Spring 2007

# Effects of Elevated Atmospheric CO2 on Root Dynamics, Biomass and Architecture in a Scrub-Oak Ecosystem at Kennedy Space Center, Florida

Daniel Benjamin Stover
*Old Dominion University*

Recommended Citation
Stover, Daniel B.. "Effects of Elevated Atmospheric CO2 on Root Dynamics, Biomass and Architecture in a Scrub-Oak Ecosystem at Kennedy Space Center, Florida" (2007). Doctor of Philosophy (PhD), dissertation, Biological Sciences, Old Dominion University, DOI: 10.25777/cac7-n592
https://digitalcommons.odu.edu/biology_etds/77

# EFFECTS OF ELEVATED ATMOSPHERIC $CO_2$ ON ROOT DYNAMICS, BIOMASS AND ARCHITECTURE IN A SCRUB-OAK ECOSYSTEM AT KENNEDY SPACE CENTER, FLORIDA

by

Daniel Benjamin Stover
B.A. Biology, May 1999, West Virginia University
B.S. Environmental Protection, May 1999, West Virginia University
B.A. Interdepartmental Studies, May 1999, West Virginia University
M.S. Environmental Plant Biology, August 2005, West Virginia University

A Dissertation Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirement for the Degree of

DOCTOR OF PHILOSOPHY

ECOLOGICAL SCIENCES

OLD DOMINION UNIVERSITY
May 2007

Approved by:

Frank P. Day (Director)

Joseph Rule (Member)

Kneeland Nesius (Member)

# ABSTRACT

## EFFECTS OF ELEVATED ATMOSPHERIC $CO_2$ ON ROOT DYNAMICS, BIOMASS AND ARCHITECTURE IN A SCRUB-OAK ECOSYSTEM AT KENNEDY SPACE CENTER, FLORIDA

Daniel Benjamin Stover
Old Dominion University, 2007
Director: Dr. Frank P. Day

A major gap in whole-plant ecology lies with our understanding of root system growth, function and distribution. Large belowground structures, in addition to fine roots, are of particular interest because of their role in carbon sequestration. Non-destructive methods, including ground-penetrating radar (GPR) and minirhizotron observation tubes, were used to investigate effects of elevated $CO_2$ on root biomass, dynamics (productivity, mortality, and turnover), root persistence and architecture in a fire dominated scrub-oak ecosystem. Open-top chambers have been exposed to elevated atmospheric $CO_2$ for the past eleven years at Kennedy Space Center, Florida. No significant sustained $CO_2$ treatment effects were observed in fine root length density, due to root closure. Root density at lower depths increased to match abundance levels observed in the upper portions of the soil profile. $CO_2$ significantly affected fine root production, mortality, and turnover during the early years of fumigation; however, this effect disappeared as fine root closure occurred. Survivorship analysis suggested the smallest fine root size classes (<0.1 mm in diameter and <0.25 mm in length) were most susceptible to mortality. In addition, root persistence increased with increasing soil depth. Coarse root biomass had a significant treatment effect ($p = 0.049$), with elevated roots having more biomass than those under ambient $CO_2$. Overall, 86% of the total biomass was belowground with 78% allocated to coarse roots and 22% to fine roots. Coarse root architecture determinations confirmed the complexity and abundance of large belowground structures in this system. Large roots with sharp angles or that transverse the study areas were most likely to be observed in the GPR images. Large root burls were readily visualized in the GPR based architecture models. The results suggest that coarse roots may play a large role in the sequestration of carbon belowground in scrub-oak ecosystems, thus having implications to carbon dynamics, $CO_2$ treatment memory, and plant regeneration following disturbances such as fire.

This dissertation is dedicated to my parents, James L. and Ella M. Long. Without their love and support, none of what was accomplished here would have been possible.

## ACKNOWLEDGMENTS

I would like to thank the members of my dissertation committee, Frank P. Day (Chair), Joseph Rule and Kneeland Nessius. I would also like to thank my guidance committee members, Rebecca Bray, William Resetarits, and Richard Whittecar. I especially thank the excellent guidance and support from my advisor Dr. Day throughout this endeavor. His insight and dedication to the field of ecology and his students will serve as an example for me throughout my career. I would also like to acknowledge John Butnor who has provided invaluable assistance in performing GPR measurements and analysis. I also acknowledge the efforts of Bert Drake of the Smithsonian Environmental Research Center, C. Ross Hinkle of Dynamac Corporation and project site staff (Dave Johnson, Tom Powell and Troy Seiler) for their assistance and reliable operation of this experiment. This work was funded by subcontract 95-59-MPOOO02 to the Smithsonian Institution's grant from the U.S. Department of Energy (DE-FG-02-95ER61993). I appreciate the field and lab assistance of Vince Bacalan, Tina Morgan, Julie Ray, Chris Day, Brad Carwile, Kadrin Getman, and Jay Bolin. I am also grateful for GPR assistance provided by Geophysical Survey Systems Inc. as well as NASA employees Steven Schindler and Lori Jones for GPR permit assistance. I also thank Jason Thorn, Kevin Fyffe, Josh Curtis, Justin and Stephanie Goodwin for their friendship and support during my tenure at Old Dominion University. Finally, I would like to thank fellow graduate students Brett McMillan and Alisha Pagel-Brown for their support, friendship, and fellow commiseration throughout this long, strange journey.

# TABLE OF CONTENTS

Page

CHAPTER

Page

# LIST OF TABLES

# LIST OF FIGURES

# CHAPTER I

# INTRODUCTION

> *"Humans are forcing the Earth's environmental systems*
> *to change at a rate that is more advanced than their*
> *knowledge of the consequences."* –Schneider, 2000

A great amount of attention has been placed on atmospheric carbon dioxide ($CO_2$) levels over the past few decades. Much of this interest lies in the expected alterations of the environment due to climate changes. Since the 1980's, a large amount of funding has been expended to examine the causes and effects of excess $CO_2$ in the atmosphere. It is generally accepted that human activities (i.e. agricultural practices, deforestation, burning of fossil fuels, industrialization, etc.) have amplified background atmospheric enrichment rates of $CO_2$. Recent evidence suggests that human activities have altered the carbon cycle resulting in conditions that have not been observed on Earth during the past several million years (Schlesinger 1991). Data from Mona Loa observatory in Hawaii and ice cores from Greenland and Antarctica suggest that human activities have increased atmospheric $CO_2$ concentrations from 270 to 350 parts per million (ppm), a 30% increase in less than 200 years (Schlesinger 1991) (Fig. 1). Biosphere models predict that with current anthropogenic trends, the $CO_2$ concentration will double within the next 50-100 years to approximately 700 ppm and mean global temperature could increase 1.4 - 5.8 $^0$C (Schlesinger 1991, IPCC 2001, Janzen 2004).

---

The journal model for this dissertation is Ecology.

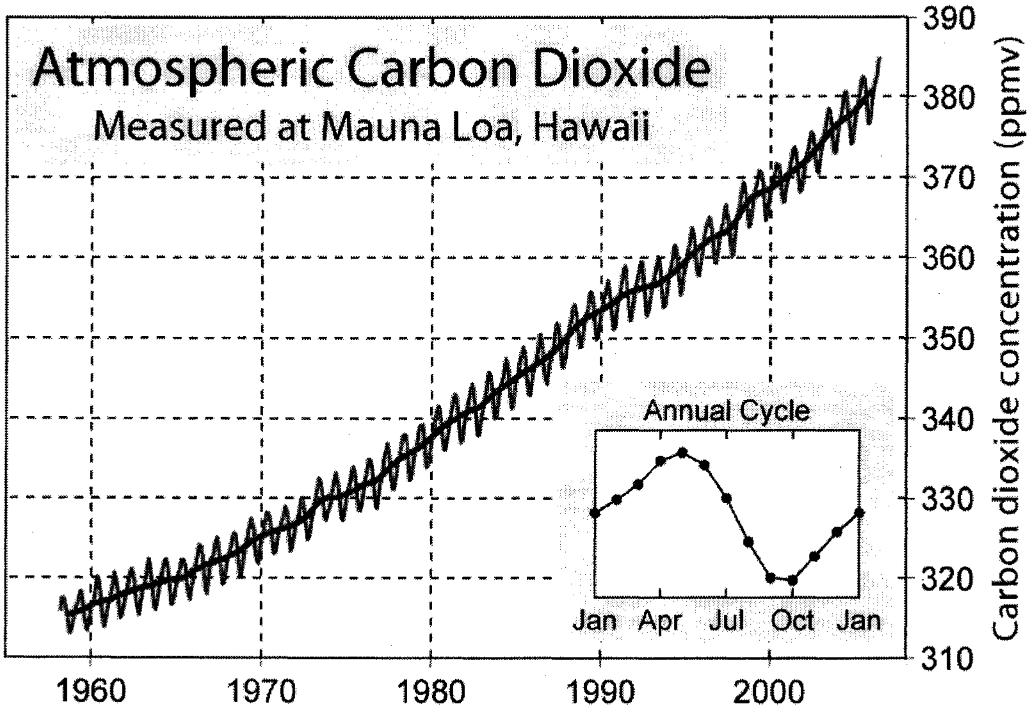


FIG. 1. Atmospheric $CO_2$ concentration (ppm) at Mauna Loa (Rohde 2007).

Expected changes from this massive alteration to the carbon cycle include rising sea levels, modification to precipitation and other weather patterns, and shifts in plant species dominance. We must actively find ways to better manage ecosystems and their services to handle the modified global carbon cycle.

Plants acquire carbon through photosynthesis by fixation of $CO_2$ molecules, primarily in the leaves. Carbon accumulated in biomass effectively immobilizes $CO_2$ from atmospheric pools, which can then be transferred to belowground structures such as roots. At this point, carbon can enter the rhizosphere by means of root turnover and decomposition, which, in effect, results in carbon loading into the lithosphere. Overall this process has potential for the sequestration of excess $CO_2$ in the environment. Although multiple other scenarios and potential solutions have been proposed, this natural option is cheap and biologically and environmentally sound. The magnitude of carbon sequestration belowground is not fully understood; however, many ecosystem level studies are beginning to address this gap in our knowledge. The goal of this research was to evaluate the effects of elevated $CO_2$ on terrestrial plant ecology, specifically focusing on belowground structures.

## CARBON CYCLE

The carbon cycle is one of the most dynamic and important biogeochemical cycles on Earth. Carbon is the backbone of life and plays a dominant role in nearly every aspect and process in all ecosystems. The largest pool of carbon dioxide resides in the ocean; however, the largest flux exists between the atmosphere and vegetation and oceans. Schlesinger (1991) suggested terrestrial vegetation could potentially consume each molecule of $CO_2$ within organic carbon in approximately 6 years, without any further atmospheric inputs. The atmosphere contains approximately 720 x $10^{15}$ g C with a mean residence time of nearly 3 years (Schelsinger 1991). Natural variations exist within atmospheric $CO_2$ concentrations due to regional, seasonal and mixing variations. Seasonality accounts for 50% of the variation in $CO_2$ in the northern hemisphere due to spring/summer growing seasons of vegetation and the resulting photosynthetic carbon fixation (D'Arrigo et al. 1987). In the southern hemisphere, $CO_2$ concentration variability is accounted for by ocean absorption and algal growth (Schlesinger 1991).

Over the past 200 years, humans have altered the steady state cycling of carbon in the biosphere. In effect, mankind has taken control of the carbon cycle (Fig. 2), changed its processes, re-routed it, and in some cases created and destroyed its functional pathways (Janzen 2004). Ecosystem models suggest oceanic processes absorb 107 x $10^{15}$ g C $yr^{-1}$; however, the ocean surface releases 105 x $10^{15}$ g C $yr^{-1}$. Schlesinger (1991) calculated that if all carbon emission halted, the oceans could sequester and therefore balance the carbon cycle again. The oceanic carbon pool is estimated to contain 38,000 x $10^{15}$ g C with a mean residence time (MRT) of nearly 350 years due to low levels of deep water mixing (Schlesinger 1991). Carbon sediment burial (0.1 x $10^{15}$ g C $yr^{-1}$) and surface flow (0.4 x $10^{15}$ g C $yr^{-1}$) have little effect on balancing the carbon cycle.

Plant biomass accounts for about 560 x $10^{15}$ g C, with 1,500 x $10^{15}$ g C in the soils (Schlesinger 1991). Gross primary production (GPP) removes nearly 120 x $10^{15}$ g C $yr^{-1}$ from the second largest carbon pool, the atmosphere (720 x $10^{15}$ g C) (Schlesinger 1991). However, plant respiration returns nearly half that amount (60 x $10^{15}$ g C $yr^{-1}$) back into the atmosphere with an average MRT of nine years. Soil respiration and organic matter decomposition also release about 60 x $10^{15}$ g C $yr^{-1}$ (Schlesinger 1991). Land use

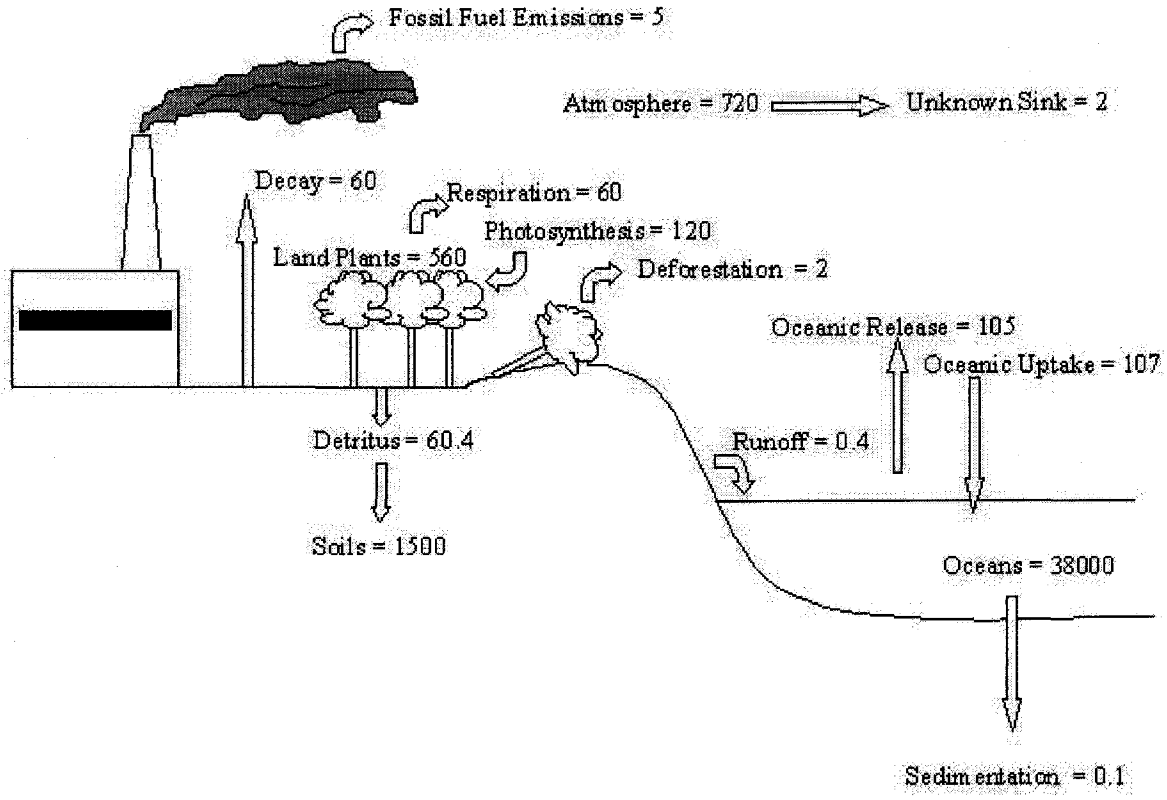


FIG. 2. The global carbon cycle with major annual transfers expressed as $10^{15}$ g C yr$^{-1}$ (modified from Schlesinger 1991, 1995).

change, particularly deforestation/land clearing and cement production, is releasing $CO_2$ (1.8-2.0 x $10^{15}$ g C $yr^{-1}$) into the air and also reducing the amount of carbon fixation, thus lowering GPP (Schlesinger 1991).

Carbon dioxide release from fossil fuels is estimated to be 5 x $10^{15}$ g C $yr^{-1}$ (approximately 0.7% per year) (Rotty and Masters 1985). Interestingly, only 0.4% (1.5 ppm or 58% of fossil fuel release) is actually present in the atmosphere and only 30-40% (1.6 x $10^{15}$ g C) present in the ocean (Tans et al. 1990, Schlesinger 1991). Using the present values of carbon pools and fluxes, the data implies that the carbon cycle is unbalanced and might contain an unaccounted sink in the system containing nearly one billion tons of C $yr^{-1}$ (2.2 x $10^{15}$ g C).

| Net Emissions | | | = | Net Changes in the Carbon Cycle | | | | |
|---|---|---|---|---|---|---|---|---|
| Fossil Fuel | + | Destruction of Land Vegetation | = | Atmospheric Increase | + | Oceanic Uptake | + | Unknown Sink |
| 5 | + | 1.8 | = | 3 | + | 1.6 | + | 2.2 |

(All values are expressed in $10^{15}$ g C $yr^{-1}$) (Schlesinger 1991)

The existence and magnitude of this missing carbon sink (also referred to as the residual terrestrial sink) has been extensively debated over the past few decades (Houghton 2003, Schimel et al. 2001). Oceanographers suggest that land release and GPP are inaccurate while others suggest that soil and vegetative carbon fixation (or potential stimulation) estimates are too low. Despite arguments accounting for carbon in the biosphere, it is agreed that fossil fuel burning for human use and industry has created a new and large biogeochemical flux that never before existed (Janzen 2004).

Over the past 25 years extensive research has been conducted on the effects of elevated $CO_2$ on plant growth and functioning (Ward and Strain 1999, Korner 2001, Bazzaz and Catovsky 2002, Poorter and Navas 2003). The results of these studies have predicted shifts in dominance within plant communities, changes in resource availability and allocation, and most importantly altered ecosystem services. The necessity for large-scale ecosystem studies on $CO_2$ enrichment is currently being addressed; however, belowground responses have traditionally been ignored or over-simplified despite the important role they might play in carbon sequestration (Mooney 1991, Mooney et al 1991, Korner and Arnone 1992, Day et al. 1996).

## ROOT ECOLOGY

Harper (1991) stated that the evolution of roots represents the most dramatic event in the evolution of the plant kingdom. However, plant roots have historically been the most ignored aspect of plant biology. Ironically, this forgotten portion is likely the most essential component of the entire plant biological system. Root systems are comprised of a congregate of several individual components that together constitute the functional "hidden half" of plants (Wilcox 1968, Bohm 1979, Feldman 1984, Waisel et al. 2002). Plant roots perform numerous biologically significant tasks including nutrient and water uptake, photosynthate storage, carbon compound release for mycorrhizal interactions, structural support and anchorage to name just a few. All of these functions are performed in a heterogeneous soil environment that presents a wide variety of obstacles to roots, constraining their functionality. These include physical (pore size, abrasion etc), chemical (diffusion, ions and buffering), and biological (microbes, parasites/insects and competition) factors.

It is estimated that roots can comprise 40-85% of net primary productivity in some ecosystems (Fogel 1985, Fitter 1987). Despite the importance of belowground structures, little research has been conducted on root systems. The inherent opaque nature of soil and the vast network of roots that are deployed into the rhizosphere make quantification extremely difficult (Fitter and Stickland 1992, Nielsen et al 1997). However, over the past decade advances in technology have permitted a better understanding of the importance of roots to overall plant growth and development. Minirhizotron tubes (Johnson et al. 2001, Day et al. 1996), ground-penetrating radar (Butnor et al. 2003) and glass plate rhizotron techniques (Gross et al. 1992) have allowed excellent, but limited quantification of root growth and development. Despite the difficulties associated with investigating root systems, an ever-increasing body of knowledge about the ecology of root systems has started to emerge (Tierney et al. 2003).

Plant root systems can be divided into two major classes: fine roots and coarse roots. Fine roots are typically defined as those roots with a diameter less than 2 mm, while coarse roots are defined as those with larger diameters. Coarse roots are long lived and associated with anchorage, carbohydrate storage and transport; whereas, short-lived fine roots are associated with water and nutrient uptake (Lyford and Wilson 1964, Lyford 1980, Vogt and Bloomfield 1991, Berntson 1994).

## FINE ROOTS

Fine roots are particularly interesting due to their functional role in acquiring resources for the plant. Studies have shown that fine roots exhibit relatively rapid turnover in the soil. Jackson et al. (1997) calculated the total fine root carbon pool to be 5% of the atmospheric pool and 33% of annual NPP. The growth and maintenance cost for this essential component of the root system can require as much as 50% of the daily photosynthate produced in crop plants (Lambers 1987, Wells and Eissenstat 2001). More importantly, fine root mortality is a major source of carbon and other essential nutrients (e.g. N, P, K, Ca and Mg) in the soil (Cox et al. 1978, Joslin and Henderson 1987, Hendrick and Pregitzer 1992, Gordon and Jackson 2000). Root mortality can deposit 18-58% more nitrogen into the soil than litterfall alone (Vogt et al 1996, Wells and Eissenstat 2001, Hendrick and Pregitzer 1992). In addition, these small diameter roots might be the most prominent sink for new carbon sequestered in terrestrial NPP (Jackson et al. 1997, Higgins et al. 2002). However, the external and internal factors and controls that govern root mortality have yet to be fully understood (Huck et al. 1987, Pritchard and Rogers 2000, Pregitzer 2002, Zobel 2003). Recent work on fine root dynamics suggests the interplay of endogenous and exogenous controls in determining root longevity (Tierney et al. 2003).

Inherently, root dynamics are controlled by environmental factors such as soil temperature (Fitter et al. 1998, 1999), soil moisture (Caldwell 1976, Hook and Lauenroth 1994, Kramer and Boyer 1995) and nutrient availability (Nadelhoffer et al. 1985, Marschner 1995, Gill et al. 2002). Tierney et al. (2003) found that root length was directly correlated with mean monthly soil temperature. Other studies have shown that in northern forests, without water limitations, fine root production peaks in the late summer and early fall when soil temperatures are highest (Burke and Raynal 1994, Steele et al. 1997, Burton et al. 2000, Tierney and Fahey 2001, Tierney et al. 2003). However, this increase in root production with increasing temperature is balanced with increases in root mortality (Forbes et al. 1997, King et al. 1999, Pregitzer et al. 2000).

Not all fine roots die at the same rate. Fine roots appear to show a great deal of variability in longevity with average root life spans ranging from less than one to more than eight years (Harris et al. 1977, Trappe and Fogel 1977, Persson 1978, 1979, 1980, Santantonio 1979, Grier et al. 1981, Keyes and Grier 1981, Joslin and Henderson 1987, Hendrick and Pregitzer 1992). Wells and Eissenstat (2001) found that fruit tree fine roots < 0.3 mm in diameter did not typically survive more than one growing season and fine roots > 0.5 mm did not survive the low temperatures during a winter period. In most deciduous trees, nearly half of the fine roots were lost during periods of leaf senescence (Hendrick and Pregitzer 1992, 1993). Since roots transport large amounts of resources into the soil, the predicted increase in temperature (1-3.5 °C by the year 2100) could have profound influences on seasonal shifts of photosynthates as well as respiration and other essential belowground processes (Houghton et al. 1995, Eissenstat et al. 2000)

The physical nature of roots also controls the longevity of the root system. For example, small diameter roots tend to have lower tissue density and C:N that make them more susceptible to mortality and decomposition (Eissenstat and Yanai 1997, Gill et al. 2002). Gordon and Jackson (2000) found that roots with diameters less than 2 mm typically had higher nutrient concentrations and lower amounts of carbon than larger roots. This size class is of particular interest due to its primary function in resource uptake and exploration (Jensen and Petersen 1980, Cunningham et al. 1989, Comas and Eissenstat 2004).

Tree roots can have a wide distribution in the environment, expanding nearly 20 m from the trunk and to depths that exceed 10 m (Pregitzer 2002). Hendrick and Pregitzer (1992) noted the difference in root distributions with relation to depth and found longer, thiner roots at shallow depths. In northern forests, these shallow root systems (less than 40 cm) can consist of up to 50% of the fine root biomass (Hendrick and Pregitzer 1996). In addition, they found that fine roots closer to the soil surface had a higher turnover rate. In $CO_2$ enriched plots, Day et al. (1996) found enhanced root proliferation near the soil surface (0-12 cm) and again at lower depths (49-61 cm). These layers of enhanced root growth correspond to zones of high nutrient and water availability (shallow) and water availability (deeper). In grassland systems, decreasing root mortality was correlated with increasing soil depth, with more than 45% of the turnover occurring in the moist top 6 cm (Arnone et al. 2000). Also, fine roots found deeper in the soil have lower respiration rates that might correlate to greater longevity. Some studies suggest that roots might acquire different resources in the soil profile at different times in their lifespan, implying alterations in functionality (Gebauer and Ehleringer 2000, Pregitzer et al. 2000). Overall, little is known about the dynamics of deep roots or about nutrient and carbon cycling in lower soil profiles (Hendrick and Pregitzer 1996).

Fine roots have a dual nutritional role in the soil. First, fine roots function as organs for nutrient uptake. Secondly, fine roots have a rapid turnover rate and replenish the soil with carbon and nutrients and account for approximately 49-56% of the total carbon and 48-58% of nitrogen cycled in the soil (Hendrick and Pregitzer 1993). The mean residence time of carbon in fine roots averages 4.2 and 1.25 years for pine and sweetgum respectively and is influenced by a number of environmental factors (Matamala et al. 2003). An essential understanding of nutrient cycling in both soil and fine roots has been limited by inadequate techniques to quantify fine root dynamics (Kurz and Kimmins 1987, Hendrick and Pregitzer 1992, Pregitzer et al. 1995). However, nutrient dynamics of the rhizosphere might be subject to change with increasing levels of atmospheric carbon dioxide.

The effect of increasing $CO_2$ on fine roots has produced a number of interesting and sometimes conflicting results. Arnone et al. (2000) found a significant increase in nutrient use efficiency under elevated $CO_2$. However, Pregitzer et al. (1995) found that increasing $CO_2$ increased fine root length with little change in litter quality despite changes in nitrogen availability; however, mean fine root lifespan decreased. Pritchard et al. (2001) found that rate of root turnover was unchanged by increasing $CO_2$. Increasing $CO_2$ is also suspected in influencing the development and deployment of roots in the soil. For example, low order fine roots have a low carbon construction cost but higher respiration, mortality and decomposition rates than higher order fine roots (Eissenstat and Yanni 1997, Pregitzer et al. 2002, Guo et al. 2004). In addition, root nitrogen concentration and biomass decreases with increasing root order, which has strong implications on the growth rate and mortality of fine roots (Guo et al. 2004).

Increasing $CO_2$ influx and the resulting changes in carbon transfer from the atmosphere through the plant to the roots could influence ecosystem carbon storage beyond what is typically sequestered in biomass and soil organic matter (Pregitzer et al. 1995). In tropical systems, it is believed that carbon will be sequestered deeper in the soil profile where nutrient limitations exist, resulting in slow decomposition and longer mean residence times (Nepstad et al. 1994, Fisher et al. 1994, Fitter et al. 1997). Another important, but poorly understood phenomena involved with carbon transfer is root exudation. Root exudation is a carbohydrate expensive process by which plants release carbon compounds that modify microenvironmental conditions making nutrients labile and encourage mycorrhizal colonization (Norby et al. 1987). A better understanding of fine root systems and their ecosystem services could help in buffering anthropogenic changes in the environment.

## COARSE ROOTS AND ROOT ARCHITECTURE

Coarse roots differ greatly from their fine root counterparts not only in size, but in function and potential ecosystem services. Large diameter roots function in carbohydrate storage, anchorage and transport for the whole plant system. Interestingly, coarse roots are suspected to be a major contributor to carbon sequestration due to their large carbon demands for long-term structural construction. However, only recent preliminary studies with ground penetrating radar (GPR) have been able to non-destructively elucidate the growth, mass, and architecture of these unique structures (Butnor et al. 2001, 2003). It is also important to note that in sclerophyllous shrublands, the root system is comprised of coarse roots, large rhizomes, underground stems and enormous burls or lignotubers (Cannadell and Zedler 1995). This suggests that the belowground ecology of a scrub ecosystem might be dominated by these large root structures, thus implying a potentially large source for carbon sequestration.

To understand roots in an ecological perspective, focus must be placed on the morphology of a root system (i.e. root system architecture) rather than on individual roots (Fitter 1987). This area of study represents one of the major gaps in our knowledge of root biology and focuses on the shape and structure of root systems. Root architecture is defined as the explicit spatial configuration of a root system (Lynch 1995, Nielsen et al. 1997). The spatial deployment of roots is critical for the growth and survival of plants due to the non-uniform distribution of resources in the environment (Nielsen et al. 1997). The shape and structure of root systems determine the ability of plants to capture and move essential resources (Caldwell 1987, Fitter et al. 1991, Thaler and Pages 1998). Past studies on root system architecture have not been successful in developing an architectural classification system due to the high degree of plasticity (Cannon 1949, Weaver 1958, Krasilnikov 1968, Fitter 1987). Until recently, there have not been adequate methods or techniques to study architecture under laboratory conditions (Fitter 1982, 1987). Studies in natural systems still elude ecologists (Fitter and Stickland 1992, Berntson 1994). Even Leonardo da Vinci recognized the importance of plant architecture in his *Notebooks*. He postulated "All of the branches of a tree at every stage of its height when put together are equal in thickness to the trunk (below them)" (Spek 1997).

To define the architecture of roots, one must consider three essential features that control the overall form of root systems: 1) balance of primary and adventitious roots, 2) degree of branching, and 3) plasticity of branching (Fitter 1987). Specifically, root system architecture can be quantified by five main characters: the distribution of branches within the system, or its topology, the lengths and diameters of internodes or links within the system, and the two angles of branching, which together can be regarded as the geometry of the system (Fitter 1987, Fitter and Stickland 1991). The overall architecture results from growth and distribution of branches (axial and radial) (Thaler and Pages 1998). However, the patterns of growth are often dependent on environmental conditions, genetics and their interplay among different species (Grime et al. 1986, Fitter 1991, Zobel 1991, Berntson 1994, Fitter and Stickland 1991).

Elevated $CO_2$ could influence the functionality of root architecture (Nielsen et al. 1994). Rogers et al. (1999) believed that a highly branched architecture will be favored in elevated $CO_2$ environments resulting in a decline in the root systems proportional exploration efficiency. Exploration efficiency (EE) is defined as the volume of soil exploited per unit volume of root or per unit carbon cost (Fitter 1991, Berntson 1994). Therefore, in high $CO_2$ environments, more carbon is available for root growth and production, which will in turn lead to increased deployment and foraging to meet additional resource demands.

Human induced changes on the environment and a global disruption of the Earth's equilibrium will have profound influences on ecosystems. Current studies on terrestrial plant communities have only begun to integrate the plant system as a whole let alone on larger scales. However, it is imperative that we examine the effects of atmospheric $CO_2$ enrichment on often-excluded belowground components of ecosystems in order to understand, predict and potentially reverse global changes.

## STUDY GOALS AND OBJECTIVES

This study will hopefully link a number of critical and fundamental aspects of root ecology in an attempt to understand how elevated $CO_2$ affects root growth and development. Increasing our understanding of root ecological processes (on multiple scales) will increase our understanding of carbon transfer and sequestration into the soil and potentially refine our estimates of the global carbon cycle.

This dissertation is a part of a larger long-term research project on Merritt Island at Kennedy Space Center, Florida. The broader study has examined the impacts of elevated atmospheric $CO_2$ on a nutrient poor scrub-oak ecosystem for the past eleven years through the use of open-top chambers receiving ambient or elevated $CO_2$ treatments. Our study ecosystem is dominated by a periodic fire disturbance. The work presented here expanded on past studies on the belowground contribution to plant productivity and carbon storage. Particularly, this project utilized non-destructive technologies (i.e. ground penetrating radar and minirhizotrons) to better understand this system.

The primary goals and questions of this study were: Does elevated carbon dioxide influence the growth and morphology of roots? Have fine root dynamics (i.e. production, mortality and turnover) changed since initial $CO_2$ fumigation? What environmental and morphological factors control fine root survival? Are there differences in the coarse root biomass between $CO_2$ treatments? Can root architecture be determined with ground-penetrating radar?

I hypothesized that fine root productivity, mortality, and turnover will be higher after several years of $CO_2$ fumigation compared to earlier findings by Dilustro et al. (2002). Also, I hypothesized that large diameter fine roots will persist longer than smaller sized roots in the scrub-oak ecosystem. In addition, fine roots exposed to elevated $CO_2$ as well as those grown deeper in the soil profile will have greater survivorship compared to those grown under ambient $CO_2$ or near the top of the soil profile. I hypothesized that GPR signal strength is correlated to root biomass and can be used to determine coarse root biomass in the scrub-oak ecosystem. Coarse roots continually fumigated with elevated $CO_2$ will also have a greater biomass compared to those treated with ambient $CO_2$ concentrations.

Chapter III presents minirhizotron results on fine root dynamics, including fine root production, mortality and turnover, as well as findings on the effects of $CO_2$ and diameter on fine root survivorship. Chapter IV addresses the effects of elevated $CO_2$ on coarse root biomass by means of ground penetrating radar (GPR) and presents a predictive relationship between mass and GPR imaging. Chapter V examines the ability to quantify coarse root architecture with GPR. Finally, Chapter VI summarizes these results in the context of the role they play in carbon sequestration and terrestrial root ecology.

# CHAPTER II

# SITE DESCRIPTION AND OVERALL EXPERIMENTAL DESIGN

## SITE DESCRIPTION

The study site for this research is located on Merritt Island in Brevard County, Florida ($28^0$ 36' 29" N; $80^0$ 40' 15" W) (Fig. 3). Merritt Island, located on the northern part of Kennedy Space Center (KSC), is a barrier island with topography ranging from sea level to about 2 m above mean sea level. The site is located in a subtropical climate with mean daily temperatures ranging from 22.3 $^0$C in January to 33.3 $^0$C in July. Mean daily minimum temperatures are 9.6 $^0$C and 21.9 $^0$C for January and August respectively (Huckle et al. 1974, Mailander 1990) (Fig. 4). The KSC site averages 131 cm precipitation per year with high year-to-year variability (Schmalzer and Hinkle 1987). The area has a pronounced wet and dry season, with the rainy season occurring from May to October, with the remainder of the year being mostly dry (Mailander 1990) (Fig. 4 and 5). Fire events, usually from lightning, are quite common during dry periods in April and May (Schmalzer and Hinkle 1991). The site experienced a severe drought in the 1998-growing season.

The soils are primarily sandy and dominated by two main types, Paola and Pomello, which were deposited in multiple sedimentation and erosion events during the Ecocene (~240,000 ya). Paola is an excessively drained Entisol (Spodic Quartzpsamment) originating from Eolian sands. Pomello is a moderately well drained Spodosol (Aernic Haplahumod) that was derived from deposited marine sands (Huckle et al. 1974, Baldwin et al. 1980, Schamlzer and Hinkle 1987). The soils located at our site

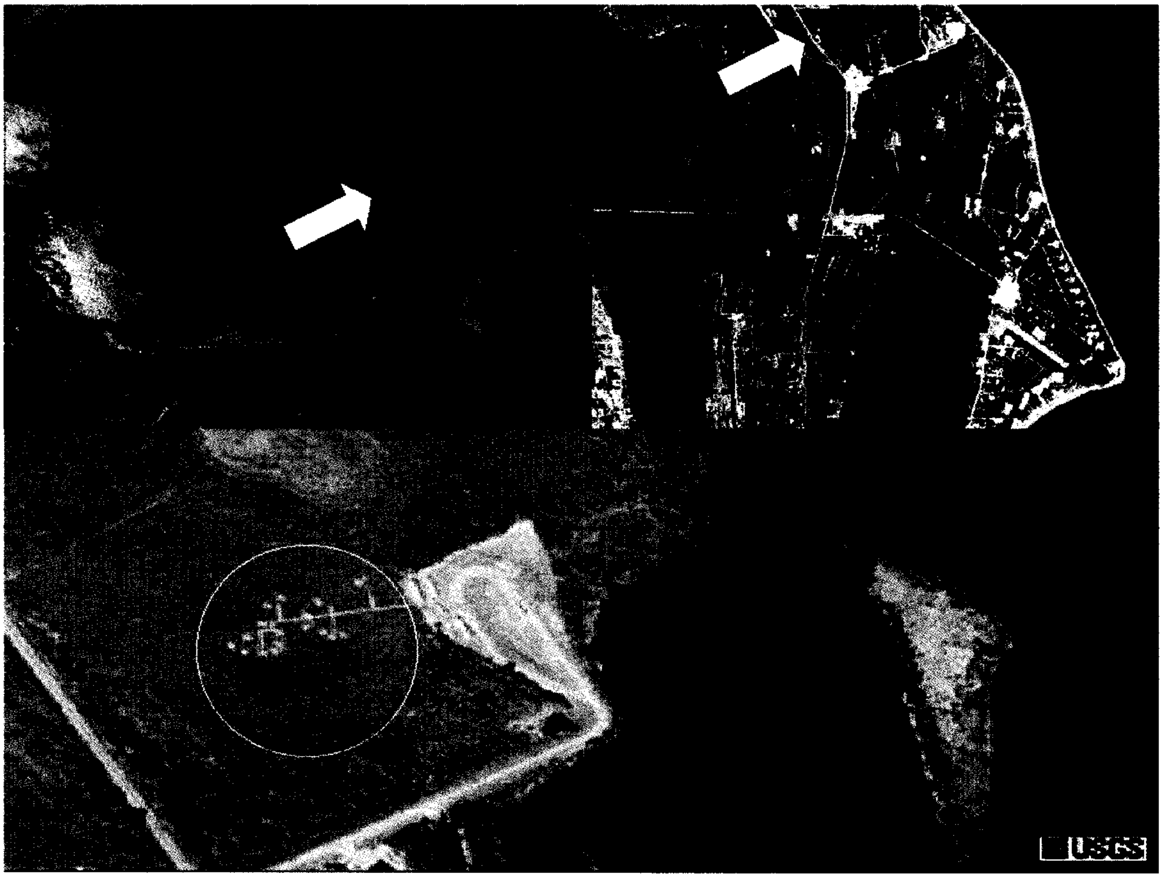


FIG. 3. Study Site on Merritt Island at Kennedy Space Center, Florida, USA.

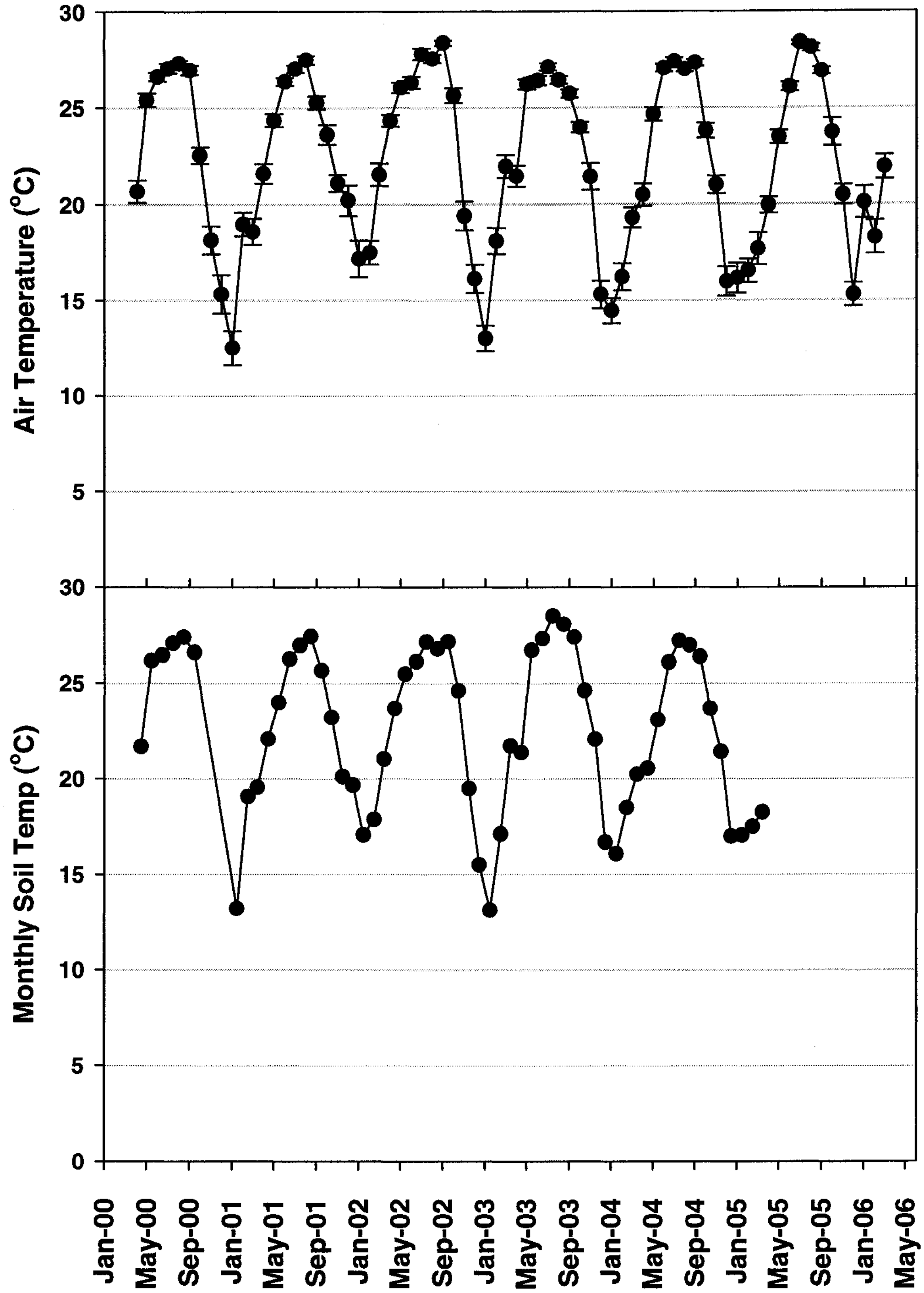


FIG. 4. Average air and soil temperature (°C) at Merritt Island at Kennedy Space Center, Florida. Temperatures were averaged every 15 days between January 2000 and May 2006. Air temperature error bars represent 1 S.E. (T. Powell, personal communication).

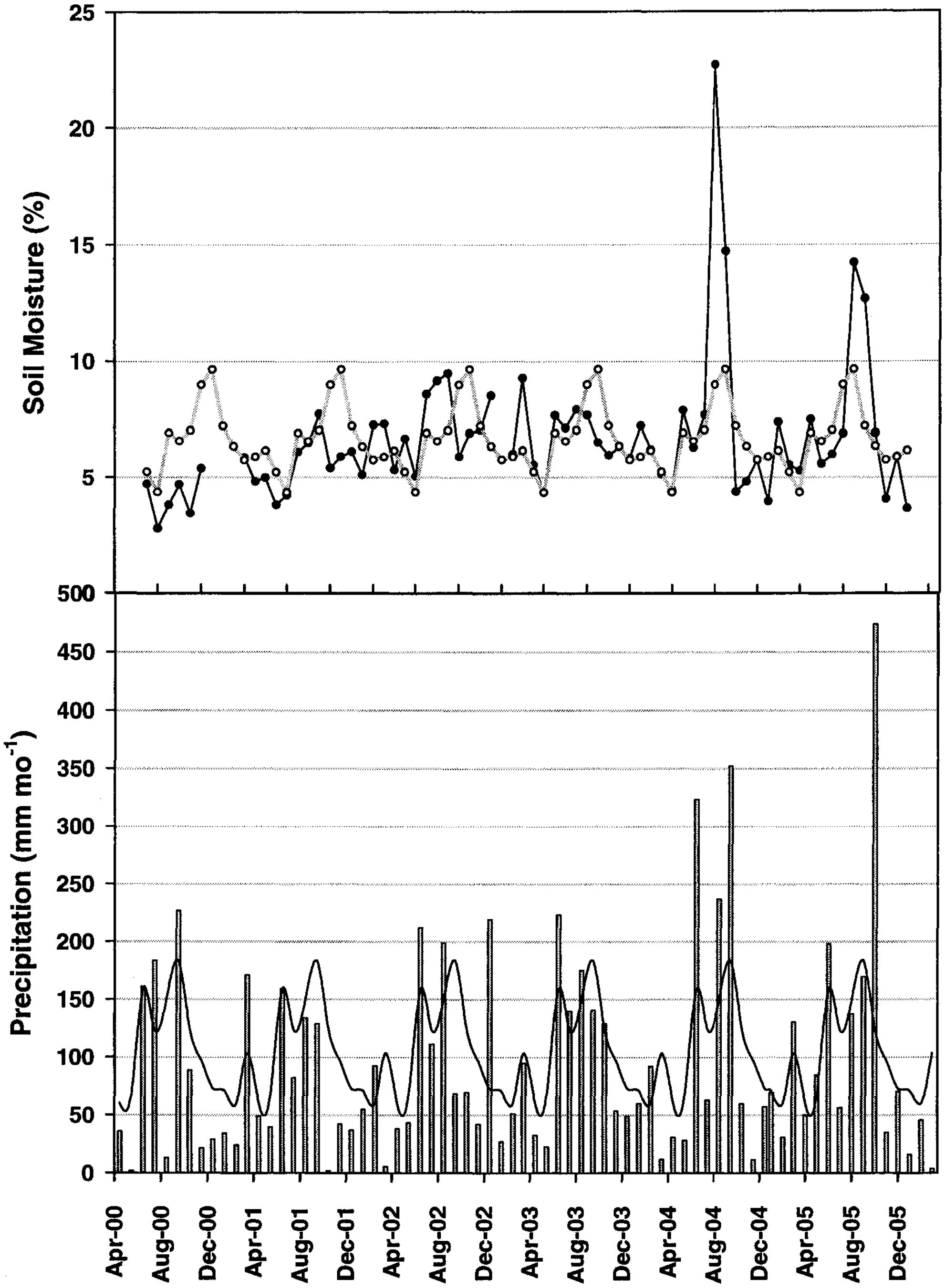


FIG 5: Average soil moisture (%) and precipitation (mm/month) at Merritt Island at Kennedy Space Center, Florida. Data points represent monthly averages between April 2000 and December 2005. Continuous grey line in soil moisture represents a rolling 10-year average trend for each month. Grey bars for precipitation represent monthly averages while the black line represents a 10-year rolling average for precipitation. (T. Powell, personal communication).

are typically acidic (pH 3-4), nutrient poor (most notably nitrogen limited), with low organic matter content.

The scrub-oak community is primarily comprised of clonal, horizontally expanding $C_3$ species *Quercus myrtifolia* Wasd. (76%), *Quercus geminata* Small (15%), *Quercus chapmanii* (7%), *Serenoa repens* (Bartram) Small and *Lyonia ferruginea* (Walt.) Nutt. Recent studies on the clonal structure of dominant *Quercus* species show that the 2 ha experimental site has a significant amount of genetic diversity (Ainsworth et al 2003). This ecosystem was chosen for its woody perennial, deciduous growth form that has a nutrient cycle similar to other forested ecosystems. The small aboveground (and resulting larger belowground) stature of scrub communities makes them ideal candidates for study with open top chambers (Fig. 6). 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).

The scrub-oak ecosystem is historically a fire-controlled system that is maintained with a natural 10-15 year fire cycle (Schmalzer and Hinkle 1991). Previous studies suggest the ecosystem requires at least three years for recovery (Schmalzer and Hinkle 1991). Following a typical burn event, soil pH and the abundance of Al, Cu, and Ca increased while nutrients such as P, K, Mg, Na, and Fe all initially decreased in abundance; however, all properties returned to pre-burn levels within 6-18 months (Schmalzer and Hinkle 1991). Nitrate and ammonium typically have a delayed (~1-2 year) increase in abundance post-burn due to recovery of soil microorganisms, leaching and volatilization (Schmalzer and Hinkle 1991). All of the dominant species resprout following a burn event, with saw palmetto having greater initial growth rates compared to

FIG. 6. Typical eastern Florida scrub-oak vegetation.

oaks. Our study site was burned in February 1996 before treatments began to examine treatment effects from the beginning of a growth cycle (Day et al. 2006).

## EXPERIMENTAL DESIGN

In this project, open top chambers (OTC's) were used to simulate elevated $CO_2$ environments (Drake et al. 1989). OTC's are considered well suited for experimental studies of ecosystems with small stature vegetation similar to that found in scrub oak communities (Mooney and Koch 1994). The OTC's are octagonal in shape with sides 139.9 cm wide, a maximum diameter of 356.6 cm, and a height of 365 cm (Fig. 7).

A total of 16 chambers were fabricated with PVC frames covered with clear mylar sheeting. Carbon dioxide treatments, initiated on May 14, 1996, included eight ambient (~350 ppm $CO_2$) chambers and eight elevated (~700 ppm $CO_2$) chambers. Carbon dioxide treatments were applied with a continuous air circulation system 24 hours a day. OTC's were constructed and treatments applied following a controlled burn of the system in late 1996. Blocks (consisting of one representative from each treatment) were assigned according to similarity of pre-existing vegetation composition (N=8) (Fig. 8).

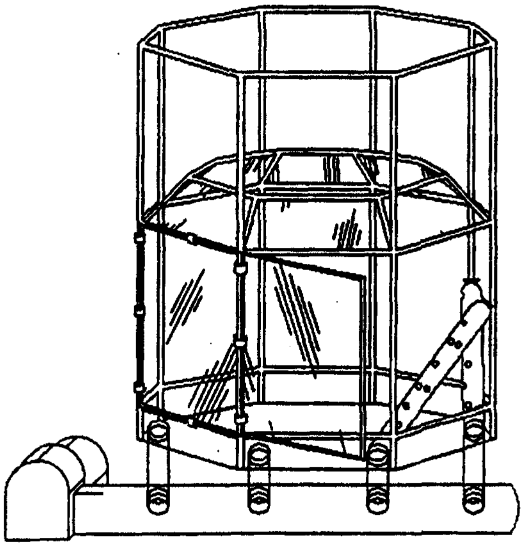
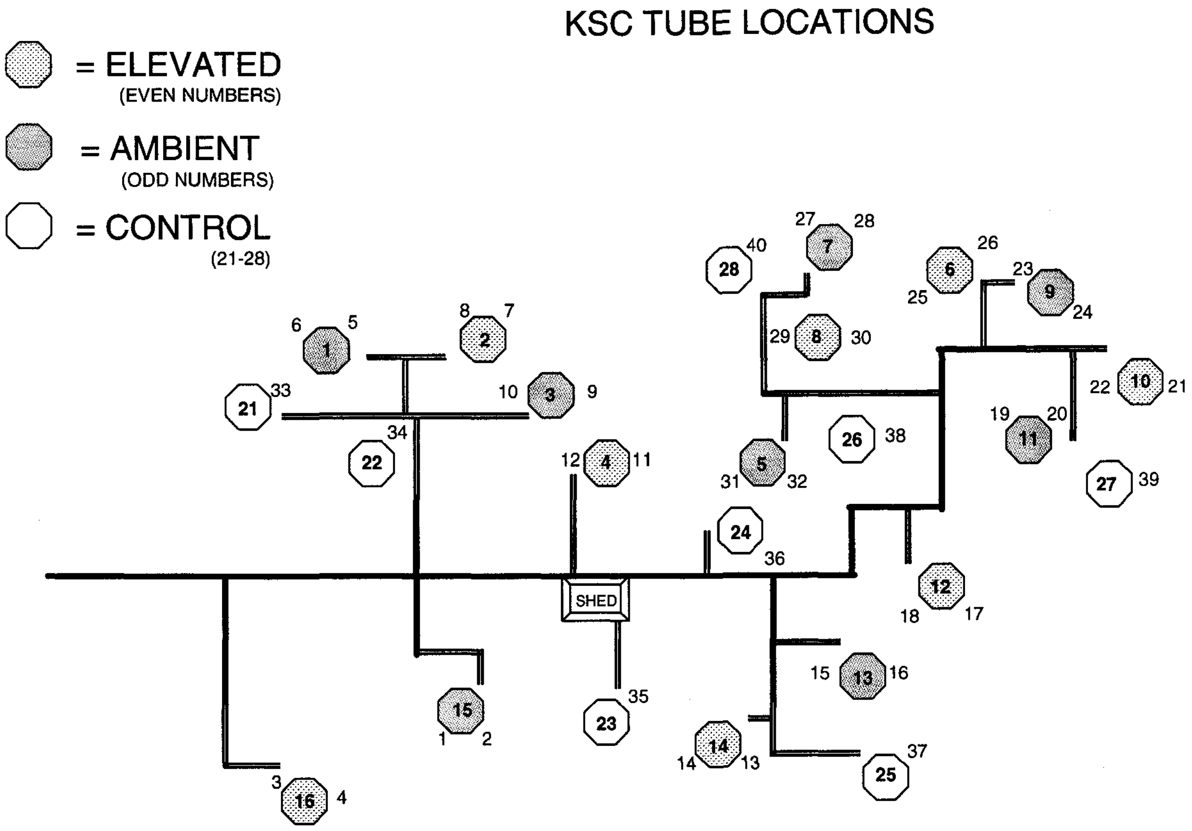


FIG. 7. Open top chamber design; air drawn into the blower exits into the chamber.



Block 1 – Chambers 1, 16, 21
Block 2 – Chambers 2, 3, 22
Block 3 – Chambers 4, 15, 23
Block 4 – Chambers 5, 14, 24
Block 5 – Chambers 12, 13, 25
Block 6 – Chambers 8, 11, 26
Block 7 – Chambers 6, 9, 27
Block 8 – Chambers 10,17, 28

FIG. 8. Map of the study site showing the layout of chambers. Elevated = ambient +350 ppm; ambient = ambient; control = chamberless ambient plots

# CHAPTER III

# FINE ROOT DYNAMICS AND SURVIVORSHIP

## INTRODUCTION

The dynamic processes of root production, mortality and turnover ensure fine root functionality by producing new roots for uptake while removing older less efficient, non-functional roots. In addition, fine roots have the potential to serve as the primary means of carbon sequestration within soil carbon pools. Caldwell (1997) suggested that annually nearly 25% of roots are replaced. Jackson et al. (1997) estimated that fine root carbon pools globally equate to approximately 5% of the atmospheric pool. Soils can potentially store nearly three times the amount of carbon than plants; however, the majority of this carbon enters the soil pools via plants (Schlesinger 1997, Dilustro et al. 2002). Therefore, the relative contribution of fine roots is important to global carbon budgeting, although the impact of root production and turnover to long and short-term sequestration is often dependent on temporal scale (Hendrick and Pregitzer 1996). Fast turnover means rapid carbon accumulation in the soil; whereas, long turnover periods mean slower sequestration. However, the processes and mechanisms are poorly understood.

A dominant control over fine roots lies in their survivorship or vitality. Recent fine root studies indicate fine root age, birth date, season, soil conditions, climate, nutrient availability, mycorrizhal infection and herbivory influence survival or longevity (See Ruess et al. 2003 for a summary). Root lifespan is quite variable depending on environmental conditions and ecosystem type, but typically fine roots persist from a few weeks to a few years (Eissenstat and Yanai 1997). In forested ecosystems, the average root turnover ranges from 0.348 – 0.362 $yr^{-1}$ in NC and Michigan (Wentz and Chamie 1980, Saterson and Vitousek 1984, Martinez et al. 1998). In a pine dominated system, Johnsen et al. (2005) found that root turnover was very slow and that 0-2 mm diameter roots had a mean residence time of 4-5 years compared to the predicted few weeks – 3 years range. The dynamic relationship that roots have with the rhizosphere and soil supports the concept that roots have different traits than their aboveground leaf counterparts; therefore, root lifespan is not correlated to leaf lifespan (Withington et al. 2006).

Root diameter also controls the survivorship of roots in the soil. Fine roots are typically more dynamic than coarse roots (Pregitzer et al. 1993, 1997). This leads to problems in accurate measurements. Soil cores are better for sampling larger roots; whereas, minirhizotrons are better at capturing smaller, more dynamic roots (Tierney and Fahey 2002). Mean turnover time for fine roots in forested ecosystems typically range from several months to several years for roots < 10 mm diameter, and finest roots (described as < 2 mm in diameter) persist 5 months to 2 years with an average of 10 months (Gill and Jackson 2000). Tierney and Fahey (2002) determined that root diameter and survivorship were related and that the probability of survival increased 43% with each 0.1 mm increase in diameter. Wells and Eissenstat (2001) indicated that fine root longevity was inversely related to diameter. The definition of a fine root (i.e. size) and selection of categorical size classes is a central and problematic issue for comparisons with other studies.

In addition to diameter, soil depth appears to control root survival. Roots in the upper 20 cm accounted for greater than 50% of the root length mortality between April and September in a northern decidous forest (Hendrick and Pregitzer 1996). Norby et al. (2004) emphasized 79% of sweet gum roots were concentrated in the upper 30 cm of the soil. Five years later the same depth had 63% of the roots; whereas, a three and four fold increase occurred in the 30-45 and 45-60 cm depth classes respectively. In a shrub ecosystem, the majority of root production occurs in the top 10 cm of the soil for the first three years of growth (Trumbore et al. 2006). The role of depth - root production interaction has profound implications for dynamics and carbon sequestration potential. Hendrick and Pregitzer (1996) reported that shallow and deep root mortality were not synchronous and that root mortality following leaf senescence was concentrated at depths greater than 50 cm. This implies that there is a temporal uncoupling between root dynamics within and among soil depths (Hendrick and Pregitzer 1996).

Over the past few decades, the effects of increasing $CO_2$ on root dynamics have received growing attention, though many of the results are conflicting. Pregitzer et al. (1995) found that elevated $CO_2$ increased root lifespan and growth rates in northern hardwoods. Conversely, Berntson and Bazzaz (1997) found that increasing $CO_2$ decreased root lifespan and therefore increased root turnover in *Betula papyrifera*. The impacts of elevated $CO_2$ might be dependent on additional environmental factors such as nutrient availability. Pregitzer et al. (1993) indicated that longevity might be inversely related to the duration of the resource supply. Therefore, lifespan would be increased if construction costs relative to maintenance costs are high and if the nutrient uptake rates are low (Eissenstat et al. 2000). However, many studies of turnover and survivorship are limited to a single growing season and lack detailed resolution.

The primary objective of this study was to examine fine root production, mortality, and survivorship in a scrub-oak ecosystem after 8 years of $CO_2$ fumigation. Shrublands represent one of the least studied and poorly understood ecosystems with respect to root dynamics (Lauenroth and Gill 2003). Specifically, we hypothesized that 1) fine root productivity, mortality, and turnover would be higher after several years of fumigation compared to early in the study, 2) larger diameter fine roots would persist longer than smaller sized roots in the scrub-oak ecosystem, 3) fine roots would persist longer with increased soil depth, and 4) fine roots exposed to elevated $CO_2$ would persist longer.

## METHODS

### *Root Measurements*

In early March 1996, two butyrate minirhizotron tubes (5.7 cm diameter) were installed in each of the 16 chambers for a total of 32 minirhizotron tubes. The tubes were installed at a 45° angle to the soil surface to a sampling depth of approximately one meter. To prevent light and water from entering the tube, the portion of the tubes extending above the soil interface was blackened and capped. Each tube was etched with 160 numbered frames (9 mm x 13 mm) along the side. Images were taped on Hi8 (mm) videotape with a Bartz Technology® BTC-2 minirhizotron camera system (Bartz Technology Co., Santa Barbara, CA, USA). The root images were captured, digitized and analyzed using ROOTS® version 2.2 (Michigan State University Remote Sensing Laboratory) to identify phenological state and measure fine root length and width. The ROOTS® software permits the comparison of individual roots between sampling periods to determine changes in length and persistence through time (i.e. survival and cohorts).

Root length density (RLD) is defined here as root length per area observed (Dilustro et al. 2002). Measurements were initiated in September 2002 and continued every three months till June 2004 when repetitive hurricane activity made it impossible to maintain the three-month measurement regime.

These measurements on root dynamics were compared to those taken during the initial three years of the study (Dilustro et al. 2002). Similar to that study, root production is defined as the new root length between sampling dates. Root mortality is defined as the measured root length at time t that has disappeared at time *t*+1. All calculations for fine root dynamics were made using data with approximately a 90-day sampling interval and are expressed as root length per area per day (mm $cm^{-2}$ $day^{-1}$).

Turnover was determined by following roots and cohorts over the duration of the experiment and interpreting the disappearance of roots as turnover. This measure was calculated on an individual root basis by computing the fraction of measured length (loss) of new roots between time t and time *t*+1. Complete disappearance of an individual root was computed as 100% turnover. If a root persisted to the next sampling period there was no mortality. This calculation on each individual root removes any bias associated with the effect of absolute root length in computing life span. The life span of roots observed in only one sampling period was assumed to be one day. Turnover is presented here as fractional root loss per day. The initial measurement date (September 2002 cohort) was used to determine the standing RLD crop and was censored since the date of birth could not be determined.

The effects of $CO_2$ enrichment on fine roots were tested using a repeated measures ANOVA. Turnover was analyzed using cohort analysis (Hendrick and

Pregitzer 1993). Frames were pooled into eight vertical depths. Depth and date were not independent, and thus were analyzed as repeated measures. The MIXED procedure was used in SAS (SAS Institute 1990) with chamber as the random effect and the fixed effects were $CO_2$ treatment and depth. Blocking was effective due to the f-values greater than 1.0 for the repeated measures analysis. The ANOVA tables calculated by PROC MIXED lack the separate error term due to the estimation of the covariance parameters via the maximum likelihood approach (Khattree and Naik 1999). When the three way interactions were calculated to be strongly non-significant ($Pr > F = 1$), the three-way interaction was removed from the model to conserve degrees of freedom (Khattree and Niak 1999).

### *Fine Root Survival*

Fine root variables, including $CO_2$ treatment, diameter, length and soil depth, were examined to determine effects on survivorship. Using measurements from the minirhizotron study, root diameter was determined and divided into 3 classes (< 0.1, 0.1 – 0.2, and > 0.2 mm). Fine root length was divided into 5 classes, which included < 0.25 mm, 0.25 – 1.0 mm, 1.0 – 2.0 mm, 2.0 mm – 1.0 cm and > 1.0 cm. Root depth was sub divided into eight 11 cm depth classes. Similar to Coleman et al. (2000), fine root survival was defined as the period from first observation till the period of last observation. Roots that persisted beyond the final observation date were coded as right-censored from the data analysis (Allison 1995).

The effects of the four covariates ($CO_2$ treatment, diameter, length and depth) on fine root survival were determined using a stratified Cox proportional hazard regression (SAS PROC PHREG). Hazard is defined as the instantaneous risk of mortality during a given time interval, conditioned on the baseline survival to $t$ (Allison 1995, Wells and Eissenstat 2001, Anderson et al. 2003). The hazard of a given individual, dependent on time $t$ is the product of two components: the baseline hazard function and the linear function of $k$ covariates. This can be mathematically expressed as

$$h_i(t) = h_0(t)\exp(\beta_1 x_{i1} + \ldots + \beta_k x_{ik})$$

(see Allison 1995 for further explanation). This method uses the partial likelihood approach to estimate the β coefficients (i.e. parameter estimates) that are correlated with each covariate in the model (Cox 1972). PHREG calculates the chi-squared statistic to test the null hypothesis that β is equal to zero. Negative and positive parameter estimates respectively indicate decreases and increases of hazard with increasing covariate value (Wells and Eissenstat 2001). In addition, the hazard risk ratio is reported for each covariate and is defined as $e^{\beta}$. An estimated percent change in hazard associated with change in covariates (controlling for other covariates) can be determined with

$$[\exp(\beta) - 1] \times 100$$

(Allison 1995, Wells and Eissenstat 2001, Wells et al. 2002). In addition, cohort survivorship was analyzed with life-table survival techniques utilizing the SAS PROC LIFETEST. Data were pooled by season among the 2002 and 2004 observations. In this analysis, the hazard function (i.e. age-specific failure rate) was defined as the probability that a root of a specific age group will die (fail) during the given time interval (Coleman et al. 2000).

## RESULTS

### *Fine Root Productivity, Mortality, and Turnover*

In the first three years of the study, a treatment effect was present where fine roots grown under elevated $CO_2$ conditions produced greater RLD than those grown under ambient $CO_2$ conditions (Dilustro et al. 2002). This treatment effect was lost as the mean RLD between treatments converged due to fine root closure (Day et al. 2006). Fine root production varied over time ($p=0.058$; Fig. 9). Early in the study, a significant $CO_2$ treatment effect was present; however, this effect has disappeared with $CO_2$ treatments converging. Interestingly, roots grown under ambient $CO_2$ fumigation had slightly higher mean production and mortality compared to elevated treated roots but this difference was not statistically significant. Additionally, significant variations in fine root productivity were observed over time within the 8 depth classes; however, no distinct pattern emerged (date*depth interaction $p=0.028$). This variability might result from fine roots exploring and filling the soil profile as root-soil carrying capacity is reached (i.e. root closure). Dilustro et al. (2002) found fine root mortality greater in late 1997 in elevated $CO_2$ treatments. Currently, the $CO_2$ treatment effect in root mortality has converged (Fig. 9); however, mortality has increased with increasing depth regardless of $CO_2$ treatment due to increased root growth in the lowers depth classes ($p< 0.001$). Fine root turnover was greater in elevated $CO_2$ treatments in 1997; however, this treatment effect was lost in the latter years of the study and is most likely attributed to root closure. Turnover increased in magnitude (fractional root loss per day), but the overall $CO_2$ treatment effect has disappeared. Similar to fine root mortality, root turnover varied significantly by depth (depth $p< 0.001$). Greatest mortality and turnover were observed in the upper 50 cm of soil.

### *Fine Root Morphology*

Fine root diameter (Fig. 10) increased over the duration of the study with an early

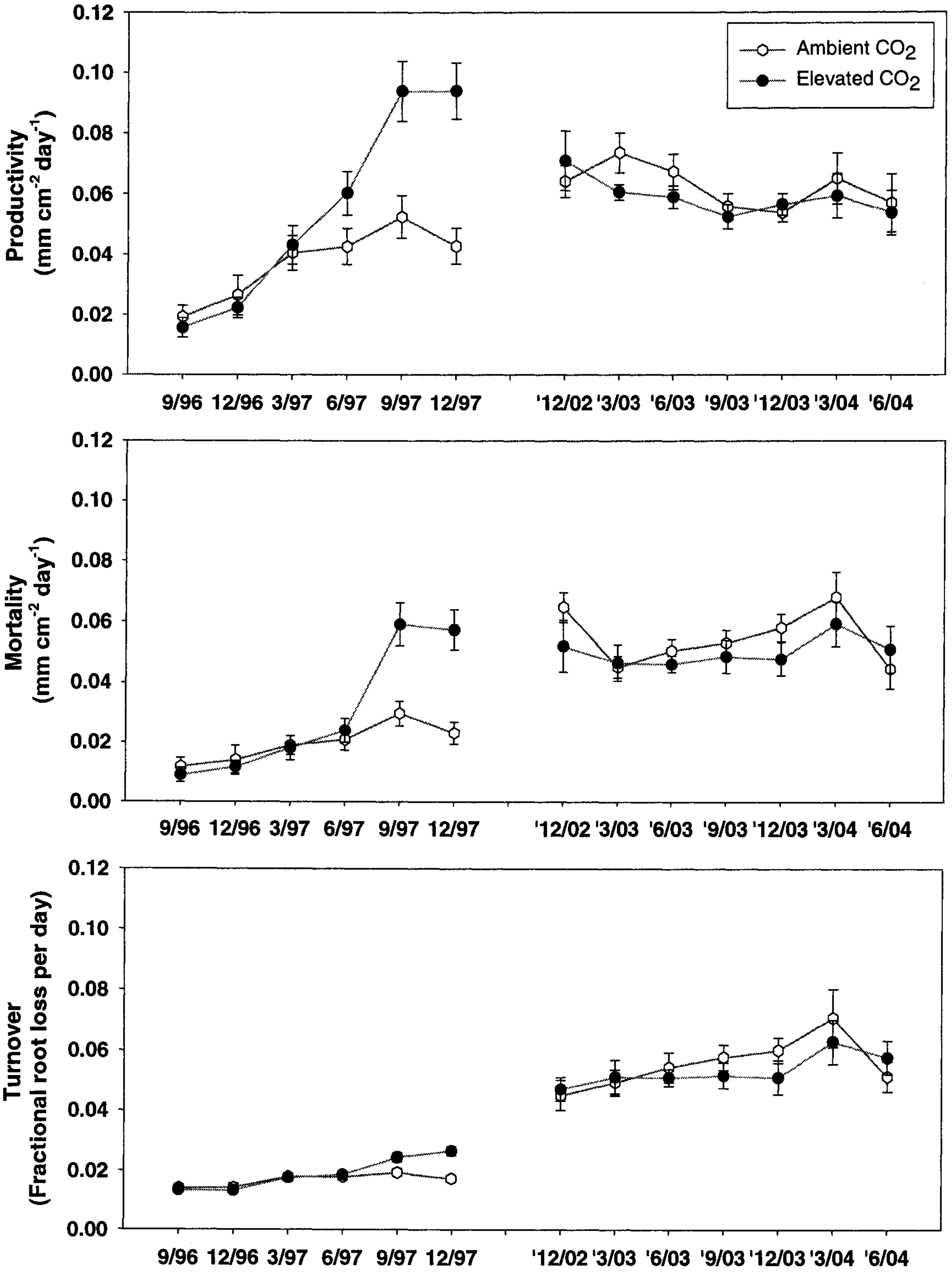


FIG. 9: The effects of $CO_2$ enrichment on fine root productivity (mm $cm^{-2}$ $day^{-1}$), mortality (mm $cm^{-2}$ $day^{-1}$) and turnover (fractional root loss per day). Error bars represent 1 S.E. Fine root data from 1996-1997 from Dilustro et al. 2002.

treatment effect dissipating later in the study (date*treatment p=0.034). Most notably, root diameters were largest in the upper 50 cm of the soil profile ($p < 0.001$) and diameters significantly increased over time among depth classes ($p < 0.001$; Fig. 11). A significant treatment by time interaction was determined over the duration of this experiment ($p < 0.001$) and is most likely a result of the maturing of roots and increased "filling" of the soil with fine roots over time. Root diameters in the uppermost depth class (0-12 cm) were significantly greater in elevated $CO_2$ treatments over time (p=0.035). When comparing just the 2002-2004 dates used in the post root closure fine root measurements, root diameters increased over time and were largest in the upper 37 cm of soil (p=0.001).

### *Fine Root Cohort Analysis*

Initial cohort analysis performed by Dilustro (et al. 2002) indicated that there were more roots per cohort in the elevated $CO_2$ treatments compared to those in the ambient chambers (Fig. 12-A). It was also interesting to note that elevated $CO_2$ tended to produce root cohorts that persisted longer in the soil. Recent cohort examination shown in Fig. 12-B indicates little difference between cohort abundance between $CO_2$ treatments. In addition, there were no significant differences between the persistence in root cohorts among treatments, although the highest mortality occurred on the observation date following study inception. However, cohort abundance and persistence, on average, tended to be greater than in the 2002-2004 study period compared to the findings of Dilsutro et al. (2002). Further examination indicated that the system reached a maximum RLD of 15 mm cm$^{-2}$.

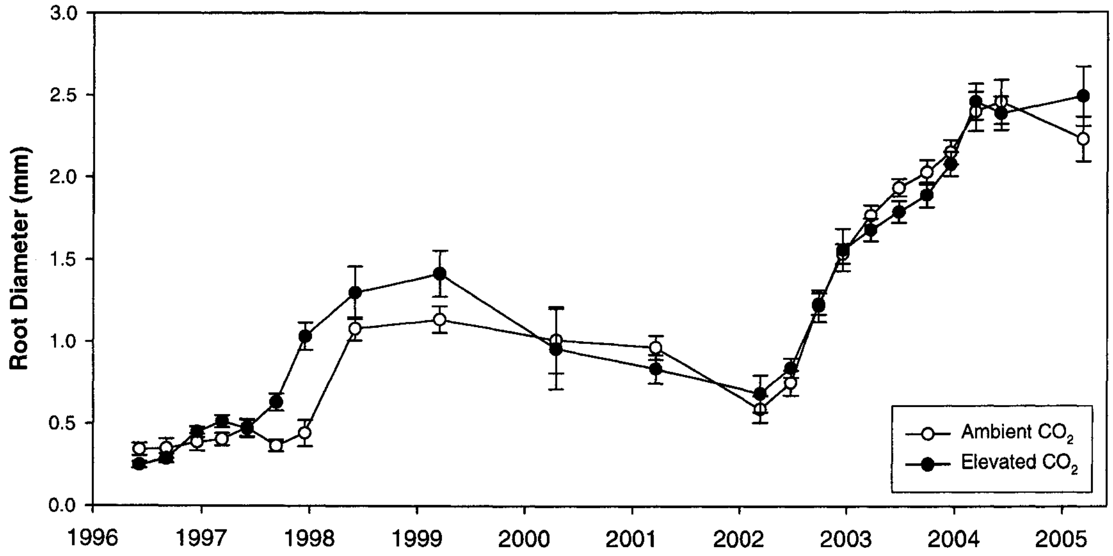


FIG. 10: The long-term effects of elevated CO2 on fine root diameter (mm) in a scrub-oak ecosystem. Values are averages of frames and replicates within treatments. Error bars are 1 S.E.

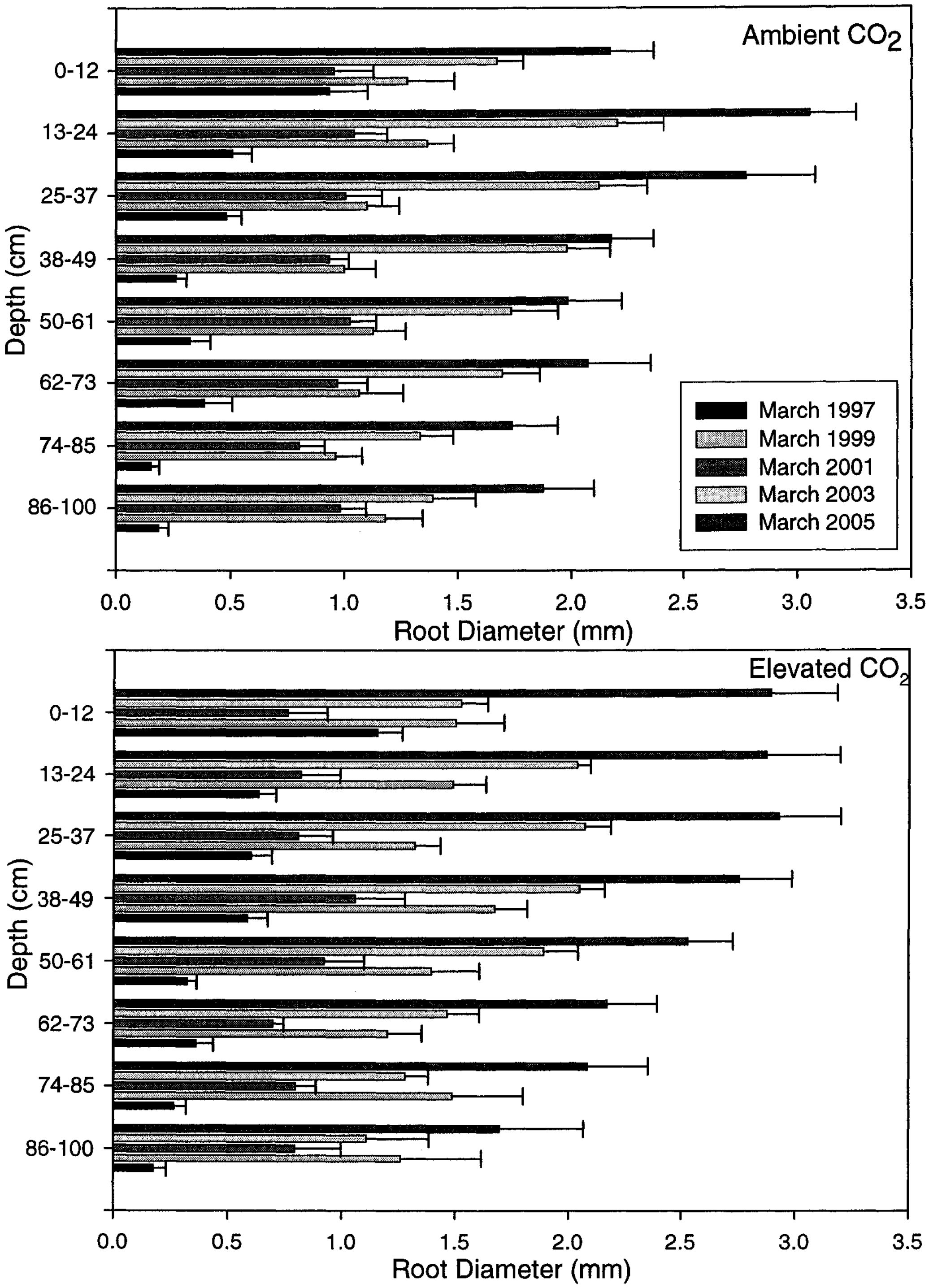


FIG. 11: The effects of soil depth on fine root diameter (cm) for alternating March observations between 1997 and 2005. Total soil depth was 101 cm and error bars represent 1 S.E.

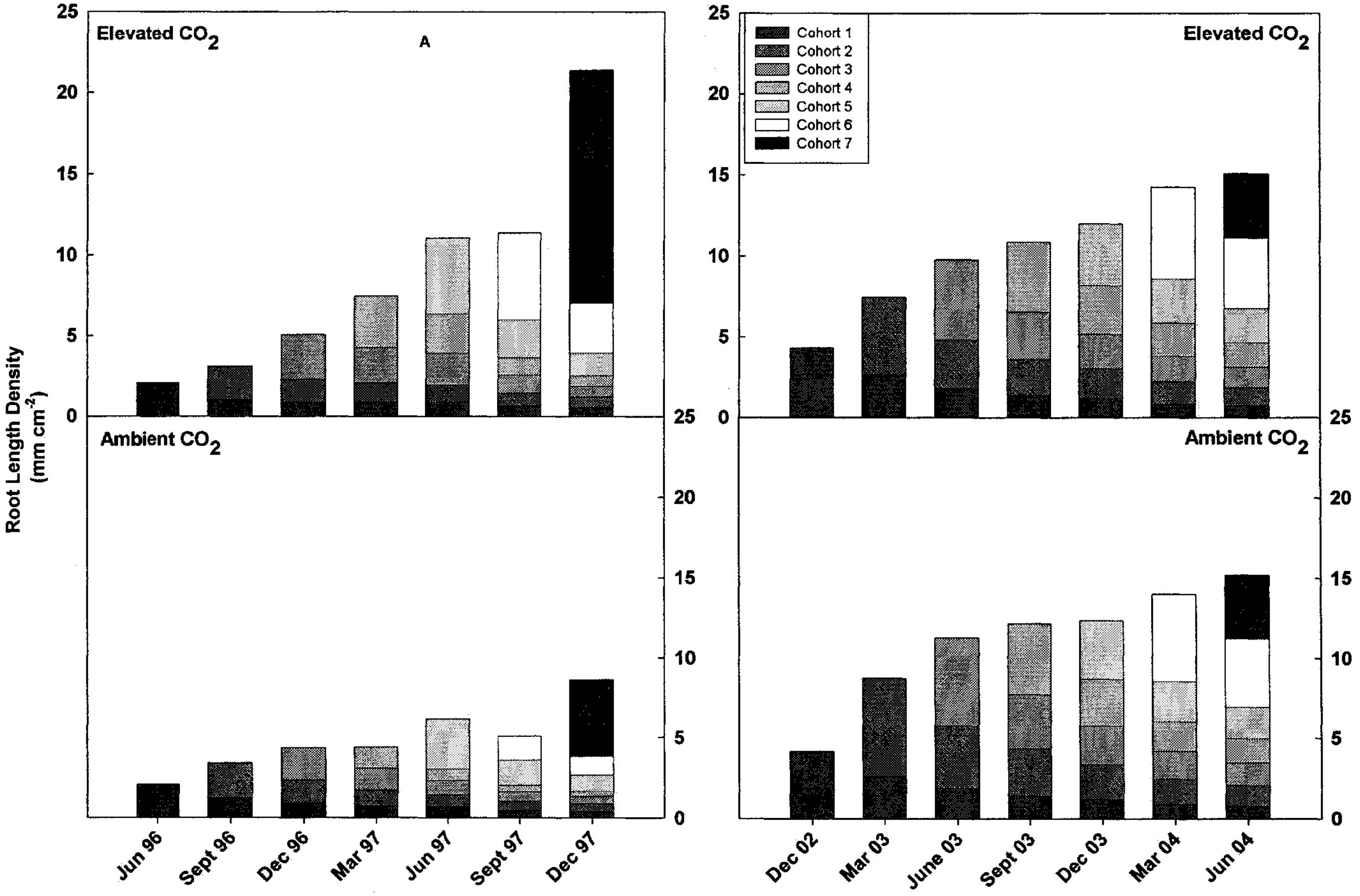


FIG. 12: Root length density (mm cm$^{-2}$) of multiple cohorts in the elevated and ambient CO$_2$ chambers during A) the initial two years, and B) again five years later in the 11-year study. Values are averages over the entire length of the minirhizotron tube and replicates within treatments. Error bars are 1 S.E. Different shades represent different cohorts through time, with the top segment representing the cohort initiated during that sample date. Data from 1996-1997 from Dilustro et al. 2002.

### *Fine Root Survivorship*

Survival analysis can be useful to better understand the various aspects of biotic and abiotic interactions on the longevity of scrub-oak fine roots. Similar to Coleman et al. (2000), I tested the controls on fine root longevity by stratifying $CO_2$ treatment, root diameter, depth class, and root length on measurements take in 2002-2004. Average root longevity was estimated between 177 and 191 days. The survival curves were similar between $CO_2$ treatments and were not significantly different ($P>\chi^2 = 0.377$). Based on my analysis, there was a 16% survival probability for roots persisting after 558 days from inception.

### *Fine Root Survival (Diameter)*

A significant relationship between fine root diameter and survivorship exists ($P> \chi^2 < 0.001$). Fig. 13 indicates that larger diameter fine roots (> 0.2 cm) persist longer than smaller diameter roots. Increased survival of the large diameter fine roots is evident where median longevity occurred at 262 days compared to 96 and 177 days for the < 0.1 and 0.1-0.2 cm diameter size classes respectively. After 558 days of monitoring, 26% of the fine roots survived in the largest size class. The midrange (16%) and smallest (9%) diameter size classes had significantly lower survival after 558 days of growth. Overall, there was a 29% decrease in mortality risk for larger roots or those roots entering the larger size classification

### *Fine Root Survival (Length Class)*

A significant relationship exists between fine root length and survival ($P> \chi^2 =0.018$). Fig. 14 indicates that the smallest size class (< 0.25 mm) had lowest survival

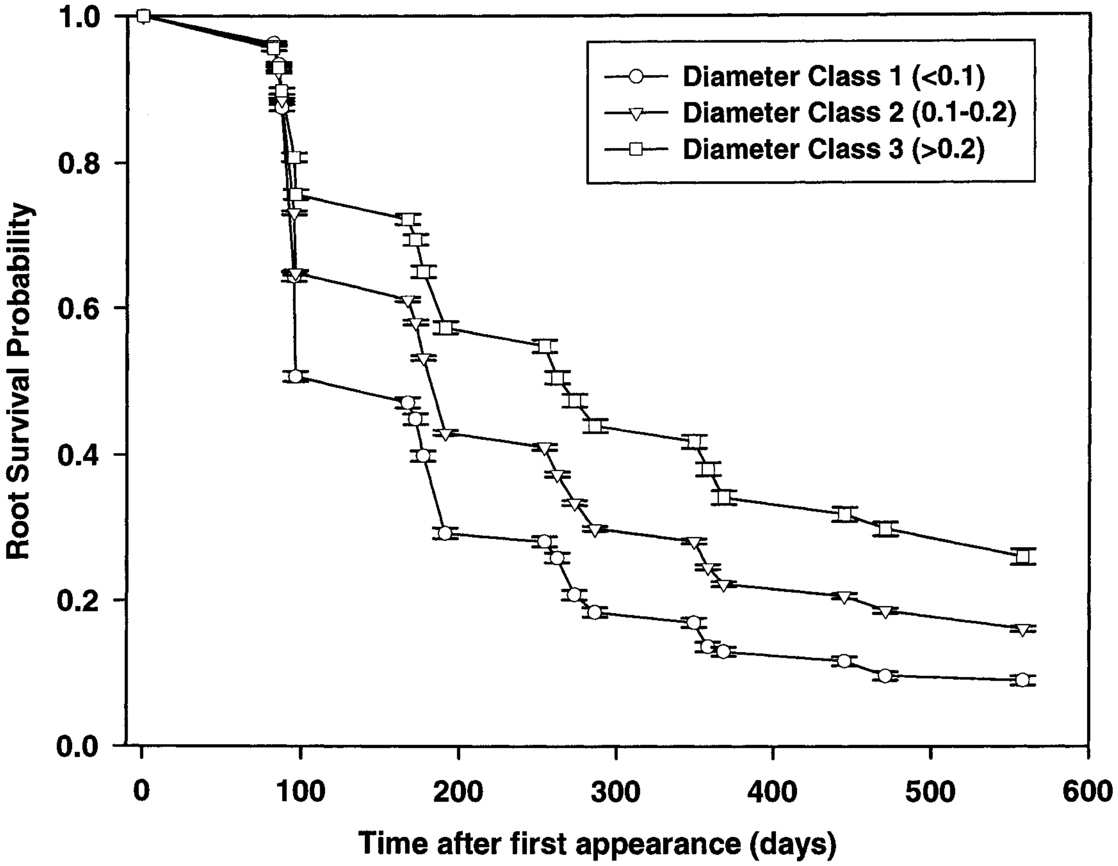


FIG. 13: The effects of fine root diameter size (mm) on root survival probability time (days) during the 2002-2004-study period. Values were determined using PROC PHREG with initial standing root crop left censored from the analysis.

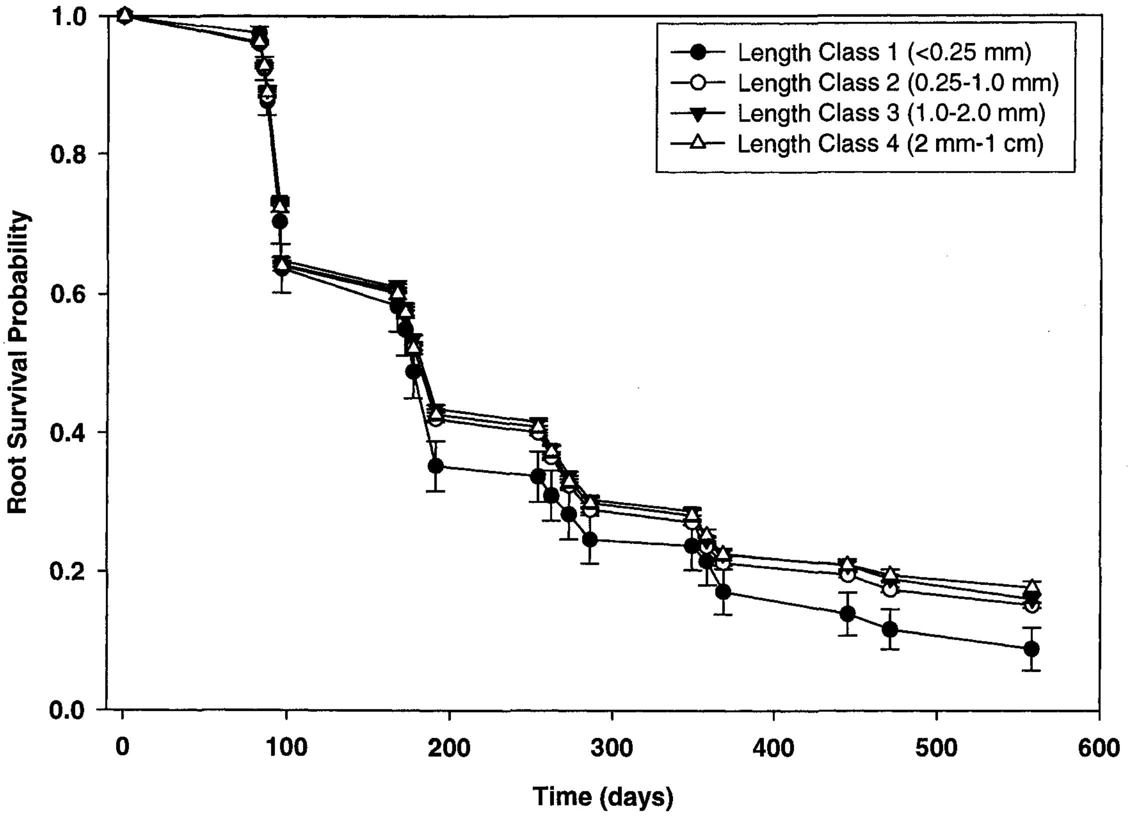


FIG. 14: The effects of fine root length (mm) on root survival probability time (days) during the 2002-2004-study period. Values were determined using PROC PHREG with initial standing root crop left censored from the analysis.

probability. Median longevity was approximately 177 days for all length classes, except for the largest size class (> 1 cm), which was 167 days. This lower median longevity in the largest size class is attributed to lower frequency of observing roots greater than 1 cm long in minirhizotron studies. At the termination of the study after 558 days, the smallest size class had only 9% survivorship, whereas the larger size classes (except the > 1 cm size class) had 15-18% survival. Overall, there was a 3.8% decreased risk of mortality as roots grew into larger size classes.

### ***Fine Root Survival (Depth)***

Fine root survival was also significantly affected by depth (Table 1) in the soil profile ($P > \chi^2 < 0.001$). The hazard ratio indicates a 3.8% decrease in fine root mortality among increasing depth classes. Highest (21%) survival was in the 100-140 cm depth classes, whereas the lowest survival (12%) was recorded in the 0-20 cm depth class.

### *Cohort survivorship*

The impact of season on fine root appearance was examined with a hazard function (Fig. 15). This function is also known as the age-specific failure rate (Coleman et al. 2000, Lee 1992). High hazard function represents high mortality in the fine roots. During the 2002-2004 analysis period, this study indicated that highest risk of mortality occurred in roots born during the spring and summer months. Spring roots had the longest survival rate of all seasons and had the highest risk of failure or death in the fall (191 days later). New roots, which first appeared in the summer observation months (June), typically had greater mortality within the next observational date 95 days later in the fall. Winter born roots had high mortality within 95 days. New fine root growth in the fall did not show a clear trend for critical mortality points, although this is most likely

TABLE 1: Fine root survival for 1 meter depth analyzed with the PROC PHREG in SAS. Average time in days until mortality is reported with the amount (%) of fine roots surviving after 558 days.

| Depth Class | Depth (cm) | N | Mortality (d) 25% | Mortality (d) 50% | Mortality (d) 75% | Final Survivorship (%) |
|---|---|---|---|---|---|---|
| 1 | 0 – 12 | 3,370 | 91 | 172 | 273 | 12 |
| 2 | 13 – 24 | 3,274 | 95 | 177 | 358 | 15 |
| 3 | 25 – 37 | 3,033 | 95 | 177 | 349 | 14 |
| 4 | 38 – 49 | 2,873 | 95 | 177 | 358 | 16 |
| 5 | 50 – 61 | 2,314 | 95 | 191 | 368 | 17 |
| 6 | 62 – 73 | 1,816 | 95 | 191 | 445 | 21 |
| 7 | 74 – 85 | 1,569 | 95 | 191 | 471 | 21 |
| 8 | 86 – 101 | 1,674 | 95 | 177 | 358 | 15 |

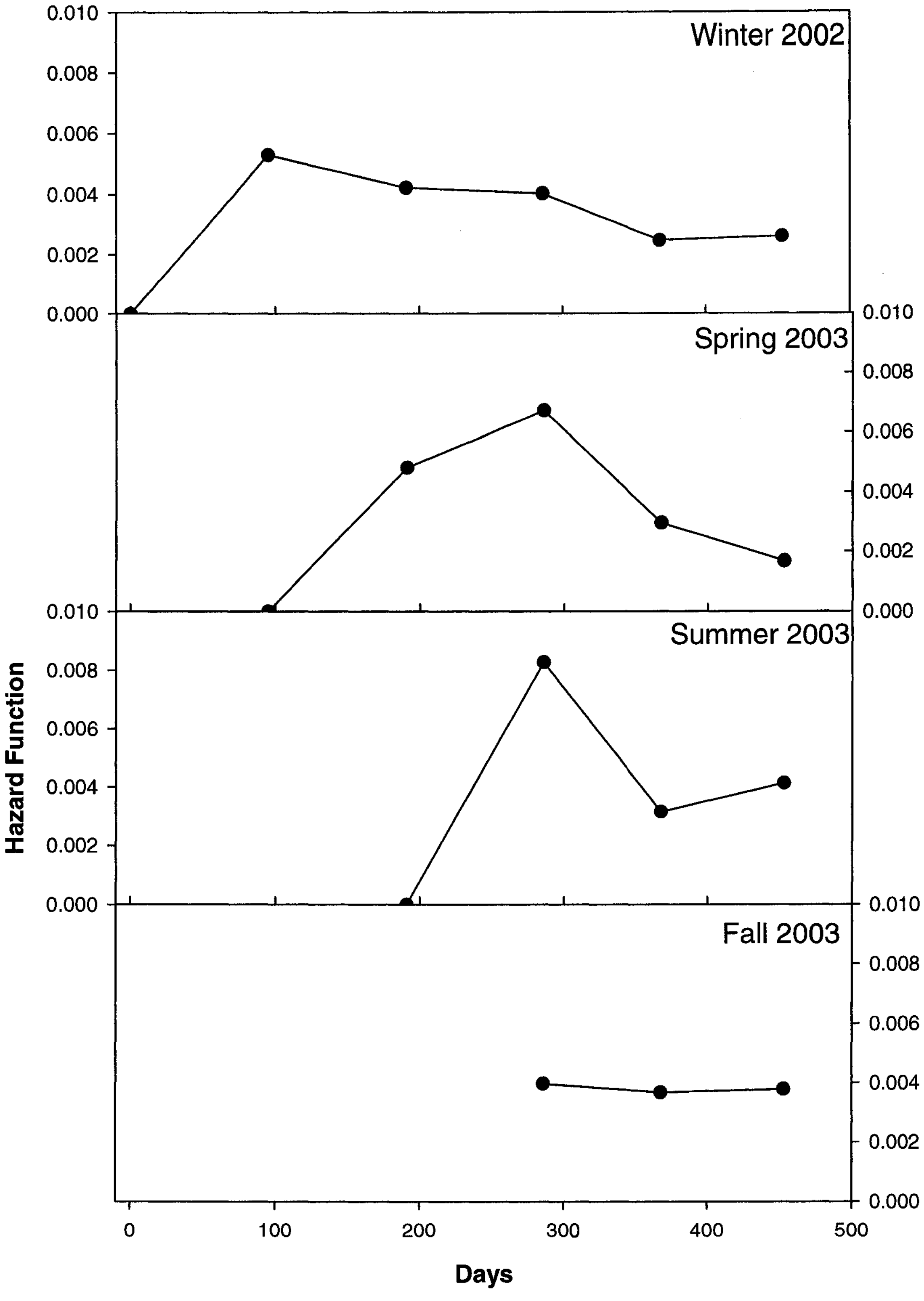


FIG. 15: Estimated hazard function versus lifespan for scrub-oak fine roots stratified by season of appearance. The hazard function is the probability that a root of a specific age will fail during the given time period (age-specific failure rate). Data points represent average midpoints of the 90-day time interval.

due to the lack of fall sample dates (one date compared to two for the other three seasons).

## DISCUSSION

### *Fine Root Production and Mortality*

The goal of this study was to examine the effects of elevated atmospheric $CO_2$ on fine root production, mortality and turnover. Dilustro et al. (2002) found that fine roots were strongly stimulated by elevated atmospheric $CO_2$ during the initial three years of the study. However, I found that this significant $CO_2$ effect had dissipated, resulting in a convergence of fine root growth and mortality among the elevated and ambient $CO_2$ treatments. These findings support the results of Day et al. (2006) that a root carrying capacity has been reached, i.e. root closure, in this scrub-oak ecosystem. They indicated that elevated $CO_2$ treatments appear to stimulate RLD allowing fine roots to reach equilibrium faster than ambient roots; however, this stimulation did not affect maximum root abundance.

Although it has taken the ambient $CO_2$ treated fine roots slightly longer to reach equilibrium, a similar maximum growth was achieved (averaging approximately 0.07 and 0.06 $mm/cm^2/day$ for root production and mortality respectively). Matamala et al. (2003) found that NPP increased 21% during the initial three years of $CO_2$ treatment in sweet gum that resulted in a carbon shift of 12-16% of the NPP from woody biomass to fine roots. In a pine ecosystem from the same study, NPP increased 25% with only a 5-7% increase to roots. Hendrick and Pregitzer (1996) documented that >50% of all root length mortality occurred in the top most 20 cm of the soil profile during the growing season and that shallow and deep root mortality wasn't synchronous. This suggests that the upper portions of the soil profile are more dynamic with higher rates of root production and mortality; whereas, roots deeper in the soil have a lower turnover rate. My data suggest that that root mortality decreases with depth, maybe due to fewer temperature extremes and reduced herbivores and pathogens (Wells et al. 2002, Andersen et al. 2003).

Pregitzer et al. (1993) found a 600% increase in fine root productivity with the addition of water and nitrogen in northern hardwoods suggesting that productivity and longevity were inversely related to resource supply. A similar response would not be unexpected in our system since the scrub-oak ecosystem is water and nutrient limited. Hungate et al. (2006) suggested that progressive nitrogen limitation (PNL) has a control on the scrub ecosystem due to increased nitrogen incorporation into biomass resulting in decreased N availability in the soil.

### *Root Turnover and Survivorship*

The third and most critical component of root dynamics is root turnover. Few studies have examined the effects of $CO_2$ enrichment on fine root turnover or longevity (Dilustro et al. 2002). Shrub ecosystems have low root turnover rates averaging 0.44 $yr^{-1}$ (Lauenroth and Gill 2003). Many studies have suggested that $CO_2$ enrichment increases root growth, mortality, and turnover (e.g. Pregitzer et al. 1995). Thus, any enrichment that increases belowground net primary productivity (BNPP) should result in increased root turnover (Lauenroth and Gill 2003). However, survivorship was not significantly affected by $CO_2$ treatment, and this is perhaps additional support for the occurrence of root closure in this system.

Dilustro et al. (2002) found a significant difference in root turnover towards the end of their study in 1997. In the current study, the elevated and ambient $CO_2$ treatments have converged to a common equilibrium (approximately 0.06 fractional root loss per day). The effects of root closure on turnover could imply that soil carbon deposition from fine roots may be at its maximum rate. The rate of carbon storage and utilization by the microbial community is limited by substrate quality and perhaps PNL (A. Pagel, Personal communication). Examination of fine root cohorts indicated that although each individual cohort of root has a higher RLD at first observation (compared to Dilustro et al. 2002), the greatest mortality occurs in the following observation period. In addition, root closure is evident and RLD values tend to reach a maximum near 15 mm/cm$^2$.

Fine root turnover was greatest in the upper 50 cm of the soil profile. A possible explanation for lower turnover with increasing soil depths might be soil moisture. Since the scrub ecosystem is water limited with the water table fluctuating between 2-3 m, deep roots could potentially serve a major role in hydraulic lift to the upper portion of the soil profile (Richards and Caldwell 1987, Dawson 1993). Finally, this regularly disturbed ecosystem has likely developed strategies to ensure that intense fires would not damage carbon reserves for future re-growth. Roots growing deeper in the soil profile could provide additional carbohydrate stockpiles for scrub-oak regrowth post disturbance. Increased root growth deeper in the soil in response to elevated $CO_2$ could also provide the greatest potential for carbon sequestration in less dynamic, more persistent carbon pools (Gill and Burke 2002)

Root length also had a significant impact on fine root survivorship, where smallest length classes had shorter lifespan compared to longer roots. Although not measured, I predict that short, small diameter roots will have median (50%) survivorship of less than 200 days and will effectively "pump" or transfer large amounts of carbon into the soil. King et al. (2002) indicated that 80% of all fine roots fall into a < 1.0 mm size category having a mean lifespan of 166 days. Longevity based on length and diameter has potentially profound implications for sequestration of atmospheric $CO_2$ in a scrub-oak ecosystem. However, environmental variables from each ecosystem will interact in a unique suite of mechanisms controlling survivorship (see Ruess et al. 2003 for review). Recent studies have noted water and temperature as having controls on turnover (Pregitzer et al. 1993, Lauenroth and Gill 2003). Decreases in soil moisture, for example, increased root mortality and therefore turnover (Klepper et al. 1973, Huck et al. 1987).

Root survivorship was also significantly affected by location in the soil profile. Lowest survival time was recorded for roots in the 0-12 cm soil depth class. Other studies have found similar findings with the upper 10 cm of soil having the highest turnover rate and lowest survival of small diameter roots (Joslin et al 2006). Fine roots located 62-85 cm deep in the soil profile had the longest survival time (approximately 21% surviving after nearly 600 days). These data imply that root survival increases with increases in soil depth. These results support earlier findings in this study that root density and dynamics increased over time in the lower portions of the observed soil profile. Increased survival deeper in the soil might be a factor of cooler soil temperatures or increased soil moisture (i.e. closer to the spodic horizon or water table). In addition, increased survival at lower depths could provide a carbon source for regrowth following periodic fire disturbance in this system.

### *Root Diameter and Survivorship*

Fine root architecture is very important to survivorship, especially in lower order roots (i.e. terminal roots) that tend to have the lowest survival rate or in higher order roots that die resulting in loss of all components below that segment (Wells et al. 2002, Gill et al. 2002, Pregitzer 2002). However, current studies note a lack of clear understanding of this trend due to limitations with the minirhizotron technique in quantifying architecture and functionality.

In an idealized system, higher order roots would have a larger diameter and be more lignified, permitting resource conduction in the root versus uptake, thus increasing its "economical" value and longevity (Eissenstat 1997, Andersen et al. 2003). A 29% increase in the survivorship for each increase in diameter class implies that fine root diameter has a strong control in carbon sequestration (i.e. large root diameters result in greater biomass and carbon storage). Tierney and Fahey (2002) indicated that fine root survival increased 43% for each 0.1 mm increase in diameter. In a loblolly pine system, King et al. (2002) observed that approximately 80% of the fine root length falls into the < 1.0 mm diameter size class and had an average lifespan of 166 days. Roots 1.0 – 2.0 mm diameter survived an estimated 294 days. Conversely, small diameter roots which have low tissue density and shorter mean lifespan tend to perform nutrient and water uptake and die rapidly (Eissenstat and Yanai 1997, King et al. 2002). Smaller roots are intrinsically vulnerable to turnover; thus, rapid cycling could provide a pathway for rapid carbon loading into the soil. A clear relationship between diameter and root survivorship is evident; however, it is important to note that smaller diameter roots are more dynamic than larger roots (King et al. 2002).

Tierney and Fahey (2002) found that root survival increased with increasing diameter. With our ecosystem reaching maturity, the dominant plant forms should be placing more carbon belowground and developing longer-lived structures that will support growth following the typical 7-15 year fire cycle. By increasing fine root diameter, scrub-oak can maximize its longevity and potential carbon storage capacity. Large diameter roots (> 2.0 mm) persisted longer than smaller diameter roots. This suggests that root mortality and turnover are lowest for fine roots with greater width and supports similar findings in other ecosystems (Coleman et al. 2000, Wells and Eissenstat 2001, Eissenstat and Yanai 1997). However, large diameter roots are less efficient at water and nutrient uptake and are more involved in conducting these resources (Nye and Tinker 1977, Eissenstat 1992, 1997). Eissenstat et al. (2000) noted that the benefit of roots with short life spans is lost in low fertility. From an economic point of view, smaller diameter roots are less likely to survive in a low fertility site and therefore it is more beneficial and efficient for larger, long-lived carbon investments especially in context of a regularly disturbed ecosystem. In addition, our findings indicate that root diameters have increased over time with increasing depth. This increase in the abundance of larger, longer-lived conducting roots might suggest that root closure is also related to a decrease in resource availability. As a result, larger roots in the upper 50 cm of the soil profile might serve as transporters for water and nutrients foraged in the lower half of the soil profile.

### *Seasonal Survivorship*

The scrub-oak ecosystem on Merritt Island has no observable seasonal fluctuations in belowground RLD. However, seasonal cohort analysis indicates that the greatest mortality (age specific failure rate) occurs in summer cohorts. Roots that were produced in the summer months tend to have the highest mortality in the fall, which corresponds to Gill et al.'s (2002) findings that small diameter fine roots tend to disappear after only one observation date. In addition, the greatest drop in survivorship of cohorts was in the following observation period, which supports the earlier findings of Dilustro et al. (2002). Roots produced in the spring also tend to have the greatest failure rate in the fall. Although our study site has a year-round growing season, there are seasonal environmental patterns, mostly relating to water. Precipitation is greatest in the summer and fall with a distinct dryer season in the winter and spring. A clear relationship as to why the wetter fall observations were a critical hazard point in the survivorship of fine roots could not be established. This apparent lack of pattern in fine root mortality resulting from seasonal controls suggests that factors controlling root death differ from those controlling root production (Eissenstat and Yanai 1997, West et al. 2004). However, the fall sampling date had the highest average soil temperature and that could suggest that soil temperature, not water, controls cohort survivorship. In a black spruce forest in Alaska, roots born in the middle of the growing season had higher survival than roots formed early or late in the summer season or in the winter months (Ruess et al. 2003). However, studies from southeastern savannas and pine forests and northern deciduous forests have observed root production and mortality occurring year round, regardless of season, from carbon reserves (Saterson and Vitousek 1984, King et al. 2002, Jones et al. 2003, West et al. 2003, Hendrick and Pregitzer 1992, West et al. 2004). Like many other studies on root longevity, my sampling regime was limited to once per season, thus limiting the resolution of my interpretation of seasonal survivorship.

### *Conclusions*

This study has supported the view that fine roots are a dynamic and important aspect of the rhizosphere. Coarse roots are less dynamic and were not included in this portion of the study; however, it is important to consider their contribution to the soil and rhizosphere. The scrub-oak ecosystem partitions 14% of its total mass aboveground while the remaining 86% is belowground (Stover et al. 2007). Belowground, 77% of the total mass is in coarse roots, which serve as a major source of carbon storage in the ecosystem and are the source of post fire regeneration. Irrespective of $CO_2$ treatment, fine roots constitute nearly a quarter of belowground mass. Although turnover is expected to be low, I have found that turnover in this scrub ecosystem was approximately 26% per year with approximately 16% survivorship after more than 500 days, implying that this system is more dynamic that previous predictions.

In this study, I hypothesized that fine root dynamics (i.e. productivity, mortality, and turnover) would be higher compared to earlier studies in this scrub-oak ecosystem. Root turnover was greater in the recent years of the study. Root mortality in ambient $CO_2$ treatments increased to match the levels from elevated $CO_2$ treated chambers. Root productivity has declined in the elevated $CO_2$ treated chambers; however, all treatment effects for all three root measurements have converged since the early years of the study. Fine root diameter increased over the course of this long-term study. Larger diameter roots had greater survivorship and were greatest in the top 50 cm of the soil profile. Root survivorship increased with increasing depth, thus supporting the third hypothesis. The final hypothesis indicating that root survivorship would be greater under elevated $CO_2$ fumigation was not supported since there was no significant difference between $CO_2$

treatment survivorship curves. This study has shown that fine roots play an essential part in belowground ecology and there are a number of environmental and morphological controls on root growth, mortality and turnover and therefore survivorship. These controls dominate the scrub-oak's ability to sequester carbon belowground into the soil. The mean residence time of carbon in the system from growth through disturbance and then recovery will help determine the amount of carbon sequestered into the soil pools as well as its mean residence time. This will determine the long-term effectiveness of this ecosystem in managing excess atmospheric carbon.

# CHAPTER IV

# APPLICATION OF GROUND-PENETRATING RADAR TO QUANTIFY THE EFFECTS OF ELEVATED $CO_2$ ON BELOWGROUND BIOMASS

## INTRODUCTION

We know little about effects on larger underground plant structures. Coarse roots and other large belowground structures have been under-represented 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 the 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 in-growth cores, soil cores and pits are destructive, thus hindering future 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. Non-destructive root analysis methods, such as minirhizotron tubes, only elucidate the fine roots within the system (Hendrick and Pregitzer 1992). A major gap in our current understanding of root systems lies with coarse roots.

Ground penetrating radar (GPR) is a thirty-year old geophysical technique that

---

This chapter has been published in Ecology. Stover, D.B., F.P. Day, J.R. Butnor, and B.G. Drake. 2007. Effect of elevated $CO_2$ on coarse root biomass in Florida scrub detected by ground-penetrating radar. Ecology *in press*.

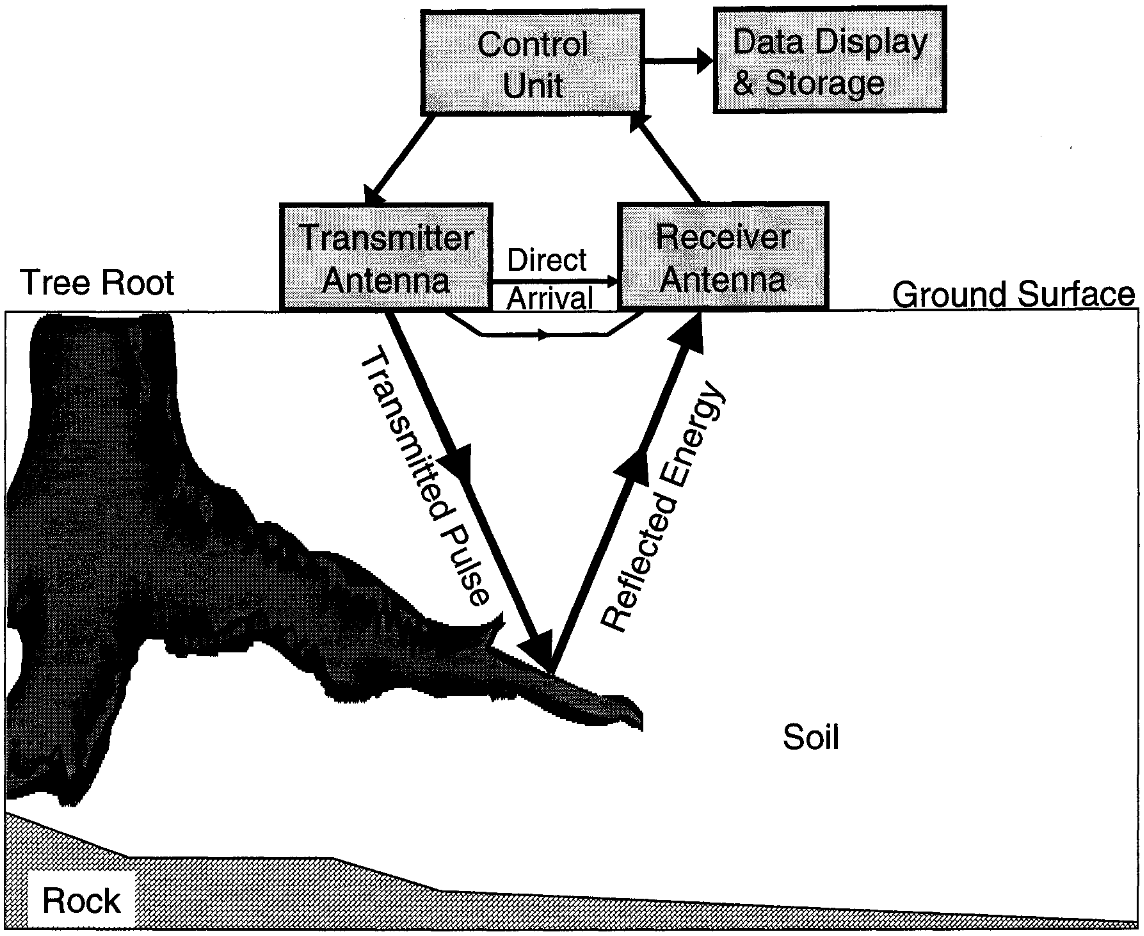


FIG. 16: Basic GPR operation. High frequency electromagnetic waves are emitted from the transmitting antenna complex into the soil and reflect off a buried object back to the receiving antenna. Time and signal strength are recorded by the control unit and produce hyperbolic shaped images on the display screen.

pulses ultra high frequency (approximately 100-1,500 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 with the propagation time (Hruska et al. 1999). GPR technology holds promise for quantifying root mass and architecture due to its rapid, non-destructive acquisition of data (approximately 90 ns collection time per scan) (Butnor et al. 2001). Recent studies with GPR using a 1,500 MHz frequency antenna have been able to distinguish roots with diameters as small as 0.5 cm (Butnor et al. 2001).

The goal of this study was to apply GPR technology to answer questions pertaining to the influence of elevated $CO_2$ on coarse roots in the scrub-oak ecosystem at Kennedy Space Center, Florida. I established a relationship between GPR signal strength and root biomass. Using this relationship, I estimated coarse root biomass in the open-top chambers where destructive measurements cannot be performed. I hypothesized that scrub-oak that has been continually fumigated with elevated $CO_2$ would have greater coarse root biomass than plants treated with ambient $CO_2$ concentrations.

## METHODS

### *Root Biomass Cores*

In June 2005 and 2006, thirty soil core sites were selected in the scrub-oak community adjacent to the chambers. Each core site was scanned with a Subsurface Interface Radar System (SIR-3000) attached to a model 5100 (1,500 MHz) GPR antenna (Geophysical Survey Systems Inc, North Salem, NH) (Fig. 17). Other studies have found a frequency tradeoff between depth of penetration and resolution (Hruska et al. 1999). Low frequency antennas (i.e. 400 MHz) penetrate deeper into the soil but have a low

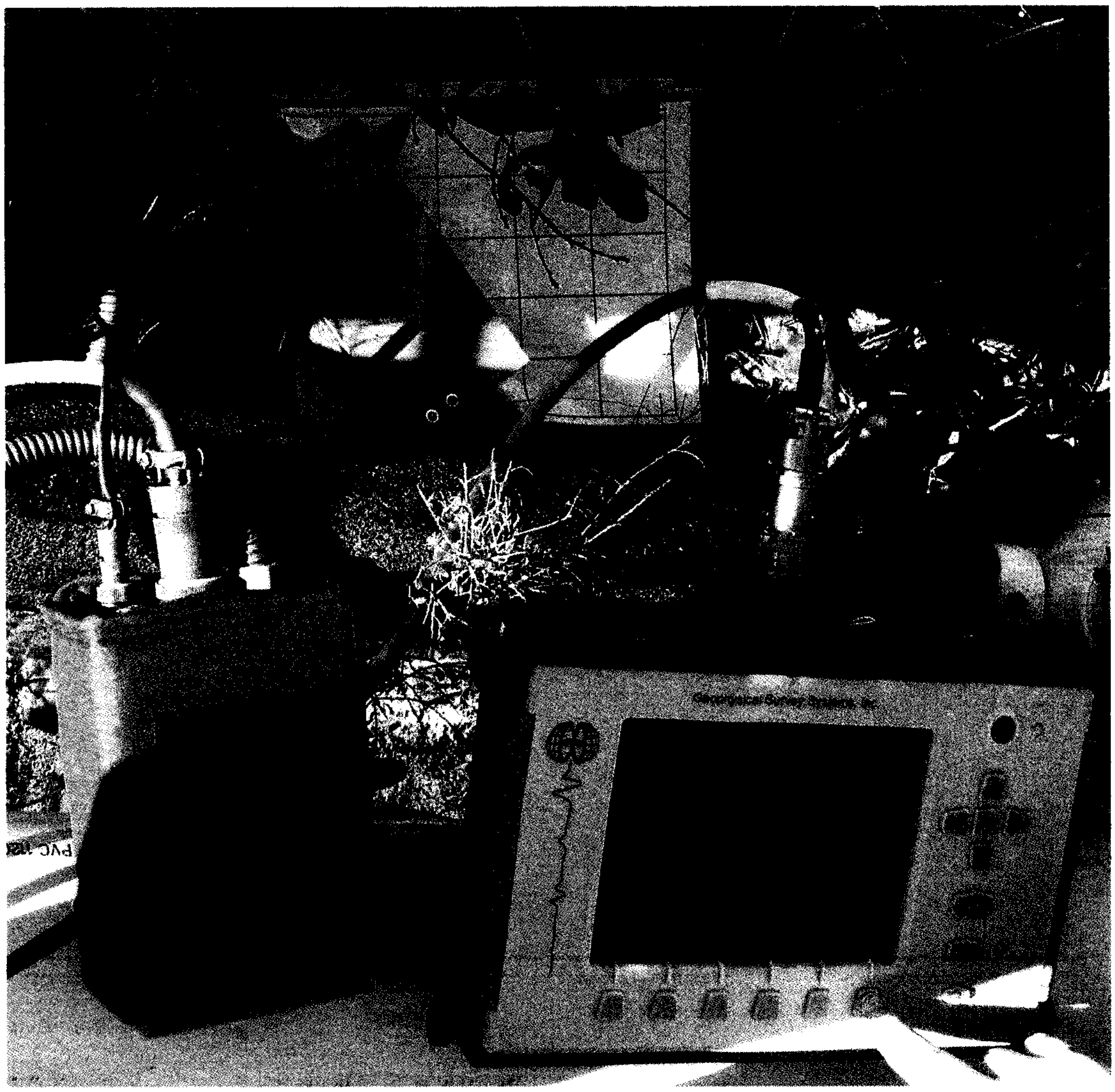


FIG. 17: Subsurface Interface Radar System (SIR-3000) attached to a model 5100 (1,500 MHz) GPR antenna. Antenna and survey wheel (red/grey), control unit (blue) and system computer and output (white/grey).

ability to resolve detailed objects. High frequency antennas tend to be better at resolving detailed objects, but are limited to a depth less than 1 m. The radar unit was calibrated prior to fieldwork similar to Butnor et al. (2003). Each scan was 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, separated into live, dead, and unidentifiable organic matter fractions, and dried at 70 $^0$C for 48 hours before weighing. Large rhizomes, burls, and belowground stems are extremely common in scrub-oak ecosystems; thus, we classified any belowground structures greater than 5 mm diameter as coarse roots.

### ***GPR Image Processing and Root Biomass Correlations***

Core scans were processed with RADAN 6.0 GPR data processing software (Geophysical Survey Systems Inc., North Salem, NH). 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. 18). 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

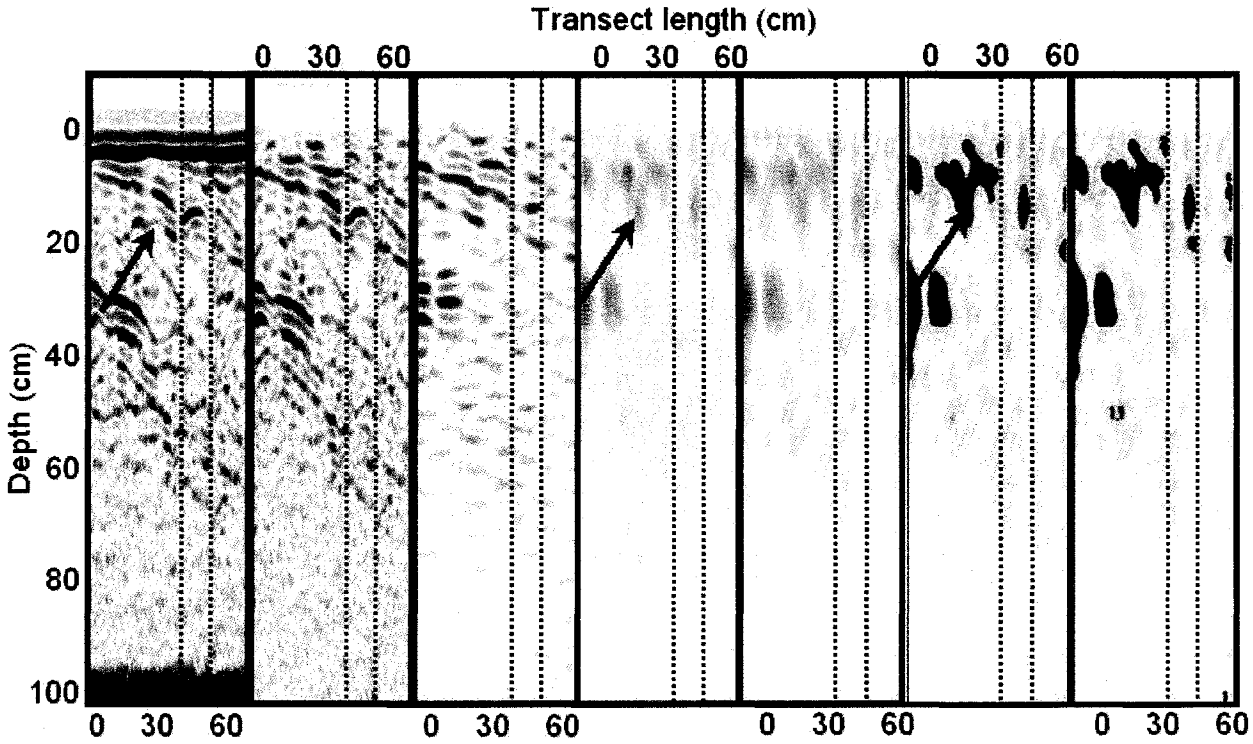


FIG. 18: Representative radar profile and standard image analysis/processing from a scrub-oak ecosystem at Kennedy Space Center, Florida. Arrows point to a common reflector (i.e. root) located at 15 cm depth. Dotted vertical lines show the relative size for an individual core that would be extracted for biomass correlation. A) Raw radar data from a 1,500 MHz GPR antanna; B) Same profile processed in RADAN software with a Finite Impulse Response Filter (FIR) background removal to reduce low frequency noise and bands; C) Kirchoff migration is used to correct the reflectors (objects) position and hyperbolic geometry; D) Radar profile after a Hilbert transformation to express the magnitude of the signal to enhance the subtle objects and reduce false "echoes"; E) Post processed images converted to grayscale; F) Roots are quantified with Sigma Scan Pro Image Analysis software by setting the image pixel intensity threshold to 60-255 range which measures each pixel's intensity on a threshold scale; G) quantification of the image is accomplished by summing the number of pixels in each grouping (given a unique number).

elucidated and reducing false "echoes" (Oppenheim and Schafer, 1975; Geophysical Survey Systems Inc., North Salem, NH). Radar profiles were converted to bitmap image files using RADAN to Bitmap Conversion Utility 1.4 (Geophysical Survey Systems, North Salem, NH).

Radar images were quantified with Sigma Scan Pro Image Analysis software (Systat, Point Richmond, CA). Each image was converted to an 8-bit grey scale image. To quantify roots within the image, pixel intensity was measured. 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, which was able to delineate roots > 0.5 cm. This technique measures the relative pixel area of interest. A linear regression was run to quantify the relationship between root biomass from the soil cores and the GPR signals.

### *Root Biomass in Chambers*

In December 2005, five random GPR scans were taken within each of the eight open-top chambers. Each scan was 15 cm long and was processed in RADAN and Sigma Scan in the same manner as the core 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 $CO_2$ enrichment on coarse root biomass were tested by ANOVA using the MIXED procedure in SAS (SAS Institute 1990). Within the statistical model, biomass was blocked within chamber and $CO_2$ was the fixed effect.

## RESULTS

### *Root Biomass/GPR Regressions*

Biomass from the cores was partitioned into live and dead roots (Table 2). The average live biomass was 290 g per core (15,395 g $m^{-2}$) and the average dead biomass was 44 g per core (2,308 g $m^{-2}$). Mean total biomass in the cores was 331 g/core (17,703 g/$m^2$). 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 approximately 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 ($R^2 = 0.68$; $r = 0.822$) (Figure 19).

***Root Biomass in Chambers***

The regression equation was applied to GPR scans from the open-top chambers. Mean root biomass in the elevated and ambient chambers was estimated at 8,971 ± 1,105 g/$m^2$ and 6,551 ± 1,295 g/$m^2$ respectively (Figure 20). There was a significant $CO_2$ 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 (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

TABLE 2: Root core dry mass (g/ 15 cm diameter core) ranges for different root fractions to 60 cm depth.

| Root Fraction | Max | Min | Mean ± S.E. | Biomass Mean ± S.E. (g $m^{-2}$) |
|---|---|---|---|---|
| Live | 1,696 | 45 | 290 ± 68 | 15,395 ± 3,717 |
| Dead | 82 | 18 | 44 ± 3 | 2,308 ± 174 |
| Total | 1,728 | 62 | 331 ± 69 | 17,703 ± 3,762 |

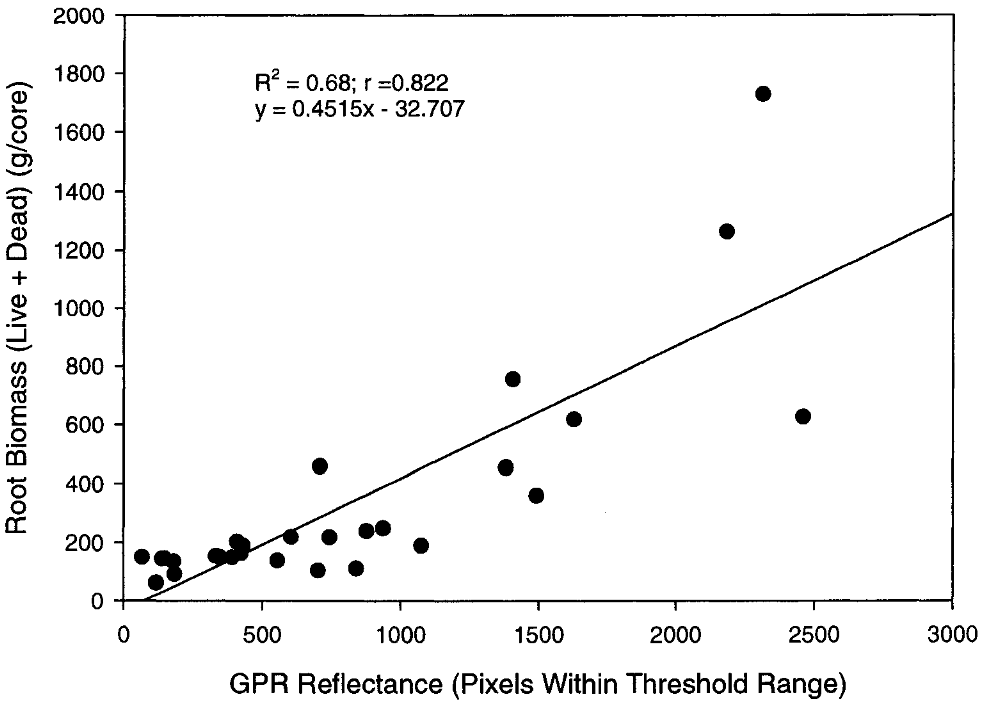


FIG. 19: Relationship between total live plus dead root biomass obtained from soil cores (g/ 15 cm core diameter to 60 cm depth) and GPR root reflectance (total pixels within threshold). Root reflectance was determined by measuring the total number of pixels (pixel intensity) within a specific intensity threshold in each GPR image. Solid line represents a significant linear regression of the mass to GPR signal reflectance

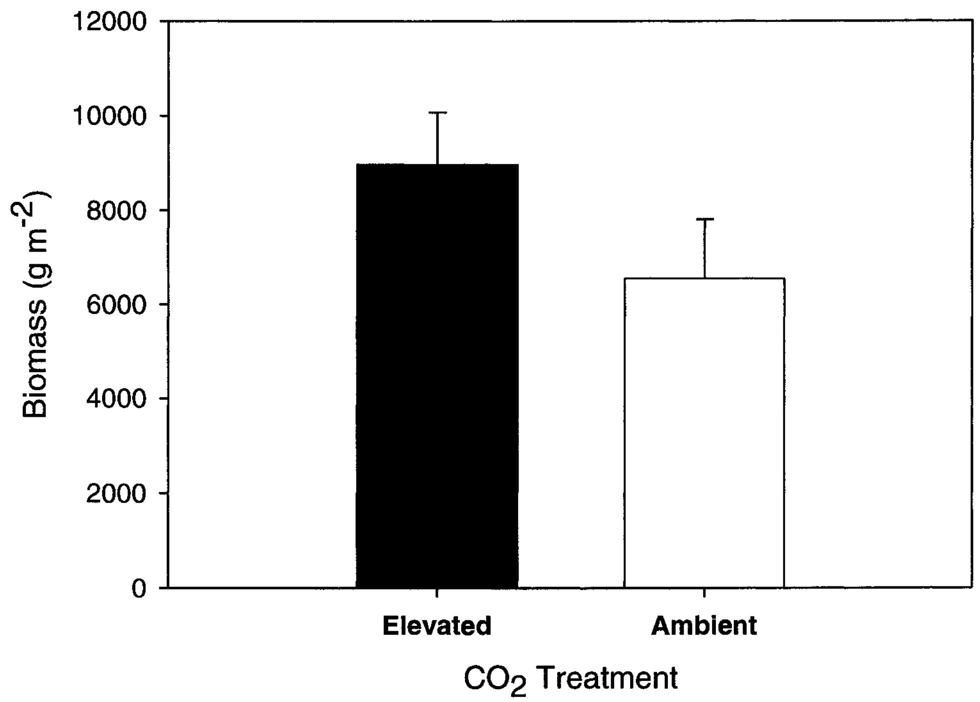


FIG. 20: Differences between coarse root biomass (g $m^{-2}$) under ambient and elevated $CO_2$ treatments (over ten years of continual fumigation) estimated from ground-penetrating radar scans.

the difficult determination of belowground effects of elevated $CO_2$ 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 $CO_2$ treatments, indicating that scrub-oak treated with elevated $CO_2$ had more coarse root biomass than areas treated with ambient atmospheric $CO_2$. This implies that more carbon sequestration is occurring in elevated $CO_2$ chambers in the form of coarse roots. Belowground carbon storage in the coarse roots probably plays an important role in the re-growth 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, 1994) implied the importance of examining $CO_2$ effects on *Quercus*, since 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 $CO_2$. 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 about 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 re-growth and are known to persist long after aboveground biomass is removed. These structures play a major role in post fire 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 $CO_2$ 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 non-destructively quantify coarse roots as affected by elevated $CO_2$. 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 3 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 $CO_2$ treatments and most notably in the belowground component. Estimated aboveground biomass (leaves and stems combined) was 1,362 g $m^{-2}$ in ambient treatments, whereas in the elevated treatments, aboveground biomass was approximately 2,037 g $m^{-2}$ (e.g. Dijkstra et al. 2002, T. Seiler, unpublished data). Belowground, the ambient chambers had 6,551 g $m^{-2}$ in coarse root biomass whereas the elevated chambers had 8,971 g $m^{-2}$ (Table 3). Fine root biomass comprised 2,226 g $m^{-2}$ and 2,203 g $m^{-2}$ in the ambient and elevated chambers, respectively (A. Pagel, unpublished data) (Table 3). Since the fine roots had apparently reached closure in both treatments, the increase in belowground biomass under elevated $CO_2$ was proportionally greater in coarse roots (75% ambient, 80% elevated) compared to the fine roots (25% ambient, 19% elevated) (Fig. 21). Based on the above and belowground estimates, total biomass was approximately 10,139 g m$^{-2}$ and 13,212 g m$^{-2}$ for ambient and elevated $CO_2$ treatments respectively, thus indicating that the increase in biomass distributed under elevated $CO_2$ 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) (Fig. 22).

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 this 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 approximately 0.5 cm diameter (Butnor et al. 2001, 2003, Cox et al. 2005, Wielopolski et al. 2000). Therefore, this technique excludes the fine root biomass component.

In conclusion, GPR based biomass estimates suggest elevated $CO_2$ chambers have greater belowground biomass, indicating a significant treatment effect in a scrub-oak ecosystem, thus supporting the 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.

TABLE 3: Average biomass (g m$^{-2}$) of major plant compartments in the scrub-oak ecosystem treated with elevated $CO_2$ (* T. Seiler, unpublished; **A. Pagel, unpublished).

| Biomass Compartment (g m$^{-2}$) | $CO_2$ Treatment | |
|---|---|---|
| | Ambient | Elevated |
| Aboveground (stems + leaves)* | 1,362 | 2,037 |
| Coarse Roots | 6,551 | 8,971 |
| Fine Roots** | 2,226 | 2,203 |
| Total | 10,139 | 13,212 |

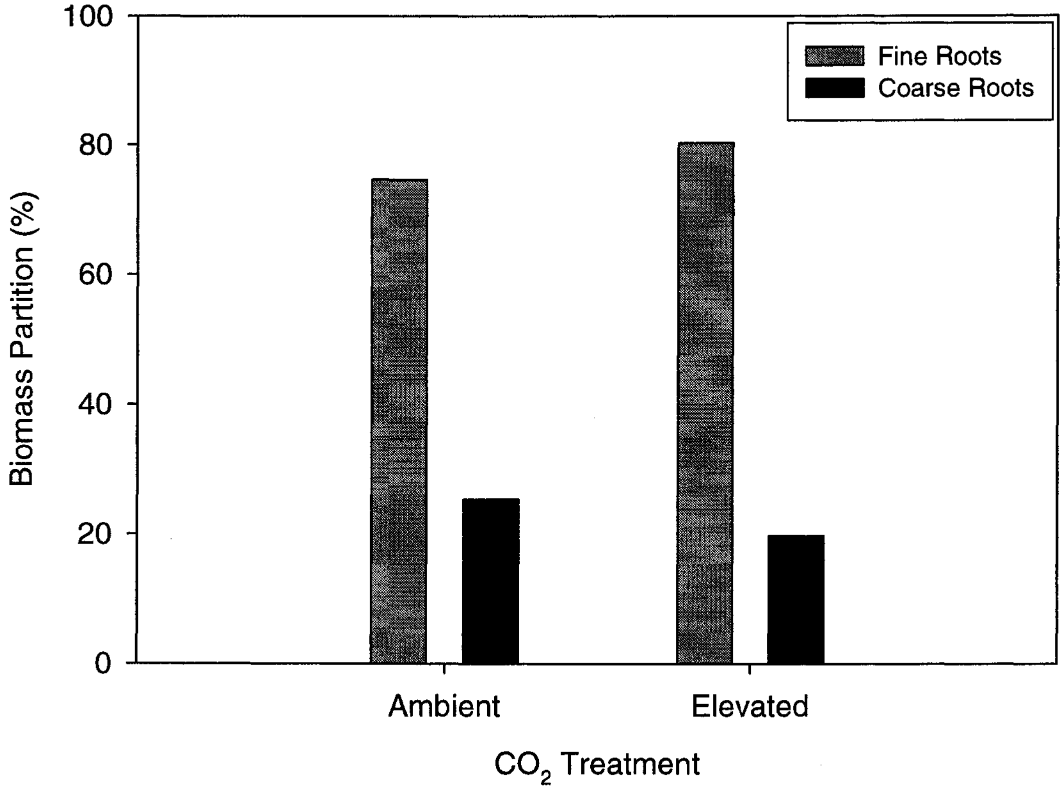


FIG. 21: Average belowground partitioning of fine and coarse root biomass following long-term $CO_2$ treatments. Coarse root estimates include all belowground structures greater than 5 mm diameter.

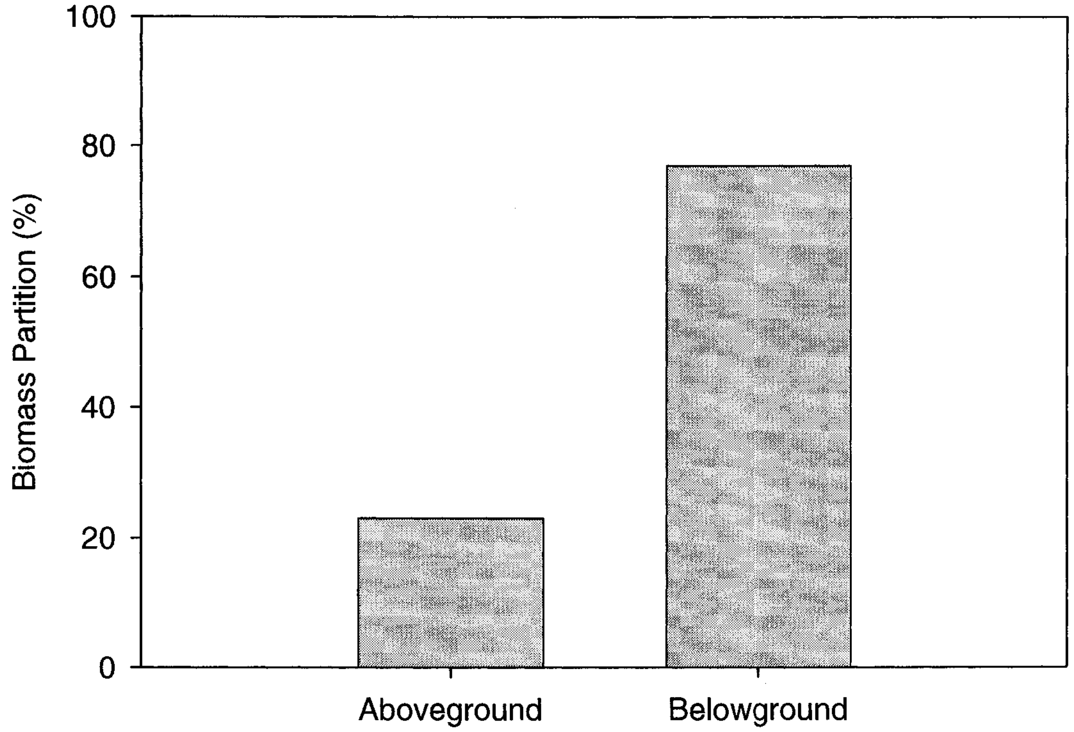


FIG. 22: Partitioning of above- and belowground biomass following long-term $CO_2$ treatments.

# CHAPTER V

# DETECTION OF SCRUB-OAK COARSE ROOT ARCHITECTURE WITH GROUND-PENTRATING RADAR

## INTRODUCTION

Increased interest in the global carbon cycle and carbon sequestration by forest ecosystems has resulted in increased demands for accurate methods of quantifying belowground biomass pools (Watson et al. 2000). This is not surprising since landscape models predict that trees within forest ecosystems account for nearly 80% of sequestered carbon (Richter et al. 1999). Forest soils and plant roots appear to be the greatest terrestrial pool of sequestered carbon. Approximately 10-65% of tree biomass is contained within the roots depending on factors such as age, species, competition, water and nutrient availability (Barton and Montagu 2004). Coarse roots (> 5 mm diameter) provide a major belowground perennial carbon sink (Johnsen et al. 2005). Compared to their aboveground counterparts, coarse roots have long residence times and are know to persist for long periods after tree harvest or disturbances such as fire (Johnsen et al. 2001, Ludovic et al. 2002, Miller et al. 2006). This significant pathway for carbon storage has been extremely difficult to accurately survey due to its complexity and growth in a heterogeneous opaque medium (Nielsen et al. 1997, Butnor et al. 2003).

Unfortunately, the study of root systems has often been hampered by inadequate methodologies that have resulted in poor or no measurements (Fitter and Stickland 1992). Common techniques such as soil cores, pits and trenches are destructive and labor intensive. They have provided useful information on root biomass but have been limited with respect to the soil volume and area that can be assessed (Butnor et al. 2003). In addition, information on root systems has been derived from two-dimensional data profiles that inaccurately describe the true natural behavior of the root system (Weaver 1919, Kutschera 1960, Coupland and Johnson 1965, Fitter and Stickland 1992). Lynch (1995) noted that spatial and temporal growth and distribution of roots are determined by dynamic interactions with chemical, physical and biological factors. This suggests the importance of overall root architecture to interactions in the environment.

"Root architecture" is commonly defined as the explicit spatial configuration of root systems comprised of a complex assemblage of subunits (Lynch 1995). The unique spatial distribution of roots has long intrigued plant biologists for over 100 years (Smith 1934). Numerous attempts have been made to develop a classification system for roots; however, due to the high degree of variability and plasticity exhibited by roots, none of these attempts have been successful (Weaver 1920, Kutschera 1960, Fitter 1991, Fitter and Stickland 1991). Many studies have generically applied the term architecture for morphology (e.g. surface features on root such as diameter or root hairs), topology (presence of roots in a positional gradient rather than orientation) or as true architecture (spatial configuration) (Lynch 1995).

Fitter (1987) suggested that three fundamental features determine plant root architecture: balance of primary and adventitious roots, degree of branching, and the plasticity of branching. Specifically, six variables should be measured to determine root architecture and include: topology, link length, branching angle, link radius, durational longevity, and symbiotic associations (Fitter 1987). The overall importance of root architecture relates to plant productivity (Lynch 1995). Resources in the soil are not evenly distributed and are often difficult to access due to physical limitations or competition. As a result, unique or complex spatial deployment of roots in the soil ensures successful foraging and exploitation of resources (Lynch 1995). As a result, there is a great deal of interest in mapping tree root systems to understand relationships between root architecture, soil volume utilization, and resource acquisition (Hruska et al. 1999, Cermak et al. 2000, Stokes et al. 2002, Butnor et al. 2005). A number of studies have shown that root architecture varies among species and ecological groups and is often dependent on water and nutrient availability (Nielsen et al. 1997). Rarely has root architecture been studied or analyzed in field grown conditions due to inherent difficulties in non-destructively measuring spatial deployment and behavior (Fitter and Stickland 1992).

Recent studies have used GPR to examine coarse root architecture with varying success (e.g. Cermak et al. 2000, Wielopolski et al. 2000, Stokes et al. 2002). Unlike the present study, low frequency antennas yielded reduced data resolution thus limiting accurate interpretations. Most importantly, advances in computer modeling software are now allowing complex three-dimensional models to be created from GPR data, which allows more powerful interpretations of coarse root architecture and spatial distribution.

Understanding root architecture is important for understanding resource acquisition as well as carbon storage. Silviculturists and forests ecologists require root biomass and architecture data to effectively and accurately evaluate effects of management practices that affect productivity (Butnor et al. 2006). The scrub-oak ecosystem utilized in this study is fire controlled with a natural 10-15 year fire cycle (Schmalzer and Hinkle 1991, 1992). Recruitment from seed is rare for most of the dominant species (Menges and Kohfeldt 1995). Dominate plant species in this community typically re-sprout from belowground structures. As a result, root carbon storage must be sufficient for re-growth until photosynthetic structures can recover (Olano et al. 2006). Therefore, it is important to examine the coarse root architecture in scrub-oak, not only to understand post-disturbance recovery, but also to understand potential carbon sequestration in scrub-oak.

The goal of this portion of the study was to determine coarse root architecture in the scrub-oak ecosystem. Using the non-destructive features of ground-penetrating radar, I expected that accurate three-dimensional models could be created and used to elucidate major structures that could be visually compared to direct excavations. If successful to appropriate levels of resolution, GPR technology will permit the quantification of coarse root architecture in the $CO_2$ study at Merritt Island. I hypothesize that coarse roots will be found to be more highly branched under elevated $CO_2$.

## METHODS

### *Root Pit Selection and Data Acquisition*

In June 2006, two 0.25 $m^2$ plots were selected adjacent to the long-term $CO_2$ study site at Kennedy Space Center, Florida. One plot was positioned over a *Quercus* stem while the second plot was positioned over a relatively bare patch. In November 2006, four adjacent 0.25 $m^2$ plots (1 $m^2$ total) were selected with varying amounts of vegetation contained within each one (Fig. 23). Vegetation and litter were removed from all plots and each was scanned with a Subsurface Interface Radar System (SIR-3000) attached to a model 5100 (1,500 MHz) GPR antenna (Geophysical Survey Systems Inc, North Salem, NH). Prior to fieldwork, the radar unit was scaled for depth by determining

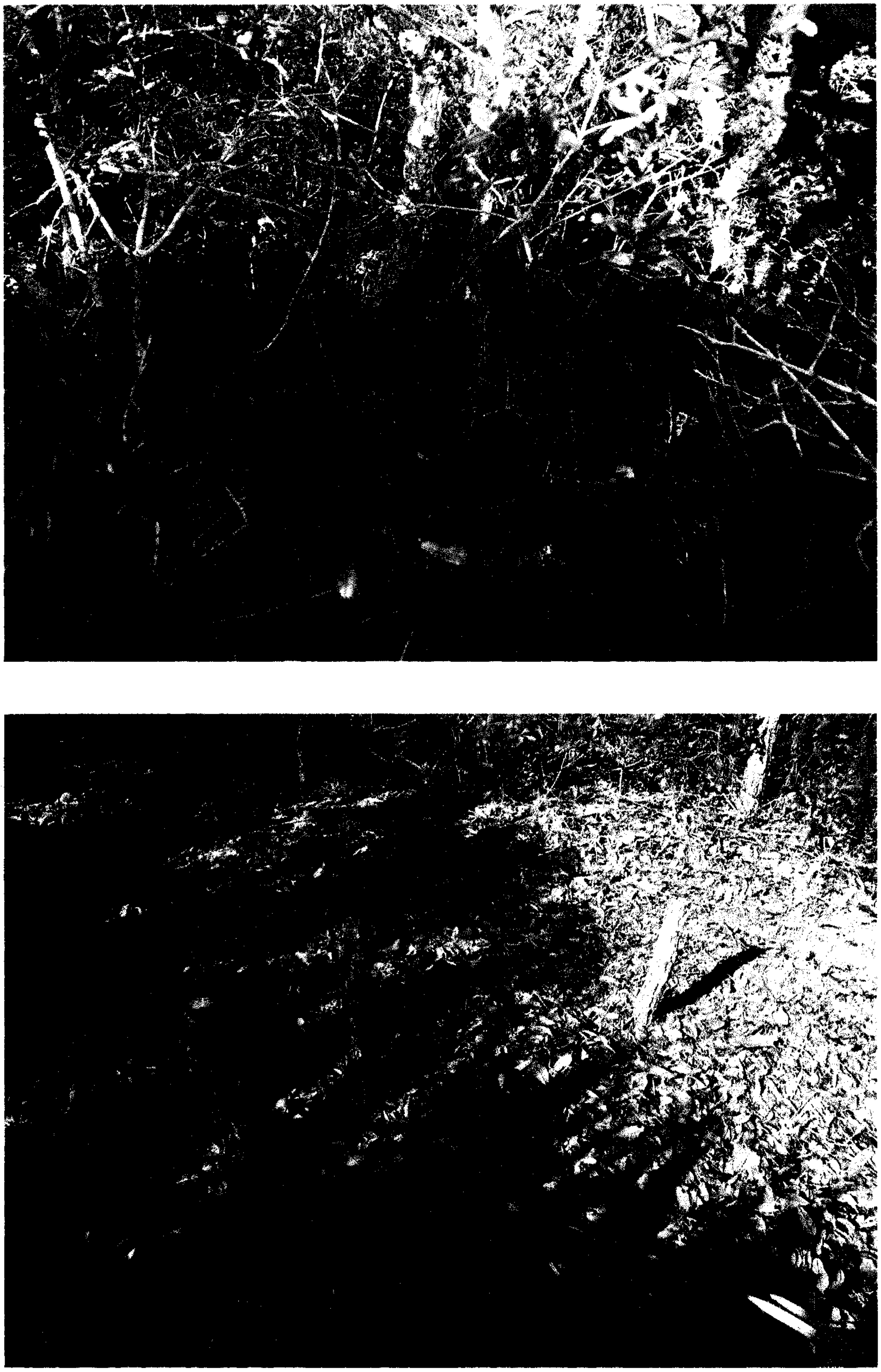


FIG. 23: Typical scrub vegetation density before (top) and after (bottom) clearing for coarse root architecture validation-excavations at Merritt Island, Florida.

the average dielectric constant of the soil with a buried metallic object at a known depth (similar to Butnor et al. 2003). Each plot was scanned every 2 cm for a total of 26 scans per each X and Y direction. Data from the 676 X-Y intersections permitted a 3-dimensional volumetric data model that allowed for spatial analysis.

### *Root Pit Excavations*

Following GPR imaging, each plot was marked with survey stakes. The fine root mat located near the soil surface was removed with hand clippers in order to access the larger roots below. Then the plots were excavated with hand trowels and shovels to loosen the soil and a shop-vac to remove loose soil. Careful attention was paid not to damage or dislocate medium and large roots during the excavation. At approximately 20-25 cm depth, images were taken with an Olympus 5-megapixel camera. At this point, all medium sized roots were removed to permit the 25-50 cm depth excavations. At 45-50 cm total depth, a second set of images were collected (Fig. 24).

### *GPR Image Processing and Root Biomass Correlations*

Root pit scans were processed with RADAN 6.5 GPR data processing software (Geophysical Survey Systems Inc., North Salem, NH). Each individual scan was cropped to ensure that only the actual 0.25 $m^2$ area of the plot was analyzed. In some cases the area was slightly smaller due to slight differences in the length of the data collection. Before quantification of the scans, several data processing steps were applied to enhance root discrimination. Root structures appear as hyperbolic reflectors, whereas parallel

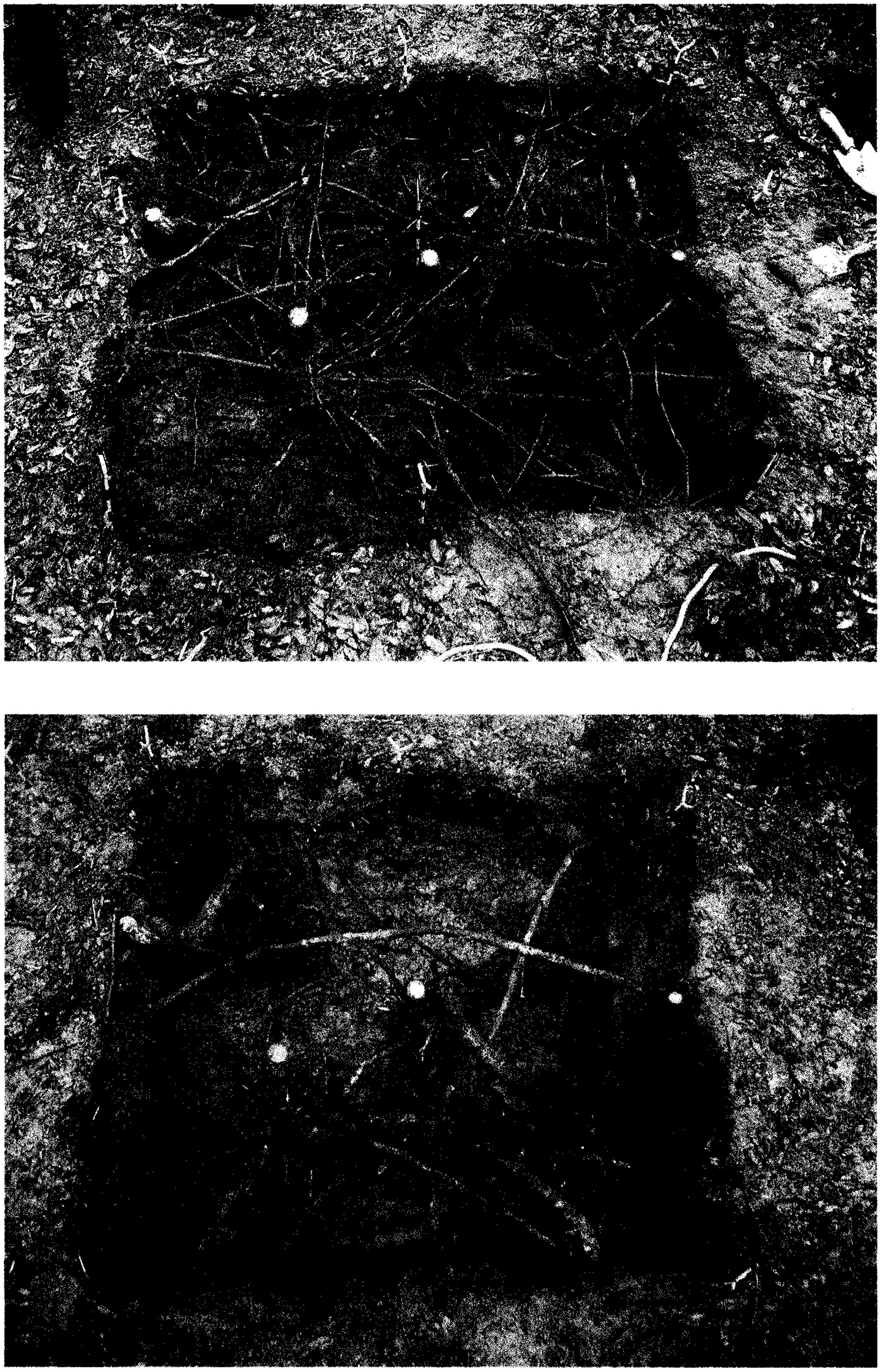


FIG. 24: Coarse root density at 20 (top) and 50 (bottom) cm depth following excavation for root architecture validation.

bands represent plane reflectors such as ground surface, soil layers and low frequency noise. 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; Geophysical Survey Systems Inc., North Salem, NH). Radar profiles were then imported into a 3-D module within RADAN with each scan location in the correct X-Y position (Fig. 25). Each 3-D profile was visually inspected at 5-15 cm depth segments identifying structures matching digital images from the ground excavation pits. RADAN software can be visualized by creating horizontal “slices” that are 13-20 cm thick and compressed to show information within that 3-D volume (Fig. 26).

## RESULTS

The results of this study demonstrate the complex assemblage of roots in a scrub-oak ecosystem. Example scrub vegetation before and after clearing in preparation for GPR imaging is shown in Fig. 23. Figure 24 shows the large amount of roots within the upper 50 cm of the soil profile. The coarse root diameters were variable in size from 1 cm to more than 15 cm. Within the excavation pit shown in Fig. 24, a number of stems were encountered along with roots with a wide array of branching and deployment angles and directions. I feel that the excavation pits used in this study adequately represent the

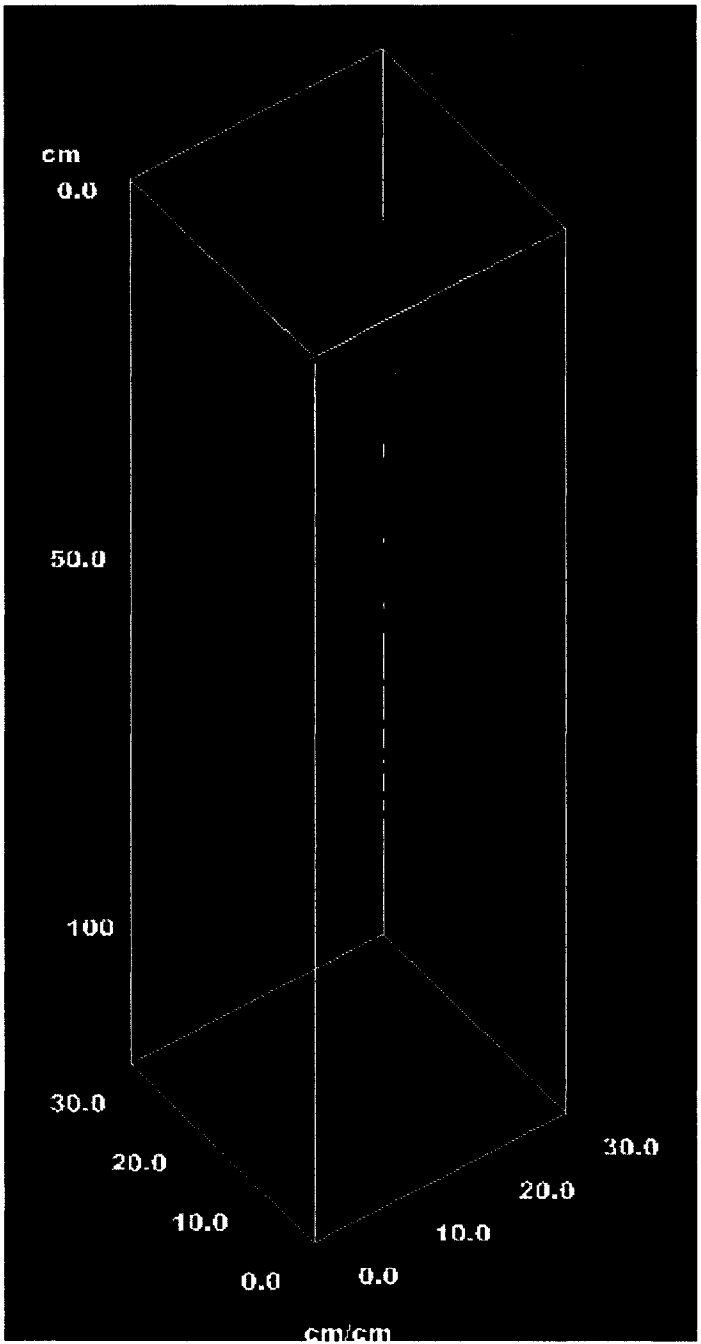


FIG. 25: Three-dimensional model of coarse roots imaged with ground-penetrating radar. Multiple X-Y intersections within the study grid were transformed into volumetric 3-D data with RADAN analysis software. GPR reflectors can be made transparent so that gross structural objects can be observed in 3-dimensional space.

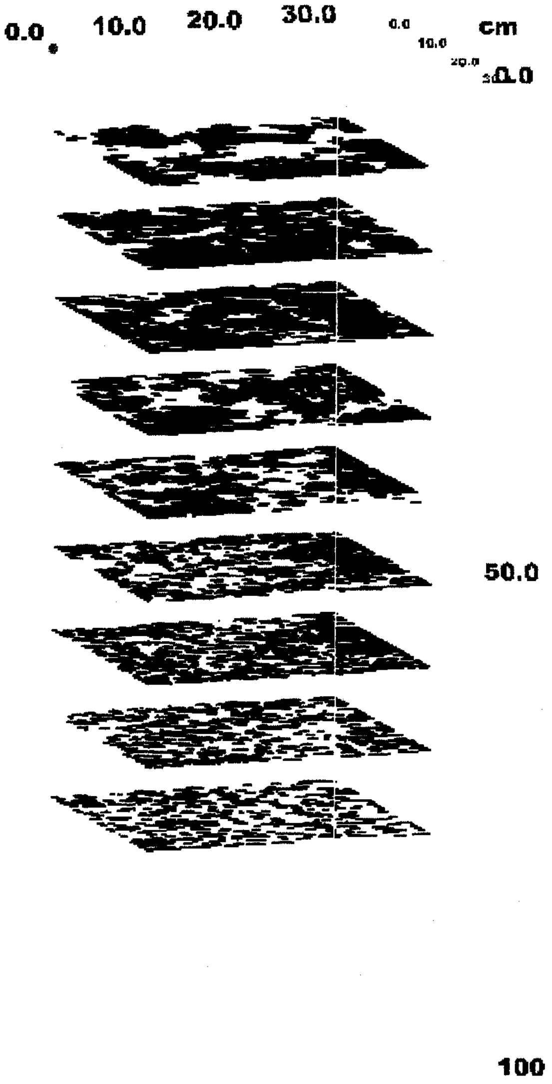


FIG. 26: Example horizontal "slice" extractions from GPR data after 3-D modeling. Each slice can be adjusted for variable thickness in order to capture entire roots at a given depth.

"typical" root matrix in the scrub community.

Three-dimensional analysis of the GPR profiles are represented in Fig. 25. GPR analysis with RADAN software permits horizontal slices at user defined depths to be extracted for visual comparisons to actual excavation images (Fig. 26). Numerous large coarse roots could be correlated with GPR images. Typically roots that spanned the entire root pit or had distinct angles were easiest to identify (Fig. 27). Roots that originated from or intersected a stem or root burl were commonly strong reflectors in the GPR data (Figs. 28 and 29). Although large roots tended to produce a strong reflectance signal in the GPR dataset, larger objects with a well defined angle that intercepted multiple scan transects tended to produced the most visually recognizable objects in the 3-D models (Fig. 30).

Large root burls appear to be common features in the belowground landscape in the scrub-oak ecosystem (Fig. 31). A large excavated root burl shown in Fig. 32 is clearly noticeable as a strong reflector in the GPR 3-D model. A minor depression appears to be present in both the root excavation photo and the GPR data slice. To the left of the root burl, two smaller roots can be identified further down in the profile (approximately 35-40 cm depth). These two parallel objects are also present in the lower horizontal slices of the 3-D GPR profile (Fig. 32).

Another feature of the three-dimensional GPR modeling software is the ability to make low energy reflection objects transparent, thus leaving the major reflectors (roots) visible. A 6 cm coarse root can be seen in both the excavation photo and the transparent 3-D model (Fig. 31). This feature is quite useful but limited to large strong reflectors, and some roots can be easily masked by obstruction of other objects and reflectors in 3-D

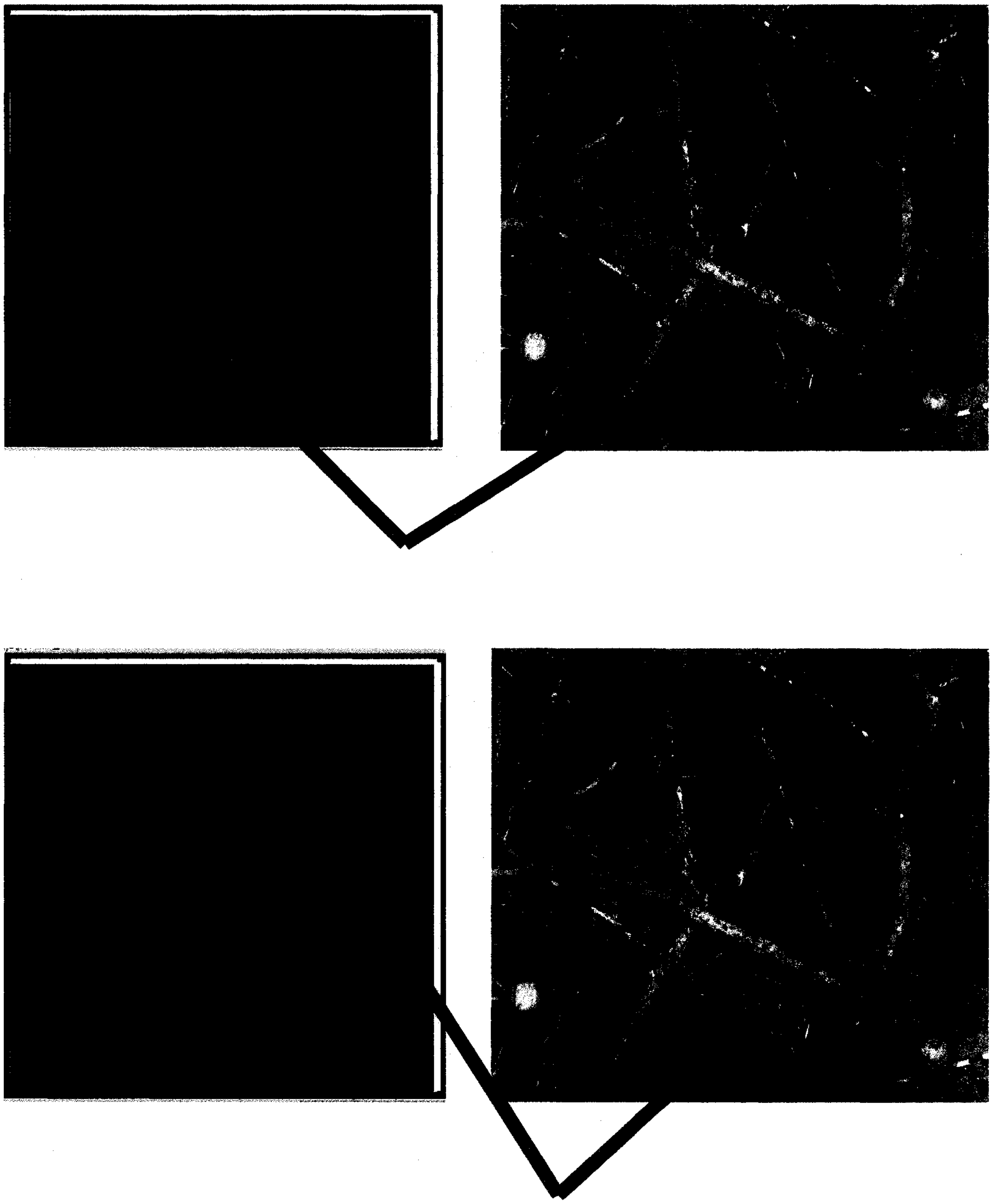


FIG. 27: Visual correlation between GPR data and coarse root excavation pits. Large roots spanning the entire length of the validation pit were most likely to be observed in the GPR data. Each GPR data image (left) represents a "slice" of the 3-D profile at the appropriately correlated depth with a viewing depth of 15-20 cm.

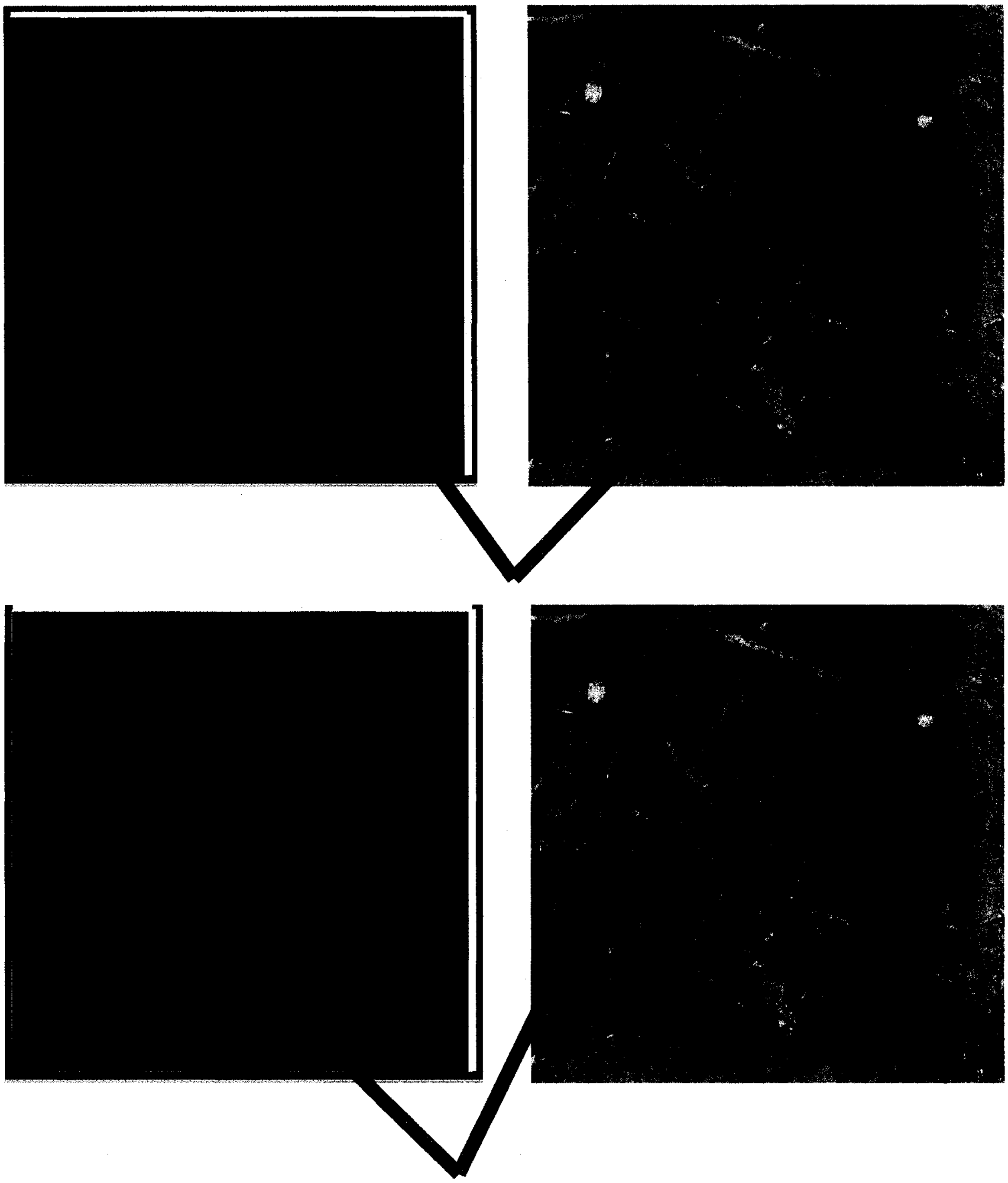


FIG. 28: Visual correlation of large roots and stem masses in GPR data and coarse root excavation pits. Large roots, stems, and burls produce strong reflectors in the GPR data. Each GPR data image (left) represents a "slice" of the 3-D profile at the appropriately correlated depth with a viewing depth of 15-20 cm.

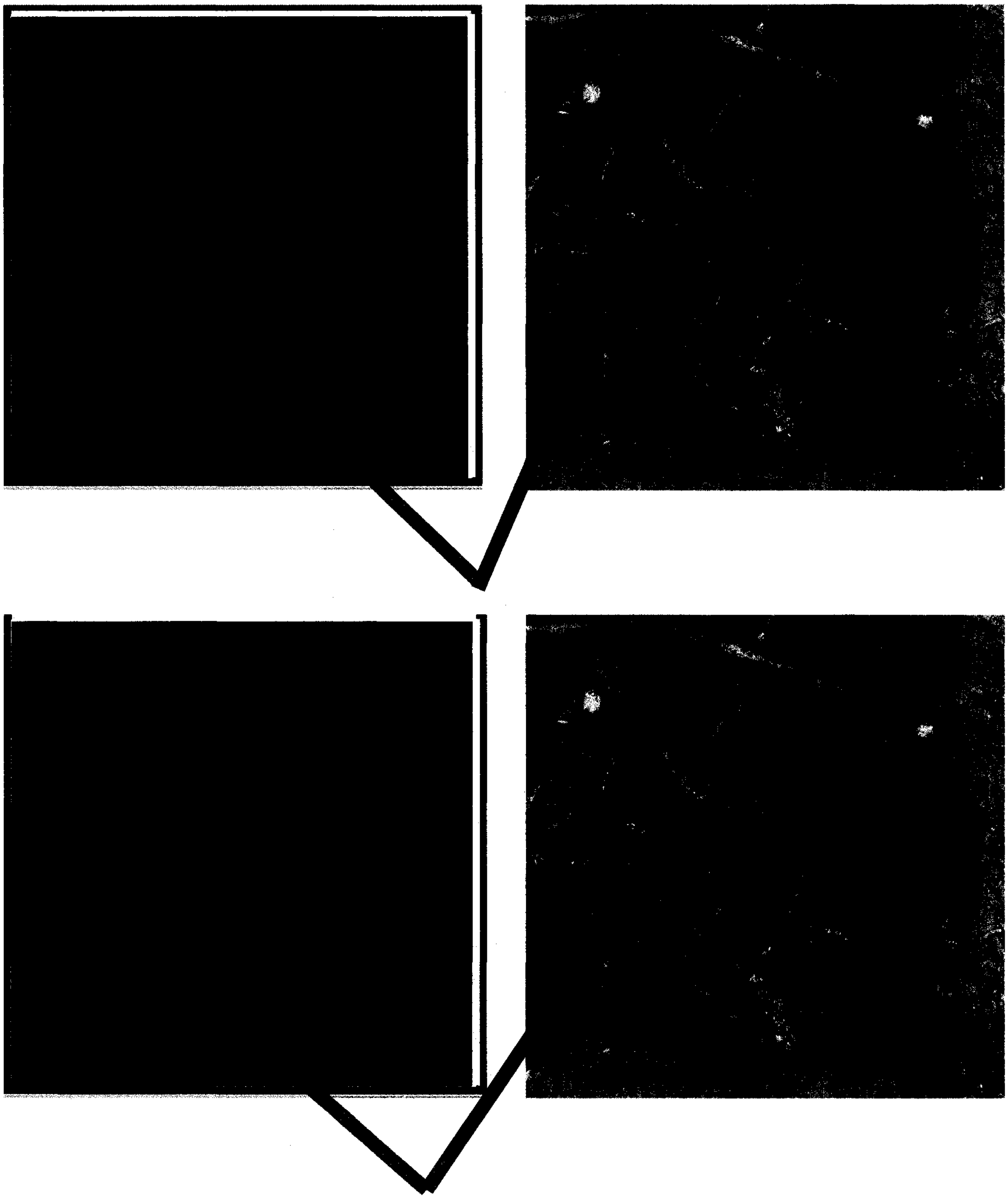


FIG. 29: Visual correlation of stems in GPR data and coarse root excavation pits. Stems typically produce a large energy reflection in the GPR data; however, smaller groups of roots or root grafts tend to produce similar large single reflections. Each GPR data image (left) represents a "slice" of the 3-D profile at the appropriately correlated depth with a viewing depth of 15-20 cm.

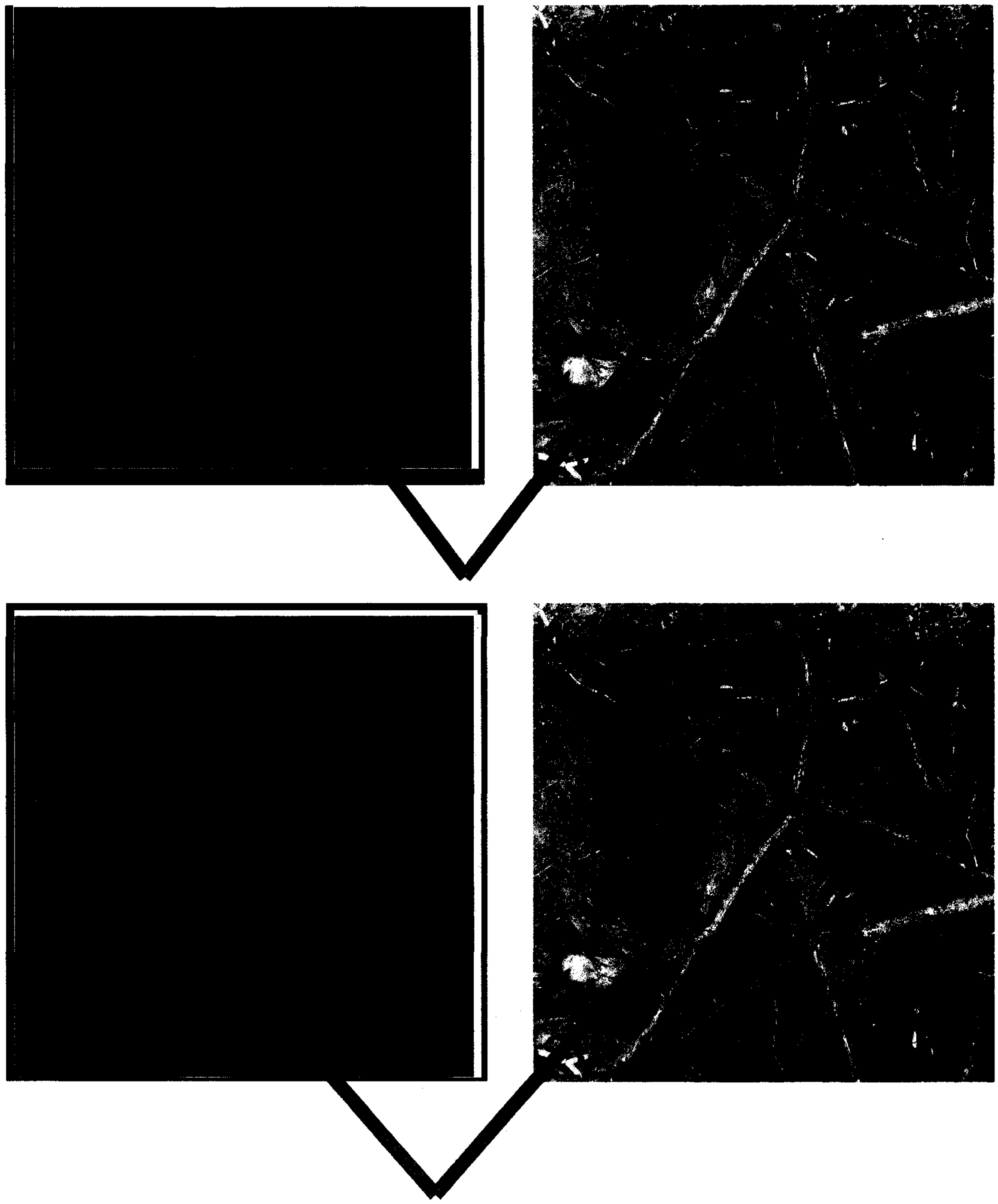


FIG. 30: Visual correlation of roots with distinct angles in GPR data and coarse root excavation pits. Roots with sharp or unique (horizontal) angles are also readily observed in the three-dimensional analysis. Each GPR data image (left) represents a "slice" of the 3-D profile at the appropriately correlated depth with a viewing depth of 15-20 cm.

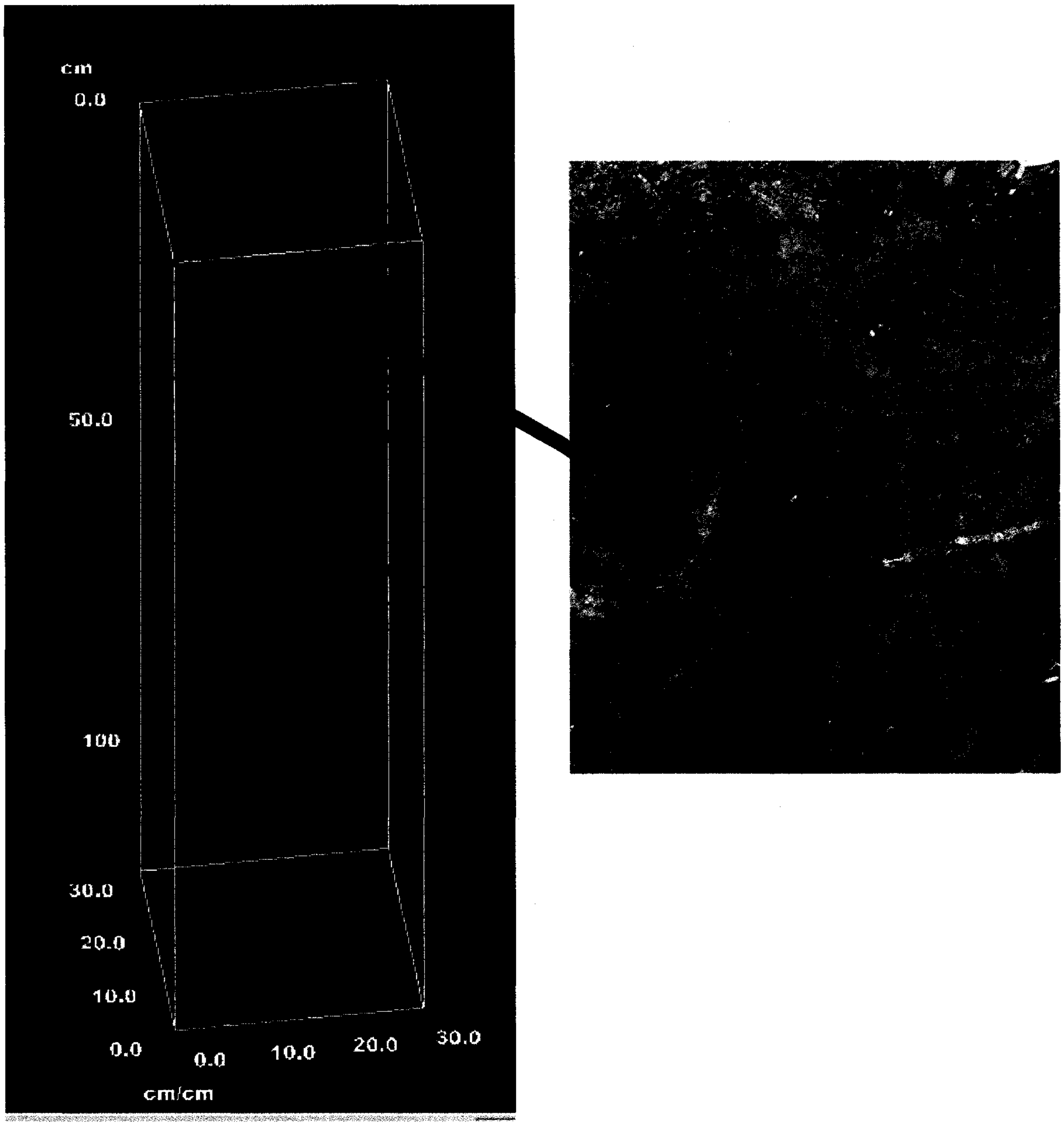


FIG. 31: Transparent visualization of coarse roots in a three-dimensional profile. Roots with sharp angles can be easily visualized and identified. Each GPR data image (left) represents a "slice" of the 3-D profile at the appropriately correlated depth with a viewing depth of 15-20 cm.

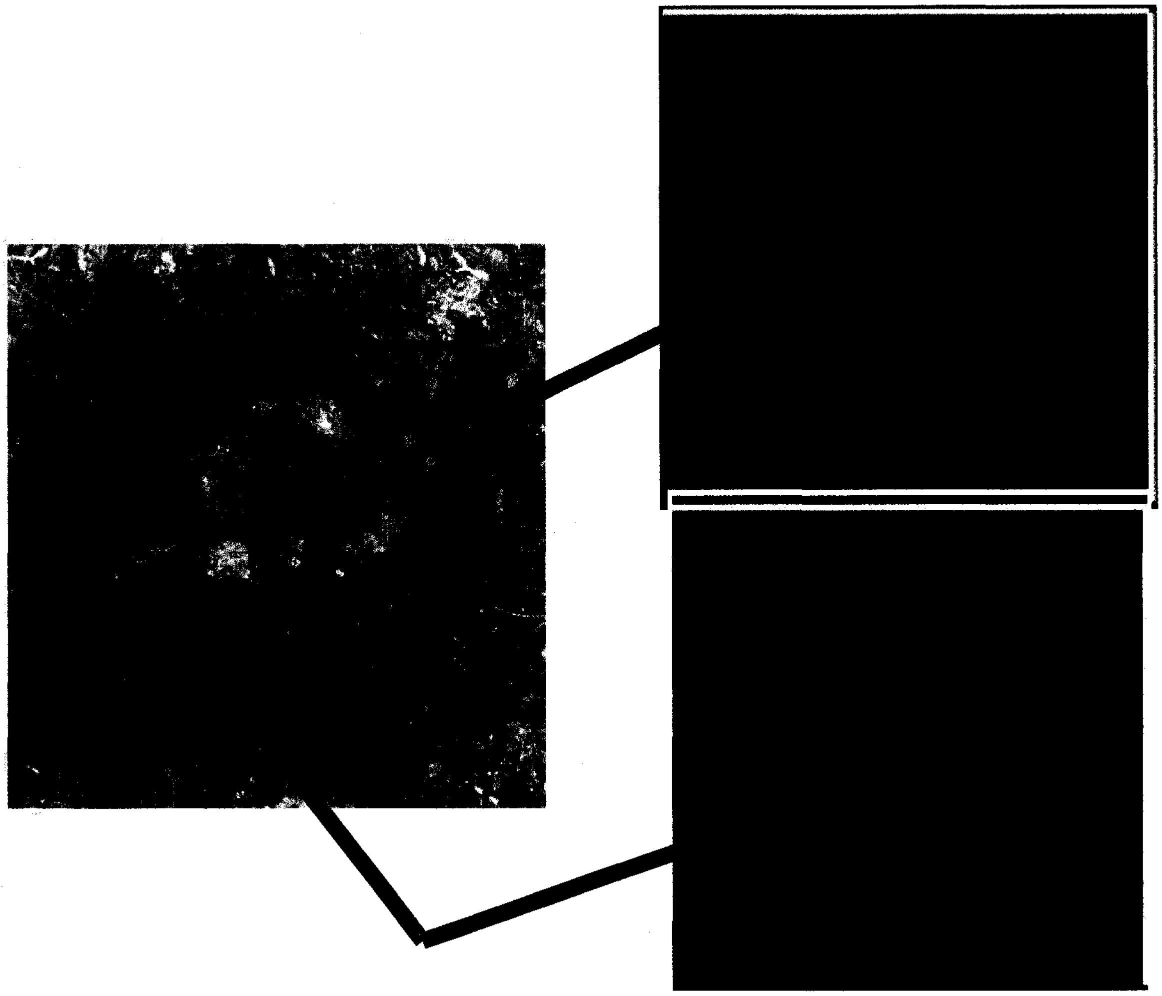


FIG. 32: Large root burls are common in the scrub-oak ecosystem. Burls produce large reflections in the GPR dataset. Note that the 3-D model appears to show the slight depression at the top of the root burl. Two parallel roots can been seen to the left of the burl and are observed in the GPR data at the correct lower depth and not in the upper portions of the model demonstrating the ability to scale depth with the 3-D model.

space. A large root burl can be observed in a transparent 3-D model, whereas smaller objects are not easily observed (Fig. 33).

## DISCUSSION

The primary goal of this portion of the study was to determine the feasibility of ground-penetrating radar to detect coarse root architecture. Due to limitations in digitizing GPR data for a quantitative comparison to root excavation pit photos, only a visual qualitative analysis could be performed. Despite this limitation, valuable information was gathered from this study. Large roots are visible in the GPR data set. Particularly, roots that transverse the entire study area are easiest to identify from the GPR data. In addition, roots that have distinct angles or branching patterns are easiest to visualize. Unfortunately, roots that fall along the same direction of a transect scan are less likely to produce a strong signal reflection. Numerous root reflectors were identified and correlated to actual roots in the root pit excavation photos in five of the six root pits. The lack of correlation in the third pit imaged and excavated in November 2006 is unclear and could be due to error in data collection or orientation recording in the 3-D model.

A few general conclusions can be made on the application of GPR to root architecture. First, although many coarse roots can be observed in the 3-D data slices and correlated to the root pit photos, a considerable number of reflectors that should have been identified were not. This could be due to lack of sufficient resolution in the data collection transects. I believe that the 2 cm distance between scan transects should be reduced to 1 cm, thus increasing the total number of intersections from 676 to 2601 within a 0.25 $m^2$ study grid. Increasing the number of transect intersections should

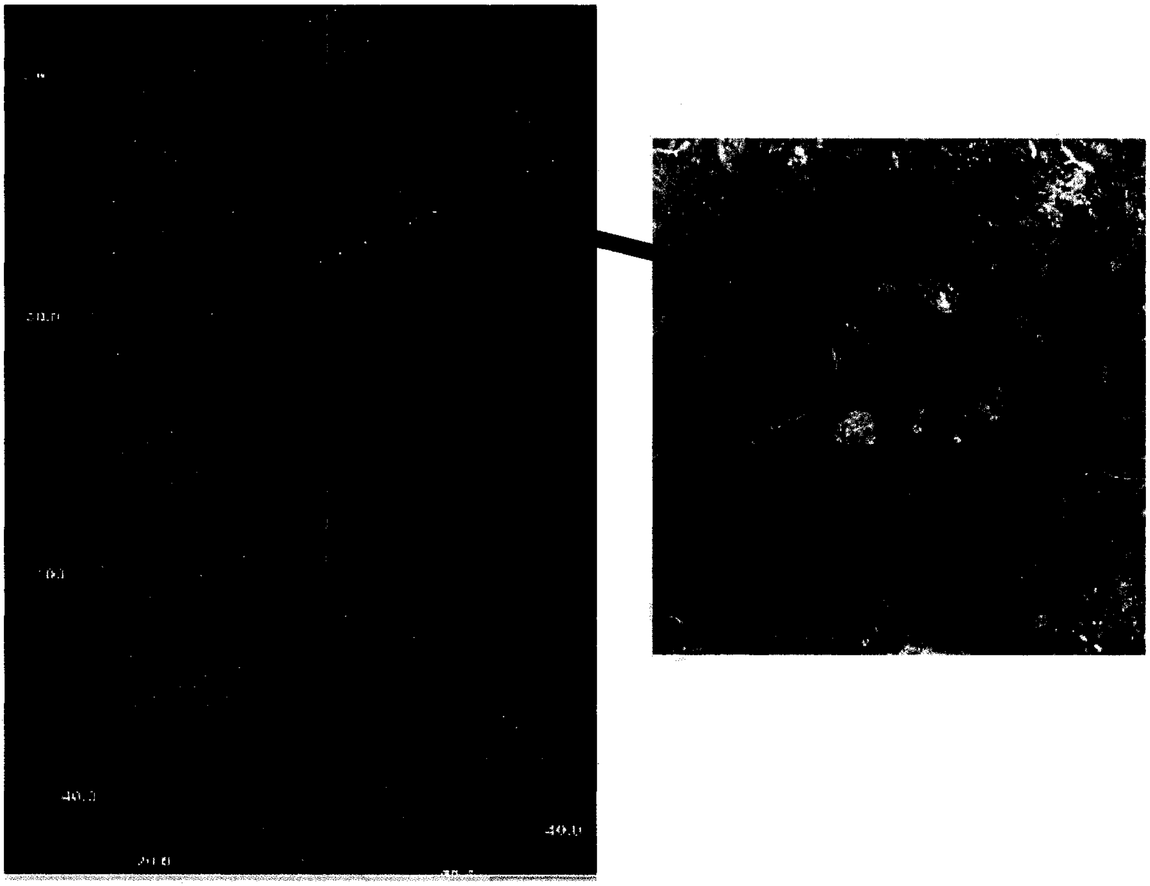


FIG. 33: Three-dimensional profile of a root burl in the scrub-oak ecosystem. The volume of each GPR data "slice" has been compressed and made partially transparent to permit viewing of additional structures throughout the profile and below the root burl.

enhance the discrimination of smaller root objects. In addition, increasing the data collection starting point (i.e. lead time or a lip space before crossing into the grid) at least 15 cm before intercepting the area of study interest should decrease any potential truncation of data on the starting edge of the validation pit. Corollary to this point is to increase the stopping distance by an equal amount. In effect, this results in scanning an additional 15 cm on each side for a total of 80 cm data transect for each “true” 50 x 50 cm pit. Increasing the scanning density and the leading and trailing edges of the grid should make smaller objects more visible in the 3-D models as well as assist in discriminating individual root reflectors from background noise.

Longer transects coupled with increased scan density should also aid in avoiding errors associated with overshadowing from large structures such as root burls. Overshadowing errors were not an issue in this study; however, due to the high frequency of burls (lignotubers) and other large reflectors at the study site, proper measures should be taken to reduce the potential for this type of data loss. Despite these added steps, roots that grow parallel on top of each other will still appear as a single larger object and cannot discriminated since the signal propagation comes from above these two reflectors. Work by Butnor et al. (2001) suggests that the utilization of borehole antennas into the soil at varying depths could help in discriminating errors of this type as well as identifying species with a taproot. Unfortunately, borehole antennas are not commercially available in the United States nor does the FCC currently permit them. In addition, increasing the transect length will permit larger data models to be created, thus creating a larger reference frame for interpretation. Larger transects should permit capture of information on the radial distribution of roots from a central stem. Johnsen et al. (2005) found that loblolly pine had lateral roots extending nearly 3-7 times further than the extension of the aboveground tree crown.

Refinement of this technique will also require additional changes to methods utilized in the validation of GPR data with root pit excavations. Since the 1,500 MHz antenna only effectively penetrates 50-75 cm in sandy soils, our data indicate that architecture analysis and interpretation must be confined to the upper 50 cm of the soil profile. Although roots can definitely penetrate deeper than 50 cm of the soil profile, other studies have found that only 2.1% of the total root biomass is found below 60 cm, such as in hardwood forests at Coweeta Basin in North Carolina (McGinty 1976, Miller et al. 2006). Mou et al. (1995) also found that coarse roots of sweet gum and loblolly pine decline steeply with depth. The use of a shop-vac to remove excess and loose soil greatly reduced excavation time and disturbance from physical digging by hand. An application of spray survey chalk would have made the visualization of roots easier in the photo correlations, especially for those roots that transverse the vertical, rather than the horizontal soil profile. Finally, future root pit photos should be digitized with a program such as MSU ROOTS that is used in the digitizing of fine roots (See Chapter 3 for details). By applying a different scale for image length, accurate measurements of root lengths and diameters could be achieved and used in a quantitative correlation.

Recent studies imply that the application of AMAPmod, a freeware spatial modeling software program, could utilize distribution data from external programs (e.g. MSU ROOTS or magnetic mapping tools) to produce 3-D spatial distribution maps of roots from the validation pits (Danjon et al. 1999). This could potentially allow RADAN transparent models to be overlaid and examine the percent correctness of the GPR identifications. The application of AMAPmod is still in it infancy with GPR data, but holds much promise. Detailed root architecture maps, such as those produced with a low frequency 400 MHz antenna by Hruska (1999), Cermak et al. (2000), and Stokes et al. (2002), seem unlikely with current limitations of GPR technologies. However, these studies have provided the conceptual foundation for the application of GPR to investigating root architecture.

Overall, the results from the current study indicate that root architecture can be examined to some degree, with the application of ground-penetrating radar. Large root burls and stems were correctly identified with some accuracy in this study. In past studies, a tradeoff between examining root system function or root system architecture coupled with insufficient methodologies have limited our understanding of the modular belowground structures (Fitter and Stickland 1991, Robinson et al. 2003). Future refinement of data collection techniques together with better validation comparison software and methodologies will permit a detailed interpretation of coarse root architecture and permit examination of the belowground carbon storage structures. Understanding coarse root architecture is important considering that carbon can be transferred among individuals via root grafts and that root quantity, size and distribution interact to determine a plant's ability to acquire soil resources (Schultz and Woods 1967, Casper et al. 2003). Finally, a better understanding of the spatial and temporal distribution of roots will permit manipulative studies that will lead to a better understanding of processes that determine root and whole plant responses (Hutchings and John 2003). Identification and quantification of these structures with GPR will refine and potentially redefine the carbon budgets of shrub ecosystems along with their contributions to sequestration in the global carbon cycle.

# CHAPTER VI

# CONCLUSIONS

Plant roots represent one of the most complex and essential components in nature. Despite their important role in resource acquisition, carbon storage and anchorage, roots have typically been ignored in the vast majority of botanical and ecological studies. Pregitzer (2002) correctly stated "...roots of perennial plants are a royal pain to study." However, over the past two decades, a large push to quantify the belowground portion of plants has resulted in major leaps in technologies and methodologies for root studies. The application of minirhizotrons, stable isotopes, x-ray tomography, and more recently ground-penetrating radar are now allowing us to quantify roots (in many cases non-destructively) and develop functional paradigms. Recent belowground studies are beginning to redefine how we look at ecosystem functions, most notably in terms of carbon sequestration.

It is estimated that roots can comprise 40-85% of net primary production in some ecosystems (Fogel 1985, Fitter 1987). Plant roots have the potential to sequester significantly large amounts of assimilated carbon belowground. Annual carbon sequestration by plant roots have major impacts on the global carbon budget. In addition, current studies might help in explaining a portion of the missing global carbon pool. The scrub-oak study system is nutrient poor sclerophyllous shrubland. Shrub ecosystems are known to produce the greatest total root biomass of any ecosystem (Jackson et al. 1996, Robinson et al. 2003).

This study is part of a larger project examining the responses of scrub-oak ecosystems to elevated atmospheric $CO_2$. The primary goals and questions of this study were: Does elevated carbon dioxide influence the growth and morphology of roots? Have fine root dynamics (i.e. production, mortality and turnover) changed since initial $CO_2$ fumigation? What environmental and morphological factors control fine root survival? Are there differences in the coarse root biomass between $CO_2$ treatments? Can root architecture be determined with ground-penetrating radar?

Fine roots appear to have reached a carrying capacity in the soil, or rather, root closure. Dilustro et al. (2002) examined fine root dynamics (i.e. productivity, mortality and turnover) during the initial 3 years of this study and found a significant $CO_2$ treatment effect. In the current study, the $CO_2$ treatment effects have converged. Fine root productivity in the elevated $CO_2$ chambers appears to have decreased slightly whereas the mean turnover has increased (non-significantly) in recent years. More interestingly, fine root diameter has significantly increased over the course of the study. Root diameters have also increased with respect to increasing depth over time. This means that greater amounts of fine roots with large diameters are now present in the lower portions of the soil profile.

In addition to fine root dynamics, root survivorship was examined in this study. Our data indicated that fine root survival increased with increasing root diameter, length, and depth. This suggests that fine roots are producing larger roots that are penetrating deeper into the soil profile perhaps to forage for resources beyond the dense root mat in the upper 40 cm of the soil. Differences in fine root persistence have strong implications on the carbon sequestration abilities of the fine root compartment. Smaller, more dynamic roots located in the upper portions of the soil profile have the potential to turnover rapidly, therefore releasing a large amount of carbon in the soil. Fine roots located deeper in the soil perhaps produce more efficient, longer-lived roots that have a large structural carbon sink. However, with the system reaching root closure, there might be limits as to how much carbon sequestration occurs post disturbance. The greatest soil "carbon-loading" by fine roots most likely occurs during the initial years post fire.

A common problem in most belowground studies centers on the ability to collect data non-destructively. Long-term ecological studies have been forced to ignore or "black-box" the coarse root compartments due to this limitation. Recently, application of ground-penetrating radar to biological systems has shown promise to image plant roots. In this study, I was able to successfully correlate data from a 1,500 MHz GPR antenna to root biomass collected adjacent to the long-term $CO_2$ experiment. A significant correlation ($R^2$=0.68) between biomass and processed GPR data permitted estimation of biomass within the $CO_2$ treatment chambers. Our findings indicate that there is a significantly greater amount of coarse root biomass within the elevated $CO_2$ chambers compared to the ambient counterparts.

Nearly 85% of the total plant biomass is located in the root compartment in the scrub-oak ecosystem. More importantly, coarse roots have the potential to be major sinks for carbon in this ecosystem. Within the root compartment, approximately 77% of root biomass is partitioned into the coarse roots. Coarse roots serve as the primary photosynthate storage compartment and source of carbon for re-growth in this ecosystem following a disturbance. The contribution of this major sink is currently redefining how we view carbon dynamics in this system as well as the relative importance of the belowground compartment.

The final study in this dissertation focused on the application of GPR to examine coarse root architecture. Large roots that had unique angles or that transverse the validation area were typically present in the 3-D modeled GPR data. Smaller roots that were close together or that were on top of one another were typically masked or appeared as a single object. Root burls, which are a common feature in this ecosystem, were readily seen in the GPR data. Although the architecture and spatial deployment of many roots were detected by GPR, many objects were not detected. The data from this study support the initial proof-in-concept for examining root architecture, but future studies should increase the sampling density from 2 cm to 1 cm. Increasing the sampling density should maximize the 3-D modeling abilities of the GPR software. Root architecture studies by Fitter and Stickland (1992) and Lynch (1995) suggest that spatial deployments of roots are correlated to the acquisition of resources in the soil. However, root architecture or deployment of the coarse roots declined with depth suggesting that most coarse roots were in the upper 50 cm of the soil. This would indicate that the majority of the carbon sequestration would be located in the upper half meter of the soil.

Overall, this study has examined both the fine and coarse root compartments of the scrub-oak ecosystem. Fine roots have reached closure while $CO_2$ treatments have converged. Currently, larger fine roots are being produced that are persisting longer and have increased in density at greater soil depths. Coarse roots have significantly greater biomass in elevated $CO_2$ treatments and comprise nearly three quarters of the total root mass. This findings are reshaping the way that we view the root compartment and the role roots play in carbon sequestration in a post fire disturbance scrub-oak ecosystem.

This experimental study is unique in that is involves a nutrient poor, mixed species costal shrub community in Florida that has been treated with elevated atmospheric $CO_2$ at levels predicted globally over the next 100 years. The results demonstrate that the $CO_2$ treatment effect was short lived for the fine roots. The impacts of $CO_2$ treatments on coarse roots early in the study are unclear; however, a significant treatment effect was present in the recent (ninth and tenth) years of the overall study. Our results indicate that non-destructive methods such as minirhizotrons and GPR will redefine our understanding of root ecology and the potential for carbon storage.

# REFERENCES

Ainsworth, E.A., P.J. Tranel, B.G. Drake, and S.P. Long. 2003. The clonal structure of *Quercus geminata* revealed by conserved microsatellite loci. Molecular Ecology **12**:527-532.

Allison, P.D. 1995. Survival Analysis Using the SAS System: A Practical Guide. SAS Institute, Cary, North Carolina.

Anderson, L.J., L.H. Comas, A.N. Lakso, and D.M. Eissenstat. 2003. Multiple risk factors in root survivorship: A 4-year study in Concord grape. New Phytologist **158**:489-501.

Arnone III, J.A., J.G. Zaller, E.M. Sphen, P.A. Niklaus, C.E. Wells, and C. Korner. 2000. Dynamics of root systems in native grasslands: effects of elevated atmospheric $CO_2$. New Phytologist **147**:73-85.

Baldwin, R., C.L. Burch, R.B. Hinton, H.H. Huckle, P. Nichols, F.C. Watts, and J.A. Wolfe. 1980. Soil survey of Volusia County, Florida. USDA Soil Conservation Service, Washington, D.C.

Barton, C.V.M., and K.D. Montagu. 2004. Detection of tree roots and determination of root diameters by ground penetrating radar under optimal conditions. Tree Physiology **24**:1323-1331.

Bazzaz, F.A., and S. Catovsky. 2002. Impact of global environmental change on plants: From cells to ecosystems. Pages 94-111 *in* H.A. Mooney and J.G. Canadell, editors. Encyclopedia of Global Environmental Change, Vol. 2. Wiley Publishers, Chichester, UK.

Berntson, G.M. 1994. Modelling root architecture: Are there tradeoffs between efficiency and potential of resource acquisition? New Phytologist **127**: 483-493.

Berntson, G.M., and F.A. Bazzaz. 1997. Elevated $CO_2$ and the magnitude and seasonal dynamics of root production and loss in *Betula papyrifera*. Plant and Soil **190**:211-216.

Bohm, W. 1979. Methods of Studying Root Systems. Springer-Verlag. Berlin.

Burke, M.R., and D.J. Raynal. 1994. Fine root growth phenology, production and turnover in a northern hardwood forest ecosystem. Plant and Soil **162**: 135-146.

Burton, A.J., K.S. Pregitzer, and R.L. Hendrick. 2000. Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. Oecologia **125**: 389-399.

Butnor, J.R., J.A. Doolittle, K.H. Johnsen, L. Samuelson, T. Stokes, and L. Kress. 2003. Utility of ground-penetrating radar as a root biomass survey tool in forest systems. Soil Science Society of America Journal **67**:1607-1615.

Butnor, J.R., J.A. Doolittle, L. Kress, S. Cohen, and K. H. Johnsen. 2001. Use of ground-penetrating radar to study tree roots in the southeastern United States. Tree Physiology **21**:1269-1278.

Butnor, J., B. Roth, and K. Johnsen. 2005. Feasibility of using ground-penetrating radar to quantify root mass in Florida's intensively managed pine plantations. Forest Biology Research Cooperative Report #38. Gainesville, Florida.

Butnor, J.R., K.H. Johnsen, P. Wikstrom, T. Lundmark, and S. Linder. 2006. Imaging tree roots with borehole radar. *in* Proceedings of the Eleventh International Conference on Ground Penetrating Radar (June 19-22, 2006, Columbus, Ohio).

Caldwell, M.M. 1976. Root extension and water absorption. Pages 63-85 *in* O.L. Lange, L. Kappen and E.-D. Schulze, editors. Water and Plant Life. Springer, Berlin.

Caldwell, M.M. 1987. Plant architecture and resource competition. Ecological Studies **61**:164-179.

Caldwell, M.M. 1997. Root structure: The considerable cost of belowground function. Pages 408-427 *in* O.T. Solbrig, editor. Topics in Plant Population Biology. Columbia University Press, New York, USA.

Canadell, J., and P.H. Zedler. 1995. Underground structures of woody plants in Mediterranean ecosystems of Australia, California, and Chile. Pages 177-210 *in* M.K.T. Arroyo, P.H. Zedler, and MD. Fox, editors. Ecology and Biogeography of Mediterranean Ecosystems in Chile, California, and Australia. Ecological Studies 108. Springer, New York, USA.

Cannon, W.A. 1949. A tentative classification of root systems. Ecology **30**:542-548.

Casper, B.B., H.J. Schenk, and R.B. Jackson. 2003. Defining a plant's belowground zone of influence. Ecology **84**:2313-2321.

Cermak, J., J. Hruska, M. Martinkova, and A. Prax. 2000. Urban tree root systems and their survival near houses analyzed using ground penetrating radar and sap flow techniques. Plant and Soil **219**:103-113.

Coleman, M.D., R.E. Dickson, and J.G. Isebrands. 2000. Contrasting fine-root production, survival and soil $CO_2$ efflux in pine and poplar plantations. Plant and Soil **225**:129-139.

Comas, L.H., and D.M. Eissenstat. 2004. Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. Functional Ecology **18**:388-397.

Coupland, R.T., and R.E. Johnson. 1965. Rooting characteristics of native grassland species in Saskatchewan. Journal of Ecology **53**:475-507.

Cox, D.R. 1972. Regression models and life tables. Journal of the Royal Statistical Society **B34**:187-220.

Cox, K.D., H. Scherm, and N. Serman. 2005. Ground-penetrating radar to detect and quantify residual root fragments following peach orchard clearing. Horttechnology **15**:600-607.

Cox, T.L., W.F. Harris, B.S. Asmus, and N.T. Edwards. 1978. The role of roots in biogeochemical cycles in an eastern deciduous forest. Pedobiologia **18**: 264-271.

Cunningham, M., M.B. Adams, R.J. Luxmoore, W.M. Post, and D.L. DeAngelis. 1989. Quick estimates of root length, using video image analyzer. Canadian Journal of Forest Research **19**:335-340.

Daniels, D.J. 2004. Ground Penetrating Radar, 2nd edition. The Institution of Electrical Engineers, London, UK.

Danjon, F., H. Sinoquet, C. Godin, F. Colin, and M. Drexhage. 1999. Characterization of structural tree root architecture using 3D digitizing and AMAPmod software. Plant and Soil **211**:241-258.

D'Arrigo, R., G.C. Jacoby, and I.Y. Fung. 1987. Boreal forest and atmosphere-biosphere exchange of carbon dioxide. Nature **329**:321-323.

Dawson, T.E. 1993. Hydraulic lift and water-use by plants – Implications for water-balance, performance and plant-plant interactions. Oecologia **95**:565-574.

Day, F.P., D.B. Stover, A.L. Pagel, B.A. Hungate, J.J. Dilustro, B.T. Herbert, B.G. Drake, and C.R. Hinkle. 2006. Rapid root closure after fire limits fine root responses to elevated atmospheric $CO_2$ in a scrub-oak ecosystem in central Florida, USA. Global Change Biology **12**:1047-1053.

Day, F.P., E.P. Weber, C.R. Hinkle, and B.G. Drake. 1996. Effects of elevated atmospheric $CO_2$ on fine root length and distribution in an oak-palmetto scrub ecosystem in central Florida. Global Change Biology **2**: 143-148.

Dijkstra, P., G. Hymus, D. Colavito, D.A. Vieglais, C.M. Cundari, D.P. Johnson, B.A. Hungate, C.R. Hinkle, and G.B. Drake. 2002. Elevated atmospheric $CO_2$ stimulates aboveground biomass in a fire-regenerated scrub-oak ecosystem. Global Change Biology **8**:90-103.

Dilustro, J.J., F.P. Day, B.G. Drake, and C.R. Hinkle. 2002. Abundance, production and mortality of fine roots under elevated atmospheric carbon dioxide in an oak-scrub ecosystem. Environmental and Experimental Botany **48**:149-159.

Drake, B.G., P.W. Lendley, W.J. Arp, D. Nassiry, and P.S. Curtis. 1989. An open top chamber for field studies of elevated atmospheric $CO_2$ concentration on salt marsh vegetation. Functional Ecology **3**:363-371.

Eissenstat, D.M. 1992. Costs and benefits of constructing roots of small diameter. Journal of Plant Nutrition **15**:763-782.

Eissenstat, D.M. 1997. Trade-offs in root from and function. Pages 173-199 *in* L.E. Jackson, editor. Ecology in Agriculture. Academic Press, San Diego, USA.

Eissenstat, D.M., C.E. Wells, R.D. Yanai, and J.L. Whitbeck. 2000. Building roots in a changing environment: Implications for root longevity. New Phytologist **147**:33-42.

Eissenstat, D.M., and R. Yanai. 1997. Ecology of root lifespan. Advances in Ecological Research **27**:1-62.

Feldman, L.J. 1984. Regulation of root development. Annual Review of Plant Physiology **35**:223-242.

Fisher, M.J., I.M. Rao, M.A. Ayarza, C.E. Lascano, J.I. Sanz, R.J. Thomas, and R.R. Vera. 1994. Carbon storage by introduced deep-rooted grasses in the South American savannas. Nature **371**:236-238.

Fitter, A.H. 1982. Morphometric analysis of root systems: Application of the technique and influence of soil fertility on root system development in two herbaceous species. Plant, Cell and Environment **5**:313-322.

Fitter, A.H. 1987. An architectural approach to the comparative ecology of plant root systems. New Phytologist **106**:61-77.

Fitter, A.H. 1991. The characteristics of root systems. Pages15-32 *in* Y. Waisel, A. Eschel and U. Kafkafi, editors. Plant Roots: The Hidden Half. Marcel Dekker, New York, USA.

Fitter, A.H., J.D. Graves, G.K. Self, T.K. Brown, D.S. Bogie, and K. Taylor. 1998. Root production, turnover and respiration under two grassland types along an altitudinal gradient: Influence of temperature and solar radiation. Oecologia **114**: 20-30.

Fitter, A.H., J.D. Graves, J. Wolfenden, G.K. Self, T.K. Brown, D. Bogie, and T.A. Mansfield. 1997. Root production and turnover and carbon budgets of two contrasting grasslands under ambient and elevated atmospheric carbon dioxide concentrations. New Phytologist **137**:247-255.

Fitter, A.H., G.K. Self, T.K. Brown, D.S. Bogie, J.D. Graves, D. Benham, and P. Ineson. 1999. Root production and turnover in an upland grassland subjected to artificial soil warming respond to radiation flux and nutrients, not temperature. Oecologia **120**:575-581.

Fitter, A.H., and T.R. Stickland. 1991. Architectural analysis of plant root systems II: Influence of nutrient supply on architecture in contrasting species. New Phytologist **118**:383-389.

Fitter, A.H., and T.R. Stickland. 1992. Architectural analysis of plant root systems III: Studies on plants under field conditions. New Phytologist **121**:243-248.

Fitter, A.H., T.R. Stickland, M.L. Harvey, and G.W. Wilson. 1991. Architectural analysis of plant root systems I: Architecture correlates of exploitation efficiency. New Phytologist **118**:375-382.

Forbes, P.J., K.E. Black, and J.E. Hooker. 1997. Temperature-induced alteration to root longevity in *Lolium perenne*. Plant and Soil **190**: 87-90.

Fogel, R. 1985. Roots as primary producers in belowground ecosystems. Pages 23-36 *in* A.H. Fitter, D. Atkinson, D.J. Read and M.B. Usher, editors. Ecological Interactions I n Soil. Special publication of the British Ecological Society, No. 4, Blackwell, Oxford, UK.

Gebauer, R.L.E., and J.R. Ehleringer. 2000. Water and nitrogen uptake patterns following moisture pulses in a cold desert community. Ecology **81**:1715-1724.

Gill, R.A., and I.C. Burke. 2002. Influence of soil depth and the decomposition of *Bouteloua gracilis* roots in the shortgrass steppe. Plant and Soil **241**:233-242.

Gill, R.A., I.C. Burke, W.K. Lauenroth, and D.G. Milchunas. 2002. Longevity and turnover of roots in the shortgrass steppe: Influence of diameter and depth. Plant Ecology **159**: 241-251.

Gill, R.A., and R.B. Jackson. 2000. Global patterns of root turnover for terrestrial ecosystems. New Phytologist **147**:13-31.

Gordon, W.S., and R.B. Jackson. 2000. Nutrient concentration in fine roots. Ecology **81**: 275-280.

Grier, C.C., K.A. Vogt, M.R. Keyes, and R.L. Edmonds. 1981. Biomass distribution and above- and belowground production in young and mature *Abies amabilis* zone ecosystems of the western cascades. Canadian Journal of Forest Research **11**:155-167.

Grime, J.P., J.C. Crick, and J.A. Rincon. 1986. The ecological significance of plasticity. Pages 5-29 *in* D.H. Jenings and A. Trewavas, editors. Plasticity in Plants. Society for Experimental Biology Symposium # 40. Cambridge University Press, Cambridge, UK.

Gross, K.L., D. Maruca, and K.S. Pregitzer. 1992. Seedling growth and root morphology of plants with different life-histories. New Phytologist **120**.535-542.

GSSI. 2004. RADAN 6.0 User's manual. Geophysical Survey Systems Incorporated, North Salem, New Hampshire, USA.

Guo, D.L., R.J. Mitchell, and J.J. Henricks. 2004. Fine root branch orders respond differently to carbon source-sink manipulations in a longleaf pine forest. Oecologia **140**:450-457.

Harper, J.L., M. Jones, and N.R.S. Hamilton. 1991. The evolution of roots and the problems of analyzing their behavior. Pages 3-22 *in* D. Atkinson, editor. Plant Root Growth, An Ecological Perspective. Blackwell, London, UK.

Harris, W.F., R.S. Kinerson, and N.T. Edwards. 1977. Comparison of belowground biomass of natural deciduous forests and loblolly pine plantations. Pedobiologia **17**:369-381.

Hendrick, R.L., and K.S. Pregitzer. 1992. The demography of fine roots in a northern hardwood forest. Ecology **73**: 1094-1104.

Hendrick, R.L., and K.S. Pregitzer. 1993. Patterns of fine root mortality in two sugar maple forests. Nature **361**:59-61.

Hendrick, R.L., and K.S. Pregitzer. 1993. The dynamics of fine root length, biomass, and nitrogen content in two northern hardwood ecosystems. Canadian Journal of Forest Research **23**:2507-2520.

Hendrick, R.L., and K.S. Pregitzer. 1996. Temporal and depth-related patterns of fine root dynamics in northern hardwood forests. Journal of Ecology **84**:167-176.

Higgins, P.A.T., R.B. Jackson, J.M. Des Rosiers, and C.B. Field. 2002. Root production and demography in a California annual grassland under elevated atmospheric carbon dioxide. Global Change Biology **8**: 841-850.

Hook, P.B., and W.K. Lauenroth. 1994. Root system response of a perennial bunchgrass to neighborhood-scale soil water heterogeneity. Journal of Ecology **82**:485-494.

Houghton, R.A. 2003. Why are estimates of terrestrial carbon balance so different? Global Change Biology **9**: 500-509.

Houghton, J.T., L.G. Meai-Filho, B.A. Callender, N. Harris, A. Kattenberg, and K. Maskel. 1995. Climate Change 1995: The Science of Climate Change. Cambridge University Press, Cambridge, UK.

Hruska, J., J. Cermak, and S. Sustek. 1999. Mapping tree root systems with ground-penetrating radar. Tree Physiology **19**:125-130.

Huck, M.G., G. Hoogenboom, and C.M. Peterson. 1987. Soybean root senescence under drought stress. Pages 109-121 *in* H.M. Taylor, editor. Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics. American Society of Agronomy, Madison, Wisconsin, USA.

Huckle, H.F., H.D. Dollar, and R.F. Pendleton. 1974. Soil survey of Brevard County, Florida. USDA Soil Conservation Service, Washington, D.C.

Hungate, B.A., D.W. Johnson, P. Dijkstra, G. Hymus, P. Stiling, J.P. Megonigal, A.L. Pagel, J.L. Moan, F.P. Day, J. Li, C.R. Hinkle, and B.G. Drake. 2006. Nitrogen cycling during seven years of atmospheric $CO_2$ enrichment in a scrub-oak woodland. Ecology **87**:26-40.

Hutchings, M.J., and E.A. John. 2003. Distribution of roots in soil, and root foraging activity. Pages 33-60 *in* H. de Kroon and E.J.W. Visser editors. Root Ecology, Springer, New York, USA.

IPCC. 2001. Climate Change 2001: The Scientific Basis. J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. Van der Linden, X. Dai, K. Maskell, and C.A. Johnson editors. Contributions of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Jackson, R.B, J. Canadell, J.R. Ehleringer, H.A. Mooney, O.E. Sala, and E.D. Schulze. 1996. A global analysis if root distributions for terrestrial biomes. Oecologia **108**:389-411.

Jackson, R.B., H.A. Mooney, and E.-D. Schulze. 1997. A global budget for fine root biomass, surface area, and nutrient contents. Proceedings of the National Academy of Science, USA. **94**: 7362-7366.

Janzen, H.H. 2004. Carbon cycling in earth systems – a soil science perspective. Agriculture, Ecosystems and Environment **104**:399-417.

Jensen, P., and S. Peterson. 1980. Nutrient uptake in roots of Scots pine. Pages 251-260 *in* T. Persson, editor. Structure and Function of Northern Coniferous Forests – An Ecosystem Study. Ecological Bulletin #32.

Johnsen, K., C. Maier, and L. Kress. 2005. Quantifying root lateral distribution and turnover using pine trees with a distinct stable carbon isotope signature. Functional Ecology **19**:81-87.

Johnsen, K.H., D. Wear, R. Oren, R.O. Teskey, R. Sanchez, R. Will, J. Butnor, D. Markewitz, D. Richter, T. Rials, H.L. Allen, J. Seiler, D. Ellsworth, C. Maier, G. Katul, and P.M. Dougherty. 2001. Meeting global policy commitments: Carbon sequestration and southern pine forests. Journal of Forestry **99**:14-21.

Johnston, C.A., P. Groffman, D.D. Breshears, Z.G. Cardon, W. Currie, W. Emanuel, J. Gaudinski, R.B. Jackson, K. Lajtha, K. Nadelhoffer, D. Nelson Jr., W.M. Post, G. Retallack, and L. Wielopolski. 2004. Carbon cycling in soil. Frontiers in Ecology and the Environment **2**:522-528.

Johnson, M.G., D.T Tingey, D.L. Phillips, and M.J. Storm. 2001. Advancing fine root research with minirhizotrons. Environmental and Experimental Botany **45**:263-289.

Jones, R., R. Mitchell, G. Stevens, and S. Pecot. 2003. Controls of fine root dynamics across a gradient of gap sizes in pine woodland. Oecologia **134**:132-143.

Joslin, J.D, J.B. Gaudinski, M.S. Torn, W.J. Riley, and P.J. Hanson. 2006. Fine-root turnover patterns and their relationship to root diameter and soil depth in a $^{14}$C-labeled hardwood forest. New Phytologist **172**:523-535.

Joslin, J.D., and G.S. Henderson. 1987. Organic matter and nutrients associated with fine root turnover in a white oak stand. Forest Science **33**: 330-346.

Khattree, R., and D. Niak. 1999. Applied Multivariate Statistics with SAS Software. SAS Institute, Cary, North Carolina.

Keyes, M.R., and C.C. Grier. 1981. Above- and belowground net production in 40-year-old Douglas-fir stands on low and high productivity sites. Canadian Journal of Forest Research **11**:599-605.

King, J., T. Albaugh, H. Allen, M. Buford, B. Strain, and P. Dougherty. 2002. Belowground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. New Phytologist **154**:389.398.

King, J.S., K.S. Pregitzer, and D.R. Zak. 1999. Clonal variation in above and belowground growth responses of *Populus tremuloides* Michaux: Influence of soil warming and nutrient availability. Plant and Soil **217**: 119-130.

Klepper, B., H.M. Taylor, M.G. Huck, and E.L. Fiscus. 1973. Water relations and growth of cotton in drying soils. Agronomy Journal **54**:307-310.

Korner, C. 2001. Biosphere responses to $CO_2$ enrichment. Ecological Applications **10**:1590-1619.

Korner, C., and J.A. Arnone. 1992. Responses to elevated carbon dioxide in artificial tropical ecosystems. Science **257**: 1672-1675.

Kramer, P.J., and J.S. Boyer. 1995. Water Relations of Plants and Soils. Academic Press, San Diego, USA.

Krasilnikov, P.K. 1968. On the classification of the root systems of trees and shrubs. Pages 106-114 *in* Methods of Productivity Studies in Root Systems and Rhizosphere Organisms. USSR Academy of Sciences. Nauka, Leningrad, USSR.

Kurz, W.A., and J.P Kimmins. 1987. Analysis of error in methods used to determine fine root production in forest ecosystems: A simulation approach. Canadian Journal of Forest Research **16**:909-912.

Kutschera, L. 1960. Wurzelatlas mitteleuropaischer Ackerunkrauter und Kulturpflanzen. DLG Verlag, Frankfurt-am-Main.

Lambers, H. 1987. Growth, respiration, exudation and symbiotic associations: The fate of carbon translocated to roots. Pages 125-145 *in* P.J.Gregory, J.V. Lake and D.A. Rose, editors. Root Development and Function. Cambridge University Press, London, UK.

Lauenroth, W.K., and R. Gill. 2003. Turnover in root systems. Pages 61-90 *in* De Kroon, H. and E.J.W Visser, editors. Root Ecology. Springer, New York, New York, USA.

Lee, E.T. 1992. Statistical Methods for Survival Data Analysis. John Wiley and Sons, Inc. New York, USA

Ludovici, K.H., S.J. Zarnoch, and D.D. Richter. 2002. Modeling in-situ pine root decomposition using data from a 60-year chronosequence. Canadian Journal of Forest Research **32**:1675-1684.

Lyford, W.H. 1980. Development of the root system of Northern red oak (*Quercus rubra* L.). Harvard Forest Paper #21. Harvard University, Cambridge, Massachusetts, USA.

Lyford, W.H., and B.F. Wilson. 1964. Development of the root system of *Acer rubrum* L. Harvard Forest Paper #10. Harvard University. Cambridge, Massachusetts, USA.

Lynch, J.P. 1995. Root architecture and plant productivity. Plant Physiology **109**:7-13.

Mailander, J.L. 1990. Climate of Kennedy Space Center and vicinity. NASA Technical Memorandum 103498. Kennedy Space Center, Florida, USA.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Academic Press, New York, USA.

Matamala, R., M.A. Gonzalez-Meleer, J.D. Jastrow, R.J. Norby, and W.H. Schlesinger. 2003. Impacts of fine root turnover on forest NPP and soil C sequestration potential. Science **302**:1385-1387.

Martinez, F., O. Merino, A. Martin, D.G. Martin, and J. Merino. 1998. Belowground structures and production in a Mediterranean sand dune shrub community. Plant and Soil **201**:209-216.

McGinty, D.T. 1976. Comparative root and soil dynamics on a white pine watershed and in the hardwood forest in the Coweeta Basin. Ph.D. thesis, University of Georgia, Athens, Georgia, USA.

Menges, E.S., and N. Kohfeldt. 1995. Life history strategies of Florida scrub plants in relation to fire. Bulletin of the Torrey Botanical Club **122**:282-297.

MSU. 2001. ROOTS 2.2. Michigan State University Center for Remote Sensing and Geographic Information Science, East Lansing, Michigan, USA.

Miller, A.T., H.L. Allen, and C.A. Maier. 2006. Quantifying the coarse-root biomass of intensively managed loblolly pine plantations. Canadian Journal of Forest Research **36**:12-22.

Mooney, H.A. 1991. Biological response to climate change: an agenda for research. Ecological Applications **1**: 112-117.

Mooney, H.A., B.G. Drake, R.J. Luxmoore, W.C. Oechel, and L.F. Pitelka. 1991. Predicting ecosystem responses to elevated $CO_2$ concentrations. Bioscience **41**: 96-104.

Mooney, H.A., and G.W. Koch. 1994. The impact of rising $CO_2$ concentration on the terrestrial biosphere. Ambio **23**:74-76.

Mou, P., R.H. Jones, R.J. Mitchell, and B. Zutter. 1995. Spatial distribution of roots in sweetgum and loblolly pine monocultures and relations with above-ground biomass and soil nutrients. Functional Ecology **9**:689-699.

Nadelhoffer, K.J., J.D. Aber, and J.M. Melillo. 1985. Fine roots, net primary production, and soil nitrogen availability: A new hypothesis. Ecology **66**: 1377-1390.

Nielsen, K.L., J.P. Lynch, A.G. Jablokow, and P.S. Curtis. 1994. Carbon cost of root systems: An architectural approach. Plant and Soil **165**:161-169.

Nielsen, K.J., J.P. Lynch, and H.N. Weiss. 1997. Fractal geometry of bean root systems: correlations between spatial and fractal dimension. American Journal of Botany **84**:26-33.

Nepstad, D.C., C.R. de Carvalho, W.A. Davidson, P.H. Jipp, P.A. Lefebvre, G.H. Negreiros, E.D. de Silva, T.A. Stone, S.E. Trumbore, and S. Vieira. 1994. The role of deep roots in the hydrological and carbon cycles of Amazonian forests and pastures. Nature **372**:666-669.

Norby, R.J. 1994. Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. Plant and Soil **165**:9-20.

Norby, R.J., and R.B. Jackson. 2000. Root dynamics and global change: Seeking an ecosystem perspective. New Phytologist **147**:3-12.

Norby, R.J., J. Ledford, C.D. Reilly, N.E. Miller, and E.G. O'Neill. 2004. Fine-root production dominates response of a deciduous forest to atmospheric $CO_2$ enrichment. Proceedings of the National Academy of Sciences **101**:9689-9693.

Norby, R.J., E.G. O'Neill, W.G. Hood, and R.J. Luxmoore. 1987. Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings grown under $CO_2$ enrichment. Tree Physiology **3**:203-210.

Norby, R.J, E.G. O'Neill, S.D. Wullscleger, C.A. Gunderson, and C.T. Nietch. 1993. Growth enhancement of *Quercus alba* saplings by $CO_2$ enrichment under field conditions. Bulletin of the Ecological Society of America **74**:375.

Nye, P.H., and P.B. Tinker. 1977. Solute movement in the soil-root system. Blackwell, Oxford.

Olano, J.M., E.S. Menges, and E. Martinez. 2006. Carbohydrate storage in five resprouting Florida scrub plants across a fire chronosequence. New Phytologist **170**:99-106.

Oppenheim, A.V., and R.W. Schafer. 1975. Digital Signal Processing. Prentice Hall, Inc. Englewood Cliffs, New Jersey, USA.

Persson, H.A. 1978. Root dynamics in a young Scots pine stand in central Sweden. Okios **30**:508-519.

Persson, H.A. 1979. Fine root production, mortality and decomposition in forest ecosystems. Vegetatio **41**:101-109.

Perrson, H.A. 1980. Death and replacement of fine roots in a mature Scots pine stand. Pages 251-260 *in* T. Persson, editor. Structure and Function of Northern Coniferous Forests – An Ecosystem Study. Ecological Bulletin **32**.

Pierret, A., C.J. Moran, and C. Doussan. 2005. Conventional detection methodology is limiting our ability to understand the roles and functions of fine roots. New Phytologist **166**:967-980.

Poorter, H., and M.L. Navas. 2003. Plant growth and competition at elevated $CO_2$: On winners, losers and functional groups. New Phytologist **157**:175-198.

Pregitzer, K.S. 2002. Fine roots of trees – A new perspective. New Phytologist **154**:267-273.

Pregitzer, K.S., J.L. DeForest, A.J. Burton, M.F. Allen, R.W. Ruess, and R.L. Hendrick. 2002. Fine root architecture of nine North American Trees. Ecological Monograph **72**:293-309.

Pregitzer, K.S., R.L. Hendrick, and R. Fogel. 1993. The demography of fine roots in response to patches of water and nitrogen. New Phytologist **125**:575-580.

Pregitzer, K.S., J.S. King, A.J. Burton, and S.E. Brown. 2000. Response of tree fine roots to temperature. New Phytologist **147**:105-115.

Pregitzer, K.S., M.E. Kubiske, C.K. Yu, and R.L. Hendrick. 1997. Relationships among root branch order, carbon, and nitrogen in four temperate species. Oecologia **111**:302-308.

Pregitzer, K.S., D. R. Zak, P.S. Curtis, M.E. Kubiske, J.A. Terri, and C.S. Vogel. 1995. Atmospheric $CO_2$, soil nitrogen and turnover of fine roots. New Phytologist **129**:579-585.

Pritchard, S.G., and H.H. Rogers. 2000. Spatial and temporal deployment of crop roots in $CO_2$-enriched environments. New Phytologist **147**:55-71.

Pritchard, S.G., M.A. Davis, R.J. Mitchell, S.A. Prior, D.L. Boykin, H.H. Rogers, and G.B. Runion. 2001. Root dynamics in an artificially constructed regenerating longleaf pine ecosystem are affected by atmospheric $CO_2$ enrichment. Environmental and Experimental Botany **46**:55-69.

Rastetter, E.B., G.L. Agren, and G.R. Shaver. 1997. Responses of N-limited ecosystems to increased $CO_2$: A balanced-nutrition, coupled-element-cycles model. Ecological Applications **7**:444-460.

Richards, J.H., and M.M. Caldwell. 1987. Hydraulic lift: Substantial nocturnal water transport between soil layers by *Artemisia tridentate* roots. Oecologia **73**:486-489.

Richter, D.D., D. Markewitz, S.E. Trumbore, and C.G. Wells. 1999. Rapid accumulation and turnover of soil carbon in a reestablishing forest. Nature **400**:56-58.

Robinson, D., A. Hodge, and A. Fitter. 2003. Constraints on the form and function of root systems. Pages 1-32 *in* H. de Kroon and E.J.W. Visser, editors. Root Ecology, Springer, New York, USA.

Rodgers, H.H., G.B. Runion, S.A. Prior, and H.A. Torbert. 1999. Responses of plants to elevated atmosphere $CO_2$: Root growth, mineral nutrition, and soil carbon. Pages 215-244 *in* L.Y. Luo and H.A. Mooney, editors. Carbon Dioxide and Environmental Stress. Academic Press, New York, NY, USA.

Rohde, R.A. "Global Warming Art – Carbon Dioxide Gallary," 2007, <http://www.globalwarmingart.com/wiki/Image:Mauna_Loa_Carbon_Dioxide_png> (9 April 2007).

Rotty, R.M., and C.D. Masters. 1985. Carbon dioxide from fossil fuel combustion: Trends, resources and technological implications. Pages 63-80 *in* J.R. Trabalka, editor. Atmospheric Carbon Dioxide and the Global Carbon Cycle. Department of Energy Technical Report ER-0239.

Ruess, R.W., R. L. Hendrick, A.J. Burton, K.S. Pregitzer, B. Sveinbjornsson, M.F. Allen, and G.E. Maurer. 2003. Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska. Ecological Monographs **73**:643-662.

Santantonio, D. 1979. Seasonal dynamics of fine roots in mature stands of Douglas-fir of different water regimes – A preliminary report. Pages 190-203 *in* A. Reidacker and J.G. Michard, editors. Root Physiology and Symbiosis. National Forest Research Center, Nancy-Champenoux, France.

SAS Institute. 1990. SAS/STAT User's Guide, Version 6, 4th Edition. SAS Institute, Cary, North Carolina, USA.

Saterson, K.A., and P.M. Vitousek. 1984. Fine-root biomass and nutrient cycling in *Aristida stricta* in a North Carolina costal plain savanna. Canadian Journal of Botany **62**:823-829.

Schimel, D.S., J.I. House, K.A. Hibbard, P.Bousquet, P. Ciais, P. Peylin, B.H. Braswell, M.J. Apps, D. Baker, A. Bondeau, J. Canadell, G. Churkina, W. Cramer, A.S. Denning, C.B. Field, P. Friedlingstein, C. Goodale, M. Heimann, R.A. Houghton, J.M. Melillo, B. More III, D. Murdiyarso, I. Nobel, S.W. Pacala, I.C. Prentice, M.R. Raupach, P.J. Rayner, R.J. Scholes, W.L. Steffen, and C. Wirth. 2001. Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. Nature **414**: 169-172.

Schlesinger, W.H. 1991. Biogeochemistry: An Analysis of Global Change. Academic Press, New York, New York, USA.

Schlesinger, W.H. 1991. Biogeochemistry: An Analysis of Global Change. Second Edition. Academic Press, New York, New York, USA.

Schlesinger, W.H. 1995. An overview of the global carbon cycle. Pages 9-25 *in* R. Lal, J. Kimble, E. Levine and B.A. Stewart, editors. Soil and Global Change. Lewis Publishers, Boca Raton, Florida, USA.

Schmalzer, P.A., and C.R. Hinkle. 1987. Effects of fire on composition, biomass and nutrients in oak scrub vegetation on John F. Kennedy Space Center, Florida. NASA Technical Memorandum 100305. Kennedy Space Center, Florida, USA.

Schmalzer, P.A., and C.R. Hinkle. 1991. Dynamics of vegetation and soils of oak/saw palmetto scrub after fire: Observations from permanent transects. NASA Technical Memorandum 103817. Kennedy Space Center, Florida, USA.

Schmalzer, P.A., and C.R. Hinkle. 1992. Recovery of oak-saw palmetto scrub after fire. Castanea **57**:158-173.

Schneider, S.H. 2000. Can we forecast climate future without climate past? Pages 230-240 *in* W.G. Ernst, editor. Earth Systems: Processes and Issues. Cambridge University Press, Cambridge, UK.

Schultz, R.P., and F.W. Woods. 1967. The frequency and implication of intraspecific root-grafting in loblolly pine. Forest Science **13**:226-239.

Smith, S.N. 1934. Response of inbred lines and crosses in maize to variations of nitrogen and phosphorus supplied as nutrients. Journal of the American Society of Agronomy **26**:785-804.

Spek, L.Y. 1997. Generation and visualization of root-like structures in a three-dimensional space. Plant and Soil **197**:9-18.

Steele, S.J., S.T. Gower, J.G. Vogel, and J.M. Norman. 1997. Root mass, net primary production and turnover in aspen, jack pine and black spruce forests in Saskatchewan and Manitoba, Canada. Tree Physiology **17**: 577-587.

Stokes, A., T. Fourcaud, J. Hruska, J. Cermak, N. Nadyezdhina, V. Nadyezhdin, and L. Praus. 2002. An evaluation of different methods to investigate root system architecture of urban trees in situ: 1. Ground-penetrating radar. Journal of Arboriculture **28**:2-10.

Stover, D.B., F.P. Day, J.R. Butnor, and B.G. Drake. 2007. Effect of elevated $CO_2$ on coarse root biomass in Florida scrub detected by ground-penetrating radar. Ecology, *In press*.

Systat. 2004. SigmaScan Pro 5.0. Systat Software, Point Richmond, California, USA.

Tans, P.P., I.Y. Fung, and T. Takahashi. 1990. Observational constraints on the global atmospheric $CO_2$ budget. Science **247**: 1431-1438.

Thaler, P., and L. Pages. 1998. Modelling the influence of assimilate availability on root growth and architecture. Plant and Soil **201**:307-320.

Tierney, G.L., and T.J. Fahey. 2001. Evaluating minirhizotron estimates of fine root longevity and production in the forest floor of a temperate broadleaf forest. Plant and Soil **229**: 167-176.

Tierney, G.L., and T.J. Fahey. 2002. Fine root turnover in a northern hardwood forest: A direct comparison of the radiocarbon and minirhizotron methods. Canadian Journal of Forest Research **32**:1692-1697.

Tierney, G.L., T.J. Fahey, P.M. Groffman, J.P. Hardy, R.D. Fitzhugh, C.T. Driscoll, and J.B. Yavitt. 2003. Environmental control of fine root dynamics in a northern hardwood forest. Global Change Biology **9**: 670-679.

Trappe, J.M., and R. Fogel. 1977. Ecosystematic functions of mycorrhizae. Pages 205-214 *in* J.K. Marshall, editor. The Belowground Ecosystem: A Synthesis of Plant Associated Processes. Range Science Department Science Series Number 26, Colorado State University, Fort Collins, Colorado, USA.

Trumbore, S., E.S. Da Costa, D.C. Nepstad, P.B. De Camargo, L.A. Martinelli, D. Ray, T. Restom, and W. Silver. 2006. Dynamics of fine root carbon in Amazonian tropical ecosystems and the contribution of roots to soil respiration. Global Change Biology **12**:217-229.

Vogt, K.A., and J. Bloomfield. 1991. Tree root turnover and senescence. Pages 287-306 *in* A.E.Y. Waisel and U. Kafkafi, editors. Plant Roots: The Hidden Half. Marcel Dekker, New York, New York, USA.

Vogt, K.A. C.C. Grier, and D.J. Vogt. 1986. Production, turnover and nutritional dynamics of above- and below- ground detritus of world forests. Advances in Ecological Research **15**: 303-307.

Waisel, Y., A. Eshel, and U. Kafkafi. 2002. Plant Roots: The Hidden Half. Marcel Deckker, New York, USA.

Ward, J.K., and B.R. Strain. 1999. Elevated $CO_2$ studies: Past, present and future. Tree Physiology **19**:211-220.

Watson, R.T., I.R. Nobel, B. Bolin, N.H. Ravindranath, D.J. Verardo, and D.J. Dokken. 2000. Land use, land-use change and forestry. Special report of the IPCC, Cambridge University Press, Cambridge, UK.

Weaver, J.E. 1919. The ecological relations of roots. Carnegie Institute Publication 286. Washington, D.C., USA.

Weaver, J.E. 1920. Root development in the grassland formation. Carnegie Institute Publication 292, Washington, D.C., USA.

Weaver, J.E. 1958. Classification of root systems of forbs of grassland and a consideration of their significance. Ecology **39**:393-401.

Wells, C.E., and D.M. Eissenstat. 2001. Marked differences in survivorship among apple roots of different diameters. Ecology **82**: 882-892.

Wells, C.E., D.M. Glenn, and D.M. Eissenstat. 2002. Changes in the risk of fine-root mortality with age: A case study in peach, *Prunus persica* (Rosaceae). American Journal of Botany **89**:79-87.

Wentz, W.A., and J.P.M. Chamie. 1980. Determining the belowground productivity of *Chamaedaphe calyculata*, a peatland shrub. International Journal of Ecology and Environmental Science **6**:1-4.

West, J.B., J.F. Espeleta, and L.A. Donovan. 2003. Differences in root longevity and phenology between two co-occurring, native sand hill savanna bunchgrasses. Functional Ecology **17**:20-28.

West, J.B., J.F. Espeleta, and L.A. Donovan. 2004. Fine root production and turnover across a complex edaphic gradient of a *Pinus palustris-Aristida stricta* savanna ecosystem. Forest Ecology and Management **189**:397-406.

Wielopolski, L., G. Hendey, and M.McGuigan. 2000. Imaging tree root systems in situ. Pages 642-626 *in* D.A. Noon, G.F. Stickley, and D. Longstaff, editors. Proceedings of the 8th International Conference on Ground-Penetrating radar, Gold Coast, Queensland, Australia, 23-26 May 2000. Proceedings of SPIE – The International Society of Optical Engineering, Bellingham, Washington, USA.

Wilcox, H. 1968. Morphological studies of the root of red pine, Pinus resinosa1: Growth characteristics and patterns of branching. American Journal of Botany **55**:247-254.

Withington, J.M., P.B. Reich, J. Oleksyn, and D.M. Eissenstat. 2006. Comparisons of structure and life span in roots and leaves among temperate trees. Ecological Monographs **76**:381-397.

Zobel, R.W. 1991. Genetic control of root systems. Pages 27-38 *in* Y. Waisel, A. Eschel and U. Kafkafi, editors. Plant Roots: The Hidden Half. Marcel Dekker, New York, USA.

Zobel, R.W. 2003. Fine roots – Discarding flawed assumptions. New Phytologist **160**:273-280.

# APPENDIX

## FINE ROOT LENGTH DENSITY

In the first three years of the study, a treatment effect was present where fine roots grown under elevated $CO_2$ produced greater RLD than those grown under ambient $CO_2$ conditions (Dilustro et al. 2002). This treatment effect was lost as the mean RLD between treatments converged due to fine root closure (Fig. 34) (Day et al. 2006). A closer examination of the distribution of fine roots over the past nine years into eight depth classes indicated that depth classes 1-3 (0-37 cm) had the highest mean RLD, whereas the deepest root classes 7 and 8 (74-101 cm) had the lowest mean RLD (Fig. 35). It is evident that RLD increased early in the study until reaching a maximum density (root closure); however, the accumulation of root density in the lower portions of the soil profile was slower within the lowest depth classes (Depth p=0.001). It is important to note that depth class 1 (surface depth class) was under sampled in the later years of the study due to soil separation and algal and organic matter deposits blocking some of the viewing frames. Compared to Day et al. (2006), fine roots have increased in density with increasing depth (more or less evenly among treatments). Overall, there is a significant depth*treat interaction (p=0.017) most likely driven by differences in the early years of the study.

## FINE ROOT SURFACE AREA

Fine root surface area was significantly different early in the study, but declined after 1999 (Fig. 36). In 2002, a general increase in fine root surface area was observed

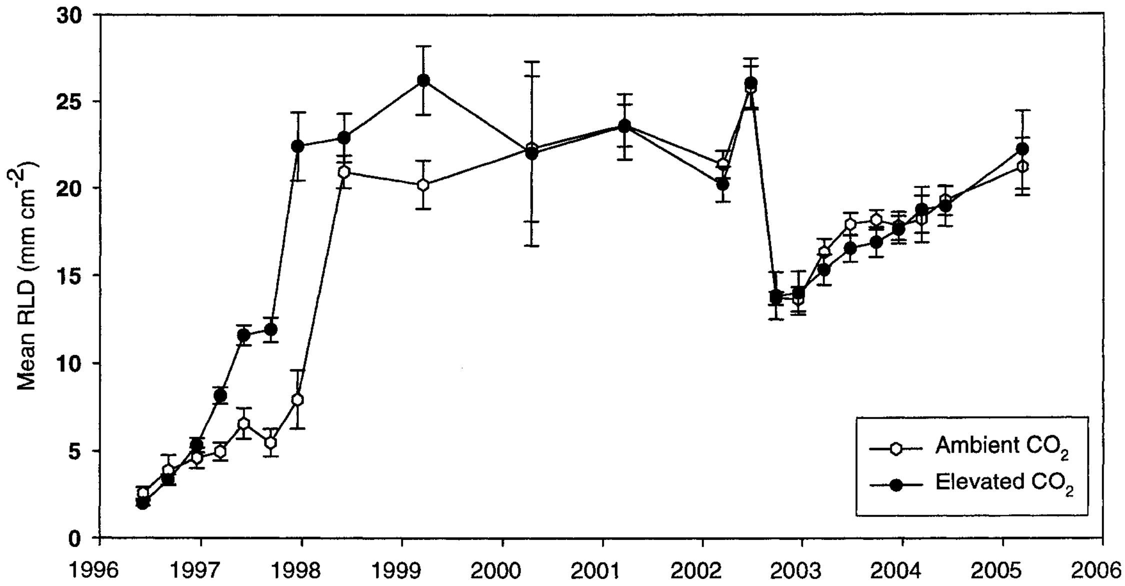


FIG. 34: Long-term minirhizotron estimates of root length density (RLD) measured in mm cm$^{-2}$. Measurements were taken for ambient and elevated $CO_2$ treatments four times yearly to a depth of 1 meter. Error bars are 1 S.E.

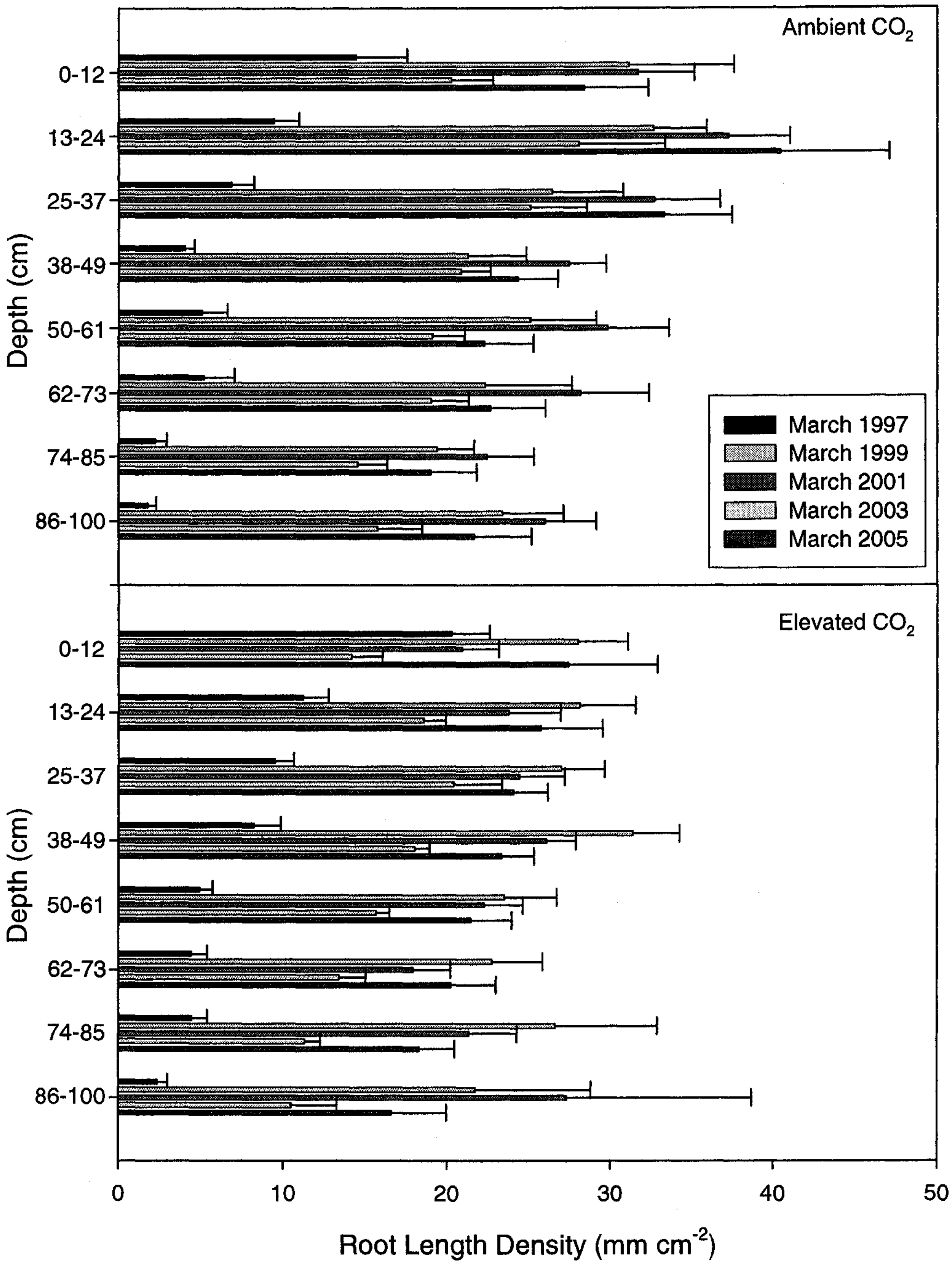


FIG. 35: The effect of $CO_2$ enrichment on root length density for eight equal depth classes to a total depth of 1 meter. Values are averages of frames within depth classes and replicates within treatments. Error bars are 1 S.E.

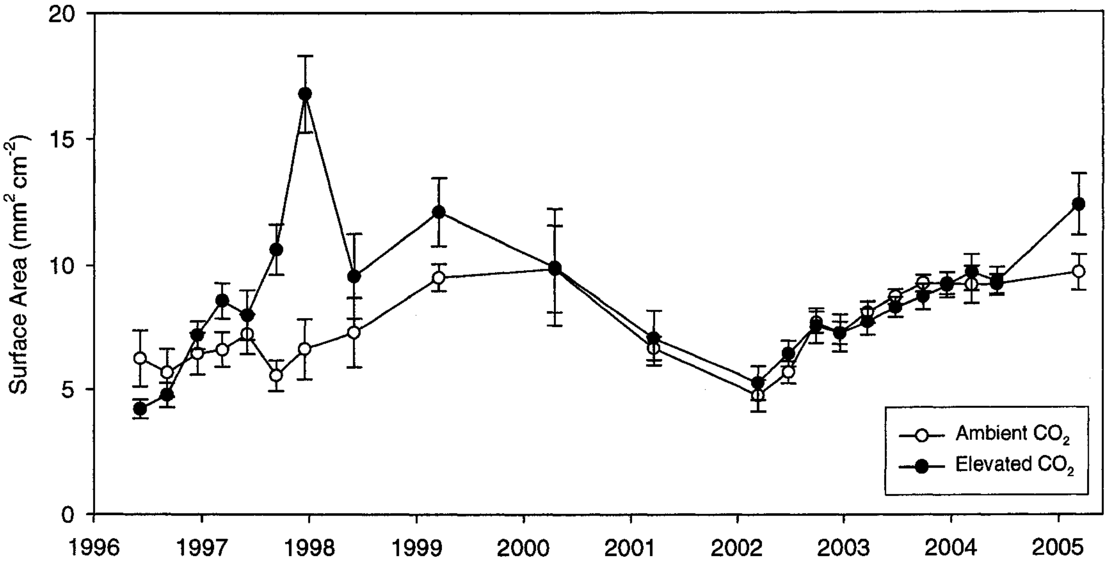


FIG. 36: The long-term effects of elevated $CO_2$ on fine root surface area (mm$^2$ cm$^{-2}$) in a scrub-oak ecosystem. Values are averages of frames and replicates within treatments. Error bars are 1 S.E.

(date*treat p=0.037). Depth ($p < 0.001$) also seems to have influenced surface area in a manner similar to root diameter. The lowest depth class had a significant $CO_2$ treatment by date interaction (p=0.043) suggesting that surface area increased over time at the lowest depths.

Fine root surface area has remained relatively constant throughout the study at approximately 10 $mm^2\ cm^{-2}$. Combined with the RLD and diameter measurements, surface area appears to indicate that fine roots were producing larger diameter roots following decline of RLD in 2002. This is supported since the average surface area increased within this period, ultimately reaching a newer and higher equilibrium of the same magnitude seen in the 1998-2000 observation periods. Increased surface area and length maximize the ability to acquire nutrients and water; however, larger diameter roots are less efficient at uptake and better at conducting these resources (Nye and Tinker 1977, Eissenstat 1992, 1997). Rastetter et al. (1997) found that as carbon allocations belowground increase in response to elevated atmospheric $CO_2$, RLD will increase while the average distance between roots will decrease in response to decreasing soil nutrients. This suggests that fine roots will begin to maximize soil foraging to a point where depletion zones begin to overlap, thus resulting in root closure.

## RELATIVE $CO_2$ RESPONSE

Relative $CO_2$ effects were calculated similar to Day (et al. 2006).

$$\frac{\text{Elevated RLD - Ambient RLD}}{\text{Ambient RLD}} \times 100$$

Relative $CO_2$ calculations were divided into four 25 cm depth increments to examine differences in relative $CO_2$ effects at different depths.

The effect of $CO_2$ on fine root abundance was significant during the initial 3 years of the study, however, this treatment effect diminished in the later years of the study (Fig. 37). By comparing the relative treatment effect by depth, a slight divergence in the $CO_2$ response is observed (Fig. 38). During the initial treatment effect, the largest response was observed 25-100 cm deep in the soil. Specifically, a large response is noted in the 25-50 and 75-100 cm depth classes, indicating a large increase in the abundance of fine roots in response to elevated $CO_2$. However, later in the study, most of the relative $CO_2$ responses became slightly negative except in the 25-50 cm depth range. This indicates that an elevated $CO_2$ response has persisted at this depth over the duration of the study.

The only consistent relative $CO_2$ response was observed in the 25-49 cm range indicating that $CO_2$ enrichment is still actively increasing RLD at this depth. Long-term relative response trends show a large response to $CO_2$ enrichment early in the study, which dissipated after the first 3-4 years.

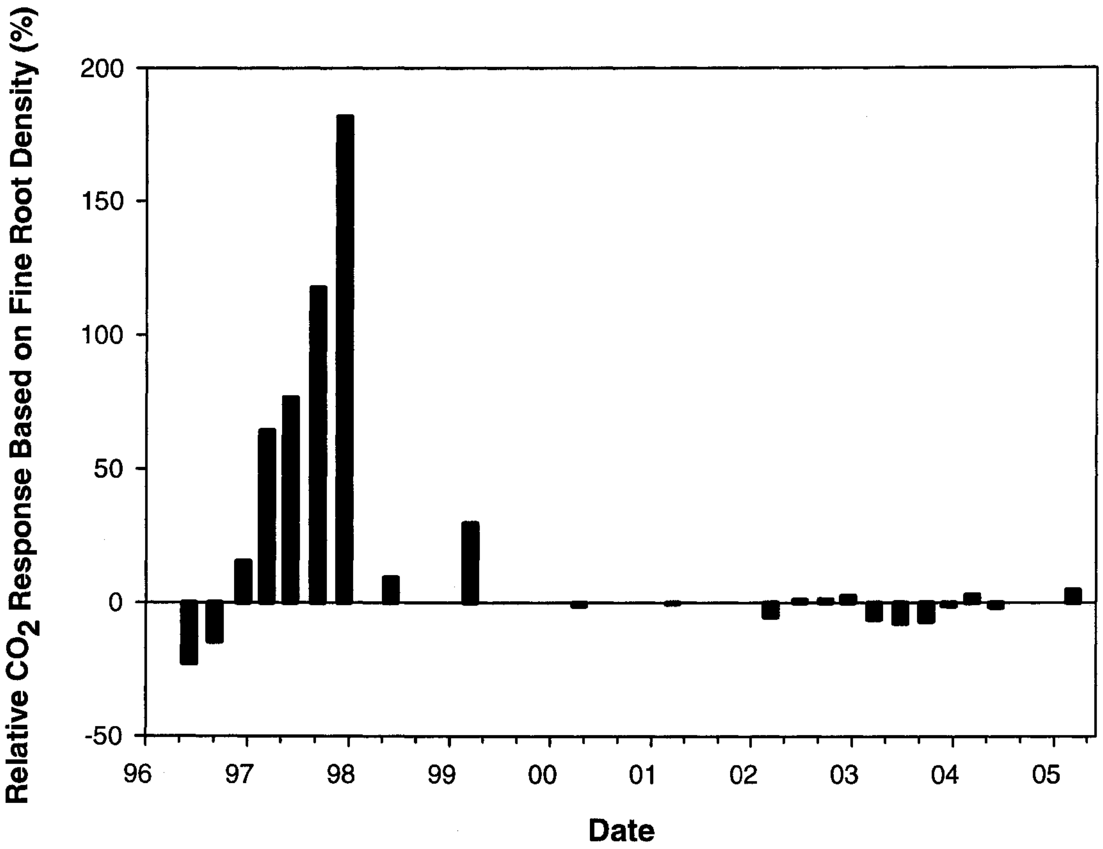


FIG.37: Relative $CO_2$ response based on fine root density from minirhizotron estimates.

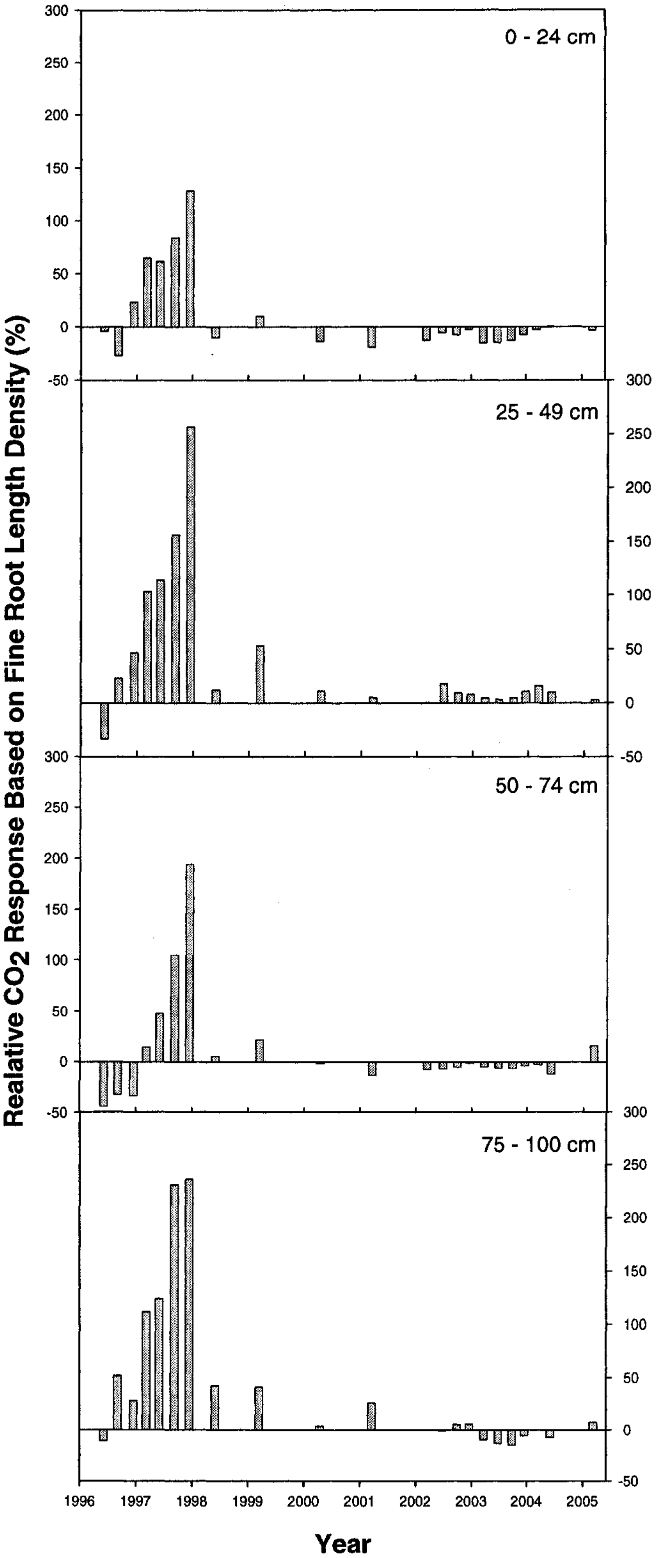


FIG. 38: Relative $CO_2$ response by depth based on fine root density from minirhizotron estimates.

# VITA

**Daniel Benjamin Stover**
Department of Biological Sciences
Old Dominion University
Norfolk, VA 23529

*Education*

Bachelor of Arts in Biology from West Virginia University, Morgantown, WV granted in May 1999.

Bachelor of Science in Environmental Protection from West Virginia University, Morgantown, WV granted May 1999.

Bachelor of Arts in Interdepartmental Studies from West Virginia University, Morgantown, WV granted May 1999.

Master of Science in Environmental Plant Biology from West Virginia University, Morgantown, WV granted August 2005.

Doctorate of Philosophy in Ecological Sciences from Old Dominion University, Norfolk, VA granted May 2007.

*Professional Experience*

Research Assistant: Fall 2002 to Spring 2007 at ODU in the lab of Dr. Frank Day examining the effects of elevated $CO_2$ on fine and coarse root growth at Kennedy Space Center, Florida.

Graduate Assistant: Spring 2005 to Spring 2006 at ODU teaching New Portals for Appreciating the Global Environment Freshman environmental survey course and assisted coordinating the global climate change themed unit.

Biological Aid: Summer 1998-1999 at U.S. Department of Agriculture, Agricultural Research Service, Beaver, WV examining the pasture ecology of novel Appalachian crops and cultivars of chicory.

*Professional Memberships*

Ecological Society of America
Association of Southeastern Biologists
International Association of Ecology (INTECOL)
Soil Science Society of America

*Publications*

Stover, D.B., F.P. Day, J.R. Butnor, and B.G. Drake. 2007. Effect of elevated $CO_2$ on coarse root biomass in Florida scrub detected by ground-penetrating radar. Ecology *in press.*

Day, F.P., D.B. Stover, A.L. Pagel, B.A. Hungate, J.J. Dilustro, B.T. Herbert, B.G. Drake, and C.R. Hinkle. 2006. Rapid root closure after fire limits fine root responses to elevated atmospheric $CO_2$ in a scrub oak ecosystem in central Florida, USA. Global Change Biology 12:1047-1053.