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 $\mu$mol 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
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\textsubscript{a} are regulated differently, the two factors often are treated as independent contributors to water loss in CO\textsubscript{2} experiments. But, ET\textsubscript{a} 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\textsubscript{a} declines as LAI increases. To the extent greater LAI reduces ET\textsubscript{a}, it also may lessen the amount by which CO\textsubscript{2} enrichment reduces water loss per unit of leaf area. Wullschleger et al. (2000) showed that the CO\textsubscript{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\textsubscript{2} by changing the CO\textsubscript{2} response of ET\textsubscript{a}.

We modified a field chamber used to regulate CO\textsubscript{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\textsubscript{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\textsubscript{2} facility, and then demonstrate the utility of the improved facility for measuring the response of ET to a continuous gradient in CO\textsubscript{2} over subambient to elevated concentrations.

Canopy LAI differed among the experimental units we studied prior to CO\textsubscript{2} exposure, but variation in LAI was relatively evenly distributed along CO\textsubscript{2} gradients. We used this initial variation in leaf area together with day-to-day changes in environmental factors during the period of CO\textsubscript{2} exposure to determine how LAI and environmental factors influence responses of ET\textsubscript{soil} and ET\textsubscript{a} to CO\textsubscript{2}. We were concerned primarily with the immediate, physiological effect of CO\textsubscript{2} enrichment on ET\textsubscript{a}, so we restricted our analysis to the 6 weeks after CO\textsubscript{2} treatment was first imposed when indirect effects of CO\textsubscript{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\textsubscript{2} treatments considered, we operated the system with an expanded CO\textsubscript{2} gradient for the first 6 weeks (200–550 µmol mol\textsuperscript{-1}, as compared to the long-term gradient of 250–500 µmol mol\textsuperscript{-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\textsubscript{4} vegetation to a continuous gradient in CO\textsubscript{2} spanning pre-Industrial to elevated concentrations. The CO\textsubscript{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\textsubscript{2} gradients. Air introduced into chambers during daylight is progressively depleted of CO\textsubscript{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\textsubscript{2} concentration of chamber air. Desired CO\textsubscript{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\textsubscript{2} is injected into air introduced into the south end of one chamber (elevated chamber; compartments 1–10) to elevate CO\textsubscript{2} concentration. The CO\textsubscript{2} level is depleted to 370 µmol mol\textsuperscript{-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\textsubscript{2} (370–200 µmol mol\textsuperscript{-1}). Night-time CO\textsubscript{2} concentrations are regulated at about 170 µmol mol\textsuperscript{-1} above daytime values along each chamber. Ambient air is introduced into the north end of the subambient chamber at night to increase the initial CO\textsubscript{2} concentration by 170 µmol mol\textsuperscript{-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\textsubscript{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\textsubscript{2} concentration, and dew point temperature of air at each sampling location were averaged over each daytime period of CO\textsubscript{2} regulation. Incident photosynthetically active radiation (PAR; 400–700 nm) is measured with silicon photodiodes mounted atop blower housings.

SOIL MONOLITHS

In order to measure water balance, we installed containerized monoliths of soil vegetated with C\textsubscript{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 Paleustolls). 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 monoliths 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.
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 × 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 × 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-(phosphonomethyl)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 albensis (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 m²) 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 occasional watering 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 × 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.
Result

ENVIRONMENTAL VARIABLES

The regressions used to model water loss have utility as prognostic tools for CO₂ effects only if means of independent variables did not change systematically with CO₂ concentration and independent variables exhibited similar variability at different CO₂ levels. There was no relationship between means of LAI or environmental variables and CO₂ concentration when monoliths of all soil types were considered ($r^2$ of linear regressions on CO₂ 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 CO₂ treatments), and LAI was smaller on average in Austin than Bastrop or Houston soils (means across days and CO₂ 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 CO₂ 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 (CO₂ 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–CO₂ 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 CO₂ treatments.

Variation in LAI and in environmental variables during the experiment was relatively evenly distributed across CO₂ treatments, although the primary source of variation differed among variables. Day-to-day change in $\varepsilon_{\text{soil}}$ of each monolith was highly correlated with variation in PAR. At the current CO₂ concentration, for example, $\varepsilon_{\text{soil}}$ 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 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 CO₂ chambers (see Supplementary Fig. S2). By contrast, LAI and soil water deficit varied relatively little at a given CO₂ concentration compared to the variation observed among CO₂ treatments (see Supplementary Fig. S3; average difference between maximum and minimum values at each CO₂ = 0·37 for LAI and 58 mm for deficit).

Consistent CO₂ levels were maintained along gradients despite day-to-day variation in PAR and temperature. Between 75% and 93% of daytime CO₂ readings at sampling locations along the elevated chamber and between 70% and 93% of CO₂ readings along the subambient chamber fell within 25 µmol mol⁻¹ of the mean for the location during the 6 weeks ET was measured. Variability in CO₂ 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.

$\varepsilon_{\text{soil}}$: REGRESSION MODEL FOR ALL SOILS COMBINED

A multiple regression explained 39% of variance in daytime means of $\varepsilon_{\text{soil}}$ when data for all soils were considered (Table 2). $F$-values for independent variables in the model were greatest for CO₂ concentration and T, indicating that these variables accounted for most of the variance in $\varepsilon_{\text{soil}}$ explained by regression, although $\varepsilon_{\text{soil}}$ also depended on LAI. Greater T increased $\varepsilon_{\text{soil}}$, but the T effect on water loss varied with changes in LAI. The CO₂ effect on $\varepsilon_{\text{soil}}$ did not depend on values of other variables. Using mean values of independent variables other than CO₂ in the regression equation, we calculate that CO₂ enrichment from 250 to 550 µmol mol⁻¹ reduced $\varepsilon_{\text{soil}}$ 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 $\varepsilon_{\text{soil}}$ increased less than proportionally with greater LAI. For example, doubling LAI from 1 to 2 increased $\varepsilon_{\text{soil}}$ by just

<table>
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<th>Parameter estimate</th>
<th>Sum of squares</th>
<th>$F$ value</th>
<th>$P$ value</th>
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<tr>
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<td>&lt;0·0001</td>
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<td>CO₂</td>
<td>−6·61 × 10⁻³</td>
<td>359·6</td>
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<td>T</td>
<td>0·394</td>
<td>356·6</td>
<td>217·1</td>
<td>&lt;0·0001</td>
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<tr>
<td>T × LAI</td>
<td>−0·160</td>
<td>157·6</td>
<td>95·9</td>
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<tr>
<td>PAR × LAI</td>
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<td>153·8</td>
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<td>Soil water deficit (mm)</td>
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<td>3·715</td>
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Table 1. Means and ranges of plant and environmental variables used with CO₂ 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 CO₂ control. Table entries represent means and ranges of daily averages calculated considering all days of ET measurements and all monoliths or CO₂ treatments ($n = 1174$)

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<th>Variable</th>
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<td>Temperature (°C)</td>
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<td>41</td>
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<td>Vapour pressure deficit (kPa)</td>
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<tr>
<td>Soil water deficit (mm)</td>
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<td>188</td>
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<tr>
<td>Leaf area index</td>
<td>1·5</td>
<td>0·4</td>
<td>4·7</td>
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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; µmol m⁻² s⁻¹), air temperature (T; °C), air vapour pressure deficit (vpd; kPa), and CO₂ concentration (µmol mol⁻¹) and all possible two-parameter and three-parameter interaction terms involving independent variables ($n = 1174, r^2 = 0·39, P < 0·0001$)
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)

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<td>PAR (\times) T (\times) CO(_2)</td>
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<td>deficit (\times) LAI (\times) CO(_2)</td>
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<td>34·6</td>
<td>28·9</td>
<td>&lt; 0·0001</td>
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<td>vpd (\times) deficit (\times) LAI</td>
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<td>34·1</td>
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<td>&lt; 0·0001</td>
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ET\(_{ia}\): REGRESSION MODEL FOR ALL SOILS COMBINED

A multiple regression model explained 62% of the variance in daytime means of ET\(_{ia}\) 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\(_{ia}\) and accounted for the bulk of variance in ET\(_{ia}\) explained by regression. A regression model with only LAI and CO\(_2\) concentration explained 51% of the variance in ET\(_{ia}\). LAI alone explained 41% of variance in ET\(_{ia}\) in simple regression. On average, ET\(_{ia}\) declined as both LAI and CO\(_2\) concentration increased and increased with greater vpd, but effects of each of these variables on ET\(_{ia}\) 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\(_{ia}\), 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\(_{ia}\). CO\(_2\) enrichment reduced ET\(_{ia}\) 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\(_{ia}\) 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\(_{ia}\) at all CO\(_2\) levels, but the absolute increase in ET\(_{ia}\) 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\(_{ia}\) 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\(_{ia}\) was more than twice as great at elevated CO\(_2\) as subambient CO\(_2\).

The LAI, like temperature, had a pronounced effect on ET\(_{ia}\) and on the response of water loss to CO\(_2\) treatment (Fig. 2). ET\(_{ia}\) 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\(_{ia}\) 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\(^{-1}\) at 550 µmol mol\(^{-1}\) CO\(_2\).
especially at elevated CO$_2$ (Fig. 3).

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 = $31 \, ^\circ C$, vpd = $3.0 \, kPa$, PAR = $1200 \, \mumol \, m^{-2} \, s^{-1}$ into the fitted regression equation.

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 $\mumol \, mol^{-1}$ 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 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. 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 = $31 \, ^\circ C$, vpd = $3.0 \, kPa$, PAR = $1200 \, \mumol \, m^{-2} \, s^{-1}$ into the fitted regression equation.](Image 76x592 to 210x720)

![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; $\mumol \, m^{-2} \, s^{-1}$), air temperature (T; $^\circ C$), air vapour pressure deficit (vpd; kPa), and CO$_2$ concentration ($\mumol \, mol^{-1}$) 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)](Image 228x593 to 363x720)
PLANT AND ENVIRONMENTAL EFFECTS ON ET

CO₂ enrichment reduced ETₐ and ET₀ 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 CO₂ effect on ET₀ did not depend on values of other variables, but the initial response of ET₀ to CO₂ change was highly dependent on plant and environmental variables for the C₄-dominated vegetation we studied. Wullschleger & Norby (2001) observed a similar trend for trees. The anti-transpiration effect of CO₂ for prairie plants was greatest at relatively low temperatures and low LAI. Higher temperatures reduced the CO₂ effect by increasing ET₀ more at elevated than subambient CO₂. Higher LAI reduced the CO₂ effect by decreasing ET₀ more at subambient than elevated concentrations. Canopy ET (ETₐ) is the product of two variables, LAI and ET₀. Surprisingly, LAI explained more of the variance in ET₀ than in ETₐ, implying that LAI regulated canopy water loss mostly by influencing ET₀.

Results indicate that water savings at elevated CO₂ may be smaller than predicted from the effects of CO₂ enrichment alone if air temperature also increases. The decrease in ET₀ on increasing CO₂ 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 CO₂ on ET₀ were smallest on warm and bright days when ET₀ was greatest.

Results also indicate that the absolute effect of CO₂ enrichment on ET₀ is smaller for well-developed grassland canopies than for canopies with lesser LAI. The LAI effect on the sensitivity of ET₀ to CO₂ entails at least three consequences. First, the relationship of ET₀ to CO₂ may vary seasonally in mesic grasslands, independently of seasonal changes in leaf age and physiological activity. The CO₂ effect on ET₀ 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 CO₂ effect on net C uptake of C₄ grasslands in our area are maximal (Mielnick et al. 2001; Polley et al. 2007). Second, ET and the response of ET to CO₂ 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 CO₂ enrichment is a negative function of LAI. Third, an increase in LAI is among the feedbacks resulting from plant exposure to elevated CO₂ that may lessen CO₂ 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 ET₀ to CO₂ may partly account for differences in CO₂ effects on water fluxes sometimes observed among experiments. Hui et al. (2001), for example, observed no effect of CO₂ on water flux per unit of leaf area from sunflower plants, especially late in their experiment when canopy LAI exceeded 4. Similarly, CO₂ 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, CO₂ enrichment reduced ET from calcareous grassland despite greater above-ground biomass at elevated CO₂ (Stocker, Leadley & Körner 1997) and reduced ET from tallgrass prairie during a period when CO₂ had no effect on above-ground biomass (Ham et al. 1995). Results from both of these grassland studies imply that CO₂ enrichment reduced ET per unit of leaf tissue. Perhaps, CO₂ effects on leaf-level water loss are best expressed on grasslands and other ecosystems where LAI is relatively small.

Plant and environmental effects on ET₀ differed among soils. As a consequence, patterns of ET₀ response observed when data from all soils were considered often failed to appear when data were analysed by soil type. For example, CO₂ enrichment reduced ET₀ 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 CO₂ effects on ET₀ 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 ET₀ 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 CO₂ (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 ET₀ in our study was most
responsive to CO₂. 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 ET₀ to CO₂.

An explanation for the disparate effects of these plant and environmental variables on CO₂ response may lie in the differing mechanisms by which variables like LAI and temperature influence transpiration. LAI and soil water deficit probably affect ET₀, mainly by influencing leaf and stomatal conductance. Stomatal conductance usually is smaller in lower-canopy leaves 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, ET₀ 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 CO₂ 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 CO₂ concentrations. CO₂ enrichment reduced stomatal conductance relatively more when temperature was low than high (Wilson & Bunce 1997), much as CO₂ enrichment in our study reduced ET₀ more at relatively low than higher temperatures. By contrast, the CO₂ 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 CO₂ 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 ET₀A

Our objective was to determine how plant and environmental variables affected the initial response of ET to CO₂ change. The response of ET₀ to CO₂ and other variables may change following long-term exposure to different CO₂ concentrations (Dugas et al. 2001), although change is not inevitable (Jones et al. 1985; Polley et al. 2007). Growth at subambient CO₂ may increase stomatal conductance at a given CO₂ level (stomatal acclimation; Maherali et al. 2002), for example, just as growth at elevated CO₂ 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 CO₂ 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 CO₂ enrichment on ecosystem water dynamics. For the C₃-dominated vegetation we studied, however, the initial response of ET₀ to CO₂ change was highly dependent on plant and environmental variables. The anti-transpiration effect of CO₂ was greatest when temperatures were moderate and LAI was small, implying that the CO₂ effect on ET₀ will be greatest early in the growing season.

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References


Response of evapotranspiration to CO2


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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 (ETla) and photosynthetically active radiation (PAR) for a vegetated monolith enclosed in an elongated chamber and exposed to the current CO2 concentration (mean = 372 µmol mol\(^{-1}\)). The line is a linear regression fit through data (ETla\(_{\text{a}}\) = \(-1.670 + 0.004 \times \text{PAR}\), \(r^2 = 0.62\), \(P < 0.0001\), \(n = 31\) 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 CO2 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 CO2 treatment. Two extreme values of LAI were excluded to clarify the plot (3.71 and 4.43 at 287 and 487 µmol mol\(^{-1}\) CO2, respectively).

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