Root responses along a subambient to elevated CO$_2$ gradient in a C$_3$–C$_4$ grassland


*Department of Biology, University of Texas, Austin, TX 78712, USA, ‡Department of Horticulture, The Pennsylvania State University, 102 Tyson Building, University Park, PA 16802, USA, §Department of Botany/Microbiology, Ohio Wesleyan University, Delaware, OH 43015, USA, ∥USDA/ARS, Grassland Soil and Water Research Laboratory, 808 East Blackland Rd., Temple, TX 76502, USA, USDA/ARS, High Plains Grasslands Research Station, 8408 Hildreth Road, Cheyenne, WY 82009-8899, USA, †Texas Parks and Wildlife Department, 4200 Smith School Road, Austin, TX 78744, USA, **Department of Biology and Nicholas School of the Environment, Duke University, Durham, NC 27708, USA

Abstract

Atmospheric CO$_2$ ($C_a$) concentration has increased significantly during the last 20,000 years, and is projected to double this century. Despite the importance of belowground processes in the global carbon cycle, community-level and single species root responses to rising $C_a$ are not well understood. We measured net community root biomass over 3 years using ingrowth cores in a natural C$_3$–C$_4$ grassland exposed to a gradient of $C_a$ from preglacial to future levels (230–550 µmol mol$^{-1}$). Root windows and minirhizotron tubes were installed below naturally occurring stands of the C$_4$ perennial grass Bothriochloa ischaemum and its roots were measured for respiration, carbohydrate concentration, specific root length (SRL), production, and lifespan over 2 years. Community root biomass increased significantly ($P<0.05$) with $C_a$ over initial conditions, with linear or curvilinear responses depending on sample date. In contrast, B. ischaemum produced significantly more roots at subambient than elevated $C_a$ in minirhizotrons. The lifespan of roots with five or more neighboring roots in minirhizotron windows decreased significantly at high $C_a$, suggesting that after dense root growth depletes soil resource patches, plants with carbon surpluses readily shed these roots. Root respiration in B. ischaemum showed a curvilinear response to $C_a$ under moist conditions in June 2000, with the lowest rates at $C_a<300$ µmol mol$^{-1}$ and peak activity at 450 µmol mol$^{-1}$ in a quadratic model. B. ischaemum roots at subambient $C_a$ had higher SRLs and slightly higher carbohydrate concentrations than those at higher $C_a$, which may be related to drier soils at low $C_a$. Our data emphasize that belowground responses of plant communities to $C_a$ can be quite different from those of the individual species, and suggest that complex interactions between and among roots and their immediate soil environment influence the responses of root physiology and lifespan to changing $C_a$.

Keywords: atmospheric CO$_2$, elevated CO$_2$, grassland, root biomass, root lifespan, root respiration, roots, subambient CO$_2$

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Introduction

As the CO$_2$ content of the atmosphere increases, plants generally respond with increased carbon fixation (e.g., Wand et al., 1999; Norby et al., 2005). The allocation of this ‘extra’ photosynthate may affect the carbon budgets of individual plants, ecosystems, and the biosphere, as well as global climate change. Some studies have documented increases in belowground plant productivity and metabolism with increased atmospheric CO$_2$ ($C_a$), suggesting that a significant portion of extra carbon is transferred to root systems, and ultimately to the soil microbial community and carbon pools (e.g., Gill et al., 2002a, 2006; Norby et al., 2004; Pendall et al., 2004; Hill et al., 2007, but see van Groenigen et al., 2006). However,
into the importance of belowground processes to the global carbon cycle and to accurate predictions of ecosystem changes with increasing $C_a$, we do not yet have a thorough understanding of root responses in this context (e.g., Norby & Jackson, 2000; LeCain et al., 2006; Jackson et al., 2009).

Plant species and communities may have different responses in terms of ‘investing’ carbon in roots and their activities with changing $C_a$. These may include producing more roots, altering root lifespan, changing single root metabolism, or a combination of approaches. The particular set of responses that occur may have significant effects on carbon cycling processes. For example, if roots become longer-lived under elevated $C_a$, but not more active or numerous, this may slow the transfer of carbon to microbial and soil pools. Simultaneous measurements of root production, lifespan, and metabolism are needed to understand the mechanisms that determine how, and if, the belowground environment will act as a carbon sink under predicted future $C_a$ levels (reviewed in Eissenstat et al., 2000).

Through evolutionary time, plants have already been exposed to substantial fluctuations in $C_a$. Ice core data indicate that $C_a$ ranged from 180 to 300 $\mu$mol mol$^{-1}$ during the last quarter million years (reviewed in Sage & Cowling, 1999), sometimes remaining below 200 $\mu$mol mol$^{-1}$ for up to 10,000 years at a time (Barnola et al., 1987; Jouzel et al., 1993). Therefore, examining root responses under subambient $C_a$ conditions, and comparing these responses to those under ambient and elevated $C_a$, may give us additional insight into the physiological capacity of plants to respond to $C_a$ increases across a range of values (Sage & Cowling, 1999; Ward et al., 2000). In addition, including a range of $C_a$ concentrations, rather than just elevated and ambient values, allows us to detect nonlinear patterns in community and ecosystem responses to $C_a$ (e.g., Ackerly & Bazzaz, 1995; Luo & Reynolds, 1999; Gill et al., 2002a; Polley et al., 2003).

Our objectives were to characterize ingrowth root biomass of the overall community, as well as root production, lifespan, respiration, carbohydrate concentration and specific root length (SRL) for the dominant $C_4$ grass, Bothriochloa ischaemum, in a natural $C_3$–$C_4$ grassland exposed to a continuous gradient of $C_a$ from preglacial (230 $\mu$mol mol$^{-1}$) to predicted future levels (550 $\mu$mol mol$^{-1}$). We predicted that community root biomass would increase with $C_a$, as has been observed in other grasslands (e.g., Hungate et al., 1997; Niklaus et al., 2001, but see Arnone et al., 2000 and LeCain et al., 2006). Root production for B. ischaemum was more difficult to predict; in earlier studies, this species showed some initial increases in aboveground biomass with $C_a$, but then decreased in abundance as $C_3$ plants increased their dominance over the course of the study (Polley et al., 2003). Therefore, we hypothesized that root production in B. ischaemum would be positively correlated with its aboveground responses to $C_a$ at the time of root sampling. Increasing C/N ratios with increasing $C_a$ had been observed for B. ischaemum roots for our system (Gill et al., 2002a), so we predicted that root carbohydrate concentrations would increase with $C_a$. Eissenstat et al. (2000) suggested that lower tissue N concentrations are related to lower single root respiration rates and consequently longer root lifespans at high $C_a$, and that these traits are associated with thicker roots. Therefore, we predicted that B. ischaemum roots at high $C_a$ would live longer, respire more slowly, and have a lower SRL than roots at low $C_a$.

Materials and methods

Study site

The experiment was carried out in a $C_3$–$C_4$ grassland at Temple, Texas, USA (31°05′N, 97°20′W). The site has been managed as grassland for 50+ years and was last grazed by cattle in 1992. The vegetation was a diverse mix of native and introduced grasses and forbs common in the region. Dominant plants included B. ischaemum (L.) Keng ($C_4$ grass), Solanum dimidiatum Raf. ($C_3$ forb), and Ratibida columnifera (Nutt.) Woot. and Standl. [previously known as R. columnaris (Sims) D. Don, $C_3$ forb]. Mean annual precipitation at the site is 877 mm (1913–1999), and the mean maximum and minimum annual temperatures are 25.9 and 13.2 °C, respectively (1914–1995, USDA/ARS Grassland Soil and Water Research Laboratory weather station records). The soil is a mollisol in the Austin series (classified as a fine-silty, carbonatic, thermic, Udomthetic Haplustoll) with 35–55% clay in the top 40 cm.

Experimental field chambers

Experimental chambers were built over two parallel plots of grassland 60 m long, 1 m wide, and 1.5 m apart. One chamber exposed plant communities to superambient $C_a$ concentrations and the other exposed them to subambient concentrations. Each chamber was 1 m tall and had ten 5 m sections, with chiller and condenser units connecting consecutive sections. Chambers were constructed of polyethylene film, which transmitted 85–95% of incident PPFD. A fan at one end of each chamber blew in ambient air. In the superambient chamber, incoming air was enriched with CO$_2$ to give a $C_a$ of 550 $\mu$mol mol$^{-1}$. As the air moved down each chamber, photosynthesis gradually reduced $C_a$ to
were 2–4 occurred during the summer months; temperatures and ambient values for temperature and VPD

average in the superambient chamber than in the sub-ambient chambers. The daytime VPD was generally lower in the chambers than in the surrounding grassland and was observed to be 0.6 kPa lower on

site, soaked to field capacity before insertion into the holes, and hand-watered immediately after insertion. They then received the same watering regime as the rest of the system.

Table 1 Measurement schedule for variables in the study

<table>
<thead>
<tr>
<th>Variable measured</th>
<th>Measurement dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community root biomass with ingrowth cores (two cores per 5 m section, 40 cores total per date)</td>
<td>1997: Mar, May, Sep, Nov 1998: Feb, May, Aug, Nov 1999: May, Sep, Nov</td>
</tr>
<tr>
<td>Specific root length for <em>B. ischaemum</em></td>
<td>1999: Jul, Sep 2000: Jun, Sep</td>
</tr>
<tr>
<td>Root total nonstructural carbohydrates (TNC) for <em>B. ischaemum</em></td>
<td>2000: Sep</td>
</tr>
</tbody>
</table>

Community ingrowth root biomass was assessed along the Cₐ gradient using two ingrowth cores in each 5 m chamber section (40 cores total, Table 1). Ingrowth cores were 6 cm diameter × 30 cm deep and made of PVC pipe and 1 mm mesh fiberglass window screen. Holes of the same size as the cores were made with a hand soil corer in March 1997. Root biomass was collected from the extracted soil (to describe initial conditions) by washing it through a 1 mm sieve. Cores were filled with a 50:50 mix of sand and sieved, root-free soil from the site, soaked to field capacity before insertion into the holes, and hand-watered immediately after insertion. They then received the same watering regime as the rest of the system.

Community ingrowth root biomass was assessed along the Cₐ gradient using two ingrowth cores in each 5 m chamber section (40 cores total, Table 1). Ingrowth cores were 6 cm diameter × 30 cm deep and made of PVC pipe and 1 mm mesh fiberglass window screen. Holes of the same size as the cores were made with a hand soil corer in March 1997. Root biomass was collected from the extracted soil (to describe initial conditions) by washing it through a 1 mm sieve. Cores were filled with a 50:50 mix of sand and sieved, root-free soil from the site, soaked to field capacity before insertion into the holes, and hand-watered immediately after insertion. They then received the same watering regime as the rest of the system.

Experimental Cₐ levels were imposed beginning in May 1997, so measurements taken on or before this date represent baseline conditions for the system.

*B. ischaemum, Bothriochloa ischaemum.*
emptied into plastic bags and refrigerated at 4 °C for up to 7 days before roots were collected by sieving and washing, as described above. Cores were reinstalled the day of harvest. The original holes in the plots were reused until the sides began to crumble. New holes were cored within 15 cm of the old in February 1998, and roots were collected from the extracted soil as described above. All roots were dried at 65 °C and weighed. A subset of each root sample was weighed, and roots were collected from the same ingrowth core in May 1997 did not differ significantly with position along the future C₃ gradient. However, there was considerable variation in ingrowth root biomass along the gradient that could mask any C₃ effect. Therefore, we calculated the ratio of ingrowth core root weights at each date to the weight of roots collected from the same ingrowth core in May 1997 to account for any differences in initial conditions.

Root respiration and carbohydrate concentrations in B. ischaemum

Root access windows were installed below naturally occurring monospecific stands of the C₄ perennial grass B. ischaemum at eight points along the C₃ gradient, representing C₃ concentrations from 231 to 534 μmol mol⁻¹. Our goal in focusing on B. ischaemum was to study effects of the C₃ gradient on root physiology without introducing variability due to species differences. Holes 45 cm deep and ~60 × 60 cm square were dug along the outer sides of the chambers in April 1999, and wooden boxes with three vertical sides were placed in the holes. The open side of each box was adjacent to the rubber liner enclosing the chamber soil. Windows (36 cm wide × 22 cm tall) were installed by cutting the liner at a depth of 25 cm (below the chamber support structures). A clear acetate window with a 2 × 2 cm grid was attached to the liner and a 50:50 mix of sand and sieved, root-free soil from the site was used to fill the gap between the existing soil and the window. The acetate was covered with pieces of liner to block light, and sand bags were put in each root box to absorb water and stabilize temperatures. Three 250 mL increments of deionized water were added to each window on May 9, 2000 to stimulate root growth. Two additional 250 mL increments were applied May 29, 2000.

Previous studies have shown that specific root respiration rates are affected by root age (e.g., Volder et al., 2005), so weekly digital photographs were taken of each window to track root age from July 27, 1999 until roots were sampled for respiration September 9–13, 1999 (Table 1). We cut the acetate windows with a razor blade, gently separated single roots from the soil, and placed each root, still attached to the plant, in a 0.7 mL microcentrifuge tube containing 1 mM CaSO₄·2H₂O + 5 mM MES buffer, pH 5.8 with 1 M KOH. After 20 min, each root was excised, rinsed in fresh buffer, and placed in a darkened oxygen electrode chamber containing the same buffer (Hansatech Instruments Ltd., Norfolk, UK). All roots were measured at 25 °C, which approximated the mean midday soil temperatures at 10 cm depth for six sites along the gradient in September 1999 (H. W. Polley, unpublished data). Slopes of oxygen depletion were measured between 10 and 20 min after the root was placed in the chamber to avoid any wounding response. Roots were then digitally scanned using WIN-RHIZO software (Regent Instruments Inc., Quebec, QC, Canada) to determine root diameter and length, dried at 60 °C and weighed. In July 1999, roots were collected for WIN-RHIZO analysis and biomass measurements only.

Roots were sampled for respiration on June 12–13 and September 21–22, 2000 using a modified procedure that did not focus on age-specific measurements of single roots, but allowed larger volumes of finer roots to be assessed. The soil in each acetate window was removed and quickly dry sorted for roots. Roots were rinsed with tap water and for respiration using the same buffer solution and oxygen electrode chamber described above (T = 24.8 °C, which again represented mean midday soil temperatures for these sampling dates). Three replicate measurements of respiration were taken for each of the eight windows in June 2000, but only six windows were used in September 2000 due to insufficient root growth. After measurement, root masses were put in petri dishes of tap water, refrigerated, and stained with red dye for 24 h to improve visibility during scanning. Roots were then scanned, dried and weighed as above. To compare our respiration rates as measured by O₂ uptake with other studies that measured respiration by CO₂ efflux, we assumed a 1:1 ratio of respiratory CO₂ release to O₂ consumption (respiratory quotient = 1). While respiratory quotients of 0.75–1.7 have been reported for roots (Lambers et al., 1996), Scheurwater et al. (1998) measured an average respiratory quotient of 1 for roots of several slow-growing grass species, suggesting that this would be an appropriate value to assume for B. ischaemum.

During the September 2000 harvest of roots for respiration measurements, 20–50 mg of root tissue was collected from the four sections with the most root growth (C₃ = 311, 332, 450, 534 μmol mol⁻¹) and frozen on dry ice, freeze dried and stored at 0 °C for later estimates of total nonstructural carbohydrate (TNC). To measure TNC, roots were ground to a fine powder and 4–5.5 mg of biomass was weighed into glass tubes with 1 mL of deionized water. Similar quantities of pure
starch were analyzed as controls. All tubes were placed in a boiling water bath for 20 min, and cooled on ice. Pairs of tubes received either 100 μL of sodium acetate solution (pH 4.8) containing starch digesting enzymes, or solutions without enzymes as controls. Tubes were incubated overnight at 30 °C, placed in a boiling water bath for 5 min to denature the enzymes, and centrifuged at 2500 rpm for 5 min. Each tube received 500 μL of Nelson’s copper reagent, followed by 10 min in a boiling water bath, and 500 μL of Nelson’s arsenomolybdate reagent, which reacts with reducing sugars (Nelson, 1944). A standard curve was generated for a spectrophotometer from glucose stock solutions ranging from 0 to 120 μg mL⁻¹, and sample solutions were diluted to fall in this range. Sample absorbances at 520 nm were recorded to give micrograms of glucose-equivalents per milliliter of solution. The original root sample weights were used to calculate milligram of glucose-equivalents per milligram of root.

Root lifespan and production in B. ischaemum

Acrylic minirhizotron tubes were installed above each root access window in May 1999 (n = 1 or 3 tubes per window, sections with three tubes were Cₐ = 231, 311, 450, 534 μmol mol⁻¹). Tubes (n = 3) were also installed in one section without a window (Cₐ = 358 μmol mol⁻¹) to assure a sampling point at ambient Cₐ. Tubes were 23 cm long × 2 cm diameter, and had two columns of etched 1 × 1 cm windows (15 windows per column, 30 windows per tube, columns were spaced 1 cm apart). The tube tops were wrapped in black electrical tape to block light, and black rubber stops were placed at the tube ends to exclude moisture and debris. Tubes were installed at a 30° angle from horizontal, 10 cm above the access window, with the etched windows oriented upwards to sample roots growing down from the plants above at a soil depth of 15–20 cm. Images of the windows were recorded bi-monthly during the growing seasons of 1999 and 2000 (July 8 through September 7, 1999, and February 29 through November 28, 2000) using the Bartz ICAP system (Bartz Technology, Carpinteria, CA, USA).

We recorded the dates when individual roots first grew against the tubes (birth date) and disappeared, based on the methods of Comas et al. (2000) and Anderson et al. (2003). Root lifespan was calculated as disappearance date minus birth date. Observation dates were recorded as the date midway between video dates. Roots were assigned one of two diameter classes (1 = < 0.4 mm, 2 = > 0.4 mm) on their birth date from direct measurements of images on the computer screen. Total numbers of roots appearing in each window during the study were recorded. As root populations need time to re-equilibrate after tube installation (e.g., Johnson et al., 2001), only roots grown in 2000 were used for root production estimates. For lifespan estimates, we included roots from 1999 with those from 2000 to expand the population of roots that we tracked from birth to death. Even so, minirhizotron work in grasslands suggests that it is unlikely that our root populations had reached equilibrium by 2000 (Milchunas et al., 2005a). Therefore, our data provide a measure of relative differences in root production and lifespan along the Cₐ gradient, rather than absolute values for this system.

Statistics

Relationships between Cₐ concentration and community ingrowth root biomass, and Cₐ and root production, respiration, SRL, and TNC concentration of B. ischaemum were explored using the Regression: Curve Estimation procedure in SPSS 13.0 and 14.0 for Windows (SPSS Inc., Chicago, IL, USA), and the Regression Wizard function in SIGMA PLOT 10.0 (Systat Software Inc., San Jose, CA, USA). When there was more than one measurement of the same variable at the same Cₐ concentration (e.g., two ingrowth cores were harvested per section of the experimental chamber), analyses were done on both the individual variates (regression with replication as described in Zar, 1996) and on the means for each Cₐ concentration. The means with standard errors and curve fits are presented graphically, and the regression results for individual variates are presented in table form. Linear, logarithmic, hyperbolic, power, and quadratic functions were fit to the data and the adjusted r² values compared with find the model with the best fit, following the methods of Anderson et al. (2001). Correlation and regression analyses done in SPSS 16.0 for Windows (SPSS Inc.) were used to explore relationships between ingrowth root biomass, root number, and previously published data on above-ground biomass for this system from Polley et al. (2003), and to examine the relationship between soil water depletion and Cₐ.

The variables Cₐ, root diameter, and total number of roots in each minirhizotron window (neighbors) were tested for their effects on root lifespan with Cox’s proportional hazards regression (CPHR) using the Cox’s Regression procedure in SPPS 15.0 (SPSS Inc.) with the forced-entry model building option. CPHR allows the effects of each covariate to be evaluated while controlling for the other covariates’ effects (Cox, 1972; Allison, 1995). Roots are evaluated for their risk of mortality based on their characteristics as specified by the covariates. The hazard ratio generated by CPHR can be interpreted as the risk of mortality of one covariate.
Table 2  Regression analyses for the relationships between C₄ concentration and root ingrowth weights (expressed as ratios with May 1997 ingrowth root weights or March 1997 soil core root weights for February 1998)

<table>
<thead>
<tr>
<th>Date</th>
<th>Model</th>
<th>Increase or decrease with C₄</th>
<th>Parameter value (a)</th>
<th>Intercept (b)</th>
<th>r²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sep 1997</td>
<td>Power</td>
<td>Increase</td>
<td>0.00003</td>
<td>1.91</td>
<td>0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nov 1997</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>Feb 1998–new holes</td>
<td>Power</td>
<td>Increase</td>
<td>0.001</td>
<td>1.20</td>
<td>0.11</td>
<td>0.044</td>
</tr>
<tr>
<td>May 1998</td>
<td>Linear</td>
<td>Increase</td>
<td>0.012</td>
<td>-1.892</td>
<td>0.18</td>
<td>0.006</td>
</tr>
<tr>
<td>Aug 1998</td>
<td>Linear</td>
<td>Increase</td>
<td>0.008</td>
<td>-2.052</td>
<td>0.18</td>
<td>0.014</td>
</tr>
<tr>
<td>Nov 1998</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>May 1999</td>
<td>Linear</td>
<td>Increase</td>
<td>0.016</td>
<td>-4.104</td>
<td>0.18</td>
<td>0.006</td>
</tr>
<tr>
<td>Sep 1999</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>Nov 1999</td>
<td>Logarithmic</td>
<td>Increase</td>
<td>3.861</td>
<td>-21.409</td>
<td>0.12</td>
<td>0.034</td>
</tr>
<tr>
<td>Means across all ingrowth dates</td>
<td>Power</td>
<td>Increase</td>
<td>0.00036</td>
<td>1.44</td>
<td>0.22</td>
<td>0.002</td>
</tr>
<tr>
<td>Means across all ingrowth dates, data at C₄ = 270 µmol mol⁻¹-excluded</td>
<td>Power</td>
<td>Increase</td>
<td>0.00006</td>
<td>1.73</td>
<td>0.37</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Linear = linear model (y = ax + b), Logarithmic = logarithmic model (y = a ln(x + b), Power = power model (y = ax²). A power model could not be fit to the November 1999 data because of observed zeros for root biomass.

category compared with the other if the variable is categorical and dichotomous, or as the percent change in mortality hazard for quantitative covariates by calculating (hazard ratio –1) × 100. See Wells & Eissenstat (2001) and Anderson et al. (2003) for more details.

The initial CPHR analysis on the full data set (n = 758 roots) identified a significant interaction between C₄ and the number of neighboring roots. Therefore, the data set was divided into roots with 0–4 neighbors (n = 395) and roots with five or more neighbors (n = 363), and CPHR was calculated for each separate data set to test the effects of C₄ and diameter on root lifespan. To ensure adequate numbers of roots at different C₄ levels, three categories were created: C₄ < 300 µmol mol⁻¹, C₄ = 300–360 µmol mol⁻¹, and C₄ ≥ 450 µmol mol⁻¹ (n ranged from 33 to 253 roots per neighbor/C₄ combination). The categories were compared using the simple contrasts option in the Cox’s Regression procedure with C₄ < 300 µmol mol⁻¹ as the reference category.

Results

Community root ingrowth biomass and B. ischaemum root production and lifespan

Root biomass in ingrowth cores increased significantly with increasing C₄ relative to initial conditions on six of the nine collection dates and when averaged across all dates (Table 2, Fig. 1). Two of the dates that showed no significant effect were November 1997 and November 1998, which represented late-season root growth when plants had mostly senesced, and tended to have low root weights for each core (data not shown). Root biomass at 270 µmol mol⁻¹ was inexplicably much higher and more variable than other parts of the gradient exposed to subambient C₄. When the relationship between C₄ and root biomass averaged across dates was reanalyzed with this section excluded, the r² value of the power function for this relationship increased from 0.22 to 0.37 (Table 2, Fig. 1).

Polley et al. (2003) reported significant or marginally significant increases in annual aboveground biomass with C₄ for this same system for 1997–1999 and our root biomass data showed the same general patterns. Annual root biomass ratios for our ingrowth cores (calculated by summing across sampling dates within a year and dividing by pretreatment biomass from May 1997) were significantly positively correlated with annual aboveground biomass values for this system in 1997 and 1999 (Pears-ons’s correlation coefficients = 0.467 and 0.542, P-values = 0.038 and 0.014 for 1997 and 1999, respectively). Root biomass ratios for 1998 were not correlated with aboveground biomass for 1998, although aboveground biomass increased significantly with C₄ for this grassland in this year (Polley et al., 2003). This may be due to the nonlinear relationship with C₄ observed for aboveground biomass in 1998, while we found linear relationships between C₄ and root biomass in this time period (Table 2).

In contrast to the root biomass data from ingrowth cores, which represent the response of the plant community to the C₄ treatment, there was a significant curvilinear decline in the number of roots produced by the C₄ grass B. ischaemum with C₄, with the greatest mean number of roots per minirhizotron tube observed at the lowest C₄ concentration of 231 µmol mol⁻¹ (Table 3, Fig. 2). There was no significant correlation between root numbers and the aboveground biomass of
B. ischaemum reported for 2000 by Polley et al. (2003). However, the last section of the C₄ gradient was one of only three (out of 20) that showed a positive change in B. ischaemum aboveground biomass from 1998 to 2000, and the last section showed the largest positive change (Polley et al., 2003); this may explain the substantial root production seen in this part of the gradient for this species.

![Diagram of relationships between C₄ and root biomass](image)

**Fig. 1** Relationships between C₄ and the ratio of ingrowth core community root biomass over that from the treatment initiation in May 1997. Mean ratios across all sample dates (a, b) and for two representative dates (c, d) are shown. Each point for individual dates is the mean of two cores from each of the chamber sections (n = 20). The r² and P-values shown in the figure correspond to the curve fits shown on the means. Curve fits for the individual cores from each section (n = 40) showed similar results (Table 2).

**Table 3** Regression analyses for relationships between C₄ concentration and root respiration, specific root length, soluble sugar and starch concentrations, and root numbers in minirhizotron tubes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Date</th>
<th>Model</th>
<th>Increase or decrease with C₄</th>
<th>Parameter value (a)</th>
<th>Intercept (b)</th>
<th>r²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resp. per g</td>
<td>Sep 1999</td>
<td>ns</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Jun 2000</td>
<td>Quadratic</td>
<td>Peak at 450</td>
<td>0.420</td>
<td>−0.0005</td>
<td>0.41</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Sep 2000</td>
<td>ns</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ns</td>
</tr>
<tr>
<td>Resp. per cm</td>
<td>Sep 1999</td>
<td>ns</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Jun 2000</td>
<td>Power</td>
<td>Increase</td>
<td>0.001</td>
<td>1.515</td>
<td>0.29</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Sep 2000</td>
<td>Quadratic</td>
<td>Peak at 311</td>
<td>0.560</td>
<td>−0.001</td>
<td>0.48</td>
<td>0.028</td>
</tr>
<tr>
<td>Resp. per cm²</td>
<td>Sep 1999</td>
<td>ns</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Jun 2000</td>
<td>Power</td>
<td>Increase</td>
<td>0.310</td>
<td>1.343</td>
<td>0.35</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Sep 2000</td>
<td>Quadratic</td>
<td>Peak at 311</td>
<td>7.080</td>
<td>−0.009</td>
<td>0.55</td>
<td>0.013</td>
</tr>
<tr>
<td>SRL cm per g</td>
<td>Jul 1999</td>
<td>ns</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Sep 1999</td>
<td>Hyperbolic</td>
<td>Decrease</td>
<td>1829.87</td>
<td>−204.307</td>
<td>0.38</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Jun 2000</td>
<td>ns</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Sep 2000</td>
<td>Hyperbolic</td>
<td>Decrease</td>
<td>5474.34</td>
<td>−153.877</td>
<td>0.13</td>
<td>0.041</td>
</tr>
<tr>
<td>Starch mg per mg</td>
<td>Sep 2000</td>
<td>Logarithmic</td>
<td>Decrease</td>
<td>0.322</td>
<td>−0.041</td>
<td>0.61</td>
<td>0.068</td>
</tr>
<tr>
<td>Sol. sugars mg per mg</td>
<td>Sep 2000</td>
<td>Logarithmic</td>
<td>Decrease</td>
<td>0.274</td>
<td>−0.029</td>
<td>0.46</td>
<td>ns</td>
</tr>
<tr>
<td>Root number (mini rhizotron)</td>
<td>2000 growing season</td>
<td>Hyperbolic</td>
<td>Decrease</td>
<td>8.821</td>
<td>−201.218</td>
<td>0.64</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Linear = linear model (y = ax + b), Logarithmic = logarithmic model (y = a ln x + b), Power = power model (y = axᵇ), Hyperbolic (y = axⁿ/b + xⁱ), Quadratic = quadratic model (y = y₀ + ax + bx²), SRL = specific root length, resp = respiration. P-values in italics indicate borderline significant results.

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Increasing \( C_a \) significantly reduced root lifespan of \( B. \) ischaemum roots with five or more neighbors (Table 4, Fig. 3). Roots produced at \( C_a > 450 \text{ mmol mol}^{-1} \) and \( C_a = 300-360 \text{ mmol mol}^{-1} \) had shorter lifespans than those grown at \( C_a < 300 \text{ mmol mol}^{-1} \), and differences between the highest and lowest \( C_a \) categories were statistically significant (Table 4). Median lifespans for the five or more neighbors group ranged from 110 days for the two higher \( C_a \) categories to 168 for \( C_a < 300 \text{ mmol mol}^{-1} \). Interestingly, \( C_a \) had no effect on root lifespan for roots growing with four or fewer neighbors, but root diameter significantly affected root lifespan for this group, with larger diameter roots having a ~40% lower risk of mortality (i.e., longer lifespan) than finer roots (Table 4). Median lifespans for this group ranged from 111 days for \( C_a > 450 \text{ mmol mol}^{-1} \) to 121 days for \( C_a = 300-360 \text{ mmol mol}^{-1} \). In total 758 roots were followed in the study and the percentage of roots censored for lifespan (i.e., roots that did not disappear or became obscured due to an obvious shift in the soil, etc.) was 21%.

**Table 4** Cox proportional hazards regression analysis results for root lifespan along the \( C_a \) gradient

<table>
<thead>
<tr>
<th>Variable</th>
<th>DF</th>
<th>Parameter estimate</th>
<th>Standard error</th>
<th>( \chi^2 ) value</th>
<th>( P )-value</th>
<th>Hazard ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Roots with 0–4 neighbors in the same minirhizotron tube window (1 cm(^2) area)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_a = 300-360 \text{ mmol mol}^{-1} ) compared with ( C_a &lt; 300 \text{ mmol mol}^{-1} )</td>
<td>1</td>
<td>0.028</td>
<td>0.140</td>
<td>0.039</td>
<td>0.844</td>
<td>1.028</td>
</tr>
<tr>
<td>( C_a &gt; 450 \text{ mmol mol}^{-1} ) compared with ( C_a &lt; 300 \text{ mmol mol}^{-1} )</td>
<td>1</td>
<td>-0.084</td>
<td>0.134</td>
<td>0.394</td>
<td>0.530</td>
<td>0.919</td>
</tr>
<tr>
<td>Root diameter</td>
<td>1</td>
<td>-0.486</td>
<td>0.170</td>
<td>8.166</td>
<td>0.004</td>
<td>0.615</td>
</tr>
<tr>
<td><strong>Roots with five or more neighbors in the same minirhizotron tube window (1 cm(^2) area)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_a = 300-360 \text{ mmol mol}^{-1} ) compared with ( C_a &lt; 300 \text{ mmol mol}^{-1} )</td>
<td>1</td>
<td>0.377</td>
<td>0.212</td>
<td>3.158</td>
<td>0.076</td>
<td>1.458</td>
</tr>
<tr>
<td>( C_a &gt; 450 \text{ mmol mol}^{-1} ) compared with ( C_a &lt; 300 \text{ mmol mol}^{-1} )</td>
<td>1</td>
<td>0.462</td>
<td>0.147</td>
<td>9.924</td>
<td>0.002</td>
<td>1.587</td>
</tr>
<tr>
<td>Root diameter</td>
<td>1</td>
<td>-0.128</td>
<td>0.198</td>
<td>0.418</td>
<td>0.518</td>
<td>0.880</td>
</tr>
</tbody>
</table>


Root respiration, SRL and carbohydrate content in \( B. \) ischaemum

Root respiration was most responsive to \( C_a \) concentration in June 2000, showing a significant curvilinear increase with \( C_a \) up to a value of 21.4 mmol O\(_2\) g\(^{-1}\)s\(^{-1}\) at 450 mmol mol\(^{-1}\), followed by a slight decrease (Table 3).
although the curve fit to the mean respiration rates per chamber section was not significant (Fig. 4a). This pattern was consistent for respiration expressed per gram, per centimeter, and per square centimeter of root, although the increase became less markedly curvilinear for the latter two (Table 3). Roots collected at \( C_a = 332 \mu \text{mol mol}^{-1} \) were particularly metabolically active at the June sampling date, with observed values of 27.9 nmol O\(_2\) g\(^{-1}\) C\(_{0}\) s\(^{-1}\) (Fig. 4a).

In September 1999 and 2000, respiration per gram of root tissue showed no consistent pattern with \( C_a \) and for September 1999 this pattern was not statistically significant regardless of whether respiration was expressed per gram, per centimeter, or per square centimeter of root (Table 3). Respiration for the single roots collected in September 1999 was also not significantly related to root age (data not shown). For September 2000, respiration expressed per centimeter or per square centimeter strongly emphasized the low respiration rates observed at the lowest \( C_a \); root respiration peaked at \( C_a = 311 \mu \text{mol mol}^{-1} \) and then declined (Fig. 4b, Table 3). This pattern was apparently due to roots at low \( C_a \) having significantly higher SRLs than roots in other parts of the gradient in September 1999 and 2000 (Fig. 5, Table 3). There were no clear trends in SRL with \( C_a \) for roots sampled in July 1999 and June 2000 (Table 3). Carbohydrate concentrations decreased slightly with increasing \( C_a \) but this pattern was not statistically significant (Fig. 6, Table 3, \( P \) for starch = 0.068, \( P \) for soluble sugar > 0.1).

Soil moisture

As root dynamics are likely to be affected by soil moisture and \( C_a \) has significant feedbacks on moisture as shown in both field and greenhouse studies, we report soil moisture in the chambers for the year 2000

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**Fig. 4** Root respiration rates for *B. ischaemum* along the \( C_a \) gradient for two sample dates in 2000 (\( n = 1–3 \) roots per section). The \( r^2 \) and \( P \)-values are for the curve fit on the June 2000 means. A curve fit was not attempted on the September 2000 means as the individual root analysis showed no significant relationship between respiration and \( C_a \). See Table 3 for complete analysis results on individual roots.

**Fig. 5** Mean specific root lengths for *B. ischaemum* along the \( C_a \) gradient for September 1999 and 2000. \( N \) for each point = 1–5. The \( r^2 \) and \( P \)-values shown are for the curve fits on the means. See Table 3 for analysis results on individual roots.

**Fig. 6** Glucose and starch concentrations for roots along the \( C_a \) gradient in September 2000. \( N \) for each point = 1–2 sets of roots. No curve fits were attempted on these means as the analyses of individual variates were not significant. See Table 3 for complete analysis results.

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to assist in data interpretation. Soil water content for the system was relatively high through the first 6 months of 2000 and then dropped dramatically, reaching its lowest point in September (Fig. 7a). This pattern is typical of the seasonal drought pattern in central Texas and has been observed in previous years in the same study (Polley et al., 2002). Soil water depletion between early season and September 2000 declined significantly as $C_a$ increased (Fig. 7b). Similar patterns were found by Polley et al. (2002) for 1997–1999.

Discussion

Increasing $C_a$ concentrations in this grassland led to increased net root biomass for the plant community throughout the study period (Fig. 1, see also Gill et al., 2002a). This result supports our root biomass hypothesis, and is consistent with other studies of ecosystems exposed to elevated $C_a$ (reviewed in Pendall et al., 2004), including deciduous forests (e.g., King et al., 2001; Norby et al., 2004), scrub (e.g., Dilustro et al., 2002), and other warm temperate grasslands (e.g., Jastrow et al., 2000; Milchunas et al., 2005a,b). This pattern of belowground biomass increases with $C_a$ is also generally consistent with the aboveground biomass increases previously reported for this system (Polley et al., 2003).

A unique aspect of our study is that, by studying a range of $C_a$ concentrations, we were able to demonstrate that ingrowth root biomass generally increases with $C_a$ in a curvilinear fashion, with slightly greater increases in belowground biomass from ambient to high $C_a$ than from subambient to ambient $C_a$. For example, the power function for the mean root biomass ratios across all dates ($y = ax^b$, Table 2), assuming ambient $C_a = 380 \mu mol mol^{-1}$, shows a 40% increase in the ingrowth root biomass ratio from 380 to 480 $\mu mol mol^{-1}$ as compared with a 36% increase from 280 to 380 $\mu mol mol^{-1}$. Using the power function for the data set excluding the most variable data point (Table 2), the contrast is even greater: a 50% increase from 380 to 480 vs. a 41% increase from 280 to 380 $\mu mol mol^{-1} C_a$. These data suggest that root biomass in grasslands may have changed markedly as $C_a$ increased since the last glacial period, but that more substantial changes are ahead if $C_a$ doubles by the end of this century as predicted. Our data also show that the shape of the response curve varies seasonally, as some sample dates showed a linear relationship between root biomass and $C_a$. This work suggests that modeling belowground responses to $C_a$ will require attention to both the shape of the relationship between root production and $C_a$ and the seasonality of root growth.

In contrast to the community pattern, the dominant $C_4$ grass *B. ischaemum* produced the greatest numbers of roots at the lowest $C_a$ (Fig. 2). While root numbers from minirhizotron tubes do not correspond directly to root biomass, these contradictory results may be explained by differing responses to $C_a$ among plant functional groups in this grassland. Polley et al. (2003) found that aboveground biomass for *B. ischaemum* increased with rising $C_a$ initially, but this trend weakened as $C_3$ forbs became dominant at elevated $C_a$ during the final 3 years of the experiment. By 2000, there was no relationship between aboveground biomass for *B. ischaemum* and $C_a$. Aboveground biomass of
B. ischaemum thus was most consistent among years at low C₃, where we observed the greatest root numbers in 2000. The positive relationships among root biomass in ingrowth cores, community aboveground biomass, and Cₛ, coupled with the fact that B. ischaemum gradually became less abundant at high Cₛ over time, suggest that much of the community root biomass at high Cₛ was from C₃ plants.

As soils are the largest carbon pool in grasslands, and grasslands contain ~30% of the global soil carbon pool (Jobbagy & Jackson, 2000), increased belowground productivity at high Cₛ raises the question of whether the extra root biomass will enter the soil organic matter pool or be recycled relatively rapidly in the soil. Gill et al. (2006) found that despite increased aboveground (Polley et al., 2003) and belowground productivity in this system, there was no net increase in soil C sequestration at elevated Cₛ after 4 years treatment, implying that decomposition had increased along the gradient to match the larger plant biomass inputs. This idea was also supported by an observed increase in ecosystem respiration per unit of net CO₂ fixation assessed through flux measurements along the Cₛ gradient (Polley et al., 2006).

Root lifespan is one of the most important variables for understanding belowground carbon cycling because it determines how quickly carbon allocated to roots is transferred to microbial and soil carbon pools (e.g., Gill & Jackson, 2000; Norby et al., 2004). Root lifespan has been shown to increase (Arnone et al., 2000; Milchunas et al., 2005a), decrease (Pregitzer et al., 1995; Fitter et al., 1996, 1997), or remain unchanged (Berntson & Bazzaz, 1996; Kubiske et al., 1998; Higgins et al., 2002) in different species at elevated Cₛ. We predicted longer root lifespans at high Cₛ for B. ischaemum, however the effects of Cₛ on root lifespan for this species were unexpectedly different for roots with different numbers of neighbors: roots growing in dense groups had significantly shorter lifespans at Cₛ > 450 μmol mol⁻¹ than roots at Cₛ < 300 μmol mol⁻¹ (Fig. 3, Table 4). The mechanism underlying this pattern is unknown, but dense root growth may indicate that the plant is locating roots in a favorable soil resource patch. Longer root lifespans at low Cₛ suggest that these plants are carbon limited and ‘save’ on root construction costs by maintaining roots even after the resource patch is depleted (Eissenstat & Yanai, 1997; Eissenstat et al., 2000). In contrast, plants at high Cₛ are presumably not carbon limited. Therefore, these roots may turn over more rapidly and plants may be foraging more efficiently by shedding roots in depleted resource patches and replacing them with more active roots in new soil sites, especially if belowground competition is more intense at high Cₛ, as suggested by the ingrowth core data. This is a fruitful area for future work.

Lifespan in roots with fewer neighbors was more strongly influenced by root diameter than by δₛ, with larger diameter roots having longer lifespans (Fig. 3, Table 4). Root diameter has been shown to have a consistent, positive relationship with root lifespan across species and habitat types (e.g., Eissenstat et al., 2000; Wells & Eissenstat, 2001; Anderson et al., 2003), including grasslands (Gill et al., 2002b). This may be because thicker roots function as conduits and initiate new laterals as well as absorbing soil resources, and so are preferentially retained by the plant (Wells & Eissenstat, 2001). However, the complex interactions we observed between diameter, neighbors and Cₛ emphasize the difficulties in isolating the effects of Cₛ on root lifespan. For example, Cₛ may indirectly influence root lifespan through its effects on SRL and root production. In addition, accurate estimates of root lifespan are difficult to obtain. Recent work by Strand et al. (2008) indicates that root longevity may be significantly underestimated when measured by short-term minirhizotron studies, and some research has indicated that root turnover dynamics require multiple years after tube installation to reach equilibrium (e.g., Milchunas et al., 2005a; Strand et al., 2008).

Root respiration for B. ischaemum appeared to peak between 400 and 500 μmol mol⁻¹ in a quadratic model fit to data from June 2000 (Table 3, Fig. 4a). Interestingly, soil respiration and microbial biomass in the Cₛ gradient also peaked between 400 and 500 μmol mol⁻¹ (Gill et al., 2006), suggesting that enhanced specific root respiration, as well as microbial activities, may contribute to increased carbon effluxes for this ecosystem at elevated Cₛ during some periods. The range of mean respiration rates we observed along the Cₛ gradient (5.6–27.9 nmol O₂ g⁻¹ s⁻¹, Fig. 4) were two to 10 times higher than rates for roots in soil cores from 11 cool temperate grassland sites in Europe (Bahn et al., 2006), but three to four times lower than those reported by Scheurwater et al. (1998) for nine species of C₃ grasses and BassiriRad et al. (1996) for a tussock sedge. In the European field study, respiration rates were reported at a reference temperature of 15 °C, while our measurements were conducted at 25 °C, as appropriate for each ecosystem. Assuming a Q₁₀ of 2, the respiration rates we observed at low Cₛ are consistent with those reported by Bahn et al. (2006). In addition, roots collected from our access windows were probably younger and therefore more active than roots collected through field coring. In the experiments by Scheurwater et al. (1998) and BassiriRad et al. (1996), plants were young and grown either in pots or hydroponically under high nutrient and moisture conditions, in contrast to our
study where mature, field grown plants in dry soils were used. Our roots were also collected below 25 cm depth, and so would experience different temperature and moisture profiles than shallower roots, and therefore show different physiological responses. Interestingly, our specific root respiration rates are quite consistent with those reported for a range of woody plants (George et al., 2003).

To our knowledge, no other studies have reported root respiration rates for plants grown at subambient $\mathrm{C}_a$. $B. \, ischaemum$ root respiration was suppressed at $\mathrm{C}_a < 300 \mu\text{mol mol}^{-1}$ in June 2000, with individual roots likely carbon limited in this low-CO$_2$ environment. However, the wide range of root respiration responses to $\mathrm{C}_a$ reported in the literature and the complexity of the response along our $\mathrm{C}_a$ gradient suggest that other variables interact to modulate the $\mathrm{C}_a$ effect. Previous studies have observed that root respiration increases (Bassiri-Rad et al., 1997), decreases (BassiriRad et al., 1996; Fitter et al., 1997), or does not change (Norby et al., 1987; Hertog et al., 1998; George et al., 2003) with elevated aboveground $\mathrm{C}_a$. Others have found, as we did, that root respiration changes in its responsiveness to $\mathrm{C}_a$ seasonally (Matamala & Schlesinger, 2000). Root age (e.g., Volder et al., 2005), soil moisture, soil temperature (Huang et al., 2005) and root nitrogen concentrations (Hertog et al., 1998, reviewed in Pendall et al., 2004) are also known to affect respiration rates. Some of these variables are themselves influenced by $\mathrm{C}_a$, and so effects of $\mathrm{C}_a$ on root respiration may be indirect. These complexities suggest that greater attention to the mechanisms driving root respiration responses in experimental $\mathrm{C}_a$ systems is needed. In our study, the strongest response of respiration to $\mathrm{C}_a$ was observed when soils were still relatively moist, early in the summer season (Fig. 7). As the soil dried, roots were probably more directly affected by moisture than $\mathrm{C}_a$.

Lack of moisture and other soil resources may have also influenced root TNC content and SRL along the gradient. We observed slightly greater TNC levels for roots grown at subambient $\mathrm{C}_a$ in September 2000 (Fig. 6). This is not consistent with our expectations that these roots would be carbon limited. However, other research has shown that when a plant’s capacity to use fixed carbon is reduced by a lack of other resources, carbon may accumulate in tissues. Studies of tissue chemistry in the legume *Lotus corniculatus* under elevated $\mathrm{C}_a$ and drought stress found that drought alone increased root TNC levels significantly, and $\mathrm{C}_a$ only enhanced TNC under drought conditions (Carter et al., 1999). Sicher (2005) found that nonstructural carbohydrates accumulated in *Hordeum vulgare* L. cv. Brant roots exposed to phosphorus limitation, regardless of $\mathrm{C}_a$ treatment. Our TNC data were collected in September, the driest part of the growing season for this plant community (Fig. 7); Milchunas et al. (2005b) found that responses of root tissue quality to elevated $\mathrm{C}_a$ in a grassland system were inconsistent over time, so it may be that our data from September are not representative of the entire growing season. In addition, plants growing at the lowest $\mathrm{C}_a$ probably experienced drought stress as an indirect effect of subambient $\mathrm{C}_a$. More negative midday water potentials for $B. \, ischaemum$ and $S. \, dimidiatum$ have been observed at subambient than elevated $\mathrm{C}_a$ for this system, as well as greater depletion of soil water in the subambient sections as recorded by neutron attenuation (Fig. 7, Polley et al., 2002). Anderson et al. (2001) documented greater stomatal conductances at subambient $\mathrm{C}_a$ in $B. \, ischaemum$ and $S. \, dimidiatum$, suggesting a potential mechanism for greater soil drying at subambient $\mathrm{C}_a$.

Root diameters may also be affected by hydration, as has been suggested for studies of fine roots in other semi-arid grassland systems (Milchunas et al., 2005a). We had predicted that roots would be thicker at high $\mathrm{C}_a$, and this pattern was evident in September 1999 and 2000. However, this trend was not seen in roots collected in July 1999 and June 2000, under wetter soil conditions (Fig. 7). Therefore, it is likely that the high SRLs for roots at subambient $\mathrm{C}_a$, like the TNC patterns, are a response to drought at this time of year being exacerbated by high transpiration rates at low $\mathrm{C}_a$, rather than a response to carbon limitations belowground.

In conclusion, this grassland responded to increased $\mathrm{C}_a$ with enhanced community root growth, and overall ingrowth root biomass responded more strongly to $\mathrm{C}_a$ increases above ambient. In contrast, the $\mathrm{C}_3$ grass $B. \, ischaemum$ had greater root numbers at low $\mathrm{C}_a$ where $\mathrm{C}_3$ aboveground biomass had decreased. Roots growing with five or more neighbors at low $\mathrm{C}_a$ lived longer than roots at high $\mathrm{C}_a$, raising interesting questions about the ratio of root construction and maintenance costs at different $\mathrm{C}_a$ levels. In June 2000, $B. \, ischaemum$ roots at high $\mathrm{C}_a$ were more metabolically active, and showed peak respiration rates at high $\mathrm{C}_a$ consistent with patterns of soil respiration for the system as a whole. $B. \, ischaemum$ roots at subambient $\mathrm{C}_a$ were thinner and tended to accumulate carbohydrates, effects that were probably related to drier soils in this part of the gradient. Our root biomass and production data emphasize that belowground responses of plant communities to $\mathrm{C}_a$ can be quite different from those of the component species, and our physiological data for $B. \, ischaemum$ roots suggest that complex interactions between and among roots and their immediate soil environment influence the responses of root physiology to changes in atmospheric $\mathrm{C}_a$. To understand the mechanisms that
will determine the role of belowground carbon sinks as carbon storage. Further studies are needed to characterize these sinks and their responses to environmental change.

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