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Root Dynamics of Crop Plants in a High Carbon Dioxide World: Effects of Elevated Versus Ambient Carbon Dioxide Levels and No-Till Versus Conventional Agricultural Management

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ROOT DYNAMICS OF CROP PLANTS IN A HIGH CARBON DIOXIDE WORLD: EFFECTS OF ELEVATED VERSUS AMBIENT CARBON DIOXIDE LEVELS AND NO-TILL VERSUS CONVENTIONAL AGRICULTURAL MANAGEMENT

by

Charlotte Jean Barker

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ABSTRACT

ROOT DYNAMICS OF CROP PLANTS IN A HIGH CARBON DIOXIDE WORLD: EFFECTS OF ELEVATED VERSUS AMBIENT CARBON DIOXIDE LEVELS AND NO-TILL VERSUS CONVENTIONAL AGRICULTURAL MANAGEMENT

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Due to the continuing increases in atmospheric carbon dioxide levels and its potential effect on food sources, there is an interest in evaluating the effect of elevated CO_2 concentration versus ambient CO_2 concentration in agricultural crop plants although more studies have focused on the aboveground portions of plants rather than the roots. Additionally, the conservation agricultural method, no-till, has been widely suggested as a possible method of increasing soil organic carbon and increasing soil moisture in a hotter world.

This research involves two major agricultural plants, *Sorghum bicolor* (sorghum), and *Glycine max* (soybean) grown under four conditions including conventional till elevated, conventional till ambient, no-till elevated, and no-till ambient. These plants, along with three rotating cover crops, were grown in open top chambers (OTCs) at the USDA-ARS National Soil Dynamics Laboratory at Auburn, Alabama as part of a series of related studies. The cover crops were not used during conventional tillage and were grown under no-till elevated and no-till ambient conditions.

The first part of the study involved comparing root growth response to the treatments of the important agricultural plants, sorghum and soybean. There was a trend toward greater standing root crop with sorghum using no-till cropping methods. There was a significantly greater average root length of sorghum in deeper soil and a trend toward elevated atmospheric CO_2 concentration being associated with increased average root length of sorghum. For soybean, there was a significant effect of no-till on average root diameter.

The second area of focus involved comparing the three legumes in the study at no-till elevated CO_2 concentration at 720 µmol mol^{-} and no-till ambient CO_2 concentrations at 365 µmol mol^{-1}conditions. This included soybean (*Glycine max* (L.) Merrill), sunn hemp (*Crotalaria juncea* L), and scarlet clover (*Trifolium incarnatum* L.). There was a species difference, as postulated previously, with sunn hemp having a significant growth response to elevated CO_2 concentration while there was less increased root growth from the other two legumes. Additionally, both sunn hemp and soybean had increased root growth in deeper soil (17 – 34 cm) which clover did not.

The third portion of the study involved a comparison of a C_3 cover crop grass, wheat (*Triticum aestivum* L.), with a C_4 grass (*Sorghum bicolor* L.), at no-till ambient and no-till elevated conditions. In this case, both members of family Poaceae had root growth response to elevated CO_2 concentration, which although not identical, did not support earlier indications of C_3 plants having an advantage in elevated CO_2 concentration.

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DEDICATION

In any major pursuit, the encouragement of family and friends is of ultimate assistance, so I wish to dedicate this dissertation first to my late husband, David Major Barker, who always encouraged me to pursue a scientific career. Secondly, I am grateful for the emotional support of my daughters, Amy Barker and Kim Barker as well as their spouses, all of whom have frequently been updated on the progress of the dissertation. Third, I appreciate the friendship and encouragement of my sister, Billie Hiett, my longtime friend, Teresa Hagen, and my scientific friend, Dr. Catherine Cone.

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CHAPTER I – GENERAL INTRODUCTION

The concentration of carbon dioxide, in chemical notation written as [CO_2], in the atmosphere has increased from 313 parts per million (ppm) on the first day that Charles Keeling took measurements at Mauna Loa in March,1958 (Peterman 2017), to a monthly average of 408.35 ppm at Moana Loa on February, 2018 (NOAA/ESRL 2018). Human activity, such as burning of fossil fuels and clearing of forested areas, is contributing to an ever upward spiral of increased [CO_2] that has resulted in global warming (Lal 2008**)** with important implications for the sustainability of food resources for a planet with a human population of some 7.6 billion **(**World Population Prospects 2017).

These observations prompt questions as to how agricultural plants will be affected by increased atmospheric carbon dioxide. Elevated atmospheric carbon dioxide increases plant growth for a number of crop plants, with enhanced root growth often exceeding that of aboveground plant structures (Kimball et al. 2002). The response of agricultural plant roots to elevated atmospheric [CO_2] is to increase in number, diameter, and length. Faster growth rates, with increased branching, is not unusual (Pritchard and Amthor 2005; Pritchard et al. 2006; Madhu and Hatfield 2013). In a meta-analysis including a number of ecosystems, Nie et al. (2013) indicate that the responses of plant roots to elevated atmospheric [CO_2] could increase absorption of water and nutrients, but they also speculate that this might not be applicable for agricultural plants due to fertilizer application which already increases the efficiency of fine roots. Leakey et al. (2009) indicate that increased root branching due to elevated [CO_2] could also decrease the roots' ability to efficiently take in nutrients and water. Although increased [CO_2] may result in a larger root area which takes in more water, the more extensively branched roots systems may be less efficient per mm of root length (Pritchard and Amthor 2005).

Fine roots (< 2mm in diameter) are directly involved in uptake of water and nutrients and, in turn, deliver carbon to the soil in the form of exudates (Pritchard 2011). The smallest (first, second, and third order) absorptive fine roots are those most closely associated with uptake of nutrients and water and are more effectively monitored by minirhizotrons than are higher order roots (McCormack et al. 2015). Fine roots are short-lived and represent a major commitment of resources on the part of the plant (Pritchard et al. 2006). An often quoted figure, based on a study of eleven biomes, is that replacement of dead fine roots by new living fine roots (root turnover) may involve as much as 30% of global terrestrial net primary production (Jackson et al.1997).

A broad based meta-analysis of a number of biomes indicated that elevated atmospheric [CO_2] increased both root production and mortality. However, for the three agricultural ecosystems included in the study, the distinction was less clear with fine root biomass showing very little increase with elevated [CO_2] (Nie et al. 2013). When the additional factor of agricultural management system is added, the results can become even more complex. In an earlier USDA study of sorghum root growth, twice ambient [CO_2] resulted in an increase in seasonal root production of 58% for the conventionally tilled plants, while root growth was unaffected by twice ambient [CO_2] with the no-till agricultural management (Pritchard et al. 2006). Alternatively, Madhu and Hatfield (2013) noted that increases in atmospheric CO_2 increases growth of plant roots, but they also speculated that the growth of fine roots and soil carbon storage may be even more affected by the adoption of no-till agricultural management than by atmospheric [CO_2].

No-till, as a category of conservation agriculture, involves a lack of mechanical soil disturbance to minimize loss of soil organic carbon (SOC), the use of cover crops and crop residues to provide mulch and contribute to soil carbon and water retention, and varied crop rotations (Corsi et al. 2012). No-till agricultural management is preferable to conventional tillage for preventing soil erosion and for carbon storage with the potential to improve soil quality and to improve soil organic carbon (SOC) (Blanco-Canqui and Lal 2008). The resulting improvement in soil carbon sequestration has a positive influence on root growth (Pritchard et al.2006). As a result of increased fine root growth, large increases in the amount of carbon stored in the soil could result from the switching from conventional to no-till agricultural management systems. The increased water retention and reduced soil erosion resulting from increased crop residue and more ground cover may be involved in the improved storage of soil organic carbon (Prior et al. 2005).

However, Madhu and Hatfield (2013) asserted that the interactive effects of increased [CO_2] and agricultural management practices upon fine root growth are incompletely understood. Pritchard (2011) has cautioned that increasing the flow of organic carbon into the soil by sloughing of root organic material and exudates would also increase decomposition and result in increased soil carbon efflux. Clearly, the combined effects of agricultural management and elevated carbon dioxide levels involve complex interactions.

A related issue is that plants with C_3 photosynthesis (C_3 plants) respond to a different extent to increased [CO_2] than do plants with C_4 photosynthesis (C_4 plants). This is relevant to agricultural concerns concerning elevated atmospheric carbon dioxide since the majority of agricultural plants are C_3 plants with 12 of the 15 crops which supply 90% of the world's calories having C_3 photosynthesis (Reddy et al. 2010). Studies have shown that C_3 plants respond to elevated [CO_2] with increased photosynthesis which translates into increased root growth (Prior et al. 2005; Pritchard and Amthor 2005).

While fewer total plant species are C_4 plants, those that undergo C_4 photosynthesis include important agricultural crop plants such as maize (corn), sorghum, sugarcane, and millet (Parry 1990). Sorghum, which is grown worldwide in temperate and tropical areas and can survive in marginal areas, uses the "malate" C_4 cycle, as do sugarcane and maize (Ruskin 1996). Additionally, many C_4 agricultural plants are of great importance in tropical areas where local farming is essential to food security (Leakey et al. 2009).

The photosynthetic response of C_4 plants to increased [CO_2] is less well known than that of C_3 plants. A number of authors indicate that C_4 plants have an increase in photosynthesis under elevated [CO_2] but less than that of C_3 plants under similar conditions (Ainsworth 2005; Runion 2009). Chaudhuri et al. (1986) found an increase in shoot and root growth of sorghum under [795 µmol mol^{-1} CO_2] while water use decreased. Pritchard and Amthor (2005) attribute the increase in productivity of C_4 plants under elevated atmospheric [CO_2] to be primarily due to increased water use efficiency (WUE). Similarly, Leakey et al. 2009 indicate that elevated [CO_2] does not directly enhance photosynthesis, but instead improves the water relations of C_4 plants which increases photosynthesis and growth in drought conditions.

Legume agricultural species, which included three of the plants utilized in this study, have a further area of interest in addition to response to [CO_2] and to agricultural management. The legumes have the ability to form a symbiotic relationship with rhizobia in which the legume provides carbohydrates to the bacteria**,** and the rhizobia move into root nodules of the legume and fix nitrogen (Rogers et al. 2006). In the case of legumes, more carbon is acquired as a result of increased photosynthesis due to elevated [CO_2], which means that more carbon could be shifted to root production. More root growth would then result in more nitrogen being available to the plant due to nitrogen fixation (Morrison and Morecroft 2006). This has led to the observation that legumes have the potential to grow more and have additional productivity in response to elevated carbon dioxide levels than non-legumes under the same conditions (Rogers et al. 2006).

Elevated carbon dioxide levels have been shown to affect growth and symbiotic activity in legume species with increased growth associated with characteristics such as larger nodule size, mass, and/or number such as was found by Prevost et al. (2010) who observed a 63% increase in nodule mass and a 50% increase in number of nodules in soybeans grown under elevated carbon dioxide. However, West et al. (2005) indicate that the amount of response of legumes to elevated [CO_2] can vary depending upon cultivar or species, soil nitrogen availability, and other conditions.

This project was designed to compare root growth of a crop plant legume (*Glycine max*) with a non-legume agricultural plant (*Sorghum bicolor*) for differing levels of atmospheric carbon dioxide and for differing agricultural management systems. The current research is associated with a series of experiments done over a span of ten years by plant scientists at the USDA-ARS National Soil Dynamics Laboratory in Auburn, Alabama, in part, to quantify the effects of increased atmospheric carbon dioxide on agricultural plant growth and physiology. As a part of those studies, Pritchard et al. (2006) had previously described the effects of elevated atmospheric carbon dioxide [CO_2]

with conventional and with conservation (no-till) agricultural management on root productivity of one crop plant, sorghum (*Sorghum bicolor*). By capturing and analyzing data for five different agricultural plants, including sorghum, during a later cropping cycle, the current research project amplified the process of quantifying agricultural plant root responses to the alternative management systems and/or ambient and twice ambient levels of carbon dioxide.

Plant root growth, under controlled conditions, was recorded using minirhizotrons and a BTC-100 microvideo camera (Prichard et al. 2006), at the USDA National Root Laboratory, while I undertook data capture and analysis as a doctoral student at the University of Southern Mississippi, Hattiesburg. Data collection methods (from previously recorded root images) and statistical analyses were similar but not identical to the earlier study. The current study used Rootfly 2.0.2 (Stanley T. Birchfield and Christina Wells, Clemson University) while the earlier sorghum study used RooTracker (Dave Tremmell, Duke University) to digitize the data. However, like the earlier study (Pritchard et al. 2006), data were considered statistically different when alpha levels were $p < 0.05$. The differences between the current study as regards data capture, analyses, and personnel involved offered an invaluable opportunity for independent observation of repeatability.

Agricultural plants grown in the open top chambers (OTCs) were chosen for their differing characteristics including a C_4 grain, *Sorghum bicolor* (sorghum), and a C_3 grain, *Triticum aestivum* (wheat). There were three leguminous C_3 plants including *Glycine max* (soybean), *Crotalaria juncea* (sunn hemp), and *Trifolium incarnatum* (crimson clover). The crop plants were soybean and sorghum, which were grown with both no-till and conventional till at ambient [CO_2] and twice normal [CO_2] (Pritchard et al. 2006). The three cover crops, grown under no-till treatment at both ambient (365 µmol CO_2 mol^{-1} air [ppmv] and twice normal [CO_2] (720 µmol CO_2 μmol^{-1} air) (Mitchell et al. 1995) were sunn hemp, wheat, and crimson clover.

The experimental design of the crop plants grown in the OTCs with two levels of carbon dioxide (ambient and twice ambient) and two agricultural management systems (conventional and no-till) was a split-plot design with three blocks (three replications). One half of each block was traditional and one half was no-till. Split-plot treatments of carbon dioxide level were randomly assigned within blocks. Clear plastic minirhizotron tubes were used that allowed repeated recording of images of root growth with six tubes for each of the four treatments (Pritchard et al. 2006). These included no-till ambient [CO_2] which was designated as NTA; No-till elevated [CO_2] which was referred to as NTE; Conventional till ambient [CO_2] which was abbreviated as CTA; and Conventional till elevated [CO_2] also referred to as CTE.

The research project involved the use of the Rootfly 2.0.2 image analysis program to quantify the length and width of roots from 0 to 34 cm vertical soil depth (Pritchard 2006)**.** The data obtained were used to calculate average root length, average root diameter, and standing root crop. Maximum root production, seasonal root production and root turnover index were calculated.

The study was designed as a repeated measures study with time being the repeated measure where root images were recorded at biweekly intervals across the growing season of each species of plant. Repeated measures MANOVA analysis (JMP 12.1) was used for statistical inference as is appropriate for complex interactions of multiple factors with more than one dependent (response) variable (SAS Institute 2012). Based on related previous studies (Prior et al. 2005; Pritchard et al. 2006), I had predicted that the results would be complex.

The two crop plants were tested at four conditions including conventional till elevated (CTE), conventional till ambient (CTA), no-till elevated (NTE), and no-till ambient (NTA). Hypotheses included, first, for the two crop plant species tested at all four conditions (CTA, CTE, NTA, NTE) (chapter two), twice ambient carbon dioxide levels will increase plant root growth of the legume more than the increase in root growth of non-legumes. Second, for the two crop plant species studied at all four conditions (CTA, CTE, NTA, NTE), no-till agricultural management will increase root growth more than will conventional till (chapter two). Third, for the legume species studied with no-till agricultural management, twice ambient CO_2 (NTE) will increase plant root growth (for one or more species) more than that with ambient CO_2 (NTA) (chapter three). Fourth, for the two grains/monocots evaluated under no-till conditions, twice ambient carbon dioxide levels (NTE) will increase root growth of the C_3 plant more than twice-ambient (NTE) conditions will increase the root growth of the plant with C_4 photosynthesis (chapter four).

CHAPTER II – SORGHUM AND SOYBEAN ROOT GROWTH AT ELEVATED CO_2

2.1 Abstract

To evaluate the effects of agricultural management and atmospheric carbon dioxide levels [CO_2] on root growth of two important agricultural crop plants, a C_4 grain, *Sorghum bicolor* (sorghum), and a C_3 legume, *Glycine max* (soybean), were grown at the USDA-ARS National Soil Dynamics Laboratory, Auburn, Alabama, USA, in silt loam soil in open top chambers under four different environmental conditions. These included: (1) no-till agricultural management and ambient [CO_2] (365 µmol mol^{-1}), (2) no-till agricultural management and elevated [CO_2] (720 µmol mol^{-1}), (3) conventional till and ambient [CO_2] (365 µmol mol^{-1}), and (4) conventional till and elevated [CO_2] (720 µmol mol^{-1}). Root growth was recorded using minirhizotrons into which a BTC-100 microvideo camera (Bartz Technologies, Santa Barbara, California) was inserted and images were recorded at specific intervals across the growing season of each plant. The image analysis program, Rootfly Version 2.0.2 (Birchfield and Wells, Clemson University), was used to measure root diameter and root length, from which standing root crop, maximum standing root crop, seasonal root production, and root turnover index could be calculated.

Statistical analysis involved repeated measures MANOVA using the JMP 12.1.0 program. Sorghum had a significantly greater average root length (m/m^2) at the deeper soil level (17–34 cm), and sorghum had significantly more roots (m/m^2) (standing root crop) in deep soil than in shallow soil. For the average root diameter of soybean (m/m^2), no-till agricultural management resulted in significantly increased values. The results

indicate that no-till agricultural management can affect the root growth of sorghum and soybean.

2.2 Introduction

In the United States conservation agriculture, of which no-till agricultural management is an integral part, dates back to a response to the dustbowl conditions of the 1930s (Baveye et al. 2011**).** No-till has advantages over conventional tillage in the prevention of soil erosion, improvement of soil structure, and retention of moisture (Wright and Hons 2004; Blanco-Canqui and Lal 2008). From a business management standpoint, the no-till agricultural management system saves time, labor, and fuel (Hobbs 2007). No-till is an agricultural innovation worldwide with the land under no-till increasing from 45 million hectares in 1999 to 105 million hectares by 2009 (Derpsch et al. 2010). By 2009 no-till agricultural management was used on 35.5 % of cropland planted with eight major crops as determined by the Economic Research Council. Among these were sorghum and soybean with close to 50% of soybeans grown using no-till agricultural management (Horowitz et al. 2010).

Atmospheric carbon dioxide concentration [CO_2] has increased from an estimated pre-industrial level of 280 parts per million (ppm) to 409.96 ppm as of March 10, 2018 as measured at NOAA's Mauna Loa Baseline Atmospheric Observatory (NOAA/ESRL 2018). No-till agricultural management has been promoted as a means of sequestering carbon from atmospheric CO_2 in the soil and as preferable to conventional tillage where cultivation releases soil organic carbon (West and Post 2002). Originally, the potential for stored amounts of carbon resulting from no-till was described as 0.57 tons of carbon per hectare per year (West and Post 2002) or 1000 kg/hectare/year (Lal et al. 2004).

Palm et al. (2014) pointed out that while increased soil carbon storage by no-till has been recorded in fewer than 50% of studies, and where it is recorded, it is primarily in the shallow layers (< 10 cm deep). While other sources have indicated that no-till only has its full effect over decades of time (Six et al. 2004), not all studies of soil organic carbon (SOC) storage have been conducted over a number of years.

The no-till agricultural management system involves not only minimal soil disturbance, the use of cover crops, and crop rotation, but it also prominently features retention of crop residue on the surface (Derpsch et al. 2010). Prior et al. (2005) indicates that the improvement in water retention and reduction in soil erosion resulting from no-till agricultural management has the potential to increase storage of SOC as does the decomposing crop residue.

Pritchard (2011) considered canopy litter and rhizodeposition to be important for accrual of SOC and pointed out that rhizodeposition includes sloughing of exterior root cells and root exudation or movement of carbon-containing molecules, such as simple sugars and amino acids, from fine roots into the soil. Kell (2012) considered that photosynthesis followed by root exudation is a major source of carbon in the complex soil ecosystem. While there are other inputs to soil carbon, such as decomposition of microorganisms and fungi (Lal 2008), root exudates may compose some 0.5–20% of net ecosystem carbon assimilation (Frank and Groffman 2009).

The turnover of roots smaller than 2 mm in diameter is part of rhizodeposition (Pritchard 2011**.** The smallest fine roots are classified as first-order, with second order fine roots beginning at the junction of first order fine roots. Lower order fine roots including first, second, and third order are typically 2 millimeters or less in diameter and are primarily absorptive in nature while slightly larger roots have more transport function (McCormack et al. 2015). Replacement of these small diameter roots, or root turnover, may involve as much as 30% of global terrestrial net primary production (Jackson et al. 1997).

It is widely agreed that elevated [CO_2] increases plant growth including plant root growth (Prior et al. 1995; Pritchard and Amthor 2005). There have been a number of studies corroborating this effect, although more of those studies have involved aboveground plant growth than belowground. Those studies which do address the growth of plant fine roots frequently involve different functional groups of plants rather than agricultural plants. Root growth in a variety of ecosystems have been studied where the effects of elevated [CO_2] on root growth has been found not to have an identical effect as that of elevated [CO_2] on the root of growth of agricultural plants (Nie et al. 2013).

Carbon dioxide is a necessary input for photosynthesis to occur, and plants with C_3 photosynthesis typically respond to elevated atmospheric carbon dioxide by more increase in root growth than do C_4 plants (Pritchard and Amthor 2005; Ainsworth 2005; Runion 2009). Plants with C_4 photosynthesis have a carbon dioxide concentrating mechanism and, at current levels of atmospheric carbon dioxide, are already undergoing photosynthesis at near maximum capacity, so they are less affected by an increase in atmospheric carbon dioxide levels (Mirkham 2011). Increased root growth of C_4 plants with elevated carbon dioxide is understood to be largely due to increased water use efficiency (WUE) (Pritchard and Amthor 2005).

Rooting depth of agricultural plants can also be affected by the treatments used in this study. No-till management is thought to encourage additional root growth at shallow levels when compared to conventional till which tends toward increased root growth at deeper levels, as was the case for sorghum (Pritchard et al. 2006). Pritchard's 2006 study, with a maximum depth of 30.4 cm, found that tillage in deep soil affected daily root length production of sorghum with root growth in conventional till with elevated [CO_2] (CTE) increased more than root growth in no-till with elevated [CO_2] (NTE).

Madhu and Hatfield (2013) have made the general assertion that agricultural management systems may be more important for root growth and soil carbon storage than elevated [CO_2]. This may vary depending upon which cultivar, species, or functional group of plant is being studied. Pritchard et al. (2006) noted that, at least for their study of root growth of *Sorghum bicolor*, agricultural management system was not more important than [CO_2].

This dissertation study compares root growth of two agricultural plants that were evaluated at all four conditions: sorghum (*Sorghum bicolor* (L). Moench), a C_4 plant, and soybean (*Glycine max* (L.) Merr.) a C_3 plant. The conditions include no-till with elevated [CO_2] (NTE), no-till with ambient [CO_2] (NTA), conventional till with elevated [CO_2] (CTE) and conventional till with ambient [CO_2] (CTA). Three other plants, used as cover crops in the no-till agricultural management at both elevated and ambient [CO_2], included: wheat (*Triticum aestivum* L.), crimson clover (*Trifolium incarnatum* L.), and sunn hemp (*Crotalaria juncea* L.)

Hypotheses addressed by this chapter include:

1) For the two crop plants tested at all four conditions, no-till agricultural management will result in an increase in root growth when compared to root growth in conventional tillage.

2) For the two crop plants evaluated at all four conditions, elevated atmospheric carbon dioxide levels will result in an increase in root growth when compared to root growth at ambient atmospheric carbon dioxide levels.

3) For the two crop plants tested at all four conditions, root turnover index will decrease with an increase in depth of soil.

2.3 Materials and Methods

Plants used in this study were grown at an outdoor soil facility (Prior et al. 2010) in several bins (2 m deep, 6 m wide, and 76 m long) located at the USDA-ARS National Soil Dynamics Laboratory, Auburn, AL, USA (32.6 ^{o} N, 85.5 ^{o} W). Each bin contained a Decatur silt loam soil (clayey, kaolinitic, thermic Rhodic Paleudults; FAO classification Haplic Acrisols) supported on a tile and gravel drainage basin in an experimentally constructed soil profile of field proportions (Batchelor 1984). In these soil bins, crops were grown from seed to maturity in open top field chambers (OTCs). The OTCs were constructed of a structural aluminum frame (3 m in diameter by 2.4 m in height) covered with a PVC (polyvinyl chloride) film panel (0.2 mm thickness) (Rogers et al. 1983). Two levels of atmospheric carbon dioxide concentrations were administered: ambient (365 µmol mol^{-1}) and twice ambient (720 µmol mol^{-1}). Carbon dioxide was supplied from a 12.7 mg liquid carbon dioxide receiver through a high volume dispensing manifold with continuous injection of carbon dioxide into plenum boxes (Mitchell et al. 1995).

While the entire series of studies covered 10 years, this study compared two crop management systems involving conventional tillage and conservation (no-till) during one crop rotation (two years). In the conventional system, grain sorghum (*Sorghum bicolor* (L.) Moench. 'Pioneer 8282') and soybean (*Glycine max* (L.) Merr. 'Asgrow 6101') were rotated each year with spring tillage after winter fallow (Prior et al. 2010). Three tillage procedures were used. These included insertion of a pitch fork at 10 cm intervals to a depth of 20 – 25 cm before heaving the soil to simulate a chisel plowing operation. In order to simulate two disking operations, a large push type PTO tiller (Model 192432, Gardenway, Inc., Troy, NY, USA) was used twice to a soil depth of 14 -20 cm. A field cultivation operation was simulated using a small push type garden cultivator (Model 410R, Ryobi Technologies, Inc., Anderson, SC, USA). In the no-tillage) system, grain sorghum and soybean were also rotated, but with three cover crops (crimson clover (*Trifolium incarnatum* L. 'AU Robin'), sunn hemp (*Crotalaria juncea* L. 'Tropic Sun'), and wheat (*Triticum aestivum* L. 'Pioneer 2684')) which were also rotated. Cover crop seeds were broadcast planted at 56 kg ha^{-1} for clover, 112 kg ha^{-1} for sunn hemp, and 168 kg ha^{-1} for wheat (Prior et al. 2005).

The seeds of the legumes including clover, soybean, and sunn hemp, were inoculated with commercial *Rhizobium* (Nitragin Co., Milwaukee, WI, USA) prior to planting (Prior et al. 2010). In both of the management systems, sorghum and soybeans seeds were planted at a density of 20 plants per meter in rows 38 cm apart. Fertilizer rates were based on standard soil tests guidelines as recommended by the Auburn University Soil Testing Laboratory (Adams et al. 1994). For grain sorghum, fertilizer N was applied at a rate of 34 kg N ha^{-1} shortly after planting and an additional 101 kg N ha^{-1} was applied 30 days after planting. For wheat, fertilizer N was applied at planting (34 kg N ha^{-1}), 3.5 months after planting (67.4 kg N ha^{-1}), and 4.5 months after planting (34 kg N ha^{-1}). In order to prevent regrowth, cover crops and sorghum were terminated with glyphosate (N-[phosphomethyl] glycine) 10 days prior to planting the following crops (Prior et al. 2010).

To study root dynamics, two minirhizotrons per open top chamber were installed. Minirhizotrons are clear plastic tubes (o.d. = 56 mm) that allow repeated non-invasive measurement of root growth. These were installed at a 45º angle from vertical to a vertical depth of 34 cm. Tubes were installed equidistant between and parallel to rows of plants. The portion of the minirhizotron tube above the ground was covered with a closed-cell polyethylene sleeve, and the end was sealed with a rubber cap to exclude light and minimize heat exchange between the air and the tube. A PVC cap was installed over the end to protect the rubber cap from UV damage, and to further protect and insulate the tube. To prevent movement, aluminum brackets were clamped to the minirhizotron tubes and anchored into the ground with 40 cm stainless steel rods (Pritchard et al. 2006).
A BTC-100 microvideo camera (Bartz Technologies, Santa Barbara, California) was inserted into minirhizotrons at specific time intervals spread across the lifespan of the particular plant. Images of roots growing along the surface of the tubes were recorded. The camera was equipped with an indexing handle allowing very precise and consistent camera placement over time (Johnson and Meyer 1998). The images were saved in jpeg format using standard Rootfly labeling for future analysis.

Manual digitization of the images on the frames involved the use of the image analysis program Rootfly Version 2.0.2 (Guang et al. 2008). Each frame represents a one-dimensional area of soil equivalent to 144 mm^2. Frames represent depths from 0 to 34 cm from all tubes at each date.

For each minirhizotron frame studied (for each sampling date/session), variables were quantified for average root diameter (m/m^2), average length of live roots (m/m^2), and standing root crop (m/m^2). Roots which were still visible were classified as live and standing root crop was quantified as the total length (m/m^2) of root that appeared live at a given session **(**McCormack et al. 2010).

Production, in repeated measures studies (repeated measure being time), can be quantified based on the length of roots that have developed since the preceding session (McCormack et al. 2010), and production for this study was quantified similarly. Seasonal root production, for these annual crop plants, included all length of roots that developed during the growing season (Pritchard et al. 2006). That is, standing root crop for day 1 plus (standing root crop of day 2 minus day 1), plus (standing root crop of day 3 minus day 2), plus, etc.

Maximum standing root crop is given as the length of roots present at the session with the greatest standing root crop present (m/m^2).

Root turnover index was seasonal root production divided by the maximum standing root production (Gill and Jackson 2000; Norby and Jackson 2000; Frank and Groffman 2009). The JMP 12.1.0 program was used for ANOVA analysis of the root turnover index for tillage, [CO_2], and depth for sorghum and for soybean.

The experimental design of the crop plants grown in the OTCs with two levels of atmospheric carbon dioxide (ambient and twice ambient) and two agricultural management systems (conventional and no-till methods) was a split-plot design with three blocks (three replications). One half of each block had conventional agricultural management and one half had no-till. Split-plot treatments of carbon dioxide level were randomly assigned within blocks. Data were analyzed as a repeated measures MANOVA (repeated measure being time) with univariate tests also included. The JMP 12.1.0 image analysis program (SAS Institute, Cary, NC) was used for analysis of the effects of agricultural management, carbon dioxide levels, and vertical soil depth (0 – 17 cm and 17 – 34 cm) with even numbered frames (2 – 36) for each session being analyzed. Data were considered statistically significant when alpha levels were < 0.05 and were indicated with an *. Statistical trends were noted at $0.05 < p < 0.15$.

Soybean was evaluated for 8 days (sessions) and sorghum for 4 sessions across the span of their respective growing seasons. The JMP program between groups results indicate comparison of the root growth of groups of plants of a particular cultivar which were grown under the four conditions (NTE, NTA, CTE, CTA). For a repeated measures experiment where time is the repeated measure, each within group results represents the recorded changes in growth of roots of plants grown in six minirhizotrons under a designated condition across the sessions (days where images were recorded). For a given response variable, such as root length, the length measured for a given species of plant for a given session under a specific condition was considered a separate variable, thereby rendering multivariate ANOVA analysis (MANOVA) appropriate (JMP Support, SAS). Response variables included root diameter, root length, and standing root crop.

Separately from MANOVA, the JMP platform for ANOVA was used to generate Tukey's Honestly Significant Difference (HISD) test as a post-hoc test.

Primary analysis of data involved JMP data tables with tillage, [CO_2], and depth recorded in three separate columns. This made it possible to separately analyze tillage, [CO_2], and depth. Where applicable, JMP data tables were divided into the four conditions at shallow soil depth and the four conditions at deep soil depth with tillage and [CO_2] in one column and depth (shallow or deep) in a second column. This enabled the analysis of NTE, NTA, CTE, and CTA at both shallow soil levels and deep soil levels.

As referred to in this dissertation, a condition under which a group of plants were grown would include tillage and [CO_2]. There were four conditions for sorghum and four conditions for soybean (NTE, NTA, CTE, CTA). One treatment would include type of tillage (conventional till or no-till) and another treatment would include [CO_2]. The two atmospheric [CO_2] treatments were ambient (365 µmol mol^{-1}), and elevated or twice-ambient (720 µmol mol^{-1}).

Additionally, as referred to in this dissertation, a group of plants would indicate those plants of a particular species (sorghum or soybean), grown in three OTCs (with two minirhizotrons per OTC) under the same condition. This correlates to the JMP terminology "subjects." For each of the three OTCs, the results of root growth observed using its two minirhizotrons were averaged to give a total of three replicates to be used in analysis of average root diameter, average root length, and standing root crop for each condition.

2.4 Results

2.4.1 Average root diameter of sorghum

For sorghum, when analyzed individually using MANOVA with univariate tests, the only significant difference was within subjects time ($p < 0.0001$*) (Table 2.1).

2.4.2 Average root length of sorghum

Sorghum with no-till agricultural management had significantly greater average root length (m/m^2) at the deep soil level (17 – 34 cm) ($p = 0.0041$*). There was a significant effect on average root length of sorghum by within (groups) time ($p = 0.0153$*) (Table 2.2). Sorghum also displayed a trend toward greater average root length at elevated [CO_2] ($p = 0.1071$).

2.4.3 Standing root crop of sorghum

For sorghum, there was a significant increase in standing root crop in the deep soil level (17 – 34 cm) ($p = 0.0009$*). There was also a within subjects time significant effect ($p = 0.0002$*). There was a trend toward an effect of [CO_2] × Depth ($p = 0.1180$) (Table 2.3).

2.4.4 Average root diameter of soybean

For soybean, no-till agricultural management resulted in a significantly greater average root diameter ($p = 0.0212$*). Soybean had a significant effect for within time ($p = 0.0004$*) (Table 2.4).

2.4.5 Average root length of soybean

For soybean, there was no significant effect of tillage, [CO_2], or depth upon average root length. There was a significant effect of (within) time upon average root length ($p = 0.0021$*) (Table 2.5).

2.4.6 Standing root crop of soybean

For standing root crop of soybean, there was a significant effect for within subjects time ($p < 0.0001$*) (Table 2.6).

2.4.7 Root Turnover Index Results

Root turnover index was calculated for roots growing in the shallow (0 cm –17 cm) and deep (17 cm – 34 cm) soil for both sorghum and soybean for all four conditions: NTE, NTA, CTE, and CTA. A root turnover index of 1.0/100% would indicate that all roots had died (Gill and Jackson 2000), which did not occur in any of these annual crop plants by the conclusion of the experiment.

The highest percent root turnover index was seen in sorghum where it ranged from 71.1% for no-till elevated (NTE) shallow to 88.5% for no till ambient (NTA) deep (Table 2.7). For all four treatments in sorghum, the deep root turnover index was higher than the shallow root turnover index. Seasonal root production for sorghum was greater for deep soil levels for all four treatments (Table 2.7).

ANOVA for depth indicated that, in sorghum, there was a significantly greater root turnover index in the deep level than in the shallow ($p = 0.0245$*) (Table 2.9). One-way analysis of root turnover index of sorghum for the four treatments was done. From greatest to least root turnover index, the treatments were CTE, NTA, CTA, and NTE with no significant difference ($p = 0.1345$). However, Tukey’s Honestly Significant Difference test showed a trend toward a difference between CTE and NTE.

One-way analysis of root turnover index of soybean for the four treatments was done ($p = 0.0481$*). For soybean, from greatest to least root turnover index, the treatments were NTA, CTE, CTA, and NTE with a significant difference between NTA and NTE (p = 0.0481*) (Table 2.10); the significantly different groups were verified by Tukey's Honestly Significant Difference post-hoc test.

In soybean, the percent root turnover indices ranged from 33.6% for no-till elevated (NTE) deep to 53.4% for no-till ambient (NTA) shallow (Table 2.8). In soybean, the shallow depths (0 – 17 cm) had a greater root turnover index than that of deep depths for three of four conditions, except for conventional till ambient (CTA) for which the results were reversed but at less than significance or trend level (Table 2.8, Table 2.10). Seasonal root production was greater for shallow depths for three out of four conditions except for no-till ambient for which the seasonal root production was greater for the deep levels (17 – 34 cm) (Table 2.8).

One-way analysis of root turnover index of soybean for the four treatments was done (p = 0.0481*) (Table 2.10). For soybean, from greatest to least root turnover index, the treatments were NTA, CTE, CTA, and NTE with a significant difference between NTA and NTE (p = 0.0481*); the significantly different groups were verified by Tukey's Honestly Significant Difference post-hoc test.

In soybean root turnover, for carbon dioxide, one way ANOVA (p = 0.1120) revealed a trend toward a greater root turnover with ambient [CO_2] than with elevated [CO_2] (Table 2.10).

2.5 Discussion

While tillage had no significant effect on average root diameter or average root length of sorghum, there was a trend toward greater standing root crop with no-till agricultural management (p = 0.1064) (Table 2.3). Standing root crop is the total length of live roots (m/m^2) present at a given session, so although the average length of fine roots was not affected, the total length of fine roots was somewhat increased with no-till. No-till agricultural management is characterized by a lack of disturbance of roots in the soil and the plant residue being allowed to remain in the field with resulting shade, soil moisture, and carbon deposition (Palm et al. 2013). These characteristics of no-till would tend to positively impact annual production which would increase the standing root crop (Frank and Groffman 2009).

Sorghum is known to be a vigorously growing, deep-rooted plant (Dial 2013). The fact that there was a significantly greater standing root crop in the deeper soil level (17 – 34 cm) ($p = 0.0009$*) could partially be a function of its innate rooting system.

There was a slight increase in average root diameter in elevated [CO_2] for all four days for which root growth was recorded for sorghum ($p = 0.4363$) (Table 2.1), but this was not to significance or trend level. While there was not a significant or trend level effect of elevated [CO_2] upon standing root crop of sorghum for each of the four session (days) that were recorded, there was slightly higher value for standing root crop with elevated [CO_2] ($p = 0.4114$) (Table 2.3).

The effect of elevated [CO_2] on C_4 plants is associated with a reduction in the effect of drought, by an improvement of water use efficiency (Pritchard and Amthor 2005; Runion 2009). Sorghum is a C_4 agricultural plant that can be grown in dry conditions in the American southwest (Fageria 2012). While there is no indication that the soil at the National Soil laboratory was under drought conditions during the study, excessive soil dryness could have slightly impacted the standing root crop. The fact that sorghum is a drought resistant and deep-rooted plant (Dial 2013), while soil dryness tends to be more pronounced at shallow soil levels, could have resulted in the effect of [CO_2] ×depth for standing root crop being at trend level ($p = 0.1180$) instead of significant.

For soybean, there was a significant effect of no-till agricultural management upon average root diameter ($p = 0.0212$*) (Table 2.4). This was the only significant effect for tillage, CO_2, or depth alone for soybean diameter, soybean root length, or soybean standing root crop. Also, there were very distinctive patterns for the effects of experimental treatments on the root growth of soybean which frequently indicated that no-till agricultural management very slightly increased root growth as did elevated carbon dioxide levels; however, these were very subtle differences at less than significance or trend level.

The repeated measures MANOVA analysis showed elevated [CO_2] as having slightly greater values than ambient [CO_2] for each of the eight sessions (days) where average root diameter of soybean was recorded. This was not at the significant level or even at trend level ($p = 0.3791$) (Table 2.4), but root diameters were consistently greater in each measurement cycle for elevated [CO_2].

Repeated measures MANOVA indicated that, for soybean average root length, no-till agricultural management had slightly higher values than conventional tillage for all eight sessions with more increase later in the season, but this was not to the significance or trend level ($p = 0.3705$) (Table 2.5). Using repeated measures MANOVA for soil depth of soybean roots revealed that the deep soil level had a slightly greater standing root crop than did the shallow soil level for the last six sessions, but this was not to the significant or trend level ($p = 0.4964$) (Table 2.6).

Elevated [CO_2] had consistently slightly greater levels for standing root crop of soybean for each of the eight sessions, but it was not to the significance or trend level ($p = 0.3376$) (Table 2.6). Soybean standing root crop for depth showed no difference for the first two sessions, yet the six sessions later in the growing season showed the deep soil level with slightly higher values, but it was not to significance or trend level ($p = 0.4964$).

Using repeated measures MANOVA for soybean the direction of influence was as was hypothesized, but the magnitude of the difference was not sufficient to reach significance level or trend. No-till agricultural management resulted in significantly higher values only for soybean average root diameter.

One hypothesis was that no-till agricultural management would result in an increased root growth for C_3 plants and for C_4 plants. The C_3 soybean’s diameter ($p = 0.0212$*) (Table 2.4) was significantly increased, and the C_4 sorghum standing root crop ($p = 0.1064$) showed a trend toward an increase, so both C_3 plants and C_4 plants showed increase in one dimension of root growth.

There were a number of interactions of factors including tillage for sorghum and soybean which resulted in significance or trend level effects. These provide additional credibility to the idea that no-till can also affect plant root growth when interacting with other factors. The results of this experiment suggest that no-till agricultural management has a positive effect on plant root growth.

Elevated [CO_2] alone had no significant effect or trend for soybean root growth, but elevated [CO_2] did have slightly greater results than ambient [CO_2] for soybean average root diameter ($p = 0.3791$) and for standing root crop of soybean ($p = 0.3371$).

Elevated [CO_2] was slightly higher for sorghum average root diameter (p = 0.4363) as it was for sorghum standing root crop (p = 0.4114).

For sorghum average root length, there was a trend (p = 0.1071) (Table 2.2) for an increase due to elevated [CO_2]. This was the only result in the trend level for an effect of elevated carbon dioxide levels alone affecting root growth, and there was no result at the significance level for the effect of elevated [CO_2] alone on plant root growth. However, there were several interactions involving carbon dioxide with other factors which added some credibility to the hypothesis that elevated atmospheric [CO_2] interacting with other factors increased plant root growth. Considering the results, even with the addition of several slightly elevated effects described above, the experiment does not prove the relationship between elevated [CO_2] and increased plant root growth. However, the results from this experiment do not rule out the probability that plant root growth is positively affected by elevated atmospheric [CO_2].

One factor that could have the potential to affect the experimental results of added atmospheric carbon dioxide is the method of dispersing the elevated [CO_2] to the plants and whether that method affects root growth by restriction of growth area. It is generally thought that open top chambers (OTCs), as used as the national soil laboratory, are better for assessing plant root growth at elevated [CO_2] than are studies involving agricultural plants grown in pots, since there is less root constriction. This would particularly be the case in studies where agricultural plants are grown to maturity across a complete growing season as was the case in the dissertation study.

However, some would say that the open top chambers are not as efficient as the free air concentration enrichment (FACE**)** method of exposing plants to elevated atmospheric [CO_2] where crops can be grown in field conditions without the restriction of growing in a partially enclosed space. One concern is the potential for elevated temperatures inside the OTCs during the middle of the day when compared to outside temperatures (Ainsworth 2008a) with one estimate of as much as 3° C in difference (Leadley 1993). It is difficult to determine whether the use of OTCs affected the results in this study especially since the OTCs used at the national soil dynamics laboratory were quite large (3 m in diameter by 2.4 m in height) (Prior et al. 2005)**.**

As regards root turnover, the dissertation study results did not agree with Pritchard et al. (2006) on the effect of soil depth on root turnover in sorghum, since the dissertation study found a significantly higher root turnover index for the deep soil level (17 – 34 cm) (p = 0.0245*) than for the shallow. There are several methods of calculating root turnover, and the dissertation study used root turnover index while the Pritchard study used a different calculation method. Whether this difference in method would enough to reverse the results is unclear. Additionally, the dissertation study was divided into shallow and deep levels with 18 frames per minirhizotron tube (McCormack et al. 2010) while the Pritchard et al. (2006) study was divided into 16 frames per minirhizotron tube at eight soil levels. This may or may not have affected the results for root turnover by depth.

However, with the treatments' effects on root turnover on sorghum, the dissertation study results did agree with Pritchard et al. (2006). For the dissertation study, sorghum plants growing under all four conditions (CTE, NTA, CTA, and NTE) were found to be group A with Tukey's HSD test indicating that there was no significant difference in root turnover index. The Pritchard study found that neither agricultural management nor CO_2 treatment had an effect on the percentage of sorghum roots to die which was interpreted to mean that root longevity was unchanged.

The dissertation hypothesis for root turnover index was that for both of the crop plants tested at all four conditions, there would be a decrease in root turnover index in the deeper soil level. For sorghum, the reverse was true. For each of the four conditions, the shallow soil level had less percent root turnover index. One reason for this could be that sorghum, being a deep-rooted plant, has more seasonal root production in the deep soil level for each of the four treatments. This could lead to more crowding and competition for nutrients at the deeper soil level, with some fine roots not surviving.

The hypothesis of a decrease in root turnover index in the deeper soil level was partially met for soybean. In soybean there was less percent root turnover index in the deep soil levels for three out of four of the conditions tested: NTE, NTA, and CTE. Only CTA had a greater value at the deep soil level (38.9%) compared to shallow soil level (34.9%).

2.6 Tables

Table 2.1

MANOVA repeated measures with univariate tests also analysis of average root diameter (m/m^2) for sorghum (*Sorghum bicolor*) grown at four conditions: no-till elevated [CO_2], no-till ambient [CO_2], conventional till elevated [CO_2], and conventional till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	7	16	0.363	0.9104
Tillage	1	16	0.014	0.9082
[CO_2]	1	16	0.638	0.4363
Depth	1	16	0.677	0.4228
Tillage× [CO_2]	1	16	0.419	0.5266
Tillage × Depth	1	16	0.586	0.4550
[CO_2] × Depth	1	16	0.037	0.8504
Tillage × [CO_2] ×Depth	1	16	0.174	0.6820
Within Subjects				
Time	3	14	46.647	<0.0001*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 µmol^{-1} and ambient [CO_2] is 720 µmol^{-1}.

Table 2.2

MANOVA repeated measures with univariate tests also analysis of average root length (m/m^2) for sorghum (*Sorghum bicolor*) grown at four conditions: no-till elevated [CO_2], no-till ambient [CO_2], conventional till elevated [CO_2], and conventional till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	7	16	2.179	0.0934
Tillage	1	16	0.883	0.3614
[CO_2]	1	16	2.915	0.1071
Depth	1	16	11.203	0.0041*
Tillage× [CO_2]	1	16	0.192	0.6671
Tillage × Depth	1	16	0.0184	0.8939
[CO_2] × Depth	1	16	0.0082	0.9291
Tillage × [CO_2] ×Depth	1	16	0.0343	0.8554
Within Subjects				
Time	3	14	4.930	0.0153*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 µmol^{-1} and ambient [CO_2] is 720 µmol^{-1}.

Table 2.3

MANOVA repeated measures with univariate tests also analysis of standing root crop (m/m^2) for sorghum (*Sorghum bicolor*) grown at four conditions: no-till elevated [CO_2], no-till ambient [CO_2], conventional till elevated [CO_2], and conventional till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	7	16	3.606	0.0159*
Tillage	1	16	2.928	0.1064
[CO_2]	1	16	0.713	0.4114
Depth	1	16	16.431	0.0009*
Tillage× [CO_2]	1	16	0.203	0.6586
Tillage × Depth	1	16	0.104	0.7509
[CO_2] × Depth	1	16	2.730	0.1180
Tillage × [CO_2] ×Depth	1	16	2.133	0.1635
Within Subjects				
Time	3	14	13.582	0.0002*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 2.4

MANOVA repeated measures with univariate tests also analysis of average root diameter (m/m^2) for soybean (*Glycine max)* grown at four conditions: no-till elevated [CO_2], no-till ambient [CO_2], conventional till elevated [CO_2], and conventional till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	7	16	1.358	0.2880
Tillage	1	16	6.525	0.0212*
[CO_2]	1	16	0.818	0.3791
Depth	1	16	0.083	0.7771
Tillage× [CO_2]	1	16	0.995	0.3333
Tillage × Depth	1	16	0.574	0.4596
[CO_2] × Depth	1	16	0.292	0.5964
Tillage × [CO_2] ×Depth	1	16	0.216	0.6485
Within Subjects				
Time	7	10	12.101	0.0004*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 µmol^{-1} and ambient [CO_2] is 720 µmol^{-1}.

Table 2.5

MANOVA repeated measures with univariate tests also analysis of average root length (m/m^2) for soybean (*Glycine max)* grown at four conditions: no-till elevated [CO_2], no-till ambient [CO_2], conventional till elevated [CO_2], and conventional till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	7	16	0.549	0.785
Tillage	1	16	0.849	0.3705
[CO_2]	1	16	0.059	0.8117
Depth	1	16	0.471	0.5023
Tillage× [CO_2]	1	16	1.221	0.2856
Tillage × Depth	1	16	0.283	0.6023
[CO_2] × Depth	1	16	0.378	0.5474
Tillage × [CO_2] ×Depth	1	16	0.583	0.4561
Within Subjects				
Time	7	10	7.940	0.0021*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 2.6

MANOVA repeated measures with univariate tests also analysis of standing root crop (m/m^2) for soybean (*Glycine max)* grown at four conditions: no-till elevated [CO_2], no-till ambient [CO_2], conventional till elevated [CO_2], and conventional till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	7	16	0.789	0.6070
Tillage	1	16	.005	0.9463
[CO_2]	1	16	0.977	0.3376
Depth	1	16	0.485	0.4964
Tillage× [CO_2]	1	16	2.088	0.1677
Tillage × Depth	1	16	1.925	0.1844
[CO_2] × Depth	1	16	0.038	0.8489
Tillage × [CO_2] ×Depth	1	16	0.0041	0.9498
Within Subjects				
Time	7	10	54.518	<0.0001*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 2.7

Sorghum root turnover index determined by seasonal root production divided by maximum standing root crop with standard error and percent root turnover.

Treatments	Seasonal root Production[a]	Max standing root crop[a]	Root turnover Index[a]	Standard error	Percent root Turnover
NTE shallow	1.75E-07	2.45908E-07	7.11E-01	0.04	71.1 %
NTE deep	3.19E-07	4.17268E-07	7.64E-01	0.07	76.4%
NTA shallow	2.01E-07	2.52444E-07	7.97E-01	0.04	79.7%
NTA deep	3.46E-07	3.9154E-07	8.85E-01	0.10	88.5%
CTE shallow	2.54E-07	3.12666E-07	8.14E-01	0.02	81.4%
CTE deep	3.06E-07	3.45594E-07	8.84E-01	0.02	88.4%
CTA shallow	2.18E-07	2.93854E-07	7.42E-01	0.02	74.2%
CTA deep	2.34E-07	2.6594E-07	8.79E-01	0.02	87.9%

[a] Values are in units of meters per meter squared. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 µmol^{-1} and ambient [CO_2] is 720 µmol^{-1}. Tillage includes conventional till and no-till.

Table 2.8

Soybean root turnover index determined by seasonal root production divided by maximum standing root crop with standard error and percent root turnover.

Treatments	Seasonal root Production[a]	Max standing root crop[a]	Root turnover Index[a]	Standard error	Percent root Turnover
NTE shallow	1.70E-07	4.62E-07	3.67E-01	0.04	36.7%
NTE deep	1.51E-07	4.48E-07	3.36E-01	0.04	33.6%
NTA shallow	2.15E-07	4.03E-07	5.34E-01	0.08	53.4%
NTA deep	2.28E-07	4.33E-07	5.27E-01	0.08	52.7%
CTE shallow	2.91E-07	6.47E-07	4.50E-01	0.09	45.0%
CTE deep	1.97E-07	4.95E-07	3.98E-01	0.07	39.8%
CTA shallow	1.57E-07	4.49E-07	3.49E-01	0.08	34.9%
CTA deep	1.14E-07	2.94E-07	3.89E-01	0.02	38.9%

[a] Values are in units of meters per meter squared. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}. Tillage includes conventional till and no-till.

Table 2.9

ANOVA analysis of sorghum (*Sorghum bicolor*) root turnover index at four conditions with mean, standard error of mean, and p-value.

Sorghum	Mean	S.E.	p-value
Between subjects			
Tillage - Conventional	0.83	0.02	0.3293
Tillage – No-till	0.79	0.03	
[CO_2] - Elevated	0.79	0.03	0.4164
[CO_2] - Ambient	0.82	0.03	
Depth - Deep	0.85	0.03	0.0245*
Depth - Shallow	0.77	0.02	
Treatments/conditions			
Conventional till elevated	0.85	0.02	0.1345
No-till ambient	0.84	0.05	
Conventional till ambient	0.81	0.03	
No-till elevated	0.74	0.04	

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth.

Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 2.10

ANOVA analysis of soybean (*Glycine max*) root turnover index at four conditions with mean, standard error of mean, and p-value.

Soybean	Mean	S.E.	p-value
Between subjects			
Tillage - Conventional	0.37	0.03	0.3147
Tillage – No-till	0.42	0.13	
[CO_2] - Elevated	0.03	0.03	0.1120
[CO_2] - Ambient	0.44	0.04	
Depth - Deep	0.40	0.04	0.9186
Depth - Shallow	0.49	0.32	
Treatments/conditions			
No-till ambient	0.51	0.05	0.0481*
Conventional till elevated	0.38	0.05	
Conventional till ambient	0.37	0.04	
No-till elevated	0.34	0.03	

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth.

Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}. Tillage includes conventional till and no-till.

Table 2.11

Comparison of Average Root Lengths between sorghum (*Sorghum bicolor*) from the Dissertation Study and the Pritchard study

Treatments	Dissertation sorghum	Pritchard sorghum
Tillage	0.3614	NS
$[CO_2]$	$p = 0.1071$	$p = 0.08$
Depth	$p = 0.0041$*****	$p = 0.02$*****
$[CO_2]$×Depth	0.9291	$p = 0.12$
Tillage×$[CO_2]$×Depth	0.8554	NS
Within Time	$p = 0.0153$*****	$P < 0.0001$*****

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated $[CO_2]$ is 365 μmol^{-1} and ambient $[CO_2]$ is 720 μmol^{-1}. Tillage includes conventional till and no-till. Results from Pritchard study from Pritchard et al 2006.

CHAPTER III – ROOT GROWTH DIFFERENCES OF THREE LEGUMES

3.1 Abstract

Rising levels of atmospheric carbon dioxide levels [CO_2] have increased the photosynthesis of all major plant groups with root growth having the potential to increase as much as or more than above ground shoots. The increased plant growth has been shown to require additional carbon and nitrogen with the nitrogen component of the C:N ratio often being the limiting element. Due to the ability of their bacterial symbionts to fix nitrogen, legumes are thought to be capable of increases in growth at higher [CO_2] without nitrogen limitation. There have been few studies comparing the root growth of legumes using no-till agricultural management at ambient [CO_2] versus no-till at twice-normal [CO_2]. As part of a larger study of agricultural plants grown at the USDA-ARS National Soil Dynamics Laboratory, Auburn, Alabama, USA, it was possible to compare the root growth of each of three leguminous species including soybean (*Glycine max* L. Merrill), sunn hemp (*Crotalaria juncea* L.), and crimson clover (*Trifolium incarnatum* L.) using the no-till cropping system with [CO_2] at 365 µmol mol^{-1}) and [CO_2] at 720 µmol mol^{-1}). This comparison of three legumes allowed an evaluation as to whether the response of legume root growth to elevated atmospheric [CO_2] is consistent across leguminous species or whether it is species dependent. Root growth was evaluated as a repeated measure study using multiple sessions across the growing season of the legumes at shallow soil depths (0 – 17 cm) and deep soil depths (17 – 34 cm). In soybean, clover, and sunn hemp, there was no effect on average root diameter of [CO_2] or depth alone. In clover, there was a significant effect of time × depth on average root diameter, as well as several multiple interactions involving time. Neither soybean nor clover showed an

effect of [CO_2] or depth alone upon average root length. Sunn hemp had a significant increase of average root length in elevated [CO_2], but had no effect of depth alone. In soybean, for average root length, there was a significant effect of (Time × [CO_2] × depth). In soybean there was no effect of [CO_2] or depth alone upon standing root crop. Clover had a significant effect toward increased standing root crop at shallow soil levels with root growth at shallow depths more prominent in the early season and root growth in deep soil depth surpassing shallow late in the season. Sunn hemp had a trend toward increased standing root crop at elevated [CO_2] and at deep soil level. There were also different responses between the agricultural plant species to multiple factor interactions and to interactions involving time. For these three species of legumes, grown in no-till conditions at ambient [CO_2] and twice ambient [CO_2], the effects of elevated [CO_2] on root growth and on depth were variable and likely species dependent.

3.2 Introduction

The family Leguminosae, or Fabaceae, is widely distributed in temperate and tropical areas of the world. It is the third largest plant family with some 630 genera and over 18,860 species (Judd et al. 2002). Most of these legume species are known to form a relationship with rhizobia and form root nodules into which the bacterial rhizobia move and where they fix nitrogen (Rogers et al. 2009).

Soybean (*Glycine max* (L.) Merrill) is a prominent leguminous agricultural plant which is an important source of oil and protein (Madhu and Hatfield 2013). Soybean is one of the top agricultural crops in the United States where it is grown on over 76 million acres. (USDA Fact sheet 2015) with an increasing percentage of acres planted with soybean farmed using no-till agricultural management (Horowitz et al. 2010).

Of the two legumes used as cover crops in this study, sunn hemp (*Crotalaria juncea* L.) is a vigorously growing, drought resistant herbaceous warm season annual of tropical origin which is widely grown as a green manure/cover crop in tropical and subtropical areas (Baligar and Fageria 2007). Sunn hemp has been studied by the USDA because of its potential as a summer cover crop with a goal of producing usable cultivars that will survive well and produce seed in the temperate southeastern United States (Mosjidis 2011). The cultivar of sunn hemp used in this study was 'Tropic Sun' which originated in Hawaii and was a joint release in 1982 by the USDA Natural Resources Conservation Service (NRCS) and the College of Tropical Agriculture and Human Resources (Valenzuela and Smith 2002).

Crimson clover (*Trifolium incarnatum* L.) is an annual clover that is widely grown in the southern U.S. as a winter pasture legume and is the most important clover used in the United States for agricultural purposes (Smith 2010). Crimson clover is also highly recommended as a cover crop because of its early season production of biomass and its nitrogen content (Mosjidis 2002). The cultivar used for this experiment was 'AU Robin,' an early-maturing strain, which was released in 1992 by the Alabama Extension Service (AES) (Smith 2010).

Most legumes have the ability to form a symbiotic relationship with soil bacteria known as rhizobia. The rhizobia move into root nodules of the legume and supply enzymes that convert atmospheric nitrogen gas (N_2) into forms such as NOx usable by the legume, while the legume provides carbohydrates to the rhizobia (Lindemann and Glover 2008; Jensen et al. 2012)**.** Nitrogen is a limiting factor in most terrestrial ecosystems (Morrison and Morecroft 2006), so the ability of legume symbionts to fix nitrogen is vitally important. Legumes are less likely to be affected by nitrogen limitation with the resulting inability to utilize additional photosynthate, so legumes may exhibit a greater growth response to elevated atmospheric [CO_2] than species that do not fix nitrogen (Bernacchi et al. 2005).

It is thought that legumes will exhibit increased growth due to elevated [CO_2] compared to non-nitrogen fixing C_3 plants (Rogers et al. 2006; Runion et al. 2009). Not all legumes respond to elevated [CO_2] to the same extent with past studies suggesting that the amount of response can vary depending upon cultivar or species and environmental factors such as availability of nitrogen and the plant's response to drought (West et al. 2005).

Fewer studies of the effects of elevated [CO_2] on agricultural plant growth have involved root growth than those evaluating aboveground plant growth (Madhu and Hatfield 2013). Quantifying root growth can be a challenge, so the opportunity to compare the root growth of cultivars of three herbaceous annual legume species grown using the same no-till agricultural management, and for each species subjects grown at ambient [CO_2] and subjects grown at twice normal [CO_2] was an additional plus to the larger study.

Patterns of root growth are of great importance in assessing the effects of increase in atmospheric carbon dioxide on agricultural plants (Pritchard et al. 2006). The majority of root biomass in annual plants occurs in the 0 – 20 cm depth, and legumes have in common a taproot system (Fageria 2012).

According to the NRCS USDA, crimson clover grows 0.4572 meters with a minimum rooting depth of 0.3048 meters, while soybean plants grow 0.0144 meters tall with minimum rooting depth of 0.3048. Sunn hemp grows 1.524 meters tall with minimum rooting depth of 0.4064 meters (NRCS USDA Plant database 2018). Crimson clover is the smallest of the plants, soybean is intermediate, and sunn hemp is the largest with their root systems reflecting this diversity. The effects of elevated [CO_2] on rooting depth could potentially vary depending upon the species of plant observed (West et al. 2005).

No-till agricultural management, with its retention of organic matter in the field, increases moisture absorption and retention in the topsoil which can increase root growth at shallow soil depths (Pritchard et al. 2006). Due to the variety of factors that affect root depth, it was worthwhile to compare the root growth of these legume species grown using no-till agricultural management under ambient [CO_2] with those grown under twice-ambient [CO2].

Hypotheses addressed by this chapter are as follows:

1) For the three legumes in the study, growth using twice ambient atmospheric carbon dioxide [CO_2] will result in differential root growth which will vary depending upon species.

2) For the three legumes in the study, growth using no-till agricultural management and twice ambient atmospheric carbon dioxide [CO_2], will result in differential root growth between shallow (0 – 17 cm) and deep soil depths (17 – 34 cm) which will vary depending upon species.

3.3 Methods

The initial growth of agricultural plants for this comparison of legume root growth was part of a larger study of root growth of five agricultural plants involved in this dissertation study. The plants were grown in an outdoor soil bin (7 m wide x 76 m long x 2 m deep) with Decatur silt loam soil at the at the USDA-ARS National Soil Dynamics Laboratory in Auburn, Alabama (32.6º N, 85.5º W).

Seeds were planted by the no-till method using standard densities with clover being broadcast planted at 56 kgha-1 and sunn hemp at 112 kgha^{-1}. Soybean, clover, and sunn hemp seeds were inoculated with *Rhizobium* (Nitragin Co., Milwaukee, Wisconsin) prior to planting (Prior et al. 2005). Fertilization rates followed guidelines recommended by the Auburn University Soil Testing Laboratory (Adams et al. 1994). Irrigation was applied as needed with a target of approximately 1 inch of water per week.

The larger study compared two cropping systems (no-till and conventional) and two levels of atmospheric carbon dioxide: ambient and twice ambient. In order to compare the root growth characteristics of a primary agricultural plant, soybean, with that of two additional legumes used as cover crops for the no-till system, this smaller study has focused on no-till agricultural management of the three legume species. Open top chambers (OTCs) which were 3 m in diameter and 2.4 m in height were used to deliver atmospheric carbon dioxide to the plants at two [CO_2] levels including ambient (365 µmol mol^{-1}) and twice ambient (720 µmol mol^{-1}).

The three legumes in this study were soybean (*Glycine max* L. Merr. **'**Asgrow 6101', crimson clover (*Trifolium incarnatum* L. 'AU robin'), and sunn hemp (*Crotalaria juncea* L. 'Tropic Sun') comprising one rotation cycle in 2004 and 2005.

The larger experiment was conducted using a split-plot design with three replicate blocks with the whole plot treatments (agricultural management system) randomly assigned to half of each block. The levels of atmospheric carbon dioxide were the split-plot treatments and were randomly assigned to two chambers within each whole plot. Of the 12 chamber plot locations, three were for no-till management and ambient [CO_2] (NTA) and three were for no-till management and twice-ambient (elevated) [CO_2] (NTE).

Each open top chamber (OTC) had two clear plastic minirhizotron tubes installed at a 45 degree from vertical. A BTC-100 microvideo camera (Bartz Technologies, Santa Barbara, California) was inserted into the minirhizotrons at appropriate days (sessions) throughout the growing seasons of the legumes. Images of the roots growing along the surface of the tubes were recorded and still images were produced from the video.

In the Davis laboratory at the University of Southern Mississippi, Hatttiesburg, Mississippi, still images of the roots were manually digitized and analyzed using the Rootfly Version 2.0.2 program. A total of 18 frames representing depths from 0 to 34 cm were analyzed from all tubes from each session. Sessions were spaced across the growing season of each plant. For soybean there were eight sessions (June to October); for clover there were nine sessions (November to March), and for sunn hemp there were four sessions (August to October).

Depths were divided into shallow (0 – 17 cm) and deep (17 – 34 cm) (McCormack et al. 2010) in order to further analyze root growth. Each frame represents a two-dimensional area of soil equivalent to 144 mm^2. Statistical analysis of the data was performed using the JMP 12.1.0 image analysis program (SAS Institute, Cary, NC). Average root diameter, average root length, and standing root crop were calculated for three replicate OTCs with two minirhizotrons each. Repeated measures MANOVA (repeated measure being time), a multivariate model, was used for the analyses due to each of the time measurements forming a variable with the multiple sessions producing multiple variables (JMP Support).

The same values were recorded for average diameter, average length, and for standing root crop for each of the three legume species. These included root growth as effected by [CO_2], deep or shallow depth, interaction of ([CO_2] × depth), interaction of (Time × [CO_2]), interaction of (Time × Depth), and interaction of (Time × [CO_2] × Depth). Also included were all between (between subjects or across-subjects effects), all within interactions (within subjects effects), and between time/intercept**.** Only the data for root growth in no-tillage conditions were included in statistics for this chapter since clover nor sunn hemp were used as cover crops during no-till agricultural management, and neither were grown using conventional tillage.

For all three legumes, one-way ANOVAS of no-till elevated [CO_2] and no-till ambient [CO_2] were done to in order to follow seasonal differences as well as the Tukey's HSD test as a post-hoc test.

3.4 Results

3.4.1 Overall Average Root Diameter

A comparison of average root diameter of the three legumes using MANOVA revealed similar results between soybean, clover, and sunn hemp in that neither [CO_2] nor depth nor ([CO_2] × depth) had a significant effect upon average root diameter. Soybean had a trend toward a within (group) time effect ($p = 0.0911$ (Table 3.1). Clover had significant effects for within time ($p = 0.0415$*) Table 3.4. Sunn hemp displayed significant results for within (group) time ($p = 0.0147$*) (Table 3.7).

3.4.2 Average root diameter across the sessions

For soybean, using ANOVA, growth in NTE conditions resulted in greater root diameter than NTA conditions for each of the eight sessions (Figure 3.1), but not to trend or significance level. For soybean average diameters grown at deep and shallow depths, while the first two sessions were very similar, sessions 3 through 8 showed greater average root diameter in deep levels (17 – 34 cm) (Figure 3.2) although not to the level of trend or significance.

For clover, although root growth was very similar during the early sessions, NTE conditions resulted in slighter greater average root diameter during sessions 5 through 9 (Figure 3.3), but not to the level of significance. For clover, there was greater average root diameter in shallow depths during sessions two through six out of nine with a trend for greater root diameter at shallow depths during day four ($p = 0.1275$) and Day 5 ($p = 0.1282$) (Figure 3.4).

For sunn hemp, average root diameter was significantly higher under NTE conditions for all four sessions than for NTA conditions (Figure 3.5) with p values for session one ($p = 0.0301$*), session two ($p = 0.0292$*, session three ($p = 0.0241$*), and session four ($p = 0.0213$*). Tukey's connecting letters report showed A/B for all four sessions. For sunn hemp, average root diameter was greater for root growth in deep soil levels than that in shallow soil levels in all four sessions (Figure 3.5) but not to the level of trend or significance.

3.4.3 Overall Average Root Length

For soybean, within (group) time had a trend toward a significant effect ($p = 0.0568$) (Table 3.2).

Clover had no significant effect of [CO_2], Depth, [CO_2] × Depth, or within (subjects) time for average root length.

In sunn hemp, elevated [CO_2] had a significant effect for increased average root length (p = 0.0370*). Within (group) time (p = 0.0236*) had significant results in sunn hemp (Table 3.8). Of the three legumes, average root length of sunn hemp displayed the most response to the treatments in the dissertation study.

3.4.4 Average root length across the sessions

Soybean average root length was greater for all eight sessions when grown at NTE conditions rather than NTA conditions (Figure 3.7) but not to the level of significance with all eight sessions recording A/A for Tukey's connecting letters report. Soybean average root length was greater at deep soil levels than shallow for all eight sessions (Figure 3.8) but not to the level of significance with Tukey's connecting letters giving values of A/A for all eight sessions.

Clover average root length was mixed with NTA conditions resulting in greater average root length for eight of nine sessions, with NTE surpassing it only at the ninth session (Figure 3.9), but there was no significant difference, with all nine sessions were recorded as A/A for the Tukey's connecting letters report. Clover average root length was greater for deep soil levels for eight out of nine sessions with shallow soil levels showing greater average root length only at the ninth session (Figure 3.10), but this was not to the level of significance with Tukey's connecting letters report recorded as A/A for all nine sessions.

Sunn hemp had significantly greater average root length at NTE conditions than at NTA conditions for all four sessions (Figure 3.11) with p values of 0.0301*, 0.0291, 0.0241, and 0.0213 respectively and A/B for the connecting letters report for all four sessions. Sunn hemp had greater average root length at deep soil conditions than at shallow soil conditions for all four sessions (Figure 3.12) but not to significance level with the Tukey's connecting letters report indicating A/A for all four sessions.

3.4.5 Standing Root Crop

Soybean had a significant within subjects effect of time (p = 0.0041*) for standing root crop (Table 3.3).

Clover had a significant effect upon standing root crop by depth (p = 0.0244*) and [CO_2] × Depth (p = 0.0474*) (Table 3.6). Clover had a trend for within time (p = 0.0961).

For standing root crop of sunn hemp, there were trends for [CO_2] (p = 0.1168), Depth (p = 0.1131), and [CO_2] × Depth (p = 0.1285). There was also a significant effect of within subjects time (p = 0.0020*) (Table3.9).

3.4.6 Standing root crop across the sessions

For soybean, while NTE growing conditions resulted in greater standing root crop during sessions two through four, NTA conditions resulted in greater standing root crop in sessions five through nine (Figure 3.13) although not to the level of significance with Tukey's connecting letter reports listed as A/A for all eight sessions. For soybean, standing root crop was greater for deep soil levels for sessions one through seven (Figure 3.15) with a trend for greater standing root crop at session two (p = 0.1910) and session three (p = 0.1223). However, there was no significant difference with all eight sessions recording A/A with the Tukey's connecting letters report.

For clover, values for standing root crop for NTA and NTE were very close across the entire nine sessions with NTE slightly greater early and NTA slightly greater during the sessions seven through nine (Table 3.15); there were no significant differences or trends for any of the sessions and the Tukey’s connecting letters report was A/A for all nine sessions. For clover standing root crop, shallow root growth was higher during sessions two through seven (Figure 3.16) with a trend for session three ($p = 0.1023$) and significantly higher for sessions four ($p = 0.0211$*), session five ($p = 0.0031$*), session six ($p = 0.0024$*), and session seven ($p = 0.0017$*). Deep standing root crop was significantly higher than shallow during session 9 ($p = 0.0463$*) with A/B showing deep as A. The connecting letters report showed A/B for sessions four through seven with shallow being the greater standing root crop.

For sunn hemp, standing root crop was greater at NTE conditions than at NTA conditions for all four sessions (Figure 3.17) at a level approaching but not quite trend level. The Tukey’s connecting letter report indicated A/A for all four sessions. For sunn hemp, standing root crop was higher at deep soil levels than at shallow soil levels for all four sessions (Figure 3.18) with p values approaching but not quite at trend level. The Tukey’s connecting letters report was A/A for all sessions indicating no significant difference.

3.5 Discussion

A comparison of the effects of the treatments of carbon dioxide concentrations and depths upon root growth of the three legumes revealed that soybean had the least trends and significant differences (trend/significant) of any of the three plants. Soybean had a trend toward a difference for average root diameter for within subjects time (p =

0.0911), a trend toward a difference with average root length for within subjects time (p = 0.568), and a significant result for within subjects time for standing root crop (p = 0.0041*).

Clover had an intermediate response to the treatments in the study with a significant result for average root diameter with within subjects time (p = 0.0415*). For standing root crop, clover had three significant results: all between (p = 0.0421*), Depth (p = 0.0244*), and [CO_2] × Depth (p = 0.0474*).

Sunn Hemp had the greatest response to the treatments with a significant result for within subjects time for average root diameter (p = 0.0147*). For average root length, sunn hemp had significant results for [CO_2] (p = 0.0370*) and within subjects time (p = 0.0236*). For standing root crop, sunn hemp had a significant result for within subjects time (p = 0.0020*). Also for standing root crop, sunn hemp had trends for all between (p = 0.0925), [CO_2] (p = 0.1168), Depth (p = 0.1131), and [CO_2] × Depth (p = 0.1285).

There is a strong indication that the three legume species do not react as a unit in the response of their root growth to elevated [CO_2] and/or depth under no-till conditions. However, the difference in response may vary as to which root growth characteristic is considered. There was less variety of response to average root diameter for all three legumes, and no significant difference in root growth for [CO_2] or for depth was recorded for any of the three without the consideration of time as a factor.

Results for another root growth characteristic, rather than average diameter, may indicate that the species in question was responsive to the difference in [CO_2] between 365 μmol mol^{-1} and 720 μmol mol^{-1} and/or depth of root growth for that particular characteristic of root growth. For average root length, while neither clover nor soybean had any significant result or trend due to either [CO_2] or root depth without the inclusion of time, sunn hemp had a significant increase in root length due to elevated [CO_2] (p = 0.0370*) (Table 3.8).

Standing root crop was the root growth characteristic showing the most increase with the treatments. For clover, there was significantly increased standing root crop at the shallow level (p = 0.0244*), and ([CO2] × Depth) (p = 0.0474*) (Table 3.6). For standing root crop, sunn hemp was clearly the most affected with trends toward effects for carbon dioxide levels (p = 0.1168), depth (p = 0.1131) and ([CO2] × Depth) (p = 0.128) (Table 3.9).

So, while there was a difference in response to the treatments by the three legumes, it also varied depending upon which characteristic of root growth was evaluated.

The discussion of legume's root growth inevitably turns to the ability of legumes to fix nitrogen. The obvious thought is that this always increases root growth, but is that so? The production of nodules by legumes requires energy with as much as 20 to 30 percent of the photosynthate produced in soybeans being used when the plant is actively fixing nitrogen. This reduces the energy-containing compounds which could otherwise be involved in plant growth (Lindemann and Glover 2008). It is unclear whether that energy requirement is a major factor in the fact that not all studies involving leguminous plants have resulted in an increased production at elevated [CO_2] (West et al. 2005).

Nitrogen is a limiting factor in the ability to use the additional carbon in elevated [CO_2] for the increased production of plant material (Rogers et al. 2006). Soybean is a plant where large quantities of Nitrogen are removed from the field when the soybeans are harvested to an extent that less soil organic carbon (SOC) is produced from the degradation of the plant material than would otherwise occur (Jensen et al. 2012). A decrease in nitrogen and SOC could decrease the response of soybean to the treatments of this study.

Conversely, green-manure legumes, such as sunn hemp, return large amounts of carbon and nitrogen to the soil particularly when they produce a large amount of plant residue, which the rapidly growing species sunn hemp does (Jensen et al. 2012).
Sunn hemp has increased soil nitrogen by 57 kg after nine to twelve weeks growth as a cover crop (Mansoer et al. 1997) while Rotar and Joy (1998) found an increase of some 60 kg of Nitrogen after growing sunn hemp as a cover crop for 60 days. Therefore, the increased root growth response to the treatments of this study could be attributed to the return of nitrogen to the soil.

While crimson clover is an effective cover crop (Smith 2010), it is a smaller plant than sunn hemp with less plant residue to be returned to the soil, which could result in an intermediate level of response to the treatments of this study.

Even though legume species are well known for nitrogen fixation, they don’t typically depend only on atmospherically fixed nitrogen instead depending partially upon soil N supply (West et al. 2005). The extent to which a species of legume depends upon soil nitrogen could contribute to the variation in legume response to [CO_2] (Lee et al. 2003a).

3.6 Tables

Table 3.1

MANOVA repeated measures with univariate tests also analysis of average root diameter (m/m^2) for soybean (*Glycine max*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	3	8	0.379	0.7711
[CO_2]	1	8	1.069	0.3314
Depth	1	8	0.065	0.8049
[CO_2] × Depth	1	8	0.0017	0.9682
Within Subjects				
Time	7	2	10.323	0.0911

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 3.2

MANOVA repeated measures with univariate tests also analysis of average root length (m/m^2) for soybean (*Glycine max*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	3	8	0.372	0.7758
Carbon Dioxide	1	8	0.609	0.4575
Depth	1	8	0.498	0.5003
[CO_2] × Depth	1	8	0.008	0.9333
Within Subjects				
Time	7	2	16.964	0.0568

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 3.3

MANOVA repeated measures with univariate tests also analysis of standing root crop (m/m^2) for soybean (*Glycine max*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	3	8	0.252	0.8580
[CO_2]	1	8	0.21	0.6596
Depth	1	8	0.48	0.5082
[CO_2] × Depth	1	8	0.07	0.8029
Within Subjects				
Time	7	2	244.66	0.0041*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 3.4

MANOVA repeated measures with univariate tests also analysis of average root diameter (m/m^2) for crimson clover (*Trifolium incarnatum*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	3	8	0.0042	0.9996
[CO_2]	1	8	0.0025	0.9611
Depth	1	8	0.0094	0.9251
[CO_2] × Depth	1	8	0.0007	0.9791
Within Subjects				
Time	8	1	347.316	0.0415*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 3.5

MANOVA repeated measures with univariate tests also analysis of average root length (m/m^2) for crimson clover (*Trifolium incarnatum*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	3	8	0.276	0.8413
[CO_2]	1	8	0.176	0.6857
Depth	1	8	0.141	0.7173
[CO_2] × Depth	1	8	0.511	0.4951
Within Subjects				
Time	1	8	0.344	0.8734

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 3.6

MANOVA repeated measures with univariate tests also analysis of standing root crop (m/m^2) for crimson clover (*Trifolium incarnatum*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	3	8	4.381	0.0421*
[CO_2]	1	8	0.010	0.9215
Depth	1	8	7.659	0.0244*
[CO_2] × Depth	1	8	5.475	0.0474*
Within Subjects				
Time	8	1	64.378	0.0961

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 3.7

MANOVA repeated measures with univariate tests also analysis of average root diameter (m/m^2) for sunn hemp (*Crotalaria juncea*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	3	8	0.991	0.4445
[CO_2]	1	8	1.025	0.3410
Depth	1	8	0.993	0.3483
[CO_2] × Depth	1	8	0.957	0.3567
Within Subjects				
Time	3	6	8.320	0.0147*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 3.8

MANOVA repeated measures with univariate tests also analysis of average root length (m/m^2) for sunn hemp (*Crotalaria juncea*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F ratio	p – value
Between Subjects				
All Between	3	8	2.732	0.1463
[CO_2]	1	8	6.249	0.0370*
Depth	1	8	0.822	0.3912
[CO_2] × Depth	1	8	0.044	0.8392
Within Subjects				
Time	3	6	6.776	0.0236*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 3.9

MANOVA repeated measures with univariate tests also analysis of standing root crop (m/m^2) for sunn hemp (*Crotalaria juncea*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F ratio	p – value
Between Subjects				
All Between	3	8	3.043	0.0925
[CO_2]	1	8	3.090	0.1168
Depth	1	8	3.165	0.1131
[CO_2] × Depth	1	8	2.874	0.1285
Within Subjects				
Time	3	6	18.345	0.0020*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 3.10

Soybean root turnover index (no till only) with [CO_2] at 365 µmol mol^{-1}) and [CO_2] at 720 µmol mol^{-1})

Treatments	Variables			
	Seasonal root production[a]	Max standing root Crop[a]	Root turnover index[a]	Percent root Turnover
NTE shallow	1.70E-07	4.62E-07	3.67E-01	36.7%
NTE deep	1.51E-07	4.48E-07	3.36E-01	33.6%
NTA shallow	2.15E-07	4.03E-07	5.34E-01	53.4%
NTA deep	2.28E-07	4.33E-07	5.27E-01	52.7%

[a] Values are in units of meters per meter squared (m/m^2)

Table 3.11

Clover root turnover index (no till only) with [CO_2] at 365 µmol mol^{-1}) and [CO_2] at 720 µmol mol^{-1})

Treatments	Variables			
	Seasonal root production[a]	Max standing root crop[a]	Root turnover index[a]	Percent root Turnover
NTE shallow	2.70E-07	4.45E-07	6.07E-01	60.7%
NTE deep	2.82E-07	3.61E-07	7.81E-01	78.1%
NTA shallow	2.72E-07	4.5E-07	6.04E-01	60.4%
NTA deep	3.07E-07	4.26E-07	7.21E-01	72.1%

[a]Values are in units of meters per meter squared (m/m^2)

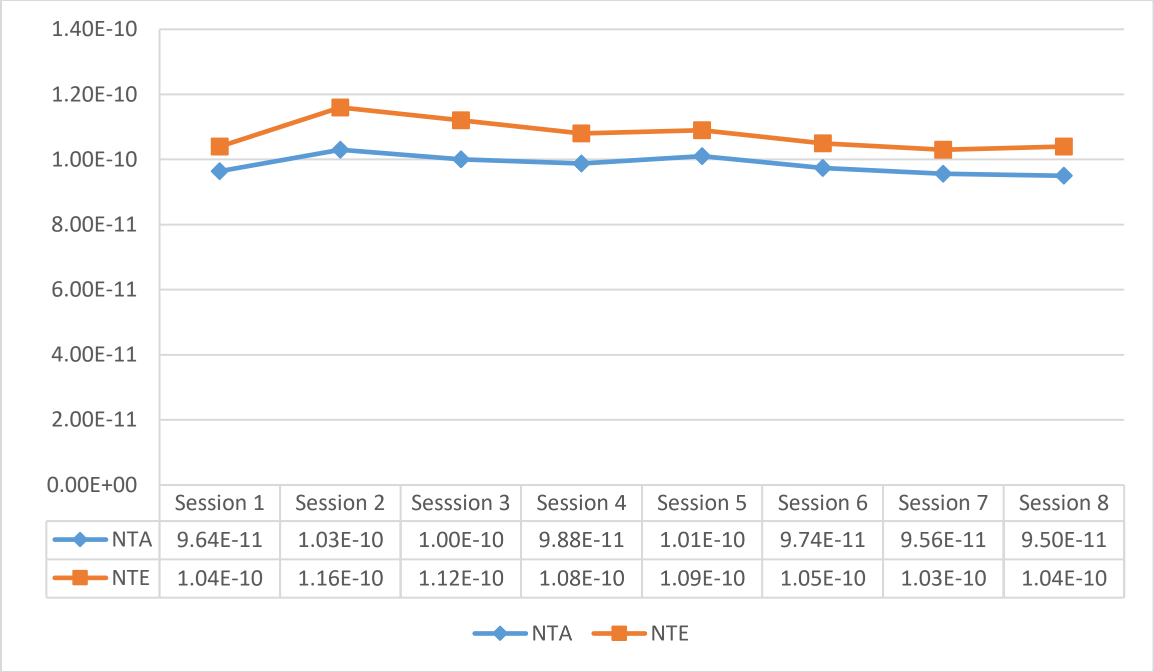
Table 3.12

Sunn hemp root turnover index (no till only) with [CO_2] at 365 µmol mol^{-1}) and [CO_2] at 720 µmol mol^{-1})

Treatments	Variables			
	Seasonal root production[a]	Max standing root crop[a]	Root turnover index[a]	Percent root Turnover
NTE shallow	3.96045E-07	4.33E-07	0.914221	91.4%
NTE deep	4.19797E-07	4.77E-07	0.880758	88.1%
NTA shallow	3.28891E-07	3.95E-07	0.832966	83.3%
NTA deep	4.29297E-07	4.67E-07	0.918587	91.9%

[a]Values are in units of meters per meter squared (m/m^2)

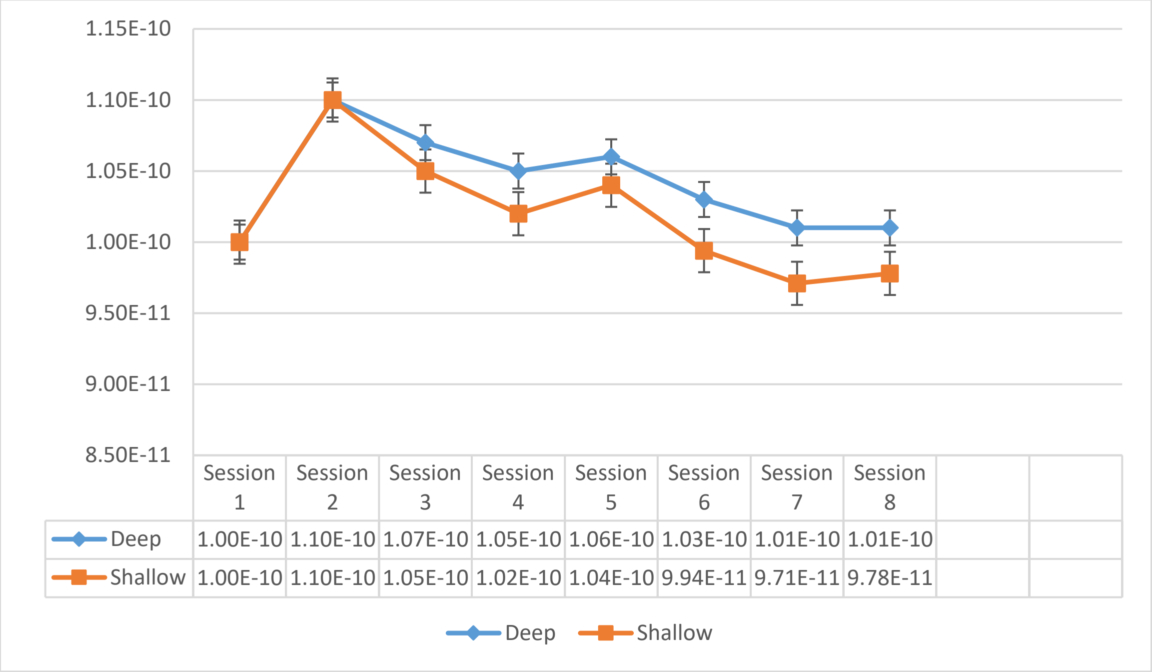
3.7 Figures



	Session 1	Session 2	Sesssion 3	Session 4	Session 5	Session 6	Session 7	Session 8
NTA	9.64E-11	1.03E-10	1.00E-10	9.88E-11	1.01E-10	9.74E-11	9.56E-11	9.50E-11
NTE	1.04E-10	1.16E-10	1.12E-10	1.08E-10	1.09E-10	1.05E-10	1.03E-10	1.04E-10

Figure 3.1 Soybean average root diameter for NTA and NTE treatments

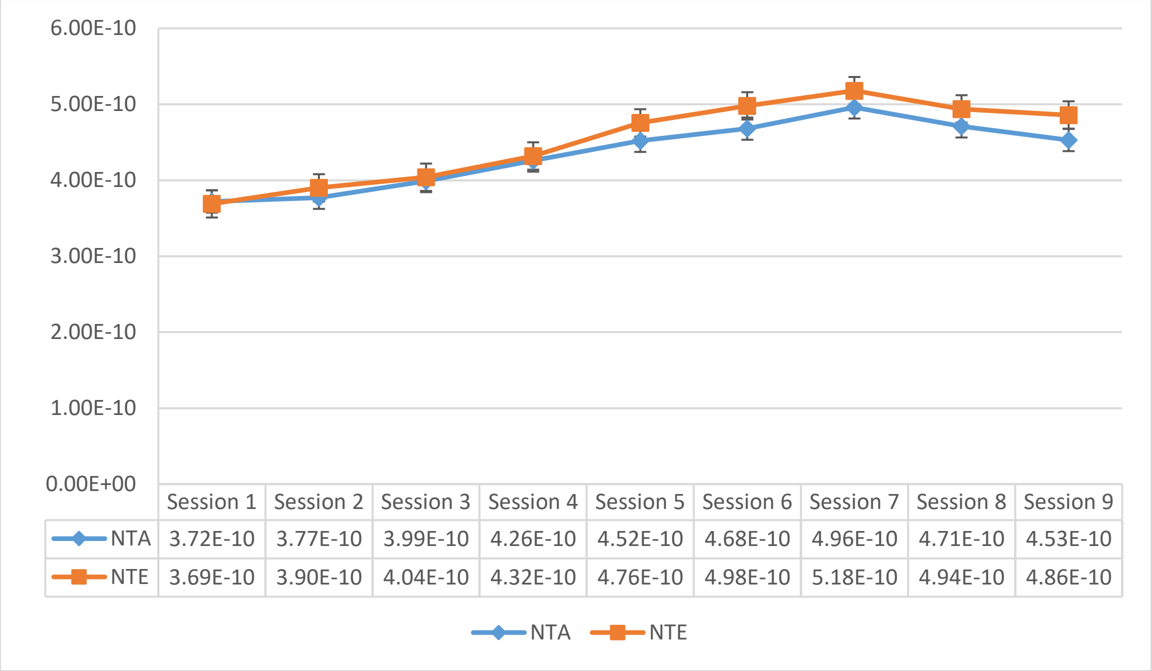
Note. Treatments are no-till ambient and no-till elevated. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8
Deep	1.00E-10	1.10E-10	1.07E-10	1.05E-10	1.06E-10	1.03E-10	1.01E-10	1.01E-10
Shallow	1.00E-10	1.10E-10	1.05E-10	1.02E-10	1.04E-10	9.94E-11	9.71E-11	9.78E-11

Figure 3.2 Soybean Average Diameter deep and shallow

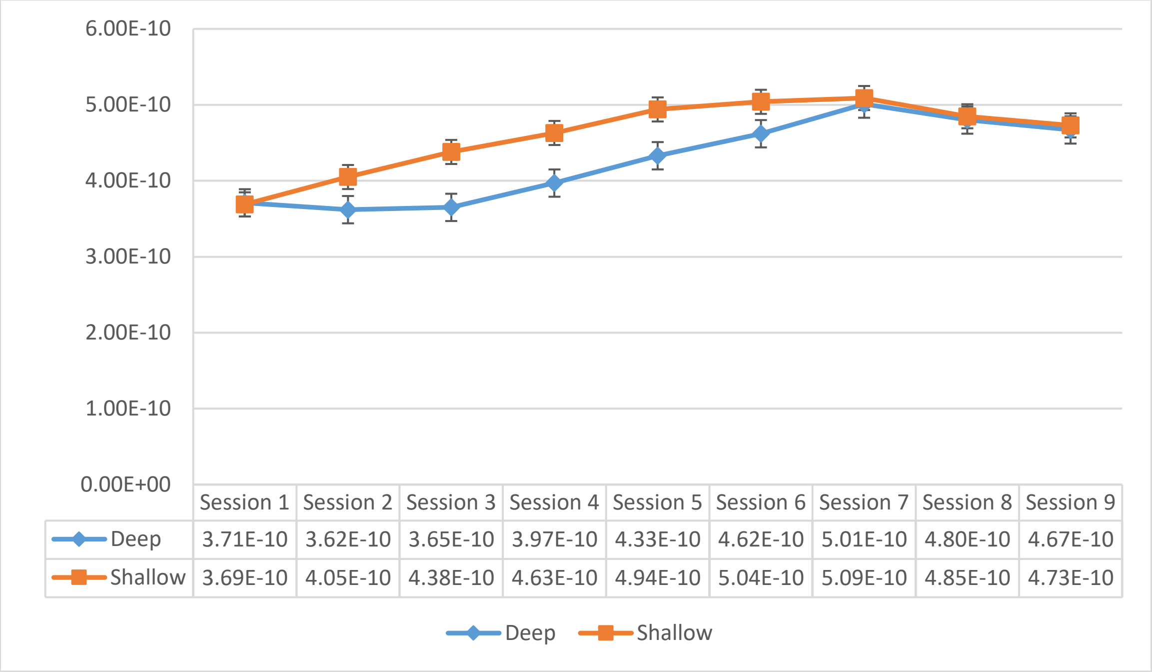
Note. Deep is 17 – 34 cm and Shallow is 0 – 17 cm. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9
NTA	3.72E-10	3.77E-10	3.99E-10	4.26E-10	4.52E-10	4.68E-10	4.96E-10	4.71E-10	4.53E-10
NTE	3.69E-10	3.90E-10	4.04E-10	4.32E-10	4.76E-10	4.98E-10	5.18E-10	4.94E-10	4.86E-10

Figure 3.3 Clover average diameter NTA and NTE

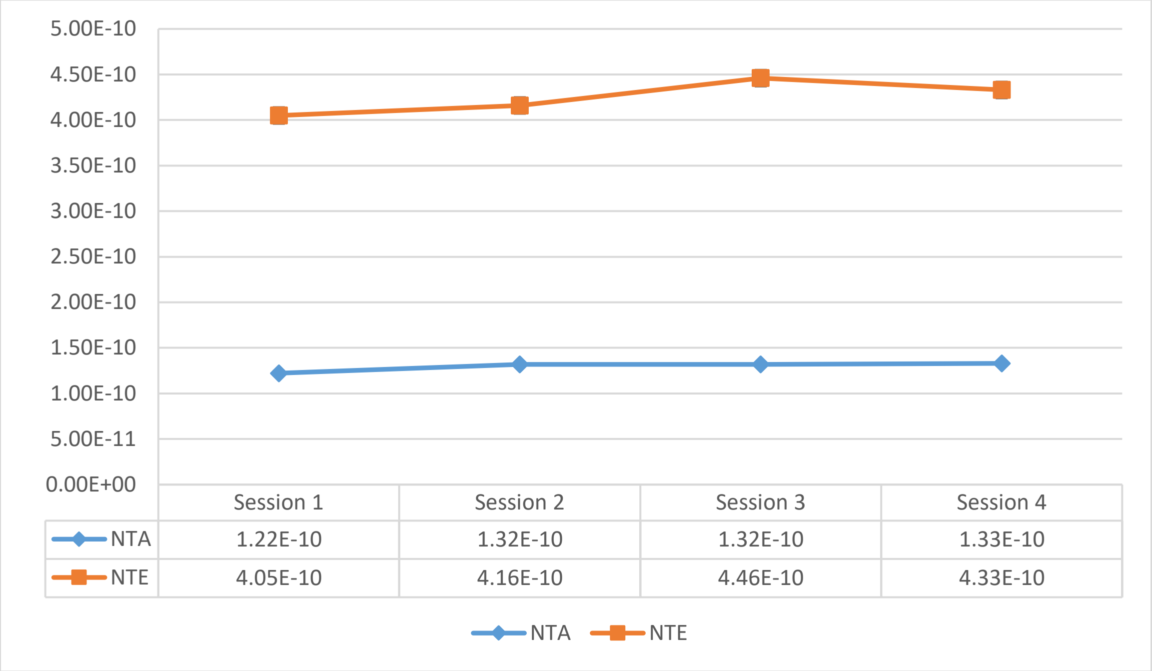
Note. NTA is no till ambient and NTE is no till elevated. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9
Deep	3.71E-10	3.62E-10	3.65E-10	3.97E-10	4.33E-10	4.62E-10	5.01E-10	4.80E-10	4.67E-10
Shallow	3.69E-10	4.05E-10	4.38E-10	4.63E-10	4.94E-10	5.04E-10	5.09E-10	4.85E-10	4.73E-10

Figure 3.4 Clover Average Diameter Deep and Shallow

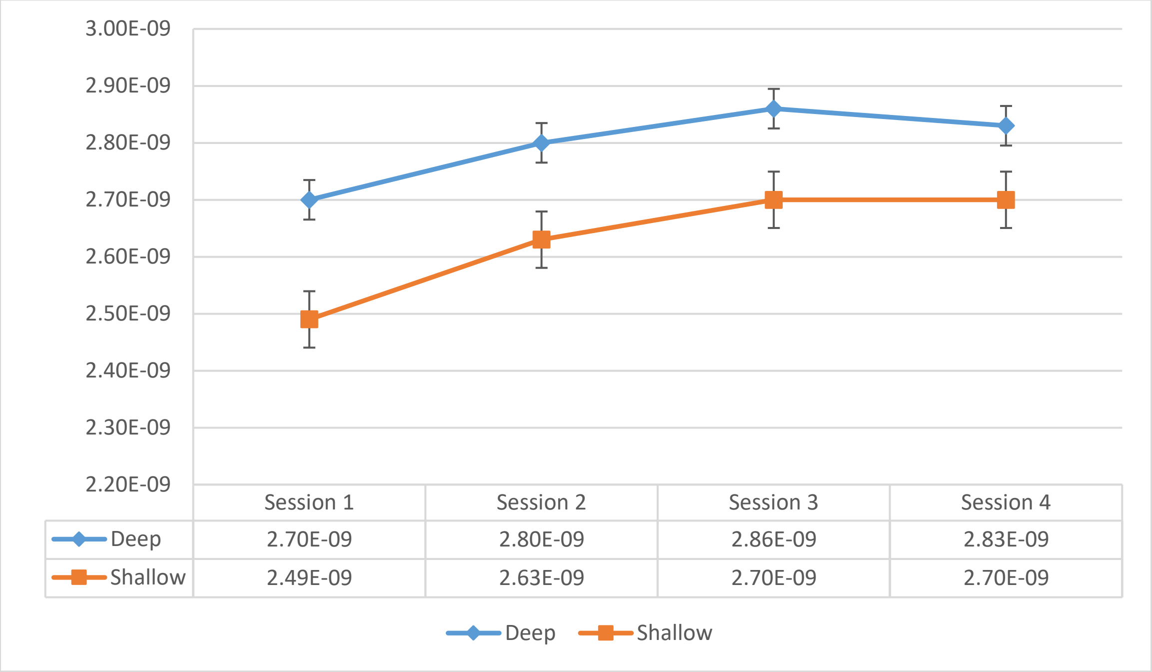
Note. Deep is 17 – 34 cm and Shallow is 0 – 17 cm. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4
NTA	1.22E-10	1.32E-10	1.32E-10	1.33E-10
NTE	4.05E-10	4.16E-10	4.46E-10	4.33E-10

Fig. 3.5 Sunn Hemp average diameter NTA and NTE

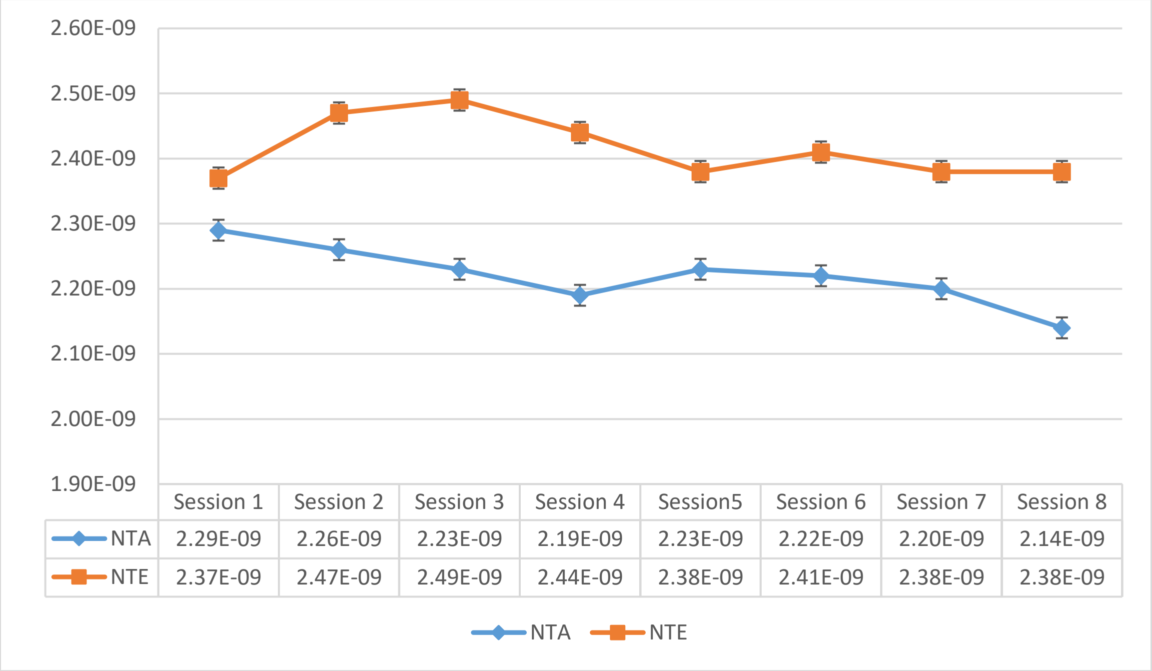
Note. NTA is no-till ambient and NTE is no-till elevated. Standrad error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4
Deep	2.70E-09	2.80E-09	2.86E-09	2.83E-09
Shallow	2.49E-09	2.63E-09	2.70E-09	2.70E-09

Figure 3.6 Chapter 3 Sunn hemp average root length deep and shallow

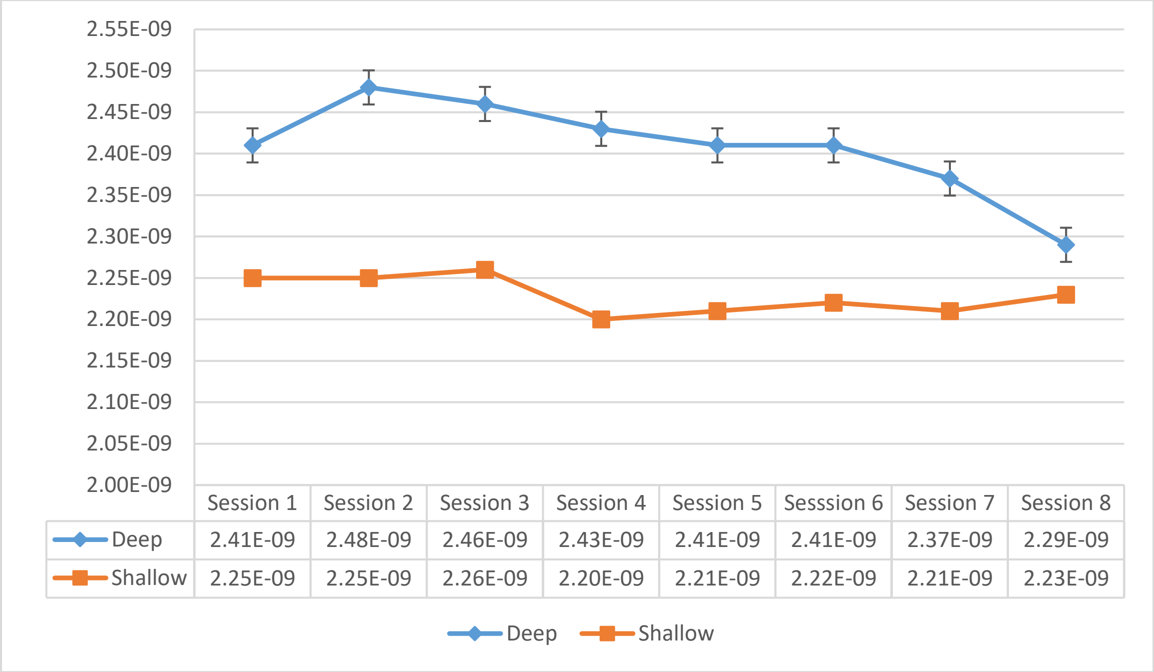
Note. Deep is 17 – 34 cm and Shallow is 0 – 17 cm. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4	Session5	Session 6	Session 7	Session 8
NTA	2.29E-09	2.26E-09	2.23E-09	2.19E-09	2.23E-09	2.22E-09	2.20E-09	2.14E-09
NTE	2.37E-09	2.47E-09	2.49E-09	2.44E-09	2.38E-09	2.41E-09	2.38E-09	2.38E-09

Fig. 3.7 Soybean Average Root Length at NTA and NTE m/m^2

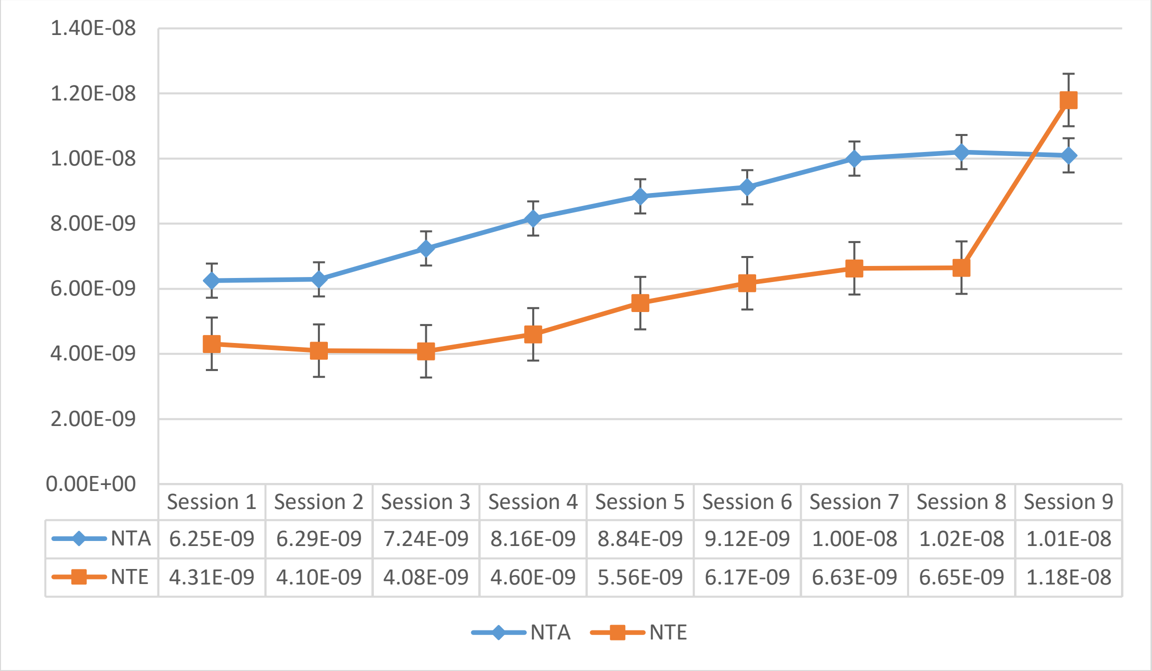
Note. Treatments include no-till ambient and no-till elevated. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4	Session 5	Sesssion 6	Session 7	Session 8
Deep	2.41E-09	2.48E-09	2.46E-09	2.43E-09	2.41E-09	2.41E-09	2.37E-09	2.29E-09
Shallow	2.25E-09	2.25E-09	2.26E-09	2.20E-09	2.21E-09	2.22E-09	2.21E-09	2.23E-09

Figure 3.8 Soybean Average Root length m/m^2 at deep and shallow

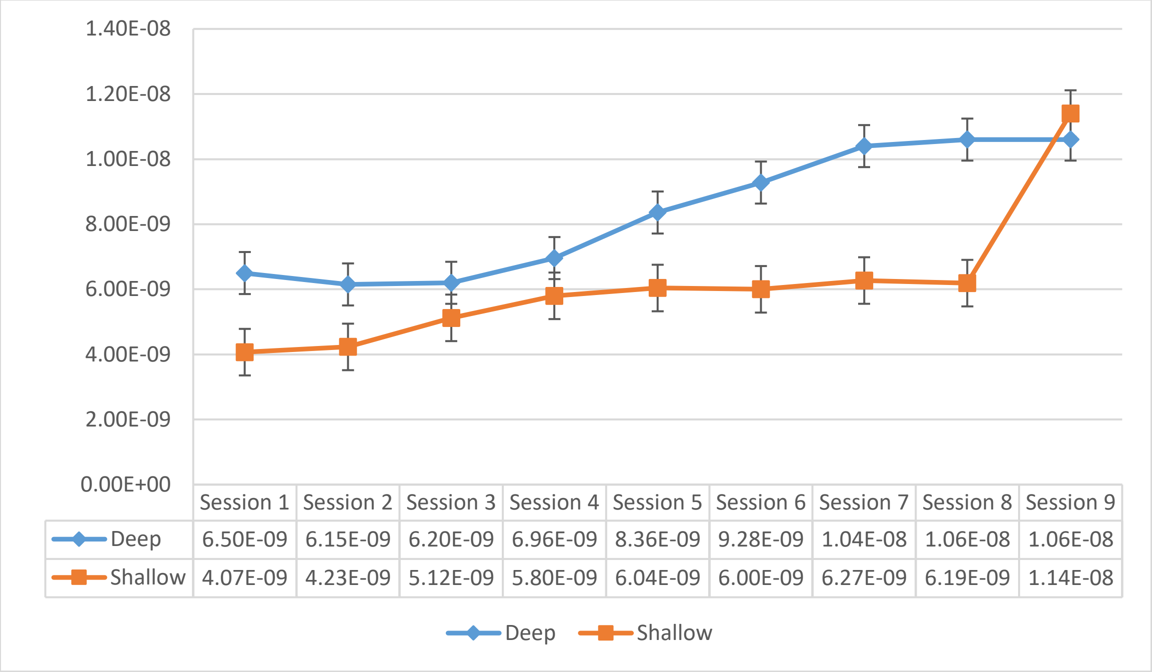
Note. Depths are 17 – 34 cm for Deep and 0 – 17 cm for shallow. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9
NTA	6.25E-09	6.29E-09	7.24E-09	8.16E-09	8.84E-09	9.12E-09	1.00E-08	1.02E-08	1.01E-08
NTE	4.31E-09	4.10E-09	4.08E-09	4.60E-09	5.56E-09	6.17E-09	6.63E-09	6.65E-09	1.18E-08

Figure 3.9 Clover average root length NTA and NTE

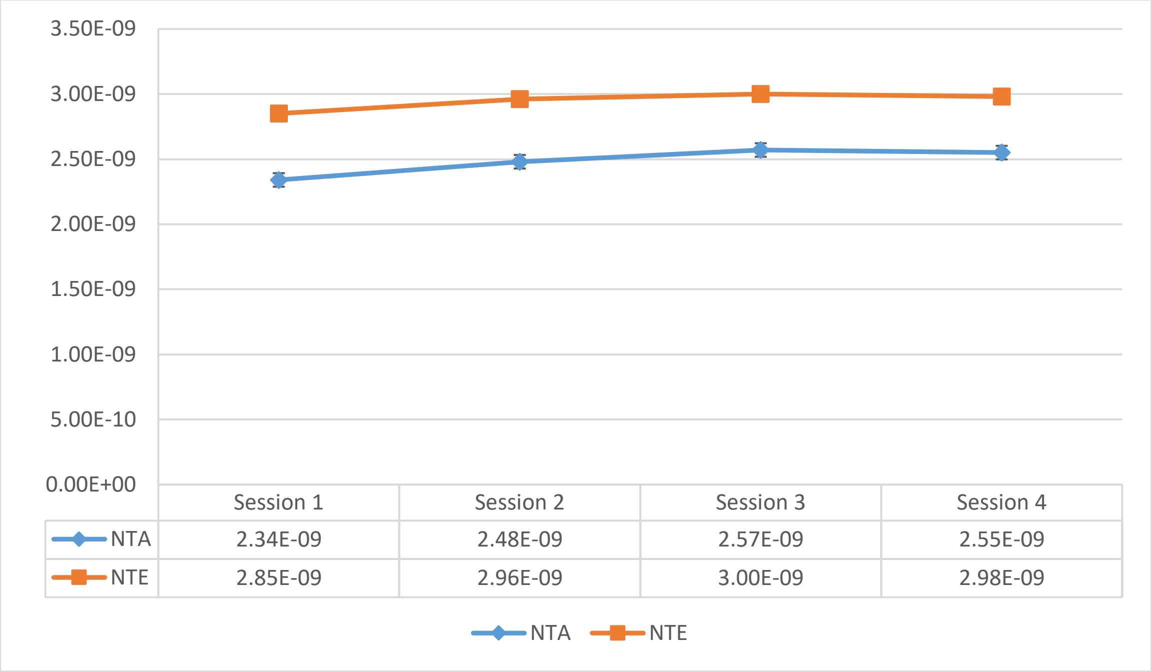
Note. NTA is no till ambient and NTE is no till elevated. Standard error bars are included. Units are m/m^2



	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9
Deep	6.50E-09	6.15E-09	6.20E-09	6.96E-09	8.36E-09	9.28E-09	1.04E-08	1.06E-08	1.06E-08
Shallow	4.07E-09	4.23E-09	5.12E-09	5.80E-09	6.04E-09	6.00E-09	6.27E-09	6.19E-09	1.14E-08

Figure 3.10 Clover average root length deep and shallow

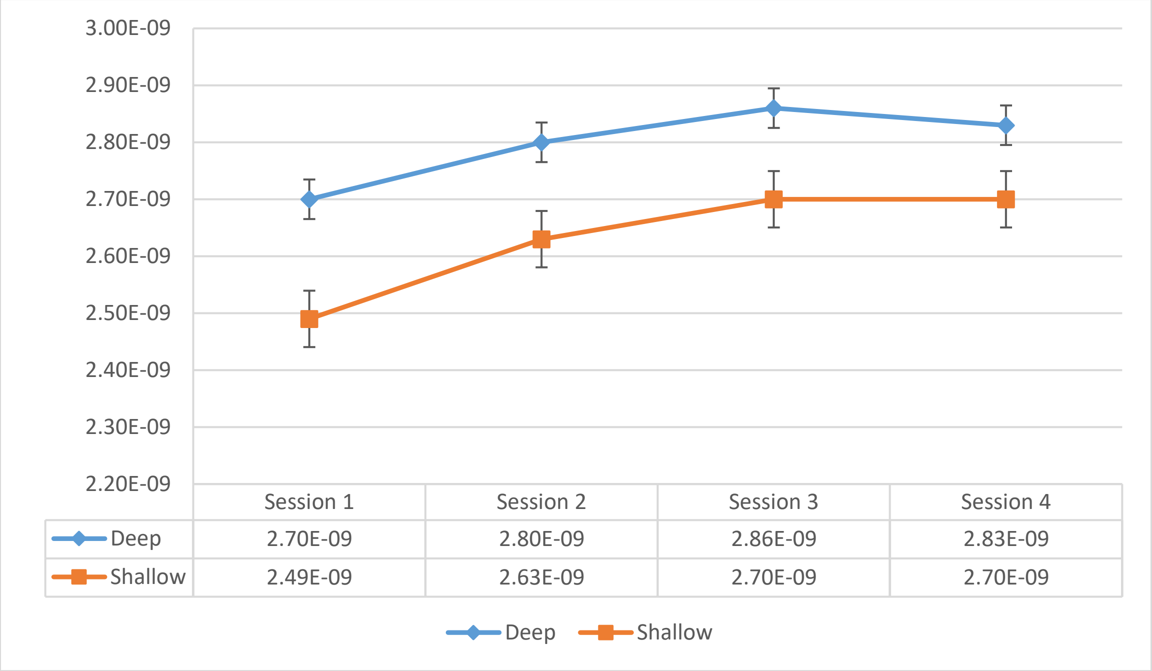
Note. Deep is 17 – 34 cm and Shallow is 0 – 17 cm. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4
◆ NTA	2.34E-09	2.48E-09	2.57E-09	2.55E-09
■ NTE	2.85E-09	2.96E-09	3.00E-09	2.98E-09

Figure 3.11 Sunn hemp average root length NTA and NTE m/m^2

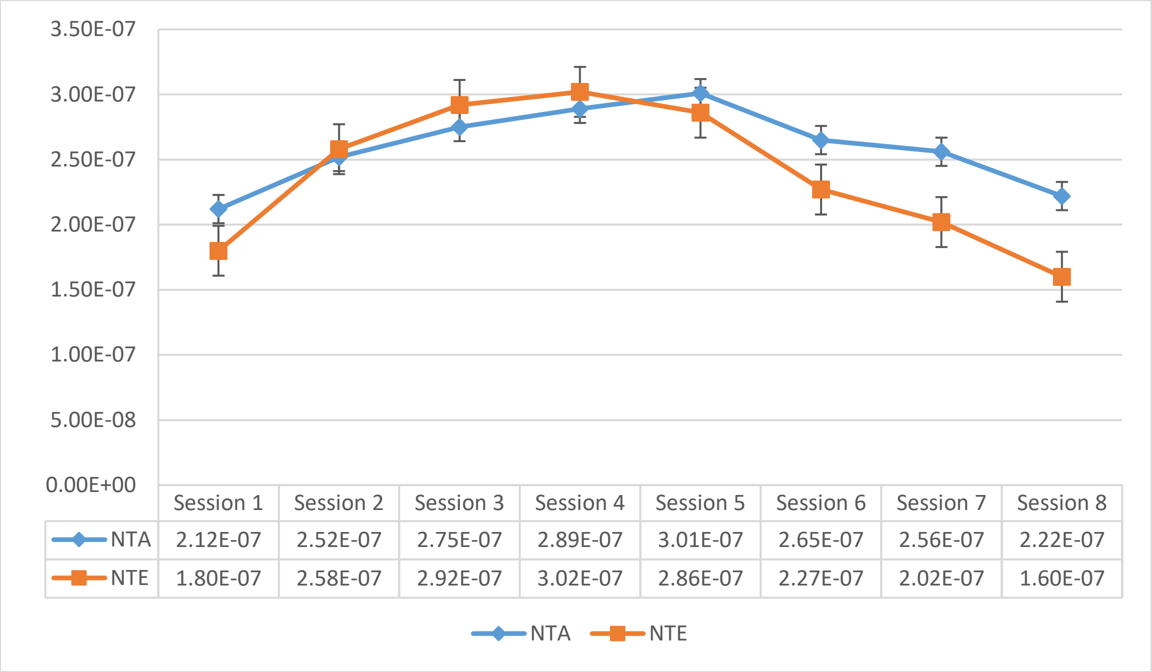
Note. NTA is no-till ambient and NTE is no-till elevated. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4
Deep	2.70E-09	2.80E-09	2.86E-09	2.83E-09
Shallow	2.49E-09	2.63E-09	2.70E-09	2.70E-09

Figure 3.12 Sunn hemp average root length deep and shallow

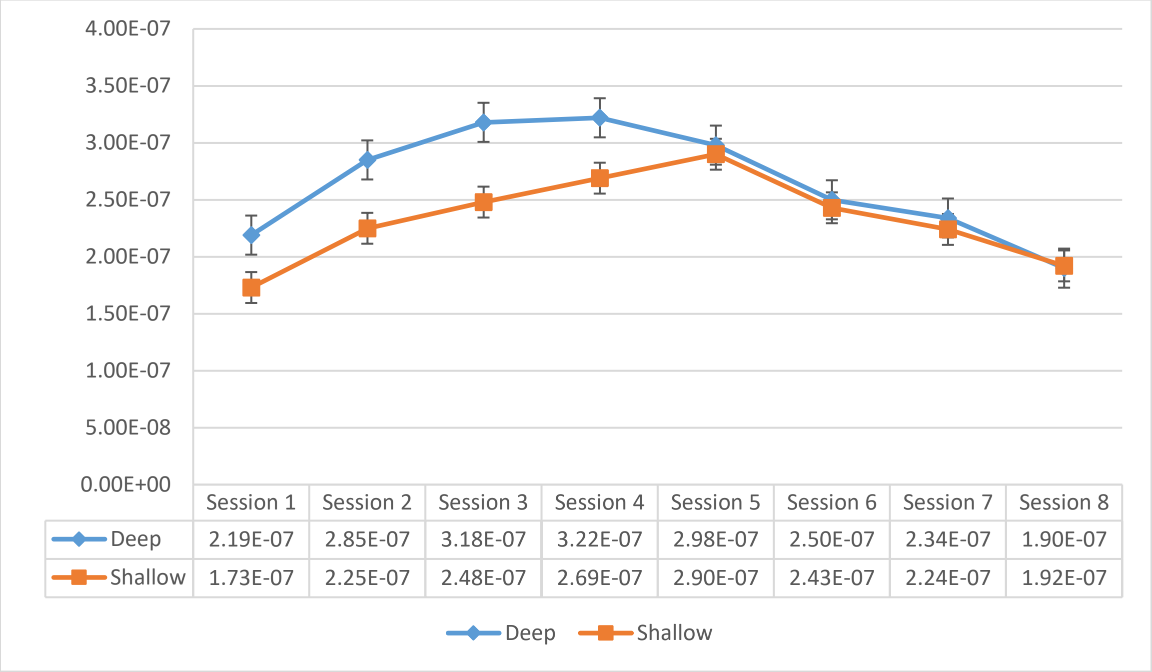
Note. Deep is 17 – 34 cm and Shallow is 0 – 17 cm. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8
NTA	2.12E-07	2.52E-07	2.75E-07	2.89E-07	3.01E-07	2.65E-07	2.56E-07	2.22E-07
NTE	1.80E-07	2.58E-07	2.92E-07	3.02E-07	2.86E-07	2.27E-07	2.02E-07	1.60E-07

Figure 3.13 Soybean standing root crop NTA and NTE in m/m^2

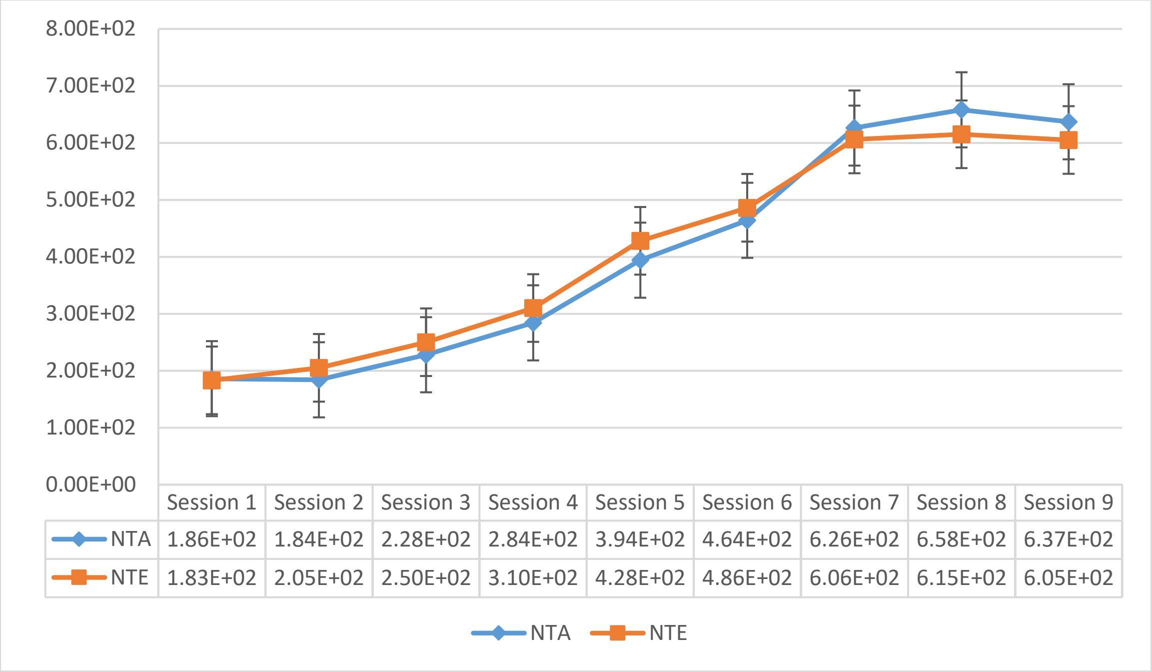
Note. Treatments are no-till ambient and no-till elevated.. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8
Deep	2.19E-07	2.85E-07	3.18E-07	3.22E-07	2.98E-07	2.50E-07	2.34E-07	1.90E-07
Shallow	1.73E-07	2.25E-07	2.48E-07	2.69E-07	2.90E-07	2.43E-07	2.24E-07	1.92E-07

Figure 3.14 Soybean standing root crop deep and shallow m/m^2

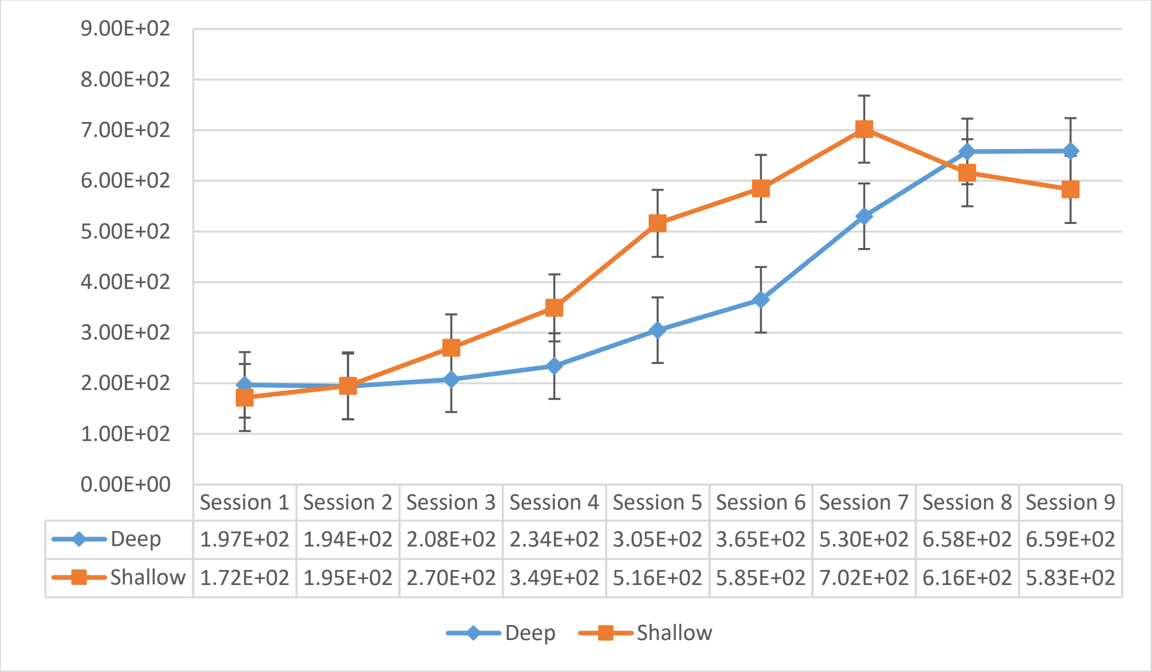
Note. Deep is 17 – 34 cm and shallow is 0 – 17 cm. Standing error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9
NTA	1.86E+02	1.84E+02	2.28E+02	2.84E+02	3.94E+02	4.64E+02	6.26E+02	6.58E+02	6.37E+02
NTE	1.83E+02	2.05E+02	2.50E+02	3.10E+02	4.28E+02	4.86E+02	6.06E+02	6.15E+02	6.05E+02

Figure 3.15 Clover Standing root crop NTA and NTE

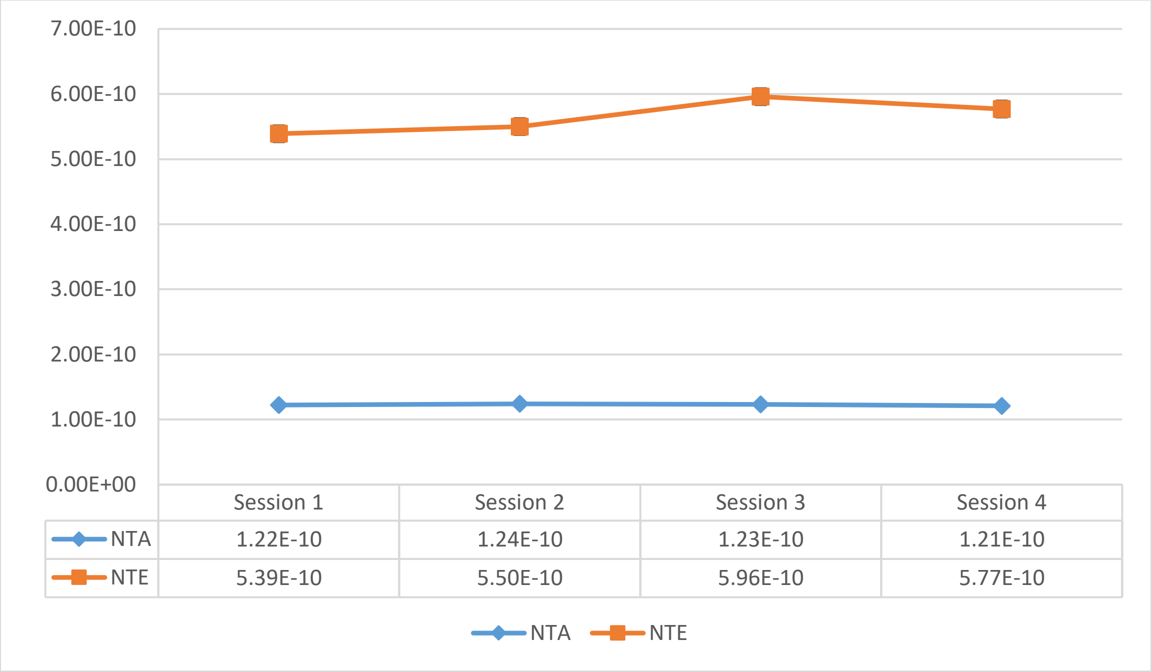
Note. NTA is no till ambient and NTE is no till elevated. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9
◆ Deep	1.97E+02	1.94E+02	2.08E+02	2.34E+02	3.05E+02	3.65E+02	5.30E+02	6.58E+02	6.59E+02
■ Shallow	1.72E+02	1.95E+02	2.70E+02	3.49E+02	5.16E+02	5.85E+02	7.02E+02	6.16E+02	5.83E+02

Figure 3.16 Clover Standing Root Crop Deep and Shallow

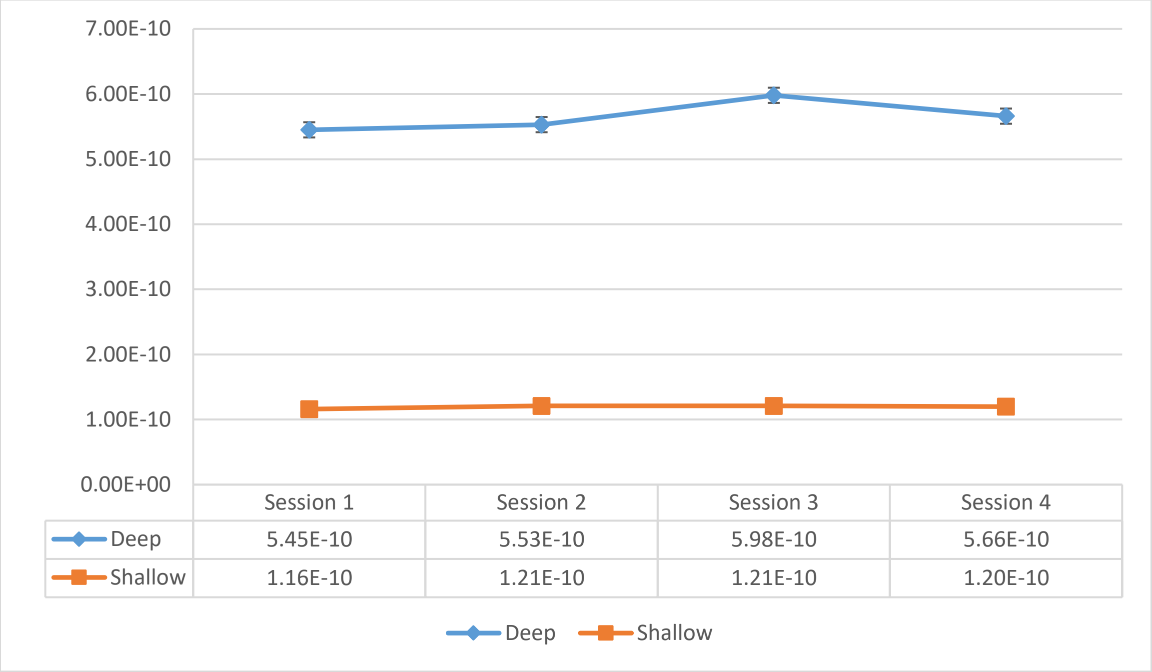
Note. Deep is 17 – 34 cm and Shallow is 0 – 17 cm. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4
NTA	1.22E-10	1.24E-10	1.23E-10	1.21E-10
NTE	5.39E-10	5.50E-10	5.96E-10	5.77E-10

Figure 3.17 Sunn hemp standing root crop NTA and NTE

Note. NTA is no-till ambient and NTE is no-till elevated. Standard error bars are included. Units are m/m^2.



	Session 1	Session 2	Session 3	Session 4
Deep	5.45E-10	5.53E-10	5.98E-10	5.66E-10
Shallow	1.16E-10	1.21E-10	1.21E-10	1.20E-10

Figure 3.18 Sunn hemp standing root crop deep and shallow

Note. Deep is 17 – 34 cm and Shallow is 0 – 17 cm. Standard error bars are included. Units are m/m^2.

CHAPTER IV – ROOT GROWTH OF C3 GRASS VERSUS C4 GRASS

4.1 Abstract

Comparison of the differential fine root growth responses of C_3 photosynthetic plants to increased carbon dioxide levels with that of C_4 photosynthetic plants has been an area of much speculation but limited research. The increasing use of no-till agricultural management, which has been advocated as a potential carbon sink, makes it an area suitable for similar research. Repeated measures MANOVA with univariate tests, was used to compare the root growth of a C_3 grass, *Triticum aestivum* L., with that of a C_4 grass, *Sorghum bicolor* L. when both species were grown under the no-till cropping system with subjects grown at (365 µmol CO_2 mol^{-1}) and subjects grown at elevated CO_2 levels (720 µmol CO_2 mol^{-1}). The plants were grown at the National Soil Dynamics Laboratory, Auburn, Alabama) using a split plot design replicated three times with the two carbon dioxide levels as split-plots. The plants were grown in Open Top Chambers in experimental bins of prepared soil. Minirhizotron cameras were used at regular intervals to record images of fine roots grown at soil depths from 0 cm to 34 cm. The Rootfly 2.1 image program was used to digitize the data and JMP 12.0 was used for statistical analysis.

For average root diameter, there were significant differences for all between, for [CO_2], for species × [CO_2], and for within subjects time. For average root length, there were significant differences for all between, species, [CO_2], species × depth, and within subjects time. For standing root crop, there were significant differences for all between, species, depth, species × depth, and within subjects time.

Both C_3 and C_4 grasses responded to the treatments used in the study but differently. While elevated [CO_2] resulted in greater average root diameter and greater average root length than ambient [CO_2], there was an increase in average root length across the season for the C_3 wheat while there was a slight decrease across the season for the C_4 sorghum. For standing root crop, while [CO_2] didn't have a significant effect, the wheat increased standing root crop across the season to a greater extent than the sorghum.

4.2 Introduction

Comparison of the differential fine root growth responses of C_3 photosynthetic plants to increased carbon dioxide levels with that of C_4 photosynthetic plants has been an area of much speculation but limited research. The increasing use of no-till agricultural management, which has been advocated as a potential carbon sink, makes it an area suitable for similar research. The root growth of a C3 grass, *Triticum aestivum* L., was compared with that of a C4 grass, *Sorghum bicolor* L. when both species were grown under the no-till cropping system with subjects grown at (365 µmol CO_2 mol^{-1}) and subjects grown at elevated CO_2 levels (720 µmol CO2 mol^{-1}). The plants were grown at the National Soil Dynamics Laboratory, Auburn, Alabama) using a split plot design replicated three times with the two carbon dioxide levels as split-plots. The plants were grown in Open Top Chambers (OTCs) in experimental bins of prepared soil. Minirhizotron cameras were used at regular intervals to record images of fine roots grown at soil depths from 0 cm to 34 cm. The Rootfly 2.0.2 image program was used to digitize the data and JMP 12.1 was used for statistical analysis.

Atmospheric carbon dioxide levels have increased from 280 parts per million (ppm) before the industrial revolution to close to 400 ppm today and are continuing to rise (Monasterksy 2013). Plant growth will be affected by the increased carbon dioxide since CO_2 is a necessary input in order for photosynthesis to take place (Prior et al. 2005). The response of agricultural plants to this increased carbon dioxide is of great interest particularly as it pertains to food security.

The majority of agricultural plants have a C_3 method of photosynthesis with 12 of the 15 plants which supply 90% of the world's calories having C_3 photosynthesis (Reddy et al. 2010). A number of studies have shown that C_3 plants respond to elevated [CO_2] with increased photosynthesis which translates into increased root growth (Pritchard and Amthor 2005). The increased root growth resulting from elevated [CO_2] can be more pronounced than that of the aboveground plant shoot (Kimball et al. 2002).

While not as many C_4 species are represented as with C_3 plants, plants that undergo C_4 photosynthesis (C_4 plants) include important agricultural crop plants. Not only are they important in the United States producing a significant proportion of grain crops, some are widely grown in tropical areas where food security is directly tied to local production of crops (Leakey, 2009).

The photosynthetic response of C_4 plants to increased [CO_2] is less well known than that of C_3 plants. It is widely thought that C_4 plant generally have an increase in photosynthesis under elevated [CO_2] but less than that of C_3 plants under similar conditions (Ainsworth 2005; Runion 2009).

Chaudhuri et al. (1986) found an increase in shoot and root growth of sorghum under [795 μmol mol^{-1}] while water use decreased. Pritchard and Amthor (2005) attribute the increase in productivity of C_4 plants under elevated atmospheric [CO_2] to be primarily due to increased water use efficiency (WUE). Pritchard et al. (2006), in an earlier study, reported root growth of sorghum (a C_4 plant) to be increased by 58% under twice normal [CO_2] and conventional till agricultural management.

4.3 Methods

This research explores the differences in response to ambient [CO_2] (365µmol mol^{-1}) and twice ambient (720 µmol mol^{-1}) between the C_4 sorghum (*Sorghum bicolor*) and the C_3 wheat (*Triticum aestivum*) while sorghum and wheat were being grown under the no-till cropping system. The no-till agricultural management also included the rotating use of three cover crops: scarlet clover (*Trifolium incarnatum*), Sunn hemp (*Crotalaria juncea*), and wheat (*Triticum aestivum*). Additionally, the roots were divided into shallow (0 – 17 cm) and deep (17 – 34 cm) soil levels.

The plants were grown at the National Soil Dynamics Laboratory, Auburn, Alabama) as per Prior et al (2005) using a split plot design replicated three times with the two carbon dioxide levels as split-plots. The plants were grown in Open Top Chambers (OTCs) in experimental bins of prepared soil. Minirhizotron cameras were used at regular intervals to record images of fine roots grown at soil depths from 0 cm to 34 cm. The Rootfly 2.0.2 image analysis program was used to digitize the data. The average root diameter, average root length, and standing root crop were determined for both wheat and sorghum. While grown at no-till conditions with elevated or ambient [CO_2]. JMP 12.1 was used for statistical analysis using repeated measures MANOVA with time being the repeated measure.

4.4 Results

4.4.1 Average root diameter

For average root diameter, there was a significant difference in all between subjects (p = 0.0141*). There was a significantly greater average root diameter at elevated [CO_2] (p = 0.0101*). There was a significant species × [CO_2] interaction (p = 0.0140*). There was also a significant difference for within subjects time (p = 0.0258*).

4.4.2 Average root length

For average root length, there was a significant result for all between (p = 0.02399*). There were also significant results for species (p = 0.0300*), for [CO_2] (p = 0.0492*), for species × Depth (p = 0.0435*), and for within subjects time (p = 0.0258*).

4.4.3 Standing root crop

For standing root crop, there was a significant result for all between (p = 0.0047*). There were also significant differences for species (p = 0.0033*), for depth (p = 0.0040*), for species × Depth (p = 0.0114*), and for within subjects time (p <0.0001*).

4.5 Discussion

It had been expected that the C_3 plant, wheat, would have a greater increase in root growth at elevated [CO_2] than that of the C_4 sorghum, but that hypothesis was not entirely supported by the data. There was no significant difference in average root diameters with elevated [CO_2], and there was no significant difference in standing root crop with elevated [CO_2]. There was a significant increase in average root length with elevated [CO_2], which doesn’t entirely outweigh the lack of a significant effect of elevated [CO_2] for the other two root growth perameters.

In C_3 plants, ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco) is the enzyme which catalyzes the initial step where CO_2 is captured (Ainsworth and Long, 2005). Carbon dioxide is combined with the five-carbon molecule, Rubisco, to form a six-carbon compound which quickly separates into two three-carbon molecules of Phosphoglycerate (3PGA). 3PGA, the first stable product of C_3 photosynthesis, then continues through the Calvin cycle where carbon is fixed into organic molecules. Rubisco alternatively catalyzes a process called photorespiration in which oxygen is taken up instead of CO_2 (Mirkham 2011). At current atmospheric carbon dioxide levels, C_3 plants undergo photosynthesis at less than peak efficiency. At elevated [CO_2], the photosynthetic fixation of C_3 can continue to increase **(**Kimball et al. 2002).

C_4 photosynthesis has an additional initial step where Phosphoenolpyruvate (PEP) is jointed with carbon dioxide to form a four carbon acid oaxaloacetate (OAA) inside the mesophyll cells of the leaf. This step is catalyzed by PEP carboxylase, which fixes carbon dioxide but does not catalyze photorespiration **(**Mirkham 2011**).** The OAA travels through strands of tissue (plasmodesmata) from the mesophyll cells to the bundle sheath cells. There it is decarboxylated and the CO_2 goes through the steps of the Calvin cycle just as in C_3 plants with the resulting incorporation of carbon into organic molecules. The concentration of carbon dioxide is much higher in the bundle sheath cells of C_4 plants, so the process is much more efficient **(**von Caemmerer and Furbank 2003). Due the concentrating mechanism, where CO_2 is concentrated around rubisco (in the bundle sheath cells), C_4 photosynthesis is saturated at current levels of atmospheric [CO_2] with photorespiration being virtually nonexistent (Von Caemmerer and Furbank 2003).

It is thought that photosynthesis of C_4 plants will be less affected by elevated [CO_2] than photosynthesis of C_3 plants (Allen 2004). The predicted result of increasing atmospheric [CO_2] is that photosynthesis of C_3 plants will increase to a point similar to that of C_4 plants (Kirkham 2011).

Elevated [CO_2] improves the photosynthetic capacity of C_3 plants relative to C_4 plants. However, increased atmospheric [CO_2] leads to increased water use efficiency (WUE) of both C_3Plants and C_4 plants (Wang 2014). WUE is the amount of dry matter (vegetative yield or grain yield) divided by the amount of water needed to produce that dry matter (Kirkham 2011). The decreased stomatal conductance seen with elevated [CO_2] and associated with improved WUE is seen in both C_3 and C_4 species (Ainsworth and Rogers 2007). The increase in photosynthesis resulting in more biomass production coupled with the increase in WUE of C_3 plants may give them a competitive edge under elevated [CO_2].

4.6 Tables

Table 4.1

MANOVA repeated measures with univariate tests also analysis of average root diameter (m/m^2) for wheat (*Triticum aestivum*) and for sorghum (*Sorghum bicolor*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	7	16	2.807	0.0414*
Species	1	16	0.546	0.4707
[CO_2]	1	16	8.507	0.0101*
Depth	1	16	2.653	0.1229
Species × [CO_2]	1	16	7.611	0.0140*
Species × Depth	1	16	0.0009	0.9766
[CO_2] × Depth	1	16	0.256	0.6201
Species × [CO_2] × Depth	1	16	0.0729	0.7907
Within Subjects				
Time	3	14	4.198	0.0258*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

Table 4.2

MANOVA repeated measures with univariate tests also analysis of average root length (m/m^2) for wheat (*Triticum aestivum*) and for sorghum (*Sorghum bicolor*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	7	16	3.071	0.02399*
Species	1	16	5.675	0.0300*
[CO_2]	1	16	4.530	0.0492*
Depth	1	16	0.250	0.6239
Species × [CO_2]	1	16	1.133	0.3030
Species × Depth	1	16	4.808	0.0435*
[CO_2] × Depth	1	16	2.677	0.1213
Species × [CO_2] × Depth	1	16	2.423	0.1391
Within Subjects				
Time	3	14	4.199	0.0258*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 µmol^{-1} and ambient [CO_2] is 720 µmol^{-1}.

Table 4.3

MANOVA repeated measures with univariate tests also analysis of standing root crop (m/m^2) for wheat (*Triticum aestivum*) and for sorghum (*Sorghum bicolor*) grown at two conditions: no-till elevated [CO_2] and no-till ambient [CO_2].

Source	Num_{DF}	Den_{DF}	F– ratio	p – value
Between Subjects				
All Between	7	16	4.761	0.0047*
Species	1	16	11.946	0.0033*
[CO_2]	1	16	0.352	0.5611
Depth	1	16	11.308	0.0040*
Species × [CO_2]	1	16	0.0012	0.9731
Species × Depth	1	16	8.178	0.0114*
[CO_2] × Depth	1	16	0.534	0.4755
Species × [CO_2] × Depth	1	16	1.006	0.3307
Within Subjects				
Time	3	16	8.809	<0.0001*

Alpha = $p < 0.05$. Deep is 17 – 34 cm vertical soil depth and shallow is 0 – 17 cm vertical soil depth. Elevated [CO_2] is 365 μmol^{-1} and ambient [CO_2] is 720 μmol^{-1}.

CHAPTER V – CONCLUSION

5.1 Chapter II Conclusion

The dissertation study included five plants, two of which were grown under all four conditions and addressed in this chapter, sorghum and soybean. A similar study was done at the USDA, National Soil Laboratory, Auburn, Alabama, with the exception that it included minirhizotron digitization and analysis of sorghum root growth without the inclusion of soybean (Pritchard et al. 2006). A comparison of average sorghum root lengths from the dissertation study versus the earlier Pritchard sorghum study revealed some interesting information (Table 2.11).

Both the dissertation study and the Pritchard study showed no significant effect of tillage on average root length of sorghum. Both studies showed significantly greater average root length in deeper soil. Both studies also showed a trend toward elevated atmospheric [CO_2] being associated with increased average root length (Table 2.11). In the Pritchard study, there was a trend toward an effect of [CO_2] ×depth on average root length of sorghum ($p = 0.120$).

The Pritchard study indicated that time was significant ($p < 0.0001$*). The dissertation study indicated that, for sorghum average root length, within (within groups) time ($p = 0.0153$*) had a significant effect. For repeated measures studies occurring across a number of weeks, the effect of time on increase of root length was not unexpected. Depth was more important than tillage or [CO_2] in both sorghum studies since only depth had a significance level effect for average root length in both studies (Table 2.11).

For average root length of sorghum, there was no significant effect of tillage in either the dissertation study or the Pritchard study; however, for [CO_2], there was a trend toward an effect in the dissertation study ($p = 0.1071$) and in the Pritchard study ($p = 0.080$) (Pritchard et al. 2006).

The correlation between average root length for the two studies was close enough to serve as a validation of both since both were conducted independently. While the minirhizotron images of roots for both studies were produced at the National Soil laboratory, the processing of those images and the statistical analyses had major procedural differences. Two different digitization programs were involved with Rootfly 2.0.2 used for the dissertation study and RooTracker (Dave Tremmel, Duke University) used for the Pritchard study.

The dissertation study was digitized at The University of Southern Mississippi, Hattiesburg, by the dissertation author, a graduate student with ten years of previous research experience.   The Pritchard study was digitized at the USDA National Soil Laboratory at Auburn, Alabama, by an experienced research associate employed by the USDA. Data from both studies were analyzed using a repeated measures (time) design, but individual analysis of each study was done independently. The statistics for the dissertation study involved MANOVA analysis using the JMP 12.1.0 program, while statistics for the Pritchard study involved mixed model procedure (PROC MIXED) analysis using the SAS program (Littell et al. 1996).

Although the sorghum plants were grown at the same facility under similar conditions, the digitization of the root images and the processing of the data as well as the statistics were done independently by different people at separate research facilities. As that is the case, it speaks well of the procedure that the sorghum results were similar.

The experimental results indicate that no-till agricultural management increases standing root crop of sorghum when analyzed individually and increases all three measures of root growth when interacting with other treatments. Elevated [CO_2] increases average root length of sorghum and increases all three measures of root growth when interacting with other treatments (Table 2.4).

The experimental results for soybean indicate that no-till agricultural management increases average root diameter when analyzed individually and increases all three measures of root growth when interacting with other treatments. Elevated [CO_2] shows a positive effect on root growth of soybean only when interacting with other treatments including depth and time (Table 2.5).

Increased root depth of sorghum, when analyzed individually, increases average root length and standing root crop and has additional effects when interacting with other treatments (Table 2.4). Increased depth increases root growth of soybean only when interacting with other treatments (Table 2.5).

5.2 Chapter III Conclusion

Hypotheses addressed by this chapter are as follows:

Firstly, for the three legumes in the study, growth using no-till agricultural management and twice ambient atmospheric carbon dioxide [CO_2], will result in differential root growth which will vary depending upon species.

Secondly, for the three legumes in the study, growth using no-till agricultural management and twice ambient atmospheric carbon dioxide [CO_2], will result in differential root growth between shallow (0-17 cm) and deep soil depths (17 – 34 cm) which will vary depending upon species.

Were the hypotheses addressed by this chapter supported by the experimental results? First, for the three species legumes in the study, using no-till agricultural management at ambient [CO_2] and twice ambient [CO_2], there was differential root growth for the values addressed with Sunn hemp (*Crotalaria juncea* L.) having the most response, soybean (*Glycine max* L.) having the least response, and crimson clover (*Trifolium incarnatum* L.) having an intermediate response. So the first hypothesis was not disproved for these three legumes.

There was differential root growth at different depths between the three legumes grown at NTE and NTA conditions. While soybean and sunn hemp had more root growth at deep conditions than shallow, it was not statistically significant. Clover, however, had greater standing root crop in the shallow depths for six out of nine sessions with four of those sessions showing significantly greater standing root crop in shallow depths. So, the second hypothesis was not disproved at least for standing root crop.

5.3 Chapter IV Conclusion

The hypothesis for this chapter was that C_3 plants would be able to take advantage of elevated [CO_2] to a greater degree than C_4 plants in the form of increased root growth. Instead, although the average root diameter of sorghum was less malleable without the addition of time as a factor, the root growth of the two members of family Poaceae were similar for with the application of carbon dioxide, depth, and various combinations of the two factors in that while wheat had two additional significant/trend results for average root length (8 versus 6), sorghum had one additional significant/trend result for standing root crop (8 versus 7). Although this research could be considered a very preliminary exploration of the topic, the hypothesis was not supported by the results of the comparison of root growth of the C_3 *Triticum aestivum* with that of the C_4 *Sorghum bicolor*.

The effect of increasing [CO_2] upon plants in general and upon agricultural crop plants specifically is a topic that has great importance for the future of a world that has some seven billion inhabitants **(**World Population Prospects 2017). Both [CO_2] levels and the population are widely predicted to continue to increase into the foreseeable future. Related research, such as that from this dissertation study on the effects of elevated [CO_2] on plant root growth, which will increase our knowledge of what to expect will be useful. The study of agricultural innovations, such as no-till agricultural management, with the potential of increasing crop yield while minimizing degradation of cropland is an area which also deserves further consideration as we strive to prepare for the conditions that will be encountered in the coming years.

BIBLIOGRAPHY

Adams JF, Mitchell, CC, Bryant, HH. 1994. Soil test recommendations for Alabama crops. agronomy and soils departmental series no. 178. Alabama agricultural experiment station, Auburn, Al

Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytologist 165(2) pp. 351-372

Ainsworth EA, Rogers A. 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant, Cell & Environment. Vol 30(3) pp. 258 – 270.

Baligar, V.C. and N.K. Fageria. 2007. Agronomy and physiology of tropical cover crops. Journal of Plant Nutrition. 30(8):1287-1339.

Batchelor JA Jr. 1984. Properties of bin soils at the national tillage machinery laboratory. Publ. 218. USDA ARS National Soil Dynamics Laboratory, Auburn, Al.

Baveye PC, Rangel D, Jacobson AR, Laba M, Darnault C, Otten W, Radukovic R, Camargo FAO. 2011. From dust bowl to dust bowl: soils are still very much a frontier of science. Soil Sci. Soc. Am. J. 75:2037-2048.

Bernachi CJ, Morgan PM, Ort DR, Long, SL. 2005. The growth of soybean under free air [CO_2] enrichment (FACE) stimulates photosynthesis while decreasing in vivo Rubisco capacity. Planta. 220: 434-446

Blanco-Canqui H, Lal R. 2008. No-tillage and soil-carbon sequestration: an on-farm assessment. SSSAJ 72(3):693-701.

Chaudhuri UN, Burnett RB, Kirkham MB, Kanemasu ET. 1986. Effect of carbon dioxide on sorghum yield, root growth, and water use. Agricultural and Forest Meteorology vol 37(2) pp 109 – 122.

Chaudhuri UN, Kirkham MB, Kanemasu ET. 1990. Root growth of winter wheat under elevated carbon dioxide and drought. Crop Sci. 30: 853-857.

Corsi S, Friedrich T, Kassam A, Pisante M, de Morales JC. 2012. Soil organic carbon accumulation and greenhouse gas emission reductions from conservation agriculture: a literature review, integrated crop management. AGP/FAO Vol 16: 101pages.

Dial HL. 2012. Plant Guide for sorghum (*Sorghum bicolor* L.) USDA – Natural resources conservation service, Tucson Plant Materials Center, Tucson, Arizona.

Derpsch R, Friedrich T, Kassam A, Hongwen L. 2010. Current status of adoption of no-till farming in the world and some of its main benefits. Int J Agric & Biol Eng 3(1):1-25

Fageria, NK. 2012. The Role of Plant Roots in Crop Production. CRC Press. Taylor and Francis Group: Boca Raton, Florida 33487.

Frank, DA, Groffman, PM. 2009. Plant rhizospheric N processes: what we don't know and why we should care. Ecology 90(6):1412-1519.

Gill RA, Jackson, RB. 2000. Global patterns of root turnover for terrestrial ecosystems. New Phytol. 147:13-31.

Guang Z, Birchfield ST, Well C. 2008. Automatic discrimination of fine roots in minirhizotron images. New Phytologist 177(2) pp 549-557.

Hobbs, PR. 2007. Conservation agriculture: what is it and why is it important for future sustainable food production? Journal of Agricultural Science 145: 127 – 137.

Horowitz J, Ebel R, Ueda K. 2010. "No-till" farming is a growing practice. USDA-ERS Economic Information Bulleting No. (EIB-70) 28 pp. November.

Jackson RB, Mooney HA, Schulze ED. 1997. A global budget for fine root biomass, surface area, and nutrient contents. Proc Natl Acad Sci USA. 94:7362-7366.

Jensen ES, Peoples MB, Boddey, RM, Gresshoff PM, Hauggaard-Nielsen H, Alves BJR, Morrison MJ. 2012. Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. Agron. Sustain. Dev. 32:329-364.

Johnson MG, Meyer PF. 1998. Mechanical advancing handle that simplifies minirhizotron camera registration and image collection. J. Environ. Qual. 27:710-714.

Judd WS, Campbell CS, Kellogg EA, Stevens PF, Donoghue MJ. 2002. Plant systematics: a phylogenetic approach, Sinauer Assoc, 287-292.

Kell, D.B. 2012. Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. Annals of Botany 108(3):407-418.

Kimball BA, Kobayashi K, Bindi, M. 2002 Responses of agricultural crops to free-air CO_2 enrichment. Advances in Agronomy Vol 77: pp 293 – 38.

Lal R, Griffin M, Apt J, Lave L, Morgan MG. 2004. Mapping soil carbon. Science. 304 - 393.

Lal R. 2008. Carbon Sequestration. Phil. Trans. R. Soc. B. 363:815-830.

Leadley PW, Drake, BG. 1993. Open top chambers for exposing plant canopies to elevated CO_2 concentration and for measuring net gas exchange. Plant Ecology. 104 -105(1) pp3-15.

Leakey ABD, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR. 2009. Elevated CO2 effects of plant cabon, nitrogen and water relations: six important lessons from FACE. Journal of Experimental Botany Vol 60(1): pages 2859 – 2876.

Lee TD, Reich PB, Tjoelker MG. 2003a. Legume presence increases photosynthesis and N concentrations of co-occurring non-fixers but does not modulate their responsiveness to carbon dioxide enrichment. Oecologia 137:22-31.

Lindemann WC, Glover, CR. 2008. Nitrogen fixation by legumes. New Mexico State University Cooperative Extension Service Guide A-129.

Littell RC, Milliken GA, Stroup WW et al. 1996. SAS system for mixed models. SAS Institute Inc., Cary, NC.

Madhu M, Hatfield JL. 2013. Dynamics of plant root growth under increased atmospheric carbon dioxide. Agronomy Journal 105A (3):657-669.

Mansoer Z, Reeves DW, Wood CW. 1997. Suitability of sunnhemp as an alternative late-summer legume cover crop. Soil Sci. Soc. Am. J. 61L246-253.

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB, Leppalammi-Kujansuu J, Mitchell RJ, Runion GB, Prior SA, Rogers HH, Amthor JS, Henning FP. 1995. Effects of nitrogen gas on *Pinus palustris* foliary respiratory responses to elevated atmospheric carbon dioxide concentration. J. Exp. Bot. 46: 1561-1567.

McCormack ML, Pritchard SG, Breland S, Davis MA, Prior SA, Runion GB, Mitchell RJ, Rogers HH. 2010. Soil fungi respond more strongly than fine roots to elevated CO_2 in a model regenerating longleaf pine-wiregrass ecosystem. Ecosystem 13:901-916.

Mirkham MB. 2011. Elevated carbon dioxide: impacts on soil and plant water relations. CRC Press:Taylor and Francis Group. Boca Raton Fla.

Mitchell RJ, Runion GB, Prior SA, Rogers HH, Amthor JS, Henning FP. 1995. Effects of nitrogen on *Pinus palustris* foliar respiratory responses to elevated atmospheric CO_2 concentration. J. Exp. Bot. 46: 1561 – 1567.

Monastersky R. 2013. Global carbon dioxide levels near worrisome milestone. Nature. Vol 497: pp. 13 – 14.

Morrison JIL, Morecroft MD, eds., 2006. Plant Growth and Climate Change. Blackwell Publishing, Ltd., 9600 Garsington Road, Oxford, UK.

Mosjidis JA. 2011. Weed control in sunn hemp and its ability to suppress weed growth. Crop Protection. 30: 70 – 73.

National oceanic and aeronatic research earth system research laboratory. 2018. Trends in atmospheric carbon dioxide. *https://www.esrl.noaa.gov/gmd/ccgg/trends*

Nie M, Lu M, Bell J, Raut S, Pendall, E. 2013. Altered root traits due to elevated CO_2: a meta-analysis. Global Ecology and Biogeography 22: 1095 – 1105.

Norby RJ, Jackson RB. 2000. Root dynamics and global change: seeking and ecosystem perspective. New Phytol. 147:3-12

Norby RJ, Phillips RP, Pregitzer KS, Pritchard SG, Reward B, Zadworny M. 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytologist. 207:505-518.

Palm C, Bllanco-Canqui H, DeClerck F, Gatere L, Grace P. 2014. Conservation agriculture and ecosystem services: An overview. Agriculture, Ecosystems and Environment 187:87-105.

Parry ML. 1990. Effects on plants, soil, pests and diseases. Climate change and world agriculture. London: earthscan publications Ltd. pp. 1 – 12**.**

Peterman KE. 2017. Climate change literacy and education: history and project overview. In: Peterman KE, Foy GP, Cordes MR, editors. Climate change literacy and education the science and perspectives from the global stage, Volume 1. American Chemical Society: pp 1 – 14.

Prevost D, Bertrand A, Juge C, Chalifour FP. 2010. Elevated CO2 induces differences in nodulation of soybean depending on bradyrhizobial strain and method of inoculation. Plant and Soil Vol 331(1 – 2) pp 115 – 127.

Prior SA, Runion GB, Rogers HH, Torbert HA, Reeve DW. 2005. Elevated atmospheric carbon dioxide effects on biomass production and soil carbon in conventional and conservation cropping systems. Glob. Change Biol. 11:657-665.

Prior SA, Runion GB, Rogers HH, Arriaga FJ. 2010. Elevated atmospheric carbon dioxide effects on soybean and sorghum gas exchange in conventional and no-tillage system. J. Environ. Qual. 39:596-608

Pritchard SG, Amthor JS. 2005. Crops and environmental change: An introduction to effects of global warming, increasing atmospheric CO2 and O3 concentrations and soil salinization on crop physiology and yield. The Haworth Press Binghamton NY.

Pritchard SG, Prior SA, Rogers HH, Davis, MA, Runion GB, Popha TW. 2006. Effects of elevated atmospheric carbon dioxide on root dynamics and productivity of sorghum growth under conventional and conservation agricultural management practices. Agriculture, Ecosystems and Environment. 113:175-183.

Pritchard SG, Strand AE, McCormack ML, Davis MA, Finzi AC, Jackson RB. 2007. Fine root dynamics in a loblolly pine forest are influenced by free-air-CO2-enrichment: a six-year-minirhizotron study. Global Change Biology Vol14 (3): pp 588-602.

Pritchard SG, 2011. Soil organisms and global climate change. Plant Pathology 60(1): 82-99.

Reddy AR, Rasineni GK, Raghavendra AS. 2010. The impact of global elevated CO_2 concentration on photosynthesis and plant productivity. Current Science. Vol 99(1), pp 46 – 57.

Rogers A, Gibon Y, Stitt M, Morgan PB, Bernacchi CJ,Ort DR, Long SP. 2006. Increased C availability at elevated carbon dioxide concentration improves N assimilation in a legume. Plant Cell Environ 29: 1651-1658

Rogers A, Ainsworth EA, Leakey ADB. 2009. Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? Plant Physiology. 141: 1009 - 1016.

Rogers HH, Heck WW, Heagle AS. 1983. A field technique for the study of plant responses to elevated carbon dioxide concentrations. Air Pollut. Control Assoc. J. 33: 42-44

Rotar PP, Joy RJ. 1983. ‘Tropic Sun’ Sunn hemp: *Crotalaria juncea* L. Research Extension Series, 02719916, University of Hawaii.

Runion GB, Torbert HA, Prior SA, Rogers HH. 2009. Soil carbon sequestration and the greenhouse effect. 2nd edition. SSSA Special Publication 57. ASA –CSSA-SSSA, 677 S. Segoe Rd., Madison WI 53711

Ruskin J. 1996. Lost crops of Africa: volume I: grains. National Research Council. Washington, DC: The National Academies Press. https://doi.org/10.17226/2305.

Six J, Ogle SM, Breidt FJ, Conant RT, Mosiers AR, Paulstian K. 2004. The potential to mitigate global warming with no-tillage management is only realized when practiced in the long term. Global Change Biology 10:155-160.

Smith, GR. 2010. History of Crimson Clover in the USA. Proceedings of Agricultural Scientific Conference. IHSC2010OralProceedings(24).pdf

Soybeans. 2015. USDA Coexistence Factsheet. USDA Office of Communications: Washington DC. oc.news@usda.gov.

Treadwell D, Klassen W, Alligood, M. 2008. Annual cover crops in Florida Vegetable systems Part 1. Objectives: Why grow cover crops? Ipm.ifas.ufl.edu.

United Nations, Department of economic and social affairs, population division. 2017. World population prospects: the 2017 revision, data booklet. ST/ESA/SER.A/401.

USDA National Resources Conservation Service. 2018. Plants profile for Crotalaria juncea (sunn hemp): Plants.usda/gov/core/profile?symbol=CRJU.

USDA National Resources Conservation Service. 2018. Plants profile for Glycine max (soybean). Plants.usda.gov/core/profile?symbol=glyma4.

USDA National Resources Conservation Service. 2018. Plants profile for Sorghum bicolor (sorghum). Plants.usda.gov/core/profile?symbol=SOBI2.

Valenzuela H, Smith J. 2002. ‘Tropic Sun’ Sunnhemp. 2002. Sustainable agriculture green manure crops, SA-GM-11. College of Tropical Agriculture and Human Resources. Honolulu, Hawaii 96822.

von Caemmeer S, Furbank RT. 2003. The C4 pathway: an efficient CO_2 pump. Photosynthesis Research Vol 77: pp 191 – 207.

Wang WF, Zong YZ, Zhang SQ. 2014. Water- and nitrogen-use efficiencies of sweet sorghum seedlings are improved under water stress. Int. J. Agric. Biol. 16:pp. 285 – 292.

West TO, Post WM. 2002. Soil organic carbon sequestration rates by tillage and crop rotations: a global data analysis. Soil Science Society of America Journal. 166:1930-1946.

West JB., HilleRisLambers J, Lee TD, Hobbie SE, Reich PB. 2005. Legume species identity and soil nitrogen supply determine symbiotic nitrogen-fixation responses to elevated atmospheric CO_2. New Phytol 167(2):523-530.

Wright AL, Hons FM. 2004. Soil aggregation and carbon and nitrogen storage under soybean cropping sequences. Soil Sci. Soc. Am. J. 68:507-513.