



Root system response of C₄ grass seedlings to CO₂ and soil water**

J.D. Derner¹, H.W. Polley, H.B. Johnson & C.R. Tischler

Grassland, Soil & Water Research Laboratory, USDA-ARS, 808 East Blackland Road, Temple, Texas 76502-9601, USA. ¹Corresponding author*

Received 10 July 2000. Accepted in revised form 2 January 2001

Key words: belowground response, carbon dioxide, C₄ grasses, fine roots, root growth, root morphology

Abstract

Aboveground growth of C₄ plants responds more strongly to atmospheric CO₂ concentration when soil water is limiting rather than abundant. Whether the same is true of root growth and morphology, however, remains to be evaluated. We investigated interactive effects of CO₂ and soil water on root growth and morphology of two C₄ grasses. Seedlings of the dominant C₄ grasses from tallgrass prairie, *Schizachyrium scoparium* and *Andropogon gerardii*, were grown for 8 weeks in an elongated, controlled environment chamber at CO₂ concentrations of 368 (ambient) and 203 (subambient) $\mu\text{mol mol}^{-1}$. Seedlings were maintained at either high (ca. 90%) or low (ca. 50%) soil relative water holding capacity (RWC). Both root and shoot systems of C₄ grass seedlings responded similarly to CO₂ enrichment irrespective of whether soil water was limiting or abundant. Root growth was affected primarily by increased RWC (40–51% increases) and secondarily by higher CO₂ (15–27% increases). The relative distribution of root surface area, number of root tips and length and volume of roots were significantly affected by CO₂ enrichment with proportional increases of 55–61%, 39–52%, 50–55% and 53–58%, respectively, occurring in very fine (0–0.3 mm) roots. The indirect effect of CO₂ enrichment on conservation of soil water in grasslands may be as important as direct photosynthetic response effects in the CO₂-induced enhancement of whole-plant growth in C₄ grasses.

Introduction

Aboveground growth of C₄ plants responds more strongly to atmospheric CO₂ concentration when soil water is limiting rather than abundant (e.g. Owensby et al., 1999), with this increase attributed directly to CO₂ enrichment (Ghannoum et al., 1997; Hunt et al., 1996; LeCain and Morgan, 1988; Morgan et al., 1994, 1988; Read et al., 1977; Sionit and Patterson, 1984) and indirectly to improved water relations resulting from 'conservation' of soil water (He et al., 1992; Hunt et al., 1996; Knapp et al., 1993; Morgan et al., 1994, 1998; Owensby et al., 1993; Sionit and Patterson, 1985). Whether the same is true of root growth and morphology, however, remains to be evaluated. In this study, we attempt to clarify the extent to which the

response of root growth of C₄ plants to an increase in CO₂ from subambient to ambient concentrations depends on soil water availability. This has implications for understanding direct and indirect impacts of global change, and important ramifications for understanding the development, global expansion and distribution of C₄ ecosystems (Cerling et al., 1997, 1998; Ehleringer et al., 1997; Jacobs et al., 1999; Pagani et al., 1999).

The few documented responses of C₄ root systems to CO₂ and soil water are inconsistent, and have been conducted using comparisons between ambient and superambient CO₂ concentrations. Unfortunately, little is known regarding the responses of C₄ root systems to subambient CO₂ concentrations. Root biomass of C₄-dominated tallgrass prairie was greater at superambient than ambient CO₂ during years when soil water was limiting, but was also increased by CO₂ enrichment during 1 of 3 years when soil water was abundant (Owensby et al., 1999). Fine root biomass

* FAX NO: +254-770-6561. E-mail: derner@brc.tamus.edu

** The U.S. Government's right to retain a non-exclusive, Royalty-free licence in and to any copyright is acknowledged.

of *Bouteloua eriopoda* increased with superambient CO₂ and abundant soil water (BassiriRad et al., 1997), while soil water content did not influence the effect of CO₂ on *B. gracilis* root growth (Hunt et al., 1996; Morgan et al., 1998). Effects of increased CO₂ on root growth are generally smaller for C₄ than for C₃ plants (Hunt et al., 1996; Morgan et al., 1998; Wand et al., 1999; Yoder et al., 2000; but see BassiriRad et al., 1997), which is consistent with aboveground responses (Poorter, 1993; Wand et al., 1999).

Widespread expansion of C₄ ecosystems at the end of the Miocene likely resulted primarily from declining CO₂ (Ehleringer et al., 1991; Jacobs et al., 1999). Cerling et al. (1998) secondarily attribute C₄ expansion to increased aridity. Pagani et al. (1999) suggest that an Asian uplift in the late Miocene, in conjunction with low CO₂ concentrations, caused changes in climate patterns that favored C₄ plant expansion. Ehleringer et al. (1997) provide support via carbon isotope signatures in sediment cores that C₄ ecosystems were more widespread during the last glacial maximum and have since decreased in abundance as CO₂ has increased. Therefore, it may be expected that recent human-induced increases in CO₂ have created conditions that are even more unfavorable for C₄ plants (Cerling et al., 1997). On the other hand, simulation studies suggest that doubling the current CO₂ concentration will result in no major changes in distributions of C₄ grasses in Australia (Howden et al., 1999). Indeed, C₄ grasses may maintain their competitive advantage over C₃ grasses with superambient CO₂ because of an increased water use efficiency response when soil water is limiting (Wand et al., 1999; Ward et al., 1999).

Our objective was to investigate the interactive effects of CO₂ and soil water on root growth and morphology of C₄ grasses. We maintained CO₂ at either subambient (ca. 200 μmol mol⁻¹) or current ambient (ca. 360 μmol mol⁻¹) concentrations and soil water at high or low levels. Subambient, rather than superambient, CO₂ was chosen to assess the possible role of low CO₂ in the global expansion of C₄ plants, and to determine the magnitude of root responses that may already have occurred as a result of past increases in CO₂.

Materials and methods

Environmental chamber

A complete description of the elongated, controlled environmental chamber used in this study is found in Mayeux et al. (1993). Briefly, the chamber consists of a tunnel formed by transparent, 100 μm polyethylene film, set upon an elongated soil container in which a subambient CO₂ gradient is created during daylight by enclosed plants [*Panicum virgatum* L., and *Stenotaphrum secundatum* (Walt.) O. Ktze.] that reduce CO₂ through photosynthesis. The chamber is located in an evaporatively cooled glasshouse at Temple, Texas, USA. Air is forced into and through the chamber with a blower, the speed of which is automatically varied by changing the DC voltage supplied to the motor. Control voltages were calculated with empirical algorithms incorporating the difference between desired (200 μmol mol⁻¹) and measured CO₂ concentration at the chamber exit and direction and the magnitude of change in photosynthetically active photon flux density (PPFD). At night, airflow rate was maintained at about 3 m³ min⁻¹ to fully exchange the volume of air every 2–5 min. This exhausts CO₂ generated by plant and soil respiration and maintains CO₂ near ambient throughout the chamber. CO₂ in the chamber was monitored with an infra-red gas analyzer (Li-Cor Model LI-6262 Li-Cor, Inc., Lincoln, Nebraska, USA). The gas analyzer was calibrated daily at noon and midnight using a CO₂ standard. Measurements alternate between the chamber exit and one of five other locations along the chamber each minute.

Air temperature along the chamber was measured at the same locations as CO₂ with fine-wire (25 μm diameter) thermocouples. PPFD was measured on the glasshouse roof with a quantum sensor (LI-190SB, Li-Cor).

Sampling methods

Schizachyrium scoparium (Michx.) Nash and *Andropogon gerardii* Vitman. seedlings were grown in a sandy loam soil (Alfisol, Udic Paleustalfs; Huckabee et al., 1977) in 0.05 m diameter × 0.60 m deep pots. Properties of the soil include: pH=7.1, organic carbon content=0.57%, 76.2% sand, 16.2% silt, 7.6% clay, field capacity=18% on a volumetric basis. Pots were constructed from polyvinyl chloride pipe cut longitudinally into two pieces of equal size to facilitate recovery of intact root systems. The two halves of each pot were taped together and secured at the base with

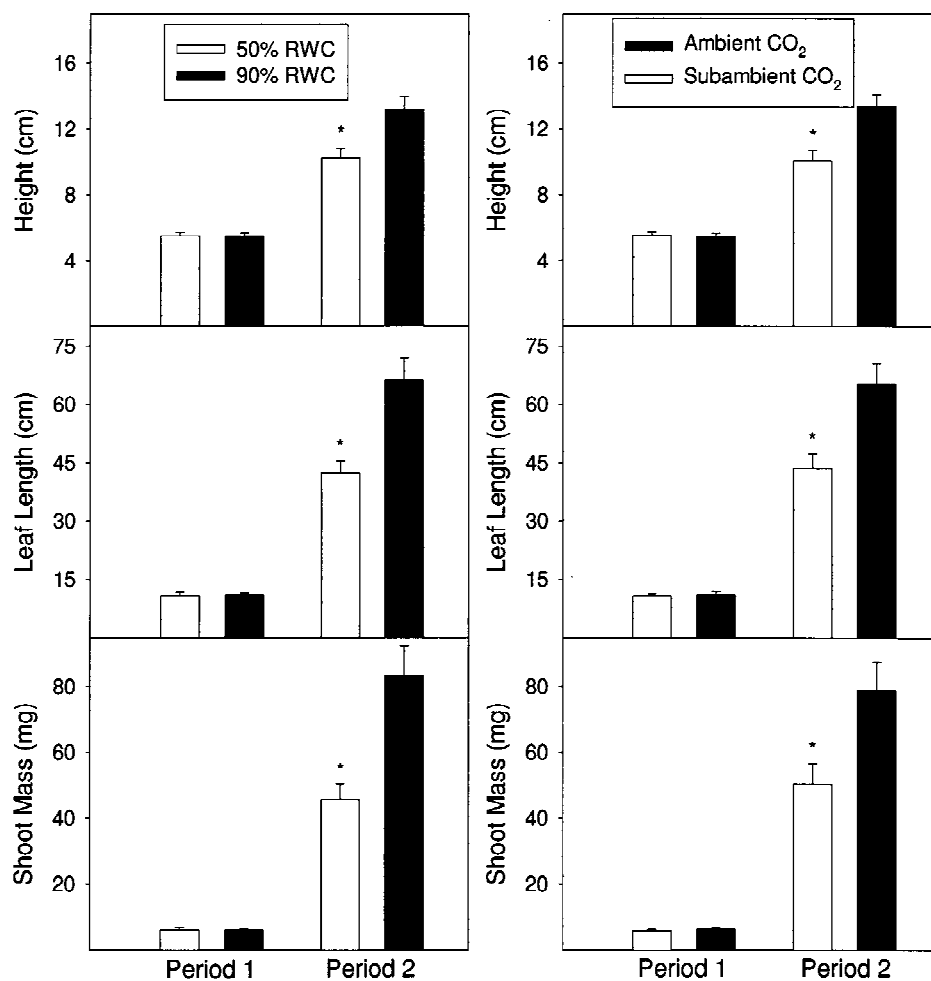


Figure 1. Mean (\pm SE, $n=192$) aboveground responses averaged across *C*₄ grass seedlings of *Andropogon gerardii* and *Schizachyrium scoparium* exposed to high and low soil relative water holding capacity (90 and 50% RWC, respectively), and ambient and subambient CO₂ (368 and 203 $\mu\text{mol mol}^{-1}$, respectively). Periods 1 and 2 are 0–4 and 4–8 wk post-emergence, respectively. Asterisks indicate a significant ($P < 0.05$) difference between RWC levels or CO₂ concentrations. See Table 1 for *F* and *P* values.

a perforated cap. Each pot was weighed when empty and after it had been filled with air-dried soil. Ten samples of air-dried soil were oven-dried at 105 °C for 72 h and weighed. The mean ratio of oven-dried–air-dried soil mass was used to calculate the mass of soil added to each pot. Soil in one-half of the pots was wetted to drip by adding 125 ml of distilled water and 125 ml of 1/3 strength Hoagland’s nutrient solution (Hoagland and Arnon, 1950), while soil in remaining pots was wetted with 125 ml of the nutrient solution.

Five seeds of *S. scoparium* and *A. gerardii* were planted separately in 192 pots for each species on April 27, 1998. Five ml of tap water was added daily to each pot until May 7, 1998, when plants in all pots were thinned to one seedling. Pots with

each species were randomly assigned in a factorial design with harvest date (2, 4, 6, or 8 weeks post-emergence), CO₂ [ambient, entrance of chamber (ca. 360 $\mu\text{mol mol}^{-1}$) and subambient (exit of chamber, ca. 200 $\mu\text{mol mol}^{-1}$)] and soil relative water holding capacity (RWC) [high (90%) and low (50%)] the main factors. Soil relative water holding capacity per pot was calculated by dividing the amount of water in the soil (mass of soil plus water on each date minus mass of oven-dried soil) by the amount of water retained in soil after drainage ceased (mass of fully wet soil minus that of oven-dried soil). Pots were weighed every 3–4 days to determine the amount of water to be added to maintain the 50% and 90% RWC treatments.

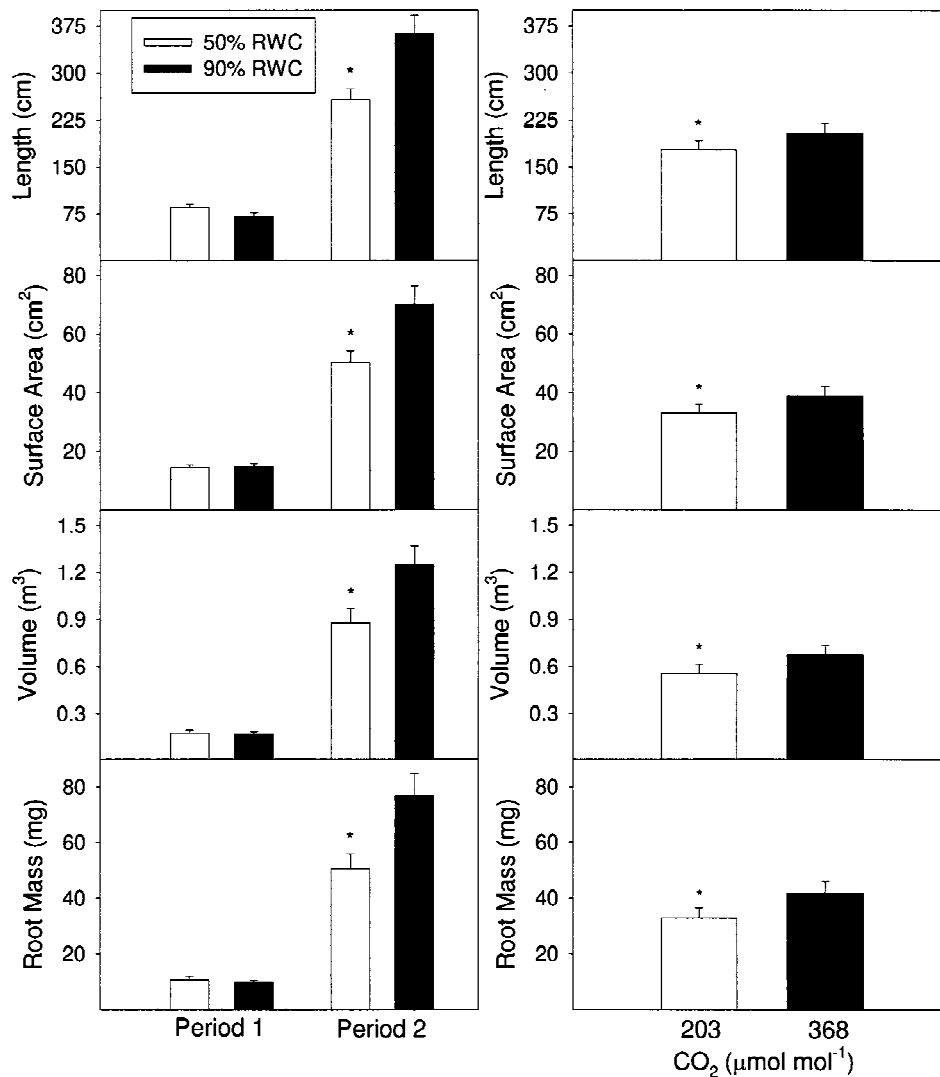


Figure 2. Mean (\pm SE, $n=192$) root responses averaged across C_4 grass seedlings of *Andropogon gerardii* and *Schizachyrium scoparium* exposed to high and low soil relative water holding capacity (90 and 50% RWC, respectively), and ambient and subambient CO_2 (368 and 203 $\mu\text{mol mol}^{-1}$, respectively). Periods 1 and 2 are 0–4 and 4–8 wk post-emergence, respectively. Asterisks indicate a significant ($P < 0.05$) difference between RWC levels or CO_2 concentrations. See Table 1 for F and P values.

At each harvest, aboveground biomass was destructively removed with shoot height, measured to the tip of the uppermost leaf, and total leaf length (sum of lengths of all individual leaf blades) measured. Soil was manually washed from roots, which were then digitally scanned using the WinRHIZO software (Regent Instruments, Inc. Quebec, Canada, version 3.9f) and hardware (Hewlett Packard ScanJet 6100C scanner). This software can determine root length, surface area and root volume. In addition, the software allows a user to specify root width classes (equal width

classes, $n=10$, increment 0.1 mm) to determine the distribution of root length, surface area, volume and number of root tips among width classes. Roots were scanned using a 10 \times 15 cm tray at high resolution (600 dpi). Because these roots were subjected to further analyses following scanning, they were not stained prior to analyses which results in underestimations (Bouma et al., 2000). This underestimation, however, was minimized by using the WinRHIZO automatic threshold (i.e. Lagarde's method) for pale roots with more sensitivity and the high resolution. Aboveground

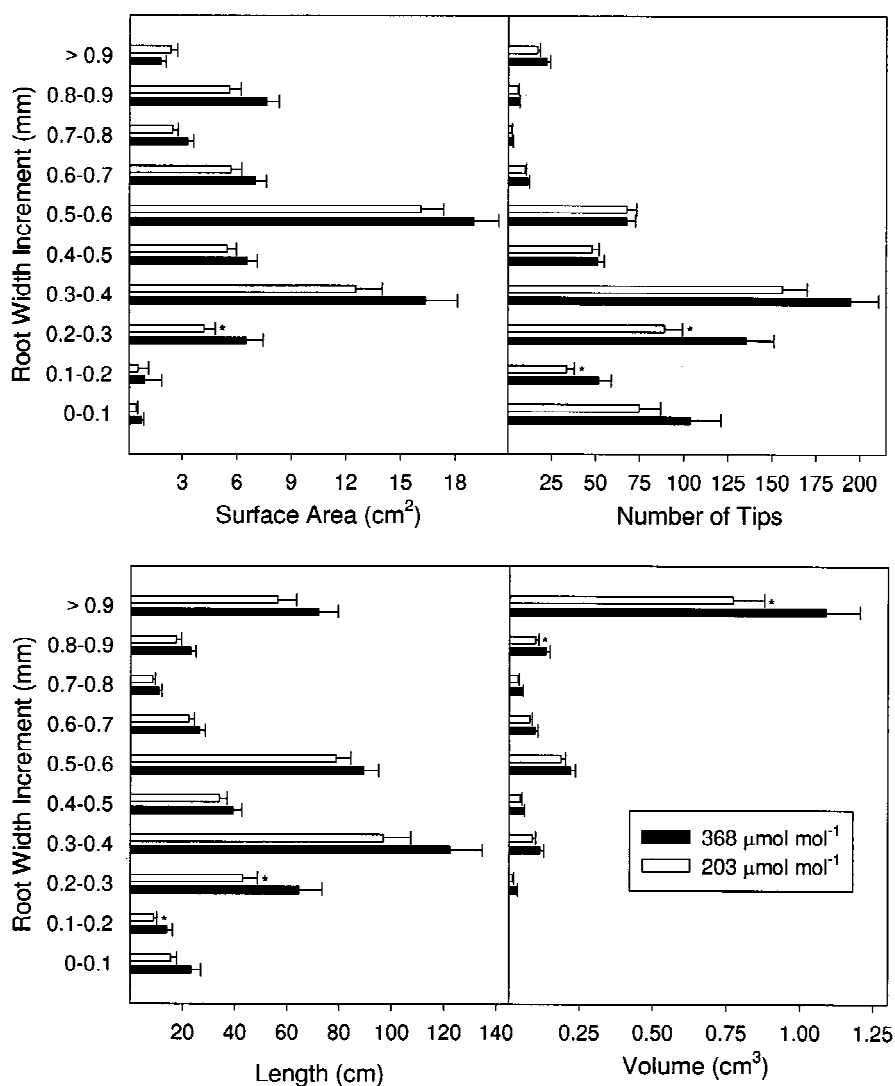


Figure 3. Mean ($n=192$) distribution of root surface area, number of root tip, total root length and root volume by root width increment averaged across soil relative water content and C_4 grass seedlings of *Andropogon gerardii* and *Schizachyrium scoparium* exposed to ambient and subambient CO_2 (368 and $203 \mu\text{mol mol}^{-1}$, respectively). Asterisks indicate a significant ($P < 0.05$) difference between CO_2 concentrations.

tissues and roots were dried at 60°C for 72 h prior to weighing.

Statistical analyses

To avoid potential confounding of treatment effects due to use of a single chamber, replication was achieved by combining the data from the four harvest dates into two sampling periods (period 1:2 and 4 week harvest; period 2:6 and 8 week harvest). Data were then analyzed using a four-way ANOVA (GLM) with period, species, RWC and CO_2 concentration as main factors. Means were separated using t -tests when

a factor was significant ($P < 0.05$). Data were transformed logarithmically before analysis when required to normalize residuals; means and standard errors are reported after back-transforming.

Results

Environmental factors

Mean daily daytime (1000–1800 h CST) CO_2 concentrations were 368 ± 2 ($n=54$) $\mu\text{mol mol}^{-1}$ for the

ambient and $203 \pm 1 \mu\text{mol mol}^{-1}$ for the subambient treatment. Daily daytime temperature for both treatments was $28.4 \pm 0.3 \text{ }^\circ\text{C}$ and mean daily nighttime (2000–800 h CST) temperature was $20.1 \pm 0.2 \text{ }^\circ\text{C}$. Daytime PPFD averaged $942 \pm 22 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Relative water holding capacity (RWC) of soils prior to adding of water to maintain the desired 50% RWC treatment was 48.6% for both CO_2 concentrations, while in the desired 90% RWC treatment it was 85.3% for ambient CO_2 and 82.1% for subambient CO_2 .

Aboveground

Significant interactions involving species did not occur for the aboveground variables, even though there were significant differences between species for all aboveground variables (Table 1). Interestingly, there were no significant $\text{CO}_2 \times \text{RWC}$ interactions for any of the aboveground variables. However, leaf length, shoot height and shoot mass did exhibit significant $\text{RWC} \times \text{period}$ and $\text{CO}_2 \times \text{period}$ interactions as differences were only observed in the latter portion of this study. Leaf length, averaged across species, was 50% greater at ambient than subambient CO_2 and 56% greater in the high than low RWC (Figure 1). Shoot height exhibited smaller increases of 33% and 29% for increased CO_2 and RWC, respectively. Shoot mass, however, increased 82% from low to high RWC, and 57% from subambient to ambient CO_2 .

Roots

In concurrence with aboveground variables, significant interactions involving species did not occur for the belowground variables; significant differences between species were observed for all aboveground variables, however (Table 1). Root variables did not display any significant $\text{CO}_2 \times \text{RWC}$ interactions which is in agreement with the aboveground variables. Root variables did exhibit significant $\text{RWC} \times \text{period}$ interactions with differences occurring only in the latter portion of the experiment (Figure 2). Root length, mass, surface area and volume averaged across species were 40–51% greater in the high than low RWC treatment. In contrast to aboveground variables, root variables did not exhibit significant $\text{CO}_2 \times \text{period}$ interactions. Rather, root length, mass, surface area and volume averaged across species and RWC were 15–27% greater at ambient CO_2 than at subambient CO_2 .

The distributions of root surface area, number of root tips, length and volume averaged across species

at the end of the experiment were significantly affected by CO_2 (Figure 3) but not RWC. Root surface area exhibited a unimodal distribution with respect to root width; greater proportional increases (55–61 vs. 18–37%) were observed at ambient CO_2 for lower (0–0.3 mm) width increments. The number of root tips was highly skewed to the lower width increments (0–0.3 mm) with similar proportional increases (39–52%) at ambient CO_2 as exhibited for surface area. The majority of root length occurred among roots of 0.3–0.6 mm width increments, but proportional increases at ambient CO_2 were less (13–26%) in these increments than at the lower width increments (53–58%). Root volume was distributed predominantly in the highest width increment (>0.9 mm) although proportional increases (53–58%) at ambient CO_2 were greatest at the lower width increments (0–0.3 mm).

Discussion

Root systems of C_4 grass seedlings responded similarly to an increase in CO_2 from subambient to ambient concentrations at limiting and abundant soil water. Root growth was affected primarily by increased RWC (40–51% increases) and secondarily by higher CO_2 (15–27% increases). Greater root length, surface area and volume with both the high RWC and ambient CO_2 treatments increase the potential for exploitation of soil nutrients and water (Norby, 1994; Rogers et al., 1994). In addition, CO_2 considerably increased the fraction of total root surface area, number of root tips and root length and volume in very fine (0–0.3 mm diameter) roots, which has important consequences for root function. Greater carbon investment in fine roots with ambient than subambient CO_2 may significantly influence nutrient cycling and longer-term changes in soil organic matter and ecosystem carbon balance (Norby and Jackson, 2000).

It is not possible to compare observed responses of roots to interactive effects of CO_2 and soil water with other investigations using C_4 grasses because others have evaluated such responses only under well-watered conditions (e.g. BassiriRad et al., 1997; Yoder et al., 2000). Similar responses to CO_2 displayed by root systems of these C_4 grass seedlings at both limiting and abundant soil water do, however, concur with results from a C_3 plant, spring wheat (*Triticum aestivum*) (Wechsung et al., 1999). A recent field study conducted on C_4 -dominated grassland revealed that both aboveground biomass and root ingrowth biomass

Table 1. Results of a 4-way ANOVA (GLM) showing the significance of the effects of period (0–4, 4–8 weeks post-emergence), species (*Schizachyrium scoparium*, *Andropogon gerardii*), CO₂ (200 $\mu\text{mol mol}^{-1}$, 360 $\mu\text{mol mol}^{-1}$), soil relative water holding capacity (RWC) (50%, 90%), and factor interactions on aboveground and root variables. Data were log transformed prior to analysis. Bold lettering indicates highest order significance ($P < 0.05$) for each factor. None of the Period \times Species, Species \times CO₂, Species \times RWC, CO₂ \times RWC, or 3- or 4-way interactions were significant.

Variable	Period	Species	CO ₂	RWC	Period \times CO ₂	Period \times RWC
<i>Aboveground</i>						
Height	<0.0001	< 0.0001	0.0007	0.0134	< 0.0001	0.0042
Leaf length	<0.0001	< 0.0001	0.0044	0.0146	0.0014	0.0292
Shoot mass	<0.0001	< 0.0001	0.0093	0.0369	0.0042	0.0451
<i>Roots</i>						
Length	<0.0001	< 0.0001	0.0475	0.6644	0.2461	0.0005
Surface area	<0.0001	< 0.0001	0.0193	0.5554	0.1394	0.0006
Volume	<0.0001	< 0.0001	0.0073	0.5296	0.0901	0.0011
Mass	<0.0001	< 0.0001	0.0004	0.5182	0.1327	0.0001

were stimulated by increased CO₂ only when soil water was limiting (Owensby et al., 1999). This discrepancy between the field study and our glasshouse study may be attributable to the absence of competitive interactions between species in our study, or comparison of seedlings to adult plants (Hunt et al., 1996).

The relative increases in root biomass (27%) observed in these C₄ grass seedlings with an increase from subambient to ambient CO₂ are similar to those measured for C₄ grasses (BassirRad et al., 1997; Hunt et al. 1996; Morgan et al., 1998; but see Yoder et al., 2000) and many crop species (Rogers et al., 1994; Wechsung et al., 1999) with an increase from ambient to superambient CO₂. C₄ grasses, therefore, may have already experienced an augmentation in root growth which is comparable to that expected with a doubling of current CO₂ concentrations.

Others have shown that biomass of C₄ grass seedlings responded to CO₂ enrichment from ambient to superambient (700 $\mu\text{mol mol}^{-1}$) concentrations (Morgan et al., 1998; but see Watling and Press, 1998). Previously unreported, however, is the finding that an increase in soil water content elicits a proportional increase in root growth similar to aboveground plant growth. Therefore, the indirect effect of CO₂ enrichment on conservation of soil water in grasslands may be as important as direct photosynthetic response effects in the CO₂-induced enhancement of whole-plant growth in C₄ grasses.

Acknowledgements

Tommy Greeson and Jeffrey Posvar monitored soil water and processed plant samples. Chris Kolodziejczyk maintained CO₂ and environmental control systems and data records.

References

- BassirRad H, Reynolds J F, Virginia R A and Brunelle M H 1997 Growth and root NO₃⁻ and PO₄³⁻ uptake capacity of three desert species in response to atmospheric CO₂ enrichment. *Aust. J. Plant. Phys.* 24, 353–358.
- Bouma T J, Nielsen K L and Koutstall B 2000 Sample preparation and scanning protocol for computerized analysis of root length and diameter. *Plant Soil* 218, 185–196.
- Cerling T E, Ehleringer J R and Harris J M 1998 Carbon dioxide starvation, the development of C₄ ecosystems and mammalian evolution. *Phil. Trans. Royal Soc. London* 353, 159–171.
- Cerling T E, Harris J M, MacFadden B J, Leakey M G, Quade J, Eisenmann V and Ehleringer J R 1997 Global vegetation change through the Miocene/Pliocene boundary. *Nature* 389, 153–158.
- Ehleringer J R, Curling T E and Helliker B R 1997 C₄ photosynthesis, atmospheric CO₂, and climate. *Oecologia* 112, 285–299.
- Ehleringer J R, Sage R F, Flanagan L B and Pearcy R W 1991 Climate change and the evolution of C₄ photosynthesis. *Trends Ecol. Evol.* 6, 95–99.
- Ghannoum O, Von Caemmerer S, Barlow E W R and Conroy J P 1997 The effect of CO₂ enrichment and irradiance on the growth, morphology and gas exchange of a C₃ (*Panicum laxum*) and C₄ (*Panicum antidotale*) grass. *Aust. J. Plant. Phys.* 24, 227–237.
- He H, Kirkham M B, Lawlor D J and Kanemasu E T 1992 Photosynthesis and water relations of big bluestem (C₄) and Kentucky bluegrass (C₃) under high concentration of carbon dioxide. *Trans. Kansas Acad. Sci.* 95, 139–152.

- Hoagland D R and Arnon D I 1950 The water-culture method for growing plants without soil. Calif. Ag. Exper. Sta. Circ. 347, 1–39.
- Howden S M, McKeon G M, Carter J O and Beswick A 1999 Potential global change impacts on C₃–C₄ grass distributions in eastern Australian rangelands. In Vth International Rangeland Congress Proceedings Vol. 1 Eds. D Eldridge and D Freudenberger. pp 41–43. Fyshwick, ACT, Australia: Elect Printing.
- Huckabee J W Jr, Thompson D R, Wyrick J C and Paulat E G 1977. Soil Survey Bell County Texas. USDA Soil Conservation Service, Washington, D.C. USA. 165 pp.
- Hunt H W, Elliott E T, Detling J K, Morgan J A and Chen D X 1996 Responses of a C₃ and C₄ perennial grass to elevated CO₂ and temperature under different water regimes. Global Change Biol. 2, 35–47.
- Jacobs B F, Kingston J D and Jacobs L L 1999 The origin of grass-dominated ecosystems. Ann. Miss. Bot. Gard. 86, 590–643.
- Knapp A K, Hamerlynck E P and Owensby C E 1993 Photosynthesis and water relations responses to elevated CO₂ in the C₄ grass *Andropogon gerardii*. Int. J. Plant. Sci. 154, 459–466.
- LeCain D R and Morgan J A 1998 Growth, leaf nitrogen and leaf carbohydrate concentrations respond differently to elevated CO₂ in NAD-ME and NADP-ME C₄ grasses. Phys. Plant. 102, 297–306.
- Mayeux H S, Johnson H B, Polley H W, Dumesnil M J and Spanel G A 1993 A controlled environment chamber for growing plants across a subambient CO₂ gradient. Funct. Ecol. 7, 125–133.
- Morgan J A, LeCain D R, Read J J, Hunt H W and Knight W G 1998 Photosynthetic pathway and ontogeny affect water relations and the impact of CO₂ on *Bouteloua gracilis* (C₄) and *Pascopyrum smithii* (C₃). Oecologia 114, 483–493.
- Morgan J A, Hunt H W, Monz C A and LeCain D R 1994 Consequences of growth at two carbon dioxide concentrations and two temperatures for leaf gas exchange in *Pascopyrum smithii* (C₃) and *Bouteloua gracilis* (C₄). Plant Cell. Environ. 17, 1023–1033.
- Norby R J 1994 Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. Plant Soil 165, 9–20.
- Norby R J and Jackson R B 2000 Root dynamics and global change: seeking an ecosystem perspective. New Phytol. 147, 3–12.
- Owensby C E, Ham J M, Knapp A K and Auen L m 1999 Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO₂. Global Change Biol. 5, 497–506.
- Owensby C E, Coyne P I, Ham J M, Auen L A and Knapp A K 1993 Biomass production in a tallgrass prairie ecosystem exposed to ambient and elevated CO₂. Ecol. Appl. 3, 644–653.
- Pagani N, Freeman K H and Arthur M A 1999 Late Miocene atmospheric CO₂ concentrations and the expansion of C₄ grasses. Science 285, 876–879.
- Poorter H 1993 Interspecific variation in the growth response of plants to an elevated CO₂ concentration. Vegetatio 104/105, 77–97.
- Read J J, Morgan J A, Chatterton N J and Harrison P A 1997 Gas exchange and carbohydrate and nitrogen concentrations in leaves of *Pascopyrum smithii* (C₃) and *Bouteloua gracilis* (C₄) at different carbon dioxide concentrations and temperatures. Ann. Bot. 79, 197–206.
- Rogers H H, Runion G B and Krupa S V 1994 Plant responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. Environ. Pollut. 83, 155–189.
- Sionit N and Patterson D T 1985 Responses of C₄ grasses to atmospheric CO₂ enrichment. II. Effect of water stress. Crop Sci. 25, 533–537.
- Sionit N and Patterson D T 1984 Responses of C₄ grasses to atmospheric CO₂ enrichment. I. Effect of irradiance. Oecologia 65, 30–34.
- Ward S J E, Midgley G F, Jones M H and Curtis P S 1999 Responses of wild C₄ and C₃ grass (Poaceae) species to elevated atmospheric CO₂ concentration: a meta-analytic test of current theories and perceptions. Global Change Biol. 5, 723–741.
- Ward J K, Tissue D T, Thomas R B and Strain B R 1999 Comparative responses of model C₃ and C₄ plants to drought in low and elevated CO₂. Global Change Biol. 5, 857–867.
- Watling J R and Press M C 1998 How does the C₄ grass *Eragrostis pilosa* respond to elevated carbon dioxide and infection with the parasitic angiosperm *Striga hermonthica*? New Phytol. 140, 667–675.
- Wechsung G, Wechsung F, Wall G W, Adamsen F J, Kimball B A, Pinter Jr P J, Lamorte R L, Garcia R L and Hartschall TH 1999 The effects of free-air CO₂ enrichment and soil water availability on spatial and seasonal patterns of wheat root growth. Global Change Biol. 5, 519–529.
- Yoder C K, Vivin P, Defalco L A, Seemann J R and Nowak R S 2000 Root growth and function of three Mojave Desert grasses in response to elevated atmospheric CO₂ concentration. New Phytol 145, 245–256.

Section editor: G.R. Shaver