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Increasing CO_2 from subambient to elevated concentrations increases grassland respiration per unit of net carbon fixation

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Abstract

Respiration (carbon efflux) by terrestrial ecosystems is a major component of the global carbon (C) cycle, but the response of C efflux to atmospheric CO_2 enrichment remains uncertain. Respiration may respond directly to an increase in the availability of C substrates at high CO_2, but also may be affected indirectly by a CO_2-mediated alteration in the amount by which respiration changes per unit of change in temperature or C uptake (sensitivity of respiration to temperature or C uptake). We measured CO_2 fluxes continuously during the final 2 years of a 4-year experiment on C_3/C_4 grassland that was exposed to a 200–560 μmol mol^{-1} CO_2 gradient. Flux measurements were used to determine whether CO_2 treatment affected nighttime respiration rates and the response of ecosystem respiration to seasonal changes in net C uptake and air temperature. Increasing CO_2 from subambient to elevated concentrations stimulated grassland respiration at night by increasing the net amount of C fixed during daylight and by increasing either the sensitivity of C efflux to daily changes in C fixation or the respiration rate in the absence of C uptake (basal ecosystem respiration rate). These latter two changes contributed to a 30–47% increase in the ratio of nighttime respiration to daytime net C influx as CO_2 increased from subamient to elevated concentrations. Daily changes in net C uptake were highly correlated with variation in temperature, meaning that the shared contribution of C uptake and temperature in explaining variance in respiration rates was large. Statistically controlling for collinearity between temperature and C uptake reduced the effect of a given change in C influx on respiration. Conversely, CO_2 treatment did not affect the response of grassland respiration to seasonal variation in temperature. Elevating CO_2 concentration increased grassland respiration rates by increasing both net C input and respiration per unit of C input. A better understanding of how C efflux varies with substrate supply thus may be required to accurately assess the C balance of terrestrial ecosystems.

Keywords: C_4 grasses, C_3 species, carbon substrate, CO_2 gradient, ecosystem C flux, residence time of C, soil water content, temperature

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Introduction

Respiration (carbon efflux) from terrestrial ecosystems is a major component of the global carbon (C) cycle and of the C balance of individual ecosystems (Valentini *et al*., 2000; Saleska *et al*., 2003). The response of respiration to atmospheric and climatic changes thus will be critical in determining the future distribution of C between atmospheric and terrestrial pools. Global change effects on terrestrial respiration remain uncertain, however, partly because of the complexity of the process (respiration involves both heterotrophic and autotrophic organisms and is regulated by factors that

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often covary) and partly because research has emphasized soil rather than ecosystem respiration and the effects of temperature (Luo *et al*., 2001a; Kirschbaum, 2004) over those of CO_2 enrichment (King *et al*., 2004), precipitation change (Harper *et al*., 2005), or multiple global change factors (Niinistö *et al*., 2004).

Respiration ultimately is a donor-controlled process that is regulated by the availability of C substrates, but the rate at which fixed C is recycled to the atmosphere also depends on how photosynthate is distributed among C pools that differ in turnover rates (Luo *et al*., 2001b; Pataki *et al*., 2003) and on environmental and soil physical properties that regulate the availability of C substrates to soil organisms (Davidson *et al*., 1998; Reichstein *et al*., 2003). Soil respiration declined abruptly when trees were girdled (Högberg *et al*., 2001) and following clipping or shading in grasslands to reduce photosynthesis (Craine *et al*., 1999; Wan & Luo, 2003), indicating a direct link between respiration and the supply of C substrates. On the other hand, soil respiration did not respond to an increase in photosynthesis during the first year of CO_2 enrichment in a loblolly pine (*Pinus taeda* L.) forest (Luo *et al*., 2001b), implying that respiration also depends on how fixed C is distributed among soil and plant pools. Both the amount of substrate and its availability to heterotrophic organisms depend, in turn, on environmental factors. Respiration from a forest soil was reduced when soil water content declined (Davidson *et al*., 1998), possibly because low water content limited the rate at which C compounds could diffuse to soil organisms. The response of soil respiration to temperature change also was reduced when soil water content declined in tallgrass prairie (Mielnick & Dugas, 2000; Harper *et al*., 2005).

By enhancing plant productivity and the availability of C substrates, CO_2 enrichment directly increases soil and ecosystem respiration rates (King *et al*., 2004; Niinistö *et al*., 2004). King *et al*. (2004), for example, found that CO_2 enrichment increased soil respiration rates and the calculated total of soil respiration in forests. The relative enhancement in respiration depended on species composition and on whether forests were aggregating or mature, suggesting the CO_2 effect was mediated partly by changes in the amount of C substrate. It is not clear, however, whether CO_2 enrichment also indirectly affects C efflux by altering the amount by which respiration changes per unit of change in temperature (sensitivity of respiration to temperature) or by changing respiration per unit of C input (the rapidity with which fixed C is respired). CO_2 enrichment did not alter the mean residence time of C in a loblolly forest (Luo *et al*., 2003), but increased the partitioning of C into labile fractions in an annual grassland (Hungate *et al*., 1997). CO_2 enrichment did not alter the temperature sensitivity of soil respiration in forests (King *et al*., 2004), but soil respiration in a tallgrass prairie was more sensitive to temperature change when soils were relatively wet than dry (Harper *et al*., 2005). CO_2 enrichment has been shown to reduce the rate at which plants deplete soil water (Polley *et al*., 2002), implying that rising CO_2 might indirectly affect the temperature sensitivity of respiration.

Few of the potential effects of CO_2 enrichment on respiration have been quantified, but it is obvious that to predict CO_2 effects will require a better understanding of how C efflux scales with substrate supply and is influenced by temperature, soil water content, and other environmental factors. One challenge in predicting CO_2 effects on respiration is to resolve the influence of factors that covary through time (are collinear). A given explanatory variable of respiration often is correlated with other variables that influence respiration. Multiple regression models do not account for the shared contributions of predictors, thus limiting their utility as explanatory models (Graham, 2003). Our understanding of how substrate supply interacts with other factors to regulate respiration at the ecosystem level also has been hampered by the paucity of continuous measurements of C uptake. Differences in C uptake frequently are inferred from differences in leaf area index or primary production (e.g. Reichstein *et al*., 2003; Campbell *et al*., 2004; Curiel Yuste *et al*., 2004), rather than measured directly.

We measured CO_2 fluxes continuously during the final two growing seasons of a 4-year experiment on C_3/C_4 grassland exposed to a 200–560 $\mu mol\,mol^{-1}$ CO_2 gradient. Here, we use flux measurements to determine whether CO_2 treatment affected ecosystem respiration at night by altering net C fixation, the sensitivity of respiration to daily changes in air temperature, or respiration per unit of net C fixation (ratio of respiration to C fixation). Assuming a linear relationship between respiration and C input, CO_2 enrichment could increase the ratio of respiration to net C fixation at a given rate of C input either by increasing the slope of the relationship between respiration and C input (sensitivity of respiration to a given change in net C fixation) or by increasing the intercept of the respiration–C fixation relationship (respiration in the absence of net C input or basal respiration rate). To distinguish unique from shared contributions of daily changes in temperature and net C fixation to changes in respiration, we used residual or sequential regression (Graham, 2003).

Biomass production and net C uptake of the mesic grassland we studied increased substantially as CO_2 concentration was increased from near 200 to 560 $\mu mol\,mol^{-1}$ (Mielnick *et al*., 2001; Gill *et al*., 2002;

Polley *et al.*, 2003). Much of the additional C that was fixed at elevated CO_2 was partitioned to rapidly cycling pools in soil, however (Gill *et al.*, 2002, 2006). Increasing CO_2 from subambient to elevated concentrations reduced soil water depletion during droughts and improved mid-day xylem potentials of dominant species, but neither effect was large (Polley *et al.*, 2002). Consistent with these results, we hypothesized that increasing CO_2 from subambient to elevated levels would increase ecosystem respiration rates by increasing net C fixation and the sensitivity of respiration to changes in both C influx and temperature, as may result from a change in the partitioning of photosynthate among C pools.

Material and methods

CO_2 chambers/research site

We used elongated field chambers to expose 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 *et al.*, 2000). The grassland had been grazed by cattle for at least 50 years. Cattle were excluded in 1992 before construction of chambers. 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 *Solanum dimidiatum* Raf. and *Ratibida columnaris* (Sims) D. Don. The contribution of *Bothriochloa* to end-of-season biomass declined from almost 60% following the first season of CO_2 treatment (1997) to 24–25% in 1999 and 2000 coincident with an increase in abundances of perennial forbs (Polley *et al.*, 2003). Annual precipitation at the study site averages 879 mm (89 years mean). Annual rainfall was greater than average during 1997, 1998, and 2000 (1143, 1043, and 903 mm), but was only 52% of the mean during 1999 (461 mm). Soils at the site are classified as fine-silty, carbonatic, thermic Udorthentic Haplustolls. The surface 0.4 m of soil is composed mostly (55%) of clay.

The CO_2 facility consisted of two transparent, tunnel-shaped chambers spaced 1.5 m apart and aligned parallel along a north–south axis (Johnson *et al.*, 2000). Each chamber consisted of a series of 10 consecutive compartments that were 1 m wide and tall and 5 m long, each separated from adjacent compartments by ducts containing chilled-water cooling coils. Air was blown through chambers with fans mounted at the north and south extremes of each chamber. During daylight, pure CO_2 was injected into air blown into the south end of one chamber to initiate a superambient CO_2 gradient (560–350 μmol mol^{-1}). Ambient air was introduced to the south end of the second chamber to initiate a subambient CO_2 gradient (365–200 μmol mol^{-1}). The direction of air flow was reversed at night, and nighttime CO_2 concentrations were regulated about 150 μmol mol^{-1} above daytime values along each chamber. Ambient air was blown 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 vapor pressure deficit (VPD) were regulated near ambient values by cooling and dehumidifying air at 5 m intervals along chambers as it passed through chilled-water cooling coils. Soil beneath chambers was separated from surrounding soil to a depth of 0.9 m with a rubber-coated fabric that was installed in a trench dug along the perimeter of each 5 m compartment.

Irrigation equivalent to rainfall was applied to the chambered grassland through a metered surface irrigation system. Volumetric soil water content was measured at 0.15 m depth increments to 1.35 m depth in the center of chamber compartments using neutron attenuation (Model 503DR, Campbell Pacific Nuclear, CA, USA). Weekly measurements of soil water were interpolated to estimate soil water content on each day.

Although CO_2 gradients were not replicated here, our approach has the advantage of providing information on the shape of ecosystem responses to a large gradient in CO_2 concentration. During daylight, the air temperature inside chambers typically was slightly cooler and the VPD of air was lower than measured outside of chambers, but neither temperature nor VPD varied systematically among chamber compartments (among CO_2 treatments; Johnson *et al.*, 2000).

CO_2 flux measurements

Net ecosystem exchange (NEE) of CO_2 was calculated for each of the twenty 5 m long compartments of chambers as the product of the CO_2 gradient and the rate of air flow (Mielnick *et al.*, 2001). The CO_2 concentration was measured once each 20 min at the air inlet and air outlet of each 5 m long compartment with an infrared gas analyzer (Model 6262, Li-Cor, Inc., Lincoln, NE, USA). The rate of air flow through each compartment was calculated from regressions relating flow rate to the number of revolutions of the fan that moved air through chambers. Regressions were developed for each 5 m compartment and direction of air flow (daytime, night) from independent measurements

of volumetric air flow rate at different fan speeds (Mielnick *et al.*, 2001). Air temperature at canopy height was measured each minute at the air inlet and air outlet of each 5 m long compartment. Twenty-minute averages of air temperature in each compartment were calculated from these measurements. Net CO_2 fluxes for each compartment were calculated every 20 min during growing seasons (days 64–335) of each of the final 2 years of CO_2 exposure (1999 and 2000). Means of nighttime air temperature (Ta) and ecosystem respiration rate (Re) were calculated by averaging 20 min measurements between 24:00 and 04:00 hours. By limiting nighttime data to the 4 h period following midnight each day, we eliminated possible confounding effects of transitions between daylight and darkness on estimates of Re. Plant photosynthesis provides the substrate for respiration, so we calculated the mean rate of daytime net CO_2 uptake (net ecosystem photosynthesis; Pn) each day by averaging 20 min measurements between 07:00 and 18:00 hours. We excluded days on which flux or temperature measurements were not available because of equipment malfunctions or because chambers were uncovered for maintenance or vegetation studies. Respiration rates for compartments located at the northern extremes of chambers, the compartments to which air was introduced at night, often were unstable and were excluded from analyses. The manifold through which air was introduced to chambers was smaller at the northern than southern end of the system. The CO_2 readings at the northern extreme of chambers thus were poorly buffered from the influence of winds and were more variable than elsewhere in chambers. Data from 215 days in 1999 and 218 days in 2000 were used in analyses (about 80% of days during the growing season).

Data analysis

Simple linear regressions were fit to relationships between seasonal means of Re and Pn across CO_2 treatments and, for each CO_2 concentration, to the relationship between daily values of Re and Pn during the preceding daylight period. Significance tests for slopes and intercepts were based on the t distribution (Weisberg, 1980). Slopes and intercepts were compared among regressions with the F statistic. For each 5 m long compartment of chambers (each CO_2 concentration), we also used multiple regression to model the relationship between daily means of Re and three explanatory variables (predictors): soil water content to 1.35 m depth, Ta during the night, and Pn during the preceding daylight period. Because each predictor was correlated with other of the predictors in our model (predictors were collinear), each explanatory variable has both a unique and shared contribution to changes in Re. The partial regression coefficients calculated in traditional multiple regression models do not account for the shared contributions of predictors. To distinguish unique from shared contributions of explanatory variables to changes in Re, we used residual or sequential regression (Graham, 2003). In sequential regression, explanatory variables must be ranked by functional importance. Variables deemed to be functionally more important than others are assigned priority over shared contributions. We assigned priority over shared contributions to the variable with the greatest unique contribution to variance in respiration. A standard partial regression coefficient (β or path coefficient; Li, 1975; Sokal & Rohlf, 1981) was calculated for each year and CO_2 treatment to define the unique contribution of each predictor variable (Ta, Pn, soil water) to Re. β coefficients were calculated for each predictor variable as the product of the conventional partial regression coefficient and the ratio of the standard deviation of the predictor variable to the standard deviation of the dependent variable (Re). Standard partial regression coefficients were compared to judge the relative effects of predictor variables on Re.

Preliminary analyses indicated that soil water content explained little of the day-to-day variance in Re, so soil water was eliminated from the sequential model. For each CO_2 treatment, Re was modeled as an exponential function of Ta and as a linear function of residuals from a regression of Pn on Ta (Pr) [$Re = B_o + ae^{bTa} + B_1 \times Pr + error$; where a, b, B_o, and B_1 are regression coefficients and Pr represents variance in Pn not explained by Ta] using the PROC NLIN procedure in SAS (SAS Institute Inc., Cary, NC, USA).

Results

Relationship between respiration and C uptake

There was considerable scatter in the relationship between seasonal means of respiration rate (Re) and CO_2 concentration in both 1999 and 2000 (Table 1). Chambers were constructed parallel along a north–south axis, so neither landscape position nor position within chambers consistently affected fluxes. Rather, variability in the Re–CO_2 relationship mirrored variability in biomass–CO_2 relationships (not shown). Aboveground biomass was a significant linear or curvilinear function of CO_2 concentration during the first 2 years of treatment (1997 and 1998; see Polley *et al.*, 2003). The variance in biomass explained by CO_2 declined significantly during 1999 and 2000, coincident with a dramatic increase in the heterogeneity of plant species composition among CO_2 concentrations that

Table 1 Seasonal means of nighttime respiration rate during 1999 and 2000 as a function of the mean daytime CO_2 concentration ($[CO_2]$) to which grassland plots were exposed

	Respiration rate ($mg\,C\,m^{-2}\,h^{-1}$)	
$[CO_2]$ ($\mu mol\,mol^{-1}$)	1999	2000
556	163.8	148.8
548	210.7	216.0
536	169.7	189.3
521	190.0	219.9
504	238.7	255.9
483	242.2	248.2
459	246.7	291.1
433	289.0	299.4
403	283.3	315.9
360	87.8	123.1
347	142.2	131.4
333	116.9	94.5
319	101.5	118.9
303	98.5	94.0
287	105.0	114.2
270	97.1	93.2
252	103.5	126.4
234	98.6	118.0

Each respiration rate is the mean of observations over 215 days in 1999 and 218 days in 2000.

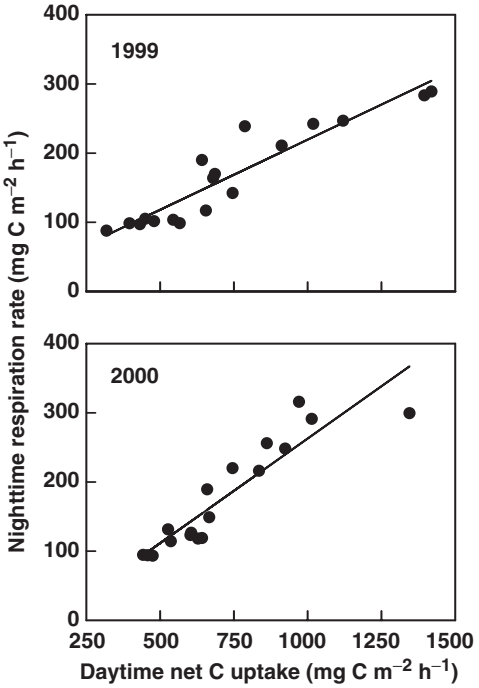

Fig. 1 Seasonal means of nighttime respiration rate as a function of the mean rate of daytime net C uptake ($n = 18$) for grassland plots exposed to mean daytime CO_2 concentrations from 234 to 556 $\mu mol\,mol^{-1}$. Each point is the mean of observations over 215 days in 1999 and 218 days in 2000. Data were fit with linear functions (respiration rate = 16.59 + 0.20 × daytime uptake, $r^2 = 0.87$, $P < 0.0001$ in 1999 and respiration rate = −39.82 + 0.30 × daytime uptake, $r^2 = 0.83$, $P < 0.0001$ in 2000).

was not related to CO_2 treatment. This increase in compositional heterogeneity as the grassland recovered from 50 years of grazing likely contributed to variability in Re–CO_2 relationships. Nevertheless, Re was greater, on average, by more than a factor of 2 at elevated ($> 360\,\mu mol\,mol^{-1}$ CO_2) than subambient ($\leq 360\,\mu mol\,mol^{-1}$ CO_2) concentrations during both 1999 and 2000 (means = 226.0 and 105.7 $mg\,C\,m^{-2}\,h^{-1}$ at elevated and subambient CO_2 in 1999 and 242.7 and 112.6 $mg\,C\,m^{-2}\,h^{-1}$ at elevated and subambient CO_2 in 2000). Nighttime temperatures were virtually identical among CO_2 treatments when averaged over each growing season, so CO_2 enrichment increased the mean Re largely by increasing Pn. Elevating CO_2 increased Pn by greater than 60% (means = 962.3 and 509.7 $mg\,C\,m^{-2}\,h^{-1}$ at elevated and subambient CO_2 in 1999 and 891.3 and 546.3 $mg\,C\,m^{-2}\,h^{-1}$ at elevated and subambient CO_2 in 2000). During both 1999 and 2000, seasonal averages of Re were linearly correlated with means of Pn across CO_2 treatments (Fig. 1).

Significantly, the ratio of the seasonal mean of Re to the mean of Pn also increased linearly with CO_2 concentration during the growing season of each year (Fig. 2). We estimate from regression that this ratio increased by 30% (from 0.196 to 0.255) in 1999 and by 47% (from 0.194 to 0.285) in 2000 as CO_2 rose from 225 to 550 $\mu mol\,mol^{-1}$. For a given linear relationship between Re and Pn, the ratio of Re to Pn will vary with Pn if the regression intercept differs from zero. The Re/Pn will decrease as Pn increases if the intercept is positive. Conversely, Re/Pn will increase as Pn increases if the intercept is negative. The intercepts of regressions of seasonal means of Re on averages of Pn (Fig. 1) did not differ significantly from zero in either 1999 or 2000 (*t*-tests, df = 16; $P = 0.30$ and 0.12, respectively), meaning that the ratio of Re to Pn did not vary consistently with Pn when evaluated across CO_2 concentrations. Clearly then, the greater ratio of Re to Pn at elevated CO_2 (Fig. 2) resulted from more than just an increase in C uptake at elevated CO_2.

CO_2 enrichment also increased both the sensitivity of Re to changes in Pn (change in Re per unit of change in Pn) and the calculated rate of C efflux in the absence of C uptake (basal ecosystem respiration rate). For each CO_2 treatment and year, we fit a simple linear regression to the relationship between daily values of Re and Pn. The intercept of each regression was significantly greater than zero (*t*-tests, df = 213 in 1999 and df = 216 in 2000; $P < 0.0001$), indicating that at each CO_2

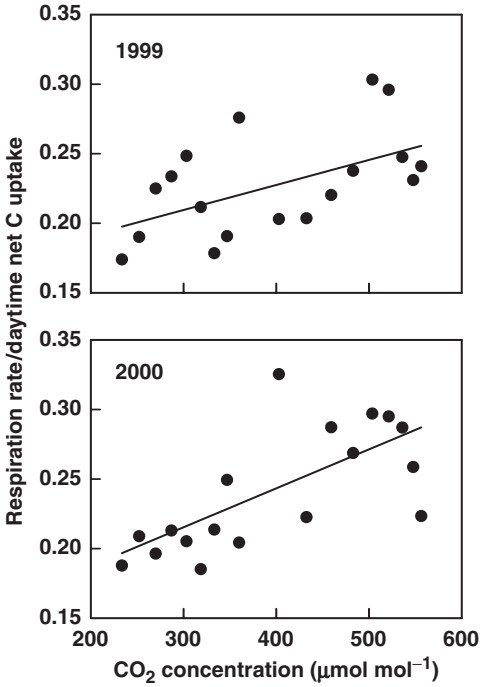

Fig. 2 The ratio of the seasonal mean of nighttime respiration rate (Re) to the mean rate of daytime net C uptake (Pn) as a function of the mean daytime CO_2 concentration to which grassland plots were exposed. The values of Re and Pn used to calculate each point are averages of observations over 215 days in 1999 and 218 days in 2000. Data were fit with linear functions ($Re/Pn = 0.1555 + 1.8 \times 10^{-4} \times CO_2$; $r^2 = 0.25$, $P = 0.02$ in 1999 and $Re/Pn = 0.1313 + 2.8 \times 10^{-4} \times CO_2$, $r^2 = 0.45$, $P = 0.001$ in 2000).

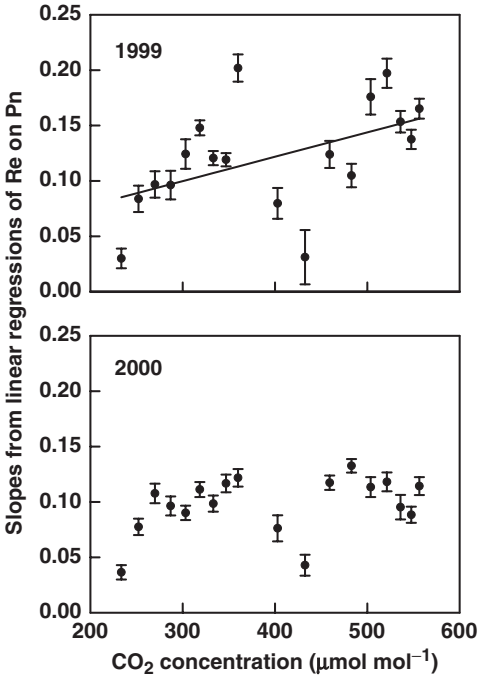

Fig. 3 Slopes from simple linear regressions of daily means of nighttime respiration rates on rates of net C uptake during the preceding daylight period (slopes are unitless; mean $n = 215$ days in 1999 and 218 days in 2000) as a function of the mean daytime CO_2 concentration to which grassland plots were exposed. Bars indicate ± 1 SE of the slope at each CO_2 concentration. Data from 1999 were fit with a linear function ($slope = 0.0338 + 2.2 \times 10^{-4} \times CO_2$; $r^2 = 0.20$, $P = 0.04$, $n = 18$). Slopes were not related to CO_2 in 2000 ($P = 0.16$).

concentration the ratio of Re to Pn decreased as Pn increased during each season. With one exception (at 433 μmol mol^{-1} in 1999), regression slopes also were significantly greater than zero (*t*-tests, df = 213 in 1999 and df = 216 in 2000; $P < 0.0008$). In both 1999 and 2000, slopes and intercepts of regressions of daily values of Re on Pn differed significantly among CO_2 concentrations [$F_{(17,3834)} > 13.3$, $P < 0.001$ in 1999 and $F_{(17,3888)} > 14.0$, $P < 0.001$ in 2000]. Regression slopes increased as a linear function of CO_2 treatment in 1999 ($P = 0.03$, $r^2 = 0.20$, $n = 18$; Fig. 3). As estimated from this linear relationship, the change in Re (mg C m^{-2} h^{-1}) per unit of change in Pn (mg C m^{-2} h^{-1}) almost doubled in 1999 as CO_2 was increased from 225 to 550 μmol mol^{-1} (from 0.083 to 0.155). The intercepts of regressions of daily values of Re on Pn were not related to CO_2 in 1999 ($P = 0.27$; not shown). The slopes of regressions of Re on Pn also did not change significantly with CO_2 in 2000 (Fig. 3), but the intercepts of regressions (basal ecosystem respiration rate) were greater on average by more than a factor of two across elevated than subambient CO_2 levels (157.0 and 61.1 mg C m^{-2} h^{-1}, respectively). At a given value of Pn, therefore, the ratio of daily values of Re to Pn also was greater on average at elevated than subambient CO_2 in 2000. As calculated from linear regressions developed for each CO_2 concentration, the ratio of Re to Pn was 72% greater across elevated than subambient CO_2 concentrations (0.31 and 0.18, respectively) at 730 mg C m^{-2} h^{-1} of Pn (approximately the seasonal mean of daytime uptake across CO_2 treatments).

Temperature and C uptake as predictors of respiration

The variance in daily means of Re explained by Pn in simple regressions could include variance that is shared with Ta if changes in Pn and Ta were correlated. Indeed, both daily values and weekly averages of Pn and Ta were highly correlated. In 1999, for example, the product–moment correlation between daily values of Pn and Ta averaged 0.56 across CO_2 treatments ($n = 18$). Ecosystem respiration rates (Re) increased during the spring of each year to a maximum between days 150 and 200 and declined during autumn, consistent with

seasonal trends of Pn and Ta but largely independent of changes in soil water content (Fig. 4).

β coefficients were calculated for each year and CO_2 treatment to determine the unique or independent contributions of Pn and Ta to variation in Re. Temperature accounted for twice the daily variance in Re as did Pn during 1999 (β coefficients averaged 0.546 and 0.273 for Ta and Pn, respectively, across CO_2 concentrations) and for >40% more of the day-to-day variance in Re than Pn during 2000 (β coefficients averaged 0.503 and 0.348 for Ta and Pn, respectively, across CO_2 treatments).

In order to determine the combined effects of Pn and Ta on Re, we used a sequential regression model in which the variance shared between predictor variables (Pn and Ta) was assigned to the variable (Ta) with the greatest unique contribution to Re. Re was modeled as an exponential function of Ta and as a linear function of residuals from a regression of Pn on Ta (Pr) [$Re = B_o + ae^{bTa} + B_1 \times Pr + error$; where Pr represents variance in Pn not explained by Ta]. This regression explained an average of 56% (1999) and 49% (2000) of the variance in daily values of Re across CO_2 concentrations ($n = 18$).

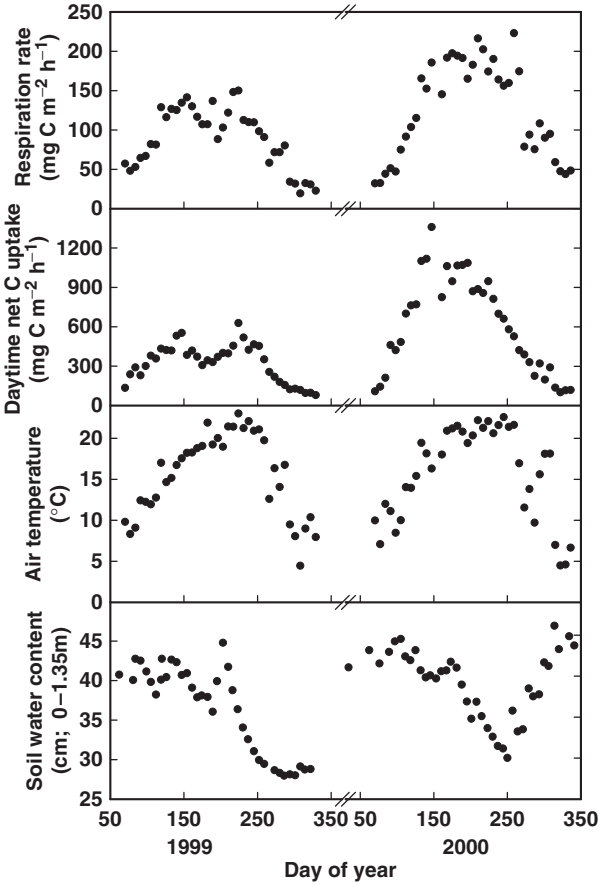

Fig. 4 Weekly averages of nighttime respiration rate, the rate of daytime net C uptake, air temperature at night, and soil water content to 1.35 m depth during the 1999 and 2000 growing seasons for a grassland plot exposed to the current CO_2 concentration (mean = 360 μmol mol^{-1}).

The coefficient describing the exponential relationship between Re and Ta in our model (b) did not vary significantly with CO_2 concentration in either year ($P > 0.26$), indicating that CO_2 did not affect the sensitivity of respiration to daily changes in temperature. The temperature coefficient (mg C m^{-2} h^{-1} °C^{-1}) averaged 0.065 in 1999 and 0.053 in 2000 ($n = 18$). The Q_{10} values calculated from temperature coefficients at each CO_2 concentration averaged 1.94 in 1999 and 1.72 in 2000 across CO_2 treatments.

Statistically controlling for collinearity between Pn and Ta reduced the effect of changes in Pn on Re, as measured by regression slopes [B_1 in our model (unitless); mean ± SE = 0.0050 ± 0.0010 in 1999 and 0.0049 ± 0.0005 in 2000], and weakened the influence of CO_2 on the response of Re to change in Pn. The slope of the regression between Re and the unique effect of Pn (B_1) generally increased as CO_2 rose to about 300 μmol mol^{-1} in both 1999 and 2000, but did not change consistently at elevated CO_2 in either year (not shown).

Discussion

One of the challenges in predicting effects of CO_2 enrichment on ecosystem-level respiration is that of resolving the multiple direct and indirect mechanisms by which respiration may be influenced by atmospheric change. As an initial step in this process, we distinguished the direct effects of CO_2 enrichment on respiration (soil plus plant) that result from changes in the availability of C substrate from indirect effects of CO_2 on the response of respiration to changes in temperature and C influx. Increasing CO_2 from subambient to elevated concentrations stimulated respiration rates of the C_3/C_4 grassland studied mainly by increasing C fixation, as evidenced by the strong, positive correlation between means of respiration rate (Re) and the net amount of C fixed during daylight (Pn). Importantly, CO_2 enrichment also stimulated Re during 1 year by increasing the sensitivity of C efflux to daily changes in Pn (by increasing the slope of the Re vs. Pn relationship) and during a second year by increasing Re in the absence of C uptake (by increasing the intercepts of linear regressions of Re on Pn). Together, these changes contributed to a linear increase in the ratio of daily values of Re to Pn as CO_2 increased from subambient to elevated concentrations. The variance in daily values of Re that was explained by Pn in simple regressions

included variance that was shared with Ta, as may have resulted because the phenology of Pn capacity tracked seasonal changes in Ta. Statistically removing this shared variance, thus, provides a conservative estimate of the influence of changes in substrate availability on rates of C efflux. Removing variance shared with Ta reduced the effect of changes in Pn on Re, as measured by regression slopes, and weakened the influence of CO_2 on the response of Re to change in Pn, indicating that variance shared with Ta contributed substantially to the overall effect of variation in Pn on Re.

The strong correlation between Re and Pn in this grassland confirms the growing appreciation that soil and ecosystem respiration are regulated by the availability of recently fixed C (Janssens *et al*., 2001; Campbell *et al*., 2004; Knohl *et al*., 2005). The link between respiration and C substrates is direct. C is the substrate for respiration, so first principles dictate that plant and microbial respiration respond, at least eventually, to a change in C availability. Indeed, an increase in C uptake at elevated CO_2 probably underlies many of the observations that elevated CO_2 increases rates of soil and ecosystem respiration (e.g. King *et al*., 2004; Niinistö *et al*., 2004). King *et al*. (2004), for example, found that CO_2 enrichment increased rates of soil respiration in forests, but that the CO_2 effect depended on the stage of forest development and, by implication, on CO_2 effects on C fixation. The link between respiration and C fixation also may be rapid, as has been demonstrated by clipping and girdling experiments (Högberg *et al*., 2001; Wan & Luo, 2003) and by the similarity between the isotopic composition of respired and recently fixed C (Pataki *et al*., 2003).

That CO_2 enrichment may increase the ratio of respiration to C uptake has not been reported previously, perhaps because few CO_2 studies have included continuous measurements of CO_2 fluxes. One explanation for the greater ratio of respiration to C fixation is that increasing CO_2 shifted partitioning toward more readily decomposable C substrates in soil and toward more-rapidly respired C substrates in plants (Hungate *et al*., 1997; Gill *et al*., 2002). Valentini *et al*. (2000) also invoked differences in C partitioning to explain latitudinal changes in the C balance of forests. NEE decreased with latitude across European forests because the fraction of GPP that was lost in respiration [total ecosystem respiration (TER)] increased with latitude (the ratio of TER to NEE increased). Valentini *et al*. (2000) suggested respiration increased with latitude because the fraction of easily decomposable organic matter is greater in soils from high-latitude than low-latitude forests. Enquist *et al*. (2003) observed a similar trend in ecosystem respiration. Across a variety of ecosystems, respiration at a given temperature increased with latitude because intercepts of linear relationships between ecosystem respiration and temperature increased with latitude. Autotrophic respiration alone may be sensitive to the partitioning of fixed C. Ludwig *et al*. (1975), for example, showed that at equivalent amounts of CO_2 fixed during the light period, dark respiration of leaves was more sensitive to changes in light levels than to changes in CO_2 concentration. Leaf respiration apparently depended not only on the amount of CO_2 that was assimilated, but also on how C was partitioned.

Greater CO_2 increased the ratio of Re to Pn during 1999 by increasing the change in Re per unit of change in Pn and during 2000 at a given value of Pn by increasing Re in the absence of Pn (basal ecosystem respiration rate). Why the two 'mechanisms' for greater Re/Pn responded differently to CO_2 between years is not clear, but may be related to late-season differences in water availability between 1999 and 2000 (see Fig. 4). Pendall *et al*. (2003) found that CO_2 enrichment stimulated soil respiration rates in shortgrass steppe by increasing decomposition rates of soil organic matter (SOM) (heterotrophic respiration) rather than by increasing rhizosphere respiration (autotrophic respiration). Decomposition rates were correlated with substrate availability, whereas variation in total soil respiration was linked to differences in soil water content. Our estimates of basal ecosystem respiration rates from intercepts of linear regressions of Re on Pn may approximate the heterotrophic component of soil respiration. Soil water content to 1.35 m depth explained little of the variance in daily values of Re at each of our CO_2 treatments, but given the results of Pendall *et al*. (2003) it is interesting to note that CO_2 enrichment stimulated basal respiration rates only during the wetter of the 2 years studied (2000) and increased the change in Re per unit of change in Pn only during the dry year of 1999 when CO_2 effects on plant water status and soil water content were relatively well expressed (Polley *et al*., 2002).

Atmospheric CO_2 also could indirectly affect the temperature sensitivity of respiration by altering the partitioning of C among plant and soil pools or the rate at which plants deplete soil water. Harper *et al*. (2005), for example, found that extending the interval between precipitation events reduced the temperature sensitivity of soil respiration in tallgrass prairie. Increasing CO_2 from subambient to elevated concentrations increased the partitioning of soil C into faster cycling pools (Gill *et al*., 2002) and sometimes reduced rates at which plants depleted soil water in the grassland we studied (Polley *et al*., 2002), but did not alter the temperature sensitivity of ecosystem respiration. Similarly, King *et al*. (2004) found that elevating CO_2 had no effect on the sensitivity of soil respiration to temperature in forests.

Ecosystem-level respiration represents the sum of CO_2 effluxes from multiple pools of C, including C located in plants, aboveground litter, decaying roots, root exudates, and SOM. The average period during which C resides in each pool before being respired differs among organic fractions (Luo *et al*., 2003). Fixed C that is allocated to rapidly cycling pools (e.g. plant respiration, root exudates) is quickly respired. C that is allocated to pools that turnover more slowly (e.g. root production, SOM) may not be respired for years or decades. If a significant fraction of C is cycled through pathways that turnover slowly, respiration will be relatively insensitive to daily variation in net C uptake. Conversely, if most C cycles through rapidly dissipated pools, short-term variation in C influx will elicit similar variation in respiration.

In sequential regressions, we used the amount of C fixed during the daylight period immediately preceding respiration measurements (Pn) as an index of the amount of C available for respiration from this grassland. As is evident in the seasonal trends in C fluxes, Pn on any 1 day was highly correlated with Pn on preceding days, weeks, and possibly months. It is difficult, therefore, to determine from these data alone the immediacy with which fixed C was respired from this grassland. Nevertheless, it is evident from regression coefficients of Re on C fixed during the preceding daylight period that the respiration rate was sensitive to recently fixed C.

Our results imply that CO_2 enrichment reduced the mean residence time of C in this grassland. Results are consistent with independent evidence from this grassland that CO_2 enrichment increased the partitioning of soil C to faster cycling pools (Gill *et al*., 2002, 2006) and with evidence from a terracosm study that elevated CO_2 stimulated the release of recently fixed C (Lin *et al*., 1999). By contrast, an inverse analysis of data from the Duke Free-air CO_2 Enrichment experiment revealed that CO_2 treatment had no effect on the mean residence time of C in a loblolly pine forest (Luo *et al*., 2003).

Our analysis does not address the issue of C sequestration directly, but the amount of organic matter that can be stored in ecosystems is strongly influenced by the residence time of C (Luo *et al*., 2003). The capacity of an ecosystem to store C depends both on the rate at which C is added to a system (C influx) and on how quickly fixed C is dissipated in respiration (the inverse of the mean residence time of C). The more rapidly C is recycled back to the atmosphere, the smaller is the potential increase in C sequestration. In the grassland we studied, CO_2 enrichment apparently reduced the residence time of C sufficiently to almost completely offset a large enhancement in C influx at elevated CO_2 (Mielnick *et al*., 2001; Gill *et al*., 2002; Polley *et al*., 2003), resulting in no significant change in soil C stocks to 15 cm depth (Gill *et al*., 2002).

Respiration rates of the C_3/C_4 grassland we studied were greater at elevated than subambient CO_2 levels because both C input and respiration per unit of C input increased with CO_2 concentration. That ecosystem respiration responds to variation in C input is well established, albeit infrequently quantified. That respiration per unit of C input also may vary with CO_2 concentration has received little attention. We suggest a better understanding of CO_2 effects on C partitioning will be required to determine how past and future changes in atmospheric composition affect ecosystem C balance.

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