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Effects of 11 Years of CO2 Enrichment on Root Biomass and Spatial Distribution in a Florida Scrub-Oak Ecosystem

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EFFECTS OF 11 YEARS OF CO_2 ENRICHMENT ON ROOT BIOMASS AND SPATIAL DISTRIBUTION IN A FLORIDA SCRUB-OAK ECOSYSTEM

by

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ABSTRACT

EFFECTS OF 11 YEARS OF CO_2 ENRICHMENT ON ROOT BIOMASS AND SPATIAL DISTRIBUTION IN A FLORIDA SCRUB-OAK ECOSYSTEM

Rachel Eilenfield Schroeder
Old Dominion University, 2011
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A Florida (USA) scrub-oak ecosystem was exposed to elevated atmospheric CO_2 in open-top chambers from 1996-2007. Minirhizotrons and ground-penetrating radar (GPR) were used to measure fine root (< 2 mm diameter) and coarse root (> 5 mm diameter) biomass, respectively. After 11 years of CO_2 enrichment, there was a trend of greater total root biomass under elevated CO_2. Fine root biomass exhibited a pattern of recovery and steady state throughout the study, with significant CO_2 stimulation observed only after disturbance. Greater root biomass under elevated CO_2 during recovery periods could result in greater carbon inputs belowground, alteration of the soil carbon cycle, and faster ecosystem recovery. At the end of the study, a greater proportion of fine root biomass was found deeper in the soil in plots exposed to elevated CO_2. The shift of biomass deeper in the soil and pattern of recovery and steady state suggest a limit on the soils' capacity to support fine roots. The dominant plants were not limited by water or nutrients, indicating that root responses to CO_2 enrichment were likely constrained by soil resource space.

At the end of the study in May 2007, all aboveground vegetation was harvested from the study plots. One month after harvest, neither fine root length density (RLD) nor biomass showed a significant decrease from pre-harvest levels. Ten months after harvest, fine root biomass increased more in plots formerly exposed to elevated CO_2 than in those

exposed to ambient CO_2, suggesting a CO_2 "legacy" effect on fine root growth. The effects of complete aboveground vegetation removal on fine roots were different from those observed after natural disturbances such as fires and hurricanes.

After the study plots were cleared of aboveground vegetation, they were scanned intensively with GPR in order to create 3-dimensional images of coarse root spatial distribution. Top-down views of root horizontal distribution (or root "cover") were quantified using pixel counts; no significant difference was found between CO_2 treatments. Belowground plant structures provide a critical carbon reservoir essential for plant recovery after disturbance in this natural ecosystem. High belowground biomass was revealed with soil cores, pit excavations, minirhizotrons, and GPR imaging. Minirhizotrons and GPR are fast and effective methods for collecting data on belowground plant structures without having to excavate the root system, which is essential in studies carried out over multiple years where nondestructive sampling methods are necessary.

I dedicate this dissertation to my husband, Keith Schroeder, for his patience and for his support of my graduate school career.

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INTRODUCTION

Background

Atmospheric carbon dioxide (CO_2) concentrations are increasing due to human activities, and this will have a direct effect on plant growth as more CO_2 is available for photosynthesis. The overall effects on plant growth will depend on many factors, such as whether other necessary resources are available (i.e. light, water, nutrients, space). If photosynthesis is stimulated by elevated CO_2, plants may respond by allocating more biomass to roots in order to acquire additional water and nutrients to support growth. Excess photosynthate may also be stored belowground in roots and other structures, providing an important sink for increasing atmospheric carbon.

This research was conducted in a natural scrub-oak ecosystem on Merritt Island, Florida to investigate the response of plants in this ecosystem to increasing atmospheric CO_2 and to explore the use of nondestructive methods for measuring roots. Roots are an important component of this ecosystem because the dominant scrub-oak species have high belowground biomass. Coarse roots, rhizomes and lignotubers contain carbohydrate reserves and bud banks that allow the plants to resprout after fire, which is a frequent ecosystem disturbance.

Atmospheric CO_2 Concentrations

CO_2 concentrations in the atmosphere remained relatively stable in the range of 275-284 ppm (parts per million) for at least a thousand years prior to the Industrial

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Revolution (Etheridge et al. 1996). Since the 1700's, industrialization and rapid human population growth resulted in increased CO_2 emissions (Neftel et al. 1985) from deforestation and fossil fuel burning. Current concentrations are higher than at any time during the past 800,000 years (Lüthi et al. 2008) and possibly the past 23 million years (Pearson and Palmer 2000). The longest continuous direct measurements of atmospheric CO_2 have been made at Mauna Loa Observatory in Hawaii since 1958 (Fig. 1). CO_2 concentrations measured at Mauna Loa increased from 316 ppm in 1959 to 385 ppm in 2008, representing an average annual increase of 1.4 ppm (Keeling et al. 2009).

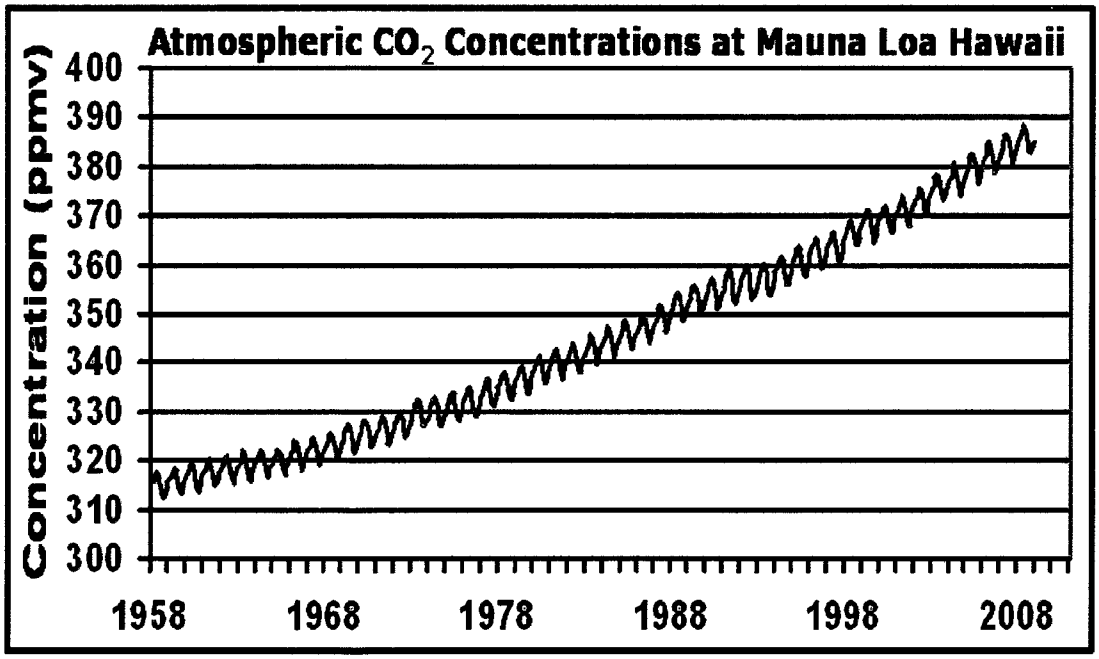

FIG. 1. Atmospheric CO_2 concentrations at Mauna Loa, Hawaii from 1958 to 2008 (Keeling et al. 2009). This data series is known as the Keeling Curve.

The mean global CO_2 concentration for December 2010 was 390 ppm, measured from a globally-distributed network of sampling sites (Tans 2011). Each year the CO_2 concentration peaks in May and is at its lowest point in October (Fig. 2, Tans 2011), demonstrating an annual cycle that is controlled by the northern hemisphere growing season of terrestrial plants. The lowest annual CO_2 concentrations correspond to the time of year when there is the most photosynthesis, resulting in a distinctive saw-tooth pattern in CO_2 records.

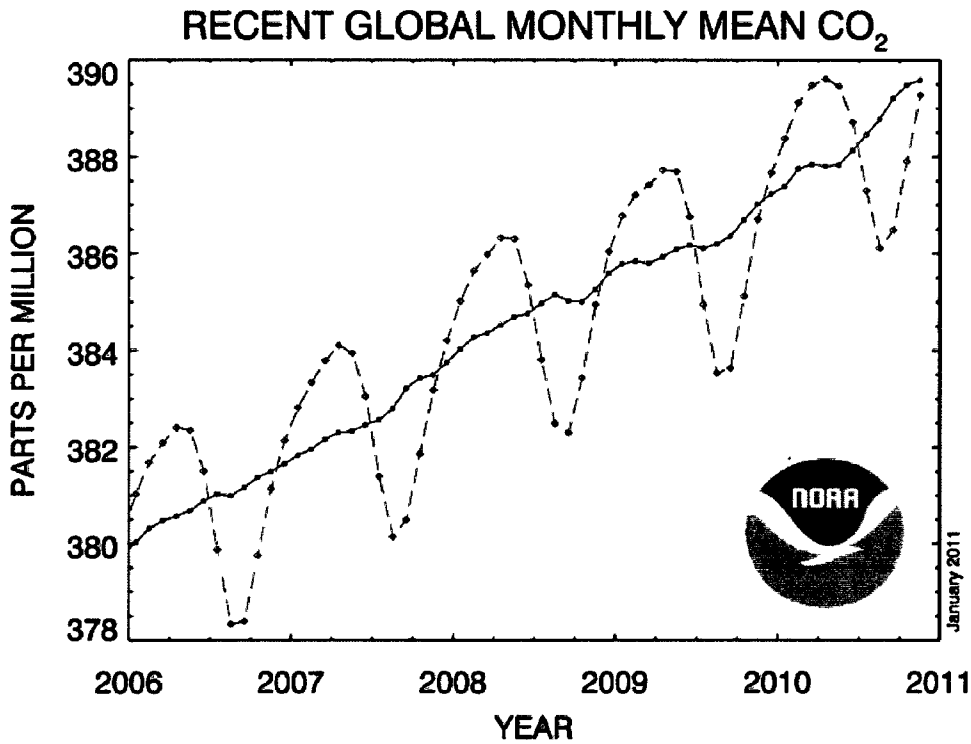

FIG. 2. Mean global monthly CO_2 concentrations averaged over marine surface sites from 1996-2010 (Tans 2011). The dashed line with diamond symbols represents data points for each monthly mean, while the solid line with square symbols represents the same data after correction for seasonal variation.

CO_2 is important because it is the second most abundant "greenhouse gas" (after water vapor) and is highly effective at absorbing thermal radiation from the Earth's surface, which results in warming of the lower atmosphere. Natural sources of CO_2 include volcanoes, combustion of organic matter, microbial decomposition, and cellular respiration. Anthropogenic sources of CO_2 include the use of fossil fuels for transportation and power generation, deforestation and decay of plant material, cement production, and biomass burning. Emissions from fossil fuel burning for energy use, which is the largest source of anthropogenic CO_2, are expected to increase 40-110% between the years 2000 and 2030 (IPCC 2007).

Effects of Increasing CO_2 on Plants

Increasing atmospheric CO_2 may stimulate growth in plants that utilize the C_3 photosynthetic pathway of carbon fixation, which is the photosynthetic system found in most terrestrial plants including trees and many crops. The primary plant responses to elevated CO_2 concentrations are increased photosynthesis and reduced stomatal conductance (Ainsworth and Rogers 2007). Additionally, plants grown under elevated CO_2 may have increased resource use efficiency (Drake et al. 1997, Leakey et al. 2009).

The overall effect of these primary responses is often an increase in plant biomass and productivity. In a meta-analysis of more than 500 studies on trees grown in chambers or greenhouses, net CO_2 assimilation and total biomass were shown to significantly increase at twice-ambient CO_2 regardless of growth conditions (Curtis and Wang 1998). In intact forests under Free-Air CO_2 enrichment (FACE), net primary productivity was stimulated by 23% across four experimental sites with a broad range of species

characteristics and environmental conditions (Norby et al. 2005). A meta-analysis of FACE and open-top chamber (OTC) experiments showed that elevated CO_2 stimulated above- and belowground plant biomass by an average of 22% and 28% respectively (de Graaff et al. 2006).

Elevated CO_2 and Roots

Photosynthetic response to elevated CO_2 is greatest under high levels of other resources such as light, water, and nutrients (Bazzaz 1990, Field et al. 1992). Progressive nutrient limitation has been proposed as a regulator of long-term plant response to elevated CO_2 (Johnson 2006, Luo et al. 2004). For example, the soil nitrogen supply may constrain vegetation responses to elevated CO_2 if the nitrogen needed to support increased plant growth is not available (Reich et al. 2006, Oren et al. 2001). In limiting soil conditions, plants may allocate more biomass to roots in order to increase water and nutrient uptake (Poorter and Nagel 2000). Low soil fertility was found to increase the fraction of root to total plant biomass for plants grown under either ambient or elevated CO_2 (Wang and Taub 2010).

Even when nutrient availability is high, root biomass may increase in plants grown under elevated CO_2 (de Graaff et al. 2006). Early studies on individual plants grown under elevated CO_2 have shown increased root length and diameter (Pritchard et al. 1999) and biomass (Rogers et al. 1994). Recent studies on woody plants in field conditions have also shown increased root production (Lukac et al. 2003, Norby et al. 2004, Pritchard et al. 2008), turnover (Lukac et al. 2003), standing crop (Jackson et al. 2009, Iversen et al. 2008, Runion et al. 2006, Pregitzer et al. 2008), and mortality

(Iversen et al. 2008, Pritchard et al. 2008). CO_2 enrichment of forests has also been found to result in deeper rooting distributions of fine roots (Iversen 2010).

Root Quantification in Long-term Studies

Assessing the impacts of elevated CO_2 on roots has been constrained by methodological limitations. Studies that are carried out over multiple years pose a challenge for vegetation sampling if the goal is to keep plants intact over the duration of the study. While nondestructive methods can be employed fairly easily to collect data about the aboveground portion of plants, it is much more difficult to collect belowground data without disturbing the system. Traditionally, root parameters were measured destructively with soil cores, excavations, soil profile walls, or ingrowth bags and cores (Atkinson 2000, Neumann et al. 2009).

Nondestructive methods have been employed with varying success. One of the most widely-used indirect methods for studying fine roots is the minirhizotron technique in which a clear tube is inserted into the ground and images of roots growing along the outside of the tube are recorded (Vogt et al. 1998). Other nondestructive methods used to study roots include multi-electrode resistivity imaging (Amato et al. 2008), computer-assisted tomography (CT), magnetic resonance imaging (MRI) (Asseng et al. 2000), X-ray imaging (Pierret et al. 2005), and ground-penetrating radar (Butnor et al. 2003).

Study Objectives

In the present study, a fire-prone scrub-oak ecosystem on the east coast of Florida was exposed to 11 years of elevated atmospheric CO_2 using OTCs following a controlled

burn in 1996. Two nondestructive methods were employed to study roots exposed to ambient and elevated CO_2 treatments: minirhizotron imaging to quantify fine roots (< 2 mm diameter) and ground-penetrating radar to quantify coarse roots (> 5 mm diameter). The specific objectives of this study were to 1) determine the effect of 11 years of CO_2 enrichment on fine and coarse root biomass, 2) determine the potential CO_2 legacy effects of aboveground vegetation removal on fine root abundance and biomass, and 3) explore the use of GPR to image coarse root spatial distribution at the study site.

Study Site Description

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 (Fig. 3). The 140,000 acre refuge was established in 1963 on NASA's Kennedy Space Center and is managed by the U. S. Fish and Wildlife Service (USFWS 2006). Elevation is 0-3 m above mean sea level on the interior of Merritt Island. The sandy soils are acidic, well-drained, and characteristically nutrient-poor; the bulk of nutrients in this system are found in live and dead vegetation instead of the mineral soil (Schmalzer and Hinkle 1987). Climate is subtropical with a wet season between late June and October and a dry season between April and early June. Sixty percent of the annual precipitation occurs during the wet season, and lightning associated with thunderstorms is responsible for igniting wildfires (USFWS 2006). While fire is the dominant ecosystem disturbance at the study site, other natural disturbances include periodic drought and severe weather from tropical storms and hurricanes.

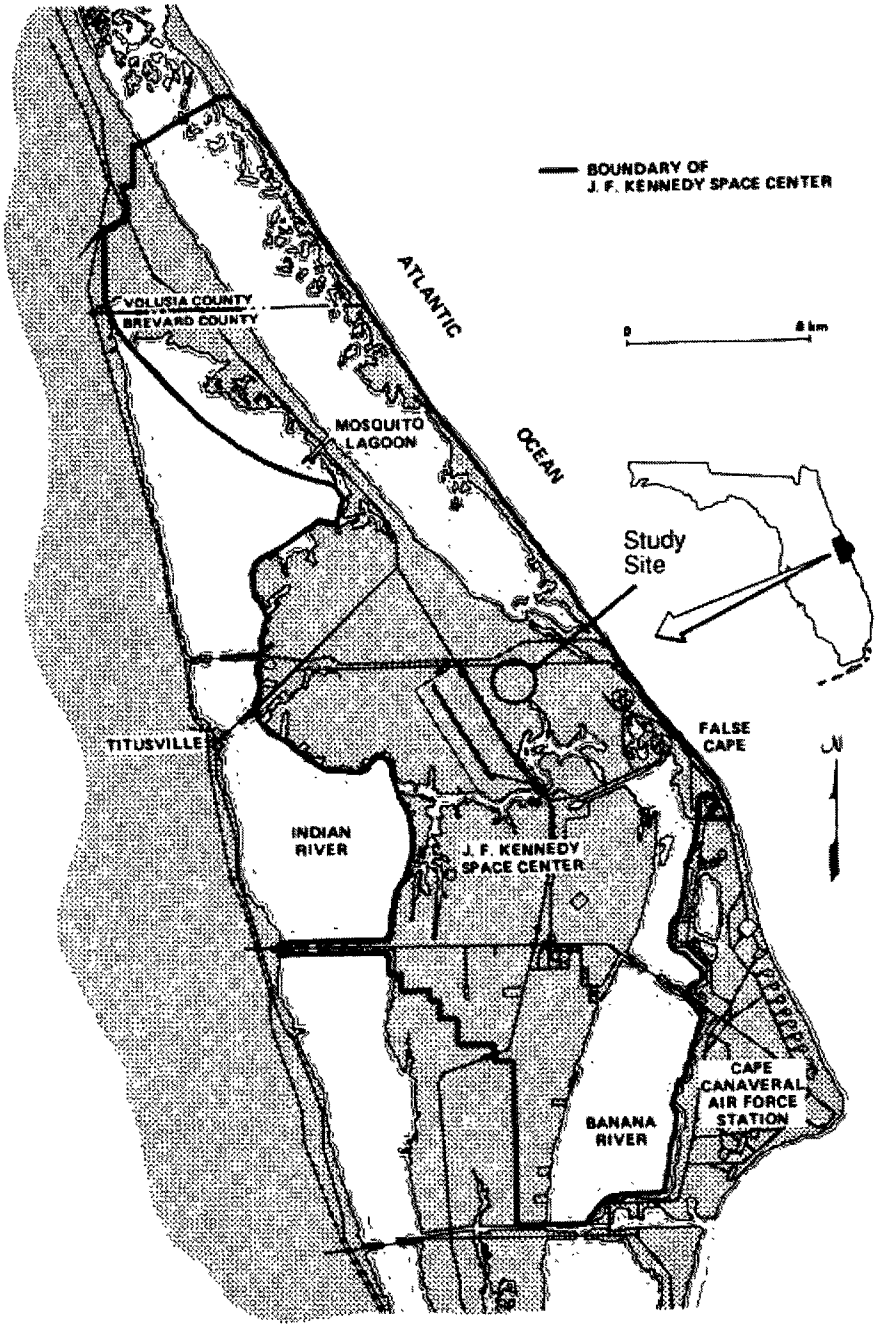

FIG. 3. Map of study site location and surrounding area (Schmalzer and Hinkle 1992b).

Scrub-oak shrublands occupy over 15,000 acres of Merritt Island NWR, and fire is essential for maintaining the vertical and horizontal structure of the plant community (USFWS 2006). The scrub-oak vegetation is dominated by woody evergreen species that have extensive belowground storage organs such as lignotubers and rhizomes that allow the plants to re-sprout after fire (Schmalzer and Hinkle 1992a, Menges and Kohfeldt 1995). *Quercus myrtifolia* Willd. (myrtle oak) and *Quercus geminata* Small (sand live oak) are the two co-dominant species; other woody species include *Quercus chapmanii* Sargenti (Chapman oak), *Serenoa repens* (Bartram) Small (saw palmetto), *Lyonia ferruginea* (Walter) Nutt. (rusty staggerbush), and *Morella cerifera* (L.) Small (wax myrtle) (Schmalzer and Hinkle 1992b, Seiler et al. 2009). Voucher specimens of the dominant plant species are kept at Kennedy Space Center, with duplicates at the University of Florida herbarium and the University of South Florida herbarium. The effects of fire on Merritt Island scrub-oak communities were studied since the 1980s in an effort to characterize the composition and structure of the ecosystem for conservation purposes (Schmalzer and Hinkle 1987, 1992a, 1992b).

CO_2 Enrichment Experiment

In 1992, a preliminary CO_2 enrichment study was initiated with funding from NASA. Six open-top chambers were used in the pilot study, which was a precursor to the experiment discussed here. Initial findings of the early study prompted an expansion with funding from the U.S. Department of Energy. The larger-scale CO_2 enrichment experiment that is the subject of this research began with a prescribed burn in late 1995 and again in early 1996. Any unburned aboveground vegetation was clipped to ground

level, and 16 plots were selected and grouped based on pre-burn vegetation into eight blocks and assigned treatment of ambient or elevated CO_2.

Sixteen open-top chambers (OTCs, Fig. 4) were constructed for the CO_2 treatments (Drake et al. 1985). Chamber frames were made with 4-inch diameter PVC pipe in an octagonal design. The sides were rectangular removable panels covered with sheets of clear Mylar film. Chambers enclosed 9.4 m^2 of ground area and were 2.5 m tall and 3.5 m wide at parallel sides. A frustum was constructed on each chamber to minimize wind intrusion, but the top of the chamber was open to the atmosphere. CO_2 addition began in May 1996 and was maintained 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_2 was ~350 ppm in 1996 and had increased to ~380 ppm in 2007. Treatment CO_2 concentrations were maintained 24 hours a day, and each chamber had an independent blower system that circulated air continuously. Air samples from each chamber were collected and analyzed with a computer-automated system, and CO_2 addition was adjusted to maintain concentrations within the desired range. CO_2 addition ended in May 2007, and all chambers were removed.

Throughout the 11 years of the study, numerous measurements were taken of various ecosystem parameters. These included, but were not limited to: photosynthesis by dominant plant species, net ecosystem exchange, aboveground biomass (from nondestructive allometric relationships), shoot density, leaf area index, soil organic matter content, soil microbial activity, nutrient dynamics (specifically nitrogen), evapotranspiration, and insect herbivore activity. Additionally, environmental data such as air temperature, precipitation, and soil water content were collected.

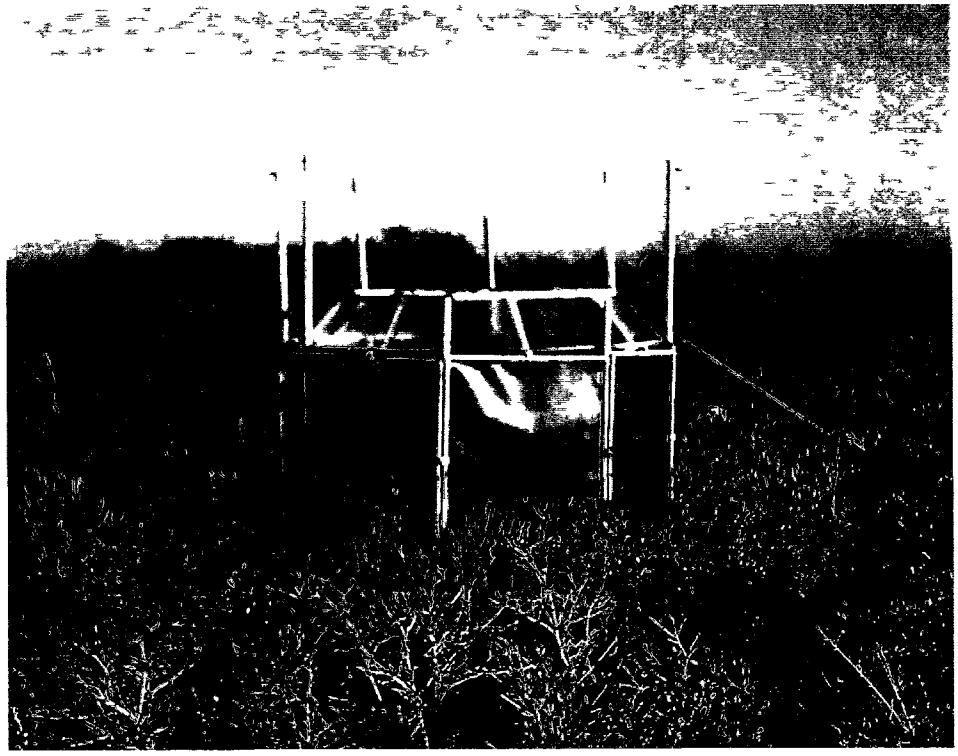

FIG. 4. Photograph of one experimental chamber and surrounding scrub-oak vegetation. Sixteen chambers were used, 8 with ambient CO_2 and 8 with elevated (ambient + 350 ppm) CO_2.

Overall, the dominant oaks responded differently to CO_2 enrichment throughout the study. Stomatal conductance and transpiration were reduced under elevated CO_2 (Hungate et al. 2002, Li et al. 2003), and both oak species had increased rates of leaf net photosynthesis (Li et al. 2007a, Hymus et al. 2002, Ainsworth et al. 2002). However, stimulation of photosynthesis in *Q. myrtifolia* (63%) was approximately twice that of *Q. geminata* (35%). Also, *Q. myrtifolia* had a strong CO_2 response in aboveground biomass throughout the study while *Q. geminata* showed no significant CO_2 effect (Seiler et al. 2009, Fig. 5). At the end of the study, total aboveground biomass was 67% higher in

elevated CO_2 chambers, driven mainly by the 128% increase in *Q. myrtifolia* biomass under elevated CO_2 (Seiler et al. 2009).

After the fire in 1996, the CO_2 effect on aboveground community biomass increased steeply for 3 years but was stable for the remainder of the experiment (Seiler et al. 2009), which was similar to the observed effect on fine root length (Day et al. 2006). Aboveground growth was correlated with annual rainfall, and shoot density dropped anomalously in 2000 following a period of drought (Li et al. 2007a, Seiler et al. 2009). In 2005, an abrupt increase in shoot density was associated with recovery after hurricane disturbance (Li et al. 2007b, Seiler et al. 2009).

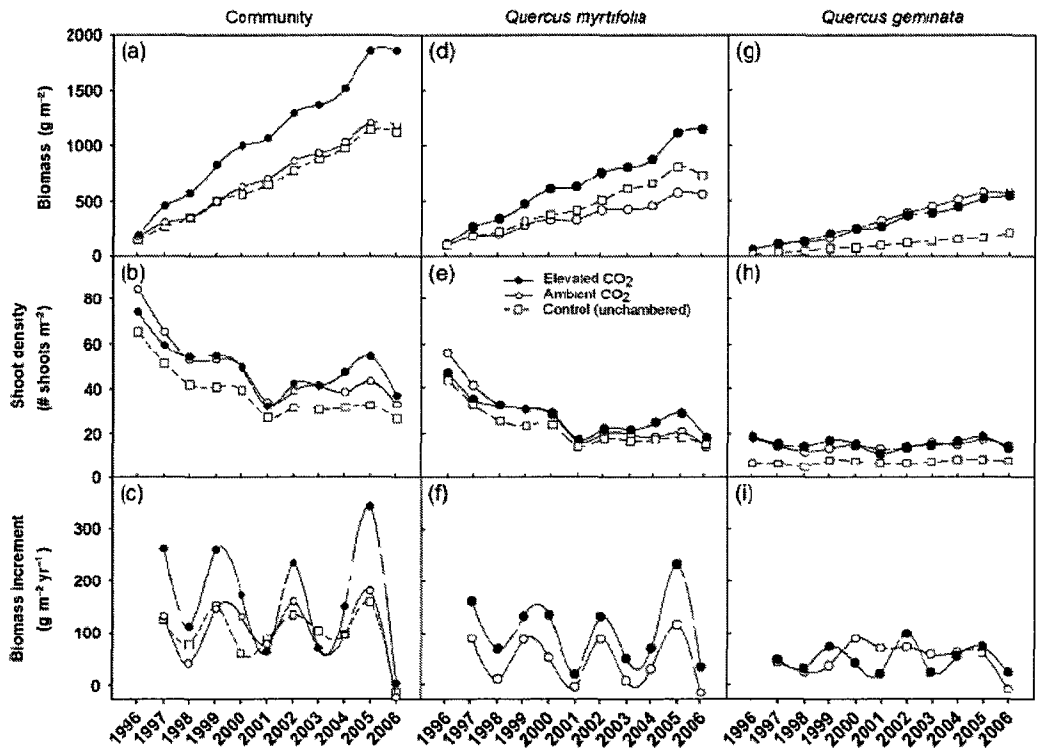

FIG. 5. Aboveground biomass, shoot density, and biomass increment from 1996-2006. Figure is from Seiler et al. 2009.

Root Methodologies Employed

Fine Roots – Minirhizotrons

Minirhizotrons were installed in the study plots in 1996 after the fire but prior to chamber construction. Minirhizotrons were clear cellulose acetate butyrate tubes, 5.7 cm in diameter, with a series of numbered 9 x 13 mm frames etched along one side of each tube. Two minirhizotrons were installed in each chamber plot at a 45° angle from the soil surface to a depth of ~ 1 m (Fig. 6). The portion of the minirhizotrons protruding above the soil surface was painted, taped, and capped. This procedure ensured that water and light could not enter the tubes and affect roots growing along the minirhizotrons.

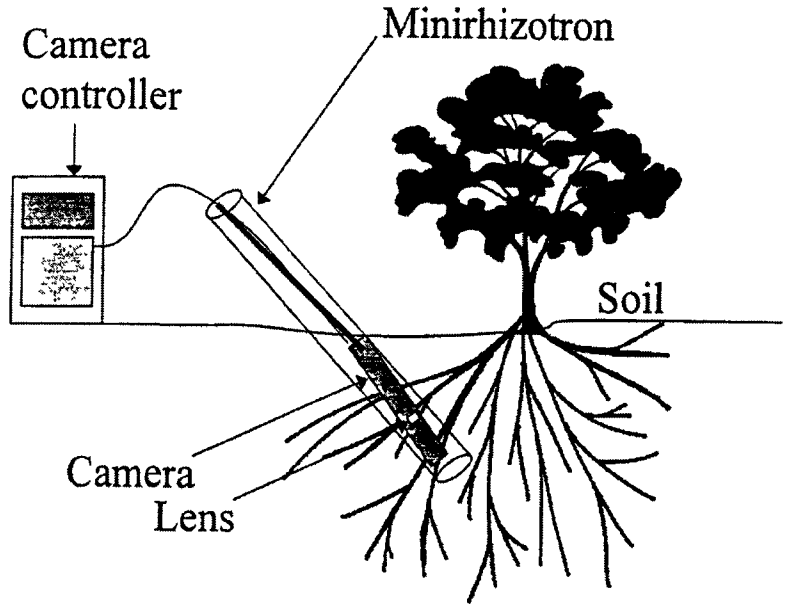

FIG. 6. Diagram of a minirhizotron installed below a shrub with camera inserted for viewing and recording root images.

To measure fine roots, a minirhizotron camera (Bartz Technology Co., Santa Barbara, CA, USA) was inserted into each tube, and images of roots within the etched frames were recorded using Hi8 videotape. Minirhizotron recordings were obtained approximately every 3 months throughout the study. In this dissertation, minirhizotron data collected in March 2007 (two months before the final aboveground harvest), August 2007 (one month after harvest), and May 2008 (10 months after harvest) are presented.

Images were converted to digital jpeg files (Fig. 7) for every fifth frame, totaling 32 frames per tube. Root lengths and widths were digitally traced for each root within the frames using the MSU Roots Tracer Program version 2.2 (Michigan State University Center for Remote Sensing and GIS, East Lansing, MI, USA). Images that were blurry, dark, filmed incorrectly, or were of frames showing incomplete soil contact with the minirhizotron tube were not included. The primary metric calculated was root length per frame area (mm/cm^2), known as root length density (RLD). Individual roots digitized from each minirhizotron image were categorized into the following size classes based on root width (diameter): <0.25 mm, 0.25-1 mm, 1-2 mm, and >2 mm.

Coarse Roots - GPR

Ground-penetrating radar (GPR) is a geophysical tool that has recently been used to study large belowground plant structures (Butnor et al. 2003, Dannoura et al. 2008, Barton and Montagu 2004, Hruska et al. 1999). With this method, an electromagnetic wave is radiated from a transmitting antenna. When the wave encounters a material with a different permittivity from that of the initial transmission, a portion of the signal is scattered and reflected back to a receiving antenna (Daniels et al. 2008). The use of GPR

to detect tree roots is illustrated in Fig. 8. GPR signal reflection and root biomass exhibited a significant linear relationship at the Florida site (Stover et al. 2007), providing a non-destructive method for estimating coarse root biomass in this system.

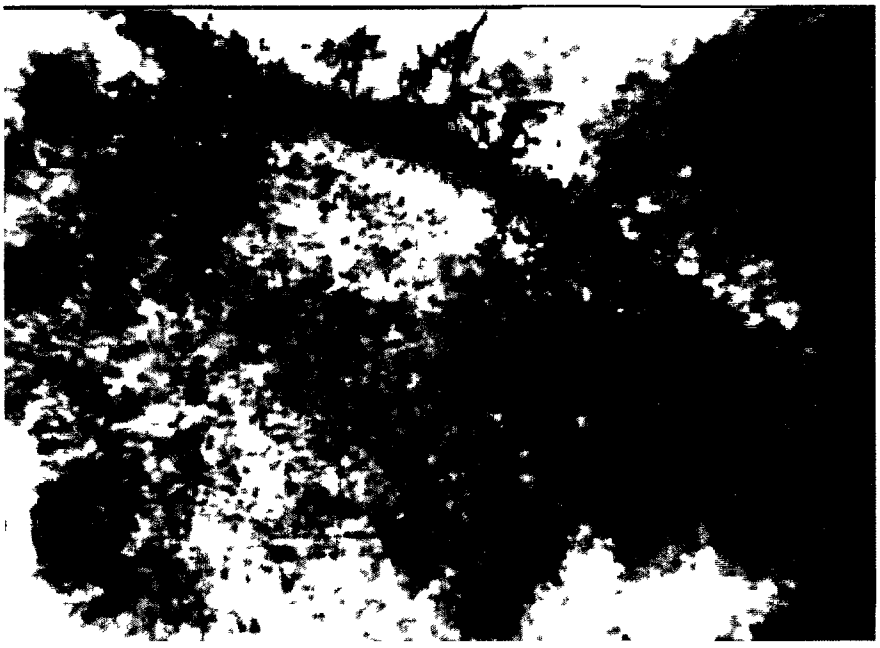

FIG. 7. Sample jpeg image showing soil and fine roots from recordings collected from minirhizotrons. Rectangular frames, each measuring 9 x 13 mm, are etched along the inside of each minirhizotron tube. Black dots demarcate corners of one frame.

After CO_2 addition ended and the chambers were removed, all aboveground vegetation was clipped to the soil surface and removed from the plots for analysis. In late June and early July 2007, less than one week after aboveground vegetation in the experimental plots was harvested, GPR was used to image roots in all experimental plots with a Subsurface Interface Radar (SIR-3000) control system and 1500 MHz (model 5100) antenna (Geophysical Survey Systems, Inc., North Salem, NH, USA).

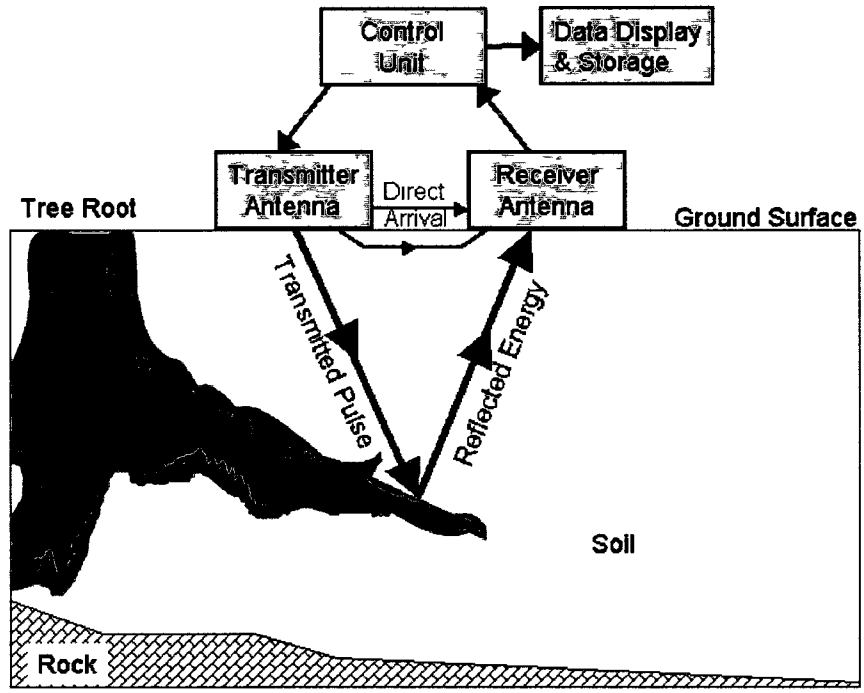

FIG. 8. Diagram of ground-penetrating radar used for detection of coarse roots. Figure is from Stover et al. (2007).

Prior to data collection, a 2 x 2 m fiberglass frame (Fig. 9) was constructed with holes drilled every 2 cm along each side of the frame. In the field, the frame was positioned within the footprint of each experimental plot. A 2-m long fiberglass beam with a freely-moving shuttle and adjustable arm was positioned on the frame using the numbered holes drilled along parallel sides. The radar antenna (with calibrated survey wheel) was attached to a plate on the shuttle arm with Velcro, and the beam and shuttle mechanism assisted with guiding the antenna along multiple 2-m transects. Plots were scanned either every 16-cm in both an x- and y-direction (resulting in a total of 26 scans per plot), or every 2-cm in both directions (totaling 200 scans per plot). Plots with fewer

scans were used strictly for estimating biomass, while those with greater scans were used for biomass as well as 3-dimensional imaging to evaluate root spatial distribution. Individual 2-m long GPR scans were processed post-collection with Radan software as described in Chapters 2 and 4.

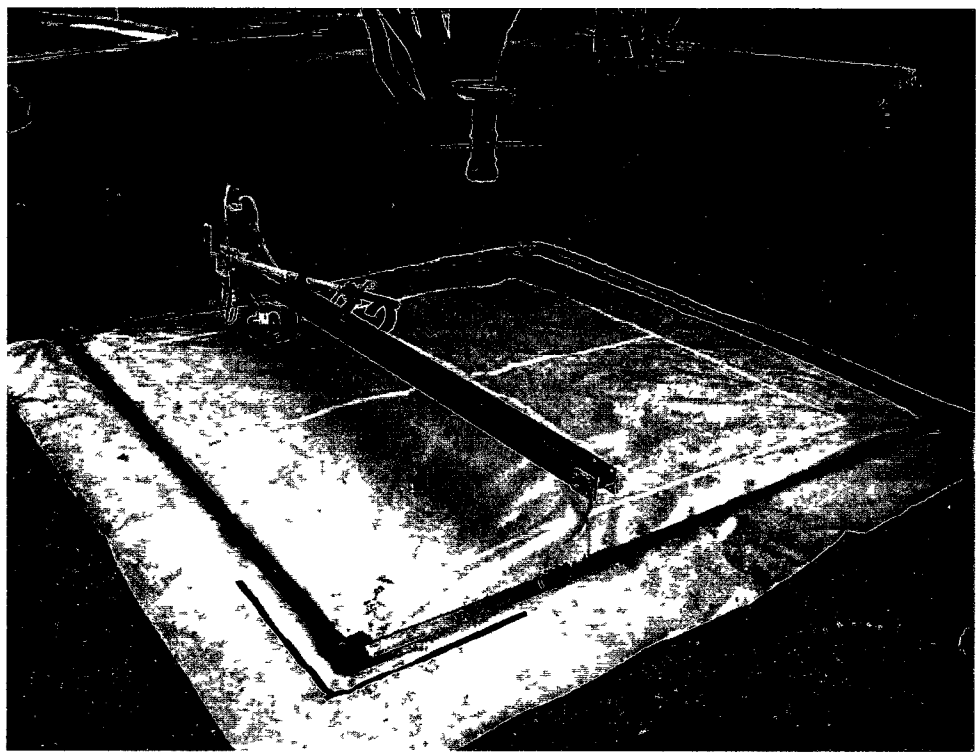

FIG. 9. Fiberglass frame constructed for scanning 2 m x 2 m plots with ground-penetrating radar. The radar antenna is the small box resting on the ground surface in the upper left quadrant of the 2 x 2 m grid.

CHAPTER 2

ROOT BIOMASS AFTER 11 YEARS OF CO_2 ENRICHMENT

Introduction

While many CO_2 enrichment experiments have been performed, few have been maintained continuously over multiple years. Within the handful of long-term enrichment studies of woody plants in field conditions, the effects of elevated CO_2 on root biomass have been mixed. A regenerating longleaf pine community had 49% greater total belowground biomass in CO_2-enriched plots after three years (Runion et al. 2006). Similarly, a poplar plantation in Italy had a 47-76% (depending on species) increase in standing root biomass after three years of FACE treatment (Lukac et al. 2003). A decade of CO_2 enrichment in a temperate loblolly pine forest doubled biomass of coarse roots and caused a 24% increase of fine roots in the top 15 cm of soil (Jackson et al. 2009). Increased root biomass was found throughout 10 years of CO_2 enrichment in mixed deciduous tree communities (Pregitzer et al. 2008). In contrast, a 30% reduction of fine root biomass was found in mature deciduous forest trees in central Europe after five years of CO_2 enrichment, with no detectable CO_2 effect after 7 years (Bader et al. 2009). No stimulation of root growth was seen during four years of FACE treatment in a long-term afforestation site at treeline in the Swiss Central Alps (Handa et al. 2008).

Early findings on the effects of CO_2 enrichment on roots at this study site were presented in previous publications examining fine root abundance, production, and mortality (1996-1997 in Dilustro et al. 2002; 2002-2004 in Stover et al. 2010); fine root abundance (1996-2004) and depth distribution (1997 in Day et al. 2006); fine root biomass (2002 core data in Brown et al. 2007; 1996-2006 minirhizotron data in Brown et

al. 2009); and coarse root biomass from GPR measurements (2005 in Stover et al. 2007). The studies by Brown et al. 2009 and Stover et al. 2007 demonstrated the effective use of minirhizotrons and GPR at this study site for estimating fine and coarse root biomass, respectively.

A key finding from previous work showed that fine root length density was significantly greater under elevated CO_2 only during the first 3 years of the study. The subsequent loss of CO_2 treatment effect was attributed to root closure (Day et al. 2006). Root closure was considered to be a dynamic equilibrium between root production and mortality. One year into the study, fine root production, mortality and turnover were higher under elevated CO_2 (Dilustro et al. 2002). Nine years after CO_2 treatment was initiated, there was no longer a treatment effect on these root parameters (Stover et al. 2010). And as in other long-term CO_2 enrichment studies, a shift in fine root distribution to deeper in the soil profile was seen over time (Stover et al. 2010). The results presented in this chapter provide updates to the long-term dataset and represent the final measurements of belowground biomass of this 11-year study.

Objectives

Following protocols that had been established previously for quantifying roots at this study site, fine (< 2 mm diameter) root biomass was measured using minirhizotrons and coarse (> 5 mm diameter) root biomass using ground-penetrating radar. These values were also compared to root biomass estimates from soil cores. Roots > 2 mm diameter could not be adequately sampled using minirhizotrons, but GPR may have detected roots < 5 mm diameter. It was hypothesized that 1) vegetation exposed to elevated CO_2 for 11

years would have greater fine and coarse root biomass than vegetation exposed to ambient CO_2, 2) greater fine root biomass would be found deeper in the soil under elevated CO_2, and 3) root biomass in 2007 would be at a low point in both treatments due to the observed trend of decreasing biomass over the previous two years.

Methods

Fine Roots

Images from minirhizotrons installed in the chamber plots were collected in March 2007 using the methods described in Chapter 1. Digital jpeg images were captured from the video recordings, and roots were then digitized from the images. Fine root biomass was calculated from root length and width values for all roots < 2 mm diameter following the methods detailed by Brown et al. (2009). The principles behind this method are described by Johnson et al. (2001) and Hendrick and Pregitzer (1996), and minirhizotrons have been used similarly to estimate fine root biomass in other studies (e.g. Jose et al. 2001, Noguchi et al. 2004, Tingey et al. 2005, Kalyn and Van Rees 2006).

In brief, fine root biomass and length from destructive cores collected from the experimental plots in 2002 were used to calculate specific root length (SRL) by dividing total root length by total mass of each size class. The SRL values were then applied to total root length data for the same size classes to estimate biomass from the minirhizotron images. A 2 mm depth-of-field was used to convert the area of the minirhizotron image to a volume for mass estimation (Brown et al. 2009, following Taylor et al. 1970).

Coarse Roots

A correlation was established between GPR signal reflection strength and belowground biomass using 15-cm diameter cores outside of the chambered plots in 2005. Twenty core locations were selected and scanned with 15-cm long scans in the x- and y-directions with a 1500 MHz GPR antenna. Cores were then excavated and root biomass was measured, and the average signal reflection area of the GPR data was plotted against the biomass of each core. The resulting correlation equation was then applied to GPR data collected in the experimental plots in June and July of 2007.

Individual 2-m long GPR scans were processed using Radan version 6.5 (Geophysical Survey Systems, Inc., North Salem, NH, USA). The processing protocol was similar to that used in Stover et al. (2007). GPR has similarly been used to estimate root biomass at other sites (e.g. Butnor et al. 2001, Butnor et al. 2003, Butnor et al. 2005, Samuelson et al. 2008). Processing steps included horizontal stretch by a factor of 2 to normalize the size of the scan file, range gain applied on a per-plot basis, background removal (FIR, boxcar type), Kirchoff migration, and Hilbert transformation (Fig. 10). The background removal processing step is designed to remove high-frequency noise by performing a simple running average on the data. Kirchoff migration is a processing method that removes signal diffraction and compresses hyperbolas to increase accuracy of reflector locations. The Hilbert Magnitude Transform further compacts reflection hyperbolas to accurately represent the size of the reflector.

For each experimental plot, 25 random intersections from the grid of 2-m long GPR scans in the x- and y-directions 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., North Salem, NH, USA) 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 were counted for each image. Pixel counts were applied to a regression equation relating pixel number and root biomass, where coarse root biomass = 0.1262 x pixel count (R^2 = 0.47); this equation is a revision of the one published in Stover et al. (2007). Biomass was extrapolated to g/m^2 based on 15-cm diameter core area.

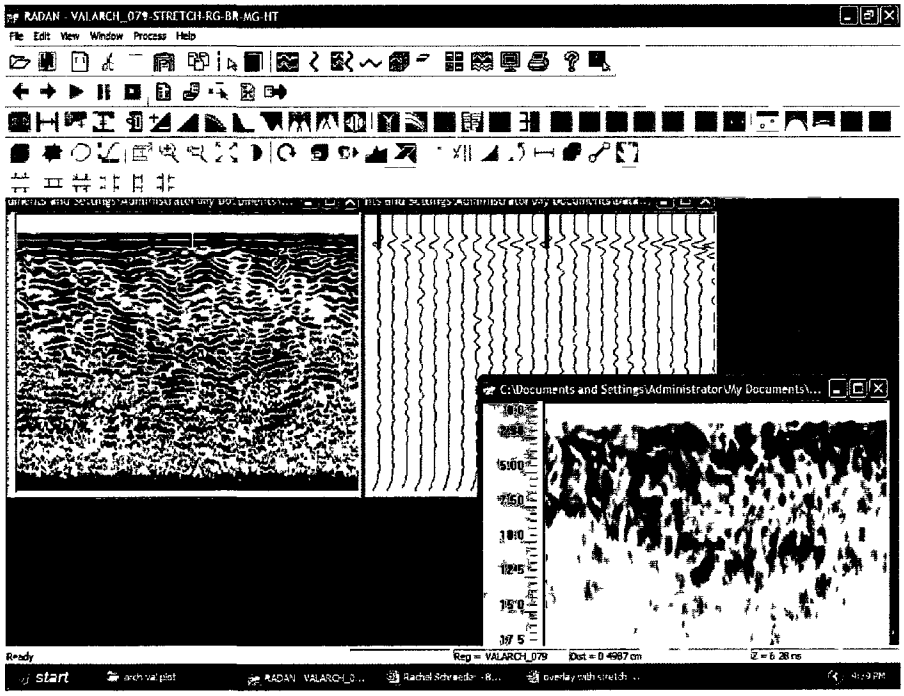

FIG. 10. Sample ground-penetrating radar data viewed with Radan software program. Top left image shows a sample unprocessed 2-m scan and bottom right image shows the same scan after processing.

While processing GPR data for the current study, it was necessary to correct the dataset presented by Stover et al. (2007). This correction produced an updated regression equation to calculate coarse root biomass estimates from data collected in 2005 from the experimental chambers. The new equation (y = 0.3416x, where x = # GPR pixels within threshold of 70-227, $R^2 = 0.50$, $N = 25$) resulted in a change in the biomass estimates from that sampling date: 8010 ± 796 g/m^2 and 6129 ± 1010 g/m^2 for elevated and ambient CO_2 chambers, respectively ($P = 0.12$). At the time those data were collected, the experimental chambers and aboveground vegetation were intact; sampling consisted of five 15-cm long GPR scans per chamber selected with a stratified-random approach to exclude existing vegetation and instruments installed in the chambers. In the current study, all chambers, instruments, and aboveground vegetation were removed and thorough GPR scans were performed.

To validate the GPR biomass estimation method, a 2 x 2 m 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 in July 2007 (Fig. 11a). Then, a 1 x 2 m pit in half of the scanned area was excavated to 60 cm depth (Fig. 11b). Roots from the soil cores and pit were extracted on site using a 6-mm mesh sieve. Root samples were transported back to Norfolk, Va., washed, dried, and weighed.

Soil Cores

Five soil cores were collected in each chamber plot with a 7-cm diameter soil corer in June/July 2007 by multiple researchers led by Bruce Hungate and Pat Megonigal. The cores were collected to the depth of the water table (~ 200 cm) and separated into the

following depth increments: 0-10 cm, 10-30 cm, 30-60 cm, 60-100 cm, 130-160 cm, 160-190 cm, albic and spodic (often > 200 cm). An additional five cores were taken for the 0-10 cm depth in each plot. Core samples were sieved with a 2 mm mesh sieve followed by a 1 mm sieve to separate roots from soil. Roots were frozen until processed at Northern Arizona University. Roots were dried at 60°C for 24 hours and sorted by hand as either fine (< 2 mm diameter) or coarse (> 2 mm diameter). Total biomass per m^2 for each depth interval was calculated by summing the root mass for all cores of a given depth per plot and dividing by total core area.

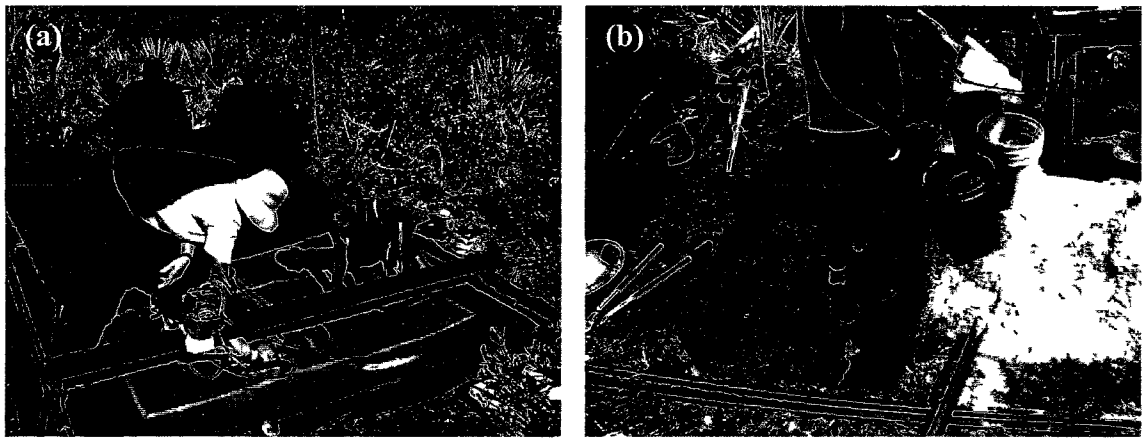

FIG. 11. GPR biomass validation pit: scanning the 2 m x 2 m plot after aboveground vegetation was cleared (a) and excavation of a 1 m x 2 m pit to 60 cm depth (b).

Statistical Analyses

CO_2 treatments were replicated using 16 experimental chambers (n = 8 for each treatment). For measurements that included subsampling within experimental plots, the model residuals were tested for normality (using Shapiro-Wilk test) and homogeneity of

variances (using Levene statistic) with PASW Statistics 17.0 (SPSS, Inc., Chicago, IL, USA). In all cases, model residuals for the raw data failed tests for normal distribution and variance homogeneity, so the data were transformed and the residuals of transformed data then met assumptions for ANOVA statistical tests. Data with subsampling included minirhizotron biomass data (log transformed), GPR data (square root transformed), and soil core data by depth (square root of biomass + 1 transformed).

Fine root biomass estimated using minirhizotrons was tested with a 3-factor mixed-model nested ANOVA using SAS Proc GLM (SAS version 9.1, SAS Institute Inc., Cary, NC, USA), with chamber as the random effect and CO_2 treatment and depth as fixed effects. Least-squares multiple-comparison tests (SAS Proc GLM) were used to determine differences among depths within each CO_2 treatment. An ANOVA was also performed for each depth interval separately to test for CO_2 treatment differences. GPR data were tested with SAS Proc GLM using a 2-factor nested ANOVA with 25 subsamples per chamber; chamber was assigned as the random effect and CO_2 treatment as the fixed effect.

Data that did not have subsampling included total root biomass from combining GPR and minirhizotron estimates, root biomass from cores, aboveground biomass, and root-to-shoot ratios. Non-parametric tests were run using SAS Proc NPAR1WAY on these data to test for differences between CO_2 treatments. All results were considered significant at $\alpha < 0.05$, but trends were recognized at $0.05 < \alpha < 0.15$ (following Runion et al. 2006).

Results

Fine Root Biomass

Biomass of fine roots estimated in March 2007 with minirhizotrons was not significantly greater under elevated CO_2 (1942 ± 168 g/m^2) than under ambient CO_2 (1644 ± 173 g/m^2) ($P = 0.31$). The roots identified by this method were separated into size categories based on root diameter, with biomass partitioned as follows: 70% and 67% in <0.25 mm diameter, 18% and 17% in 0.25–1 mm diameter, and 12% and 16% in 1–2 mm diameter roots for ambient and elevated CO_2 respectively. Using minirhizotrons, there were no roots observed in the ambient CO_2 plots with diameter > 2 mm for this sampling date and there were only two roots observed in the elevated plots. These roots were not included in total biomass measurements since they were not adequately sampled.

Coarse Root Biomass

Coarse root biomass was 5830 ± 487 g/m^2 under elevated and 5105 ± 418 g/m^2 under ambient CO_2, and was not significantly different between treatments ($P = 0.26$). Biomass of roots > 2 mm diameter could not be accurately determined using minirhizotrons, but GPR may have detected a portion of the roots < 5 mm diameter. Dense mats of near-surface fine roots and clusters of fine roots may be captured by GPR. High coarse root biomass in this system and the validity of GPR biomass estimates were confirmed by the validation plot with an estimate of 7770 g/m^2 roots using GPR, compared to an actual biomass of 8222 g/m^2 to 60 cm depth. It is likely that the

difference between actual biomass and the GPR estimate was due to fine roots not detected by GPR.

Total Root Biomass

Combining the minirhizotron estimate of fine root biomass measured in March 2007 (prior to the harvest) and the GPR estimate of coarse root biomass from June 2007, total root biomass for plots exposed to elevated CO_2 was 7772 g/m^2, compared to 6749 g/m^2 for ambient plots ($P = 0.11$; Table 1). The combined total root biomass from these two methods is likely an underestimate given the possible methodological gap in detecting roots between 2 and 5 mm diameter.

Live aboveground biomass at the end of the study was significantly greater ($P < 0.01$) under elevated CO_2 (2103 ± 184 g/m^2) than ambient CO_2 (1257 ± 107 g/m^2), representing an 846 g/m^2 difference (Seiler et al. 2009), comparable to the observed difference of 1023 g/m^2 in root biomass estimated from minirhizotron and GPR methods. Root-to-shoot ratios averaged 3.9 ± 0.4 for elevated CO_2 plots, significantly less than the average of 5.5 ± 0.5 for ambient CO_2 plots ($P = 0.02$, Fig. 12).

Biomass Estimated From Cores

Total root biomass estimated using cores was lower than the combined total from the minirhizotron and GPR sampling methods (Table 1). The CO_2 effect on total root biomass to a depth of 100 cm estimated from cores was not significant ($P = 0.27$), but the 870 g/m^2 difference in means between treatments was comparable to that observed for

minirhizotrons and GPR (Table 1). The majority of root biomass from cores consisted of coarse roots > 2 mm diameter (74% in ambient and 77% in elevated CO_2).

TABLE 1. Root biomass (g/m^2; means ± SE) measured using three sampling methods: minirhizotron image analysis, ground-penetrating radar (GPR), and 7-cm diameter soil cores. *P*-values represent results of non-parametric or ANOVA tests for difference in biomass between ambient and elevated CO_2 plots.

	Fine roots (< 2 mm diameter)	Coarse roots (> 5 mm diameter)	**Total**
	Minirhizotron	GPR	
Ambient CO_2	1644 ± 173	5105 ± 418	**6749 ± 422**
Elevated CO_2	1942 ± 168	5830 ± 487	**7772 ± 422**
	$P = 0.31$	$P = 0.26$	$\boldsymbol{P = 0.11}$
	Fine roots (< 2 mm diameter)	Coarse roots (> 2 mm diameter)	**Total**
	7-cm diameter cores		
Ambient CO_2	1230 ± 170	3414 ± 168	**4643 ± 280**
Elevated CO_2	1258 ± 359	4255 ± 721	**5513 ± 697**
	$P = 0.94$	$P = 0.28$	$\boldsymbol{P = 0.27}$

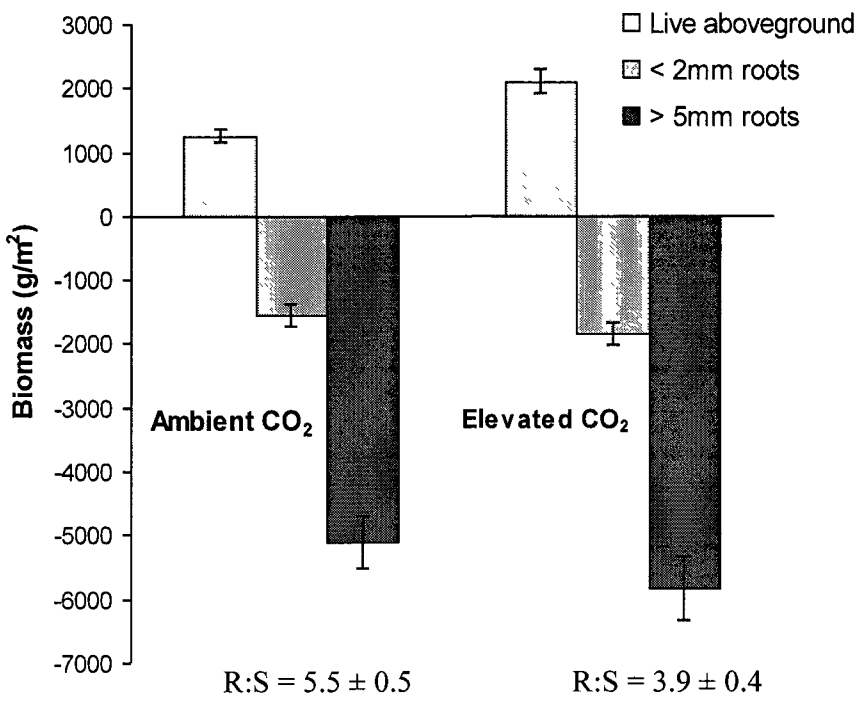

FIG. 12. Live aboveground biomass (above the horizontal line) and root biomass (below the horizontal line) in $g/m^2 \pm$ SE for the CO_2 treatments. Root-to-shoot ratios (R:S) are also listed for each treatment.

Fine Root Biomass Depth Profiles

Minirhizotron biomass estimates for roots < 2 mm diameter showed no significant difference among depths within either CO_2 treatment with the exception of the 0-10 cm depth (Fig. 13). The biomass estimates for the 0-10 cm depth were significantly lower than all other depths, and this is most likely due to the ineffectiveness of the minirhizotron method in quantifying roots near the soil surface. There was a significant CO_2 treatment effect observed in the 38-48 cm ($P = 0.04$) and 76-86 cm ($P = 0.05$) depths with greater biomass under elevated CO_2 (Fig. 13).

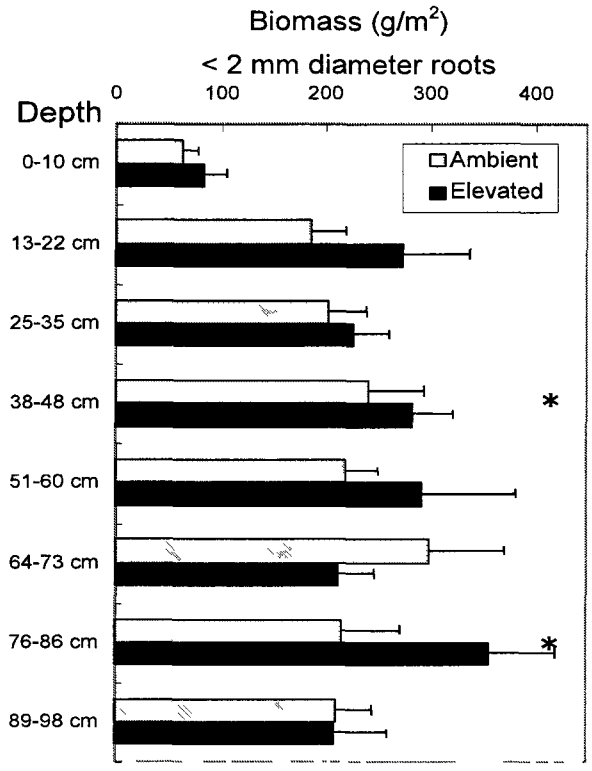

FIG. 13. Fine root biomass (mean + SE) of roots < 2 mm diameter estimated from minirhizotron observations and categorized by depth. * denotes $P < 0.05$ result in ANOVA test for difference in mass between CO_2 treatments for given depth.

Root biomass estimated from cores was not significantly different among the depth categories 0-10 cm, 10-30 cm, 30-60 cm, and 60-100 cm for either CO_2 treatment (Fig. 14). However, biomass abruptly decreased to almost zero below 100 cm in both treatments (Fig. 14). For roots > 2 mm diameter, there was a trend ($P < 0.15$) of greater biomass in ambient CO_2 plots at the 0-10 cm depth, but greater biomass in elevated CO_2 plots at 30-60 cm and 60-100 cm depths (Fig. 14). There were no treatment effects observed for roots < 2 mm diameter in the core depth data.

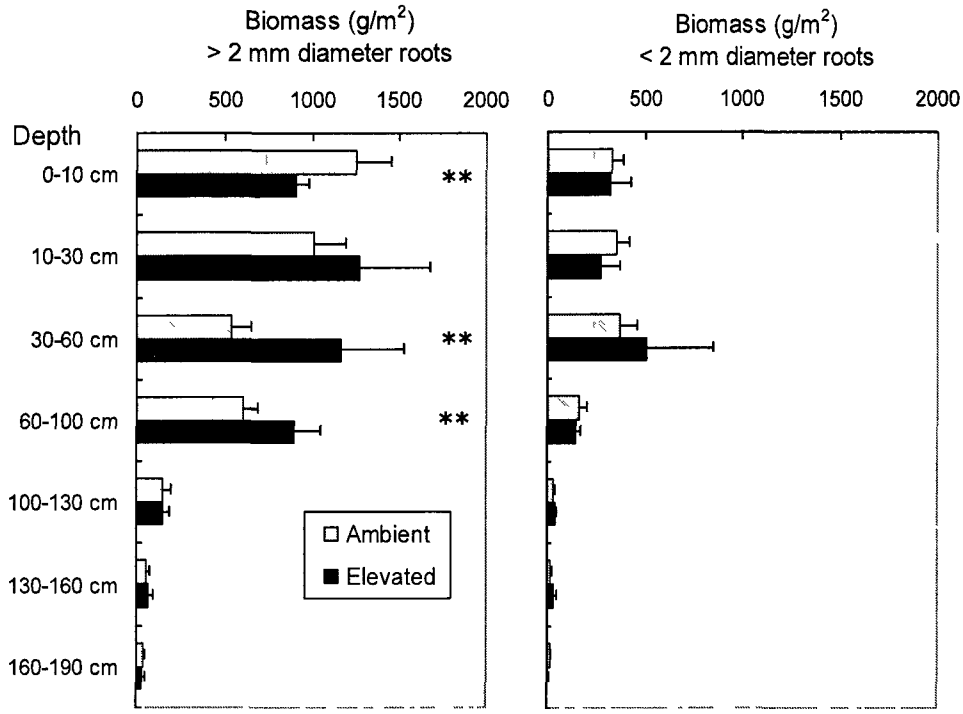

FIG. 14. Root biomass (mean + SE) for chambers under ambient and elevated CO_2 estimated from cores and categorized by depth. Cores were collected and processed by a team from Northern Arizona University led by Bruce Hungate. ** denotes $P < 0.15$ result in ANOVA test for difference in mass between CO_2 treatments for given depth.

Fine Root Patterns over the Course of the Study

Fine root biomass over the 11-year study period estimated from minirhizotron measurements showed a pattern of recovery and steady state following disturbance (Fig. 15). Fine root abundance (Day et al. 2006) and biomass increased in both CO_2 treatments during the first three years of the study as the ecosystem recovered from fire. A strong CO_2 stimulation was observed 1 to 3 years after CO_2 enrichment began with a peak in

late 1997, but by 2000, fine root biomass under elevated CO_2 had declined to ambient treatment levels and no treatment effect was seen over the next 4 years.

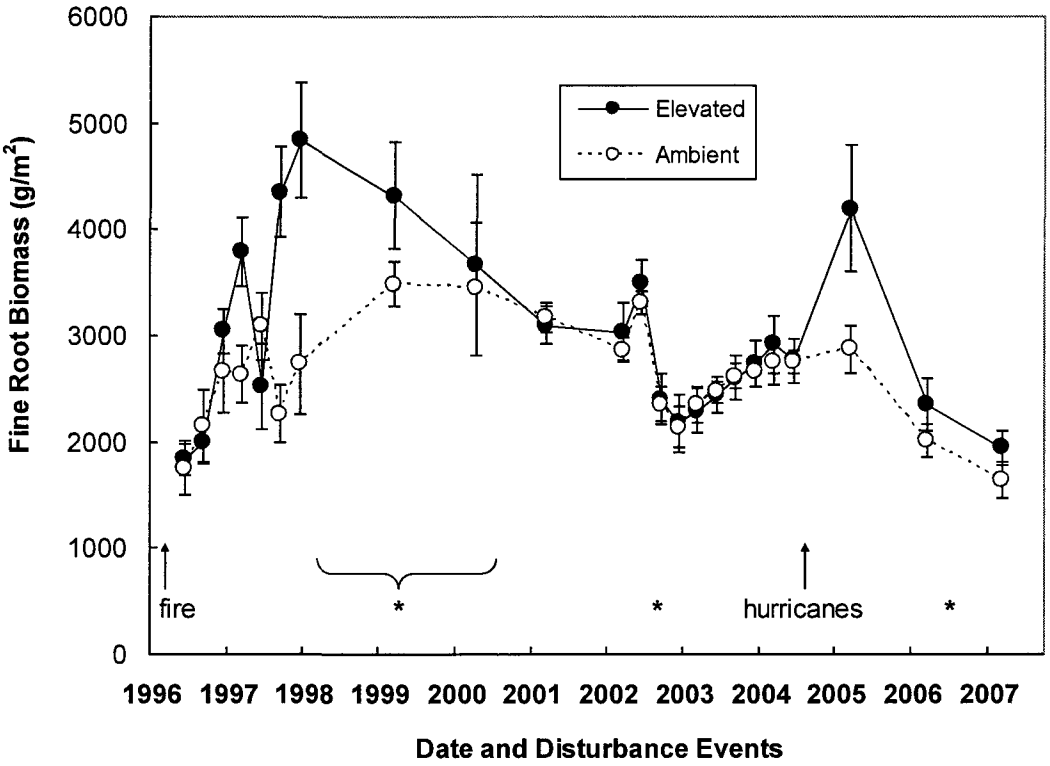

FIG. 15. Fine (< 2 mm diameter) root biomass to 100 cm depth estimated using minirhizotrons over the 11-year study period. Values are means ± SE. Results presented are revised from those previously published by Brown et al. (2009). Disturbance events are also noted; asterisks denote years with low or atypical precipitation.

After a severe drought in 1998 and low summer precipitation in 1999-2000, there was a substantial decline in fine roots in both treatments, followed by a recovery period from 2002-2004. The drought also caused a significant decrease in shoot density in 2001

followed by recovery over the next 4 years (Li et al. 2007a, Seiler et al. 2009). In fall of 2004 three hurricanes impacted the study site, the strongest of which was Hurricane Frances, which resulted in significant defoliation and a 21% reduction in leaf area index (Li et al. 2007b). In 2005, there was again a large CO_2 stimulation of fine root biomass, which coincided with increased shoot density under elevated CO_2 (Seiler et al. 2009). The response may be at least partially attributed to a pulse of water (precipitation) and nutrients (from defoliation/litterfall) as a result of the hurricanes (Seiler et al. 2009, Li et al. 2007b). One year later the CO_2 treatment effect was no longer evident, and fine root biomass in both treatments declined until the end of the study in 2007.

Discussion

Fine Root Biomass

Although the absolute value for fine root biomass was greater for elevated CO_2 plots at the end of the study in 2007, there was no significant CO_2 treatment effect. This was not entirely unexpected because strong treatment effects were only seen at the beginning of the study and again in 2005 (Fig. 15). The minirhizotron method was not suitable for sampling roots greater than 2 mm in diameter, and the vast majority of roots sampled were less than 0.25 mm diameter.

Coarse Root Biomass

While fine root biomass was monitored throughout the study, coarse root biomass was only measured after 2005. Limits on destructive sampling at long-term study sites may affect observed CO_2 stimulation of root biomass; for example, the doubling of

coarse roots under elevated CO_2 presented 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_2 plots).

The validation pit dug near the study plots yielded over 8 kg/m^2 of root biomass to a depth of 60 cm. This is considerably higher than the upper range of 5 kg/m^2 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, e.g. the scrub-oak of the garrigue in southern France in which large belowground structures (> 5 mm diameter) constituted 85% of the 7200 g/m^2 total root biomass (Kummerow et al. 1990).

Robinson (2007) suggested that most global carbon models substantially underestimate the size of the root component. Data presented here and collected from the site previously indicate that coring techniques, especially those using small diameter corers such as the 7-cm one used in this study, likely under-sample coarse roots. In a study comparing actual (using whole-tree harvest extraction) and estimated (using 5-cm diameter soil cores) lateral-root density measurements, soil cores consistently underestimated root density, at times by half the actual value (Retzlaff et al. 2001).

GPR-based estimates of coarse root biomass were considerably greater than those obtained from coring, but comparable to coarse root biomass directly sampled in the 1 x 2 m validation pit. Thus, the GPR data accurately reflect destructive sampling on larger spatial scales (2 m^2) and suggest that the smaller areal coverage of cores (~ 0.02 m^2) underestimated coarse root biomass. Additionally, low frequency encounter of large roots

during coring biases the data toward values of "zero" that may lead to underestimation of coarse root biomass (B. Hungate, unpublished data).

Aboveground vs. Belowground Responses

Elevated CO_2 stimulated production of aboveground biomass throughout the duration of the study; 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 years of enrichment (Seiler et al. 2009). The absolute difference between elevated and ambient CO_2 plots in belowground biomass was 1023 g/m^2, comparable to the absolute difference of 846 g/m^2 in live aboveground biomass, although the treatment effect for belowground biomass was only marginally significant.

At the end of this study, average root-to-shoot ratio was higher in plots exposed to ambient CO_2. This does not support the hypothesis that elevated CO_2 would result in increased root-to-shoot ratio in order to enhance acquisition of belowground resources to support increased growth. The contrasting responses of the dominant oaks to CO_2 enrichment may have affected the patterns of biomass allocation above- and belowground. Because roots were not quantified by species, the direct contribution of each species to root biomass cannot be determined, but it is possible that differences in biomass partitioning could have affected total root biomass at the end of the study.

Biomass partitioning under CO_2 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_2, there are many studies where that is not the case. Stulen and den Hertog (1993) thought that 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 and Taub (2010) found that abiotic stresses (i.e. drought or exposure to ozone) had a greater effect on the fraction of root to total biomass than did exposure to elevated CO_2.

Soil Resources

It does not appear that low water availability limited plant growth over the long-term as the dominant oak species under elevated CO_2, *Q. myrtifolia*, had reduced transpiration (Li et al. 2003), and both dominant oaks were found to take up most of their water from the water table (Hungate et al. 2002). Although progressive nitrogen limitation was initially thought to affect plant response to CO_2 in this system (Hungate et al. 2006), analysis of soil cores at the end of the study showed increased nitrogen mineralization in the 10-30 cm depth under elevated CO_2, which may have increased nitrogen availability (Langley et al. 2009). A considerable amount of nitrogen in deep soils at the water table was also thought to provide a source of nitrogen for plant uptake in this system (McKinley et al. 2009).

Soil nutrient measurements after five years of CO_2 enrichment indicated that nutrients such as potassium, calcium and magnesium may have also been taken up by plants from groundwater or deep soil horizons (Johnson et al. 2003). Because water and nutrient availability did not appear to be long-term controlling factors in plant growth and biomass storage above- and belowground, alternative mechanisms must have been

responsible for the observed higher root-to-shoot ratio under ambient CO_2.

Fine Roots with Depth

A change in the vertical distribution of fine roots was observed for plots under elevated CO_2. A review of root biomass studies showed an average of 67% of root biomass in the upper 30 cm of soil for sclerophyllous shrublands (Jackson et al. 1996). Minirhizotron data showed an increase in biomass of roots < 2 mm diameter down to 90 cm under elevated CO_2 (Fig. 13). A pilot study conducted in 1992-1993 at this site showed an increase in fine root abundance at 50-60 cm depth under elevated CO_2 (Day et al. 1996). Other long-term CO_2 enrichment studies have also reported deeper rooting distributions under elevated CO_2 (Iversen 2010, Norby et al. 2004, Pritchard et al. 2008).

Possible causes for increased root abundance and proportion of biomass deeper in the soil under elevated CO_2 may include: increased demand for nutrients and water as plant production is stimulated that leads to mining for deeper soil resources (although this does not appear to be the case in the Florida scrub-oak system), increased carbon allocation belowground to support new root growth, and increased competition for resources in shallower soil depths (Iversen 2010). Occupation of soil space is of primary importance in belowground competition (Casper and Jackson 1997).

In the Florida study, the estimate of fine root biomass in the 0-10 cm depth using minirhizotrons is likely an underestimate. Coring and pit excavations at the site yielded abundant fine roots (often including the presence of a "root mat", a dense layer of fine roots immediately below the soil surface) at this depth compared to lower depths. After long-term deployment in the field, minirhizotron frames located in the upper 10 cm of

soil can become occluded with organic matter, making root identification more difficult for these frames and leading to lower biomass estimates. The discrepancy between fine root data quantified with minirhizotrons in the top portions of the soil profile compared to other sampling methods was documented in other studies (e.g. Franco and Abrisqueta 1997, Samson and Sinclair 1994, Wiesler and Horst 1994, Ephrath et al. 1999).

Patterns of Root Response over Time

Stimulation of root biomass as a result of CO_2 enrichment may decrease over time, and this phenomenon could be due to a number of factors including acclimation of photosynthesis to elevated CO_2, increased resource use efficiency, or limits on soil resource space. A decrease in fine root response after long-term CO_2 enrichment has been observed in other studies as well. In the study by Bader et al. (2009), the loss of a treatment effect after 7 years of enrichment was attributed to increased soil moisture (through reduced transpiration) under elevated CO_2 that may have caused the trees to reduce biomass allocation to fine roots. The lack of 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 CO_2 are strongly controlled by ecosystem successional state and plant demography was explored by Körner (2006).

In contrast, some long-term CO_2 enrichment studies have shown sustained root biomass stimulation under elevated CO_2 over more than a decade of CO_2 enrichment, e.g. Jackson et al. (2009). Fine root peak standing crop was approximately doubled across all years in a 9-year FACE study in a sweetgum plantation (Iversen et al. 2008, Norby et al.

2004). Averaged over six years of FACE treatment in a loblolly pine plantation, root standing crop was increased by 23% (Pritchard et al. 2008).

Also, fine roots are temporally dynamic; root biomass, production and mortality vary seasonally (McClaugherty et al. 1982, Hendrick and Pregitzer 1992) and inter-annually (Lopez et al. 2001, Espeleta and Clark 2007). In CO_2 enrichment studies, the CO_2 treatment effect on fine root biomass can vary 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_2 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 a CO_2 effect was not apparent and when biomass was low. Conclusions based on fine root "lows" may differ from those based on the "highs" as the CO_2 effects were strongest during periods of recovery. The difference between root estimates measured using GPR from 2005 and 2007 indicates a decrease in coarse root biomass over that time period; it is possible that this also included a decrease in fine roots that may be detected by GPR. Fine roots observed using minirhizotrons decreased substantially during this period.

The largest CO_2 effects on fine root biomass occurred at the beginning of the Florida study during recovery from fire, and again in 2005 following a drought-recovery period and a major hurricane. Day et al. (2006) proposed that fine roots reached dynamic equilibrium in the study system three years after the experiment began, and that this root closure was reached shortly before canopy closure. Disturbances such as fire or drought

appear to reduce fine roots to levels below their carrying capacity. During the recovery phase, fine roots are capable of responding to CO_2 fertilization. One important implication of this finding is that elevated CO_2 may result in greater carbon inputs to soils following disturbance. After root closure, limited resource space results in equilibration and loss of the fertilization effect.

CHAPTER 3

EFFECTS OF DISTURBANCE AND CO_2 TREATMENT LEGACY ON FINE ROOTS

Introduction

Severe ecosystem disturbances such as fires, hurricanes, herbivory, disease outbreaks or land clearing may result in damage to or removal of aboveground vegetation. Following severe injury, woody plants may either be killed or may re-sprout from vegetative tissue, and plants that can re-sprout after disturbance are favored where disturbance is frequent (Bond and Midgley 2003). Plants that re-sprout following disturbance mobilize belowground nutrient stores to support fine roots during initial re-establishment instead of allocating "new" photosynthate to root growth (Fig. 16, Langley et al. 2002). This allows plants to regenerate quickly and not be constrained by low or no photosynthesis immediately after the disturbance. In ecosystems subjected to periodic disturbances, plants store resources in areas of the plant least likely to be damaged. Belowground organs such as lignotubers, burls and rhizomes are woody structures that contain dormant buds, carbohydrates and nutrients for the primary function of sprouting (James 1984, Canadell and Lopez-Soria 1998).

While large belowground storage structures are capable of surviving disturbance, fine roots may be negatively impacted, depending on the severity of damage to the plant. For example, experimental gap creation from removal of aboveground vegetation in a wet subtropical forest resulted in a 40% decline in live fine roots two months after disturbance (Silver and Vogt 1993). In the same study, a major hurricane affecting the study site resulted in a significant and sustained decrease in live fine roots in both

experimental gap plots and control plots (Silver and Vogt 1993). Gap creation in a temperate hardwood forest caused a reduction in fine root biomass in the top 15 cm of soil late in the growing season following canopy gap formation (Wilczyinski and Pickett 1993). Fine root recovery after disturbance differs among ecosystems. In a wet tropical forest, fine root systems had almost complete recovery one year after forest felling (Raich 1980). Fine root biomass in a northern hardwood forest with experimental clearcutting recovered to almost 71% of pre-disturbance levels after 4 years of regrowth (Fahey and Hughes 1994).

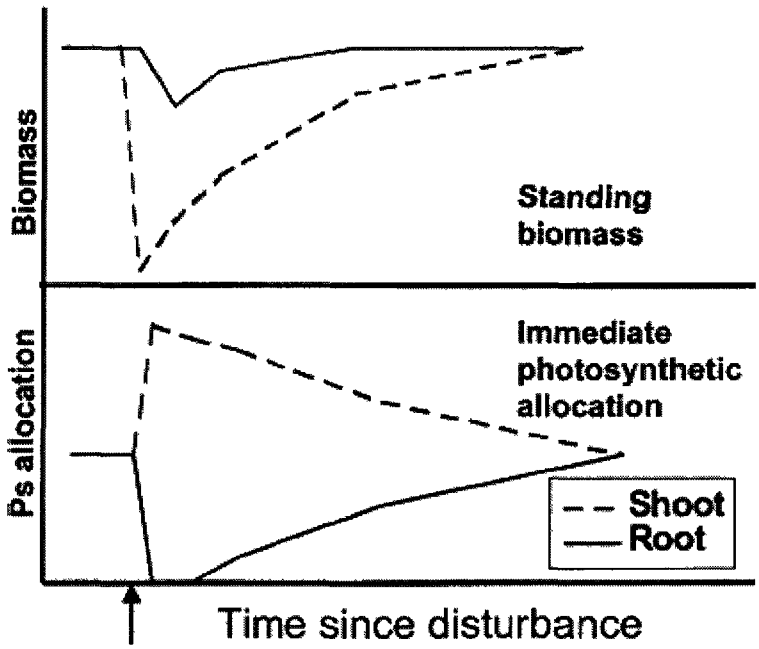

FIG. 16. Hypothesized effects of aboveground disturbance (arrow) on shoot and root biomass and photosynthate allocation. Even though allocation of photosynthate to roots is low after disturbance, root biomass remains greater than shoot biomass. Graph is from Langley et al. (2002).

The type of disturbance impacting an ecosystem affects its eventual recovery (Turner et al. 1997). Fires, depending on their severity, may result in partial to complete combustion of aboveground vegetation, roots, and soil organic matter. Fires release nutrients back into the system and are essential for germination of some fire-adapted plant species. Similarly, hurricanes are also short-term disturbances that vary in the amount of damage to vegetation, ranging from defoliation to complete uprooting of plants. Litterfall from hurricane defoliation may also result in a pulse of nutrients to the system as well as short-term water inputs from heavy rainfall (Seiler et al. 2009). In contrast to the natural disturbance regimes of fires and hurricanes, complete vegetation removal by harvest does not result in a pulse of nutrients or changes in water availability.

Disturbance at the Study Site

Florida scrub-oak communities are controlled by intense, stand-replacing wildfires with a natural fire return interval between 5 and 7 years (Adrian 2006). The dominant oak species (*Quercus myrtifolia* and *Q. geminata*) are fire-adapted and regenerate from sprouting and clonal spreading rather than seeds (Menges and Kohfeldt 1995). Vegetation at the Merritt Island study site recovered following a burn in 1986 with little change in species composition or species richness post-fire (Schmalzer and Hinkle 1992a). After the study site was burned in 1996 and CO_2 enrichment treatments began, fine root biomass and root length density (RLD) increased in both treatments for 3 years post-fire (Fig. 15, Day et al. 2006). During this initial recovery phase after fire and complete aboveground vegetation removal, fine root biomass and RLD were significantly greater under elevated CO_2 (Fig. 15, Day et al. 2006, Brown et al. 2009).

Objectives

When aboveground vegetation was harvested in July 2007, it was hypothesized that fine root biomass and abundance (RLD) would initially decline and then recover in the former experimental plots regardless of CO_2 treatment. Additionally, at the time of the harvest there was a trend of greater total root biomass under elevated CO_2. After aboveground disturbance, plants with greater belowground carbohydrate and nutrient stores should be able to recover faster than those with less root biomass. Using carbon isotope labeling from CO_2 addition at the study site, it was determined that in the first 3 years post-fire, oak roots received < 20% of their carbon from recent photosynthesis and that an estimated half of their carbon was residual from "old" roots (Langley et al. 2002). After harvest, it was also hypothesized that a CO_2 treatment "legacy" effect would be apparent with greater fine root growth under plots that had been exposed to elevated CO_2 for 11 years, and that plots previously treated with elevated CO_2 would continue to have a greater proportion of roots deeper in the soil.

Methods

Root Measurements

Images from minirhizotrons installed in the former chamber plots were collected in August 2007 (~ 1 month after aboveground vegetation removal) and May 2008 (~ 10 months after removal) using the methods described in Chapter 1. Digital jpeg images were captured from the video recordings. Fine root abundance (RLD, mm/cm^2) was calculated as the total root length per area of an individual frame, averaged over all frames in each minirhizotron tube. Fine root biomass (g/m^2 to a meter depth) was

calculated from root length and width values for all roots < 2 mm diameter following the methods detailed by Brown et al. (2009) and described in Chapter 2. GPR measurements of coarse roots were attempted in May 2008, but due to technical problems, the GPR data were unusable.

Statistical Analyses

CO_2 treatments were replicated using 16 plots ($n = 8$ for each former CO_2 treatment). Model residuals were tested for normality (using Shapiro-Wilk test) and homogeneity of variances (using Levene statistic) with PASW Statistics 17.0 (SPSS, Inc., Chicago, IL, USA). The data were log-transformed to meet the assumptions for ANOVA statistical tests. Fine root biomass and RLD were tested with a 4-factor repeated measures ANOVA using SAS Proc GLM (SAS version 9.1, SAS Institute Inc., Cary, NC, USA), with plot as the random effect and CO_2 treatment, depth, and date as fixed effects. A 3-factor nested ANOVA was run on each individual date to test for CO_2 treatment effects; plot was the random effect and treatment and depth were fixed effects. An ANOVA was also performed for each depth interval separately for each sampling date to test for CO_2 treatment differences. All results were considered significant at $\alpha < 0.05$, but trends were recognized at $0.05 < \alpha < 0.15$ (following Runion et al. 2006).

Results

Fine Root Abundance

Fine root abundance (RLD) in ambient CO_2 plots was 13.43, 13.31, and 14.90 mm/cm^2 for March 2007, August 2007, and May 2008, respectively (Table 2). In elevated

CO_2 plots, RLD was 14.05, 13.42, and 15.40 mm/cm^2 for the same time series (Table 2). Within each individual sampling date, no statistically significant CO_2 treatment effect was detected.

TABLE 2. Fine root abundance (RLD, mm/cm^2; means ± SE) for sampling dates before and after complete aboveground vegetation removal. *P*-values represent results of ANOVA test for difference in RLD between ambient and elevated CO_2 plots.

RLD	March 2007	August 2007	May 2008
Ambient CO_2	13.43 ± 1.08	13.31 ± 0.92	14.90 ± 0.97
Elevated CO_2	14.05 ± 1.13	13.42 ± 1.11	15.40 ± 1.39
	$P = 0.83$	$P = 0.83$	$P = 0.98$

When the data from all 3 dates were pooled for analysis there was no significant CO_2 treatment effect observed ($P = 0.84$), but RLD values were consistently higher in plots previously under elevated CO_2 for all 3 sample dates. There was no interaction between treatment and date ($P = 0.47$), but there was a significant difference among the 3 sampling dates ($P < 0.0001$). RLD was not significantly different 1 month after aboveground harvest compared to 4 months prior to the harvest (Fig. 17). However, changes in fine root abundance during the 5-month sampling interval may have obscured immediate responses to vegetation removal. One year after harvest, RLD was higher in

plots formerly exposed to both ambient and elevated CO_2, indicating similar fine root growth regardless of previous CO_2 treatment (Fig. 17).

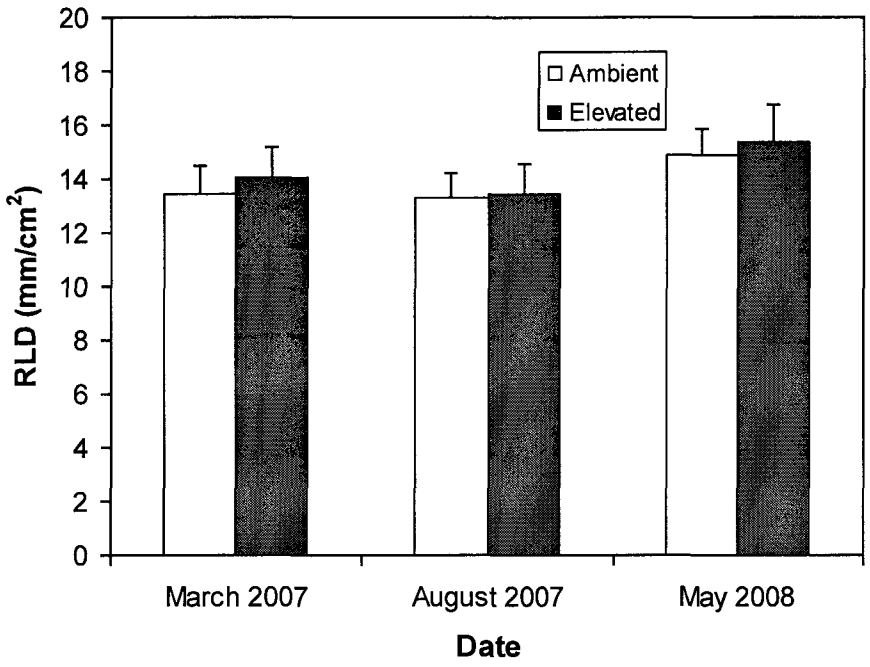

FIG. 17. Fine root abundance (RLD, mm/cm^2) estimated using minirhizotrons for sampling dates before and after complete aboveground vegetation removal. Values are means + SE.

Fine Root Biomass

Biomass of fine roots in ambient CO_2 plots was 1644, 1620, and 1687 g/m^2 for March 2007, August 2007, and May 2008, respectively (Table 3). In former elevated CO_2 plots, fine root biomass was 1942, 1852, and 2078 g/m^2 for the same time series (Table 3). As with RLD, no statistically significant CO_2 treatment effect was detected within

individual sampling dates, although there was a tendency toward higher biomass under elevated CO_2 for all three dates (Fig. 18).

TABLE 3. Fine root biomass (g/m^2; means ± SE) for sampling dates before and after complete aboveground vegetation removal. *P*-values represent results of ANOVA test for difference in biomass between ambient and elevated CO_2 plots.

BIOMASS	**March 2007**	**August 2007**	**May 2008**
Ambient CO_2	1644 ± 173	1620 ± 133	1687 ± 133
Elevated CO_2	1942 ± 168	1852 ± 163	2078 ± 205
	$P = 0.31$	$P = 0.57$	$P = 0.39$

Pooling the fine root biomass data from all 3 dates, no significant CO_2 treatment effect was observed ($P = 0.56$) and there was no significant interaction between treatment and date ($P = 0.59$). There was a significant difference among dates ($P < 0.0001$), although this was not apparent in plots formerly exposed to ambient CO_2. In elevated CO_2 plots, however, biomass appeared to decrease slightly 1 month after aboveground harvest and then increase significantly 10 months later (Fig. 18).

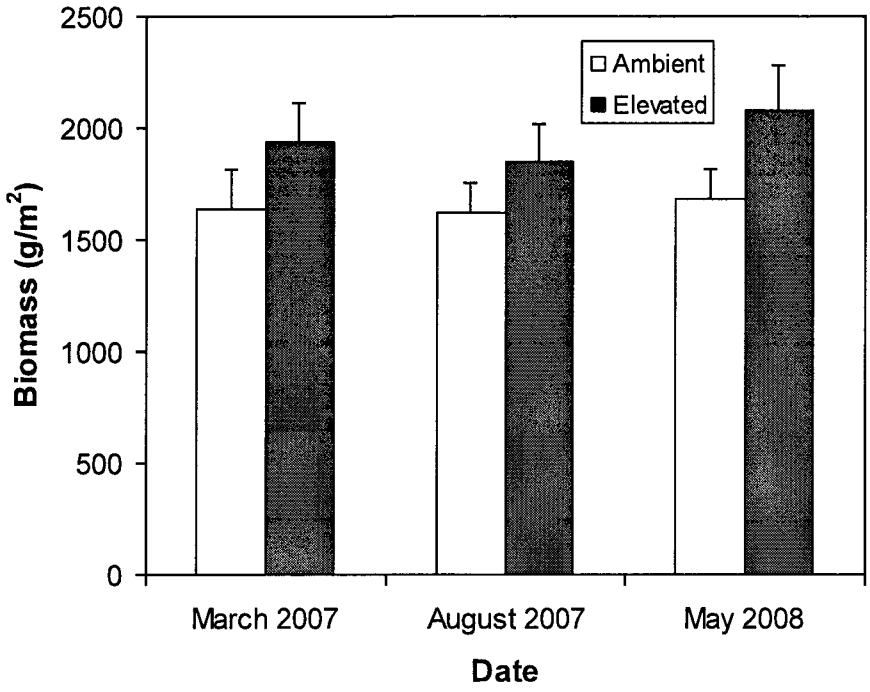

FIG. 18. Fine root biomass (g/m^2) to 100 cm depth estimated using minirhizotrons for sampling dates before and after complete aboveground vegetation removal. Values are means + SE.

Fine Root Parameters With Depth

Fine root abundance (RLD) was significantly greater under elevated CO_2 in the 38-48 cm depth in March 2007 ($P = 0.05$) and August 2007 ($P = 0.02$), but not in May 2008 ($P = 0.19$) (Fig. 19). Fine root biomass was greater under elevated CO_2 in the 38-48 cm depth for all three sampling dates (March 2007, $P = 0.04$; August 2007, $P = 0.02$; May 2008, $P = 0.08$) (Fig. 20). It was also higher under elevated CO_2 in the 76-86 cm depth in March 2007 ($P = 0.05$). There were no treatment differences at any other depth in either biomass or RLD.

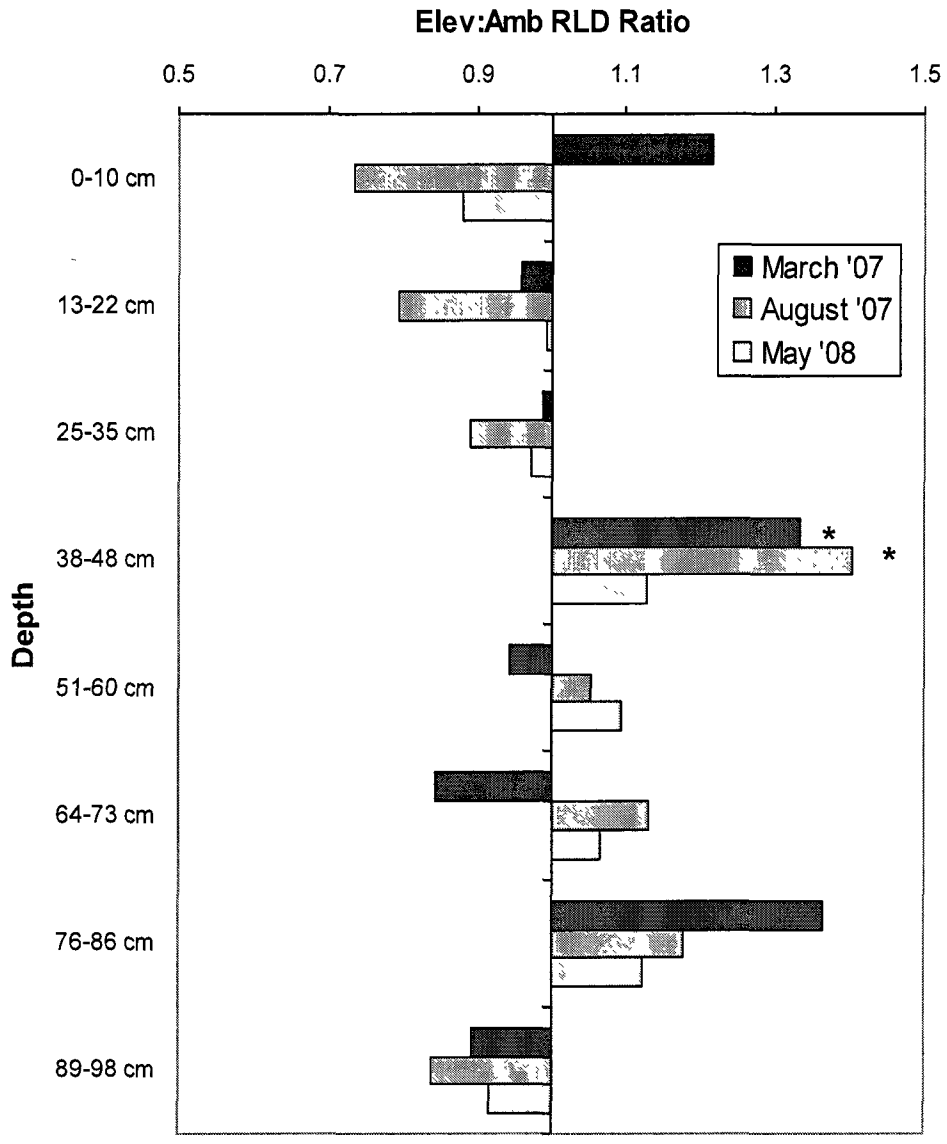

FIG. 19. Ratio of fine root abundance (RLD, mm/cm^2) in elevated vs. ambient CO_2 plots at eight depth intervals measured using minirhizotrons. * represents P-value < 0.05, indicating greater fine root abundance at that depth under elevated CO_2.

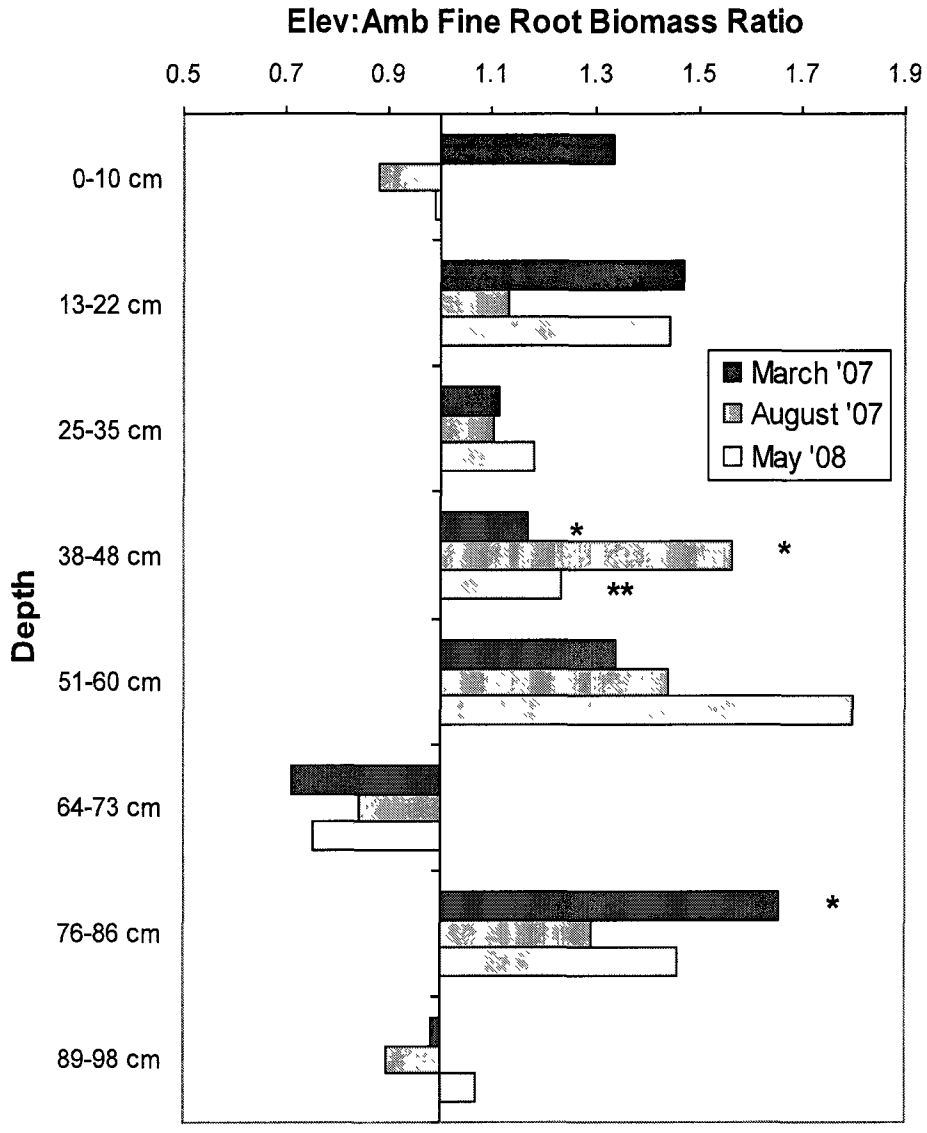

FIG. 20. Ratio of fine root biomass (g/m^2) in elevated vs. ambient CO_2 plots at eight depth intervals measured using minirhizotrons. * represents P-value < 0.05 and ** represents P-value < 0.15, indicating greater fine root biomass at that depth under elevated CO_2.

Discussion

It was initially hypothesized that disturbances such as fire, hurricanes and aboveground vegetation removal would all result in initial decreases in fine root biomass and abundance followed by recovery of these parameters. At the beginning of the study in 1996, fine root biomass and RLD were low, indicating that fire and the resulting shoot removal had reduced fine roots substantially prior to the start of the experiment. Fine roots increased dramatically over the first 3 years post-fire and there was a significant CO_2 stimulation of root growth during that time. These observations indicated that fine roots recovered after fire until root closure was reached, and it is possible that nutrients made available from the fire supported this recovery.

A significant CO_2 treatment effect on fine root biomass was observed only at one other point during the study, which was after the hurricane that occurred in September 2004. This coincided with a spike in aboveground shoot density (Seiler et al. 2009) that was attributed to nutrient input from litterfall during the hurricane. However, there was no CO_2 stimulation of RLD at any point after the first 3 years of the experiment; patterns in RLD after 1999 were similar in ambient and elevated CO_2 plots (Day et al. 2006).

It is puzzling that neither the hurricane (resulting in severe defoliation) nor the harvest at the end of the study resulted in a significant decrease in fine root biomass or RLD. It is possible that there was a decrease in fine roots immediately after disturbance followed by a quick recovery that was masked by the 5-month interval in sampling. It is also possible that fine root abundance and biomass were higher immediately before harvest than they were in March, and they subsequently decreased back to the March levels following the harvest.

Ten months after complete vegetation removal, fine root abundance was higher than it had been pre-disturbance in both CO_2 treatments indicating no significant effect of previous CO_2 treatment ("legacy") on RLD. In 2005 after the hurricanes, there was also no CO_2 treatment effect on RLD (Day et al. 2006). In contrast, plants in former elevated CO_2 plots had greater fine root biomass one year after vegetation removal. The increase in fine root biomass in former elevated CO_2 plots after harvest was not nearly as dramatic as the spike in biomass following the hurricanes or 2 years post-fire (Fig. 15). This suggested that CO_2 stimulation of fine roots after fire and hurricanes was bolstered by nutrient inputs as a result of those events and the lack of a nutrient pulse after harvest prevented a similar response. In this way, complete aboveground harvest did not accurately represent a natural disturbance, which could explain why fine roots did not respond as expected.

The eventual increase in fine root biomass after fire, hurricanes, and harvest was probably also due to biomass not being at maximum levels at the time the disturbances occurred. Day et al. (2006) attributed the loss of the CO_2 stimulation of root growth early in the experiment to root closure, which can be thought of as the carrying capacity of the soil for fine roots. As discussed in Chapter 2, root growth can only be stimulated until soil capacity is reached. If root biomass was already at capacity at the time of disturbance, it is likely that the observed increases would not have been possible.

With regard to fine root depth distribution, the only statistically significant CO_2 treatment effect on fine roots for the 3 sampling dates presented in this chapter was greater biomass and RLD in elevated CO_2 plots at the 38-48 cm depth, which seemed to diminish by May 2008. The significantly greater biomass and abundance of fine roots

under elevated CO_2 at that depth may have been due to increased competition at shallower depths that caused roots to grow deeper in the soil (Iversen 2010). There was a tendency toward greater fine root biomass in elevated CO_2 plots at almost all depths for the 3 sampling dates (Fig. 20), but there was no clear trend indicating a change in depth distribution following aboveground harvest.

CHAPTER 4

COARSE ROOT SPATIAL DISTRIBUTION IMAGED WITH GROUND-PENETRATING RADAR

Introduction

In studies that involve plant root measurements, parameters of interest may include root mass, abundance, surface area, length, branching pattern, and vertical and horizontal distribution. Root spatial distribution is one factor that affects plant acquisition of soil resources such as water and nutrients as well as the ability to compete with other plants for those resources (Casper and Jackson 1997). The distribution of large belowground structures such as coarse roots, rhizomes, and lignotubers may be controlled by space availability as well as soil type, moisture, and depth to water table. Traditionally, root distribution was studied by excavation methods that were destructive and not suitable for long-term projects where minimal disturbance to the study system was necessary. Ground-penetrating radar (GPR) is a nondestructive method for assessing root spatial distribution in the field.

Ground-penetrating Radar

With the GPR method, high-frequency electromagnetic energy is propagated from a transmitting radar antenna into the ground. When the transmitted wave intercepts material with a different electrical permittivity, part of the signal is transformed at the boundary. The transformation (scattering) and reflection of electromagnetic waves detected by a receiving radar antenna is used to locate buried objects and can be used to create a 3-dimensional pseudo-image of the subsurface (Fig. 21, Daniels et al. 2008).

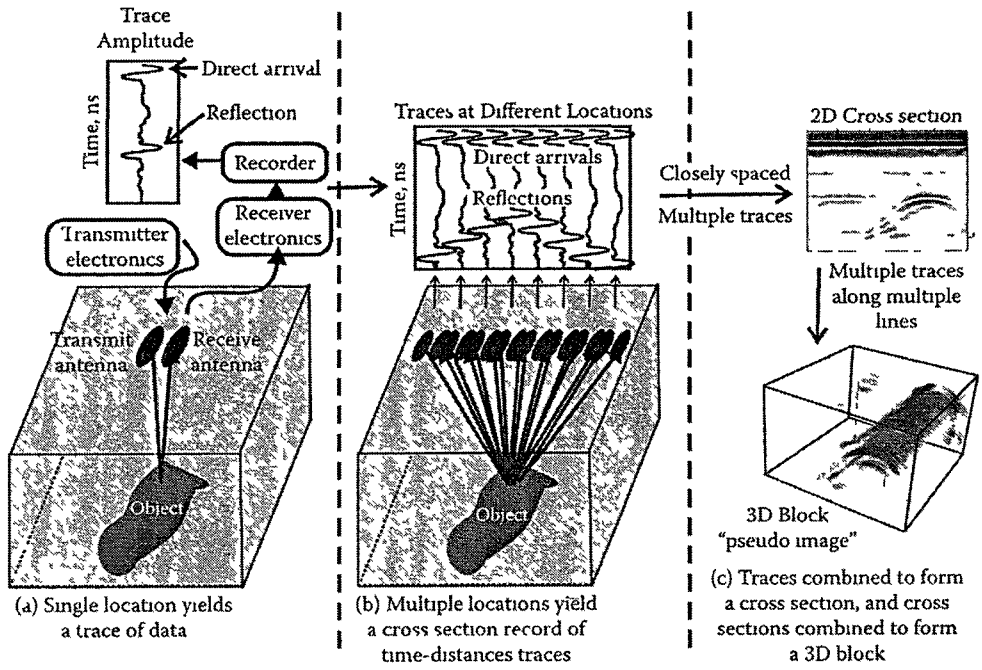

FIG. 21. Process for using ground-penetrating radar to construct a 3-dimensional pseudo-image of a buried object. From Daniels et al. (2008).

GPR has been used to study plant roots for over 10 years. Controlled experiments to explore the capabilities of the GPR method have shown that a twig as small as 0.25 cm diameter buried in sand can be detected with a 1500 MHz antenna (Wielopolski et al. 2000). Tree roots 1-10 cm in diameter buried in a pit filled with damp sand were detected with radar antennas of various frequencies down to 155 cm depth (Barton and Montagu 2004). Buried roots 1.1-5.2 cm in diameter were clearly detected in sandy granite soil with a 900 MHz antenna (Dannoura et al. 2008). Hirano et al. (2009) determined that root diameter, root water content, and the interval between roots were all important factors in GPR detection of roots under optimal sandy soil conditions.

Soils with high water, clay, or gravel content can be problematic for root detection (Zenone et al. 2008, Butnor et al. 2001). However, GPR has been used successfully in the field to study roots of pines and oaks in Florida (Butnor et al. 2008), pines in Georgia (Samuelson et al. 2008), peach trees in Georgia (Cox et al. 2005), oaks in Sobesice, Czech Republic (Hruska et al. 1999), and maples in Brno, Czech Republic (Cermak et al. 2000). GPR was used to recreate top-down and cross-sectional views of coarse tree roots growing *in situ* by Cermak et al. (2000), Hruska et al. (1999), and Stokes et al. (2002), although the exact method for drawing root systems from the GPR data was unclear. Previous work at the Merritt Island, Florida study site showed that GPR could be used to create top-down images in close agreement to the actual orientation of coarse roots from 0.25 m^2 pits (Stover 2007).

GPR was an ideal tool for imaging belowground plant structures at this site because a) all aboveground vegetation was removed from the study plots in order to measure standing biomass at the end of the 11-year CO_2 enrichment study, allowing for intensive scanning of the study plots; b) it was necessary to leave the belowground biomass intact so that the plants could resprout after harvest for further research; c) the well-drained, sandy soils were ideal for use with GPR; and d) the size of the experimental plots was large enough to sample a wide distribution of roots but not so large as to make intensive GPR scanning impossible.

Objectives

A 4-m^2 "validation" plot was used to explore the effectiveness of GPR in describing coarse root spatial distribution on a larger scale. Several approaches to

processing the GPR data were examined to determine the best processing protocol for imaging coarse roots. Additionally, GPR data collected from the CO_2 treatment plots were used to create top-down GPR images of coarse roots; these images were then used to test for differences in the spatial extent and distribution of roots after 11 years of ambient or elevated CO_2 treatment.

Methods

Data Collection

To determine the effectiveness of using GPR to image coarse root spatial distribution, a 2 x 2 m plot separate from the experimental plots was cleared of aboveground vegetation and scanned with the 1500 MHz GPR antenna using 100 scans in the x- and y-directions using methods described in Chapter 1. All plant litter, fine roots, organic matter and soil were removed from the plot while leaving the coarse root system intact. Roots estimated to be less than 5 mm in diameter were clipped and removed. Soil was loosened with hand spades and removed with a Shop-vac down to a depth of 30 cm. Photographs were taken of the remaining roots. The photographs were compared to processed GPR images of the validation plot to determine whether GPR could be used to approximate the spatial distribution of coarse roots. Additionally, GPR data collected in the 16 experimental plots at the end of the study in 2007 were used to examine the spatial distribution of roots growing in the treatment chambers; 8 plots were scanned with 13 scans in the x- and y-directions (5 ambient and 3 elevated CO_2 plots) and 8 were scanned with 100 scans in each direction (3 ambient and 5 elevated CO_2 plots).

Image Processing

For each plot, a 3-d GPR file was created in Radan version 6.5 (Geophysical Survey Systems, Inc., North Salem, NH, USA). Individual GPR scans can be considered 2- dimensional "cross-sections" of data collected along a transect with the z-dimension of depth. Multiple cross-sections in perpendicular directions (x- and y- scans) can then be combined to create a 3-dimensional image from the GPR data (Fig. 21). Once a 3-d file is created in Radan it can be enhanced with post-processing algorithms to optimize the appearance of belowground signal reflectors. For the validation plot, a series of separate processing treatments was performed on the raw 3-d file that included:

- no processing
- gain equalization
- gain equalization + position correction
- gain equalization + position correction + variable velocity migration
- background removal
- background removal + Hilbert Transform
- background removal + constant velocity migration + Hilbert Transform
- position correction + constant velocity migration + Hilbert Transform

After each processing protocol was performed, the data were viewed using the 3-d mode in Radan from a top-down perspective. Each image was then converted to a bitmap and all areas of the image except the red high-reflectance areas were made transparent using Microsoft Photo Editor. The bitmaps were then 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 were counted for each image.

For comparison, roots were digitized from the validation plot photograph using Microsoft Paint by converting the jpeg image into a bitmap and manually tracing all coarse roots using the paintbrush function. The enhanced root image was then processed with SigmaScan Pro in the same manner as the GPR images to obtain pixel counts of the digitized root area. Pixel counts for each GPR processing protocol were compared to the pixel counts of digitized coarse roots from the validation plot photograph. Additionally, individual GPR images for each processing treatment were viewed as overlays on top of the enhanced root photograph to compare distribution of GPR signal reflections to actual root distribution from the validation plot. The GPR image that had closest agreement with actual root distribution and pixel count of coarse roots was chosen as the best protocol for this project.

The gain equalization + position correction protocol had the best agreement. Gain equalization is a processing step recommended for correcting datasets that have gain differences from profile to profile; it removes visual artifacts (such as striping and mosaics) resulting from differences in gain between transects. Position correction is a process in which the user identifies the actual ground surface location in order for the program to accurately calculate the velocity of radar energy in the ground. 3-d files were then created in Radan for the 16 experimental plots and processed with this protocol to obtain top-down images of coarse root spatial distribution for each plot. Pixel counts were then obtained for each plot and compared between CO_2 treatments and between scanning intensities (i.e. 26 vs. 200 scans per plot). Non-parametric tests were run using SAS Proc NPAR1WAY on these data to test for differences.

Results

Validation Plot

The coarse root distribution for the 2 x 2 m validation plot is shown in Fig. 22. A large woody lignotuber measuring ~ 30 cm in diameter was present in quadrant C, along with other substantial belowground structures such as those located in quadrant D. While coarse roots were not identified by species, palmetto roots could be identified by the reddish-brown color and flaky texture. Roots were digitized from the photograph (Fig. 23), which resulted in a pixel count of 34,443. An example of raw 2-m long GPR scans from the validation plot is shown in Fig. 24 with their scan locations indicated. Roots intercepted by the GPR signal created hyperbolic reflectors in the data, and the GPR processing software was used to identify all hyperbolas in the 3-d dataset (Fig. 25).

After post-processing the collected data with gain equalization and position correction, strong signal reflections appeared as red "blobs" in the 3-d image (Fig. 26). Strong signal reflections were seen in the GPR data in areas where large roots or lignotubers were present, suggesting that GPR detected many of the large belowground structures present in the plot. The pixel count for the GPR image was 35,830; this was in close agreement to the number of pixels in the digitized roots from the photograph.

Experimental Plots

Qualitatively, there was a pronounced difference in GPR image resolution between scanning intensities. Plots that were scanned with 100 scans in each direction had greater signal resolution while those scanned with 13 scans in each direction had lower resolution and striping (Fig. 27). The 8 experimental plots that were intensively

scanned averaged 35,691 pixels per GPR image while those with fewer scans averaged 32,606 pixels per image ($P = 0.12$). Within each scanning intensity, the number of pixels in ambient vs. elevated CO_2 plots was not significantly different (intensively scanned plots, $P = 0.95$; partially scanned plots, $P = 0.91$) (Table 4).

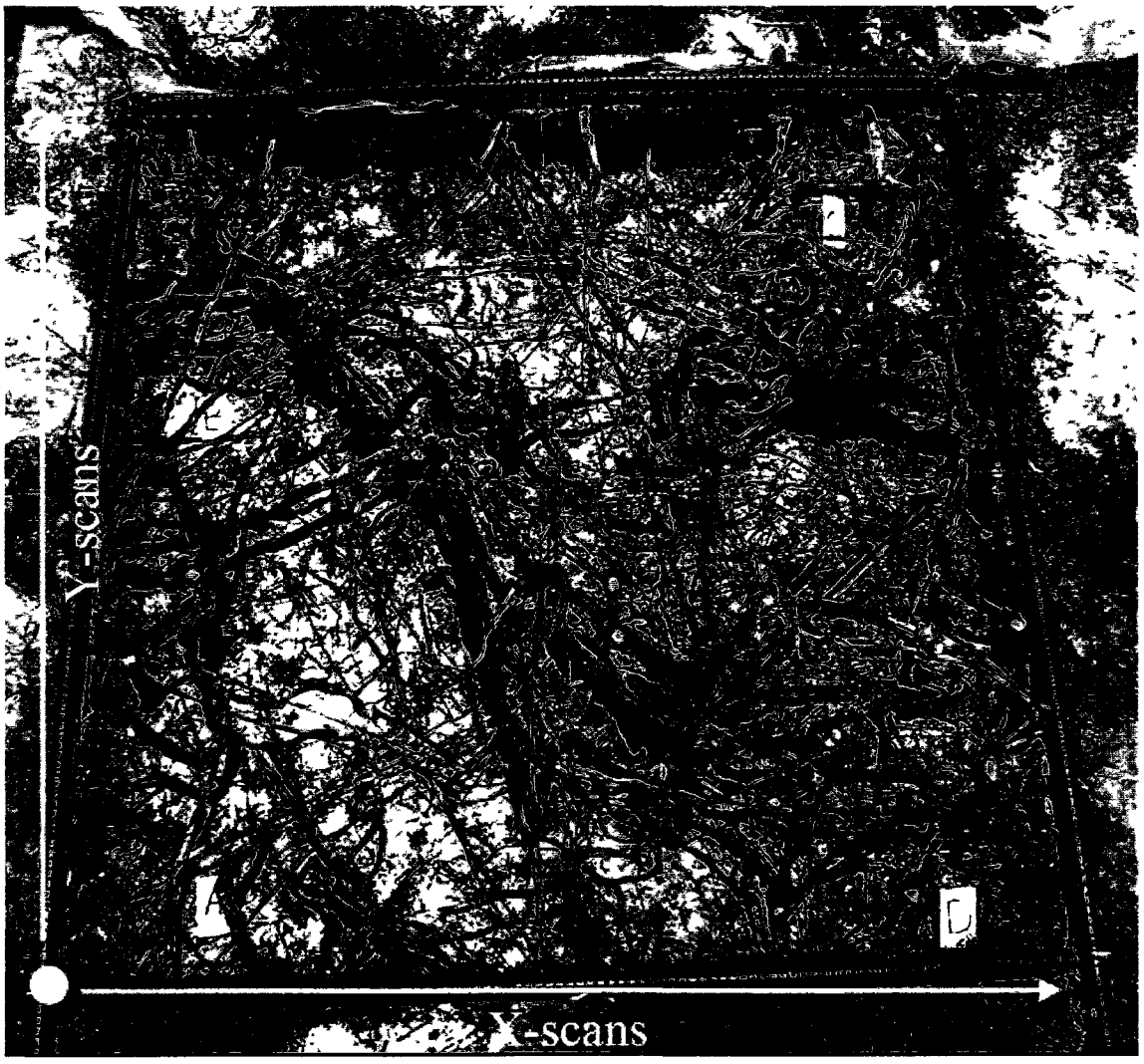

FIG. 22. GPR validation plot after scanning showing the intact coarse root system. Fine roots, organic matter and soil were removed to a depth of 30 cm, leaving large belowground structures intact for comparison with GPR images.

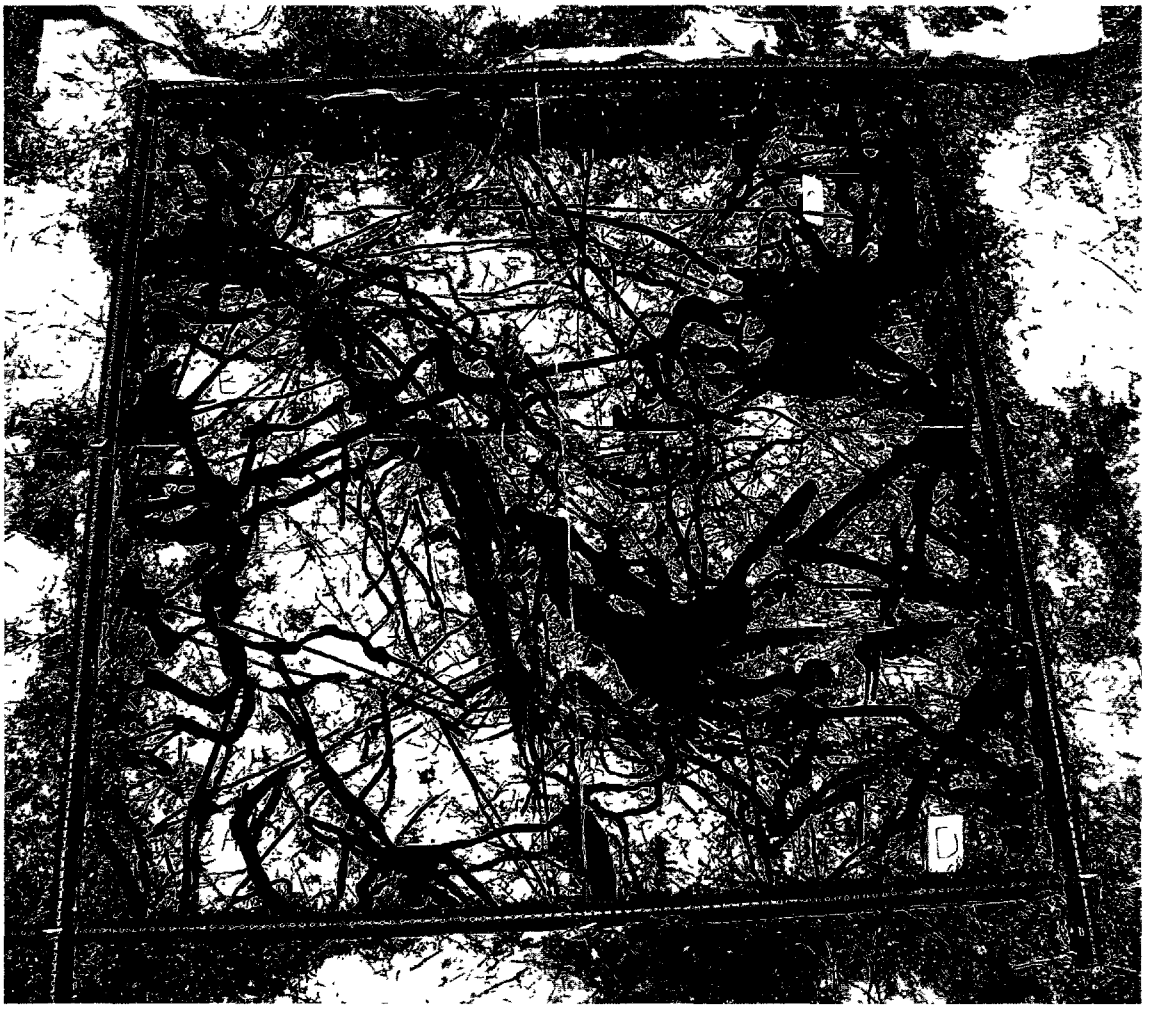

FIG. 23. Spatial distribution of coarse roots digitized for the 2 m x 2 m GPR validation plot. Woody oak roots are shown in blue and palmetto roots are shown in green.

Discussion

In this study, the potential use of GPR to image the spatial distribution of coarse roots and other large belowground structures was explored. Signal reflections from coarse roots were readily identifiable in both 2-d and 3-d GPR data. Processed 3-d images showed strong signal reflections that coincided with the location of large belowground structures in the validation plot. These images provided a view of the horizontal

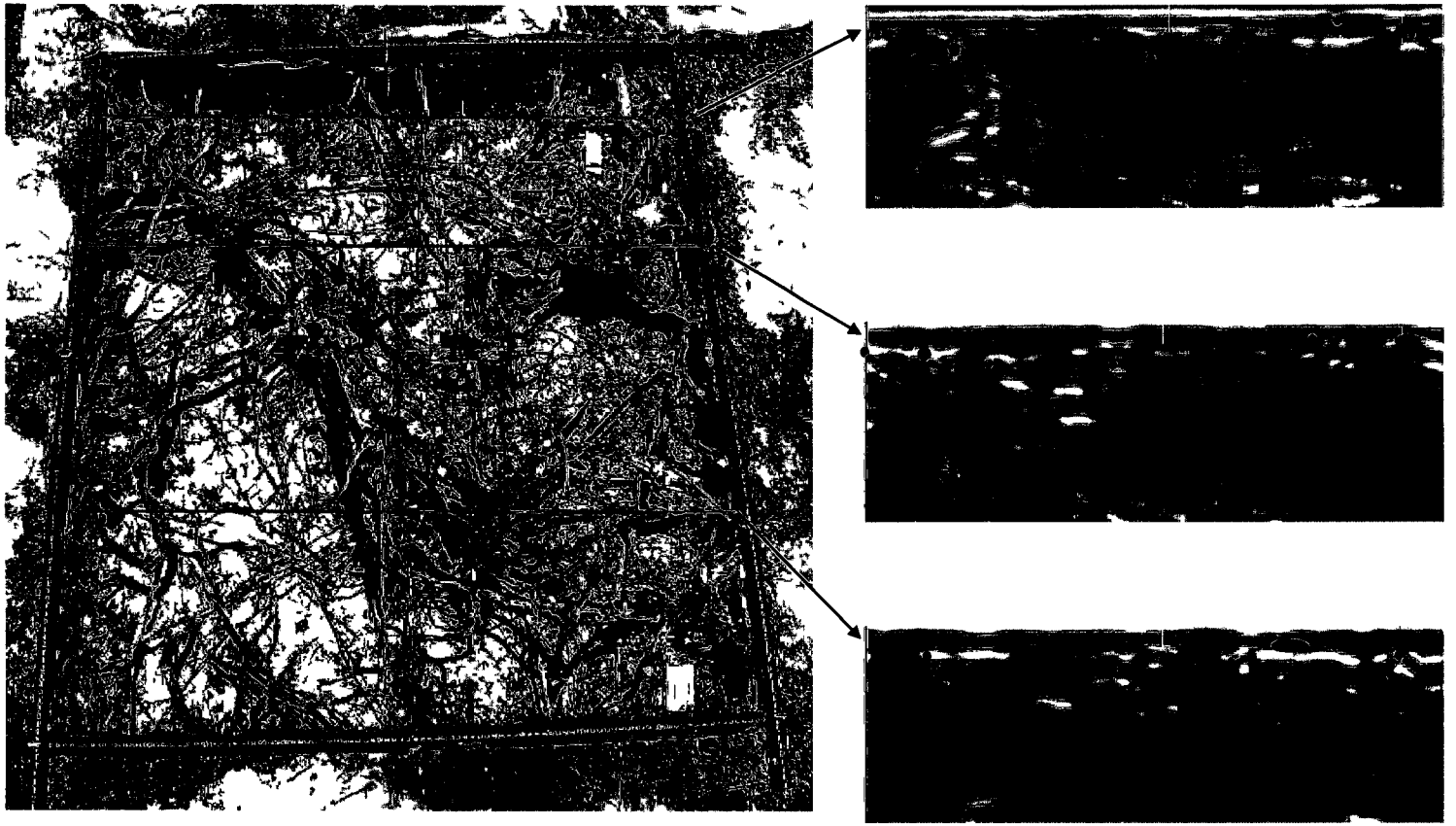

FIG. 24. Unprocessed 2-m long GPR scans from the validation plot with hyperbolas indicated with red dots.
Hyperbolas are generated when a belowground object (such as a root) scatters part of the GPR signal back to the receiving antenna.

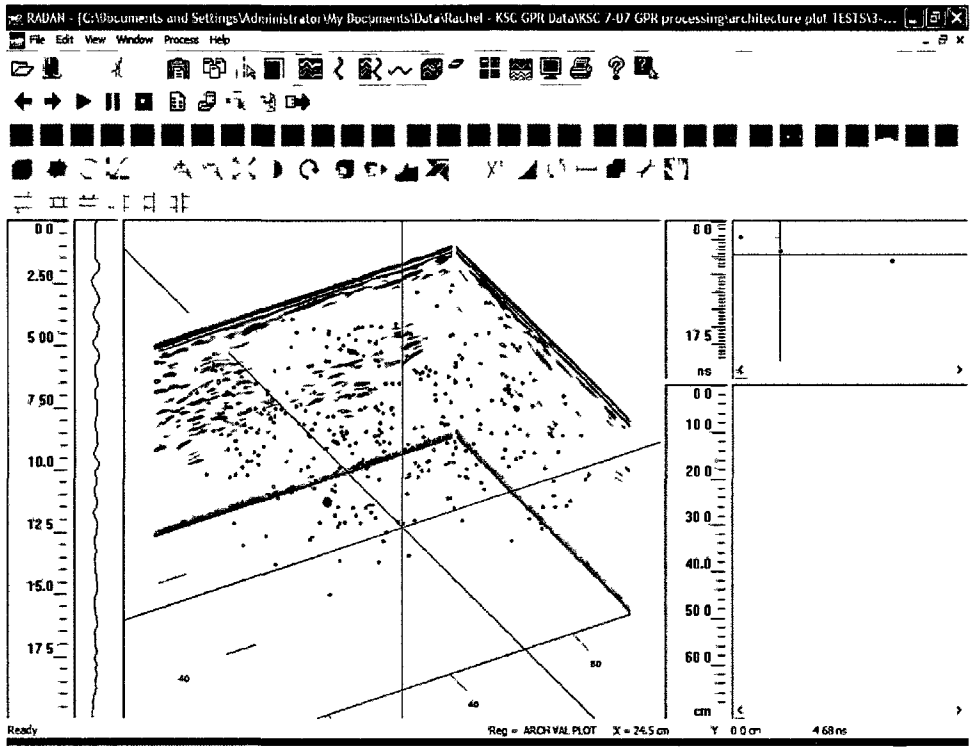

FIG. 25. Example screen from Radan GPR processing software showing 3-d view and auto target function to locate all hyperbolas in the 3-d dataset. Data shown are from the GPR validation plot with no post-collection processing. Images on right-hand side of screen show 2-d GPR scans corresponding to point location in 3-d cube on the left.

distribution of coarse roots, which is similar to the concept of plant cover in aboveground plant measurements. "Root cover" is typically not measured due to the difficulty in sampling root systems over large areas. The pixel count method of comparing GPR 3-d root images provided a means of quantifying root "cover" in the study plots. This allowed for comparisons among plots, between treatments, and potentially over multiple sampling dates to detect changes over time. At the end of the 11-year study, no significant difference in pixel counts was seen between CO_2 treatments.

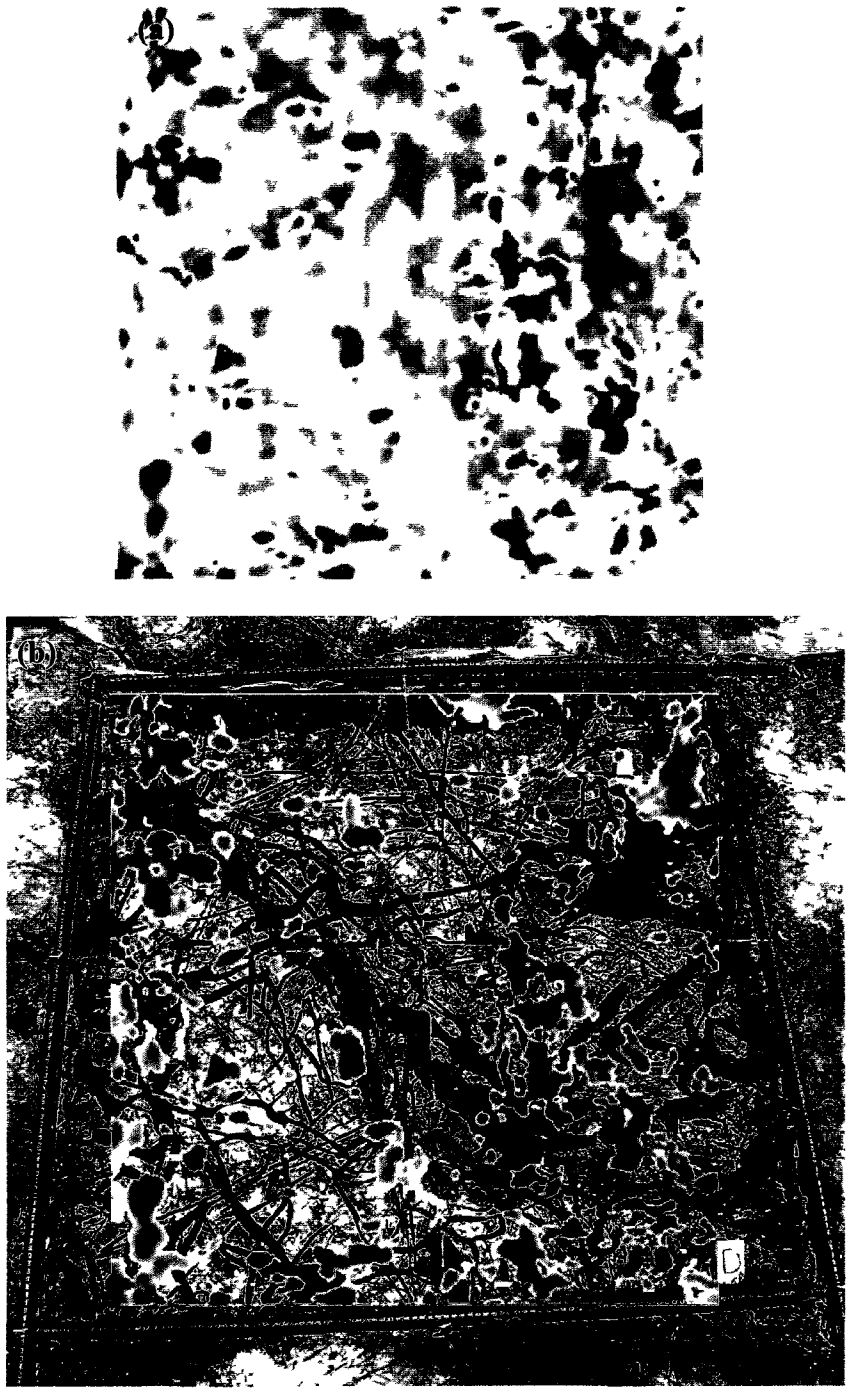

FIG. 26. Top-down view of 3-d GPR data file for the validation plot processed with gain equalization and position correction (a); red areas indicate strong signal reflections. The same GPR image set as a transparent overlay on top of digitized coarse root system from the validation plot (b).

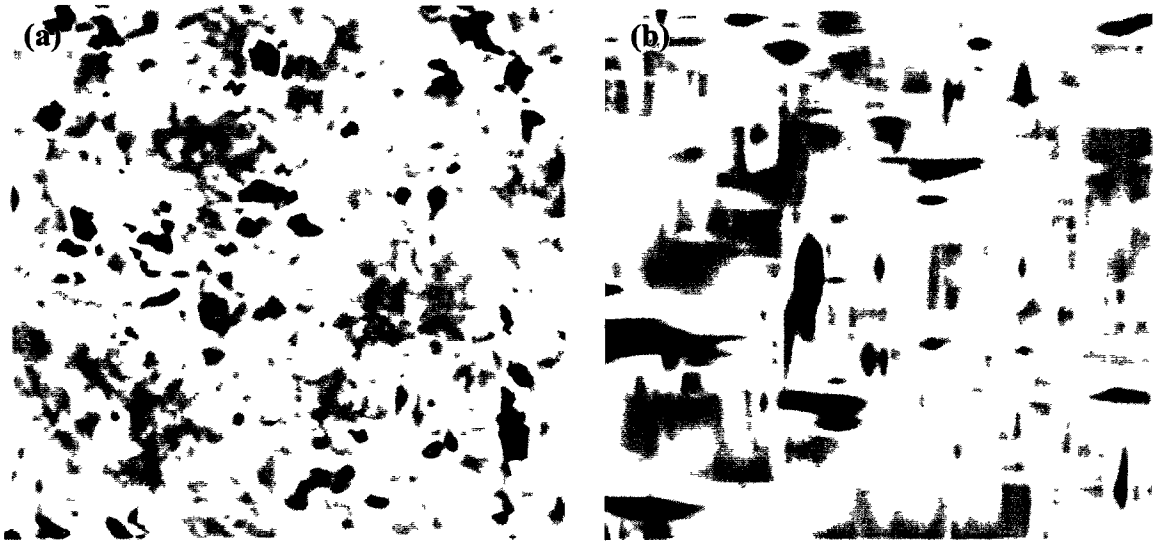

FIG. 27. Sample top-down views of 3-d GPR files from the experimental plots: Plot 12 (a) was scanned with 100 scans in the x- and y-directions while Plot 13 (b) was scanned with only 13 scans in each direction. Red areas indicate strong signal reflections.

TABLE 4. Average pixel counts for GPR images created from either full (100 scans in the x- and y- directions) or partial (13 scans in the x- and y-directions) scans of the experimental plots. *P*-values represent results of non-parametric test for differences in pixel counts between ambient and elevated CO_2 plots.

	Full GPR Scans	**Partial GPR Scans**
Ambient CO_2	35832	32713
Elevated CO_2	35606	32427
	$P = 0.95$	$P = 0.91$

Root excavations are labor-intensive and time-consuming, and not possible in systems where disturbance to the plants is detrimental. The GPR method eliminates the need for excavations and provides a non-destructive means for sampling root systems. 3-d GPR imaging was also useful for determining the location of large roots and belowground structures, which would be beneficial for targeted sampling of these structures. Another potential use is for determination of the most appropriate places to collect soil cores if large roots need to be avoided. Due to the speed, ease of use, and non-invasive nature of data collection, GPR is an appropriate method for surveying coarse root systems in experimental, natural, and agricultural settings. The Florida study was ideal for testing the GPR method because all aboveground vegetation had been removed from the study plots. However, GPR can also be used around plant stems in areas where vegetation is not exceedingly dense; it has been used successfully to study roots in forests and tree plantations where scans were made around tree trunks (Butnor et al. 2003, Butnor et al. 2005, Zenone et al. 2008).

This is the only study to use GPR to view intact root systems as a 3-d horizontal image and quantify the "cover" of roots in study plots. Previous research using GPR to study root systems was focused on testing the ability of GPR to detect roots (i.e. Barton and Montagu 2004, Cox et al. 2005, Dannoura et al. 2008, Hirano et al. 2009) or to determine root locations or biomass (Butnor et al. 2001, Butnor et al. 2005, Stover et al. 2007, Samuelson et al. 2008). GPR has been used to map tree root systems by Hruska et al. (1999) and Cermak et al. (2000), but this was done by drawing the root system by hand from individual 2-d radar scan data. Zenone et al. (2008) analyzed 3-d top-down GPR images in relation to partially excavated root systems similar to the method used in

the Florida study. However, GPR scans were collected only every 25 cm and image resolution was low; images were also only compared qualitatively instead of quantitatively.

Other than GPR, nondestructive methods for assessing root spatial distribution include high-resolution computed tomography ("CT") scanning and magnetic resonance imaging (MRI). CT and MRI methods are useful only for small potted plants due to size and cost constraints (Danjon and Reubens 2008), and therefore are not suitable for field applications. X-ray technology has been used to image intact root systems from soil monoliths collected in the field, but this is a destructive technique and does not allow for repeated measurements of the same roots (Pierret et al. 2005). Multi-electrode resistivity imaging is the only technique other than GPR that allows for non-destructive visualization of root systems *in situ*, and 2-d images were used to detect spatial variability of roots and quantify biomass in a tree stand in Italy (Amato et al. 2008). Zenone et al. (2008) used both electrical resistivity tomography (ERT) and GPR approaches to study tree roots in the field and found that ERT was more useful for measuring water content and soil parameters while GPR was capable of detecting distribution of tree roots with higher resolution than ERT.

Off-the-shelf GPR post-processing software enables a wide range of processing options to view 3-d data. Further research into the best data collection settings, scan intensity regimes, and processing protocols would add to this field of knowledge. One limitation to this study was that not all plots were sampled with the intensive scanning regime due to time constraints and physical properties of some of the experimental plots. There was a noticeable difference in 3-d image quality depending on the intensity of

scanning; greater scan density yielded better image resolution. In future work, a greater scan density is recommended when possible.

The top-down view of the 3-d GPR data was useful for examining coarse root horizontal distribution. With further exploration of the data collection and processing methods it may be possible to image root architecture more precisely from both top and side views. Specifically, the Gain, Scans Per Unit, and Range data collection settings should be carefully tested. Manual gain settings customized to the study site and soil dielectric properties will ensure consistent signal readings from plot to plot, and Gain is essential for accurate visualization of belowground objects. The Scans Per Unit setting controls the vertical signal density during data collection that in turn impacts scan resolution. The Range function determines the depth of signal penetration and should be customized to each study site in order to conserve bandwidth and not waste part of the signal on depths that are not important for study. Post-collection processing protocols may also be individually adapted to each site, and combinations other than those presented earlier in this chapter can be attempted to maximize the information return from GPR data.

CHAPTER 5

CONCLUSIONS

Summary of Results

High root biomass in the natural Florida scrub-oak ecosystem provides a critical carbon reservoir essential for plant recovery after disturbance. Root biomass was 3 to 5 times greater than aboveground biomass at the end of the study in 2007, and coarse roots constituted the majority of root biomass in this system. During 11 years of CO_2 enrichment, strong CO_2 effects on fine root biomass were seen only after disturbance during periods of recovery, followed by steady state where CO_2 effects diminished. Greater root biomass under elevated CO_2 during recovery periods could result in greater carbon inputs belowground and an alteration of the soil carbon cycle. Over time, there was a shift in fine root biomass deeper in the soil under elevated CO_2. These findings suggest that fine roots reached closure or a limit to the soils' capacity to support additional fine roots.

At the end of the study, there was a trend of greater total root biomass under elevated CO_2. However, root biomass was at a low point in both CO_2 treatments at that time. One month after all aboveground vegetation in the experimental plots was harvested, fine root abundance (RLD) and biomass did not significantly decrease. Ten months later, fine root biomass increased significantly in former elevated CO_2 plots but not in former ambient CO_2 plots. The observed effects of vegetation harvest on fine roots were different than those observed after fire and hurricanes. Complete removal of vegetation is different from natural disturbances at the site where damage to aboveground vegetation is also accompanied by a pulse of nutrients.

Ground-penetrating radar (GPR) was used successfully to image coarse root horizontal distribution in 4-m^2 plots. No difference in root spatial distribution was detected between plots exposed to ambient or elevated CO_2 for 11 years, although this was not entirely unexpected since there was not a significant difference in coarse root biomass between treatments at the end of the study. GPR proved to be a fast and effective method for collecting data on belowground plant structures without having to excavate the root system.

Implications and Applications

This study is unique because roots were measured for 11 years using non-destructive methods. Excavation of root systems is labor- and time-intensive and not feasible when it is necessary to keep the plants intact. Reliable methods for measuring root parameters indirectly are needed to advance current knowledge of this under-studied component of ecosystems. The Florida study site was ideal for minirhizotron and GPR methods because the soil was homogeneous, well-drained, and free of rocks and other belowground objects that would interfere with root measurements.

One limitation of the minirhizotron method was that over time the viewing frames at 0-10 cm depth became stained dark by organic matter, leading to undersampling of roots at that depth. This is a common problem with long-term deployment of minirhizotrons, and the undersampling of roots at shallow depths is well-documented (e.g. Samson and Sinclair 1994, Ephrath et al. 1999). Also, roots > 2 mm diameter were not adequately measured using minirhizotrons. The GPR method may have detected clumps of fine roots, but the amount of sampling overlap for roots in the 2 to 5 mm

diameter range between the two methods was not investigated.

This study is also novel because it was performed in a natural ecosystem that experienced periodic disturbances such as fire, drought, and hurricanes. No other CO_2 enrichment experiment has had a similar disturbance regime. The dominant plants in this system recovered quickly following disturbance and had a short maturation time (Schmalzer and Hinkle 1992a). An important finding of the study was that there was an upper limit to the amount of roots the soil can support (termed "root closure", first described in Day et al. 2006); disturbance may reduce root stocks below this capacity which then allows fine root recovery and eventual return to steady state. During recovery periods, elevated CO_2 boosted fine root biomass which could lead to changes in the soil carbon cycle. This also indicated potential for faster ecosystem recovery after disturbances under future atmospheric CO_2 concentrations.

In the broader field of CO_2 enrichment studies, the lack of a significant increase in fine root biomass under elevated CO_2 at the end of the study is not unique (e.g. Bader et al. 2009, Handa et al. 2008), although there are examples of sustained stimulation over multiple years (e.g. Lukac et al. 2003, Jackson et al. 2009, Pregitzer et al. 2008). The quick maturation time of this system most likely limits the potential stimulation of plant biomass by elevated CO_2. The idea that ecosystem response to CO_2 enrichment is primarily affected by the ecological state of the system was discussed by Körner (2006); he postulated that plant-soil interactions were more important than plant-atmosphere coupling when analyzing ecosystem responses. In the Florida study, plants were not found to be constrained by long-term nutrient or water limitations (Hungate et al. 2002, Langley et al. 2009, McKinley et al. 2009, Johnson et al. 2003). Root responses to CO_2

enrichment were likely constrained by restrictions on soil resource space.

Recommendations for Future Work

Some root sampling could have been done differently to better address the research questions. First, minirhizotron measurements of fine roots would have been more informative immediately prior to the harvest (instead of 4 months before) and immediately post-harvest (instead of 1 month after). The long sampling interval may have masked changes in fine root abundance and biomass that were due to aboveground vegetation removal instead of some other factor. Also, if time had allowed, it would have been best to sample all experimental plots with the intensive GPR scanning regime at the end of the study. The GPR data collection settings used in 2007 also presented a challenge for comparison with previously-collected datasets that used different settings.

Further testing of the GPR method will advance the study of coarse roots. The limitations of this method make it unusable in some field conditions such as waterlogged soils, soils with high clay or gravel content, and areas with closely-spaced stems or dense vegetation. However, in areas not limited by those conditions, GPR can be an invaluable tool for assessment of root parameters. As discussed in Chapter 4, site-specific testing of the best data collection and post-processing steps is essential to maximize the information obtained from GPR data analysis. Another important area that needs further testing is the determination of the degree to which GPR detects fine roots, as well as the minimum root diameter of individual roots that can be detected at each site. There was evidence at the Florida site that clumps of fine roots were detected by GPR, which could complicate root biomass estimates if used in conjunction with fine root estimates from other methods.

These findings can be applied to future work in several ways. First, belowground carbon budgets and predictions regarding the effect of increasing atmospheric CO_2 on root biomass will need to take into account root closure as a limit on the amount of carbon that can be sequestered in mature ecosystems. Second, belowground biomass is temporally dynamic and undergoes natural cycles that are strongly affected by ecosystem disturbances. The change in root biomass over time means that one-time sampling may not give an accurate representation of root parameters over the long-term, and this must be taken into consideration. Third, elevated CO_2 may enhance root growth following disturbance and potentially speed recovery and return to steady state, which has major implications for ecosystem recovery as a whole. Fourth, the minirhizotron and GPR methods for sampling root biomass and spatial distribution are not without limitations, but can be invaluable tools nonetheless. The successes and failures of these methods, as presented here, will hopefully guide future researchers in their efforts to quantify roots nondestructively.

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- "The long-term effects of elevated atmospheric CO_2 on root biomass in a Florida scrub-oak ecosystem". Penn State Plant Biology Symposium. University Park, Pennsylvania. May 2009.
- "Coarse root biomass and architecture under elevated CO_2 in a Florida scrub-oak ecosystem determined by ground-penetrating radar". Meeting of the Association of Southeastern Biologists. Spartanburg, South Carolina. April 2008.
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