Direct inhibition of leaf dark respiration by elevated CO_2 is minor in 12 grassland species

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Summary

• Direct inhibition of dark respiration by elevated atmospheric concentrations of CO_2 could alter the carbon balance of plants and ecosystems. The short-term response of leaf dark respiration to elevated CO_2 concentrations are reported here in 12 grass and forb species of a North American grassland community.

• Specific respiration rates at 25°C and a range of measurement CO_2 concentrations were determined for detached leaves of each species field-grown in monoculture.

• On average, respiration rates were 1.8% lower at 700 than at 360 μmol mol^{-1} CO_2. Among species, responses ranged from a 6.4% inhibition to a 2.4% stimulation and were generally not statistically significant. Across a range of CO_2 concentrations from 360 to 1300 μmol mol^{-1}, respiration rates declined linearly and were 11% lower at 1300 than 360 μmol mol^{-1} CO_2.

• Direct inhibition of leaf respiration is small compared with other longer-term, indirect effects of CO_2 on carbon exchange. The direct effects of rising atmospheric CO_2 concentrations on respiration rates should result in minimal effects on plant carbon exchange in grasslands.

Key words: respiration, elevated CO_2, direct inhibition, grasses, forbs.

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Introduction

The magnitude and causes of a direct inhibitory effect of CO_2 on rates of dark respiration in plants have been the subject of an extended, but so far inconclusive debate. Rates of leaf dark respiration were directly and reversibly inhibited by increasing concentrations of CO_2 in some studies (Reuveni & Gale, 1985; Bunce, 1990; Amthor *et al.*, 1992; Mousseau, 1993; Thomas & Griffin, 1994; Ziska & Bunce, 1994; Teskey, 1995; Gonzàlez-Meler *et al.*, 1996; Griffin *et al.*, 1996), but not others (Ryle *et al.*, 1992; Wullschleger *et al.*, 1994; Ziska & Bunce, 1994; Mitchell *et al.*, 1995; Tjoelker *et al.*, 1999b). High concentrations of CO_2 inhibited rates of dark respiration in roots of *Pseudotsuga menziesii* (Qi *et al.*, 1994), *Acer saccharum* (Burton *et al.*, 1997), and *Pinus strobus* (Clinton & Vose, 1999), but not in *Citrus volkameriana* (Bouma *et al.*, 1997) or five boreal tree species (Tjoelker, 1997).

Although dark fixation of CO_2 by phosphoenolpyruvate carboxylase (Amthor, 1991) and suppression of the alternative pathway of plant respiration are candidate mechanisms of a direct inhibitory effect of CO_2 on respiration (Drake *et al.*, 1999), there is little evidence that these mechanisms are involved in the response of leaf respiration to CO_2 (Reuveni *et al.*, 1995; Gonzàlez-Meler *et al.*, 1996). However, in roots of hydroponically grown tomato, increases in dissolved inorganic carbon concentrations were found to inhibit CO_2 efflux through increased phosphoenolpyruvate carboxylase activity (Van der Westhuizen & Cramer, 1998). In the same study, oxygen consumption was stimulated in roots supplied with nitrate and inhibited in roots supplied with ammonium (Van der Westhuizen & Cramer, 1998), suggesting an interaction of nitrogen metabolism with respiratory enzyme regulation under elevated CO_2 concentrations.

There is evidence that elevated CO_2 concentrations inhibit the activity of mitochrondrial enzymes succinate dehydrogenase and cytochrome c oxidase (Gonzàlez-Meler *et al.*, 1996; Gonzàlez-Meler & Siedow, 1999). However, direct inhibition of this enzyme by doubling CO_2 concentrations (eg 350–

700 μmol mol^{-1}) fails to account for the 15–20% inhibition of rates of respiration observed with a doubling of CO_2 concentration in many studies (Gonzàlez-Meler & Siedow, 1999). By comparison, the direct inhibitory effect of CO_2 concentration on leaf respiration in nine temperate deciduous tree species was small, averaging a 1.5% reduction in rate at 800 compared with 400 μmol mol^{-1} CO_2 (Amthor, 2000). Other metabolic mechanisms await experimental testing (Drake *et al.*, 1999). In addition, the potential role of systematic errors in influencing measured respiratory responses to CO_2 remains unresolved (Drake *et al.*, 1999; Amthor, 2000).

The global implications of a direct suppression of rates of dark respiration in terrestrial plants by elevated concentrations of CO_2 are important given rising atmospheric concentrations of CO_2 (Houghton, 1997) and the key role dark respiration plays in plant and ecosystem-level carbon balance. Respiratory CO_2 efflux from plants may constitute over 50% of net CO_2 fixed in photosynthesis (Farrar, 1985; Tjoelker *et al.*, 1999a). At the global scale, a relatively small suppression of respiration in an elevated concentration of CO_2 would have potentially large effects on the net carbon gain by plants and ecosystems and the balance between CO_2 fluxes to terrestrial and atmospheric sinks (Gifford, 1994). For example, a 15% reduction in foliar respiration in response to a doubling of current ambient atmospheric CO_2 concentrations was estimated to contribute 3 Giga tonnes (Gt) of carbon per year to the biosphere sink in a carbon model (Drake *et al.*, 1999).

The objectives of this study were to determine if CO_2 concentration directly affected leaf dark respiration and whether taxonomically diverse grass and forb species of a grassland community differed in this regard. To this end, we selected 12 species common to temperate prairie grassland communities of North America and part of a free-air carbon dioxide enrichment (FACE) experiment in Minnesota, USA. The question of direct effects of CO_2 on plant respiration is important for this FACE experiment, since atmospheric CO_2 concentrations are enriched only during daylight hours. We tested the hypothesis that a short-term increase in concentration of CO_2 would directly and reversibly inhibit rates of leaf dark respiration. We distinguish the direct short-term effect of CO_2 examined here from potential indirect effects of CO_2 that may occur as the result of acclimation of leaf structure and function when grown under elevated CO_2.

Materials and Methods

Species monoculture field plots

We obtained our plant material from field plots of a FACE experiment (BioCON, http://www.swan.lter.umn.edu/biocon/) that is exploring the response of prairie grassland species to biodiversity, elevated carbon dioxide, and soil nitrogen (Lee *et al.*, 2001; Reich *et al.*, 2001a,b). The study is at the Cedar Creek Natural History Area, a National Science Foundation, Long-Term Ecological Research site in Minnesota, USA. The region has a continental climate with cold winters, warm summers (mean January and July temperatures of −10 and 25°C), and annual precipitation averaging 660 mm. The soils are derived from a glacial outwash sand plain and are sandy and nitrogen poor.

The 12 grassland species we chose for this study included three species of each of the following groups: C_3 grasses: *Agropyron repens* (L.) Beauv., *Bromus inermis* Leysser, *Koeleria cristata* Pers.; C_4 grasses: *Andropogon gerardii* Vitman, *Schizachyrium scoparium* (Michaux) Nash, *Sorghastrum nutans* (L.) Nash; nonleguminous forbs: *Achillea millefolium* L., *Anenome cylindrica* A. Gray, *Solidago rigida* L.; legumes: *Amorpha canescens* Pursh, *Lespedeza capitata* Michaux, *Petalostemum villosum* Nutt. Bare field plots (2 × 2 m) of the various treatment combinations were seeded in July of 1997 in six large circular plots (20-m diameter), three of which serve as the FACE rings. To compare species at common growing conditions, we selected the monoculture plots of the unamended soil (without added nitrogen) and ambient CO_2 (*c.* 360 μmol mol^{-1}) treatment combination from the larger study. Thus, two replicate monoculture plots of each species from among the three ambient-CO_2 treatment rings were available for our study.

Measurement of leaf dark respiration

To control for potential diurnal variation in leaf carbohydrate status, we collected leaf samples from field plots at the same time each morning (between 07.30 and 08.30 hours) on eight dates beginning 8 July and ending 18 August, 1999. From each species plot we collected five mature leaves from the upper canopy, enclosed the leaves in a plastic bag with a moistened paper towel, and placed the bags in a cooler at 11.5°C (within the range of night-time temperatures at this site). In the laboratory we transferred the sample bags to a darkened controlled-environment chamber (Conviron E15, Controlled Environments, Inc., Winnipeg, Man., Canada) at 25°C at least 30 min before measurement.

Individual species were sampled repeatedly across the eight measurement days. On a given measurement date one plot was sampled from each of 11 species or subset. Across days, four to six samples were obtained for each species, except for *Lespedeza capitata* ($n = 2$) for which fewer plants were available. Plots were selected, sampled, and analysed in random order. We completed all measurements the same day, on average, 6 h since sampling and within an average maximum of 9 h (range 4–13 h across days). Same-slopes analysis of covariance of the pooled data revealed that time since sampling had no effect on specific respiration rates ($P = 0.13$) or response to CO_2 concentration ($P = 0.99$).

We conducted a separate study of specific respiration rates of leaves collected from the same plots and other plots of the larger study on 6 and 7 July, 1999. Using the same sampling protocol described above including the period of dark storage,

we measured specific respiration rates at a standard CO_2 concentration (360 μmol mol^{-1}) and temperature (25°C). Specific respiration rates were comparable with those of the present study. After measurement of specific respiration rates, measured values of mean soluble sugar concentration of the same samples ranged from 3.7 to 14.3% of leaf dry mass among the species (data not shown), values at least as high as commonly observed in other field studies. These data confirm that carbohydrates had not been depleted in the dark using our sampling protocol. In addition, linear regression analysis revealed that the mean response of respiration to CO_2 concentration appeared unrelated to mean soluble sugar concentration among species ($P = 0.88$).

Rates of net CO_2 exchange were measured using a portable photosynthesis system, equipped for automatic CO_2 control (CIRAS-1, PP Systems, Hitchin, UK) and operated in the differential mode. We used a standard cuvette (PLC-conifer, PP Systems). Samples were placed into the cuvette in the darkened growth chamber at 25°C. For two samples of each species we determined rates of dark CO_2 efflux at each of five target CO_2 concentrations of 360, 550, 700, 1000, and 1300 μmol mol^{-1} in random order for each sample. For the other samples ($n = 2–4$) of each species, rates were determined only at 360 and 700 μmol mol^{-1} CO_2. Samples were exposed to each CO_2 concentration for typically 10–12 min before recording a measurement. After measurement, we dried the tissues in a forced-air oven (65°C), determined their masses, and calculated specific respiration rates on a dry mass basis (Rs, nmol g^{-1} s^{-1}).

Rates of respiratory CO_2 efflux from individual leaves are low, increasing the difficulty of accurately measuring small changes in CO_2 flux, especially in differential analysis of CO_2 concentrations. To check for leaks, we periodically confirmed that the net flux rates of CO_2 in an empty and dry cuvette were zero throughout the range of measurement CO_2 concentrations. By measuring detached leaves, we controlled for potential diffusive leaks of CO_2 by completely sealing the sample in the cuvette during measurement (Amthor, 2000). In addition, measuring multiple leaves from each plot together rather than individually increased the measured CO_2 differentials, therefore increasing the signal to noise ratio.

Environmental factors, such as temperature, or leaf water and carbohydrate status could influence specific rates of respiration and perhaps responsiveness to CO_2 concentration. By sampling leaves at the same time early each morning, we controlled for potential diurnal variation in carbohydrate status. By darkening, cooling, and enclosing the samples in plastic bags, the leaves remained turgid and respiratory carbon losses minimized. Measuring the leaves in the laboratory in a growth chamber ensured identical temperature treatments prior to and during respiration measurements, controlling for both the short-term temperature sensitivity of respiration and temperature acclimation of specific respiration rates (Tjoelker *et al.*, 1999b; Atkin *et al.*, 2000). Prior checks in our laboratory and elsewhere have revealed that measured rates of dark CO_2 efflux do not differ between attached and detached foliage of a number of species, and may remain stable even after 12 h in the dark (Mitchell *et al.*, 1999).

Data analysis

In comparing specific respiration rates determined at 360 and 700 μmol mol^{-1} CO_2, we included data of the samples measured at the five CO_2 concentrations. To compare the effect of CO_2 concentration on respiration rates, we calculated the proportional change (%) in rates at 700 compared with the rates determined at 360 μmol mol^{-1} CO_2 for each sample ((700 − 360) : 360 *100). We used two-tailed *t*-tests to test the null hypothesis ($\mu = 0$) of no difference in respiration rates between the paired measurements (360 and 700 μmol mol^{-1} CO_2) for each species separately.

We used ANOVA and regression to examine respiration response to CO_2 across the five target measurement concentrations (360–1300 μmol mol^{-1}). Since measures at the various CO_2 concentrations were repeated on individual samples, the ANOVA included both between-sample and within-sample variation. We used separate and same-slopes analysis of covariance to test for homogeneity of slopes and equality of intercepts in examining species differences in the response of specific respiration rates to CO_2 concentration.

Results

Among the 12 species the response of leaf dark respiration to a short-term change in CO_2 concentration between 360 and 700 μmol mol^{-1} ranged from an apparent 6.4% inhibition in *P. villosum* to 2.4% stimulation in *Agropyron repens* (Table 1). However, with the exception of *P. villosum*, none of the individual species responses at these CO_2 concentrations were statistically significant based on two-tailed *t*-tests of percent change in the paired measurements ($P > 0.23$). Pooling all data, mean specific respiration rate was 1.8% lower when measured at 700 compared with 360 μmol mol^{-1} CO_2 (Table 1, $n = 57$, $P > |t| = 0.02$). Species did not differ in response of specific respiration rate to CO_2 concentration (ANOVA, Species × CO_2 effect, $P = 0.51$).

Mean specific rates of respiration of all species pooled differed among the wider range of five target measurement CO_2 concentrations from 360 to 1300 μmol mol^{-1} (ANOVA, CO_2 effect, 4 df, $P < 0.0001$). The interaction effect of species and measurement CO_2 concentration was not statistically significant ($P = 0.69$). Moreover, separate-slopes analysis of covariance revealed that slopes of the CO_2-response relationships were homogeneous among species ($P = 0.41$), suggesting that the species responded similarly to the measurement CO_2 concentrations. The slope estimate was negative ($P > |t| < 0.0001$), indicating that respiration rates declined linearly with increasing measurement CO_2 concentration.

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Table 1 A test of direct inhibition (% change) of specific dark respiration rates (Rs) in leaves at elevated (700 μmol mol^{-1}) compared with ambient (360 μmol mol^{-1}) CO_2 concentrations for 12 grassland species

		Rs (nmol g^{-1} s^{-1})		% change* at 700 vs 360 μmol mol^{-1} CO_2 with 95% confidence intervals			
Species	*n*	360	700	Mean	Lower	Upper	$P > \|t\|$
C_3 grass							
Agropyron repens	4	15.7	16.1	2.4	−10.1	14.9	0.58
Bromus inermis	5	13.5	13.3	−1.5	−6.3	3.4	0.45
Koeleria cristata	4	12.4	12.4	0.1	−10.1	10.4	0.97
C_4 grass							
Andropogon gerardii	6	17.4	17.4	−0.3	−5.4	4.8	0.89
Schizachyrium scoparium	5	15.3	14.5	−4.9	−14.6	4.7	0.23
Sorghastrum nutans	6	16.8	16.5	−2.0	−8.4	4.4	0.46
Forb							
Achillea millefolium	6	15.0	14.7	−1.9	−6.5	2.7	0.33
Anenome cylindrica	4	13.1	12.8	−2.3	−13.8	9.3	0.58
Solidago rigida	6	12.1	12.0	−1.4	−4.7	1.8	0.31
Legume							
Amorpha canescens	5	17.4	17.2	−1.5	−7.5	4.6	0.54
Lespedeza capitata	2	26.6	26.0	−2.0	−63	59	0.75
Petalostemum villosum	4	20.7	19.4	−6.4	−11.9	−0.9	0.03
Mean	57	15.8	15.5	−1.8	−3.2	−0.3	0.02

*Percent change in specific respiration rates measured at 360 and 700 μmol mol^{-1} CO_2 and 25°C were calculated for each sample as: (700–360) : 360 *100. Positive values indicate an apparent stimulation, whereas negative values indicate an apparent inhibition of respiration. A two-tailed *t*-test based on paired comparisons was used.

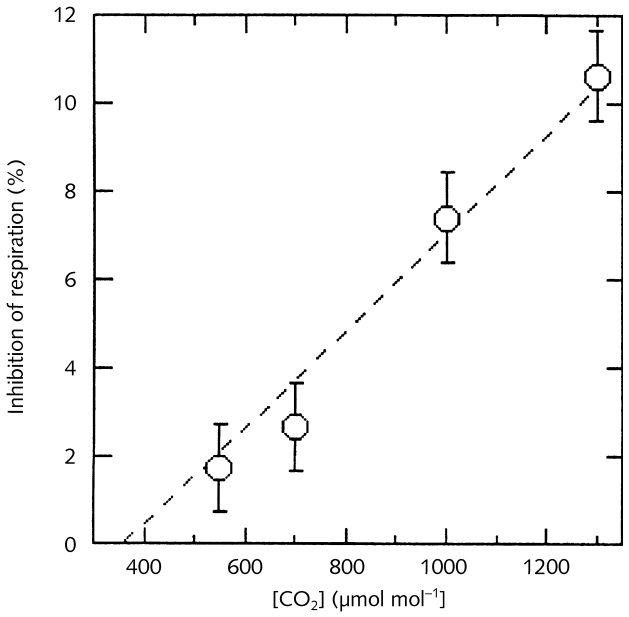

Fig. 1 Apparent direct inhibition of leaf dark respiration by elevated CO_2 concentrations in 12 grassland species. Observations consisted of two replicate samples of each of 12 species measured at each of five target CO_2 concentrations of 360, 550, 700, 1000, and 1300 μmol mol^{-1}. Shown are least squares means (± 1 SE) of inhibition (% decrease) in respiration rates relative to rates measured at 360 μmol mol^{-1} CO_2. The linear regression fit without an intercept is: $y = 0.0110*([CO_2]-360)$.

The apparent inhibitory effect of CO_2 on specific respiration rates for all species together was a linear function of external, measurement CO_2 concentration. Mean specific respiration rates were lower by 1.7% at 550, 2.7% at 700, 7.4 at 1000 and 10.6% at 1300 compared with 360 μmol mol^{-1} CO_2. We used linear regression forced through the origin (ie 360 μmol mol^{-1} CO_2) to estimate the slope of the relationship between percent inhibition and CO_2 concentration relative to rates determined at 360 μmol mol^{-1} CO_2. Overall, inhibition of respiratory CO_2 efflux increased linearly with increasing CO_2 concentration from 360 to 1300 μmol mol^{-1} (Fig. 1).

Discussion

By contrast to a number of studies showing a substantial direct inhibitory effect of CO_2 concentration on specific rates of leaf respiration, we found evidence for a small inhibition detectable only at very high CO_2 concentrations. Recent reviews suggest that a doubling of current atmospheric CO_2 concentrations, on average, may reduce specific respiration rates by 15–20% through a short-term direct effect (Drake *et al.*, 1999; Gonzàlez-Meler & Siedow, 1999). In the 12 grassland species in our study, the direct effects of CO_2 on respiration at twice current ambient concentrations were small, averaging less than a 2% inhibition overall, and were well within the range of sampling error and not statistically significant for all but one species. At 1300 μmol mol^{-1} CO_2, specific respiration rates were inhibited

by 11% compared with rates at 360 μmol mol^{-1} CO_2. The magnitude of the effects observed here concur with those of Amthor (2000) who reported a 1.5% decrease in specific respiration rates of nine temperate deciduous tree species at 800 compared with 400 μmol mol^{-1} CO_2. Direct inhibitory effects of CO_2 concentration on dark respiration of this magnitude are perhaps reconcilable to observed direct effects of CO_2 in inhibiting cytochrome c oxidase (Gonzàlez-Meler & Siedow, 1999).

Given the CO_2 treatments in the BioCON FACE experiment of 368 and 550 μmol mol^{-1} CO_2, a 2.1% decrease in specific respiration rate was predicted (mean of 1.7% ± 1.0 SE) at 550 compared with 368 μmol mol^{-1} CO_2, based on the linear regression relationship across the wide range of measurement CO_2 concentrations (Fig. 1). Compared at 700 μmol mol^{-1} CO_2, the mean value of inhibition (% decrease) was 1.8% (0.3, 3.2, 95% confidence interval (CI)) overall (Table 1) and 2.7% (0.8, 4.7, 95% CI) in the regression dataset (Fig. 1). However, these estimates did not differ statistically, owing to large confidence intervals. Thus, we conclude that the lack of CO_2 enrichment at night likely has small short-term direct effects on plant respiration and carbon balance in BioCON (Reich *et al.*, 2001a,b). However, our findings do not preclude the potential for longer term, indirect effects of night-time CO_2 concentration on plant growth, as observed, for example, in soybean (*Glycine max*; Griffin *et al.*, 1999).

Our protocol used detached leaves to standardize sampling time, control temperature, and reduce potential errors owing to leaks and low flow rates. Might detached leaves differ from attached leaves in response of specific respiration rates to CO_2 concentration? We are not aware of a direct comparison in the literature. The minimal response of specific respiration to CO_2 concentration we found was comparable with at least one other study that measured attached leaves and controlled for potential cuvette leaks (Amthor, 2000). Detached leaves could differ in their carbon and water status compared with attached leaves. However, diurnal changes in leaf carbon status as well as air temperature influence respiration rates of attached leaves, even when measured at a standard temperature (Atkin *et al.*, 2000). We confirmed that the cooled and darkened detached leaves were not depleted in nonstructural carbohydrates and appeared turgid at measurement. Despite a wide range in mean soluble carbohydrate concentrations among species in these plots in a prior check, species did not differ in response of respiration to CO_2 concentration, suggesting that differences in carbohydrate concentrations among species were unrelated to direct effects of CO_2 on respiration.

In summary, we found rather small, if any, direct effects of CO_2 concentration on rates of leaf dark respiration rate among 12 grassland species, and species did not differ in their responsiveness to short-term changes in CO_2 concentrations across a wide range. By comparison, acclimation of photosynthesis and respiration rates to growth in an elevated CO_2 environment has demonstrably larger effects on rates of net CO_2 exchange in a wide range of species (eg Poorter *et al.*, 1992; Curtis & Wang, 1998; Tjoelker *et al.*, 1998), including the present study (Lee *et al.*, 2001). Given the rather minor direct effects reported here compared with potential indirect effects on carbon exchange rates, it is unlikely that a direct inhibitory effect of CO_2 will substantially influence rates of respiratory CO_2 exchange in these grassland species in a future, higher CO_2 world.

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