CARBON STOCKS IN PERENNIAL BIOFUEL FEEDSTOCK MANAGEMENT SYSTEMS

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CHAPTER I

LITERATURE REVIEW

Abstract:

Demand for alternatives to fossil fuels and the desire to mitigate CO_2 emissions has driven interest in cellulosic biofuel and methods of sequestering carbon (C). Perennial grasses have the potential to meet both of these needs. However, little is known about how management practices influence soil C sequestration and a method is needed to determine changes in C stocks to accurately represent the changes in soil C over time. Soil C storage is expressed as a mass of C per unit area measured to a specific depth. This is not ideal as the soil surface can due to shrink/swell of clays. This can manifest as changes in bulk density. Because many Oklahoma soils have high shrink/swell capacities, their bulk densities may change with soil moisture. Therefore 3 studies were conducted to 1) evaluate the impact of management practices on soil C stocks and 2) evaluate the use of a fixed mass method to calculate soil C stocks in a high shrink/swell soil. In Experiment 1, switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus* spp.) and eastern gamagrass *(Tripsacum dactyloides)* were evaluated with harvest frequency to determine the best management practices to achieve energy production and C sequestration in the Southern Great Plains. Soil samples were collected from experiment 2 evaluating 9 switchgrass varieties. The data for experiment 1 showed no difference in soil C stocks between switchgrass, miscanthus, or eastern gamagrass, nor was a difference in C stocks found between harvest frequency treatments. Data from experiment 2 showed that soil C stocks were not proportional to yield. The data suggest that the upland varieties allocate a greater proportion of carbon to belowground carbon stock when compared to the upland varieties. Experiment 3 showed that under moist conditions swelling of clays did not decrease bulk density. On the contrary, moist conditions resulted in compression at discrete depth increments. This compression resulted in increases in bulk density, which increased C stock estimates at these depth increments when fixed depth was used to calculate C stocks. Utilization of a fixed mass method removed this error and provided a more precise measurement of C stocks.

Introduction:

Energy-related activities (production, transmission, storage, distribution and combustion of fossil fuels) accounted for 86.7% or 5,377.3 teragrams (Tg) CO_2 equivalents (eq) of total greenhouse gas (GHG) emissions in the United States in 2009; combustion of fossil fuels alone resulted in CO_2 emissions of 5,209.0 Tg (US Environmental Protection Agency, 2011). Recently, the US government proposed the Waxman-Markey bill to reduce CO_2 emissions or sequester CO_2 in an environmentally neutral manner; while this bill was not passed some states are adopting their own bills to mitigate GHG emissions. California presented the Pavley bill to cap emissions for motor vehicles and signed it into law in 2002 and in 2005 the regulations were adopted. This law requires a reduction of 31.7 Tg CO_2 eq in 2020 (CA ARB, 2008). Then in October 2011, California voted to adopt a Cap-and-Trade regulation that will now provide a market for carbon (C) credits generated by practices which sequester C (CA ARB, 2011).

Additionally, rising demand for cleaner, renewable and sustainable fuel sources has led to much research into the use of cellulosic biofuel as an alternative to fossil fuels to reduce net CO_2 emissions. The production of perennial bioenergy feedstocks also promises to sequester C in the form of soil organic matter (SOM), which will accumulate after the conversion of cropland to a perennial production system. Research is underway to develop and evaluate perennial bioenergy feedstock production systems however; few efforts have been made to assess soil C sequestration under these production systems.

Perennial Cellulosic Biofuel:

Crop selection for cellulosic biofuel tends to favor species of perennial grasses with large amounts of biomass production and deep root systems. These perennial grasses are mostly bunch-type grasses that are harvested at the end of the growing season once the plant has senesced. These species are generally persistent and can go long periods between plantings (>15 years) (Lemus & Lal, 2005).

Farrell et al. (2006) used data from six previously published studies that focused on ethanol production to model and compare GHG emissions and primary energy inputs. For all three cases reviewed, the production of one mega joule (MJ) of ethanol required less petroleum input (measured as MJ petroleum/MJ ethanol) than that needed to produce one MJ of gasoline, but the net GHG varied greatly depending on the production process. The petroleum inputs required for the production of gasoline were 1.1 (measured as MJ petroleum/MJ fuel) whereas the inputs required for the production of cellulosic ethanol from switchgrass were a mere 0.08. The model created by Farrell et al. (2006) showed that, overall, cellulosic ethanol production required less nonrenewable resource inputs and released fewer GHG's than gasoline or corn (*Zea mays* L.) ethanol. Cellulosic ethanol produced net GHG emissions of 11 kg CO_2 eq MJ^{-1} fuel compared to 94 kg CO_2 eq MJ^{-1} fuel for gasoline and 81 and 96 kg CO_2 eq MJ^{-1} fuel for corn ethanol and CO_2 intensive corn ethanol, respectively. The primary focus for each of these studies was the net GHG emissions of various ethanol production methods.

Cellulosic biofuel can be produced on lands that are considered marginal or highly degraded agricultural soils. In Oklahoma, approximately 126,700 hectares of land (mine land and severely eroded cropland) are in need of rehabilitation (Lemus & Lal, 2005). Herbaceous biofuel crops may be an effective way to rehabilitate degraded and marginal hectares within the state. These marginal areas are not well suited for food production, but growing cellulosic biofuel may provide much needed energy and make these areas productive again. Varvel et al. (2008) compared the use of corn and switchgrass for biofuel and found that using corn residue (stover) is not a sustainable option for marginal soils in Nebraska. Corn and switchgrass were grown in the same trial in order to directly compare the two crops. In the first 5 years of the long term study, it was found that the removal of approximately half of the corn stover significantly reduced corn grain, stover, and total above-ground biomass yield, while no reduction in yields were found in the switchgrass. This study also directly compared predicted ethanol yield from corn and switchgrass and found that switchgrass fertilized at the same rate (120 kg N ha^{-1}) as corn would provide as much or more ethanol than the total amount of ethanol yield from corn grain and the harvested corn stover combined. In fact, on average the authors estimated that the switchgrass could produce approximately 3500 L ha^{-1} of ethanol and that the corn system would produce approximately 3200 L ha^{-1}. The efficiency of the perennial grass production system is appealing due to the amount of yield that can be obtained compared to the yields seen from corn systems that require management practices that are potentially harmful from both a yield and soil conservation standpoint.

Soil Organic Carbon:

Soil organic carbon (SOC) is the largest terrestrial pool for C storage globally, containing between 1200 and 1600 Gt (Post et al., 1990). Soil is an attractive medium for mitigating atmospheric C because it appears to be responsive to modification (no-till/conservation tillage). Davidson and Ackerman (1993) estimated that approximately 20-40% of soil C is lost after previously uncultivated soil is tilled. Additionally, Girma et al. (2007) found that grassland converted to continuous wheat (*Triticum aestivum*) lost around 20 g OM kg^{-1} of soil over a period of 100 years. Blame for the loss of SOC is often placed on plowing the soil, resulting in oxidation and loss to the atmosphere as CO_2 (Follett, 2001).

Baker et al. (2007) reviewed several studies that compared conventional tillage systems to conservation and no-till systems for C sequestration. These researchers posed alternative explanations for the loss of C in agricultural soils. The main difference between agricultural lands and the ecosystems that preceded them is that agricultural lands are dominated by annual crops compared to the perennial grasses and forested systems that were in place before the lands were settled. Perennial grasses assimilate C for a larger portion of the growing season than annual crops do, leading to a difference in the amount of C delivered to the soil system on an annual basis. The greater amount of organic C produced in perennial systems is responsible for the larger stocks of SOC compared to annual cropping systems. An alternative explanation for loss of SOC following cultivation is the change in hydrology. Many wetlands were drained for conversion to agricultural fields which in turn stimulated the oxidation of SOC.

Glover et al. (2010) conducted a long-term study and a short-term conversion study to examine field scale impacts of perennial and annual production systems on soil nutrient content and environmental quality. In the long-term study, unfertilized grasslands, annually harvested for approximately 75 years (management information based on landowner/manager interviews), offered the opportunity to examine the ecosystem associated with an agricultural system that receives few anthropogenic inputs. These fields were compared with adjacent fields of similar soil types that were either primarily or exclusively planted to winter wheat over a similar time frame that had been cultivated regularly. During the course of the study, wheat straw was not removed from the fields after harvest. The conversion study was established in 2003 to study in greater detail the soil and ecosystem properties following the conversion of perennial grass plots to annual cropping systems using no-till practices. The levels of SOC to a depth of 1 m were significantly different between the long term perennial fields (182.2 Mg ha^{-1}) and the annual fields (138.8 Mg ha^{-1}), indicating a loss of C from the wheat field. This difference could be explained by the greater root mass of the perennial system when compared to the annual system. In fact, the results of the short-term conversion study found that plant root C was 6.7 times greater, and the rooting depth was 1m deeper in the perennial treatments than in the adjacent wheat treatments. Plant roots provide much of the C inputs to the soil ecosystem and differences in root characteristics have great influence on soil C and nitrogen pools.

By restoring or limiting SOC losses, the soil can become more fertile and more productive, and the accumulation or sequestration of SOC will help offset CO_2 emissions to the atmosphere. Various studies have shown that converting previously cultivated cropland to grassland is an effective way to sequester SOC. Conant et al. (2001) analyzed 115 studies and found that conversion of cultivated land to pasture had a mean annual increase in C concentration of 5% and >3% annual increase in soil C stocks to a mean depth of 32.5 cm. Frank et al. (2004) found that 4 years after planting switchgrass SOC (to a depth of 90 cm) increased linearly from 13.5 kg m^{-2} at planting in 1999 to 16.5 kg m^{-2} in 2002, with an average net system C gain of 758 g C m^{-2}. Ma et al. (2000a) found no difference in SOC two years after planting, but 10 years after planting SOC was 45% higher in the 0-15 cm depth and 28% higher in the 15-30 cm depth compared to the adjacent fallowed soil. These findings show that while SOC losses occur rapidly, it takes several years of a perennial grass system to sequester soil C.

Billings et al. (2006) found that accumulating a significant amount of C in grassland soil may be more difficult than expected using normal fertilization and haying management in well established, long lived grasslands. Plots with this treatment did not show soil C increases after 5 years of treatment. However, fertilization without haying or grazing did increase soil C stocks significantly, but this is not a realistic management practice, nor was the C stored very stable. In contrast, a study in Russia found that in unfertilized grasslands SOC was not reduced after more than 50 years of annual harvesting when compared to unharvested grasslands (Mikhailova et al., 2000; Mikhailova and Post, 2006), indicating that aboveground biomass harvest had no influence on soil C stocks. Soil C sequestration may offset some C emissions from agriculture, but Billings, et al. (2006) suggested that significantly mitigating C emissions from fossil fuels is not very realistic in traditional long-lived grassland management systems.

Soil Organic Carbon and Root Growth under Perennial Cellulosic Biofuel Crops:

In Europe, miscanthus is being studied as potential crop for C sequestration and biofuel. Hansen et al. (2004) found that C concentration was greater in a 16 year old miscanthus stand at all soil depths (0-100 cm) compared to a 9 year old stand, indicating that miscanthus can continue to accumulate SOC after 9 years of production. Hansen et al. (2004) estimated that between 26-29% of cumulative C assimilated by the miscanthus stands was retained in the soil.

Root biomass can represent a significant soil C pool under perennial grass systems. Miscanthus has many more roots than other arable crops, because as a perennial crop miscanthus builds up a root system at depth that can be used for more than one season. Shimoda et al. (2009) found that miscanthus produced 2.5 times more belowground biomass than aboveground biomass. Root length density is lower in the topsoil for miscanthus when compared to annual crops like winter wheat or sugar beets (*Beta vulgaris*). However, miscanthus has a greater rooting depth which allows the plant to potentially take up nutrients and water from the subsoil thereby overcoming periods of drought or low nutrient availability in the topsoil, especially during times of rapid above-ground biomass growth. The extensive root system also has the potential to reduce nutrient (especially nitrate) leaching throughout the year (Neukirchen et al., 1999).

Eastern gamagrass *(Tripsacum dactyloides)* is a perennial C_4 grass native to the eastern US that provides a high quality forage but is sensitive to overgrazing. Eastern gamagrass has become popular recently for a variety of purposes, as a forage crop, a grass for vegetative hedges, and a crop to improve soils with high clay content that can reduce root growth. Gilker et al. (2002) found that neither low pH nor high soil strength treatments had an adverse effect on root growth for eastern gamagrass, meaning eastern gamagrass roots are capable of penetrating clay pans or compacted soil layers in non-water saturated soils. This makes eastern gamagrass an attractive crop for marginal lands with high clay content and low pH.

Switchgrass is a native perennial grass species to the US that is still at the forefront of biofuel feedstock research. Similar to miscanthus and eastern gamagrass, switchgrass has an extensive root system even at depth. A one year study of a 4 year old switchgrass stand found that changes in belowground biomass follow a seasonal trend with most of the belowground biomass produced in the last half of the growing season (Garten et al., 2010). Garten et al. (2010) concluded that the rapid turnover and net production of live fine roots are likely an important input to SOC under switchgrass. Consequently, a two harvest system could hinder soil C sequestration by forcing C allocation to aboveground biomass production during a time when it would otherwise be allocated to root biomass production and turnover. Garten et al. (2010) found that maximum belowground production took place during the end of the growing season (mid-summer to fall), increasing total live belowground biomass from 11960 kg ha^{-1} and 11680 kg ha^{-1} in April and July, respectively, to 16210 kg ha^{-1} in October. Therefore, this research suggests that in a system with dual goals of biofuel feedstock production and C sequestration, a single harvest management plan would most likely be ideal.

Despite the availability of data suggesting that perennial cellulosic biofuel feedstock crops can sequester SOC, there is little data available to assess the impact of management on the soil C stocks, especially in the Southern Plains region of the US. Basic information regarding the impact of management decisions such as harvest frequency, species selection, variety selection, and fertilizer application on soil C stocks is needed to properly assess the potential for soil C sequestration under these systems. This information will better characterize the global climate change mitigation potential of the cellulosic bioenergy production system currently under development in the US.

Soil Bulk Density and Carbon Stock Estimates:

Currently, soil profile C storage is generally expressed as a mass of C per unit area measured to a specific soil depth. This spatial coordinate method requires accurate soil bulk density measurements. However, changes in soil bulk density can occur due to tillage or removal of tillage, crop residue presence or absence, reforestation/deforestation, switching from annual crops to perennials and many other reasons (Brady and Wiel, 2002; Lemus and Lal, 2005).

Because soil bulk density is a dynamic property, researchers have recently proposed alternatives to the spatial coordinate method for assessment of soil C stocks. Gifford and Roderick (2003), propose that for accuracy when determining soil C stocks, sampling should refer to a fixed dry soil mass per unit ground area (cumulative mass coordinates), instead of a fixed depth. The use of a fixed depth requires that the surface be used as a reference. However, the practice of using the soil surface as a reference is not ideal as the soil surface can fluctuate for a variety of reasons. Gifford and Roderick (2003) give the following examples of how the surface elevation may change; drainage of wetlands and the oxidation of peat, erosion or deposition of material on the surface, shrink/swell and compaction. Due to this capacity for movement, it may be necessary to calculate soil C stocks in a manner that is less subject to fluctuations of the soil surface, especially for soils with high shrink/swell. However, no research data are available to evaluate the use of the fixed mass method proposed by Gifford and Roderick (2003) in high shrink/swell soils. Wuest (2009) did compare the use of an equivalent sample depth method to an equivalent sample mass method when calculating available water content of soils. Wuest (2009) found that by using the equivalent mass method, fluctuations in water content caused by equivalent depth method were corrected. This allows for more accurate comparisons between sites with varying bulk densities. The equivalent mass method was also found to correct for differences in sampling equipment and sampling conditions, allowing for a broader basis for comparisons of soil constituents between sites, conditions, times and researchers.

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CHAPTER II

IMPACT OF PERENNIAL BIOFUEL FEEDSTOCK MANAGEMENT ON SOIL CARBON

Abstract:

Demand for alternatives to fossil fuels and the desire to mitigate CO_2 emissions has driven interest in cellulosic biofuel and various methods of sequestering carbon (C). Perennial grasses have the potential to meet both of these needs. However, little is known about how management practices influence soil C sequestration. Samples were collected from 2 studies to evaluate the impact of management practices on soil C stocks. In the first study (Experiment 1), switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus* spp.) and eastern gamagrass *(Tripsacum dactyloides)* were evaluated with harvest frequency to determine the best management practices to achieve these dual goals of energy production and C sequestration in the Southern Great Plains. Soil samples were also collected from a second study (Experiment 2) evaluating 9 switchgrass varieties. Soils from both experiments were analyzed for organic C, bulk density and moisture. The data showed no difference in soil C stocks between switchgrass, miscanthus, or eastern gamagrass, nor was a difference in C stocks found between harvest frequency treatments in experiment 1. No change in soil C was detected during the 1 year between sampling, implying no sequestration of C during this period. Data from experiment 2, in which the 3 year mean biomass yields ranged from 9 to 19 Mg ha^{-1}, showed that soil C stocks were not proportional to yield. The data suggests that the upland varieties allocate a greater proportion of carbon to the belowground carbon stock when compared to the upland varieties. The inconsistent results from the lowland varieties suggest differences among the varieties in the allocation of carbon to the

belowground pool.

Introduction:

With an ever increasing public awareness of environmental issues caused by fossil fuel consumption, demand for cleaner, renewable and sustainable fuel sources has led to research into the use of cellulosic biofuel as an alternative fuel to reduce net CO_2 emissions. Farrell et al. (2006), estimated that the production of one mega joule (MJ) of ethanol required less petroleum input (0.08 MJ MJ) than that needed to produce one MJ of gasoline (1.1 MJ MJ). Estimates of GHG emissions of cellulosic ethanol were 11 kg CO_2 Eq MJ fuel^{-1} compared to 94 kg CO_2 Eq MJ fuel^{-1} for gasoline and 81 and 96 kg CO_2 Eq MJ fuel^{-1} for corn (*Zea mays*) ethanol and CO_2 intensive corn ethanol, respectively (Farrell et al., 2006). The production of perennial bioenergy feedstocks holds the promise of sequestering carbon (C) in the form of soil organic matter (SOM), which will accumulate after the conversion of cropland to a perennial production system. Research is underway to develop and evaluate perennial biofuel feedstock production systems. However, few efforts have been made to assess soil C stocks under these production systems. Soil is an attractive medium for mitigating atmospheric C because it appears to be responsive to modification (no-till/conservation tillage). Thus, pairing the goals of growing perennial grasses for biofuel feedstock production and sequestering C in the soil appears to be a solution.

Switchgrass (*Panicum virgatum*) and eastern gamagrass (*Tripsacum dactyloides*) are both native grasses to the US and the Central Plains region. Switchgrass has been identified by the US Department of Energy's Bioenergy Feedstock Development Program (Wright, 2007) as the preferred species for cellulosic biofuel feedstock production due to the low input requirements, potential for high biomass production on low quality sites, reliable yields and the potential for sequestering C. Eastern gamagrass produces high quality forage but is sensitive to overgrazing. It has become popular recently for a variety of purposes: as a forage crop, a grass for vegetative hedges, and a crop to improve soils with high clay content that can reduce root growth (Polk and Adcock, 1964; Gilker et al., 2002). In Europe, miscanthus (*Miscanthus* spp.) is being studied as potential crop for C sequestration and biofuel (Lewandowski, 2003).

Each of these grasses has attributes that make them desirable as biofuel feedstocks. Similar to miscanthus and eastern gamagrass, switchgrass has an extensive root system even at depth. A one year study of a 4 year old switchgrass stand found that changes in belowground biomass follow a seasonal trend with most of the belowground biomass produced in the last half of the growing season (Garten et al., 2010). It was found that maximum belowground production took place during the end of the growing season (mid-summer to fall) increasing total live belowground biomass from 11960 kg ha^{-1} and 11680 kg ha^{-1} in April and July, respectively, to 16210 kg ha^{-1} in October. Root C was significantly increased in the 0-5 cm depth(□ 175 g C m^{-2} in April and July and □ 325 g C m^{-2} in October), and the 15-30 cm depth (□ 75 g C m^{-2} in April and July and □ 110 g C m^{-2} in October) in the October samples over the April and July samples. Garten et al. (2010) concluded that the rapid turnover and net production of live fine roots are likely an important input to SOC under switchgrass and as such, a two harvest system could hinder soil C sequestration by forcing C allocation to aboveground biomass production during a time when it would otherwise be allocated to root biomass production and turnover. Therefore, in a switchgrass production system with dual goals of biofuel feedstock production and C sequestration, a single harvest management plan would most likely be ideal. However, Ma et al. (2000b) found that variety selection can influence rooting characteristics such as root mass, which Garten et al. (2010) concluded was an important source for SOC. When evaluating three varieties of switchgrass (Alamo, Cave-in-Rock and Kanlow), it was found that Cave-in-Rock produced significantly greater root mass than Alamo or Kanlow (14.48, 8.80 and 7.89 mg cm^{3} respectively).

Gilker et al. (2002) found that neither low pH nor high soil strength treatments had an adverse effect on root growth for eastern gamagrass, meaning eastern gamagrass roots are capable of penetrating clay pans or compacted soil layers in non-saturated soils. This makes it an attractive crop for marginal lands with high clay content and low pH. However, there is currently no data available to evaluate the impact of eastern gamagrass production on soil C.

Hansen, et al. (2004) found that C concentration was greater in a 16 year old miscanthus stand at all soil depths (0-100 cm) compared to a 9 year old stand. Additionally, Hansen et al. (2004) estimated that between 26-29% of cumulative C assimilated by the miscanthus stands was retained in the soil.

Currently, there are no data available to assess the soil C sequestration potential of perennial biofuel feedstocks in the Southern Great Plains. Furthermore, there are currently no data available to evaluate the impact of management decisions that may influence soil C stocks in the Southern Great Plains. The goal of this study was to explore the impact of perennial biofuel feedstock production management practices on soil C stocks and the potential to sequester C to mitigate CO_2 emissions by providing insight on how management decisions such as harvest frequency, species selection, and variety selection influence soil C stocks.

Materials & Methods:

This research utilized field plots established to evaluate production management practices of various perennial biofuel crops in Oklahoma. The first experiment (Exp. 1) was initiated in June 2002 to evaluate the impact of species and harvest frequency on biomass yield. The experiment was established on a Kirkland silt loam (Fine, mixed, superactive, thermic Udertic Paleustoll) at the Oklahoma State University, Agronomy Farm in Stillwater, OK. The experimental design is a randomized complete block design with 6 treatments and 4 replicates. Each plot is 3 m by 6 m. The treatments include 3 species, switchgrass (Alamo), miscanthus and eastern gamagrass. The harvest frequency treatments are a single harvest at the end of the growing season after frost kill (October-November) and a two harvest treatment where the biomass is harvested at mid-season (July) and again at the end of the growing season.

Yield data collection was discontinued during the 2006-2008 growing seasons. The plots were burned after frost kill to remove standing biomass growth from these growing seasons. Also, fertilizer was not applied during the 2006-2008 growing seasons.

In the spring of 2009, management of these crops was reestablished with the application of 90 kg N ha^{-1} as urea ammonium nitrate (UAN) liquid fertilizer (28-0-0). This fertilizer application was applied in the spring of 2010 as well. Composite soil samples collected from the experimental area to a depth of 15 cm were used to determine that pH, P and K levels were sufficient for crop growth according to the Oklahoma State University soil test recommendations.

In the 2009 and 2010 growing seasons, yields were determined by harvesting a 1 m by 6 m area of each plot using a chute forage harvester (Carter Manufacturing, Brookston, IN). Subsamples were taken and dried at 60°C to determine water content of the biomass. Residue in each plot was taken from a 1 m^2 area, dried and weighed to determine water content of the residue. Both grass and residue samples were analyzed for total C and N using a TrueSpec CN analyzer (LECO, Inc. St. Joseph, MI). Management information and yield data during the 2002-2005 growing season for the miscanthus and switchgrass treatments can be found in Aravindhakshan et al. (2010)

The second experiment (Exp. 2) was established on a Kirkland silt loam (Fine, mixed, superactive, thermic Udertic Paleustoll) at the Oklahoma State University, Agronomy Farm in Stillwater, OK. in June 2006 to evaluate the impact of switchgrass variety selection on biomass yield. The experimental design is a randomized complete bock with 9 treatments and 4 replicates. The 9 varieties included in this study are listed in Table 1. The plots were 1 m by 6 m in length. These plots receive 90 kg N ha^{-1} as urea annually in the spring prior to green up. Biomass yields were determined by harvesting a 1 m by 4.5 m area of each plot using chute forage harvester (Carter Manufacturing, Brookston, IN). Subsamples were taken and dried at 60°C to determine water content of the biomass after frost kill.

Soil sample collection:

Soil samples used for the determination of soil C stocks were collected from Exp. 1 in May, 2009 and March, 2010. All samples were collected with a tractor mounted hydraulic probe to a depth of 80 cm. In May 2009, a single core with a cutting tip diameter of 7.6 cm was collected from each plot. In March 2010, 2 cores with diameters of 3.9 cm were collected from each plot. At both sample times cores were also collected from the alleys and border areas of the experimental area. In May 2009, 8 cores were collected and in March 2010, 12 cores were collected from the alleys and border areas. These areas are kept free of plant growth using applications of glyphosate as needed.

These samples were collected to provide a comparison of C stocks in the treatment plots to that found in soil without perennial grass production. The experimental areas were in cultivated crop production prior to grass establishment therefore these samples are meant to represent C stocks prior to grass establishment in cultivated cropland. This requires the assumption that C stocks have not changed significantly in the alleys and borders since establishment. Although this is not ideal, the comparison of these samples to the samples collected in the treatment plots will be used to estimate C sequestration rates.

Soil samples were collected from Exp. 2 in March 2010, again using the 3.9 cm diameter core. Two cores were collected from each plot.
All cores were cut into sections of 0-10, 10-20, 20-40, and 40-80 cm at the time of core collection. The cores were placed in a cradle made from 10 cm (inside diameter) PVC pipe such that the core could be cut without loss of soil from each section. The individual sections were placed in a plastic bag and stored in an ice chest until they were transported back to the laboratory and stored in a refrigerator at 4°C.

Sample analysis:

Each soil core section was initially weighed to determine bulk density. After the initial weight was determined the sample was mixed and a subsample (20 g) was dried at 110°C to determine the moisture content. The bulk density was then adjusted to a dry weight basis. The remaining sample was transferred to a paper bag and placed in a greenhouse to air dry. Each sample was then ground using a Bico disc pulverizer (Bico Braun International, Burbank, CA). Each sample was analyzed for total C and N using a TrueSpec CN analyzer (LECO, Inc. St. Joseph, MI). Soil pH was determined on a 1:1, soil: deionized H_2O mixture after a 30 min equilibration period. Soil inorganic C was determined on soil samples with a $pH > 7.0$ using a pressure calcimeter method (Sherrod et al., 2002). Soil organic C was determined by the difference between total C and inorganic C.

Analysis of variance and contrast analysis were performed using the SAS PROC GLM procedure (SAS Institute, 2001) to determine significant treatment effects on measured response variables. Repeated measure analysis to determine the significance of year was conducted using multivariate analysis of variance (MANOVA) with the SAS PROC GLM procedure. Fisher's protected LSD was used to separate treatment means. Regression analyses were conducted using the SAS PROC REG procedure.

Results and Discussion:

Experiment 1:

Biomass and Residue:

In 2009, analysis of variance found no significant interaction between species and harvest frequency for cumulative yield, harvested biomass C, residue mass or residue C; therefore contrast analyses were used to compare treatments (Table 2). These analyses show that grass species did not significantly influence cumulative yield or harvested C. The switchgrass treatments did result in less residue and residue C after the end of season harvest compared to the miscanthus and eastern gamagrass treatments. The contrast analysis also showed that the cumulative yield, harvested C, end of season residue, and residue C were all significantly higher in the single harvest treatments compared to the dual harvest treatments. The grass plots received very little rainfall (Figure 1) from the time they were fertilized in May 2009 until the first harvest in July. While the plots did receive more rainfall following the first harvest, it apparently occurred after these warm-season grasses initiated translocation of carbohydrates to their roots, as has been observed by Garten et al. (2010). Comparisons of yields measured in the first harvest to those measured in the second harvest suggest that the single harvest treatment was capable of utilizing the late season rainfall to compensate for the early-season water stress and produce additional biomass. In contrast, the mid-season harvest limited photosynthesis such that the 2 harvest treatment was not able to take advantage of the late season rainfall to produce aboveground biomass.

The yields of all species in 2010 were approximately twice the yields of 2009. This resulted from more adequate rainfall received in the spring of 2010 in comparison to 2009. Analysis of variance of yield and residue data collected in 2010 revealed a significant interaction between species and harvest frequency for cumulative yield and harvested C, therefore LSD's were used to separate these means (Table 4). This analysis shows that harvesting miscanthus once at the end of the growing season resulted in 11,171 Kg ha^{-1}, which was significantly greater than yields collected from the remaining treatments which were not significantly different from each other with an average yield of 7,489 Kg ha^{-1}. Harvested C followed the same trend with the single harvest miscanthus maximizing the amount of C in harvested biomass.

In general yield data collected during this two year study suggest that harvest frequency has a limited impact on seasonal yields, except in the case of miscanthus under favorable conditions. Thus, following a single harvest plan is more likely to remain economically preferable for producers looking for maximum yield (Aravindhakshan et al, 2010) and C assimilation. The data also indicated that miscanthus can outperform switchgrass and eastern gamagrass under favorable conditions in a single harvest system, but that switchgrass assimilates the most C of the three species under low yield conditions. In addition, switchgrass leaves behind the least residue after harvest which could affect soil C sequestration.

Soil Moisture and Bulk Density:

Analysis of variance showed no species by harvest frequency interaction for soil moisture, therefore contrast analyses were used to assess the treatment affects in each year. The switchgrass plots were significantly drier (0.23 g g^{-1}) in the spring of 2009 than the other two species (0.26 g g^{-1} for both miscanthus and eastern gamagrass) in the surface 10 cm (Table 4). Soil moisture was not significant for any of the species at any other depth for either year. The soil samples for 2009 were collected in May, after the grasses had come out of winter dormancy. The difference in soil moisture between switchgrass and the other two species may be the result of differences in early season water use. Repeated measure analysis using MANOVA showed no significant difference in soil moisture among years.

Analysis of variance of bulk density data found no interaction between harvest frequency and species in either year of the study. Therefore, contrast analysis was used to compare main treatment effects (Table 5). Overall, the only significant difference in bulk density between species was found at the 0-10 cm depth in 2009 and 2010. The mean bulk density for switchgrass was significantly higher than the mean bulk density for miscanthus and eastern gamagrass in both years and miscanthus in 2010. There were no significant differences in bulk density between species for the 10-20, 20-40 or 40-80cm depths for either year.

Switchgrass had a higher bulk density than miscanthus and eastern gamagrass possibly due to it being a more drought tolerant species that can still be high yielding with low inputs (Heaton et al., 2004). Under the dry conditions in 2009, the switchgrass may have been putting resources into producing aboveground biomass, while the miscanthus and eastern gamagrass were utilizing their resources by producing roots to seek out water and nutrients within the soil profile (Coyne and Bradford, 1985). These hypothesized different strategies could result in root growth patterns which alter bulk density. Specifically, the switchgrass may have had less rhizome biomass in the surface 0-10 cm of soil, therefore bulk density was higher as was found by Dohleman, (2001) when comparing switchgrass to miscanthus.

Multivariate analysis of variance for repeated measures found no interaction between treatment effect and year. However, this analysis did show that the bulk density of the 0-10 and 10-20 cm depth increments in 2010 (1.49 and 1.66 g cm^{-3}, respectively) were significantly ($p=0.05$) higher than that found in 2009 in the 0-10 and 10-20 cm increments (1.39 and 1.58 g cm^{-3}, respectively). The difference in bulk density between the 2009 and 2010 samples is likely due to differences in compressions of soil cores during sampling. Recall that in 2009 a 7.6 cm diameter core was used and in 2010 a 3.9 cm core was used. The smaller core likely caused greater compression in 2010 resulting in greater measured bulk densities.

Carbon Concentration:

Statistical analysis of inorganic C values resulted in no significant differences among treatments at any depth in each year, therefore inorganic C was only used to determine organic C by subtraction from total C.

Analysis of variance showed no species by harvest frequency interaction for organic C concentration, therefore contrast analyses were used to assess the main treatment effects.

No significant differences were observed among harvest frequency or species treatments for organic C concentration (Table 6). It is noteworthy that the organic C concentration in the switchgrass treatments was 2.6 g kg^{-1} lower than that found in the miscanthus and eastern gamagrass treatments at the 0-10 cm depth in 2009. The C concentration at 0-10 cm under the switchgrass was also numerically lower than that in the miscanthus in 2010. This is consistent with the previous assertion that the switchgrass treatments contain less root biomass in the surface 10 cm of soil.

Repeated measure analysis using MANOVA again found no year by treatment response but did show significant changes in soil organic C concentrations between 2009 and 2010. Specifically, the organic C concentrations in the 20-40 cm depth was significantly ($p=0.05$) lower in 2010 (5.5 g kg^{-1}) compared to 2009 (5.8 g kg^{-1}). This may have resulted from decreased root growth during the 2009 growing season due to limited spring rainfall compared to previous years.

Organic Carbon Stocks:

Analysis of variance showed no species by harvest frequency interaction for organic C stocks; therefore contrast analyses were used to assess the treatment effects. Table 7 presents the organic C stocks in each treatment combination. Again, no significant treatment effects were observed, indicating that harvest frequency and species do not have an impact on soil C stocks. This is in contrast to assertions of previous research by Garten et al. (2010) that concluded that a two harvest management would negatively impact soil C sequestration by forcing C allocation aboveground when it would normally be directed to belowground biomass. Additionally, yield data for the first three years of this study (2003-2005) presented by Aravindhakshan et al (2010) showed that the average annual miscanthus yields were 12.7 Mg ha^{-1}, whereas switchgrass yields were 15.6 Mg ha^{-1} with no significant difference in yield between harvest frequencies (eastern gamagrass yields were not presented). Our data suggests that these historic yield differences were insufficient to result in significant differences in soil C stocks. It is possible however that the lack of management during the 2006-2008 growing seasons eliminated treatment differences and is responsible for the lack of significant difference in soil C stocks observed in the 2009 soil samples. Despite the uncertainty regarding this lack of management, data collected in 2009 does show that any differences in soil C stocks that may have resulted from yield differences observed in the first three years of the study were either short lived or nonexistent.

Recall that the 2009 biomass yield data showed no difference between species but the single harvest resulted in greater yield compared to the 2 harvest frequency (Table 2). Again, this difference of 1028 Kg ha^{-1} was insufficient to exert a significant difference in soil C mass in soil samples collected the following spring of 2010.

Multivariate analysis of variance for repeated measures found no significant difference in organic C stocks from the 2009 to 2010 samples indicating no significant sequestration of soil organic C during this 1 year period. This may be a result of a stabilization of the organic C content of the soil. As was indicated by Billings et al. (2006), accumulating a significant amount of C in long lived grassland is not rapid, as the soil in their study did not show a significant increase in C after 5 years of treatment. The low yielding conditions experienced in 2009 may have limited the accumulation of belowground biomass C stocks compared to previous years, therefore no accumulation of C was observed.

The equivalent mass method (as described in Chapter 3) was used to calculated C stock because of the significant increase in measured bulk density between years 2009 and 2010 in the surface 20 cm. Despite this correction, analysis of variance of soil C stocks calculated using the equivalent mass method again found no significant differences among treatments nor did it find any interactions among treatment factors. Table 1A (found in Appendix A) provides the contrast analyses, which again found no significant differences. Multivariate analysis of variance for repeated measures also found no significant differences in C stocks between the 2009 and 2010 data. For clarification, the soil masses, 2000, 3000, 6000, and 13000 Mg are equivalent to 14, 20, 39, and 82 cm in 2009 and 13, 19, 37, and 80 cm in 2010. These depths are based on the relationships between cumulative soil mass and actual soil depth presented in Figures 1A and 2A. Using this data to estimate C sequestration we find that in 13,000 Mg of soil the C stocks, when averaged across treatments decreased by 2.5 Mg C ha^{-1} between the 2009 and 2010 sampling dates. Whereas, the C stock averaged across treatments decreased by 1.9 Mg C ha^{-1} when calculated on an equivalent depth of 80 cm as presented in Table 2, indicating that both methods provided similar results despite the significant increase in bulk density at 0-20 cm between 2009 and 2010.

Comparison of organic C stocks found in alleyways and those found in the treatment plots also provided a C sequestration estimate. However, this estimate assumes that the C stock in the alleyways has not changed since the initiation of the study. Figure 2 shows the soil organic C profile for the alleyways and the average organic C profiles for treatment plots in 2010. Assessment of this data suggests that the treatment plots accumulated organic C to a depth of 40 cm. In fact, the average C stock found to a depth of 80 cm in the grass treatment plots was 63.1 Mg ha^{-1}, whereas the alleyways contained 56.7 Mg ha^{-1}. The experiment was planted in the spring of 2002 therefore the average annual sequestration rate would be 0.8 Mg C ha^{-1}. The current estimate for C sequestration rates in OK for cropland converted to grassland is 0.67 Mg C ha^{-1} (Oklahoma Conservation Commission, 2011). This analysis does suggest that soil organic C has increased as a result of planting the perennial grasses. However, the numeric decline in soil organic C between 2009 and 2010 suggests that this accumulation did not occur at a constant rate. This illustrates the need for long-term monitoring with periodic sampling of C stocks to accurately assess the changes in C stocks after establishment of perennial grasses for biofuel feedstock systems.

Experiment 2:

Soil Moisture and Bulk Density:

Analysis of variance in the soil moisture data found significant differences in soil moisture at the 20-40 cm and 40-80 cm depth increments (Table 8). Briefly, subsoil moisture was highest under Alamo and the soil moisture under NSU 95-2001 was significantly lower at these depths. These differences are interesting to note and are likely due to variability in the previous season’s crop water use. However, they do not relate significantly to yield and further discussion is beyond the scope of this study. They are presented in combination with the bulk density data to illustrate that the difference in subsoil moisture did not significantly influence the measured bulk density in this experiment (Table 9).

Soil Carbon:

No significant treatment effects were observed for inorganic C (data not shown). In fact, inorganic C was found only at the 40-80 cm depth increment. Therefore, it was only used to calculate organic C by subtraction from total C at this depth.

Organic C concentrations were also not significantly influenced by switchgrass variety (Table 10). However, significant differences in organic C stocks in the surface 10 cm were found (Table 11). Specifically, the two lowland varieties; NSL 2001-1 and NL 93-2 contained significantly more C in the surface 10 cm compared to the SL-93-2001-1, NL 94-2001-1, and Kanlow varieties which are also lowland types. The three upland varieties contained intermediate amounts of C in the surface 10 cm.

Table 12 shows that significant differences biomass were found between varieties. The three upland varieties (Blackwell, Cave-in-Rock, and NSU 95-2001-1) produced lower yields than all of the lowland varieties except for Kanlow and Alamo in 2008. The 3-year average yields show that Kanlow was generally the lowest yielding lowland type and SL 93-2001-1 and NSL 2001-1 were the highest yielding varieties, having significantly higher 3-year average yields than 5 of the nine varieties evaluated, including two commercially available lowland varieties, Kanlow and Alamo.

Figures 3 shows the mean average annual yields and mean soil C contents in the surface 10 cm. This graphic representation shows that the soil organic carbon content of the surface 10 cm was not proportional to the average annual aboveground harvested yield. In fact, the lowland variety SL 93-2001-1 produced the highest yields and the lowest soil carbon content; however NSL 2001-1 produced the second highest yield and contained the highest mass of carbon in the surface 10 cm. Additionally this graphical presentation shows that the upland varieties, NSU 95-2001-1, Blackwell, and Cave-in-Rock had disproportionally higher carbon contents relative to yield when compared to the lowland varieties. This is consistent with the results of Ma et al. (2000b) that showed Cave-in-Rock produced more root biomass compared to Kanlow and Alamo. However, the data from Ma et al. (2000b) showed no difference in root biomass between Kanlow and Alamo. Suggesting that the difference in soil organic carbon observed in the current study did not result solely from differences in root biomass but perhaps from surface residue deposition or soil respiration. Figure 4 shows the mean 2009 biomass yields and soil organic C stocks in the surface 10 cm. Here again, soil carbon stocks were not directly proportional to yield, confirming that neither the average annual yield nor the previous year's yield explained these differences in soil carbon stocks.

The data collected is unique in that it allows for an evaluation of the relationship between historic yield as affected by variety and soil carbon stocks. It is generally accepted that soil carbon stocks vary as a result of differences in carbon input or soil respiration (Ellert et al., 2001). Therefore, the inconsistent results from the lowland varieties suggest that differences exist among the varieties in the allocation of carbon to the belowground pool. These differences may include the shoot:root ratio, root turnover, or soil respiration. Future efforts to quantify soil carbon sequestration under switchgrass production systems must consider these potential differences among varieties.

Conclusions:

Soil C was not affected by species or harvest frequency, suggesting that these factors have limited influence on soil C stocks in the short term. In terms of soil C sequestration, either a single harvest or split harvest will yield similar results. From a yield perspective, utilizing a 2 harvest system has the potential to suppress yields in years were spring rainfall is less than optimum. Additionally in 2010, when rainfall was more optimal, the 2 harvest system did not significantly increase yields. Therefore a single harvest system is likely the more efficient method for bioenergy production.

Switchgrass variety selection can influence soil C stocks. However, this influence is not proportional to yield. In fact, significant differences in soil carbon stocks were only found between lowland varieties with similar average annual yields. This indicates differences in the allocation of carbon below and aboveground for these lowland varieties. These findings should be considered during future efforts to develop switchgrass varieties for biofuel feedstock, if an objective of the system is to maximize the mitigation of CO_2 emissions. It also suggests that variety is an important factor to consider when evaluating soil carbon sequestration under switchgrass biofuel production systems.

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List of Figures

Table 1: Varieties and type included in Exp. 2

Variety	Type
NL 93-2, 10-parent synthetic	Lowland
SL 93-2001-1	Lowland
NSL 2001-1	Lowland
NL 94-2001-1	Lowland
Alamo	Lowland
Kanlow	Lowland
NSU 95-2001-1	Upland
Blackwell	Upland
Cave-in-Rock	Upland

Table 2: The 2009 yield, biomass carbon, end of season residue mass, and residue carbon collected from experiment 1 evaluating species and harvest frequency interactions.

Species	**Harvest Frequency**	**----------Biomass Yield----------**			**Biomass Carbon Harvested**	**Residue mass**	**Residue Carbon**
		1st Harvest	2nd Harvest	Cumulative			
		---------------------------------Kg ha^{-1}------------------------------------					
Treatment Means							
Switchgrass	1 Harv.		4267	4267	1933	3029	1146
Switchgrass	2 Harv.	3379	745	4124	1804	2290	808
Miscanthus	1 Harv.		5150	5150	2267	4302	1622
Miscanthus	2 Harv.	2126	706	2832	1208	3324	1184
E. Gamagrass	1 Harv.		3555	3555	1576	4181	1505
E. Gamagrass	2 Harv.	2447	486	2933	1279	3715	1196
	LSD(0.05)	NS	1529	NS	NS	1228	471
Contrast Comparisons of Species							
Switchgrass				4196a†	1869a	2659b	977b
Miscanthus				3991a	1737a	3813a	1403a
E. Gamagrass				3244a	1428a	3948a	1350a
Contrast Comparisons of Harvest Frequency							
	1 Harv.			4324a	1925a	3837a	1424a
	2 Harv.			3296b	1430b	3110b	1063b

† Different letters within each contrast comparison indicate a significant difference at the 0.05 probability level. Contrast comparisons with no letter beside means were not analyzed due to interactions between species and harvest frequency.

Table 3: The 2010 yield, biomass carbon, end of season residue mass, and residue carbon collected from experiment 1 evaluating species and harvest frequency interactions.

Species	Harvest Frequency	----------Biomass Yield----------			Biomass Carbon Harvested	Residue Mass	Residue Carbon
		1st Harvest	2nd Harvest	Cumulative			
		---------------------------------Kg ha^{-1}					-----------------------------------
Treatment Means							
Switchgrass	1 Harv.		7496	7496	3456	4809	1728
Switchgrass	2 Harv.	6901	853	7754	3472	3645	1189
Miscanthus	1 Harv.		11171	11171	5098	6679	2353
Miscanthus	2 Harv.	7128	393	7521	3358	4045	1472
E. Gamagrass	1 Harv.		6869	6869	3141	5307	1979
E. Gamagrass	2 Harv.	6946	857	7804	3487	5947	2042
	LSD(0.05)	NS	1088	1869	835	NS	NS
Contrast Comparisons of Species							
Switchgrass				7625	3464	4227a	1459a
Miscanthus				9346	4228	5362a	1912a
E. Gamagrass				7336	3314	5627a	2011a
Contrast Comparisons of Harvest Frequency							
	1 Harv.			8512	3899	5598a	2020a
	2 Harv.			7693	3439	4546a	1568a

† Different letters within each contrast comparison indicate a significant difference at the 0.05 probability level. Contrast comparisons with no letter beside means were not analyzed due to interactions between species and harvest frequency.

Table 4: Contrast analysis of soil moisture to compare effects of species and harvest frequency in experiment 1.

Species	Harvest Frequency	-------------Spring 2009-----------				-------------Spring 2010------------			
		0-10cm	10-20cm	20-40cm	40-80cm	0-10cm	10-20cm	20-40cm	40-80cm
		------------------------------------g g^{-1}------------------------------------							
Contrast Comparisons of Species									
Switchgrass		0.23b †	0.22a	0.25a	0.22a	0.24a	0.21b	0.24a	0.22a
Miscanthus		0.26a	0.22a	0.25a	0.23a	0.25a	0.21b	0.24a	0.22a
E. Gamagrass		0.26a	0.23a	0.24a	0.22a	0.24a	0.22a	0.24a	0.22a
Contrast Comparisons of Harvest Frequency									
	1 Harv.	0.25a	0.22a	0.25a	0.23a	0.24a	0.21a	0.24a	0.22a
	2 Harv.	0.24a	0.22a	0.24a	0.22a	0.25a	0.21a	0.24a	0.22a

† Different letters within each contrast comparison indicate a significant difference at the 0.05 probability level. Contrast comparisons with no letter beside means were not analyzed due to interactions between species and harvest frequency.

Table 5: Contrast analysis of soil bulk density to compare effects of species and harvest frequency in experiment 1.

Species	Harvest Frequency	-------------Spring 2009-----------				-------------Spring 2010------------			
		0-10cm	10-20cm	20-40cm	40-80cm	0-10cm	10-20cm	20-40cm	40-80cm
		------------------------------------g cm^{-1}------------------------------------							
Contrast Comparisons of Species									
Switchgrass		1.50a†	1.57a	1.59a	1.67a	1.54a	1.67a	1.60a	1.67a
Miscanthus		1.36b	1.61a	1.58a	1.65a	1.43b	1.66a	1.61a	1.67a
E. Gamagrass		1.29b	1.55a	1.58a	1.67a	1.49ab	1.65a	1.61a	1.67a
Contrast Comparisons of Harvest Frequency									
	1 Harv.	1.40a	1.57 a	1.58a	1.65a	1.52a	1.67a	1.61a	1.67a
	2 Harv.	1.37a	1.59 a	1.58a	1.67a	1.45a	1.66a	1.60a	1.67a

† Different letters within each contrast comparison indicate a significant difference at the 0.05 probability level. Contrast comparisons with no letter beside means were not analyzed due to interactions between species and harvest frequency.

Table 6: Contrast analysis of organic carbon concentration to compare effects of species and harvest frequency in experiment 1.

Species	Harvest Frequency	Spring 2009				Spring 2010			
		0-10cm	10-20cm	20-40cm	40-80cm	0-10cm	10-20cm	20-40cm	40-80cm
		g kg^{-1}							
Contrast Comparisons of Species									
Switchgrass		11.2a†	6.9a	6.1a	2.8a	11.3a	6.7a	5.6a	2.6a
Miscanthus		13.7a	6.9a	5.6a	3.1a	12.0a	6.6a	5.5a	2.8a
E. Gamagrass		13.9a	7.1a	5.7a	2.3a	11.2a	6.6a	5.3a	2.6a
Contrast Comparisons of Harvest Frequency									
	1 Harv.	12.8a	6.9a	5.6a	2.6a	11.1a	6.6a	5.4a	2.5a
	2 Harv.	13.0a	7.0a	5.9a	2.9a	11.9a	6.7a	5.5a	2.8a

† Different letters within each contrast comparison indicate a significant difference at the 0.05 probability level. Contrast comparisons with no letter beside means were not analyzed due to interactions between species and harvest frequency.

Table 7: Contrast analysis of organic carbon stocks to compare effects of species and harvest frequency in experiment 1.

Species	**Harvest Frequency**	**Spring 2009**				**Spring 2010**			
		0-10cm	0-20cm	0-40cm	0-80cm	0-10cm	0-20cm	0-40cm	0-80cm
		Mg ha^{-1}							
Contrast Comparisons of Species									
Switchgrass		17a†	28a	47a	65a	17a	29a	46a	64a
Miscanthus		18a	29a	47a	67a	17a	28a	46a	64a
E. Gamagrass		18a	29a	47a	62a	16a	27a	44a	62a
Contrast Comparisons of Harvest Frequency									
	1 Harv.	18a	29a	46a	64a	17a	28a	45a	62a
	2 Harv.	18a	29a	47a	67a	17a	28a	46a	65a

† Different letters within each contrast comparison indicate a significant difference at the 0.05 probability level. Contrast comparisons with no letter beside means were not analyzed due to interactions between species and harvest frequency.

Table 8: Soil moisture content of soil samples collected from experiment 2.

Variety	0-10cm	10-20cm	20-40cm	40-80cm
	--------------------	----------g g^{-1}	----------	--------------------
NL 93-2	0.25	0.21	0.24	0.20
SL 93-2001-1	0.25	0.21	0.26	0.22
NSL 2001-1	0.25	0.21	0.24	0.23
NL 94-2001-1	0.25	0.21	0.25	0.22
Alamo	0.25	0.21	0.26	0.24
Kanlow	0.25	0.22	0.24	0.22
NSU 95-2001-1	0.25	0.20	0.23	0.21
Blackwell	0.24	0.21	0.25	0.22
Cave-in-Rock	0.25	0.21	0.24	0.21
LSD(0.05)†	NS	NS	0.02	0.02

†LSD is the least significant difference between means at the 0.05 probability level.

Table 9: Bulk Density of soil samples collected from experiment 2.

Variety	0-10cm	10-20cm	20-40cm	40-80cm
	--------------------	----------g cm^{-1}	----------	--------------------
NL 93-2	1.39	1.47	1.43	1.54
SL 93-2001-1	1.29	1.50	1.46	1.57
NSL 2001-1	1.32	1.52	1.46	1.54
NL 94-2001-1	1.33	1.53	1.46	1.54
Alamo	1.37	1.52	1.48	1.56
Kanlow	1.38	1.49	1.49	1.60
NSU 95-2001-1	1.38	1.55	1.46	1.59
Blackwell	1.42	1.51	1.44	1.59
Cave-in-Rock	1.43	1.51	1.47	1.58
LSD(0.05) †	NS	NS	NS	NS

†LSD is the least significant difference between means at the 0.05 probability level.

Table 10: Soil organic carbon concentrations of soil samples collected from experiment 2.

Variety	0-10cm	10-20cm	20-40cm	40-80cm
	---g kg^{-1}---			
NL 93-2	12.2	8.9	7.8	4.9
SL 93-2001-1	11.3	8.9	8.0	5.0
NSL 2001-1	12.9	8.7	7.8	5.3
NL 94-2001-1	11.3	8.8	8.1	4.7
Alamo	12.1	8.6	8.1	5.2
Kanlow	11.0	8.9	7.1	4.7
NSU 95-2001-1	11.7	8.6	8.0	5.4
Blackwell	11.7	8.7	7.7	6.0
Cave-in-Rock	11.3	8.7	8.1	5.2
LSD(0.05) †	NS	NS	NS	NS

†LSD is the least significant difference between means at the 0.05 probability level.

Table 11: Soil organic carbon mass from the surface to specified depth for experiment 2.

Variety	0-10cm	0-20cm	0-40cm	0-80cm
	---Mg/ha^{-1}---			
NL 93-2	16.9	30.0	41.1	48.7
SL 93-2001-1	14.6	27.9	39.7	47.5
NSL 2001-1	17.1	30.3	41.7	49.8
NL 94-2001-1	14.9	28.3	40.1	47.3
Alamo	16.6	29.6	41.5	49.6
Kanlow	15.2	28.6	39.2	46.6
NSU 95-2001-1	16.1	29.4	41.2	49.7
Blackwell	16.4	29.5	40.6	50.2
Cave-in-Rock	16.1	29.2	41.2	49.4
LSD(0.05) †	1.7	NS	NS	NS

†LSD is the least significant difference between means at the 0.05 probability level.

Table 12: Biomass yield from experiment 2.

Variety	2007	2008	2009	3-yr average
	------------------Mg ha^{-1}			-----------------
NL 93-2	24.1	11.4	16.1	17.2
SL 93-2001-1	26.1	13.9	16.7	18.9
NSL 2001-1	24.3	13.5	17.7	18.5
NL 94-2001-1	22.8	13.2	16.5	17.5
Alamo	23.3	11.7	13.5	16.2
Kanlow	21.0	10.8	14.7	15.5
NSU 95-2001-1	12.1	9.2	10.0	10.4
Blackwell	11.3	8.4	11.3	10.3
Cave-in-Rock	11.6	8.0	8.6	9.4
LSD(0.05) †	3.1	2.2	3.4	1.9

†LSD is the least significant difference between means at the 0.05 probability level.

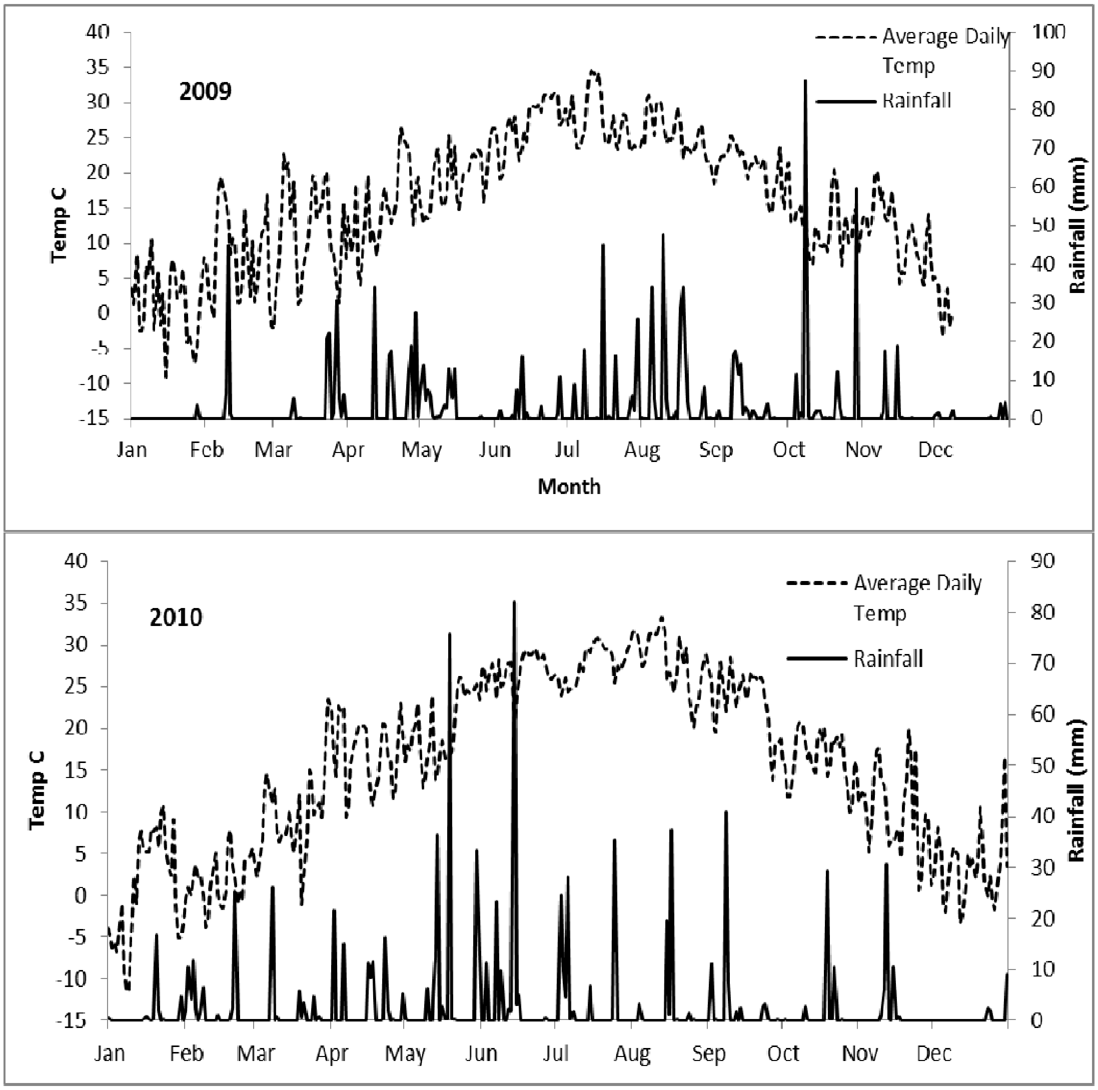

Figure 1: Average daily temperature and daily rainfall for 2009 and 2010.

Figure 2: Soil organic carbon profile in the treatment plots and alleyways for experiment 1 in which grass species and harvest frequency effects were tested.

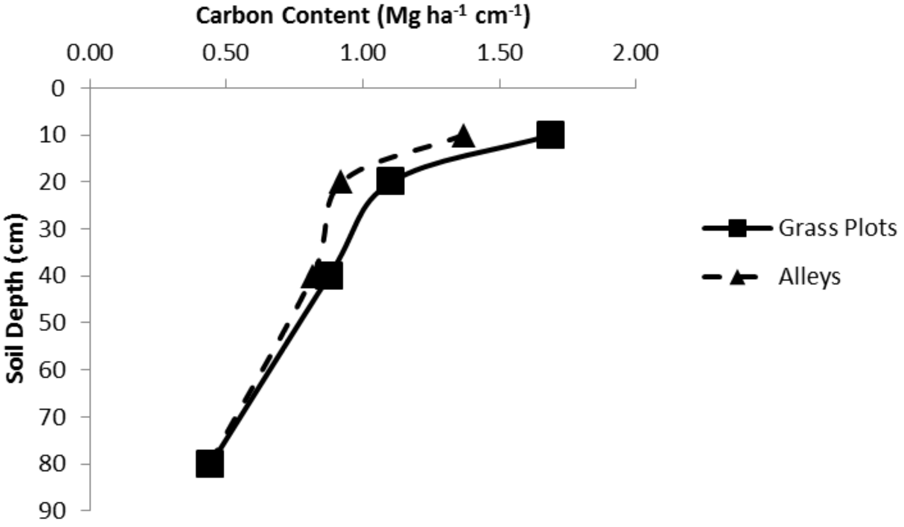

Figure 2: Soil organic carbon profile in the treatment plots and alleyways for experiment 1 in which grass species and harvest frequency effects were tested.

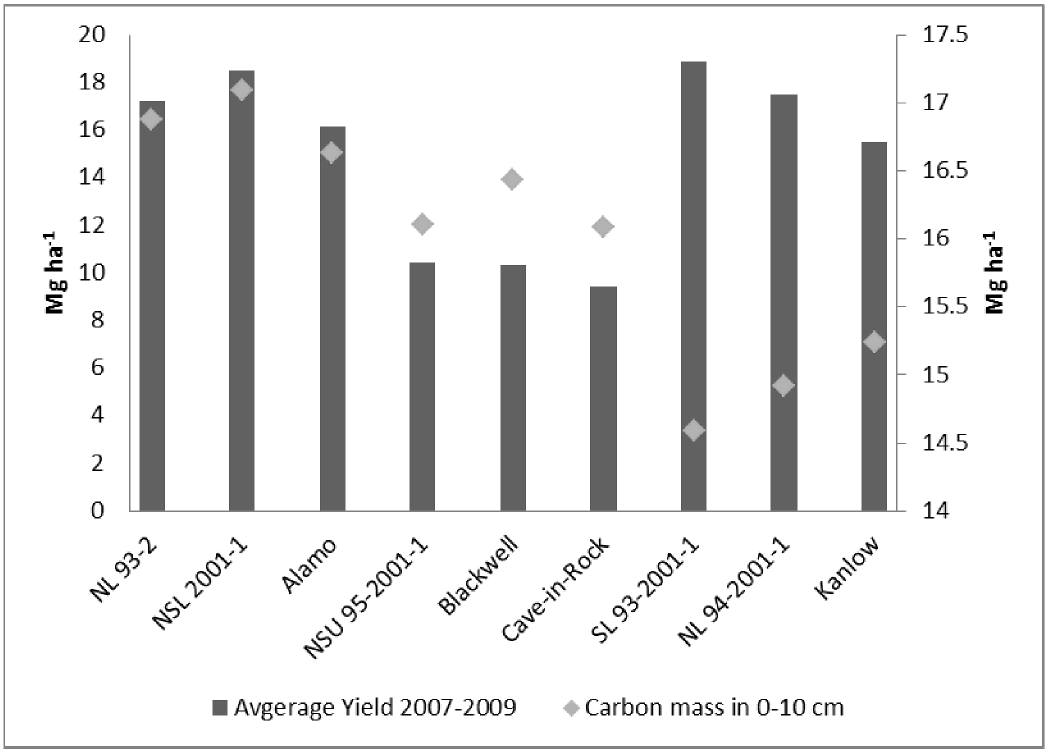

Figure 3: The average yield from 2007-2009 and soil C stocks (0-10cm) in soils collected in spring 2010 from experiment 2.

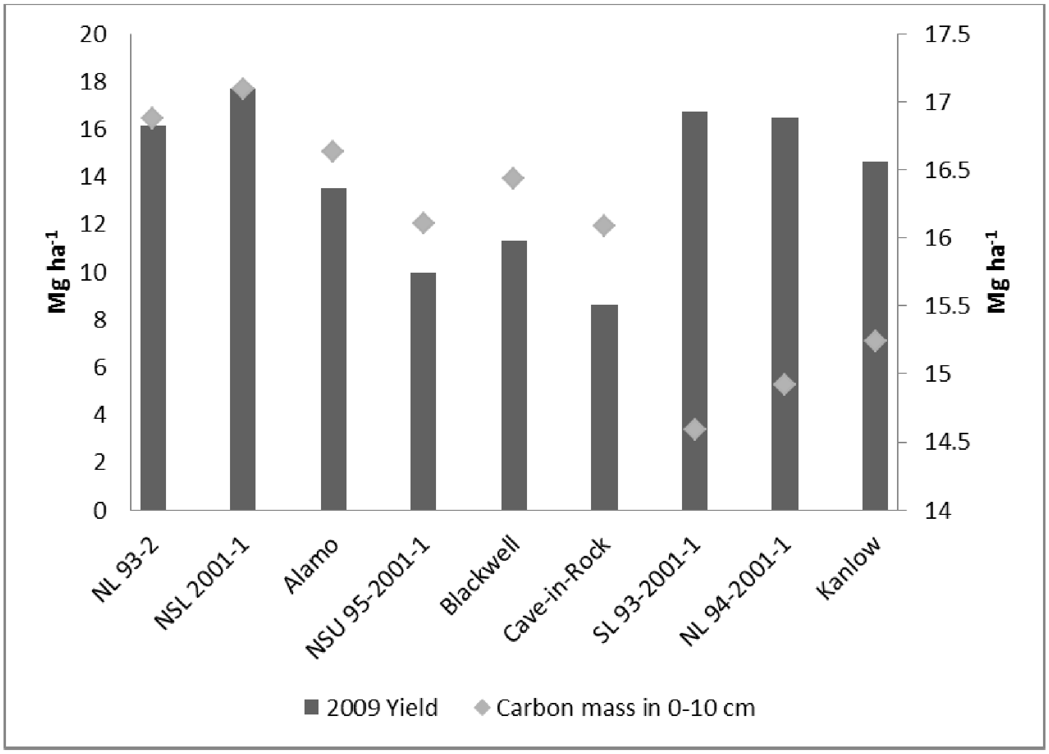

Figure 4: The 2009 mean yield and soil C stocks (0-10cm) in soils collected in spring 2010 from experiment 2.

CHAPTER III

BULK DENSITY AND CARBON STOCK ESTIMATES

Abstract:

Soil C storage is generally expressed as a mass of C per unit area measured to a specific depth. This practice is not ideal as the soil surface can fluctuate for several reasons including compaction, tillage, and shrink/swell of clays. These changes manifest as changes in bulk density. Because many Oklahoma soils have high shrink/swell capacities their bulk densities may change as a function of soil moisture. This change in bulk density may cause the calculated C stocks in a soil to change from one sampling period to the next, regardless of soil management. This could present severe limitations to efforts to monitor changes in C stocks in high shrink/swell soils. Therefore, a study was conducted in a Kirkland silt loam (Fine, Mixed, Superactive, Thermic Udertic Paleustoll) with high shrink/swell capacity which was sampled to a depth of 90 cm at 3 different moisture conditions and analyzed for bulk density and organic C at 10 cm increments. Organic C stocks were analyzed using the current “fixed depth” method and also with a “fixed mass” method. The analysis showed that under moist conditions the swelling of clays did not decrease bulk density. On the contrary, moist conditions resulted in compression at discrete depth increments. This compression resulted in significant increases in bulk density, which in turn increased C stock estimates at these depth increments when fixed depth was used to calculate C stocks. Utilization of a fixed mass method removed this error and provided more precise measurements of C stocks which should be used for monitoring soil C stocks.

Introduction:

Currently, soil profile C storage is generally expressed as a mass of C per unit area as recommended by the International Panel on Climate Change (IPCC). This spatial coordinate or fixed depth method requires accurate soil bulk density measurements. However, changes in soil bulk density can occur due to tillage or removal of tillage, crop residue presence or absence, reforestation/deforestation, switching from annual crops to perennials and many other reasons (Brady and Wiel, 2002, Lemus and Lal, 2005).

"Without details on soil erosion or deposition, comparisons among soils of stored C should be based on an equivalent soil mass, otherwise it is unclear whether calculated differences represent actual C changes or haphazard differences in soil mass (as defined by soil density and depth at sampling)(Ellert et al., 2002)." Grossman and Reinsch (2002) point out that obtaining an unbiased measurement of soil bulk density is difficult bordering on impossible. They go on to mention that different methods of measurement for soil bulk density yield different results. Ellert et al. (2002) reported that in their evaluation of the fixed volume or fixed depth method that when looking at C content even small, seemingly minor, changes in bulk density can distort C calculations. This poses a problem when trying to compare results from multiple sources or even multiple years.

Because soil bulk density is a dynamic property researchers have recently proposed alternatives to the spatial coordinate method for assessment of soil C stocks. Gifford and Roderick (2003), propose that for accuracy when determining soil C stocks, sampling should refer to a fixed dry soil mass per unit ground area (cumulative mass coordinates), instead of a fixed depth. The use of a fixed depth requires that the surface be used as a reference. However, the practice of using the soil surface as a reference is not ideal as the soil surface can fluctuate for a variety of reasons. Gifford and Roderick (2003) give the following examples of how the surface elevation may change; drainage of wetlands and the oxidation of peat, erosion or deposition of material on the surface, shrink/swell and compaction. Shrink/swell is of particular importance to Oklahoma soils as greater than 60% of the state's soils contain more than 35% montmorillonite clay (OSU Tech. Bull.). Montmorillonite clay minerals have the most shrink/swell capacity and can expand from 10-15 Å per unit cell increasing the volume by 50%. This gives Oklahoma soils a tremendous capacity to move the soil surface depending on soil moisture or dryness. Due to this capacity for shrink/swell, it may be necessary to calculate soil C stocks in a manner that is less subject to fluctuations in response to soil moisture content.

Wuest (2009) compared the use of an equivalent sample depth method (fixed depth) to an equivalent sample mass method (fixed mass) when calculating available water content of soils. Wuest (2009) found that by using the fixed mass method, fluctuations in water content caused by the fixed depth method were corrected. Another advantage to this method is that if a core is fractured or if settling or compaction occurs in the core it does not affect precision, and there is no need for precise core length or depth measurements. This allows for more accurate comparisons between sites with varying bulk densities. It was also found to correct for differences in sampling equipment and sampling conditions, allowing for a broader basis for comparisons of soil constituents between sites, conditions, times and researchers.

Few studies are currently available to evaluate the utility of using the fixed mass method to improve the precision of soil C stock measurements. In fact, there are no studies available to evaluate its impact on the precision of soil C stock measurements in soils with high shrink/swell capacities. Therefore this study was conducted to determine if using the fixed mass method would improve the precision of soil C stock measurements in a high shrink/swell soil under variable soil moisture conditions. This research will be useful in determining if changing from the current fixed depth method to the fixed mass method is needed in order to monitor soil C stocks for the purpose of determining soil C sequestration rates in shrink/swell soils.

Materials & Methods:

This experiment was located in Stillwater, OK on a Kirkland silt loam (Fine, Mixed, Superactive, Thermic Udertic Paleustoll). This soil was chosen for its high clay content and corresponding shrink/swell that causes cracks in the soil for some time during most years. This soil was selected using the NRCS Soil Characterization database that provided bulk density data demonstrating that the bulk density, as determined using the clod method (Brasher et al., 1966; Blake and Hartge, 1986; Grossman and Reinsch, 2002), of this soil can change by as much as 30 % between field capacity and permanent wilting point. This experimental location was planted to soybeans under conventional tillage. An area measuring 5 m by 7 m was sectioned into 32 individual sample areas. The experimental area was sampled 3 times to provide 3 different soil moisture conditions. Therefore, the sample areas were randomly assigned to a sample time (treatmeant) such that 10 samples would be collected during each treatment. This left 2 sample areas remaining which would not be sampled unless an error was made in collecting from the other areas. Due to the short experimental period (2 weeks) it is assumed that real changes in organic C stocks would be minimal.

Soil samples were collected August 13th, 25th and 30th of 2010 to capture various soil moisture conditions. Sampling dates were chosen to represent a range from very dry to moist soil conditions. August 13th was quite dry, between August 13th and 25th the location received approximately 5.7 cm of rainfall and on the 24th the plots were irrigated with approximately 2.5 cm of water and sampled on the 25th. On the 28th the plots were again irrigated with approximately 2.5 cm of water and sampled two days later. These sampling dates are referred to as T1 (13th), T2 (25th) and T3 (30th).

Soil samples were collected using a tractor mounted hydraulic probe with a cutting diameter of 7.45 cm. The probe was pushed to a depth of approximately 125 cm. Soil from the bottom of some of the cores fell out as the core was extracted from the soil. Therefore, only 90 cm of soil was used for this analysis because this depth was consistently extracted throughout each sampling time. Each hole created after sampling was measured for depth. The depth of the hole created was then compared to the length of the core to gauge compression if any. The cores were placed in a cradle made from PVC pipe with a diameter of 10 cm and cut into 10 cm sections using a curved knife, such that soil was not lost from each section. Soil samples were then placed in a plastic bag and stored in an ice chest until they were delivered to a refrigerator for storage at 4°C. Each soil core section was initially weighed to determine bulk density. After the initial weight was determined the sample was mixed and a subsample (20 g) was dried at 110°C to determine the moisture content. The bulk density was then adjusted to a dry weight basis. The remaining sample was transferred to a paper bag and placed in a greenhouse to air dry. Each sample was then ground to pass through a 2 mm sieve. Each sample was analyzed for total C and N using a TrueSpec CN analyzer (LECO, Inc. St. Joseph, MI). Soil pH was determined on a 1:1, soil: deionized H_2O mixture after a 30 min equilibration period. Soil inorganic C was determined on soil samples with a pH > 7.0 using a pressure calcimeter method (Sherrod et al., 2002). Soil organic C was determined by the difference between total C and inorganic C.

In addition, the coefficient of linear extensibility (COLE) was determined on three randomly selected soil samples from each depth using the method of Schafer and Singer (1976).

Two methods were used to calculate C stocks for each sampling period. The first method is the commonly used spatial coordinate method. In this method, the sampling depth, z, is specified and therefore constant. The soil volume, V (m^3), contains a dry mass, m_s (kg), and total mass, m_t (kg), including water. The C mass within the volume is

$$c_{s=}\frac{c_s}{m_s}\frac{m_s}{V}V = f_c \rho_b V, \tag{1}$$

where $f_c = c_s/m_s$ is the mass fraction of C within the total dry mass, and $\rho_b = \frac{m_s}{V}$(kg m^{-3}) is the mass concentration of the dry material ('dry bulk density' or 'bulk density'). Since V equals area (A) times depth (z), soil C per unit area is

$$\frac{c_s}{A} = f_c \rho_b \frac{V}{A} = f_c \rho_b \frac{A_z}{A} = f_c \rho_b z. \tag{2}$$

The second method used is the cumulative mass method proposed by Gifford and Roderick (2003) as an alternative to the spatial coordinate method. The cumulative mass method calculated C stocks found in a constant mass of soil instead of a constant depth and therefore may reduce errors associated with changes in bulk density resulting from shrink swell. In this method, depth varies so that each samples contains the same dry mass per unit area (m_s/A). In Eq. 2, $\rho_b z$ is equivalent to the dry soil mass per unit area. Therefore, in the cumulative mass method, as ρ_b increases, the sampling depth (z) is reduced, thereby maintaining the product of the two terms as a constant.

In order to find the cumulative mass of soil C, Gifford and Roderick (2003) use linear interpolation to allow for variation with depth in both the mass fraction of C and bulk density. This is accomplished by dividing the core into two sections, for example, a core taken to 40 cm would be divided into one section of 0-20 cm and another of 20-40 cm. The total length of the core is represented by z_b and the surface subsection is represented by z_a with the cumulative dry soil masses to the respective depths denoted by $m_s(z_b)$ and $m_s(z_a)$ and the cumulative mass of soil C, $c_s(z_b)$ and $c_s(z_a)$. The target or 'standard' cumulative mass of dry soil is denoted by $m_s(t)$ and the corresponding cumulative mass of soil C that we are looking for is denoted as $c_s(t)$. Through linear interpolation, the resulting equation is

$$c_s(t) = c_s(z_a) + \frac{c_s(z_b) - c_s(z_a)}{m_s(z_b) - m_s(z_a)} \left(m_s(t) - m_s(z_a)\right). \qquad (3)$$

Analysis of variance and contrast analysis were performed using the SAS PROC GLM procedure (SAS Institute, 2001), to determine significant treatment effects on measured response variables.

Results and Discussion:

COLE values increased with depth (Figure 5). The COLE values of 0.10 or greater found below 20 cm in this profile indicate very high potential for shrink-swell in a soil. The values obtained for this sample site meet the criteria for a Vertic suborder classification as defined by the Soil Survey Staff (2010).

Soil Moisture:

Analysis of variance found that all sample dates had significantly different soil moisture at the 0-10 cm increment, with T1 being the driest at 0.08 g g^{-1} moisture, T3 being the intermediate moisture level at 0.15 g g^{-1} and T2 being the wettest with 0.19 g g^{-1} soil moisture. Analysis of the 10-20 cm, 30-40 cm and 60-70 cm increments all revealed T1 to be significantly drier than T2. The T3 soil was not significantly different from either T1 or T2 at any of these increments. No significant differences were found at depth increments below 70 cm. (Figure 6).

Soil Bulk Density:

Analysis of variance showed no significant differences in mean bulk density for the surface 30 cm. At 30-40 cm, T1 was found to have a significantly lower bulk density of 1.35 g cm^{-3} than either T2 or T3 with bulk densities of 1.49 and 1.50 g cm^{-3}, respectively. From 40-60 cm, no significant differences were found. At 60-70 cm the bulk density of T1 was again significantly lower at 1.46 g cm^{-3} compared to the T2 and T3 dates which both had bulk densities of 1.62 g cm^{-3}. The bulk density of T1 was significantly higher than the remaining sampling dates in the 70-80 cm increment. No significant differences were found at the 80 to 90 cm depth. (Figure 7)

The differences in soil moisture help to explain the differences found in the bulk densities. The T1 soil profile was generally drier than the T2 and T3 profiles. At depths where significantly different bulk densities were observed the T1 soils had lower bulk densities. This is contrary to the hypothesis that shrinkage of soils upon drying would result in an increase in bulk density in these soils with apparent shrink/swell capacity as indicated by measured COLE values. It appears that the differences in bulk density found at 30 -40 and 60-70 resulted from compression of the T2 and T3 cores.

Table 13 shows the average measured whole core lengths and the average depth of holes created during sampling. Notice that whole core lengths were approximately 1 cm longer than the depth of holes for T1 and T2 and that the core length was approximately equal to hole depth for T3. Recall that during the sampling process the probe was pushed to an approximate depth of 125 cm. At T1 and T2 portions of the core fell out of the probe tube before the tube could be lifted from the hole. This did not apparently occur at T3. Therefore, it appears that the section of soil falling back down the hole did not set firmly back from where it came, which explains the fact that, on average, the hole depth is shallower than the length of core for T1 and T2. This illustrates the difficulty in estimating small amounts of compression by measuring core length and hole depth. Also recall that the bulk density for T1 was significantly lower than T2 and T3 at 70-80 cm. This may have offset the apparent compression occurring at 30-40 and 60-70 cm. In fact, when the compression of the T2 and T3 cores is calculated from the average bulk density values measured to 90 cm it is found that equivalent mass of soil in T2 and T3 would be

0.57 and 1.27 cm shorter than T1. Wuest (2009) states that the same soil sampled when bulk density is higher will remove more soil than when the soil has a lower bulk density. In other words if the soil is measure to specific depth more soil will be removed. However, if a soil could be measure to a specific mass a shorter core would be extracted.

Carbon Concentration:

Analysis of variance found no significant differences in total C, soil inorganic C or soil organic C between the treatments. Figure 8 shows that C concentrations generally decreased with depth and given the short experimental period, one would not expect to see significant differences between the sampling dates.

Carbon Stocks:

Analysis of variance of the mean C stocks in each depth increment showed that at 30-40 cm the C stocks in the T1 samples were 11.9 Mg C ha^{-1}, which was significantly lower than the 12.8 and 13.3 Mg ha^{-1} found at this depth in T2 and T3 respectively. At the 60-70 cm increment the T1 samples contained 12.6 Mg C ha^{-1}, which was significantly lower than 13.9 Mg C ha^{-1} found in T3 but not different from the 13.3 Mg C ha^{-1} found in T2 (Figure 9). Despite these significant differnces found at each depth increment, no significant differences were found in the cumulative C stocks when calculated on a fixed depth basis (Table 14).

Calculating the soil C stocks on a fixed mass basis Table 15 shows the C stocks found in a range of soil masses corresponding to depth increments from 16 to 90 cm. Here again there were no differences among the three sampling dates. However, the absolute differences in C stocks when calculated using the fixed depth method (Table 14) are greater than the absolute differences when C stocks are calculated using the fixed mass method (Table 15). The fixed mass method removed error associated with the significantly different bulk densities found at 30-40 and 60-80 cm. The remaining variability could be due to spatialvariability or analytical variability in the C analysis. In fact, when the fixed mass method was used to calculate C stocks the largest difference between sample dates, was 2.6% found in the surface 5000 Mg of soil (Table 15). In contrast, when the fixed depth method was used the maximum difference observed in the

0-10cm depth was 9.3 % of the average C stock found in this depth. The difference observed at 0-40 cm was 5.5 % of the average C stock (Table 14). This analysis is consistent with the findings of Ellert and Bettany (1995) who stated that use of the fixed mass method eliminates sensitivity to bulk density.

Figure 11 shows the C mass in each depth increment normalized based on equivalent mass of soil. Notice that significant differences found in Figure 9 at 30-40, and 60-70 cm are eliminated when equivalent mass is used to calculate C stocks within each soil layer. This supports the findings of previous research (Gifford and Roderick, 2003; VandenBygaart and Angers, 2005) that small differences in bulk density can change how much C mass is reported. If scientists are to understand global climate change then accurate and standardized reporting of soil C stocks is essential. Currently, the Oklahoma Carbon Program estimates C sequestration to be 0.3 Mg C ha^{-1} yr^{-1} after conversion to no-till in Western OK. This data demonstrates that the magnitude of error that can occur when monitoring C is quite large compared to the potential average annual changes. Using the fixed mass method to calculate C can at least reduce variability associated with changes in measured bulk densities. Therefore, it may be a more appropriate method than the current fixed depth method suggested by the IPCC, particularly for monitoring C changes over time or when data from different sources or methods is to be compared.

Conclusions:

Soil bulk density did not increase with decreasing soil moisture as was expected in this high shrink/swell soil. Alternatively, under moist soil conditions, discrete depth increments were susceptible to compaction during sampling, presumably because internal structure was compressed in these depth increments. This compaction did result in significant difference in soil C stocks at these depth increments. Because there were only 2 weeks between sampling dates in this experiment, these changes in C stocks must be attributed to error imposed by the compression of these soil layers while moist. The fixed mass method removed these errors and provided a more precise estimate of soil C stocks.

Methods of analysis while generally standardized can still have a huge impact on soil C measurements. The method tested here, the fixed mass method, as proposed by Gifford and Roderick (2003), allows for correction of biases imposed by differences in sampling equipment and sampling conditions that result in different measured bulk densities. This may allow for a broader basis for comparisons of soil C measurements between sites, conditions, times and researchers.

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List Figures:

Table 13: The date of sample collection, the average measure whole length of soil cores extracted and the average depth of holes after core extraction.

Sample Date	Sample Date ID	Core Length	Hole Depth
		-------cm---------	
8/13/2010	T1	104.8	103.7
8/25/2010	T2	105.1	104.1
8/30/2010	T3	122.0	122.2

Table 14: The cumulative carbon stocks as measured to each depth on a fixed depth basis for each sample date (T1, T2, and T3) and the maximum difference among sampling dates.

Depth	T1	T2	T3	LSD†	Max Difference
Cm	-------------------------	-Mg ha^{-1}-		-------------------------	
10	14.2	15.5	15.6	ns	1.4
20	32.0	32.5	33.6	ns	1.5
30	47.1	47.0	48.9	ns	1.9
40	58.9	59.9	62.2	ns	3.3
50	71.6	72.7	74.3	ns	2.7
60	85.7	85.5	86.7	ns	1.1
70	98.7	98.8	100.6	ns	1.9
80	109.5	109.9	112.1	ns	2.6
90	117.7	118.3	121.2	ns	3.6

†LSD, Least significant difference at the 0.05 probability level.

Table 15: The cumulative carbon stocks as measured in each increment of soil mass on a fixed mass basis for each sample date (T1, T2, and T3) and the maximum difference among sampling dates.

Fixed Mass	Estimated Depth T1†	Estimated Depth T2	Estimated Depth T3	T 1	T 2	T 3	LSD‡	Max. Difference
Mg	------------cm------------			------------------------Mg ha^{-1}-----------------------				
2000	16	16	16	25.0	25.5	25.2	ns	0.5
3000	23	23	23	36.9	36.8	37.1	ns	0.3
4000	29	29	29	47.5	46.8	47.9	ns	1.1
5000	36	36	36	56.2	55.6	57.1	ns	1.5
6000	43	42	42	64.5	64.4	65.5	ns	1.1
7000	50	49	49	73.4	72.1	73.2	ns	1.3
8000	56	55	55	81.3	80.1	80.8	ns	1.2
9000	63	61	61	89.5	88.0	88.7	ns	1.5
10000	70	68	68	97.6	96.2	97.3	ns	1.4
11000	76	74	74	104.4	103.7	105.0	ns	1.3
12000	83	81	81	110.3	110.2	111.9	ns	1.7
13000	90	87	87	115.5	115.6	117.9	ns	2.4

†The depth was estimated from the relationship between cumulative mass and depth (Figure 10).
‡ LSD, Least significant difference at the 0.05 probability level.

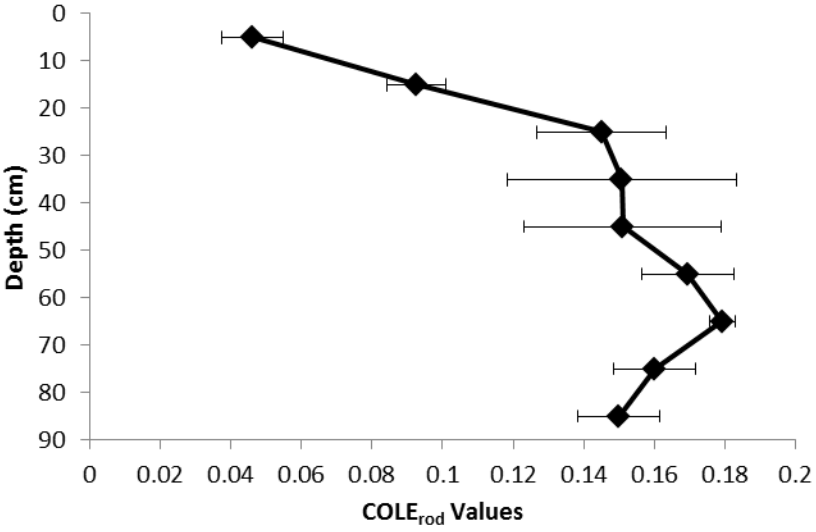

Figure 5: The average COLE$_{rod}$ values for 3 randomly selected samples from each depth.

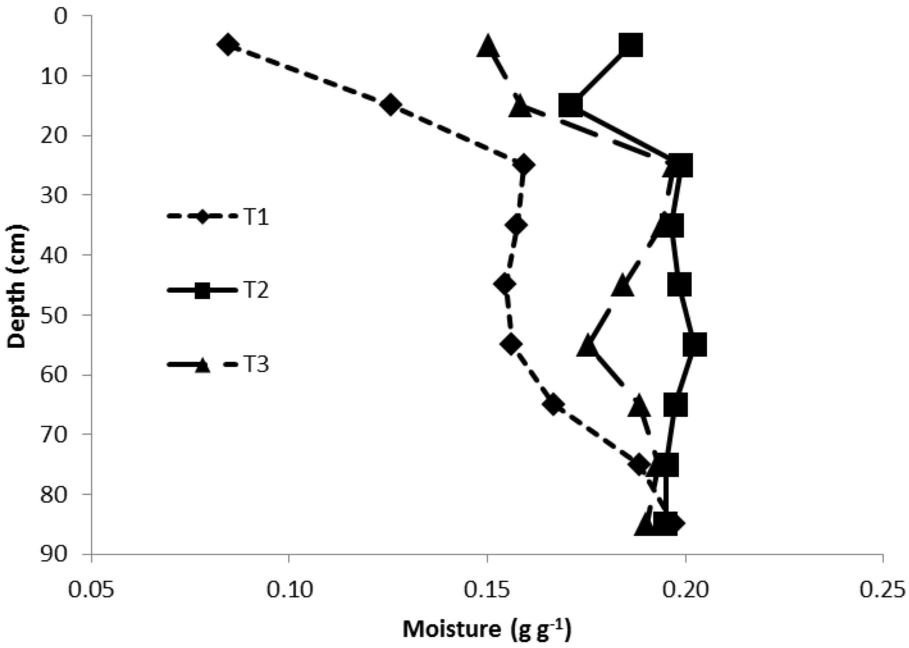

Figure 6: Soil moisture as measured at sample date T1, T2 and T3. Values right of data points are least significant differences (LSD) at the 0.05 probability level. Data points without LSD values were not significantly different.

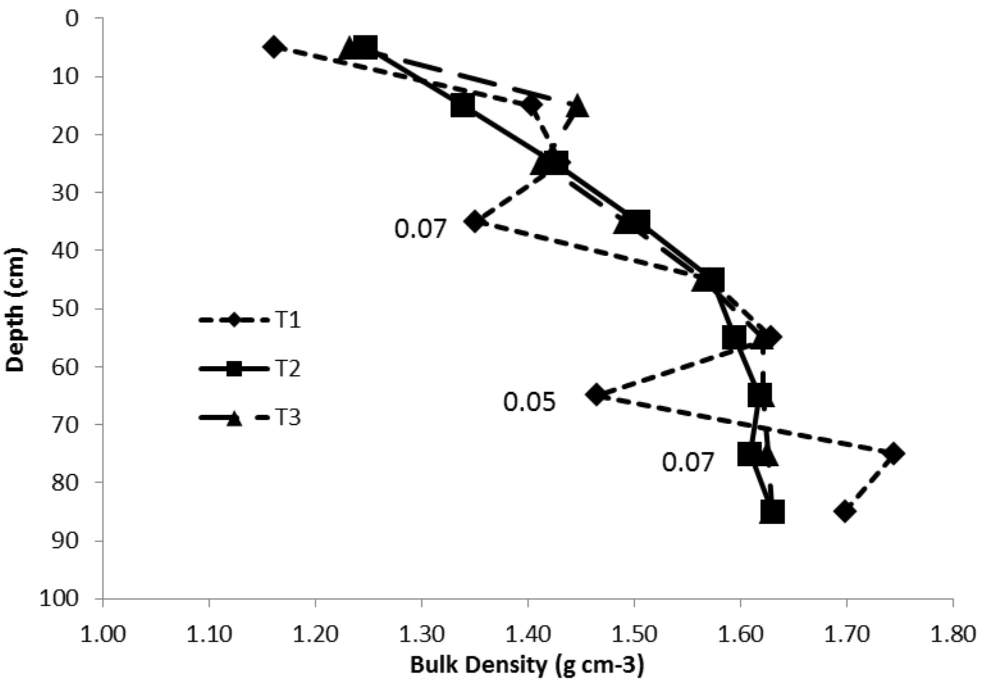

Figure 7: Soil bulk density as measured at sample date T1, T2 and T3. Values left of data points are least significant differences (LSD) at the 0.05 probability level. Data points without LSD values were not significantly different.

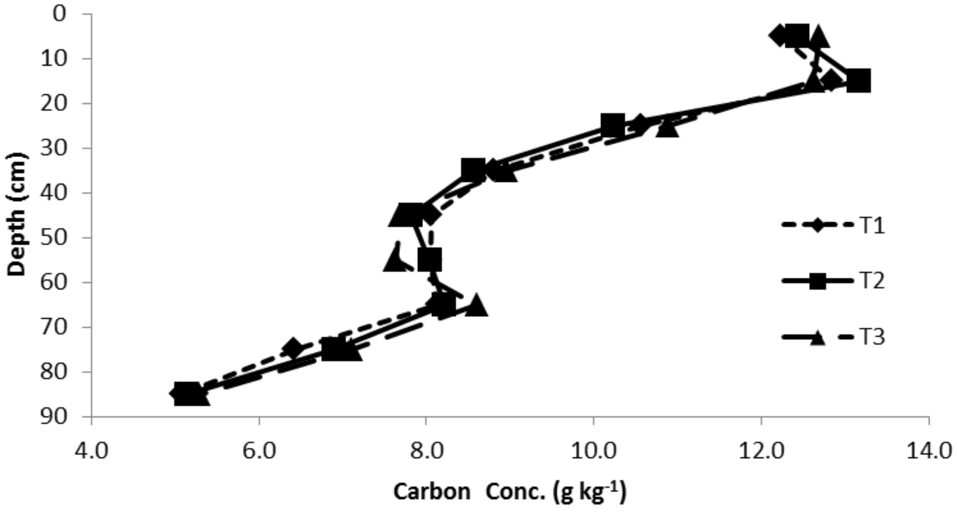

Figure 8: Organic carbon concentrations as measured at sample date T1, T2 and T3. Values left of data points are least significant differences (LSD) at the 0.05 probability level. Data points without LSD values were not significantly different.

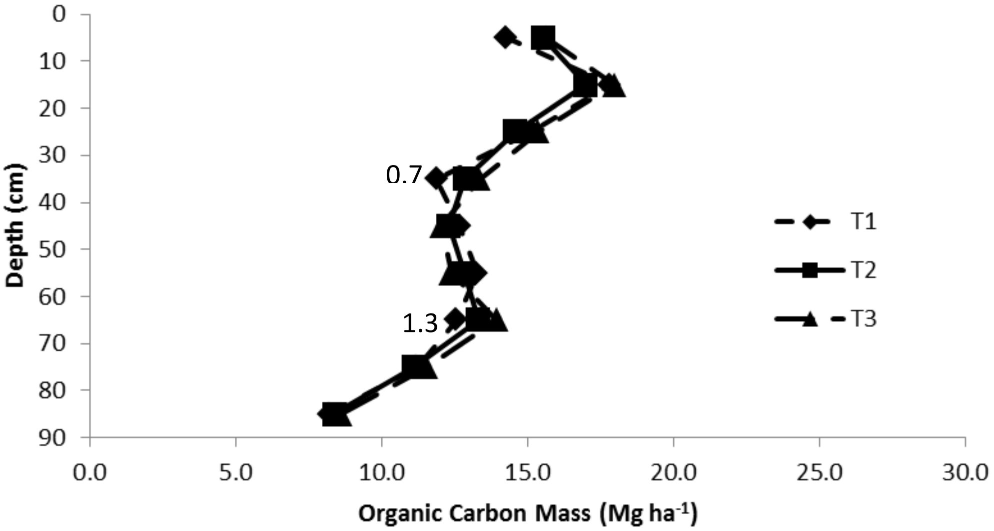

Figure 9: Organic carbon mass in each depth increment as measured at sample dates T1, T2 and T3. Values left of data points are least significant differences (LSD) at the 0.05 probability level. Data points without LSD values were not significantly different.

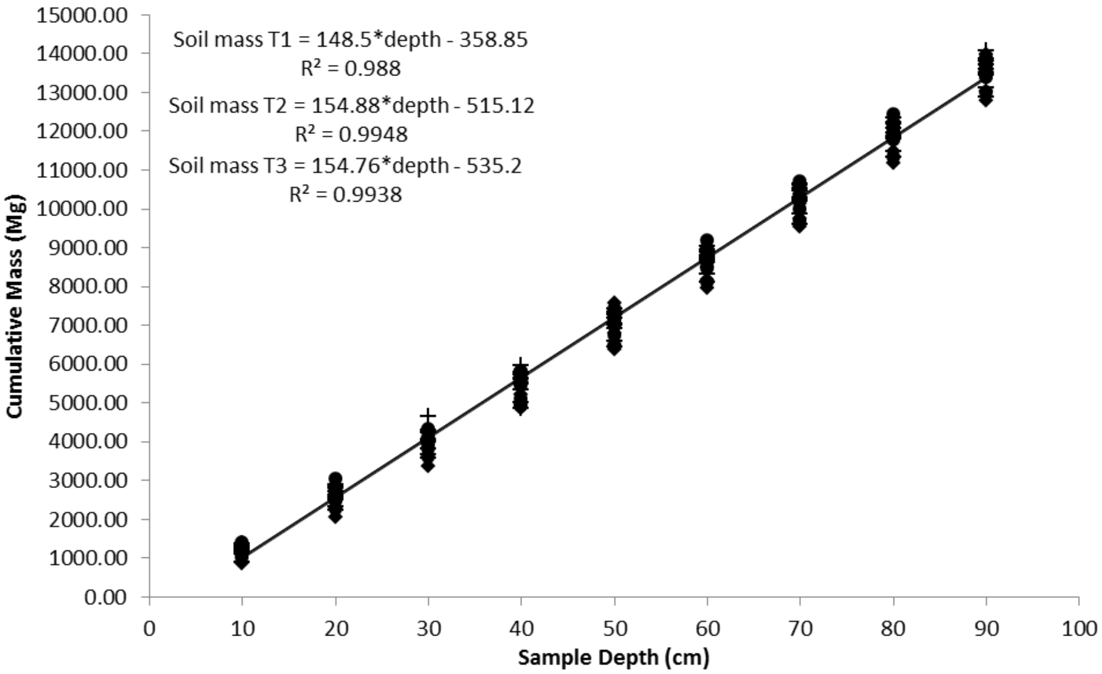

Figure 10: Relationship between cumulative mass of soil and sampling depth, data includes all sample dates.

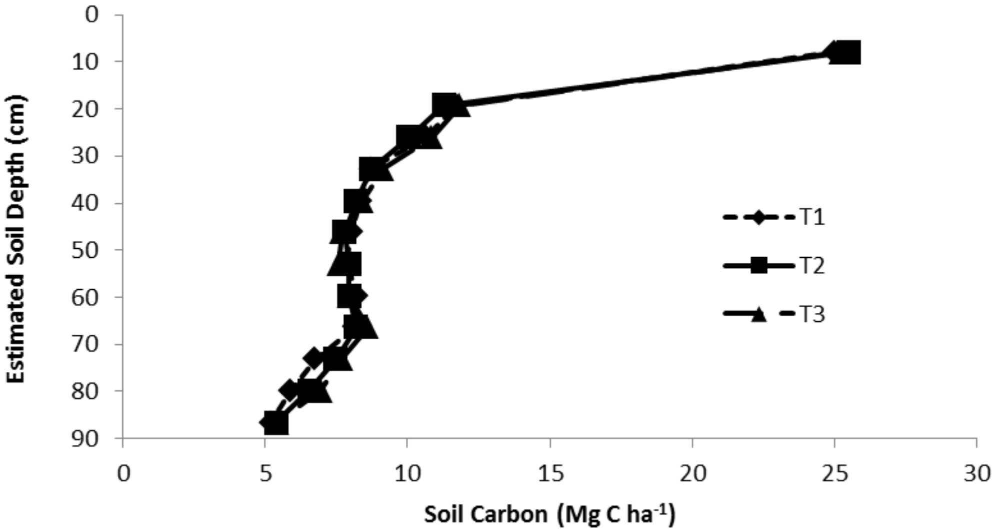

Figure 11: Organic carbon mass in each estimated depth increment (increments were created from relationship between cumulative mass and soil depth in Figure 10) as measured at sample date T1, T2 and T3. Data points without LSD values were not significantly different at the 0.05 probability level.

APPENDIX

Table 1A: Contrast analysis of organic carbon stocks calculated using the equivalent mass method to compare effects of species and harvest frequency.

Species	**Harvest Frequency**	**-------------Spring 2009-----------**				**-------------Spring 2010------------**			
		2000Mg	3000Mg	6000Mg	13000Mg	2000Mg	3000Mg	6000Mg	13000Mg
		------------------------------------Mg ha^{-1}-------------------------------------							
Contrast Comparisons of Species									
Switchgrass		20a†	27a	45a	65a	20a	27a	44a	63a
Miscanthus		23a	30a	46a	68a	21a	27a	44a	64a
E. Gammagrass		23a	30a	47a	63a	20a	26a	42a	62a
Contrast Comparisons of Harvest Frequency									
	1 Harv.	22a	29a	46a	64a	20a	27a	43a	62a
	2 Harv.	22a	29a	47a	67a	21a	27a	44a	65a

† Different letters within each contrast comparison indicate a significant difference at the 0.05 probability level. Contrast comparisons with no letter beside means were not analyzed due to interactions between species and harvest frequency.

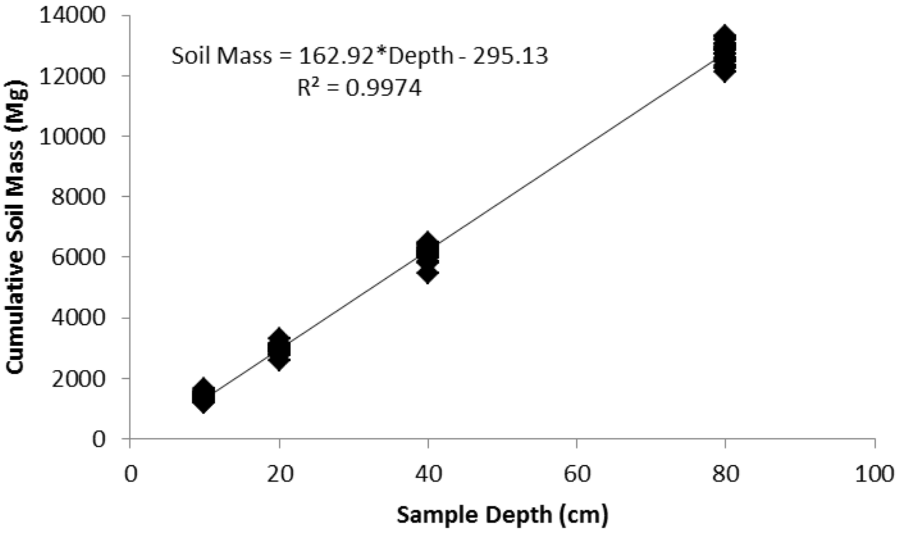

Figure 1A: Relationship between cumulative mass of soil and sampling depth for soils collected in 2009 from experiment 1 in Chapter II.

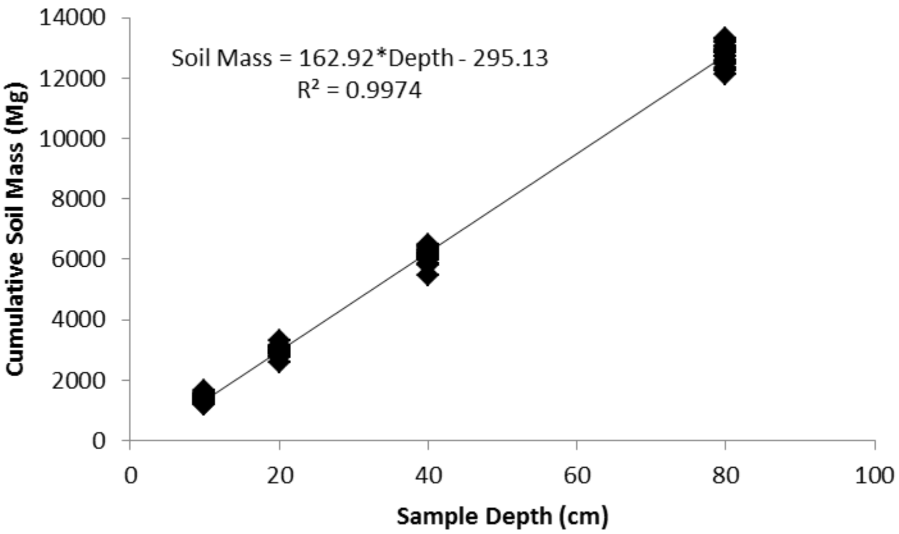

Figure 2A: Relationship between cumulative mass of soil and sampling depth for soils collected in 2010 from experiment 1 in Chapter II.

VITA

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Master of Science

Thesis: CARBON STOCKS IN PERENNIAL BIOFUEL FEEDSTOCK MANAGEMENT SYSTEMS

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Major Field: Soil Science

Scope and Method of Study:

Demand for alternatives to fossil fuels and the desire to mitigate CO_2 emissions has driven interest in cellulosic biofuel and methods of sequestering carbon (C). Perennial grasses have the potential to meet both of these needs. However, little is known about how management practices influence soil C sequestration and a method is needed to determine changes in C stocks to accurately represent the changes in soil C over time. Soil C storage is expressed as a mass of C per unit area measured to a specific depth. This is not ideal as the soil surface can due to shrink/swell of clays. This can manifest as changes in bulk density. Because many Oklahoma soils have high shrink/swell capacities, their bulk densities may change with soil moisture. Therefore 3 studies were conducted to 1) evaluate the impact of management practices on soil C stocks and 2) evaluate the use of a fixed mass method to calculate soil C stocks in a high shrink/swell soil. In Experiment 1, switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus* spp.) and eastern gamagrass *(Tripsacum dactyloides)* were evaluated with harvest frequency to determine the best management practices to achieve energy production and C sequestration in the Southern Great Plains. Soil samples were collected from experiment 2 evaluating 9 switchgrass varieties.

Findings and Conclusions:

The data for experiment 1 showed no difference in soil C stocks between switchgrass, miscanthus, or eastern gamagrass, nor was a difference in C stocks found between harvest frequency treatments. Data from experiment 2 showed that soil C stocks were not proportional to yield. The data suggest that the upland varieties allocate a greater proportion of carbon to belowground carbon stock when compared to the upland varieties. Experiment 3 showed that under moist conditions swelling of clays did not decrease bulk density. On the contrary, moist conditions resulted in compression at discrete depth increments. This compression resulted in increases in bulk density, which increased C stock estimates at these depth increments when fixed depth was used to calculate C stocks. Utilization of a fixed mass method removed this error and provided more precise measurements of C stocks.

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