EFFECT OF ELEVATED CO₂ ON COARSE-ROOT BIOMASS IN FLORIDA SCRUB DETECTED BY GROUND-PENETRATING RADAR

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Abstract. Growth and distribution of coarse roots in time and space represent a gap in our understanding of belowground ecology. Large roots may play a critical role in carbon sequestration belowground. Using ground-penetrating radar (GPR), we quantified coarse-root biomass from an open-top chamber experiment in a scrub-oak ecosystem at Kennedy Space Center, Florida, USA. GPR propagates electromagnetic waves directly into the soil and reflects a portion of the energy when a buried object is contacted. In our study, we utilized a 1500 MHz antenna to establish correlations between GPR signals and root biomass. A significant relationship was found between GPR signal reflectance and biomass ($R^2 = 0.68$). This correlation was applied to multiple GPR scans taken from each open-top chamber (elevated and ambient CO₂). Our results showed that plots receiving elevated CO₂ had significantly ($P = 0.049$) greater coarse-root biomass compared to ambient plots, suggesting that coarse roots may play a large role in carbon sequestration in scrub-oak ecosystems. This nondestructive method holds much promise for rapid and repeatable quantification of coarse roots, which are currently the most elusive aspect of long-term belowground studies.

Key words: carbon dioxide; coarse-root biomass; ground-penetrating radar; roots; scrub oak.

INTRODUCTION

Atmospheric carbon dioxide (CO₂) has been increasing in concentration globally over the past century and current biosphere models predict CO₂ concentration will double within the next 50–100 years to ~700 ppm with a likely mean global temperature increase of 1.4–5.8°C (Schlesinger 1991, IPCC 2001). It is unclear how plant communities will respond to this change in CO₂ concentration. However, many studies suggest changes in species dominance, resource availability and allocation, invasiveness, and, most importantly, alteration of ecosystem services (Ward and Strain 1999, Korner 2001, Bazzaz and Catovsky 2002, Poorter and Navas 2003). Belowground responses have traditionally been ignored or oversimplified (Mooney 1991, Mooney et al. 1991, Korner and Arnone 1992, Day et al. 1996). The allocation of carbon to the root system has implications for long-term sequestration and storage of excess atmospheric CO₂ in the rhizosphere (Pregitzer et al. 1995, Johnston et al. 2004, Day et al. 2006).

Long-term data from an elevated CO₂ experiment on Merritt Island at Kennedy Space Center, Florida indicate that fine root growth was higher during the initial years of elevated CO₂ treatment (postburn) (Dilustro et al. 2002). However, as the community matured, the CO₂ treatment effect appears to have disappeared (Day et al. 2006). In addition, we have suggested that this system has reached root closure (i.e., carrying capacity) in the fine root component of belowground biomass (Day et al. 2006). We know little about effects on larger underground plant structures. Coarse roots and other large belowground structures have been underrepresented in most studies (Norby 1994), but are expected to play a critical role in regrowth in the scrub-oak ecosystems, which are fire controlled with a 7–15 year natural burn cycle (Schmalzer and Hinkle 1991).

Historically, root systems have been the most understudied aspect of plant biology (Waisel et al. 2002). This is due to the opaque nature of soils and difficulties in repeatability of quantitative measures of root systems (Fitter and Stickland 1992, Nielsen et al. 1997). Most root quantification methods, such as ingrowth cores, soil cores, and pits are destructive, thus hindering repeated assessments in long-term studies (Norby and Jackson 2000, Pierret et al. 2005). In addition, these methods are often labor intensive and limited by manageability of size and number of samples. Nondestructive root analysis methods, such as minirhizotron tubes, only elucidate the fine roots within the
A major gap in our current understanding of root systems involves coarse roots and other large belowground structures. Ground-penetrating radar (GPR) is a 30-year-old geophysical technique that pulses ultra high-frequency (100–1500 MHz) electromagnetic waves into the ground (Wielopolski et al. 2000, Daniels 2004). Electromagnetic waves travel through the soil and reflect off buried objects, such as roots, back to a receiving antenna (Fig. 1). The GPR control unit measures the intensity and velocity of the signal along with the propagation time (Hruska et al. 1999). GPR technology holds promise for quantifying root mass and architecture due to its rapid, nondestructive acquisition of data (~90-ns collection time per scan; Butnor et al. 2001). Recent studies with GPR using a 1500-MHz frequency antenna have distinguished roots with diameters as small as 0.5 cm (Butnor et al. 2001).

The goal of this study was to apply GPR technology to questions pertaining to the influence of elevated CO2 on coarse roots in a scrub-oak ecosystem at Kennedy Space Center, Florida. We established a relationship between GPR signal strength and root biomass. Using this relationship, we estimated coarse-root biomass in open-top chambers in an elevated CO2 experiment where destructive measurements cannot be performed. We hypothesized that scrub oak that has been continually fumigated with elevated CO2 would have greater coarse-root biomass than plants exposed to ambient CO2 concentrations.

**METHODS**

**Site description**

The study site for this research is located on Merritt Island in Brevard County, Florida, USA (28°36'29" N; 80°40'15" W). Merritt Island, located on the northern part of Kennedy Space Center (KSC), is a subtropical barrier island with topography ranging from sea level to ~2 m above mean sea level (Huckle et al. 1974, Mailander 1990).

The soils are primarily sandy and dominated by the Pomello soil type. Pomello is a moderately well-drained Spodosol (Aeric Haplhumod) derived from deposited marine sands (Huckle et al. 1974, Baldwin et al. 1980, Schmalzer and Hinkle 1987). Soils located at our site are typically acidic (pH 3–4), nutrient poor (most notably nitrogen limited), and low in organic matter content. GPR is best suited for dry sandy conditions; wet or high clay content soils usually exhibit signal attenuation, resulting in diminished resolution for accurate interpretation (Doolittle et al. 2002, Leckebusch 2003, Daniels 2004). Doolittle et al. (2002) mapped relative soil suitability for GPR work for the conterminous United States.

The scrub-oak community is primarily composed of *Quercus myrtifolia* Wasd. (76%), *Quercus geminata* Small (15%), *Quercus chapmani* (7%), *Serenoa repens* (Bartram) Small, and *Lyonia ferruginea* (Walt.) Nutt. These scrub communities are historically fire-controlled with a natural 10–15 year fire cycle (Schmalzer and Hinkle 1991, 1992). Our study site was control burned in February 1996 before treatments were initiated. The
scrub-oak ecosystem was selected because it represents a perennial, subtropical woody community with high evaporative demand and low nutrient availability (Day et al. 1996).

**CO₂ treatment**

Open-top chambers (OTCs) were used to create elevated CO₂ environments (Drake et al. 1989). A total of 16 chambers were fabricated with PVC frames covered with clear mylar sheeting. Each octagonal chamber was designed with sides of 139.9 cm, a maximum diameter of 356.6 cm, and a height of 365 cm. Treatments were initiated following a controlled burn of the system. Blocks (consisting of one representative from each elevated and ambient treatment) were assigned according to similarity of preexisting vegetation composition (N = 8 chambers per treatment). Carbon dioxide treatments initiated on May 1996 and continually treated to date, include eight ambient (~350 ppm CO₂) chambers and eight elevated (~700 ppm CO₂) chambers. This represents >10 years of continuous CO₂ fumigation. Carbon dioxide treatments are applied with a continuous air circulation system 24 hours a day.

**Root biomass cores**

In June 2005 and September 2006, 30 soil core locations were selected in the scrub-oak community adjacent to the chambers. Prior to coring, each core location was scanned with a Subsurface Interface Radar System (SIR-3000) attached to a model 5100 (1500 MHz) GPR antenna (Geophysical Survey Systems, North Salem, New Hampshire, USA). Other studies have found a frequency trade-off between depth of penetration and resolution (Hruska et al. 1999, Cox et al. 2005). Low-frequency antennas (i.e., 400 MHz) penetrate deeper into the soil but have a low ability to resolve detailed objects. High-frequency antennas are smaller and tend to be better at resolving detailed objects, but are limited to a depth <1 m. Prior to fieldwork, the radar unit was scaled for depth by determining the average dielectric constant of the soil with a buried metallic object at a known depth (similar to Butnor et al. 2003). Each coring location was scanned with the centerline of the antenna moving 15 cm in length. Once the GPR data were collected, a 15 cm diameter corer was used to extract a soil core to a depth of 60 cm. Each core was divided into three 20-cm segments, which were dry sieved, refrigerated, separated one week later into live, dead (based on lack of flexibility and loss of structural integrity), and unidentifiable organic matter fractions, and dried at 70°C for 48 hours before weighing. Larger roots were dried longer until there was no change in mass after 24 hours. Large rhizomes, burls (lignotubers), and belowground stems are extremely common in scrub-oak ecosystems; thus, we classified any belowground structures >5 mm diameter as coarse roots.

**GPR image processing and root biomass correlations**

Core location scans were processed with RADAN 6.5 GPR data processing software (Geophysical Survey Systems, North Salem, New Hampshire, USA). Each individual scan was cropped to ensure that only the actual 15 cm area of the core was analyzed. Before quantification of the scans, several data processing steps were applied to enhance root discrimination. Root structures appear as hyperbolic reflectors, whereas parallel bands represent plane reflectors such as ground surface, soil layers, and low-frequency noise (Fig. 2). Parallel bands were removed with a horizontal finite impulse response filter (FIR) filtration method called background removal (Oppenheim and Schafer 1975, Butnor et al. 2003). We used the Kirchoff migration to correct the position of objects and collapse hyperbolic diffractions based on signal geometry (Daniels 2004). Finally, we performed a Hilbert transformation on the radar data. Hilbert transformations express the relationship between magnitude and the phase of the signal allowing the phase of the signal to be reconstructed from its amplitude, thus allowing subtle properties and objects to be elucidated and reducing false “echoes” (Oppenheim and Schafer 1975, GSSI 2004). Radar profiles were converted to bitmap image files using Radan to Bitmap Conversion Utility 1.4 (Geophysical Survey Systems, North Salem, New Hampshire, USA).

Radar images were quantified with Sigma Scan Pro Image Analysis software (Systat 2004). Each image was converted to an 8-bit gray scale image. To quantify roots within the image, pixel intensity was measured (Cox et al. 2005). Intensity is a relative measure of how light or dark an individual pixel is on a scale of 0 (black) to 255 (white). We used an intensity threshold range of 60–255 pixels, which was able to delineate roots >0.5 cm. This technique measures the relative pixel area of interest (Cox et al. 2005). Past studies suggest that large diameter dead roots will persist for substantial periods of time in the soil matrix and therefore should be combined with live roots to estimate total belowground biomass (Butnor et al. 2005, Cox et al. 2005). Therefore, a linear regression was performed to quantify the relationship between total root biomass (live and dead) from the soil cores and the GPR signals (pixels within the threshold range).

**Root biomass in chambers**

In December 2005, five stratified-random GPR scans were taken within each of the 16 open-top chambers (eight ambient and eight elevated). There were restrictions on sample locations due to installed instruments and sampling devices, and the space occupied by plant stems could not be scanned. Each scan was 15 cm long and was processed in RADAN and Sigma Scan in the same manner as the core location scans previously.
described. The sum of each scan’s intensity threshold was used to estimate biomass with the regression equations developed from the biomass cores. The effects of CO₂ enrichment on coarse-root biomass were tested by ANOVA using the MIXED procedure in SAS (SAS Institute 1990). Within our statistical model, biomass was blocked within chamber and CO₂ was the fixed effect.

**RESULTS**

**Root biomass/GPR regressions**

Biomass from the cores was partitioned into live and dead roots (Table 1). The average live biomass was 290 g (15 395 g/m²) and the average dead biomass was 44 g (2308 g/m²). Mean total biomass in the cores was 331 g/core (17 703 g/m²). Root diameters varied among cores, but large diameter roots were captured by the 15-cm core. Roots and other belowground structures with the largest diameters (7.9–15 cm) were primarily found in the top 20 cm of the soil profile. A noticeable decline in diameter size was observed with increasing depth. At the 40–60 cm depth, the largest roots measured 2–5 cm diameter. The regression between root biomass (live plus dead components) and the total number of pixels within the thresholds from the GPR images yielded a significant relationship ($R^2 = 0.68; r = 0.822$; Fig. 3).

**Table 1. Root core dry mass (g/15 cm diameter core) ranges for different root fractions to 60 cm depth.**

<table>
<thead>
<tr>
<th>Root fraction</th>
<th>Root core dry mass (g/core)</th>
<th>Biomass (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max.</td>
<td>Min.</td>
</tr>
<tr>
<td>Live</td>
<td>1696</td>
<td>45</td>
</tr>
<tr>
<td>Dead</td>
<td>82</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>1728</td>
<td>62</td>
</tr>
</tbody>
</table>

The regression equation was applied to GPR scans from the open-top chambers. Root biomass in the elevated and ambient chambers was estimated at 8971 ± 1105 g/m² (mean ± SE) and 6551 ± 1295 g/m², respectively (Fig. 4). There was a significant CO₂ treatment effect for coarse-root biomass in the scrub-oak system ($P = 0.049$). The effects of soil moisture on GPR signal penetration were examined at our field site.
by scanning multiple locations following the addition of increasing amounts of water. Soil moisture was recorded with a handheld TDR probe at 20 cm depth. The sandy soils at Merritt Island drain rapidly, thus producing little change in our interpretation of scans except in the case of saturated field moisture conditions (D. B. Stover, unpublished data). As a result, we feel confident that our results from GPR data interpretation and predictive root biomass equations are not limited to just conditions present at the time of core collection.

**Discussion**

The goal of this study was to apply recent advances in ground-penetrating radar to the difficult determination of belowground effects of elevated CO2 in a long-term study. The results indicate that properly processed GPR data can reasonably detect root biomass in the upper 60 cm of the soil profile. Significant relationships between the combined live and dead coarse-root biomass and the total number of pixels within the radar image provide a basis for a robust measurement of root biomass.

Processed GPR data applied to our biomass regression equation showed significantly different biomass between CO2 treatments, indicating that scrub oak treated with elevated CO2 had more coarse-root biomass than areas treated with ambient atmospheric CO2. This implies that more carbon sequestration is occurring in elevated CO2 chambers in the form of coarse roots. Belowground carbon storage in the coarse roots probably plays an important role in the regrowth of the scrub-oak community following fire. It is estimated that roots can comprise 40-85% of net primary production in some ecosystems (Fogel 1985, Fitter 1987). Norby et al. (1993) and Norby (1994) implied the importance of examining CO2 effects on Quercus because root mass increased more than twice that of stem mass. These studies showed 77–136% increase in root mass in Q. alba grown under elevated compared to ambient CO2. The ecosystem in the current study is dominated by scrub oaks and saw palmetto, which are clonal and produce large amounts of coarse roots, lignotubers, and belowground stems. Sclerophyllous shrublands and tropical evergreen forests tend to produce the greatest total mass of roots, on average ~5 kg/m^2 (Jackson et al. 1996, Robinson et al. 2003). Large root burls are very common in this system, thus making it difficult to separate the belowground stem from the root system. These structures provide major carbohydrate storage for regrowth and are known to persist long after aboveground biomass is removed. These structures play a major role in postfire recovery and are a critical component of belowground storage and ecology in this shrub system.

Numerous studies (e.g., Norby 1994, Miller et al. 2006) have suggested that coarse roots are large carbon sequestration sites and predicted that greater biomass should be present in elevated CO2 treatments, but few if any have been able to quantify this effect. Our finding provides support for these predictions. This finding is unique in that this study is one of the first to nondestructively quantify coarse roots as affected by elevated CO2. The methods utilized in this study can be applied to long-term studies to refine our understanding of coarse roots as well as to examine changes in biomass of larger belowground structures over time.

Previous belowground studies at our site have focused only on fine roots via minirhizotron methodology (Day et al. 1996). Early in the study, a treatment effect was reported; however, after about three years, this effect had dissipated (Day et al. 2006). Many woody plants in fire-controlled systems develop large belowground structures. In this system, biomass is promoted in elevated CO2 treatments and most notably in the...
belowground component. Estimated aboveground biomass (leaves and stems combined) was 1362 g/m² in ambient treatments, whereas in the elevated treatments, aboveground biomass was ~2037 g/m² (e.g., Dijkstra et al. 2002; T. Seiler, unpublished data). Belowground, the ambient chambers had 6551 g/m² in coarse-root biomass whereas the elevated chambers had 8971 g/m². Fine root biomass comprised 2226 g/m² and 2203 g/m² in the ambient and elevated chambers, respectively (A. Pagel, unpublished data). Because the fine roots had apparently reached closure in both treatments, the increase in belowground biomass under elevated CO₂ was proportionally greater in coarse roots (75% ambient, 80% elevated) compared to the fine roots (25% ambient, 19% elevated). Based on the above and belowground estimates, total biomass was ~10139 g/m² and 13212 g/m² for ambient and elevated CO₂ treatments, respectively, thus indicating that the increase in biomass distributed under elevated CO₂ was distributed in the same proportion above and belowground as in the ambient treatments. The contribution of belowground biomass to system carbon storage was massive (85% of total biomass).

While using GPR for root analysis is still in its infancy, this technique shows great promise for elucidating belowground plant structures. Very few studies have applied GPR to biological systems, especially to roots. GPR based estimates require substantial ground-truthing to ensure accurate quantification and repeatability of the technique. Potential future applications of GPR to our system will focus on determining coarse-root growth patterns temporally (growth rates) and spatially (root architecture). Similar to other studies, our method only identifies roots greater than ~0.5 cm diameter (Wielopolski et al. 2000, Butnor et al. 2001, 2003, Cox et al. 2005). Therefore, this study excludes the fine root biomass component.

In conclusion, GPR based biomass estimates suggest elevated CO₂ chambers have greater belowground biomass, indicating a significant treatment effect in a scrub-oak ecosystem, thus supporting our central hypothesis. In addition, GPR appears to be a rapid and feasible method to quantify and examine coarse roots and is redefining our understanding of the role larger belowground structures play in ecosystem dynamics.

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LITERATURE CITED


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