Root dynamics and demography in shortgrass steppe under elevated CO_2, and comments on minirhizotron methodology

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Abstract

The dynamics and demography of roots were followed for 5 years that spanned wet and drought periods in native, semiarid shortgrass steppe grassland exposed to ambient and elevated atmospheric CO_2 treatments. Elevated compared with ambient CO_2 concentrations resulted in greater root-length growth (+ 52%), root-length losses (+ 37%), and total pool sizes (+ 41%). The greater standing pool of roots under elevated compared with ambient CO_2 was because of the greater number of roots (+ 35%), not because individuals were longer. Loss rates increased relatively less than growth rates because life spans were longer (+ 41%). The diameter of roots was larger under elevated compared with ambient CO_2 only in the upper soil profile. Elevated CO_2 affected root architecture through increased branching.

Growth-to-loss ratio regressions to time of equilibrium indicate very long turnover times of 5.8, 7.0, and 5.3 years for control, ambient, and elevated CO_2, respectively. Production was greater under elevated compared with ambient CO_2 both below- and aboveground, and the above- to belowground ratios did not differ between treatments. However, estimates of belowground production differed among methods of calculation using minirhizotron data, as well as between minirhizotron and root-ingrowth methods. Users of minirhizotrons may need to consider equilibration in terms of both new growth and disappearance, rather than just growth.

Large temporal pulses of root initiation and termination rates of entire individuals were observed (analogous to birth–death rates), and precipitation explained more of the variance in root initiation than termination. There was a dampening of the pulsing in root initiation and termination under elevated CO_2 during both wet and dry periods, which may be because of conservation of soil water reducing the suddenness of wet pulses and duration and severity of dry pulses. However, a very low degree of synchrony was observed between growth and disappearance (production and decomposition).

Keywords: belowground decomposition–production synchrony, belowground net primary production, carbon dioxide, drought, life span of roots, methods for belowground production, root diameter and branching, root turnover, semiarid grassland, water stress

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Introduction

Root carbon inputs and storage in ecosystems is often greater than aboveground inputs and storage (Coleman, 1976; Sims & Singh, 1978). This is particularly true

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for temperate semiarid grasslands with a long evolutionary history of grazing where both abiotic and biotic forces shape the belowground dominance (Milchunas *et al.*, 1988). More than 90% of plant biomass in the shortgrass steppe of the North American Great Plains is belowground (Milchunas & Lauenroth, 2001). The concentration of atmospheric CO_2 has been increasing during the past two centuries and is predicted to

continue to increase well into the next century (Alcamo *et al.*, 1996). Root dynamics will be an important component in the trajectory of these ecosystems in response to elevated CO_2, yet this remains one of the least understood of the more basic response variables (Curtis *et al.*, 1994; Norby, 1994; Norby & Jackson, 2000) because of both the difficulty in studying roots and the biases associated with various methods (Milchunas & Lauenroth, 1992; Arnone *et al.*, 2000; Norby & Jackson, 2000; Milchunas *et al.*, 2005a).

Concentrations of atmospheric CO_2 can affect root dynamics both in terms of production, life span, and mortality and decomposition rates. Increases in total plant production with elevated CO_2 are a common response, but differences in the aboveground vs. belowground response can occur (Norby, 1994; Rogers *et al.*, 1994; Arnone *et al.*, 2000). Elevated CO_2 can often result in higher C : N ratios of plant tissue and in other ways potentially lower rates of decomposition by microorganisms or digestibility by consumers (Owensby *et al.*, 1993; Jackson & Reynolds, 1996; Fitter *et al.*, 1997; Pregitzer *et al.*, 2000; Milchunas *et al.*, 2005b). However, increased levels of soil water (Field *et al.*, 1995; Freden *et al.*, 1997; Owensby *et al.*, 1999; Morgan *et al.*, 2001, 2004; Wullschleger *et al.*, 2002) could result in increased decomposition rates, and retranslocation of solubles during senescence (O'Neill & Norby, 1996; Owensby *et al.*, 1996) may temper potential changes in decomposition rate, although our understanding of these processes is heavily based on aboveground observations. Zak *et al.* (2000) reviewed the literature on root mortality responses to elevated CO_2 and concluded that it can increase, decrease, or not change depending on species and/or environmental conditions.

We exposed a native shortgrass steppe site to 5 years of elevated CO_2 treatment. Results from this study thus far show that under elevated compared with ambient CO_2 there were periods of greater soil moisture (Nelson *et al.*, 2004) because of reduced stomatal conductance (Morgan *et al.*, 2001; LeCain *et al.*, 2003) and because of lower evaporative losses with the greater canopy development (Ferretti *et al.*, 2003). Aboveground net primary production (ANPP) (Morgan *et al.*, 2004) and belowground net primary production (BNPP) based on root ingrowth cylinders (Milchunas *et al.*, 2005a) were greater under elevated compared with ambient CO_2 concentrations. At the same time, root biomass did not significantly differ among CO_2 treatments (King *et al.*, 2004; Nelson *et al.*, 2004), suggesting that decomposition had increased. Nutritional quality of aboveground (King *et al.*, 2004; Morgan *et al.*, 2004; Milchunas *et al.*, 2005b) and belowground plant tissue (Milchunas *et al.*, 2005a) was lower under elevated compared with

ambient CO_2 treatment. Soil respiration and stable isotope analyses provide support for an increase in belowground decomposition rates under elevated CO_2 (Pendall *et al.*, 2003; Pendall *et al.*, 2005), although decomposition of aboveground material was slower (King *et al.*, 2003).

We report here on root dynamics based on minirhizotron observations during 5 years of CO_2 treatments. Differences among methods are a problem in comparing and synthesizing results from CO_2 studies in different ecosystems (Arnone *et al.*, 2000; Norby & Jackson, 2000). We compare BNPP estimates obtained from this study site using the minirhizotron with those reported earlier using root ingrowth cylinders, and examine methods of calculating BNPP from minirhizotron data. Minirhizotron methodology has the advantage of providing information in addition to estimates of turnover and BNPP, including root loss (related to decomposition), root life spans, initiation and termination of individuals (demography of births and deaths), and root size and architecture (length, diameter, branching).

Materials and methods

The study was conducted at the USDA-ARS Central Plains Experimental Range (lat. 40°49′N, long. 104°46′W) in north central Colorado. Mean annual precipitation is 321 mm, with 71% occurring during the May–September growing season (Lauenroth & Milchunas, 1991). Total vegetative basal cover at the site is typically 25–35% (Milchunas *et al.*, 1989). Vegetation has *Bouteloua gracilis* (H.B.K.) Lag., *Stipa comata* (Trin and Rupr.) and *Pascopyrum smithii* (Rybd.) as codominants (88% of total biomass), with a subfrutescent shrub (*Artemesia frigida* [Willd.]) also abundant (Morgan *et al.*, 2001). Soil is a fine sandy loam (Ustollic camborthids).

A portion of a pasture previously moderately grazed was divided into three blocks, with each including ambient ($360 \pm 20 \, \mu$mol mol^{-1}) and elevated ($720 \pm 20 \, \mu$mol mol^{-1}) CO_2 large open-top chambers (4.5 m diameter by 3.8 m high, 15.5 m^2 ground area) and a nonchambered control of equal area. Each year, chambers were placed on plots just before first vegetation green-up (late March), and removed after senescence (late October). Precipitation was returned to the chambers by an automated system, with inefficiencies in capture supplemented back to the plots. See Morgan *et al.* (2001) for details of design and operation of the chambers. Comparisons of microclimatic and plant responses indicated significant chamber effects (Morgan *et al.*, 2001, 2004). Canopy air and soil temperatures averaged 2.6 °C and 1.25 °C warmer inside than outside chambers.

The shortgrass steppe has a long evolutionary history of grazing by large herds of bison, (Milchunas *et al.*, 1988), and current primary land-use is grazing by domestic livestock. We simulated grazing on plants growing above the minirhizotron tubes sampled in this study (see Morgan *et al.*, 2004 for details), and data were used to estimate ANPP. Root dynamics represent those under the nominal grazed regime, but possibly do not differ much from the ungrazed condition as Milchunas & Lauenroth (1989) found only small effects of heavy grazing on root biomass and no grazing by CO$_2$ interactions were observed for aboveground production or forage nutritive quality during this study (Milchunas *et al.*, 2005b).

Two minirhizotron tubes were installed in each treatment-replicate (18 tubes) the summer of the year before the start of CO$_2$ exposure in order to allow for roots to establish around the tubes prior to start of the experiment. A number of studies suggest that a single growing season may allow for equilibration of roots around tubes (Polomski & Kuhn, 2002, and references in Gill *et al.*, 2002). Tubes were installed at a 23° angle to the surface using a Bartz coring guide (Bartz Technology Co., Santa Barbara, CA, USA). Tube holes were cored out using a core size slightly smaller than the tube size, followed by a reamer core (straight outside bit and wall) that cut hole sides without compacting soil to the outside of the core. The belowground portion of the 4.4 cm inside-diameter tubes spanned a horizontal distance of 140 cm, and went to a depth of 59 cm. Aboveground portions of tubes were insulated with water-pipe fiberglass tape secured with reflectance duct tape, further fully covered with a capped PVC pipe. Rubber stoppers were glued to tube bottoms, and rubber stoppers closed tops. These covers kept light from entering and minimized temperature differentials in tubes.

Video images were taken to a depth of 40 cm. Fifty video images (12.5 mm vertical by 18 mm horizontal) were taken along each length of tube using a Bartz BCT-100X minirhizotron video camera system with a square indexing handle (Bartz Technology, Santa Barbara, CA, USA). There were from four to five sampling dates from April through October each year for years 1997–2001, depending on green-up and senescence timing of the vegetation. This number of dates per year was decided based on the very slow root dynamics observed in this semiarid system, where 5.4 years turnover was estimated based on ^{14}C decay methodology (Milchunas & Lauenroth, 2001).

Video images were processed using RooTracker software (David Tremmel, Duke University, Durham, NC, USA). Roots were classified as live, live with root hairs, or dead. However, the dead classification could not be reliably determined in this semiarid grassland. Programs were written to calculate new root growth or root loss between dates and record each individual's beginning and ending length and current diameter. The 40 cm depth of images was divided into 0–20 and 20–40 cm depth increments for analyses. Demographic analyses included initiation dates, termination dates, and life span of individuals. The terms initiation and termination of individual roots can be viewed analogous to demography of births and deaths of animal individuals in a population. Initiation and termination dates were considered the mid-point of each particular period, except for roots that survived to the end of the experiment for which the last day (day 1700) of observation was used. A camera malfunction and replacement during the winter of the first year of treatment meant that observations of specific individuals for life span analyses had to begin at the start of year 2. Only roots present in year 2 were analyzed for life span, because the long life span of roots in this slow-moving semiarid environment was such that 23% of roots present at sometime during year 2 were still present at the end of year 5 of the experiment. Life spans reported here are therefore conservative, because roots present during year 2 could have been present the year before the start of the treatments just after tube instillation (2 years old) or grown around the tube the first year of treatment (1 year old) and could have survived long after the end of the experiment.

CO$_2$ treatment effects were analyzed using SAS PROC MIXED (SAS Institute Inc., Cary, NC, USA) with 'Year' or 'Period' as a repeated measure variable; 'block' as a random effect (thereby removing the variability because of blocking); and block*CO$_2$ treatment as the error term for CO$_2$ treatment comparisons. Where significant treatment effects were detected, treatment comparisons were conducted utilizing the Tukey's means comparison test at the 0.05 level of confidence. Turnover was calculated in three ways: (1) new length growth for each individual root was summed over periods within each year, with spring considered the start of a year, and divided by the sum of the maximum length (total length, not growth) for each individual root during that same year, (2) the same new length growth was divided by the total root length averaged across periods for each year, and (3) by regressing the ratio of new length growth to new disappearance within each period over time, where time in this case starts the year prior to the start of the experiment at the time of tube installation, and calculating the X-axis intercept at $Y = 1$, where the X-axis is time in years. The X-axis intercept in this latter method is the time of equilibrium between new growth around a tube and loss of roots along a tube, because

growth and decomposition should oscillate around a ratio of one when equilibrium is reached. Turnover is then equal to $1/(X$ years), where X is the intercept. This method is similar to the one illustrated by Milchunas & Lauenroth (1992, 2001) for calculating turnover from their ^{14}C-decay method of estimating BNPP by regressing isotope loss over time to the time of stabile residual isotope. Note that the 'average' method (number 2 above) is the same as the average as described in Norby & Jackson (2000) and Eissenstat & Yanai (2002), but maximum is not, and is also not the same 'maximum' as defined in Gill & Jackson (2000) and Norby & Jackson (2000). Their maximum is the maximum for a single period within a year, whereas maximum in this paper is the maximum for an individual root at any period within the year, (i.e.) the maximum length for root number 1001 may have occurred on the second sampling date that year, and for root number 1002 may have occurred on the last sampling date. Estimates of BNPP were then calculated by multiplying turnover coefficient by root biomass for each year. Root biomass estimates were obtained from two 20.3 cm diameter cylinders collected from each treatment-replicate chamber each year in autumn (King *et al.*, 2004). Initial conditions at the start of the experiment indicated no difference between ambient and elevated CO_2 treat-

ments in numbers of roots per tube (458 vs. 433, respectively), total length per tube (1027 vs. 1008 mm), or average diameter (0.191 vs. 0.188 mm), but roots on the control tubes were fewer, of less total length, and slightly larger diameter. We focus comparisons on the ambient and elevated CO_2 chamber treatments.

Results

Weather variability

Precipitation during the 5 years of study varied from 84% above average to 7% below average (Fig. 1a), and the seasonal pattern of precipitation also varied within both wet and dry years (Fig. 1b). The fourth and fifth years of the study both had periods of severe drought that were more intense than either the annual or seasonal amounts may indicate. The fourth year had a very dry spring and the drought lasted through late in the growing season, when a short period of very intense storms raised the late season and annual totals to near average. The fifth year was a drought year as well, but seasonally the opposite of the fourth year, with a wet spring followed by a long drought through the rest of the year. The two wet years (first and third) also varied seasonally; the first having a wet late season and the
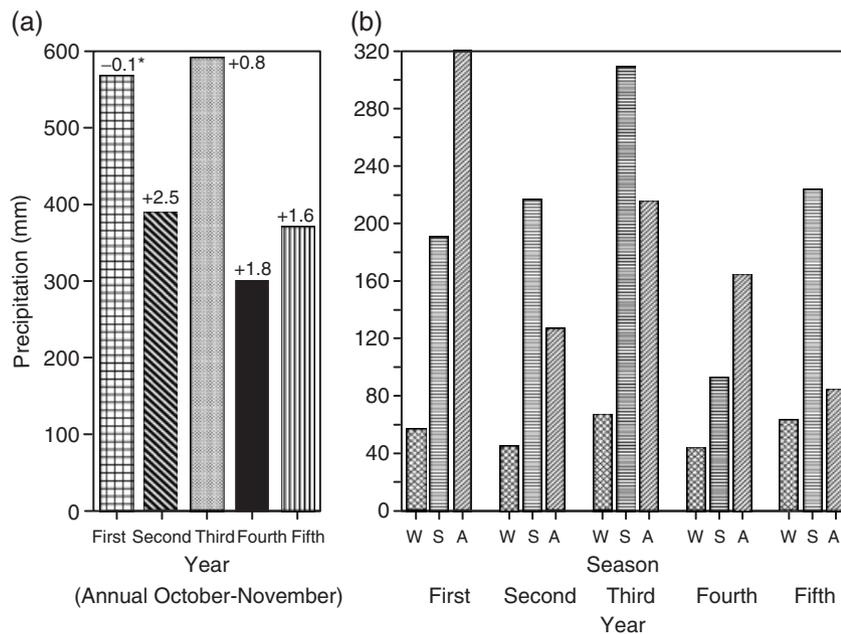

Fig. 1 (a) Annual and (b) seasonal precipitation (mm) (W, winter; S, spring, early growing season; A, autumn, late growing season) during the 5 years of CO_2 treatments at a shortgrass steppe site. Long-term average annual precipitation is the dotted line. Annual precipitation was calculated from November through October, as chambers were removed in October after plant senescence. Winter was considered November–March, early growing season April–July 15, and late growing season July 15–October. July 15 is the approximate time of peak-green-standing crop. Year 1–5 were 1997–2001. * = Difference between elevated and ambient CO_2 treatments in the percent gravimetric soil water overwinter carryover from the previous year ((i.e.), the differences between soil moisture of the treatments in early spring right after moving open-top chambers onto plots). Soil water data is from Nelson *et al.* (2004).

third having a wet early season. Thus, the years of study spanned a wide variety of conditions. The broad longer-term general trend was one of an overall relatively wet period of years prior to and during early years of the study followed by a period of dry years later in the study. Periods of drought within years, and longer-term multi-year wet–dry cycles are common in this semiarid environment (Lauenroth & Milchunas, 1991).

Root length production and loss

For data compiled on an annual basis, a significant effect of CO$_2$ treatment by depth in the soil profile, and main effect of year, was observed for length of new root growth (Fig. 2a). Elevated CO$_2$ increased root growth over ambient conditions in the upper-profile, but the increase was smaller and nonsignificant at the deeper depth. No differences in growth were observed between control and ambient treatments. Root growth was greatest in the first year (Fig. 2b), but this may be at least partially because equilibrium in growth around the tube may not have been reached even though tubes had been installed in summer, the year before starting treatments (see also equilibrium data by period presented later). The very wet third year did not show more root production than the previous year, which had one-third less precipitation, or than the last year, when drought occurred during the latter half of the year. Root growth was lowest in the fourth year when drought extended throughout the first half of the year and late into the growing season.

Root length disappearance was also greater in the elevated compared with ambient CO$_2$ treatment (Fig. 2c), but the difference was not as large as for growth and there was no CO$_2$ by depth interaction. Root disappearance was also greater in ambient than control CO$_2$ treatment. Depth interacted with year in terms of root length disappearance (Fig. 2d). Disappearance was greater in the upper compared with lower depth in the second and the wet third year. Depth was not a factor in root disappearance during the two drought years (years 4 and 5). The very wet third year showed a huge pulse in root disappearance. The lack of a similarly large amount of root disappearance in the first year, which was also wet, may be because of the long life span of roots in this system (data presented later). The drought during the fourth year had a smaller effect on disappearance than it had on growth (Fig. 2b, d).

The large differences among years in growth and loss of roots, and the periods of very high root initiations and terminations (see Fig. 6 and root demography section below), raises questions concerning the degree of control of production and decomposition by precipitation, and the synchrony of the two processes. For the control treatment, annual precipitation explained 59% of the variance among years in annual root length growth (Fig. 3a). In contrast, annual precipitation explained only 35% of the variance among years in annual root length loss. Regressions by seasonal rather than by annual time increments lowered explanatory power for both root growth ($r^2 = 0.26$) and disappearance ($r^2 = 0.04$). Synchrony of root growth and disappearance was low, with an $r^2 = 0.05$ for all treatments combined or $r^2 = 0.04$ for the control alone. Synchrony of root growth and disappearance was also low when calculated on an annual basis ($r^2 = 0.01$).

Root characteristics

The number of roots per tube shows a similar pattern with depth and CO$_2$ treatment as root length, with elevated CO$_2$ greater than ambient, and the difference greatest in the upper than lower depth (Fig. 4a). The numbers of roots were similar in upper and lower depths in the elevated CO$_2$ treatment, but were greater in the lower depth in the control and ambient treatment. A CO$_2$ treatment by year interaction was also significant. In general, the difference in root numbers between elevated compared with ambient CO$_2$ treatment increased through time, whereas the difference between ambient compared with control CO$_2$ treatment decreased through time (Fig. 4b). Root numbers generally increased from year 1–3, declined dramatically during the fourth year drought, and recovered slightly during the fifth year that had drought but a moist spring.

The average diameter of roots was larger under elevated compared with ambient CO$_2$ treatment in the upper soil profile, but not at the deeper depth (Fig. 4c). Root diameters were smaller in the lower compared with upper depth only in the control treatment. Root diameters showed a large decline from previous widths during the fourth and fifth year drought (Fig. 4d), in conjunction with the large decline in root numbers during the same period (Fig. 4b).

The sum of maximum lengths of individual roots within an annual period was greater in elevated compared with ambient CO$_2$ treatment in all years, and the difference was greater during the latter 3 years compared to the first couple years (Fig. 5a). Maximum root length on ambient compared with control CO$_2$ treatment progressed from greater in the first year to less by the last year of the experiment. Root length peaked during the third wet year, and was much shorter during the fourth and fifth two drought years. The average amount of standing root length during a year showed similar relative differences among CO$_2$ treatments as sums of maximum lengths of individuals,
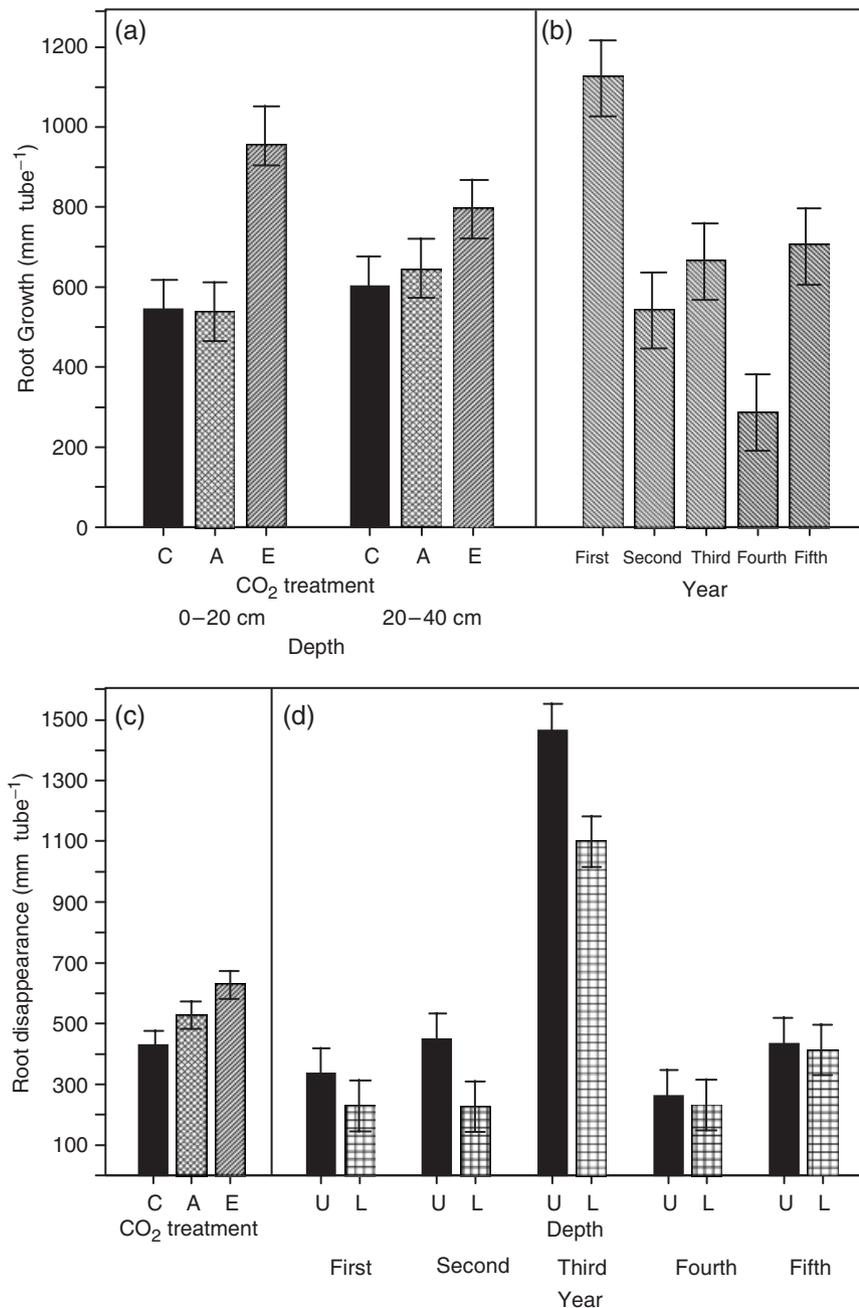
Fig. 2 Root new length growth (mm tube^{-1}) for (a) control (C) ambient (A), and elevated (E) CO_2 treatments by 0–20 and 20–40 cm depths in the soil profile, (b) for 5 years averaged over CO_2 treatments, and root length disappearance (mm tube^{-1}) and for (c) CO_2 treatments, and (d) upper (U) and lower (L) depths in the soil profile over 5 years of CO_2 treatments. Confidence intervals (Tukey's HSD, $P = 0.05$) are for CO_2 treatment or year within other factors. Values were summed over several sampling periods within each year. The insert in the upper right corner shows the pattern of growth or disappearance based on data for each period, averaged over all treatments and depths.

with overall means of 1313, 1407, and 1980 mm tube^{-1} (standard deviation (SD) 358, 408, 415 mm tube^{-1}) for the control, ambient, and elevated CO_2 treatments, respectively. However, the average length per individual root did not differ between elevated (2.93 mm, SD

1.53 mm) and ambient (2.97 mm, SD 2.01 mm), but both were slightly longer than roots in the control plots (2.85 mm, SD 1.6 mm).

The number of roots classified within a screen-view according to branching level showed a greater number
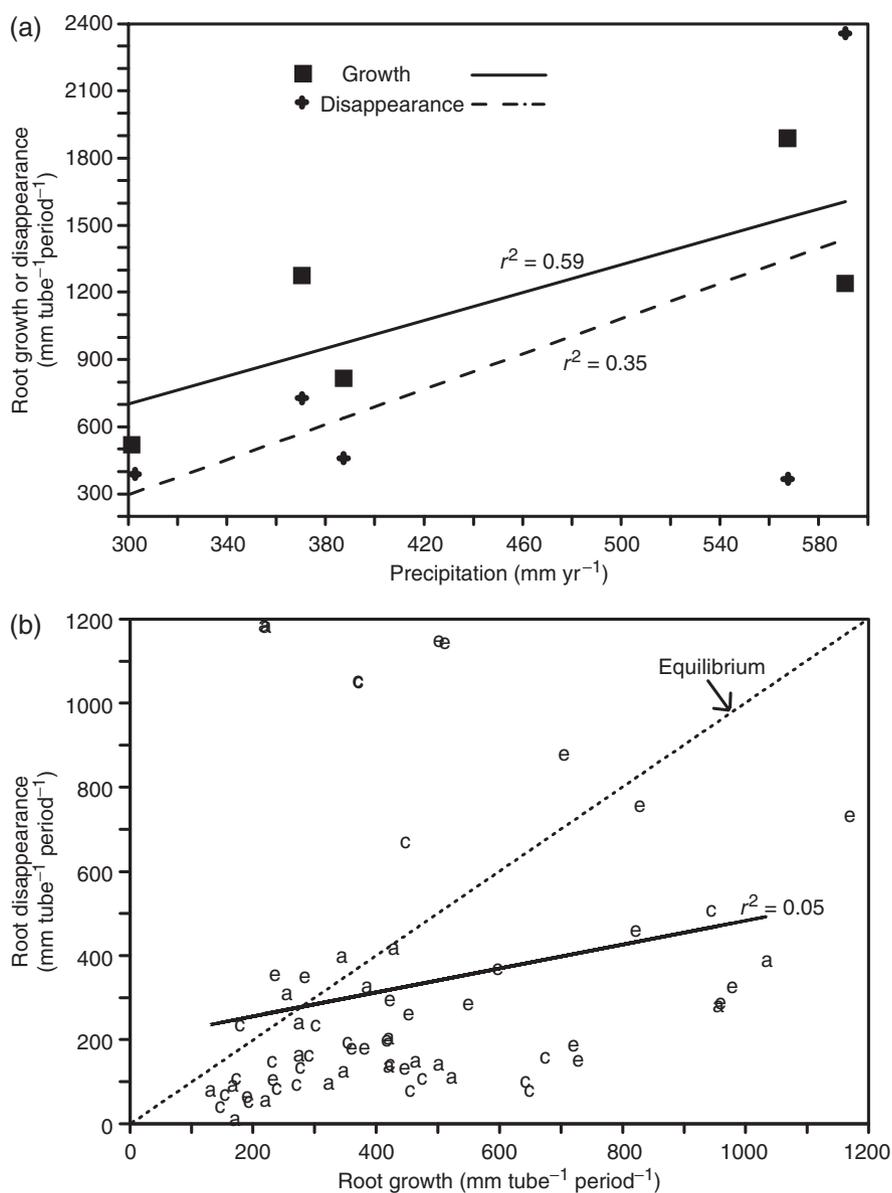
Fig. 3 (a) Root length growth and root length disappearance (mm tube^{-1} yr^{-1}) in relation to annual precipitation (mm yr^{-1}) for the control treatment. (b) Root length disappearance in relation to root length growth (mm tube^{-1} period^{-1}). *c*, control; *a*, ambient; *e*, elevated CO$_2$ treatment.

and proportion of secondary compared with primary roots in the elevated compared with ambient CO$_2$ treatment (Fig. 5b). There were as many secondary lateral roots as there were primaries in the elevated CO$_2$ treatment. The number of roots with root hairs was greater in elevated than ambient CO$_2$ treatment (data not shown), but the number of total roots was also greater (Fig. 4a, b) and, therefore, the proportion with roots hairs was not greater. The average percentages of roots with root hairs were 2.19%, 2.29%, and 2.64% (SD 0.17%, 0.58%, 1.8%) for the control, ambient, and elevated treatments, respectively.

Root demography

Initiation and termination of individual roots was assessed within each period from the spring of the second year (camera breakdown and replacement occurred the winter of the first year) through the end of the experiment. Termination of an individual root is defined as disappearance, as live vs. dead roots could not be distinguished in this system (see 'Methods'). The dynamics of both initiations and terminations appear to be dominated by periods of high peaks, surrounded by periods of low initiation and termination rates (Fig. 6a, c).
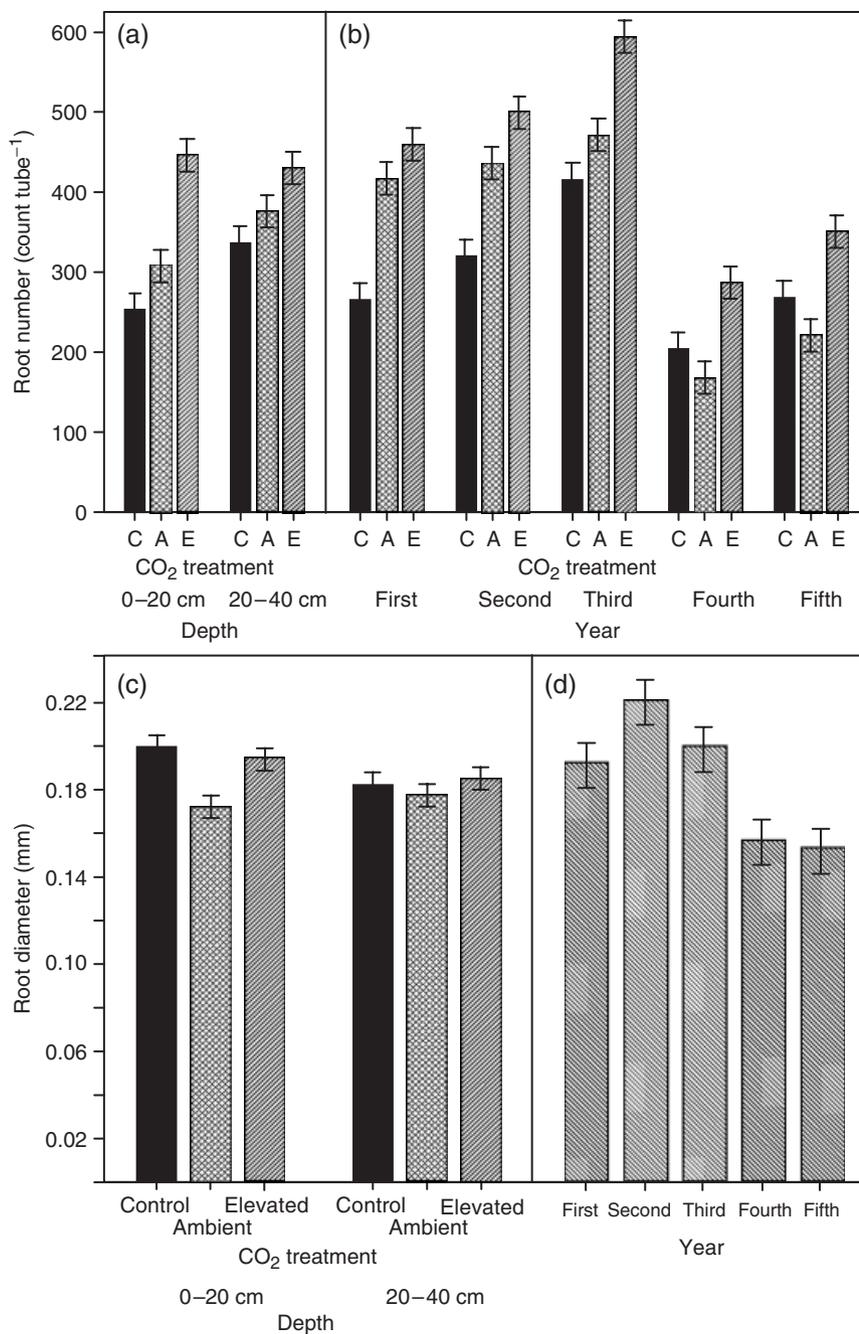
Fig. 4 Number of roots (count tube^{-1}) for (a) control (C) ambient (A), and elevated (E) CO_2 treatments by 0–20 and 20–40 cm depths in the soil profile, (b) for CO_2 treatments over 5 years, and root diameter (mm) and for (c) CO_2 treatments by 0–20 or 20–40 cm depths, and (d) for 5 years averaged over CO_2 treatments. Confidence intervals (Tukey's HSD, $P = 0.05$) are for CO_2 treatment within other factors (a–c) or for year (d). Values were averaged over several sampling periods within each year.

Peaks in initiations occurred under several situations: (1) early in the second year after the very wet second half of the first year with a large over-winter carryover of moisture, (2) the later half of the third year with the prior very wet spring, and (3) a smaller but extended period of increased initiations during the early parts of year 5 after the previous years' late-

season storms and earlier drought and the relatively wet spring that year (Figs 1a, b and 6a). However, noteworthy is the relatively lower root initiation rates on the elevated compared with the other CO_2 treatments during two of the peaks, and a general trend of greater initiation rates during other time periods, especially during the year four drought. Overall, root
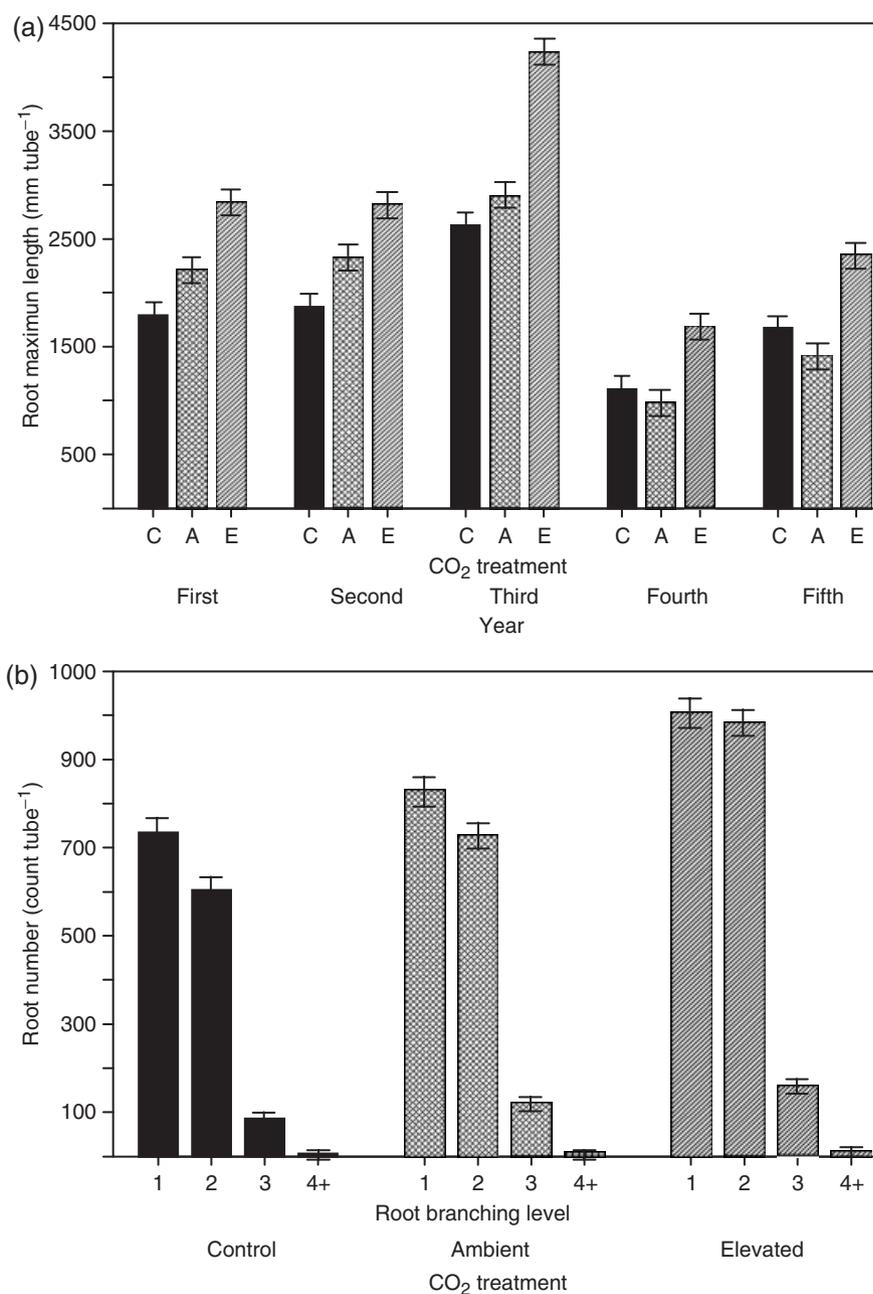
Fig. 5 (a) Root maximum length (mm tube^{-1}) for control (C) ambient (A), and elevated (E) CO_2 treatments over 5 years and (b) number of roots classified as first, second, third, or fourth and greater order branching level for each CO_2 treatment. Maximum length was the sum of each individual root's maximum length during that year. Branching order was classified within each individual frame (~ 50 frames tube^{-1}) and, therefore, do not represent actual branching order throughout the entire soil volume occupied by an individual plant. Confidence intervals are for CO_2 treatment within other factors.

initiations were lower on the elevated compared with ambient or control CO_2 treatments (Fig. 6b). High termination rates (Fig. 6b) coincided with high initiation rates of roots during some of the peaks (third and early fifth years), but not others (second and late fifth). Root terminations were greater under control and ambient compared with elevated CO_2 treatment at the

end of the fourth year with the earlier drought, and termination rates were delayed and lower under elevated CO_2 during the third year peak in terminations (Fig. 6c). Thus, elevated CO_2 sometimes resulted in an amelioration of both initiations and terminations during extreme wet and dry cycles. In contrast to root initiation rates, however, CO_2 treatment was not
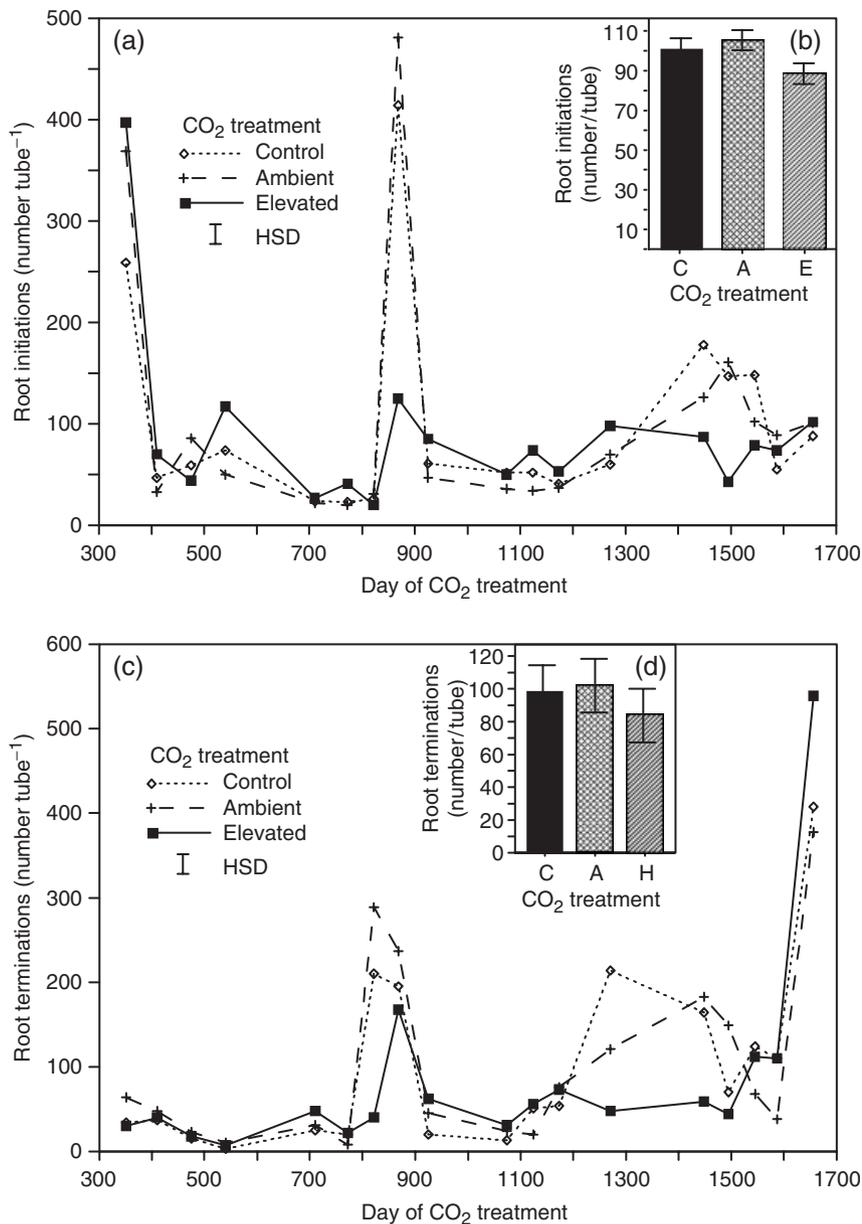
Fig. 6 Root initiations (number tube^{-1}) (a) during each sampling period over the last 4 years of control, ambient, and elevated CO_2 treatments, (b) root initiations for CO_2 treatments averaged over all periods, (c) root terminations (number tube^{-1}) during each sampling period over the last 4 years of control, ambient, and elevated CO_2 treatments, and (d) root terminations for CO_2 treatments averaged over all periods. Initiations are those occurring from the previous sampling date to the day of CO_2 treatment shown. The first year was not included in life span analyses because of a camera breakdown during the first winter of the experiment, which precluded tracking of a specific individual root from year one to later years. Termination here refers to the death plus total disappearance of an individual root, because only date of disappearance of an individual could be determined (see 'Methods'). Confidence intervals are for CO_2 treatment.

significant as a main effect for root termination rates (Fig. 6d). This was in contrast to the greater root-length disappearance observed under elevated compared with other CO_2 treatments (Fig. 2c).

Root life spans (period of presence of individuals) were significantly affected by CO_2 treatment, with longer life spans observed under elevated compared with either ambient or control treatments (Fig. 7a). Root life span was also greater in the lower compared with the upper depth in the soil profile (Fig. 7b), but no CO_2 treatment by depth interaction was observed. The life span of individual roots was not related to root diameter when assessed by either mean diameter of life span groups (Fig. 7c) or when plotted for 36 777
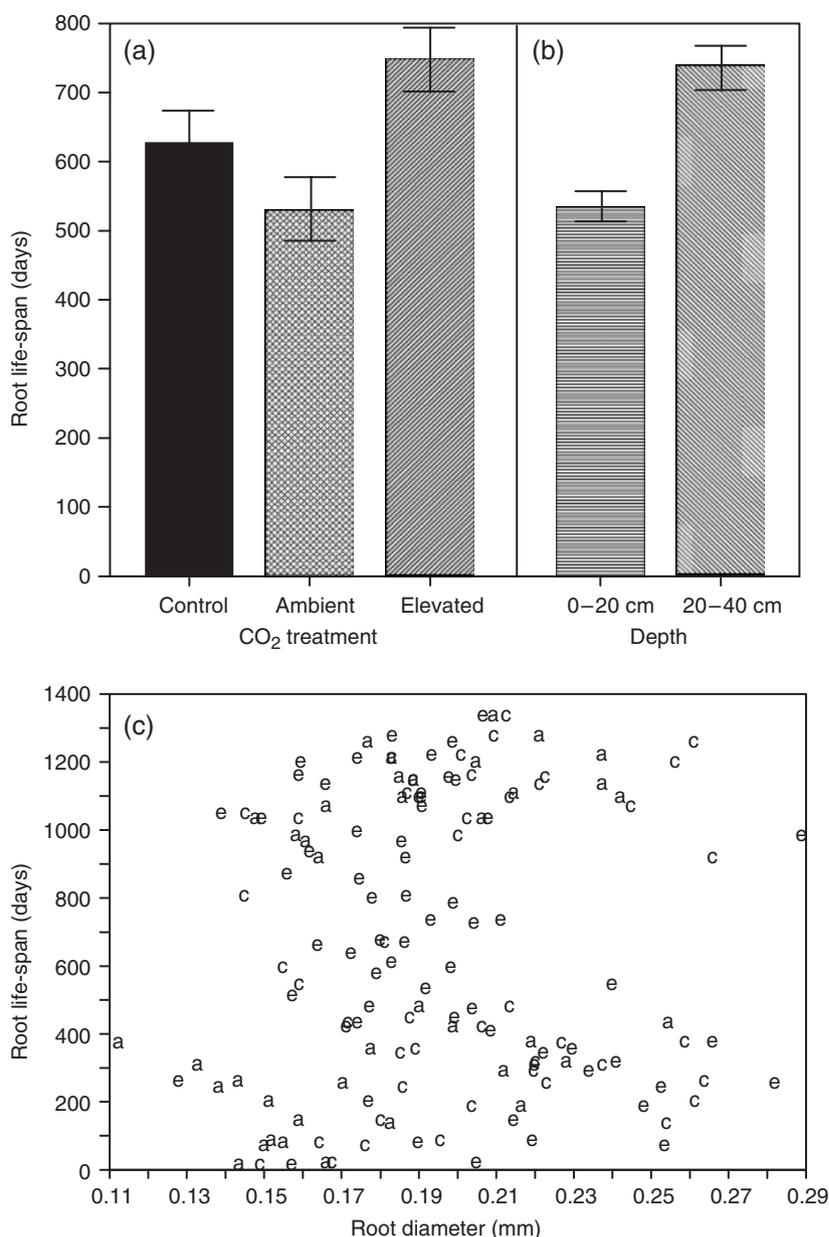
Fig. 7 Root life span (days) for (a) control, ambient, and elevated CO$_2$ treatments, (b) 0–20 and 20–40 cm depth increments in the soil profile, and (c) as a function of root diameter (*c*, control; *a*, ambient; *e*, elevated CO$_2$ treatment). Life span was calculated only for roots present during year 2 of the experiment, with initiation-day for each individual root calculated as the midpoint of the period of appearance and termination-day as the midpoint of the period of disappearance (except for roots that survived from year 2 until the end of the experiment, where the end day was used as the termination day). Life spans are an underestimate of actual maximum life span because roots present at the start of year 2 could have been present as long as two additional years ago (1 year of acclimation, and 1 year of prior CO$_2$ treatment) and 23% of roots present at some point in year 2 survived until the end of the experiment at the end of year 5. Each point in part c is an average diameter of from 2 to 955 individual roots (mean = 75 roots), depending on the number of individuals in a particular life span group (initiations and terminations were not continuously monitored, but represent mid-points of the sample periods; see 'Methods').

individual roots (data not shown). Grouping all 36 777 year-two roots into those that lived less than 300 days, 300–1000 days, and greater than 1000 days also yielded

very similar diameters of 0.2018, 0.2012, and 0.2068 mm (SD 0.023, 0.021, 0.027 mm), respectively. Diameters differed only when roots were grouped into very short-

lived (<100 days) and very long-lived (>1200 days), with average diameters of 0.1761 and 0.2038 mm (SD 0.018, 0.021 mm), respectively.

Turnover coefficients and BNPP

Part of the reason for the low synchrony between root growth and disappearance may be because of the general slow temporal dynamics in this semiarid system. At equilibrium, root growth and loss around the minirhizotron tube should oscillate temporally around a value of 1, and should have a slope of 0. Plots of root length growth to disappearance ratios over time indicate that equilibrium was barely reached over the 5 years of CO_2 treatment and nearly six years after minirhizotron-tube installation, depending on treatment (Fig. 8). Root turnover determined by regressing the root growth to disappearance ratio over time and calculating the time of intercept at Y (ratio) = 1 resulted in estimates of 5.8, 7.0, and 5.3 years for the control, ambient, and elevated CO_2 treatments, respectively.
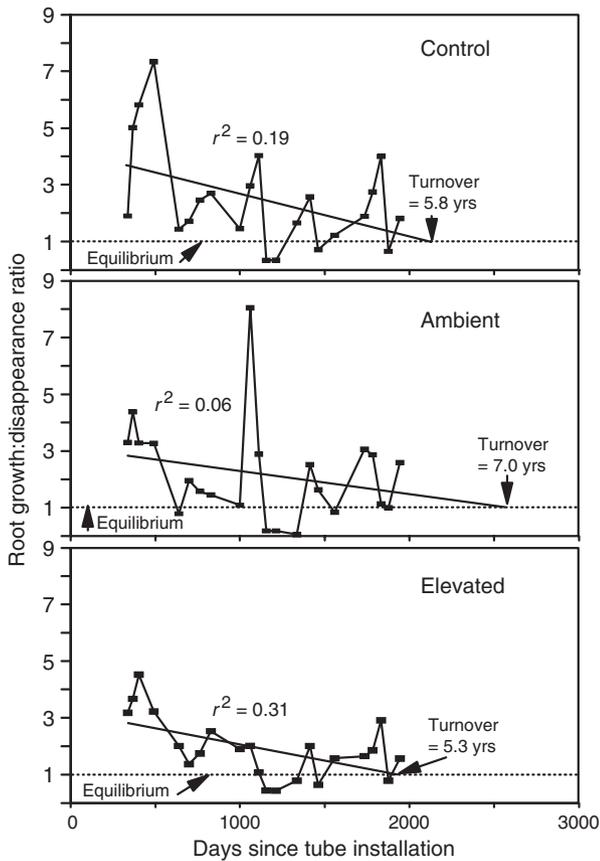

Fig. 8 Root length growth to disappearance ratio over time as minirhizotron tube installation for control, ambient, and elevated CO_2 treatments. Arrows indicate the estimated time for complete turnover of roots for the three treatments.

Table 1 Root turnover coefficients (annual averages) based on three methods of calculation from minirhizotron root length data

	Root turnover coefficient (annual average)		
	Method of calculation		
CO_2 treatment	Maximum length	Average length	Regression
Control	0.2125[a]	0.4474[a]	0.1719[b]
Ambient	0.2102[a]	0.4801[b]	0.1410[a]
Elevated	0.2119[a]	0.4423[a]	0.1889[b]

The maximum length method used the sum of annual new growth divided by the sum of the maximum length for each individual root during that same year, the average length method used new length growth divided by the total root length averaged across periods for each year, and the regression method used 1 (turnover years)$^{-1}$ from Fig. 8 of the regression of growth to disappearance ratio over time. Different superscript letters within a column indicate significant differences.

The regressions were generally noisy, but highest fit was obtained for the elevated CO_2 treatment. The lower temporal variance in the growth-to-disappearance ratio on the elevated CO_2 treatment further suggests an amelioration of extremes of the two processes during wet and dry cycles as similarly seen for individual root initiation and termination rates (Fig. 6a, c).

Turnover coefficients were calculated based on the regressions in Fig. 8 and by dividing annual new root growth by either the sum of the individual root's maximum lengths that year or the total root length averaged across periods for that year (Table 1, and see 'Methods' for detailed definition of maximum and average). The maximum length and the regression method generally gave similar turnover coefficients, and the coefficient was higher under elevated compared with ambient CO_2 treatment for the regression method. The average length method gave much higher turnover coefficients, and the coefficient was lower rather than higher under elevated compared with ambient CO_2 treatment.

Annual turnover coefficients were multiplied by annual estimates of root biomass from the treatment-replicates to obtain estimates of BNPP. We present data for the regression method and the individual root maximum lengths method because the sum of individual root maximum lengths during each year was a more similar parameter to the sum of individual length growth for the year than was period averages for the year. Main effects of year and CO_2 treatment were observed for BNPP, ANPP, and ANPP-to-BNPP ratios. BNPP by maximum length was very low in both the
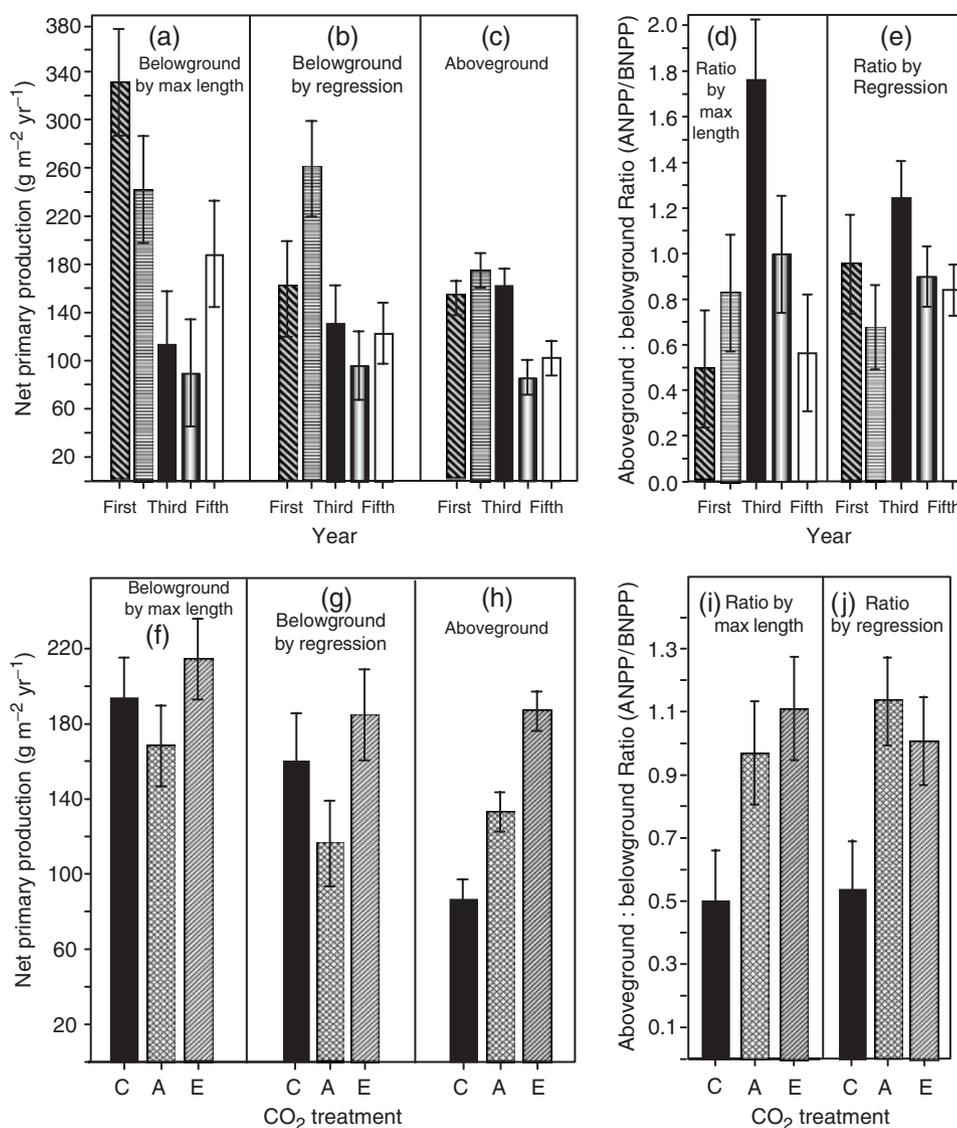
Fig. 9 Net primary production ($g\,m^{-2}\,yr^{-1}$) (a) belowground by the maximum length method, (b) belowground by the regression method, (c) aboveground, and aboveground to belowground ratios based on (d) the maximum length method and (e) the regression method, all for 5 years of study averaged over CO$_2$ treatments. Net primary production (f) belowground by the maximum length method, (g) belowground by the regression method, (h) aboveground, and aboveground to belowground ratios based on (i) the maximum length method, and (j) the regression method, all for elevated CO$_2$ treatment averaged over years.

wet third year as well as the dry fourth year, and rebounded somewhat during the fifth year with a wet spring followed by drought (Fig. 9a). BNPP by regression was significantly higher in the second year and other years were not significantly different (Fig. 9b). ANPP was low during both drought years, but also did not respond to a large degree to the all-season wet third year (Fig. 9c). ANPP-to-BNPP ratios based on the maximum length method were greatest during the third year, and did not differ significantly among other years (Fig. 9d). ANPP-to-BNPP ratios based on regressions were also highest in the third year (Fig. 9e, but the range in values was less than that observed for the

maximum length method (Fig. 9d). A similar relationship among CO$_2$ treatments was observed for BNPP based on maximum length vs. regression, although values were lower based on regression and the difference between ambient and elevated CO$_2$ treatments was greater based on regression (Fig. 9f, g). Both BNPP and ANPP were greater under elevated compared with ambient CO$_2$ treatment, but whether the difference was greater for ANPP than BNPP depended upon the method used to calculate BNPP (Fig. 9f–h). BNPP estimates based on annual average lengths were 414, 400, and 432 $g\,m^{-2}\,yr^{-1}$ (SD83, 80, 91 $g\,m^{-2}\,yr^{-1}$) for the control, ambient, and elevated CO$_2$ treatment,

respectively, with no significant CO_2 treatment effect. ANPP-to-BNPP ratios did not differ significantly between elevated and ambient treatments, although control values were lower than both (Fig. 9i, j).

Discussion

Dynamics of root growth and loss

While absolute estimates of BNPP by minrhizotron and other methods may have biases, much interesting information can be generated using the minirhizotron method. Of particular interest is the dynamics of the root pool with respect to gains and losses, how this changes under different seasonal and annual abiotic conditions, and how the demography of individuals affects these dynamics. In general, elevated compared with ambient atmospheric CO_2 concentrations resulted in much greater root-length growth ($+52\%$), relatively smaller but still positively greater root-length losses ($+37\%$), with total pool sizes intermediately greater ($+41\%$). The greater standing pool of roots under elevated compared with ambient CO_2 was because of the greater number of roots ($+35\%$), not because individuals were longer. Loss rates increased relatively less than growth rates because life spans were longer ($+41\%$). There was an immediate response of an increase in root numbers with elevated CO_2, and this perpetuated the increased growth rates, and possibly was also the reason for the inhibited initiation rates during subsequent years. Root diameters were also greater under elevated CO_2, even though roots also branched more under elevated CO_2. The increase in the root-length pool under elevated CO_2 did not match measured nonsignificant increase in root biomass pools ($+41\%$ vs. $+18\%$) possibly because root numbers were just coming into equilibrium around the minirhizotron tubes 6 years after installation at the end of the experiment while new biomass growth was only gradually being added to the existing, large root biomass pool. Root biomass estimates were obtained by hand picking roots from the soil, so the larger diameter of roots under elevated CO_2 should have tended to increase not decrease the biomass differences if this had been a factor because hand picking should favor larger roots.

Some studies have found changes in the vertical distribution of roots in response to elevated CO_2 (Day *et al.*, 1996; Thomas *et al.*, 1999; Arnone *et al.*, 2000) while others have not (Higgins *et al.*, 2002). In this study, increases in root new length growth and diameter with elevated CO_2 were greatest in upper compared with deeper depths in the profile. However, architectural biases may be different between minirhizotron tubes that are inserted at an angle to the ground surface and root-ingrowth cores or cylinders that are generally inserted vertically. At the same CO_2 site, we observed greater increases in root production with elevated CO_2 at deeper depths using root-ingrowth donuts (Milchunas *et al.*, 2005a) compared with a greater effect of elevated CO_2 on growth in the upper depth using the minirhizotron. Soil water increases with elevated CO_2 at this site were greatest at deeper depths (Nelson *et al.*, 2004). Disappearance of roots was greater in the upper soil profile and life spans were greater in deeper depths regardless of CO_2 treatment.

No consistent seasonal patterns in root growth and loss or demography were observed, which is consistent with 13 years of seasonal root biomass data from a nearby study site Milchunas & Lauenroth (2001). Interestingly, dramatic peaks and troughs were similarly observed in both studies in this semiarid grassland. Root initiations appeared to follow very wet periods and to a lesser extent good spring precipitation after a previous drought. Large termination (disappearance) rates appeared to follow a very wet period (but not the first wet period possibly because roots were too young at the time) during drought and following a good spring after a drought. Very long-term studies would be necessary to better define the abiotic controls of these dynamics, but this system does appear to be subject to strong pulsing events. There also appears to be a dampening of the pulsing under elevated CO_2 during both wet and dry periods, which may be because of conservation of soil water at this site (Nelson *et al.*, 2004) reducing the suddenness of the wet pulses and the duration and severity of the dry pulses. Smoothing of endogenous pulses in a system can lead to altered states, and this is an aspect of CO_2 enrichment in semiarid environments that may deserve greater theoretical and empirical attention. Precipitation explained more of the variance in root growth than it did for root disappearance, and this may or may not be because of time lags that may be involved in death and then the disappearance of roots. The very low degree of synchrony of the two processes can have implications for timing of nutrient availability for plants and consumers, as well as for carbon biomass dynamics and estimates of BNPP by sequential coring methods.

Root demography and morphology

Reviews of fine-root (<2 mm) turnover rates generally indicate values of 3 years or less, and values of 1 or 2 years are often used as a general value (Gill & Jackson, 2000; Gaudinski *et al.*, 2001), although isotope decay methods have yielded estimates of 5–7 years in

grasslands (Milchunas & Lauenroth, 2001), 3–18 years in eastern US forests (Gaudinski et al., 2001), and 4.2 years mean residence time for roots <1 mm in a loblolly pine forest (Matamala et al., 2003). Eissenstat & Yanai (1997) reviewed 16 minirhizotron or rhizotron studies and showed the longest median root life span reported was 340 days for sugar maple in Michigan and the shortest was 16 days for Volkamer lemon in Florida. We were not able to distinguish live from dead roots in this study (see 'Methods'), so life span as defined here represents the time for death and decomposition or consumption, as is generally the case for isotope decay studies and many minirhizotron studies. Twenty-three percent of roots present during the year when life span analyses began (second year, with a total of approximately 11 000 individual roots) survived until the end of the experiment at the end of the fifth year. Twenty-six percent of roots present the spring of year two (day 351 of CO$_2$ treatment) were present on day 1656 at the end of the experiment, or 3.6 years. Some roots in this system must be present for very long periods of time, because some of the roots could very well have been present 2 years earlier just after the tubes were installed, and some would have undoubtedly lived longer. Turnover time from the growth-to-loss ratio equilibrium regressions indicate a turnover time of 5.8 years under control conditions. Mean life span in this study based on disappearance of individuals, a very limited and conservative estimate in this study (see 'Results' section), was a little over 600 days under control conditions and approximately 750 days under elevated CO$_2$ treatment. Root life span in this semiarid grassland is long compared with many other studies, and elevated CO$_2$ increased life spans. This is consistent with earlier considerations of root strategies in relation to resource availability in forests, where there is evidence for relatively high longevity in nutrient-poor environments (Schoettle & Fahey, 1994).

Root diameter has recently been established as a potentially important factor in life span or turnover times of roots (Eissenstat & Yanai, 1997). Gill & Jackson (2000) found that root life span doubled as diameter increased from <1 to <2 mm. Wells & Eissenstst (2001) observed that apple-tree roots of <0.3, 0.3–0.5, and 0.5–1.1 mm diameters had median life spans of <2, 3–5, and >7 months, respectively. Gaudinski et al. (2001) found 2–3 year differences in average root ages for small vs. larger diameter fine roots from three different eastern US forests. Almost all roots sampled in this study were fine roots; only 2 of 221 611 total roots were >2 mm and the mean was 0.19 mm. In this study, we found more rapid turnover only when roots were grouped into extreme classes of relatively very fine and relatively very coarse. Many studies of life spans in relation to root diameter come from forests or more mesic grassland. Under semiarid conditions, adaptations to desiccation loss may play a role in increasing structural integrity of roots through periods of drought (Lauenroth et al., 1987) and decomposition rates are slow, both of which may decrease disappearance rates and minimize differences among diameter classes. The range in root diameters for fine, intensively distributed roots of the grass life form may also be narrower than for other life forms. Elevated compared with ambient CO$_2$ treatment in this study resulted in greater root diameters and longer life spans. The increased soil moisture with elevated CO$_2$ may increase life span through the drought periods during this study, but would tend to increase decomposition rates. Characteristics of roots sampled from ingrowth cylinders at this same site showed some subtle differences in terms of nutritional quality for microorganisms and consumers. Root soluble concentrations increased and lignin and nitrogen concentrations decreased with elevated CO$_2$ (Milchunas et al., 2005a). However, quality decreased significantly in only 1 year as indexed by an increase in C : N ratios, and lignin : N ratios showed only trends of an increase. Fitter et al. (1997) observed increased root mortality under elevated CO$_2$ in UK grasslands, but to a lesser degree in less fertile soils. Species from fertile habitats may have shorter life spans than species from low fertility sites independent of a CO$_2$ factor (Van der Krift & Berendse, 2002). However, drought has been observed to increase fine root mortality in forests (Schoettle & Fahey, 1994). All possible directions of response in life span to CO$_2$ have been observed with forest species (reviewed in Zak et al., 2000). In this study, disappearance (decomposition) of roots was greater under elevated compared with ambient CO$_2$ because of the greater total root pool even though average life spans were longer. The longer life span of roots was probably not because of the greater diameter, but may have been because of the lower N concentrations as predicted by Eissenstat et al. (2000). Wheat has been observed to produce coarser roots under higher nutrient and water supply (Manski & Vlek, 2002), suggesting that the greater soil water under elevated CO$_2$ in this study (Nelson et al., 2004) may have overridden any CO$_2$ effect on soil nutrients. The large decline in root diameters because of the latter drought years during this study is consistent with this root diameter relationship with soil moisture.

The branching aspect of root architecture is another factor reported to change with CO$_2$ treatment, and has implications concerning the efficiency of exploiting the soil volume and concerning competition because of root overlap among adjacent individuals (reviewed in Pritchard et al., 1999). An increase in branching has

most often been reported (Del Castillo *et al.*, 1989; Bernston & Woodward, 1992; Wechsung *et al.*, 1999), but increased length of penetration is another means of increasing soil exploration under elevated CO_2 (Chaudhuri *et al.*, 1990; Rogers *et al.*, 1992; Pritchard *et al.*, 1999; Wechsung *et al.*, 1999). Within the limitation of the small screen of view provided by minirhizotron images, we observed greater branching with elevated CO_2, while average length of roots remained unchanged. In this study, the increased branching was not likely in response to soil water as levels were greater under elevated CO_2 (Nelson *et al.*, 2004), but more likely was related to lower available nitrogen because uptake of nitrogen by resin bags was lower (Milchunas unpublished data). The greater branching may not be because of greater drought survival of smaller diameter secondary roots because the greater soil water would have the effect of lessening periods of drought and root diameters were greater under elevated CO_2. A possibility not considered in CO_2 studies is that root diameters may be increased simply because of water status.

BNPP and root/shoot partitioning

Estimates of BNPP from the same last four years of this study based on a modified root ingrowth method (root ingrowth donuts) resulted in BNPP estimates of 372, 404, and $610 \, g \, m^{-2} \, yr^{-1}$ for the control, ambient, and elevated CO_2 treatments, respectively (Milchunas *et al.*, 2005a). For the same latter 4 years, this compares with respective treatment values of 159, 135, and $181 \, g \, m^{-2} \, yr^{-1}$ for the minirhizotron maximum length method and 161, 112, and $184 \, g \, m^{-2} \, yr^{-1}$ for the minirhizotron growth:disappearance regression method. The increased production for elevated compared with ambient CO_2 for the three respective methods was, therefore, 51%, 34%, and 64%. These values compare with a 59% increase in new root length growth for elevated compared with ambient CO_2 treatments. As new length growth is not subject to the bias the long life span of roots in this system may have on pool size, this would suggest that the root ingrowth method and the minirhizotron growth:disappearance regression method gave similar relative differences between elevated and ambient CO_2 treatments.

Estimates of BNPP by all minirhizotron methods had much larger confidence intervals than other data estimated directly (such as new length growth), possibly because variances associated with both estimates of root biomass and root length turnover were multiplicative to some extent. No significant differences were observed between ambient and elevated CO_2 treatments in ANPP to BNPP ratio, although variance associated with these estimates were also large. The

greater allocation to BNPP compared with ANPP suggested by earlier literature of CO_2 effects, based to a large extent on pot studies, has not held as a generality for field experiments (Norby, 1994; Rogers *et al.*, 1994), particularly for grasslands (Arnone *et al.*, 2000; Nowak *et al.*, 2004). This may be because of a tradeoff in the effect of CO_2 on nitrogen vs. water relations. CO_2 can make nitrogen more limiting, which may increase root to shoot ratios. But, CO_2 can also increase water availability, which may tend to decrease root to shoot ratios. The end result in dry systems, where water is important, can be no change in the ratio.

Root production techniques

The problems and biases associated with methods of estimating BNPP are generally well known (Singh *et al.*, 1984; Milchunas & Lauenroth, 1992; Pages & Bengough, 1997; Eissenstat & Yanai, 2002). These problems can interfere with syntheses concerning the effects of elevated atmospheric CO_2 concentrations even within biomes (Arnone *et al.*, 2000; Norby & Jackson, 2000), but many may underestimate the magnitude of the problem. In this study, we used one primary method, the minirhizotron, and show that differences among methods of calculating BNPP can greatly affect absolute values, relative differences between BNPP and ANPP, and even differences among CO_2 treatments. To some extent, this may be magnified in this particular study because of the slow temporal rate of processes in this semiarid system. A relative, oscillating equilibrium of root growth around the minirhizotron tubes may or may not have been reached when the study started the year after installing the tubes. The ratios of growth to disappearance over the 5 years of study indicate that an oscillating equilibrium for the two processes together would take from 5 to 7 years depending on the CO_2 treatment. Estimates of root turnover based on ^{14}C turnover at a site several kilometers from this study suggest a similarly long turnover time of 6.9 years compared with 5.8 years by regression for the control in this study (Milchunas & Lauenroth, 2001). The shorter time for the minirhizotron equilibrium regression compared with ^{14}C turnover may be because of a small amount of roots that turn over rapidly and are missed by waiting for ^{14}C-solubles to equilibrate in the ^{14}C turnover method (Milchunas & Lauenroth, 1992), or that the low r^2 for the minirhizotron regressions and the potential sensitivity of the $Y = 1$ intercept value to slope may introduce error into the minirhizotron regression method we illustrate here. Regardless, the generally accepted method of waiting a year for growth to equilibrate around minirhizotron tubes appears to be flawed.

Regardless of when growth equilibrates, death and decomposition processes can affect total length pools, and in turn also influence turnover coefficients that are used to multiply root biomass to obtain BNPP estimates. Users of minirhizotrons need to be aware of these equilibration issues, and that the method for calculating turnover can affect estimates of BNPP. Our results show how regressions of growth to disappearance ratios can be used as a means to estimate equilibration time as well as BNPP. Turnover estimates based on regression, however, are the same for all years within a treatment. Thereby, annual BNPP estimates are based entirely on difference in biomass, with treatment differences representing differences in biomass and differences in the regressions for each treatment. Estimates based on length-growth over pool-maximum lengths should be viewed with caution, and may be the reason that Gill et al. (2002) estimated root turnover at a nearby site as $0.86 \, yr^{-1}$ based on growth and maximum root length using the same minirhizotron as used in this study. While Hendrick & Pregitzer (1993) and Johnson et al. (2001) indicate that time for sufficient recolonization of roots around tubes may take longer than a year under some environmental conditions, the time necessary for total pool size to equilibrate can also affect turnover estimates, and this has been overlooked by minirhizotron researchers. Equilibration in loss of roots may affect total pool size, which is the second factor in the calculation of turnover. We suggest that regressions of growth to disappearance be used to assess level of equilibration to better define absolute estimates of BNPP for users of control data for comparative purposes, unless just relative treatment differences are of interest.

Implications for C cycling in the shortgrass steppe

Key to understanding the implications of these results for carbon dynamics the shortgrass steppe ecosystem under elevated CO_2 are the differentials in the increases of root inputs and losses. With unchanged ANPP-to-BNPP ratios, roots represent $\sim 70\%$ of carbon inputs in this system (Milchunas & Lauenroth, 2001). While both inputs and losses of roots increased with elevated CO_2, the inputs increased to a greater degree than the losses, so standing pools also increased. Stable C isotope partitioning of soil respiration also indicated that decomposition rates at this site increased under elevated CO_2, but that turnover of new soil C decreased (Pendall et al., 2003, 2004, 2005). The lower nutrient quality of roots (Milchunas et al., 2005) slower turnover of new soil C, and increased life span of roots suggest an increased storage of C under elevated CO_2. A short-term priming effect of increased labile-C inputs (Diaz et al., 1993) on losses of old slow and passive pool soil-C

may eventually slow as nitrogen becomes more limiting. There is evidence of a decrease in N availability with elevated CO_2 at this site from both trace-gas studies (Mosier et al., 2003) and lower N-capture in resin bags (Milchunas unpublished data). Long-term studies would be necessary to determine how these dynamics play out. An additional important uncertainty of climate change is suggested by the high amplitudes of root initiations and terminations induced by extreme wet and dry periods for this system, and by the interactions of CO_2 with root growth and loss. Changes in amounts or pulsing of precipitation will have large consequences for arid systems and the manner in which they respond to elevated CO_2.

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