C₃–C₄ composition and prior carbon dioxide treatment regulate the response of grassland carbon and water fluxes to carbon dioxide

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Summary

1. Plants usually respond to carbon dioxide (CO₂) enrichment by increasing photosynthesis and reducing transpiration, but these initial responses to CO₂ may not be sustained.

2. During May, July and October 2000, we measured the effects of temporarily increasing or decreasing CO₂ concentration by 150–200 µmol mol⁻¹ on daytime net ecosystem CO₂ exchange (NEE) and water flux (evapotranspiration, ET) of C₃–C₄ grassland in central Texas, USA that had been exposed for three growing seasons to a CO₂ gradient from 200 to 560 µmol mol⁻¹. Grassland grown at subambient CO₂ (< 365 µmol mol⁻¹) was exposed for 2 days to an elevated CO₂ gradient (> 365 µmol mol⁻¹). Grassland grown at elevated CO₂ was exposed for 2 days to a subambient gradient. Our objective was to determine whether growth CO₂ affected the amount by which grassland NEE and ET responded to CO₂ switching (sensitivity to CO₂).

3. The NEE per unit of leaf area was greater (16–20%) and ET was smaller (9–20%), on average, at the higher CO₂ concentration during CO₂ switching in May and July. The amount by which NEE increased at the higher CO₂ level was smaller at elevated than subambient growth concentrations on both dates, but relationships between NEE response and growth CO₂ were weak. Conversely, the effect of temporary CO₂ change on ET did not depend on growth CO₂.

4. The ratio of NEE at high CO₂ to NEE at low CO₂ during CO₂ change in July increased from 1·0 to 1·26 as the contribution of C₃ cover to total cover increased from 26% to 96%. Conversely, in May, temporary CO₂ enrichment reduced ET more in C₄-dominated grassland.

5. For this mesic grassland, sensitivity of NEE and ET to brief change in CO₂ depended as much on the C₃–C₄ composition of vegetation as on physiological adjustments related to prior CO₂ exposure.

Key-words: acclimation, CO₂ enrichment, net ecosystem CO₂ exchange, subambient CO₂

Introduction

Plants in most ecosystems respond initially to an increase in atmospheric CO₂ concentration with faster rates of photosynthesis and growth and a decrease in stomatal conductance (Drake, González-Meler & Long 1997; Long et al. 2004). However, initial responses to CO₂ enrichment may not be sustained, complicating the task of predicting CO₂ effects on carbon (C) and water exchange. Nutrient limitations and increased carbohydrate levels in plant leaves (Long et al. 2004) are among the factors that can induce downward regulation of canopy-level photosynthesis (Baker et al. 1990; Oechel et al. 1994; Drake et al. 1996) and transpiration at elevated CO₂ (Dugas et al. 2001). Conversely, an increase in leaf area or a shift in plant composition to more responsive species may reinforce or sustain stimulatory effects of CO₂ enrichment.

Photosynthesis at a given CO₂ concentration is often lower for plants grown at elevated than ambient CO₂ concentrations (acclimation or downregulation;
Baker et al. 1990; Oechel et al. 1994; Drake et al. 1996). When expressed per unit of above-ground biomass, for example, net ecosystem exchange of CO$_2$ (NEE) was reduced by 50% in a calcareous C$_4$ grassland exposed for 2 years to elevated CO$_2$ (Stocker, Leadley & Körner 1997).

The response of stomatal conductance (Polley, Johnson & Mayeux 1997) and of plant transpiration rates (Dugas et al. 2001) to CO$_2$ also may depend on the CO$_2$ concentration at which plants were grown. When measured at a given CO$_2$ concentration, transpiration per unit of leaf area of Acacia farnesiana plants was more than halved by growth at 980 compared with 385 µmol mol$^{-1}$ (Dugas et al. 2001).

However, CO$_2$ enrichment need not alter plant sensitivity to CO$_2$. CO$_2$ enrichment had no effect on photosynthetic potential of field-grown soybean (Campbell, Allen & Bowes 1990), wheat (Kimball et al. 1995), or rice (Baker et al. 1997). Similarly, CO$_2$ stimulation of NEE persisted for 6 years in a scrub-oak ecosystem (Hymus et al. 2003) and from years 3 to 17 following enrichment in a salt marsh (Rasse, Peresta & Drake 2005).

We measured the effects of temporarily altering the CO$_2$ concentration by 150–200 µmol mol$^{-1}$ on the daytime net ecosystem CO$_2$ exchange (NEE) and evapotranspiration (ET) of C$_3$–C$_4$ grassland exposed for more than three growing seasons to a continuous gradient in CO$_2$ from subambient to elevated concentrations (200–560 µmol mol$^{-1}$). CO$_2$ enrichment substantially increased biomass production and net C uptake of this grassland (Mielenick et al. 2001; Polley, Johnson & Derner 2003), but also altered the sensitivity of leaf C and water fluxes to CO$_2$. Leaf photosynthesis of the dominant C$_4$ grass and C$_3$ forb (Anderson et al. 2001) and stomatal conductance of the dominant forb displayed up-regulation in plants grown at subambient compared with ambient CO$_2$ (Maherali et al. 2002). These changes should diminish the sensitivity of NEE and ET in CO$_2$-enriched grassland to short-term variation in CO$_2$. However, biomass of C$_4$ species increased at the expense of C$_3$ grass biomass, irrespective of CO$_2$ treatment (Mielenick et al. 2001; Polley et al. 2003).

This shift towards physiologically more-responsive C$_3$ than C$_4$ plants (Anderson et al. 2001; Maherali et al. 2002) would be expected to sustain grassland response to CO$_2$ enrichment. Our objective in temporarily altering CO$_2$ was to determine whether a multyear CO$_2$ treatment affected the amount by which grassland NEE and ET responded to CO$_2$ change (sensitivity to CO$_2$). Consistent with previous results, we predicted that sensitivity of NEE and ET to CO$_2$ would depend more on the relative cover of C$_3$ plants than on the CO$_2$ concentration at which the grassland had been maintained for 3 years. We know of no previous study in which responses of NEE and ET to short-term change in CO$_2$ concentrations have been examined in an intact ecosystem exposed for several years to both subambient and elevated CO$_2$ concentrations.

Materials and methods

**EXPERIMENTAL FACILITY**

We used elongated field chambers to expose a C$_3$–C$_4$ grassland in central Texas, USA (31°05’ N, 97°20’ W) to a continuous gradient in CO$_2$ from 200 to 560 µmol mol$^{-1}$ (Johnson, Polley & Whitis 2000). The CO$_2$ gradient was maintained during growing seasons (March–November) of 1997–2000 on grassland dominated by the C$_4$ perennial grass Bothriochloa ischaemum (L.) Keng and C$_3$ perennial forbs such as Solanum dimidiatum Raf. Annual precipitation at the site averages 879 mm (89 years mean).

The CO$_2$ facility consisted of two transparent, tunnel-shaped chambers, each with 10 consecutive compartments 1 m wide × 1 m tall × 5 m long (Johnson et al. 2000). During daylight, pure CO$_2$ was injected into air introduced into the south end of one chamber (elevated chamber; compartments 1–10) to initiate a 560–350 µmol mol$^{-1}$ gradient in CO$_2$. Ambient air was introduced into the south end of the second chamber (subambient chamber; compartments 11–20) to initiate a 365–200 µmol mol$^{-1}$ gradient in CO$_2$. Night-time CO$_2$ concentrations were regulated at about 150 µmol mol$^{-1}$ above daytime values along each chamber. Ambient air was introduced into the north end of the subambient chamber at night. Pure CO$_2$ was injected into air blown from the north of the superambient chamber at night to increase the initial CO$_2$ concentration to 500 µmol mol$^{-1}$. Desired CO$_2$ concentration gradients were maintained by automatically varying the rate of air flow through chambers in response to changes in photosynthetic (daylight) or respiration rates (night). Air temperature and vapour pressure deficit were regulated near ambient values by cooling and dehumidifying air at 5-m intervals along chambers. Irrigation equivalent to rainfall was applied to the chambered grassland through a metered surface irrigation system.

**FLUX MEASUREMENTS**

Daytime totals of NEE and ET were calculated for each 5-m compartment for three periods during the 2000 growing season using the CO$_2$ or water vapour gradient measured each 20 min in each compartment with an infra-red gas analyser (Model 6262, Li-Cor, Inc., Lincoln, NE, USA) and the volumetric rate of air flow (Mielenick et al. 2001). The rate of air flow through each compartment was a linear function of the number of revolutions of the fan that moved air through chambers ($r^2 > 0.99$). Net fluxes of CO$_2$ and ET calculated every 20 min were summed for each daylight period. Fluxes for compartments located at the north end of the chambers (air exit; compartments 10 and 20) were highly variable because the air exit was poorly buffered from the influence of winds. Data from these compartments were excluded.
We altered the CO₂ concentration of air input to chambers for 2 days during early season (May) and late-season in 2000 (October) and for 4 days during mid-season (July) to determine the grassland response to a short-term change in CO₂. Grassland maintained (grown) for 3 years at subambient CO₂ (< 365 µmol mol⁻¹) was exposed for 2 days during July (days 206–207) and October 2000 (days 277–278) to an elevated CO₂ gradient (> 365 µmol mol⁻¹; Fig. 1a). Plants in each compartment of the subambient chamber thus were briefly exposed to a CO₂ level that was 150–200 µmol mol⁻¹ greater than the ‘growth’ concentration (Fig. 1b). Grassland grown at elevated CO₂ was exposed for 2 days during May (days 137–138) and July 2000 (days 208–209) to a subambient gradient. Grassland in each compartment of the elevated chamber thus was briefly exposed to a CO₂ level that was 150–180 µmol mol⁻¹ lower than the growth concentration. The CO₂ was altered (switched) when clear skies were predicted for several days.

The influence of prior CO₂ exposure on physiological sensitivity to CO₂ usually is assessed at a common CO₂ concentration. This approach was not feasible in elongated chambers. Instead, we standardized the absolute difference between the lowest and highest CO₂ levels to which grassland in each chamber compartment was exposed during each of the three periods of CO₂ switching. In the absence of acclimation, the absolute change in NEE or ET for a given differential in CO₂ should be similar irrespective of the absolute flux rate provided fluxes respond approximately linearly to CO₂.

Daily totals of photosynthetic photon flux density (PPFD) during CO₂ switching varied from 45 to 54 mol m⁻² day⁻¹ on days 135–138, 42–49 mol m⁻² day⁻¹ on days 206–209, and 38–42 mol m⁻² day⁻¹ on days 275–278. Diurnal variation in PPFD likely affected the amount by which CO₂ switching changed NEE and ET. Variation in PPFD should not have compromised our ability to detect effects of prior CO₂ exposure on fluxes, however, because on a given day, the grassland in treatment and control chambers was exposed to the same light environment and CO₂ gradient (Fig. 1a).

**PLANT AND ENVIRONMENTAL MEASUREMENTS AND ANALYSES**

Rates of NEE also vary as a function of within-day change in PPFD. In order to determine whether the response of NEE to CO₂ switching depended on within-day changes in PPFD, we calculated the effect of short-term CO₂ treatment on parameters derived from regressions fit to relationships between NEE and PPFD. For each day and for each 5-m compartment, we fit a hyperbolic regression to the NEE-PPFD relationship (Ruimy et al. 1995),

\[
\text{NEE} = \frac{\alpha \text{PPFD} \ NEE_{\text{max}}}{\alpha \text{PPFD} + NEE_{\text{max}}} - R(\text{hyperbolic})
\]

where \(\alpha\) is the initial slope of the light–response curve, \(NEE_{\text{max}}\) is maximum NEE, and \(R\) is respiration rate (NEE at PPFD = 0). For each hyperbolic equation, we calculated NEE at 800 and 1600 µmol (quanta) m⁻² s⁻¹ (NEE₈₀₀, NEE₁₆₀₀). The absolute differences in parameter values between high and low CO₂ levels then were calculated for each period of CO₂ switching.

Leaf area index (LAI) in each 5-m compartment was measured on days 145 (early season), 199 (mid-season), and 285 (late-season) using a SunScan canopy analysis system (Delta-T Devices Ltd, Burwell, Cambridge, UK), and averaged 2·5, 3·4 and 3·2 at elevated CO₂ and 2·4, 3·1 and 2·6 at subambient CO₂ during early, mid and late season, respectively. Percentage ground cover by plant species was estimated visually.

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**Fig. 1.** (a) Depiction of the application of CO₂ switching treatments. Grassland grown at subambient CO₂ concentration [CO₂] was exposed for 2 days in October and July 2000 to an elevated CO₂ gradient. Grassland grown at elevated CO₂ was exposed for 2 days in May and July 2000 to a subambient gradient. (b) Average daytime CO₂ concentration on days 206 and 207 (solid bars) and 208 and 209 (open bars) in consecutive 5-m long compartments of CO₂ chambers during CO₂ switching in July 2000. Grassland had been exposed to a gradient in elevated CO₂ (compartments 1–9) and in subambient CO₂ (compartments 11–19) for three seasons (indicated by bars with no arrows) when CO₂ concentration was increased in compartments 11–19 on days 206 and 207 (indicated by upward arrow) and reduced in compartments 1–9 on days 208 and 209 (indicated by downward arrow). CO₂ also was increased in compartments 11–19 for 2 days in October 2000 and reduced in compartments 1–9 for 2 days in May 2000.
from photographs of the centre 4 m of each 5-m long compartment each day on which LAI was measured. Cover of C3 grasses and litter plus standing dead material was near zero in all compartments (results not shown), so we used the ratio of C3 cover (mostly forbs or broadleaf herbaceous plants) to total [C3 plus C4 (grasses)] cover to characterize vegetation differences among compartments. Absolute responses of NEE and ET (each expressed per unit of leaf area) and of parameters derived from fitted light–response curves to CO2 change were analysed as a function of the growth CO2 concentration and C3/total cover using regression (Sigma Plot 2000, SPSS Inc., Chicago, IL, USA). Relationships between fluxes measured prior to each period of CO2 switching and growth CO2 were also subject to regression analysis.

Results

NEE and ET measured at growth CO2

Daytime NEE per unit of leaf area increased sharply at higher growth CO2 concentrations during the 6 days prior to CO2 switching in May (DOY 129–134; Fig. 2). As estimated from regression analysis, increasing growth CO2 from 235 to 550 µmol mol–1 increased early season NEE by an average of 145% (from 12·3 to 30·2 g CO2 m–2 leaf area day–1). As the growing season progressed and the contribution of C3 species to plant cover decreased from an average of 79% in May to 69% in July and 58% in late September, NEE per unit of leaf area declined and linear relationships between daytime NEE and growth CO2 weakened (Fig. 2). By contrast, there was no relationship between growth CO2 and daytime ET measured prior to either the early season (May), mid-season (July) or late-season (late September) period of CO2 switching (not shown; F < 0·34, P > 0·56, n = 6; mean ± SE = 921 ± 70 g H2O m–2 leaf area day–1 in May and 1783 ± 127 g H2O m–2 leaf area day–1 in July).

Response of NEE and ET to CO2 switching: relationship to growth CO2

Temporarily increasing CO2 usually increased NEE and reduced ET. The ratio of daytime NEE at the higher compared to lower CO2 concentration during CO2 switching averaged 1:20 in May across plots grown at elevated CO2 (n = 9), 1:29 in October across plots grown at subambient CO2 (n = 9), and 1:16 in July across plots grown at subambient and elevated concentrations (n = 18). The ratio of ET at high CO2 to ET at low CO2 during switching was ≤ 1:0 at all growth concentrations in May and July (data not shown) and averaged 0·80 (May; n = 9) and 0·91 (July; n = 18) across long-term treatments. The ratio of ET at high compared with low CO2 during switching averaged 0·97 in October (n = 9). During early to mid-season there-
Sensitivity of grassland carbon and water flux to CO₂

Fig. 3. The difference in the daytime total of net ecosystem exchange (NEE) between days at elevated CO₂ concentrations and days at subambient concentrations (E-S) during CO₂ switching in May and July 2000 as a function of the growth CO₂ concentration. Data from May were fit with a linear function [NEE(E-S) = 13 070 – 0·018 × growth CO₂, r² = 0·31, P = 0·07]. Data from July were fit with an exponential function following deletion of the outlying point at the highest growth concentration [NEE(E-S) = 5·8762 × e⁻⁰·⁰⁰⁰³⁵ growth CO₂], r² = 0·21, P = 0·04].

outlier, the effect of growth CO₂ on change in NEE depended on a single data point at the lowest growth concentration without which the regression of NEE response on growth CO₂ was not significant (F = 1·77, P = 0·20, n = 16). There was no relationship between change in NEE during late-season switching (October) and growth CO₂ (F = 3·77, P = 0·10, n = 9), nor did the decline in ET at the higher CO₂ level during switching depend on growth CO₂ on any sampling date (F = 0·18, 0·06, 0·16; P = 0·69, 0·80, 0·70; n = 9, 18, 9 in May, July and October, respectively).

During CO₂ switching in both May and July, NEE-PPFD relationships were strongly curvilinear whether measured at high (average r² of hyperbolic functions = 0·88) or at low CO₂ concentrations (average r² = 0·87; Fig. 4). The trend in July for NEE to increase more following a short-term increase in CO₂ concentration at subambient than elevated growth concentrations (Fig. 3) resulted largely from a similar trend in the response of NEE1600 to CO₂ change (Table 1). Growth CO₂ concentration did not affect the response of other parameters from light–response curves (NEE800, R, α) in July to CO₂ change (F < 0·09, P > 0·77, n = 18) or the response of any parameter from curves in May to CO₂ change (F < 0·54, P > 0·48, n = 9).

RESPONSE OF NEE AND ET TO CO₂ SWITCHING: RELATIONSHIP TO C₃ COVER
Flux responses to CO₂ switching did not depend consistently on growth CO₂, implying that factors other than or in addition to physiological acclimation

Table 1. Significant regressions between the change in gas exchange from elevated to subambient CO₂ concentrations (E-S) during CO₂ switching and either the growth CO₂ concentration (CO₂) or the ratio of C₃ plant cover to total cover (C₃ cover). NEE800, NEE1600 = Rates of daytime net ecosystem exchange (µmol CO₂ m⁻² leaf area s⁻¹) at 800 and 1600 µmol (quanta) m⁻² s⁻¹ as calculated from light–response curves in July (n = 18). ET = daytime rate of evapotranspiration in May (g H₂O m⁻² leaf area day⁻¹; n = 9). Linear (y = ax + b) or exponential (y = a × e⁻bx) functions were fit to relationships between dependent and independent variables

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<th>Independent variable</th>
<th>Model type</th>
<th>Slope or a-value</th>
<th>Intercept or b-value</th>
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explained the limited sensitivity of NEE (Fig. 2) and ET to growth CO$_2$, especially during mid- and late-season. In this grassland, CO$_2$ response depended partly on the photosynthetic pathway of dominant plants. The amount by which NEE was stimulated at the higher CO$_2$ level during switching in July increased linearly as the contribution of C$_3$ species to plant cover rose from 26% to 96% (Fig. 5). The greater response of NEE to CO$_2$ enrichment in C$_3$- than C$_4$-dominated plots resulted largely from a similar trend in the response of NEE$_{1600}$ to CO$_2$ change (Table 1). By contrast, there was no relationship between C$_3$/total cover and the change in other parameters from light–response curves (NEE$_{1600}$, R, α) following a CO$_2$ change in July ($F < 2.25, P > 0.14, n = 18$). Nor, did C$_3$/total cover affect the response of any parameter from light–response curves in May to CO$_2$ change ($F < 0.31, P > 0.60, n = 9$). Surprisingly, higher CO$_2$ during switching reduced ET more in C$_3$- than in C$_4$-dominated grassland in May. The amount by which ET declined at higher CO$_2$ in May decreased linearly from 580 g H$_2$O m$^{-2}$ leaf area day$^{-1}$ when C$_3$ plants comprised 40% of plant cover to 330 g H$_2$O m$^{-2}$ leaf area day$^{-1}$ when C$_3$ cover approached 100% (Table 1). Effects of CO$_2$ change on ET did not depend on C$_3$ cover in either July ($F = 0.06, P = 0.80, n = 18$) or October ($F = 2.88, P = 0.13, n = 9$).

Daytime NEE responded more to CO$_2$ change in C$_3$- than C$_4$-dominated grassland during mid-season (Fig. 5), implying that compartment-to-compartment differences in C$_3$ cover were at least partly responsible for weak relationships between grassland NEE and long-term CO$_2$ treatment in July and September (Fig. 2). To lessen the influence of variation in C$_3$ cover, we averaged fluxes measured at growth CO$_2$ levels across either elevated or subambient concentrations (average CO$_2$ difference = 195 µmol mol$^{-1}$). C$_3$ plants contributed a similar fraction to total cover in grassland grown at elevated and subambient CO$_2$ (mean of 64% and 73% of total cover, respectively, in July and 55% and 63% of total cover, respectively, in September). Over the 6 days prior to CO$_2$ switching, the ratio of NEE at elevated growth CO$_2$ to NEE at subambient growth CO$_2$ averaged 1.40 in July and 1.34 in September. Excluding two extreme values of NEE in July (> 20 g CO$_2$ m$^{-2}$ leaf area day$^{-1}$; Fig. 2) reduced the ratio of NEE at elevated to subambient CO$_2$ to 1.18, a value similar to the average ratio of 1.16 derived by dividing the NEE measured in each compartment at high CO$_2$ by the NEE measured at low CO$_2$ during CO$_2$ switching. When CO$_2$ was switched in early October, the ratio of NEE at high CO$_2$ to NEE at low CO$_2$ averaged 1.29. This value is similar to the average of 1.34 derived by dividing NEE measured at elevated growth concentrations by NEE measured at subambient growth concentrations prior to switching (Fig. 2). The sensitivity of NEE to CO$_2$ thus apparently depended more on C$_3$–C$_4$ composition of vegetation than on physiological acclimation during mid- and late-season.

Discussion

Plant and ecosystem responses to short-term changes in CO$_2$ concentration may depend on the CO$_2$ concentration at which plants have been grown (Stocker et al. 1997; Dugas et al. 2001). We varied CO$_2$ by 150–200 µmol mol$^{-1}$ on C$_3$–C$_4$ grassland that had been exposed for three seasons to a 200–550 µmol mol$^{-1}$ CO$_2$ gradient. The amount by which NEE increased at high compared with low CO$_2$ during switching declined at higher growth concentrations on two dates (May, July), but relationships between NEE response and growth CO$_2$ were weak. For only one date (July) could we identify an explanation from light–response curves for reduced sensitivity of NEE to CO$_2$ at higher growth concentrations. Similarly, we found no relationship between change in ET following CO$_2$ switching and growth CO$_2$. If physiological acclimation occurred in this grassland, it was difficult to detect at the canopy level amid other factors regulating CO$_2$ response. During the mid- and latter-part of the growing season, the C$_3$–C$_4$ composition of vegetation proved a more important regulator of NEE and ET sensitivity to CO$_2$ than growth CO$_2$.

It often has been reported that canopy-level gas exchange is more sensitive to CO$_2$ enrichment in C$_3$ than C$_4$ plants (Drake & Leadley 1991). Elevated CO$_2$ had little effect on NEE of C$_3$-dominated tallgrass prairie until late in the growing season when CO$_2$ enrichment stimulated C uptake by delaying senescence (Ham et al. 1995), for example, but CO$_2$ enrichment increased the seasonal total of NEE per unit of aboveground biomass of a C$_3$, Scirpus olneyi (Grey) stand by 48% during the second year of treatment (Drake & Leadley 1991). At least initially following CO$_2$ treatment, biomass production of the dominant C$_4$ grass (Bothriochloa ischaemum) in our study was highly responsive to growth CO$_2$ (Polley et al. 2003). Leaf net
photosynthesis of *B. ischaemum* remained a linear function of growth CO₂ throughout the experiment (Anderson et al. 2001), but biomass of this short-statured grass decreased sharply during the final 2 years of CO₂ exposure as taller grasses and forbs proliferated (Polley et al. 2003). As a fraction of C₄ grass production, biomass of *B. ischaemum* also declined from 94% in 1997 to 50% in 2000 across CO₂ treatments. Our finding that daytime NEE responded little to short-term variation in CO₂ in C₄-dominanted grassland implies *B. ischaemum* was replaced by less-responsive C₃ grasses.

Neither the ratio C₃ to total (C₃ + C₄) plant cover nor the CO₂ treatment at which grassland had been grown consistently affected the CO₂ response of ET, implying that short-term CO₂ enrichment reduced ET in both C₃ and C₄ species. Indeed at the May CO₂ switching, the higher CO₂ concentration reduced ET more in C₄ than C₃-dominated grassland. CO₂ enrichment reduced stomatal conductance of both C₃ and C₄ species in this grassland, but the absolute decline in conductance from subambient to elevated concentrations was greater by more than a factor of 2 for the C₃ grass *Bromus japonicus* and by more than an order of magnitude for the C₄ forb *Solanum dimitiatum* than for the C₃ grass *Bothriochloa* (Anderson et al. 2001; Maherali et al. 2002). ET rates have been measured in relatively few CO₂ studies on intact ecosystems, but CO₂ enrichment has been shown to reduce rates of soil water depletion, and by inference to reduce ET, in both C₃- and C₄-dominated ecosystems (Freedeen et al. 1997; Owensby et al. 1997; Nelson et al. 2004).

In this mesic grassland, carbon and water fluxes remained responsive to short-term change in CO₂ after three growing seasons at different CO₂ levels. NEE was greater (16–20%) and ET was smaller (9–20%), on average, at high than low CO₂ during CO₂ switching in May and July. However, the amount by which NEE and ET responded to CO₂ change depended on both growth CO₂ and the C₃-C₄ composition of vegetation. Thus, our results imply that sensitivity of this grassland to CO₂ depends both on management and other influences on C₃–C₄ dynamics (e.g. C₄ dominance may be increased by spring fires and reduced by grazing ungulates) and on prior CO₂ exposure.

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**References**


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