BIOFUEL FEEDSTOCKS: IMPLICATIONS FOR SUSTAINABILITY AND ECOSYSTEM SERVICES

by

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B.S., Universite Cheikh Anta Diop Dakar, 2000
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Abstract

Biofuel feedstocks such as grains and cellulose are gaining increased attention as part of the U.S. portfolio of solutions to address climate change and improve energy security. As the future of biofuels unfolds, major concerns are emerging, including the sustainability of the soil resource in bioenergy cropping system. With a clear understanding of the sustainability risks that exist within the agricultural soil resources, it is now essential to develop metrics that document the soil health as well as the total biomass production of different cropping system. We tested the effectiveness of eight bioenergy plant species grouped between annual and perennial crops. Our main objective was to determine the sustainability of bioenergy cropping systems. There was significantly greater soil structural stability plus greater root biomass under the perennial crops but greater aboveground biomass in the annual crop. Differences in soil carbon measured to 1.2 m were not significant between energy crops after five years. A transparent, unbiased method to identify possible change in soil characteristics under bioenergy cropping practice was offered. Our next metrics were soil aggregate stability and microbial community structure as indicators of soil ecosystem health and environmental stability. The effects 24 years of differing levels of residue and fertilizer inputs on soil aggregate stability, aggregate C and microbial community structure were evaluated. A native, undisturbed prairie site, located nearby was used as a reference in this study. The results showed that greater inputs of inorganic N and increased returns of crop residues did not cause a proportionately greater increase in SOC. The abundance of microbial parameters generally followed their potential carbon pool in cultivated soils but a strong mismatch was observed in the native prairie site. Our results showed for the first time a clear disconnect between decomposers and macroaggregates; highlighting the role of soil

structure in protecting organic matter. Soil carbon sequestration is one of the mechanisms that have been proposed as temporary measure to mitigate global climate change. However, there was a particularly large risk of negative effects of mitigation measures related to the increased removal of crop residues from cropping systems for use in bioenergy, if this means that soil carbon is reduced. Effective measurement of soil C at the field scale requires an understanding of the spatial variability of soil C on a landscape scale. Recent technological advances in soil C measurement offer new opportunities in this area. Our surface measurements of soil C by near infrared spectroscopy (NIRS) provided a quick assessment of soil C and, soil C predicted by NIRS and measured by dry combustion laboratory measurements was correlated with and R-squared of 0.84.

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Approved by:

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Charles W. Rice

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Table of Contents

List of Figures xi
List of Tables xvi
Acknowledgements xviii
Dedication xix
Chapter 1 - Introduction 1
Literature review 4
Research Study 6
References 7
Chapter 2 - Measuring Soil Sustainability: Index for Bioenergy Cropping Systems 10
Introduction 10
Materials and Methods 17
Soil Sampling and Analysis 18
Soil Organic C and N: 19
Microbial Biomass Carbon-Fumigation Incubation (MBC-FI) 20
Soil Aggregates 20
Microbial Community Structure: Phospholipid Fatty Acid Analysis 21
Statistical analysis 22
Sustainability index 22
Results 24
Biomass production 24
Soil microbial biomass 25
Soil microbial community structure 25
Soil structural stability 27
Soil organic carbon 27
Sustainability index 27
Discussion 29
Economic and environmental considerations. 31
Conclusion 35

References 36
Chapter 3 - Soil Aggregates, C and Microbial Parameters As Affected by 24 Years of Differing Levels of Crop Residue and Fertilizer Inputs 63
Abstract 63
Introduction 64
Materials and Methods 67
Soil Sampling and Analysis 68
Soil Aggregates: 69
Microbial Community Structure: Phospholipid Fatty Acid Analysis: 69
Modeling effort 70
Statistical analysis: 71
Results 72
Soil aggregate distribution and stability 72
Carbon distribution in the aggregate fractions 72
Soil Carbon 74
Simulating soil carbon change over time 75
Grain and residue yield response to residue management and fertilizer treatment 75
Soil microbial community structure, aggregation and organic matter composition 76
Discussion 76
Conclusion 81
References 83
Chapter 4 - Understanding spatial variability: Measuring and monitoring soil organic carbon. 113
Abstract 113
Introduction 114
Materials and methods 117
Sampling design and methodology 117
Laboratory analyses 118
Data analysis 118
Results 119
Drummond 120
Kerj 121

Gypsum 122
Lund 122
Lund CT 123
Markley 124
Tarn 125
SOC variability at different spatial scales 125
Discussion 126
Conclusion 129
References 130
Chapter 5 - General Conclusion 167
Appendix A - 170
Appendix B - Chapter3 180
Appendix C - 183

List of Figures

Figure 2.1. Total biomass yield for various feedstocks. 44
Figure 2.2. Root dry weight (RDW) in (0-120 cm) soil profile of different biofuel crops. Phot.Srgh = Photoperiod sorghum, SweetSrgh = Sweet sorghum GrainSrgh = Grain sorghum, Corn Rot. = Rotated corn, SwitchgrassK = Switchgrass Kanlow..... 45
Figure 2.3. Root distribution of perennials versus annual crops within the upper 120cm 46
Figure 2.4. Soil microbial biomass C determined by fumigation-incubation method in different bioenergy crops (mean value ± standard error of four replicates). 47
Figure 2.5. Soil microbial biomass N determined by fumigation-incubation method in different bioenergy crops (mean value ± standard error of four replicates). 48
Figure 2.6. Estimated relative abundance of Gram (+) under different energy crops. 49
Figure 2.7. Estimated relative abundance of gram-negative bacteria ($Gram^-$) under different energy crops. 50
Figure 2.8. Estimated relative abundance of total bacteria. 51
Figure 2.9. Estimated relative abundance of total fungi. 52
Figure 2.10. Effects of different energy crops of soil fungi: bacteria ratio, both fungal and bacterial communities was measured by PLFAs analysis. 53
Figure 2.11. Sand-free water-stable aggregates in different size fractions within the 0-5 cm depth as affected by different energy crops. 54
Figure 2.12. Soil organic carbon (SOC) in the 0-120 cm soil depth profile after 5 yrs of crop residue study under different biofuel feedstocks. 55
Figure 2.13. Effects of energy crops on arbuscular mycorrhizal fungi (AMF) and saprophytic fungi. Error bars are standard errors of the mean. RC = Rotated corn, Misc = Miscanthus, GS = Grain sorghum, SgK = Switchgrass Kanlow, CC = Continuous corn, SgC = Switchgrass Ceres, PS = Photoperiod sorghum, BBS = Big bluestem, SwtS = Sweet sorghum..... 56
Figure 2.14. Relative fractional abundance of microbial functional lipid and associated ratio. Capital letters represent significant differences in fungal subgroup; while lowercase letters represent significant difference in bacterial subgroup. Error bars are standard errors of the

mean. SgK = Switchgrass Kanlow, SgC = Switchgrass Ceres, Misc = Miscanthus, BBS = Big bluestem, GS = Grain sorghum, PS = Photoperiod sorghum, CC = Continuous corn, RC = Rotated corn, SwtS = Sweet sorghum 57
Figure 3.1. Distribution of the sand-free water stable aggregates (SFWSA) in top 5 cm. Values followed by a different letter within the same aggregate fraction are significantly different at (P<0.05). 88
Figure 3.2. Total SOC contents (0-5 cm) in different aggregate size fractions in silt loam under different crop residue management with a native prairie. Values followed by a different letter within the same aggregate fraction are significantly different at (P<0.05). 89
Figure 3.3. Total SOC contents (5-15 cm) in different aggregate size fractions in silt loam under different crop residue management with a native prairie. Values followed by a different letter within the same aggregate fraction are significantly different at (P<0.05). 90
Figure 3.4. Total SOC contents (15-30 cm) in different aggregate size fractions in silty clay loam under different crop residue management with a native prairie. Values followed by a different letter within the same aggregate fraction are significantly different at (P<0.05)... 91
Figure 3.5. Relationship between carbon in aggregate and the amount of SFWSA in the top 5 cm according to residue management, using mean values of all treatments (Ottawa, KS)..... 92
Figure 3.6. Effects of crop residue management and level of fertility on SOC after 24 years of study (0-5cm). 93
Figure 3.7. Carbon stratification ratios in top soil compared from different residue management (Ottawa, KS). 94
Figure 3.8. DNDC simulations of total soil organic C for a silt loam soil with different crop residue management and different fertility levels (0-30 cm). 95
Figure 3.9. Wheat residue management effects on grain yield over time (Ottawa, KS). 96
Figure 3.10. Wheat residue management effects on residue yield over time (Ottawa, KS). 97
Figure 3.11. Effects of fertilization on wheat grain yield over time (Ottawa, KS). 98
Figure 3.12. Effects of fertilization rate on wheat residue yield over time (Ottawa, KS). 99
Figure 3.13. Effect of fertilization rate on sorghum residue yield over time (Ottawa, KS). 100
Figure 3.14. Effects of fertilization rate on sorghum grain yield over time (Ottawa, KS). 101
Figure 3.15. Effects of fertilization rate on corn residue yield over time (Ottawa, KS)..... 102
Figure 3.16. Effects of fertilization rate on corn grain yield over time (Ottawa, KS). 103

Figure 3.17. Soybean residue management effects on residue yield over time (Ottawa, KS).... 104
Figure 3.18. Soybean residue management effects on grain yield over time (Ottawa, KS). 105
Figure 3.19. Depth profiles of microbial parameters under crop residue removal treatment. 106
Figure 3.20. Depth profiles of microbial parameters under crop residue maintenance 107
Figure 3.21. Depth profiles of microbial parameters under crop residue addition 108
Figure 3.22. Depth profiles of microbial parameters under native prairie reference site 109
Figure 4.1. Sampling design and methodology. 136
Figure 4.2. Average whole soil profile (0-75 cm) and top 30 cm SOC stocks in various triangles across field. Error bars represent standard error. 137
Figure 4.3. Estimated SOC at each individual depth increments. 138
Figure 4.4. Drummond variogram of SOC stocks (dots) and exponential model fitted to the variograms (solid blue lines). Variogram represents soil layer 15-30 cm. 139
Figure 4.5. Average whole soil profile (0-75cm) and top 30cm SOC stocks in various triangles across field. Error bars represent standard error. 140
Figure 4.6. Estimated SOC at each individual depth increments. 141
Figure 4.7. Kerj variograms of SOC stocks (dots) and exponential model fitted to the variograms (solid blue lines). Top variogram represents soil layer 30-45 cm; middle variogram is for 45-60cm and bottom variogram represents 60-75 cm soil layer..... 142
Figure 4.8. Average whole soil profile (0-75 cm) and 0-30 cm SOC stocks in various triangles across field. Error bars represent standard error. 143
Figure 4.9. Estimated SOC at each individual depth increments. 144
Figure 4.10. Average whole soil profile (0-75cm) and 0-30cm SOC stocks in various triangles across field. 145
Figure 4.11. Estimated SOC at each individual depth increments. 146
Figure 4.12. Lund variogram of SOC stocks (dots) and exponential model fitted to the variogram (solid blue lines). Variogram represents soil layer 15-30cm. 147
Figure 4.13. Average whole soil profile (0-75cm) and 0-30cm SOC stocks in various triangles across field. 148
Figure 4.14. Estimated SOC at each individual depth increments. 149

Figure 4.15. Lund CT variograms of SOC stocks (dots) and exponential model fitted to the variograms (solid blue lines). Top variogram represents soil layer 5-15 cm; middle variogram is for 30-45 cm and bottom variogram represents 45-60 cm soil layer. 150
Figure 4.16. Average whole soil profile (0-75 cm) and top 30 cm SOC stocks in various triangles across field. Error bars represent standard error. 151
Figure 4.17. Estimated SOC at each individual depth increments. 152
Figure 4.18. Markley variograms of SOC stocks (dots) and exponential model fitted to the variograms (solid blue lines). Top semi-variogram represents soil layer 15-30 cm middle semi- variogram correspond to 30-45 cm and bottom variogram represents 45-60 cm soil layer. Note at 45-60 cm soil layer semi-variogram analysis indicated the distribution of sampling points made binning difficult, therefore results for this particular soil layer were little unstable and did not reach sill. 153
Figure 4.19. Average whole soil profile (0-75 cm) and top 30 cm SOC stocks in various triangles across field. Error bars represent standard error. 154
Figure 4.20. Estimated SOC at each individual depth increments. Within depths, means followed by the same or no letter are not significantly different ($P<0.05$) by Tukey's test. Lowercase letters represent differences within soil layer; capital letters represent differences between triangles. 155
Figure 4.21. Tarn variogram of SOC stocks (dots) and exponential model fitted to the variogram (solid blue lines). Top variogram represents soil layer 30-45 cm with significant spatial autocorrelation but dispersed. Bottom variogram represents 45-60 cm soil layer. 156
Figure 4.22. Carbon predicted by NIRS and measured by dry combustion. 157
Figure 4.23. Vertical variations in soil carbon content for the various fields. Much of the variation in soil C between soils types occurred in the top 30 cm. 158
Figure A.1. Mean annual precipitation at Manhattan, KS biofuel site over the first five years of crop establishment. 172
Figure A.2. Maxima and minima temperatures during the growing season of 2009. Flat thick line 173
Figure A.3 Total PLFA 174
Figure B.1. Effects of crop residue management and fertility level on total soil N after 24 years of study in 3 depths increments (0-5; 5-15 and 15-30 cm). 180

Figure C.1. Location of different study sites (1 = Drummond; 2 = Kerj; 3 = Markley; 4 = Lund; 5 = Lund CT; 6 = T. 183

List of Tables

Table 2.1. Analysis of variance significant levels for total fungi, total bacteria, fungal:bacterial ratio, and Gram$^-$ and Gram$^+$ bacteria from soil under different energy crops evaluated over three years. 58
Table 2.2. Nutrient (N, P, K) concentration of perennial crops from Propheter et al. (2010). 59
Table 2.3. Total nutrient credits in dollars per dry ton of perennial energy crop. 60
Table 2.4. Feedstock yields and prices for biofuel crops in Kansas. 61
Table 2.5. Sustainability index for the different bioenergy cropping systems in Manhattan Kansas. 62
Table 3.1. Crop residue management and fertilizer effects on sand-free water stable aggregates and total carbon inside associated with the aggregates after 24 years experiment (P-Values). 110
Table 3.2. Statistical significance of crop residue management and fertilizer N treatment effects on selected soil characteristics after 24 years experiment (P-Values). 111
Table 3.3. Statistical significance of crop residue management and fertilizer N treatment effects on grain and residue yields of different after 24 years experiment (P-Values). 112
Table 4.1. Characteristics of the various fields. 159
Table 4.2. Statistical analysis for within field SOC, comparison between triangle, depth and their interactions. 160
Table 4.3. Statistical analysis for infield bulk density, comparison between triangle, depth and their interactions. 161
Table 4.4. Classical statistical analyses of within triangles variability of SOC, for composites depth increments; 0-30 and 0-75 cm in various fields from northeast Kansas. 162
Table 4.5. Coefficients of variation in SOC based on field stratification. Test results are from three different homogeneity tests of variance (Levene-Test, Brown-Forsythe-Test and Barlett-Test). Test results were declared significant when at least two or all three test results were significant. 164
Table 4.6. Coefficients of variation (%) in SOC based on 3m triangle sampling design at major soil type from seven different fields in Northeast Kansas. Test results are from three

different homogeneity tests of variance (Levene-Test, Brown-Forsythe-Test and Barlett-Test). Test results were declared significant when at least two or all three test results were significant. 165
Table 4.7. Coefficients of variation (%) based on field stratification by soil type. Test results are from three different homogeneity tests of variance (Levene-Test, Brown-Forsythe-Test and Barlett-Test). Test results were declared significant when at least two or all three test results were significant. 166
Table A.1 Farm chemical inputs. 170
Table A.2. Statistical significance of bioenergy cropping systems, depth and their interaction on yield, MBC, MBN and root biomass. 171
Table A.3. Effects of different bioenergy cropping systems on soil microbial community after three growing seasons 175
Table A.4. Effects of different bioenergy cropping systems on soil microbial community after four growing seasons 176
Table A.5. Effects of different bioenergy cropping systems on soil microbial community after five growing seasons. 177
Table A.6. Sustainability index for a range of bioenergy cropping systems in Manhattan, KS: An alternative classification. 178
Table A.7. Economic return between different bioenergy cropping systems. 179
Table B.1. Relative fractional abundance of different microbial groups at different depths. Comparison between residue removal (0 X) maintenance (1 X) and addition (2 X). 182

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Dedication

In memory of Khadydiatou Dembelé AKA Diatou Diop and Fary Diop

Chapter 1 - Introduction

Harvesting crop residues, especially of cereal crops, is considered by industry as one of the sources of biofuel feedstock. However, soil quality and the ecosystem services they provide could be reduced if crop residues are harvested regularly. Consequently, harvesting crop residues would have strong adverse impacts on soil quality. The demand for bioenergy as a means to provide clean energy that can potentially offset future petroleum needs is the driving force behind the move to harvest crop residues (Ragauskas et al., 2006). While producing renewable energy from biomass is necessary, impacts of harvesting crop residue on soil quality, agricultural productivity, and environmental quality must be carefully and objectively assessed. As we change from a feed/food system to fuel system, the goals and outcomes change. Will we remove all of the biomass in a given crop? Will we remove all of the biomass from every crop in the rotation? How do we manage cropping systems and biomass removal for biofuel feedstocks for a particular region? It is, therefore, important to understand the short and long term implications of extractive agricultural practices on sustainability of production and soil quality. What are the consequences for sustainability related to feedstock production? These are some of the few questions that will guide our analysis. The most efficient outcome occurs when crops are located where they are best suited to the local resource conditions.

The current paradigm for bioenergy feedstock is derived from corn grain. However, cellulosic fermentation allows for a much greater diversity of feedstock sources. Perennial grasses and annual forage crops may be more energy efficient and environmentally beneficial. Perennial grasses may offer lower inputs and greater adaptation to specific regions (Tilman et al., 2006). Perennial crops species have been reported to have higher nutrient use efficiency than annual crops. This results in lower fertilizer requirements by perennials. Reduced fertilizer use

results in lower N_2O emissions or contributions to greenhouses gases. The extensive root systems developed by perennial crops also create greater water use efficiency and lower risk of soil losses through erosion. It has also been reported that perennials have a negative greenhouse gas emissions potential compared with annual cropping systems (Robertson et al., 2000). Perennial grasses as a biofuel feedstock have the potential to improve soil quality, reduce erosion, improve water quality and wildlife habitat throughout the Great Plains and Midwest if areas now in annual crop production are converted to perennial grass production.

Sorghum's drought and temperature tolerance make it an ideal crop for bioenergy feedstock production on the Central Great Plains. The use of annual forage and grain crops, such as sorghum, also allow producers to maintain the diverse cropping systems necessary to minimize the impact of insects, diseases, and weeds often found in continuous monoculture cropping systems. Corn is also planted throughout the region under both dry land and irrigation. Although widely believed to provide significant contributions to the biofuel feedstock supplies, corn will not likely produce higher yields than forage sorghum in more arid environments (Table 1). Targeting biomass production for different agro-ecozones will optimize biomass production and environmental benefits. However, the analysis requires a systems approach to support existing and future policies for bioenergy production. Integration of agroenergy crops (Ragauskas et al., 2006) and a multifunctional production system offers the potential for development of sustainable bioenergy.

An additional issue must be addressed regarding sustainable biomass feedstock production systems. What are the impacts of biomass removal on soil, and other ecosystems services? These issues require systems research to determine the appropriate management strategies (species selection, cropping system, and harvest intensity) for sustainable and

compatible agro-ecosystems. To date there is no study explicitly linking biofuel and ecosystem services.

Many countries around the world have also set or are planning ambitious biofuel targets. The Energy Independence and Security Act of 2007 (EISA) mandates at least an annual production of 16 billion gallons of cellulosic biofuel in the US by 2022. Countries from the European Union also have a set target of 10% of their fuel transportation to come from biofuel by 2020. The combined impact of these targets on global food and feed markets as well as on the rate of agricultural expansion into virgin grasslands and forests needs to be analyzed in order to assess the likely impact of current or future bioenergy production. According to a recent review by the World Bank, the potential environmental benefits of biofuels including their impacts on biodiversity, air, water and soil qualities cannot be generalized and need to be assessed on a case-by-case basis, evaluating cropping and land use patterns as well as the type of crop used for biofuel production (World Development Report, 2008). Increasing the use of biomass for food, feed, energy and manufacturing purposes brings with it a potential for competition between various end-use streams (Wuppertal-Institut für Klima, 2007). An example of that can be seen in the recent increase in world cereal prices, which in part was induced by additional demand for agricultural output for biofuel production (FAO, 2007). Given the likely future impacts from climate change (IPCC, 2007a); a careful reflection is needed on the issue of how human needs can best be met from the available land area, without endangering its future productivity and ecological functions. Recent studies focusing on ethanol production show that the carbon emissions from the conversion of forests or grasslands to energy crops or for replacing food production area that has been converted to biomass crops, lead to higher greenhouses gases (GHG) emissions compared with fossil fuels over a period of 50 years or longer in most cases

(Fargione et al., 2008; Searchinger et al., 2008). The overall GHG balance and environmental impact of different bioenergy pathways is therefore strongly influenced by their effect on direct or indirect land use change from carbon rich land cover types (e.g. virgin grasslands or forests) to energy crops.

Literature review

In the last ten years, many papers have been written on the impacts of residue extraction for bioenergy on soil (Blanco-Canqui and Lal, 2009; Wilhelm et al., 2007; Wilhelm et al., 2004). However, none or very few authors cover sustainability strategies of the future bioenergy industry. Most research have focused on the interrelated tillage-residue-cropping management implications where the effects of residue management are mix up with those of tillage and cropping systems (Mann et al., 2002; Wilhelm et al., 2007; Wilhelm et al., 2004). There have been proposal of harvesting crop residue and dumping into the deep sediments as a way to mitigate the elevated carbon dioxide level in the atmosphere (Strand and Benford, 2009). This misguided thinking made many soil scientists and soil conservation policy experts to write a response and highlight seventeen ecosystem services that crop residue provide and the list is not exhaustive (Karlen et al., 2009). Strand and Benford (2009) considered plants' unique capacity to capture CO_2 and by dumping it into the deep ocean; the chemistry preventing its decomposition. The problem is more complex. Our research will make data on biomass available for energy on a sustainable basis for various potential energy crops. For bioenergy to become fully integrated into the U.S. economy, it must be economically, environmentally, and socially sustainable (Robertson et al., 2000). Sustainability depends on ensuring the long-term provision of an adequate food, feed, and fiber supply. Information about the sustainability of much higher domestic production of biofuels can help guide national and international policies concerning

energy, the environment, and agriculture. It can also help set priorities for research programs and improve the operation of the biofuel energy sector. This research will addresses the uncertainty surrounding the use of additional feedstocks, particularly, what types of feedstocks and, grown where, and with what implications for soil quality and sustainability; identify implications and priorities for further research. A wide array of feedstocks will lead to more geographic diversity, less resource pressure on any one location, and greater resilience to drought, pests, and other production shocks.

Aggregate stability and size distribution are two physical soil properties most sensitive to crop residue removal on soil quality (Arshad and Coen, 1992). Aggregate stability decreases with decreases in surface residue cover. Without residue cover, surface aggregates dispersion has been related to water erosion and runoff (Stern et al., 1991). While most studies have reported a large decrease in aggregate stability with increasing rates of crop residue removal (Blanco-Canqui et al., 2006) some have not (Karlen et al., 1994; Roldán et al., 2003). Soil carbon content has been suggested as a soil quality indicator because decrease in this parameter can be directly related to decreased water stability of both macro-and micro-aggregates (Tisdall and Oades, 1982).

Wilhelm et al. (2004) reported soil compaction with harvesting of residues. The removal of residues impacts soil compaction in 2 ways; removal of organic matter on or near the soil surface and the increased field traffic during collection and removal. Soil organic matter can help soil resist the huge compactive forces of modern tillage and harvest equipment. The impacts of organic matter on reducing soil compaction are important but difficult to quantify as review by (Soane, 1990). Generally, there is a direct relationship between soil-incorporated organic matter

content and the stability of soil structure and an opposite relationship with soil bulk density. However, the ability of surface residue to buffer force of wheel traffic may be limited.

Crop residue removal also influences the dynamics of soil microorganisms. (Karlen et al., 1994) reported higher fungal biomass in soils with 200% of residue cover compared with a total residue removed control, and the decrease in fungal biomass with residue removal partly explained the lower aggregate stability in bared soils. Higher microbial activity stabilizes soil aggregates by producing organic binding agents (Wright and Upadhyaya, 1996).

Research Study

Chapter 1-Measuring soil sustainability: Index for bioenergy cropping systems

Our objectives are (1) to evaluate soil physical, chemical and biological properties under different bioenergy systems to assess soil sustainability. (2) Quantify above and belowground biomass of different energy crops. (3) Combine data to develop a soil sustainability index that includes key soil parameters for potential bioenergy crops (SSI).

Chapter 2-Soil aggregate C, N and microbial parameters as affected by 24years of differing levels of crop residue and fertilizer input.

Aggregation is a major soil process, which controls the flow of energy and the cycling of matters in soil. Aggregates can also serve as habitats for the large community of soil microorganisms. Understanding the relationship between aggregates and the community of soil microorganisms is crucial for the long-term stability of ecosystems and the services that they provide. Long term data provide an excellent database to evaluate the sustainability of the soil resource under a bioenergy cropping system.

Chapter 3-Understanding spatial variability-Measuring and monitoring soil carbon.

Measuring and monitoring soil carbon is an important issue for sustainable soil use, protection and management. Key research needs in bioenergy include finding ways to lower adoption barriers for bioenergy cropping system. This includes developing information related to SOM status, and applying the information to develop better knowledge and tools for using ecological processes to enhance bioenergy production.

The results from this research will provide a more complete understanding of biomass removal impacts on soil quality and as a result ecosystem services. This study will provide the basis for recommendations which will pertain to which species are most appropriate for the region and how these crops need to be managed to maintain soil carbon levels.

References

Arshad M.A., and G.M. Coen. 1992. Characterization of soil quality: physical and chemical criteria. Am. J. Alt. Agric. 7:25-31. DOI: doi:10.1017/S0889189300004410.

Blanco-Canqui H., and R. Lal. 2009. Corn stover removal for expanded uses reduces soil fertility and structural stability. Soil Sci. Soc. Am. J. 73:418-426. DOI: 10.2136/sssaj2008.0141.

Blanco-Canqui H., R. Lal, W.M. Post, R.C. Izaurralde, and L.B. Owens. 2006. Corn stover impacts on near-surface soil properties of no-till corn in Ohio. Soil Sci. Soc. Am. J. 70:266-278. DOI: 10.2136/sssaj2005.0137.

Fargione J., J. Hill, D. Tilman, S. Polasky, and P. Hawthorne. 2008. Land clearing and the biofuel carbon debt. Sci. 319:1235-1238. DOI: 10.1126/science.1152747.

Karlen D.L., N.C. Wollenhaupt, D.C. Erbach, E.C. Berry, J.B. Swan, N.S. Eash, and J.L. Jordahl. 1994. Crop residue effects on soil quality following 10-years of no-till corn. Soil Till. Res. 31:149-167. DOI: Doi: 10.1016/0167-1987(94)90077-9.

Karlen D.L., R. Lal, R.F. Follett, J.M. Kimble, J.L. Hatfield, J.M. Miranowski, C.A. Cambardella, A. Manale, R.P. Anex, and C.W. Rice. 2009. Crop residues: The rest of the story. Environ. Sci. Tech. 43:8011-8015. DOI: 10.1021/es9011004.

Mann L., V. Tolbert, and J. Cushman. 2002. Potential environmental effects of corn (Zea mays L.) stover removal with emphasis on soil organic matter and erosion. Agric. Ecosys. Environ. 89:149-166. DOI: Doi: 10.1016/s0167-8809(01)00166-9.

Ragauskas A.J., C.K. Williams, B.H. Davison, G. Britovsek, J. Cairney, C.A. Eckert, W.J. Frederick, J.P. Hallett, D.J. Leak, C.L. Liotta, J.R. Mielenz, R. Murphy, R. Templer, and T. Tschaplinski. 2006. The path forward for biofuels and biomaterials. Sci. 311:484-489. DOI: 10.1126/science.1114736.

Robertson G.P., E.A. Paul, and R.R. Harwood. 2000. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. Sci. 289:1922-1925. DOI: 10.1126/science.289.5486.1922.

Roldán A., F. Caravaca, M.T. Hernández, C. Garcia, C. Sánchez-Brito, M. Velásquez, and M. Tiscareño. .2003. No-tillage, crop residue additions, and legume cover cropping effects on soil quality characteristics under maize in Patzcuaro watershed (Mexico). Soil Till. Res. 72:65-73.

Searchinger T., R. Heimlich, R.A. Houghton, F. Dong, A. Elobeid, J. Fabiosa, S. Tokgoz, D. Hayes, and T.-H. Yu. 2008. Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. Sci. 319:1238-1240. DOI: 10.1126/science.1151861.

Soane B.D. 1990. The role of organic matter in soil compactibility: A review of some practical aspects. Soil Till. Res. 16:179-201. DOI: http://dx.doi.org/10.1016/0167-1987(90)90029-D.

Stern R., B.E. Eisenberg, and M.C. Laker. 1991. Correlation between micro-aggregate stability and soil surface susceptibility to runoff and erosion. South African J. Plant Soil 8:136-140. DOI: 10.1080/02571862.1991.10634821.

Strand S.E., and G. Benford. 2009. Ocean sequestration of crop residue carbon: Recycling fossil fuel carbon back to deep sediments. Environ. Sci. Tech. 43:1000-1007. DOI: 10.1021/es8015556.

Tilman D., J. Hill, and C. Lehman. 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. Sci. 314:1598-1600. DOI: 10.1126/science.1133306.

Tisdall J.M., and J.M. Oades. 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33:141-163. DOI: 10.1111/j.1365-2389.1982.tb01755.x.

Wilhelm W.W., J.M.F. Johnson, D.L. Karlen, and D.T. Lightle. 2007. Corn stover to sustain soil organic carbon further constrains biomass supply. Agron. J. 99:1665-1667. DOI: 10.2134/agronj2007.0150.

Wilhelm W.W., J.M.F. Johnson, J.L. Hatfield, W.B. Voorhees, and D.R. Linden. 2004. Crop and soil productivity response to corn residue removal. Agron. J. 96:1-17. DOI: 10.2134/agronj2004.1000.

Wright S.F., and A. Upadhyaya. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci. 161:575-586.

World Development Report. 2008. Biofuels: The Promise and the Risks. pp. 70-71.

Wuppertal-Institut für Klima U., Energie. 2007. Towards a sustainable biomass strategy: What we know and what we should know ; a discussion paper of the Wuppertal Institute Wuppertal-Inst. für Klima, Umwelt, Energie.

Chapter 2 - Measuring Soil Sustainability: Index for Bioenergy Cropping Systems

ABSTRACT

To ensure the sustainability of the soil resource under a bioenergy cropping system, a field experiment was conducted in northeast Kansas. We tested the effectiveness of eight bioenergy plant species grouped between annual and perennial crops. Our main objective was to determine the sustainability of bioenergy cropping systems. There was significantly greater soil structural stability plus greater root biomass under the perennial crops but greater aboveground biomass for the annual crops. Differences in soil carbon measured to 1.2 m were not significant between energy crops after five years. Assessment of soil microbial diversity can be a sensitive indicator to management and can be used to illustrate the effect of management decisions on productivity and soil quality. Soil microbial community composition was assessed using phospholipids fatty acid (PLFA) analysis. To assess the sustainability of bioenergy feedstocks, there is an urgent need to develop a transparent unbiased method to identify possible change in soil characteristics under bioenergy cropping practice. Although the methods and sustainability criteria used in this investigation provided key insights about different feedstocks, it is difficult to assess how well they will predict the long term sustainability of the soil given the short-term of the project.

Introduction

Biofuel feedstocks from grains and cellulosic material are gaining attention as part of the U.S. portfolio of solutions to address climate change issues and improve energy security. Under the Energy Independence and Security Act (EISA) of 2007, the Renewable Fuel Standard program ((RFS2), 2010) lays the foundation for achieving significant reduction in greenhouse

gas (GHG) emissions from the use of biofuel (U.S.EPA, 2011). As biorefinery conversion technologies become commercial, major concerns about the sustainability of the soil resource in bioenergy production system are emerging. A clear understanding of the sustainability risks for soil resources is needed within the US and globally. It is essential to develop metrics that assess soil health as well as the biomass production of different cropping systems. Sustainable cellulosic bioenergy production systems should include a range of potential energy crops because the environmental, economical and societal consequences will depend on which crops are grown where and how they are managed. The long-term sustainability of biofuel energy crops resources will depend on how well we research, plan and allocate resources. So assessing the economic, ecological and environmental aspects together is a necessary part of determining biofuel feedstocks' viability. This approach can highlight the tradeoffs involved in making choices about which bioenergy crops need more attention. The perceived benefits of biofuel (e.g. local availability, energy independence, greenhouse gas reduction, economic and rural development) must be evaluated against the potential stress to the environment (e.g. habitat and biodiversity lost, increase GHG emissions) cause by an intensive global biofuel program. Biofuels were thought as a green alternative to fossil fuel; however, Fargione et al. (2008) argue that the energy savings gained from biofuels depend heavily on where and how the energy crops are produced. If rainforest, peatland, savannas, or grasslands are converted into agricultural use for the production of biofuels, more CO_2 could be released than the annual GHG reductions biofuels displace fossil fuels. Searchinger et al. (2008) wrote that the GHG savings by substituting biofuels for fossil fuel could be negated if biofuels are produced from edible crops or from perennial grasses grown on lands formerly used for corn production. Both studies based their calculations on the assumption that crop yields and the biofuel demand are met by

continued increased in agricultural area. Other scientists are more optimistic recognizing that land use is a dynamic process, influenced by biophysical, demographics, economic, technological and social forces (Kline et al., 2009). The global economic models employed by Searchinger et al. (2008) and others that attribute widespread deforestation to biofuels have not been corroborated by empirically observed land use changes (Kline et al., 2009). (Kline et al., 2009) wrote that adequate land is available for energy crops using previously cleared lands which benefit both human livelihood and the environment while reducing pressures on forests. Subsequently, a report from the MIT Joint Program on the Science and Policy of Global Change found that: an aggressive global cellulosic biofuels program could contribute substantially to future global-scale energy needs, but could have significant unintended environmental consequences (Melillo et al., 2009). Their study uses a global modeling system that links economic and biogeochemistry data and examined the effects of direct and indirect land use on GHG emissions as production of biofuels increases over the 21st century. One of their main findings was C loss stemming from the displacement of food crops and pastures for biofuels crops may be twice as much as the CO_2 emissions from land dedicated to biofuels production. The study also predicts that increased fertilizer use for biofuel production will increase N_2O emissions. Interest on producing biofuels at the global scale offers the opportunity to design ways to select locations and management plans that are best suited to meet human needs while protecting biodiversity and the environment.

The development of a sustainable bioenergy cropping system is triggered by escalating energy demand and the need to find alternatives to fossil fuel achieve energy security. Despite the significant body of knowledge coming from the various fields of agricultural (soil sciences, crop sciences, agronomy), biological, engineering, economical and social sciences, there is little

integration of this crucial information. Gasparatos et al. (2011) proposed the use of ecosystem services as a consistent language to compare biofuel's diverse trade-offs and to facilitate the integration of biofuels' fragmented knowledge to assess sustainability. Using bioenergy can be beneficial to achieve environmental objectives to reduce CO_2 emissions compared to fossil fuels and support rural development. In the context of global climate change, the agricultural sector could contribute much to climate change mitigation by providing bioenergy to substitute fossil fuel (Smith and Olsen, 2010; Werner et al., 2012). Modern agriculture, while providing most of our vital commodities comes at the high cost of soil, air and water quality degradation, reduced ecosystem services and increased agrochemical use (Power, 2010). Including bioenergy crops in agricultural landscapes brings the opportunity to reduce these costs by providing ecosystems services, such as carbon sequestration, reduced soil erosion and increased water holding capacity (Robertson et al., 2008; Tilman et al., 2006). Innovative management systems (e.g. reduced disturbance and intensified crop rotations) such as those used in southern Brazil today, hold promise for a more viable and environmental friendly cropping system (Delgado et al., 2011; SÁ et al., 2009).

McBride et al. (2011) asserted that indicators are needed to assess environmental sustainability of bioenergy systems. Heink and Kowarik, (2010) defined environmental indicators as environmental metrics that provide information about potential or realized effects of human activities on environmental phenomena of concern. The United States, the European Union and many countries around the world have enacted policies that call for the expansion of the biofuel sector and increased use of liquid fuels. However, no consensus have yet emerged on what experts consider as critical sustainability indicators (Buchholz et al., 2009). Therefore, the legislative interest to support sustainable biofuel production must give rise to a coordinated

research effort, in which soils should occupy a central part. Soil is at the center of every major global grand challenge, from food security, protecting biodiversity, ecosystem services, and environmental quality to climate change. Therefore, a major part of meeting those challenges involves managing the soil. The long-term sustainability supply of biofuel feedstocks around the world will depends on how well we research, plan and anticipate the implications of biofuel feedstocks on soils and the environment.

Sustainability at the site level includes plant production (both above- and below-ground) inputs, and soil. Sustainability analysis of bioenergy cropping system also requires information on plant roots, however there is still little information on the rooting system of potential energy crops (Frank et al., 2004; Liebig et al., 2008). There is a growing research interest on the significance of energy crop with regards to carbon accumulation (Cannell, 2003; Monti and Zatta, 2009; Zan et al., 2001) and the root biomass as a significant contributor to carbon sequestration (Rasse et al., 2005; Rice et al., 1998). In addition, Garten and Wullschleger, (2000) showed that root biomass of switchgrass reflect soil C accumulation.

There have been many studies involving indices for soil quality around the world. Most of them are developed around agro-ecosystem soils from: U.S. (Andrews et al., 2002; Glover et al., 2000; Hussain et al., 1999; Karlen et al., 1994; Liebig et al., 2001), Southeast Asia (Kang et al., 2005; Masto et al., 2007; Mohanty et al., 2007; Sharma and Arora, 2010), China (Li et al., 2013; Wang and Gong, 1998), Africa (Erkossa et al., 2007), Europe (Koper and Piotrowska, 2003) and Latin America (Alvarenga et al., 2012). Other are developed around soils from natural and pristine environments from: Europe (Armas et al., 2007; Bastida et al., 2006; Trasar-Cepeda et al., 1998; Zornoza et al., 2007), U.S. (Burger and Kelting, 1999), Brazil (Freitas et al., 2012) and China (Pang et al., 2006). A few indices are developed for polluted soils, from:

Canada (Bécaert et al., 2006), Italy (Puglisi et al., 2006; Puglisi et al., 2005), UK (Dawson et al., 2007), and China (Chen et al., 2005). The objective of these metrics is to reduce soil degradation and a general assumption is indices can contribute in monitoring ecosystem status. Most of the time the status of soil characteristics and the risk of negative effect on them are the central concept of soil quality index development.

Examining soil sustainability requires first the answer to two questions: (1) how should sustainability be measured and monitored? (2) Which indicators are most useful and for what purpose? Larson and Pierce (1991) proposed that a minimum data set should be adopted to assess the health of our soil around the world. Basic soil quality indicators should be sensitive to management and climate (Doran and Parkin, 1994). Assessment microbial biomass and mineralizable C and N through time should be measured (Rice et al., 1996). Filip (2002) considered that physical and physic-chemical parameters are of little use since they alter only when the soil undergoes dramatic changes. On the contrary biology and biochemical parameters are sensitive to slight modifications that soil can undergo in the presence of any stressing or disturbing agents. Therefore, whenever the sustainability of soil natural function and the impact of soil different uses have to be evaluated; key indicators must include biological and biochemical parameters (Nannipieri et al., 1990). Since we cannot use all ecosystem or attribute of soil quality, there is a need to select specific indicators having high discriminating potential and high value to account for actual soil quality status of agroecosystem (Karlen and Andrews, 2000).

The need to identify soil microbial community parameters which are the engine of many ecosystem services, is becoming more urgent, due to the desire to integrate microbial community parameters within ecosystem models (Allison and Martiny, 2008; Stromberger et al., 2011; Wall

et al., 2010; Wieder et al., 2013). Soil microorganisms are key attributes of long-term sustainability of ecosystems in that they control the cycling of nutrients in ecosystems through mineralization, immobilization and decomposition (Nannipieri et al., 2003). Almost no study has been conducted in comparing perennial bioenergy crops and traditional annual crops to determine how these different cropping systems affect soil microbial community structure and other soil biochemical characteristics, and soil structural stability.

Phospholipids fatty acids (PLFAs) are potentially useful biomarkers for assessment of the soil microbial community. The concentration of total PLFA expressed provides quantitative information on the viable soil microbes (White and Rice, 2009; White et al., 2007). The use of these compounds to identify specific subgroups of microorganisms, e.g. gram-positive or gram-negative bacteria, mycorrhizal or saprophytic fungi and actinomycetes is attractive for profiling the abundance of microbial groups. This technique has been evaluated and successfully used to evaluate microbial communities in heavy metal polluted soils (Hinojosa et al., 2005). However, the only index that takes into account the PLFAs was established by (Puglisi et al., 2005), but their soil alteration index based on a sole technique, is not enough to evaluate the sustainability of the soil resource. Soil microorganisms can further be examined by comparing the changes in microbial parameters through time, reflecting the changes in soil quality due to changes in land use or management. Understanding the integrated environmental and management factors that drive the microbial community structure patterns and associated soil properties under various energy crops will help improve our capacity to predict the sustainability of these cropping systems.

Our objectives were to evaluate soil physical, chemical and biological properties under different bioenergy systems to assess soil sustainability. Quantify root biomass of different energy crops. The independent study described here summarizes the ongoing research in north-east Kansas that is quantifying differences in above and belowground biomass, soil chemical, physical and biological properties with production of biofuel feedstock crops.

Materials and Methods

Study Site: This study was conducted on an ongoing bioenergy feedstock field study established at a dryland location in northeast Kansas; the Kansas State University Agronomy Research Farm at Manhattan. The soil type was an Ivan, Kennebec, and Kahola silt loam complex (fine-silty, mixed, superactive, mesic Cumulic Hapludolls) (200g kg^{-1} clay, 700g kg^{-1} silt, and 100g kg^{-1} sand). Plots were established in soybean residue in 2007 (Propheter and Staggenborg, 2010). The experiment was a randomized complete block design with four replications. Annual crops studied were corn (*Zea mays*) grown continuously and in rotation, three different type of sorghum (*Sorghum bicolor*); grain sorghum, sweet sorghum, and photoperiod sorghum. The photoperiod sorghum never produces grain at this latitude because of the short day length. The perennial crops were miscanthus (*Miscanthus giganteus*) originated from southeast Asia, two varieties (Kanlow and Ceres) of switchgrass (*Panicum virgatum*) a native warm-season grass indigenous to the North American tallgrass prairie and big bluestem, another warm-season grass that characterized the tallgrass prairie of North America. Switchgrass Ceres was planted in 2008, a year after experiment was established. Soybean was planted for rotational purposes but was not part of the evaluation. Soybean was established within the plot area as rotation in subsequent years for rotated corn and for all sorghums. Continuous corn and rotated corn were planted after the second week of April every year, while

sorghums were planted after the second week of May ever year. Switchgrass and big bluestem were established in the middle of May, 2007 while miscanthus was established in mid-June of the same year. All crops were no-till planted throughout the study. For fertilizer application rates and herbicides used for each crop, (appendix A.1).

Soil Sampling and Analysis

Soil samples were taken in 2009, after the third growing season, during the crops physiological maturity. A bulk soil sample was collected from each treatment from the 0-5, 5-15, 15-30 30-45, 45-60, 60-75, 75-90 and 90-120 cm depths for the determination of the soil organic C, total soil N, and bulk density. Three samples were randomly taken from the 0-5; 5-15 and 15-30 cm layers of each plot for aggregate distribution and stability. Microbial community analyses were conducted using Phospholipid Fatty Acid Analysis (PLFA), samples were collected from the 0-5 and 5-15 cm depths. Root biomass analysis as well as aboveground biomass production was investigated to obtain a total biomass production. In late September and October for annual and perennial crop respectively, at plant physiological maturity; three soil cores (8cm diameter by 120 cm depth) were collected in each replicate by a Giddings hydraulic probe. The probe was first positioned at the center of the plant (p1), (p2) at the midway between two rows and (p3) at the midway between the first two sampling position (p1 and p2). Once extracted, root biomass was quantified in increments of 0-5, 5-15, 15-30, 30-45, 45-60, 60-75, 75-90 and 90-120 cm for a total of 864 subsamples. The root biomass per unit soil volume was obtained using a root washer that can accommodate relatively large soil samples for washing (Benjamin and Nielsen, 2004). The root washer has a rotary design and can accommodate up to 24 samples (100 mm diameter by 240 mm long) at one time, the filter body consists of a stainless steel screen cylinder with 300µm openings. After separating the roots from soil, living root were

manually picked from the soil organic debris using Teflon tweezers, rinse soil and detritus through a sieve then placed into separate containers. Picking the roots in the very top surface layers of soil was more tedious because it has more roots and the amount of organic debris was largest. Root samples from deeper soil horizons had fewer roots and much less debris than the surface samples and therefore were cleaned relatively easily. Root samples were dried at 65ºC for 48 h followed by weighing. Root dry weight per area (RDW; $Mg.ha^{-1}$) was determined, assuming that each of the three sampling positions (P1, P2 and P3) was representative for a specific area. Since we did not sampled P2 and P3 in the non row crops, the root samples from the same position P1 and depths were compared between the different energy crops. Aboveground biomass was sampled and harvested at the end of September for corn and the last week of October for sorghum. Perennial crops were sampled and harvested on the last week of November after the plants went into dormancy. Aboveground biomass determination for the annual crops was based on harvest of two 4.6 m rows located at the center of the plot at a stubble height of 10 cm. Total wet biomass was obtained first and corn ears and sorghum heads were separated from the crop residue. Wet grain weight was measured and dried at 65ºC for 48 h followed by weighing. Perennial grasses were sampled at the center of each plot in a 1.2 by 10.7 m swath using conventional hay equipment at 10 cm plant height. Harvested biomass was collected and weighed. A subsample was further dried at 65ºC for 48 h followed by weighing again for dry matter determination.

Soil Organic C and N:

Soil organic C and N were determined from soil samples that were dried and ground with a mortar and pestle to pass through a 500-µm sieve eliminating identifiable root particles and

plant and animal debris and analyzed on a Carlo-Erba C and N analyzer (Thermo Finnigan Flash EA 1112 Series, Milan, Italy). Soil C concentrations were converted to soil C stocks and expressed in Mg C ha^{-1} by multiplying bulk density and layer thickness.

Microbial Biomass Carbon-Fumigation Incubation (MBC-FI)

Microbial biomass C and N represents the amount C and N in the microbial biomass. Microbial biomass C and N was done by the chloroform-fumigation procedure (Jenkinson and Powlson, 1976). Duplicate 25 g field moist equivalent samples were used (Rice et al., 1994; Rice et al., 1996). Samples were pre-incubated for 5 days. At the end of the pre-incubation, one of the samples were fumigated with chloroform for 24 h inside desiccators at room temperature. At the end of the 24 h, each desiccator were connected to a vacuum pump hose and chloroform residue was evacuated 10 times for 3 minutes. Both fumigated and unfumigated samples were placed inside 940 mL mason jars containing enough water to maintain a highly humidified environment. Jars were closed tightly and incubated for 10 days at 25ºC. At the end of the incubation period, CO_2-C was measured using a Shimadzu GC-8A gas chromatograph (Shimadzu Scientific Instruments Inc., Columbia, MD) and N was extracted by adding 100 mL of 1M KCl. The extractant was analyzed for NH_4^+ and NO_3^- using a calorimetric analysis on an autoanalyzer (Alpkem Corp., Clackamas, OR).

Soil Aggregates

Soil aggregation was assessed according to the methods of Mikha and Rice (2004). Soil fractions were separated by slaking air-dry soil followed by wet-sieving (Elliott, 1986) through a series of four sieves (2000, 250, 53, and 20µm). Air-dried soil (50 g) from 0-5, 5-15, and 15-30 cm depths were placed on the top of the sieve of each nest. To slake the air-dried soil, 1 L of water was rapidly added until soil was covered with water. The samples were submerged in

water for 10 min following the 10 min of wet sieving. Four aggregate size classes were collected from each treatment >2000, 250-2000, 53-250, and <20 μm diameter. Water stable aggregates were dried and a subsample were used to determine sand content of each fraction Mikha and Rice (2004). Large macroaggregates were defined as >2000 μm, small macroaggregates 250-2000 μm, microaggregates 250-53 μm, and silt plus clay by <53 μm size fraction. The sand-free water stable aggregates was calculated using the mass of aggregated soil remaining on the sieve after 10 min slaking and the 100 g soil used at the beginning. The initial and final weights of aggregates were corrected for sand (>53 μm). The proportion of SFWA differed significantly among different energy crops. Total C and N, were determined in each sand-free, water-stable aggregate size fraction.

Microbial Community Structure: Phospholipid Fatty Acid Analysis

Phospholipids lipid fatty acids (PLFA) analysis was determined following a modification of the methods by Bligh and Dyer (1959) and White et al. (1997). Lipids were extracted with single phase chloroform: methanol: phosphate buffer solution (Blight and Dyer, 1959) for 2 h from 5 g of freeze-dried soil. Total lipid extracts were separate into neutral lipids, polar lipids, and glycolipids using preconditioned silica gel disposable extraction columns (J.T. Baker, Phillipsburg, NJ, USA). Neutral and polar lipids are subject to alkaline methanolysis to cleave the fatty acids from the glycerol molecule replacing it with methyl groups thus creating fatty acid methyl esters. FAMEs were analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer with a DB5-MS column (30 m × 250 μm i.d. × 0.25 μm film thickness). Helium was the carrier gas (1.0 mL min^{-1} constant flow). The temperature program was: 50 to 170°C at 20°C per min^{-1}; from 170 to 270°C at 2°C min^{-1}. The injector temperature was 220°C. Analysis was conducted in the electron impact (70 eV) mode and mass spectrometer scanning m/z^{+} was

from 200 to 400. Bacterial acid methyl esters mix (BAME; Matreya 1114) was used to identify peaks. Tentative assignments of methyl ester peaks not present in the BAME mix were made by mass spectral interpretation. The internal standard methyl nonadecanoate was used to quantify the data. Peaks are identified using retention times of fatty acid standards and by comparing spectra from a library (Wiley 138K mass spectral database). Samples peak are quantified based on comparison of the abundance with an internal standard nonadecanoic acid methyl ester (19:0) in terms of nmol g^{-1} dry soil or mol %. Fatty acids are grouped into Gram positive bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative (16:1w7, Cy17:0, 18:1w7), actinomycetes (10Me18:0 and 10Me17:0), and fungi (18:3ω6,9,12; 16:1w5 and 18:1ω9c) (McKinley et al., 2005).

Statistical analysis

Treatment effects on measured variables were tested by analysis of variance and all statistical procedures were carried out using SAS 9.2 SAS institute, Cary NC (Institute, 2009). The means were compared using Tukey's HSD. Unless otherwise stated, all differences discussed are significant at the $P < 0.05$ probability level. We did not see any treatment differences in the soil layers below 5 cm.

Sustainability index

The proposed sustainability index uses five parameters for its calculation. Key sustainability parameters associated with biofuel production, from an ecosystem services perspective were selected for the index. To our knowledge, there is no study linking the production of biofuel and ecosystem services. The logic that guides our choice of parameters was, we selected indicators that gave us insights on some of the ecosystem processes and services that society care about when dealing with bioenergy cropping systems and understand

bioenergy sustainability. Total biomass production gives insights on the provisioning services that can go to the refinery, the amount of carbon that can be sequestered in soil and, along with total energy input gives insights on the profitability of the bioenergy cropping system. Also, crop species that have the highest biomass yield may require less land because the cropping area needed for cultivation would be minimal. With the high cost of fertilizers, feedstocks that have the lowest energy requirement may have huge economic implications; plus crops species that have the greatest nitrogen use efficiency could be the most viable because the carbon footprint associated with the fertilizer would be minimal. Soil structural stability gives insights on how much each bioenergy cropping systems will conserve the soil resource. The measurement of soil structural stability also reflects the resistance of soil to erosion; favors high infiltration rates and appropriate aeration for root growth but also regulates the flow of energy and matter within the soil ecosystem. Soil carbon sequestration provides information on GHG mitigation and soil quality. Lastly, soil microorganisms are drivers of soil nutrients cycling, contributing to critical ecosystem functions such as decomposition, disease suppression, regulation of plant growth and primary productivity (Wurst et al., 2012) and in turn affect many of the ecosystem services. We adopted a standardization procedure to estimate the changes in outcomes causes by different bioenergy cropping systems. Basically, we took the opportunity of one useful property of the standard deviation, which is unlike the variance; it is expressed in the same unit as the data. We estimated a sub-index for each parameter based on the objective quantitative data collected in the field, subtracted the mean within each sustainability parameter; the whole was then divided by the standard deviation. Soil sustainability was calculated as cumulative changes in the different sub-indices (Andrews and Carroll, 2001; Inskip, 2005; Karlen and Stott, 1994).

Results

Biomass production

Aboveground biomass. There was significant variability of biomass production among feedstock every year (Fig. 2.1). Perennial biomasses were significantly lower ($p < 0.0001$) at the beginning of the experiment which was attributed to time required for establishment (see ANOVA, Appendix A.2). Aboveground biomass from year to year also varied partly in response to inter annual variation in temperature and precipitation (Appendix A.3 mean annual precipitation, Appendix A.4 maxima and minima temperatures during growing season). The grasses were more consistent in their production and, after the fifth year, had similar biomass yield as the annual energy crops. The most productive crops were photoperiod sorghum and sweet sorghum with yields close to 30 Mg ha^{-1} in the first two years.

Belowground biomass. Root dry weight (RDW) was significantly higher ($p = 0.0672$; see ANOVA in Appendix A.2) for the perennial energy crops compared to the annual crops in their third production year (Fig. 2.2). Total RDW in the profile to 120 cm was 14.4; 13.8 and 13.0 Mg ha^{-1} for switchgrass Kanlow, miscanthus, and big bluestem, respectively compared to 6.8; 5.5; 4.1 and 3.6 Mg ha^{-1} for rotated corn, photoperiod sorghum, grain sorghum, and sweet sorghum, respectively. Among the annual energy crops, RDW was significantly lower under sweet sorghum. Root distribution decreased with sampling depth for all energy crops. The root distribution (Fig. 2.3) for all annual crops exhibited a similar pattern of root distribution and had 90 % of their belowground biomass in the top 15 cm. The root pattern of the perennial grasses was more evenly distributed with depth. For sake of simplicity we used two annuals and two perennials energy crops in our comparison. Miscanthus had 30% of its root biomass below 30 cm, while switchgrass had 15% of its total root biomass below 30 cm. The average root biomass

from 30-120 cm was 66 and 11 times greater under miscanthus than sweet sorghum and photopheriod sorghum, respectively. Switchgrass K had 25 and 4 time greater root biomass below 30 cm than sweet sorghum and photoperiod sorghum, respectively. The lower rooting depth associated with the perennial energy crops may have positive ramifications with regards to C sequestration because of deeper deposition of C in the soil.

Soil microbial biomass

Soil microbial biomass C (SMBC) was significantly ($p < 0.0001$; also see Appendix A.2) affected by cropping systems (Fig. 2.4). Three years after establishment, SMBC decreased significantly under annual crops compared to the perennial crops in the 0-5 cm soil layer. In the soils under perennial bioenergy crops, SMBC varied from 86 to 183 µg C g^{-1}, whereas in the soils under the annual crops values ranged from 50 to 94 µg C g^{-1}. SMBC was lowest in soil under sweet sorghum.

Soil microbial biomass N (SMBN) was also significantly ($p = 0.03$; also see Appendix A.2) affected by the different energy crops with significantly lower values in annual cropping systems compared to perennials (Fig. 2.5). The values ranged between 5.83-12 µg N g^{-1} and 17-21 µg N g^{-1} in annual and perennial cropping systems respectively (Fig. 2.5). In all energy crops, SMBN was highest under switchgrass Ceres and lowest under sweet sorghum.

Soil microbial community structure

The response of soil microbial community to different cropping systems was accomplished using PLFA. Total PLFA, the sum of all the fatty acids that were identified in the soils, were significantly ($p < 0.0001$) higher in the perennial crops and showed a similar trend as microbial biomass C. To avoid redundancy total PLFA data are shown in Appendix A.3. In general, bacterial fatty acids (Gram-positive: i15:0, a15:0, i16:0, i17:0, a17:0) (e.g. Tables

Appendix 1.5, 1.6, 1.7) represented the highest proportion of total PLFA. The relative fractional abundance of Gram (+) bacteria calculated as the ratio between the measured biomarkers (i15:0, a15:0, i16:0, i17:0, and a17:0) and total bacteria, were significantly ($p=0.0073$) different between treatments and across years (Table 2.1). The proportion of Gram (+) organisms were significantly greater ($p = 0.0044$) in the annual cropping systems over all years compared to the perennial cropping systems (Fig. 2.6). There was a clear segregation between the annual and perennial energy crops with regards to their relative abundance of Gram (+) bacteria. No significant differences between the different energy crop were observed with regards to their relative abundance of Gram (-) (Table 2.1). However, when the concentrations of the specific PLFA attributed to Gram (-) among the different energy crops were compared throughout the years, significant differences were found ($p=0.0017$). The PLFA associated with the Gram (-) bacteria were less variable throughout the year. Analysis of the lipid biomarker from the total bacterial community indicated that there were no significant differences between the different energy crops (Fig. 2.8). In the surface soil layers total fungi were significantly (0.0063) reduced in soils under annual energy crops. The abundance of soil fungi is crucial in enhancing soil structure thus increasing carbon sequestration, as the abundance of arbuscular mycorrhizal fungi (AMF) and saprophytic fungi are often correlated with macro aggregation (Wilson et al., 2009). Therefore, soil management practices that increase or maintain soil fungi should be encouraged (Rice and Angle, 2003). There was a clear differentiation among cropping systems according to their total fungal biomass (Fig. 2.9). The fungal:bacterial biomass ratio determined directly from the measurement of fungus-specific and bacterium-specific PLFAs indicated significant plant effect ($p < 0.02$). The ratios were significantly higher in soil from perennial cropping systems than those in annual cropping soils (Fig. 2.10).

Soil structural stability

We restricted our analysis to the macroaggregates (>2000 μm and 2000-250 μm), since this fraction is sensitive to short term management. After 3 years, macroaggregate formation was greatly enhance in switchgrass and miscanthus compared to the other treatments (Fig. 2.11). Continuous corn and grain sorghum exhibited the lowest soil structural stability. Switchgrass Ceres was planted a year prior to our sampling for soil aggregate stability and therefore did not show good soil structural development.

Soil organic carbon

There was no significant (P>0.05) energy crop effect on soil organic C after 5 years averaging 260 Mg C ha^{-1} to a depth of 120 cm (Fig. 2.12). Total N also did not show any difference between the bioenergy cropping systems evaluated after 5 years.

Sustainability index

Although biomass production is an important indicator for bioenergy feedstock, the cost of cellulosic biomass in dollars per dry matter better illustrate the economic value of the bioenergy crop. The annual costs in dollars per metric ton for Kansas annual crops were obtained from the National Biomass Energy Report (Agricultural Marketing Service, 2013). The prices of grain and stover for all annual crops are listed in (Table 2.4). For the perennial crops, since almost no data are available in the literature, we use the following estimation:

According to a study on nutrient removal from this site (Propheter and Staggenborg, 2010), one ton of dry biomass of miscanthus, big bluestem and switchgrass K contents (9.6, 5.6 and 5.9 kg N); (0.8, 1.3 and 1.5kg P) and (11, 11.7 and 12.5 kg K) respectively (Table 2.2). Using the five years average (2007-2012) of fertilizer prices (Agricultural Prices National

Agricultural Statistics Service, 2013). Based on $2/kg N, 1.6/kg P and 1.75/kg K (Agricultural Prices National Agricultural Statistics Service, 2013) the fertilizer value equates to $11.55, $10.18 and $10.97 fertilizer replacement credit for miscanthus, big bluestem and switchgrass K (Table 2.3). If we add $20 profit value estimated from wheat straw (Wheat value, University of Wisconsin-Extension electronic publication), then the grower would be asking $32, $30 and $31/ton of dry biomass for miscanthus, big bluestem and switchgrass respectively. The buyer would need to consider the cost of harvesting, which varies depending on harvest method. Large bale harvesting are preferred (Brownell and Liu, 2011) since they are the cheapest approximately $30/ton (Wheat value, University of Wisconsin-Extension electronic publication). This figure leads us to a final price of $62/ton for miscanthus, $60/ton for big bluestem and $61/ton for switchgrass (Table 2.4). These results are close to price of ($65/ton) for large scale sources of cellulosic biomass (Sokhansanj et al., 2009).

Our energy estimates were calculated from farm chemical inputs based on established standards obtained from the American Society of Agricultural Engineering and the American Agricultural Economic Association (Nelson et al., 2009). Infield energy values were compared with regional no-till corn, sorghum; infield energy requirements for perennial crops were compared with energy values from GREET. Our energy estimates were consistent with literature evidences (Boehmel et al., 2008; Lewandowski and Schmidt, 2006; Smeets et al., 2009; Venturi and Venturi, 2003) for all our crops. Important considerations should include the time period for which yield data for each crop were collected, because prices and industry energy efficiency have changed over time. A second tentative of evaluating the different cropping system was performed by ranking the different sub-indices to obtain non parametric normalized values (appendix A Table A.6). The resulting ordinal sub-indices when cumulated consistently ranked

switchgrass and miscanthus at the top, while continuous corn and grain sorghum obtained the lowest rank. However, photoperiod sorghum ranks better than sweet sorghum and consistently follows rotated corn. The different bioenergy cropping systems were also evaluated based on their revenues and production costs (net economic return) by cumulating the sub-indices of revenues and energy saving (appendix Table A.7). Economics favored big blue stem, switchgrass K and rotated corn bioenergy cropping systems, while photoperiod sorghum and sweet sorghum showed no return at least for our location and conditions during the 5 yr study. Miscanthus, grain sorghum and continuous corn showed a net negative return. In the different bioenergy cropping systems studied, sustainability index was highest for switchgrass Kanlow and miscanthus, while continuous corn and grain sorghum showed the lowest values (Table 2.5)

Discussion

Maintaining a high level of soil health is the ultimate goal for a sustainable production system (Govaerts et al., 2007). Dedicated perennial energy crops hold the potential to provide diverse and abundant belowground microbial complexity and habitats than traditional annual crops. Some perennial (switchgrass and miscanthus) have more potential than others, for soil structural stability enhancement. The different bioenergy crops result in differences in (above and belowground), microbial biomass and community structure, and soil structure. These findings highlight the importance of considering the impacts of land use change on soil. Perennial cropping systems and annual cropping systems impacted aggregation with the perennial cropping systems resulting in greater macro aggregation. Greater aggregation should lead to improve soil parameters including infiltration and greater carbon storage (Rillig et al., 2007; Rillig et al., 1999; Wilson et al., 2009).

Perennial species in our study were associated with greater aggregation (Jastrow, 1987; Wilson et al., 2009). These differences among energy crops provided us with an opportunity to examine which factors were responsible for bringing about these changes in macroaggregation. We particularly focused on differences in key soil biochemical properties. All energy crops that we have investigated were host plant for AMF. AMF have been shown to differ in production of glomalin per hyphal length (Wright and Upadhyaya, 1996), in physiological and ecological traits (Giovannetti and Gianinazzi-Pearson, 1994) and in promoting aggregate stability (Schreiner and Bethlenfalvay, 1995). Perennial energy crops harboring a greater abundance of AMF could enhance macroaggregation as was shown in our study. Despite the similarities in abundance in AMF and fungal:bacterial ratio between perennial energy crops, we observed significantly greater macroaggregates formation in switchgrass Kanlow and miscanthus compared to big bluestem and switchgrass Ceres. This variation can partly be explained by the fact that aggregate formation is more likely linked to microbial activity than microbial abundance (Harris et al., 1966; Metzger et al., 1987). The production of extracellular polysaccharide from fungi, bacteria and root mucilages acting as a binding agent; therefore enhancing aggregate formation, is controlled by microbial activity rather than microbial abundance (De Gryze et al., 2005).

The soil microbial community structure consistently changed as an effect of different cropping systems. When comparing annual versus perennial cropping systems, there was a clear segregation in Gram (+) bacteria, total fungi and fungal:bacterial ratio. The Gram (+) dominated over the rest of community of soil microorganisms in the annual cropping systems, while the fungi dominated in the perennial cropping systems. Fungi might have been favored in the perennial cropping systems, perhaps due to an increase quantity of root exudates. Fungi are also favored in less disturbed system (Rice and Angle, 2003; Rice et al., 2004; Six et al., 2006; White

and Rice, 2009). These observations raise the question of whether microbial community compositional shifts will affect ecosystem processes and services under these bioenergy cropping systems. In addition, differences in microbial biomass were found between cropping systems. These evidences indicate that cropping system have an important role in regulating microbial community dynamics. Franzluebbers et al. (1995) noted that crop growth could play an important role in soil microbial community dynamic in competing with microorganisms for substrates and altering the spatial distribution of organic inputs from roots and residues. Results of many studies suggest that in agroecosystem soils, fungal biomass dominance leads to greater accumulation of stable sequestered carbon (Rice and Angle, 2003; Rice et al., 2004; Six et al., 2006; White and Rice, 2009). We want to be able to predict what future cropping systems are going to do to critical ecosystem services like carbon storage, soil structural stability and nutrient cycling. All these services are driven by the action microorganisms. All these microbial characteristics are very important and affect the ecosystem services delivered by the soil. It will be crucial to conduct the same measurement over time that allows us to detect differences due to management.

Results presented here provide an original contribution to the issue of sustainable bioenergy feedstock by (1) linking biofuel feedstocks production and ecosystem services (2) highlighting key biochemical variables possibly influencing the level of risk associated to certain feedstock (3) highlighting tradeoffs associated with attractive highly productive annual cropping systems (4) highlighting the possible policy response to these challenges.

Economic and environmental considerations.

Switchgrass, miscanthus, and big bluestem are considered to be promising sources of cellulosic ethanol but planting decisions rely on the expected economic return of the crop.

Perennial grasses are less expensive to produce because they do not have to be replanted each year. Also traditional annual crops tend to require more farm chemical inputs to control pest and maintain yields (Power, 2010). Some perennials show great promise as bioenergy crop at this site because they seems to be more consistent in their productivity and better adapted to the specific climatic conditions of our site and thus are less expensive to manage. Cellulosic ethanol is expected to be less expensive and more energy efficient than today's ethanol because it can be made from low cost feedstocks. Perennial energy crops may need innovative management techniques in order to be sustainable. Traditional annual energy crop such as corn and soybean, from which biofuel can be produced, have received considerable attention and investments; thus showed dramatic increases in productivity. But these crops are approaching their limits (Tester and Langridge, 2010); similar advances are possible in many perennial dedicated energy crops (Cox et al., 2006). Additionally, the perennial energy crops offer some environmental advantages compared to traditional annual crops. Perennial crops by enhancing soil structure would increase soil organic matter, reduce pollution of groundwater (with their more extensive rooting system); of surface water (by stabilizing soil against erosion). Perennial energy crops also, with their greater rooting depth may have some positive ramifications, this characteristic would help sequester large quantities of CO_2 playing a key role on climate change mitigation. High-yielding dedicated annual energy crops are also very important because they can help reduce the cropping area needed for cultivation. However, the ecological price may be high under certain annual energy crops if we do not adjust our management practices. Microbial biomass C, soil aggregate stability and other key soil biochemical parameters were consistently and significantly lower under one of our most productive feedstock (sweet sorghum). This high-

yielding annual crop may require some agronomic adjustments (intensified rotation, implement proper use of nutrients, farm chemical inputs).

The more efficient cropping system is the one that has the higher ranking (Table 2.5). The index ranked the bioenergy cropping systems: switchgrass Kanlow > miscanthus > big bluestem > sweet sorghum > rotated corn > photoperiod sorghum > continuous corn > grain sorghum. An alternative ranking consistently gave the same order, except, that photoperiod sorghum rank better than sweet sorghum. Perennial energy crops along with photoperiod sorghum appears to be more viable bioenergy cropping systems based on conditions maximizing productivity and protecting the soil resources. Additionally, photoperiod sorghum with its high biomass production and relative yield stability requiring less land; seems to provide less conflict with food security compared to rotated corn. In the present study, the calculations are based on key sustainability parameters associated to the production of biofuel: Annual biomass production of potential feedstocks from 2007 to 2012; energy requirement based from the farm chemical inputs; total soil carbon measured at establishment, three and five years after establishment; soil structural stability evaluated three year after establishment and total microbial biomass estimated from the total PLFA analysis. One of the main critics of index approach is, weighing of the individual parameters or indicators can be subjective (Stenberg, 1999); we tried to overcome that issue by standardizing all of our parameters in one of the most transparent and widely accepted method of standardization. The index was supported by key observations on soil biochemical parameters such as the PLFAs of different subgroups (AMF), sustainability predictor such as fungal:bacterial ratio or even consistent trends and shifts in microbial communities. Although the parameters selected have individual meaning, their integration within the index offers the opportunity of sustainability analyses for decision support. However,

the index could be management and site specific. For example, if one of the bioenergy cropping system being evaluated is replaced by another cropping system, this could change the sub-indices which will likely affect the index as well. Similarly, if climatic conditions change to the extent that soil C is affected or shift in the microbial community occurs; this will like affect the index. The choice of crop and management practices can also affect the structural stability of the soil, particularly for the cropping system requiring tillage, impacting the energy saving thus affecting the final index. Therefore, the main drawback of our soil sustainability index is, its age; it is very young. It has not been tested in different locations or under conditions other than those for which it was designed. Therefore, intensive coordinated efforts need to be carried out in potential major bioenergy feedstock belts around the world. Until the same measurements are conduct, under different climatic conditions, different management or choice of crop; to make sure that key indicators have not changed, or in what proportions changes have occurred, it would be prudent to adopt a conservative approach and assume that potential energy crops will not be replaced by trees or other grasses.

Conclusion

Our results show that SOC did not change over five years of complete removal across the different bioenergy crops. Also, there may be possibility of harvesting biomass for the production of biofuel under certain energy crops without adversely impacting the soil aggregate stability. The annual cropping system seems to be at risk and it will be crucial to find ways to return it to a state that is more capable of providing ecosystem services. The perennial systems do hold potential that should be further explored. The use of sorghum and corn grown in a rotation with soybean, were more effective for the production of bioenergy feedstock, but there are environmental benefits associated with the perennial cropping systems that may potentially merit the decrease in yield. The lower biomass productions associated with the perennial grasses were due to the time required for establishment. Our results highlight that research and development should be intensified for perennial energy crops but more research efforts is needed on the impacts of high-yielding energy crops such as sorghum on key soil characteristics. Annual crops in general need more research on how to return, maintain or even improve soil health for a bioenergy sustainable system. Perhaps by intensifying the rotation while keeping the soil disturbance low (e.g. Brazilian success), we can achieve, approach or be on a path of an annual sustainable bioenergy cropping system. Future research of these cropping systems for bioenergy feedstocks production should focused at enhancing the soil productivity. Our objective must be productive agroenergy systems that operate in concert with the natural environmental systems rather than polluting them.

References

(RFS2) R.f.s.p. 2010. Renewable fuel standard program (RFS2) regulatory impact analysis assessment and standards division, office of transportation and air quality, U.S. Environmental Protection Agency, Ann Arbor, MI.

Agricultural marketing service. 2013, in: USDA (Ed.), National biomass energy report, Ames, IA.

Agricultural prices national agricultural statistics service. 2013.

Allison S.D., and J.B.H. Martiny. 2008. Resistance, resilience, and redundancy in microbial communities. Proc. Natl. Acad. Sci. U.S.A. 105:11512-11519. DOI: 10.1073/pnas.0801925105.

Alvarenga C.C., C.R.d. Mello, J.M.d. Mello, A.M.d. Silva, and N. Curi. 2012. Índice de qualidade do solo associado à recarga de água subterrânea (IQSRA) na Bacia Hidrográfica do Alto Rio Grande, MG. Rev. Brasil. Ciênci. Solo 36:1608-1619.

Andrews S.S., and C.R. Carroll. 2001. Designing a soil quality assessment tool for sustainable agroecosystem management. Ecol. App. 11:1573-1585. DOI: 10.2307/3061079.

Andrews S.S., D.L. Karlen, and J.P. Mitchell. 2002. A comparison of soil quality indexing methods for vegetable production systems in Northern California. Agric. Ecosys. Environ. 90:25-45.

Armas C.M., B. Santana, J.L. Mora, J.S. Notario, C.D. Arbelo, and A. Rodríguez-Rodríguez. 2007. A biological quality index for volcanic andisols and aridisols (Canary Islands, Spain): Variations related to the ecosystem degradation. Sci. Tot. Environ. 378:238-244.

Bastida F., J. Luis Moreno, H. Teresa, and C. García. 2006. Microbiological degradation index of soils in a semiarid climate. Soil Biol. Biochem. 38:3463-3473.

Bécaert V., R. Samson, and L. Deschênes. 2006. Effect of 2,4-D contamination on soil functional stability evaluated using the relative soil stability index (RSSI). Chemos. 64:1713-1721.

Benjamin J.G., and D.C. Nielsen. 2004. A method to separate plant roots from soil and analyze root surface area. Plant Soil 267:225-234. DOI: 10.1007/s11104-005-4887-3.

Bligh E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physio. 37:911-917. DOI: 10.1139/o59-099.

Boehmel C., I. Lewandowski, and W. Claupein. 2008. Comparing annual and perennial energy cropping systems with different management intensities. Agric. Syst. 96:224-236.

Buchholz T., V.A. Luzadis, and T.A. Volk. 2009. Sustainability criteria for bioenergy systems: results from an expert survey. J. Cleaner Prod. 17, Supplement 1:S86-S98.

Burger J.A., and D.L. Kelting. 1999. Using soil quality indicators to assess forest stand management. Forest Ecol. Man. 122.

Cannell M.G.R. 2003. Carbon sequestration and biomass energy offset: Theoretical, potential and achievable capacities globally, in Europe and the UK. Biomass Bio. 24:97-116.

Chen T.-B., Y.-M. Zheng, M. Lei, Z.-C. Huang, H.-T. Wu, H. Chen, K.-K. Fan, K. Yu, X. Wu, and Q.-Z. Tian. 2005. Assessment of heavy metal pollution in surface soils of urban parks in Beijing, China. Chemos. 60:542-551.

Cox T.S., J.D. Glover, D.L. Van Tassel, C.M. Cox, and L.R. DeHaan. 2006. Prospects for developing perennial grain crops. BioSci. 56:649-659. DOI: 10.1641/0006-3568(2006)56[649:pfdpgc]2.0.co;2.

Dawson J.J.C., E.J. Godsiffe, I.P. Thompson, T.K. Ralebitso-Senior, K.S. Killham, and G.I. Paton. 2007. Application of biological indicators to assess recovery of hydrocarbon impacted soils. Soil Biol. Biochem. 39:164-177.

De Gryze S., J. Six, C. Brits, and R. Merckx. 2005. A quantification of short-term macroaggregate dynamics: influences of wheat residue input and texture. Soil Biol. Biochem. 37:55-66.

Delgado J.A., P.M. Groffman, M.A. Nearing, T. Goddard, D. Reicosky, R. Lal, N.R. Kitchen, C.W. Rice, D. Towery, and P. Salon. 2011. Conservation practices to mitigate and adapt to climate change. J. Soil Water Conserv. 66:118A-129A. DOI: 10.2489/jswc.66.4.118A.

Doran J.W. and T.B. Parkin. 1994. Defining and assessing soil quality, in: J. W. Doran, et al. (Eds.), Defining soil quality for a sustainable environment, Soil Sci. Soc. Am. J. Madison, Wisconsin. pp. 3-21.

Elliott E.T. 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils1. Soil Sci. Soc. Am. J. 50:627-633. DOI: 10.2136/sssaj1986.03615995005000030017x.

Erkossa T., F. Itanna, and K. Stahr. 2007. Indexing soil quality: A new paradigm in soil science research. Aus. J. Soil Res. 45:129-137.

Filip Z. (2002) International approach to assessing soil quality by ecologically-related biological parameters. Agric. Ecosys. Environ. 88:169-174.

Frank A.B., J.D. Berdahl, J.D. Hanson, M.A. Liebig, and H.A. Johnson. 2004. Biomass and carbon partitioning in switchgrass. Crop Sci. 44:1391-1396. DOI: 10.2135/cropsci2004.1391.

Franzluebbers A.J., F.M. Hons, and D.A. Zuberer. 1995. Tillage and crop effects on seasonal soil carbon and nitrogen dynamics. Soil Sci. Soc. Am. J. 59:1618-1624. DOI: 10.2136/sssaj1995.03615995005900060016x.

Freitas D.A.F.d., M.L.N. Silva, E.L. Cardoso, and N. Curi. 2012. Índices de qualidade do solo sob diferentes sistemas de uso e manejo florestal e cerrado nativo adjacente. Rev. Ciênci Agron. 43:417-428.

Garten C.T., and S.D. Wullschleger. 2000. Soil carbon dynamics beneath switchgrass as indicated by stable isotope analysis. J. Environ. Qual. 29:645-653. DOI: 10.2134/jeq2000.00472425002900020036x.

Gasparatos A., P. Stromberg, and K. Takeuchi. 2011. Biofuels, ecosystem services and human wellbeing: Putting biofuels in the ecosystem services narrative. Agric. Ecosys. Environ. 142:111-128.

Giovannetti M., and V. Gianinazzi-Pearson. 1994. Biodiversity in arbuscular mycorrhizal fungi. Myc. Res. 98:705-715.

Glover J.D., J.P. Reganold, and P.K. Andrews. 2000. Systematic method for rating soil quality of conventional, organic, and integrated apple orchards in Washington State. Agric. Ecosys. Environ. 80:29-45.

Govaerts B., M. Mezzalama, Y. Unno, K.D. Sayre, M. Luna-Guido, K. Vanherck, L. Dendooven, and J. Deckers. 2007. Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. Appl. Soil Ecol. 37:18-30.

Harris R.F., G. Chesters, and O.N. Allen. 1966. Dynamics of soil aggregation, in: A. G. Norman (Ed.), Adv. in Agron., Academic Press. pp. 107-169.

Heink U., I. Kowarik. 2010. What are indicators? On the definition of indicators in ecology and environmental planning. Ecol. Indic. 10:584-593.

Hinojosa M.B., J.A. Carreira, R. García-Ruíz, and R.P. Dick. 2005. Microbial response to heavy metal–polluted soils. J. Environ. Qual. 34:1789-1800. DOI: 10.2134/jeq2004.0470.

Hussain I., K.R. Olson, M.M. Wander, and D.L. Karlen. 1999. Adaptation of soil quality indices and application to three tillage systems in southern Illinois. Soil Till. Res. 50:237-249.

Inskip H. 2005. Standardization Methods, Encycl. Biostat., John Wiley & Sons, Ltd.

Institute S. 2009. SAS 9.2 Output delivery system user's guide SAS Inst., Cary, NC.

Jastrow J.D. 1987. Changes in soil aggregation associated with tallgrass prairie restoration. Am. J. Bot. 74:1656-1664. DOI: 10.2307/2444134.

Jenkinson D.S., and D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil—I. Fumigation with chloroform. Soil Biol. Biochem. 8:167-177.

Kang G.S., V. Beri, B.S. Sidhu, and O.P. Rupela. 2005. A new index to assess soil quality and sustainability of wheat-based cropping systems. J. Biol. Fert. Soils 41:389-398.

Karlen D.L., and D.E. Stott. 1994. A framework for evaluating physical and chemical indicators of soil quality., in: J. W. Doran, et al. (Eds.), Defining soil quality for a sustainable environment, Soil Sci. Soc. Am. J. Madison, Wisconsin, USA. pp. 52-72.

Karlen D.L., and S.S. Andrews. 2000. The soil quality concept: a tool for evaluating sustainability, in: S. Elmholt, et al. (Eds.), Soil stresses, quality and care, Danish Institute of Agricultural Science (DIAS), Tjele, As, Norway. pp. 15-26.

Karlen D.L., N.C. Wollenhaupt, D.C. Erbach, E.C. Berry, J.B. Swan, N.S. Eash, and J.L. Jordahl. 1994. Crop residue effects on soil quality following 10-years of no-till corn. Soil Till. Res. 31:149-167. DOI: Doi: 10.1016/0167-1987(94)90077-9.

Kline K., V.H. Dale, R. Lee, and P. Leiby. 2009. In defense of biofuels, done right. Issues in Sci. Tech. 25:75-84.

Koper J., and A. Piotrowska. 2003. Application of biochemical index to define soil fertility depending on varied organic and mineral fertilization. Elec. J. Pol. Agric. Universities. Vol.6 (1), online http://www.ejpau.media.pl/volume6/issue1/agronomy/art-06.html. Access 11.9.2013.

Larson W.E., and F.J. Pierce. 1991. Conservation and enhancement of soil quality., in: J. Dumanski, et al. (Eds.), Evaluation for sustainable land management in the developing world, International Board for Soil Research and Management, Bangkok, Thailand, Chiang Rai, Thailand. pp. 175-203.

Lewandowski I., and U. Schmidt. 2006. Nitrogen, energy and land use efficiencies of miscanthus, reed canary grass and triticale as determined by the boundary line approach. Agric. Ecosys. Environ. 112:335-346.

Li P., T. Zhang, X. Wang, and D. Yu. 2013. Development of biological soil quality indicator system for subtropical China. Soil Till. Res. 126:112-118.

Liebig M.A., G. Varvel, and J. Doran. 2001. A simple performance-based index for assessing multiple agroecosystem functions. Agron. J. 93:313-318.

Liebig M.A., M.R. Schmer, K.P. Vogel, and R.B. Mitchell. 2008. Soil carbon storage by switchgrass grown for bioenergy. BioEn. Res. 1:215-222. DOI: 10.1007/s12155-008-9019-5.

Masto R.E., P.K. Chhonkar, D. Singh, and A.K. Patra. 2007. Soil quality response to long-term nutrient and crop management on a semi-arid Inceptisol. Agric. Ecosys. Environ. 118:130-142.

McBride A.C., V.H. Dale, L.M. Baskaran, M.E. Downing, L.M. Eaton, R.A. Efroymson, C.T. Garten Jr, K.L. Kline, H.I. Jager, P.J. Mulholland, E.S. Parish, P.E. Schweizer, and J.M. Storey. 2011. Indicators to support environmental sustainability of bioenergy systems. Ecol. Indic. 11:1277-1289.

McKinley V.L., A.D. Peacock, and D.C. White. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biol. Biochem. 37:1946-1958.

Melillo J.M., A.C. Gurgel, D.W. Kicklighter, J.M. Reilly, T.W. Cronin, B.S. Felzer, S. Paltsev, C.A. Schlosser, A.P. Sokolov, and X. Wang. 2009. Unintended environmental consequences of a global biofuels program

Metzger L., D. Levanon, and U. Mingelgrin. 1987. The Effect of sewage sludge on soil structural stability: Microbiological aspects. Soil Sci. Soc. Am. J. 51:346-351. DOI: 10.2136/sssaj1987.03615995005100020016x.

Mikha M.M., and C.W. Rice. 2004. Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. Soil Sci. Soc. Am. J. 68:809-816. DOI: 10.2136/sssaj2004.8090.

Mohanty M., D.K. Painuli, A.K. Misra, and P.K. Ghosh. 2007. Soil quality effects of tillage and residue under rice-wheat cropping on a Vertisol in India. Soil Till. Res. 92:243-250.

Monti A., and A. Zatta. 2009. Root distribution and soil moisture retrieval in perennial and annual energy crops in Northern Italy. Agric. Ecosys. Environ. 132:252-259.

Nannipieri P., B. Ceccanti, and S. Grego. 1990. Ecological significance of biological activity in soil, in: J. M. Bollag and G. Stotzky (Eds.), Soil Biochemistry. Marcel Dekker, New York. pp. 293-355.

Nannipieri P., J. Ascher, M.T. Ceccherini, L. Landi, G. Pietramellara, and G. Renella. 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54:655-670. DOI: 10.1046/j.1351-0754.2003.0556.x.

Nelson R.G., C.M. Hellwinckel, C.C. Brandt, T.O. West, D.G. De La Torre Ugarte, and G. Marland. 2009. Energy use and carbon dioxide emissions from cropland production in the United States, 1990–2004. J. Environ. Qual. 38:418-425. DOI: 10.2134/jeq2008.0262.

Pang X.Y., W.K. Bao, and Y.M. Zhang. 2006. Evaluation of soil fertility under different Cupressus chengiana forests using multivariate approach. Pedosphere 16:602-615.

Power A.G. 2010. Ecosystem services and agriculture: tradeoffs and synergies. Phil. Trans. Roy. Soc. B: Biol. Sci. 365:2959-2971. DOI: 10.1098/rstb.2010.0143.

Propheter J.L., and S. Staggenborg. 2010. Performance of annual and perennial biofuel crops: nutrient removal during the first two years. Agron. J. 102:798-805. DOI: 10.2134/agronj2009.0462.

Puglisi E., A.A.M. Del Re, M.A. Rao, and L. Gianfreda. 2006. Development and validation of numerical indexes integrating enzyme activities of soils. Soil Biol. Biochem. 38:1673-1681.

Puglisi E., M. Nicelli, E. Capri, M. Trevisan, and A.A.M. Del Re. 2005. A soil alteration index based on phospholipid fatty acids. Chemos. 61:1548-1557.

Rasse D., C. Rumpel, and M.-F. Dignac. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. Plant Soil 269:341-356. DOI: 10.1007/s11104-004-0907-y.

Rice C., F. Garcia, C. Hampton, and C. Owensby. 1994. Soil microbial response in tallgrass prairie to elevated CO2. Plant Soil 165:67-74. DOI: 10.1007/bf00009963.

Rice C.W., and J.S. Angle. 2003. A role for genetically modified organisms in soil carbon sequestration. In: N. J. M. Rosenberg, F. B.;Izaurralde, R. C. (Ed.), Applications of biotechnology to mitigation of greenhouse warming. Proceedings of the St. Michaels II Workshop, April 2003. pp. 61-78.

Rice C.W., T.B. Moorman, and M.H. Beare. 1996. Role of microbial biomass carbon and nitrogen in soil quality. In: J. Doran and J. A. Jones (Eds.), Methods for assessing soil quality. Soil Sci. Soc. Am. J. Special Publication, Madison, WI. pp. 203-215.

Rice C.W., P.M. White, K.P. Fabrizzi, and G.W.T. Wilson. 2004. Managing the microbial community for soil carbon management. SuperSoil: 3rd Australian New Zealand Soils Conference, University of Sydney, Sydney (Australia).

Rice C.W., T.C. Todd, J.M. Blair, T.R. Seastedt, R.A. Ramundo, and G.W.T. Wilson. 1998. Belowground biology and processes. In: J. M. B. A K Knapp, D C Hartnett and S L Collins. (Ed.), Grassland dynamics: Long-term ecological research in tallgrass prairie., Oxford University Press New York. pp. 244-264.

Rillig M., B. Caldwell, H.B. Wösten, and P. Sollins. 2007. Role of proteins in soil carbon and nitrogen storage: controls on persistence. Biogeochem. 85:25-44. DOI: 10.1007/s10533-007-9102-6.

Rillig M.C., S.F. Wright, M.F. Allen, and C.B. Field. 1999. Rise in carbon dioxide changes soil structure. Nature 400:628-628.

Robertson G.P., V.H. Dale, O.C. Doering, S.P. Hamburg, J.M. Melillo, M.M. Wander, W.J. Parton, P.R. Adler, J.N. Barney, R.M. Cruse, C.S. Duke, P.M. Fearnside, R.F. Follett, H.K. Gibbs, J. Goldemberg, D.J. Mladenoff, D. Ojima, M.W. Palmer, A. Sharpley, L. Wallace, K.C. Weathers, J.A. Wiens, and W.W. Wilhelm. 2008. Sustainable biofuels redux. Science. 322:49-50. DOI: 10.1126/science.1161525.

Sà J.C.M., L. Séguy, M.F.M. Sà, A.O. Ferreira, C. Briedis, J.B. Santos, and L. Canalli. 2009. Intensificação da rotação de culturas como estratégia para a construção e manutenção da matéria orgânica e da fertilidade do solo. In: simposio sobre plantio direto na palha, foz do Iguaçu,, Ponta Grossa, Federação Brasileira de plantio direto na palha. pp. 39-46.

Schreiner R.P., and G.J. Bethlenfalvay. 1995. Mycorrhizal interactions in sustainable agriculture. Crit. Rev. Biotech. 15:271-285. DOI: doi:10.3109/07388559509147413.

Sharma A., and S. Arora. 2010. Soil quality indices and relative production efficiency for maize and wheat crops in agroclimates of Northwest India. Soil Sci. 175:44-49.

Six J., S.D. Frey, R.K. Thiet, and K.M. Batten. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70:555-569. DOI: 10.2136/sssaj2004.0347.

Smeets E.M.W., I.M. Lewandowski, and A.P.C. Faaij. 2009. The economical and environmental performance of miscanthus and switchgrass production and supply chains in a European setting. Renewable and Sust. En. Rev. 13:1230-1245.

Smith P., and J.E. Olsen. 2010. Synergies between the mitigation of, and adaptation to, climate change in agriculture. J. Agric. Sci. 148:543-552. DOI: doi:10.1017/S0021859610000341.

Sokhansanj S., S. Mani, A. Turhollow, A. Kumar, D. Bransby, L. Lynd, and M. Laser. 2009. Large-scale production, harvest and logistics of switchgrass (Panicum virgatum L.) – current technology and envisioning a mature technology. Biof. Bioprod. Bioref. 3:124-141. DOI: 10.1002/bbb.129.

Stenberg B. 1999. Monitoring soil quality of arable land: Microbiological indicators. Acta Agric. Scandinavica, B 49:1-24.

Stromberger M.E., Z. Shah, and D.G. Westfall. 2011. High specific activity in low microbial biomass soils across a no-till evapotranspiration gradient in Colorado. Soil Biol. Biochem. 43:97-105.

Tester M., and P. Langridge. 2010. Breeding technologies to increase crop production in a changing world. Science 327:818-822. DOI: 10.1126/science.1183700.

Tilman D., J. Hill, and C. Lehman. 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. Science 314:1598-1600. DOI: 10.1126/science.1133306.

Trasar-Cepeda C., C. Leirós, F. Gil-Sotres, and S. Seoane. 1998. Towards a biochemical quality index for soils: An expression relating several biological and biochemical properties. Biol. Fert. Soils 26:100-106.

U.S.EPA., 2011. Biofuels and the environment: The first triennial report to congress, U.S. Environmental Protection Agency, , Washington, DC.

Venturi P., and G. Venturi. 2003. Analysis of energy comparison for crops in European agricultural systems. Biom. Bio. 25:235-255.

Wall D.H., R.D. Bardgett, and E. Kelly. 2010. Biodiversity in the dark. Nat. Geosci 3:297-298.

Wang X., and Z. Gong. 1998. Assessment and analysis of soil quality changes after eleven years of reclamation in subtropical China. Geod. 81:339-355.

Werner C., E. Haas, R. Grote, M. Gauder, S. Graeff-Hönninger, W. Claupein, and K. Butterbach-Bahl. 2012. Biomass production potential from Populus short rotation systems in Romania. GCB Bioenergy 4:642-653. DOI: 10.1111/j.1757-1707.2012.01180.x.

White D.C., D.B. Ringelberg, S.J. Macnaughton, S. Alugupalli, and D. Schram. 1997. Signature lipid biomarker analysis for quantitative assessment *in situ* of environmental microbial ecology, molecular markers in environmental geochemistry. Am. Chem. Soc. pp. 22-34.

White P.M., and C.W. Rice. 2009. Tillage effects on microbial and carbon dynamics during plant residue decomposition. Soil Sci. Soc. Am. J. 73:138-145. DOI: 10.2136/sssaj2007.0384.

White P.M., C.W. Rice, J.A. Baldock, and M.R. Tuinstra. 2007. Soil biological properties following additions of bmr mutant grain sorghum. Soil Biol. Biochem. 39:1518-1532.

Wieder W.R., G.B. Bonan, and S.D. Allison. 2013. Global soil carbon projections are improved by modelling microbial processes. Nat. Clim. Change advance online publication. DOI: 10.1038/nclimate1951

Wilson G.W.T., C.W. Rice, and M.C. Rillig, A. Springer, and D.C. Hartnett. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol. Let. 12:452-461. DOI: 10.1111/j.1461-0248.2009.01303.x.

Wright S.F., and A. Upadhyaya. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci. 161:575-586.

Wurst S., G.B. De Deyn, and K. Orwin. 2012. Soil biodiversity and functions. In: D. H. Wall (Ed.), Soil ecology and ecosystem services, Oxford Univ. Press, Oxford, United Kingdom. pp. 28-44.

Zan C.S., J.W. Fyles, P. Girouard, and R.A. Samson. 2001. Carbon sequestration in perennial bioenergy, annual corn and uncultivated systems in southern Quebec. Agric. Ecosys. Environ. 86:135-144.

Zornoza R., J. Mataix-Solera, C. Guerrero, V. Arcenegui, F. García-Orenes, J. Mataix-Beneyto, and A. Morugán. 2007. Evaluation of soil quality using multiple lineal regression based on physical, chemical and biochemical properties. Sci. Total Environ. 378:233-237.

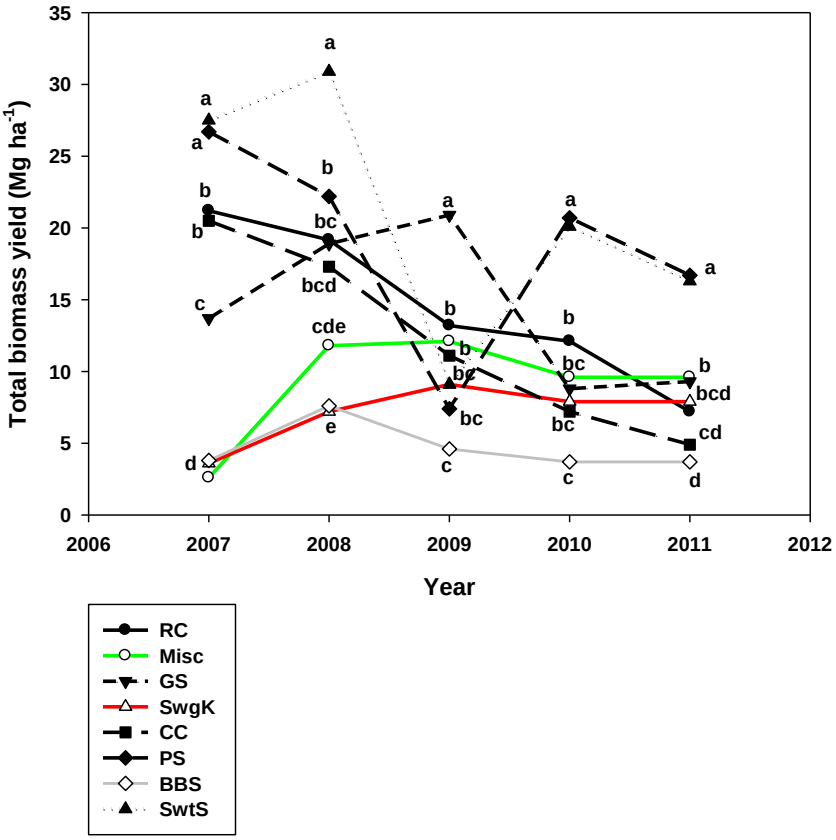


Figure 2.1. Total biomass yield for various feedstocks.
Means within a year followed by different letters are significantly different, as measured by Tukey's HSD ($p<0.01$). CC = Continuous corn, GS = Grain sorghum, RC = Rotated corn, Swts = Sweet sorghum, PS = Photoperiod sorghum, Misc = Miscanthus, BBS = Big bluestem, SgK = Switchgrass Kanlow

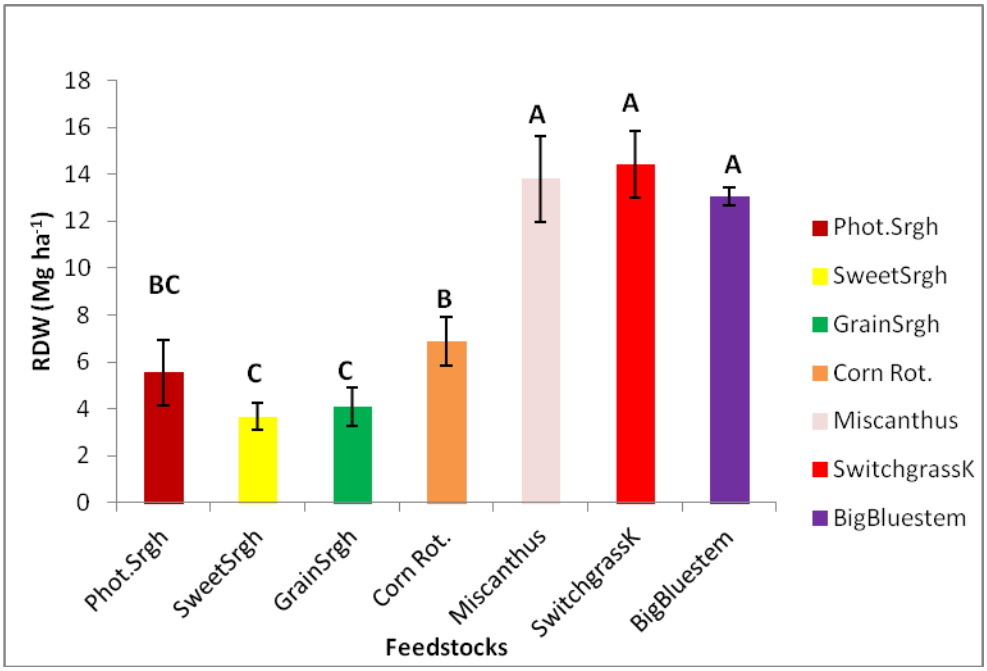


Figure 2.2. Root dry weight (RDW) in (0-120 cm) soil profile of different biofuel crops. (mean value ± standard error of four replicates). Phot.Srgh = Photoperiod sorghum, SweetSrgh = Sweet sorghum GrainSrgh = Grain sorghum, Corn Rot. = Rotated corn, SwitchgrassK = Switchgrass Kanlow

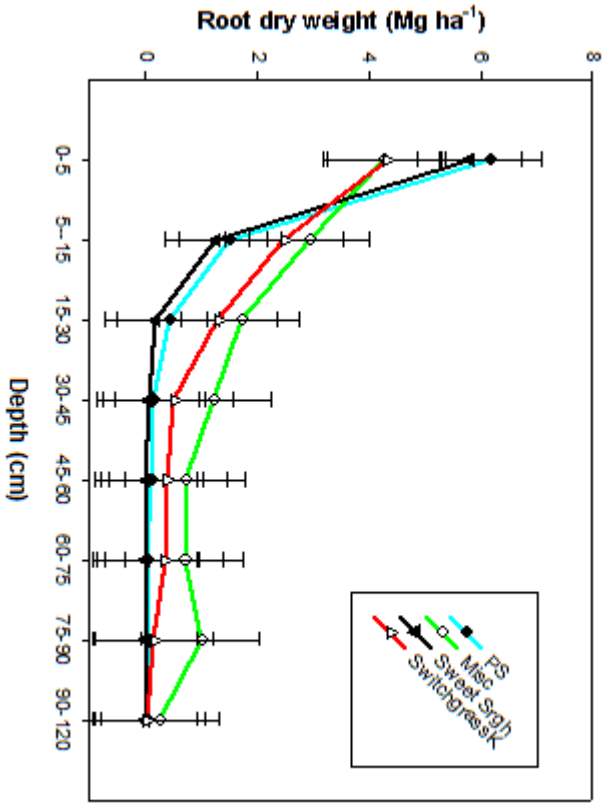


Figure 2.3. Root distribution of perennials versus annual crops within the upper 120cm. (mean value ± standard error of four replicates). Switchgrass K = switchgrass kanlow; S. Sorghum = sweet sorghum; P. Sorghum = photoperiod sorghum.

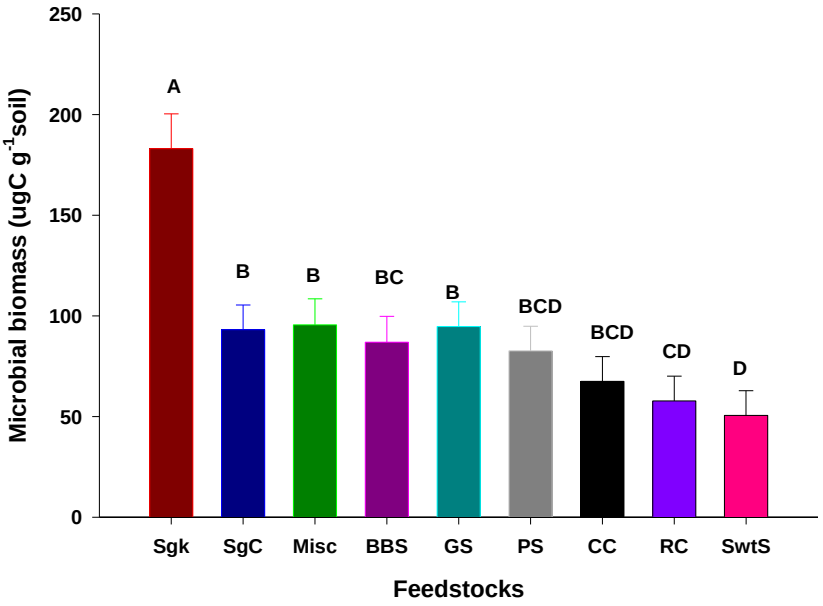


Figure 2.4. Soil microbial biomass C determined by fumigation-incubation method in different bioenergy crops (mean value ± standard error of four replicates).
Different letters indicate significant differences among energy crops ($p<0.05$). SgK = Switchgrass Kanlow, SgC = Switchgrass Ceres, Misc = Miscanthus, BBS = Big bluestem, GS = Grain sorghum, PS = Photoperiod sorghum, CC = Continuous corn, RC = Rotated corn, SwtS = Sweet sorghum.

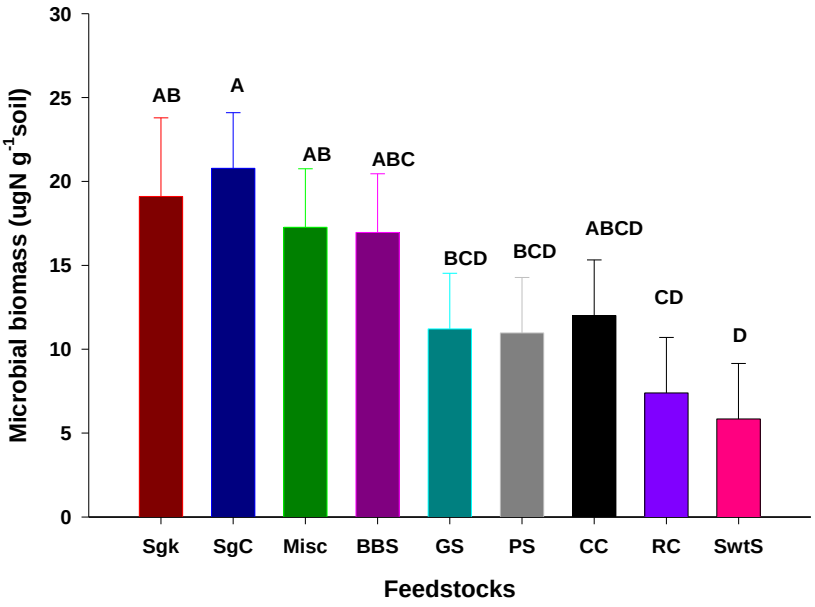


Figure 2.5. Soil microbial biomass N determined by fumigation-incubation method in different bioenergy crops (mean value ± standard error of four replicates).
Different letters indicate significant differences among energy crops ($p<0.05$). SgK = Switchgrass Kanlow, SgC = Switchgrass Ceres, Misc = Miscanthus, BBS = Big bluestem, GS = Grain sorghum, PS = Photoperiod sorghum, CC = Continuous corn, RC = Rotated corn, SwtS = Sweet sorghum

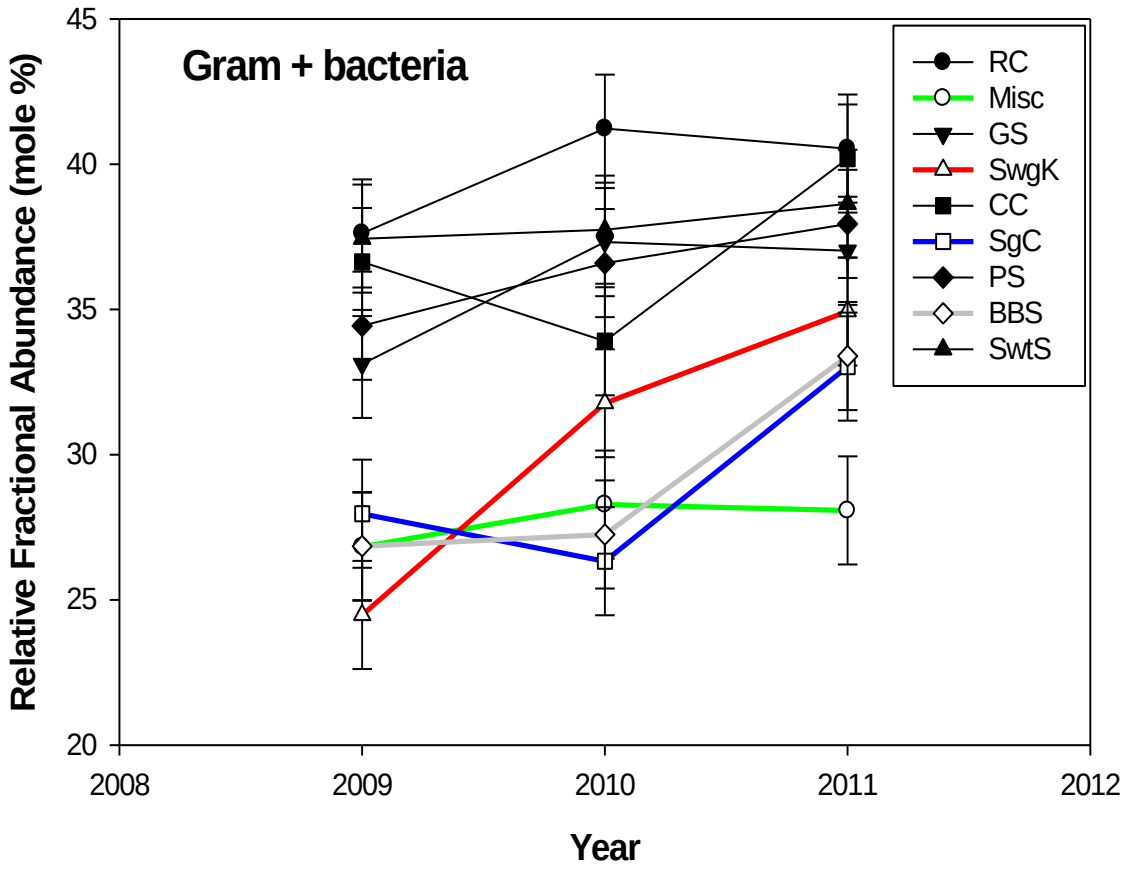


Figure 2.6. Estimated relative abundance of Gram (+) under different energy crops.
The relative abundance is calculated as the ratio between the measured value of Gram (+) and the total number of bacteria. RC = Rotated corn, Misc = Miscanthus, GS = Grain sorghum, SgK = Switchgrass Kanlow, CC = Continuous corn, SgC = Switchgrass Ceres, PS = Photoperiod sorghum, BBS = Big bluestem, SwtS = Sweet sorghum

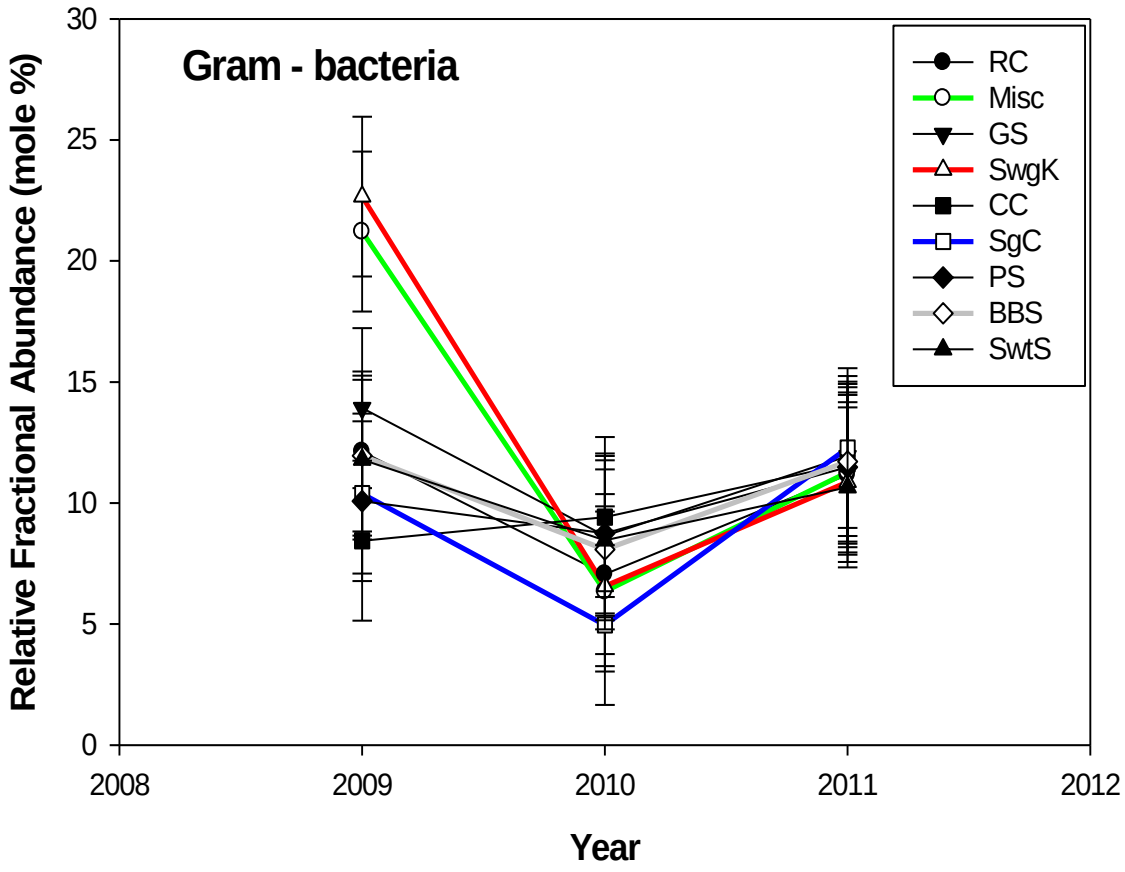


Figure 2.7. Estimated relative abundance of gram-negative bacteria (Gram$^-$) under different energy crops.

The relative abundance is calculated as the ratio between the measured value of Gram (-) and the total number of bacteria. RC = Rotated corn, Misc = Miscanthus, GS = Grain sorghum, SgK = Switchgrass Kanlow, CC = Continuous corn, SgC = Switchgrass Ceres, PS = Photoperiod sorghum, BBS = Big bluestem, SwtS = Sweet sorghum.

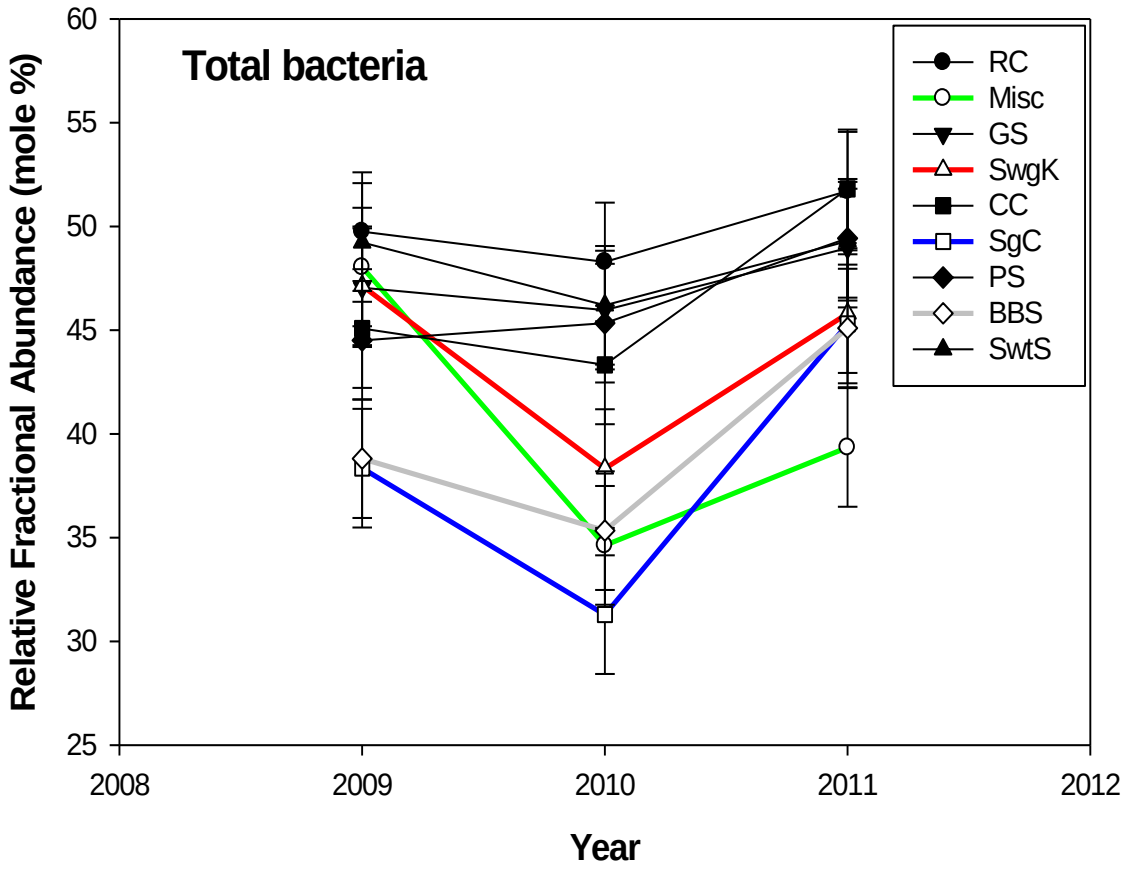


Figure 2.8. Estimated relative abundance of total bacteria.
The fractional abundance is calculated as the ratio between the measured value for all bacteria and the total lipid biomass. RC = Rotated corn, Misc = Miscanthus, GS = Grain sorghum, SgK = Switchgrass Kanlow, CC = Continuous corn, SgC = Switchgrass Ceres, PS = Photoperiod sorghum, BBS = Big bluestem, SwtS = Sweet sorghum.

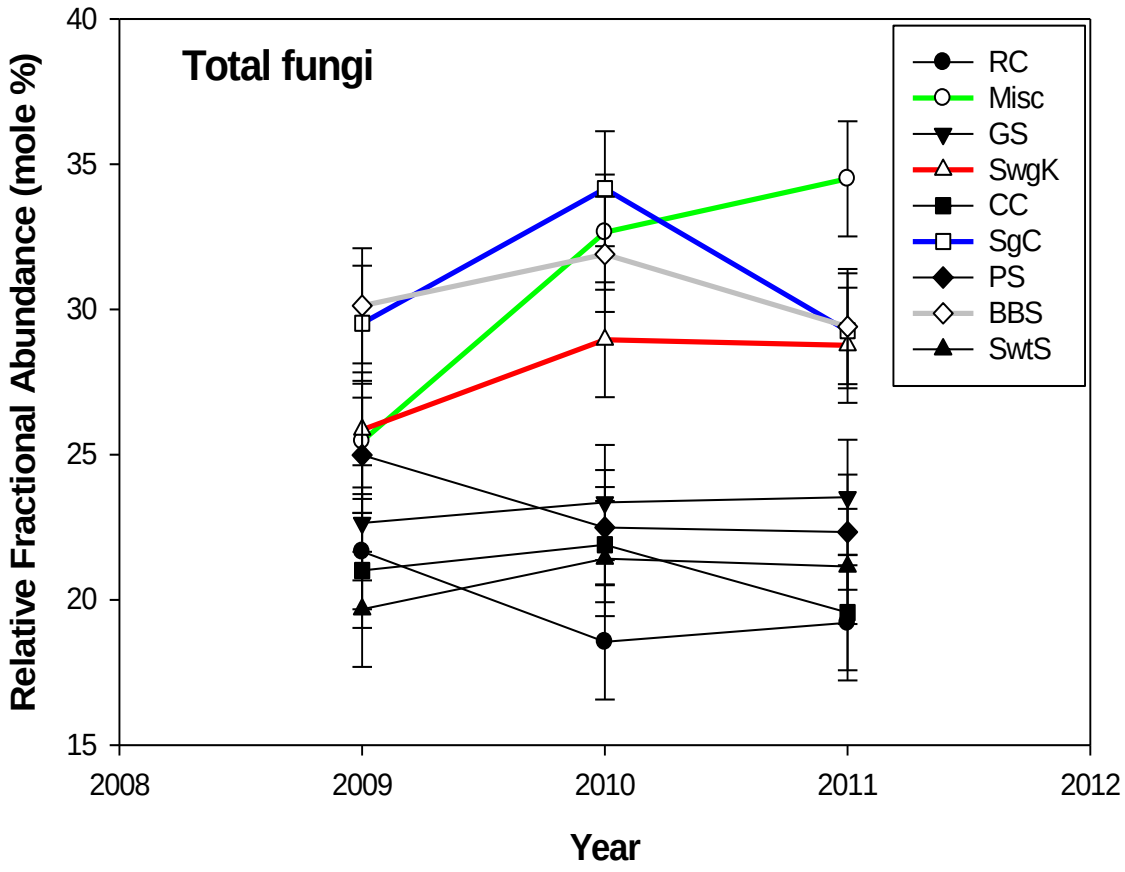


Figure 2.9. Estimated relative abundance of total fungi.
The fractional abundance is calculated as the ratio between the measured value for all fungi and the total lipid biomass. RC = Rotated corn, Misc = Miscanthus, GS = Grain sorghum, SgK = Switchgrass Kanlow, CC = Continuous corn, SgC = Switchgrass Ceres, PS = Photoperiod sorghum, BBS = Big bluestem, SwtS = Sweet sorghum.

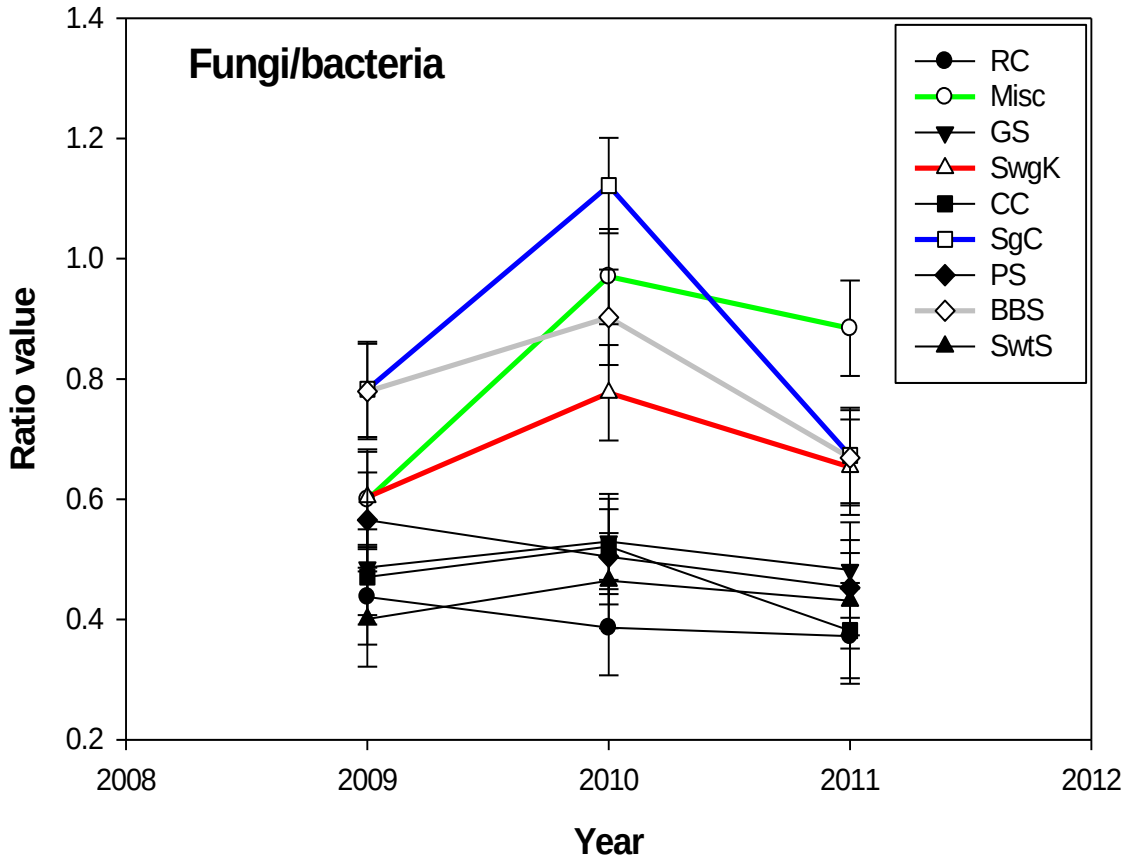


Figure 2.10. Effects of different energy crops of soil fungi: bacteria ratio, both fungal and bacterial communities was measured by PLFAs analysis.
RC = Rotated corn, Misc = Miscanthus, GS = Grain sorghum, SgK = Switchgrass Kanlow, CC = Continuous corn, SgC = Switchgrass Ceres, PS = Photoperiod sorghum, BBS = Bigbluestem, SwtS = Sweet sorghum.

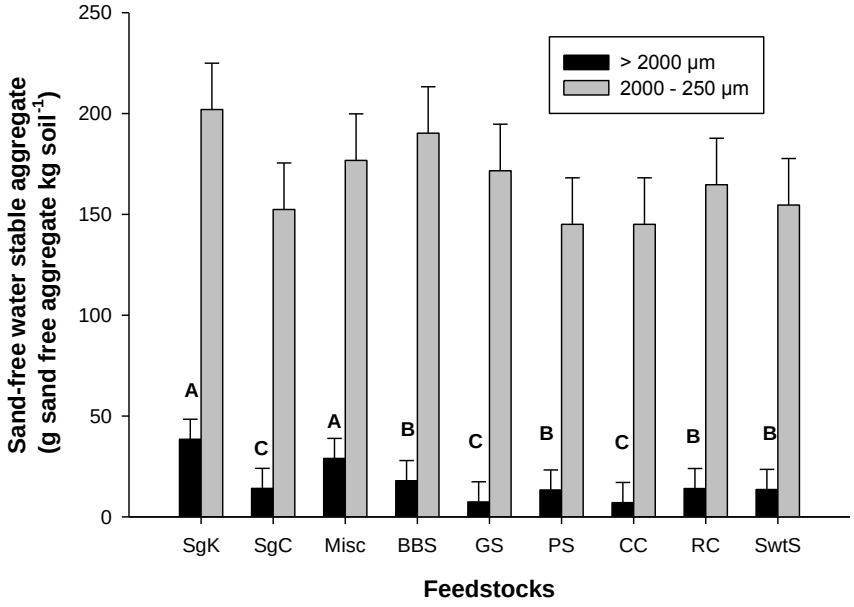


Figure 2.11. Sand-free water-stable aggregates in different size fractions within the 0-5 cm depth as affected by different energy crops.
Capital letters represent significant differences ($P<0.05$) among energy crop at the same aggregate size fraction. The error bars represent standard errors of the mean. SgK = Switchgrass Kanlow, SgC = Switchgrass Ceres, Misc = Miscanthus, BBS = Big bluestem, GS = Grain sorghum, PS = Photoperiod sorghum, CC = Continuous corn, RC = Rotated corn, SwtS = Sweet sorghum

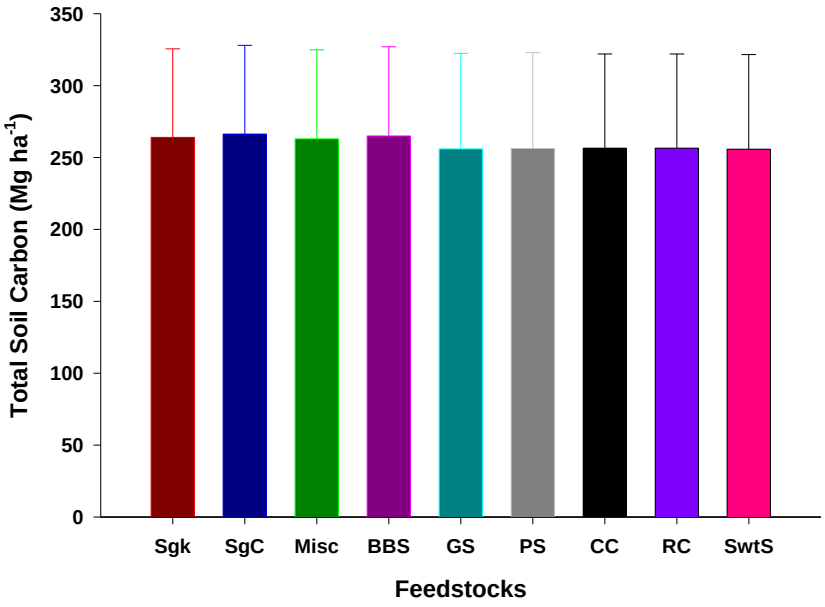


Figure 2.12. Soil organic carbon (SOC) in the 0-120 cm soil depth profile after 5 yrs of crop residue study under different biofuel feedstocks.
SgK = Switchgrass Kanlow, SgC = Switchgrass Ceres, Misc = Miscanthus, BBS = Big bluestem, GS = Grain sorghum, PS = Photoperiod sorghum, CC = Continuous corn, RC = Rotated corn, SwtS = Sweet sorghum

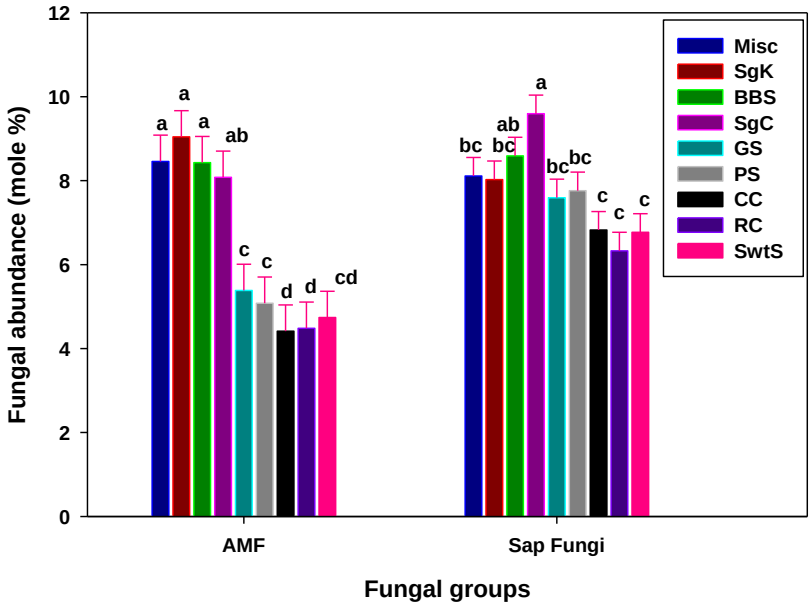


Figure 2.13. Effects of energy crops on arbuscular mycorrhizal fungi (AMF) and saprophytic fungi. Error bars are standard errors of the mean. RC = Rotated corn, Misc = Miscanthus, GS = Grain sorghum, SgK = Switchgrass Kanlow, CC = Continuous corn, SgC = Switchgrass Ceres, PS = Photoperiod sorghum, BBS = Big bluestem, SwtS = Sweet sorghum.
Means follow by different letters are significantly different, as measured by Tukey's HSD ($p<0.05$).

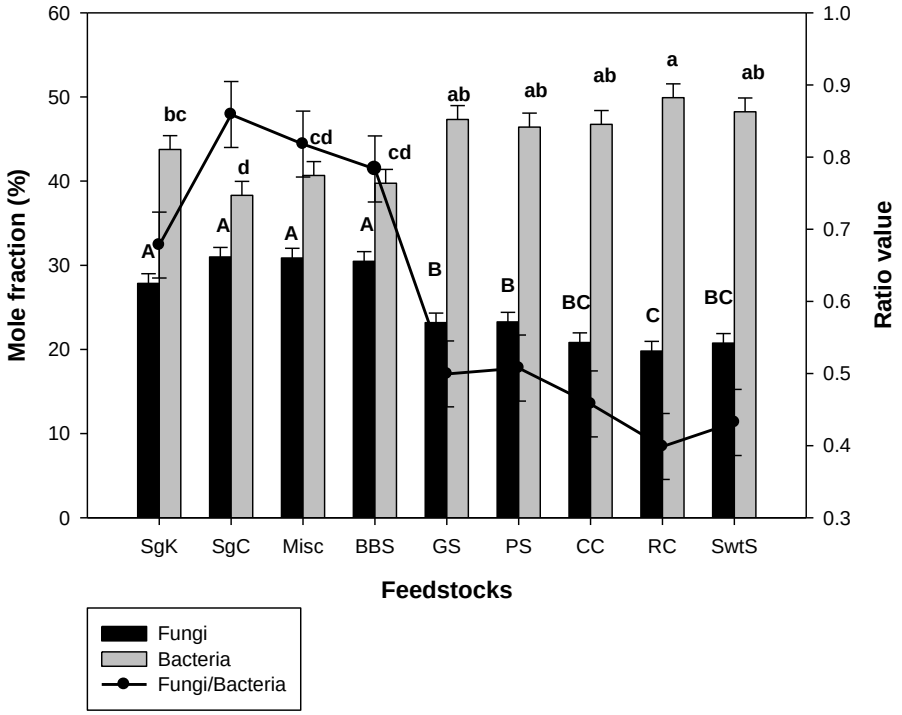


Figure 2.14. Relative fractional abundance of microbial functional lipid and associated ratio. Capital letters represent significant differences in fungal subgroup; while lowercase letters represent significant difference in bacterial subgroup. Error bars are standard errors of the mean. SgK = Switchgrass Kanlow, SgC = Switchgrass Ceres, Misc = Miscanthus, BBS = Big bluestem, GS = Grain sorghum, PS = Photoperiod sorghum, CC = Continuous corn, RC = Rotated corn, SwtS = Sweet sorghum

Table 2.1. Analysis of variance significant levels for total fungi, total bacteria, fungal:bacterial ratio, and Gram$^-$ and Gram$^+$ bacteria from soil under different energy crops evaluated over three years.

EFFECT	DF	TOTAL FUNGI	TOTAL BACTERIA	FUNGI/BACTERIA	GRAM-	GRAM+
Year	2	NS	0.0019	NS	0.0017	0.0044
Plant	8	0.0063	NS	0.02	NS	0.0073
Plant*Year	16	NS	NS	NS	NS	NS

Table 2.2. Nutrient (N, P, K) concentration of perennial crops from Propheter et al. (2010).

Feedstocks	N	P	K
Miscanthus	9.6	0.8	11
Big blue-stem	5.6	1.3	11.7
Switchgrass	5.9	1.5	12.5

Table 2.3. Total nutrient credits in dollars per dry ton of perennial energy crop.

PERENNIALS	N	P	K	FERTILIZER CREDIT ($)
Miscanthus	4.8	0.48	6.27	11.55
Big blue stem	2.8	0.78	6.6	10.18
Switchgrass K	2.95	0.9	7.12	10.97

All values are in dollars, calculated by multiplying nutrient concentrations in each crop by the nutrient value obtain from Agricultural Prices National Agricultural Statistics Services (2013).

Table 2.4. Feedstock yields and prices for biofuel crops in Kansas.

Feedstock	*Grain price($ per dry biomass)	#Stover price ($ per dry biomass)	Grain earnings	Stalks earnings	Total earnings
			$USD		
Rotated corn	177	62	1529	541	2070
Continuous corn	177	62	1304	461	1766
Grain sorghum	161	80	1044	634	1678
Sweet sorghum	161	80	1332	1174	2506
Photo sorghum	N/A	80	0	1824	1824
Miscanthus	N/A	62	0	680	680
Big blue stem	N/A	60	0	393	393
Switchgrass K	N/A	61	0	545	545

*Average grain prices from 2007-2011 estimated from

http://www.agmanager.info/marketing/basis/tools/default.asp

[#] Estimated stover price for the state of Kansas from the National Biomass Energy Report. Except for the perennials (see text for calculation).

All values are in dollars, calculated by multiplying the price of grain and stover, and the dry biomass for each crop.

Table 2.5. Sustainability index for the different bioenergy cropping systems in Manhattan Kansas.

Feedstock	Revenue	Energy saving	C storage	Aggregation	Total PLFA	Index	Rank
Switchgrass Kanlow	-1.13	1.5	1.10	1.92	0.84	4.23	1
Miscanthus	-0.95	0.36	0.92	1.04	2.10	3.47	2
Big blue stem	-1.32	1.5	1.43	0.03	0.07	1.71	3
Sweet sorghum	1.36	-1.5	-0.96	-0.36	-0.55	0.99	4
Rotated corn	0.81	-0.79	-0.78	-0.32	-0.53	-1.61	5
Photoperiod sorghum	0.49	-0.59	-0.83	-0.39	-0.56	-1.88	6
Continuous corn	0.42	-0.79	-0.08	-0.96	-0.63	-2.04	7
Grain sorghum	0.31	-0.59	-0.78	-0.93	-0.74	-2.73	8

Chapter 3 - Soil Aggregates, C and Microbial Parameters As Affected by 24 Years of Differing Levels of Crop Residue and Fertilizer Inputs

Abstract

A residue harvest study was conducted at the Kansas State University's East Central Experiment field near Ottawa, KS on a 0-1% slope, Woodson silt loam soil (fine, montmorillonitic, thermic, Abruptic Argiaquoll). Residue levels were 0X, 1X, and 2X and three fertilizer levels were maintained for 24years. Soil samples collected to a depth of 30 cm were analyzed for aggregate stability and SOC and SON. The macroaggregates (>2000 µm and 2000-250 µm) were highest (100-250 g kg^{-1}) and (95-230 g kg^{-1}) in 2X and 1X respectively and lowest (50-200 g kg^{-1}) in the 0X residue level. The most abundant aggregate size fraction was that (53-250 µm) in diameter at 0-5 and 5-15cm but at 15-30cm; the maccroaggregates (250-2000 µm) were the most abundant aggregate size fraction. This fact was independent of the residue treatments, fertility level or cropping succession. After 24 years, soil organic carbon was lower in the 0X level in the top 5cm compared to 1X and 2X. These slowly occurring effects are potentially a threat to the sustainability of the soil in the long-term. Maintaining or doubling crop residues in combination with fertilizer inputs did not statistically show significant difference for SOC and N. The results show that greater inputs of fertilizer N and increased returns of crop residues did not cause a proportionately greater increase in SOC. Tillage masks the benefits of the residue. Cultivation reduces the OM content in the top 5 cm of soil by 48%, 36% and 47% in the respective C pools: >2000µm, 2000-250µm, and 250-53µm compared to an undisturbed native site located 50 m from the experimental plots.

Introduction

Management of crop residue and soil organic carbon (SOC) is of primary importance in maintaining soil fertility, productivity and maintaining soil quality. One of the biggest current environmental issues is that of global climate change. Globally, soils represent one of the largest terrestrial reservoir of carbon on Earth (Jobbagy and Jackson, 2000; Post et al., 1982). Bioenergy reduces fossil based fuels and CO_2 emissions (Dufey, 2006). However, removal of plant residues from agricultural systems may have long-term implications on sustainability of soil resources. Therefore it is critical to understand the consequences of harvesting biomass on soil properties as biofuel feedstock production expands. Long term experiments provide an excellent database to examine the impacts of residue management on carbon and nutrients dynamics. This database in combination with models could be a very important tool when formulating complex but comprehensive research or policy questions. Despite the numerous benefits and services provided by crop residue, many reports show that their effects are highly variable. For instance, Karlen et al. (1994) found no impacts after 10 yr of crop residue removal on soil structural stability in silt loam soils from Iowa. While Blanco-Canqui et al. (2006) noted a rapid decrease in soil aggregate stability after 1 yr of crop residue removal in Ohio. Therefore, the impact of crop residue removal on soil needs to be elucidated. Recent reports noted that residue removal may impact soil particulate organic matter (POM) more rapidly than total C (Hammerbeck et al., 2012). They found a decrease in labile POM which can rapidly reduce soil aggregate stability and carbon storage. Other reports suggested that crop residue do not contribute significantly to SOC (Gale and Cambardella, 2000). Lafond et al. (2009) found no difference in SOC after 50 yr provided that <40% of cereal straw was removed in a fallow-spring wheat-spring wheat rotation. On the contrary, Clapp et al. (2000) observed a change in SOC at

both 0-15 and 15-30 cm depths after only 13 yr of stover. Prior to large scale residue removal for biofuel production, further characterization of these varied effects of crop residue on soil properties must be elucidated. Understanding the factors that stabilized or destabilized SOC may lead to better soil management strategies that help maintain current levels or increase the SOC. Retention of crop residues to the soil in combination with less soil disturbance is associated with an increase SOC concentration (Govaerts et al., 2009; Karlen et al., 1994). Residue management and agronomic practices are keys determinants of soil C (Allmaras et al., 2004; West and Post, 2002). New insights on soil organic matter (SOM) stabilization mechanisms (Schmidt et al., 2011) reported that the degradation rate of SOM is not determine by the molecular structure of the OM itself but by the soil environment in which the degradation takes place. It has been hypothesized that increased inputs control SOM formation and stabilization. Gentile et al. (2011) confirm that litter quality controls shorter term dynamics of C decomposition and accumulation in soil but longer term SOM formation and stabilization is controlled by the quantity of litter input. Studies on the effects of residue management on soil characteristics are highly variables. For examples, many long-term studies in the have shown that residue removal reduces SOC (Allmaras et al., 2004; Barber, 1979; Karlen et al., 1994; Larson et al., 1972), whereas others have shown no change (Hooker et al., 2005). Likewise, several studies reported SOC decrease under continuous grain corn and silage removal versus moldboard plow incorporation of corn stover at different levels of fertility (Huggins et al., 1998; Reicosky et al., 2002; Robinson et al., 1996). Barber (1979) for example observed that 30 years of moldboard plowing diminished SOC both when only grain was harvested and when silage was harvested. Similarly, Barnhart et al. (1978) found that continual removal of corn silage on a silt loam

resulted in decreased SOC when compared where only grain was removed. Both studies suggest that SOC would decrease irrespective of residue management if soil were moldboard plowed.

Crop residues are of great benefit in agro-ecosystems, they are substantial reservoir of plant nutrients and therefore are of great value for soil fertility (Scholes and Scholes, 2013). In comparing different crop residue removal rate between no till and conventional till (with incorporated residues), Karlen et al. (1984) found that removing residues increased macronutrient removal. They concluded that some residue could safely be harvested for biofuel production purposes, if the nutrients from the residue were replaced by additional fertilizer. Besides the prevention of wind and water erosion, crop residue have gained attention for the many ecosystem services they provide, such as sustaining crop productivity, reducing non-point source risks; sedimentation and anoxia (Karlen et al., 2009). Additionally, reduce tillage, N fertilization and crop residues can positively interact to improve SOC, total soil nitrogen TSN, and soil structure. Furthermore, reduced disturbance help control SOC by reducing the rate of crop residues decomposition and aggregate degradation.

Because of the uncertainties and the complex interactions associated with residue management and SOC dynamics, computer simulations models are very often used to evaluate the potential effects of agricultural inputs such as residue and N fertilizer on SOC. However, no single simulation model incorporates all aspects of these of these complex interactions. In evaluating the applications of the EPIC and CENTURY models, Parton (1996) and Parton et al. (1996) examine the potential effects of reduced disturbance on SOC sequestration in agro-ecosystems and realized that there was considerable uncertainty about the impact of tillage and residue inputs on SOC and nutrient dynamics due to a lack of data. Therefore, the objectives of this study were to 1) analyze the effects of crop residue management and fertilizer on selected

soil characteristics and yield; and (2) predict soil C and biomass yield of the different treatments using the DNDC process based model. We hypothesized that at greater residue and fertilizer addition rate, soil organic carbon would increase due to increased inputs.

Materials and Methods

Site characteristics: The residue harvest study was conducted at the Kansas State University's East Central Experiment Field near Ottawa, (38^{o} 32' 14" N and 95^{o} 15' 14" W) on a nearly level, 0-1% slope, Woodson silt loam soil (fine, montmorillonitic, thermic, Abruptic Argiaquoll). Duration of the study was 24 years (1982 through 2005).

Experimental design: The crop residue and fertilizer treatments evaluated were: 1) crop residue harvested annually (0X); 2) normal amounts of crop residues returned to the soil and incorporated (1X); and 3) twice (2X) normal amounts of crop residue returned and incorporated (which was accomplished by spreading evenly the crop residues from the corresponding residue harvest treatments). The fertilizer treatments were zero, low, average, and high rates of N, P, and K applied at levels ranging from 0-120 kg N ha^{-1}, 0-50 kg ha^{-1} P and 0-84 kg ha^{-1} K for individual crops. These levels of fertilizer were applied to measure the effects of increased fertility on grain and residue biomass production and to determine the fertility needs of crops when both grain and residue yields were harvested. The crop residue and the fertilizer treatments were replicated four times in a randomized complete block split plot experiment design. The residue treatments were established as whole plots and the fertilizer treatments as subplots.

The crops grown, initially, were wheat, grain sorghum and soybean in a 3 yr rotation with one crop grown each year. Beginning in 1994, corn was substituted for grain sorghum in the rotation. In 2000, the cropping sequence was changed to a corn-soybean rotation to match

changes in cropping practices that had occurred in the area. Also, in some years, the cropping sequences were altered because of weather. In 1994, corn was planted in place of wheat because of a wet fall and not being able to plant wheat. In 2003, grain sorghum was planted in place of corn because of a prolonged wet spring.

The fertilizer treatments for wheat were applied prior to planting wheat in the fall and for grain sorghum and corn prior to planting in the spring. No fertilizer was applied in the years when soybeans were grown. Tillage each year consisted of a tandem disk-harrow operation (10-13 cm depth) immediately after the crop residues were harvested, followed by a second disk-harrow operation as needed, and a pre-plant field-cultivation (8-10 cm depth) immediately after the fertilizer materials were applied and before planting. Labeled rates of herbicides were applied to control weeds.

Soil Sampling and Analysis

Soil samples were collected in 2009, 4 years after termination of the experiment. After termination of the experiment plots were bulk planted with no till corn. The samples were obtained from the first 30 cm in three depth increments: 0-5, 5-15, and 15-30 cm for analyses. Soil samples consisted of ten soil cores collected randomly from each plot, all ten cores were combined to make a composite sample for each soil layer and replication. We also sampled an undisturbed native prairie site located nearby (50 m) from the field experiment; whose land use type matches that of the site at the time of establishment. Triplicate soil cores and intact soil were taken for bulk density determination and aggregate stability respectively. Subsamples of the first two layers of soil from the general analyses were stored immediately at -20ºC for

phospholipid fatty acids (PLFAs), after sampling to avoid modifications of the composition of microbial communities.

Soil Aggregates:

Soil aggregation was assessed according to the methods of (Mikha and Rice, 2004). Soils fractions were separated by slaking air-dry soil followed by wet-sieving (Elliott, 1986) through a series of four sieves (2000, 250, 53, and 20 μm). Air-dried soil (50 g) from 0-5, 5-15, and 15-30 cm depths were placed on the top of the sieve of each nest. To slake the air-dried soil, 1 L of water was rapidly added until soil was covered with water. The samples were submerged in water for 10 min following the 10 min of wet sieving. Four aggregate size classes were collected from each treatment >2000, 250-2000, 53-250, and <20 μm diameter. Water stable aggregates were dried and a subsample used to determine sand content of each fraction (Mikha and Rice, 2004). Large macroaggregates are defined as >2000 μm, small macroaggregates 250-2000 μm, microaggregates 250-53 μm, and silt plus clay by <53 μm size fraction. Total C and N were determined in each sand-free, water-stable aggregate size fractions.

Microbial Community Structure: Phospholipid Fatty Acid Analysis:

Phospholipids and neutral lipid fatty acids (PLFA and NLFA) analysis were determined following a modification of the Bligh and Dyer (1959) method (White and Ringelberg, 1997). Lipids were extracted with a single phase chloroform:methanol:phosphate buffer solution (Blight and Dyer, 1959) for 2 h from 5 g of freeze-dried soil. Total lipid extracts were separate into neutral lipids, polar lipids, and glycolipids using preconditioned silica gel disposable extraction columns (J.T. Baker, Phillipsburg, NJ, USA). Neutral and polar lipids were subject to alkaline methanolysis to cleave the fatty acids from the glycerol molecule replacing it with methyl groups thus creating fatty acid methyl esters. FAMEs were analyzed using a Thermo Scientific Trace

GC-ISQ mass spectrometer with a DB5-MS colum (30 m × 250 µm i.d. × 0.25 µm film thickness). Helium was the carrier gas (1.0 mL min^{-1} constant flow). The temperature program was: 50 to 170°C at 20°C per min^{-1}; from 170 to 270°C at 2°C min^{-1}. The injector temperature was 220°C. Analysis was conducted in the electron impact (70 eV) mode and mass spectrometer scanning m/z^{+} was from 200 to 400. Bacterial acid methyl esters mix (BAME; Matreya 1114) was used to identify peaks. Tentative assignments of methyl ester peaks not present in the BAME mix were made by mass spectral interpretation. The internal standard methyl nonadecanoate was used to quantify the data. Peaks are identified using retention times of fatty acid standards and by comparing spectra from a library (Wiley 138K mass spectral database). Samples peak are quantified based on comparison of the abundance with an internal standard nonadecanoic acid methyl ester (19:0) in terms of nmol g^{-1} dry soil or mol %. Fatty acids are designated ***a:b***, where a is the total number of carbons and ***b*** are the number of double bonds. An ***ω*** refers to the position of the double bond from the aliphatic end of the fatty acid. The prefixes ***a*** and ***i*** refer to ***anteiso*** and ***iso*** branching, the suffixes ***c*** and ***t*** indicate ***cis*** and ***trans*** conformations. Methyl groups are indicated by ***aMe***, where ***a*** indicates the position of the methyl group. Fatty acids were grouped into Gram positive bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram negative bacteria (16:1ω7c, 18:1ω7c, 2-OH 12:0, 3-OH 12:0, 2-OH 14:0, 3-OH 14:0, 2-OH 16:0 and cyclic cy17:0, cy19:0), actinomycetes (10Me18:0 and 10Me17:0), and fungi (18:2ω6,9c, 16:1ω5c arbuscular mycorrhizal and 18:1ω9c saprophytic fungi) (McKinley et al., 2005).

Modeling effort

In this study, DNDC was used to simulate SOC dynamics in our long-term experiment with the different crop residue management, fertilizer treatments and crop rotations. The model

contains four interacting sub-models of denitrification, decomposition, soil climate and plant growth (Li, 1996). DNDC is a process-based model that stimulates the effects of climate change, anthropogenic activities including agricultural management practices on SOC and N dynamics (Li, 1996). The model uses meteorological data as input variables and it works on a daily time step. Daily mean air temperature and precipitation as well as solar radiation from 1984 to present were extracted from Kansas State University Weather Library data Library. SOC change was calculated by tracking biomass production and losses through decomposition (Li et al., 1997). Soil input variables include pH, moisture, temperature and Eh. Management variables include tillage (all operations and dates). Observed SOC stocks used for testing the model were samples from 2007 (0-30 cm) and 2009 (0-30 cm) at Ottawa, Kansas.

Statistical analysis:

Treatment effects on measured variables were tested by analysis of variance and all statistical procedures were carried out using SAS 9.2 SAS institute, Cary NC (Institute, 2009). The 24 yr field experimental was analyzed as a split plot design using Proc MIXED from the SAS statistical software 9.2 (SAS Institute, Cary NC). The main effects of crop residue inputs and N fertilizer and their interaction were treated as fixed effects, while block and block * crop residue were considered random effects. Treatments comparisons were considered significant at $P< 0.05$ for all analyses and mean separations were performed using Pdiff option of the LSMEANS statement. The means were compared using Tukey's HSD. Unless otherwise stated, all differences discussed are significant at the $P < 0.05$ probability level.

Results

Soil aggregate distribution and stability

Wet aggregate size distribution data showed a higher proportion of the whole soil in the micro aggregates (53-250 μm) (Fig. 3.1). Residue management significantly ($P<0.05$) effected the percentage of both micro and macro-aggregates (Table 3.1). However, there was no significant interaction effect of residues and fertilizer on the proportion of different aggregates size. The two main factors explaining the variation in aggregates was residue management and depth of the profile (Table 3.1). In the top 5 cm soil, the greatest proportion of large macroaggregates was found in the 2X and 1X, while the lowest was found in the 0X. In contrast the 0X residue management had a greater fraction of soil in the greater microaggregates (less desirable and more erodible) size fractions compared to the 2X and 1X (Fig.3.1). There were no treatment effects on aggregate distribution and stability in the 5-15 and 15-30 cm but the small macroaggregates represented the greatest proportion of the whole soil below 15cm.

Carbon distribution in the aggregate fractions

In the 0-5 cm soil layer, the carbon content associated with the aggregates was greater in soil where crop with residue returned and incorporated to the soil regardless of the aggregate fraction (Fig. 3.2). In other words, when residue was removed, C was lost in all the aggregate fractions. No treatment differences were found between residue maintenance and addition for the small macroaggregates and the larger microaggregates. The amount of residue significantly affected ($P<0.05$) C in the small and large macroaggregates as well as in the microaggregates. In the treatments with residue maintenance or addition, the organic C content in the small microaggregates was greater (2.4%) than where residue was removed (2.08%). In the large

macroaggregates, the greatest organic C was found for the higher residue return (2.1%), while the lowest organic C was found for the soil without residue (1.7%). The C content associated with aggregates decrease with cultivation compared to the native prairie reference. The decline was mainly due to the loss with the greater microaggregates and the larger macroaggregates. The undisturbed native prairie had 48%; 36% and 47% more C, in the respective pools of C >2000 µm, 2000-250 µm, and 250-53µm compared to the cultivated soil.

In the 5-15 cm soil layer, we observed a similar trend as the upper 5cm of soil but with a little attenuation. The C content associated with the aggregates was significantly greater in treatments with residue addition compared to when residue was removed for all aggregate fractions (Fig. 3.3). Residue maintenance and removal did not show a difference for any aggregate size fractions at this particular depth. Except for the greater microaggregates, residue maintenance and addition did not show treatments differences at this soil depth. The undisturbed native prairie had 40%; 33% and 39% more C, in the respective pools of carbon >2000 µm, 2000-250 µm, and 250-53µm compared to the cultivated soil.

In the 15-30 cm soil layer, C content associated with the aggregates did not show any treatment effect in the small macroaggregates but was significantly greater in treatments with residue addition compared to when residue was removed (Fig. 3.4). Residue maintenance and removal did not show a difference for any aggregate size fractions at this particular depth. Except for the greater microaggregates, residue maintenance and addition did not show treatments differences at this soil depth. The undisturbed native prairie had 27%; 24% and 28% more C, in the respective pools of C >2000 µm, 2000-250 µm, and 250-53µm compared to the cultivated soil. In all 3 soil layers, 0-5cm, 5-15 cm, and 15-30 cm, the C content in the small macroaggregates contributed most to the organic C averaged 69%.

The relationship between intra C aggregates associated to the different fractions of aggregates; for the different residue treatment is shown in (Fig. 3.5). In the larger macroaggregates, the positive linear relationship between intra C aggregate associated to the larger macroaggregate was highly significant ($R^2 = 0.99$, $P = 0.05$). However, no correlation between C in aggregate and their associated small macroaggregate was found. The responsiveness of the carbon in aggregate associated to the great microaggregates was negative. The C content associated with the aggregates decreased with the amount of residue that was returned. The negative correlation between the C concentration of the aggregates and the greater microaggregates (53-250 µm) suggests lower affinity between the carbon storage and micro aggregates in these soils.

Soil Carbon

After 24 years of study, residue management had no significant effect on soil organic carbon in the 0-5, 5-15, and 15-30 cm. Crop residue management and fertility level interactions were not significant at $P < 0.05$ (Table 3.2). The comparison of crop residue being removed or added in combination with fertility showed a small decline in SOC where residue was removed in the 0-5 cm (Fig. 3.6). These slowly occurring effects could have significant impacts in the sustainability of the soil resource. In the top 30 cm layer, the SOC stratification ratio was significantly higher with residue addition (1.24) compared to residue removal (1.12). There were no significant differences in stratification ratio values between residue maintenance (1.18) and removal or, maintenance and addition (Fig. 3.7). These values were generally consistent with other studies (Franzluebbers, 2002; Sá and Lal, 2009).

Simulating soil carbon change over time

Simulation of soil C trends in the 0-30 cm depth over the duration of the study based on the DNDC model and displaying measured values 23 and 25 years after establishment is shown in (Fig. 3.8). Generally, the DNDC model simulated the soil C effectively in all 12 treatment combinations (RMSE = 3.82) but we used just four treatments (maintaining and removing residue in combination with normal and high fertility) for practical reason. The simulations suggest that crop residue removal or maintenance in combination with normal and high fertilizer should not result in a big difference after 25 years under current practices, and this was supported by the measurements.

Grain and residue yield response to residue management and fertilizer treatment

The yearly average residue and grain yield as influence by the different treatments considered in this study are shown in (Fig.3.9- 3.18). Grain and straw yield show a decreasing trend across the years (Fig.3.9-3.12). Despite the decreasing grain and residue yield with time, slight or non-significant grain yield increases due to application of crop residues occurred in most years. As expected, fertilizer application increased wheat yields. Residue management had no effect on grain and sorghum residue yields. Grain and residue yield of sorghum followed the same trend and increased with fertilizer (Fig. 3.13-3.14). Compared to low and no fertilizer, high fertilizer produced greater grain and residue for sorghum. Sorghum residue yield for the first 3years (1983-1986) averaged 3.8 Mg ha^{-1} but in some years was as high as 6.5 Mg ha^{-1}. Sorghum residue yield were greatest in 1989 (7 Mg ha^{-1}), but sorghum grain yields were greatest in 1992 (9 Mg ha^{-1}). Both sorghum grain and residue yields decrease in 2002 by about 45% and 60% for residue and grain yields, respectively. Residue management had no effect on corn grain and residue yields. The beneficial effect of fertilizer appeared to be relatively greater under corn

(Fig. 3.15-3.16). Each year; there were significant differences in corn grain and residue yield due to fertilizer input. Soybean did not respond to crop residue management. However, both soybean grain and residue showed an increasing trend over the duration of our experiment (Fig. 3.17-18).

Soil microbial community structure, aggregation and organic matter composition

The soil C:N ratio was least for 0X (Fig. 3.19) and highest for 2X (Fig. 3.21). The C:N was generally not different between the 5-15 and the 15-30 cm depths and among all crop residue management. The C:N ratio declined in the 0-5 cm relative to the lower depths under 1X and 2X. The C:N declined faster under the residue maintenance (1X) or addition (2X) compared to the residue removal. The profiles in the cultivated soils were very similar with respect to the evolution of microbial parameters and their associated macroaggregates. However, we observed a strong mismatch of microbial abundance and the larger macroaggregates as well as the C:N ratio in the native prairie soil. The clear physical disconnection between the community of decomposers and the macroaggregates indicates that the microorganisms were spatially isolated from the large macroaggregates.

Discussion

After 24 years of crop residue management and fertilizer treatments, there were slight but non-significant changes in the level of soil C in the top 0-30 cm of soil for the different treatments combination. Total N did not show any difference in residue management or fertilizer treatments at any depth (Appendix Fig. B.1).Our results confirmed earlier observations after 11 years which indicate that despite the different amount of residue returned there were no significant difference in residue management or fertilizer treatment (Janssen and Whitney, 1995). This was not to be expected in view of the increased crop residue addition and fertilizer input.

Several other studies have shown that SOC was controlled by crop residue input, fertilization, and climate (Reicosky and Lindstrom, 1995). Likewise, SOC was reported to increase by 14% in residue returned and N fertilization subjected to 13 years experiment in Minnesota (Clapp et al., 2000); and in a study by (Karlen et al., 1994). Doubling of corn residue for 10 years significantly increase SOC and aggregate stability. However, few studies have surprisingly shown small differences in SOC as a function of increased C inputs (Campbell et al., 1991; Soon, 1998). Paustian et al. (1997) suggested that soils initially high in SOC are less responsive to changes in C input and may not gain measurable amounts of C independent of the input rate. Twenty four years experiment of removing, maintaining and doubling the amount of residue combined with a tandem disk harrow had a little measurable effect on SOC content. Apparently, tillage was masking the effects of residue inputs. This is similar to findings from (Reicosky et al., 2002) after 30 years of moldboard tillage with different levels of inputs yielded no difference in SOC. They concluded that tillage was causing a rapid release of CO_2 masking the effects of fertilizer and residue. Both simulated and observed data showed a modest decreasing trend where residue removed and these slowly occurring effects may have big impacts for the sustainability of the soil resource in the long-term. Our simulated results regarding the influence of crop residue management and fertilizer inputs to SOC dynamic are consistent with modeling studies (Lemke et al., 2010) which suggest that residue removal treatment should result in a modest change in soil C when all residue was maintained for the fertilized treatment but the effect might not be detected *in situ* due to large spatial variability in their measured values. One reason for the similarity of findings may be related to the fact that the DNDC model estimates C inputs from the measured yields in the same way than the Introductory Carbon Balance Model. In general, crop residue management did not have any effect on grain or residue production,

whenever an increase occurs; it was attributable to increased nutrient supply. Overall the results suggest that there were no significant changes in yield due to crop residue management. Changes in relation to productivity were more evident with fertilization in wheat and corn grain and residue yields; probably due to significant of fertilization on microbial transformations such as decomposition, mineralization, nitrification, decreasing N availability to crops. It is clear that the residue removal maintenance or addition had a rather small effect on soil carbon; however, we saw a much larger impact on the aggregate stability and the carbon inside the aggregate. Aggregate stability may be used as an early warning sign in soil carbon change.

Despite the lack of residue inputs on SOC, the effect of treatments on the aggregate stability and the distribution of C inside the aggregate were significant. Rice and Smith (1984) reported that residue incorporation resulted in a more uniform distribution of surface C and N throughout the surface. Mechanical tillage can influence microbiological, chemical and physical properties of soil by modifying organic matter, enhancing mineralization, reducing aggregate stability and water exchange (Carter and Steed, 1992; Gupta and Germida, 1988; Tisdall and Oades, 1982). Plowing stimulates decomposition of organic matter by oxidation, breaking aggregates and incorporating crop residues into the soil; increasing the contact between soil decomposers and crop residue. Therefore, it is evident that with disturbance, the microorganisms will have access and metabolized the organic matter. Additionally, the frequent tillage continually exposes soil to wet-dry cycles at the soil surface (Beare et al., 1994) further increasing the susceptibility of aggregates to disrupt (Mikha et al., 2005). Soil microorganisms represent a small portion of SOM (<5%) but due to its dynamic nature acts as a sink or source of nutrients. Microbial biomass is responsible for 85-90% of OM decomposition and about 10-15% of the energy of organic carbon are utilized by soil animals (Wolters, 2000). Abiotic chemical

oxidation accounts for only 5% of decomposition (Lavelle et al., 1993), so it is safe to say that C cycling and decomposition are mainly driven by biological mechanisms. There is growing evidence that the degradation rate is not controlled by the molecular structure of the SOM but by the soil environment in which the degradation takes place (Kleber and Johnson, 2010; Kögel-Knabner et al., 2008; Marschner et al., 2008; Schmidt et al., 2011). It is now evident that physical inaccessibility of organic substrate to decomposer microbes or extracellular enzymes can arise for numerous reasons and appropriate substrates are sparsely and heterogeneously distributed in the soil matrix (Bachmann et al., 2008). In many soils, inaccessibility is caused by aggregation of the soil at multiple spatial scales, creating diffusional limitations to enzyme and oxygen movement. The low effect of crop residue on SOC was probably due to increased accessibility of soil organisms caused by the constant tillage (Barnhart et al., 1978; Reicosky et al., 2002). The lack of difference in effect of treatment (residue and fertility) on soil C was also probably a function of the lack of difference in crop residue yield response after different levels of fertility and crop residue was superimposed. The tillage mixed the soil and the residue; therefore, masking the effect of the residue. The increased aggregate stability in residue maintenance and addition compared to residue removal was significant but not sufficient to cause a difference in SOC. Six et al. (2004) reported that the stability of a soil is related to the proportion of large macroaggregates, normally accumulating most of the C in soil. The greater microaggregates (53-250 µm) comprise the greatest proportion of the soil (55%) followed by 24% for the smaller macroaggregates (250-2000 µm) and only 0.7% for the larger macro aggregates (>2000µm). The small macroaggregates contained more C than the large macroaggregates contrary to the concept of aggregate hierarchy where the large macroaggregates tend to be richer in organic C compared to smaller aggregates (Balesdent et al., 2000; Oades,

1984; Tisdall and Oades, 1982). A possible explanation of the lower C content in the large macroaggregates compared to the smaller macroaggregates in our study is that multiple disturbances caused greater loss of C in the larger macroaggregates. In a system with degradation and reduction of the macroaggregates it make sense to find greater carbon content in smaller than larger macro aggregates. Previous studies have reported increasing organic carbon content associated with increasing sizes of macro aggregates (Elliott, 1986; Wilson et al., 2009). Studies in Iowa (Barnhart et al., 1978; Larson et al., 1972; Robinson et al., 1996), and in Indiana (Barber, 1979) have indicated that with intensive tillage, soil C decreases with continued removal of residue or show small increases with continued large input of crop residue. There were two new insights to be gained from our observations, (1) the abundance of soil microorganisms in intensely cultivated soils generally follow their potential carbon food source; (2) there is a widespread evidence that soil structure protect organic matter from decomposition (Lützow et al., 2006) but all the findings are based on the fact that carbon mineralization is enhanced when soil aggregates are disrupted (Craswell and Waring, 1972; Elliott, 1986; Gupta and Germida, 1988; Powlson, 1980; Reicosky et al., 1997; Rovira and Greacen, 1957; Six et al., 2002; Sørensen, 1983; Tebrügge and Düring, 1999). Our study showed a clear physical disconnection between the decomposers and their potential carbon source e.g. depth profiles of microbial parameters (Fig. 3.19 – 3.22). There is a considerable body of knowledge about the biology (Elliott, 1986; Wilson et al., 2009), chemistry and physics (Mikha et al., 2005) of soil aggregate formation and its stability (Balesdent et al., 2000; Oades, 1984; Tisdall and Oades, 1982) . However, detailed measurements are not available and in complex non-linear systems such as soils and its attributes, details are important. One possible implications of these findings is; current soil C models do not considered C protection in aggregate or implement physical

protection in an oversimplify way by using the amount of clay to partition C between slow and passive pool Parton (1996) and Parton et al. (1996). Detailed understandings of aggregate dynamic such as those highlighted above are needed in order to model more realistically how soil C is stabilized in a soil. PLFA data are shown in appendix Table B.1. Our study support the idea that gains in SOC could be limited in cropping systems that employed intensive tillage (Reicosky et al., 2002). Any attempt to embark on bioenergy programs that require the use of crop residue should considered the adoption of no-till or reduced disturbance for these soils. We also recommend that studies are conducted in any region where crop residue is being considered as primary feedstock for bioenergy production.

Conclusion

Soil carbon would decrease irrespective of the combinations of crop residue management and fertilizer treatment if the soil were plowed. Undisturbed native vegetation had 48%; 36% and 47% more carbon, in the respective pools of carbon; > 2000 µm, 2000-250 µm, and 250-53 µm. Therefore, it appears safe to conclude that reduce tillage, when combined with an adequate residue and fertilizer management strategy, can increase soil organic carbon. Significantly greater gain of carbon could be expected by reducing the disturbance of these soils, thus contributing to the sustainability of the soil resource. DNDC successfully predicted the potential long-term effects of the various combinations of crop residue and fertilizer management on soil carbon. The phospholipids and fatty acids (PLFA) analyses used to investigate composition of the soil microbial communities in these agricultural soils by comparing treatments such as residue removal (0X), maintenance (1X), or double (2X) and the native prairie site revealed how soil microorganisms and their potential food sources are distributed in disturbed or undisturbed

soils. Our results show that no matter how important the amount of residue returned and incorporated to the soil, if we continually disturbed these soils, we will experience the similar dramatic effects caused by removing the entire crop residue from the field.

References

Allmaras R.R., D.R. Linden, and C.E. Clapp. 2004. Corn-residue transformations into root and soil carbon as related to nitrogen, tillage, and stover management. Soil Sci. Soc. Am. J. 68:1366-1375.

Bachmann J., G. Guggenberger, T. Baumgartl, R.H. Ellerbrock, E. Urbanek, M.-O. Goebel, K. Kaiser, R. Horn, and W.R. Fischer. 2008. Physical carbon-sequestration mechanisms under special consideration of soil wettability. J. Plant Nutrit. Soil Sci. 171:14-26. DOI: 10.1002/jpln.200700054.

Balesdent J., C. Chenu, and M. Balabane. 2000. Relationship of soil organic matter dynamics to physical protection and tillage. Soil Till. Res. 53:215-230

Barber S.A. 1979. Corn residue management and soil organic matter. Agron. J. 71:625-627. DOI: 10.2134/agronj1979.00021962007100040025x.

Barnhart S.L., W.D. Shrader, and J.R. Webb. 1978. Comparison of soil properties under continuous corn grain and silage cropping systems. Agron. J. 70:835-837. DOI: 10.2134/agronj1978.00021962007000050031x.

Beare M.H., P.F. Hendrix, and D.C. Coleman. 1994. Water-stable aggregates and organic matter fractions in conventional- and no-tillage soils. Soil Sci. Soc. of Am. J. 58:777-786. DOI: citeulike-article-id:7292043.

Campbell C.A., G.P. Lafond, R.P. Zentner, and V.O. Biederbeck. 1991. Influence of fertilizer and straw baling on soil organic matter in a thin black chernozem in western Canada. Soil Biol. Biochem. 23:443-446.

Carter M., and G. Steed. 1992. The effects of direct drilling and stubble retention on hydraulic properties at the surface of duplex soils in north-eastern Victoria. Soil Res. 30:505-516.

Clapp C.E., R.R. Allmaras, M.F. Layese, D.R. Linden, and R.H. Dowdy. 2000. Soil organic carbon and 13C abundance as related to tillage, crop residue, and nitrogen fertilization under continuous corn management in Minnesota. Soil Till. Res. 55:127-142.

Craswell E.T., and S.A. Waring. 1972. Effect of grinding on the decomposition of soil organic matter—II. Oxygen uptake and nitrogen mineralization in virgin and cultivated cracking clay soils. Soil Biol. Biochem. 4:435-442.

Dufey A., 2006. Biofuels production, trade and sustainable development: emerging issues., International Institute for Environment and Development (IIED), , London.

Dungait J.A.J., D.W. Hopkins, A.S. Gregory, and A.P. Whitmore. 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Glob. Change Biol. 18:1781-1796. DOI: 10.1111/j.1365-2486.2012.02665.x.

Dungait J.A.J., S.J. Kemmitt, L. Michallon, S. Guo, Q. Wen, P.C. Brookes, and R.P. Evershed. 2011. Variable responses of the soil microbial biomass to trace concentrations of 13C-labelled glucose, using 13C-PLFA analysis. Eur. J. Soil Sci. 62:117-126. DOI: 10.1111/j.1365-2389.2010.01321.x.

Elliott E.T. 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils1. Soil Sci. Soc. Am. J. 50:627-633. DOI: 10.2136/sssaj1986.03615995005000030017x.

Franzluebbers A.J. 2002. Soil organic matter stratification ratio as an indicator of soil quality. Soil Till. Res. 66:95-106.

Govaerts B., N. Verhulst, A. Castellanos-Navarrete, K.D. Sayre, J. Dixon, L. Dendooven. 2009. Conservation agriculture and soil carbon sequestration: between myth and farmer reality. Crit. Rev. Plant Sci. 28:97-122. DOI: 10.1080/07352680902776358.

Gupta V.V.S.R., and J.J. Germida. 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biol. Biochem. 20:777-786.

Hooker B.A., T.F. Morris, R. Peters, and Z.G. Cardon. 2005. Long-term effects of tillage and corn stalk return on soil carbon dynamics. Soil Sci. Soc. Am. J. 69:188-196. DOI: 10.2136/sssaj2005.0188.

Huggins D.R., C.E. Clapp, R.R. Allmaras, J.A. Lamb, and M.F. Layese. 1998. Carbon dynamics in corn-soybean sequences as estimated from natural carbon-13 Abundance. Soil Sci. Soc. Am. J. 62:195-203. DOI: 10.2136/sssaj1998.03615995006200010026x.

Institute S. 2009. SAS 9.2 Output delivery system user's guide SAS Inst., Cary, NC.

Janssen K.A., and D.A. Whitney. 1995. Crop residue removal and fertilizer effects on crop yield and soil sustainability. Bet. Crops 79:4-6.

Karlen D.L., P.G. Hunt, and R.B. Campbell. 1984. Crop residue removal effects on corn yield and fertility of a norfolk sandy loam. Soil Sci. Soc. Am. J. 48:868-872. DOI: 10.2136/sssaj1984.03615995004800040034x.

Karlen D.L., N.C. Wollenhaupt, D.C. Erbach, E.C. Berry, J.B. Swan, N.S. Eash, and J.L. Jordahl. 1994. Crop residue effects on soil quality following 10-years of no-till corn. Soil Till. Res. 31:149-167. DOI: Doi: 10.1016/0167-1987(94)90077-9.

Karlen D.L., R. Lal, R.F. Follett, J.M. Kimble, J.L. Hatfield, J.M. Miranowski, C.A. Cambardella, A. Manale, R.P. Anex, and C.W. Rice. 2009. Crop residues: The rest of the story. Environ. Sci. Tech. 43:8011-8015. DOI: 10.1021/es9011004.

Kladivko E.J. 2001. Tillage systems and soil ecology. Soil Till. Res. 61:61-76.

Kleber M., and M.G. Johnson. 2010. Chapter 3 - Advances in understanding the molecular structure of soil organic matter: Implications for interactions in the environment. In: L.S. Donald (Ed.), Adv. Agron, Academic Press. pp. 77-142.

Kögel-Knabner I., K. Ekschmitt, H. Flessa, G. Guggenberger, E. Matzner, B. Marschner, and M. von Lützow. 2008. An integrative approach of organic matter stabilization in temperate soils: Linking chemistry, physics, and biology. J. Plant Nutrit. Soil Sci. 171:5-13. DOI: 10.1002/jpln.200700215.

Larson W.E., C.E. Clapp, W.H. Pierre, and Y.B. Morachan. 1972. Effects of increasing amounts of organic residues on continuous corn: II. Organic carbon, nitrogen, phosphorus, and sulfur. Agron. J. 64:204-209. DOI: 10.2134/agronj1972.00021962006400020023x.

Lavelle P., E. Blanchart, A. Martin, S. Martin, and A. Spain. 1993. A hierarchical model for decomposition in terrestrial ecosystems: Application to soils of the humid tropics. Biotrop. 25:130-150. DOI: 10.2307/2389178.

Lemke R.L., A.J. VandenBygaart, C.A. Campbell, G.P. Lafond, and B. Grant. 2010. Crop residue removal and fertilizer N: Effects on soil organic carbon in a long-term crop rotation experiment on a udic boroll. Agric. Ecosys. Environ. 135:42-51. DOI: DOI: 10.1016/j.agee.2009.08.010.

Lützow M.v., I. Kögel-Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner,and H. Flessa. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – A review. Eur. J. of Soil Sci. 57:426-445. DOI: 10.1111/j.1365-2389.2006.00809.x.

Marschner B., S. Brodowski, A. Dreves, G. Gleixner, A. Gude, P.M. Grootes, U. Hamer, A. Heim, G. Jandl, R. Ji, K. Kaiser, K. Kalbitz, C. Kramer, P. Leinweber, J. Rethemeyer, A. Schäffer, M.W.I. Schmidt, L. Schwark, and G.L.B. Wiesenberg. 2008. How relevant is recalcitrance for the stabilization of organic matter in soils? J. of Plant Nutrit. Soil Sci. 171:91-110. DOI: 10.1002/jpln.200700049.

McKinley V.L., A.D. Peacock, and D.C. White. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biol. Biochem. 37:1946-1958.

Mikha M.M.,and C.W. Rice. 2004. Tillage and manure effects on soil and aggregate-associated carbon and nitrogen contribution No. 03-86-J of Kansas Agric. Exp. Stn. Soil Sci. Soc. Am. J. 68:809-816. DOI: 10.2136/sssaj2004.8090.

Mikha M.M., C.W. Rice, and G.A. Milliken. 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biol. Biochem. 37:339-347.

Oades J.M. 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76:319-337. DOI: 10.1007/bf02205590.

Parton W.J. 1996. The CENTURY model. In: D.S. Powlson, et al. (Eds.), Evaluation of soil organic matter models using existing long-term datasets. Springer, New York. pp. 284-291.

Parton W.J., D.S. Ojima, and D.S. Schimel. 1996. Models to evaluate soil organic matter storage and dynamics. In: M.R. Carter and B.A. Stewart (Eds.), Structure and organic matter storage in agricultural soils, CRC Press, Lewis Publishers, New York. pp. 421-448.

Paustian K., H.P. Collins, and E.A. Paul. 1997. Management controls on soil carbon. In: E. A. Paul et al. (Eds.), Soil organic matter in temperate ecosystems: Long-term experiments in North America, CRC Press, New York. pp. 15-49.

Powlson D.S. 1980. The effect of grinding on microbial and non-microbial organic matter in soil. J. Soil Sci. 31:77-85. DOI: 10.1111/j.1365-2389.1980.tb02066.x.

Reicosky D.C., and M.J. Lindstrom. 1995. The impact of fall tillage on short-term carbon dioxide flux. In: R. Lal (Ed.), Soils and global change, Lewis Publishers, Chelsea, MI. pp. 177-187.

Reicosky D.C., W.A. Dugas, and H.A. Torbert. 1997. Tillage-induced soil carbon dioxide loss from different cropping systems. Soil Till. Res. 41:105-118.

Reicosky D.C., S.D. Evans, C.A. Cambardella, R.R. Allmaras, A.R. Wilts, and D.R. Huggins. 2002. Continuous corn with moldboard tillage: Residue and fertility effects on soil carbon. J. Soil Water Cons. 57:277-284.

Rice C.W., and M.S. Smith. 1984. Short-term immobilization of fertilizer nitrogen at the surface of no-till and plowed soils. Soil Sci. Soc. Am. J. 48:295-297. DOI: 10.2136/sssaj1984.03615995004800020013x.

Robinson C.A., R.M. Cruse, and M. Ghaffarzadeh. 1996. Cropping system and nitrogen effects on mollisol organic carbon. Soil Sci. Soc. Am. J. 60:264-269. DOI: 10.2136/sssaj1996.03615995006000010040x.

Rovira A., and E. Greacen. (1957.) The effect of aggregate disruption on the activity of microorganisms in the soil. Aus. J. Agric. Res. 8:659-673.

Sá J.C.d.M., and R. Lal. 2009. Stratification ratio of soil organic matter pools as an indicator of carbon sequestration in a tillage chronosequence on a Brazilian Oxisol. Soil Till. Res. 103:46-56.

Schmidt M.W.I., M.S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I.A. Janssens, M. Kleber, I. Kogel-Knabner, J. Lehmann, D.A.C. Manning, P. Nannipieri, D.P. Rasse, S. Weiner, and S.E. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478:49-56.

Scholes M.C., and R.J. Scholes. 2013. Dust unto dust. Science 342:565-566. DOI: 10.1126/science.1244579.

Six J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Till. Res. 79:7-31.

Six J., C. Feller, K. Denef, S.M. Ogle, J.C.d.M. Sa, and A. Albrecht. 2002. Soil organic matter, biota and aggregation in temperate and tropical soils - Effects of no-tillage. Agron. 22:755-775.

Soon Y.K. 1998. Crop residue and fertilizer management effects on some biological and chemical properties of a Dark Grey Solod. Can. J. Soil Sci. 78:707-713. DOI: 10.4141/s98-016.

Sørensen L.H. 1983. Size and persistence of the microbial biomass formed during the humification of glucose, hemicellulose, cellulose, and straw in soils containing different amounts of clay. Plant Soil 75:121-130. DOI: 10.1007/bf02178619.

Tebrügge F., and R.A. Düring. 1999. Reducing tillage intensity — a review of results from a long-term study in Germany. Soil Till. Res. 53:15-28.

Tisdall J.M., and J.M. Oades. 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33:141-163. DOI: 10.1111/j.1365-2389.1982.tb01755.x.

West T.O., and W.M. Post. 2002. Soil organic carbon sequestration rates by tillage and crop rotation. Soil Sci. Soc. Am. J. 66:1930-1946. DOI: 10.2136/sssaj2002.1930.

Wilson G.W.T., C.W. Rice, M.C. Rillig, A. Springer, and D.C. Hartnett. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments. Ecol. Let. 12:452-461. DOI: 10.1111/j.1461-0248.2009.01303.x.

Wolters V. 2000. Invertebrate control of soil organic matter stability. Biol. Fert. Soils 31:1-19.

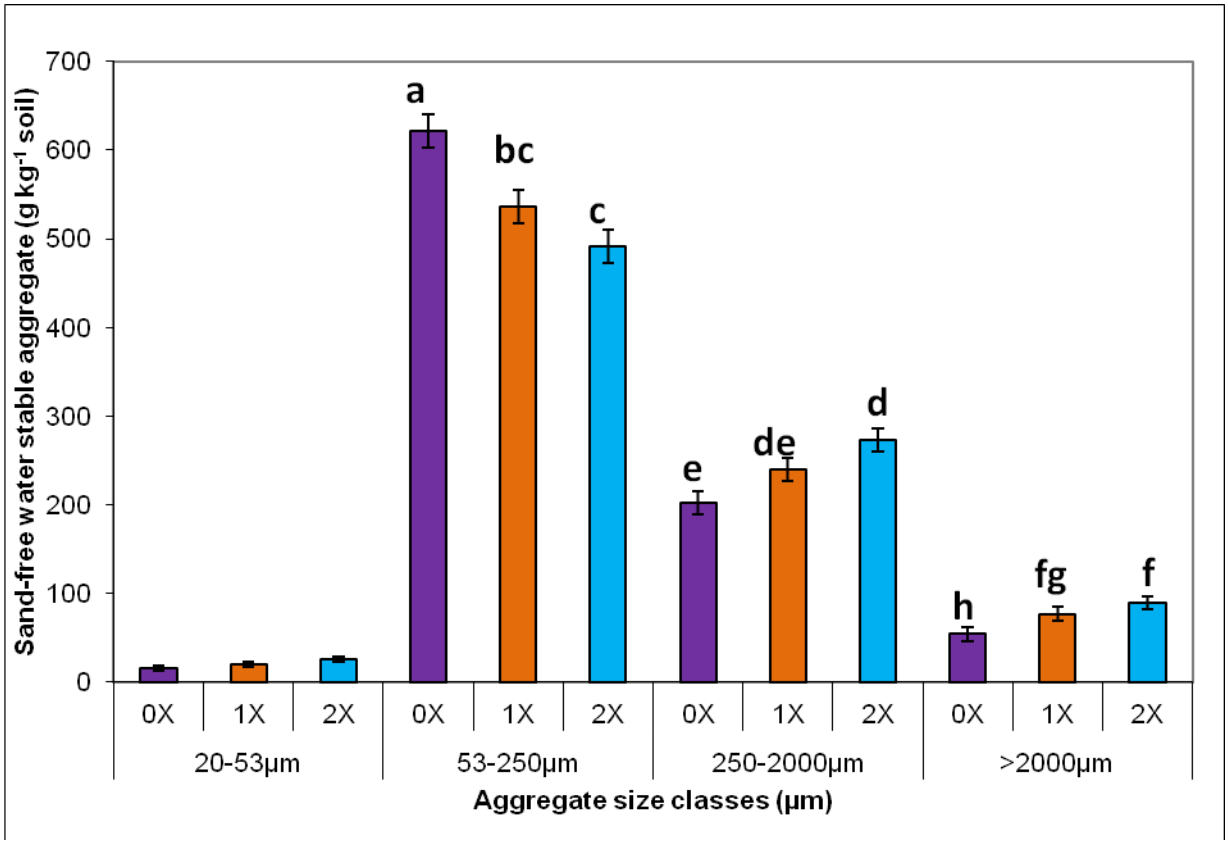


Figure 3.1. Distribution of the sand-free water stable aggregates (SFWSA) in top 5 cm. Values followed by a different letter within the same aggregate fraction are significantly different at ($P<0.05$).

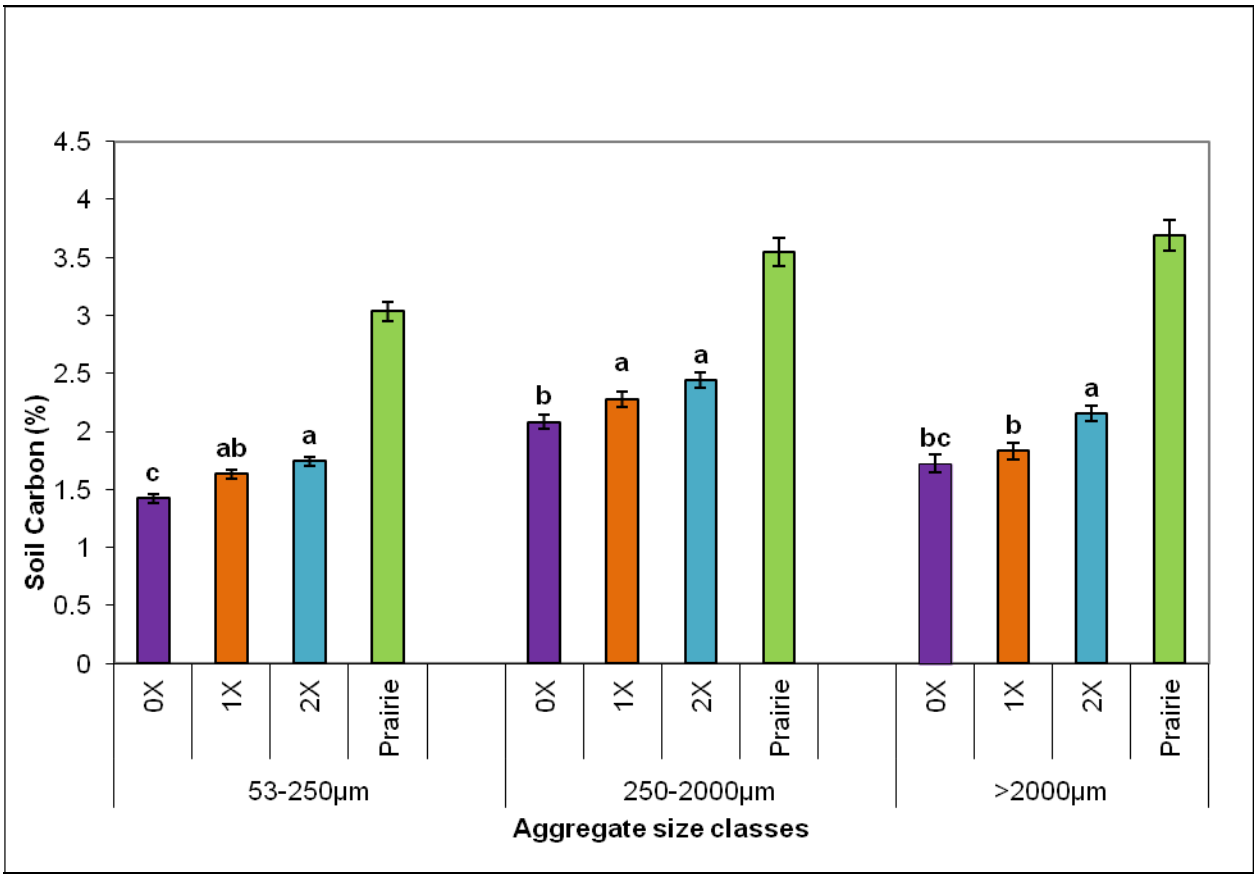


Figure 3.2. Total SOC contents (0-5 cm) in different aggregate size fractions in silt loam under different crop residue management with a native prairie. Values followed by a different letter within the same aggregate fraction are significantly different at ($P<0.05$).

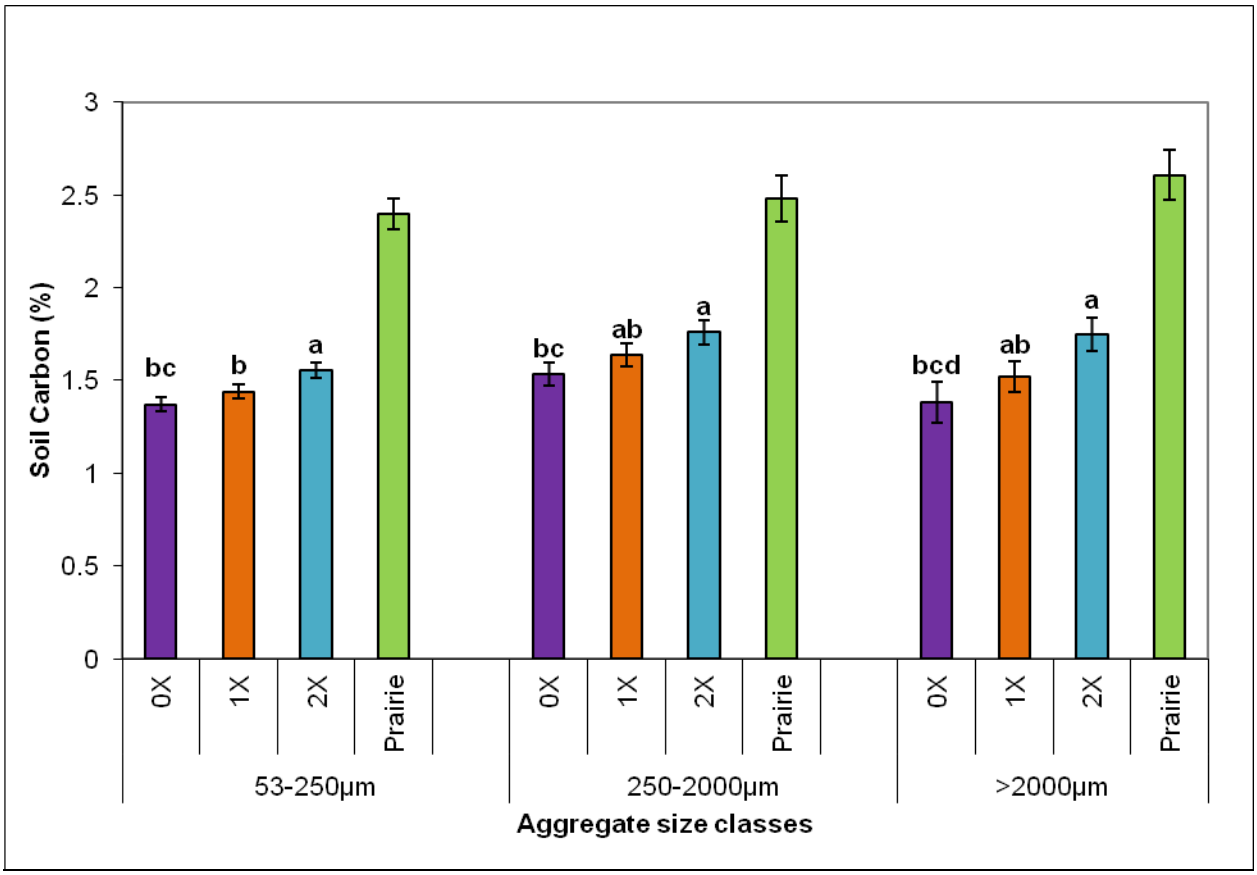


Figure 3.3. Total SOC contents (5-15 cm) in different aggregate size fractions in silt loam under different crop residue management with a native prairie. Values followed by a different letter within the same aggregate fraction are significantly different at ($P<0.05$).

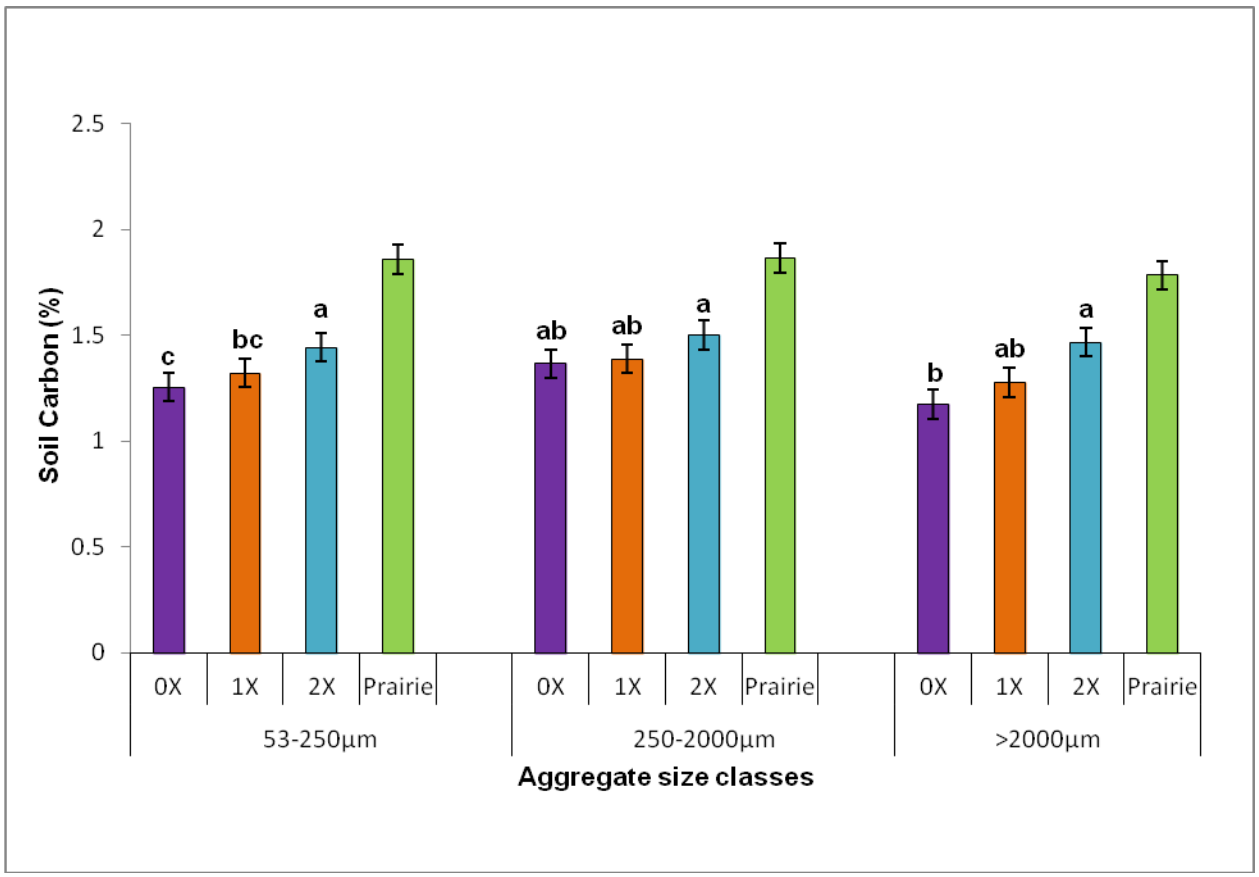


Figure 3.4. Total SOC contents (15-30 cm) in different aggregate size fractions in silty clay loam under different crop residue management with a native prairie. Values followed by a different letter within the same aggregate fraction are significantly different at ($P<0.05$).

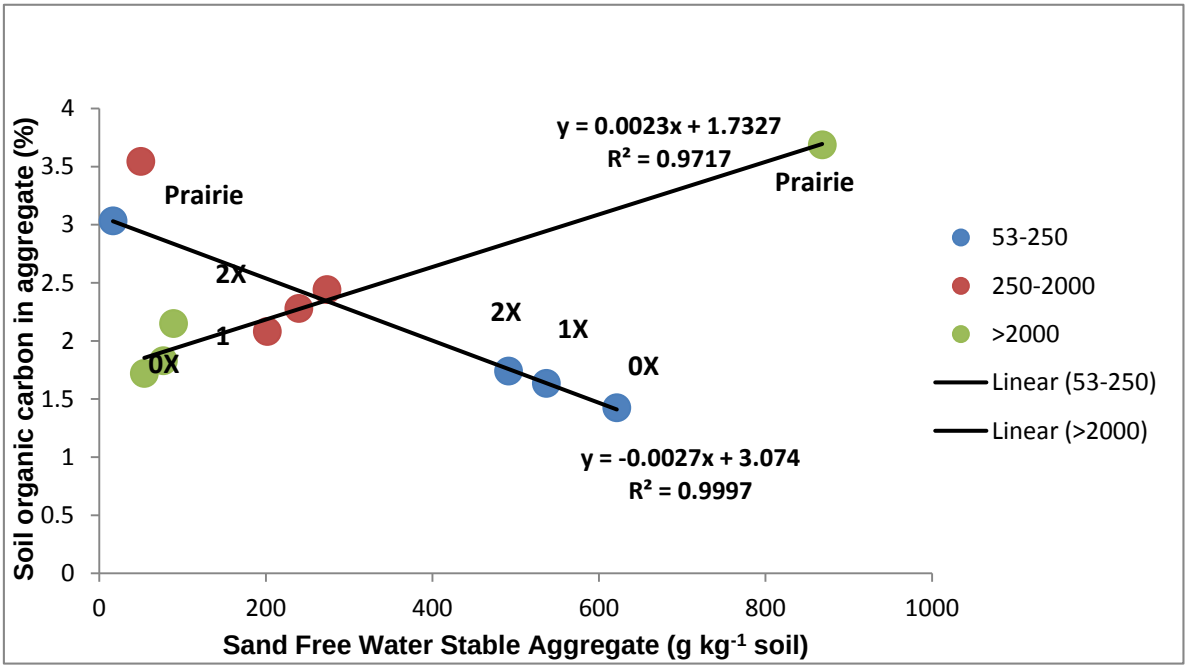


Figure 3.5. Relationship between carbon in aggregate and the amount of SFWSA in the top 5 cm according to residue management, using mean values of all treatments (Ottawa, KS)

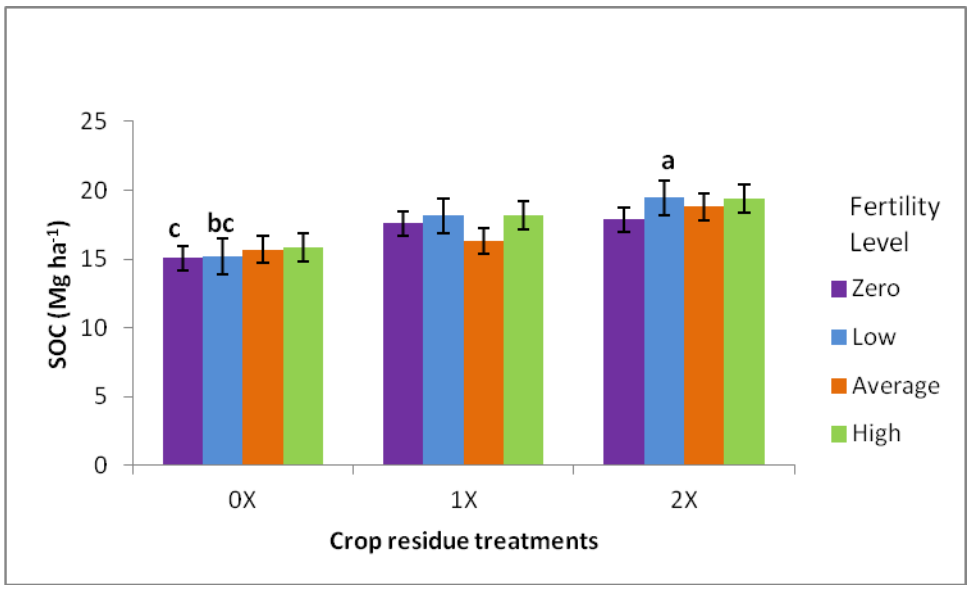


Figure 3.6. Effects of crop residue management and level of fertility on SOC after 24 years of study (0-5cm). Mean value ± standard error of four replicates. Means follow by different letters are significantly different, as measured by Tukey's HSD ($p<0.05$).

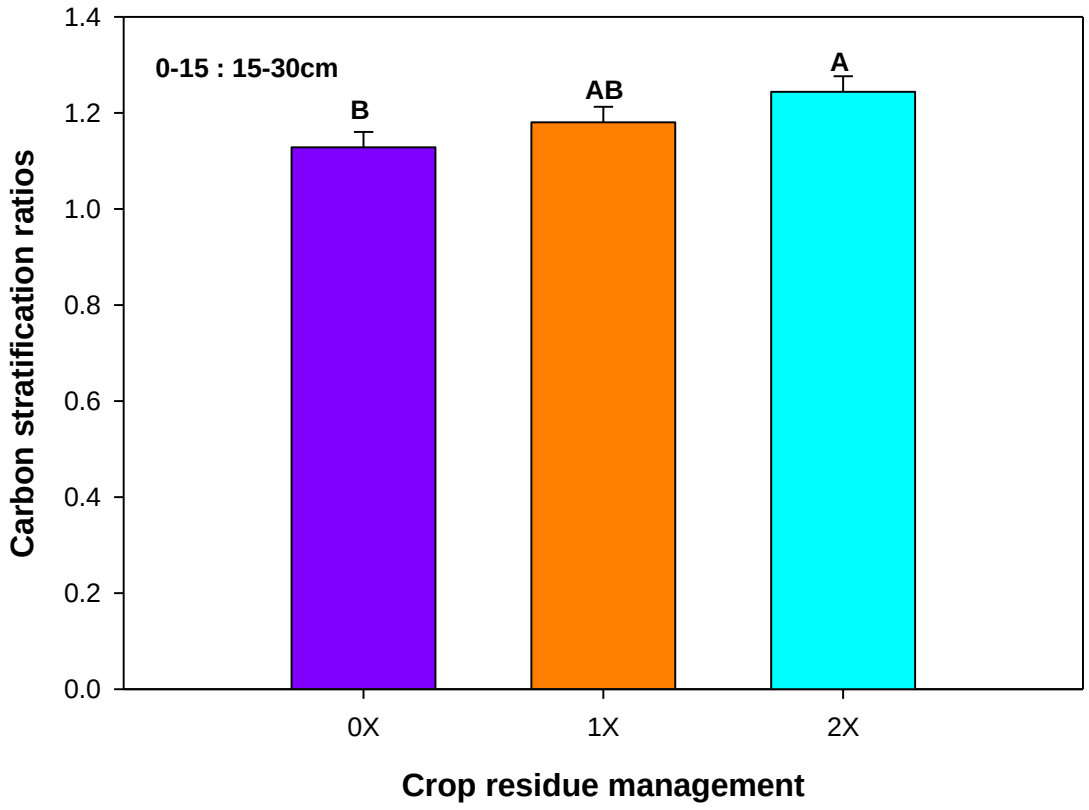


Figure 3.7. Carbon stratification ratios in top soil compared from different residue management (Ottawa, KS). Mean value ± standard error of four replicates. Means follow by different letters are significantly different, as measured by Tukey's HSD ($p<0.05$).

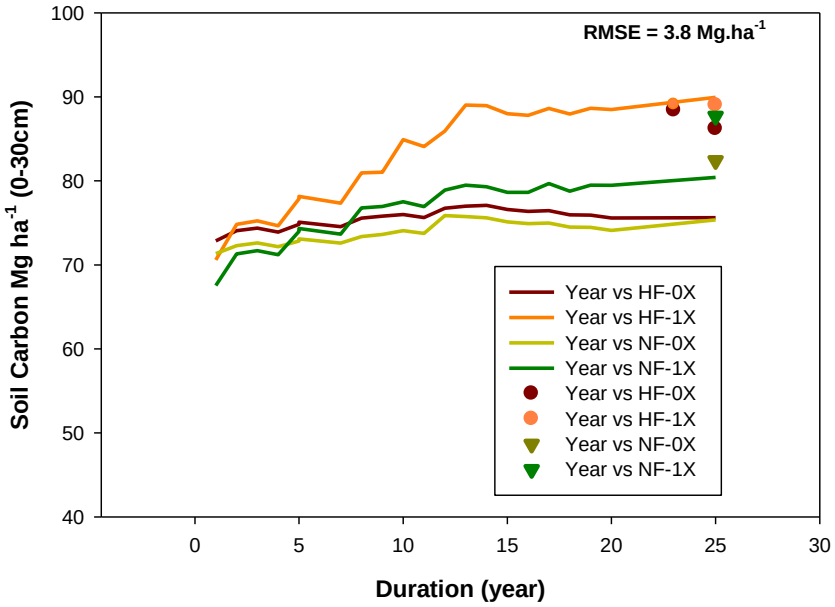


Figure 3.8. DNDC simulations of total soil organic C for a silt loam soil with different crop residue management and different fertility levels (0-30 cm).
Dots represent observed values, lines represent simulated values HF = high fertility; NF = normal fertility; 0X = residue removed; 1X = residue maintained. RMSE = root mean square error of the model.

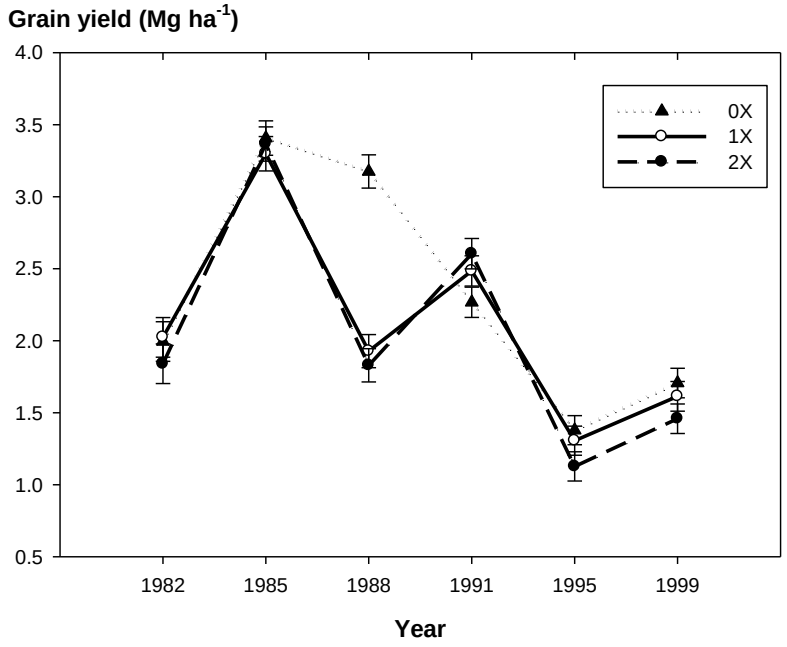


Figure 3.9. Wheat residue management effects on grain yield over time (Ottawa, KS). 0X = residue removed; 1X = residue maintained; 2X = residue double.

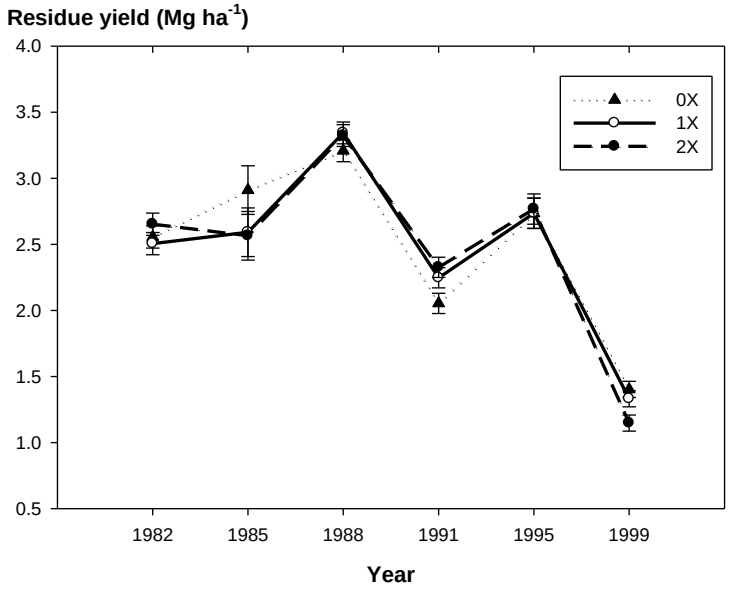


Figure 3.10. Wheat residue management effects on residue yield over time (Ottawa, KS). 0X = residue removed; 1X = residue maintained; 2X = residue double.

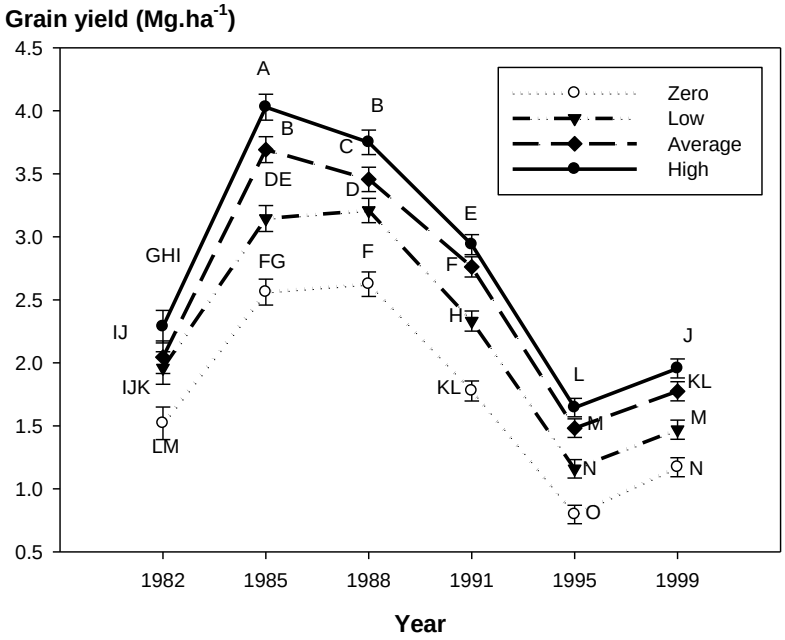


Figure 3.11. Effects of fertilization on wheat grain yield over time (Ottawa, KS).
Zero, low average and high represents the different levels of fertility. Mean value ± standard error of four replicates. Means follow by different letters are significantly different, as measured by Tukey's HSD ($p<0.05$).

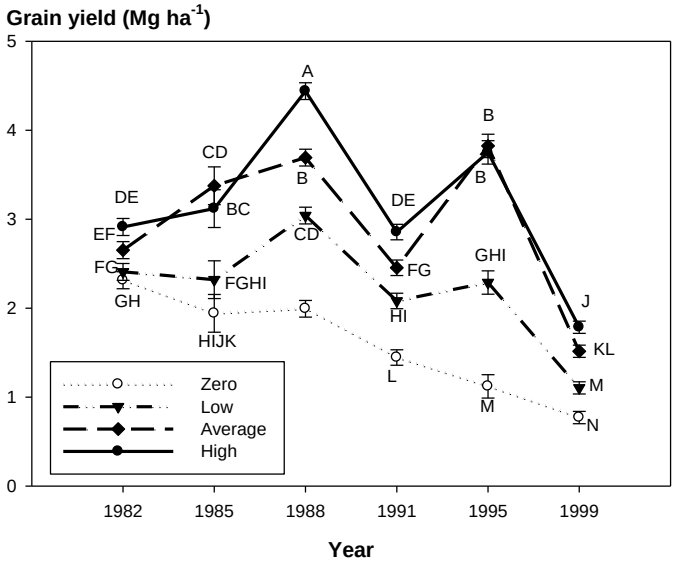


Figure 3.12. Effects of fertilization rate on wheat residue yield over time (Ottawa, KS). Zero, low average and high represents the different levels of fertility. Mean value ± standard error of four replicates. Means follow by different letters are significantly different, as measured by Tukey's HSD ($p<0.05$).

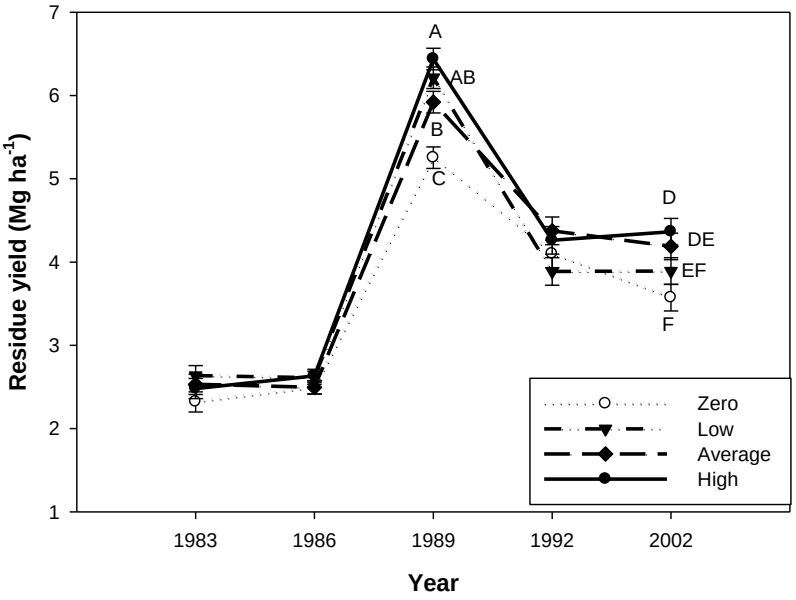


Figure 3.13. Effect of fertilization rate on sorghum residue yield over time (Ottawa, KS). Zero, low average and high represents the different levels of fertility. Mean value ± standard error of four replicates. Means follow by different letters are significantly different, as measured by Tukey's HSD ($p<0.05$).

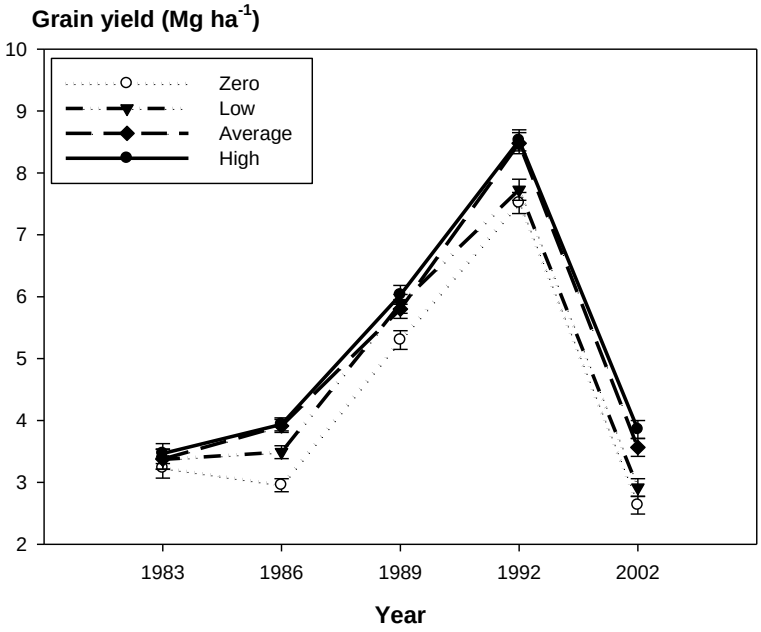


Figure 3.14. Effects of fertilization rate on sorghum grain yield over time (Ottawa, KS). Zero, low average and high represents the different levels of fertility.

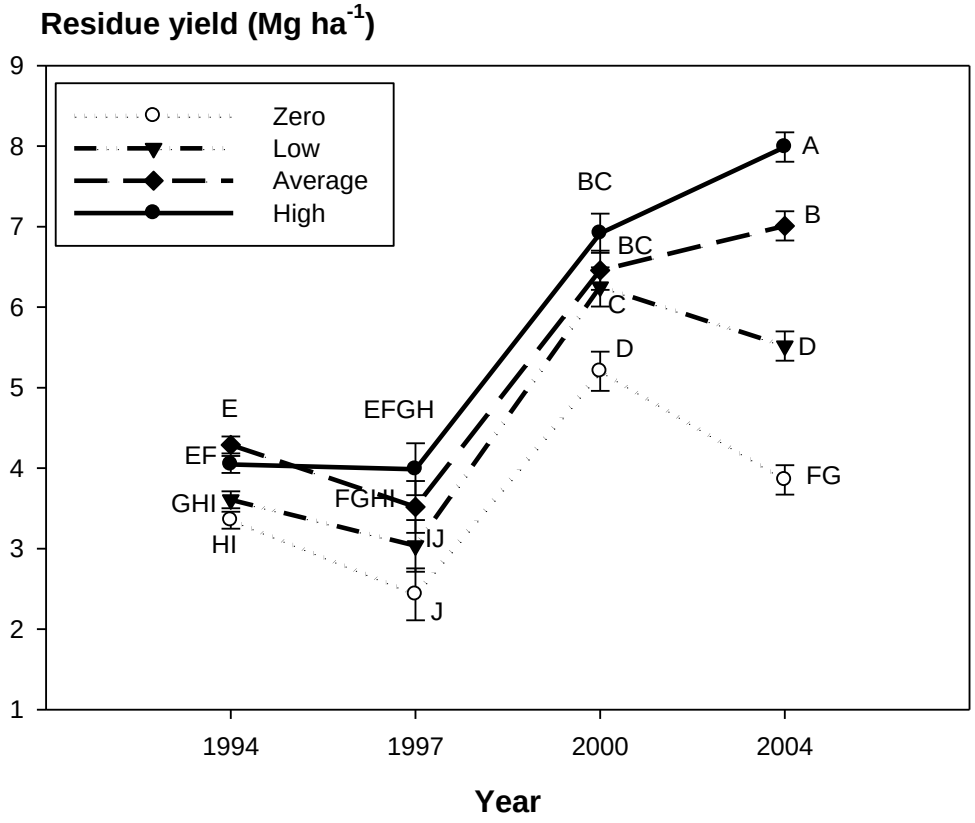


Figure 3.15. Effects of fertilization rate on corn residue yield over time (Ottawa, KS). Zero, low average and high represents the different levels of fertility. Mean value ± standard error of four replicates. Means follow by different letters are significantly different, as measured by Tukey's HSD ($p<0.05$).

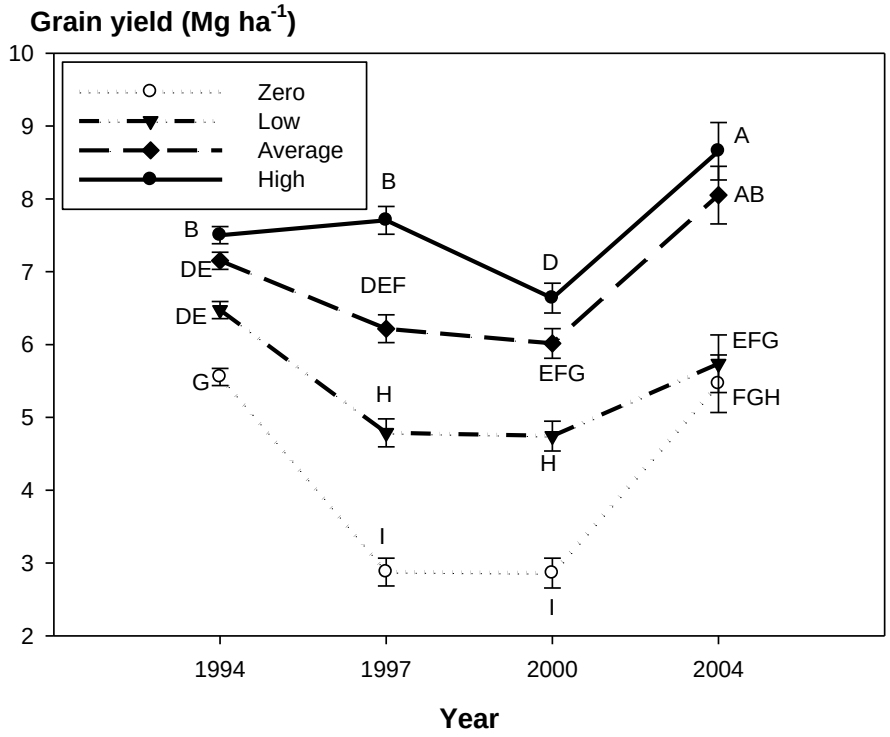


Figure 3.16. Effects of fertilization rate on corn grain yield over time (Ottawa, KS).
Zero, low average and high represents the different levels of fertility. Mean value ± standard error of four replicates. Means follow by different letters are significantly different, as measured by Tukey's HSD ($p<0.05$).

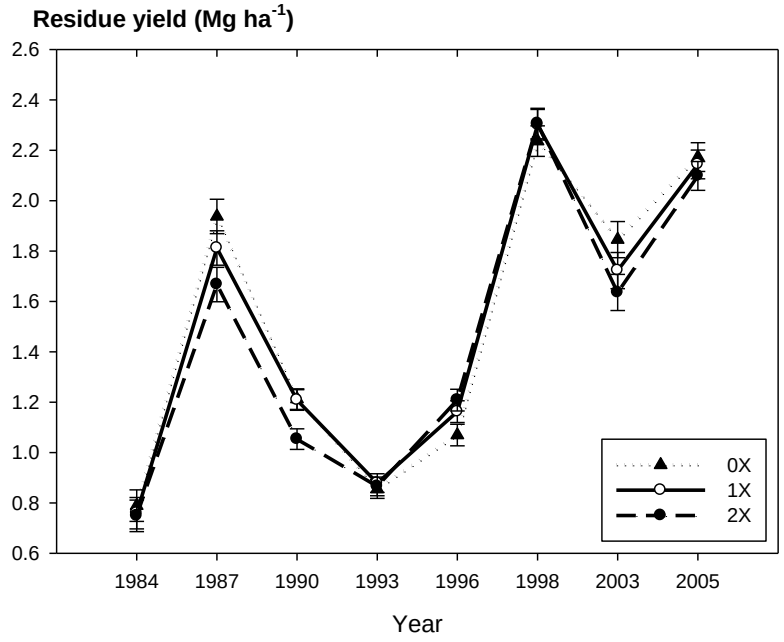


Figure 3.17. Soybean residue management effects on residue yield over time (Ottawa, KS). 0X = residue removed; 1X = residue maintained; 2X = residue double.

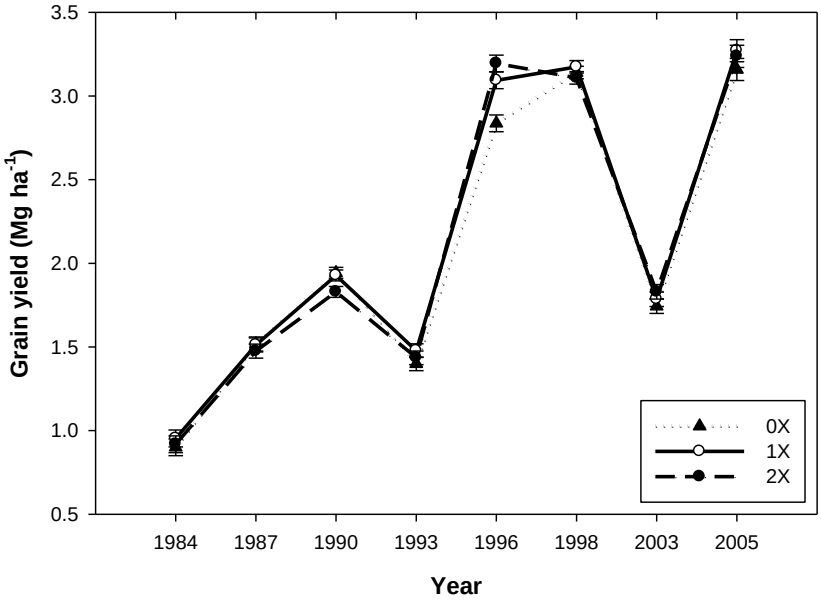


Figure 3.18. Soybean residue management effects on grain yield over time (Ottawa, KS). 0X = residue removed; 1X = residue maintained; 2X = residue double.

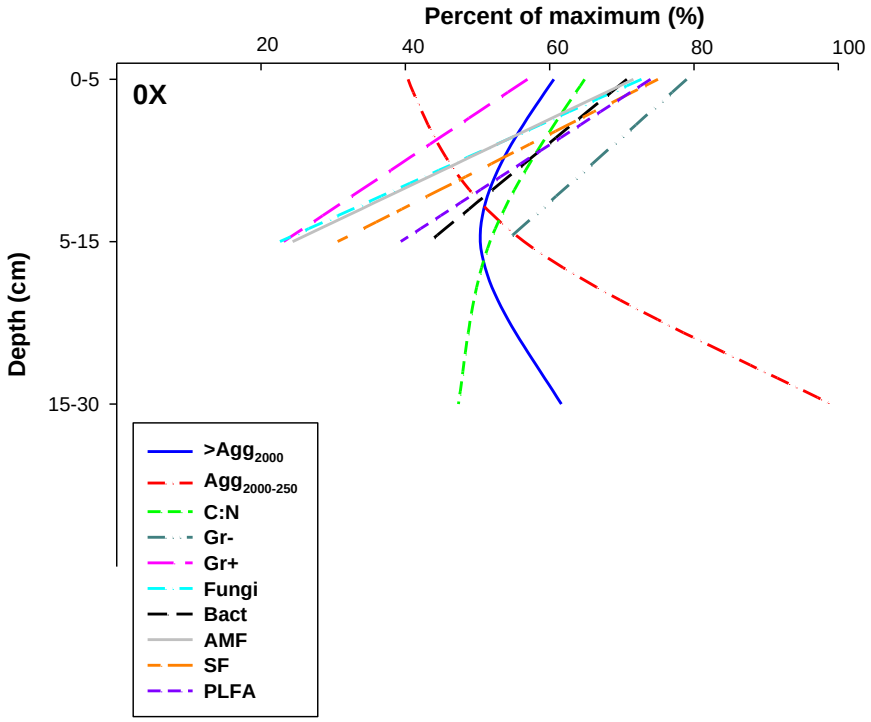


Figure 3.19. Depth profiles of microbial parameters under crop residue removal treatment. Agg_{2000} = large macroaggregate greater than 2000 µm; $Agg_{2000-250}$ = small macroaggregate C:N = C/N ratio; Gr- = gram negative bacteria; Gr+=gram positive bacteria; Fungi=total fungi; Bact=total bacteria; AMF = arbuscular mycorrhizal fungi; SF=saprophytic fungi and PLFA = phospholipid fatty acid.

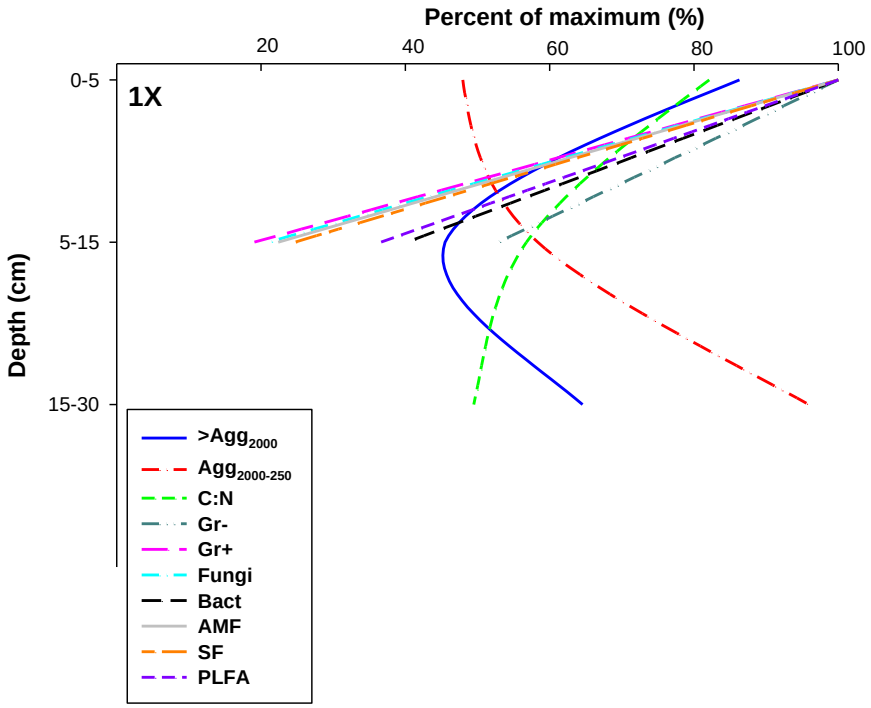


Figure 3.20. Depth profiles of microbial parameters under crop residue maintenance

Agg_{2000} = large macroaggregate greater than 2000 µm; $Agg_{2000-250}$ = small macroaggregate C:N = C/N ratio; Gr- = gram negative bacteria; Gr+=gram positive bacteria; Fungi=total fungi; Bact=total bacteria; AMF = arbuscular mycorrhizal fungi; SF=saprophytic fungi and PLFA = phospholipid fatty acid.

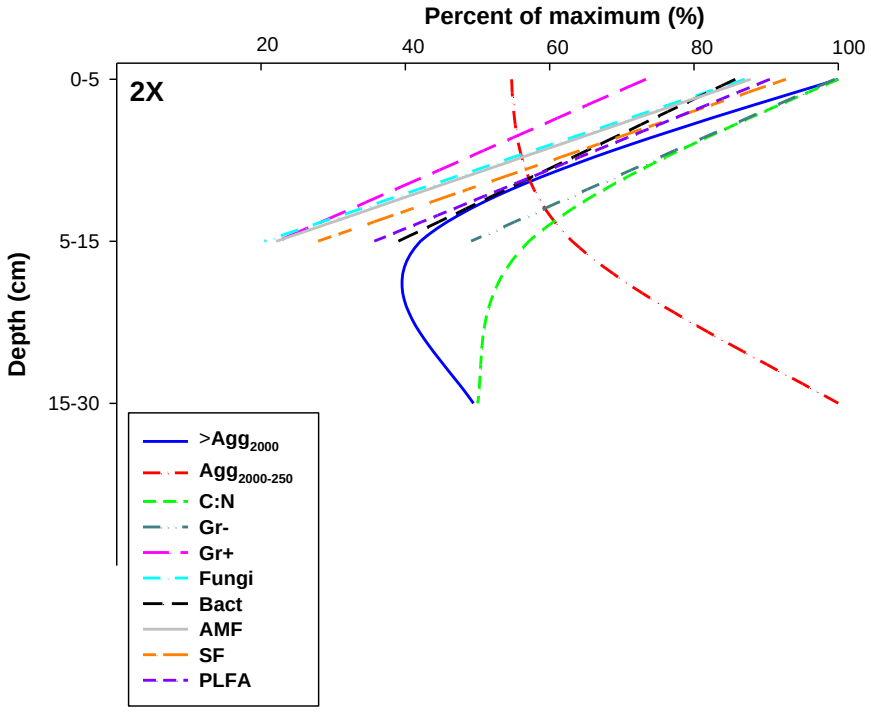


Figure 3.21. Depth profiles of microbial parameters under crop residue addition

Agg_{2000} = large macroaggregate greater than 2000 µm; $Agg_{2000-250}$ = small macroaggregate C:N = C/N ratio; Gr- = gram negative bacteria; Gr+=gram positive bacteria; Fungi=total fungi; Bact=total bacteria; AMF = arbuscular mycorrhizal fungi; SF=saprophytic fungi and PLFA = phospholipid fatty acid.

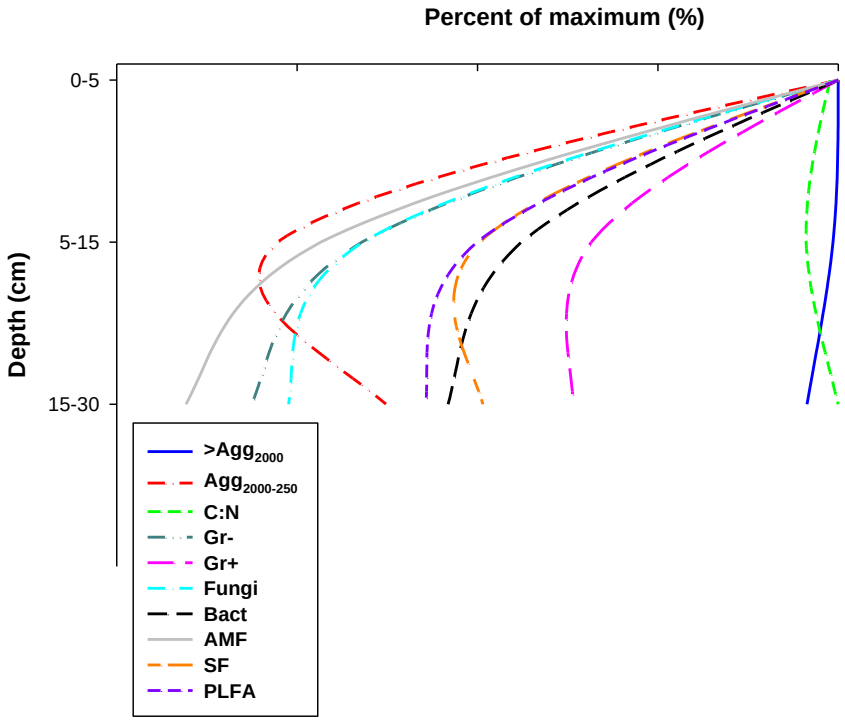


Figure 3.22. Depth profiles of microbial parameters under native prairie reference site

Agg_{2000} = large macroaggregate greater than 2000 µm; $Agg_{2000-250}$ = small macroaggregate C:N = C/N ratio; Gr- = gram negative bacteria; Gr+=gram positive bacteria; Fungi=total fungi; Bact=total bacteria; AMF = arbuscular mycorrhizal fungi; SF=saprophytic fungi and PLFA = phospholipid fatty acid.

Table 3.1. Crop residue management and fertilizer effects on sand-free water stable aggregates and total carbon inside associated with the aggregates after 24 years experiment (P-Values).

	SFWSA (g kg^{-1})				Total C in Aggregates (%)		
Factors	……(µm)…….				……..(µm)……		
	20-53	53-250	250-2000	>2000	53-250	250-2000	>2000
Residue	0.1570	**0.0556***	**0.0519***	0.5219	**<0.0001***	**<0.0001***	**<0.0001***
Fertility	0.8466	0.9889	0.8697	0.9066	**0.0458***	**0.0887****	**0.0305***
Res*Fert	0.9370	0.3347	0.4589	0.2490	- -	- -	- -
Depth	**<0.0001***	**<0.0001***	**<0.0001***	**<0.0001***	**<0.0001***	**<0.0001***	**<0.0001***
Res*Depth	0.2738	**0.0039***	0.1233	**0.0276***	0.2599	0.5040	0.9045
Fert*Depth	0.1409	0.3069	0.1830	0.8297	0.9152	0.1483	0.4002
Res*Fert*D	0.6171	0.6134	0.8118	0.2438	- -	- -	- -

Table 3.2. Statistical significance of crop residue management and fertilizer N treatment effects on selected soil characteristics after 24 years experiment (P-Values).

	TOC	C:N	C stratification	BD	MWD	pH
Factors	Mg ha^{-1}	……ratio…..……		g cm^{-3}		
Residue	0.2092	**0.0588***	**0.0334***	0.3241	**0.0094***	0.1728
Fertility	**0.0744****	0.1818	**0.0752****	0.5953	0.6323	**0.063****
Res*Fert	0.7856	**0.0287***	0.1482	0.2020	0.4699	0.1487
Depth	**<.0001**	**<.0001***	- -	**0.0026***	- -	**0.0008***
Res*Depth	0.3345	**<.0001***	- -	0.6591	- -	0.9865
Fert*Depth	0.4763	0.4563	- -	0.1020	- -	**0.0007***
Res*Fert*D	0.8703	0.8190	- -	0.3253	- -	0.3636

Note: TOC-Total Organic Carbon; C:N- Carbon:Nitrogen ratios; BD-Bulk-Density; MWD-Mean Weight Diameter

Table 3.3. Statistical significance of crop residue management and fertilizer N treatment effects on grain and residue yields of different crops after 24 years experiment (P-Values).

Factors	Crop							
	Wheat		Sorghum		Soybean		Corn	
	Grain	Residue	Grain	Residue	Grain	Residue	Grain	Residue
Residue	NS	NS	NS	NS	NS	NS	NS	NS
Fertility	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Res*Fert	NS	NS	NS	NS	NS	NS	NS	NS
Year	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Year*Res	**<0.0001**	**0.0139**	NS	NS	**<0.0001**	**0.0003**	NS	**0.077**
Year*Fert	**0.0004**	**<0.0001**	**0.0024**	**0.0002**	**<0.0001**	**0.091**	**<0.0001**	**<0.0001**
Yr*Res*Fert	NS	NS	NS	NS	NS	NS	0.093	NS

Chapter 4 - Understanding spatial variability: Measuring and monitoring soil organic carbon

Abstract

Effective measurement of soil C at the field scale requires an understanding of the spatial variability of soil C at a landscape scale. Recent technological advances in soil C measurements offer new opportunities for cost effective landscape measurements. Our objectives were to (1) characterize soil C vertically and horizontally, and (2) evaluate near infrared spectroscopy (NIRS) as a tool to measure soil C. Six fields were studied; each field was divided into 5 to 8 electrical conductivity (EC) zones having similar soil properties within 3 to 4 m distance. Fields were mapped on 20 m transects at 8-10 km/hour and probed to 60 cm depth using both Veris NIR (500 to 2200 nm) Spectrophotometer shank and NIR Spectrophotometer Probe. Within each zone, three soil profiles were sampled at an equal distance of 3 m for examining total carbon, total nitrogen and bulk density. Samples were analyzed for total C and N with a Thermo-Finnigan Flash EA 1112. Spatial variability of these soil properties was evaluated using regression procedures and Proc Mixed from SAS 9.2. Soil C varied considerably in some fields even with a similar soil type (30% coefficient of variation). However in some fields, soil C had low spatial variation <10% coefficient of variation. Soil Organic Carbon (SOC) was more variable at depths >30 cm than in the surface 30 cm. The field by depth interaction was significant at both depth composites, top 30 cm and 0-75 cm. Small (3 m triangles) geo-referenced sampling points reduced spatial variation. Surface measurements of soil C by NIR provided a quick assessment of soil C and soil C predicted by NIRS and measured by dry combustion laboratory measurements was correlated with an R-squared of 0.836. Stratifying

fields by soil type and surface soil C mapped by NIRS may help reduce variability for measuring and monitoring soil carbon but may not always explain SOC.

Introduction

Measuring and monitoring SOC is important for the scientific understanding of the C cycle but also critical for a variety of policy objectives such as mitigation of greenhouse gas emissions, food and energy security, biodiversity protection and assessing the feedbacks between soil C and climate change. In the past few years there has been an increasing demand for new technologies to provide more cost effective measures SOC (Ebinger et al., 2006; Izaurralde et al., 2013; Reeves III et al., 2006) and to understand SOC spatial variability (Gehl and Rice, 2007; Wielopolski et al., 2010). Knowledge on the spatial distribution of soil C is crucial for many tasks in agricultural and environmental management, monitoring and modeling but, measuring soil carbon is a real challenge. SOC is a key property for climate change mitigation as well as an attribute of soil health that can influence the relative importance of many ecosystem services (Delgado et al., 2011; Halpern et al., 2010; Rice and Angle, 2003). Most of the time soil use is associated with agriculture but soils provide many other ecosystem services such as soil biodiversity, regulation of the water exchange characteristics, control pathogens and toxins in the environment (Pepper et al., 2009; Sanchez et al., 2009) plus climate regulation. Soils are the basis for the production of food, fiber and fuel (Smith et al., 2013). Most of these services are dependent on SOC. Among the strategies to manage SOC, greater emphasis needs to be placed on SOC assessment. The rationale being, we cannot manage what we cannot measure. However, quantifying soil C presents a number of challenges, many of which associated with the high degree of spatial and temporal variability. Despite the long history on soil C research and numerous recent technological advances, we still lack a comprehensive, quantitative

understanding of soil C changes (Conen et al., 2003; Grimm et al., 2008). This is in part due to the difficulty in understanding C spatial variability in soils. Understanding spatial variability of SOC will be crucial in current efforts to model ecosystems responses to global climate change, since such an understanding can help developed targeted and cost effective interventions (Gehl and Rice, 2007; Wieder et al., 2013). Soil carbon measurements have been mostly investigated under laboratory conditions.

Spatial variation at the field scale potentially contributes the greatest to uncertainty of SOC measurement, given the complexity of soil carbon spatial variation; measuring soil C content poses several challenges. Therefore, special attention must be given to soil sampling design to capture that variability (Izaurralde and Rice, 2006). Soil variability has been assessed in many studies; Watson and Rice (2006) showed that grid sampling can determine soil C stocks with low errors but the cost is prohibitive. In an attempt to measure a change in soil C over a period of 4-8 years (Ellert et al., 2000) proposed a high-resolution sampling method using specific points or microsites. New in situ methods (Doetterl et al., 2013), using soil probes and field measuring devices such as near-infra-red spectroscopy (NIRS) and other spectrographic methods (Bricklemyer and Brown, 2010; Knadel et al., 2011; Stevens et al., 2013) offer hope for more rapid (Stenberg et al., 2010), nondestructive and less expensive measurements (O' Rourke and Holden, 2011) in the future (Christy et al., 2006; McCarty et al., 2002; Rossel and McBratney, 2008; Wetterlind et al., 2013). At present, these new technologies are not yet at an operational stage for widespread monitoring of soil C stocks. Some *in situ* soil analytical methods such as inelastic neutron scattering (INS), laser induced breakdown spectroscopy (LIBS) are still under development (Ebinger et al., 2006; Reeves III et al., 2006; Wielopolski et al., 2008). NIRS has been very useful in many fields such as food science (Christy and

Kvalheim, 2006; Osborne et al., 2006; Williams et al., 2006), pharmaceutical and medicine (2002a; 2002b) and has also proven valuable to provide the large amount of spatial data required for soil monitoring (Al-Abbas et al., 1972; Bowers and Hanks, 1965; Kooistra et al., 2001; Mouazen et al., 2005; Shepherd and Walsh, 2002; Sudduth and Hummel, 1993; Vågen et al., 2006). There is a need to build a global and standardized soil spectral library (Brown, 2007; Brown et al., 2006; Rossel and Behrens, 2010). Field soil core sampling and automated dry combustion analyses (Izaurralde and Rice, 2006; Post et al., 2001) constitute the standard methodology for measuring and monitoring soil carbon. Numerous strategies for estimating soil C spatial variability have been reported. Earlier studies proposed the division of fields by soil type (Ball and Williams, 1968; Mahinakbarzadeh and Veneman, 1991) or delineating fields into relatively homogeneous landscape areas to be sampled as microsites and thus minimizing the SOC spatial variability (Ellert et al., 2002; McConkey et al., 2000). Izaurralde and Rice (2006) raised the challenge that the "use of classical statistics, microsite sampling, and landscape fragmentation produces the same answer of SOC changes at the field scale as would be obtained with more intensive sampling techniques". Explicitly designed sampling across the relatively homogeneous strata within a field and maintaining high sampling intensity through NIRS can greatly enhance our understanding of SOC spatial variability. Therefore, the objectives of this study were to (1) characterize soil carbon vertically and horizontally, and (2) evaluate NIRS as a tool to measure SOC. A good agreement between direct field carbon measurements and dry combustion measurements was hypothesized if:

-NIRS estimates of soil C were effective as a tool to determine soil carbon

-Within field triangles sampling at dominant soil type reduced soil carbon spatial variability.

Materials and methods

Sampling design and methodology

All fields were located in East central and North East Kansas, Drummond and Kerj were located in Ottawa County; Markley, Lund and Lund CT belong to Saline County while Tarn was located in Dickinson (appendix Fig. C.1). The sampling design is presented in (Fig. 4.1). For description of the different fields and their various soils types see Table 4.1. A commercially available system from Veris Technologies (Salina, Kansas) that maps the surface and the soil profile to a depth of 75 cm was used for this study. The system was comprised of two modules: an on-the-go shank for collecting measurements at a discrete depth as it maps transects across a field, and a probe for collecting measurements of the profile to a depth of 75 cm. Fields were mapped at 6 cm depth using Veris NIR (500-2200 nm) Spectrophotometer shank. The system collects NIR measurements through a sapphire window pressed directly against the soil, at a rate of 20 spectra per second with an 8 nm resolution. The field was mapped on 20 m transects at 8-10 km h^{-1}. Data were collected at five second intervals on transects spaced approximately 10 m apart, which resulted in a data density of about 40-50 measurement points per hectare. Fields were probed to 75 cm depth with Veris NIR Spectrophotometer Probe utilizing the same spectrometers and sapphire window methodology as the shank unit. It has a force sensor to measure insertion force. Insertion speed was 2.5 cm sec^{-1}. The sampling design allows a comprehensive investigation into the spatial variability of SOC by dividing the whole fields into smaller homogeneous zones.

Five to eight locations per field were identified using either a soil electrical conductivity (EC) or NIRS map as having similar soil properties within a 3-4 m distance. At these locations, three soil profiles were sampled at an equal distance of 3 m for an estimation of small scale

spatial variability of SOC. Each soil type was represented with at least one geo-referenced triangle. Soil profiles (0-75 cm) were collected using a Giddings hydraulic probe, and NIR/EC/force probe (Salina, KS). At each corner of the triangle, one 0-75 cm core was retained in a plastic liner for future analysis, and one 0-75 cm core was segmented into 0-5, 5-15, 15-30, 30-45, 45-60 and 60-75 cm segments.

Laboratory analyses

Soil cores were passed through a sieve of 2 mm screen diameter and oven dried at 60ºC for 48 h for SOC determination. After drying, soil samples were ground to fine powder before weighing into tin capsules (5 × 9 mm) using a micro scale. Soil C was determined by dry combustion using a Flash EA 1112 elemental analyzer (Milan, Italy). Bulk densities (BD) were estimated with the moisture content of sub sample, wet weight and the volume of the core. Percent SOC concentration were converted into carbon stock (Mg ha^{-1}) using the BD for each core.

C (Mg ha^{-1}) = [(Soil BD, (g cm^{-3}) × soil depth (cm) × % C)] × 100.

In this equation % C was expressed as a decimal fraction; for example, 2.5 % C should be expressed as 0.025.

Data analysis

Soil C was first characterized by classical statistics. Analysis of variance (ANOVA) using the total number of samples was conducted to compare SOC stock at different spatial scales. Mean, variance and coefficient of variation (CV) of SOC were calculated to examine the spatial variability of SOC in each field. Semi-variogram of geostatistics was also conducted to test the spatial autocorrelation of SOC that was not explained by classical statistics. Standard geostatistical methods were used to analyze the spatial autocorrelation and amounts of carbon

within each field. Spatial autocorrelation was studied using semi-variograms. To construct experimental semi-variogram, the data are analyzed by plotting sample variability versus separation distance between samples (Franklin and Mills, 2007). The measure of the sample variability used to construct the experimental semi-variogram is the semi-variance (ϒ) which can be expressed as:

$$\gamma(h) = \frac{1}{2nh} \sum \left(y_{i+h} - y_{(i)}\right)^2$$

Where nh, the number of pairs of observations is separated by a distance h from one another; y are the observed values. The analyses were performed in Arc GIS version 10.1 (ESRI, 2010), to fit the semi-variance. The geostatistical parameters used to characterize the variability include C_0 which is the y intercept of the semi-variogram and represent the variability occurring from measurement errors or spatial sources of variation at distances smaller than the sampling interval (e.g. 3m triangles in the present study); C (sill) or the y value at which the semi-variogram levels off and the correlation length or range (a) which can be derived from the fitted semi-variogram (Goovaerts, 1999).

Results

Of the variables studied, percent C varied most strongly among the different fields (Table 4.2.) Uncertainty analysis allows quantifying the relative contribution of each of the variables in estimating the SOC stock. Percent C contributed as much as 77% of the total variance of C stock, BD (Table 4.3) contributed about 9% of the total variance, while sampling depth contributed about 14% of the total variance. Bulk density would be the soil characteristic requiring the least intensive sampling for reliable estimates for field means values. Classical

statistical analyses of SOC contents for the various fields included the variance standard deviation coefficient of variation and mean are shown in (Table 4.2). Among these, CV represents the overall variation or heterogeneity of SOC and is the most discriminating factor for describing SOC variability.

Drummond

Within spatial variability of the triangle (average variation of three sampling points) of SOC ranged from a CV of 1.09 to 9.84% and 1.81 to 5.77% in the surface 30 cm and the whole profile (0-75 cm) respectively. Results of the ANOVA indicate significant differences in mean SOC contents for the different triangles at both composite depth increments (0-30cm, $P= 0.0032$) and (0-75 cm, $P< 0.0001$) (Table 4.2). The range in CVs at both depths was low in this field; meanwhile, this value was greater in the surface. The variability among triangles in SOC was two times greater at the surface than the entire profile for this field. The estimated SOC at each individual depth increments for the five triangles, corresponding to 15 geo-referenced sampling locations showed a fairly homogeneous SOC distribution (Fig. 4.2). Spatial variation was less pronounced at the surface (0-30 cm) than in subsoil (below 30 cm). For the surface 5 cm soil, four out of five triangles had similar SOC stocks. This observation was the same at 5-15 cm soil layer. At 15-30, 30-45 and 45-60 cm, three out of five triangles had similar SOC stocks. SOC had the greatest variability (Fig. 4.3) at the lowest depth (60-75 cm) with only two triangles displaying similar SOC contents. The extent of SOC variability was low at all depths in this field. No strong spatial autocorrelation in SOC stocks was found, except in soil layer spanning 15-30 cm; outside this layer, this pattern seemed to disappear or showed a very weak spatial autocorrelation (Fig. 4.4).

Kerj

Triangle-scale spatial variability of SOC ranged from a CV of 4.88 to 103% and 4.91 to 83.2% in the top 30 cm and the whole profile (0-75 cm) respectively (Table 4.2). The ANOVA showed no significant differences in mean SOC stocks for the different triangles for the surface 30 cm regardless of the five different soil types (Fig. 4.5). However, there were statistically significant differences in mean SOC stocks for the different triangles when considering the whole profile (P= 0.0083). The range in CVs at both depths were high and moderate in the top 30 cm and the whole profile (0-75 cm) respectively (Table 4.2). The estimated SOC at each individual depth increments for the eight triangles, corresponding to 24 geo-referenced sampling locations; had high spatial variability (Fig. 4.6**)**. Spatial variation of SOC was the same in the 0-5 and 5-15 cm soil layers, with seven out of the eight triangles having similar SOC contents. At 15-30 and 30-45 cm, the SOC variability increased with six out of the eight triangles having same SOC stocks. At 45-60 and 60-75 cm, SOC became even more variable, with only four out of eight triangles having similar SOC stocks (Fig. 4.6**)**. The extent of SOC variability was low, in 3 out of the 8 triangles at both composite depths. Variability was moderate in 4 out of the 8 triangles and 5 out 8 triangles for the top 30 cm and the whole profile (0-75 cm) respectively. Only one triangle had high SOC spatial variability in the top 30 cm soil layer. A moderate but significant clustered spatial autocorrelation of SOC was found starting at soil layer 30-45 cm. This pattern displayed strong spatial autocorrelation at deeper depths 45-60 and 60-75 cm with an increasing estimated range parameter (Fig. 4.7). There were no significant spatial autocorrelation in the top 30 cm, which agrees with the results from the classical statistic that found no significant differences in SOC at the top 30 cm for this field.

Gypsum

Within triangle variability of SOC ranged from a CV of 3.37 to 16.2% and 1.7 to 9.62% in the top 30 cm and the whole profile (0-75 cm) respectively (Table 4.2). Significant differences existed among the 6 different triangles at both depths ($P<0.0001$). Mean SOC stocks showed significant differences within similar and distinct soil types. The ranges in CVs were moderately low in the top 30 cm to low when considering the whole profile (0-75 cm) (Fig. 4.8). The estimated SOC at each individual depth increments for the six triangles, corresponding to 18 geo-referenced sampling locations; had high spatial variability (Fig. 4.9). Three out of six triangles showed similar SOC stocks in the first three soil layers (0-5, 5-15 and 15-30 cm) but SOC spatial variability increase in the lower depths (30-45 and 45-60cm) with only two triangles out of six having similar SOC stocks. At the lowest depth (60-75cm), three out of six triangles showed similar SOC stocks. Triangle and depth as well as the interaction was significant ($P<0.0001$). The extent of SOC variability was low in all 6 triangles when considering the 0-75 cm. However, SOC spatial variation was low in 3 out 6 triangles and moderately low in the rest of the triangles. Geostatistical analyses were not performed for this field because of a geo-referencing error that occurred in one of the locations.

Lund

Triangle scale spatial variability of SOC ranged from a CV of 1.57 to 18.0% and 4.34 to 56.3% in the top 30 cm and the whole profile (0-75 cm) respectively (Table 4.2). Only the composite depth profile of 0-30 cm ($P= 0.0583$) was significant in means SOC stocks for the different triangles. There were no significant differences when considering the whole profile (0-75 cm); mean SOC stocks showed no significant differences despite the different soil types at this composite depth profile (Fig. 4.10). The range in CVs were moderately low to moderate in

the top 30 cm and the whole profile (0-75 cm) respectively. The estimated SOC at each individual depth increments for the five different triangles, corresponding to 15 geo-referenced sampling locations; showed a fairly homogeneous SOC distribution (Fig. 4.11). Spatial variation of SOC was similar in the different soil layers across all five triangles from 0-60 cm. The deepest layer had the greatest variation, with one triangle having high SOC stock. The extent of SOC variability was low in 4 out of the 5 triangles and, the last triangle showed a moderately low SOC spatial variation in the top 30 cm. When considering the whole profile (0-75 cm), SOC had low spatial variation in 2 out of the 5 triangles; 2 other triangles had a moderately low spatial variation, while only one triangle displayed moderate SOC spatial variability. Geostatistical analyses showed a clustered pattern and strong spatial autocorrelation in only 15-30 cm soil layer (Fig. 4.12).

Lund CT

Triangle scale spatial variability of SOC ranged from a CV of 2.42 to 19.0% and 5.68 to 13.1% in the top 30 cm and the whole profile (0-75 cm) respectively (Table 4.2). Means SOC stocks for the different triangles at both composite depths 0-30 cm (P= 0.0246) and 0-75 cm (P= 0.0235) were significant (Table 4.2) (Fig. 4.13). The ranges in CVs were moderately low to low in the top 30 cm and the whole profile (0-75 cm) respectively. The estimated SOC at each individual depth increments for the five different triangles, corresponding to 15 geo-referenced sampling locations; showed fairly homogeneous SOC distribution (Fig. 4.14.). The first two depths, (0-5 and 5-15 cm) as well as the 45-60 cm depth showed similar SOC spatial variation with all five triangles having similar SOC stocks. At 15-30 and 60-75 cm four out of five triangles had similar SOC stocks. At 30-45 cm, three out of five triangles had similar SOC stocks. The extent of SOC variability was low in 3 out of 5 triangles and the last 2 triangles had

a moderately low SOC spatial variation in the top 30 cm. When considering the 0-75 cm depth profile, SOC had low and moderately low variability in 3 and 2 different triangles respectively. Spatial variability of SOC was spatially structured as shown in the semi-variograms (Fig. 4.15). The presence of SOC spatial autocorrelation persisted in soil layers 5-15, 30-45 and 45-60 cm. However, at 5-15 cm soil layer semi-variogram analysis indicated the distribution of sampling points made binning difficult, therefore results for this soil layer did not reach sill. Stronger spatial structure was observed at 30-45 cm and this pattern was weakened at 45-60 cm with the reduction of the range parameter.

Markley

Triangle scale spatial variability of SOC ranged from a CV of 6.73 to 23.8% and 2.86 to 13.4% in the top 30 cm and the whole profile (0-75 cm) respectively (Table 4.2). SOC stocks for the different triangles was significant at both composite depths 0-30 cm ($P= 0.0013$) and 0-75 cm ($P< 0.0001$) (Fig. 4.16). The range in CVs were moderately low and low in the top 30 cm and the whole profile (0-75 cm) respectively. The estimated SOC at each individual depth increments for the six triangles, corresponding to 18 geo-referenced sampling locations, had a high spatial variability in SOC associated with different soil types but fairly homogeneous in triangles within the same soil type (Fig. 4.17). All six triangles had similar SOC stocks in the in the 0-5 and 5-15 cm soil layers, with four out of six triangles showing similar SOC stocks. At 15-30, 30-45, and 45-60 cm, three out of six triangles had similar SOC stock confirming the highest variability at lower depths. At 60-75 cm, four out six triangles had similar SOC stocks. The extent of SOC spatial variability in the top 30 cm was low in 2 out of 4 triangles, moderately low in 3 out of 6 triangles and one triangle displayed a moderate SOC variability. When considering the 0-75 cm depth profile, SOC had low variability in 5 out of 6 triangles with one

triangle having moderately low SOC variability. SOC was spatially structured **(**Fig. 4.18). The spatial correlation ranges of the residual semi-variograms decreased and ratios between variance parameter at shortest separation distance (x-axis) and sill decrease with depth.

Tarn

Triangle scale spatial variability of SOC ranged from a CV of 1.52 to 26.5% and 0.67 to 30.1% in the top 30 cm and the whole profile respectively (Table 4.2). SOC stocks for the different triangles was significant at both composite depths 0-30 cm (P= 0.0121) and 0-75 cm (P= 0.0808) (Fig. 4.19). The range in CVs were moderate at both composite depths. Despite the similar soil type, the estimated SOC at each individual depth increments for the six triangles, corresponding to eighteen geo-referenced sampling locations; showed a great spatial variability in SOC (Fig. 4.20). All six triangles displayed similar SOC stocks at 0-5, 5-15 and 30-45 cm. At 15-30 and 60-75 cm, four out of six triangles had similar SOC stocks; while at 30-45 cm three out of six triangles displayed similar SOC stocks. The extent of SOC spatial variability in the 0-30 cm was low in 5 out of 6 triangles, and only one triangle displayed a moderate SOC spatial variation. When considering the 0-75 cm depth profile, SOC showed low variability in 3 out of 6 triangles, 2 triangles had moderately low SOC spatial variability and only one triangle displayed a moderate SOC spatial variation.

SOC variability at different spatial scales

SOC distribution as stratification by field was not uniform (P <0.0001) based on the 3 homogeneity test (Table 4.3). The measurements based on field stratification were characterized by significant heterogeneity of variance. One field that was under no-till showed the greatest spatial variability (Lund), followed by Kerj which have the most complex landscape (five different soil type and three different topographic levels). Between-triangle variability was

homogenous of variance (homoscedasticity) in four out of the seven fields' case study (Table 4.4). SOC variability between triangles showed heterogeneity of variance (heteroscedasticity), in three fields out of seven with probability ranging between (0.01-0.05) for Drummond and (0.05-0.001) for Markley and Gypsum. Between triangles variability was greater at the Kerj field where there was a great landscape complexity. SOC did not differ significantly in their variance when stratifying by soil type (Table 4.5).

The NIRS results confirm findings of earlier studies from (Lund, 2010) and show the potential for assessing SOC contents directly using NIRS. Overall, the prediction accuracy obtained, with R^2 value of 0.836 (Fig. 4.22) was sufficient for discriminating between low and high concentration of SOC where spatial variability is high. Correlation coefficient (R^2) was very similar with the one found by another lab using the same set of NIRS samples. However, predictions of SOC showed somewhat poorer performance at higher SOC concentration, as data points seem to spread at higher SOC values (Fig. 4.22). These findings indicate that after calibrations reliable measurements of soil carbon can be done, thus reducing the need for dry combustion analysis.

Discussion

Many studies have shown that NIRS can be used for the determination of number of soil characteristics, including SOC, TN, EC, pH, water holding capacity, soil moisture (Dalal and Henry, 1986; Sudduth and Hummel, 1993; Zornoza et al., 2008); and many other measures of soil biological activity. According to Zhang et al. (2007) a variable is considered to have low variability if the CV is less than 10% and moderate variability if the CV is between 10% and 90%; and if CV is more than 90% it shows a strong variability. Most studies on SOC estimation, considered the top 30 cm (West and Post, 2002). More recent studies recommend an SOC stocks

to depths greater than 30 cm, when estimating changes in SOC to document potential change in the entire profile (Syswerda et al., 2011).

In most cases, greater ranges in CV were observed in surface (0-30 cm) than the whole profile (0-75 cm). However, no significant change in the top 30 cm and significant difference of SOC measured when considering the whole profile (0-75cm) at Kerj, suggest that increase in SOC is occurring at greater depth at this particular field. The great SOC spatial variation found in the upper 30 cm of soil at Lund was probably due to management (no-till). In cases where we have fairly homogeneous fields such as Drummond, Gypsum, Lund CT and Markley, stratified sampling may not be efficient if the same number of samples have to be taken within each stratum. Smith (2001) defined efficient sampling as taking relevant number of samples to minimize the error associated to the mean estimate of soil carbon to an acceptable level. Stratification can increase the efficiency of SOC monitoring only if blocking the variance in different strata is made such that it reduces the variability within each stratum. On average, our sampling strategy of blocking population variance by soil type was adequate to obtain mean estimates of SOC at 95% confidence interval. Twenty six out of 41 triangles showed low variability of SOC in the upper 30 cm, 9 showed a moderately low variability, 5 indicated a moderate variability and only one indicated high variability of SOC. When considering the whole profile (0-75 cm), 27 triangles displayed low SOC spatial variation, 8 triangles showed a moderately low SOC spatial variation; only 6 triangles showed a moderate variability in SOC spatial variation. No triangle had high SOC spatial variability when considering the 0-75 cm soil. The best fitted semi-variogram model for SOC was exponential, with high goodness of fit which could be attributed to the small separation distance of sampling points to capture spatial dependence. This is consistent with review findings from (McBratney and Pringle, 1999) who

noted that spatial correlation of soil C within a field is between 20 and 300 m. They concluded that this short range spatial variation is important for field scale mapping and requires an efficient sampling strategy to capture this variation.

Results of this study generally supported the hypothesis that triangles sampling at dominant soil type within each field reduced soil carbon spatial variability. Testing of this hypothesis was complicated by the greater SOC variance found in some fields. The converted SOC stocks showed a great spatial variation across each individual field. Between 5-6 triangles sampling locations per field, one out of 6 fields showed similar infield soil carbon content to a depth of 30 cm but with significant difference in SOC to a depth of 75 cm (Fig.4.5; Kerj). Also, one out of 6 fields showed similar SOC content to a depth of 75 cm but with significant differences in SOC at 30 cm (Fig.4.10; Lund). Within field SOC spatial variability was significantly different between triangles (Table 4.2). SOC show significant variation across the various field. The least variation was observed with the Drummond site which consists of similar soil type. However, the distribution of SOC did not appear to correspond closely with soil type.

In most of the field case studies (except Drummond), SOC spatial autocorrelation was found, which reveals that triangle scale spatial variability needs to be taken into account in sampling design (by locating sampling points at adequate intervals) when mean carbon stocks are estimated. Therefore in these cases, the observed spatial pattern has an influence on the average variation of the three sampling points of different soil layers. However, in most cases, the observed spatial pattern seems to disappear in the top 30 cm surface layers.

Almost no study describing SOC acknowledges the greater spatial variability at lower depths. In trying to document that carbon gains in surface soils are offset by losses lower in the

profile, (Syswerda et al., 2011) identified the greater variability of SOC pool at depth. In such context the low SOC concentration with depth, combined with the high spatial variability led to very few statistically significant treatment differences at depth. One implication of failing to document this spatial variability at depth is not the differential carbon loss from mitigation stand point but the risk of overlooking significant carbon gain at depth due to inadequate sampling intensity. There was strong spatial autocorrelation in the subsoil layers for all the field case studies, confirming that the SOC spatial variability at lower depth was not independent. Without paying attention to such small scale spatial variability and their drivers (processes that cause them), changes in SOC can be attributed to random variation as noted earlier (Wilding and Drees, 1983). Understanding the high degree of spatial heterogeneity in soil C and including such feature in model simulations could help improve poor spatial correlations that exist between modeled soil C pools and observational data, particularly; the recently improved global soil carbon projections by modeling microbial processes (Wieder et al., 2013). Soil sampling at depth is important but will not likely to yield difference in soil C without an exhaustive sampling strategy. Soil C variability between triangles was minimal. Soil C was not significantly different between triangles in 4 out of seven fields (Table 4.6.).

Conclusion

Understanding spatial variability of SOC at the field and landscape scale is crucial to help monitor SOC with high degree of precision at regional scale. Knowledge on SOC distribution at different scales and its impact on sampling intensity will enhance the use of this information for designing appropriate sampling techniques for monitoring SOC. The magnitude of SOC variability appeared to be different from the surface and the subsoil. Findings gave clear

indication that sampling in smaller extent such as 3 m triangles or at field scale, provide better understanding of SOC spatial variability than stratifying sampling by soil type. Results show a general decrease in soil carbon concentration with depth as well as significant variation in carbon concentration throughout the profile. Soil C varied considerably in some fields even with a similar soil type. However in some fields, soil C had low spatial variation. SOC pool showed greater spatial variability at lower depths as materialized by lower number of triangles having same SOC stocks. Small (3 m triangles) geo-referenced sampling points reduced spatial variation, the amount of variability that can be explained by considering the spatial separation of sampling locations was quite low to moderate. There was a good agreement between NIRS and carbon concentration from dry combustion. Surface measurements of soil C by NIR provided a quick assessment of soil C with an R^2 of 0.836. Further research is required to better understand the drivers for spatial variability of soil. Soil type may help stratified sampling but does not always explain the spatial variability.

References

Al-Abbas A.H., P.H. Swain, M.F. and Baumgardner. 1972. Relating organic matter and clay content to the multispectral radiance of soils. Soil Sci. 114:477-485.

Ball D.F., and W.M. Williams. 1968. Variability of soil chemical proprieties in two uncultivated brown earths. J. Soil Sci. 19:379-391. DOI: 10.1111/j.1365-2389.1968.tb01548.x.

Bowers S.A., and R.J. Hanks. 1965. Reflection of radiant energy from soils. Soil Sci. 100:130-138.

Bricklemyer R.S., and D.J. Brown. 2010. On-the-go VisNIR: Potential and limitations for mapping soil clay and organic carbon. Comp. Electron. Agric. 70:209-216.

Brown D.J. 2007. Using a global VNIR soil-spectral library for local soil characterization and landscape modeling in a 2nd-order Uganda watershed. Geoderma 140:444-453.

Brown D.J., K.D. Shepherd, M.G. Walsh, M.D. Mays, and T.G. Reinsch. 2006. Global soil characterization with VNIR diffuse reflectance spectroscopy. Geoderma 132:273-290.

Christy A.A., and O.M. Kvalheim. 2006. Latent-variable analysis of multivariate data in infrared spectrometry. In: Y. Ozaki et al. (Eds) Near-infrared spectroscopy in food science and technology. John Wiley & Sons, Inc. pp. 145-162.

Christy C., P. Drummond, and E. Lund. 2006. Precision agriculture applications of an on-the-go soil reflectance sensor. In D.J. Mulla (ed.) Proc. eighth Intern. Conf. Precision Agric [CD-ROM]. July 23-26. Minneapolis, MN, USA.

Conen F., M.V. Yakutin, and A.D. Sambuu. 2003. Potential for detecting changes in soil organic carbon concentrations resulting from climate change. Glob. Change Biol. 9:1515-1520. DOI: 10.1046/j.1365-2486.2003.00689.x.

Dalal R.C., and R.J. Henry. 1986. Simultaneous determination of moisture, organic carbon, and total nitrogen by near infrared reflectance spectrophotometry. Soil Sci. Soc. Am. J. 50:120-123. DOI: 10.2136/sssaj1986.03615995005000010023x.

Delgado J.A., P.M. Groffman, M.A. Nearing, T. Goddard, D. Reicosky, R. Lal, N.R. Kitchen, C.W. Rice, D. Towery, and P. Salon. 2011. Conservation practices to mitigate and adapt to climate change. J. Soil Water Cons. 66:118A-129A. DOI: 10.2489/jswc.66.4.118A.

Doetterl S., A. Stevens, K. Van Oost, and B. van Wesemael. 2013. Soil organic carbon assessment at high vertical resolution using closed-tube sampling and Vis-NIR spectroscopy. Soil Sci. Soc. Am. J. 77:1430-1435. DOI: 10.2136/sssaj2012.0410n.

Ebinger M.H., D.A. Cremers, C.M. Meyer, and R.D. Harris. 2006. Laser-induced breakdown spectroscopy and applications for soil carbon measurement., In: R. Lal et al. (Eds.), Carbon sequestration in soils of Latin America The Haworth Press, Inc., New York, NY. pp. 407-421.

Ellert B.H., H.H. Janzen, B.G. McConkey. 2000. Measuring and comparing soil carbon storage., in: R. Lal, et al. (Eds.), Assessment methods for soil carbon., Lewis imprint of the CRC Press, , Boca Raton. pp. 131-146.

Ellert B.H., H.H. Janzen, and T. Entz. 2002. Assessment of a method to measure temporal change in soil carbon storage. Soil Sci. Soc. Am. J. 66:1687-1695. DOI: 10.2136/sssaj2002.1687.

ESRI. 2010. ArcGIS. Environmental System Research Institute, Redlands.

Franklin R.B., and A.L. Mills. 2007. Statistical analysis of spatial structure in microbial communities. In: R. B. Franklin and A. L. Mills (Eds.) The spatial distribution of microbes in the environment, Springer, Dordrecht, The Netherlands. pp. 31-60.

Gehl R., and C. Rice. 2007. Emerging technologies for in situ measurement of soil carbon. Climatic Change 80:43-54. DOI: 10.1007/s10584-006-9150-2.

Goovaerts P. 1999. Geostatistics in soil science: State-of-the-art and perspectives. Geoderma 89:1-45.

Grimm R., T. Behrens, M. Märker, and H. Elsenbeer. 2008. Soil organic carbon concentrations and stocks on Barro Colorado Island — Digital soil mapping using random forests analysis. Geoderma 146:102-113.

Halpern M.T., J.K. Whalen, and C.A. Madramootoo. 2010. Long-term tillage and residue management influences soil carbon and nitrogen dynamics. Soil Sci. Soc. Am. J. 74:1211-1217. DOI: 10.2136/sssaj2009.0406.

Izaurralde R.C., and C.W. Rice. 2006. Methods and tools for designing a pilot soil carbon sequestration project. In: R. Lal et al. (Eds.) Carbon sequestration in soils of Latin America, The Haworth Press, New York, NY. pp. 457-476.

Izaurralde R.C., C.W. Rice, L. Wielopolski, M.H. Ebinger, J.B. Reeves, III, A.M. Thomson, R. Harris, B. Francis, S. Mitra, A.G. Rappaport, J.D. Etchevers, K.D. Sayre, B. Govaerts, and G.W. McCarty. 2013. Evaluation of three field-based methods for quantifying soil carbon. PLoS ONE 8:e55560. DOI: 10.1371/journal.pone.0055560.

Knadel M., A. Thomsen, and M.H. Greve. 2011. Multisensor on-the-go mapping of soil organic carbon content. Soil Sci. Soc. Am. J. 75:1799-1806. DOI: 10.2136/sssaj2010.0452.

Kooistra L., R. Wehrens, R.S.E.W. Leuven, and L.M.C. Buydens. 2001. Possibilities of visible-near-infrared spectroscopy for the assessment of soil contamination in river floodplains. Analytica Chimica Acta 446:97-105.

Lund E. 2010. Can field-based spectroscopic sensors measure soil carbon in a regulated carbon trading program?, 19th World Congress of Soil Science, soil solutions for a changing world, Brisbane, Australia.

Mahinakbarzadeh S.S., and P.L.M. Veneman. 1991. Spatial variability of organic matter content in selected Massachusetts map units. In: M. J. Mausbach and L. P. Wilding (Eds.) Spatial variabilities in soils and landforms, Soil Sci. Soc. Am. pp. 231-242.

McBratney A.B., and M.J. Pringle. 1999. Estimating average and proportional variograms of soil properties and their potential use in precision agriculture. Prec. Agric. 1:125-152. DOI: 10.1023/a:1009995404447.

McCarty G.W., J.B. Reeves, V.B. Reeves, R.F. Follett, and J.M. Kimble. 2002. Mid-infrared and near-infrared diffuse reflectance spectroscopy for soil carbon measurement. Soil Sci. Soc. Am. J. 66:640-646. DOI: 10.2136/sssaj2002.6400.

McConkey B.G., B.C. Liang, G. Padbury, and R. Heck. 2000. Prairie soil carbon balance project: Carbon sequestration from adoption of conservation cropping practices. Final report to GEMCo, Agriculture and Agri-Food Canada, Swift Current, SK.

Mouazen A.M., W. Saeys, J. Xing, J. De Baerdemaeker, and H. Ramon. 2005. Near infrared spectroscopy for agricultural materials: An instrument comparison. J. Near Infrared Spectros. 13:87-97.

O' Rourke S.M., and N.M. Holden. 2011. Optical sensing and chemometric analysis of soil organic carbon – a cost effective alternative to conventional laboratory methods? Soil Use Man. 27:143-155. DOI: 10.1111/j.1475-2743.2011.00337.x.

Osborne B.G., S.E. Kays, F.E. Barton, D. Cozzolino, R. Giangiacomo, and T.M.P. Cattaneo. 2006. Applications to foodstuffs, near-infrared spectroscopy in food science and technology, John Wiley & Sons, Inc. pp. 279-340.

Pepper I.L., C.P. Gerba, D.T. Newby, and C.W. Rice. 2009. Soil: A public health threat or savior? Critical Reviews in Environ. Sci. Tech. 39:416-432. DOI: 10.1080/10643380701664748.

Post W.M., R.C. Izaurralde, L.K. Mann, and N. Bliss. 2001. Monitoring and verifying changes of organic carbon in soil. Clim. Change 51:73-99. DOI: 10.1023/a:1017514802028.

Reeves III J.B., G.W. Mccarty, R.F. Follett, and J.M. Kimble. 2006. The potential of spectroscopic methods for the rapid analysis of soil samples. In: R. Lal et al. (Eds.) Carbon sequestration in soils of Latin America, The Haworth Press, Inc., New York, NY. pp. 423-442.

Rice C.W., and J.S. Angle. 2003. A role for genetically modified organisms in soil carbon sequestration, in: N. J. M. Rosenberg, F. B.;Izaurralde, R. C. (Ed.), Applications of biotechnology to mitigation of greenhouse warming: Proceedings of the St. Michaels II Workshop, April 2003. pp. 61-78.

Rossel R.A.V., and A.B. McBratney. 2008. Diffuse reflectance spectroscopy as a tool for digital soil mapping. In: A. Hartemink et al. (Eds.) Digital soil mapping with limited data. Springer Netherlands. pp. 165-172.

Rossel R.A.V., and T. Behrens. 2010. Using data mining to model and interpret soil diffuse reflectance spectra. Geoderma 158:46-54.

Sanchez P.A., S. Ahamed, F. Carré, A.E. Hartemink, J. Hempel, J. Huising, P. Lagacherie, A.B. McBratney, N.J. McKenzie, M.d.L. Mendonça-Santos, B. Minasny, L. Montanarella, P. Okoth, C.A. Palm, J.D. Sachs, K.D. Shepherd, T.-G. Vågen, B. Vanlauwe, M.G. Walsh, L.A. Winowiecki, and G.-L. Zhang. 2009. Digital soil map of the world. Science 325:680-681. DOI: 10.1126/science.1175084.

Shepherd K.D., and M.G. Walsh. 2002. Development of reflectance spectral libraries for characterization of soil properties. Soil Sci. Soc. Am. J. 66:988-998. DOI: 10.2136/sssaj2002.9880.

Smith G.R. 2001. Toward an efficient method for measuring soil organic carbon stocks in forests. In: R. Lal et al. (Eds.) Assessment methods for soil carbon, Lewis Publishers, Boca Raton,, FL. pp. 293-310.

Smith P., H. Haberl, A. Popp, K. Erb, C. Lauk, R. Harper, F. Tubiello, A. de Siqueira Pinto, M. Jafari, S. Sohi, O. Masera, H. Böttcher, G. Berndes, M. Bustamante, H. Ahammad, H.

Clark, H. Dong, E.A. Elsiddig, C. Mbow, N.H. Ravindranath, C.W. Rice, C. Robledo-Abad, A. Romanovskaya, F. Sperling, M. Herrero, J.I. House, and S. Rose. 2013. How much land based greenhouse gas mitigation can be achieved without compromising food security and environmental goals? Glob. Change Biol. Vol. 19:2285-2302.

Stenberg B., R.A. Viscarra Rossel, A.M. Mouazen, and J. Wetterlind. 2010. Visible and near infrared spectroscopy in soil science, in: L. S. Donald (Ed.), Adv. Agron., Academic Press. pp. 163-215.

Stevens A., M. Nocita, G. Tóth, L. Montanarella and B. van Wesemael. 2013. Prediction of soil organic carbon at the European scale by visible and near infrared reflectance spectroscopy. PLoS ONE 8:e66409. DOI: 10.1371/journal.pone.0066409.

Sudduth K., and J. Hummel. 1993. Soil organic matter, CEC, and moisture sensing with a portable NIR spectrophotometer. Trans. ASAE 36.

Syswerda S.P., A.T. Corbin, D.L. Mokma, A.N. Kravchenko, and G.P. Robertson. 2011. Agricultural management and soil carbon storage in surface vs. deep layers. Soil Sci. Soc. Am. J. 75:92-101. DOI: 10.2136/sssaj2009.0414.

Vågen T.-G., K.D. Shepherd, and M.G. Walsh. 2006. Sensing landscape level change in soil fertility following deforestation and conversion in the highlands of Madagascar using Vis-NIR spectroscopy. Geoderma 133:281-294.

West T.O., and W.M. Post. 2002. Soil organic carbon sequestration rates by tillage and crop rotation. Soil Sci. Soc. Am. J. 66:1930-1946. DOI: 10.2136/sssaj2002.1930.

Wetterlind J., B. Stenberg, and R.V. Rossel. 2013. Soil analysis using visible and near infrared spectroscopy. In: F. J. M. Maathuis (Ed.) Plant Mineral Nutrition. Humana Press. pp. 95-107.

Wieder W.R., G.B. Bonan, and S.D. Allison. 2013. Global soil carbon projections are improved by modelling microbial processes. Nature Clim. Change advance online publication. DOI: 10.1038/nclimate1951

Wielopolski L., K. Johnsen, and Y. Zhang. 2010. Soil analysis based on samples withdrawn from different volumes: Correlation versus calibration. Soil Sci. Soc. Am. J. 74:812-819. DOI: 10.2136/sssaj2009.0205.

Wielopolski L., G. Hendrey, K.H. Johnsen, S. Mitra, S.A. Prior, H.H. Rogers, and H.A. Torbert. 2008. Nondestructive system for analyzing carbon in the soil. Soil Sci. Soc. Am. J. 72:1269-1277. DOI: 10.2136/sssaj2007.0177.

Wilding L.P., and L.R. Drees. 1983. Spatial variability and pedology, pedogenesis and soil taxonomy. I. Concepts and interactions. Elsevier Science Publishers B.V., Amsterdam, The Netherlands. pp. 83-116.

Williams P., S. Saranwong, S. Kawano, T. Isaksson, and V.H. Segtnan. 2006. Applications to agricultural and marine products, near-infrared spectroscopy in food science and technology, John Wiley & Sons, Inc. pp. 163-277.

Zhang X.-Y., Y.-Y. Sui, X.-D. Zhang, K. Meng, and S.J. Herbert. 2007. Spatial variability of nutrient properties in black soil of northeast China. Pedos. 17:19-29. DOI: Zornoza R., C. Guerrero, J. Mataix-Solera, K.M. Scow, V. Arcenegui, and J. Mataix-Beneyto. 2008. Near infrared spectroscopy for determination of various physical, chemical and biochemical properties in Mediterranean soils. Soil Biol. Biochem. 40:1923-1930.

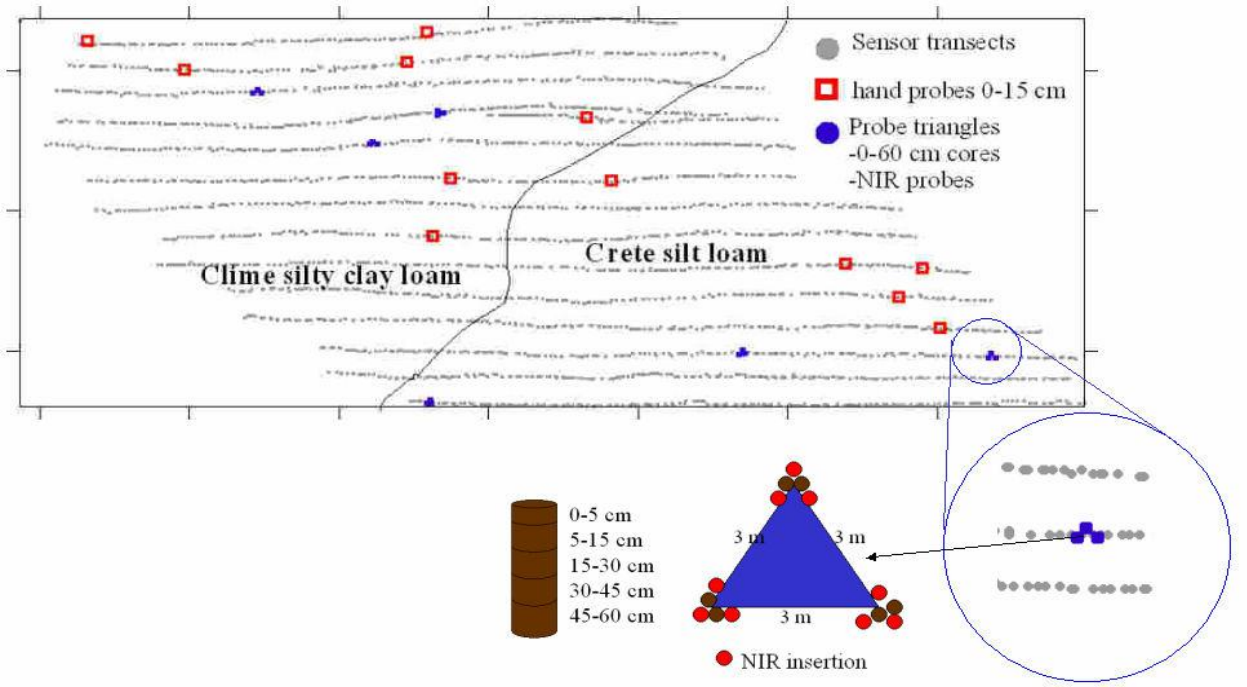


Figure 4.1. Sampling design and methodology.

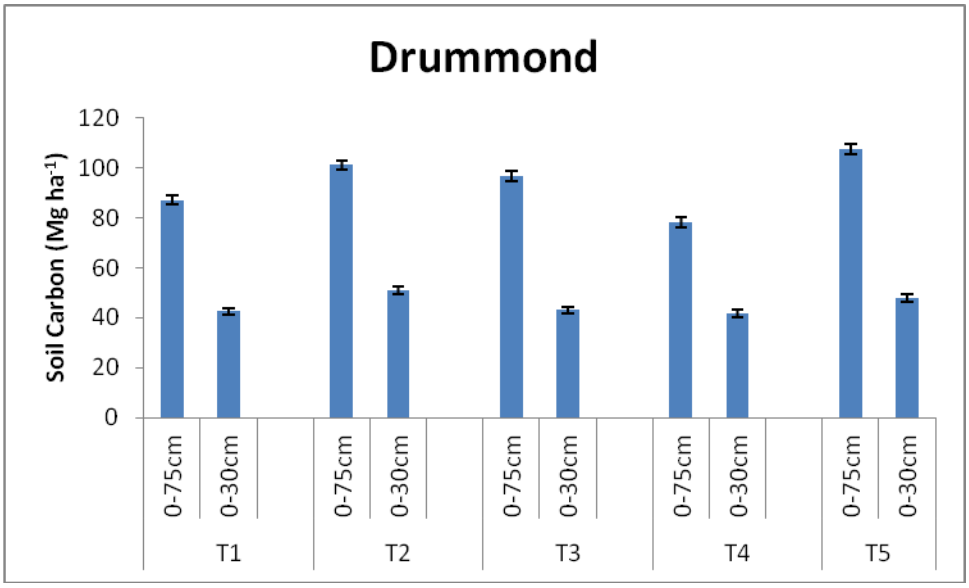


Figure 4.2. SOC stock at two sampling depths (0-75 cm) and (0-30 cm) in various triangles across field. Error bars represent standard error.T1 to T5 represent five triangles, the five triangles correspond to 15 geo-referenced sample locations. All triangles are within the same soil type (Crete silt loam).

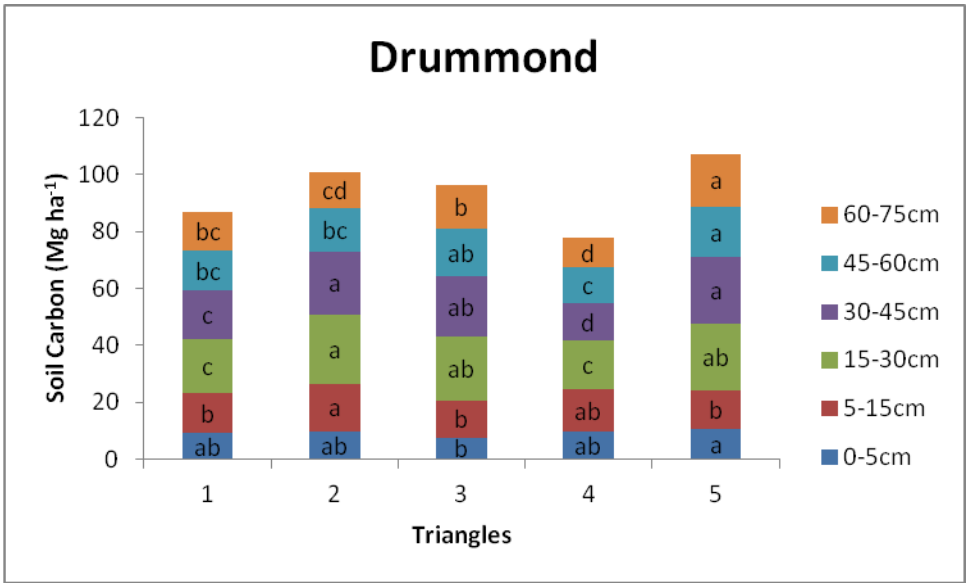


Figure 4.3. Estimated SOC at each individual depth increments.
Within depths, means followed by the same or no letter are not significantly different ($P<0.05$) by Tukey's test. Lowercase letters represent differences within soil layer; capital letters represent differences between triangles.

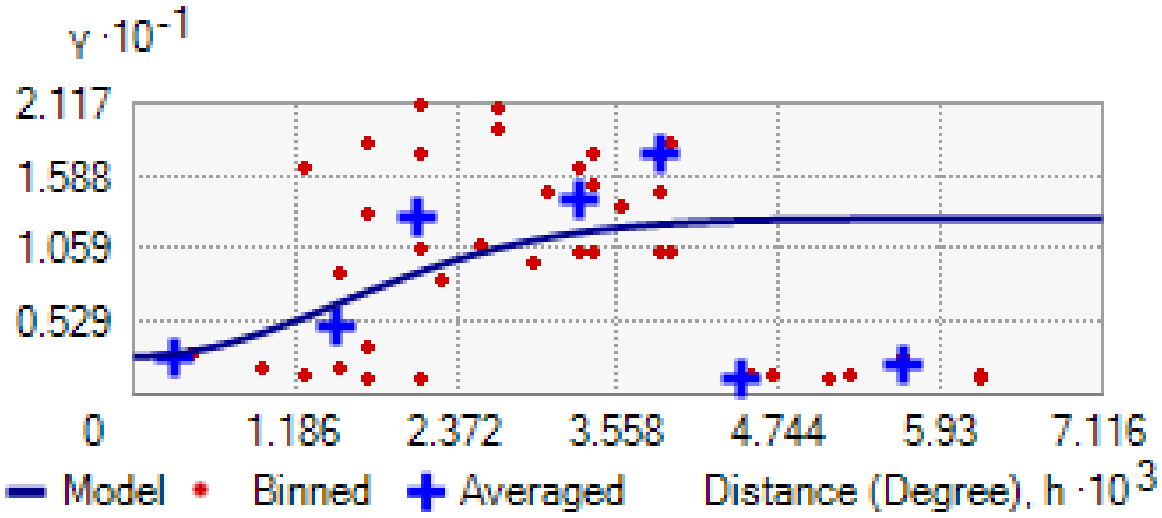


Figure 4.4. Drummond variogram of SOC stocks (dots) and exponential model fitted to the variograms (solid blue lines). Variogram represents soil layer 15-30 cm.

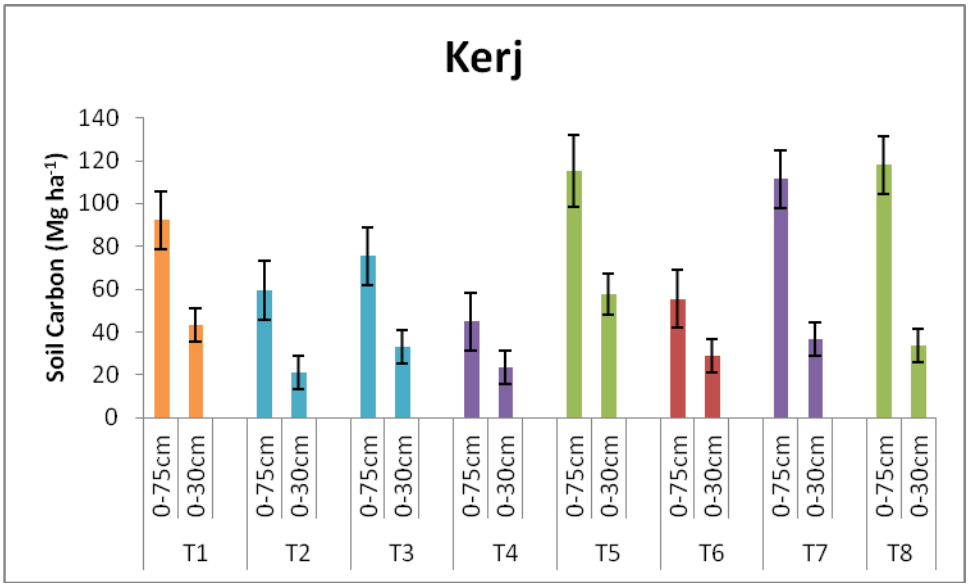


Figure 4.5. SOC stock at two sampling depths (0-75 cm) and (0-30 cm) in various triangles across field. Error bars represent standard error.T1 to T8 represent eight triangles, the eight triangles correspond to 24 geo-referenced sample locations. The different colors represent different soil types.

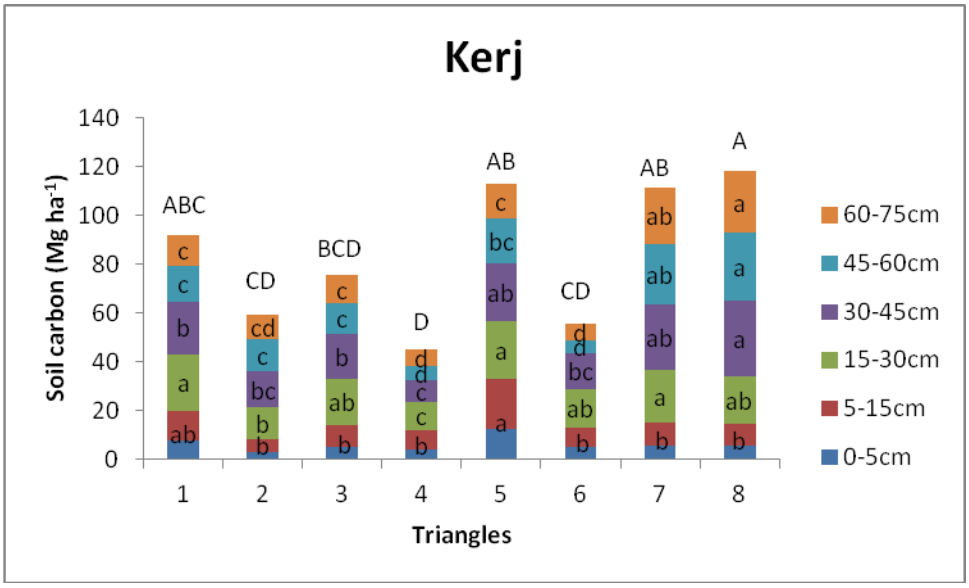


Figure 4.6. Estimated SOC at each individual depth increments.
Within depths, means followed by the same or no letter are not significantly different ($P<0.05$) by Tukey's test. Lowercase letters represent differences within soil layer; capital letters represent differences between triangles.

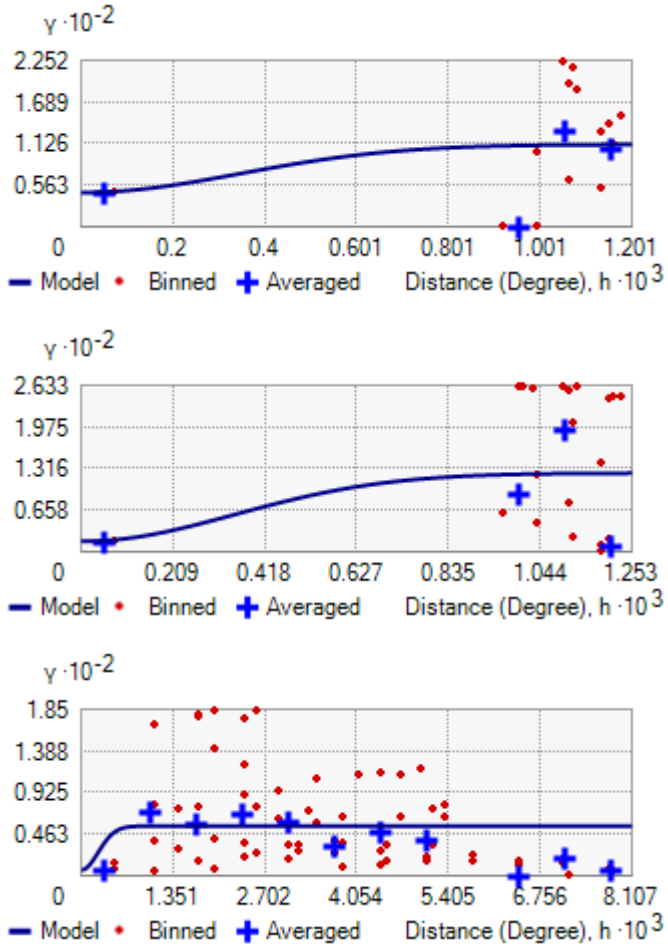


Figure 4.7. Kerj variograms of SOC stocks (dots) and exponential model fitted to the variograms (solid blue lines). Top variogram represents soil layer 30-45 cm; middle variogram is for 45-60cm and bottom variogram represents 60-75 cm soil layer.

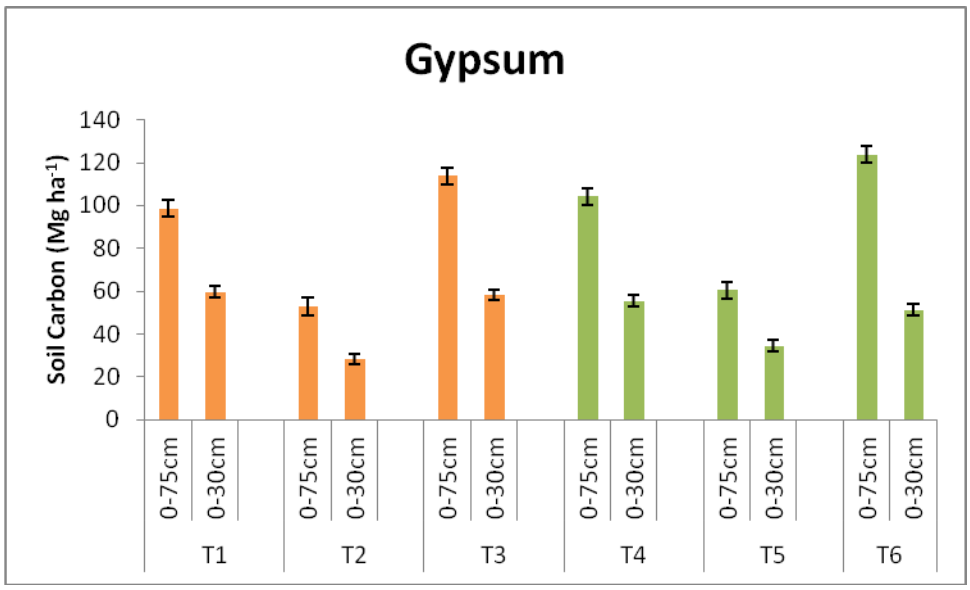


Figure 4.8. SOC stock at two sampling depths (0-75 cm) and (0-30 cm) in various triangles across field. Error bars represent standard error.T1 to T6 represent six triangles, the six triangles correspond to 18 geo-referenced sample locations. Different colors represent different soil types. Triangles 1, 2 and 3 representing the Hord silt loam displayed great SOC spatial variability. The triangles 4, 5 and 6 representing the Detroit silty clay loam soil showed great variability in SOC.

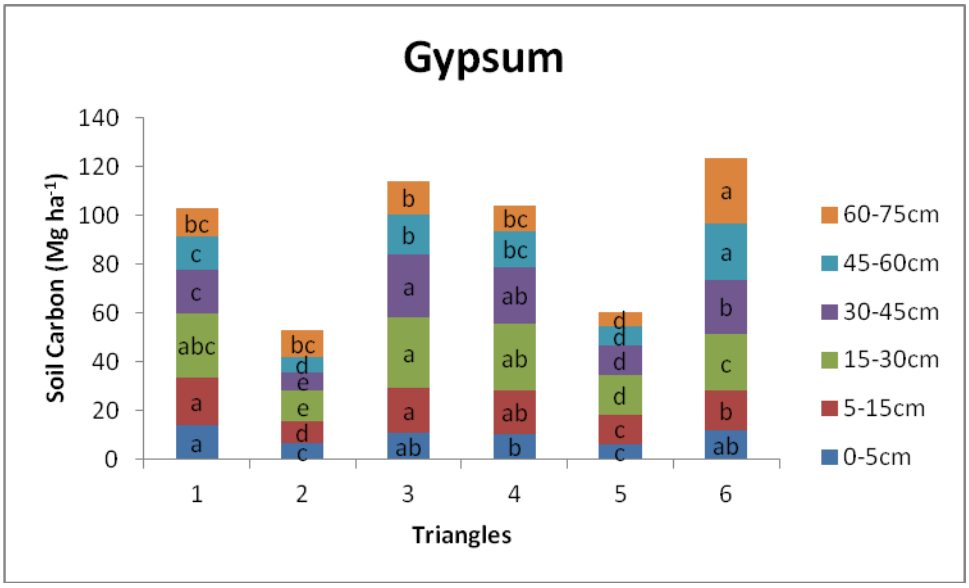


Figure 4.9. Estimated SOC at each individual depth increments.
Within depths, means followed by the same or no letter are not significantly different ($P<0.05$) by Tukey's test. Lowercase letters represent differences within soil layer; capital letters represent differences between triangles.

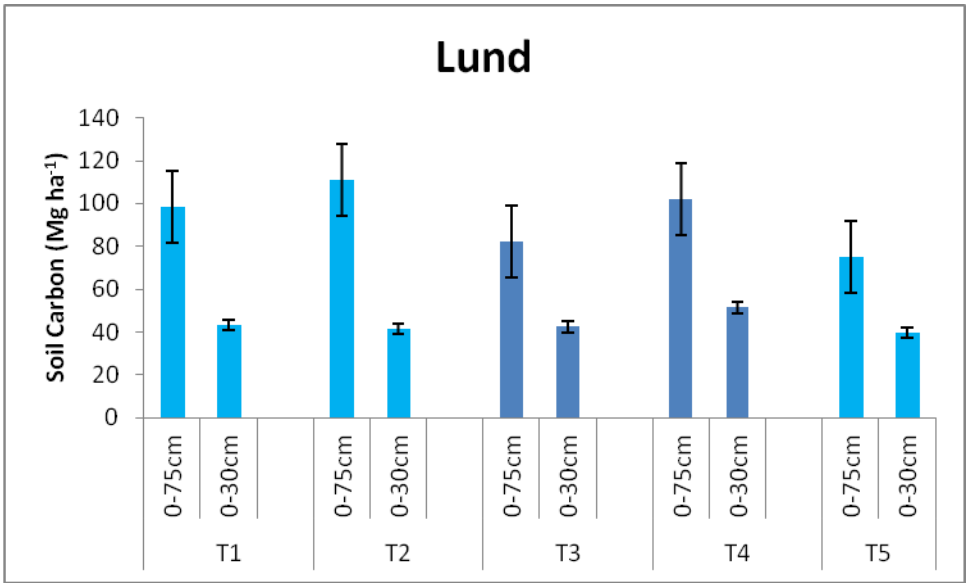


Figure 4.10. SOC stock at two sampling depths (0-75 cm) and (0-30 cm) in various triangles across field. Error bars represent standard error.T1 to T5 represent five triangles, the five triangles correspond to 15 geo-referenced sample locations. Triangles 1, 2 and 5 representing the Longford silt loam showed a homogeneous SOC distribution. The triangles 3 and 4 representing the Crete silt loam showed a homogeneous SOC distribution.

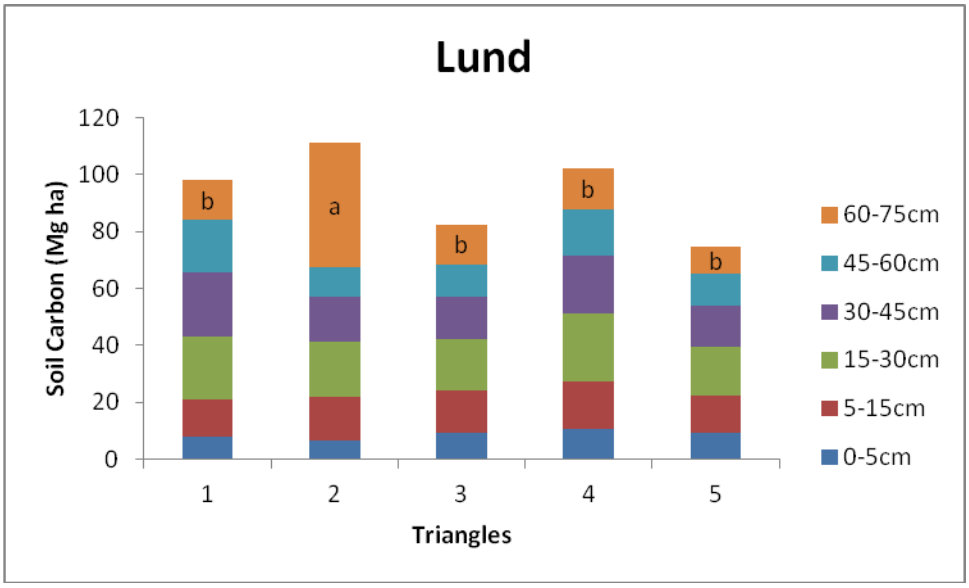


Figure 4.11. Estimated SOC at each individual depth increments.
Within depths, means followed by the same or no letter are not significantly different ($P<0.05$) by Tukey's test. Lowercase letters represent differences within soil layer; capital letters represent differences between triangles.

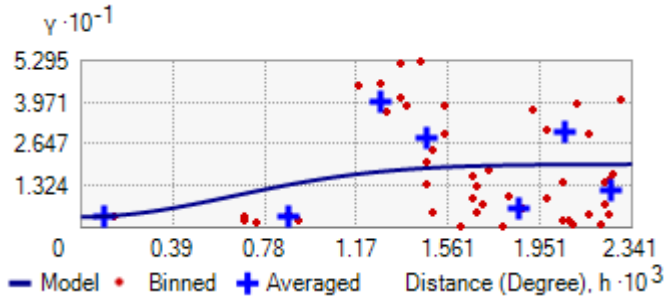


Figure 4.12. Lund variogram of SOC stocks (dots) and exponential model fitted to the variogram (solid blue lines). Variogram represents soil layer 15-30cm.

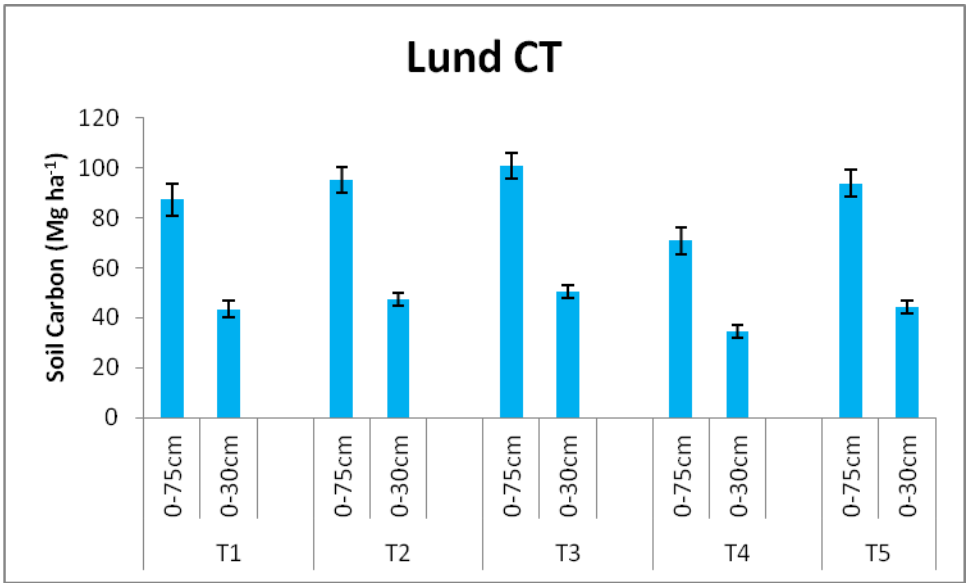


Figure 4.13. SOC stock at two sampling depths (0-75 cm) and (0-30 cm) in various triangles across field. Error bars represent standard error.T1 to T5 represent five triangles, the five triangles correspond to 15 geo-referenced sample locations. All triangles are within the same soil type (Longford silt loam).

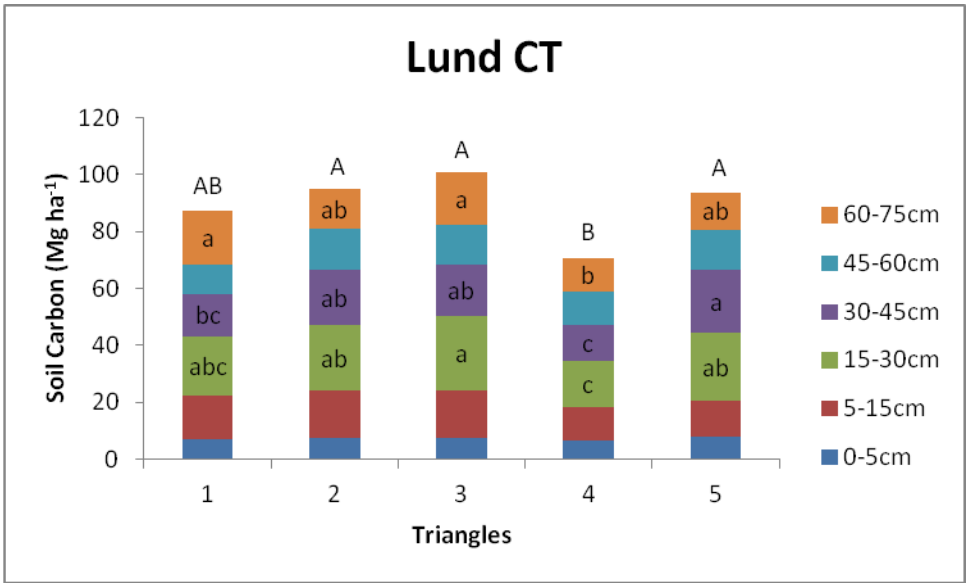


Figure 4.14. Estimated SOC at each individual depth increments.
Within depths, means followed by the same or no letter are not significantly different ($P<0.05$) by Tukey's test. Lowercase letters represent differences within soil layer; capital letters represent differences between triangles.

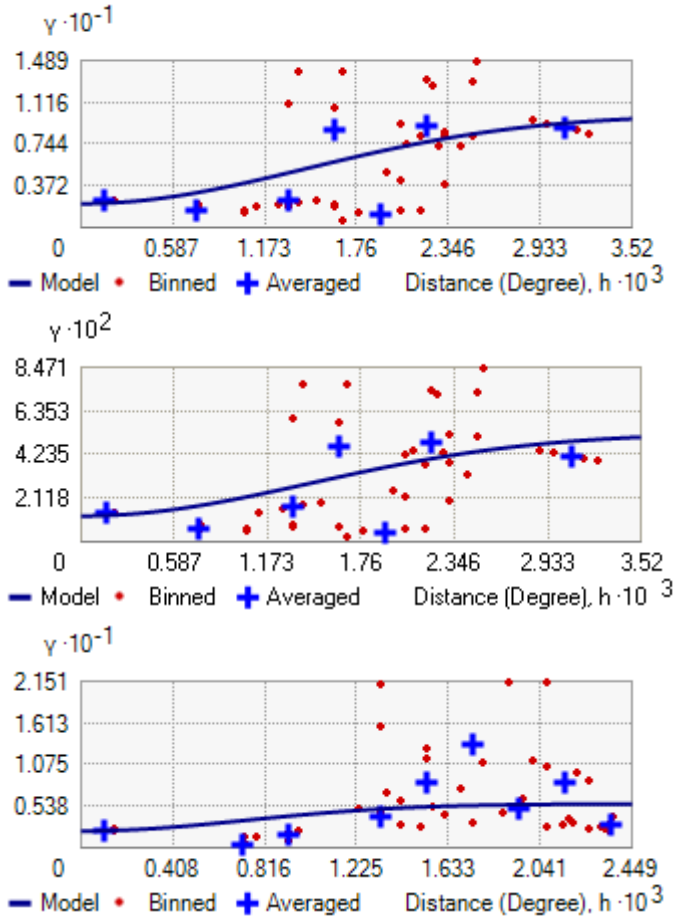


Figure 4.15. Lund CT variograms of SOC stocks (dots) and exponential model fitted to the variograms (solid blue lines). Top variogram represents soil layer 5-15 cm; middle variogram is for 30-45 cm and bottom variogram represents 45-60 cm soil layer.

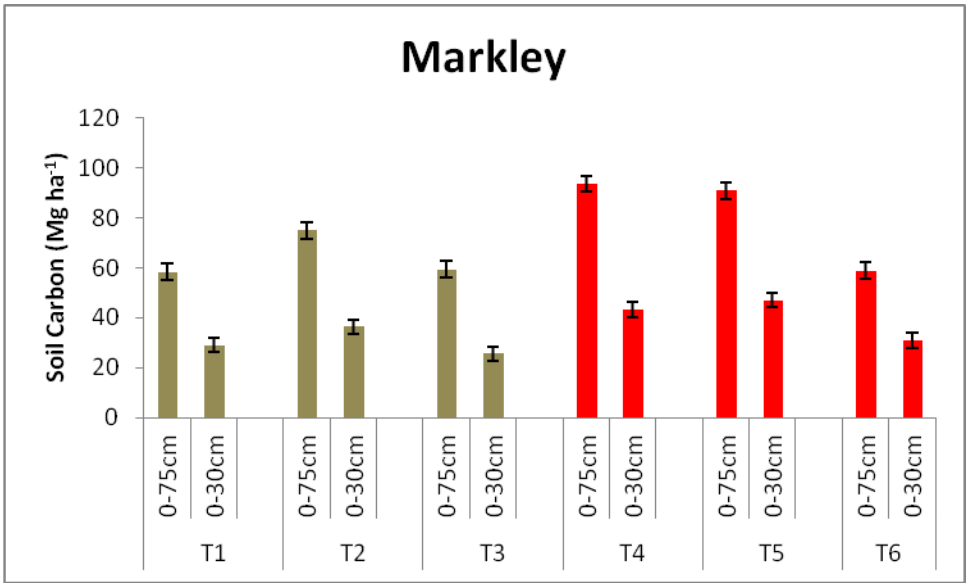


Figure 4.16. SOC stock at two sampling depths (0-75 cm) and (0-30 cm) in various triangles across field. Error bars represent standard error.T1 to T6 represent six triangles, the six triangles correspond to 18 geo-referenced sample locations. Triangles 1, 2 and 3 represent the Clime soil. The triangles 4, 5 and 6 represent the Crete soil.

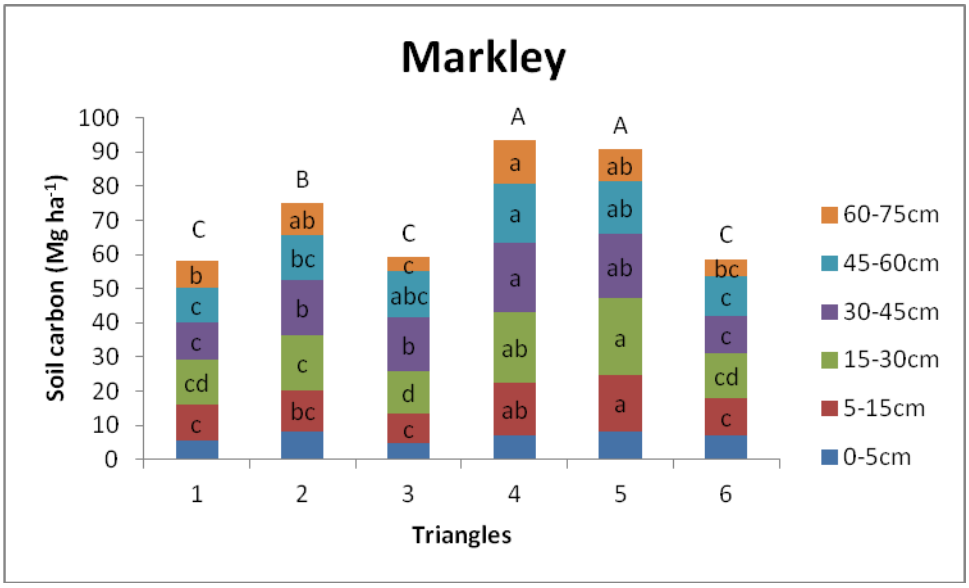


Figure 4.17. Estimated SOC at each individual depth increments.
Within depths, means followed by the same or no letter are not significantly different (P<0.05) by Tukey's test. Lowercase letters represent differences within soil layer; capital letters represent differences between triangles.

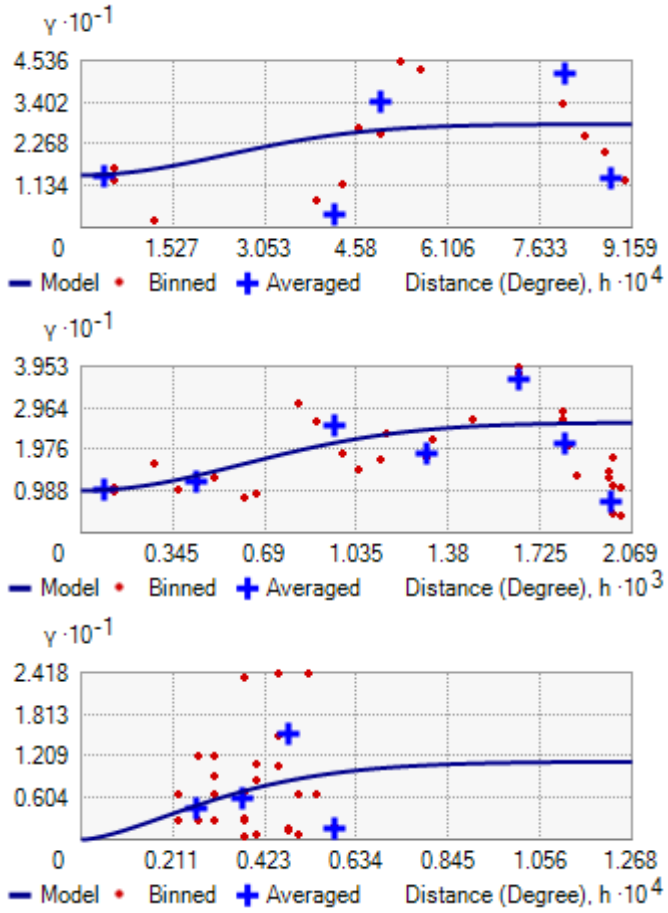


Figure 4.18. Markley variograms of SOC stocks (dots) and exponential model fitted to the variograms (solid blue lines). Top semi-variogram represents soil layer 15-30 cm middle semi-variogram correspond to 30-45 cm and bottom variogram represents 45-60 cm soil layer. Note at 45-60 cm soil layer semi-variogram analysis indicated the distribution of sampling points made binning difficult, therefore results for this particular soil layer were little unstable and did not reach sill.

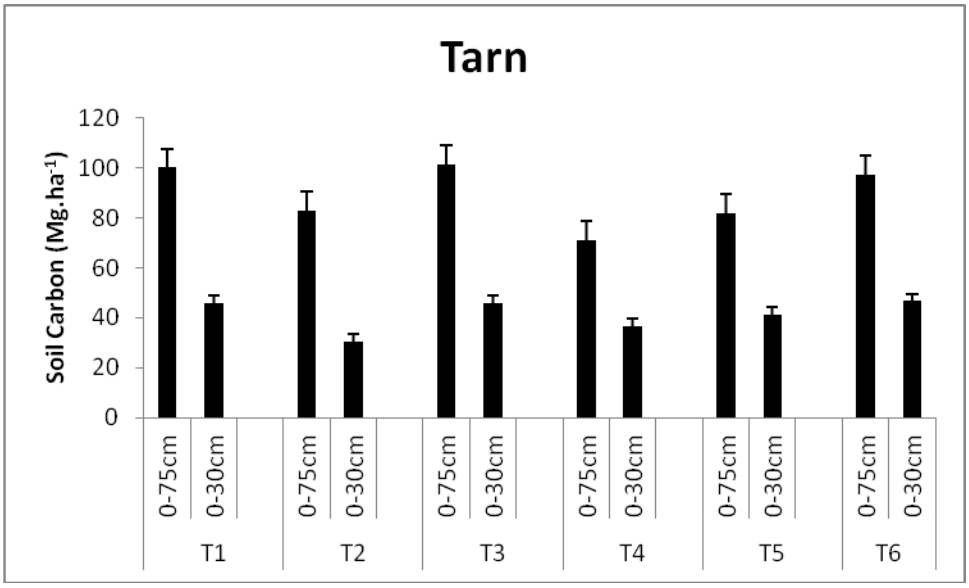


Figure 4.19. SOC stock at two sampling depths (0-75 cm) and (0-30 cm) in various triangles across field. Error bars represent standard error.T1 to T6 represent six triangles, the six triangles correspond to 18 geo-referenced sample locations. The soil C content between the six different triangles within the same soil type showed greater variability in this field/soil type than the Drummonds field. All triangles are within the same soil type (Irwin silty clay loam).

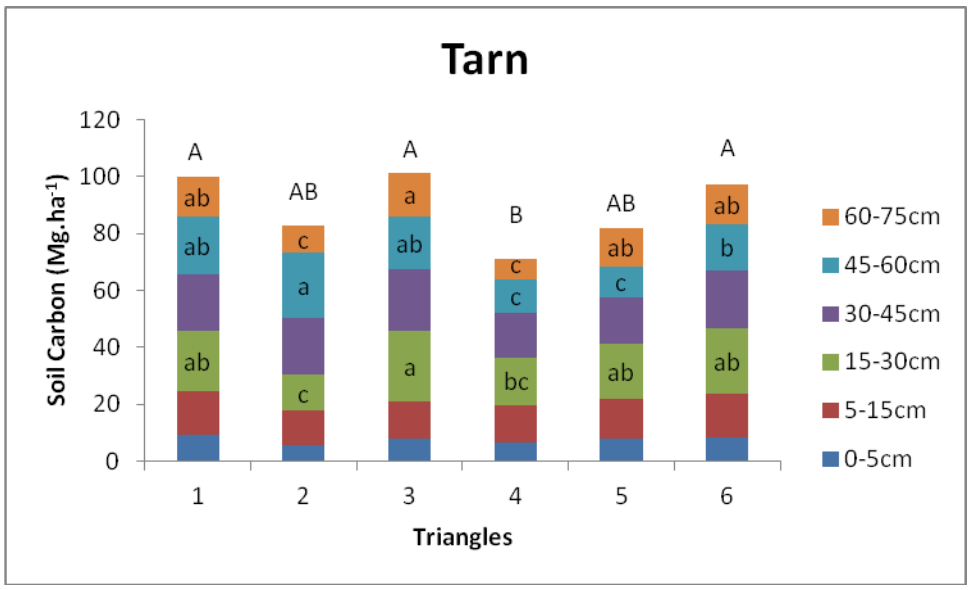


Figure 4.20. Estimated SOC at each individual depth increments. Within depths, means followed by the same or no letter are not significantly different (P<0.05) by Tukey's test. Lowercase letters represent differences within soil layer; capital letters represent differences between triangles.

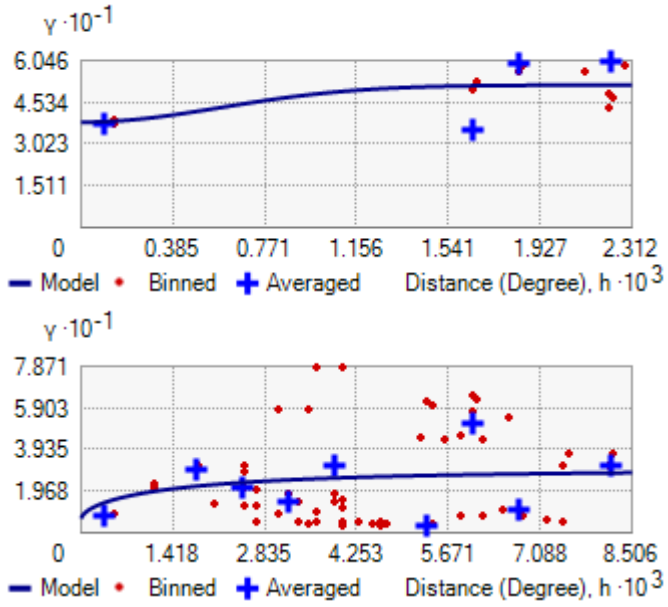


Figure 4.21. Tarn variogram of SOC stocks (dots) and exponential model fitted to the variogram (solid blue lines). Top variogram represents soil layer 30-45 cm with significant spatial autocorrelation but dispersed. Bottom variogram represents 45-60 cm soil layer.

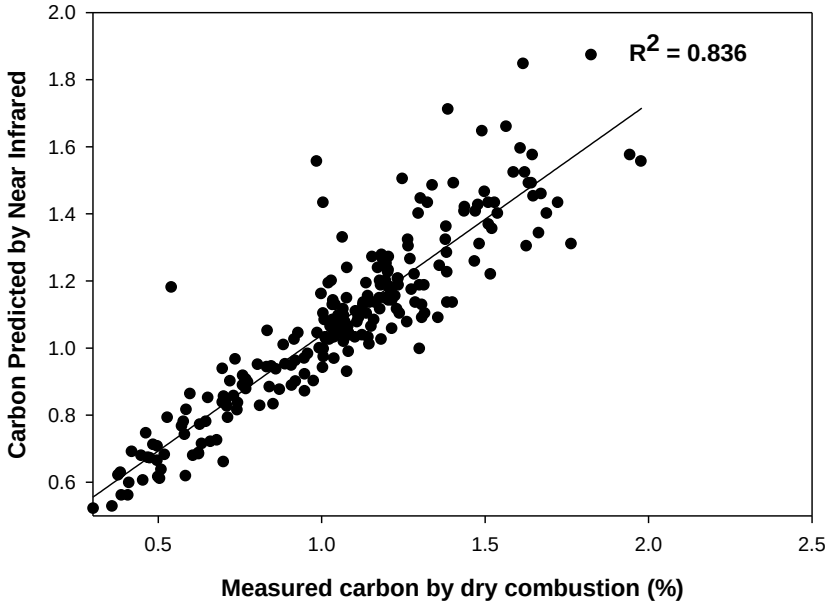


Figure 4.22. Carbon predicted by NIRS and measured by dry combustion.

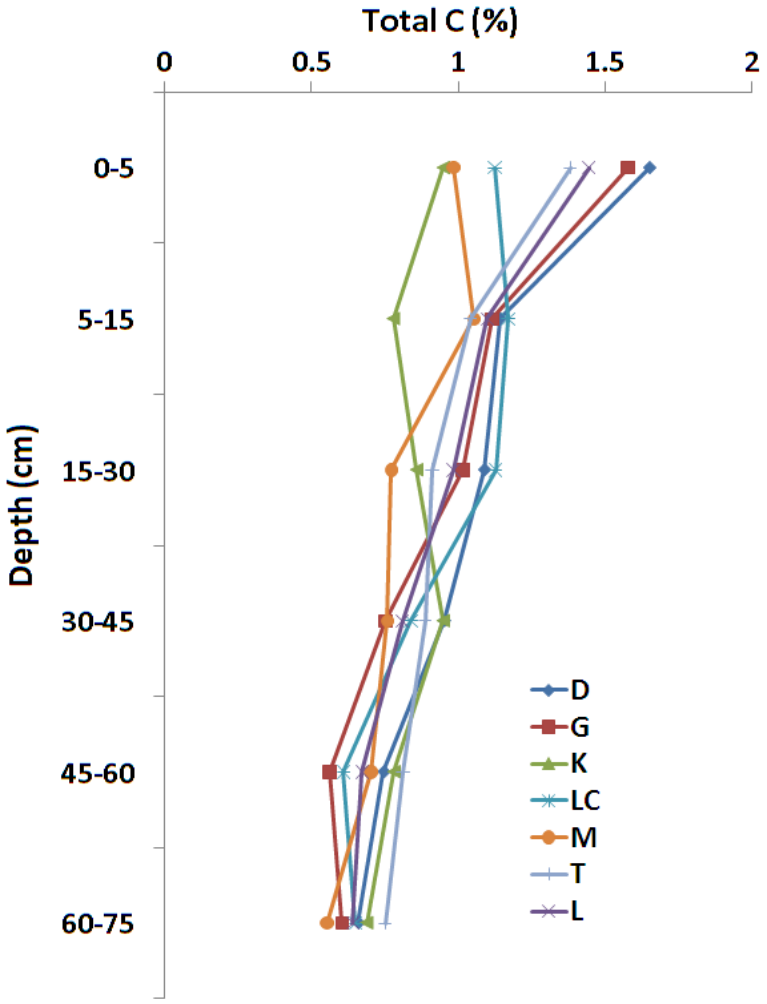


Figure 4.23. Vertical variations in soil carbon content for the various fields. Much of the variation in soil C between soils types occurred in the top 30 cm. D = Drummond; G = Gypsum; K = Kerj; LC = Lund CT; M = Markley; T = Tarn and L = Lund.

Table 4.1. Characteristics of the various fields.

Fields	Soil textural class , topography, triangles
Drummond	Crete Silt loam, 0 to 1% slope, triangles 1 to 5
Gypsum	Hord silt loam, triangles 1 to 3 Detroit silty clay loam, 0 to 1% slope, triangles 4 to 6
Kerj	Hord silt loam, triangle 1 Wells sandy loam, 3 to 7% slopes, triangles 2 to 3 Carwile fine sandy loam, 0 to 1% slope, triangles 4 and 7 Detroit silty clay loam, triangle 5 and 8 Pratt loamy sand, 5 to 12% slopes, triangles 6
Lund Lund CT	Longford silt loam, 0 to 1% slope, triangles 1,2 and 5 Crete silt loam 0 to 1% slope, triangles 3 and 4 Longford silt loam, 0 to 1% slope, triangles 1 to 5
Markley	Clime silty clay loam, 3 to 7% slopes, triangles 1 to 3 Crete silt loam, 3 to 7% slopes, triangles 4 to 6
Tarn	Irwin silty clay loam, 3 to 7% slopes, triangles 1, 2 and 6 Irwin silty clay loam, 1 to 3% slopes, triangles 3, 4 and 5

Table 4.2. Statistical analysis for within field SOC, comparison between triangle, depth and their interactions.

Fields	Drummond	Kerj	Gypsum	Lund	Lund CT	Markley	Tarn
Effect	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Triangle	<0.00001	0.0084	<0.0001	0.4172	0.0235	<0.0001	0.0808
Depth	<0.00001	<0.0001	<0.0001	0.2595	<0.0001	<0.0001	<0.0001
Triangle*Depth	<0.00001	0.0001	<0.0001	0.4137	0.1946	0.0504	0.0771

Table 4.3. Statistical analysis for infield bulk density, comparison between triangle, depth and their interactions.

Fields	Drummond	Kerj	Gypsum	Lund	Lund CT	Markley	Tarn
Effect	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Triangle	<0.00001	0.0084	<0.0001	0.4172	0.0235	<0.0001	0.0808
Depth	<0.00001	<0.0001	<0.0001	0.2595	<0.0001	<0.0001	<0.0001
Triangle*Depth	<0.00001	0.0001	<0.0001	0.4137	0.1946	0.0504	0.0771

Table 4.4. Classical statistical analyses of within triangles variability of SOC, for composites depth increments; 0-30 and 0-75 cm in various fields from northeast Kansas.

Fields	0-30cm				0-75cm		
D	**Triangles**	**Std. Dev.**	**CV**	**Mean**	**Std.Dev.**	**CV**	**Mean**
	1	2.10	4.96	42.48	2.19	2.52	87.09
	2	0.76	1.50	51.02	1.83	1.81	101.15
	3	1.36	3.15	43.09	4.02	4.16	96.59
	4	0.45	1.09	41.73	4.51	5.77	78.20
	5	4.71	9.84	47.90	3.51	3.27	107.53
K							
	1	2.10	4.88	43.16	5.028	5.45	92.15
	2	5.47	25.88	21.14	14.54	24.43	59.51
	3	21.29	64.42	33.05	41.78	55.46	75.34
	4	24.06	102.7	23.43	37.34	83.20	44.87
	5	3.38	5.85	57.76	20.64	17.90	115.26
	6	15.69	54.52	28.79	22.02	39.77	55.37
	7	3.18	8.70	36.59	5.46	4.91	111.36
	8	6.50	19.20	33.86	8.87	7.51	118.03
G							
	1	7.08	11.87	59.69	8.07	8.18	98.65
	2	1.35	4.79	28.19	0.90	1.70	52.86
	3	2.25	3.86	58.18	4.48	3.93	113.861
	4	1.87	3.37	55.49	6.37	6.11	104.24
	5	5.56	16.16	34.44	5.81	9.62	60.49
	6	5.40	10.56	51.20	11.10	8.97	123.73
L							
	1	7.79	17.97	43.36	12.48	12.71	98.16
	2	4.27	10.34	41.32	62.62	56.38	111.07
	3	0.66	1.57	42.39	3.88	4.71	82.48
	4	3.28	6.39	51.37	11.83	11.60	102.05
	5	2.00	5.03	39.70	3.24	4.346	74.74

K= Kerj; D = Drummond; G = Gypsum; L = Lund; LC = Lund CT; Std. Dev. = Standard deviation; CV = Coefficient of variation.

Table 4.4. continues within triangles variability for composite depth increments 0-30 cm and 0-75 cm in various fields from northeast Kansas.

Fields	**0-30cm**				**0-75**		
Markley	**Triangles**	**Std.Dev.**	**CV**	**Mean**	**Std.Dev.**	**CV**	**Mean**
	1	3.52	12.13	29.03	7.77	13.35	58.26
	2	8.66	23.85	36.30	2.92	3.89	74.99
	3	4.11	16.02	25.66	6.27	10.59	59.25
	4	2.90	6.73	43.15	2.67	2.86	93.59
	5	3.44	7.32	47.05	6.62	7.28	90.88
	6	5.09	16.48	30.89	6.22	10.60	58.69
Tarn							
	1	4.01	8.75	45.89	0.67	0.67	100.14
	2	2.28	7.51	30.43	14.89	17.95	82.93
	3	3.88	8.48	45.78	11.99	11.83	101.41
	4	0.55	1.52	36.47	6.34	8.90	71.22
	5	10.89	26.46	41.15	24.64	30.06	81.98
	6	1.41	3.03	46.56	4.15	4.28	97.12
LC							
	1	1.06	2.45	43.30	7.56	8.66	87.32
	2	6.23	13.20	47.24	7.76	8.16	95.07
	3	1.88	3.74	50.34	12.97	12.86	100.85
	4	6.54	18.95	34.55	9.26	13.08	70.81
	5	3.73	8.43	44.28	5.33	5.68	93.85

Table 4.5. Coefficients of variation in SOC based on field stratification. Test results are from three different homogeneity tests of variance (Levene-Test, Brown-Forsythe-Test and Barlett-Test). Test results were declared significant when at least two or all three test results were significant.

Field	Coefficient of Variation
Drummond	30
Gypsum	44.5
Kerj	62.7
Lund	73.1
Lund CT	38.8
Markley	41.6
Tarn	40.4
Homogeneity test	<0.0001

Table 4.6. Coefficients of variation (%) in SOC based on 3m triangle sampling design at major soil type from seven different fields in Northeast Kansas. Test results are from three different homogeneity tests of variance (Levene-Test, Brown-Forsythe-Test and Barlett-Test). Test results were declared significant when at least two or all three test results were significant.

Triangle	1	2	3	4	5	6	7	8	Test
Fields	Coefficient of Variation (%)								
Drum	22.5	30.7	32	21.6	29.3	---	---	---	*
Kerj	39	59.2	69.5	88.8	29.3	79.53	45	51.3	ns
Lund	43.5	127.3	22.6	32.9	24.9	---	---	---	ns
LundCT	41.7	34	41.2	30.5	38.2	---	---	---	ns
Markley	35	33.7	48.6	33.6	35.57	33.6			**
Tarn	35	33.7	48.6	33.6	35.5	33.6	---	---	ns
Gypsum	31	29.5	35.8	37.6	41.4	27.3	---	---	**

Table 4.7. Coefficients of variation (%) based on field stratification by soil type. Test results are from three different homogeneity tests of variance (Levene-Test, Brown-Forsythe-Test and Barlett-Test). Test results were declared significant when at least two or all three test results were significant.

Fields	Soil Type	Coefficient of variation (%)	Homogeneity Test
Drummond	Crete silt loam	30	N/A
Kerj	Hord silt loam	43.5	ns
	Detroit silty clay loam	39	
	Wells sandy loam	66.3	
	Carwile fine sandy loam	71.5	
	Pratt loamy sand	79.5	
Lund	Crete silt loam	30.7	ns
	Longford silt loam	90.5	
Lund CT	Longford silt loam	38.8	N/A
Markley	Crete silt loam	39.6	ns
	Clime silty clay loam	40.1	
Tarn	Irwin silty clay loam	40.4	N/A
Gypsum	Hord silt loam	43.8	ns
	Detroit silty clay loam	45.5	

Chapter 5 - General Conclusion

In this study we examined bioenergy cropping systems, it implications for sustainability and ecosystem services. This is addressed not only by measuring the biomass production and changes in key soil attributes following the first 5 years of various bioenergy cropping systems, but also by sampling and analyzing soil from 24 years crop residue experiment. Simulations of soil C also were performed to help quantify long-term effects of crop residue removal. An important issue for sustainable use of the soil has been addressed, by measuring soil C to understand its spatial variability. Aboveground biomass was lower with the perennial bioenergy cropping systems compared to annual crops but yielded similar to corn 5 yr after establishment. Also, key soil biochemical attributes, which are useful indicators of sustainable cropping systems, were significantly influenced by the different bioenergy cropping systems. Soil microbial biomass C, soil fungal:bacterial ratio and abundance of AMF were significantly greater under perennial bioenergy cropping systems. Overall, perennial crops provide a promising hope for improving the sustainability of future bioenergy cropping systems, but some annual crops such as photoperiod sorghum do hold potential that should perhaps be further explored. There may be possibility of harvesting crop residue for the production of biofuel under certain energy crops without adversely impacting the soil structural stability. This work also integrates key sustainability parameters relative to bioenergy cropping system and provides a sustainability index for various bioenergy cropping systems. An alternative classification was also offered but, sustainability index consistently gave higher score to switchgrass and miscanthus and ranks photoperiod and sweet sorghums as promising hope for potential bioenergy crop. Research conducted here provides critical information to validate ecosystem models on sustainability related issues. Data presented in this study also show that SOC would

decrease irrespective of the crop residue addition and fertilizer treatment if the soils are plowed. Significantly greater gain of C could be expected by reducing the disturbance of the soil, thus contributing to the sustainability of the soil resource. Cropping system, in which crop residue are completely removed may not be sustainable in the long-term, particularly when employed with constant tillage. Aggregate stability appears quite sensitive to level of residue management; generally, soil structural stability is affected more rapidly than SOC when soils are in use. Therefore, aggregate stability can be used as an early warning sign to monitor any change in soil quality when soils are exploited. Increasing residue addition has an increased C associated with the aggregate, particularly in larger macroaggregates of the surface soil. Soil under complete residue removal contains less aggregate associated C in all fractions. More C was concentrated in the small macroaggregates, where no correlations were found between C associated aggregates and the sand-free water stable aggregate. Hence, this aggregate fraction should be the focus of future studies attempting to understand the stabilization of organic C accumulation in agricultural soil. Future studies should investigate the physical protection inside the small macroaggregates to determine which material is the dominant binding agent. Such information can help maximized nutrients cycling, improved carbon storage which should be the paramount of sustainable cropping systems. Promoting a sustainable use of the soil requires an understanding of soil C spatial variability for accurate estimate of SOC. Soil C need to be cost effectively monitored for a sustainable soil use protection and management. Future research is required to better understand the drivers for soil C spatial variability at lower depths, soil type may help stratified the sampling but does not always explain the variability. With regard to the impacts of land use change, the continuation of this study is critical for long-term evaluation of soil microbial communities and other soil parameters as sensitive indicators of soil ecosystem

sustainability. The increased demand for biofuel will have direct impacts on soil and its ability to deliver ecosystem services. There is an urgent need support research to decrease risks and challenges associated with bioenergy cropping systems. Finally, soil scientists, agronomists, social scientists and engineers need to work together to address this issue of bioenergy sustainability not as a single soil resource issue or single feedstocks issue but rather from a multiple management approach that involve the agroecosystem as a whole.

Appendix A -

Table A.1 Farm chemical inputs.

Feedstocks	Herbicide (lb/ac)			Fertilizer (Kg/ha)---lb/ac	Percent Energy Use
	2007	2008	2009		Onsite----Total
Continuous corn	1.602 Atrazine; 1.246 S-metholachor	1.691S-metholachor; 0.178 mesotrione; 0.623 atrazine	0.5 glyphosate; 1.25 lunax	180---160.2	40----------8.41
Rotated corn	1.602 Atrazine; 1.246 S-metholachor	1.691S-metholachor; 0.178 mesotrione; 0.623 atrazine	0.5 glyphosate; 1.25 lunax	180---160.2	40----------8.41
Sweet sorghum	1.602 Atrazine; 1.246 S-metholachor	2.599 Atrazine; 1.246 S-metholachor; 0.623 glyphosate; 0.374 dicamba	0.5 glyphosate	168---149.52	54.39----------13.7
Photoperiod sorghum	1.602 Atrazine; 1.246 S-metholachor	2.599 Atrazine; 1.246 S-metholachor; 0.623 glyphosate; 0.374 dicamba	0.5 glyphosate	168---149.52	54.39----------13.7
Grain sorghum	1.602 Atrazine; 1.246 S-metholachor	2.599 Atrazine; 1.246 S-metholachor; 0.623 glyphosate; 0.374 dicamba	0.5 glyphosate	168---149.52	54.39----------13.7
Switchgrass K	0.979 Glyphosate 0.498 2,4 D dimethylamine salt; 0.94L/ha COC	0.374 diglycolamine salt; 2.3L/ha COC		45---40.05	
Bigbluestem	0.979 Glyphosate 0.498 2,4 D dimethylamine salt; 0.94L/ha COC	0.374 diglycolamine salt; 2.3L/ha COC	1.5 atrazine	45---40.05	
Miscanthus	0.979 Glyphosate 0.498 2,4 D dimethylamine salt; 0.94L/ha COC			112---99.68	

Table A.2. Statistical significance of bioenergy cropping systems, depth and their interaction on yield, MBC, MBN and root biomass.

Effect	*Yield*	*Microbial biomass C*	*Microbial biomass N*	*Root biomass*
Plant	<0.0001	<0.0001	0.03	0.0672
Depth	N/A	<0.0001	<0.0001	<0.0001
Plant*Depth	N/A	<0.0001	0.9582	<0.0001

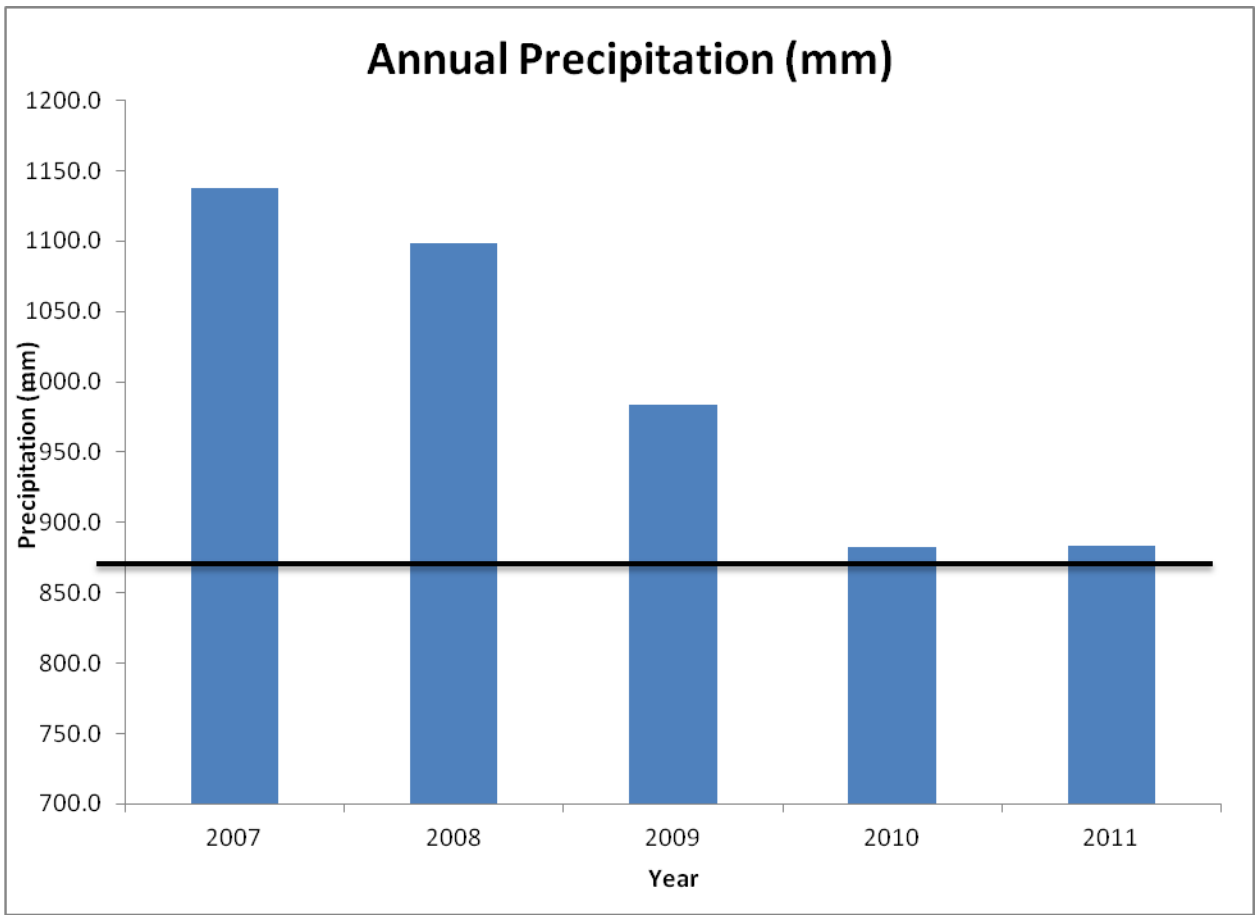


Figure A.1. Mean annual precipitation at Manhattan, KS biofuel site over the first five years of crop establishment. Black thick line represents the normal annual precipitation.

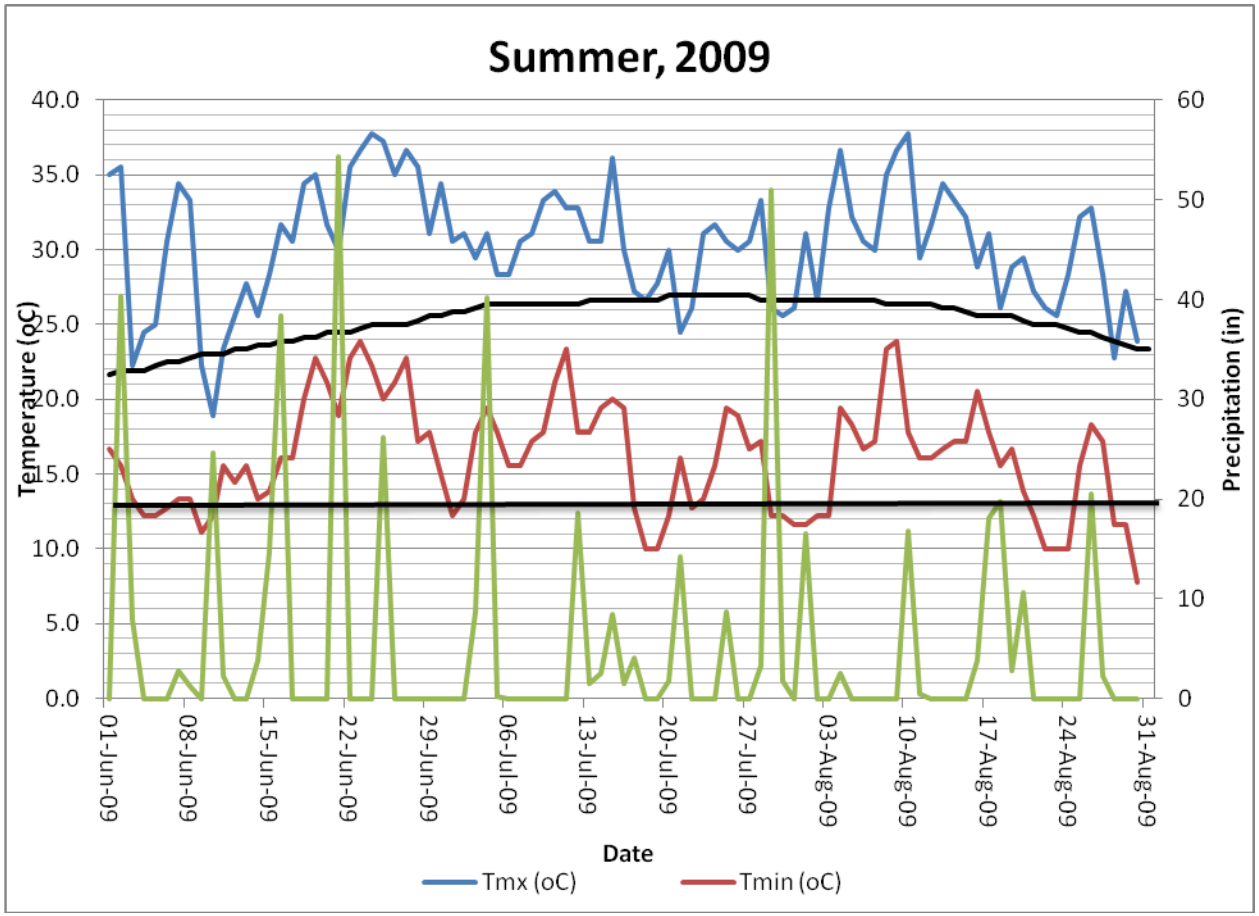


Figure A.2. Maxima and minima temperatures during the growing season of 2009. Flat thick line represents the minimal temperature limit of tolerance for sorghum.

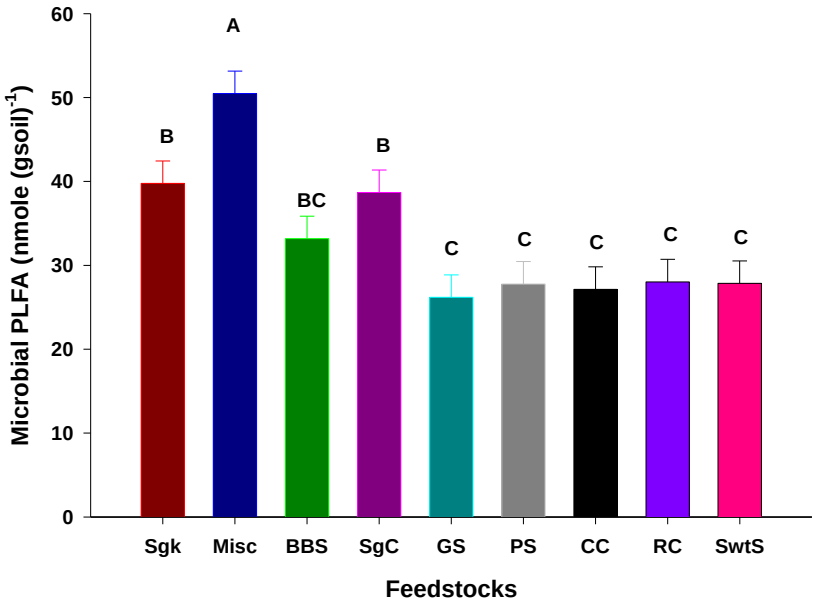


Figure A.3 Total microbial lipid determined by PLFA method in different bioenergy cropping systems. Different letters indicate significant differences among energy crops ($p<0.05$). SgK = Switchgrass Kanlow, Misc = Miscanthus, BBS = Big bluestem, SgC = Switchgrass Ceres, GS = Grain sorghum, PS = Photoperiod sorghum, CC = Continuous corn, RC = Rotated corn, SwtS = Sweet sorghum.

Table A.3. Effects of different bioenergy cropping systems on soil microbial community after three growing seasons
Phospholipid fatty acid (PLFA) composition (mean mole %) found in the top 0-5 cm of the soils from different energy crops in 2009.
Lowercase letters indicate significant differences of p <0.05 in Tukey's HSD and Kolmogorov-Smirnov tests
Abbreviations: Misc = Miscanthus; SgK = Switchgrass Kanlow; RC = Rotated Corn; SwtS = Sweet Sorghum; GS = Grain Sorghum; PS = Photoperiod Sorghum; BBS = Big Bluestem; SgC = Switchgrass Ceres; CC = Continuous Corn.

Fatty acid	Misc	SgK	RC	SwtS	GS	PS	BBS	SgC	CC	**Marker**
10-me18:0	1.12	0.97	1.76	1.75	1.42	1.41	1.04	1.19	1.61	Actinomyctes
i15:O	11.47**bc**	10.20**c**	15.48**a**	15.48**a**	13.58**abc**	14.48**ab**	11.02**bc**	12.44**bc**	13.82**abc**	Gram(+)
a15:O	6.10	5.32	7.80	7.56	7.13	7.49	6.17	6.44	7.86	Gram(+)
i16:O	4.11	3.82	6.32	6.11	5.35	5.54	4.09	3.49	6.70	Gram(+)
i17:O	2.4887	2.49	4.03	4.35	3.50	3.46	2.67	2.77	4.12	Gram(+)
a17:O	2.63	2.62	3.97	3.90	3.54	3.43	2.88	2.80	4.11	Gram(+)
16:1w7	17.06**a**	18.30**a**	6.72**bc**	6.98**bc**	9.41**b**	5.01**cd**	7.78**bc**	5.49**cd**	1.87**d**	Gram(-)
Cy17:0	2.77	2.70	3.93	3.42	3.19	3.47	2.94	2.98	4.31	Gram(-)
2-OH12:0	0.09	0.09	0.07	0.07	0.06	0.08	0.10	0.09	0.09	Gram(-)
3-OH12:0	0.09	0.01	0.04	0.13	0.04	0.07	0.01	0.02	0.03	Gram(-)
2-OH14:0	0.30	0.27	0.40	0.37	0.34	0.44	0.24	0.42	0.39	Gram(-)
3-OH14:0	0.42	0.66	0.24	0.37	0.33	0.37	0.19	0.78	1.41	Gram(-)
2-OH16:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.009	0.01	Gram(-)
18:1w7	0.45	0.59	0.69	0.42	0.51	0.60	0.66	0.57	0.30	Gram(-)
18:1w9c	6.58	6.79	6.54	5.72	6.58	6.66		7.23		Fungi, Sapro
18:2w9,12c	4.54**ab**	4.37**ab**	2.32**b**	2.85**b**	3.45**ab**	3.78**ab**	4.10**ab**	6.28**a**	3.24**ab**	Fungi
16_1w5c	7.74**ab**	7.88**ab**	6.25**b**	5.37**b**	6.02**b**	7.86**ab**	7.76**ab**	8.76**a**	6.33	Fungi, AMF

Table A.4. Effects of different bioenergy cropping systems on soil microbial community after four growing seasons
Phospholipid fatty acid (PLFA) composition (mean mole %) found in the top 0-5 cm of the soils from different energy crops in 2010. Lowercase letters indicate significant differences of p <0.05 in Tukey's HSD and Kolmogorov-Smirnov tests
Abbreviations: Misc = Miscanthus; SgK = Switchgrass Kanlow; RC = Rotated Corn; SwtS = Sweet Sorghum; GS = Grain Sorghum; PS = Photoperiod Sorghum; BBS = Big Bluestem; SgC = Switchgrass Ceres; CC = Continuous Corn.

Fatty acid	Misc	SgK	RC	SwtS	GS	PS	BBS	SgC	CC	**Marker**
10-me18:0	1.21	1.32	2.29	2.07	1.84	1.84	1.24	1.28	1.67	Actinomyctes
i15:O	11.19**d**	13.13**c**	17.09**a**	15.43**ab**	14.77**bc**	14.60**bc**	10.63**de**	9.92**de**	13.44**c**	Gram(+)
a15:O	5.98**c**	6.90**abc**	8.03**a**	7.77**ab**	8.40**a**	8.01**a**	5.57**c**	5.18**c**	6.87**ab**	Gram(+)
i16:O	5.03**c**	5.39**bc**	7.78**a**	6.91**ab**	6.77**ab**	6.19**abc**	4.61**c**	5.14**c**	6.64**ab**	Gram(+)
i17:O	2.9177**b**	3.48**ab**	4.72**a**	4.40**ab**	4.52**ab**	4.20**ab**	3.16b	3.22**b**	3.92**ab**	Gram(+)
a17:O	3.15	2.84	3.58	3.19	2.82	3.55	3.26	2.84	3.01	Gram(+)
16:1w7	1.49**b**	1.44**b**	1.28**bc**	3.24a	3.12**ab**	3.40a	3.16**ab**	-355E-17**c**	4.62**a**	Gram(-)
Cy17:0	3.60	3.61	4.07	3.80	3.89	3.81	3.56	3.54	3.55	Gram(-)
2-OH12:0	0.04	0.05	0.04	0.05	0.05	0.02	0.05	0.03	0.03	Gram(-)
3-OH12:0	0.02	0.01	0.01	0.07	0.06	0.04	0.01	0.01	0.02	Gram(-)
2-OH14:0	0.30	0.32	0.36	0.35	0.40	0.34	0.29	0.28	0.29	Gram(-)
3-OH14:0	0.21	0.26	0.66	0.31	0.38	0.32	0.15	0.22	0.32	Gram(-)
2-OH16:0	0.009	0.009	0.01	0.02	0.02	0.03	0.02	0.008	0.01	Gram(-)
18:1w7	0.65	0.82	0.61	0.60	0.72	0.78	0.83	0.85	0.55	Gram(-)
18:1w9c	8.11**b**	8.02**bc**	6.32**cd**	6.76**c**	7.59**bc**	7.76**bc**	8.59**ab**	9.59**a**	6.82**c**	Fungi, Sapro
18:2w9,12c	7.97**a**	3.86**c**	1.41**d**	3.14**cd**	2.78**cd**	1.88**d**	6.28**ab**	6.88**ab**	3.83**c**	Fungi
16_1w5c	8.45**a**	9.04**a**	4.48**cd**	4.74**cd**	5.38**c**	5.07**c**	8.42**a**	8.07**ab**	4.41**d**	Fungi, AMF

Table A.5. Effects of different bioenergy cropping systems on soil microbial community after five growing seasons.
Phospholipid fatty acid (PLFA) composition (mean mole %) found in the top 0-5 cm of the soils from different energy crops in 2011.
Lowercase letters indicate significant differences of p <0.05 in Tukey's HSD and Kolmogorov-Smirnov tests
Abbreviations: Misc = Miscanthus; SgK = Switchgrass Kanlow; RC = Rotated Corn; SwtS = Sweet Sorghum; GS = Grain Sorghum; PS = Photoperiod Sorghum; BBS = Big Bluestem; SgC = Switchgrass Ceres; CC = Continuous Corn.

Fatty acid	Misc	SgK	RC	SwtS	GS	PS	BBS	SgC	CC	**Marker**
10-me18:0	1.11	1.21	2.19	2.07	1.45	1.96	1.28	1.11	1.98	Actinomyctes
i15:O	10.82**d**	14.00**abc**	14.54**ab**	13.40**bc**	13.26**bc**	12.78**c**	13.13**c**	12.92**c**	15.03**a**	Gram(+)
a15:O	6.26	7.61	8.25	8.04	8.22	7.53	7.33	7.21	8.32	Gram(+)
i16:O	5.28**c**	6.31**bc**	8.91**a**	8.37**a**	7.59**ab**	8.73**a**	5.98**c**	6.26**bc**	8.13**a**	Gram(+)
i17:O	2.52	3.31	4.31	4.33	3.84	4.12	3.26	2.96	4.04	Gram(+)
a17:O	3.18	3.68	4.50	4.47	4.08	4.75	3.66	3.65	4.64	Gram(+)
16:1w7	6.91	5.78	6.14	5.85	6.97	6.41	6.93	7.49	6.55	Gram(-)
Cy17:0	3.14	3.61	3.81	3.71	3.73	3.91	3.46	3.44	3.68	Gram(-)
2OH12:0	0.04	0.04	0.04	0.03	0.04	0.02	0.05	0.03	0.05	Gram(-)
3OH12:0	0.03883	0.02	0.06	0.06	0.05	0.03	0.02	0.06	0.06	Gram(-)
2OH14:0	0.2588	0.29	0.31	0.30	0.31	0.29	0.24	0.32	0.35	Gram(-)
3OH14:0	0.26	0.35	0.44	0.39	0.40	0.42	0.32	0.25	0.44	Gram(-)
2OH16:0	0.007553	0.01	0.01	0.03	0.01	0.01	0.01	0.004	0.02	Gram(-)
18:1w7	0.5958	0.74	0.33	0.29	0.41	0.36	0.65	0.65	0.46	Gram(-)
18:1w9c	8.64**a**	8.44**a**	6.39**c**	6.66**c**	7.68**ab**	7.34**bc**	8.50a	8.12ab	6.56**c**	Fungi, Sapro
18:2w9,12c	9.10**a**	3.99**cd**	2.88**d**	4.47**c**	3.72**cd**	4.32**c**	5.83**bc**	6.24**b**	2.70**d**	Fungi
16_1w5c	8.09**a**	7.88**a**	3.54**c**	3.34**c**	4.43**c**	3.31**c**	6.56**b**	6.76**a**	3.72**c**	Fungi, AMF

Table A.6. Sustainability index for a range of bioenergy cropping systems in Manhattan, KS: An alternative classification.

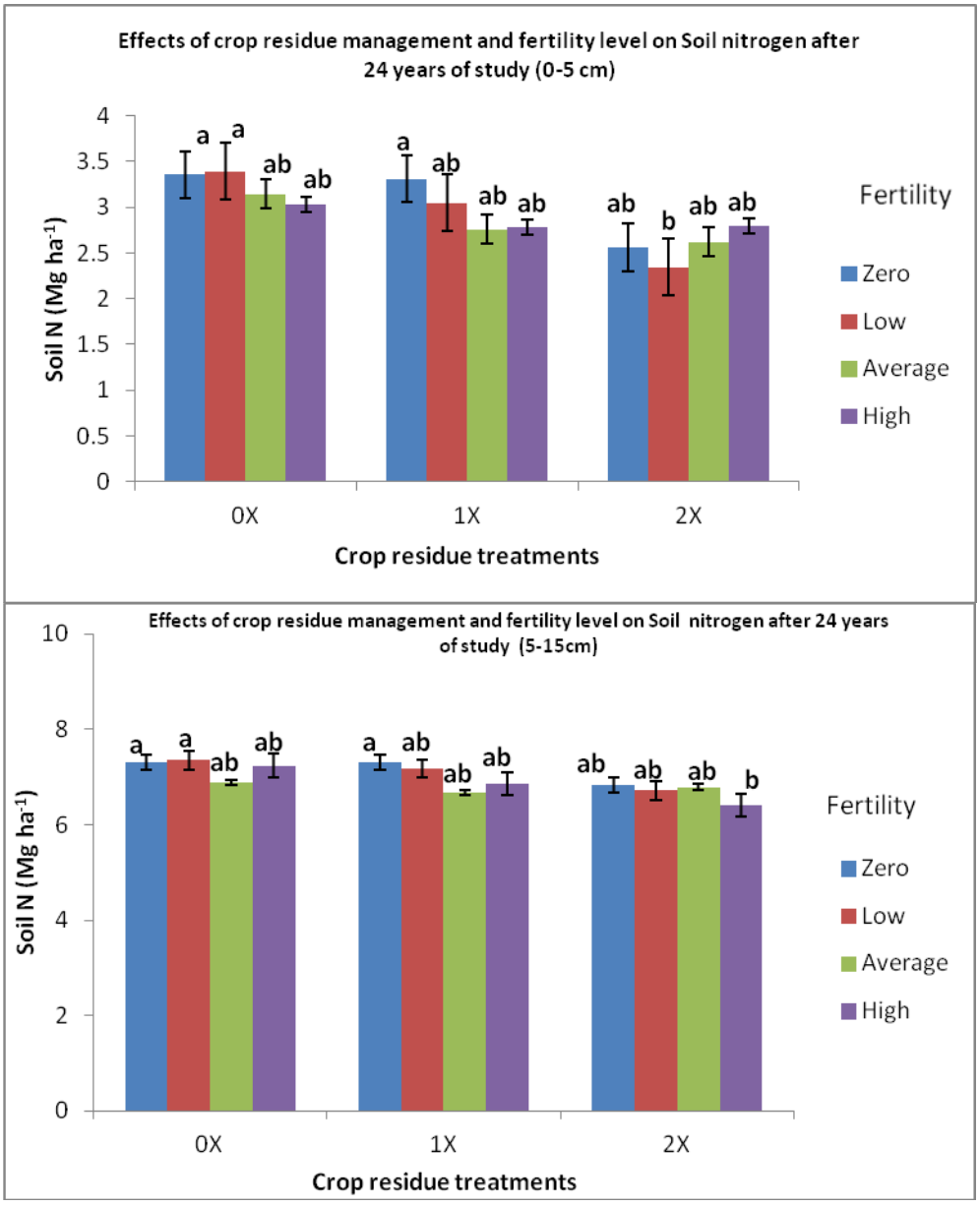
Feedstock	Revenue	Energy saving	C storage	Aggregation	Total PLFA	Index	Rank
Switchgrass Kanlow	7	1	2	1	2	13	1
Miscanthus	6	3	3	2	1	15	2
Big blue stem	8	1	1	3	3	16	3
Rotated corn	2	6	5	4	4	21	4
Photoperiod sorghum	3	4	7	6	6	26	5
Sweet sorghum	1	8	8	5	5	27	6
Continuous corn	4	6	4	8	7	29	7
Grain sorghum	5	4	5	7	8	29	8

Table A.7. Economic return between different bioenergy cropping systems.

Feedstock	Revenue	Energy saving	Economic return	Rank
Big blue stem	-1.32	1.5	0.18	1
Switchgrass Kanlow	-1.13	1.5	0.02	2
Rotated corn	0.81	-0.79	0.02	2
Photoperiod sorghum	0.49	-0.59	-0.1	4
Sweet sorghum	1.36	-1.5	-0.14	5
Grain sorghum	0.31	-0.59	-0.28	6
Continuous corn	0.42	-0.79	-0.37	7
Miscanthus	-0.95	0.36	-0.59	8

Appendix B - Chapter3

Figure B.1. Effects of crop residue management and fertility level on total soil N after 24 years of study in 3 depths increments (0-5; 5-15 and 15-30 cm).



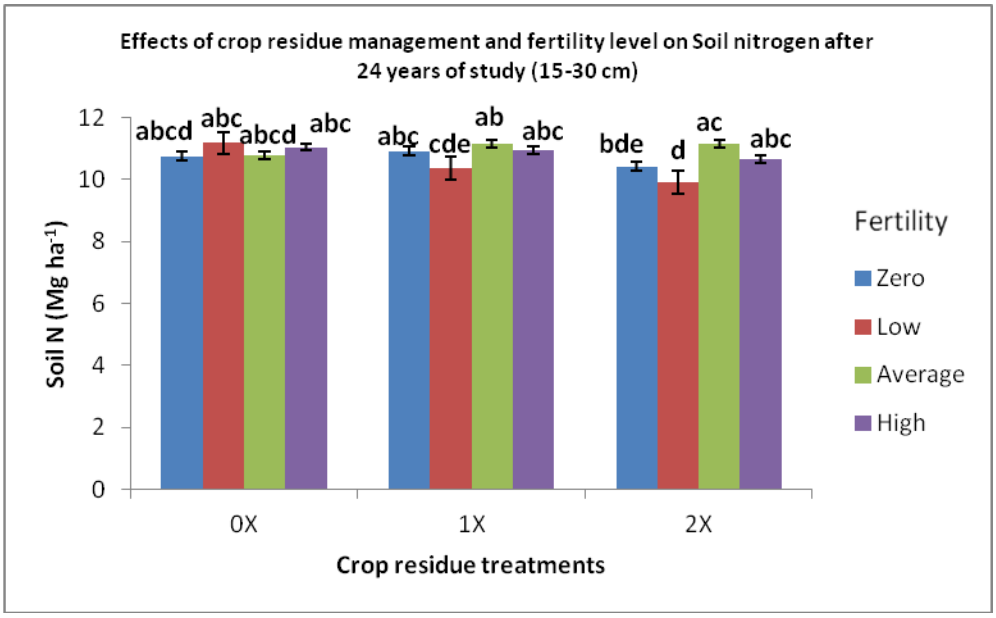

Effects of crop residue management and fertility level on Soil nitrogen after 24 years of study (15-30 cm)
Soil N (Mg ha-1)
12
10
8
6
4
2
0
abcd
abc
abcd
abc
abc
cde
ab
abc
bde
d
ac
abc
0X
1X
2X
Crop residue treatments
Fertility
Zero
Low
Average
High

Table B.1. Relative fractional abundance of different microbial groups at different depths. Comparison between residue removal (0 X) maintenance (1 X) and addition (2 X).

	Relative fractional abundance (mol. %)					Relative fractional abundance (mol. %)		
Factors	Bacteria PLFA		Fungal PLFA		Total PLFA	Gram+ Bacteria		
	0-5 (cm)	5-15 (cm)	0-5 (cm)	5-15 (cm)	0-5 (cm)	5-15 (cm)	0-5 (cm)	5-15 (cm)
0 X	23.12	13.85	11.16	3.47	46.24	24.61	16.13	11.01
1 X	32.7	13.14	15.34	3.22	62.5	22.9	20.43	10.8
2 X	29.33	12.76	13.35	3.14	56.57	22.32	20.34	10.03

Appendix C -

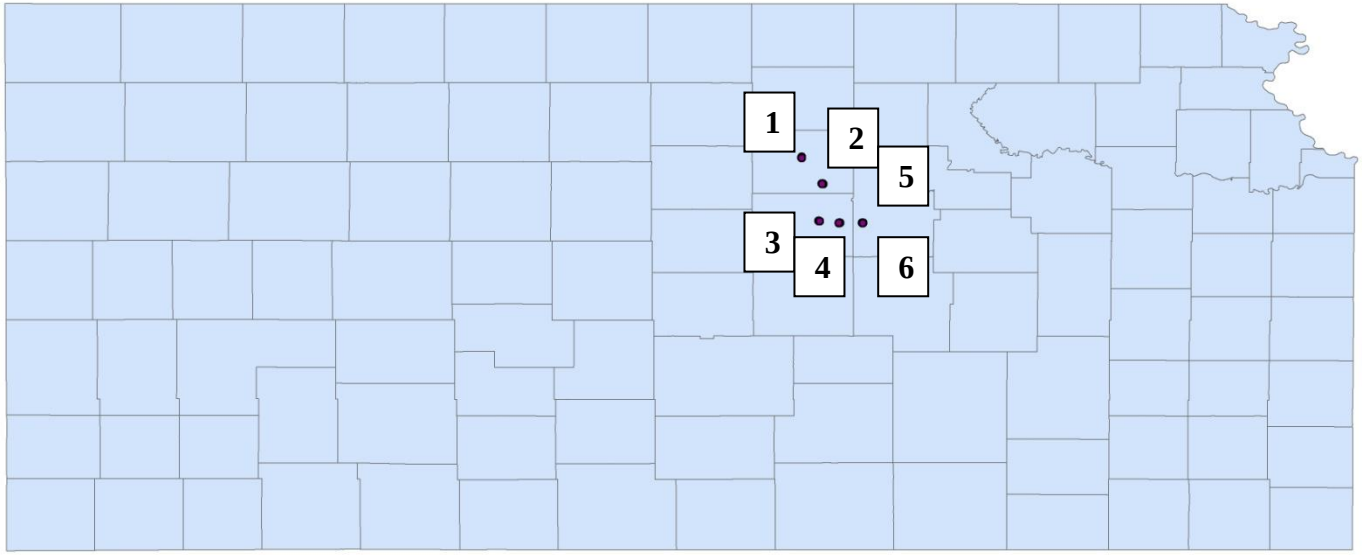


Figure C.1. Location of different study sites (1 = Drummond; 2 = Kerj; 3 = Markley; 4 = Lund; 5 = Lund CT; 6 = T.