus

Table 2. Results from forward stepwise regression analysis in which daily means of ET rates per unit of soil surface area ($mmol\ m^{-2}\ s^{-1}$) from grassland vegetation grown in three soils were modelled as a linear function of daily values of soil water deficit from field capacity (deficit; mm water), LAI, photosynthetically active radiation (PAR; $\mu mol\ m^{-2}\ s^{-1}$), air temperature (T; °C), air vapour pressure deficit (vpd; kPa), and CO_2 concentration ($\mu mol\ mol^{-1}$) and all possible two-parameter and three-parameter interaction terms involving independent variables ($n = 1174$, $r^2 = 0.39$, $P < 0.0001$)

Variable	Parameter estimate	Sum of squares	F value	P value
Intercept	−5·275	63·8	38·9	< 0·0001
CO_2	-6.61×10^{-3}	359·6	218·9	< 0·0001
T	0·394	356·6	217·1	< 0·0001
$T \times LAI$	−0·160	157·6	95·9	< 0·0001
$PAR \times LAI$	1.49×10^{-3}	153·8	93·6	< 0·0001
deficit	-7.85×10^{-3}	85·1	51·8	< 0·0001
LAI	3·715	80·4	49·0	< 0·0001

was exposed to most of the observed range of variation in T and vpd, although maximum and minimum values of both variables tended to increase with distance along subambient and elevated CO_2 chambers (see Supplementary Fig. S2). By contrast, LAI and soil water deficit varied relatively little at a given CO_2 concentration compared to the variation observed among CO_2 treatments (see Supplementary Fig. S3; average difference between maximum and minimum values at each CO_2 = 0·37 for LAI and 58 mm for deficit).

Consistent CO_2 levels were maintained along gradients despite day-to-day variation in PAR and temperature. Between 75% and 93% of daytime CO_2 readings at sampling locations along the elevated chamber and between 70% and 93% of CO_2 readings along the subambient chamber fell within 25 $\mu mol\ mol^{-1}$ of the mean for the location during the 6 weeks ET was measured. Variability in CO_2 increased slightly with distance from the air entrance of chambers, but did not differ among sampling locations over the 20–35 m of chambers nearest air exits.

ET_{soil}: REGRESSION MODEL FOR ALL SOILS COMBINED

A multiple regression explained 39% of variance in daytime means of ET_{soil} when data for all soils were considered (Table 2). *F*-values for independent variables in the model were greatest for CO_2 concentration and T, indicating that these variables accounted for most of the variance in ET_{soil} explained by regression, although ET_{soil} also depended on LAI. Greater T increased ET_{soil}, but the T effect on water loss varied with changes in LAI. The CO_2 effect on ET_{soil} did not depend on values of other variables. Using mean values of independent variables other than CO_2 in the regression equation, we calculate that CO_2 enrichment from 250 to 550 $\mu mol\ mol^{-1}$ reduced ET_{soil} by 35% (from 5·63 to 3·64 $mmol\ m^{-2}\ s^{-1}$). The LAI effect on water loss depended on values of T and PAR, but on average ET_{soil} increased less than proportionally with greater LAI. For example, doubling LAI from 1 to 2 increased ET_{soil} by just

Table 3. Results from forward stepwise regression analysis in which daily means of ET rates per unit of leaf area (mmol m^{-2} s^{-1}) from grassland vegetation grown in three soils were modelled as a linear function of daily values of soil water deficit from field capacity (deficit; mm water), LAI, photosynthetically active radiation (PAR; μmol m^{-2} s^{-1}), air temperature (T; °C), air vapour pressure deficit (vpd; kPa), and CO_2 concentration (μmol mol^{-1}) and all possible two-parameter and three-parameter interaction terms involving independent variables ($n = 1174$, $r^2 = 0.62$, $P < 0.0001$)

Variable	Parameter estimate	Sum of squares	F value	P value
Intercept	6·206	328·6	274·6	< 0·0001
LAI	−1·409	212·7	177·8	< 0·0001
CO_2	−0·011	210·7	176·1	< 0·0001
PAR × T × CO_2	$2·028 \times 10^{-7}$	170·0	142·1	< 0·0001
deficit × LAI × CO_2	$2·427 \times 10^{-5}$	38·9	32·5	< 0·0001
vpd × deficit × LAI	$−2·740 \times 10^{-3}$	34·6	28·9	< 0·0001
vpd	0·732	34·1	28·5	< 0·0001
vpd × deficit × T	$1·239 \times 10^{-4}$	24·2	20·2	< 0·0001
vpd × T × CO_2	$−3·516 \times 10^{-5}$	17·3	14·5	0·0002
vpd × deficit × CO_2	$−1·048 \times 10^{-5}$	13·1	10·9	0·001

12% (from 4·56 to 5·10 mmol m^{-2} s^{-1}) at 370 μmol mol^{-1} CO_2 when calculated using mean values of independent variables other than LAI in the regression equation. This trend implies ET_{la} declined as LAI increased.

ET_{LA}: REGRESSION MODEL FOR ALL SOILS COMBINED

A multiple regression model explained 62% of the variance in daytime means of ET_{la} when data from all soils were considered (Table 3). Of the six independent variables entered into the stepwise regression, LAI and CO_2 correlated most highly with ET_{la} and accounted for the bulk of variance in ET_{la} explained by regression. A regression model with only LAI and CO_2 concentration explained 51% of the variance in $ET_{la}$. LAI alone explained 41% of variance in ET_{la} in simple regression. On average, ET_{la} declined as both LAI and CO_2 concentration increased and increased with greater vpd, but effects of each of these variables on ET_{la} depended on values of other environmental parameters. Four of the six three-variable interaction terms in the final regression model included CO_2 treatment. Of these terms, the interaction involving PAR and temperature explained the greatest fraction of variation in ET_{la}, followed by the interaction of CO_2 with soil water deficit and LAI.

We used the fitted multiple regression equation and mean values of variables other than temperature and PAR to examine interactive effects of CO_2, temperature and PAR on ET_{la}. CO_2 enrichment reduced ET_{la} more at low than higher temperatures at all values of mean PAR encountered during the 6 weeks of measurements (Fig. 1). For example, enriching CO_2 from 250 to 550 μmol mol^{-1} reduced ET_{la} by 52% (from 4·28 to 2·07 mmol m^{-2} s^{-1}) at 26 °C, but by only 37% (from 4·85 to 3·05 mmol m^{-2} s^{-1}) at 36 °C when calculated at the average value of PAR observed during measurements (1200 μmol m^{-2} s^{-1}). Higher temperatures increased ET_{la} at all CO_2 levels, but the absolute increase in ET_{la} at high temperature was greater at elevated than subambient CO_2. High temperature countered the CO_2 effect more when PAR was high. The absolute and relative amount by which CO_2 enrichment from 250 to 550 μmol mol^{-1} reduced ET_{la} at 36 °C declined from 2·24 mmol m^{-2} s^{-1} (50%) at 1000 μmol m^{-2} s^{-1} to 1·36 mmol m^{-2} s^{-1} (26%) at 1400 μmol m^{-2} s^{-1}. At this high temperature, the amount by which increasing PAR from 1000 to 1400 μmol m^{-2} s^{-1} increased ET_{la} was more than twice as great at elevated CO_2 as subambient CO_2.

The LAI, like temperature, had a pronounced effect on ET_{la} and on the response of water loss to CO_2 treatment (Fig. 2). ET_{la} declined substantially as LAI increased at all CO_2 levels, but the absolute decrease was greater at subambient than elevated concentrations. Increasing LAI from 1 to 2·4 reduced ET_{la} by 2·16 mmol m^{-2} s^{-1} (from 5·34 to 3·18 mmol m^{-2} s^{-1}; by 40%) at 250 μmol mol^{-1} CO_2, but by just 1·54 mmol m^{-2} s^{-1}
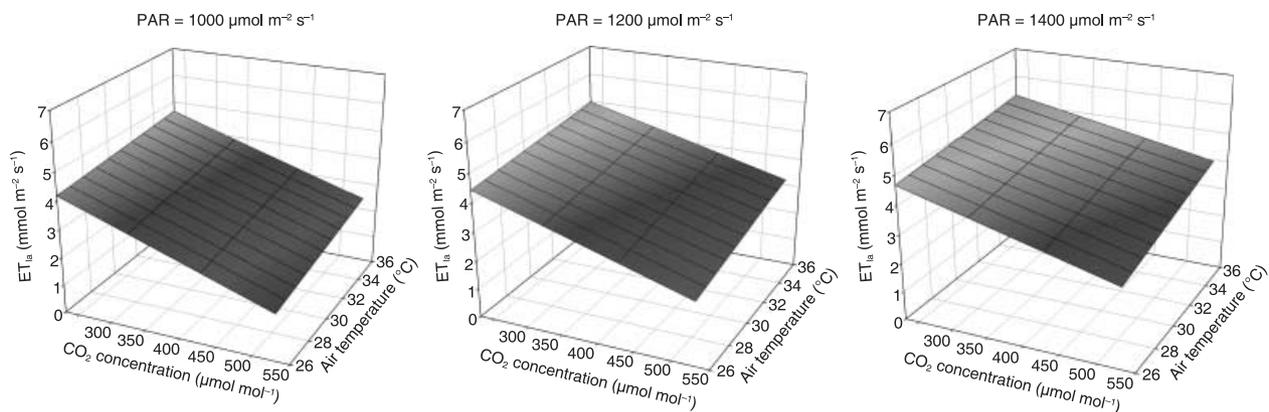
Fig. 1. Response of the daytime mean of evapotranspiration rate per unit of leaf area (ET_{la}) from vegetated monoliths to CO_2 concentration and air temperature at different daytime means of photosynthetically active radiation (PAR). Six independent variables, and all possible two-way and three-way cross-product interaction terms were entered into a stepwise regression analysis to predict ET_{la}. The significant interaction term involving PAR, temperature and CO_2 illustrated here explained the greatest fraction of variation in ET_{la}. Values of ET_{la} were calculated at each of three values of PAR by entering mean values of variables other than temperature and CO_2 concentration (vpd = 3·0 kPa, LAI = 1·5, soil water deficit = 60 mm) into the fitted regression equation.
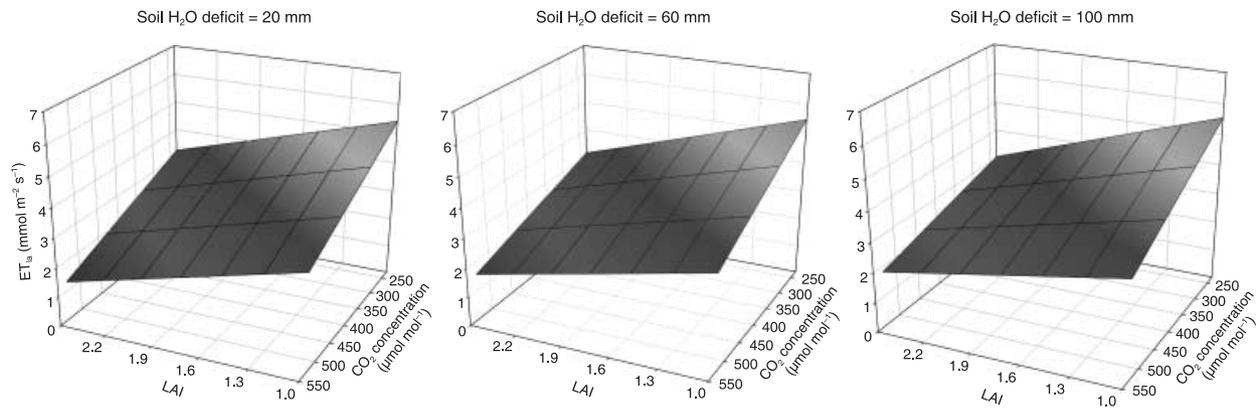
Fig. 2. Response of the daytime mean of evapotranspiration rate per unit of leaf area (ET_{la}) from vegetated monoliths to CO_2 concentration and leaf area index (LAI) at different mean values of soil water deficit. Six independent variables, and all possible two-way and three-way cross-product interaction terms were entered into a stepwise regression analysis to predict ET_{la}. A significant interaction term involving deficit, LAI and CO_2 is illustrated here. Values of ET_{la} were calculated at each of three values of soil water deficit by entering mean values of variables other than LAI and CO_2 concentration (air temperature = 31 °C, vpd = 3·0 kPa, PAR = 1200 µmol m^{-2} s^{-1}) into the fitted regression equation.

(from 3·11 to 1·57 mmol m^{-2} s^{-1}; by 50%) at 550 µmol mol^{-1} CO_2 when calculated at the mean value of soil water deficit observed during measurements (60 mm). CO_2 enrichment reduced ET_{la} at LAI levels from 1 to > 2·4, but the anti-transpiration effect of CO_2 was greatest early in the season when LAI was small. The CO_2 effect on ET_{la} increased slightly as soils dried when LAI was small, but decreased as soils dried when LAI was large.

ET_{LA}: REGRESSION MODELS FOR EACH SOIL

Multiple regression models differed among soils (Table 4). Among the six independent variables considered, LAI correlated mostly highly with ET_{la} in all soils. LAI explained between 37% (Austin and Houston soils) and 48% (Bastrop soil) of variance in ET_{la} in simple regression. The ET_{la} for the Austin soil also was highly correlated with CO_2 concentration in the multiple regression model. In this soil, ET_{la} declined by 0·78 mmol m^{-2} s^{-1} per 100 µmol mol^{-1} increase in CO_2 concentration. The anti-transpiration effect of CO_2 enrichment in other soils and of greater LAI in all soils depended on values of other environmental parameters, however. Interaction terms of CO_2 with air temperature and with LAI and soil water deficit explained large fractions of variation in ET_{la} in Bastrop and Houston soils, respectively.

The significant interaction effect involving CO_2, LAI and water deficit that was observed when ET_{la} from all soils combined was considered (Fig. 2) resulted largely because ET_{la} from Houston monoliths depended strongly on the $CO_2 \times$ LAI $\times$ deficit interaction. One consequence of this interaction is that the CO_2 effect on ET_{la} depended significantly on LAI only in monoliths of Houston soil. For vegetation in this soil, CO_2 enrichment reduced ET_{la} more at low than higher LAI. At LAI > 2, ET_{la} was larger for Houston than other monoliths, especially at elevated CO_2 (Fig. 3).

Air temperature influenced the response of ET_{la} to CO_2 when data from all soils were considered (Fig. 1), but significantly

Table 4. Results from forward stepwise regression analysis in which daily means of ET rates per unit of leaf area (mmol m^{-2} s^{-1}) from grassland vegetation in each of three soils were modelled as a linear function of daily values of soil water deficit from field capacity (deficit; mm water), LAI, photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}), air temperature (T; °C), air vapour pressure deficit (vpd; kPa), and CO_2 concentration (µmol mol^{-1}) and all possible two- and three-parameter interaction terms involving independent variables ($r^2 = 0·58$, $n = 423$ for the Austin soil; $r^2 = 0·72$, $n = 395$ for the Bastrop soil; $r^2 = 0·62$, $n = 356$ for the Houston soil; $P < 0·0001$)

Soil/variable	Parameter estimate	Sum of squares	F value	P value
Austin soil				
Intercept	9·525	1947·6	1472·9	< 0·0001
LAI	−3·848	229·1	173·3	< 0·0001
CO_2	−7·82 × 10^{-3}	195·7	148·0	< 0·0001
PAR × T × LAI	4·96 × 10^{-5}	78·6	59·4	< 0·0001
Bastrop soil				
Intercept	3·285	101·8	116·5	< 0·0001
PAR × LAI	−1·44 × 10^{-3}	636·8	729·3	< 0·0001
PAR × T	1·63 × 10^{-4}	302·9	346·8	< 0·0001
T × CO_2	−3·01 × 10^{-4}	56·0	64·1	< 0·0001
deficit × CO_2	4·75 × 10^{-5}	7·4	8·5	0·004
deficit	−0·013	5·2	6·0	0·015
Houston soil				
Intercept	−1·535	7·9	8·3	0·004
T	0·203	93·9	98·2	< 0·0001
T × LAI	−0·049	63·6	66·6	< 0·0001
PAR	2·69 × 10^{-3}	27·6	28·8	< 0·0001
PAR × CO_2	−3·05 × 10^{-6}	24·4	25·6	< 0·0001
deficit × CO_2	−5·42 × 10^{-5}	23·0	24·1	< 0·0001
deficit × LAI × CO_2	2·0 × 10^{-5}	13·4	14·0	0·0002

affected the ET_{la}–CO_2 relationship only among Bastrop monoliths. For vegetation in this sandy soil, CO_2 enrichment reduced ET_{la} more at high than low air temperature (not shown), opposite the trend that emerged when data from all soils were combined prior to analysis.
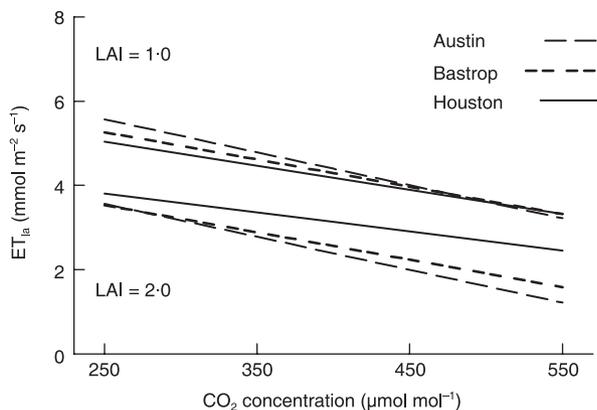
Fig. 3. Response of the daytime mean of evapotranspiration rate per unit of leaf area (ET$_{la}$) from vegetation growing on three soil types (Austin, Bastrop and Houston series) to CO$_2$ concentration at low and high values of leaf area index (LAI). For each soil type, six independent variables and all possible two-way and three-way cross-product interaction terms were entered into a stepwise regression analysis to predict ET$_{la}$. The ET$_{la}$ was calculated at LAI values of 1·0 (upper three lines) and 2·0 (lower three lines) by entering mean values of remaining variables (air temperature = 31 °C, vpd = 3·0 kPa, PAR = 1200 μmol m^{-2} s^{-1}, soil water deficit = 60 mm) into the fitted regression equation.

Discussion

PLANT AND ENVIRONMENTAL EFFECTS ON ET

CO$_2$ enrichment reduced ET$_{soil}$ and ET$_{la}$ of tallgrass prairie vegetation, and together with air temperature and LAI accounted for most of the variance in water loss explained by multiple regression models. The CO$_2$ effect on ET$_{soil}$ did not depend on values of other variables, but the initial response of ET$_{la}$ to CO$_2$ change was highly dependent on plant and environmental variables for the C$_4$-dominated vegetation we studied. Wullschleger & Norby (2001) observed a similar trend for trees. The anti-transpiration effect of CO$_2$ for prairie plants was greatest at relatively low temperatures and low LAI. Higher temperatures reduced the CO$_2$ effect by increasing ET$_{la}$ more at elevated than subambient CO$_2$. Higher LAI reduced the CO$_2$ effect by decreasing ET$_{la}$ more at subambient than elevated concentrations. Canopy ET (ET$_{soil}$) is the product of two variables, LAI and ET$_{la}$. Surprisingly, LAI explained more of the variance in ET$_{la}$ than in ET$_{soil}$, implying that LAI regulated canopy water loss mostly by influencing ET$_{la}$.

Results indicate that water savings at elevated CO$_2$ may be smaller than predicted from the effects of CO$_2$ enrichment alone if air temperature also increases. The decrease in ET$_{la}$ on increasing CO$_2$ from 250 to 550 μmol mol^{-1} declined from 2·0 (44%) to 1·71 mmol m^{-2} s^{-1} (37%) when air temperature at the elevated concentration increased from 31 °C to 34 °C. Both the absolute and relative effects of CO$_2$ on ET$_{la}$ were smallest on warm and bright days when ET$_{la}$ was greatest.

Results also indicate that the absolute effect of CO$_2$ enrichment on ET$_{la}$ is smaller for well-developed grassland canopies than for canopies with lesser LAI. The LAI effect on the

sensitivity of ET$_{la}$ to CO$_2$ entails at least three consequences. First, the relationship of ET$_{la}$ to CO$_2$ may vary seasonally in mesic grasslands, independently of seasonal changes in leaf age and physiological activity. The CO$_2$ effect on ET$_{la}$ should be greater early in the growing season when temperatures are mild and the plant canopy is re-establishing than later in the season when temperatures are warmer and the plant canopy is fully developed. It also is during early season that C fixation and the CO$_2$ effect on net C uptake of C$_4$ grasslands in our area are maximal (Mielnick *et al.* 2001; Polley *et al.* 2007). Second, ET and the response of ET to CO$_2$ depend both directly and indirectly on LAI. Water loss is a positive, direct function of the amount of transpiring surface present, but the water savings effect of CO$_2$ enrichment is a negative function of LAI. Third, an increase in LAI is among the feedbacks resulting from plant exposure to elevated CO$_2$ that may lessen CO$_2$ effects on ET (Field *et al.* 1995). The impact of a given increase in LAI on grassland ET declined as LAI increased, however, implying that the negative feedback of increased LAI on water savings is smaller than sometimes thought.

Canopy-level feedbacks on the sensitivity of ET$_{la}$ to CO$_2$ may partly account for differences in CO$_2$ effects on water fluxes sometimes observed among experiments. Hui *et al.* (2001), for example, observed no effect of CO$_2$ on water flux per unit of leaf area from sunflower plants, especially late in their experiment when canopy LAI exceeded 4. Similarly, CO$_2$ enrichment had little effect on annual estimates of stand-level ET from a stand of sweetgum (*Liquidambar styraciflua*) trees (Wullschleger *et al.* 2002). By contrast, CO$_2$ enrichment reduced ET from calcareous grassland despite greater above-ground biomass at elevated CO$_2$ (Stocker, Leadley & Körner 1997) and reduced ET from tallgrass prairie during a period when CO$_2$ had no effect on above-ground biomass (Ham *et al.* 1995). Results from both of these grassland studies imply that CO$_2$ enrichment reduced ET per unit of leaf tissue. Perhaps, CO$_2$ effects on leaf-level water loss are best expressed on grasslands and other ecosystems where LAI is relatively small.

Plant and environmental effects on ET$_{la}$ differed among soils. As a consequence, patterns of ET$_{la}$ response observed when data from all soils were considered often failed to appear when data were analysed by soil type. For example, CO$_2$ enrichment reduced ET$_{la}$ more at low than high LAI in the full data set, but the same was true for only the Houston soil when data were analysed by soil type. Why CO$_2$ effects on ET$_{la}$ depended more on LAI in this heavy clay soil than in other soils is not evident from our data, but may reflect differences in canopy development and self-shading among leaves or in rates at which physiological activity declined in ageing leaves. In any case, our results indicate that soils may affect ET$_{la}$ response, perhaps by influencing plant growth patterns and physiology.

Conditions that maximize conductance and transpiration rates per unit of leaf area usually also maximize the anti-transpiration effect of CO$_2$ (Wullschleger *et al.* 2002). Conductances and transpiration rates per unit of leaf area typically are high when LAI is small and soil water is plentiful. It was under these conditions that ET$_{la}$ in our study was most

responsive to CO_2. Transpiration rates also typically increase as temperature and PAR levels rise. High values of temperature and PAR promoted more rapid water loss in our experiment, but reduced the responsiveness of ET_{la} to CO_2.

An explanation for the disparate effects of these plant and environmental variables on CO_2 response may lie in the differing mechanisms by which variables like LAI and temperature influence transpiration. LAI and soil water deficit probably affect ET_{la} mainly by influencing leaf and stomatal conductance. Stomatal conductance usually is smaller in lower-canopy than upper-canopy leaves (Wullschleger *et al.* 2002) because leaves within plant canopies are exposed to lower light levels, contain lower nitrogen concentration, and are older and less physiologically active than younger leaves in the upper canopy. As a result, ET_{la} increases less than proportionally with greater LAI. Indeed, the rate of ET from herbaceous stands is relatively insensitive to increases in LAI at high values (Schulze *et al.* 1994). By contrast, air temperature may affect the CO_2 responses of both conductance (Wilson & Bunce 1997; Bunce 2000) and water loss per unit of conductance (Jones, Allen & Jones 1985). Higher temperatures often lead to greater rates of stomatal conductance (Bunce 2000), but may not do so uniformly across CO_2 concentrations. CO_2 enrichment reduced stomatal conductance relatively more when temperature was low than high (Wilson & Bunce 1997), much as CO_2 enrichment in our study reduced ET_{la} more at relatively low than higher temperatures. By contrast, the CO_2 effect on stomatal conductance of wheat and barley was better expressed at low than higher temperatures (Bunce 2000). In addition to effects on conductances, leaf and air temperatures influence transpiration rates per unit of conductance (Jones *et al.* 1985). As air temperature rises, leaf temperature and vapour pressure inside the leaf increase resulting in an increase in the vapour pressure difference between leaves and air, and faster transpiration rates for a given level of conductance. At air temperatures observed in this experiment, the saturation vapour pressure increases by about 6% for each 1 °C increase in temperature. Partial stomatal closure at elevated CO_2 also reduces latent heat flux, leading to a rise in leaf temperature at a given air temperature (Idso *et al.* 1993; Kimball *et al.* 1995). The resulting increase in vapour pressure difference between air and the plant canopy promotes water loss, and partially offsets the anti-transpiration effect of stomatal closure (Polley, Johnson & Derner 2002).

LONGER TERM CONTROLS ON ET_{LA}

Our objective was to determine how plant and environmental variables affected the initial response of ET to CO_2 change. The response of ET_{la} to CO_2 and other variables may change following long-term exposure to different CO_2 concentrations (Dugas *et al.* 2001), although change is not inevitable (Jones 1985; Polley *et al.* 2007). Growth at subambient CO_2 may increase stomatal conductance at a given CO_2 level (stomatal acclimation; Maherali *et al.* 2002), for example, just as growth at elevated CO_2 sometimes reduces the sensitivity of stomatal conductance to change in the leaf-to-air vapour

pressure difference (Wilson & Bunce 1997; Maherali, Johnson & Jackson 2003). These and similar changes in physiology as well as possible feedbacks resulting from CO_2 effects on plant growth (including an increase in LAI and soil water content, and shift in species composition) must be considered to predict impacts of CO_2 enrichment on ecosystem water dynamics. For the C_4-dominated vegetation we studied, however, the initial response of ET_{la} to CO_2 change was highly dependent on plant and environmental variables. The anti-transpiration effect of CO_2 was greatest when temperatures were moderate and LAI was small, implying that the CO_2 effect on ET_{la} will be greatest early in the growing season.

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Supplementary material

The following supplementary material is available for this article:

Fig. S1. Relationship between daytime means of evapotranspiration rate per unit of leaf area (ET_{la}) and photosynthetically active radiation (PAR) for a vegetated monolith enclosed in an elongated chamber and exposed to the current CO_2 concentration (mean = 372 µmol mol^{-1}). The line is a linear regression fit through data ($ET_{la} = -1.670 + 0.004 \times$ PAR, $r^2 = 0.62$, $P < 0.0001$, $n = 31$ days).

Fig. S2. Minimum and maximum values of daytime means of air vapour pressure deficit and air temperature to which grassland vegetation grown in three soils was exposed plotted as a function of CO_2 treatment.

Fig. S3. Mean values of LAI and soil water deficit from field capacity for grassland vegetation grown in three soils plotted as a function of CO_2 treatment. Two extreme values of LAI were excluded to clarify the plot (3·71 and 4·43 at 287 and 487 µmol mol^{-1} CO_2, respectively).

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