

# The effects of 11 yr of CO<sub>2</sub> enrichment on roots in a Florida scrub-oak ecosystem

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## Summary

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- Uncertainty surrounds belowground plant responses to rising atmospheric CO<sub>2</sub> because roots are difficult to measure, requiring frequent monitoring as a result of fine root dynamics and long-term monitoring as a result of sensitivity to resource availability.
- We report belowground plant responses of a scrub-oak ecosystem in Florida exposed to 11 yr of elevated atmospheric CO<sub>2</sub> using open-top chambers. We measured fine root production, turnover and biomass using minirhizotrons, coarse root biomass using ground-penetrating radar and total root biomass using soil cores.
- Total root biomass was greater in elevated than in ambient plots, and the absolute difference was larger than the difference aboveground. Fine root biomass fluctuated by more than a factor of two, with no unidirectional temporal trend, whereas leaf biomass accumulated monotonically. Strong increases in fine root biomass with elevated CO<sub>2</sub> occurred after fire and hurricane disturbance. Leaf biomass also exhibited stronger responses following hurricanes.
- Responses after fire and hurricanes suggest that disturbance promotes the growth responses of plants to elevated CO<sub>2</sub>. Increased resource availability associated with disturbance (nutrients, water, space) may facilitate greater responses of roots to elevated CO<sub>2</sub>. The disappearance of responses in fine roots suggests limits on the capacity of root systems to respond to CO<sub>2</sub> enrichment.

## Introduction

Increased interest in the global carbon cycle and carbon storage by forest ecosystems demands accurate methods of quantification of belowground biomass (Watson *et al.*, 2000). Vegetation accounts for nearly 80% of carbon stored in forest ecosystems (Richter *et al.*, 1999; Barton & Montagu, 2004). Coarse roots (> 5 mm in diameter) constitute a major belowground perennial carbon sink and, compared with their aboveground counterparts, often persist for long periods after tree harvest or disturbances such as fire (Johnsen *et al.*, 2001, 2005; Ludovici *et al.*, 2002; Miller *et al.*, 2006). More carbon may be stored in roots in tropical and temperate forests, shrublands and savannas than previously thought, with recent estimates of at least 268 Pg of carbon stored in roots in these ecosystems (Robinson, 2007). Thus, there is a critical need to reassess belowground carbon storage by roots.

In shrub ecosystems that are fire prone and subject to drought, the unique belowground morphology of the dominant plants includes large rhizomes, underground stems and lignotubers

(Canadell & Zedler, 1995). Estimates of belowground biomass in our study in a Florida scrub-oak ecosystem suggest that biomass and carbon pools are much greater (*c.* 8000 g m<sup>-2</sup> of root mass to 60 cm depth) than previously reported for forested systems (*c.* 5000 g m<sup>-2</sup> for sclerophyllous shrublands and tropical evergreen forests; Jackson *et al.*, 1996; Robinson *et al.*, 2003). We attribute this primarily to large structures that do not typically exist in many forests (lignotubers and large rhizomes). These massive structures potentially serve as one of the largest sinks of carbon in the system (Stover *et al.*, 2007). They are the primary means of plant regeneration in fire-dominated ecosystems (Schmalzer & Hinkle, 1992a,b).

Root abundance and biomass production are often stimulated by elevated CO<sub>2</sub> (Rogers *et al.*, 1994; Jongen *et al.*, 1995; Bernston & Bazzaz, 1996; Pritchard *et al.*, 1999, 2008; Matamala & Schlesinger, 2000; Norby *et al.*, 2004; Iversen *et al.*, 2008; Pregitzer *et al.*, 2008; Jackson *et al.*, 2009). In a 25-yr-old stand of *Pinus taeda*, CO<sub>2</sub> enrichment increased fine root biomass by 24% in the top 15 cm of soil, with no sign of this response

diminishing after more than a decade (Jackson *et al.*, 2009). This response may be driven by the need to acquire additional below-ground resources to support more rapid biomass accumulation, such that plants exposed to elevated CO<sub>2</sub> invest more carbon in root systems for expanded exploitation of the soil (Matamala & Schlesinger, 2000).

Plant responses to elevated CO<sub>2</sub> are often greater under higher levels of other resources, such as space, light and nutrients (Bazzaz, 1990; Field *et al.*, 1992; Oren *et al.*, 2001; Körner, 2006; Reich *et al.*, 2006; Garten *et al.*, 2011). Therefore, changes in resource availability could shape responses of plants to CO<sub>2</sub> over time. For example, progressive nutrient limitation has been postulated to reduce long-term plant response to elevated CO<sub>2</sub> driven by changes in nutrient cycling (Luo *et al.*, 2004; Johnson, 2006). The influences of exogenous phenomena that increase resource availability, such as disturbance, are not as well characterized, but would be expected to have the opposite effect, enhancing CO<sub>2</sub> responses by causing resource pulses (Körner, 2006; Li *et al.*, 2007). The investigation of both above- and belowground responses to elevated CO<sub>2</sub> is especially important because biomass allocation may shift in response to relative resource limitation (Poorter & Nagel, 2000).

### Complementary and comprehensive sampling

The substantial pool of carbon in roots has been difficult to quantify because of shortcomings in methodologies for the measurement of belowground biomass and root dynamics in general (Fitter & Stickland, 1992; Butnor *et al.*, 2003). Most studies on elevated CO<sub>2</sub> have sampled only fine roots near the soil surface, providing a limited picture of root responses to increased CO<sub>2</sub>. Roots of different diameters can vary in functions, and so lumping roots together may miss key responses (Pregitzer *et al.*, 2002; Keel *et al.*, 2012).

Estimates of root carbon stocks may be substantially low (40% of actual root mass on average) because of incomplete sampling (e.g. small cores missing large roots and inefficient recovery of the smallest roots; Robinson, 2007). Long-term experiments that involve belowground measurements pose a particular challenge, because these studies can tolerate only minimal disturbance to the soil (Bledsoe *et al.*, 1999; Fahey *et al.*, 1999). Thus, destructive sampling in the form of pit excavations and soil core extractions, although more direct, is severely limited. Large belowground structures are frequently not sampled. For this reason, most inferences regarding belowground responses to elevated CO<sub>2</sub> are based on measurements of fine roots (Hendrick & Pregitzer, 1992; Norby, 1994; Day *et al.*, 2006). Ground-penetrating radar (GPR; Stover *et al.*, 2007; Butnor *et al.*, 2008) and minirhizotrons offer viable options for the quantification of both coarse and fine root biomass throughout the course of a long-term experiment without the major disturbances that are associated with pits and cores and without the inherent sampling problems suggested by Robinson (2007). In this study, we employed minirhizotrons to quantify fine roots and GPR to quantify coarse roots. Soil cores were also periodically taken to compare with the other two methods.

### Florida study

A fire-maintained scrub-oak ecosystem on the east coast of Florida was exposed to 11 yr of elevated atmospheric CO<sub>2</sub> using open-top chambers (OTCs) following a controlled burn in 1996. Earlier findings on the effects of CO<sub>2</sub> enrichment on roots at this study site included reports on fine root abundance and turnover for 1996–1997 (Day *et al.*, 1996; Dilustro *et al.*, 2002) and 2002–2004 (Stover *et al.*, 2010), fine root abundance for 1996–2004 and distribution by depth for 1997 (Day *et al.*, 2006), fine root biomass for 2002 based on core data (Brown *et al.*, 2007) and for 1996–2006 based on minirhizotron data (Brown *et al.*, 2009), and coarse root biomass from GPR measurements for 2005 (Stover *et al.*, 2007).

Here, we bring together these past results, together with new data from the final harvest in 2007 and previously unpublished data from soil cores taken throughout the duration of the experiment. We also compare fine root and leaf responses to CO<sub>2</sub> enrichment over time to assess responses in allocation to above- and belowground resource acquisition. Our synthesis tests the following hypotheses: (1) elevated CO<sub>2</sub> stimulates fine and coarse root biomass; and (2) elevated CO<sub>2</sub> causes larger responses in fine root biomass relative to leaf biomass. We are particularly interested in the influence of disturbance on CO<sub>2</sub> responses. Unlike typical mature forested systems that experience infrequent disturbances, the Florida scrub-oak woodland is frequently disturbed by fire and coastal storms (hurricanes), similar to Mediterranean shrublands (e.g. chaparral) and savannahs.

## Materials and Methods

### Study site

The study site was located at Kennedy Space Center on Merritt Island National Wildlife Refuge (28°38'N, 80°42'W) on the east coast of Florida, USA. The sandy soils are acidic, well drained and nutrient poor (Schmalzer & Hinkle, 1992a,b). The climate is subtropical with a wet season between late June and October and a dry season between April and early June. Lightning associated with thunderstorms is responsible for igniting wildfires. Although fire is the dominant ecosystem disturbance at the study site (7–15-yr fire cycle), other natural disturbances include periodic drought and severe weather from tropical storms and hurricanes.

Scrub-oak shrublands occupy over 15 000 acres of Merritt Island, and fire is essential for maintaining the community. The scrub-oak vegetation is dominated by woody evergreen species with extensive belowground storage organs, such as lignotubers and rhizomes, that allow the plants to re-sprout after fire (Schmalzer & Hinkle, 1992a; Menges & Kohfeldt, 1995). The two co-dominant species are *Quercus myrtifolia* Willd. (myrtle oak) and *Quercus geminata* Small (sand live oak).

### Experimental design

The study site was burned in early 1996 before the installation of the chambers. Sixteen plots were grouped on the basis of

pre-burn vegetation into eight blocks and randomly assigned to a treatment of ambient CO<sub>2</sub> or elevated CO<sub>2</sub>. Chamber frames were made with 4-in-diameter PVC pipe in an octagonal design with panels covered with clear Mylar film. The chambers enclosed 9.4 m<sup>2</sup> of ground area and were 2.5 m tall and 3.5 m wide. CO<sub>2</sub> addition began in May 1996 and was controlled at 350 ppm above ambient throughout the experiment, except for brief periods in 1999 and 2004 during repairs to the chambers after damaging storms. Ambient CO<sub>2</sub> was *c.* 350 ppm in 1996 and had increased to *c.* 380 ppm in 2007. CO<sub>2</sub> addition was stopped in May 2007.

### Fine root measurements

Minirhizotrons were installed in the study plots in 1996 after fire, but before chamber construction. Details of the minirhizotron design, installation and sampling protocol are described in Day *et al.* (2006). Minirhizotron recordings were obtained approximately every 3 months throughout the study, but, because of time constraints, some dates were not digitized. Images were converted to jpeg files and digitized following the protocol of Day *et al.* (2006). The primary metric was root length per frame area (mm cm<sup>-2</sup>; root length density). Root length density was used to calculate fine root biomass following the methods detailed by Brown *et al.* (2009). The principles behind this method are described by Johnsen *et al.* (2001), Hendrick & Pregitzer (1996) and Taylor *et al.* (1970).

Previously reported minirhizotron estimates of fine root biomass for this system (Brown *et al.*, 2009) included roots > 2 mm in diameter. Here, we restricted the analyses to roots < 2 mm in diameter because an extremely small number of roots > 2 mm in diameter indicated inadequate sampling by minirhizotrons (discussed in Brown *et al.*, 2009). For example, in the minirhizotron images, there were no > 2-mm-diameter roots in ambient CO<sub>2</sub> plots in March 2007 and there were only two roots in this size class in elevated plot images. Thus, we recalculated fine root biomass (roots < 2 mm in diameter) for all minirhizotron sampling dates, and these revised minirhizotron fine root biomass data presented here are different from those reported by Brown *et al.* (2009). Further details of the methods can be found in Dilustro *et al.* (2002), Day *et al.* (2006), Brown *et al.* (2009) and Stover *et al.* (2010).

### Coarse root measurements

After CO<sub>2</sub> addition had ended and the chambers had been removed, all aboveground vegetation was clipped to the soil surface, dried and weighed to obtain total aboveground biomass (Seiler *et al.*, 2009). In June 2007, < 1 wk after the aboveground vegetation in experimental plots was harvested, GPR was used to image roots in all plots with a Subsurface Interface Radar (SIR-3000) and 1500-MHz antenna (Geophysical Survey Systems Inc., North Salem, NH, USA). The high-frequency antenna provides high resolution to a depth of 60 cm. Soil cores taken to a depth of 200 cm indicate that GPR captured at least 80% of total coarse roots in the top 100 cm of soil. GPR signal reflection was correlated with coarse root biomass estimates from soil cores, resulting in a linear relationship, which was then used to estimate

root biomass nondestructively from the study plots. In order to obtain a more complete picture of total root biomass by combination with the minirhizotron data (to a depth of 100 cm), we extrapolated the GPR data to 100 cm (see Soil cores methods section below).

A 2 × 2-m<sup>2</sup> fiberglass frame was positioned within the footprint of each experimental plot. A 2-m-long beam with a freely moving shuttle was positioned on the frame. The radar antenna (with calibrated survey wheel) was attached to the shuttle arm, and the shuttle guided the antenna along 2-m transects. Plots were scanned every 16 cm in an *x* and *y* direction (resulting in a total of 26 scans per plot). Individual 2-m-long GPR scans were processed using Radan version 6.5 (Geophysical Survey Systems Inc.). The processing protocol was similar to that used in Stover *et al.* (2007). For each experimental plot, 25 random intersections from the grid of GPR scans were selected. After digital processing of the corresponding scan, a 15-cm-wide section was cropped at each intersection. This was equivalent to the size of the cores used to establish the relationship between GPR signal intensity and root biomass (Stover *et al.*, 2007). Cropped GPR images were converted to bitmaps using Radan to Bitmap Conversion Utility 2.1 (Geophysical Survey Systems Inc.) and converted to 24-bit grayscale with SigmaScan Pro Image Analysis software version 5.0 (SPSS Inc., Chicago, IL, USA). Pixels within an intensity range of 70–227, representing live roots, were counted for each image. Pixel counts were applied to a regression equation relating pixel number and root biomass, where coarse root biomass per 15-cm-diameter area = 0.1262 × pixel count ( $R^2 = 0.47$ ).

To validate the GPR biomass estimation method, a 2 × 2-m<sup>2</sup> plot separate from the experimental plots was cleared of aboveground vegetation and scanned with the 1500-MHz GPR antenna using 13 scans in the *x* and *y* directions. A 1 × 2-m<sup>2</sup> pit in half of the scanned area was excavated to a depth of 60 cm. Roots from the pit were extracted on site using a 6-mm mesh sieve. Root samples were washed, oven dried at 70°C until the weight loss was stable and weighed.

### Soil cores

Fine roots in the uppermost soil layer (0–10 cm) were sampled using cores throughout the study. On several dates in 1998, 1999 and 2001, three cores were removed from each plot using a 1.9-cm-diameter punch auger. The cores were composited and hand picked to remove fine roots, which were subsequently washed, dried and weighed. Core samples were collected in May 2002 using a 7-cm-diameter soil corer, as described in Brown *et al.* (2009). The 2002 sampling included exhaustive treatment of the depth distribution of fine and coarse root biomass. We used these data to develop plot-specific models of root depth distribution, using the model of Gale & Grigal (1987), where the proportion of total root biomass (*Y*) at depth (*d*, cm) is equal to

$$Y = 1 - \beta^d, \quad \text{Eqn 1}$$

where  $\beta$  is a fitted parameter. To fit the model, we calculated the cumulative proportion of fine or coarse root biomass at each

depth interval to 1 m based on the 2002 sampling, and compared this with modeled estimates using Eqn 1. We minimized the sum of squared differences between measured and modeled values of  $Y$  using the Solver function in Microsoft Excel. Values of  $\beta$  based on coarse roots from 2002 for each plot were used to estimate coarse root biomass for the 60–100-cm soil depth in 2007, the fraction of the top meter which the GPR readings could not assess. Additional coarse root biomass estimated using this method amounted to, on average, only 7% of the total coarse root biomass in each plot, and so biomass estimates using this approach do not substantially alter the calculations of total root mass. Nevertheless, the inclusion of this fraction of coarse root biomass in our estimates provides a more complete picture of the total root biomass in the top meter of soil in this ecosystem.

At the end of the experiment (June–July 2007), five soil cores were collected in each chamber plot with a 7-cm-diameter soil corer. The cores were collected in 10-cm increments for the top meter of soil, and then in 30-cm increments until the water table was reached (180–330 cm). Core samples were sieved with a 2-mm mesh sieve, followed by a 1-mm sieve to separate roots from mineral soil. Coarse roots were hand picked from the fraction retained on the sieve, and fine roots were picked from a subsample, scaled to the entire mass of retentate by weight. Roots were washed, oven dried and weighed to determine biomass. Total biomass per square meter was calculated by summing the root mass for all cores per plot and dividing by the total core area.

### Statistical analyses

CO<sub>2</sub> treatments were replicated ( $n=8$  for each treatment). For measurements that included subsampling within experimental plots, the model residuals were tested for normality (using the Shapiro–Wilk test) and homogeneity of variances (using the Levene statistic) with PASW Statistics 17.0 (SPSS Inc.). Model residuals for the data failed tests for normal distribution and variance homogeneity, and so the data were transformed. Data with subsampling included minirhizotron biomass data (log transformed) and GPR data (square root transformed). Fine root biomass estimated using minirhizotrons was tested with a repeated measures ANOVA (SAS version 9.1; SAS Institute Inc., Cary, NC, USA). GPR data were tested with SAS Proc GLM using a two-factor nested ANOVA with 25 subsamples per chamber; chamber was assigned as the random effect and CO<sub>2</sub> treatment as the fixed effect.

Data that did not have subsampling included total root biomass from the combination of GPR and minirhizotron estimates, root biomass from cores, aboveground biomass and root-to-shoot ratios. Nonparametric tests were run on these data using SAS Proc NPAR1WAY to test for differences between CO<sub>2</sub> treatments. All results were considered to be significant at  $\alpha < 0.05$ , but trends were recognized at  $0.05 < \alpha < 0.15$  (following Runion *et al.*, 2006).

We compared responses to CO<sub>2</sub> by fine roots and leaves in order to assess the relative responses to elevated CO<sub>2</sub> of resource-acquiring organs above- and belowground. Total leaf biomass

was estimated from measurements of stem diameter and allometric relationships developed for the three co-dominant oak species, as described in Dijkstra *et al.* (2002) and Seiler *et al.* (2009). Total fine root biomass was estimated on the basis of the minirhizotron measurements and, where available, the soil cores. For each year of the study, 1996–2007, we used all available data estimating total fine root biomass and total leaf biomass in each plot. We used bootstrapping to estimate confidence intervals for the difference in log response ratio below- vs aboveground:

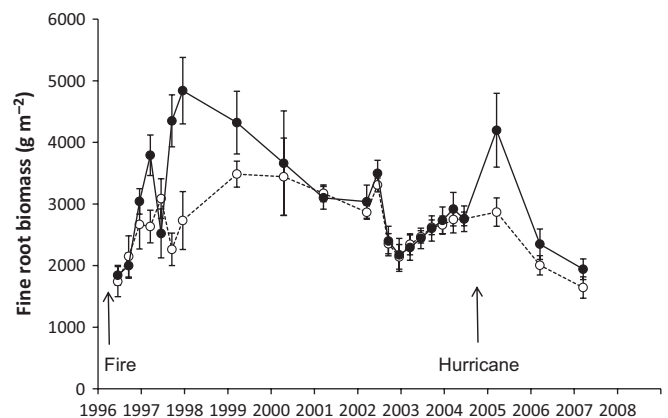
$$\text{Log}_e(E_r/A_r) - \text{log}_e(E_l/A_l),$$

where  $E$  is the mean for the elevated CO<sub>2</sub> treatment,  $A$  is the mean for the ambient CO<sub>2</sub> treatment, the subscript 'r' indicates fine roots and the subscript 'l' indicates leaves. In this way, positive values indicate larger relative responses belowground, and negative values show larger relative responses aboveground. The use of log response ratios facilitates comparison of relative responses with symmetrical distributions regardless of sign.

## Results

### Fine roots

Fine root biomass varied over time from  $< 2000 \text{ g m}^{-2}$  at the beginning and end of the study to nearly  $5000 \text{ g m}^{-2}$  during year 3 (Fig. 1). There were significant ( $P < 0.001$ ) increases during the first 2 yr, followed by a general decline until a significant increase ( $P < 0.001$ ) from 2003 to 2005. Increases in fine root biomass during the first year or two probably represent regrowth after tube installation (Strand *et al.*, 2008). Changes over time did not show the sustained increase after fire disturbance that we postulated, but rather showed strong temporal variation apparently associated with multiple disturbances. Fine root biomass showed a repeating pattern of CO<sub>2</sub> stimulation after or coincident with disturbance, followed by declining biomass and diminution of the CO<sub>2</sub> effect (Fig. 1). The fine root biomass increase during the

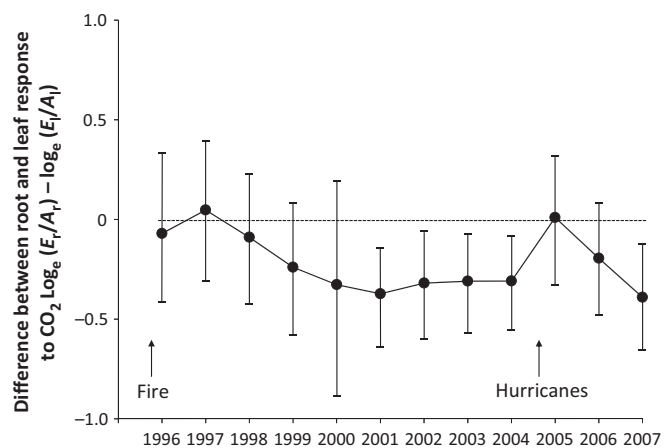


**Fig. 1** Fine (< 2 mm in diameter) root biomass to a depth of 100 cm estimated using minirhizotrons over the 11-yr study period in a scrub-oak shrubland community in Florida, USA. Values are means  $\pm$  SE. Results presented are revised from those published previously by Brown *et al.* (2009). Disturbance events are noted. Ambient, white circles. Elevated, black circles.

first 3 yr of the study was greater under elevated CO<sub>2</sub>, but subsequently peaked and then declined to levels found in the ambient chambers. Surface soil core data, although obtained at different intervals from the minirhizotron observations, nonetheless showed the high in fine root biomass under elevated CO<sub>2</sub> in early 1998, followed by a decline (Table 1). Declines in root mass were generally associated with periods of below average rainfall (1999–2002 and 2006). In 2005, following a hurricane, which occurred in September 2004 and severely reduced leaf area (Li *et al.*, 2007), fine root biomass peaked again and was significantly greater in elevated CO<sub>2</sub> plots. This was again followed by a decline that persisted until the end of the study. At the end of the study in March 2007, fine root biomass was not significantly greater under elevated CO<sub>2</sub> than under ambient CO<sub>2</sub> (Table 2, Fig. 2). These values were lower than at any time since the beginning of the study in 1996. Estimates of fine root biomass obtained through soil cores also showed low biomass at the end of the study compared with other time periods (Tables 1, 2).

Fine root production, mortality and turnover were higher in the elevated chambers during the first 2 yr of the study (Dilustro *et al.*, 2002). However, our findings 5 yr later revealed that treatment differences in productivity, mortality and turnover were no longer present (Stover *et al.*, 2010). The results indicated that CO<sub>2</sub> enrichment was no longer driving changes in fine root dynamics.

After fire disturbance at the beginning of the study, responses of fine roots and leaves to elevated CO<sub>2</sub> were roughly comparable (Fig. 2). Later, elevated CO<sub>2</sub> usually stimulated leaf growth more than fine root growth (negative response values indicate greater CO<sub>2</sub> effects on leaves). This was the trend for 1999, 2000 and 2006, and was significant for 2001–2004 and 2007. In 2005, the



**Fig. 2** Relative effect of CO<sub>2</sub> on fine roots vs leaves for the 11-yr study period in a scrub-oak shrubland community in Florida, USA. Values are means  $\pm$  90% confidence intervals estimated by bootstrapping. The y axis is the difference in log response ratios to elevated CO<sub>2</sub>, belowground – aboveground. Thus, negative values indicate greater relative responses to elevated CO<sub>2</sub> aboveground relative to belowground. Disturbance events are noted.

year after the hurricane, responses in fine roots and leaves were comparable, as found in the early years associated with fire disturbance.

### Coarse roots

Coarse root biomass estimated using GPR averaged 5476 g m<sup>-2</sup> for the top 0–60 cm (Table 2, Fig. 3). Scaled to the top meter of soil (after Gale & Grigal, 1987; see Materials and Methods), this average increased to 5949 g m<sup>-2</sup> (Table 2).

**Table 1** Surface fine root biomass (top 10 cm) from soil cores in a scrub-oak shrubland community in Florida, USA

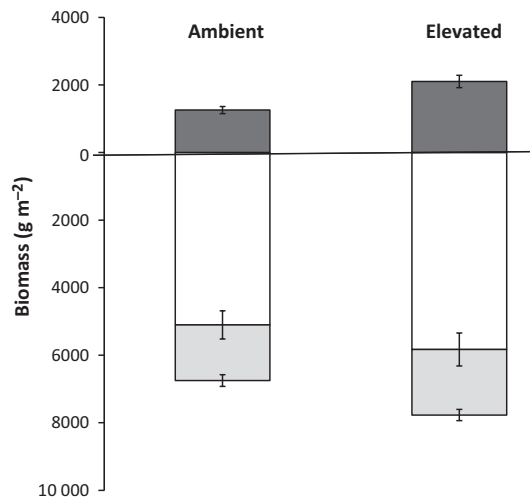
	June 1998	July 1998	September 1998	December 1998	September 1999	May 2002	July 2007
Ambient	1400 $\pm$ 162	1273 $\pm$ 118	1268 $\pm$ 183	1406 $\pm$ 276	1249 $\pm$ 186	1700 $\pm$ 244	675 $\pm$ 147
Elevated	2096 $\pm$ 194	1973 $\pm$ 309	1182 $\pm$ 158	948 $\pm$ 120	1377 $\pm$ 191	1178 $\pm$ 134	472 $\pm$ 61
<i>P</i> value	0.011	0.040	0.709	0.126	0.614	0.222	0.036

Values are biomass (g m<sup>-2</sup>) plus or minus one standard error of the mean.

**Table 2** Root biomass (g m<sup>-2</sup>; means  $\pm$  standard errors) measured using minirhizotron image analysis, ground-penetrating radar (GPR) and 7-cm-diameter soil cores in a scrub-oak shrubland community in Florida, USA

	Minirhizotron (MR)		GPR		MR + GPR	Cores		
	< 2 mm	> 5 mm	> 5 mm		Total	< 2 mm	> 2 mm	Total
	0–100 cm		0–60 cm	60–100 cm	0–100 cm	0–100 cm	0–100 cm	0–100 cm
Ambient CO <sub>2</sub>	1644 $\pm$ 173	5105 $\pm$ 418	345 $\pm$ 84	5451 $\pm$ 371	7094 $\pm$ 381	2800 $\pm$ 189	2690 $\pm$ 246	5490 $\pm$ 307
Elevated CO <sub>2</sub>	1942 $\pm$ 168	5830 $\pm$ 487	617 $\pm$ 166	6448 $\pm$ 615	8390 $\pm$ 552	2932 $\pm$ 612	3014 $\pm$ 467	5947 $\pm$ 942
<i>P</i> value	0.236	0.277	0.174	0.191	0.076	0.842	0.552	0.657

Root diameter categories are indicated for each method. GPR estimates are reported for a depth of 0–60 cm, imaged directly, and for a depth of 60–100 cm, estimated using the model of Gale & Grigal (1987). Totals are shown for the sum of the two indirect measures (Minirhizotron + GPR) and the sum of diameter categories for the cores. Note that the core sampling includes the roots between 2 and 5 mm in diameter, a range that may not be adequately detected by the indirect measurements. All of these data were collected during the final harvest in 2007. *P* values are reported for two-tailed *t*-tests assuming unequal variance.



**Fig. 3** Live aboveground biomass (above the horizontal line) and root biomass to a depth of 60 cm (below the horizontal line) in  $\text{g m}^{-2} \pm \text{SE}$  for the  $\text{CO}_2$  treatments at the time of the final harvest in 2007 in a scrub-oak shrubland community in Florida, USA. Below the horizontal line, the white bar represents coarse root biomass and gray represents fine root biomass.

These estimates were 22% lower than those from 2005, where the average coarse root biomass for 0–60 cm was estimated at  $7070 \text{ g m}^{-2}$  (calculated from Stover *et al.*, 2007). The reliability of GPR biomass estimates was confirmed at the end of the study by the validation plot ( $1 \times 2 \times 0.6\text{-m}^3$  pit (length  $\times$  width  $\times$  depth) in July 2007), with an estimate of  $7770 \text{ g m}^{-2}$  roots to a depth of 60 cm using GPR, compared with an actual biomass of  $8222 \text{ g m}^{-2}$ .

Coarse root biomass was not significantly different between treatments after more than a decade of exposure to experimentally increased atmospheric  $\text{CO}_2$  concentration (Table 2), although coarse root biomass was generally higher in the elevated treatment plots. Average coarse root biomass was  $726 \text{ g m}^{-2}$  higher in the elevated  $\text{CO}_2$  treatment for the top 0–60 cm and  $997 \text{ g m}^{-2}$  for the top meter of soil (Table 2). The previous estimate of coarse root biomass (Stover *et al.*, 2007) suggested a greater treatment difference of  $1881 \text{ g m}^{-2}$  ( $P=0.12$ ) 2 yr earlier ( $8010 \pm 796 \text{ g m}^{-2}$  elevated and  $6129 \pm 1010 \text{ g m}^{-2}$  ambient for the top 0–60 cm).

### Total root biomass

Combining the minirhizotron estimate of fine root biomass measured in March 2007 and the GPR estimate of coarse root biomass from June 2007, total root biomass for plots exposed to elevated  $\text{CO}_2$  was  $8390 \pm 552 \text{ g m}^{-2}$ , compared with  $7094 \pm 381 \text{ g m}^{-2}$  for ambient plots ( $P=0.076$ ; Table 2). The combined total root biomass from these two methods is probably an underestimate given the methodological gap in detecting roots between 2 and 5 mm in diameter. Minirhizotrons are limited to roots  $< 2$  mm in diameter and the GPR detection limit is assumed to be roots  $> 5$  mm in diameter in this study. Although the biomass of roots  $> 2$  mm in diameter could not be accurately determined using minirhizotrons, GPR may have detected a portion of the roots  $< 5$  mm in diameter. Dense mats of near-

surface fine roots and clusters of fine roots may be captured by GPR (R. Schroeder, pers. obs.). The  $\text{CO}_2$  fertilization effect aboveground was significant and proportionally greater than that belowground (Figs 2, 3). However, belowground biomass, particularly coarse roots, was the major contributor to total plant biomass (84% in ambient and 78% in elevated  $\text{CO}_2$ ), and the absolute difference between elevated and ambient biomass was greater belowground:  $846 \text{ g m}^{-2}$  aboveground (Seiler *et al.*, 2009) and  $1296 \text{ g m}^{-2}$  belowground (Table 2, Fig. 3). The ratio of total belowground to total aboveground biomass averaged  $3.9 \pm 0.4$  for elevated  $\text{CO}_2$  plots, significantly less than the average of  $5.5 \pm 0.5$  for ambient  $\text{CO}_2$  plots ( $P=0.02$ ).

### Biomass estimated from cores

Total root biomass estimated using 7-cm-diameter soil cores was 26% lower than the combined total from the minirhizotron and GPR sampling methods, an average across treatments of  $5719 \text{ g m}^{-2}$  for the cores compared with  $7742 \text{ g m}^{-2}$  for the imaging methods (Table 2). The  $\text{CO}_2$  effect on total root biomass estimated from cores was not significant (Table 2), and the  $456 \text{ g m}^{-2}$  difference in means between treatments was smaller than that observed for minirhizotrons and GPR, probably in part because the cores underestimated coarse root biomass by *c.* 50%.

## Discussion

### Fine root response over time in a frequently disturbed woodland ecosystem

The stimulation of fine root biomass as a result of  $\text{CO}_2$  enrichment was transient, and appeared to be associated with disturbance: responses were strongest at the beginning of the experiment after fire, and again during year 8 after hurricanes, periods during which aboveground biomass was either completely removed (fire) or severely reduced (hurricane). This phenomenon could be a result of increased resource use efficiency, or limits on soil resources over time. A decrease in fine root response after long-term  $\text{CO}_2$  enrichment was observed by Bader *et al.* (2009), a finding that contrasts with the majority of empirical studies. The loss of a treatment effect after 7 yr of enrichment was attributed to increased soil moisture (through reduced transpiration) under elevated  $\text{CO}_2$  which may have caused reduced biomass allocation to fine roots. The lack of  $\text{CO}_2$  stimulation of root growth reported by Handa *et al.* (2008) was thought to be evidence that mature ecosystems may not show a belowground treatment effect as much as an expanding or early successional community. The idea that vegetation responses to elevated  $\text{CO}_2$  are strongly controlled by ecosystem successional state and plant demography was explored by Körner (2006). Körner (2006) identified several ecosystem types based on nutrient cycling properties, but he indicated that some systems have unique combinations of these properties. We suggest that the Florida scrub-oak woodland may represent such a system.

Unlike most forests, the scrub-oak system is frequently impacted by disturbances (fire and hurricanes). Key drivers in a disturbance-prone system are likely to be different from those in

systems that are disturbed infrequently. Fire and hurricanes produce resource (nutrient and water) pulses that interact with CO<sub>2</sub> to produce unexpected responses. An elevated CO<sub>2</sub> experiment in a desert ecosystem revealed sporadic CO<sub>2</sub> treatment effects over time (Ferguson & Nowak, 2011). Periodic rain events in this system apparently act as an environmental signal eliciting CO<sub>2</sub> responses. Elevated CO<sub>2</sub> had greater effects on fine root dynamics during certain phenological events influenced by soil moisture (Sonderregger *et al.*, 2013). Similar pulse drivers occur in savanna ecosystems (Chen *et al.*, 2003; February & Higgins, 2010; Tomlinson *et al.*, 2012). We suggest that nutrient availability is the primary pulse driver in the scrub-oak system. Fires mobilize nutrients and can increase nutrient availability to plants (Ojima *et al.*, 1994; Turner *et al.*, 1997), which could enhance the CO<sub>2</sub> response. The hurricanes defoliate the trees, resulting in increased nutrient input to the soil via increased leaf fall, and reduce water demand by the canopy. Several months following the hurricane, concentrations of soil extractable micronutrients increased, and then declined over the next 2 yr (B. A. Hungate *et al.*, unpublished). Thus, both nutrient and water pulses may have enhanced the CO<sub>2</sub> response after the hurricanes.

Day *et al.* (2006) proposed that fine roots reached a dynamic equilibrium ('root closure') in the study system 3 yr after the experiment began, and that this root closure was reached shortly before canopy closure. However, the analogy to closure aboveground is limited, because resource availability belowground is far more dynamic. In other words, light and CO<sub>2</sub>, the major aboveground resources, do not vary over space and time as much as belowground resources (water and nutrients). This might explain the large fluctuations in fine root biomass. The concept of root 'closure' may be resource (other than just space) dependent. Disturbances, such as fire or drought, appear to reduce fine root biomass to levels below the soil's carrying capacity for fine roots. During the recovery phase, after disturbance and root die-back, fine roots respond to CO<sub>2</sub> fertilization (a resource pulse). One important implication of this finding is that elevated CO<sub>2</sub> may result in greater carbon inputs to soils following disturbance. After root closure, limited resources (space, water and nutrients) result in a loss of the CO<sub>2</sub> fertilization effect.

Fine roots are temporally dynamic. Root biomass, production and mortality vary seasonally (McClaugherty *et al.*, 1982; Hendrick & Pregitzer, 1992) and interannually (Espeleta & Clark, 2007). In CO<sub>2</sub> enrichment studies, the CO<sub>2</sub> treatment effect on fine root biomass varied over the course of a year (Norby *et al.*, 2004; Jackson *et al.*, 2009) and between years (Norby *et al.*, 2004). Natural variations in root biomass over time may complicate the evaluation of CO<sub>2</sub> treatment effects in long-term studies. At the end of the Florida study, fine root biomass was lower than it had been at any time since the beginning of the study; thus, the biomass values and differences between treatments from 2007 represent a point in time when fine root biomass was low and CO<sub>2</sub> effects were not apparent. Thus, the end-of-study results may have underestimated the CO<sub>2</sub> response by roots. Conclusions based on fine root 'lows' may differ from those based on the 'highs', as the CO<sub>2</sub> effects were strongest during periods of recovery. The value of long-term studies is that

they provide a time series that encompasses such variability and provides the opportunity to assess relationships among ecosystem response variables with environmental drivers or pulses that may not be apparent from short-term studies or snapshot or end-of-study results. This may explain the differences in 2005 and 2007 estimates of root mass by GPR. The biomass validation plot data and the interannual fine root biomass data both support the idea that a substantial portion of roots of < 2 mm in diameter are detected by GPR. They may exist as clumps or as part of the dense surface mat. The decrease in coarse root estimates from 2005 to 2007 measured using GPR is not likely to be the result of an actual decrease in coarse root biomass, but may just reflect the decrease in fine root biomass over that time period. Additional testing of GPR is needed to evaluate the sensitivity to fine root clusters.

By contrast, some long-term CO<sub>2</sub> enrichment studies have shown sustained root biomass stimulation under elevated CO<sub>2</sub> over more than a decade of CO<sub>2</sub> enrichment (Jackson *et al.*, 2009). Fine root peak standing crop was approximately doubled across all years in a 9-yr free air carbon dioxide enrichment (FACE) study in a sweetgum plantation (Norby *et al.*, 2004; Iversen *et al.*, 2008). Averaged over 6 yr of FACE treatment in a loblolly pine plantation, root standing crop was increased by 23% (Pritchard *et al.*, 2008). Resource availability, including space, may explain these differences.

### Coarse roots in a frequently disturbed woodland ecosystem

Although fine root biomass was monitored throughout the study, coarse root biomass was only measured during the last few years of the experiment. Limits on destructive sampling in long-term experiments using small-diameter cores may limit observations of CO<sub>2</sub> stimulation of root biomass. The doubling of coarse roots under elevated CO<sub>2</sub>, reported by Jackson *et al.* (2009), was not observed until pits were dug in 2008. This poses a significant problem for biomass estimates, because belowground biomass, particularly coarse roots, constituted the majority of total plant biomass at the Florida site (84% in ambient and 79% in elevated CO<sub>2</sub> plots).

The data presented here and previously collected from the site indicate that coring techniques, especially those using small-diameter corers, probably underestimate coarse root biomass. In a study comparing actual (whole-tree harvest extraction) and estimated (5-cm-diameter soil cores) lateral root density, the soil cores underestimated root density by as much as half the value determined by whole-tree harvest (Retzlaff *et al.*, 2001). Our GPR-based estimates of coarse root biomass were greater than those obtained from coring, but comparable with coarse root biomass sampled directly in the 1 × 2-m<sup>2</sup> validation soil pit. Thus, the GPR data accurately reflected destructive sampling on a larger spatial scale (2 m<sup>2</sup>), and suggested that the smaller area of the cores (*c.* 0.02 m<sup>2</sup>) underestimated coarse root biomass. The small number of cores allowed in this long-term study is not sufficient to cover the spatial heterogeneity of coarse root distribution, and results in bias towards values of zero, which may lead to an underestimation of coarse root biomass (B. A. Hungate, unpublished).

The validation pit dug near the study plots yielded over 8000 g m<sup>-2</sup> of root biomass to a depth of 60 cm. This is considerably higher than the upper range of 5000 g m<sup>-2</sup> of root biomass in global biomes analyzed by Jackson *et al.* (1996). Studies in systems with large belowground structures, such as rhizomes and lignotubers, have found high root biomass similar to the Florida site, for example the scrub-oak of the garrigue in southern France, in which large belowground structures (> 5 mm in diameter) constituted 85% of the 7200 g m<sup>-2</sup> total root biomass (Kummerow *et al.*, 1990).

### Root-to-shoot ratios

Elevated CO<sub>2</sub> stimulated the production of aboveground biomass; however, this response was species specific with *Q. myrtifolia* increasing in aboveground biomass by 128% and *Q. geminata* displaying no significant treatment effect after 11 yr of enrichment (Seiler *et al.*, 2009). Live aboveground biomass values were 1257 ± 107 and 2103 ± 184 g m<sup>-2</sup> for ambient and elevated CO<sub>2</sub> plots, respectively (Seiler *et al.*, 2009; Fig. 3). At the end of the study, the average root-to-shoot ratio was higher in plots exposed to ambient CO<sub>2</sub>. The contrasting responses of the dominant oaks to CO<sub>2</sub> enrichment may have affected the patterns of biomass allocation above- and belowground. Because roots were not quantified by species, we cannot determine the direct contribution of each species to root biomass, but it is possible that differences in biomass partitioning among species could have affected total root biomass at the end of the study.

Biomass partitioning under CO<sub>2</sub> enrichment does not seem to follow a predictable pattern. Although a meta-analysis by Luo *et al.* (2006) showed slightly higher root-to-shoot ratios in plants grown under elevated CO<sub>2</sub>, there are many studies in which this is not the case. Stulen & den Hertog (1993) believed that the determination of root-to-shoot ratios was highly susceptible to experimental error, such as the subjectivity surrounding the point at which shoots end and roots begin. The lack of a consistent pattern in biomass partitioning found in a literature review by BassiriRad *et al.* (2001) was attributed to variations in experimental protocol and/or interspecific differences. Wang & Taub (2010) found that abiotic stresses (i.e. drought or exposure to ozone) had a more pronounced effect on the fraction of root to total biomass than did exposure to elevated CO<sub>2</sub>.

The relatively greater CO<sub>2</sub> stimulation of leaf biomass relative to fine root biomass contrasts with earlier speculation that increasing CO<sub>2</sub> should promote relatively stronger responses in roots (Norby *et al.*, 2004). Two phenomena could account for this response: (1) elevated CO<sub>2</sub> increases the functional efficiency of fine roots; or (2) elevated CO<sub>2</sub> stimulates nutrient availability. We have no direct evidence of increased physiological efficiency of fine roots (e.g. nutrient uptake kinetics), and responses in other studies have been mixed (BassiriRad, 2000). As an extension of the nutrient scavenging surface, mycorrhizae can effectively increase the functional efficiency of fine roots; in the scrub-oak system, elevated CO<sub>2</sub> stimulated ecto-mycorrhizal biomass and infection (Langley *et al.*, 2003), soil fungal markers consistent with mycorrhizae (Klamer *et al.*, 2002) and the ratio of fungi

to bacteria in soil (Carney *et al.*, 2007), suggesting that increased mycorrhizal infection, a general response to elevated CO<sub>2</sub> (Treseder, 2004), may have contributed to the relatively smaller response to CO<sub>2</sub> of fine roots. Second, increased nutrient availability may also partly explain the smaller response of fine roots to elevated CO<sub>2</sub>: we found that elevated CO<sub>2</sub> stimulates the turnover of soil organic matter in this system (Carney *et al.*, 2007; Langley *et al.*, 2009; B. A. Hungate *et al.*, unpublished), and increases plant nitrogen uptake (B. A. Hungate *et al.*, unpublished), a pattern also found in a pine forest (Drake *et al.*, 2011). In the first several years of CO<sub>2</sub> exposure in the Florida scrub-oak system, fine root turnover increased with elevated CO<sub>2</sub>, but this response was no longer observed later in the study (Dilustro *et al.*, 2002; Stover *et al.*, 2010). Additional observations are required to fully address this question.

### Conclusions

Data from this 11-yr CO<sub>2</sub> enrichment study in a fire-maintained, shrub-dominated woodland ecosystem marginally supported the hypothesis that elevated CO<sub>2</sub> would stimulate root biomass; however, fine root biomass was at a low point at the end of the study and, although statistically insignificant (*P*=0.07), there was a substantial absolute difference in belowground biomass in elevated vs ambient CO<sub>2</sub> plots. Strong CO<sub>2</sub> effects on fine root biomass were seen after disturbance by fire and hurricane during periods of recovery, followed by periods in which CO<sub>2</sub> effects diminished. Possibly, disturbance increased resource availability and altered plant sink strength above- and belowground, modulating belowground responses to CO<sub>2</sub> in this ecosystem. Total root biomass was as much as five times greater than aboveground biomass in this system, reflecting the importance of belowground structures as a carbon reservoir. This study suggests that both minirhizotrons and GPR are effective and compatible in covering the range of root size classes, but there are unknown areas of overlap that need resolution. Analysis and interpretation of the entire 11-yr dataset was necessary to fully elucidate the root response to long-term CO<sub>2</sub> enrichment at this site.

These findings can be applied to future work in several ways. First, belowground carbon budgets and predictions regarding the effect of increasing atmospheric CO<sub>2</sub> on root biomass will need to take into account root closure as a possible limit on carbon soil dynamics in mature ecosystems. Second, belowground biomass is temporally dynamic and undergoes natural cycles that are affected by ecosystem disturbances in systems with strong disturbance regimes. Similar responses have been observed in desert plants, with CO<sub>2</sub> effects dependent on soil moisture (Ferguson & Nowak, 2011; Sonderegger *et al.*, 2013). The change in root biomass over time means that one-time sampling will not give an accurate representation of root parameters over the long term. Third, elevated CO<sub>2</sub> may enhance root growth following disturbance and potentially speed up the recovery.

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