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Root responses along a subambient to elevated CO_2 gradient in a C_3–C_4 grassland

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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,

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despite 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, Udorthentic 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

360 μmol mol^{-1} at the end of the 10th section in the superambient chamber and to 200 μmol mol^{-1} at the end of the subambient chamber. Air flows were automatically adjusted by changing fan speeds to accommodate photosynthetic fluctuations. C_a gradients were maintained on >90% of growing season days, even during severe droughts. Consistent C_a concentrations were also maintained within different sections of the gradient, with the daily standard errors of daytime C_a concentrations ranging from 0.9–2.7 at the air entrance of a 5 m section to 2.1–4.8 μmol mol^{-1} at the air exit point (Polley *et al*., 2002). At night, C_a concentrations were maintained at 150 μmol mol^{-1} above daytime levels by reversing air flow and using respiratory CO_2 releases to create the gradient.

A barrier of rubber-coated fabric extended 0.9 m deep into the soil along the chamber sides and between 5-m long chamber sections. This prevented surface roots of plants outside the system from growing into the chamber soil. The chambers were activated in May 1997, and operated from mid-February to mid-November through 2000. The plastic covers were removed from the chambers for 1 day each month for data collection and harvests, and when the vegetation was dormant in winter. All vegetation was clipped to 5 cm for aboveground biomass measurements each December. After drying, sorting by species, and weighing, the plant material was shredded with a wood chipper and returned to the 1 m section from which it was harvested in early January (Polley *et al*., 2003).

Air temperature and vapor pressure deficits (VPD) along the gradient were controlled to near-ambient values by cooling and drying the air at 5 m intervals using the chiller/condenser units mentioned above. This approach created a series of smaller gradients in temperature and VPD within the larger C_a gradient, but allowed for consistent atmospheric conditions among C_a treatments. The largest differences between chambers and ambient values for temperature and VPD occurred during the summer months; temperatures were 2–4 °C cooler in the chambers than outside for May–September, and were similar between the super- and subambient 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 average in the superambient chamber than in the subambient chamber for June–August 1998 (Johnson *et al*., 2000; Polley *et al*., 2002).

As evapotranspiration varied with C_a it was expected that soil water dynamics would change accordingly along the gradient. Soil water content was measured weekly to 1.35 m depth in the center of each 5 m section by neutron attenuation (Polley *et al*., 2002). Initially, irrigation was applied equally to each 5 m section to match rainfall outside, but this over-watered the system, as plants also accessed outside water flowing laterally at depth. After July 1999, irrigation was applied equally to each to each section such that soil water content in the ambient C_a sections matched that of adjacent grassland as measured by neutron attenuation. The total water added was 349 and 381 mm in the very dry years of 1999 and 2000, respectively. More details on chamber operation and sampling methods are given in Johnson *et al*. (2000), Polley *et al*. (2002, 2003).

Community root biomass

Community ingrowth root biomass was assessed along the C_a 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.

Ingrowth cores were harvested every 2–4 months from May 1997 through November 1999. The cores were

Table 1 Measurement schedule for variables in the study

Variable measured	Measurement dates
Community root biomass with ingrowth cores (two cores per 5 m section, 40 cores total per date)	1997: Mar, May, Sep, Nov 1998: Feb, May, Aug, Nov 1999: May, Sep, Nov
Root respiration for *B. ischaemum* (individual roots in 1999, root bunches in 2000)	1999: Sep 2000: Jun, Sep
Specific root length for *B. ischaemum*	1999: Jul, Sep 2000: Jun, Sep
Root total nonstructural carbohydrates (TNC) for *B. ischaemum*	2000: Sep
Root lifespan and production for *B. ischaemum* (tubes installed in May 1999)	1999: videotaped Jul 8–Sep 7 2000: videotaped Feb 29–Nov 2000

Experimental C_a 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, ashed in a muffle furnace at 550 °C for 5 h, and reweighed to calculate ash-free total sample weights.

Pretreatment data for root weights from cores collected in March and May 1997 did not differ significantly with position along the future C_a gradient. However, there was considerable variation in ingrowth root biomass along the gradient that could mask any C_a 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 C_4 perennial grass *B. ischaemum* at eight points along the C_a gradient, representing C_a concentrations from 231 to 534 μmol mol^{-1}. Our goal in focusing on *B. ischaemum* was to study effects of the C_a 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 $CaSO_4 \cdot 2H_2O$ + 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 measured 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 O_2 uptake with other studies that measured respiration by CO_2 efflux, we assumed a 1 : 1 ratio of respiratory CO_2 release to O_2 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 (C_a = 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 $\mu g\,mL^{-1}$, 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 $C_a = 231$, 311, 450, 534 $\mu mol\,mol^{-1}$). Tubes ($n = 3$) were also installed in one section without a window ($C_a = 358\,\mu mol\,mol^{-1}$) to assure a sampling point at ambient C_a. 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 stoppers 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 C_a gradient, rather than absolute values for this system.

Statistics

Relationships between C_a concentration and community ingrowth root biomass, and C_a 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 C_a 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 C_a 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 r^2 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 aboveground biomass for this system from Polley *et al*. (2003), and to examine the relationship between soil water depletion and C_a.

The variables C_a, 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 C_a concentration and root ingrowth weights (expressed as ratios with May 1997 ingrowth root weights or March 1997 soil core root weights for February 1998)

Date	Model	Increase or decrease with C_a	Parameter value (a)	Intercept (b)	r^2	P-value
Sep 1997	Power	Increase	0.00003	1.91	0.40	<0.001
Nov 1997	ns	–	–	–	–	ns
Feb 1998–new holes	Power	Increase	0.001	1.20	0.11	0.044
May 1998	Linear	Increase	0.012	−1.892	0.18	0.006
Aug 1998	Linear	Increase	0.008	−2.052	0.18	0.014
Nov 1998	ns	–	–	–	–	ns
May 1999	Linear	Increase	0.016	−4.104	0.18	0.006
Sep 1999	ns	–	–	–	–	ns
Nov 1999	Logarithmic	Increase	3.861	−21.409	0.12	0.034
Means across all ingrowth dates	Power	Increase	0.00036	1.44	0.22	0.002
Means across all ingrowth dates, data at $C_a = 270\,\mu mol\,mol^{-1}$ excluded	Power	Increase	0.00006	1.73	0.37	<0.001

Linear = linear model ($y = ax + b$), Logarithmic = logarithmic model ($y = a \ln x + b$), Power = power model ($y = ax^b$). 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 C_a 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 C_a and diameter on root lifespan. To ensure adequate numbers of roots at different C_a levels, three categories were created: $C_a < 300\,\mu mol\,mol^{-1}$, $C_a = 300$–$360\,\mu mol\,mol^{-1}$, and $C_a \geq 450\,\mu mol\,mol^{-1}$ (n ranged from 33 to 253 roots per neighbor/C_a combination). The categories were compared using the simple contrasts option in the Cox's Regression procedure with $C_a < 300\,\mu mol\,mol^{-1}$ 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 C_a 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 $270\,\mu mol\,mol^{-1}$ was inexplicably much higher and more variable than other parts of the gradient exposed to subambient C_a. When the relationship between C_a and root biomass averaged across dates was reanalyzed with this section excluded, the r^2 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 C_a 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 (Pearsons'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 C_a for this grassland in this year (Polley *et al*., 2003). This may be due to the nonlinear relationship with C_a observed for aboveground biomass in 1998, while we found linear relationships between C_a 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 C_a treatment, there was a significant curvilinear decline in the number of roots produced by the C_4 grass *B. ischaemum* with C_a, with the greatest mean number of roots per minirhizotron tube observed at the lowest C_a concentration of $231\,\mu mol\,mol^{-1}$ (Table 3, Fig. 2). There was no significant correlation between root numbers and the aboveground biomass of

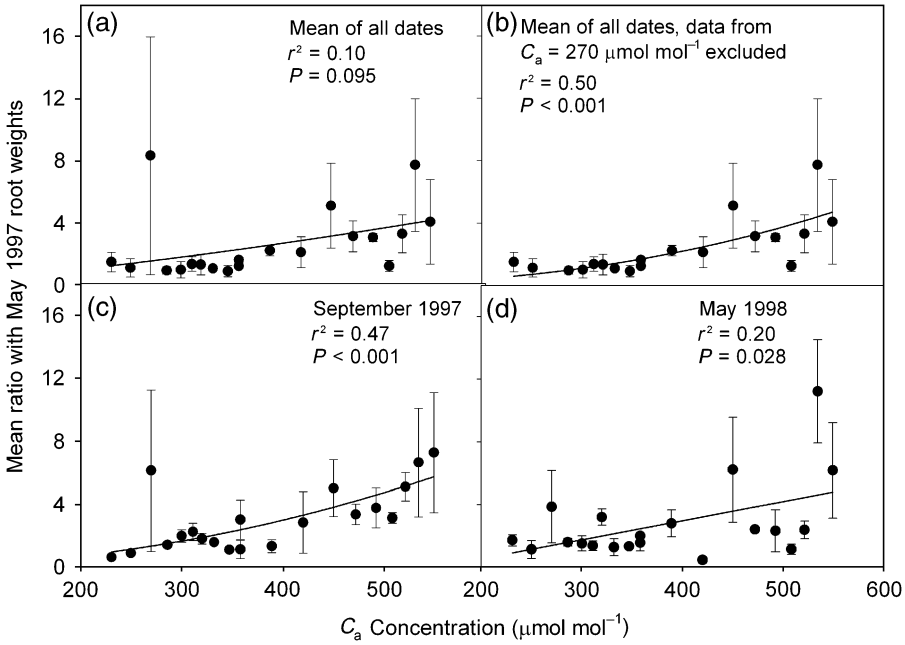

Fig. 1 Relationships between C_a and the ratio of ingrowth core community root biomass over that from the treatment initiation in May 1997. Mean ratios across all sample dates (a, b) and for two representative dates (c, d) are shown. Each point for individual dates is the mean of two cores from each of the chamber sections ($n = 20$). The r^2 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 C_a concentration and root respiration, specific root length, soluble sugar and starch concentrations, and root numbers in minirhizotron tubes

Variable	Date	Model	Increase or decrease with C_a	Parameter value (a)	Intercept (b)	r^2	P-value
Resp. per g	Sep 1999	ns	–	–	–	–	ns
	Jun 2000	Quadratic	Peak at 450	0.420	−0.0005	0.41	0.009
	Sep 2000	ns	–	–	–	–	ns
Resp. per cm	Sep 1999	ns	–	–	–	–	ns
	Jun 2000	Power	Increase	0.001	1.515	0.29	0.011
	Sep 2000	Quadratic	Peak at 311	0.560	−0.001	0.48	0.028
Resp. per cm^2	Sep 1999	ns	–	–	–	–	ns
	Jun 2000	Power	Increase	0.310	1.343	0.35	0.005
	Sep 2000	Quadratic	Peak at 311	7.080	−0.009	0.55	0.013
SRL cm per g	Jul 1999	ns	–	–	–	–	ns
	Sep 1999	Hyperbolic	Decrease	1829.87	−204.307	0.38	0.009
	Jun 2000	ns	–	–	–	–	ns
	Sep 2000	Hyperbolic	Decrease	5474.34	−153.877	0.13	0.041
Starch mg per mg	Sep 2000	Logarithmic	Decrease	0.322	−0.041	0.61	*0.068*
Sol. sugars mg per mg	Sep 2000	Logarithmic	Decrease	0.274	−0.029	0.46	ns
Root number (mini rhizotron)	2000 growing season	Hyperbolic	Decrease	8.821	−201.218	0.64	0.0001

Linear = linear model ($y = ax + b$), Logarithmic = logarithmic model ($y = a \ln x + b$), Power = power model ($y = ax^b$), Hyperbolic ($y = ax/(b + x)$), Quadratic = quadratic model ($y = y_0 + ax + bx^2$), SRL = specific root length, resp = respiration. P-values in italics indicate borderline significant results.

B. ischaemum reported for 2000 by Polley *et al.* (2003). However, the last section of the C_a 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.

Increasing C_a significantly reduced root lifespan of *B. ischaemum* roots with five or more neighbors (Table 4, Fig. 3). Roots produced at $C_a > 450\,\mu mol\,mol^{-1}$ and $C_a = 300–360\,\mu mol\,mol^{-1}$ had shorter lifespans than those grown at $C_a < 300\,\mu mol\,mol^{-1}$, 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 $C_a < 300\,\mu mol\,mol^{-1}$. 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 $\sim 40\%$ lower risk of mortality (i.e., longer lifespan) than finer roots (Table 4). Median lifespans for this group ranged from 111 days for $C_a > 450\,\mu mol\,mol^{-1}$ to 121 days for $C_a = 300–360\,\mu mol\,mol^{-1}$. 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%.

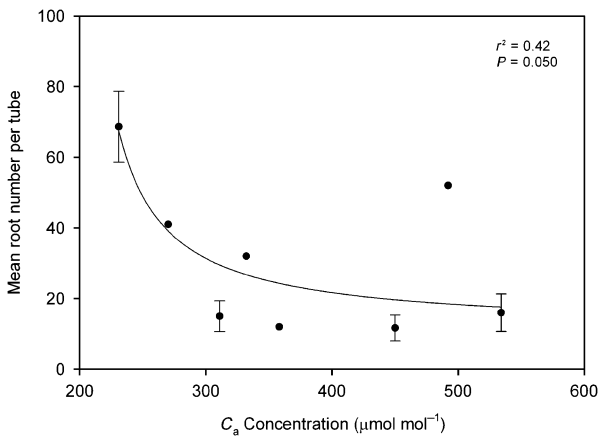

Fig. 2 Mean root number per minirhizotron tube by chamber section for *Bothriochloa ischaemum* plants along the C_a gradient in 2000 (n = 1–3 tubes per section). The r^2 and P-value shown is for the curve fit on these means. See Table 3 for analysis results on individual tubes.

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 $21.4\,nmol\,O_2\,g^{-1}\,s^{-1}$ at $450\,\mu mol\,mol^{-1}$, followed by a slight decrease (Table 3),

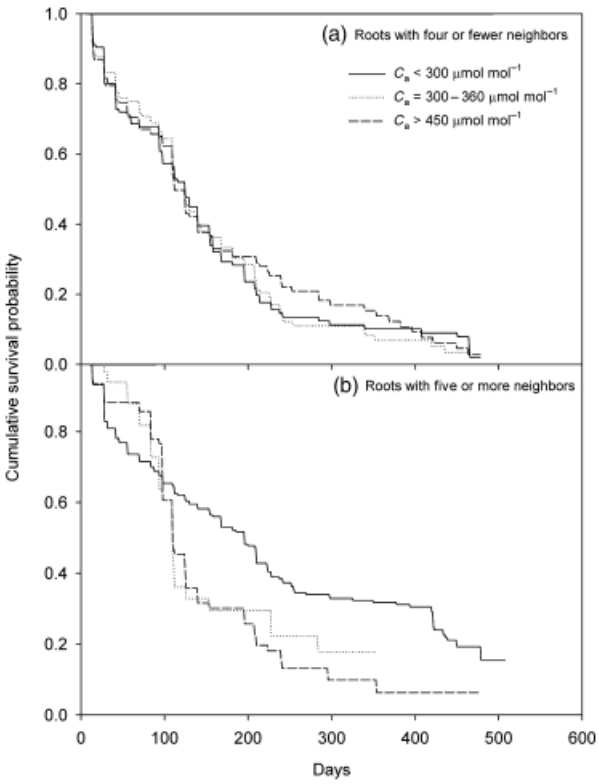

Fig. 3 Survival probability curves for roots born in 1999 and 2000 at three C_a levels. These curves account for diameter effects on lifespan, and show that lifespan is not affected by C_a for roots with four or fewer neighbors. For roots with five or more neighbors, roots grown at C_a levels above $300\,\mu mol\,mol^{-1}$ have shorter lifespans than roots grown below $300\,\mu mol\,mol^{-1}$. See Table 4 for proportional hazards regression results.

Table 4 Cox proportional hazards regression analysis results for root lifespan along the C_a gradient

Variable	DF	Parameter estimate	Standard error	χ^2 value	P-value	Hazard ratio
Roots with 0–4 neighbors in the same minirhizotron tube window (1 cm^2 area)						
$C_a = 300–360\,\mu mol\,mol^{-1}$ compared with $C_a < 300\,\mu mol\,mol^{-1}$	1	0.028	0.140	0.039	0.844	1.028
$C_a > 450\,\mu mol\,mol^{-1}$ compared with $C_a < 300\,\mu mol\,mol^{-1}$	1	−0.084	0.134	0.394	0.530	0.919
Root diameter	1	−0.486	0.170	8.166	0.004	0.615
Roots with five or more neighbors in the same minirhizotron tube window (1 cm^2 area)						
$C_a = 300–360\,\mu mol\,mol^{-1}$ compared with $C_a < 300\,\mu mol\,mol^{-1}$	1	0.377	0.212	3.158	0.076	1.458
$C_a > 450\,\mu mol\,mol^{-1}$ compared with $C_a < 300\,\mu mol\,mol^{-1}$	1	0.462	0.147	9.924	0.002	1.587
Root diameter	1	−0.128	0.198	0.418	0.518	0.880

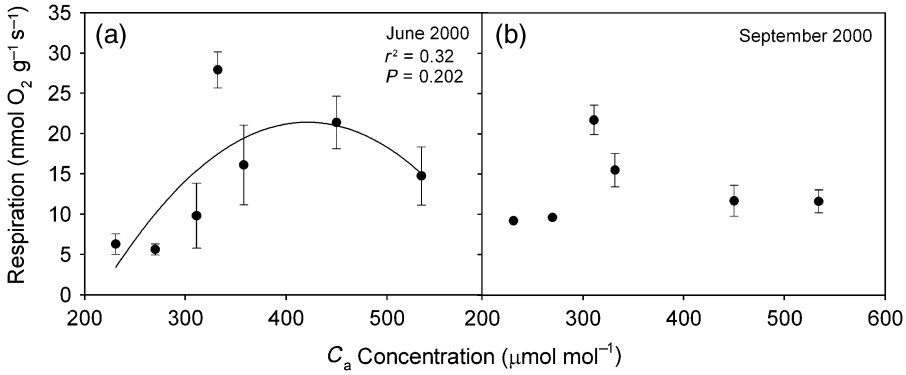

Fig. 4 Root respiration rates for *B. ischaemum* along the C_a gradient for two sample dates in 2000 (n = 1–3 roots per section). The r^2 and P-values are for the curve fit on the June 2000 means. A curve fit was not attempted on the September 2000 means as the individual root analysis showed no significant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

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 μmol mol^{-1} were particularly metabolically active at the June sampling date, with observed values of 27.9 nmol O_2 g^{-1} 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 μmol 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

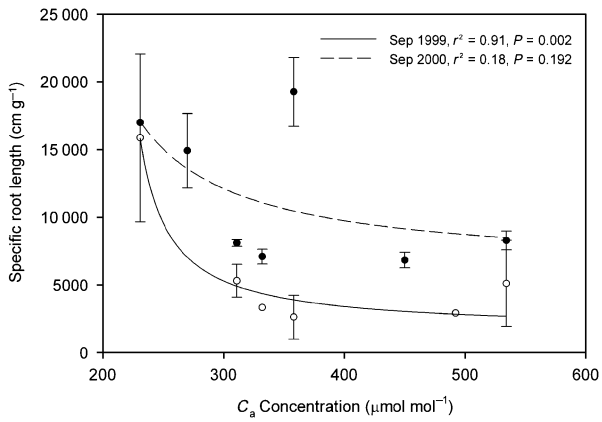

Fig. 5 Mean specific root lengths for *B. ischaemum* along the C_a gradient for September 1999 and 2000. N for each point = 1–5. The r^2 and P-values shown are for the curve fits on the means. See Table 3 for analysis results on individual roots.

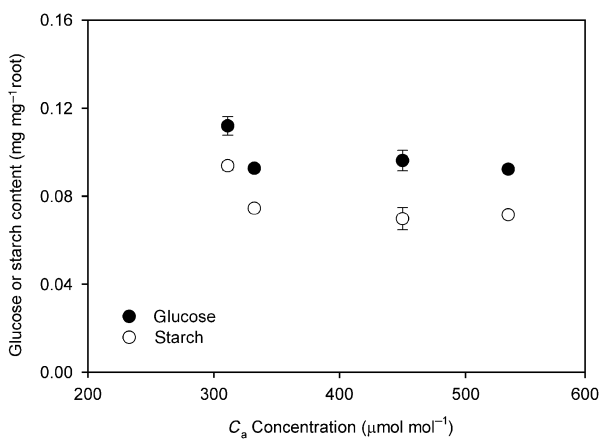

Fig. 6 Glucose and starch concentrations for roots along the C_a gradient in September 2000. N for each point = 1–2 sets of roots. No curve fits were attempted on these means as the analyses of individual variates were not significant. See Table 3 for complete analysis results.

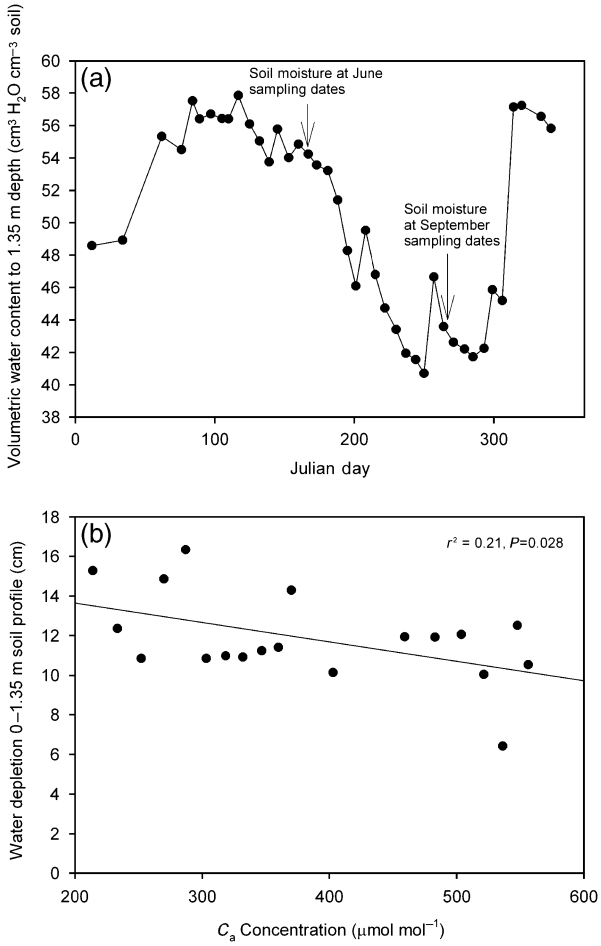

Fig. 7 (a) Volumetric soil water content for one of the ambient C_a sections ($C_a = 370\,\mu mol\,mol^{-1}$) of the gradient for the year 2000 as measured by weekly neutron attenuation. The sampling dates referred to are for root respiration. (b) Maximum soil water depletion along the C_a gradient. Maximum depletion was calculated as the difference between the average maximum water content measured by neutron attenuation following initiation of C_a control and the average minimum soil water content (Polley *et al.*, 2002). Each point was derived from one set of neutron probe measurements to 1.35 m depth in the center of one 5 m section ($n = 19$). One section ($C_a = 432\,\mu mol\,mol^{-1}$) was excluded because of unusual soil characteristics. Regression equation: $y = 15.607–0.010(x)$.

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 C_a where we observed the greatest root numbers in 2000. The positive relationships among root biomass in ingrowth cores, community aboveground biomass, and C_a, coupled with the fact that *B. ischaemum* gradually became less abundant at high C_a over time, suggest that much of the community root biomass at high C_a was from C_3 plants.

As soils are the largest carbon pool in grasslands, and grasslands contain $\sim 30\%$ of the global soil carbon pool (Jobbágy & Jackson, 2000), increased belowground productivity at high C_a 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 C_a 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 CO_2 fixation assessed through flux measurements along the C_a gradient (Polley *et al*., 2006).

Root lifespan is one of 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 C_a. We predicted longer root lifespans at high C_a for *B. ischaemum*, however the effects of C_a 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 $C_a > 450\,\mu mol\,mol^{-1}$ than roots at $C_a < 300\,\mu mol\,mol^{-1}$ (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 C_a 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 C_a 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 C_a, 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 C_a, 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 C_a emphasize the difficulties in isolating the effects of C_a on root lifespan. For example, C_a 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 $500\,\mu mol\,mol^{-1}$ in a quadratic model fit to data from June 2000 (Table 3, Fig. 4a). Interestingly, soil respiration and microbial biomass in the C_a gradient also peaked between 400 and $500\,\mu mol\,mol^{-1}$ (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 C_a during some periods. The range of mean respiration rates we observed along the C_a gradient (5.6–27.9 $nmol\,O_2\,g^{-1}\,s^{-1}$, 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 C_3 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 Q_{10} of 2, the respiration rates we observed at low C_a 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 C_a. *B. ischaemum* root respiration was suppressed at $C_a < 300\,\mu mol\,mol^{-1}$ in June 2000, with individual roots likely carbon limited in this low-CO_2 environment. However, the wide range of root respiration responses to C_a reported in the literature and the complexity of the response along our C_a gradient suggest that other variables interact to modulate the C_a effect. Previous studies have observed that root respiration increases (BassiriRad *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 C_a. Others have found, as we did, that root respiration changes in its responsiveness to 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 C_a, and so effects of C_a on root respiration may be indirect. These complexities suggest that greater attention to the mechanisms driving root respiration responses in experimental C_a systems is needed. In our study, the strongest response of respiration to 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 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 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 C_a and drought stress found that drought alone increased root TNC levels significantly, and 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 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 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 C_a probably experienced drought stress as an indirect effect of subambient C_a. More negative midday water potentials for *B. ischaemum* and *S. dimidiatum* have been observed at subambient than elevated 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 C_a in *B. ischaemum* and *S. dimidiatum*, suggesting a potential mechanism for greater soil drying at subambient 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 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 C_a, like the TNC patterns, are a response to drought at this time of year being exacerbated by high transpiration rates at low C_a, rather than a response to carbon limitations belowground.

In conclusion, this grassland responded to increased C_a with enhanced community root growth, and overall ingrowth root biomass responded more strongly to C_a increases above ambient. In contrast, the C_4 grass *B. ischaemum* had greater root numbers at low C_a where C_3 aboveground biomass had decreased. Roots growing with five or more neighbors at low C_a lived longer than roots at high C_a, raising interesting questions about the ratio of root construction and maintenance costs at different C_a levels. In June 2000, *B. ischaemum* roots at high C_a were more metabolically active, and showed peak respiration rates at high C_a consistent with patterns of soil respiration for the system as a whole. *B. ischaemum* roots at subambient 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 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 C_a. To understand the mechanisms that

will determine the role of belowground carbon sinks as C_a continues to increase throughout this century, more simultaneous measurements of root parameters for multiple species are needed in field experiments.

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References

Ackerly DD, Bazzaz FA (1995) Plant growth and reproduction along CO_2 gradients: non-linear responses and implications for community change. *Global Change Biology*, **1**, 199–207.

Allison PD (1995) *Survival analysis using the SAS system: a practical guide*. SAS Institute Inc., Cary, NC, USA.

Anderson LJ, Comas LH, Lakso AN, Eissenstat DM (2003) Multiple risk factors in root survivorship: a four year study in Concord grape. *New Phytologist*, **158**, 489–501.

Anderson LJ, Maherali H, Johnson HB, Polley HW, Jackson RB (2001) Gas exchange and photosynthetic acclimation over subambient to elevated CO_2 in a C_3-C_4 grassland. *Global Change Biology*, **7**, 693–707.

Arnone JA, Zaller JG, Spehn E, Niklaus PA, Wells CA, Körner C (2000) Dynamics of root systems in intact native grasslands: effects of elevated atmospheric CO_2. *New Phytologist*, **147**, 73–85.

Bahn M, Knapp M, Garajova Z, Fahringer NP, Cernusca A (2006) Root respiration in temperate mountain grasslands differing in land use. *Global Change Biology*, **12**, 995–1006.

Barnola JM, Raynaud D, Dorotkevich YS, Lorius CD (1987) Vostok ice core provides a 160,0000-year record of atmospheric CO_2. *Nature*, **329**, 408–414.

BassiriRad H, Griffin KL, Reynolds JF, Strain BR (1997) Changes in root NH_4^+ and NO_3^- absorbtion rates of loblolly and ponderosa pine in response to CO_2 enrichment. *Plant and Soil*, **190**, 1–9.

BassiriRad H, Tissue DT, Reynolds JF, Chapin FS III (1996) Response of *Eriophorum vaginatum* to CO_2 enrichment at different soil temperatures: effects on growth, root respiration and PO_4^{-3} uptake kinetics. *New Phytologist*, **133**, 423–430.

Berntson GM, Bazzaz FA (1996) The allometry of root production and loss in seedlings of *Acer rubrum* (Aceraceae) and *Betula papyrifera* (Betulaceae): implications for root dynamics in elevated CO_2. *American Journal of Botany*, **83**, 608–616.

Carter EB, Theodorou MK, Morris P (1999) Responses of *Lotus corniculatus* to environmental change. 2. Effect of elevated CO_2, temperature and drought on tissue digestion in relation to condensed tannin and carbohydrate accumulation. *Journal of the Science of Food and Agriculture*, **79**, 1431–1440.

Comas LH, Eissenstat DM, Lakso AN (2000) Assessing root death and root system dynamics in a study of grape canopy pruning. *New Phytologist*, **147**, 171–178.

Cox DR (1972) Regression models and life tables. *Journal of the Royal Statistical Society*, **B34**, 187–220.

Dilustro JJ, Day FP, Drake BG, Hinkle CR (2002) Abundance, production and mortality of fine roots under elevated atmospheric CO_2 in an oak-scrub ecosystem. *Environmental and Experimental Botany*, **48**, 149–159.

Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL (2000) Building roots in a changing environment: implications for root longevity. *New Phytologist*, **147**, 33–42.

Eissenstat DM, Yanai RD (1997) The ecology of root lifespan. *Advances in Ecological Research*, **27**, 1–60.

Fitter AH, Graves JD, Wolfenden J, Self GK, Brown TK, Bogie D, Mansfield TA (1997) Root production and turnover and carbon budgets of two contrasting grasslands under ambient and elevated atmospheric carbon dioxide concentrations. *New Phytologist*, **157**, 247–255.

Fitter AH, Self GK, Wolfenden J *et al*. (1996) Root production and mortality under elevated atmospheric carbon dioxide. *Plant and Soil*, **187**, 299–306.

George K, Norby RJ, Hamilton JG, DeLucia EH (2003) Fine-root respiration in a loblolly pine and sweetgum forest growing in elevated CO_2. *New Phytologist*, **160**, 511–522.

Gill RA, Anderson LJ, Polley HW, Johnson HB, Jackson RB (2006) Potential nitrogen constraints on carbon sequestration in a grassland exposed to subambient and elevated atmospheric CO_2. *Ecology*, **87**, 41–52.

Gill RA, Burke IC, Lauenroth WK, Milchunas DG (2002b) Longevity and turnover of roots in the shortgrass steppe: influence of diameter and depth. *Plant Ecology*, **159**, 241–251.

Gill RA, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. *New Phytologist*, **147**, 13–31.

Gill RA, Polley HW, Johnson HB, Anderson LJ, Maherali H, Jackson RB (2002a) Nonlinear grassland responses to past and future atmospheric CO_2. *Nature*, **417**, 279–282.

Hertog JD, Stulen I, Posthumus F, Poorter H (1998) Interactive effects of growth-limiting N supply and elevated atmospheric CO_2 concentration on growth and carbon balance of *Plantago major*. *Physiologia Plantarum*, **103**, 451–460.

Higgins PAT, Jackson RB, Des Rosiers JM, Field CB (2002) Root production and demography in a California annual grassland under elevated atmospheric carbon dioxide. *Global Change Biology*, **8**, 841–850.

Hill PW, Marshall C, Williams GG, Blum H, Harmens H, Jones DL, Farrar JF (2007) The fate of photosynthetically-fixed carbon in *Lolium perenne* grassland as modified by elevated CO_2 and sward management. *New Phytologist*, **173**, 766–777.

Huang X, Lakso AN, Eissenstat DM (2005) Interactive effects of soil temperature and moisture on Concord grape root respiration. *Journal of Experimental Botany*, **56**, 2651–2660.

Hungate BA, Holland EA, Jackson RB, Chapin FS III, Mooney HA, Field CB (1997) On the fate of carbon in grasslands under carbon dioxide enrichment. *Nature*, **388**, 576–579.

Jackson RB, Cook CW, Pippen JS, Palmer SM (2009) Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. *Ecology*, in press.

Jastrow JD, Miller RM, Owensby CE (2000) Long-term effects of elevated atmospheric CO_2 on below-ground biomass and transformations to soil organic matter in grassland. *Plant and Soil*, **224**, 85–97.

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, **10**, 423–436.

Johnson HB, Polley HW, Whitis RP (2000) Elongated chambers for field studies across atmospheric CO_2 gradients. *Functional Ecology*, **14**, 388–396.

Johnson MG, Tingey DT, Phillips DL, Storm MJ (2001) Advancing fine root research with minirhizotrons. *Environmental and Experimental Botany*, **45**, 263–289.

Jouzel J, Barkov NI, Barnola JM *et al*. (1993) Extending the Vostok ice-core record of paleoclimate to the penultimate glacial period. *Nature*, **364**, 407–412.

King JS, Pregitzer KS, Zak DR *et al*. (2001) Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO_2 and tropospheric O_3. *Oecologia*, **128**, 237–250.

Kubiske ME, Pregitzer KS, Zak DR, Mikan CJ (1998) Growth and C allocation of *Populus tremuloides* genotypes in response to atmospheric CO_2 and soil N availability. *New Phytologist*, **140**, 251–260.

Lambers H, Atkin OK, Schuerwater I (1996) Respiratory patterns in roots in relation to their functioning. In: *Plant roots: the hidden half*, 2nd edn (eds Waisel Y, Eshel A, Kafkafi U), pp. 323–362. Marcel Dekker Inc., New York.

LeCain DR, Morgan JA, Milchunas DG, Mosier AR, Nelson JA, Smith DP (2006) Root biomass of individual species, and root size characteristics after five years of CO_2 enrichment on native shortgrass steppe. *Plant and Soil*, **279**, 219–228.

Luo Y, Reynolds JF (1999) Validity of extrapolating field CO_2 experiments to predict carbon sequestration in natural ecosystems. *Ecology*, **80**, 1568–1583.

Matamala R, Schlesinger WH (2000) Effects of elevated atmospheric CO_2 on fine root production and activity in an intact temperate forest ecosystem. *Global Change Biology*, **6**, 967–979.

Milchunas DG, Morgan JA, Mosier AR, LeCain DR (2005a) Root dynamics and demography in shortgrass steppe under elevated CO_2, and comments on minirhizotron methodology. *Global Change Biology*, **11**, 1837–1855.

Milchunas DG, Mosier AR, Morgan JA, LeCain DR, King JY, Nelson JA (2005b) Root production and tissue quality in a shortgrass steppe exposed to elevated CO_2: using a new ingrowth method. *Plant and Soil*, **268**, 111–122.

Nelson N (1944) A photometric adaptation of the Somogyi's method for the determination of glucose. *Journal of Biological Chemistry*, **153**, 375–380.

Niklaus PA, Glöckler E, Siegwolf R, Körner C (2001) Carbon allocation in calcareous grassland under elevated CO_2: a combined ^{13}C pulse-labelling/soil physical fractionation study. *Functional Ecology*, **15**, 43–50.

Norby RJ, DeLucia EH, Gielen B *et al*. (2005) Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences*, **102**, 18052–18056.

Norby RJ, Jackson RB (2000) Root dynamics and global change: seeking an ecosystem perspective. *New Phytologist*, **147**, 3–12.

Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG (2004) Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. *Proceedings of the National Academy of Sciences*, **101**, 9689–9693.

Norby RJ, O'Neill EG, Hood WG, Luxmoore RJ (1987) Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO_2 enrichment. *Tree Physiology*, **3**, 203–210.

Pendall E, Bridgham S, Hanson PJ *et al*. (2004) Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. *New Phytologist*, **162**, 311–322.

Polley HW, Johnson HB, Derner JD (2002) Soil- and plant-water dynamics in a C_3/C_4 grassland exposed to a subambient to superambient CO_2 gradient. *Global Change Biology*, **8**, 1118–1129.

Polley HW, Johnson HB, Derner JD (2003) Increasing CO_2 from subambient to superambient concentrations alters species composition and increases above-ground biomass in a C-3/C-4 grassland. *New Phytologist*, **160**, 319–327.

Polley HW, Mielnick PC, Dugas WA, Johnson HB, Sanabria J (2006) Increasing CO_2 from subambient to elevated concentrations increases grassland respiration per unit of net carbon fixation. *Global Change Biology*, **12**, 1390–1399.

Pregitzer KS, Zak DR, Curtis PS, Kubiske ME, Teeri JA, Vogel CS (1995) Atmospheric CO_2, soil nitrogen and turnover of fine roots. *New Phytologist*, **129**, 579–585.

Sage RF, Cowling SA (1999) Implications of stress in low CO_2 atmospheres of the past: are today's plants too conservative for a high CO_2 world? In: *Carbon Dioxide and Environmental Stress* (eds Luo Y, Mooney HA), pp. 289–308. Academic Press, San Diego.

Scheurwater I, Cornelissen C, Dictus F, Welschen R, Lambers H (1998) Why do fast- and slow-growing grass species differ so little in their rate of root respiration, considering the large differences in rate of growth and ion uptake? *Plant, Cell and Environment*, **21**, 995–1005.

Sicher RC (2005) Interactive effects of inorganic phosphate nutrition and carbon dioxide enrichment on assimilate partitioning in barley roots. *Physiologia Plantarum*, **123**, 219–226.

Strand AE, Pritchard SG, McCormack ML, Davis MA, Oren R (2008) Irreconcilable differences: fine root life spans and soil carbon persistence. *Science*, **319**, 456–458.

van Groenigen KJ, Six J, Hungate BA, de Graaff MA, van Breemen N, van Kessel C (2006) Element interactions limit soil carbon storage. *Proceedings of the National Academy of Sciences*, **103**, 6571–6574.

Volder A, Smart DR, Bloom AJ, Eissenstat DM (2005) Rapid decline in nitrate uptake and respiration with age in fine lateral roots of grape: implications for root efficiency and competitive effectiveness. *New Phytologist*, **165**, 493–502.

Wand SJE, Midgley GF, Jones MH, Curtis PS (1999) Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmo-

spheric CO_2 concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology*, **5**, 723–741.

Ward JK, Antonovics J, Thomas RB, Strain BR (2000) Is atmospheric CO_2 a selective agent on model C_3 annuals? *Oecologia*, **123**, 330–341.

Wells CE, Eissenstat DM (2001) Marked differences in survivorship among apple roots of different diameters. *Ecology*, **82**, 882–892.

Zar JH (1996) *Biostatistical Analysis*, 3rd edn. Prentice Hall, Upper Saddle River, NJ.

