Root system response of C_4 grass seedlings to CO_2 and soil water[**]

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Abstract

Aboveground growth of C_4 plants responds more strongly to atmospheric CO_2 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 CO_2 and soil water on root growth and morphology of two C_4 grasses. Seedlings of the dominant C_4 grasses from tallgrass prairie, *Schizachyrium scoparium* and *Andropogon gerardii*, were grown for 8 weeks in an elongated, controlled environment chamber at CO_2 concentrations of 368 (ambient) and 203 (subambient) μmol 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 C_4 grass seedlings responded similarly to CO_2 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 CO_2 (15–27% increases). The relative distribution of root surface area, number of root tips and length and volume of roots were significantly affected by CO_2 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 CO_2 enrichment on conservation of soil water in grasslands may be as important as direct photosynthetic response effects in the CO_2-induced enhancement of whole-plant growth in C_4 grasses.

Introduction

Aboveground growth of C_4 plants responds more strongly to atmospheric CO_2 concentration when soil water is limiting rather than abundant (e.g. Owensby et al., 1999), with this increase attributed directly to CO_2 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 C_4 plants to an increase in CO_2 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 C_4 ecosystems (Cerling et al., 1997, 1998; Ehleringer et al., 1997; Jacobs et al., 1999; Pagani et al., 1999).

The few documented responses of C_4 root systems to CO_2 and soil water are inconsistent, and have been conducted using comparisons between ambient and superambient CO_2 concentrations. Unfortunately, little is known regarding the responses of C_4 root systems to subambient CO_2 concentrations. Root biomass of C_4-dominated tallgrass prairie was greater at superambient than ambient CO_2 during years when soil water was limiting, but was also increased by CO_2 enrichment during 1 of 3 years when soil water was abundant (Owensby et al., 1999). Fine root biomass

Figure 1. Mean (±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 CO_2 (368 and 203 μmol 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.

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), CO_2 [ambient, entrance of chamber (ca. 360 μmol mol^{-1}) and subambient (exit of chamber, ca. 200 μmol 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.
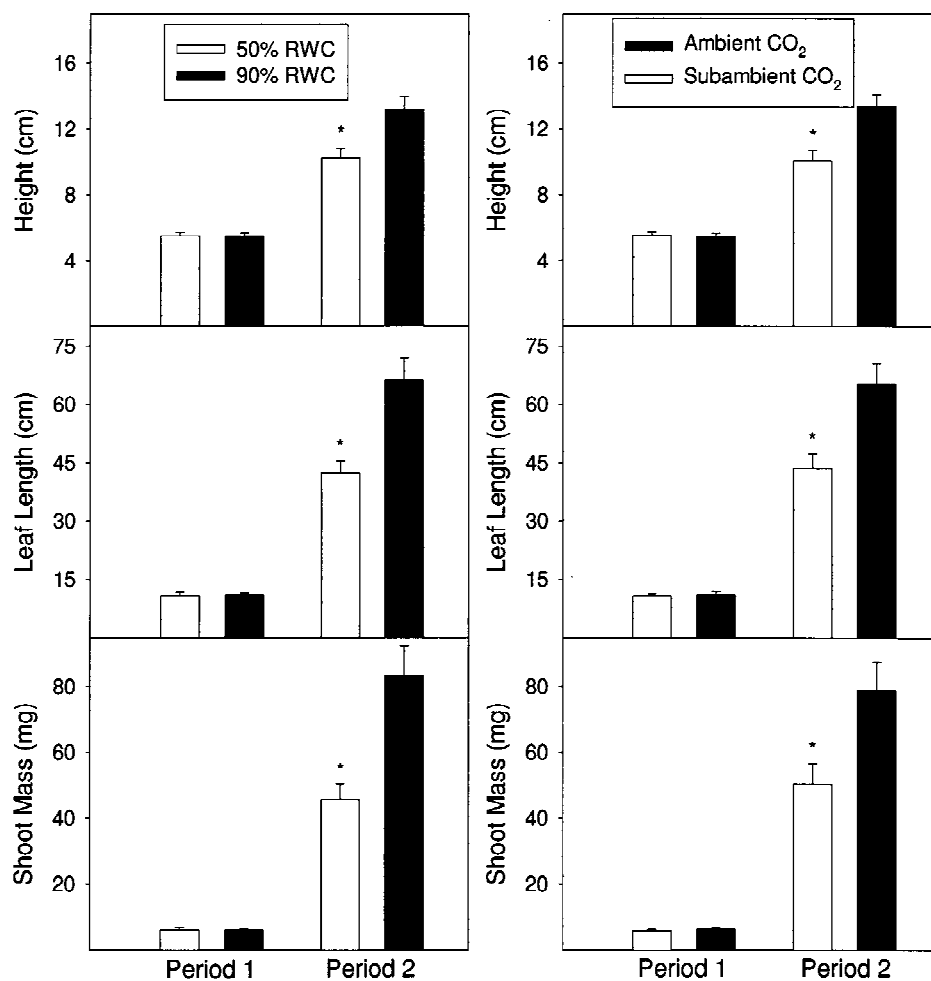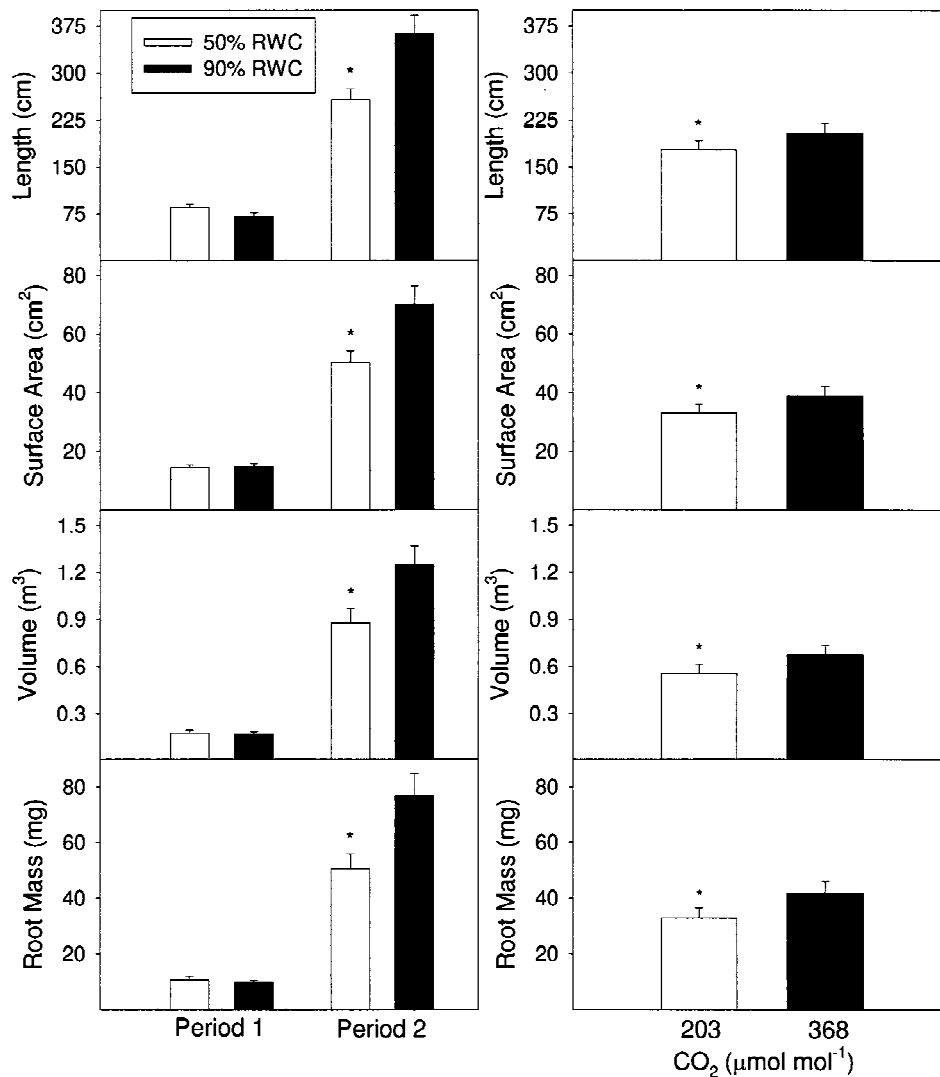
Figure 2. Mean ($\pm$SE, $n=192$) root responses averaged across C_4 grass seedlings of *Andropogon gerardii* and *Schizachyrium scoparium* exposed to high and low soil relative water holding capacity (90 and 50% RWC, respectively), and ambient and subambient CO_2 (368 and 203 μmol mol^{-1}, respectively). 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 10×15 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
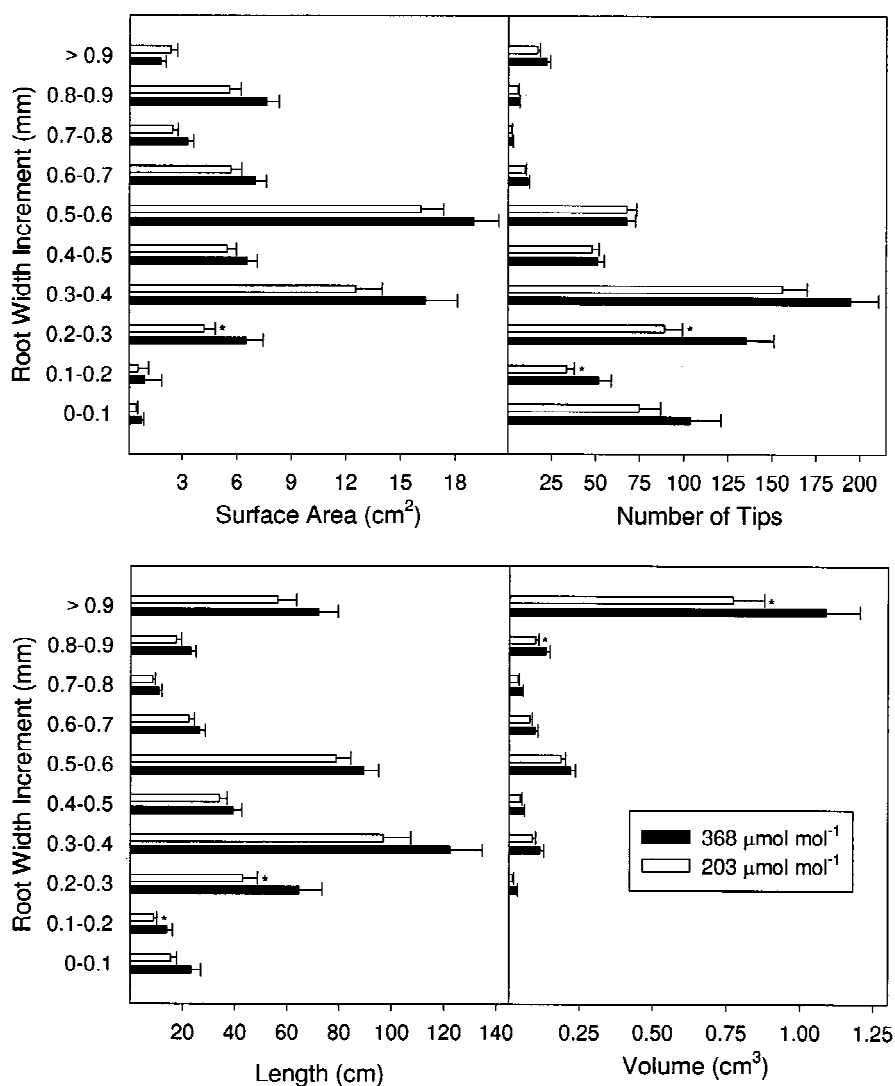
Figure 3. Mean (*n*=192) distribution of root surface area, number of root tip, total root length and root volume by root width increment averaged across soil relative water content and C_4 grass seedlings of *Andropogon gerardii* and *Schizachyrium scoparium* exposed to ambient and subambient CO_2 (368 and 203 μmol 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) μmol mol^{-1} for the

ambient and 203 ± 1 μmol mol^{-1} for the subambient treatment. Daily daytime temperature for both treatments was 28.4 ± 0.3 °C and mean daily nighttime (2000–800 h CST) temperature was 20.1 ± 0.2 °C. Daytime PPFD averaged 942 ± 22 μmol m^{-2} 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 $CO_2\times$RWC interactions for any of the aboveground variables. However, leaf length, shoot height and shoot mass did exhibit significant RWC$\times$period and $CO_2\times$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 $CO_2\times$RWC interactions which is in agreement with the aboveground variables. Root variables did exhibit significant RWC$\times$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 $CO_2\times$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_4grass 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_4grass 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*), CO_2 (200 μmol mol^{-1}, 360 μmol 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×Species, Species×CO_2, Species×RWC, CO_2×RWC, or 3- or 4-way interactions were significant.

Variable	Period	Species	CO_2	RWC	Period×CO_2	Period×RWC
Aboveground						
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**
Roots						
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 CO_2 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 C_4 grass seedlings with an increase from subambient to ambient CO_2 are similar to those measured for C_4 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 CO_2. C_4 grasses, therefore, may have already experienced an augmentation in root growth which is comparable to that expected with a doubling of current CO_2 concentrations.

Others have shown that biomass of C_4 grass seedlings responded to CO_2 enrichment from ambient to superambient (700 μmol 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 CO_2 enrichment on conservation of soil water in grasslands may be as important as direct photosynthetic response effects in the CO_2-induced enhancement of whole-plant growth in C_4 grasses.

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