ABSTRACT

The Quantification and Characterization of Soil Carbon in Switchgrass Plots under Fertilization and Harvesting Practices

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The shift toward renewable energy resources through agricultural biofuel production provides an opportunity to study and implement sustainable land management practices that improve soil health and a deeper understanding of mechanisms involved in terrestrial carbon cycling. Agricultural land management strategies such as fertilization, tillage, and crop selection affect soil organic carbon (SOC) storage through the quantity and quality of soil organic matter (SOM) fractions. A small perturbation in the SOC pool due to climate or land use change has major implications for food production, greenhouse gas emissions, and ecosystem health and stability. The goal of this research was to assess the impacts of fertilization and harvesting practices on whole soil C and N and develop approaches to quantifying soil health, using spectroscopic and thermal analyses of SOM fractions at a bioenergy field-scale plot at the Kellogg Biological Station in Hickory Corners, MI. This is one of the first comprehensive studies of twice-annual harvesting impacts on SOC and SOM fractions in agricultural bioenergy plots. Twice-annual harvesting resulted in smaller C stocks in root and low-density fraction of the soil organic

matter (LF OM). The roots and LF OM are the primary substrate for organisms in the soil food web. Therefore, this dissertation also quantifies roots and LF OM in terms of Gibbs free energy and macronutrient inventories. The plots with the highest rates of nitrogen (N) fertilizer application (196kg N/ha) contained smaller SOC and TN stocks than the unfertilized control plots, particularly in the dense fraction of the soil (>1.8 g/cm3). Carbon and N in the dense fraction is associated with mineral particles and is more stable in soil than C and N of the LF OM. This fertilization rate increased N content in root and LF OM and reduced decomposability indices (lignin/N and C/N) and likely increased their susceptibility to decomposition. Our results indicate that fertilizer and harvesting practices affect SOC storage with a direct impact on short-term C cycling and measurable effects on the Gibbs free energy. The lessons learned in this research suggest that land management practices can be optimized for carbon storage, soil health, and sustainability outcomes.

The Quantification and Characterization of Soil Carbon Plots under Fertilization and Harvesting Practices

by

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A Dissertation

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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DEDICATION

To those that guide me within.
Jeremy, Mom, and Angel.
May your laughter, light, and love shine through me

CHAPTER ONE

Introduction

Attribution

I would like to acknowledge the contributions from my co-authors and the work they provided to ensure this research reached submission and publication. Dr. William Hockaday was my overall adviser and guided me through the development and organization of this research plan. Dr. Hockaday garnered the funding and contacted the co-authors to ensure their shared interest and input in the final product before publishing. Dr. Hockaday was essential in the editing process required to get these manuscripts to publications and coordinating with our co-authors for their input. Dr. Carrie Masiello and Dr. Phil Robertson provided expertise in the editing and clarification of the manuscript text as well as resource support for the experimentation. Dr. Morgan Gallagher assisted proofreading and providing feedback for published and submitted articles.

Dissertation Organization

The pursuit of this research was an analysis of nitrogen fertilization and harvest management strategies on biofuel soil carbon pools and refining the stability metrics and energetic reservoirs of root and light fraction organic matter (LF OM). We chose to shift from studying the relationship between SOM origin and stability to a molecular and thermodynamic approach to energy, stability, and microbial biodegradeability of labile SOM pools in reference to the larger concepts of soil health and the food web. This was accomplished through combustion analysis, molecular characterization, and thermal

oxidation of whole soil, root, LF OM. Chapter One is written as a general introduction to concepts and motivations important for understanding soil biogeochemistry and carbon cycling in agroecosystems. Chapter Two evaluates the effect of harvesting and fertilization treatments on the size of C and N stocks in whole soil, roots, labile (LF OM), and passive (dense fraction OM) SOC pools across multiple depths to 60cm and seasonal changes occurring between harvests. Chapter Three provides a qualitative look at the chemical composition of the roots and LF OM, and a novel approach to assessing soil health and SOM stability by calculating biomolecular and energetic reservoirs. Chapter Four empirically examines the energy and stability of LF OM by thermal analysis to validate previous stability metrics and build a quantitative understanding of microbial biodegradeability. Chapter Five concludes with general insights gained from this research.

Soil Carbon Cycling and Environmental Health

Carbon is one of the most common elements in the universe and a critical component of life on Earth. The bonding properties of C atoms provide multiple opportunities for other elements to construct complex molecules and form the building blocks, down to the DNA, of plants and animals. Therefore, it seems fitting the carbon cycle is studied in deep time within our rock records, in its movement laterally and vertically across our atmosphere, throughout trophic levels, across landscapes, and within soils. In fact, soils contain the largest active-cycling portion of organic carbon on Earth. Soil organic carbon (SOC) is considered the main component stored in soil organic matter (SOM) through incompletely decomposed or living plant and animal residues and living and dead microorganisms in a variety

of particle sizes, C content, and turnover times. Fractionation procedures have been used to classify active and passive pools of SOC and determine their persistence in soil from months to millennia (Schmidt 2011). The mineralization of SOM, as a main source of energy for microbial communities, is controlled through climatic and edaphic conditions, physical protection by aggregates, chemical composition, and microbial community adaptation and utilization. Therefore, SOM is often in various stages of a transformation or stabilization with distinct pools providing various soil ecosystem services as a function of soil health.

The understanding of soil biogeochemical cycles is fundamental to sustainable management of soils ecosystem services. Soil organic matter is crucial to soil health and an essential component of ecosystem services such as: 1) supporting nutrient cycling, soil formation, and gas exchange; 2) regulating climate, water, and air quality; 3) providing food, fuel, and fiber for humans, plants, and animals; 4) the preservation of cultural and recreational landscapes. SOM management has broad implications for environmental health and maintaining growing global human population.

Land management practices influence the production of goods and services with significant impacts on C cycling between soil, plants, water, and the atmosphere. Management practices affect how much and what type of SOM is created and cycled through the ecological system with variable effects on the biological, physical, and chemical properties used to assess ecosystem health. Highlighting the effect of agricultural land management on soil ecosystem services, as the dominant form of land use change on Earth's most productive soils, is essential to maintaining and building

sustainable practices toward ecosystem resilience. The adoption of best land use practices can increase the size SOM pools which improves aboveground productivity through changes in soil structure, water and nutrient holding capacity. The increased and improved storage of SOM can also provide a method to mitigate greenhouse gas emissions (GHG), such as atmospheric Carbon Dioxide (CO^2). The proper management of SOM can increase ecosystem resilience to future changes in climate and land-use to continue to provide ecosystem services inherent to environmental health.

Soil carbon cycling is sensitive to climatic and land use changes with the potential to store or release C, however uncertainty remains in the SOC dynamics to forecast and parameterize impacts to climate and agricultural productivity models. The processes that affect SOC composition, size, and fate for future climate and land management scenarios are contingent on soil health indicators such as soil C content, pH, and microbial community structure.

The interactions of ecosystem processes, such as photosynthesis and decomposition are vital to the determination of the size and composition of SOC pools. The transformation of SOC to an energy resource within terrestrial ecosystems can provide a quantitative understanding of the relationship between soil, plant and microbial communities as a model for soil health. The larger SOC pools will contain larger energetic reservoirs and are likely to be healthier and more resilient. Land management practices can affect the size and composition SOM and influence the distribution of distinct energy pools associated with the SOC cycle.

The elemental and chemical analysis of soil carbon pools can provide information on the molar composition of OM to calculate the functional groups and biochemistry within distinct pools. A molecular mixing model developed by Baldock et al. (2001) combines the elemental and chemical analysis of organic matter to link functional groups such as O-Alkyl and di-O-Alkyl to carbohydrates or breakdown the protein content into a combination of Alkyl, N-Alkyl, Amides, and other compounds. These compounds can also be defined by the number of available electrons to obtain their carbon oxidation state as a function of the metabolic energy available to perform work on the system, or Gibbs free energy (ΔG). A direct thermodynamic quantity is calculated through loss of weight and heat released under constant temperature change using thermogravimetric analysis (TGA). These exothermic reactions provide data for internal thermal energy (ΔE) released with changes in temperature, and through measuring the change in weight in TGA programs we can calculate decay rate (k) and activation energy (E_a). These values are essential in developing C and energy budgets to illustrate the nutritional and ecosystem value as part of a soil health assessment.

Thermal stability of SOM has been closely related to biological decomposability (Plante et al., 2005) Labile SOM was shown to decompose at temperature ranges between 300 and 350°C, and stable SOM with greater aromatic content decomposed between 400 and 450°C (Lopez-Capel et al., 2005).Thermally labile and resistant SOM fractions contain different exothermic regions correlated with weight loss and change in energy that can be related to their decomposition at a certain temperature ranges (Plante et al., 2009). From a thermodynamic perspective, the low quality, complex SOM will require higher activation energies and higher

initial energy costs for enzyme breakdown than high quality, labile SOM during metabolic reactions (Bosatta & Ågren, 1999, Manzoni et al., 2012).

The ability to gauge soil quality for different SOM pools with a qualitative and quantitative accuracy across climate, management practices, depth, and ecosystems provides a detailed scope SOC cycling and ecosystem health. The application and communication of scientific knowledge towards practical metrics for various SOM pools under management practices is essential to providing a sustainable approach for land managers and farmers to increase productivity and land stewardship.

Potential of Biomass-Based Energy and Fuels

Biomass converted to a renewable energy alternative in the form of liquid or solid fuel can reduce CO_2 emissions by fixing atmospheric CO_2 into plant biomass and potentially reduce NO_x emissions through the reduction of nitrogen-based fertilizer usage (Pauly, et al., 2008). Different types of biomass, or feedstock, are harvested and converted to biomass-based fuels in the form of a solid, liquid, or gas to offset coal, gasoline, oil, and other petrochemicals. First generation bioenergy crops originate from food/grain sources that compete for limited land and water resources, such as ethanol made from corn. Second generation bioenergy sources are produced from plant and non-food sources on marginal lands less suitable for food production, and can generate a similar range of energy products from the feedstock with thermo- or bio-chemical fuel conversion (Naik, et al., 2010). The C footprint from bioenergy production should combine less intensive management practices with second generation bioenergy feedstock to reduce the demand on water

resources, fertilizer, and pesticides while improving soil and ecosystem health (Hill et al., 2006). Innovative research to develop sustainable second-generation bioenergy crops is essential for soil carbon storage strategies and future energy production (Sanchez DL 2015).

Perennial grasses are a promising source of 2nd generation biomass for advanced bioenergy feedstock, due to complex root systems allowing successive years of growth without intensive soil management (e.g. tilling, planting, cultivation). The C_4 carbon fixation pathway in perennial grasses generally has better water-use-efficiency (WUE) than the C_3 photosystem that tolerate conditions of drought and high temperatures. Temperatures are predicted to increase 2- 4 °C from 20th-century averages and the concentration of CO_2 is set to exceed 500 ppm by the mid-21st century (Romero Lankao et al. 2014, Melillo et al. 2016). These changes will impact the ecology and biogeochemistry of all ecosystems, and therefore policies and land use decisions are needed to manage climate feedbacks effectively.

Switchgrass (Panicum virgatum L.) is a native North American C_4 perennial bunchgrass selected by the United States Department of Energy as a "model" energy crop due to its coverage area, large aboveground yields on marginal lands, deep root system, and low required inputs (fertilizer, water, pesticides) (McLaughlin and Kszos, 2005; Wright and Turnhollow, 2010; Georgescu, 2011). Two major cytotypes of switchgrass are native to almost the entire North American continent (lowland and upland cultivars) and flourish under a range of environments. These characteristics met the economic and environmental criteria for crop selection when the renewable fuel standard was being considered through the late 1980's (Wright, 2007 ORNL). A

restructured agricultural ecosystem dominant throughout the Midwest of annual crops and tilling, such as maize and soybean, to perennial cellulosic biofuel crops, such as switchgrass, could provide economic and environmental benefits. Switchgrass agriculture can provide the opportunity to improve the management of natural resources (carbon, nitrogen, and water) while simultaneously providing soil conservation, sequestration of atmospheric CO_2, and a renewable energy biomass product for (Robertson et al., 2011).

Carbon and Nitrogen Management Opportunities in Switchgrass Agrisystems

The transfer rate (or flux) of carbon between the atmosphere, plant, and soil systems has an effect on local atmospheric CO_2 concentrations. Agricultural land-use occupies about 34% of possible land surface area globally (Leff et al., 2004). Seemingly minor differences in land use change or agricultural systems can change the size or stability of soil carbon with implications for soils to act as a source or sink for atmospheric CO_2. The transformation and production of belowground C dynamics in switchgrass agricultural plots can provide a source of C storage (passive SOM pools) and/ or sustainable nutrient development and energetic reservoirs (labile SOM pools).

Ammonium nitrate (NH_4-NO_3) is the most widely used nitrogen fertilizer in industrialized agriculture to supplement nutrients in cultivated soils, but it has a litany of environmental implications (Lemus and Lal, 2005). Industrial N fertilizer is manufactured via the Haber-Bosch process—an energy intensive process powered by fossil fuel combustion. The production and application of N fertilizer accounts for

over 50% of the total modern agricultural method C footprint, and creates the main source of nitrous oxide (N_2O) emissions into the atmosphere (Gan YT et al., 2011, Metz et al. 2007). Nitrous oxide is a powerful GHG with a mean lifetime over 100 years and a global warming potential 250 times greater than CO_2 ([IPCC 2007]). The topsoil runoff of chemical fertilizers also increases nitrogen deposition into surface waters and can create eutrophication in water basins and hypoxic zones downstream (Burkart MR 1999; Smith et al., 1999). The environmental impacts due to fertilization should be a consideration when developing the bioenergy portfolio.

Perennial grasses, like Switchgrass, generally have greater nitrogen-use-efficiency than the annual crops like maize (corn) and may require less fertilization while developing large belowground C stocks. Switchgrass planted as a riparian buffer was shown to reduce nitrogen leaching and runoff from an agricultural system while reducing N_2O emissions, when compared to traditional intensive row crop agriculture (Smith, C.M. et al, 2013). The management practices for sustainable bioenergy agricultural systems should be optimized for biomass production and C storage while reducing GHG emissions and conserving natural resources. The use of sustainable practices on perennial bioenergy crops and improved fuel-conversion systems in alternative renewable agricultural crop systems can provide future economic and environmental benefits.

Impacts of Land Management on Energy Consumption

The development of environmentally-sustainable energy for an increasing global population can help improve quality of life, economic stability, and alleviate future energy complications. The International Energy Outlook projects a 48% increase in world

energy consumption by the year 2040, and more than 71% of that increase is estimated to occur in developing countries [IEO 2016]. Greenhouse gas (GHG) emissions in the form of carbon dioxide (CO_2), methane (CH_4), and Nitrous Oxide (N_2O) have increased by 40%, 150%, and 22% respectively from 1750 to 2011. Between the years of 2002- 2011 atmospheric CO_2 concentrations increased at an average annual rate of 2.0 ± 0.1 parts per million (ppm) [IPCC 2013]. The report stated with a "very high level of confidence" that the leading cause for increased atmospheric CO_2 emissions is a product of anthropogenic influence through activities such as fossil fuel burning and land use change. The growing global demand for energy, food, and fuel require a diversification of the energy resources and sustainable land use management strategies to offset the GHG emissions of fossil fuel energy and land use change. It is imperative to understand the impact of current and prospective energy sources on environmental health and global climate to inform future policy decisions.

The central aim of my dissertation research was to quantify and qualify the effects of fertilization and harvesting treatments on belowground C pools in switchgrass field-scale plot in SW Michigan. We focused on soil C and N stocks for various fractionated pools, the molecular characterization of root and LF OM pools, and the thermal degradation patterns of LF OM to derive various stability and energetic characteristics that affect the trajectory of belowground C storage. The intention of this research framework was to connect the various sections from each chapter, and although each project was independently assessed the results collectively have both comparable and indistinct products.

CHAPTER TWO

Soil Carbon and Nitrogen Responses to Nitrogen Fertilizer and Harvesting Rates in Switchgrass Cropping Systems

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Abstract

The environmental sustainability of bioenergy cropping systems depends upon multiple factors such as crop selection, agricultural practices, and the management of carbon (C), nitrogen (N), and water resources. Perennial grasses, such as switchgrass (Panicum virgatum L.), show potential as a sustainable bioenergy source due to high yields on marginal lands with low fertilizer inputs and an extensive root system that may increase sequestration of C and N in subsurface soil horizons. We quantified the C and N stocks in roots, free-particulate, and mineral-associated soil organic matter pools in a four-year-old switchgrass system following conversion from row-crop agriculture at the W.K. Kellogg Biological Station in southwest Michigan. Crops were fertilized with nitrogen at either 0, 84, or 196 kg N ha-1 and harvested either once or twice annually. Twice-annual harvesting caused a reduction of C and N stocks in the relatively labile roots and free-particulate organic matter pools. Nitrogen fertilizer significantly reduced total soil organic C and N stocks, particularly in the stable, mineral-associated C and N pools at depths greater than 15 cm. The largest total belowground C stocks in biomass and soil occurred in unfertilized plots with annual harvesting. These findings suggest that

fertilization in switchgrass agriculture moderates the sequestration potential of the soil C pool.

Introduction

Managing the soil carbon cycle could help the bioenergy industry to deliver environmental benefits and mitigate the pace of climatic change. In addition to direct fossil fuel offsets, bioenergy cropping systems provide biogeochemical services such as the biological sequestration of atmospheric CO_2 in soil carbon reservoirs and biophysical services such as reduced latent heating from evapotranspiration (Margaret S. Torn et al. 1997; Trumbore 2000; Paul, Collins, and Leavitt 2001). Carbon sequestration occurs when soil organic carbon (SOC) accumulates more rapidly than it is respired (as CO_2 or CH_4) by soil heterotrophs. Deeply-rooted perennial grasses offer high annual net primary productivity (NPP) and the potential to promote the accrual of SOC (Lal et al. 2004; Liebig et al. 2005).

Switchgrass is a perennial, warm-season C_4 bunchgrass that is native to North America, and is a promising bioenergy feedstock due to large aboveground yields and hardiness across climate zones, soil types, and landscapes (Bransby, McLaughlin, and Parrish 1998; Sanderson et al. 2006; Wright and Turhollow 2010). Switchgrass is also suitable for marginal lands with low soil quality (Wright and Turhollow, 2010). The extensive rooting system of switchgrass and its C_4 photosystem efficiently use water and nutrients and reduce soil erosion (Vogel et al. 2002; Jung et al. 2011). Switchgrass rooting depths >1 meter may also promote the accrual of deep SOC pools in soils where SOC has been depleted by conventional row crop agriculture (Garten and Wullschleger 2000; Frank et al. 2004).

The stability of SOC can be viewed as an ecosystem property with physical, chemical, and biological controls. For the purpose of estimating relative stability, SOC pools can be divided into protected and unprotected pools. Aggregate-protected and/or mineral-associated SOC can be isolated and quantified by size or density separation procedures (Baldock and Skjemstad 2000; Kleber et al. 2005; von Lützow et al. 2007; M. S. Torn et al. 2013). The unprotected or free-particulate organic matter in the low-density light fraction (LF, < 1.8 g cm^{-3}) predominantly contains plant necromass (leaf and root litter) with typical turnover times < 10 years (Gregorich and Janzen 1996; Six et al. 1998). The mineral-associated and aggregate-protected dense fraction (DF, > 1.8 g cm^{-3}) of SOC has mean residence times on the order of 10 to greater than 100 years (Janzen et al. 1992; Baisden et al. 2002; von Lützow et al. 2008).

Soil C storage in switchgrass plantations is a biogeochemical service that can be directly influenced through management practices, such as fertilization and harvesting rates. The responses of soil C and N pools to management practices are key indicators of the role that bioenergy landscapes can play in greenhouse gas abatement strategies (G Philip Robertson 2011). Varied responses of SOC to switchgrass agriculture demonstrate the complexity in plant-soil interaction, and the need to study mechanisms of SOC accrual and stability (Table 2.1). Both fertilizer application rate and harvesting frequency can affect the accrual and long-term stability of SOC by modifying the extent to which organic matter enters protected and unprotected C pools (Stewart et al. 2014; Tiemann and Stuart Grandy 2014). In this study, we investigated soil C and N stocks in organic matter fractions of differing depth and stability (roots, LF, and DF) in response to two treatments: N fertilization rate and harvesting frequency, applied individually and in

combination. We hypothesized that more frequent harvesting would reduce belowground C and N stocks due to preferential allocation of resources to aboveground biomass at the expense of root development, while applications of N-fertilizer to the soil surface would reduce the growth of roots deep into the mineral soil profile, and therefore attenuate the SOC and TN stocks in the unprotected and protected fractions (LF and DF).

Materials and Methods

Field Site

The experiment was established at the W.K. Kellogg Biological Station (KBS) Long-term Ecological Research (LTER) site in southwest Michigan, USA (42° 249 N, 85° 249 W, elevation 288m), as part of the Great Lakes Bioenergy Research Center (GLRBC). Mean annual temperature at KBS is 10.1 °C; mean annual precipitation is 1027 mm (Robertson and Hamilton 2015). The soil is the Kalamazoo soil series, a mixed, mesic-Typic Hapudalf developed on glacial outwash with a fine and coarse-loamy texture comprising 85% sand and silt (Crum, J.R. and Collins 1995). Cropping history included corn-soybean and alfalfa rotations under conventional tillage prior to the planting of an upland switchgrass variety, "Cave-in-Rock", on July 11th, 2008 at a seeding rate of 7.84 kg/ha.

The experimental design was a randomized split-plot arrangement: 4 replicate blocks each containing 8 plots measuring 4.6 m by 15.2 m. Each plot comprised one fertilization rate that was split into two harvest intensity treatments for a total of 64 plots, each with dimensions of 4.6 m by 7.6 m. Eight fertilization treatments were applied in 28 kg N/ha increments, from 0 to 196 kg N/ha once per year between 2009-2011. The recommended N application rates for warm season grass crops in this area is

approximately 50-120 kg N/ha (Brejda 2000; Warnke, Dahl, and Jacobs 2009). Granular urea 46 % N (wt/wt) was broadcast on 17 June 2009, one year after plant establishment. In subsequent years, liquid urea ammonium nitrate (40% NH_4NO_3, 30% $CO(NH_2)_2$, 30% H_2O) was applied as a foliar spray at a concentration of 28 % N (wt/wt) in May 2010 and 2011. The plots sampled for this study were those fertilized once annually at rates of 0, 84, and 196 kg N/ha. Harvest intensity treatments were once per year (in November, after a killing frost) or twice per year (July and November) (lter.kbs.msu.edu/datatables/375).

Sample Collection and Analysis

Soil samples for this study were collected in July and November of 2011, immediately following the biomass harvest. In 2011 the mean annual temperature and total annual precipitation were 9.6 °C and 1125 mm (http://lter.kbs.msu.edu/datatables/7). Two soil cores from each plot were collected by first removing the litter layer and then pushing a 5cm steel tube (5 cm diameter with plastic liner) to a soil depth of 60 cm using a hydraulic GeoProbe ™. A total of 8 cores per treatment (2 cores per each of 4 replicate blocks) were extracted and capped in the field. The liners were split on-site, sectioned into four depth intervals (0 - 5, 5 - 15, 15 -30, 30 - 60 cm), and sealed in separate plastic bags before being packed with ice in coolers and shipped to Baylor University where they were stored at -20°C until processed. Each soil sample bag was allowed to warm to room temperature and then weighed as an initial step before handling. Each depth interval for all bulk soil cores were individually homogenized before being processed and analyzed separately. An initial sub sample (50 - 100g) was oven dried at 50 °C for at least 24 hours (to constant mass) to determine soil dry weight for bulk density calculations. A subset of the soils were also oven dried at 105ºC to quantify any potential bias in soil masses

obtained at 50 °C (Table S2.6). Soil bulk density was calculated by dividing the oven-dried weight by the soil core volume for each depth interval after correcting for the mass of the gravel fraction (>2 mm) (http://lter.kbs.msu.edu/datatables/308).

The remaining soil used to calculate SOC and TN stocks was air dried, picked for roots, and sieved to 2 mm. Roots were hand-picked with tweezers, lightly brushed of any adhered soil and placed in an aluminum tray for drying. Roots and a subsample of the sieved soil was placed in the drying oven at 50 °C for at least 24 hours, weighed, and stored for further analysis. Approximately 20 g of the soil subsample was placed in a 50mL centrifuge tube with approximately 30 mL of sodium iodide (NaI) solution (density =1.8 g/cm^3). After shaking for 30 seconds by hand, the tubes were centrifuged at 82 × g for 20 minutes. The solution was then allowed to settle before the floating LF was decanted onto glass fiber filters (Whatman, GFF) under vacuum. The LF was rinsed with deionized water to remove residual NaI, then dried in the oven at 50°C for 24 hours before being transferred to a glass vial for storage until C and N elemental analysis. The DF (> 1.8 g cm^{-3}) remaining in the centrifuge tube was drained and rinsed of residual NaI solution, dried, and stored for future analysis.

The remaining subsample of root-free, oven-dried soil (< 2 mm) was homogenized in a planetary ball-mill before determining weight percent C and N. The roots were pulverized and homogenized using dry ice and a Scienceware™ Micro-mill grinder. An initial group of soils treated with 10% hydrochloric acid (HCl) to remove inorganic C produced no detectable carbonate at any sampled depth interval. Therefore, HCl pretreatment was deemed unnecessary for the remaining samples. The soil, root, and LF samples were weighed into tin capsules and combusted in a Thermo Scientific Flash

EA 1112 Series NC Soil Analyzer to obtain total organic C and total N concentrations. SOC and TN stocks (kg m^{-2}) were calculated from the elemental concentration, soil layer bulk density, and soil layer depth (Stock = concentration (g/g) x soil density (g/cm^3) x depth interval (cm)). The C and N stocks in the mineral-associated, dense fraction (C_{DF} and N_{DF}, respectively) were calculated as the difference between whole soil and the free light fraction (C_{LF} and N_{LF}, respectively) stocks: C_{DF} = (SOC –C_{LF}); N_{DF} = (total N – N_{LF}).

The aboveground switchgrass C and N stocks were estimated as the product of biomass yield and C and N concentrations obtained from KBS LTER datatables (KBS LTER Datatables: Costech Elemental Combustion System CHNS-O, 2004; Total Soil Carbon and Nitrogen, 2009; Plant Carbon and Nitrogen, 2012). Total ecosystem carbon stocks were calculated from the sum of above and below ground stocks as: Total ecosystem C stock = (total aboveground biomass C + standing root biomass C + soil CLF + soil CDF). For plots harvested twice annually, the total aboveground biomass C was calculated from the sum of the July and November biomass C yields.

Deep soil core samples were collected immediately prior to switchgrass establishment in June 2008 by KBS staff, and sectioned at depth intervals of: 0 - 10cm, 10 – 25 cm, 25 – 50 cm, and 50 – 100 cm. These samples were passed through a 2mm sieve, oven dried at 60 ^{o}C, and stored in air-tight glass jars at room temperature. Subsamples were sent to Baylor University in 2016 for C and N elemental analysis. Soil C and N stocks were calculated, as described above, using elemental concentration values measured at Baylor and KBS bulk soil density values from the GLBRC Sustainability Data Catalog (KBS LTER Datatables: Soil Bulk Density, 2013). The initial (pre-

switchgrass) soil C and N stocks provide a meaningful baseline against which to evaluate the switchgrass treatment effects. However, differences in sampling depth intervals preclude direct quantitative comparisons of initial soil C and N stocks to those for switchgrass treatments using statistical analysis methods.

Statistical Analysis

To test for treatment effects on C and N stocks, we used a 3-way analysis of variance (ANOVA) General Linear Model Univariate. The fixed factors in this analysis were fertilization rate, harvest frequency, and depth intervals. Homoscedasticity of data was checked by the Levene's test prior to ANOVA. The p-value < 0.05 was chosen as the significance level in testing for differences between experimental treatments. The 84 kg N/ha fertilization rate was omitted from the ANOVA due to a lack of data for the November sampling of the twice-annual harvest treatment. Analyses were performed with IBM SPSS statistics 21.0 software (SPSS Inc., Chicago, IL).

Results

Ecosystem Carbon and Nitrogen Stocks were Highest in Unfertilized Switchgrass Treatments

Treatment plots with the combination of twice-annual harvesting and high rates of N fertilization generated the largest aboveground biomass C and N stocks, however the root C stock in the annually harvested treatments were significantly larger than twice-annually harvested plots ($p = 0.018$) (Figure 2.1, Table S2.1). The SOC and TN stocks were highest in unfertilized plots (Figure 2.2). The SOC stocks were 13% higher in unfertilized plots than in plots fertilized at a rate of 196 kg N ha-1 ($p = 0.004$, Figure 2.2a), and 85% of the change in SOC stocks occurred below 15cm between these

treatments. The soil TN stocks were also higher in unfertilized plots both in annually harvested ($p = 0.006$, Figure 2.2b) and twice-annually harvested treatments ($p = 0.055$). In our accounting of the total ecosystem C stock, Figure 2.3, the C_{DF} was the largest contributor to SOC stocks. Most notably, high N fertilization rates attenuated the total ecosystem C stocks (Figure 2.3) due to smaller soil C_{DF} stocks.

Treatment Effects on Soil C and N Pools

Fertilization reduced C and N in the dense fraction. The addition of N-fertilizer reduced C_{DF}($p=0.003$) and N_{DF} ($p = 0.005$) stocks by 14% relative to unfertilized controls through the 60 cm soil profile (Figure 2.4). The fertilizer treatments did not significantly affect C_{LF} and N_{LF} stocks ($p = 0.725$ and $p = 0.261$, respectively) or the root C and N stocks ($p = 0.253$ and $p = 0.225$, respectively).

Twice-annual harvesting increased C and N in the dense fraction. Soil N_{DF} stocks were 12 % larger in the twice-annually harvested plots ($p = 0.037$). The C_{LF} stocks were 32 % larger and N_{LF} stocks were 18 % larger in twice-annually harvested plots ($p = 0.049$ and $p = 0.073$, respectively), compared to annually-harvested plots (Figure 2.5a, b). No major differences were observed between harvest treatments for overall LF mass. The C_{LF} and N_{LF} stocks declined significantly with depth in all treatments ($p \leq 0.01$) and on average 70% of these stocks were located in the upper 15cm (Figure 2.5a, b). The root C and N stocks were considerably more variable between treatments than other C and N pools. Nevertheless, twice-annual harvesting significantly reduced standing root biomass and root C stocks through the 60 cm soil profile ($p = 0.026$, $p = 0.018$, respectively; Table S2.1; Figure 2.5c).

Soil C and N Pools Changed Seasonally

The SOC and TN stocks declined by 9 % from July to November, and SOC stocks were also significantly smaller with N fertilization for both seasons ($p=0.025$, Table S2.3). The late season decline in SOC and TN were driven by a reduction in C_{DF} and N_{DF} stocks, which occurred between the July and November harvests (Table S2.4). The LF mass was 28 % larger with N-fertilizer application ($p = 0.043$, Table S2.3), however the C_{LF} and N_{LF} stocks showed no significant seasonal changes between July and November harvest dates. Root N stocks increased from July to November ($p = 0.008$, Table S2.4), but no other significant changes were apparent between harvest dates and among fertilization treatments for root biomass, root C stocks, and root N stocks.

Discussion

A review of recent publications on switchgrass agriculture shows substantial variability in the response of SOC stocks to N fertilizer applications (Table 2.1). The complex interplay of substrate quality (plant residue chemistry), nutrient availability, soil redox gradients, microbial enzyme capacity/activity and community structure, soil mineralogy and available surface area may contribute to disparate responses of SOC and the effects of N-fertilization across switchgrass field trials. In this study, we found several important changes in soil C and N with harvesting and fertilizer treatments. The SOC and TN stocks were significantly larger in unfertilized switchgrass stands. Approximately half of the SOC and TN stocks are found at depths >15 cm (Figure 2.2), and predominantly in the mineral–associated dense fraction (Figure 2.3, Figure 2.4). Additionally, twice-annual harvesting caused a reduction in the root C and free-particulate C_{LF} stocks.

Changes in Soil Carbon and Nitrogen Stocks

The unfertilized SOC stocks to 60 cm depth measured 0.78 kg C m-2 larger than the fertilized treatment over the course of the study (3.7 years), corresponding to steady-state change of 0.21 kgC m^2 yr^{-1}. The annualized rate of 0.21 kg C m-2 y-1 to 60 cm depth is similar to those reviewed by Anderson-Teixeira et al. 2009, where the average SOC accrual was 0.1 kgC m^2 y^{-1} to 30 cm for fertilized sites. None of the perennial grass sites they reviewed were unfertilized. Follett et al. 2012 also observed an accrual rate of 0.2 kgC m^2 y^{-1} to 150 cm, where half of the SOC accumulated at depths below 30 cm. These relative rates of SOC change are relatively modest, and we note that Ruan et al. 2016 found no significant changes in SOC at the KBS GLBRC site but took fewer samples and did not fractionate nor include root biomass. Nevertheless, modest SOC accrual rates can lead to significant C sequestration if the accrual occurs within protected soil pools with potential for long-term stability. The N fertilizer treatment may be detrimental to long-term sequestration potential by affecting both the accrual depth and mineral association of C and N stocks (Liebig et al. 2005; Schrumpf et al. 2013).

Nitrogen Fertilizer Reduced Soil C_{DF} and N_{DF} Stocks

The N fertilizer treatment plots had significantly lower C_{DF} and N_{DF} stocks compared to the unfertilized control, mainly at depths > 15cm (Figures 2.2 and 2.3). This result is important because deeper soil C pools have longer mean residence times, which can be attributed to lower O^2 availability and slower rates of decomposition and mineralization (Trumbore 2000; Gill and Burke 2002; Rumpel and Kögel-Knabner 2010). The residence time (radiocarbon age), and the thermodynamic stability of SOM

typically increases with soil depth (Wang et al., 1996; LaRowe and Van Cappellen 2011; Keiluweit et al. 2016). Radiocarbon dating and laboratory incubation studies indicate that SOM associated with soil minerals (both mineral-bound and aggregate-occluded) has greater stability against biodegradation than free-particulate SOM (Margaret S. Torn et al. 1997; Trumbore 2000; Paul, Collins, and Leavitt 2001).

The causal mechanism for the rapid response of C_{DF} to N fertilizer remains unclear, but we consider two likely mechanisms. First, molecular level studies of grassland SOM suggest that roots and microbial biomass are the predominant sources of organic matter in the dense fraction (or humin fractions) (Otto et al., 2005; Rasse et al., 2005; Simpson et al. 2007). Our measurements at KBS indicate that root biomass C is ~30% lower in the fertilized plots (196 kg N ha^{-1}) than the unfertilized plots, though the effect was not statistically significant in 2011 samples ($p = 0.25$, Table S2.1). Nevertheless, a reduction in root C inputs may have contributed to lower C_{DF} and N_{DF} over the 3.7-year duration of the study. Second, N fertilization may reduce SOM accrual in the dense fraction by indirect effects on SOM decomposition rates, caused by changes to SOM chemical composition and/or microbial activity. For instance, high rates of N fertilization can increase root decomposability through the reduction of root C:N ratios (Garten Jr. et al. 2011). Furthermore, soil nutrient availability can affect microbial community structure and activity and promote or retard the decomposition of SOM (Chen et al. 2014; Nottingham et al. 2015). Chen et al. 2014 demonstrate that N fertilizer added to soil in combination with fresh plant residues tends to accelerate the mineralization of organic matter. Acceleration of the decomposition rate may reduce the accrual of SOC and TN.

Twice-Annual Harvesting Reduced LF and Root C and N Stocks

Mechanisms for the reduction in C_{LF} and N_{LF} pools with twice-annual harvesting (Figure 2.5a and b) could be due to a more efficient removal of aboveground biomass and therefore less incorporation into the soil C and N pools, or the increased exposure at the soil surface favoring increased erosion (physical transport) and aerobic (biotic) or photic (chemical) decomposition of surface residues and associated LF organic matter. In the present study, root C stocks below 15 cm represented 30-45% of total root C to 60 cm for all samples collected in November. The smaller root C and N stocks observed in the twice-annually harvested treatment (Figure 2.5c and d; Table S2.1) may be from the mid-season harvesting disturbance which could modify resource allocation to aboveground biomass. The 12% reduction in root C stocks with fertilization at the deepest depth (30-60cm) may be a function of nutrient availability at the surface. The reduced root C and N inputs may also have contributed to the lower C_{LF} and N_{LF} pools in the twice-annual harvesting treatments, as root biomass can be transformed into LF SOM (Ma, Wood, and Bransby 2000).

Soil Dense Fraction C and N Declined Rapidly Between Summer and Fall Harvests

The rapid decline of the C_{DF} and N_{DF} pools over the intervening months between July and November harvests is surprising, given the presumed stability of this fraction (Table S2.3, S2.4). There are several mechanisms that might explain such a rapid reduction of C_{DF} and N_{DF} stocks between harvests. (1) Seasonal soil aggregate stability could diminish between seasons as a function of increased autumn precipitation and cooler temperatures (Dimoyiannis 2009; Bach and Hofmockel 2016). (2) The priming of microorganisms by surface residues additions during the mid-season harvesting and the

soil disturbance associated with that harvest could accelerate the mineralization C_{DF} (Kuzyakov, Friedel, and Stahr 2000). (3) Alternatively (or additionally), mid-season harvesting could cause a reallocation of photosynthate from root growth to shoot growth, leading to a decline in the substrates supporting mineral-associated microbial biomass, thus diminishing the C_{DF} and N_{DF} between harvests (De Vries et al. 2015). The reduction in C_{DF} was larger in the unfertilized treatments between harvests, however the unfertilized plots had significantly larger C_{DF} and N_{DF} stocks at both harvest dates. This implies that high rates of N fertilization and harvesting, which reduce the production of roots, C_{LF}, and N_{LF} stocks, may also affect inputs to the C_{DF} and N_{DF} pools (Kallenbach et al. 2015).

Summary

Although a primary objective in bioenergy production is maximizing aboveground biomass for use as feedstock for energy and fuel, energy conservation and soil C storage are also valuable biogeochemical services (Robertson et al., 2008) that can further reduce the carbon intensity of bioenergy systems. Our results show that the largest total ecosystem C stocks (above + below ground) were achieved with the least energy-intensive agricultural practices: no N fertilizer and a single postseason harvest. Harvest intensity and N-fertilizer rates affected the magnitude of soil C and N storage, as well as the depth and relative stability of the C and N pools. The changes in SOC occurred primarily at depths greater than 15 cm and in the dense fraction of the SOC pool where organo-mineral associations provide a mechanism for long-term soil C storage. The N-fertilizer treatments caused a reduction in soil C stocks, particularly in the mineral-associated fraction, while the combination of annual harvesting and N-fertilization

reduced soil N stocks in the mineral-associated fraction. The twice-annual harvest treatment reduced LF and root C pools. Unfertilized switchgrass plots contained 15% more SOC, on average, 4 years after planting than did plots under high fertilization rates. Ruan et al., 2016 recently demonstrated the high carbon cost of fertilizing biomass crops such as switchgrass. Our findings demonstrate that management practices that minimized carbon emissions from N fertilization and mechanical harvesting also enhanced the magnitude and longevity of soil carbon storage.

Acknowledgments

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Table 2.1 Summary of literature on soil C pool response to N fertilizer in switchgrass plots

	Location	Stand Age	Soil Depth interval (cm)	N Fertilization Rate (kg N ha^{-1})	Soil C Response to Fertilization
(Liebig et al., 2008)	Ten sites in NE, ND, SD	5 years	0 – 30	31 to 104	Linear increase (P=0.03) *
			0 – 120	31 to 104	Linear increase (P=0.07)
(Jung and Lal, 2011)	Three sites in OH	6 years	10 – 20	0, 50, 100, 200	Increase in SOC (P = 0.05) *
			0 – 30	0, 50, 100, 200	No change in SOC
(Stewart et al., 2014)	NE	9 years	0 – 5	60	Increase in SOC (P=0.05) *
			0 – 30	60, 120	Increase in SOC (P<0.01) *
(Follett et al., 2012)	NE	9 years	0 – 30	60	Increase in SOC (P=0.10)
			0 – 15	60	Increase in SOC (P=0.06)
(Heggenstaller et al., 2009)	IA	3 years	0 – 100	65	Increase in roots
			0 – 100	140	Increase in roots
			0 – 100	220	No change in roots
(Lee et al., 2007)	SD	4 years	0 – 60	112, 224	Increase in SOC
(Ruan et al., 2016)	KBS, MI	3 years	0 – 100	0 to 196	No change in SOC
(Ma et al., 2000)	AL	4 years	0 – 225	112	No change in SOC
			0 – 225	224	No change in SOC

* P values for significant treatment effects

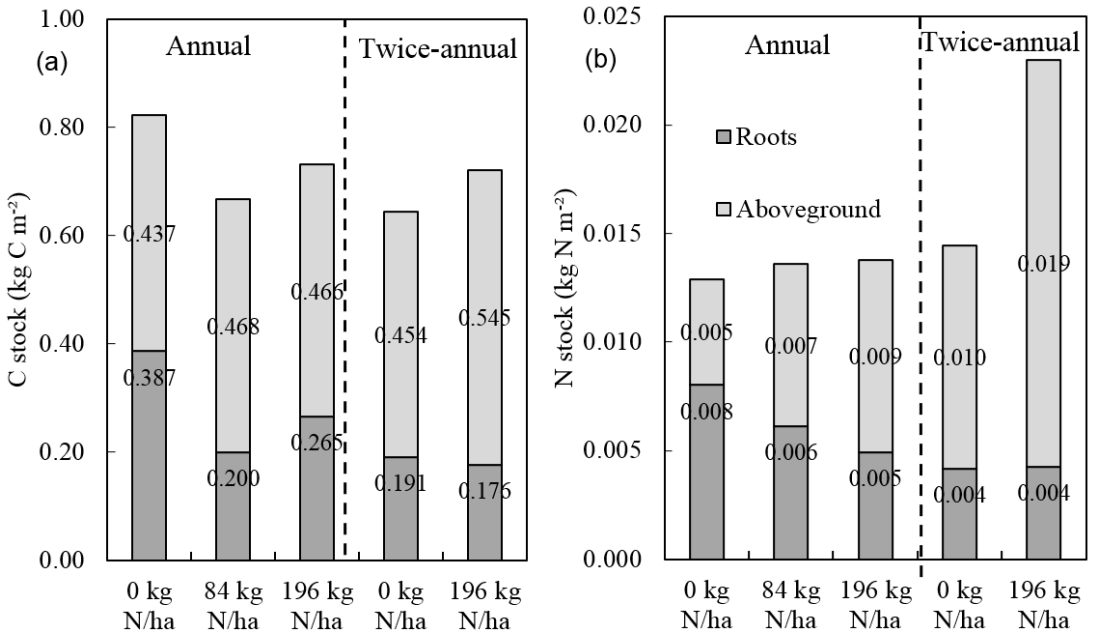

Figure 2.1 Carbon (a) and Nitrogen (b) stocks in total annual aboveground (twice annual: sum of 2011 July and November harvests) and belowground (root) biomass after 3 full growing seasons under different harvesting and fertilizer treatments. Standing root biomass C and N were measured in November.

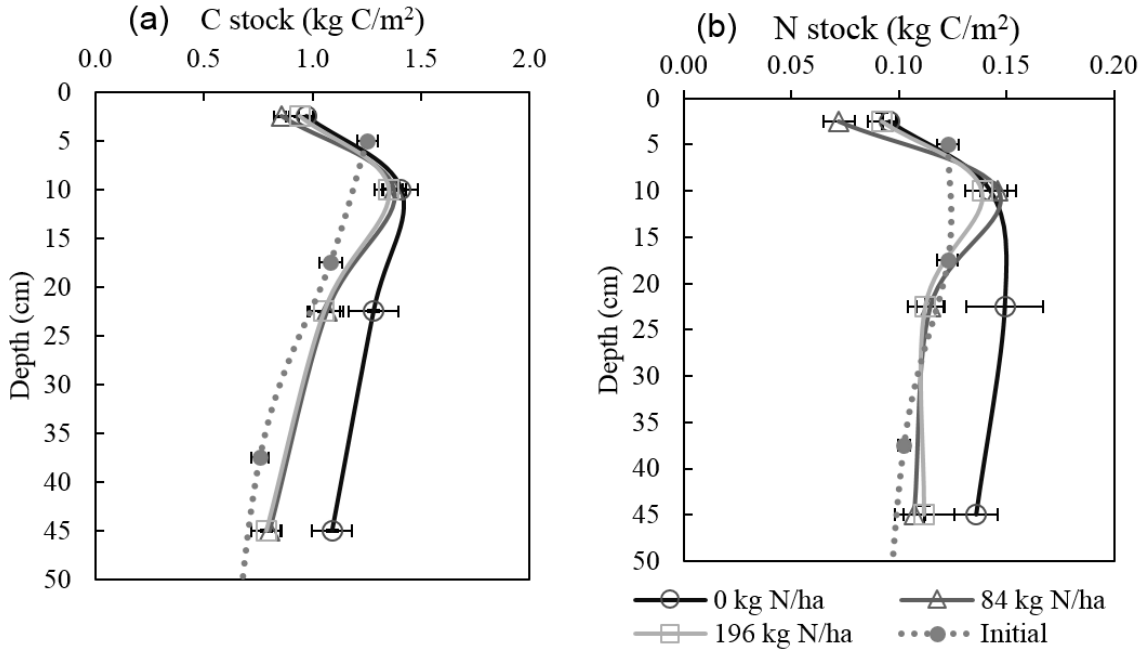

Figure 2.2 Soil C (a) and TN (b) stocks (roots, LF, DF) at different fertilization rates (open symbols) in Fall 2011. Initial soil C and TN stocks (closed symbols, n = 4) were sampled adjacent to the experimental plots at time of switchgrass establishment. Plotted values are averages across harvest treatments for 0 and 196 kg N/ha (n = 8) and the single annual harvest data for the 84 kg N/ha (n = 4) fertilization rate at each soil depth interval. Horizontal bars are standard error for replicated field plots.

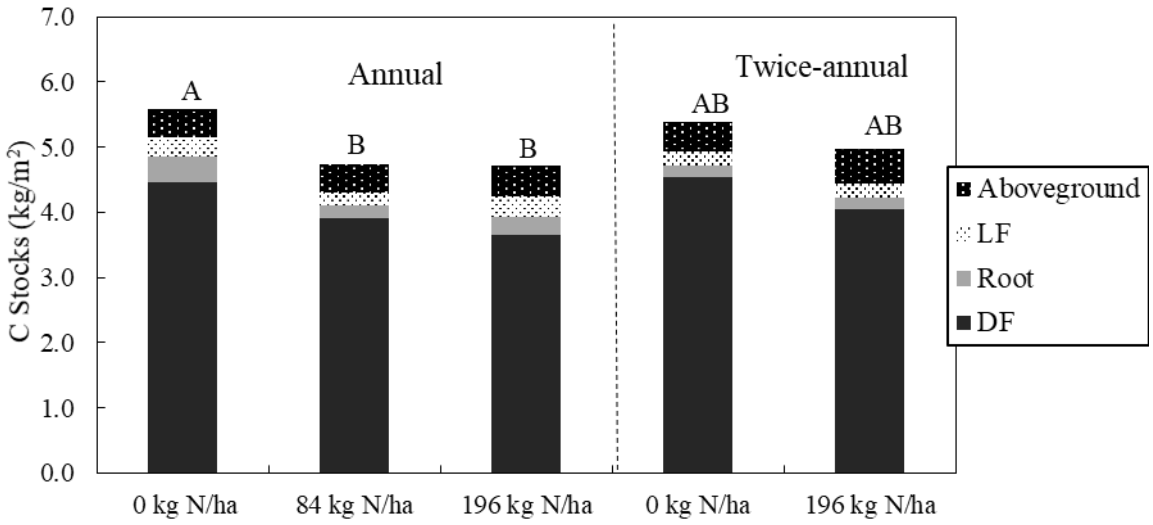

Figure 2.3 Total ecosystem C stocks for switchgrass cropping systems after the 3rd full growing season under fertilizer and harvest intensity treatments. Total Ecosystem C stock = (total aboveground biomass + root C stock + soil C stock (light + dense fraction). Upper case letters represent significant differences ($P < 0.10$) between Total Ecosystem C stocks.

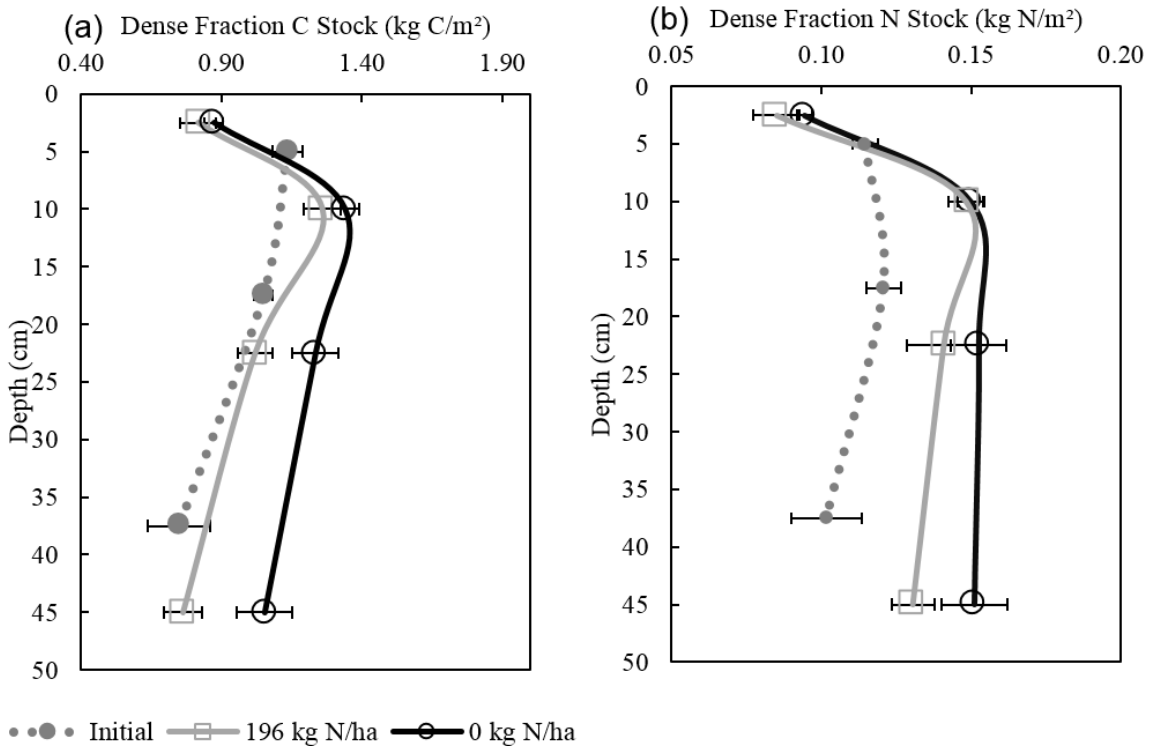

Figure 2.4 Averaged Dense Fraction C (a) and N (b) stocks by depth in 0 and 196 kg N/ha (open symbols) treatments sampled in November 2011 with harvest intensities of annual and bi-annual pooled by depth interval (n=8). Initial stocks (closed symbols, n=4) were sampled adjacent to the experimental plots at time of switchgrass establishment at different depth intervals. Horizontal bars are standard errors for replicated field plots.

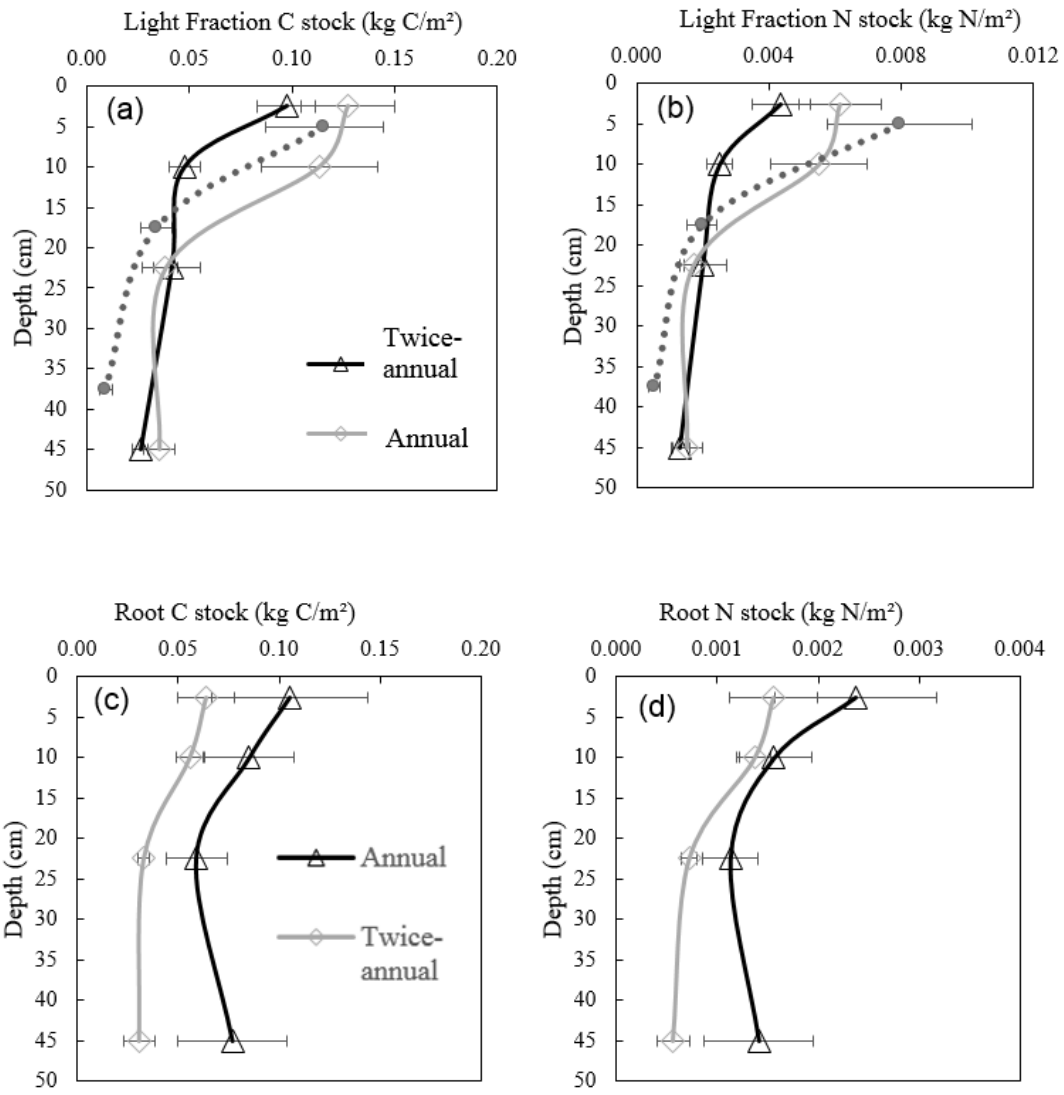

Figure 2.5: End of season distribution of Light Fraction (a, b) and Root (c, d) C and N stocks for annual and twice-annual harvest frequencies with initial LF stocks shown where measured (closed circles). Horizontal bars are standard error for replicated field plots (n=8, annual and twice-annual harvest; n=4, time zero).

CHAPTER THREE

Nutrition Facts for Soil Organic Matter: Agricultural Effects on Gibbs Free Energy and Macronutrient Inventories

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Abstract

The decompostion of organic matter in soils is an ensemble of biochemical reactions, and the energy yield to decomposer organisms can be represented as the Gibbs free energy, $\Delta G°$. $\Delta G°$ is the sum of energy invested in activation/oxidation (electron donating) and reduction (electron accepting) reactions. Previous studies have shown that the energy of oxidation reactions, $\Delta G°_{ox}$, exhibits a linear relationship with the oxidation state of carbon, C_{ox} (LaRowe and Van Cappellen 2011). We used solid-state ^{13}C nuclear magnetic resonance spectroscopy to estimate the C_{ox}, $\Delta G°_{ox}$, $\Delta G°$, and macronutirent inventories of root tissues and organic matter in the unprotected light fraction of soil (LF-SOM) in a switchgrass (*Pannicum virgatum*) system to quantify the effects of agricultural practices upon energy and nutritional content of labile substrates entering the soil food web. Field agricultural treatments included two nitrogen fertilizer application levels and two harvest rates—once and twice annually. Harvesting twice per year caused a >40 % decrease in the $\Delta G°$ of switchgrass roots. Combined with N-fertilizer, twice annual harvesting significantly reduced root tissue stability as measured by $\Delta G°_{ox}$ and the lignin / N ratio. The relationship between root energy and stability depended stongly on management. The senstivity (slope) of the energy-stability curve for roots predicted the

decomposability of the LF-SOM carbon pool. Soils from plots with a high energy-for-stability tradeoff in roots exhibitied LF-SOM pools with more extensive decomposition and lower $\Delta G°$. We interpret the relationships of energy, stability, and decomposition in the root-SOM continuum as measures of the extent to which agricultural management can affect the function of the soil food web. We propose SOM Nutrition Facts as a tool for data-based land management decisions and a quantitive means of communicating SOM quality.

Introduction

Carbon and nutrient cycling in soils are regulated by feedbacks between primary plants and heterotrophic organisms in the soil food web. The metabolic activities of soil heterotrophs determine the balance between mineralization and storage of carbon and nutrients by affecting the rate, extent, and mechanisms of organic matter decomposition (Hector et al. 2009, Gessner et al. 2010). The energy and nutrients provided by soil organic matter (SOM) affects the feeding behaviors, biodiversity and functioning of soil organisms across trophic levels (Laakso and Setälä 1999, Wardle 1999, Moore et al. 2004, Gessner et al. 2010, Wickings et al. 2012). Linkages between SOM composition and soil biological function are acknowledged in recent appeals for a shift in focus from quantifying soil carbon stocks toward process-level studies of SOM decomposition (Lehmann and Kleber 2015). Likewise, agricultural extension and education programs of the USDA NRCS emphasize the principle that soil health can best be promoted by practices that provide energy and nutrition to organisms of the soil food web (Romig et al. 1995, Doran et al. 1996, Doran and Zeiss 2000, Kibblewhite et al. 2008), a long held postulate of organic agriculture (Robertson and Harwood 2013). Although there is broad

recognition of the benefits (i.e. ecosystem services) derived from the flow of energy through the soil food web, there are few quantitative data on the Gibbs free energy (ΔG°) in soil organic matter and few methods for making these measurements.

We provide here a brief synthesis of theory on chemical and thermodynamic state variables for describing the composition and decomposition of organic matter in soil. We then present novel Gibbs free energy values and macronutrient data from a switchgrass field trial in Michigan, USA. Although thermodynamic and chemical variables are well-suited and widely used for modelling rates and processes, we demonstrate further their potential utility for assessing the impact of agricultural practices on SOM dynamics, and therefore the potential utility for thermodynamic measures for quickly assessing an important component of soil health. Data-based agricultural decision making often does not require complex models. We propose that the familiar nutrition facts labels of the US Food and Drug Administration (FDA) and the Food and Agriculture Organization (FAO Codex standard) serve the purpose of conveying Gibbs free energy and macronutrient data for quantitative assessment of agricultural practices relevant to soil health outcomes.

Thermodynamics of SOM Decomposition

Thermodynamic state variables, including the activation energy (E_a), enthalpy (ΔH), and potential energy (ΔE) for the oxidation of SOM, can be measured by calorimetry, during which, SOM is heated in the presence of oxygen while monitoring heat and CO_2 fluxes (Plante et al. 2009). Alternatively, apparent activation energy can be determined by measuring respiration rates in soil incubations conducted across a range of temperatures in the laboratory. E_a itself has utility in numerical models via the Arrhenius equation for predicting microbial respiration rate constants as a function of temperature

(Craine et al. 2010, Lefèvre et al. 2013, Yanni et al. 2017). Harvey et al. (2016) also showed that soil carbon mineralization rates were correlated to a return-on-investment value, which is a unitless quantity from the quotient of the potential energy of organic matter oxidation and the activation energy ($ROI = \Delta E / E_a$). Gibbs free energy (ΔG) is a similar measure of heterotrophic return-on-investment. Gibbs free energy is the quantity of energy (Joules, Calories, etc.) available for performing work in the ecosystem, and provides a measure of the thermodynamic driving force for SOM decomposition (LaRowe and Van Cappellen 2011, LaRowe et al. 2012, Keiluweit et al. 2016).

The use of Gibbs free energy is prevalent in marine C cycle modeling frameworks because it provides a convenient means of accounting for the important control of redox potential upon the availability of terminal electron acceptors (TEAs), and thereby, the metabolic energy yields and rate constants (Arndt et al. 2013). Natural variation in redox potential of soil and sediment arise from limitations in the availability of O_2 as an oxidant (electron acceptor) during microbial oxidation of SOM. In models of soil C cycling, resource limitations are a key feature (e.g., Parton et al. 1988, Schmidt et al. 2011, Bailey et al. 2018). However, soil C cycle models do not typically represent resource limitations in terms of redox potentials, nor are SOM decomposition reactions characterized as redox (electron transfer) reactions with predictable energy yields. Nevertheless, SOM decomposition in soils and subaqueous sediments occur by common processes and common organisms. Indeed, Sylvain and Wall (2011) emphasized that many soil microbiota are aquatic organisms, even in arid soil environments. The bound water layers on soil particle surfaces and the water filled inter- and intra-aggregate pores have highly restricted O_2 and NO_3 concentrations due to diffusion limited transport and microbial

consumption (Sexstone et al. 1985, Hojberg et al. 1994, Sierra and Renault 1996). Evidence of anaerobiosis in many upland soils is exhibited as redoximorphic features within aggregates and peds, and in the selective stabilization of lipid-like SOM, which is parsimonious with their thermodynamic stability in the absence of powerful oxidants (O_2, NO_3) (Keiluweit et al. 2016, Keiluweit et al. 2017).

Redox-based models of the organic matter decomposition are theoretically appropriate. However, there are challenges in model parameterization (Arndt et al. 2013), including accurate representations of the oxidation half reactions (electron donation by SOM) and reduction half reactions (by a terminal electron acceptor). It also remains challenging to characterize the spatial and temporal variability in the availability of electron donors acceptors (Hojberg et al. 1994, Blagodatsky and Smith 2012, Wanzek et al. 2018b), although recent work deploying spatial arrays of platinum electrodes shows promise (Wanzek et al. 2018a).

This paper uses the carbon oxidation state (C_{ox}) to simplify the representation of the electron donation (oxidation) reactions for SOM (Masiello et al. 2008). We apply the methods of LaRowe and Van Cappellen (2011) to relate carbon oxidation state to the Gibbs energy of oxidation, ΔG°_{ox}. Although we have not measured redox potentials at the field site, figure 3.2 quantifies the influence of the common terminal electron acceptors upon the Gibbs energy of reduction, ΔG°_{red}.

Carbon Oxidation State and Gibbs Energy of Oxidation

A common approach to evaluating the stability of SOM is to quantify the activation energy required for oxidation, E_a. An equivalent quantity is the Gibbs energy of oxidation, ΔG°_{ox} which can be estimated from the covalent bond types present in the electron-donating organic molecule. LaRowe and Van Capellen (2011) showed that the

Gibbs free energy of oxidation can be estimated from the organic carbon oxidation state (C_{ox}), using Equation 1. The C_{ox} is a unitless quantity that captures covalent bond type and abundance. The stochastic average C_{ox} value of SOM can be measured by spectroscopy such as ^{13}C NMR, or calculated from the elemental stoichiometry ($C_xH_yO_zN_w$) using Equation 2 (from Masiello et al. 2008). The relationship of SOM biomolecular composition to C_{ox} and $\Delta G°_{ox}$ are shown in Figure 3.1.

Equation 1 $$\Delta G°_{ox} \text{ (kJ mol}^{-1} \text{ C}^{-1}) = 60.3 - 28.5 \times C_{ox}$$

Equation 2 $$C_{ox} = [(2 \cdot z + 3 \cdot w - y) / x]$$

The $\Delta G°_{ox}$ values for organic matter are positive in magnitude (Figure 3.1), indicating that oxidation is endothermic—energy input is required to withdraw electrons from chemical bonds. Smaller $\Delta G°_{ox}$ values indicate less energy for electron withdrawal, and therefore a lower investment of energy by heterotrophic decomposers. Therefore, C_{ox} and $\Delta G°_{ox}$ values are describing the inherent average stability of the chemical bonds in SOM, which plays a role in the biochemical stability of SOM. The C_{ox} of plant residue and SOM are sensitive to ecological changes (Randerson et al. 2006) including plant species distribution (Gallagher et al. 2017), agricultural crop selection and nitrogen application (Gallagher et al. 2011, Gallagher et al. 2014), fire (Hockaday et al. 2009), and atmospheric CO_2 concentration (Hockaday et al. 2015). Here, we test the hypothesize that $\Delta G°_{ox}$ (kJ/mol C) has utility as state variable for comparing the inherent thermodynamic stability of SOM pools in switchgrass fields under differing agricultural management practices.

Redox and C_{ox} are Controls on Gibbs Free Energy of SOM Decomposition

The microbial decomposition of organic matter can be represented as electron transfer reactions comprising two halves—electron donating (oxidation) and electron accepting (reduction). The Gibbs free energy ($\Delta G°$) is the sum of Gibbs energy of the oxidation and reduction half reactions ($\Delta G° = \Delta G°_{ox} + \Delta G°_{red}$). The calculation of $\Delta G°$ requires knowledge of the terminal electron acceptors (TEAs) used during metabolism. The major TEAs used by soil biota are O_2, NO_3^-, Mn^{4+}, Fe^{3+} (as ferrihydrite, hematite, and goethite) and SO_4^{2-} (Stumm and Morgan 1996, LaRowe and Van Cappellen 2011). Figure 3.1 depicts the wide range of $\Delta G°_{red}$ values for various TEAs and illustrates the great importance of the redox potential of soil microenvironments to the energetics of soil C dynamics. The TEA determines the magnitude of the exergonic reduction reactions (ΔG^o_{red} in Figure 3.1). Therefore, TEA availability is an important factor in greenhouse gas composition and emission rates, as demonstrated by Hall and Silver (2015).

To determine the sensitivity of Gibbs free energy yields to the TEA availability and C_{ox}, we evaluated ΔG^o for all of the electron donor-acceptor pairs shown in Figure 3.1. The results are shown in Figure 3.2. The $\Delta G°$ Gibbs free energy of SOM mineralization can range from -3 to -44 kJ g^{-1} C^{-1} depending upon the donor-acceptor pair. In Figure 3.2, the $\Delta G^o - C_{ox}$ coordinate space occupied by SOM been shaded in gray, and several features of the TEA trend lines are noteworthy (Equation 3a – 3f). First, the slope of the ΔG^o vs. C_{ox} trendlines decreases as conditions become more reducing. Thus, C_{ox} of the organic matter exerts more thermodynamic control over decomposition under oxidizing conditions, and C_{ox} becomes less important under reducing conditions. The molecular composition of organic matter at the extremes of the redox gradient in

Figure 3.2 provides important insight to the phenomena of selective preservation and selective decomposition (Hatcher et al. 1983, Hedges et al. 2001, Zonneveld et al. 2010). Under the highly reducing conditions of sulfate reduction, the thermodynamic driving force is lowest (i.e. more positive values of ΔG^o) for the oxidation of lipids. Under oxidizing conditions where O_2, Mn^{4+}, and NO_3^- are dominant electron acceptors, peptide oxidation is less exergonic than lipids. These trends are broadly manifested in soils and sediments. Lipid-like polymethylenic C are predominant under highly reducing conditions (Gelinas et al. 2001, Keiluweit et al. 2016, Keiluweit et al. 2017), whereas intensively cultivated and aerated soils exhibit C/N values approaching those of protein ($C/N \leq 10$) (Rillig et al. 2007).

The second important feature of Figure 3.2 is the similar free energy yields from the use of O_2, Mn^{4+}, and NO_3^- as electron acceptors for SOM oxidation. The $\Delta G°$ of microbial metabolic processes using O_2, Mn^{4+}, and NO_3^- (Equations 3a – 3c) are indistinguishable within the natural variations in $\Delta G°$ caused by structural diversity (e.g. constitutional isomerism). This is consistent with the simultaneous production of CO_2 and NO_x by soils. Therefore, in oxidizing soil environments where strong electron acceptors (O_2, Mn^{4+}, NO_3^-) are prevalent, the $\Delta G°$ of SOM decomposition can be approximated using Equation 3a. We applied this approximation throughout this study because the soils under study (Alfisols) lack redoximorphic features are the iron-bearing minerals are predominantly sesquioxides, suggesting that Fe^{3+} reduction is not common. We hypothesize that Equations 3a – 3g represent state functions describing the thermodynamic driving force for decomposition. We tested this hypothesis by assessing

the extent to which ΔG° values calculated by Equation 3a predicted the extent of SOM decomposition.

Equation 3a $\Delta G^\circ_{O2} = 7.85 \times C_{ox} - 35.9$

Equation 3b $\Delta G^\circ_{Mn(4+)} = 7.63 \times C_{ox} - 35.0$

Equation 3c $\Delta G^\circ_{NO3(1-)} = 7.48 \times C_{ox} - 34.4$

Equation 3d $\Delta G^\circ_{Fe(3+)ferrihydrite} = 5.52 \times C_{ox} - 26.5$

Equation 3e $\Delta G^\circ_{Fe(3+)goethite} = 3.95 \times C_{ox} - 20.3$

Equation 3f $\Delta G^\circ_{Fe(3+)hematite} = 3.84 \times C_{ox} - 19.8$

Equation 3g $\Delta G^\circ_{SO4(2-)} = -0.38 \times C_{ox} - 2.98$

Methods

Switchgrasss field trial. The experiment was established at the W.K. Kellogg Biological Station (KBS) Long-term Ecological Research (LTER) site in southwest Michigan, USA (42° 249 N, 85° 249 W, elevation 288m), as part of the Great Lakes Bioenergy Research Center (GLRBC). Mean annual temperature at KBS is 10.1 °C; mean annual precipitation is 1027 mm (Robertson and Hamilton 2015). The soil is the Kalamazoo soil series, a mixed, mesic-Typic Hapudalf developed on glacial outwash with a fine and coarse-loamy texture comprising 85% sand and silt (Crum and Collins 1995). Cropping history included corn-soybean and alfalfa rotations under conventional tillage prior to the planting of an upland switchgrass variety, “Cave-in-Rock”, on July

11th, 2008 at a seeding rate of 7.84 kg/ha. The design was a randomized split-plot arrangement with one fertilization rate split between two harvest treatments.

Soil sampling. Soil samples were collected in November 2011, 3 years after switchgrass establishment. Two soil cores were taken from each of 4 replicated plots for arrangements of 0 and 196kg N/ha fertilization rate (combination of granular 46% urea and liquid 28% urea ammonium nitrate spray) and annual and twice-annual harvest frequencies. Each soil core was separated into four depth intervals (0-5, 5-15, 15-30, 30-60 cm), the material from each depth was independently homogenized, and oven-dried to constant mass at 50°C to determine soil dry weight for bulk density calculations.

Soil processing. In processing the soils we isolated root biomass and labile particulate organic matter because these are the source of most soil greenhouse gases emissions in agricultural soils (Trumbore 2000, 2006), are most sensitive to cultural practices on annual timescales, and most directly influence the diversity, and function of the soil food web (Brussaard 1997, Kuyper 2011, Griffiths and Philippot 2013). Coarse roots were picked from the soil by hand and fine roots were recovered while passing the soil through a 2mm sieve. Density separations were performed with 20 grams of dried, sieved soil placed in a 50mL centrifuge tube with approximately 30mL of sodium iodide (NaI) solution (density = 1.8g cm^3). The solution was hand shaken and centrifuged at 82 g for 20 minutes. The light fraction of soil organic matter (LF-SOM) was decanted with the supernatant liquid into vacuum filter funnel fitted with a glass fiber filter (Whatman GF/F). The LF-SOM on the filter was rinsed copiously with deionized water to remove residual NaI and oven-dried at 50°C for 24 hours before being transferred to a glass vial

for storage until further analysis. For more information regarding experimental design, see Valdez et al. (2017).

Elemental analyses. Analyses of LF-SOM was restricted to depth intervals to 0-5 and 5-15cm, but roots were analyzed at all depth intervals (0-5, 5-15, 15-30, 30-60cm). Root and LF-SOM samples were massed and combusted in tin capsules using a Thermo Scientific Flash EA 1112 Series NC Soil Analyzer to obtain total organic C and total N concentrations. Four samples were tested for each treatment of root and LF OM.

Spectrochemical analysis. We characterized the molecular composition of root and LF-SOM samples by solid-state ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy. The ^{13}C NMR analyses were conducted on a standard bore 300 MHz Bruker Avance III spectrometer equipped with a 4 mm magic angle-spinning (MAS) probe operating at ^{13}C resonance frequency of 75 MHz and spin rate of 12kHz. The pulse angles and Hartman-Hahn matching conditions for ^{1}H-^{13}C polarization transfer were optimized using glycine as an external standard. We acquired the cross-polarization data with a 90-degree proton pulse length of 2.6 μs and variable-amplitude contact pulse of 2 ms with a 3 second recycle delay. The number of scans acquired varied by sample and signal was acquired until a minimum signal-to-noise ratio of 10 was obtained. The signal region selected for signal-to-noise ratio determination was the 110 – 165 ppm (aromatic and phenolic) which was typically the region of lowest signal intensity. One-thousand twenty-four data points were acquired during each scan. Signal processing included zero-filling to 16,384 data points and Fourier transformation with 40 Hz line broadening, manual phasing, and application of a linear baseline. We defined the functional groups by integrating signal magnitude across seven chemical shift regions

associated with the following C functional groups: (1) alkyl C (0-45ppm), (2) N-alkyl and Methoxyl C (45-60ppm), (3) O-alkyl C (60-95ppm), (4) Di-O-alkyl C (95-110ppm), (5) Aryl C (110-145ppm), (6) O-Aryl C (145-165ppm), (7) Amide and Carboxyl C (165-215ppm).

Molecular mixing model. We calculated the relative proportion of organic biomolecules (lignin, protein, carbohydrate, and lipids) from the ^{13}C NMR spectra by applying the molecular mixing model (Baldock et al. 2004, Nelson and Baldock 2005) with a modification of the lignin composition to 2:3 syringyl to guaiacyl monomer ratio that best describes switchgrass (Yan et al. 2010). The molecular mixing model predictions were further constrained by the molar C/N ratios measured by combustion elemental analysis. The model-based estimate of each biomolecule's molar proportions were converted to a biochemical C stock (i.e. root Carbohydrate C stock (g C/m^2)) by multiplying the biochemical mole fraction by the C stocks, previously determined by Valdez et al. (2017). The mean carbon oxidation state (C_{ox}) of in root and LF-SOM was calculated from the measured C/N and the mixing model-based estimates of H/C and O/C, according to Eqn. 2 (Baldock et al. 2004, Hockaday et al. 2009).

Statistical Analysis

To test for treatment effects on proportions and stocks of biochemicals and $\Delta G°$, and stability indices, we used a 3-way analysis of variance (ANOVA) General Linear Model Univariate. The fixed factors in this analysis were fertilization rate, harvest frequency, and depth intervals. Homoscedasticity of data were checked by the Levene's test prior to ANOVA. The p-value < 0.05 was chosen as the significance level in testing

for differences between experimental treatments. Analyses were performed with IBM SPSS statistics 21.0 software (SPSS Inc., Chicago, IL).

Results and Discussion

The C_{ox} and ΔG^{o}_{ox} as Predictors of Stability

We postulated that the C_{ox} parameter and $\Delta G°_{ox}$ (kJ/mol C) are useful state variables for quantifying the inherent biochemical stability of C pools. We use the term inherent stability to refer to persistence in the absence of mineral association or other physico-chemical protection. We tested this idea by measuring C_{ox} and G^{o}_{ox} for comparison to established indices of stability (lignin / N) and decomposition (alkyl C / O-alkyl C) in soils under switchgrass trials at KBS GLBRC where nitrogen fertilizer and harvesting treatments have been shown to affect the size and stability of soil C pools (Valdez et al., 2017). Our hypothesis predicts two specific outcomes. First, the $\Delta G°_{ox}$ of switchgrass root biomass should be positively correlated to the lignin / N ratio index of stability. Second, plots having less stable root biomass (lower $\Delta G°_{ox}$ values) should decompose more extensively, leaving behind a detrital residue of LF-SOM that is more decomposed and retains only the most stable constituents (high G^{o}_{ox}). Therefore, the $\Delta G°_{ox}$ of LF-SOM fraction should exhibit a positive correlation with the decomposition index (alkyl C/ O-alkyl C).

Consistent with the first prediction, the $\Delta G°_{ox}$ of roots were positively correlated to the lignin/N ratio stability index ($R^2 = 0.15$, $P = 0.02$, Fig. S3.1). The positive correlation between the two proxies indicates that reduced forms of C with high $\Delta G°_{ox}$ (kJ/mol C) values require more energy to initiate decomposition (i.e. greater activation energy), therefore, making them more stable on short timescales. The second prediction

of our hypothesis was supported by a strong positive correlation of $\Delta G°_{ox}$ values with the alkyl C / O-alkyl C ratios of the LF-SOM ($R^2 = 0.57$, $P = 6.3 \times 10^{-7}$, Figure 3.3a). We interpret this trend as the progressive decomposition of labile constituents (low $\Delta G°_{ox}$) leading to a progressive increase in the stability (increasing $\Delta G°_{ox}$) of the residual LF-SOM.

Agricultural management practices are driving the variation in switchgrass root stability and LF-SOM decomposition. Figure 3.3b shows that plots harvested twice-annually had LF-SOM with significantly higher $\Delta G°_{ox}$ than annually harvested plots. A plausible explanation of the trends in Figure 3.3 is that twice-annual harvesting caused more extensive decomposition due to the timing of litter production. The first harvest occurred in July when soil temperatures were highest. The decomposition of litter in the soil during the intervening months between the first and second harvest (July to November) likely allowed for more extensive decomposition of the LF-SOM than in plots harvested exclusively in November when soil temperatures were colder. This may explain why Figure 3.3 indicates that the LF-SOM in annually harvested plots was less stable yet less decomposed (lower $\Delta G°_{ox}$ and lower alkyl C / O-alkyl C). This explanation agrees with soil incubation experiments in which the apparent activation energy (E_a) of SOM decomposition increases with incubation time (i.e. extent of SOM decomposition) (Craine et al. 2010).

The ΔG°ox as a proxy for activation energy. We suggest that $\Delta G°_{ox}$ has promise as a predictor of SOM decomposition, based upon the relationships we observed between $\Delta G°_{ox}$ and the chemical indices of stability (Figure 3.3, S3.1) as well as the sensitivity of $\Delta G°_{ox}$ to agricultural practices (Figure 3.3b). These relationships are not surprising

because $\Delta G°_{ox}$ is theoretically equivalent to the activation energy (E_a). In the context of SOM, the E_a represents the energetic barrier to SOM mineralization. Several studies have reported values for a similar parameter known as the apparent E_a obtained by measuring microbial respiration rates during laboratory incubations of a soil at multiple different temperatures (e.g., Craine et al. 2010, Lefèvre et al. 2013). The E_a and $\Delta G°_{ox}$ values for SOM are useful in the context of modeling C cycle climate feedbacks because the Arrhenius expression (Eqn 4a-b) can be used to estimate the temperature-dependence of the decomposition rate constant (k) and the mean residence time of SOM (MRT = 1 / k). In Eqn 4a-b, A is an empirical constant, R is the gas constant (8.314 J / mol · K) and T is temperature in Kelvin. The SOM decomposition rate (respiration rate) can then be calculated as the product of the rate constant and the SOM concentration.

Equation 4a $$k = A \times e^{-E_a/RT}$$

Equation 4b $$k = A \times e^{-\Delta G^o_{ox}/RT}$$

The root and LF-SOM collected in this study are unprotected by mineral associations, making them biologically accessible on timescales similar to those of laboratory incubation experiments. Therefore, $\Delta G°_{ox}$ values reported here should be comparable to apparent E_a values from the literature. Our $\Delta G°_{ox}$ values ranged from 63 to 68 kJ / mol C, which is consistent with grassland soils studied by Lefèvre et al. (2013) where the initial 50 mg CO_2-C respired had E_a values ranging from 49 to 68 kJ / mol C. Craine et al. (2010) measured apparent E_a of soils from 28 sites across North-Central America and found values ranging from 53 to 139 kJ / mol during the initial 15 days of incubation. However, Lefèvre et al. (2013) surmised that high E_a values (>>68 kJ / mol) were inflated by water limitations under the relatively dry incubation conditions of 35%

water holding capacity. Even so, apparent E_a values obtained by incubating bulk soils with mineral-associated SOM are expected to exhibit a broader range of than $\Delta G°_{ox}$ values from isolated LF-SOM fractions. The $\Delta G°_{ox}$ of LF-SOM is inherent to the chemical composition of the SOM and the range of values is constrained by the chemical bonding patterns that control the carbon oxidation state. Therefore, we suggest that the $\Delta G°_{ox}$ value might best be considered an "inherent" $E_{a,}$ in contrast to apparent E_a values, which reflect other ecosystem variables such as microbial resource availability including physical-chemical protection or occlusion of SOM.

The Arrhenius constants (k) and SOM decomposition rates calculated from apparent E_a (Eqn 4a) should differ from those calculated from ΔG^o_{ox} (Eqn 4b). The differences likely provide a meaningful measurement of the extent to which microbial resource limitations can attenuate or accelerate the temperature sensitivity of soil respiration. Thus, direct comparison of the apparent rate versus inherent rates may provide mechanistic insight to the soil food web and soil C cycle feedbacks to warming.

Gibbs Free Energy of the Root System

The aboveground switchgrass biomass was mechanically harvested, with no residue addition to the soil, leaving the live roots and root detritus as the main substrates in the soil food web. Root-based food chains and detritus-based food chains consist of different communities of organisms (Glavatska et al. 2017). Therefore, we separated roots from detrital (LF-) SOM and independently determined their mass, carbon, and energy budgets.

Root energy budgets are a function of management. We used the product of root C stocks and ΔG^o (from Eqn 3a) to calculate Gibbs free energy budget for the standing

crop of roots. The use of Eqn 3a assumes that the dominant metabolic processes are those using the most energetic electron acceptors (O_2, NO_2, and MnO_2). Figure 3.4A shows the effects of harvesting rate and N fertilizer upon the distribution of root-derived ΔG^o in the soil profile. The combination of treatments (N fertilizer x twice-annual harvesting) caused a 55% decrease in the Gibbs free energy of the root system, compared to the control, with the greatest losses occurring at depths >15cm. We note that switchgrass has bunch-type growth habit, and we did not collect soil cores directly through the crown of the plant where root biomass is greatest. Therefore, the root energy budgets in Figure 3.4A are conservative estimates. The lower root ΔG^o values in twice-annually harvested plots can be attributed to the allocation of carbon to regrowth of aboveground biomass following the midseason harvest. Indeed, root biomass C was 45% lower in plots harvested twice annually (Table S3.4). The greatest root biomass C and ΔG^o occurred in the unfertilized control plot. A review by Iversen (2010) demonstrated that nitrogen limitations caused redistribution of roots and root-associated fungal mycorrhizas deeper within the soil profile. Although the species included in the review were arboreal, we speculate that our observation of "deeper" root energy distributions in the unfertilized switchgrass plots may represent a similar physiological to response N-limitation.

Energy-stability relationships in root systems. Changes in root tissue stability also contributed to the management effects on root ΔG^o values in Figure 3.4A. The C / N and lignin / N ratios of roots were significantly higher in the annually harvested plots ($p = 0.038$) and at depths >15cm ($p = 0.025$), suggesting that annual harvesting may increase the stability of roots. Additional insight to the energy-stability relationship was gained by

interrogating the relationship of ΔG^o with the ratio lignin / N. Figure 3.4B shows the cumulative ΔG^o of root tissues and lignin / N for each soil depth interval. The lignin / N of the root tissues generally increased with depth, from values of 25 in the upper 5 cm to values approaching 70 for the 30 – 60 cm soil depth interval. The three-fold increase of the lignin / N ratio was caused by both increasing lignification and declining N concentration in the root tissue with depth (Fig S3.1, S3.2).

The ΔG^o of the root systems decreases with increasing root tissue stability. The four curves in Figure 3.4B represent energy-stability relationships for switchgrass root biomass in the four agricultural treatments. The energy-stability curves had the general linearized form: $\ln(-\Delta G^o) = -m \cdot (\text{lignin / N}) + b$. Regression models are listed in Table S3.2 with figures of merit. The slope values, $-m$, are a measure of the tradeoff between root energy input to the soil food web and the stability of root tissues for the purposes of soil C storage. The slope of the energy-stability curves became increasingly negative with increasing management intensity, having values -0.03 in the control plot and -0.15 in plots that received nitrogen fertilizer and twice-annual harvesting (Table S3.2). Therefore, when considering management practices as a means by which to affect soil C sequestration, Figure 3.4B gives several insights. First, Gibbs free energy in the soil food web declines exponentially with increasing root C stability. Second, root C pools can be "managed" through cultural practices to optimize for stability or energy provision.

Does Gibbs Free Energy Budget of SOM Reflect Trophic Status?

Root necromass is the dominant source of detrital SOM inputs to agricultural grasslands and serves as a biogeochemical interface between primary producers and heterotrophic organisms (Jackson et al. 1997, Rasse et al. 2005, Russell et al. 2009).

Therefore, our second hypothesis posited that energy-stability relationships identified in root biomass (Figure 3.4b) should govern the Gibbs free energy of the detrital (LF-) SOM pool. Specifically, in plots where root systems had higher oxidation energy (ΔG^o_{ox}) and high biochemical stability (Lignin / N), the LF-SOM pool should have more Gibbs free energy and exhibit less decomposition. Applying the hypothesis to the data in Figure 3.4B leads to the prediction that the root inputs in annually-harvested plots (low slope in the energy-stability curve*)* should sustain an LF-SOM pool with more negative ΔG^o and lower alkly C / O-alkyl C ratio than plots harvested twice annually (high slope in the energy-stability). To test this hypothesis, we used Eqn 3a to estimate ΔG^o and then determined the free energy budget (kJ m^{-2}) of the LF-SOM pool by taking the product of ΔG^o (kJ g^{-1} C^{-1}) and the C stock (g C m^{-2}). The values are given in Table 3.1. In support of our hypothesis, the ΔG^o budgets of LF-SOM pool in the annually harvested plots were 50% and 75% higher ($P \leq 0.022$) than in plots harvested twice-annually (Figure 3.5, Table 3.1). Furthermore, the decomposition state variable (alkyl C / O-alkyl C) indicates that the LF-SOM is less extensively decomposed in the annual harvest than in the twice-annual harvest treatment ($P = 0.006$), and also less decomposed in the in the surface 0-5 cm soil than the 5-15 cm soil ($P = 0.016$).

Agricultural management of energy in the soil food web. The test of the second hypothesis is presented schematically in Figure 3.5. Figure 3.5 depicts the principle of trophic transfer, which states that energy is progressively lost with successive trophic interactions (metabolic reactions). When applied to SOM decomposition, the principle of trophic transfer predicts that that free energy budget (ΔG^o) should progressively diminish as organic matter undergoes progressive decomposition by the heterotrophic community.

In Figure 3.5 we used the alkyl C / O-alkyl C as the reaction coordinate for SOM decomposition reactions. Our interpretation of the root energy-stability trends (Figure 3.4B) together with the SOM energy-decomposition trend (Figure 3.5) is that the chemical composition of LF-SOM was controlled by primary production (i.e. root inputs) when harvested annually and became increasingly controlled by decomposers with greater management intensity.

The energy and nutritional quality of LF-SOM are determinants of the diversity, abundance, viability, and stability of the community of soil biota (Moore et al. 2004). If Gibbs free energy is a currency of soil biological function, then Figure 3.5 is describing the impact of crop management upon soil biological function (i.e. soil health). The regression model shown in Figure 3.5 [$\Delta G^o = 4117 * (Alkyl\ C/O\ Alkyl\ C) - 2117$] is useful as a simple a predictor of biological function. The equation predicts that decomposition would cease when organisms depending upon SOM become energetically limited (i.e. ΔG^o = 0 kJ m^{-2}). The ΔG^o = 0 kJ m^{-2} when the alkyl C / O-alkyl C reaches a value of 0.51. Fresh switchgrass root biomass had values ranging 0.12 to 0.17, thus, a value of 0.51 indicates extensive decomposition. To provide context to the unitless alkyl C / O-alkyl C index of decomposition, we compare our values for switchgrass to those of another C4 grass litter—corn. Baldock et al. (2004, Table 3.1) presented the alkyl C / O-alkyl C values for corn litter in mesh litter bags buried at 15 cm depth in a similar climate and soil type for a period of 2 years. The decomposition followed the expression [(alkyl C / O-alkyl C) = (0.000494 × t_{days}) + 0.105; R^2 = 0.875; P = 0.019)]. Substituting this expression into the regression model from Figure 3.5 and solving for the turnover time (where ΔG^o = 0 kJ m^{-2}) corresponds to approximately 840 days or 2.2 years. A turnover

time of 2.2 yr for the LF-SOM is highly consistent with the turnover times for grassland soil particulate OM fractions (f-POM density < 1.6 $g.cm^3$) which range from 1 to 22 years (von Lützow et al. 2007). These simple estimates demonstrate that LF-SOM energy budget (Figure 3.5) upon which the soil food web depends, is dynamic on annual timescales and sensitive to cultural practices.

Combining Energy Budgets and Biochemical Inventories: Nutrition Facts for Roots and SOM

Nutrition is the study of the relationships between diet and health. The health of a soil is defined as the ability of the soil and soil organisms to support plant and animal productivity (Doran and Zeiss 2000). The diet of soil organisms and the health of the soil ecosystem are linked by the nutritional quality of soil substrates (roots and SOM). Across trophic levels from microbes to predators, soil organisms have specific energy and macronutrient requirements (intake targets) that affect their development, physiology, behavior, and reproductive success (Schoener 1971, Raubenheimer et al. 2009, Chen et al. 2014). The macronutrient and stoichiometric targets of soil organisms place boundary conditions upon SOM decomposition rates, soil gas emissions, and nutrient availability to plants (Schoener 1971, Raubenheimer et al. 2009, Chen et al. 2014, Meyer et al. 2018, Zhu et al. 2018), and therefore biochemical inventories should be considered together with the Gibbs free energy as possible descriptors of these processes. Here we focus on the utility of energy budgets and biochemical inventories in the assessment of agricultural management practices. We propose that the energy budget and macronutrient inventory as presented on the "Nutrition Facts" label of the FDA might be easily adopted as a soil health assessment tool because of its simplicity and familiarity.

Biochemical inventories – measuring macronutrients in the soil food web.

Biochemical inventories are presented in grams C per biochemical class per square meter to a specified soil depth. Table 3.1 is the nutrition facts label for LF-SOM and roots in the upper 15cm soil depth. The roots and LF-SOM have nearly identical C inventory and ΔG^o budget in the control plots (unfertilized and harvested annually). However, the compositional differences between roots and LF-SOM (Table S3.3) result in substantially different biochemical inventories. Figure 3.6 shows that responses of the soil biochemical inventory to fertilizer and harvest treatments differed in both magnitude and direction. Figure 3.6A clearly shows that twice annual harvesting significant and non-uniform decrease of macronutrients in LF-SOM, while N-fertilization led to differential macronutrient gains (Figure 3.6B). Differential changes in macronutrient inventory (Figure 3.6) require changing macronutrient ratios. For example, the protein / lipid ratio of LF-SOM ranged from 1.9 to 4.0 as a result of combined fertilizer and harvesting effect, while the protein / carbohydrate decreased from 4.3 to 2.7 due to N fertilization.

The significance of macronutrient ratios (i.e. nutrition facts) to insects was recently reviewed by Raubenheimer and Simpson, (2018). In some insects, the protein / lipid ratio of their diet was a determinant of egg production, and therefore affected the fecundity of the species. Additionally, the carbohydrate / protein ratio of the insect diet affected the tradeoff between fecundity and lifespan. Across trophic levels (herbivores and predators), insects demonstrate the ability to learn and modify macronutrient-specific feeding behaviors to mitigate nutrient limitations (Raubenheimer and Simpson 2018). Therefore, the macronutrient distribution in dietary substrates (root, detritus, etc) may act as bottom-up controls on the fitness and feeding behavior of insect consumers. In turn,

the feeding behaviors of insects are a top-down control on the nutritional quality of detrital SOM, thereby affecting the population dynamics of organisms at lower trophic levels in the soil food web.

Microbial community responses to macronutrient availability are generally considered to occur at the community or rhizosphere level. For example, the microbial 'priming effect' that occurs with the addition of carbohydrates to soil is increasingly interpreted as a community response to nitrogen- and/or phosphorous-limitation (Murphy et al. 2015, Meyer et al. 2018, Zhu et al. 2018). Under positive priming, the addition of labile carbohydrate provides the activation energy necessary for N and P mineralization from the pre-existing SOM – a theory known as microbial mining (Craine et al. 2007, Kuzyakov 2010).

Microbial mining theory. The microbial mining theory predicts that N fertilizer application to an N-limited microbial community should slow the rate of microbial mining (extracellular enzyme production), allowing labile substrates like carbohydrates to accumulate in the SOM. The nutrition facts (Table 3.1) are useful for testing this prediction of microbial mining theory. Figure 6B shows the effect of N fertilizer on the macronutrients in LF-SOM. As mining theory predicts, the carbohydrate inventory of the LF-SOM was higher (23 % to 28 %) in plots receiving N-fertilizer. Protein inventories were also 40 % to 98 % higher in plots receiving N-fertilizer (Figure 3.6B, Table 3.1). The carbohydrate and protein inventories of root biomass showed no increase and typically decreased with N-fertilizer (Table 3.1). Therefore, an increased supply of carbohydrate and protein from roots to the LF-SOM is unlikely. The observed carbohydrate and protein accruals in LF-OM are more consistent with an increase in

microbial peptide nitrogen (i.e. protein) and passivation of microbial mining due to N-fertilizer addition.

The absence of root protein accrual for plots receiving N fertilizer (Table 3.1, Table S3.4, and Figure 3.6B) suggests that belowground primary production was not limited by N-availability in unfertilized plots. This is surprising because agronomic studies of switchgrass recommend N fertilizer applications of 78 kg N ha^{-1} for Michigan (Withers 2009). However, episodic N-fixation by diazotroph communities in the rhizosphere soil and roots of switchgrass was recently discovered at this field site (Roley et al. 2019). Roley and colleagues hypothesized that the episodic N-fixation is sufficient to meet switchgrass N-deficits on annual timescales. The absence of root protein accrual with N fertilizer is consistent with their hypothesis.

Conclusions

This paper made three new contributions. First, we summarize the theory and methods for estimating Gibbs oxidation energy, ΔG^o_{ox}, and Gibbs free energy, ΔG^o, of plant biomass and soil carbon pools. Second, we quantified the impact of specific agricultural practices upon ΔG^o_{ox} of switchgrass root systems showed that intensive practices, such as twice-annual harvesting diminished the ΔG^o of SOM pool. We identified two lines of evidence in support of the hypothesis that ΔG^o_{ox}, is a predictor of organic matter stability in soil. The ΔG^o_{ox} of root tissues were positively correlated with the lignin / N stability proxy. Additionally, the ΔG^o_{ox} of root tissues predicted the extent of decomposition of LF-SOM pool, as measured by the mass of the C pool and the alkyl C / O-alkyl C index. Energy-for-stability tradeoffs in the root system were sensitive to cultural practice, and consistent with declining Gibbs free energy budget of the LF-SOM.

We hypothesize that correspondence between the energy-stability tradeoff in roots and the Gibbs free energy budget of the LF-SOM is a consequence of trophic interactions in the soil food web. Therefore, the third outcome of the study is the development of the nutrition facts label for soil organic matter. Soil nutrition facts labelling conveys chemical and thermodynamic data on energy and nutrition of food web substrates in terms that are familiar to practitioners. Future work is necessary to test the relationships of ΔG^o and nutrition facts to biological function.

Implications and Future Directions

This study calculated the quantity and chemical composition of root and light-fraction organic matter to interpret inherent energy and stability relationships that occurred because of land management practices and translated this information into practical models for data-based land managers to consider within the greater soil health and food web structure. We observed a positive correlation between known state variables (lignin / N and Alkyl C / O-Alkyl C) and C_{ox} and ΔG_{ox} values as decomposition/ stability indices. inherent stability of soil C pools. The comparison of ΔG_{ox} to activation energy (E_a) showed good results to utilize the former as an "inherent" activation energy for use outside of ecosystem contributions to organic matter decomposition. We noticed significant changes to the energy budget as a function of standing root C stock and root tissue stability and explain the potential trade-off between stable soil C pools and energy driving force for the provisions of ecosystem services rendered by soil biota.

This study observed a positive relationship for energy and stability state variables between management practices for the transition of root to LF-SOM, where high energy less decomposed roots resulted in similar LF-SOM pools. This energy-stability trend was

further investigated with the energy decomposition trend to uncover a pattern of increasing management practices changing the control of LF-SOM composition from primary producers to decomposers. This study also leads to the creation of biochemical inventories and the production of a nutrition data label for soil C pools in which major bio-macromolecules (Carbohydrates, Proteins, Lignin, Lipids) and total energy were calculated with changes to agricultural treatments to reduce complexity in data-based decision making about soil health and sustainability.

The long-term goal of agricultural management in the face of climate change and increased demand should be the sustainable production of food, fuel, and fiber. In our attempt to answer questions regarding the quantitative relationships between energy, stability, and nutrition within soil C pools under agricultural practices we suggest the quantity of active SOM inputs is paramount for short term C and energy flows as a direct measure of soil health (Dou et al., 2009; Culman et al., 2013). Simplistic models for characterization and measurement multiple variables within SOC pools facilitates the transfer of data-led science to standardized information for practical assessments to ensure sustainability across management practices, climates, and ecosystems. We propose that the nutritional quality and energy availability from SOM are systematically linked through chemical bonding and thermodynamics, and therefore could be used to predict from first principles biogeochemical outcomes such as soil greenhouse gas emissions (Bolinder et al. 1999; Herrick, 2000; Smith et al., 2015). Under the energy-stability relationship, future studies may consider the temporal and spatial distribution of roots and their connection to the rhizophere (changes in emissions, C and N mineralization rates), as well as further investigations on the role of stable energy sources and higher energetic

sources within soils under major climate (drought, fire, floods, etc.) disturbances. This study aims to progress the accessibility and precision with new calculations and tools to recognize predict changes in soil C systems under various conditions to build resilience and sustainability.

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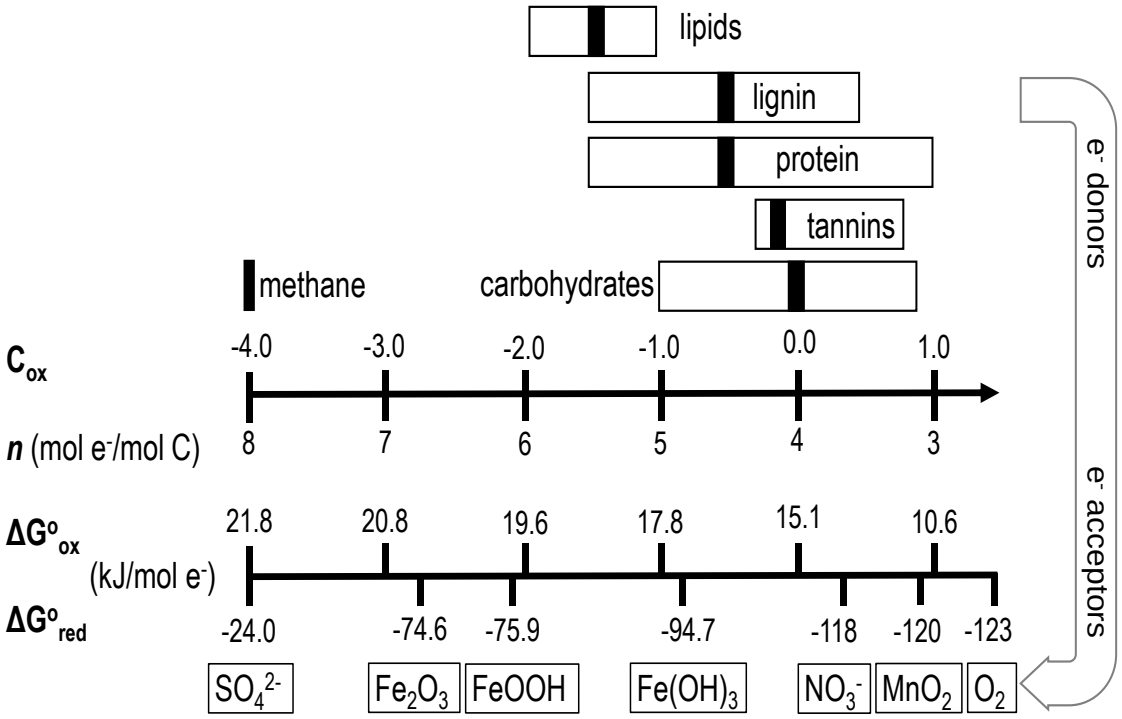

Figure 3.1. The carbon oxidation state, C_{ox}, of biochemicals in SOM have a linear relation to the Gibbs free energy of oxidation, $\Delta G°_{ox}$. The $\Delta G°_{ox}$ values were derived using the expression [$\Delta G°_{ox} = (60.3 - 28.5 \cdot C_{ox})/n$] (LaRowe and Van Cappellen, 2011), where n is moles of electrons liberated per mole C ($n = -C_{ox} +4$). The free energy of reduction, $\Delta G°_{red}$, values for common terminal electron acceptors were taken from Arndt et al. (2013). The free energy yield for a donor-acceptor pair were calculated in units of kJ/mol e^- using the expression [$\Delta G° = \Delta G°_{red} + \Delta G°_{ox}$] or in units of kJ/mol C as [$\Delta G° = n \cdot (\Delta G°_{red} + \Delta G°_{ox})$]. All ΔG values are for standard temperature and pressure. The thermodynamic driving force for SOM oxidation increases to the right of the diagram.

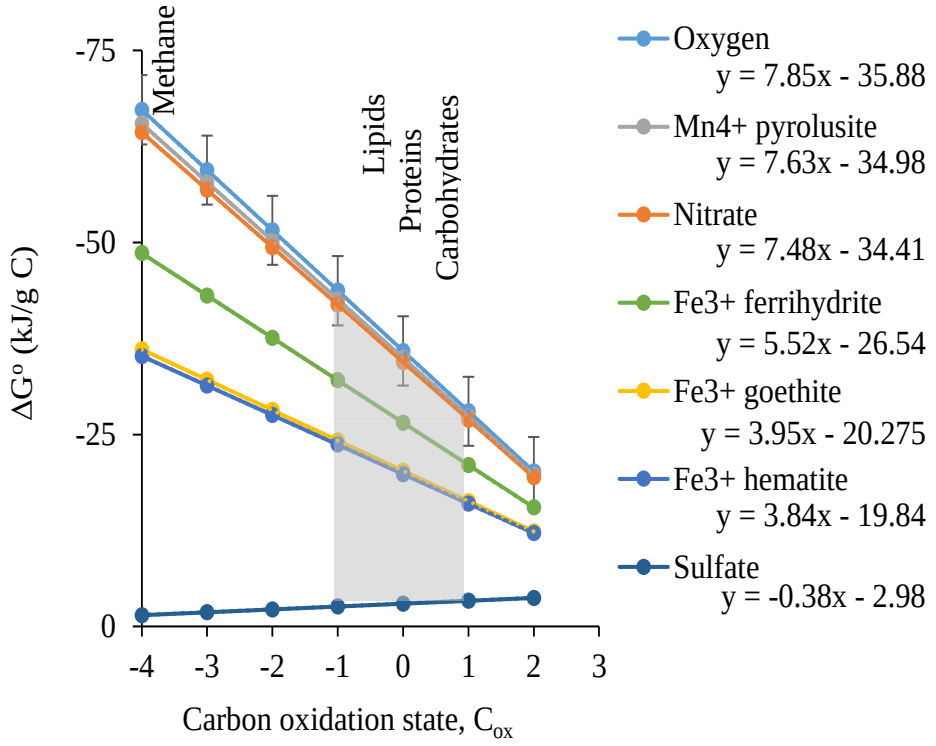

Figure 3.2. The thermodynamic cascade of terminal electron acceptors (TEAs) as a function of carbon oxidation state. The shaded region is the range of typical ΔG^o values for soil organic matter (-3 to -44 kJ/g C). Error bars represent the uncertainty (± 5 kJ / g C) due to structural diversity among molecules of equal C_{ox}. For oxidizing soils environments where the dominant TEAs are O_2, Mn^{4+}, and NO_3^-, the energy yield of SOM decomposition can be approximated as $\Delta G° = 7.6 \cdot C_{ox} - 35$.

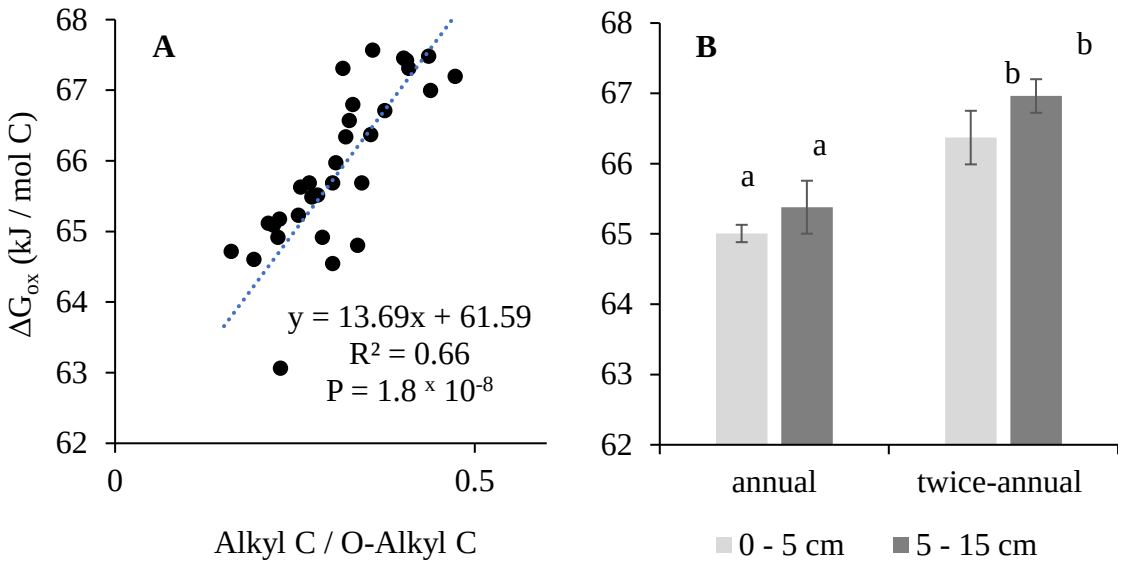

Figure 3.3. (A) The molar Gibbs energy of oxidation (ΔG^{o}_{ox}) for the light fraction of soil organic matter (LF-SOM) is positively correlated with the extent of decomposition (Alkyl C / O-Alkyl C). (B) The ΔG^{o}_{ox} of LF-SOM is greater in the twice-annually harvested treatments.

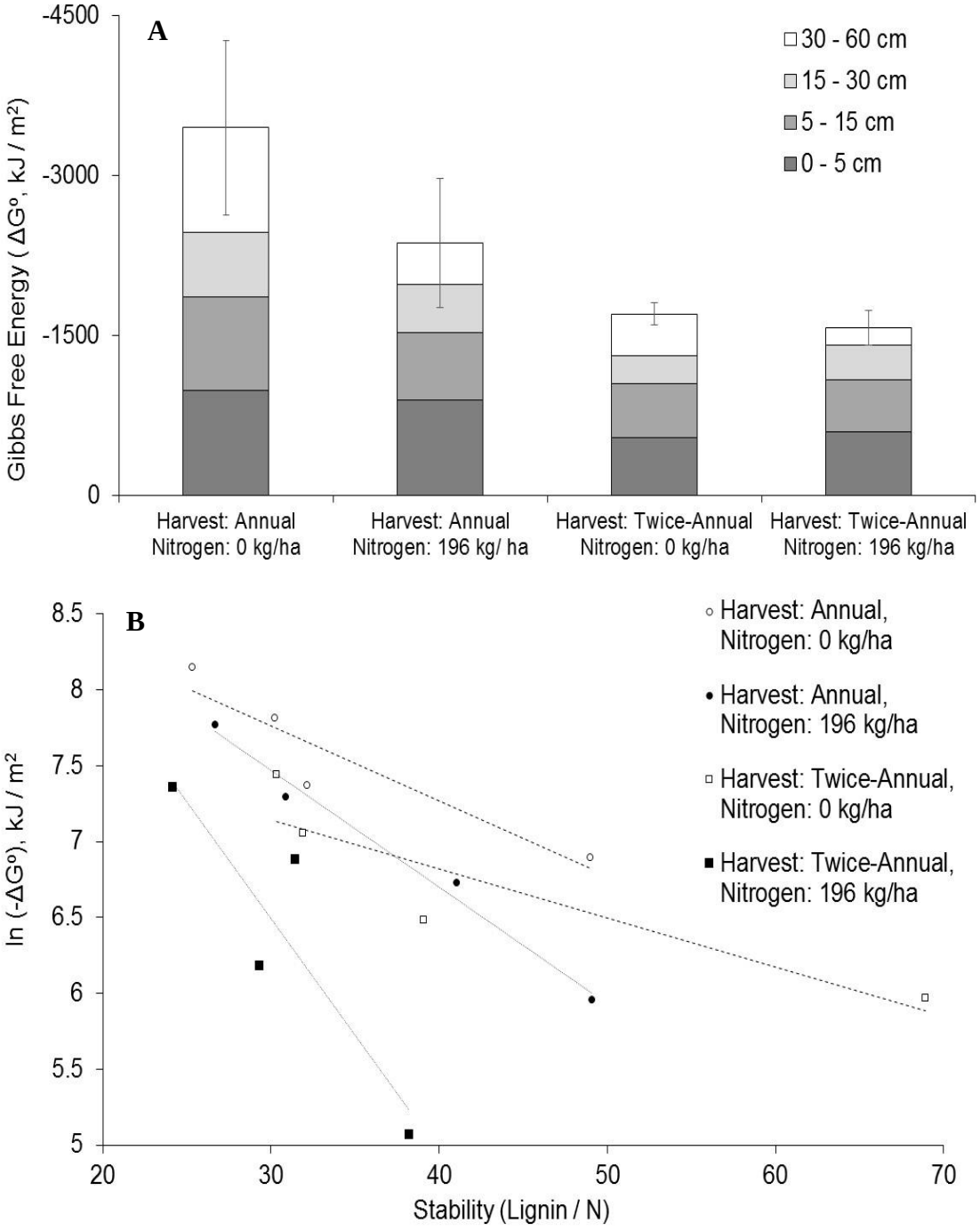

Figure 3.4. The Gibbs free energy in the switchgrass root system. Effects of harvesting and nitrogen fertilization rates upon (A) the depth distribution of ΔG^o, and (B) the energy-stability relationships. The slope steepness in (B) is the energy-for-stability tradeoff. Error bars represent one standard error of the mean. Regression models and figures of merit are listed in Table S3.2.

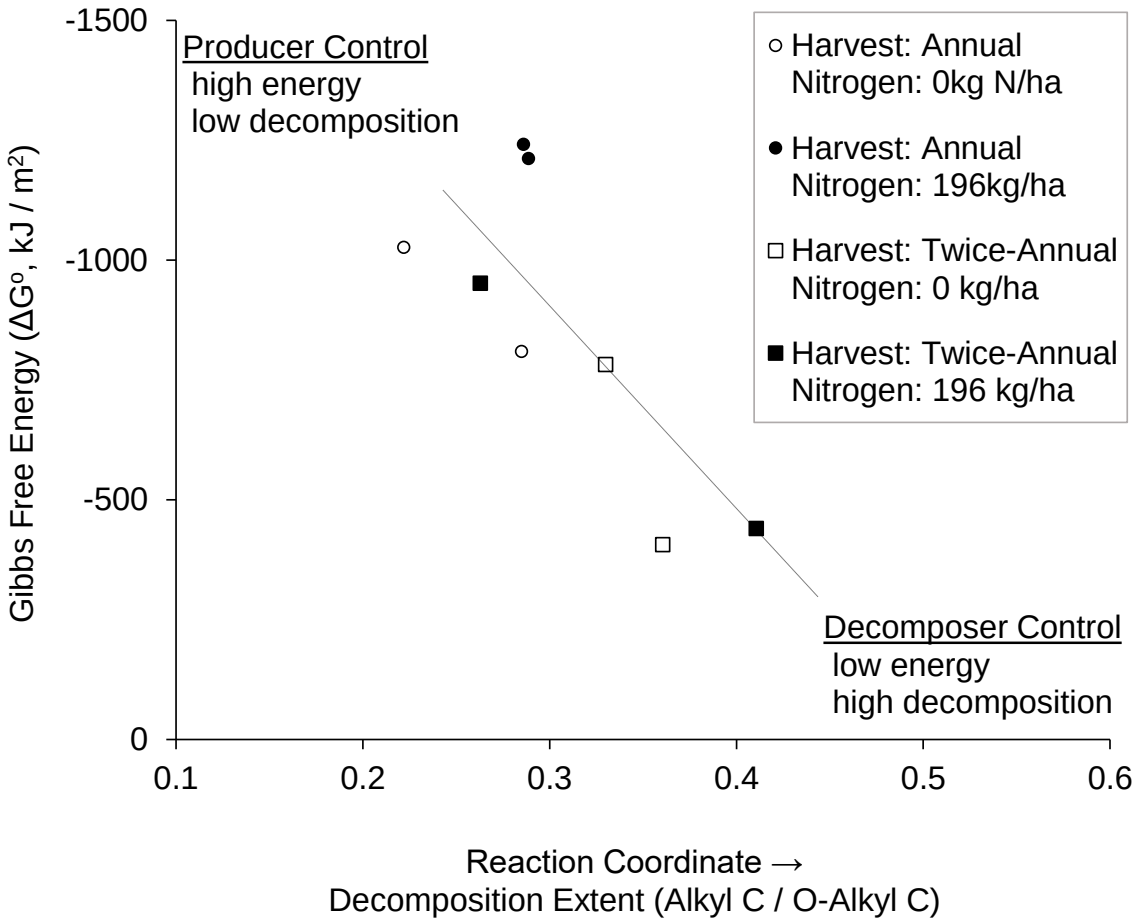

Figure 3.5. Free energy reaction coordinate for the energy budget of the LF-SOM pool at soil depth intervals (0 – 5 cm and 5 – 15 cm). The reaction coordinate is the unitless index of decomposition (Alkyl C / O-Alkyl C). Differences in the Gibbs free energy budget of the soil C pool are due to soil depth as well as harvesting and fertilizer treatments. The fitted linear equation is $\Delta G^o = 4117 \cdot (Alkyl\ C / O\ Alkyl\ C) - 2117$ (R^2 = 0.60, P = 0.024). The high energy, low decomposition state is consistent with high input of fresh root and shoot necromass, while the composition of low energy C pools are increasingly controlled by decomposer activity.

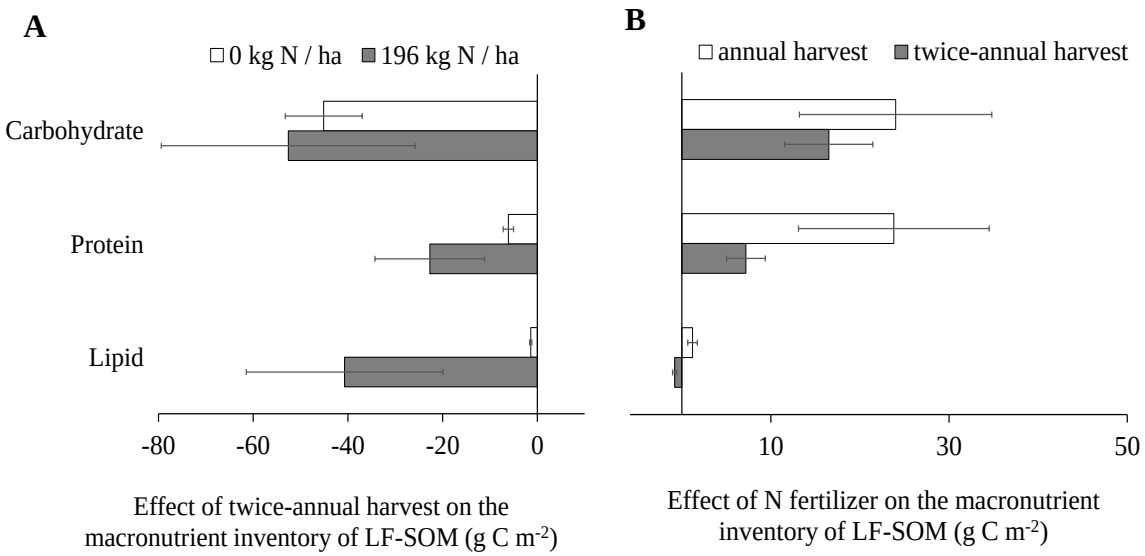

Figure 3.6. Changes in the macronutrient inventory of LF-SOM from 0 cm to 15 cm soil depth caused by (A) twice-annual harvesting and (B) N-fertilizer application. (Change = treatment plot mean – control plot mean). Data are from Table 1 with propagated errors.

CHAPTER FOUR

Thermal Stability Assessment of Switchgrass Light Fraction Organic Matter under Different Fertilization and Harvest Treatments

Abstract

The assessment of soil organic matter (SOM) biodegradability can elucidate the processes of biogeochemical cycles affecting Carbon and energy flows within terrestrial ecosystems. The development of quantitative values to complement qualitative characteristics in characterizing short-term effects of land management decisions on SOM. We compared thermal analysis data with chemical composition to understand the impact fertilization and harvesting agricultural practices could have on light fraction organic matter (LF OM; <1.8g cm^{-3}) stability through quantifying the energetic characteristics. We observed the LF OM under annual harvest and at the 0-5cm soil interval were significantly more thermally stable with larger C-normalized energy content that corresponded with less proportions of Alkyl and Aromatic C. The fluctuating net energy (ΔE) and activation energy (E_a) values we calculated did not reveal significant differences in the return on energy investment (ROI; $\Delta E/E_a$) biodegradability assessment between treatments, but followed the likely trajectory when compared with previous studies. The ability to quantify SOM biodegradability and distinguish effects across land management practices, crops, and climates can inform policy makers and land managers how to model future agricultural decisions to promote sustainability and ecosystem services.

Introduction

The productivity and health of terrestrial ecosystems is dependent on the role of soil organic matter (SOM) and how it chemically, physically, and biologically functions within the carbon cycle. The framework of soil formation factors (climate, land use, parent material, etc.) influence SOM stability and vulnerability to decomposition, which affects nutrient cycling, greenhouse gas (GHG) emissions, soil aggregation and erosion, and overall soil health (Lal 2004, 2009, 2011, Bolinder 2010; Hati 2007). The turnover of SOM is regulated by accessibility to decomposer communities, environmental constraints, and the quantity and quality of the SOM fraction (Davidson et al 2006; Jastrow 2007, Conant 2011; Dungait 2012). Labile SOM fractions with a mean residence time (MRT) of days to months are more readily mineralized and require less complex interpretations for chemical and physical protection mechanisms associated with decomposition dynamics and stability assessments in soil C cycling. This unprotected, labile SOM fraction can serve as an early indicator for changes due to land management and climate to help refine terrestrial carbon and climate modelling, build ecosystem resilience, and develop sustainability efforts (Haynes, 2005; Smith, 2005; Schuur et al., 2008; Kögel-Knabner et al., 2008).

Agriculture, forestry, and other land use change account for over one quarter of global GHG emissions (IPCC 2016). A net increase in the exchange of GHGs from terrestrial ecosystems to the atmosphere due to decomposition and increased metabolic rates in soil heterotrophs is a potential factor in temperature related climate system feedbacks with potential implications on soil health (Kirschbaum 1995; Trumbore et al., 1996; Schlesinger and Andrews 2000; Davidson et al., 2006; IPCC 2016). The sensitivity

of labile SOM fractions to small perturbations in temperature and land use change is of high interest to studies focused on soil health, climate change, and global C cycles (Conteh et al., 1998; Alvarez and Alvarez, 2000; Carter, 2002 Lal 2004). Small changes in climate variables, such as temperature and moisture, can change the metabolic pathways of decomposer communities that affect SOC storage. Land use practices, such as increased tillage or fertilization also affect substrate availability for nutrient and energy flows within the C cycle. Measuring and modelling fluctuations in SOC pools alongside changes in climate and land use practices is important to inform policy-makers and land managers of methods to improve sustainability and environmental health. Furthermore, the development of stable SOM fractions with sustainable land use practices may increase the ability for soils to act as a C sink mitigating GHG emissions, improve soil fertility, and expand ecosystem services.

The quantity, quality, and turnover time of SOM fractions in agricultural systems are controlled by crop selection, climate and edaphic conditions, management practices, and microbial community structure (Leifeld 2006; Gao 2015; Valdez et al., 2017). The quality of SOM has been defined by its ability to resist decomposition, or recalcitrance, and is characterized using spectral, chemical, and thermal analysis across landscapes, management practices, and plant types (Lopez-Capel et al. 2005, 2006; Barros et al. 2007; Leifeld 2007; Rovira et al. 2008). The physical fractionation of SOM can distinguish high quality (labile, unprotected, and easily decomposed) and low fractions (stable, chemically or physically protected, and less easily decomposed) (Denef et al., 2009; von Lützowet al., 2007). Recent studies suggest abiotic and biotic factors affect SOM availability for microbial decomposition as well as the chemical and structural

features of SOM (Kemmitt et al., 2008; Plante 2009; Schmidt 2011; Bruelmann et al 2014; Leifeld 2014). The labile SOM fractions exhibit less protection by physical and chemical association with soil minerals that are known to restrict decomposition and therefore obscure the apparent relationships between substrate composition and concentration, enzyme affinity, and temperature sensitivity effects on decomposition dynamics. In this study we seek chemical and energetic descriptors of SOM decomposition, and therefore, focus on the SOM fraction that is not protected by mineral-associations.

Calorimetry and other thermal analysis techniques have established a connection between combustion temperatures and biological stability of soil carbon pools, with energy requirements proxies for thermal oxidation and temperature classifications of labile (high quality) and stable (low quality) C fractions (Grisi et al. 1998; Plante et al., 2005, 2009; Gregorich et al., 2015; Lopez-Capel et al., 2005; Peltre et al., 2013; Kuzyakov et al., 2006; Helfrich et al., 2010; Katsumi et al., 2016; Ma et al., 2016). These studies generally define biologically labile, high quality SOM as thermally oxidized at peak temperatures below 400°C (DIN Standards Committee Water Practice, 2015). This fraction likely has a more immediate effect on the health and energy of an ecosystem due to climate change sensitivity compared to stable SOM that requires higher temperatures for thermal oxidation. Studies have found correlations between peak temperature and total energy with thermal analysis and the molecular structure of SOC using ^{13}C nuclear magnetic resonance spectroscopy (NMR) (Lopez-Capel et al., 2005; Barros, 2011; Merino et al 2014). Thermal analysis studies observed a relationship between high quality, labile SOM and high energy content, and calculated quantitative values for

activation energy (E_a) and recalcitrance that characterize the biodegradability of C pools in soils (Rovira et al 2008; Harvey et al. 2012; Leifeld 2014).

More recently, Harvey et al. (2016) combined these tools to describe an energetic return on investment principle (ROI) for the short -term biodegradability of charcoal (PyOM). In that study Harvey et al. proposed that biodegradability of PyOM is a function of microbial ROI, which is expressed as the quotient of net energy value and activation energy ($\Delta E/E_a$) (Table 4.1). Therefore, the ROI quotient ($\Delta E/E_a$) approximates microbial utilization of SOM fractions as the exchange between energy input and energy output, with higher ROI predicting greater susceptibility to decomposition. The use of thermodynamic and kinetic properties to study SOM fractions stability using laboratory calorimetry measurements is a potentially rapid and quantitative alternative to traditional microbial incubations and respiration measurements for assessing SOM biodegradability and decomposition kinetics (Craine et al., 2010; LeFevre et al., 2013).

One goal of this study was to quantify and compare the calculated E_a and net energy (ΔE) for the ROI principle in relation to the results of Harvey et al. (2016) for charcoal. The effects of agricultural treatments upon SOM thermal properties and ROI results are also discussed. Thermal analyses of LF OM stability and energy content were compared to previous spectroscopic analyses of sampled aliquots measured herein to further compare the relationship between chemical and thermal stability (Valdez, in review). The biodegradability of low-density, light fraction soil organic matter (< 1.8 g cm-3, LF OM) in a switchgrass field trial under different fertilization and harvest treatments after three years. Our goal was to create a comprehensive approach to understand the effect of management practices in short-term C cycling pools with

implications for soil health and climate change with qualitative and quantitative measurements.

Our hypothesis includes: (1) the LF OM in plots under twice annual harvest composed of stable structures will exhibit more thermally stable properties and oxidation at higher temperatures resulting in a lower ROI; (2) fertilization will increase ΔE and ROI due to increased nutrient availability for microbial utilization; (3) the biochemical composition of LF OM will align with distinct thermal stability parameters due to management practices.

Methods

Experimental design. Low-density light fraction organic matter (LF OM) samples were collected from 0-60cm soil cores from the nitrogen gradient switchgrass field trial as part of the Great Lake Bioenergy Research Center long-term ecological research site at the Kellogg Biological Station (KBS) in southwest Michigan, USA (42° 249 N, 85° 249 W, elevation 288m). The sampled plots were previously corn-soybean and alfalfa rotations under conventional tillage prior to the planting of an upland switchgrass variety, "Cave-in-Rock", on July 11th, 2008 at a seeding rate of 7.84 kg/ha. Mean annual temperature at KBS is 10.1 °C; mean annual precipitation is 1027 mm (Robertson and Hamilton 2015). The soil is the Kalamazoo soil series, a mixed, mesic-Typic Hapudalf developed on glacial outwash with a fine and coarse-loamy texture comprising 85% sand and silt (Crum, J.R. and Collins 1995). The experimental design was a randomized split-plot arrangement with one fertilization rate split between two harvest treatments. Two replicate soil cores were taken from 4 individual plots for arrangements of 0 and 196kg N/ha fertilization rate (combination of granular 46% urea and liquid 28% urea

ammonium nitrate spray) and annual and twice-annual harvest frequencies. Soil cores were separated into four depth intervals (0-5, 5-15, 15-30, 30-60cm).

Density fractionation and molecular characterization. Density separations were performed with 20 grams of soil previously dried, sieved to 2mm, and picked for roots. The individually sieved soil samples were placed in a 50mL centrifuge tube with approximately 30mL of sodium iodide (NaI) solution (density = 1.8g cm3), gently shaken and centrifuged before aspirating and rinsing with deionized water to remove residual NaI. These LF OM samples were further ground and homogenized separately using mortar and pestle for thermal analysis. Sample mass was combined for each plot, and 0-5 and 5-15cm depths were the only intervals with sufficient LF OM mass for this study.

Complete experimental design, set-up, elemental and chemical characterization of the LF OM fraction was previously analyzed by solid-state 13C nuclear magnetic resonance (NMR) spectroscopy with further detail and parameters described in Valdez (2018) (in review): 75.4 MHz resonance frequency, 12kHz spin rate, cross-polarization program with square pulse program, 2ms contact pulse, 3 second recycle delay relaxation time, 40 Hz line broadening, and scans acquired for a minimum signal-to-noise ratio threshold of 10 at the 110-165ppm region. We calculated organic biomolecule (lignin, lipid, protein, carbohydrate) content from a modified molecular mixing model (MMM) adopted from Baldock (2004) for switchgrass using a 2:3 syringyl to guaiacyl monomer ratio and the following distribution of NMR functional groups: (1) Alkyl C (0-45ppm), (2) N-Alkyl and Methoxyl C (45-60ppm), (3) O-Alkyl C (60-95ppm), (4) Di-O-Alkyl C (95-110ppm), (5) Aryl C (110-145ppm), (6) O-Aryl C (145-165ppm), (7) Amide and Carboxyl C (165-215ppm).

Net energy value and thermal stability assessment. Thermal analyses of finely ground, dry LF OM samples were conducted under controlled combustion experiments with differential scanning calorimetry (DSC, TA Instruments Q20) and thermogravimetric analysis (TGA, TA Instruments Q50). Differential scanning calorimetry measures the differential heat flow of a sample relative to a control as a function of temperature, which can be transformed to a measure of energy (kJ). Thermogravimetric analysis measures the mass loss or gain as a function of change in temperature. Both were calibrated with standard indium of 99.99% purity. Sample mass for all analyses measured between 0.15 to 0.45mg of C, which totaled about 10mg of LF OM sample. Samples were placed into an open Aluminum pan (DSC) or platinum carrier (TGA). An empty aluminum pan was used as a reference for DSC samples. The same combustion program was run on DSC and TGA to normalize the effect of weight loss in the TGA with heat release in the DSC. All samples used a 10°C min^{-1} heating rate for valid and reproducible peak resolution and minimal peak overlap (Fernandez et al., 2011). All samples were heated under a synthetic air flow rate of 40ml min^{-1}. Due to instrumental constraints we were limited in our ability to run all samples for each treatment (Table 4.2).

The following DSC and TGA program was conducted for all samples: (a) samples were held for 5 min isothermally at room temperature; (b) samples were heated to 110°C and held isothermally for 10 minutes, to minimize the effect of endothermic to exothermic transitions between water evaporation and OM oxidation (Rovira et al., 2008); (c) samples were heated from 110°C to 550°C, where complete loss on ignition (LOI) of OM is assumed in non-calcareous soils (Howard and Howard, 1990; de Vos, B

et al., 2005). The net energy (ΔE; kJ g−1) was calculated as the normalized area under the DSC curves obtained before 550°C and after 190°C, to correct for inorganic carbon, water and volatiles, or ash content (Figure 4.1). For this reason, the region above 190°C was also set as the baseline. The DSC curves were converted to energy (kJ) from power (watts) by switching from a temperature to time parameter:

kJ = Watts * sec/1000

The ΔE was normalized to energy per organic carbon content (J mg^{-1} C) using previously measured values from combustion elemental analysis. The dry combustion elemental analysis of C and N and estimated C/H and C/O ratios were previously recorded in the molecular mixing model results conducted for these samples, and therefore Oxygen and Hydrogen content were estimated from those ratios. These values were used to convert ΔE and activation energy (E_a) units of kJ mol-1 of CHNO. The thermal stability measurements included: (1) Exotherm 1, area of peak representing heat released between 190°C and 375°C (EXO1); (2) Exotherm 2, area of peak representing heat released between 375°C and 550°C (EXO2); (3) Exo total (total area representing heat released between 190°C and 550°C (ExoTOT); (4) the temperature and heat flux at each exotherm peak; (5) temperature and percent weight where half of the exothermic mass loss occurred (TG_T50); (6) energy density calculated as the energy total divided by thermogravimetric mass loss between 190-550°C (Table 4.2). As explained by Lopez-Capel et al (2005), Exo1 is associated with labile, easily decomposable SOM (O-alkyl C, aliphatic C, carbohydrates, cellulose), while thermally and biologically stable SOM dominates Exo2 (aromatic C, lignin).

Activation energy and microbial return on investment principle. Controlled thermal oxidation reaction experiments at different temperatures have been used to calculate activation energy (Ea) related to the temperature sensitivity of SOM properties (Leifeld, 2014; Harvey et al., 2016). A stepwise-isothermal thermogravimetric analysis was conducted separately to assess thermal decomposition by change in weight of LF OM at various temperature levels to determine the decay rate (k) and Ea for the ROI principle developed by Harvey et al. (2016). The samples were run in ambient air flow (40mL min-1) at 5 isothermal steps under the following program: (a) samples were brought to 110°C and held isothermally for 15 minutes; (b) samples were heated at a rate of 10°C min-1 to 250°C and between each isothermal step (300, 350, 400, 450°C); the LF OM sample was held isothermally for 60 minutes at each isothermal step (Figure 4.2). Ten minutes of initial and five minutes of final data at each isotherm step was removed for over temperature effects and plotted as a function of time to determine the thermal oxidation rate constant (k) for each isothermal step using a first-order model equation developed by Harvey et al. (2016):

$$W=W_o e\,(-kt) + \beta$$

where W is the weight (%) at time t, W_o is the initial weight of the sample (100%), k is the first-order decay rate constant, and β is the remaining weight after a given isoTemp step. The activation energy was derived from the Arrhenius equation using the decay rate, k:

$$\ln(k) = \ln(A) - Ea/RT$$

where k denotes decay rate; A is the frequency factor; T is the isoTemp (in K); R is the gas constant (0.008314 kJ mol−1K−1). The Ea was taken as the average slope of all

samples of the straight line when plotting ln (k) and -1/RT (Figure 4.3). The ROI value was calculated from the quotient of net energy and activation energy (ROI = ΔE/Ea).

Statistical Analysis

Activation energy was evaluated from the variation in reaction rate coefficients as a function of temperature. The samples were analyzed using the general linear model univariate analysis of variance, taking the depth intervals, harvest frequency, and fertilization rates as fixed factors. All variables were checked for equal variance with Levene's Test and log transformed as necessary. The corresponding Pearson's correlation and ANOVAs were carried out with SPSS v. 23.0 (IBM).

Results and Discussion

Thermal Stability of Light Fraction Organic Matter Using Differential Scanning Calorimetry

A DSC and TGA trace for the LF OM is shown in Figure 4.1 along the 190°C baseline used to calculate the net energy values (area under the curve) to the 550°C mark with the break point minimum separating EXO1 and EXO2. The starting mass at 190°C ranged from 88-95% of the initial mass and 20 to 64% of the mass remained at 550°C. The total LF OM mass loss ranged from 68-31% with half of the average combustible OM by weight remaining at a temperature of 350°C. The peaks for EXO1 occurred between 295-327°C and have been attributed to aliphatic compounds and cellulose decarboxylation (300-340°C) with variability in peak heights (2.42-6.92W g-1) (Leinweber et al. 1992a, 1992b; Dell'Abate et al. 2000, 2002). EXO2 exhibited a larger range of peak heights (3.73-9.07W g-1) and peak temperatures occurring between 398-478°C, possibly signifying increased lignin content (410-430°C). Energy lost at the

higher temperature range could be an artifact of char formation from cellulose decomposition as observed in by Kaloustian et al. (2001) and Lopez-Capel (2005). The large variation in peak ranges and heights for EXO1 and EXO2 are likely a function of the heterogeneity of LF OM and effects of the decomposition continuum on thermal stability across increasing temperatures. EXO2 contained 2.5 times the energy of EXO1 on average suggesting most of the energy released consisted of thermally stable material (Figure 4.4).

The larger peak areas and peak heights in EXO2 may encompass a third exotherm suggested in previous studies, however we did not observe consistent and distinct shoulder peaks between 450 and 500°C to warrant a third exotherm for this study (Dell'Abate et al., 2000; Liefeld et al., 2007; Lopez-Capel 2006; Fernandez et al., 2008). The energetic values included above 450°C have been proposed as the end product of broad lignin decomposition, the increase in polyromantic components as a function of OM decomposition, or the combustion of char produced during cellulose combustion (Dell Abate 2002, Lopez –Capel et al 2006). The samples were unprotected, labile SOM fractions under field scale decomposition plots with variable enzyme affinity possibly occurring throughout the growing season. The thermogravimetric values we obtained for each treatment at their corresponding depths are listed in Table 1.

Harvesting and Fertilization Treatment Effects on Light Fraction Organic Matter

The annual harvest contained a significantly greater energy within the stable form in the EXO2 areas and greater peak temperatures than twice annual harvests ($p = 0.046$, $p = 0.016$; respectively). We suggest this indicates the annually harvested treatment contained larger E reservoirs (Figure 4.4). We assume larger EXO2 peaks and areas

across all depths and treatments specifies a predominance of thermally stable OM, especially in the annually harvested treatment where the large EXO2 peak heights occur in conjunction with greater energy density ($p = 0.012$). The carbon use efficiency of the microbial community preferentially mineralize high quality, labile SOM fractions associated with lower temperatures in the EXO1 range to increase the benefit for energy spent. The changes in EXO2 areas due to between harvest practices may be a function of fresh inputs incorporated into the soil from a midseason harvest from twice annual harvests. The microbial community preference for available high-quality substrate, EXO1, before mineralization of stable OM may be an explanation for the significantly larger EXO2 areas found in annual harvest, where no fresh inputs were included until end of season harvest when samples were collected. We suggest the larger energetic reservoirs and EXO2 values across all 0-5cm depths are a function of proximity and distribution of aboveground inputs increasing OM concentration and active microbial communities increasing the complexity of residue remnants along the decomposition spectrum leading to larger thermally stable energy values in the LF OM. This process would increase the formation of polyaromatic groups as a product of decomposition and account for an increased EXO2 signal within the thermal analysis (De la Rosa et al., 2008).

The thermogravimetric (TG) data used in conjunction with LF OM C stocks was used to calculate the energy stock of LF OM (Figure 4.5a). The C stocks for these samples were not statistically different between treatments, however the energy stocks were significantly larger in the annually harvested plots ($p = 0.037$) (Figure 4.5a, b). When normalized for C content the annually harvested LF OM contained greater total

energy (ExoTOT; p = 0.011) and larger C content (%C; p = 0.034). The normalization of OM using C content was completed because of its significance as the main substrate in soil food webs and soil health. Our findings indicate the annual harvests tend to have more stable and larger energy stocks with higher C content, which supports similar findings by Leifield (2014).

The isothermal TGA procedure used to calculate activation energy (E_a) and the decay rate (*k*) of LF OM samples across each treatment did not return any significant differences between treatments or depths. This is surprising given the significant differences between EXO2 and harvest treatments. There was a slightly larger average E_a value for 0kg N/ha fertilization treatments (P = 0.106) which correlated with ExoTOT and therefore EXO2. This likely and correctly suggests thermally stable fractions of LF OM are associated with larger E_a. Insufficient sample size in certain treatments may be the result of ambiguous relationships between net energy and E_a, or the 15-35% calculated variability in E_a standard deviations which ranged from 30.53 to 42.66 kJ mol^{-1}. The connection between management practices, thermal stability, and the required energy for the mineralization is essential to building sustainable land management practices, building soil health assessments, and developing soil resilience with stable SOC pools.

Correlations between Molecular Composition and Thermal Stability Measurements

The results from TG were correlated with the structural and biochemical composition of LF OM from the same aliquot of samples obtained previously using ^{13}C NMR analysis and a molecular mixing model (MMM). We observed the annually harvested samples contained significantly less alkyl C and lipid abundance (p = 0.042,

0.021), and the 0-5cm depth interval had a larger abundance of alkyl C and aromatic groups than the 5-15cm depth ($p = 0.037, 0.022$) (figure 4.6). The twice-annual harvest and 5-15cm depth had significantly larger Alkyl/O-Alkyl C ratios, which was observed previously and interpreted as an index for greater extent of decomposition. We found these results surprising considering they are contrary to our TG interpretations with larger EXO2 in annual harvest treatments representing thermally stable and complex SOM. These results suggest the TG and molecular data may not correlate well for these samples, possibly due to increased heterogeneity. We used a two-tailed Pearson's correlation between molecular composition and TG data. The percent weight at which half of the exothermic loss occurred (TG_T50) was lower with increases in alkyl C ($r = -.664$), aromatic C ($r = -.582$), and protein ($r = -.623$) content (Figure S4.1a - c). These results indicate 'recalcitrant' molecular structures lowered TG_T50 values. The OM with larger amounts of biochemically stable molecules, such as aromatic C and Alkyl C, reduces rapid thermal breakdown and provides C and energy sources likely to persist longer in the soil C cycle. The TG_T50 value may be a possible characteristic in proxy development for molecular stability as the NMR and TG experiments aligned best with this quantification of thermal stability

Calculation of Return on Investment Principle (ROI)

The conversion of ΔE to kJ mol^{-1} provided a range of energies from 263 to 997 kJ mol^{-1} and coupling ΔE with the E_a values from 18.7 to 45.2 kJ mol^{-1} provided ROI calculations between 6.3 and 35.1 (Table 4.3). The values for ΔE were larger and E_a

smaller than the pyrolysis samples obtained by Harvey et al. (2016). This resulted in larger ROI values and predicts the LF OM samples would be more available to the microbial community as an energy source and preferentially decomposed over the charcoal samples measured by Harvey et al. (2016). This confirms the method for biodegradeability for ROI is an available proxy to differentiate between C pools.

No treatments or depths contained significant differences among ROI values, although at the 0-5cm depth ROI was 15% larger when calculated across all treatments. The ROI principle used to understand the range of biodegradability in LF OM did not distinguish between the heterogeneity of the samples found among thermal and chemical stability characterizations. This may be molecular differences relating to stability that were not found in the thermal experiments, greater net energy values, or an inability to directly correct for ash content or weight by non-aromaticity, as done in the Harvey et al. (2016) experiment (we estimated H and O concentrations from NMR data).

Conclusion

We compared thermal analysis data with chemical composition to understand the impact fertilization and harvesting agricultural practices could have on LF OM stability through quantifying the energetic characteristics. Our data indicates the annual harvest treatment was more thermally stable, due to larger EXO2 values, and contained more energy when normalized by C content. The larger energy stocks in thermally stable fractions suggest that annual harvesting techniques may be a beneficial way to increase long-term energy sources into soil. This may promote further resilience to changes in climate conditions through a reduction in CO_2 release as a product of decomposition. No significant differences were observed among activation energies, decay rates, or ROI

values throughout treatments. Therefore, the increases in energy reservoirs due to land management may provide a best practice for improving soil health. Our ROI values confirmed the biodegradability of LF OM over previously calculated pyrogenic OM by Harvey et al. (2016), but we understand that due to the estimation of Oxygen and Hydrogen content using NMR data our calculations are best estimates. The biological mineralization of C sources will differ with the enzymatic processes that occur as microbes have adapted certain carbon-use-efficiencies to target various substrates, and our calculations using thermal oxidation are conservative estimates of the microbial degradation pathways. We note the ROI principle may not be sufficiently accurate to distinguish land management practices within an agricultural species for LF OM, but could likely prioritize and categorize multiple energy sources within the soil food web to understand how they interact within the larger soil C cycling system and potential changes in climate and land use.

Table 4.1 Definitions of TGA and DSC values

Net Energy ΔE	energy content released as a function of DSC curves converted to $kJmol^{-1}$ (exothermic)
Activation Energy E_a	energy investment for reaction to proceed; indicates sensitivity of reaction rate to temperature (endothermic)
Exotherm 2 EXO1	total area representing mass loss during TGA, expressed as energy, between 190°C and 375°C (exothermic)
Exotherm 2 EXO2	total area representing mass loss during TGA, expressed as energy, between 375°C and 550°C (exothermic)
Energy Total ExoTOT	total area representing mass loss during TGA, expressed as energy, between 190°C and 550°C (exothermic)
Energy Density	energy content divided by thermogravimetric mass loss between 190-550°C
Energy Stock	amount of energy (kJ) contained within volume of organic matter (cm3)
TG_T50	form of recalcitrance; percent weight remaining when half of the exothermic mass loss occurred

Table 4.2: Sample size and thermal properties of light fraction organic matter for each depth and treatment.

Harvest	Fertilizer rate	Depth	Sample	EXO1	EXO2	ExoTOT	TG_T50	Energy Density
	kg N/ha	cm	size	kJ/g C	kJ/g C	kJ/g C	%	kJ/g C
Annual	0	0-5	3	0.92±0.27	2.92±1.12	3.84±1.39	61 ± 3	0.06±0.02
		5-15	2	0.52±0.06	2.36±0.66	2.88±0.71	64 ± 5	0.05±0.01
	196	0-5	4	0.75±0.25	2.02±1.25	2.77±1.42	64 ± 7	0.05±0.01
		5-15	4	0.98±0.38	1.93±0.45	2.91±0.74	67 ± 4	0.06±0.01
Twice-Annual	0	0-5	2	1.02±0.05	2.01±0.40	3.03±0.36	63 ± 3	0.05±0.01
		5-15	2	0.36±0.21	1.26±0.37	1.63±0.58	74 ± 4	0.05±0.02
	196	0-5	4	0.68±0.23	1.13±0.31	1.81±0.41	68 ± 4	0.04±0.01
		5-15	3	0.49±0.10	1.18±0.20	1.67±0.29	71 ± 2	0.04±0.01

Table 4.3: Calculated Net Energy, Activation Energy, and ROI for LF OM and Harvey et al. (2016) pyrogenic organic matter (PYOM) (values are averages of treatments ± standard deviation).

Harvest	Fertilizer Rate (kg N/ha)	Depth (cm)	Activation Energy (kJ mol-1)	Net Energy (kJ mol-1)	ROI
Annual	0	0-5	37.79 ± 5.47	515.21 ± 59.79	13.97 ± 3.48
		5-15	*30.53 ± 7.03*	*533.89 ± 65.3*	*17.71 ± 1.94*
	196	0-5	31.72 ± 9.56	487.17 ± 87.24	16.72 ± 6.14
		5-15	34.98 ± 5.67	515 ± 76.65	15.14 ± 3.99
Twice-annual	*0*	*0-5*	*31.76 ± 4.03*	*659.58 ± 477.19*	*21.9 ± 17.8*
		5-15	*42.66 ± 0.96*	*363.66 ± 141.83*	*8.49 ± 3.13*
	196	0-5	29.81 ± 8.02	529.97 ± 195.33	19.91 ± 13.13
		5-15	30.77 ± 10.67	459.76 ± 5.9	16.51 ± 6.8
Average of study		*Current study*	*33.75 ± 4.47*	*507.5 ± 82.66*	*15.04*
		Harvey et al. (2016)	*82.23 ± 23.65*	*413.89 ± 249.4*	*5.03*

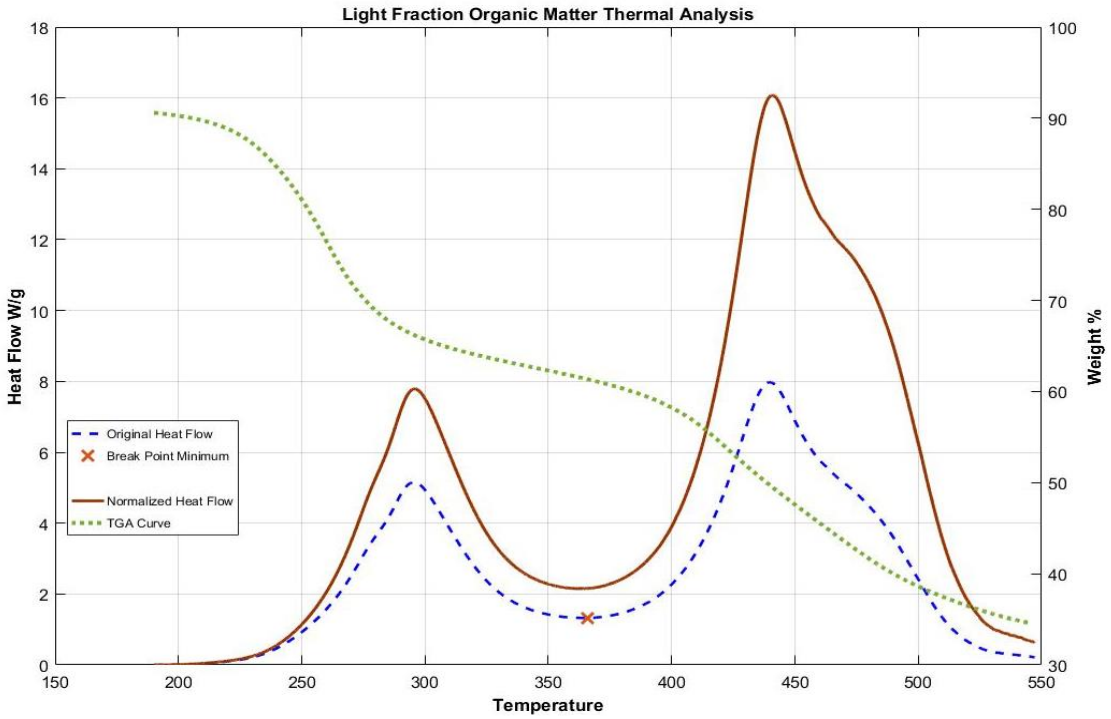

Figure 4.1: Normalized (solid line) and original (dashed line) curves from Differential Scanning Calorimetry to account for weight loss during heating program. Break point (X) determines cutoff point between EXO1and EXO2. The thermogravimetric analysis curve ((dotted line) show change in weight loss with increasing temperature

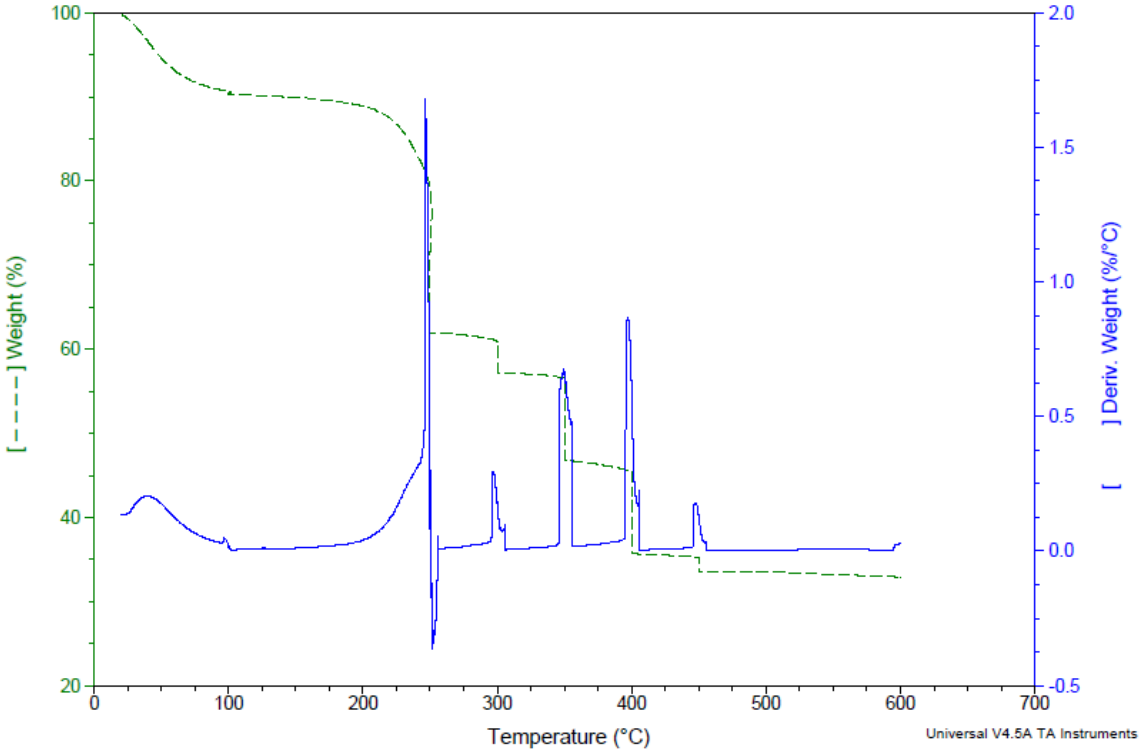

Figure 4.2: TGA data for isothermal experiment with total weight (dashed green line) and derived weight (solid blue line).

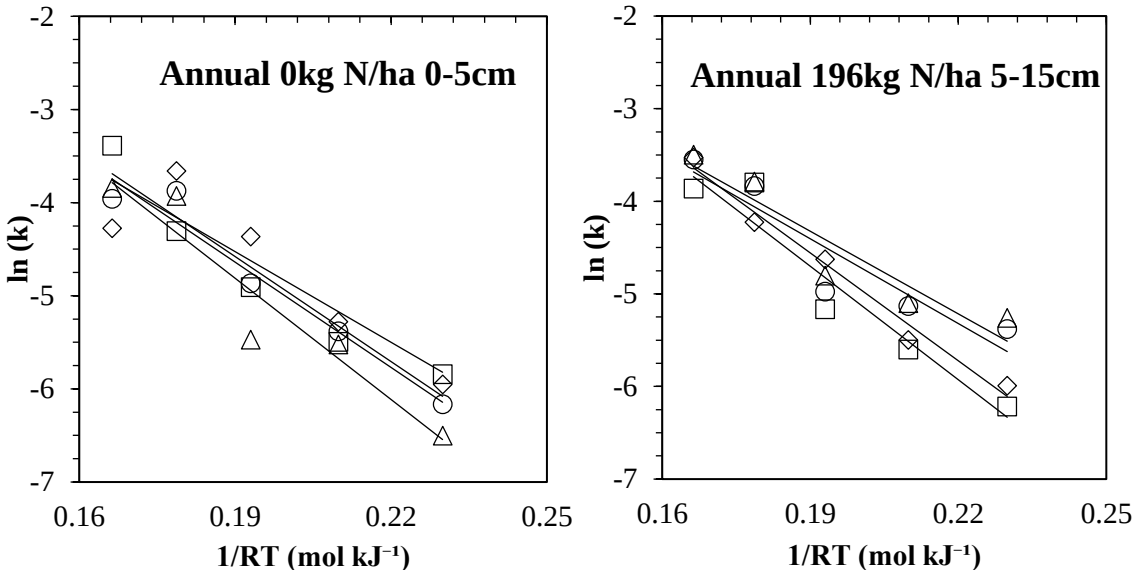

Figure 4.3: Arrhenius plots (ln(k) versus 1/RT) used to determine activation energy for light fraction organic matter. Units for the gas constant, R, were kJ mol^{-1} K^{-1} and that for isothermal temperature, T were Kelvin (K). Each symbol represents one sample for a particular temperature for each replicate.

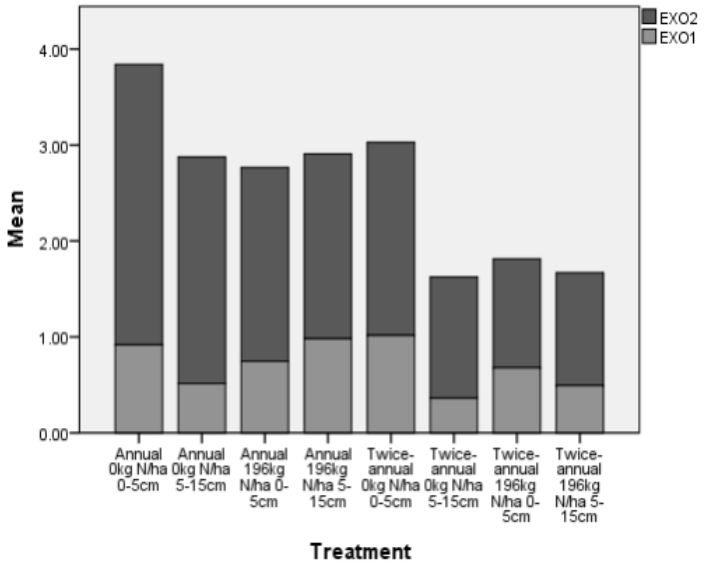

Figure 4.4: Average energy total of each exotherm for all treatments where EXO2 (dark gray) is 2.5 larger than EXO1 (light gray).

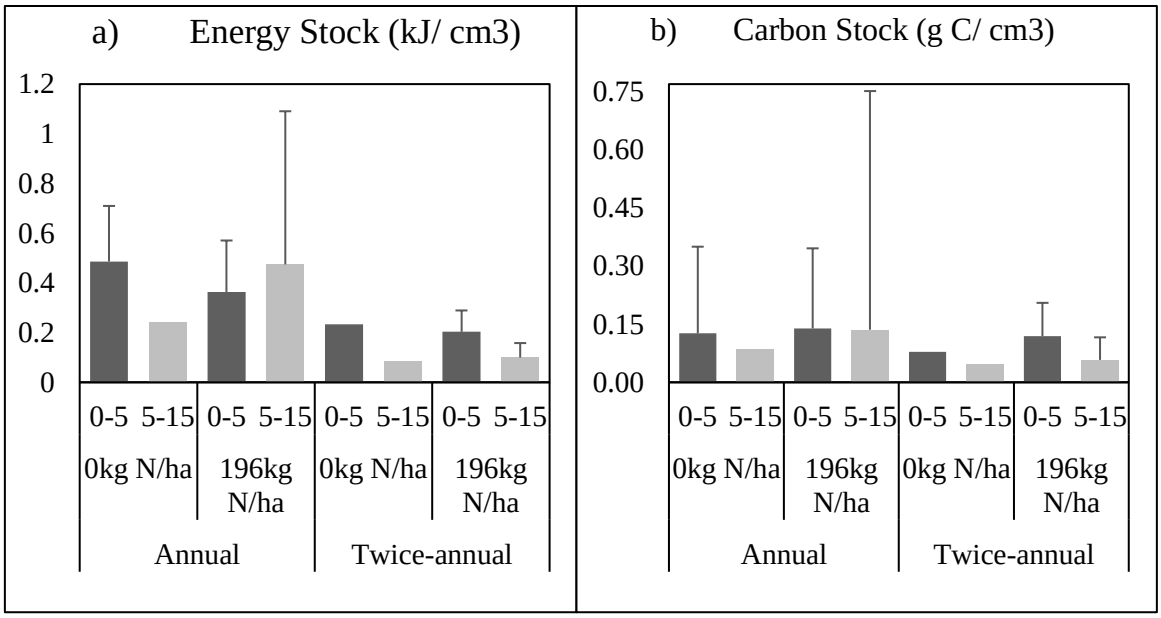

Figure 4.5: Energy (a) and Carbon (b) stocks of LF OM by treatment with bars representing standard deviation (n>2).

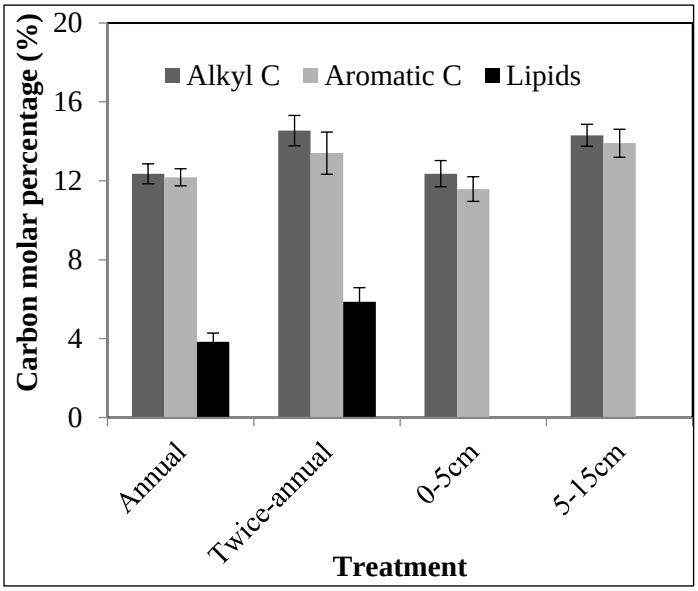

Figure 4.6: Light fraction organic matter percentage alkyl C (dark gray), aromatic C (light gray), and lipid composition with standard error bars for harvest treatment and depth

CHAPTER FIVE

Summary of Findings

We studied whole soil, roots, and light fraction organic matter (LF OM) at multiple depth intervals, from 0 to 60cm, to elucidate the effect of fertilization and harvest treatments on switchgrass field plots at the Kellogg Biological Station in western Michigan after three treatment years from 2009-2011. In each chapter we established different quantitative or qualitative calculations of soil C fractions for use as state variables in models for soil C cycling, soil C storage, or overall soil health assessments. The identification of multiple ways to characterize soil C sources can improve management practices for future climate or land use changes resilience, so land managers and biogeochemical modelers may identify how to sustainably approach agricultural development while improving ecosystem services. We have attempted to demonstrate how each chapter can reduce uncertainty about the effects of harvesting and fertilization on soil C pools and potential consequences on belowground energy storage and C-cycling through a robust and thorough data set.

In Chapter two, combustion analysis on the whole soil, roots, and LFOM revealed dense fraction organic matter (DF OM, >1.8 g/cm^3) averaged 95% of the soil matrix. The significant reductions to the DF OM C and N stocks due to the 196kg N/ha fertilization rate drove the total soil C and N stocks down, mostly in depths below 15cm. This is important because DF OM is considered relatively stable with more physical and chemical protection mechanisms from mineralization than LF OM, especially at depth

where lower O_2 availability leads to longer residence times. We also observed significant increases in LF OM and roots C and N stocks above 15cm in annually harvested plots, which can often be the most available source of nutrients and energy for soil heterotrophs. These findings indicate that the least intensive land management practices (no fertilization and annual harvesting) stored more soil C and N in key areas to improve soil health and C storage. These are key findings to increase sustainable terrestrial systems while potentially providing a net positive C storage budget for alternative biofuels.

We utilized ^{13}C Nuclear Magnetic Resonance Spectroscopy (NMR) and a molecular mixing model (MMM) to calculate various chemical, biochemical, and energetic responses of LF OM and roots to fertilization and harvesting practices in Chapter three. We applied methods with these instruments to calculate the OM decomposition state variables and energetic values to present a biochemical inventory (lipids, proteins, carbohydrates, and lignin) and energy budget, analogous to "nutrition facts" for soil organic matter to assess soil health. The annually-harvested switchgrass roots contained a more energetic substrate, distributed deeper within the soil profile, and were less altered by decomposition, than the twice-annually harvested plots. The fertilization management practice increased root and LF OM N content and reduced C:N and lignin:N ratios accordingly, resulting in a substrate more available for decomposition. The fertilization treatment increased the amount of available energy in LF OM and decreased the energetic value in roots, especially below 15cm. These measurements and calculations are crucial inputs to develop and inform biogeochemical models and the

conception of energetic values to understand the mechanisms of soil C cycling, but also provide a unique and innovative method to measure energy in soil organic C pools.

We took an empirical approach to calculating the energetic values as heat released, enthalpy, from thermogravimetric (TGA) experiments on LF OM. We isolated the LF OM above 15cm for each treatment and observed 2.5 times the amount of energy in thermally stable portions (EXO 2, 375-550°C) than the labile portions (EXO1; 190-375°C). LF OM with larger EXO2 created larger total energy (ExoTOT) given off as heat especially in the 0-5cm depth interval and with annual harvesting; the samples also contained less Alkyl C and Aromatic C content and a lower Alkyl C/O-Alkyl C ratio, signifying less decomposed material. The annual harvest also increased the total energy, energetic stocks, energy density, and net energy measured as enthalpy. We employed TGA experiments to estimate the activation energy (E_a) of the OM using decay rates at various temperatures and found LF OM from unfertilized plots contained a greater E_a. We then associated the quotient of net energy and E_a as a biodegradeability component labelled as the return on investment (ROI) for the microbial decomposition of the OM. This research did not find any significant changes to the ROI based on treatments likely due to the heterogeneity of the material and large variation in values. This research is essential to future developments of energetic budgets and decomposability assessments for land managers to have a quantitative measure for how their agricultural practices affect the ecosystem.

Contributions and Future Work

This research provides a novel and robust data set from a field scale experiment on well-studied soils that is accessible for local and global biogeochemical modelling

efforts. The data provided is a thorough representation of the changes due to management practices, in which harvest frequency has not been studied in switchgrass, with archived samples and those used within this research catalogued and stored for further examination. The broad range of data we have collected can be introduced as new variables in a host of biogeochemical models, and provides a wealth of novel and quantitative approaches to measuring soil C and terrestrial energy budgets. This research can be coupled with aboveground data from collaborators at Rice University and other measurements taken at the field site in Michigan to offer a comprehensive approach to modelling the natural system.

Our global soils are increasingly tasked to provide more goods on less land with less resources. In large areas of the globe the value of water is dramatically higher and will be a major consideration when selecting crops. The development of advanced bioenergy crops that provide a reprieve from the amount of water and fertilization used to create more traditional ethanol-based biofuels is key to sustainable biofuel production. This research provides a measure to quantify best practices to reduce fertilizer use as a means to increase soil C and energy budgets. These measures can supply a measure to conserve water and more importantly reduce the effects of fertilization on the release of harmful GHGs, NO_x and CO_2, and the contamination of local and regional water ways. This research takes steps to develop sustainable practices and improve ecosystem benefits in agricultural environments through identifying and labelling robust and quantifiable values to incorporate across crop and climate regimes.

APPENDIX A

Chapter Two Supplemental Information

Soil Carbon and Nitrogen Responses to Nitrogen Fertilizer and Harvesting Rates in Switchgrass Cropping Systems

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Chapter Two Supplemental Tables

Table S2.1. Carbon and Nitrogen Stocks (kg m^{-2}) for Soil and Roots. Values are averages for 4 replicated plots. ANOVA results for harvesting and fertilizer treatment effects.

Harvesting frequency		Annual	Annual	Biannual	Biannual		3 way ANOVA	
Fertilization rate:		0kg N/ha	196 kg N/ha	0kg N/ha	196 kg N/ha		F Value	P Value
	Soil Depth (cm)	(kg/m^2)	(kg/m^2)	(kg/m^2)	(kg/m^2)			
Soil C stock	0-5	0.968	0.859	0.978	1.020	Harvest	0.416	0.522
	5-15	1.440	1.305	1.375	1.396	Fertilizer	**9.409**	**0.004**
	15-30	1.336	1.011	1.225	1.093	Depth	**16.958**	**0.000**
	30-60	1.022	0.808	1.160	0.765	H x F	0.618	0.436
	0-60	4.766	3.983	4.737	4.274	H x F x D	0.739	0.534
Soil N Stocks	0-5	0.093	0.081	0.098	0.104	Harvest	3.879	0.055
	5-15	0.140	0.134	0.145	0.143	Fertilizer	**8.213**	**0.006**
	15-30	0.139	0.109	0.159	0.115	Depth	**11.227**	**0.000**
	30-60	0.128	0.106	0.144	0.118	H x F	0.011	0.917
	0-60	0.501	0.430	0.546	0.480	H x F x D	0.339	0.797
Root Biomass	0-5	1.552	1.549	0.914	0.885	Harvest	**5.243**	**0.026**
	5-15	1.385	0.956	1.134	0.981	Fertilizer	2.075	0.156
	15-30	0.955	0.911	0.523	0.609	Depth	0.991	0.405
	30-60	2.039	0.747	0.899	0.411	H x F	0.525	0.472
	0-60	5.930	4.163	3.470	2.886	H x F x D	0.195	0.899
Root C stock	0-5	0.110	0.100	0.061	0.067	Harvest	**5.96**	**0.018**
	5-15	0.099	0.071	0.057	0.055	Fertilizer	1.340	0.253
	15-30	0.067	0.051	0.030	0.036	Depth	1.393	0.256
	30-60	0.111	0.043	0.044	0.018	H x F	0.827	0.368
	0-60	0.387	0.265	0.191	0.176	H x F x D	0.035	0.991
Root N Stock	0-5	0.0027	0.0020	0.0015	0.0016	Harvest	3.347	0.074
	5-15	0.0018	0.0013	0.0014	0.0013	Fertilizer	1.511	0.225
	15-30	0.0012	0.0010	0.0006	0.0009	Depth	2.49	0.071
	30-60	0.0022	0.0006	0.0007	0.0005	H x F	1.714	0.197
	0-60	0.0080	0.0049	0.0042	0.0043	H x F x D	0.137	0.937

1 tailed ANOVA. Items bolded $P < 0.05$

Table S2.2. Total mass, carbon, and nitrogen stocks (kg m^{-2}) of the low-density (LF) and the high- density (DF) fractions of the soil organic matter. Values are averages for 4 replicated plots. ANOVA results for harvesting and fertilizer treatment effects.

Harvesting frequency:		Annual	Annual	Biannual	Biannual	ANOVA		
Fertilization rate:		0kg N/ha	196 kg N/ha	0kg N/ha	196 kg N/ha		F Value	P Value
	Soil depth (cm)	(kg/m2)	(kg/m2)	(kg/m2)	(kg/m2)			
DF C stock	0-5	0.853	0.720	0.891	0.913	Harvest	1.327	0.255
	5-15	1.349	1.169	1.329	1.347	Fertilizer	10.071	0.003
	15-30	1.288	0.982	1.180	1.056	Depth	16.979	0.000
	30-60	0.973	0.786	1.132	0.740	H x F	0.617	0.417
	0-60	4.463	3.657	4.532	4.056	H x F x D	0.907	0.445
DF N stock	0-5	0.089	0.072	0.095	0.098	Harvest	4.608	0.037
	5-15	0.137	0.127	0.142	0.140	Fertilizer	8.671	0.005
	15-30	0.137	0.108	0.157	0.114	Depth	12.105	0.000
	30-60	0.126	0.104	0.142	0.117	H x F	0.036	0.850
	0-60	0.489	0.412	0.536	0.469	H x F x D	0.386	0.764
LF mass	0-5	0.102	0.167	0.087	0.137	Harvest	1.739	0.194
	5-15	0.049	0.072	0.024	0.034	Fertilizer	2.036	0.160
	15-30	0.021	0.016	0.027	0.014	Depth	24.462	0.000
	30-60	0.014	0.010	0.013	0.006	H x F	0.232	0.632
	0-60	0.186	0.265	0.151	0.190	H x F x D	0.020	0.996
LF C Stock	0-5	0.115	0.139	0.088	0.107	Harvest	4.081	0.049
	5-15	0.091	0.136	0.046	0.049	Fertilizer	0.125	0.725
	15-30	0.048	0.029	0.045	0.037	Depth	8.895	0.000
	30-60	0.048	0.022	0.028	0.025	H x F	0.014	0.908
	0-60	0.302	0.326	0.206	0.218	H x F x D	0.309	0.819
LF N stock	0-5	0.0041	0.0082	0.0035	0.0052	Harvest	3.371	0.073
	5-15	0.0037	0.0072	0.0024	0.0027	Fertilizer	1.292	0.261
	15-30	0.0023	0.0012	0.0025	0.0015	Depth	6.087	0.001
	30-60	0.0018	0.0012	0.0014	0.0012	H x F	0.619	0.435
	0-60	0.0119	0.0179	0.0098	0.0106	H x F x D	0.441	0.725

Table S2.3. Seasonal effects on soil and root carbon and nitrogen stocks (kg m^{-2}) within the biannual harvesting treatment. Values are averages for 4 replicated plots. ANOVA results for harvesting and fertilizer treatment effects.

Sampling month:		July	July	November	November			
Harvesting frequency:		Biannual	Biannual	Biannual	Biannual		3 way ANOVA	
Fertilization rate:		0kg N/ha	196 kg N/ha	0kg N/ha	196 kg N/ha		F Value	P Value
	Soil Depth (cm)	(kg/m^2)	(kg/m^2)	(kg/m^2)	(kg/m^2)			
Soil C stock	0-5	1.000	0.766	0.978	1.020	Harvest	2.971	0.091
	5-15	1.600	1.493	1.375	1.396	Fertilizer	**5.316**	**0.025**
	15-30	1.429	1.483	1.225	1.093	Depth	**14.914**	**0.000**
	30-60	1.276	0.847	1.160	0.765	H x F	0.245	0.623
	0-60	5.305	4.588	4.737	4.274	H x F x D	0.570	0.638
Soil N Stocks	0-5	0.097	0.076	0.098	0.104	Harvest	3.191	0.080
	5-15	0.159	0.161	0.145	0.143	Fertilizer	2.378	0.130
	15-30	0.149	0.169	0.159	0.115	Depth	**17.799**	**0.000**
	30-60	0.159	0.145	0.144	0.118	H x F	1.091	0.302
	0-60	0.565	0.552	0.546	0.480	H x F x D	2.171	0.104
Root Biomass	0-5	0.748	0.658	0.914	0.885	Harvest	2.481	0.122
	5-15	0.439	0.921	1.134	0.981	Fertilizer	0.96	0.332
	15-30	0.415	0.903	0.523	0.609	Depth	1.925	0.138
	30-60	0.196	0.735	0.899	0.411	H x F	**5.535**	**0.023**
	0-60	1.797	3.216	3.470	2.886	H x F x D	1.141	0.342
Root C stock	0-5	0.044	0.049	0.061	0.067	Harvest	1.59	0.213
	5-15	0.027	0.057	0.057	0.055	Fertilizer	1.109	0.298
	15-30	0.018	0.048	0.030	0.036	Depth	**2.882**	**0.045**
	30-60	0.016	0.030	0.044	0.018	H x F	2.302	0.136
	0-60	0.104	0.184	0.191	0.176	H x F x D	0.327	0.806
Root N Stock	0-5	0.0010	0.0008	0.0015	0.0016	Harvest	**7.585**	**0.008**
	5-15	0.0004	0.0010	0.0014	0.0013	Fertilizer	1.218	0.275
	15-30	0.0003	0.0008	0.0006	0.0009	Depth	**5.121**	**0.004**
	30-60	0.0002	0.0006	0.0007	0.0005	H x F	0.900	0.347
	0-60	0.002	0.003	0.004	0.004	H x F x D	0.504	0.681

Table S2.4. Seasonal effects on total mass, carbon, and nitrogen stocks (kg m^{-2}) of the low-density (LF) and the high- density (DF) fractions of the soil organic matter within the biannual harvesting treatment. Values are averages for 4 replicated plots. ANOVA results for harvesting and fertilizer treatment effects.

Sampling month:		July	July	November	November		ANOVA	
Harvesting frequency:		Biannual	Biannual	Biannual	Biannual			
Fertilization rate:		0kg N/ha	196 kg N/ha	0kg N/ha	196 kg N/ha		F Value	P Value
	Soil depth (cm)	(kg/m^2)	(kg/m^2)	(kg/m^2)	(kg/m^2)			
DF-C stock	0-5	0.920	0.670	0.891	0.913	Harvest	**4.515**	**0.039**
	5-15	1.566	1.403	1.329	1.347	Fertilizer	**8.144**	**0.006**
	15-30	1.408	1.456	1.180	1.056	Depth	**17.359**	**0.000**
	30-60	1.370	0.825	1.132	0.740	H x F	0.617	0.417
	0-60	5.264	4.355	4.532	4.056	H x F x D	0.636	0.595
DF-N stock	0-5	0.094	0.072	0.095	0.098	Harvest	3.494	0.068
	5-15	0.157	0.157	0.142	0.140	Fertilizer	2.865	0.097
	15-30	0.148	0.168	0.157	0.114	Depth	**19.495**	**0.000**
	30-60	0.160	0.145	0.142	0.117	H x F	0.897	0.348
	0-60	0.560	0.541	0.536	0.469	H x F x D	2.134	0.108
LF mass	0-5	0.101	0.140	0.087	0.137	Harvest	0.100	0.753
	5-15	0.026	0.060	0.024	0.034	Fertilizer	**4.321**	**0.043**
	15-30	0.009	0.012	0.027	0.014	Depth	**55.322**	**0.000**
	30-60	0.007	0.005	0.013	0.006	H x F	0.407	0.527
	0-60	0.142	0.217	0.151	0.190	H x F x D	0.00	0.817
LF C stock	0-5	0.080	0.096	0.088	0.107	Harvest	0.204	0.653
	5-15	0.034	0.090	0.046	0.049	Fertilizer	1.907	0.174
	15-30	0.020	0.026	0.045	0.037	Depth	**16.537**	**0.000**
	30-60	0.029	0.022	0.028	0.025	H x F	0.962	0.334
	0-60	0.164	0.234	0.206	0.218	H x F x D	0.785	0.508
LF N stock	0-5	0.0034	0.0046	0.0035	0.0052	Harvest	0.184	0.670
	5-15	0.0018	0.0047	0.0024	0.0027	Fertilizer	2.041	0.160
	15-30	0.0009	0.0012	0.0025	0.0015	Depth	**8.524**	**0.000**
	30-60	0.0020	0.0009	0.0014	0.0012	H x F	0.735	0.396
	0-60	0.008	0.011	0.010	0.011	H x F x D	0.721	0.544

1 tailed ANOVA. Items bolded $P < 0.05$

TABLE S2.5 Initial and end-of-season values for each treatment by depth for bulk density, soil C%, and soil C stock.

					Annual				Biannual			
Bulk Density		Initial			0 kg N/ha		196 kg N/ha		0 kg N/ha		196 kg N/ha	
(g/cm^3)	Depth	Average	*S.E.*	Depth	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*
	0-10cm	1.131	*0.037*	0-5cm	1.697	*0.256*	1.522	*0.185*	1.664	*0.227*	1.656	*0.261*
	10-25cm	1.301	*0.030*	5-15cm	1.791	*0.101*	1.579	*0.216*	1.707	*0.156*	1.645	*0.098*
	25-50cm	1.328	*0.016*	15-30cm	1.854	*0.070*	1.686	*0.082*	1.678	*0.146*	1.690	*0.107*
	50-100cm	1.284	*0.009*	30-60cm	1.851	*0.084*	1.732	*0.082*	1.709	*0.077*	1.737	*0.065*
Soil C%												
(wt. percent)	Depth	Average	*S.E.*	Depth	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*
	0-10cm	1.104	*0.070*	0-5cm	1.141	*0.032*	1.129	*0.080*	1.176	*0.057*	1.232	*0.075*
	10-25cm	0.555	*0.050*	5-15cm	0.804	*0.050*	0.826	*0.048*	0.805	*0.041*	0.849	*0.030*
	25-50cm	0.228	*0.029*	15-30cm	0.480	*0.041*	0.400	*0.036*	0.487	*0.047*	0.431	*0.023*
	50-100cm	0.089	*0.012*	30-60cm	0.184	*0.017*	0.155	*0.012*	0.226	*0.018*	0.147	*0.013*
Soil C Stock												
($kg\ C/m^2$)	Depth	Average	*S.E.*	Depth	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*	Average	*S.E.*
	0-10cm	1.252	*0.079*	0-5cm	0.968	*0.047*	0.859	*0.062*	0.978	*0.027*	1.020	*0.061*
	10-25cm	1.083	*0.097*	5-15cm	1.440	*0.070*	1.305	*0.049*	1.375	*0.090*	1.396	*0.077*
	25-50cm	0.758	*0.095*	15-30cm	1.336	*0.118*	1.011	*0.059*	1.225	*0.113*	1.093	*0.092*
	50-100cm	0.568	*0.077*	30-60cm	1.022	*0.091*	0.808	*0.070*	1.160	*0.092*	0.765	*0.063*

TABLE S2.6 Soil dry weight determinations at 50 and 105 °C drying temperatures.

	Annual Harvest				Twice-annual (July samples)			
Soil Depth (cm)	196 kg N/ha		0 kg N/ha		196 kg N/ha		0 kg N/ha	
Drying Temp	50°C	105°C	50°C	105°C	50°C	105°C	50°C	105°C
0-5cm	9075	9053	4212	4196	10158	10107	11426	11371
5-15cm	10488	10446	10618	10558	14685	14626	18385	18307
15-30cm	13284	13201	14535	14358	15311	15236	12200	12122
30-60cm	11885	11782	8552	8499	9298	9238	17282	17161

Weight in mg.

	percent difference between 50C and 105C			
	0-5cm	5-15cm	15-30cm	30-60cm
h1 401	-0.2%	-0.4%	-0.6%	-0.9%
h1- 408	-0.4%	-0.6%	-1.2%	-0.6%
July 401	-0.5%	-0.4%	-0.5%	-0.6%
July 408	-0.5%	-0.4%	-0.6%	-0.7%

Chapter Two Supplemental Figures

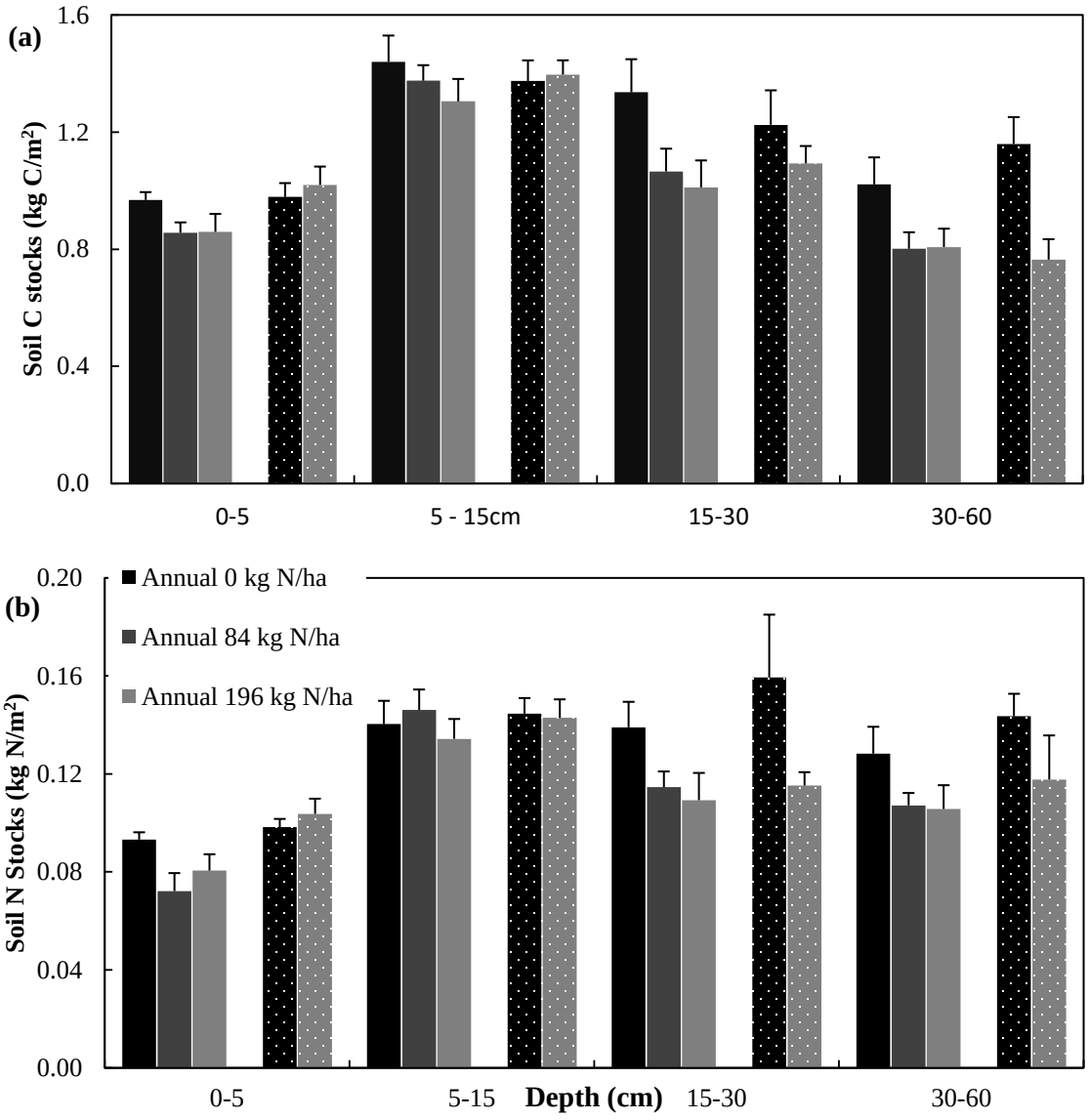

Figure S2.1: Depth effect of harvesting and fertilizer treatments on soil N and C stocks

APPENDIX B

Chapter Three Supplemental Information

Nutrition Facts for Soil Organic Matter: Agricultural Effects on Gibbs Free Energy and Macronutrient Inventories

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1.0 INTRODUCTION

Describing SOM Composition and Decomposition [Previously section 1.3 main text]

Previous studies have established the use of chemical state variable as valid predictors of organic matter quality in terms of stability (resource ratios) and/or extent of decomposition. In this paper we use the previously-established state variables to test the hypothesis that agricultural management practices are deterministic in the energy and stability of organic matter entering the soil by altering the quantity and chemical quality of roots. Second, we use the decomposition state variable to show that the Gibbs free energy budget declines with SOM decomposition. Finally, we introduce the nutrition facts labelling scheme for SOM as a familiar format through which to convey spectroscopic and thermodynamic data to land managers for the purpose of assessing impacts of agricultural management practices.

Stability: The C/N and Lignin/N ratios. The C/N and lignin/N ratios for living and non-living C pools are indices of litter quality. The C/N is a coarse index for stability. Though C content of fresh plant residues is relatively consistent across ecosystems and tissue types (Palm and Rowland 1997), N is a limiting resource and litter with higher N tends to decompose more rapidly (Austin and Ballaré 2010). Lignin is moderately resistant to degradation by extracellular enzymes in surface soils, requires a distinct microbial community for breakdown, and the abundance of lignin is generally negatively correlated to the initial decay rate of plant necromass, especially roots and woody structures (Meentemeyer 1978). The C/N and lignin/N of plant residues were thus developed as decomposability indices (Melillo et al. 1982, Wang et al. 2004, Bonanomi et al. 2013). Plant residues with lower C/N or lignin/N are likely to undergo more rapid or more extensive decomposition. Multiple studies have shown the relationship between C/N and lignin/N in plant residues related to biological decomposition (Tian et al. 1992, Moore et al. 1999). Both ratios are used to scale decomposition rates in models of organic matter dynamics (e.g., Parton et al. 1988).

Extent of Decomposition: The Alkyl-to-O-Alkyl ratio. The ratio of Alkyl C to O-Alkyl C is directly calculated from ^{13}C NMR spectra and was developed by (Baldock et al. 2004) as an index of organic matter decomposition. The Alkyl C / O-Alkyl C is the quotient of stable and insoluble, aliphatic macromolecules in Alkyl structures (e.g., plant waxes, cutin and suberin) to labile, O-Alkyl compounds (e.g. polysaccharides, cellulose and hemicellulose) (Baumann et al. 2013, Dou et al. 2013). Alkyl C compounds that likely decompose slowly include membrane lipids, epicuticular waxes, and cuticle polymers such as cutin, and suberin (Tegelaar et al. 1989, Baldock et al. 1992, Lorenz

and Lal 2005, Poirier et al. 2005, Winkler et al. 2005, Quénéa et al. 2006, Li et al. 2015). A consistent pattern of increasing Alkyl C / O-Alkyl C is observed in SOM with increasing decomposition time and SOM mass loss (Baldock et al. 1992, Lorenz et al. 2000, Leifeld et al. 2005, Baldock 2007, Preston et al. 2009).

Biochemical Inventory: Nutrition Facts for the Soil Food Web. In many agricultural systems, the aboveground biomass is harvested for food, fiber, or fuel, leaving the plant roots and detritus as the main sources of nutrition for the soil ecosystems. Root-based food chains and detritus-based food chains consist of different communities of organisms (Glavatska et al. 2017) which interact such that shared energy and nutrient flows affect the structure and function of the whole food web (Moore et al. 2004). Therefore, this study separately isolated roots from detritus from the soil and independently determined the energy and nutritional quality of roots and detrital (light fraction, LF) SOM.

Nutritional analysis of the soil food web substrates requires biochemical inventory of roots and detrital OM. The major biochemical constituents of these substrates are carbohydrates, lignins, protein, and lipids. Carbohydrates include cellulose, hemicellulose, starches, and sugars. Lignin is a structural cell wall polymer made of ether-linked proplyphenols. Protein, as defined for our purpose, include amino acids and peptides and comprise the main nitrogen-containing substrate of SOM. Lipids include the oxygen-substituted hydrocarbons of cell membranes and cuticles, as well as oils, pigments, and vitamins. These biochemical classifications are based upon gross chemical structure. Since chemical structure and bonding also exert control over the mechanism of oxidation and depolymerization during digestion by organisms, the biochemical

classifications have significance to community structure in the soil food web. Finally, as shown in Fig 1, chemical structure determines the activation energy (ΔG^{o}_{ox}), and therefore affects the free energy available to soil heterotrophs (ΔG^{o}).

The biochemical inventory and the Gibbs free energy budget provide a quantitative basis for evaluating the management practices impact upon energy and nutritional quality of roots and detrital SOM. However, this information will only find utility if land managers are able to understand and attribute energy and nutrition to outcomes such as productivity (i.e. yields) or inputs (fertilizer cost). Therefore, we present energy and biochemical inventory data in the format of Nutrition Facts Labels (FDA, WHO), reasoning that familiarity with the nutritional implications for personal health allows for intuitive and logical connections between complex chemical data and the nutrition of the community of soil heterotrophs.

2.0 RESULTS

Compositional Differences in Root and LF-SOM. We quantified separately the C stock and biochemical composition of roots and LF-SOM. The roots and LF-SOM represent C pools of similar size, but differ significantly in biochemical composition. The average biochemical composition of the root C pool across all treatments was 70.8 ± 0.2 % carbohydrate C, 20.2 ± 0.2 % lignin C, 5.65 ± 0.18 % protein C, and 3.3 ± 0.1 % lipid C. The LF-SOM had an average composition of 55.4 ± 1.3 % carbohydrate C, 27.1 ± 1.1 % lignin C, 12.8 ± 0.6 % protein C, and 4.4 ± 0.4 % lipid C. The effects of N fertilizer application upon roots at depths >15cm was a 27% increase in lignin and a corresponding decrease in carbohydrates. (Fig. S2). The chemical differences between root and LF-SOM are consistent with differences in decomposition extent. For instance, the

preferential depletion of carbohydrate in LF-SOM compared to root biomass is consistent with carbohydrates having the lowest energy barrier to decomposition (ΔG_{ox}) among the major classes of biochemicals (Fig 1).

Chapter Three Supplemental Tables

Table S3.1. Terminal electron acceptors, half reactions, reduction potentials, and Gibbs free energy values

		ΔG^{o}_{red} (kJ/mol e^-)[a]	E^o (Volts)[b]
Oxygen	$O_2 + 4e^- + 4H^+ \Leftrightarrow 2H_2O$	-125	1.27
Nitrate	$NO_3^- + 5e^- + 6H^+ \Leftrightarrow 1/2N_2 + 3H_2O$	-95.2	1.22
Mn^{4+} pyrolusite	$MnO_2 + 4H^+ + 2e^- \Leftrightarrow Mn^{2+} + 2H_2O$	-195	1.24
Fe^{3+} goethite	$FeOOH + 3H^+ + e^- \Leftrightarrow Fe^{2+} + 2H_2O$	-75.9	0.786
Fe^{3+} hematite	$Fe_2O_3 + 6H^+ + 2e^- \Leftrightarrow Fe^{2+} + 3\ H_2O$	-74.6	0.773
Fe^{3+} ferrihydrite	$Fe(OH)_3 + 3H^+ + e^- \Leftrightarrow Fe^{2+} + 3H_2O$	-94.7	0.981
Sulfate	$SO_4^{2-} + 8e^- + 9H^+ \Leftrightarrow HS^- + 4H_2O$	-24.0	0.248

[a] from Arndt et al., 2003.

[b] reduction potential for standard hydrogen electrode at 25ºC, calculated from the expression $\Delta G^{o}_{red} = -nFE^o$, where Faraday's constant, F = 96.48 kJ / mol $e^- \cdot$ V

Table S3.2. Gibbs free energy-stability curves for switchgrass root biomass

Harvesting rate	Nitrogen Fertilizer (kg N/ha)	Best-fit Equation	Correlation Coefficient (R^2)
Annual	0	$\ln(-\Delta G^o) = -0.049(lignin/N) + 9.24$	0.87
Annual	196	$\ln(-\Delta G^o) = -0.032(lignin/N) + 8.12$	0.81
Twice-Annual	0	$\ln(-\Delta G^o) = -0.076(lignin/N) + 9.77$	0.98
Twice-Annual	196	$\ln(-\Delta G^o) = -0.154(lignin/N) + 9.24$	0.81

Table S3.3. Biochemical composition of roots and LF-SOM (carbon molar percentages)

Harvest frequency	Annual		Annual		Twice-Annual		Twice-Annual	
Fertilizer (kg N ha^{-1})	0		196		0		196	
Soil depth interval (cm)	0 – 5	5 – 15	0 – 5	5 – 15	0 – 5	5 – 15	0 – 5	5 – 15
Roots								
Carbohydrate (%)	69.32	72.2	71.78	72.63	69.28	71.57	70.0	70.62
Protein (%)	6.47	5.14	6.01	4.82	6.12	6.54	7.14	6.48
Lignin (%)	21.18	19.13	19.24	20.38	20.59	19.59	19.29	19.71
Lipid (%)	2.73	3.53	2.77	2.00	4.01	2.30	3.59	3.18
Carbonyl (%)	0.30	0.00	0.20	.018	0.00	0.00	0.00	0.00
C_{ox}	-0.145	-0.158	-0.140	-0.131	-0.172	-0.135	-0.158	-0.153
LF- SOM								
Carbohydrate (%)	60.97	54.55	55.60	56.31	51.32	54.06	63.70	46.48
Protein (%)	9.64	10.64	15.00	15.05	10.50	13.64	12.87	15.14
Lignin (%)	24.95	29.70	26.45	25.45	31.99	25.91	19.53	32.72
Lipid (%)	3.64	4.27	2.82	3.11	5.39	6.39	3.90	5.66
Carbonyl (%)	0.80	0.83	0.12	0.09	0.79	0.00	0.00	0.00
C_{ox}	-0.168	-0.193	-0.163	-0.164	-0.225	-0.229	-0.157	-0.239

Table S3.4. Nutrition facts for roots to 60 cm depth

Nutrition Facts: Roots

Serving size

Soil area: 1 square meter (m^2)

Soil depth: 60 centimeters (cm)

Harvest frequency **Fertilizer (g N m^{-2})**	Annual 0	Annual 19.6	Twice-Annual 0	Twice-Annual 19.6
Amount per serving				
Gibbs Free Energy (kJ)	-3,452	-2,368	-1,702	-1,571
Total Fat, g C	255	172	121	111
Total Carbohydrate, g C	25.0	15.3	13.1	13.3
Total Lignin (Fiber), g C	87.9	67.1	47.8	43.4
Protein, g C	18.3	10.7	9.00	7.83
Total Organic Carbon, g C	386	265	191	176
Standard error of the mean, %	± 41	± 49	± 21	± 27

Chapter Three Supplemental Figures

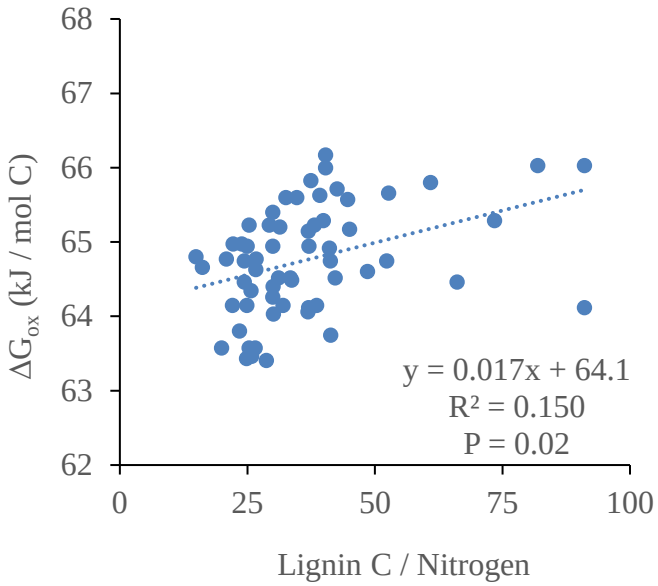

Figure S3.1. Relationships of Gibbs Free Energy of oxidation (ΔG^{o}_{ox}) with state variables for (left panel) stability of switchgrass roots (Lignin C/ N). The positive correlations suggest that roots with larger ΔG^{o}_{ox} values are more stable.

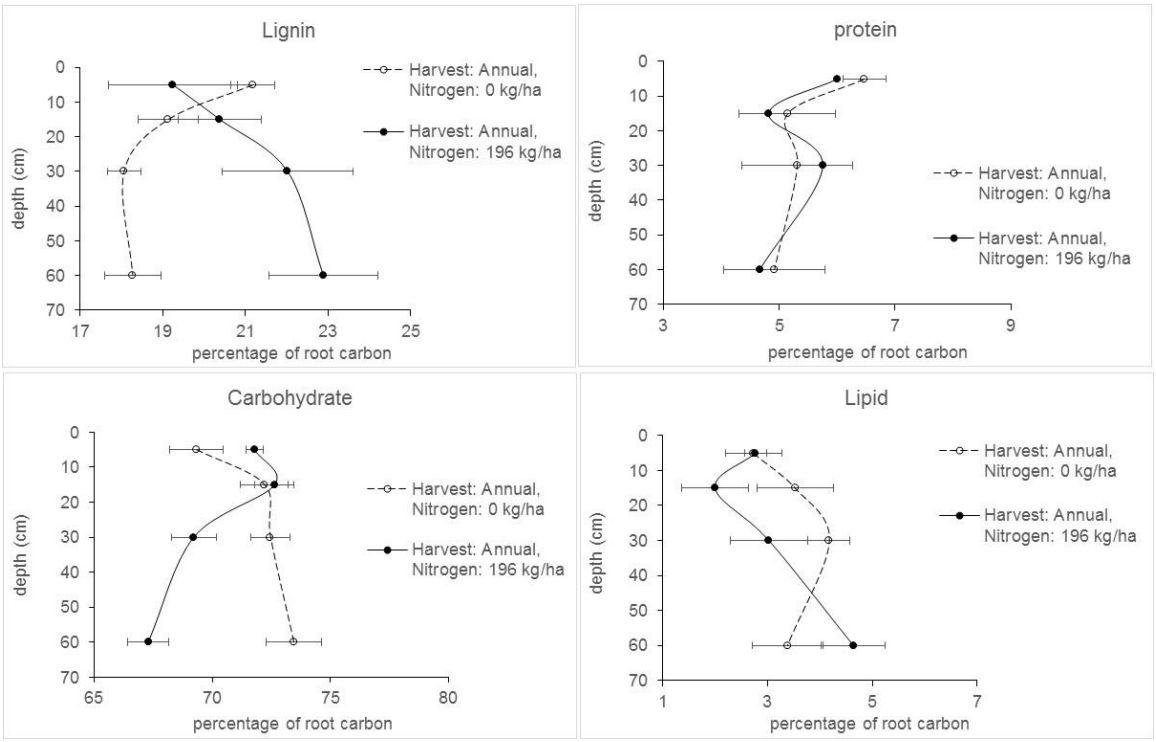

Figure S3.2**.** Effect of nitrogen fertilizer on switchgrass root biochemistry in annually harvested plots. The greater lignification of roots at depths >15 cm ($P < 0.05$) in fertilized plots on occurs at the expense of carbohydrates (e.g., cellulose and hemicellulose).

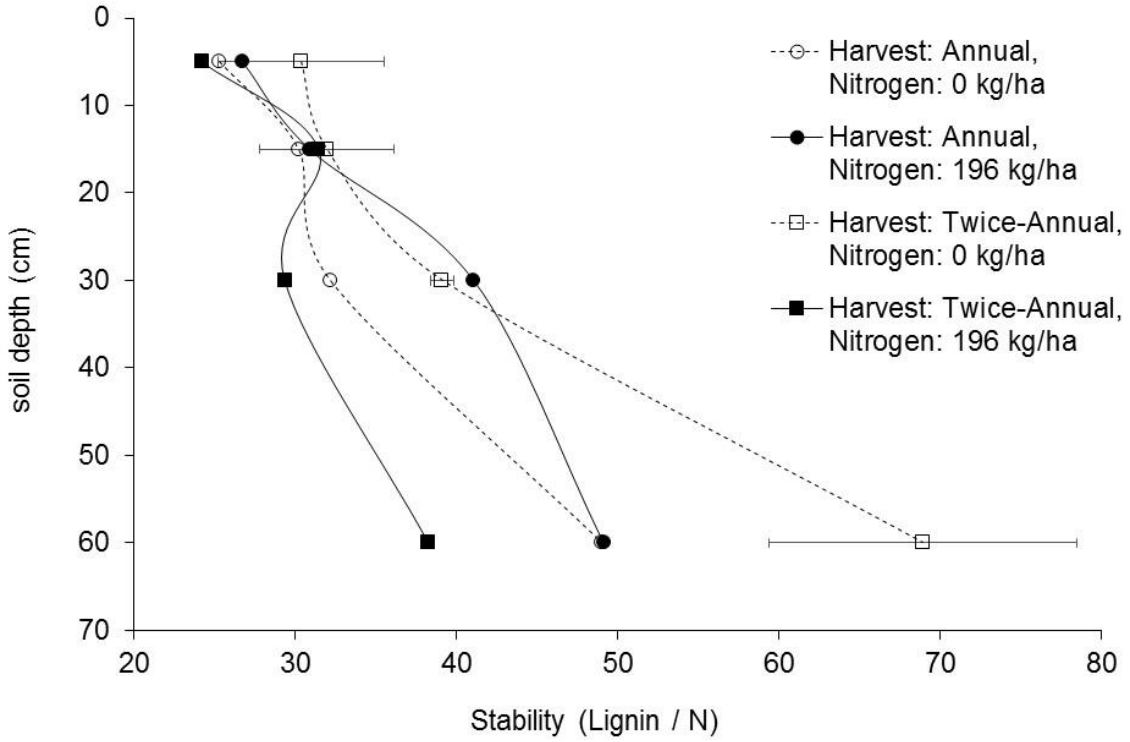

Figure S3.3 The digestibility index (Lignin / N) for standing root biomass depth profiles.

APPENDIX C

Chapter Four Supplemental Information

Thermal Stability Assessment of Switchgrass Light Fraction Organic Matter under Different Fertilization and Harvest Treatments

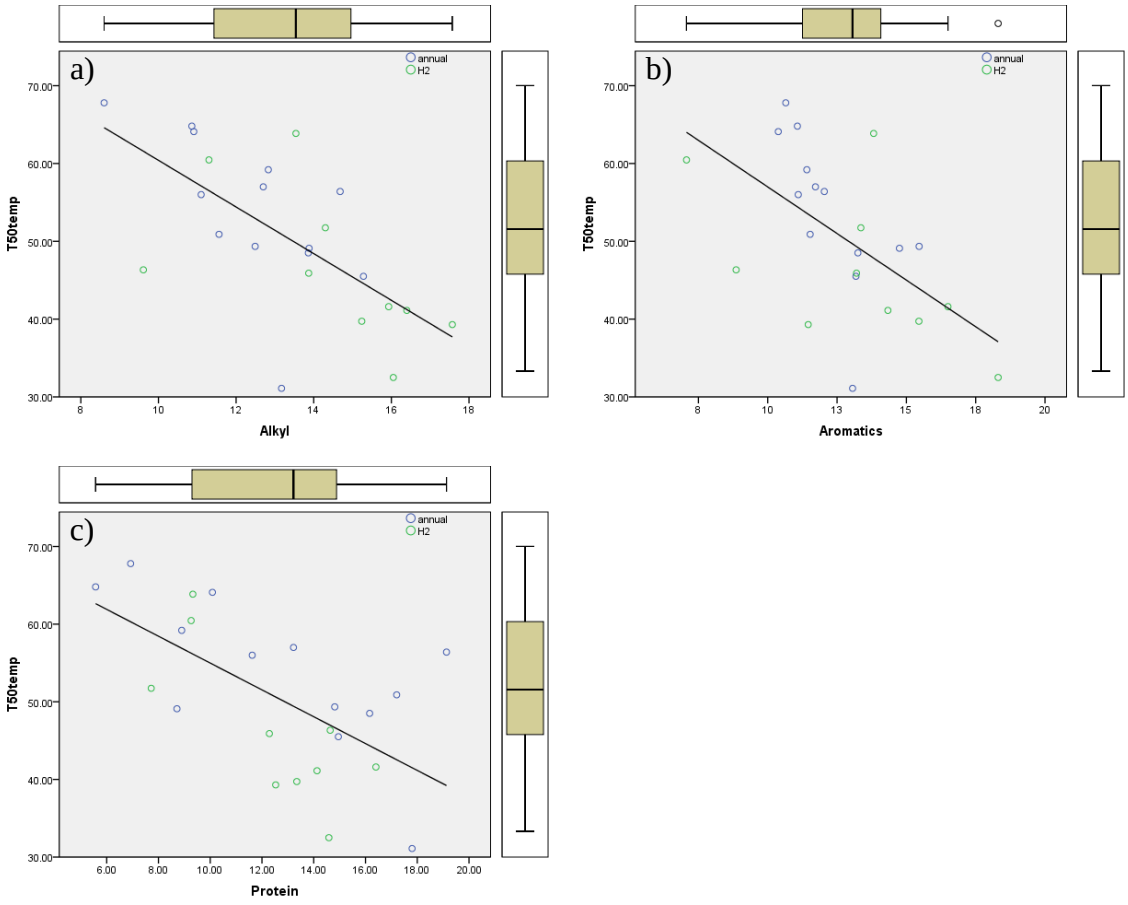

Figure S4.1. Cross plots of annual (blue) and twice-annual (green) harvest regimes and corresponding bar-whisker plots of percent weight at which half of the exothermic loss occurred (TG_T50) and alkyl C, aromatic C, and protein content.

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