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# C$_3$–C$_4$ 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 (CO$_2$) enrichment by increasing photosynthesis and reducing transpiration, but these initial responses to CO$_2$ may not be sustained.

**2.** During May, July and October 2000, we measured the effects of temporarily increasing or decreasing CO$_2$ concentration by 150–200 $\mu$mol mol$^{-1}$ on daytime net ecosystem CO$_2$ exchange (NEE) and water flux (evapotranspiration, ET) of C$_3$–C$_4$ grassland in central Texas, USA that had been exposed for three growing seasons to a CO$_2$ gradient from 200 to 560 $\mu$mol mol$^{-1}$. Grassland grown at subambient CO$_2$ (< 365 $\mu$mol mol$^{-1}$) was exposed for 2 days to an elevated CO$_2$ gradient (> 365 $\mu$mol mol$^{-1}$). Grassland grown at elevated CO$_2$ was exposed for 2 days to a subambient gradient. Our objective was to determine whether growth CO$_2$ affected the amount by which grassland NEE and ET responded to CO$_2$ switching (sensitivity to CO$_2$).

**3.** The NEE per unit of leaf area was greater (16–20%) and ET was smaller (9–20%), on average, at the higher CO$_2$ concentration during CO$_2$ switching in May and July. The amount by which NEE increased at the higher CO$_2$ level was smaller at elevated than subambient growth concentrations on both dates, but relationships between NEE response and growth CO$_2$ were weak. Conversely, the effect of temporary CO$_2$ change on ET did not depend on growth CO$_2$.

**4.** The ratio of NEE at high CO$_2$ to NEE at low CO$_2$ during CO$_2$ change in July increased from 1·0 to 1·26 as the contribution of C$_3$ cover to total cover increased from 26% to 96%. Conversely, in May, temporary CO$_2$ enrichment reduced ET more in C$_4$- than C$_3$-dominated grassland.

**5.** For this mesic grassland, sensitivity of NEE and ET to brief change in CO$_2$ depended as much on the C$_3$–C$_4$ composition of vegetation as on physiological adjustments related to prior CO$_2$ exposure.

*Key-words*: acclimation, CO$_2$ enrichment, net ecosystem CO$_2$ exchange, subambient CO$_2$

## Introduction

Plants in most ecosystems respond initially to an increase in atmospheric CO$_2$ concentration with faster rates of photosynthesis and growth and a decrease in stomatal conductance (Drake, Gonzàlez-Meler & Long 1997; Long *et al*. 2004). However, initial responses to CO$_2$ enrichment may not be sustained, complicating the task of predicting CO$_2$ 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 CO$_2$ (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 CO$_2$ enrichment.

Photosynthesis at a given CO$_2$ concentration is often lower for plants grown at elevated than ambient CO$_2$ concentrations (acclimation or downregulation;

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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 $C_3$ 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 $\mu$mol 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 $\mu$mol 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 $\mu$mol mol$^{-1}$). $CO_2$ enrichment substantially increased biomass production and net C uptake of this grassland (Mielnick *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 $C_3$ species increased at the expense of $C_4$ grass biomass, irrespective of $CO_2$ treatment (Mielnick *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 multiyear $CO_2$ 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.

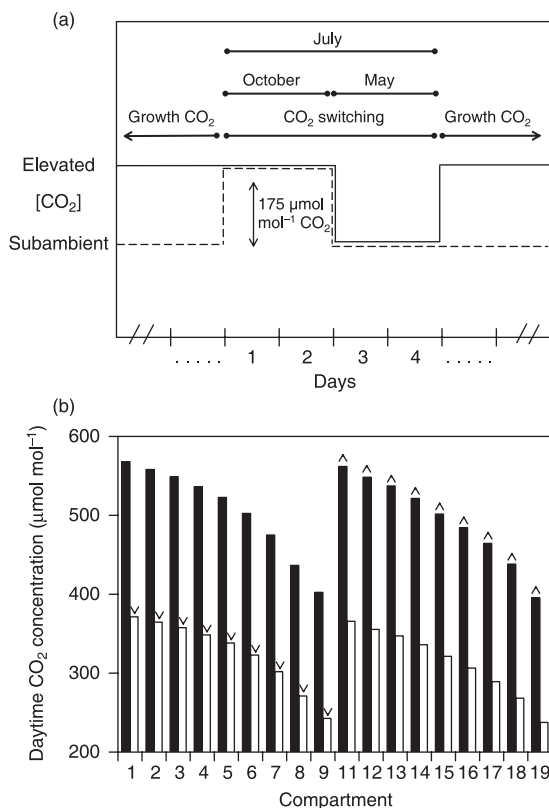## Materials and methods

### 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 $\mu$mol 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 $\mu$mol 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 $\mu$mol mol$^{-1}$ gradient in $CO_2$. Night-time $CO_2$ concentrations were regulated at about 150 $\mu$mol 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 $\mu$mol 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 (Mielnick *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 $CO_2$ 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 $CO_2$. Grassland maintained (grown) for 3 years at subambient $CO_2$ (< 365 µmol mol$^{-1}$) was exposed for 2 days during July (days 206–207) and October 2000 (days 277–278) to an elevated $CO_2$ gradient (> 365 µmol mol$^{-1}$; Fig. 1a). Plants in each compartment of the subambient chamber thus were briefly exposed to a $CO_2$ level that was 150–200 µmol mol$^{-1}$ greater than the 'growth' concentration (Fig. 1b). Grassland grown at elevated $CO_2$ 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 $CO_2$ level that was 150–180 µmol mol$^{-1}$ lower than the growth concentration. The $CO_2$ was altered (switched) when clear skies were predicted for several days.

The influence of prior $CO_2$ exposure on physiological sensitivity to $CO_2$ usually is assessed at a common $CO_2$ concentration. This approach was not feasible in elongated chambers. Instead, we standardized the absolute difference between the lowest and highest $CO_2$ levels to which grassland in each chamber compartment was exposed during each of the three periods of $CO_2$ switching. In the absence of acclimation, the absolute change in NEE or ET for a given differential in $CO_2$ should be similar irrespective of the absolute flux rate provided fluxes respond approximately linearly to $CO_2$.

Daily totals of photosynthetic photon flux density (PPFD) during $CO_2$ 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 $CO_2$ switching changed NEE and ET. Variation in PPFD should not have compromised our ability to detect effects of prior $CO_2$ exposure on fluxes, however, because on a given day, the grassland in treatment and control chambers was exposed to the same light environment and $CO_2$ 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 $CO_2$ switching depended on within-day changes in PPFD, we calculated the effect of short-term $CO_2$ 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),

$$NEE = \frac{\alpha PPFD\ NEE_{max}}{\alpha PPFD + NEE_{max}} - R(hyberbolic)$$

where $\alpha$ is the initial slope of the light–response curve, $NEE_{max}$ 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$^{-1}$ ($NEE_{800}$, $NEE_{1600}$). The absolute differences in parameter values between high and low $CO_2$ levels then were calculated for each period of $CO_2$ 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 $CO_2$ and 2·4, 3·1 and 2·6 at subambient $CO_2$ during early, mid and late season, respectively. Percentage ground cover by plant species was estimated visually



**Fig. 1.** (a) Depiction of the application of $CO_2$ switching treatments. Grassland grown at subambient $CO_2$ concentration [$CO_2$] was exposed for 2 days in October and July 2000 to an elevated $CO_2$ gradient. Grassland grown at elevated $CO_2$ was exposed for 2 days in May and July 2000 to a subambient gradient. (b) Average daytime $CO_2$ concentration on days 206 and 207 (solid bars) and 208 and 209 (open bars) in consecutive 5-m long compartments of $CO_2$ chambers during $CO_2$ switching in July 2000. Grassland had been exposed to a gradient in elevated $CO_2$ (compartments 1–9) and in subambient $CO_2$ (compartments 11–19) for three seasons (indicated by bars with no arrows) when $CO_2$ 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). $CO_2$ 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 $C_3$ grasses and litter plus standing dead material was near zero in all compartments (results not shown), so we used the ratio of $C_3$ cover (mostly forbs or broadleaf herbaceous plants) to total [$C_3$ plus $C_4$ (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 $CO_2$ change were analysed as a function of the growth $CO_2$ concentration and $C_3$/total cover using regression (Sigma Plot 2000, SPSS Inc., Chicago, IL, USA). Relationships between fluxes measured prior to each period of $CO_2$ switching and growth $CO_2$ were also subject to regression analysis.

## Results

### NEE AND ET MEASURED AT GROWTH $CO_2$

Daytime NEE per unit of leaf area increased sharply at higher growth $CO_2$ concentrations during the 6 days prior to $CO_2$ switching in May (DOY 129–134; Fig. 2). As estimated from regression analysis, increasing growth $CO_2$ from 235 to 550 $\mu$mol mol$^{-1}$ increased early season NEE by an average of 145% (from 12·3 to 30·2 g $CO_2$ m$^{-2}$ leaf area day$^{-1}$). As the growing season progressed and the contribution of $C_3$ 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 $CO_2$ weakened (Fig. 2). By contrast, there was no relationship between growth $CO_2$ and daytime ET measured prior to either the early season (May), mid-season (July) or late-season (late September) period of $CO_2$ switching (not shown; $F < 0·34$, $P > 0·56$, $n = 6$; mean $\pm$ SE = 921 $\pm$ 70 g $H_2O$ m$^{-2}$ leaf area day$^{-1}$ in May and 1783 $\pm$ 127 g $H_2O$ m$^{-2}$ leaf area day$^{-1}$ in July).

### RESPONSE OF NEE AND ET TO $CO_2$ SWITCHING: RELATIONSHIP TO GROWTH $CO_2$

Temporarily increasing $CO_2$ usually increased NEE and reduced ET. The ratio of daytime NEE at the higher compared to lower $CO_2$ concentration during $CO_2$ switching averaged 1·20 in May across plots grown at elevated $CO_2$ ($n = 9$), 1·29 in October across plots grown at subambient $CO_2$ ($n = 9$), and 1·16 in July across plots grown at subambient and elevated concentrations ($n = 18$). The ratio of ET at high $CO_2$ to ET at low $CO_2$ during switching was $\leq 1·0$ 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 $CO_2$ during switching averaged 0·97 in October ($n = 9$). During early to mid-season there-
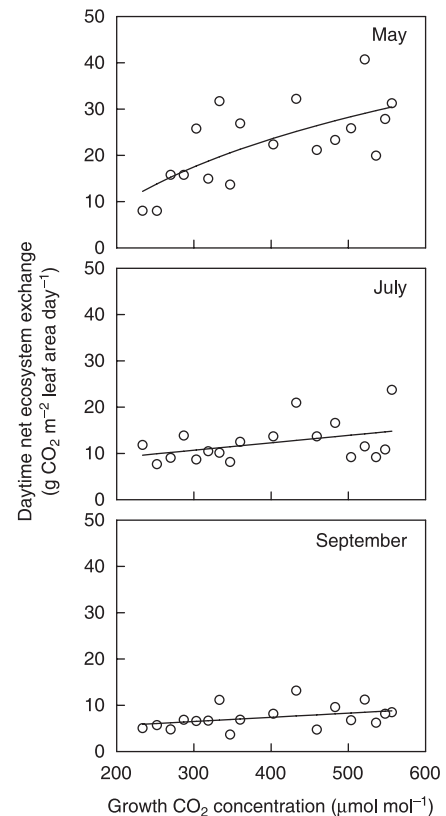
**Fig. 2.** Relationships between means of daytime net ecosystem $CO_2$ exchange (NEE) over 6 days in May (DOY 129–134), July (DOY 200–205), and late September 2000 (DOY 270–274) prior to $CO_2$ switching and the $CO_2$ concentration to which grassland had been exposed for three growing seasons (growth $CO_2$). Data from May were fit with a logarithmic function (NEE $= -102·3 + 21·0 \times \ln$(growth $CO_2$), $r^2 = 0·44$, $P = 0·002$). Data from July and September were fit with linear functions (NEE $= 5·883 + 0·016 \times$ growth $CO_2$, $r^2 = 0·11$, $P = 0·09$ in July; NEE $= 3·788 + 0·009 \times$ growth $CO_2$, $r^2 = 0·11$, $P = 0·10$ in September).

fore temporarily increasing $CO_2$ by 150–200 $\mu$mol mol$^{-1}$ increased NEE per unit of leaf area by 16–20% and reduced ET per unit of leaf area by 9–20%. Yet, the absence of relationships between ET and growth $CO_2$ and the weakening of NEE-growth $CO_2$ relationships during mid- and late-season (Fig. 2) imply that the sensitivity of fluxes to $CO_2$ differed among growth concentrations during at least part of the growing season.
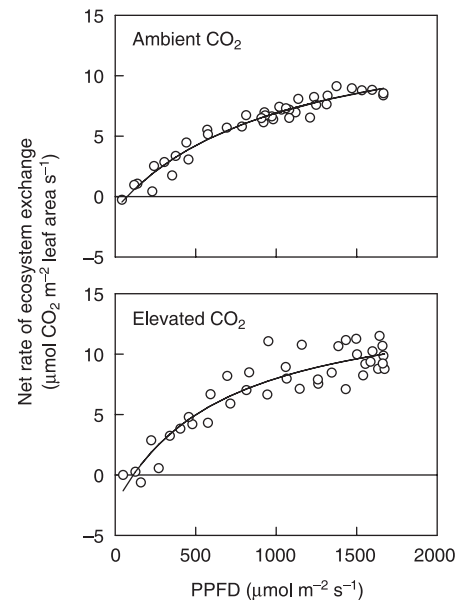
The effect of a temporary change in $CO_2$ on fluxes did not depend consistently on the growth concentration, however. The amount by which NEE was greater at the higher $CO_2$ concentration during switching was smaller at elevated than subambient growth concentrations in both May and July, but relationships between NEE response and growth $CO_2$ were weak (Fig. 3). The relationship between NEE response to switching and growth $CO_2$ was significant at the $P = 0·07$ level in May ($F = 4·59$, $n = 9$) and declined significantly in July only following exclusion of an outlying data point at the highest growth concentration ($F = 5·27$, $P = 0·04$, $n = 17$). Even after excluding this
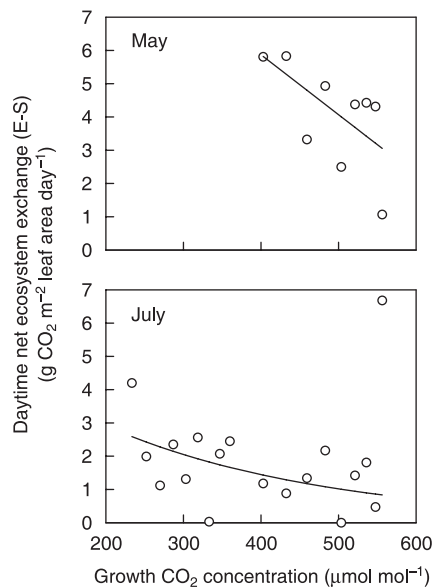
**Fig. 3.** The difference in the daytime total of net ecosystem exchange (NEE) between days at elevated $CO_2$ concentrations and days at subambient concentrations (E-S) during $CO_2$ switching in May and July 2000 as a function of the growth $CO_2$ concentration. Data from May were fit with a linear function [NEE(E-S) = 13·070 − 0·018 × growth $CO_2$, $r^2 = 0.31$, $P = 0.07$]. 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 × $e^{(-0.0035 \times \text{growth } CO_2)}$, $r^2 = 0.21$, $P = 0.04$].



**Fig. 4.** Net rate of ecosystem $CO_2$ exchange (NEE) as a function of photosynthetic photon flux density (PPFD) for grassland grown at the current $CO_2$ concentration (360 µmol mol⁻¹) and exposed for 2 days in July 2000 to an elevated $CO_2$ concentration (556 µmol mol⁻¹). Illustrated are data from single days at ambient and elevated $CO_2$ that were fit with hyperbolic functions, where NEE = [($\alpha \times b \times$ PPFD)/($b + \alpha \times$ PPFD)] − R and $\alpha$ = 0·0158 and 0·0262, $b$ = 16·2161 and 17·5054, R = 1·0861 and 2·5058, and $r^2$ = 0·95 and 0·85 at ambient and elevated $CO_2$, respectively ($P < 0.0001$).

outlier, the effect of growth $CO_2$ on change in NEE depended on a single data point at the lowest growth concentration without which the regression of NEE response on growth $CO_2$ 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 $CO_2$ ($F = 3.7$, $P = 0.10$, $n = 9$), nor did the decline in ET at the higher $CO_2$ level during switching depend on growth $CO_2$ 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 $CO_2$ switching in both May and July, NEE-PPFD relationships were strongly curvilinear whether measured at high (average $r^2$ of hyperbolic functions = 0·88) or at low $CO_2$ concentrations (average $r^2 = 0.87$; Fig. 4). The trend in July for NEE to increase more following a short-term increase in $CO_2$ concentration at subambient than elevated growth concentrations (Fig. 3) resulted largely from a similar trend in the response of $NEE_{1600}$ to $CO_2$ change (Table 1). Growth $CO_2$ concentration did not affect the response of other parameters from light–response curves ($NEE_{800}$, R, $\alpha$) in July to $CO_2$ change ($F < 0.09$, $P > 0.77$, $n = 18$) or the response of any parameter from curves in May to $CO_2$ change ($F < 0.54$, $P > 0.48$, $n = 9$).

### RESPONSE OF NEE AND ET TO $CO_2$ SWITCHING: RELATIONSHIP TO $C_3$ COVER

Flux responses to $CO_2$ switching did not depend consistently on growth $CO_2$, 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 $CO_2$ concentrations (E-S) during $CO_2$ switching and either the growth $CO_2$ concentration ($CO_2$) or the ratio of $C_3$ plant cover to total cover ($C_3$). $NEE_{800}$, $NEE_{1600}$ = Rates of daytime net ecosystem exchange (µmol $CO_2$ 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 $H_2O$ m⁻² leaf area day⁻¹; $n = 9$). Linear ($y = ax + b$) or exponential ($y = a \times e^{(-bx)}$) functions were fit to relationships between dependent and independent variables

| Dependent variable | Month | Independent variable | Model type | Slope or $a$-value | Intercept or $b$-value | $r^2$ | $P$-value |
|---|---|---|---|---|---|---|---|
| $NEE_{1600}$ (E-S) | July | $CO_2$ | Exponential | 4·595 | 0·003 | 0·20 | 0·04 |
| $NEE_{800}$ (E-S) | July | $C_3$ cover | Linear | −0·691 | 2·108 | 0·35 | 0·006 |
| ET (E-S) | May | $C_3$ cover | Linear | −751·15 | 427·63 | 0·39 | 0·04 |
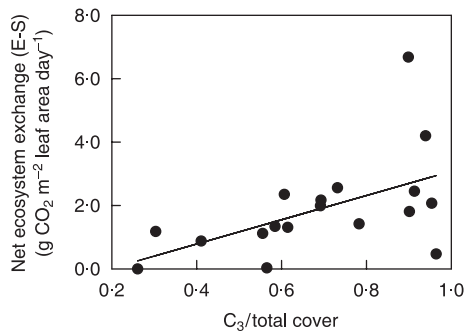
**Fig. 5.** The difference in the daytime total of net ecosystem $CO_2$ exchange (NEE) between days at elevated $CO_2$ concentrations and days at subambient concentrations (E-S) during $CO_2$ switching in July 2000, vs. the ratio of $C_3$ plant cover to total cover. Data were fit with a linear function [NEE(E-S) = −0·743 + 3·822 × ($C_3$/total cover), $r^2 = 0·24$, $P = 0·02$, $n = 18$].

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 $NEE_{800}$ 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, $\alpha$) 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 $C_4$- than in $C_3$-dominated grassland in May. The amount by which ET declined at higher $CO_2$ in May decreased linearly from 580 g $H_2O$ m$^{-2}$ leaf area day$^{-1}$ when $C_3$ plants comprised 40% of plant cover to 330 g $H_2O$ 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 $C_4$-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 $CO_2$ throughout the experiment (Anderson *et al.* 2001), but biomass of this short-statured grass decreased sharply during the final 2 years of $CO_2$ exposure as taller grasses and forbs proliferated (Polley *et al.* 2003). As a fraction of $C_4$ grass production, biomass of *B. ischaemum* also declined from 94% in 1997 to 50% in 2000 across $CO_2$ treatments. Our finding that daytime NEE responded little to short-term variation in $CO_2$ in $C_4$-dominated grassland implies *B. ischaemum* was replaced by less-responsive $C_4$ grasses.

Neither the ratio $C_3$ to total ($C_3 + C_4$) plant cover nor the $CO_2$ treatment at which grassland had been grown consistently affected the $CO_2$ response of ET, implying that short-term $CO_2$ enrichment reduced ET in both $C_3$ and $C_4$ species. Indeed at the May $CO_2$ switching, the higher $CO_2$ concentration reduced ET more in $C_4$- than $C_3$-dominanted grassland. $CO_2$ enrichment reduced stomatal conductance of both $C_3$ and $C_4$ 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 $C_3$ grass *Bromus japonicus* and by more than an order of magnitude for the $C_3$ forb *Solanum dimidiatum* than for the $C_4$ grass *Bothriochloa* (Anderson *et al.* 2001; Maherali *et al.* 2002). ET rates have been measured in relatively few $CO_2$ studies on intact ecosystems, but $CO_2$ enrichment has been shown to reduce rates of soil water depletion, and by inference to reduce ET, in both $C_3$- and $C_4$-dominated ecosystems (Fredeen *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 $CO_2$ after three growing seasons at different $CO_2$ levels. NEE was greater (16–20%) and ET was smaller (9–20%), on average, at high than low $CO_2$ during $CO_2$ switching in May and July. However, the amount by which NEE and ET responded to $CO_2$ change depended on both growth $CO_2$ and the $C_3$–$C_4$ composition of vegetation. Thus, our results imply that sensitivity of this grassland to $CO_2$ depends both on management and other influences on $C_3$–$C_4$ dynamics (e.g. $C_4$ dominance may be increased by spring fires and reduced by grazing ungulates) and on prior $CO_2$ exposure.

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