SOIL CARBON DYNAMICS IN WETNESS-PRONE MARGINAL SOILS UNDER PERENNIAL GRASS BIOENERGY CROPS OF NORTHEASTERN UNITED STATES

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Srabani Das

August 2017

SOIL CARBON DYNAMICS IN WETNESS-PRONE MARGINAL SOILS UNDER PERENNIAL GRASS BIOENERGY CROPS OF NORTHEASTERN UNITED STATES

Srabani Das, Ph. D.

Cornell University 2017

Perennial bioenergy crops are projected to reduce US reliance on fossil fuels, bring down greenhouse gas emissions and enhance rural economies. In the Northeastern United States, seasonally-wet underutilized marginal lands are being considered as a resource base for the renewable bioenergy sector. Studies incorporating soil moisture and other field characteristics are needed for commercial production of bioenergy. The objective of this study is, therefore, to investigate the impact of converting wetness-prone fallow lands to perennial grass cultivation and specifically to examine the effect of moisture controls on soil carbon dynamics.

In the first study, quadruplicate treatments were established in a wetness-prone marginal fallow grass site that was converted to perennial grass bioenergy feedstock production, consisting of fallow-control, reed canarygrass with nitrogen (N) fertilizer, switchgrass without and with N fertilizer in a four-year study. After three years, the loss of organic matter (OM) and soil organic carbon (SOC) due to plowing of formerly fallow land had not been repaid. The wettest soils had the greatest OM, SOC, and active carbon, independent of the cropping treatment, while driest soils had the lowest pH. During the last year, the wettest soils had significantly greater SOC and total nitrogen (TN) than drier soils, and fallow soils had significantly greater SOC than

soils of switchgrass and switchgrass + N. In the relatively short timeframe of this study, neither crop species photosynthetic pathway (C3 vs. C4) nor moderate rates of N fertilization had a significant impact on soil properties. The second study found that surface volumetric water contents had a stronger correlation with soil properties (OM, SOC, TN, bulk density and $\delta^{13}C$) down to 120 cm depth, than did shallow perched water depth. Correlation between loss-on-ignition (LOI) derived C and combustion elemental analysis measured C contents to soil depths of 60 cm (using New York State soil testing conversions of LOI), was very good. The third study showed that in a 371-day incubation experiment, C mineralizability was significantly less with high field moisture compared to low field moisture, indicating possible stabilization mechanism facilitated (through mineral interactions of SOC) under high water content.

BIOGRAPHICAL SKETCH

Srabani was born in Sindri, a small township housing India's first fertilizer mega-plant and grew up in the crowded, humid setting of Kolkata on a high freshwater fish diet! Her dad's work in agricultural extension excited her and she followed his footsteps by taking up biological sciences as an area of study. She made an entry into the world of sustainable development, with coordinating grassroots environmental and civil society movements. While working for the Dialogue for Water, Food and Environment (World Wildlife Fund-Consultative Group on International Agricultural Research), Srabani worked alongside stalwarts of India's environmental movement, including Medha Patkar and among the poorest of citizens, which left a deep impression on her. In between her work in the development sector, she completed a Masters in Life Sciences (Jawaharlal Nehru University, New Delhi) and a Masters in Environmental Engineering (Indian Institute of Technology Bombay; thesis title 'Biodegradation of terephalates'). Srabani was a recipient of the British Chevening Fellowship (at the University of Wales, Bangor). Srabani moved to Rochester, NY to join her husband in 2007 and worked as substitute teacher in Henrietta School District and as adjunct faculty in Environmental Policy at the Rochester Institute of Technology.

At Cornell, Srabani has worked with the Sustainable Bioenergy Project at BEE http://www.cornell.edu/video/sustainable-bioenergy-production and as TA of Undergraduate Biology from 2011-2016, serving as the Head TA in 2015-2016 and receiving the CALS Outstanding TA award the same year. She was also a Provost's Diversity Fellow in 2016-2017 and a Graduate School Dean's Scholar.

Srabani plans to carry on her academic focus in environmental and soil sciences and hopes her passion for environment and sustainable development will guide her to engage in meaningful work in this area.

For my father

ACKNOWLEDGMENTS

My heartfelt thanks to Tammo Steenhuis for being my Advisor. Thanks to him for his guidance, help, support, confidence, and for being the best mentor ever. Many thanks to Brian Richards for taking me under his tutelage, inducting me into the project, initiating me to hands-on research and data analysis, providing day to day guidance, helping to plan and complete all my experiments, being there for all field activities, for his unending patience and for help with expanding vocabulary! It has been a great opportunity to learn from the finest of teachers, Johannes Lehmann. Many thanks for his patience and continuous help to bring out the best. Many thanks to Todd Walter for his friendly words, support and constant enthusiasm. While I have been extremely lucky for the opportunity to work with such exceptional scientists and gain immense intellectual insight, it also has been fun interacting with such fantastic people.

My heartfelt thanks to Jennifer Blesh for being instrumental to get back to the PhD program and being an extraordinary mentor and friend. I am also indebted to Darlene Campbell and the Office of Undergraduate Biology for hiring me and helping to complete this program. I am grateful to Mike Walter for admitting me to this program and for his help over the years. Thanks to Sarah Zayarath Hernandez for supporting this work.

Many thanks to Kevin Packard and Francoise Vermeylen for their help and patience, which has been crucial for completing this work. Thanks to Bob Schindelback, Mike Rutzke, Kelly Hanley, Heather Scott and Kim Sparks for their help and assistance with lab work and for answering numerous questions.

The best part of research was probably participating in all the massive N_2O chamber campaigns at the CRC site - Brian moderated, Cathjeline executed, walkie-talkie enabled, military-style field operations with Cedric, Ryan, Jamie, Ani and the entire Bioenergy Team. Thanks for including me in the excitement and for your help! Many thanks to all Bioenergy project assistants and summer interns for their immense help and contribution towards all pieces of this work - Karin, Irwin, Max, Sarah, KB, Dhanya, Wenjie, Anita, Rachael, Lara, and Cristina.

Many thanks to my colleagues and friends Allison, Erin, Sheila, Lauren, Chelsea, Bahar, Christian, Jiang, Tigist, Shree, Larry, Steve, Brenda, Leilah, Maria, Anne, Biz, Ali, Marissa, James, and others. Thanks to my friends in Ithaca and Rochester, Tonima, Grace, Kamal auntie, Anila, and Shivani for being there for me. Many thanks to Biksham, Sanjoy, Madhumita, Ritu, Manish and Ina for always supporting me to pursue a career and for being pillars of strength. Thanks also to others not mentioned here, who have supported me over the years.

Many thanks to my husband Prakash, for his continued patience and taking the best care of our children. Thanks to Mahika and Ruthvik for being the bravest kids and being part of this journey from their womb days! My heartfelt thanks to all my babysitter friends and to Dr. Ash Lal and Dr. Craig Mullen for all their support. Nothing would have been possible without the life-support and blessings of my parents Mrs. Sova Das and Dr. P.K. Das and my brother Spandan, his wife Sudeshna, and his family. Thanks to them for their unswerving confidence and for being part of all tears and joys. And Thank you God.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Active carbon: permanganate-oxidizable (labile) carbon, POXC

C3 grass: Cool-season grass with C3 photosynthetic pathway

C4 grass: Warm-season grass with C4 photosynthetic pathway

CTRL: fallow control

LOI: loss-on-ignition assay

Q1: soil moisture/wetness quintile 1, Q2: soil moisture/wetness quintile 2, Q3: soil moisture/wetness quintile 3, Q4: soil moisture/wetness quintile 4, Q5: soil moisture/wetness quintile 5

RCG: reed canarygrass + fertilizer N treatment

SOC: soil organic carbon

SWG: switchgrass treatment

SWGN: switchgrass +fertilizer N treatment

Crop treatment and cropping system have been used interchangeably

Moisture quintile and wetness group/level have been used interchangeably

CHAPTER 1

INTRODUCTION

Perennial bioenergy crops are projected to reduce US reliance on fossil fuels, bring down greenhouse gas emissions and enhance rural economies. In the Northeast US, soil moisture status renders about 20% soils poorly or very poorly drained (Stout *et al.,* 1994, Stoof *et al.,* 2014). With perennial bioenergy crops poised to contribute significantly towards future energy needs of the region, there is an increased interest in converting such wet marginal lands to bioenergy cropping systems. Land use change through such agricultural conversion can significantly alter carbon (C) cycling processes in these agroecosystems.

Though the capacity of perennial grasses to tolerate (and potentially improve) suboptimal soil properties, reduce erosion, sequester soil organic carbon (SOC), and improve soil biodiversity are well-known (McLughlin and Walsh, 1998; Lemus and Lal, 2005; Blanco-Cancui, 2010; Schmer *et al*., 2011), research on the impacts of dedicated bioenergy perennial crops at the field scale under varied soil conditions is yet needed. Several soil physical, chemical and biological parameters are commonly used as indicators of soil health (Moebius-Clune *et al*., 2016). The impact of soil moisture conditions, vegetation and land use changes on soil parameters warrant further detailed studies.

Soil respiration represents one of the largest C fluxes in the global C cycle, ten times larger than the CO_2 released from fossil fuel burning (Raich and Tufekcioglu, 2000). Soil respiration consists of an autotrophic component from roots and their associated rhizosphere and a heterotrophic component from free-living soil micro-and macro organisms that decompose SOC (Kutsch *et al.,* 2009; Stockmann *et al*., 2013). While initial decomposition rates of plant residues in surface soils correlate with indices of bulk chemical composition of plant materials, long-term stabilization of OM is essentially an ecosystem property (Schmidt *et al.,* 2011), much under the control of parameters such as soil moisture. While temperature impacts on SOC are better understood, processes relating soil moisture to SOC stabilization are poorly understood and poorly quantified.

Chapter 2 examines the impact of conversion (via tillage) of the previously fallow field to perennial grass, over a four-year period after bioenergy cropping was established. Specifically, we investigated the effect of soil moisture and vegetation on soil properties including organic matter (OM), active carbon, wet aggregate stability, soil pH, SOC, and total nitrogen (TN). In addition, the relationships between the various soil properties and crop parameters (cumulative yields and root biomass) were evaluated.

Chapter 3 examines the influence of surface volumetric water content and perched water table depth on the distribution of OM, SOC, TN, C/N ratio, bulk density, and $\delta^{13}C$ for six depth increments to 120 cm, in the bioenergy field that was for decades minimally-managed before conversion to perennial grasses. Additionally, we tested the suitability of using loss-on-ignition (LOI) for soil analytical studies by comparing it to dry combustion elemental C analysis.

Chapter 4 provides a mechanistic understanding of the role of moisture on carbon mineralization for soils that were exposed to different moisture levels. With a 371-day incubation experiment, we investigated if legacy effects from different field moisture levels (viz plant above-ground /below-ground biomass inputs or SOC stabilization and therefore the form of SOC) determined the subsequent C mineralizability under varying incubation moisture contents.

REFERENCES

Kutsch WL, Bahn M, Heinemeyer A (2009) Soil carbon relations:an overview In: *Soil carbon dynamics: an integrated methodology* (eds Kutsch WL, Bahn M, Heinemeyer A), pp. 1-2, Cambridge University Press, Cambridge, UK.

Lemus R, Lal R (2005) Bioenergy crops and carbon sequestration. *Critical Reviews in Plant Science,* 24, 1–21.

McLaughlin SB, Walsh ME (1998) Evaluating environmental consequences of producing herbaceous crops for bioenergy. *Biomass and Bioenergy,* 14, 317-324.

Moebius-Clune BN, Moebius-Clune DJ, Gugino BK, Idowu OJ, Schindelbeck RR, Ristow AJ, van Es HM, Thies JE, Shayler HA, McBride MB, Wolfe DW, Abawi GS (2016) Comprehensive Assessment of Soil Health. In: *The Cornell Framework Manual: Third Edition,* pp. 51-52, Cornell University, Geneva, United States.

Raich JW, Tufekcioglu A (2000) Vegetation and soil respiration; correlations and controls, *Biogeochemistry* 48,71-90.

Schmer MR, Liebig MA,Vogel KP, Mitchell RB (2011) Field-scale soil property changes under switchgrass managed for bioenergy. *Global Change Biology Bioenergy,* **3**, 439–448.
Raich,J.W. and Tufekcioglu, A. 2000 Vegetation and soil respiration; correlations and controls, *Biogeochemistry* 48,71-90.

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens I, Kleber, M, Kogel-Knabner I, Lehmann J, Manning DAC, Nannipieri, P, Rasse DP, Weiner S, Trumbore S (2011) Persistence of soil organic matter as an ecosystem property. *Nature.* http://dx.doi.org/10.1038/nature10386.

Stockmann U, Adams M, Crawford J, Field, D, Henakaarchchi N, Jenkins M, Minasny B, McBratney, A, De Remy De Courcelles V, Singh K, Wheeler I, Abbott L, Angers DA, Baldock J, Michael Birde M, Brookes PC, Chenug C, Jastrow JD, Lal R, Lehmann J, O’Donnell AG, Partonl WJ, Whitehead D, Zimmermannn M (2013) The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems and Environment*, 164, 80-99.

Stoof CR, Richards BK, Woodbury PB, Fabio PB, Brumbach ES, Cherney J, Das S, Geohring L, Hansen J, Hornesky J, Mayton H, Mason C, Ruestow G, Smart LB, Volk TA, Steenhuis TS (2014) Untapped potential: Opportunities and challenges for sustainable bioenergy production from marginal lands in New York and the Northeast. *Bioenergy Research*, 8, 482-501. DOI: 10.1007/s12155-014-9515-8

Stout W (1994) Soil drainage influence on biomass and nitrogen accumulation by ryegrass. *Agronomy Journal*, **86,** 111-116.

CHAPTER 2

PERENNIAL GRASS BIOENERGY CROPPING ON WET MARGINAL LAND: IMPACTS ON SOIL PROPERTIES, SOIL ORGANIC CARBON AND ABOVE-GROUND BIOMASS DURING INITIAL ESTABLISIHMENT.[1]

Abstract

The control of soil moisture, vegetation type and prior land-use on soil health parameters of perennial grass cropping systems on marginal lands is not well known. A fallow wetness-prone marginal site in New York (USA) was converted to perennial grass bioenergy feedstock production. Quadruplicate treatments were fallow-control, reed canarygrass (*Phalaris arundinaceae* L. Bellevue) with nitrogen (N) fertilizer (75 kg N ha^{-1}), switchgrass (*Panicum virgatum* L. Shawnee), and switchgrass with N fertilizer (75 kg N ha^{-1}). Based on periodic soil water measurements, permanent sampling locations were assigned to various wetness groups. Surface soil (0-15 cm) organic matter (OM), active carbon, wet aggregate stability, pH, soil organic carbon (SOC), total nitrogen (TN), root biomass and harvested above-ground biomass were measured annually (2011-2014). Multi-year decreases in OM, wet aggregate stability and pH followed plowing in 2011. For all years, wettest soils had the greatest OM, SOC, and active carbon, while driest soils had the greatest wet aggregate stability and lowest pH. In 2014, wettest soils had significantly ($p<0.0001$) greater SOC and TN than drier soils, and fallow soils had 14% to 20% greater SOC than soils of reed canarygrass+N, switchgrass, and switchgrass + N. Crop type and N fertilization did not result in significant differences in SOC, OM, active carbon or wet aggregate stability. Cumulative three-year above-ground biomass yields of driest switchgrass+N soils (18.8 Mg ha^{-1}) were 121% greater than the three wettest switchgrass (no N) treatments.

[1] Manuscript submitted to *BioEnergy Research* BERE-S-17-00301-1, Srabani Das, Karin Teuffer, Cathelijne R. Stoof, M. Todd Walter, Mike F. Walter, Tammo S. Steenhuis, Brian K. Richards*

Overall, soil moisture status must be accounted for when assessing soil dynamics during feedstock establishment.

Keywords Wetness-prone marginal land, bioenergy cropping, soil organic carbon(SOC), active carbon, wet aggregate stability, above-ground biomass.

Introduction

Perennial bioenergy crops are projected to reduce US reliance on fossil fuels, reduce greenhouse gas emissions, and enhance rural economies. In the Northeast US (NE), fallow marginal lands are cited as a primary resource base for the perennial bioenergy sector (Stoof *et al*., 2014; Baxter and Calvert, 2017). Many soils in this region are not well-suited (hence marginal (Richards *et al.*, 2014)) for row crop agriculture due to seasonal water saturation or near-saturation, which commonly results from presence of shallow restrictive layers. Soil moisture status renders about 20% of NE soils poorly or very poorly drained (Stout *et al*., 1994). Though the capacity of perennial grasses to tolerate (and potentially improve) suboptimal soil properties, reduce erosion, sequester soil organic carbon (SOC), and improve soil biodiversity are well-known (McLaughlin and Walsh, 1998; Lemus and Lal, 2005; Blanco-Cancui, 2010; Schmer *et al*., 2011), research on the impacts of dedicated bioenergy perennial crops at the field scale under varied soil conditions is yet needed. Additionally, there is little representation of the Northeast US in most reviews of C sequestration potential of bioenergy crops (Blanco-Canqui, 2010; Skinner *et al.*, 2012). The extent of potential carbon (C) sequestration as a ecosystem service of perennial bioenergy systems is dependent on soil texture, initial SOC, climate, drainage, cropping history and management practices (Skinner *et al.*, 2012). Though conversion of croplands to grassland (pastures or bioenergy plantations) results in accumulation of SOC (Knops & Tilman, 2000; Schnabel *et al*., 2001; Guo and Gifford, 2002; Hansen *et al*., 2004; Grandy & Robertson, 2007), an initial loss of SOC is observed when previously uncultivated fallow lands are converted to perennial grasses (Davidson and Ackerman 1993; Corre *et al*.,1999; Anderson-Teixeira *et al*., 2009; Skinner *et al.,* 2012; Stockmann *et al*., 2013). Cultivation of undisturbed/untilled soils usually depletes organic carbon stocks by releasing stored carbon to the atmosphere and hence impacts both CO_2 fluxes and soil fertility (Burke *et al.*, 1995; Grandy and Robertson, 2007). Tillage results in significant fracturing of protective soil aggregates and peds, mixing of soil horizons, disruption of plant roots and mycorrhizae, and increased priming effects. This incurred "carbon debt" is often repaid over a period of years or decades before any net sequestration occurs.

Soil moisture is one of the most important environmental controls of plant growth (Sims & Singh, 1978; Sala *et al*., 1988; Knapp *et al*., 1993; Rodriguez-Iturbe & Poporato, 2004) and soil microbial activity, hence affecting both C inputs and outputs of soil (Linn & Doran, 1984; Raich & Schlesinger, 1992; Parton *et al*., 2007; O'Brien *et al*., 2010). As the rate of decomposition relative to production is low in cold and wet climates, soil C stocks are globally greater in such areas (Trumbore, 1997; Jobba´gy and Jackson, 2000; Fissore *et al*., 2008; Moyano *et al*., 2013). Though its overall control for buildup in grasslands is well established (Jelinski and Kucharik, 2009; O'Brien *et al*., 2010), site specific variations in drainage impacting SOC and TN accrual in perennial grasslands need to be determined.

Switchgrass (*Panicum virgatum* L) is a common warm-season (C4) perennial selected as a model system for cellulosic biomass production. Apart from placing organic C in deeper soil layers due to rooting that is deeper than C3 grasses, switchgrass is thought to increase the proportion of stable soil aggregates, which aids in long term C sequestration (Ma *et al.,* 2000). Reed canarygrass (*Phalaris arundinacea* L) is a herbaceous cool-season (C3) perennial long used for forage production in wetness-prone soils. Because of its adaptation to wet conditions, it is regarded as an invasive species in riparian fringes and wetlands throughout North America (Miller and Dickerson, 1999). Its capacity to accumulate large amounts of C in biomass within a short growing season is thought to cause significant alterations in SOC dynamics (Bills, 2008; Shurpali *et al*., 2008). Due to the differing mechanisms of carbon fixation of the two photosynthetic pathways (Hatch-Slack cycle [C4 plants] vs Calvin cycle [C3 plants]), warm-season C4 plants have approximately 50% greater photosynthetic efficiency than cool-season C3 plants (Wang *et al.*, 2012). It is generally assumed that C4 grasses will have greater long-term C sequestration rates due to below-ground productivity that is greater than C3 species (Knops and Tilman, 2000; Kucharik *et al*., 2001; Baer *et al.*, 2002; Camill *et al.*, 2004; Ampleman *et al.*, 2014).

Work by Corre *et al.* (1999) at six sites in the Northeast US showed that SOC in moderately drained soil was influenced by stand age and temperature variations when C3 and C4 grasses were grown on fallow lands. However, there was no significant difference in SOC values between C3 and C4 grasses at all depths, and conversion of C3 to C4 grasslands resulted in SOC loss that required 16-18 years to recover. Studies with pasture mixtures (perennial C3/C4 grasses and legumes) in well-drained soils in Pennsylvania showed no relationship between changes in soil C and root biomass (Skinner, 2006) between 5 and 60 cm. In southeastern Pennsylvania (Sanderson, 2008), conversion of hayfield/pastureland to switchgrass (and subsequent grazing activities) on well drained, deep soils did not result in any change (minor loss) in net SOC after 5 years at soil depth increments of 0-5, 5,15 and 15-30 cm depth and there were no differences in SOC levels under different cultivars.

Given that inter-annual changes are small in comparison to the large reservoir of SOC (Powlson *et al*., 1987; Kutsch *et al.*, 2009), SOC shifts are difficult to measure in the short-term. However, the rapid, simple, and inexpensive permanganate-oxidizable carbon assay (more commonly known as active carbon, sometimes abbreviated as POXC) estimates a processed and stabilized pool of labile soil C derived from total microbial biomass (Culman *et al*., 2012; Culman *et al*., 2013; Geng *et al*., 2014; Mizin, 2014). As such, it is sensitive to management and/or environment (Weil *et al*., 2003; Culman *et al*., 2012; Lucas and Weil, 2012; Mizin, 2014; Tiemann and Grandy, 2015) and may be effective over shorter timeframes as a leading indicator of SOC trends and is accordingly used in soil health analytical assessments (Moebius-Clune *et al*., 2016).

Perennial cropping promotes increased root production and exudates which are thought to promote aggregate formation and SOC stabilization. With reorientation of soil particles through physical and biological (Six *et al*. 2002, Jastrow *et al*., 2007; Tiemann and Grandy, 2015) disturbances, SOC becomes more protected within aggregates. The extent to which soil aggregates resist disintegration when wetted via simulated rainfall is measured as the wet aggregate stability of the soil, which is also often used as a soil health indicator (Moebius-Clune *et al*., 2016). It is a key factor of soil resistivity to mechanical stresses and is correlated to soil organic matter content (Cañasveras *et al.*, 2010; Tiemann and Grandy, 2015).

In this perennial grass field study, we used four years (2011, 2012, 2013 and 2014) of soil properties data (OM, active carbon, wet aggregate stability, soil pH; also, SOC and TN in 2014) and cumulative above-ground biomass yields to explore 1) the impact of the conversion via tillage of the previously fallow field to perennial grass during the early establishment phase, and 2) the degree of soil moisture and vegetation control on soil properties. Finally, the association of soil and crop productivity parameters (cumulative above-ground biomass and root biomass) were also evaluated. Above-ground biomass yields and below-ground root biomass were measured, but three year cumulative values were used for analyses, given that switchgrass typically reaches full yield potential only after several growing seasons (McLaughlin and Kszoz, 2005; Parrish and Fike, 2005).

We hypothesized that a) there would be loss in OM due to the initial plowing, b) that soil properties and above-ground biomass yields would be influenced by moisture gradient of the field, and c) that at this early stage there would be no detectable differences in OM or SOC levels because of plant photosynthetic pathway (C3 vs. C4) or use of N fertilization.

Methods

Experimental site The primary research site was a 10ha field at Ithaca, New York, USA (42N28.20', 76W25.94') (Fig.2.1a) with a predominant drainage catena comprised of three soil series: well-drained Canaseraga (coarse-silty, mixed, active, mesic Typic Fragiudept), somewhat poorly drained Dalton (Coarse-silty, mixed, active, mesic Aeric Fragiaquept), and poorly drained Madalin (fine, illitic, mesic Mollic Endoaqualf). Small areas of associated Rhinebeck (fine, illitic, mesic Aeric Endoaqualfs) and Langford (fine-loamy, mixed, active, mesic Typic Fragiudepts) soils were also present. As reflected in their taxonomic classifications, the Dalton, Canaseraga and Langford series silt loams characterized by a dense subsoil fragipan (Fig.2.1b). The field topography is undulating, with slopes in the sampled areas varying from 0 and 8%, with a small area with short slopes up to 15% on the eastern edge of the field (Fig.2.1b). Perched water tables recur seasonally, resulting from shallow restrictive layers (fragipan and/or dense basal till). At such spots, significant quantities of lateral interflow atop the restrictive layer saturate depressions and concave areas (Steenhuis *et al*., 1995; Zollweg *et al*., 1996; Frankenberger *et al*., 1999; Walter *et al*., 2000), a characteristic of many wetness-prone soils of the region. The field is marginal for row crop or alfalfa production (Richards, *et al*., 2014) due to this recurring wetness in many areas. Before perennial grasses were established in July 2011, the field had effectively been fallow for circa 50 years, with occasional mowing (and rarely hay harvest) used to prevent reversion to shrub and tree growth. The site's fallow vegetation in 2011 was dominated by legacy reed canarygrass and mixed forbs, including goldenrod (*Solidago* sp.) and, in the wettest areas, hemp dogbane (*Apocynum cannabinum*).

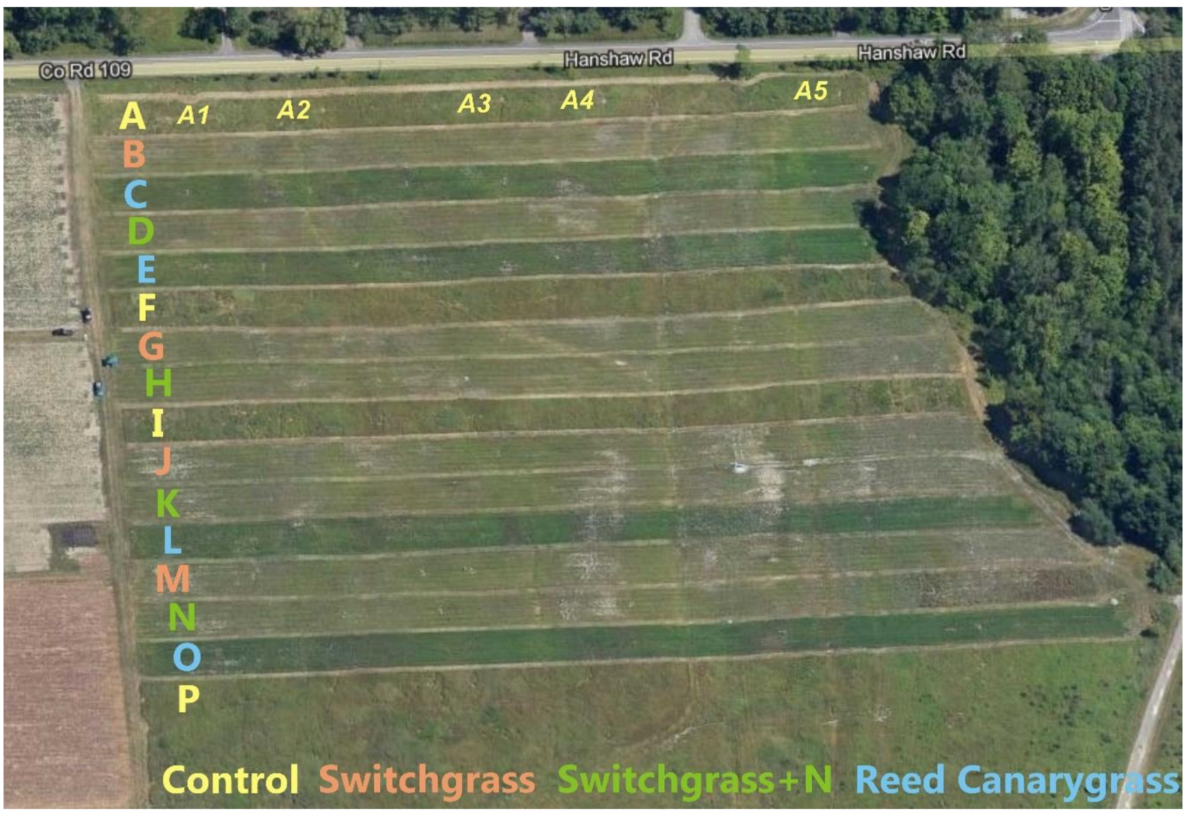

Figure 2.1a. Field site layout depicting strip plots: fallow-control (A, F, I, P); switchgrass (B, G, J, M); switchgrass + fertilizer 75 kg N ha^{-1} (D, H, K, N), reed canarygrass + fertilizer 75 kg N ha^{-1} (C, E,L,O). Modified from July 2012 GoogleEarth image.

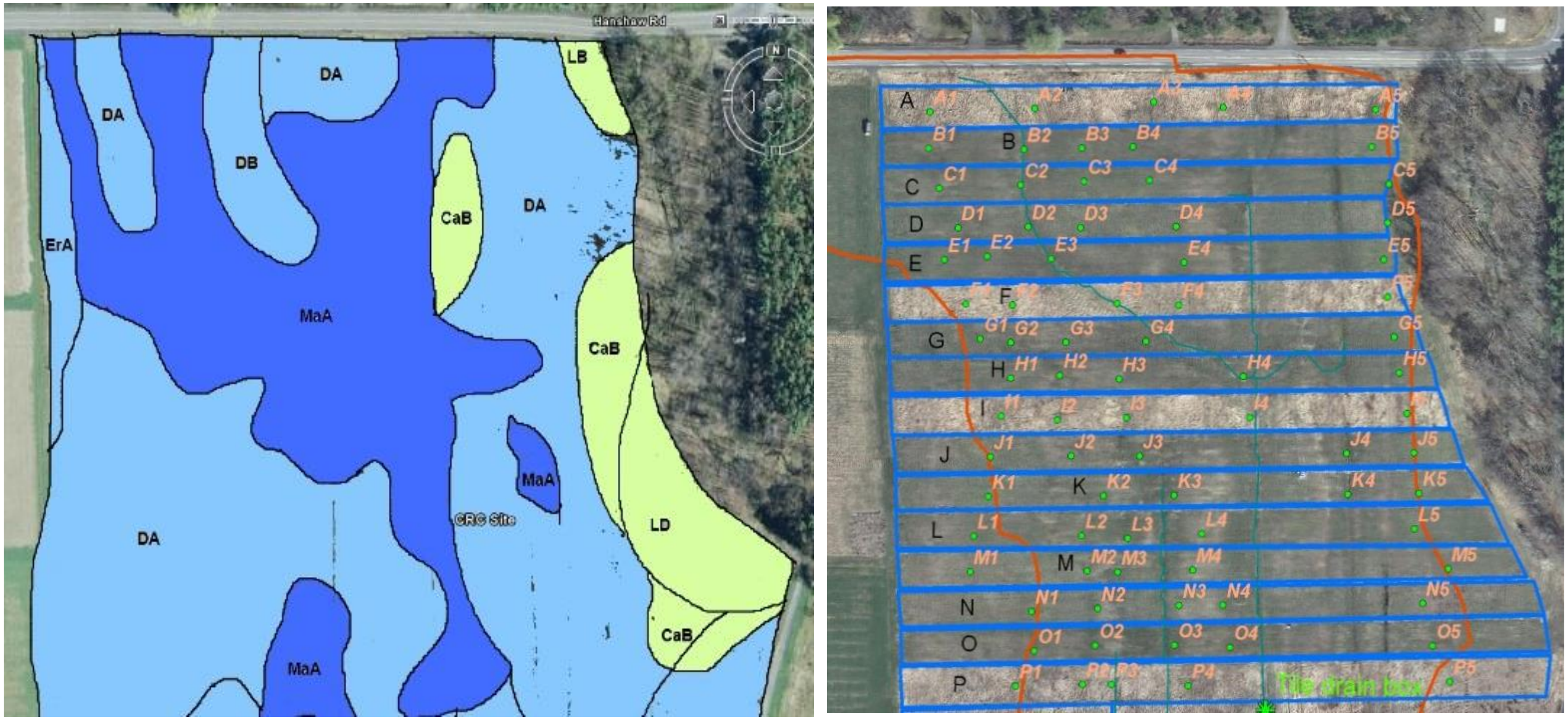

Figure 2.1b. Soil series *(left)* and soil wetness-targeted distribution of the sampling subplots *(right)* denoted with suffixes 1 through 5. Soil series mapping adapted from Cline and Bloom, 1965: CaB – Caneseraga 3-8% slopes, DA – Dalton 0-3% slopes, DB – Dalton 3-8% slopes LB – Langford 3-8% slopes, LD – Langford 8-15% slopes, MaA – Madalin 0-3% slopes. (Yellow –moderately well-drained; light blue – somewhat poorly drained; dark blue – poorly drained.)

Table 2.1 Monthly total rainfall (mm) during the growing season for switchgrass and reed canarygrass (Mar–Sep) for the years 2011-2014.

Monthly total rainfall (mm)							
Year	**March**	**April**	**May**	**June**	**Jul**	**Aug**	**Sep**
2011	92	188	170	66	51	118	266
2012	44	81	74	47	40	91	97
2013	30	77	59	105	177	133	96
2014	77	62	113	131	98	154	56
29 yr. mean	67	84	81	101	97	92	94

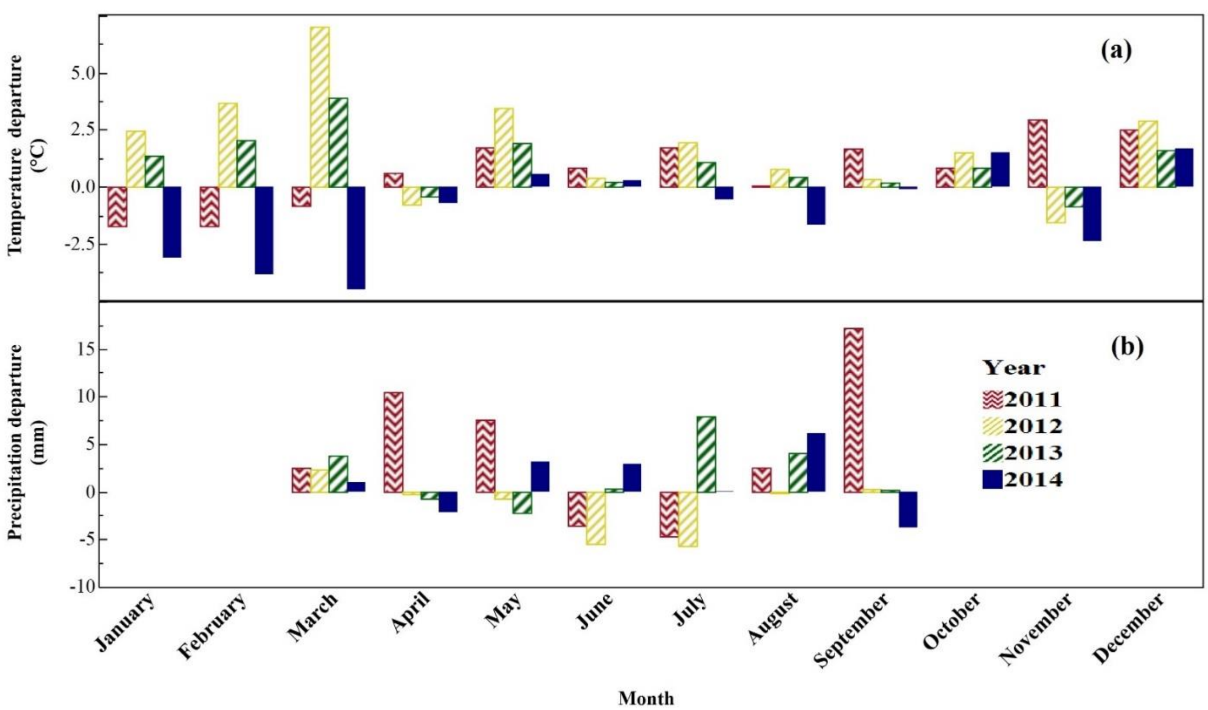

Figure 2.2. Monthly departure of a) air temperature and b) precipitation from the 29-year mean (1981-2010) values for the four-year study period (2011-2014) at Cornell University, Ithaca, obtained from the Northeastern Regional Climate Center database. The precipitation data spans the growing season for both reed canarygrass and switchgrass (March-September).

The total monthly precipitation for the study period (2011 through 2014) is shown in Table 2.1, while the total departure from 29-year average values is seen in Figures 2.2b (precipitation) and

2.2a (temperature). The crops were not irrigated. Subsurface tile drains (paralleling the main and lateral legacy surface drainage swales, Fig.2.1a) were installed in June 2011 to help ensure trafficability of the wettest areas at harvest.

A randomized complete block design was used for 16 large strip plots (denoted A through P, Fig. 2.1a, 2.1b) that comprised quadruplicate plots of the four cropping treatments: switchgrass (v. Shawnee, a selection from upland ecotype Cave-in-Rock) (SWG), switchgrass+ fertilizer N (SWGN), reed canarygrass (cv. Bellevue) +fertilizer N (RCG), and pre-existing fallow control (CTRL). Where used, the N fertilization rate was 75 kg N ha^{-1} of ammonium sulfate ($(NH4)_2SO_4$), surface-applied once annually in the spring, starting in 2012 for reed canarygrass + fertilizer and delayed (as is typical for slow-to-establish switchgrass) until 2013 for SWGN. Concurrent research at the site monitored nitrous oxide emissions from these same treatments (Mason *et al*., 2016, 2017).

Sampling subplots The trial is unique as the strip plots (each between 0.34-0.44 ha) were intentionally laid out to capture the continuum of soil moisture conditions that vary naturally from moderately well-drained to poorly drained. In June 2011, the fallow land was prepared for perennial grass production by successively mowing, spraying regrowth with glyphosate herbicide, conventional moldboard plowing, disking, and harrowing prior to planting. Five permanent sampling subplots were established along the natural moisture gradients of each strip-plot (Fig.2.1b) based on an initial intensive survey (June 1-2, 2011) of surface layer volumetric water content measurements by time-domain reflectometry (TDR, Campbell Scientific Hydrosense meter using 12 cm sensor rods). This subplot approach thus yielded eighty permanent sampling points where soil (including SOC and other soil health parameters) and harvested above-ground biomass yields (among other parameters) are sampled yearly. Frequent periodic surface soil moisture measurements (by TDR) were used to characterize the relative soil moisture status of each subplot. For each measurement event, a field average volumetric water content of all 80 subplots was calculated, and each subplot's value was normalized relative to the field mean (yielding a "relative soil moisture ratio" for that subplot and time point). These relative values were averaged over the entire study period for each subplot, and each subplot's characteristic wetness (relative to the field average) was thus established over 40 such measurement events cumulatively representing thousands of readings at the site. Finally, these multi-years mean values for the 80 subplots were aggregated into "soil wetness quintiles" for the entire study period (Table 2.3).

Therefore, 20 subplots of each cropping system and 16 subplots of each wetness quintile (Table 2.2a) formed the basis of comparison in this study. Given that soil moisture did not vary uniformly among the total population of subplots as divided among cropping treatments, *N* for each wetness quintile under a cropping system varied between 2 and 6 (Table 2.2b). Thus, the unintended consequence of incorporating greater precision to predict wetness and crop interaction, was an unbalanced sample size for each wetness quintile (Table 2.2b). The driest subplots represented in wetness quintile 5 (Q5) had mean volumetric water contents approximately 0.8 times the field mean, whereas the more variable wettest subplots, wetness quintile 1 (Q1) averaged 1.3 times the field mean (Richards *et al*., 2013). These quintiles were used for categorizing soil moisture status with respect to other parameters.

Calculated for one sampling event (Aug 2014) but not shown were corresponding values in gravimetric units, based on a subset of soils collected and analyzed for a measurement event in 2014. With these data, the volumetric water content values (TDR) were regressed against values of the gravimetric water content, and the equation was used to compute the mean water content values corresponding to the high, mid and low TDR moisture quintiles from the field. The multi-year mean growing season gravimetric water contents corresponding to Q1 (highest), Q3 and Q5 (lowest) were 0.5, 0.4, and 0.3 g g^{-1} respectively. As the gravimetric soil moisture equivalent from a single time point would not be able to correctly reflect the variability of the soil moisture content over the study period, we have instead used the quintile-based relative volumetric wetness to compare SOC levels.

Table 2.2a. Distribution schematic for the for the 80 subplots of the quadruplicate cropping systems spanning over fallow-control (CTRL), reed canarygrass + fertilizer 75 kg N ha^{-1} (RCG), switchgrass (SWG) and switchgrass + fertilizer 75 kg N ha^{-1}(SWGN) and the wetness quintiles (Q1 wettest-Q5 driest, water content variation between 0.3 g g^{-1} and 0.5 g g^{-1})

Distribution of subplots at the field site								
		Cropping system		Multi-year wetness quintile rank (Q1, wettest - Q5, driest)				
CTRL	RCG	SWG	SWGN	Q1	Q2	Q3	Q4	Q5
20	20	20	20	16	16	16	16	16

Table 2.2b. Distribution schematic of the number of subplots of each wetness quintile (Q1 wettest-Q5 driest, water content variation between 0.3 g g^{-1} and 0.5g g^{-1}) for each cropping system, fallow-control (CTRL), reed canarygrass + fertilizer 75 kg N ha^{-1} (RCG), switchgrass (SWG) and switchgrass + fertilizer 75 kg N ha^{-1}(SWGN)

Multi-year wetness quintile rank	**Distribution of wet quintile representatives for each cropping system**			
(Q1, wettest- Q5, driest)	**CTRL**	**RCG**	**SWG**	**SWGN**
Q1	5	3	4	4
Q2	6	2	4	4
Q3	4	5	3	4
Q4	2	6	5	3
Q5	3	4	4	5

Sampling protocol Soil sampling in June 2011 took place prior to any tillage and thus represented the preconversion fallow state. Soils from the surface Ap layer were sampled each year in June/July. A flat shovel was used to dig to 15 cm depth at two locations equidistant (1.2 m) from the center of each subplot (as marked by a permanent subplot flag). Approximately 4 kg of soil was dug from each of the two locations, mixed and composited in a bucket, with circa1 kg transferred to labeled polyethylene bag. The soils were initially air dried and later oven dried at 55°C for several days until constant weight was achieved. The soil samples were stored for later processing.

Biomass sampling For this study, above-ground yield from each subplot was determined for the years 2012, 2013 and 2014 using hand-harvesting of replicate 1 m^2 quadrants, followed by crop vs. weed separation, weighing and dry matter analysis. Results were added to obtain a cumulative yield value for each subplot used in the analysis. After drying, each bag of soil was weighed, and coarse roots were removed by hand picking and kept separately. Root crowns were not sampled. The roots were then passed through a 2mm-sieve to remove the associated dirt particles and then weighed. This procedure (of non-washing and handpicking) was undertaken to preserve soil samples for further analysis, avoiding destructive processing. This approach recovers ~60% of the root mass typically obtained through more extensive soil washing techniques (Matamala *et al*., 2008). These conservative underestimates have been used for correlations with soil properties. The coarse root biomass estimation from each subplot was thus undertaken for the years 2012, 2013 and 2014. Subsequently, the cumulative root biomass for each subplot was obtained by adding the values from each year.

Table 2.3. All-year wetness quintile ranks of the 80 subplots based on numerous measurement events; field average volumetric water content of all 80 subplots calculated during each measurement event and each subplot's value normalized relative to field mean (yielding a "relative soil moisture ratio" for that subplot and time point). These relative values were averaged over the entire study period for each subplot, and each subplot's all-year characteristic wetness (relative to the field average) was thus established. Finally, the multi-year mean values for 80 subplots were aggregated into "soil wetness quintiles" for the entire study period. The four cropping systems are fallow-control (CTRL), reed canarygrass + fertilizer 75 kg N ha^{-1} (RCG), switchgrass (SWG) and switchgrass + fertilizer 75 kg N ha^{-1}(SWGN). The wetness quintile ranks are Q1, Q2, Q3, Q4 and Q5(Q1, wettest, Q5 driest).

Strip	Subplot	Cropping system	All-year, all-field mean volumetric water content (normed)	Std. dev. (normed)	All-year, all-field, wetness quintile rank	Strip	Subplot	Cropping system	All-year, all-field mean volumetric water content (normed)	Std. dev. (normed)	All-year, all-field, wetness quintile rank
A	1	CTRL	1.0	0.1	Q2	**I**	1	CTRL	0.9	0.1	Q5
	2	CTRL	1.4	0.2	Q1		2	CTRL	1.0	0.2	Q2
	3	CTRL	1.1	0.1	Q2		3	CTRL	1.0	0.2	Q2
	4	CTRL	1.2	0.2	Q1		4	CTRL	1.2	0.1	Q1
	5	CTRL	0.9	0.1	Q3		5	CTRL	0.7	0.1	Q5
B	1	SWG	1.0	0.1	Q2	**J**	1	SWG	0.8	0.1	Q4
	2	SWG	1.4	0.4	Q1		2	SWG	1.0	0.1	Q3
	3	SWG	0.9	0.1	Q4		3	SWG	1.2	0.1	Q1
	4	SWG	1.1	0.1	Q2		4	SWG	1.6	0.3	Q1
	5	SWG	1.0	0.1	Q4		5	SWG	0.8	0.1	Q5
C	1	RCG	1.1	0.1	Q1	**K**	1	SWGN	0.7	0.1	Q5
	2	RCG	1.4	0.3	Q1		2	SWGN	0.9	0.1	Q3
	3	RCG	0.8	0.1	Q5		3	SWGN	1.0	0.1	Q2
	4	RCG	1.0	0.1	Q3		4	SWGN	1.1	0.2	Q1
	5	RCG	0.9	0.1	Q4		5	SWGN	0.8	0.1	Q5
D	1	SWGN	1.1	0.1	Q2	**L**	1	RCG	0.9	0.1	Q4
	2	SWGN	1.5	0.4	Q1		2	RCG	0.9	0.1	Q4
	3	SWGN	0.9	0.1	Q4		3	RCG	1.0	0.1	Q3
	4	SWGN	0.9	0.1	Q3		4	RCG	0.8	0.1	Q5
	5	SWGN	1.1	0.2	Q2		5	RCG	0.8	0.1	Q5
E	1	RCG	0.9	0.1	Q4	**M**	1	SWG	0.8	0.1	Q5
	2	RCG	1.1	0.1	Q2		2	SWG	1.1	0.1	Q2
	3	RCG	1.2	0.2	Q1		3	SWG	1.0	0.1	Q3
	4	RCG	0.9	0.1	Q4		4	SWG	0.8	0.1	Q5
	5	RCG	1.0	0.1	Q3		5	SWG	0.8	0.1	Q5
F	1	CTRL	0.8	0.1	Q4	**N**	1	SWGN	0.9	0.1	Q4
	2	CTRL	1.0	0.1	Q2		2	SWGN	1.1	0.1	Q1
	3	CTRL	1.2	0.1	Q1		3	SWGN	0.8	0.1	Q5
	4	CTRL	1.0	0.1	Q3		4	SWGN	1.0	0.1	Q3
	5	CTRL	1.1	0.2	Q2		5	SWGN	0.9	0.1	Q4
G	1	SWG	0.9	0.1	Q4	**O**	1	RCG	0.7	0.1	Q5
	2	SWG	1.0	0.1	Q3		2	RCG	1.1	0.1	Q2
	3	SWG	1.1	0.1	Q2		3	RCG	1.0	0.1	Q3
	4	SWG	1.3	0.1	Q1		4	RCG	1.0	0.1	Q3
	5	SWG	0.9	0.1	Q4		5	RCG	0.9	0.1	Q4
H	1	SWGN	0.8	0.1	Q5	**P**	1	CTRL	1.0	0.1	Q3
	2	SWGN	1.0	0.1	Q3		2	CTRL	1.0	0.1	Q3
	3	SWGN	1.1	0.1	Q2		3	CTRL	1.2	0.1	Q1
	4	SWGN	1.3	0.2	Q1		4	CTRL	0.9	0.1	Q4
	5	SWGN	0.8	0.1	Q5		5	CTRL	0.8	0.1	*Q5*

Laboratory analyses Each soil sample (dried, with roots removed) was passed through a soil mill (Dynacrush DC-5 Soil Crusher, Custom Laboratory Eqpt, Orange City, Florida) with 10 mesh sieve (2mm openings) three times before being finally sieved with a 1.8 mm sieve, in accordance with Garten and Wullschleger (1999). The coarse fragments were then weighed to help when converting analytical results (which based on fine soil only) to a realistic areal basis, since said fragments remain present during bulk density determinations. The mineral soil fraction (including any fine roots that passed the sieve) was thus prepared and elemental C and N analysis was carried out on oven dried (60°C) 0.4 g soil samples by combustion infrared detection [LECO TruMac CN, (LECO Corp, St. Joseph, MI) with analytical precision of 0.01 mg or 0.3% RSD (whichever is greater) for N and 0.01mg or 0.4% RSD for C (whichever is greater)]. Without access to LECO during the first year, the loss on ignition (LOI) method was used to estimate OM for 2011 samples, and OM estimations for all years are also reported here. For LOI, mass losses from ignition (2 h at 500°C) are determined gravimetrically on oven dried (105°C for at least 4 h) ground and sieved soil samples. New York State empirical relationships of fractional OM content = 0.7(LOI)-0.23. (R^2= 0.94) and OM=1.724 (Soil organic C) have been used for calculations of % C (Moebius-Clune *et al*., 2016).

As pH values of all soil samples were below 7, the total soil C was considered equivalent to SOC (Propheter and Staggenborg, 2010; Bonin and Lal, 2014), with no carbonate presence assumed, which was further confirmed using 5 M HCl that resulted in no effervescence on a subset of 16 soil samples. SOC data reported here on a mass basis (g C kg^{-1}), as the switchgrass establishment was still in process after 3 years, and post-establishment bulk density measurements had not yet been done by 2014.

Soil pH and texture analyses as outlined in Moebius-Clune *et al*., 2016 are presented in Table 2.4. Active carbon analysis was carried out via permanganate oxidation and spectrophotometry (Weil *et al*., 2003, Culman *et al*., 2012, Moebius-Clune *et al*., 2016) and wet aggregate stability was measured using a sprinkle infiltrometer that steadily rains on a sieve containing a known weight of soil aggregates between 0.25 mm and 2 mm (Moebius-Clune *et al*., 2016); while the unstable aggregates fall apart and pass through the sieve, the fraction of the soil that remains on the sieve is used to calculate the percent aggregate stability.

Statistical Analyses Hierarchical linear mixed-effects models were fit using OM, active carbon, wet aggregate stability, soil pH for all years (2011, 2012, 2013, 2014), 2014 SOC, 2014 TN and 3-year cumulative above-ground biomass as response variables, to analyze the impact of soil moisture and cropping system on these variables. In all the models, wetness quintile rank, cropping system and the interaction between wetness quintile rank and cropping system were fixed effects, with subplot nested within strip plot treated as random effect. In model selection, the interaction between fixed effects was removed when insignificant (p value > 0.05).

Changes in soil properties (OM, active carbon, wet aggregate stability, soil pH) from the baseline were calculated by subtracting initial (2011) values from values after 3 years (2014) for each sampling location (subplot). Similar structured linear mixed-effects models were used to explain differences among moisture quintiles or cropping systems for changes in these soil parameters from baseline. Additionally, to understand whether changes from baseline were significant within a moisture quintile or cropping system, paired *t*-tests were performed. Post-hoc treatment comparisons were made by using Tukey’s HSD method to control for multiple comparisons.

Pearson correlation coefficients were computed between the analyzed variables of 2014 (SOC, TN, active carbon, wet aggregate stability), edaphic factors (silt content, clay content, and soil pH) and cumulative above-ground biomass and cumulative root biomass. Correlation coefficients were also computed between change in soil properties (OM, active carbon, wet aggregate stability, soil pH) from 2011-2014 and cumulative above-ground biomass and cumulative root biomass. All statistical analyses were carried out using JMP Pro 12 (SAS Inc., Cary, NC).

Results

We first present yearly trends of OM, active carbon, wet aggregate stability and soil pH as impacted by moisture or cropping system as well as the change in these parameters from 2011 baseline levels. Thereafter, we present SOC, TN, soil texture and pH from 2014, and cumulative above-ground biomass. Whenever results are expressed on the basis of *wetness quintiles*, parameter values are averaged over all cropping systems; conversely, when represented on the basis of *cropping systems*, results are averaged over all wetness quintiles. This is because the interaction of cropping system x wetness quintile rank was not a significant effect for any soil parameter during any sampling year nor for changes from preplow baseline. Finally, we present relationships among soil properties (2014 only) and cumulative above-ground biomass and cumulative root biomass.

Organic matter, active carbon, wet aggregate stability, soil pH from baseline to 2014 OM levels in the wettest soils (Q1) were significantly ($p<0.0001$) greater than in soils of the drier quintiles (Q2, Q3, Q4, Q5) during each sampling year (Fig.2.3a) with the only exception being Q2 soils in 2012. Among the cropping systems, OM values were significantly ($p<0.0001$) greater for CTRL soils than for that of RCG in 2012 and SWGN in 2013 (Fig.2.3b). Decrease in OM from 2011 to 2014 was significant for each moisture quintile group and cropping system (Table 2.6a). Though the loss from baseline levels was not significant among the wetness quintiles, the loss for CTRL was significantly ($p=0.009$) lower than those of SWG and SWGN (Fig. 2.4a, 2.4b, Table 2.6b).

The wettest Q1 soils displayed greater active carbon values than those of drier soils during different years, being significantly greater that those of the driest quintile (Q5) for the years 2011 ($p=0.004$) 2012 ($p=0.0001$), 2013 ($p=0.04$) and 2014 ($p=0.001$) (Fig.2.3c). Among the cropping systems, active carbon was significantly greater in CTRL than all other treatments in 2012 ($p=0.0002$) (Fig. 2.3d). Increases in active carbon over time were significant for CTRL and SWG cropping systems only; change from baseline in RCG or SWGN or any of the 5 wetness groups (Q1,Q2,Q3, Q4 and Q5) was not significant (Table 2.6a). Though the change from baseline levels was not significant among the wetness quintile groups, the increase in CTRL was significantly ($p=0.007$) greater than those of SWG and SWGN (Fig.2.4c, 2.4d, Table 2.6b).

Wet aggregate stability values for the driest Q5 soils were greatest among the wetness quintiles during all years and were significantly ($p=0.03$) greater than those of Q1 in 2013 (Fig.2.3e). Cropping system was a significant main effect impacting wet aggregate stability, with CTRL being greater than all other treatments in 2012 ($p=0.0001$), 2013 ($p=0.0003$) and 2014 ($p=0.005$) (Fig.2.3f). Loss in wet aggregate stability of soils from baseline levels for each moisture quintile and cropping system was significant (Table 2.6a). Though loss from baseline levels was not significant among the wetness quintile groups, that for CTRL was significantly ($p=0.01$) lower than those for RCG and SWGN (Fig.2.4e,2.4f, Table 2.6b).

Soil pH was significantly ($p<0.0001$) lower in the driest soils (Q5), than soils of all wetter quintiles (Q4, Q3, Q2, Q1) for all sampling years (Fig.2.3g). Among the cropping systems, CTRL was significantly ($p=0.008$) greater than RCG in 2012, significantly ($p<0.0001$) greater than all other treatments (RCG, SWG and SWGN) in 2013, and significantly ($p=0.009$) greater than SWGN in 2014 (Fig.2.3h). Reductions in soil pH from 2011 to 2014 were significant for each moisture quintile and cropping system (Table 2.6a). The loss from initial baseline levels were not significant among the wetness quintiles or cropping systems (Fig.2.4g,2.4h, Table 2.6b).

SOC and TN in 2014 In 2014, mean SOC values varied between 29.6±4 (Q5) and 39.5±6.5 g C kg^{-1} (Q1) (Fig.2.5a), those of Q1 soils being significantly ($p<0.0001$) greater than those of all other wetness group soils (Fig.2.5a, Table2.5). Mean values for the cropping systems varied between 31.2±1.4 (SWGN) and 37.4±1 g C kg^{-1}(CTRL), with CTRL being significantly ($p=0.03$) greater than SWGN (Fig.2.5b, Table 2.7). 2014 TN values varied between 2.9±0.3 (Q5) and 3.9±0.6 g kg^{-1} (Q1) those of Q1 soils being significantly ($p<0.0001$) greater than all the other wetness quintiles (Fig.2.5c, Table 2.8). Cropping system was not a significant main effect impacting 2014 TN (Table 2.7).

Soil texture and soil pH For soils of the four cropping systems, the mean value for silt contents varied between 62.5±1.3 and 69.4±1.2 %), clay contents varied between 14.0±0.7 and 19.7±2.1 % (Table 2.4), with driest sites (Q5) being significantly more sandy and wettest sites (Q1) having higher silt and clay contents. Soil pH varied between 5.1±0.1 and 6.2 ±0.1 (Fig. 2.3).

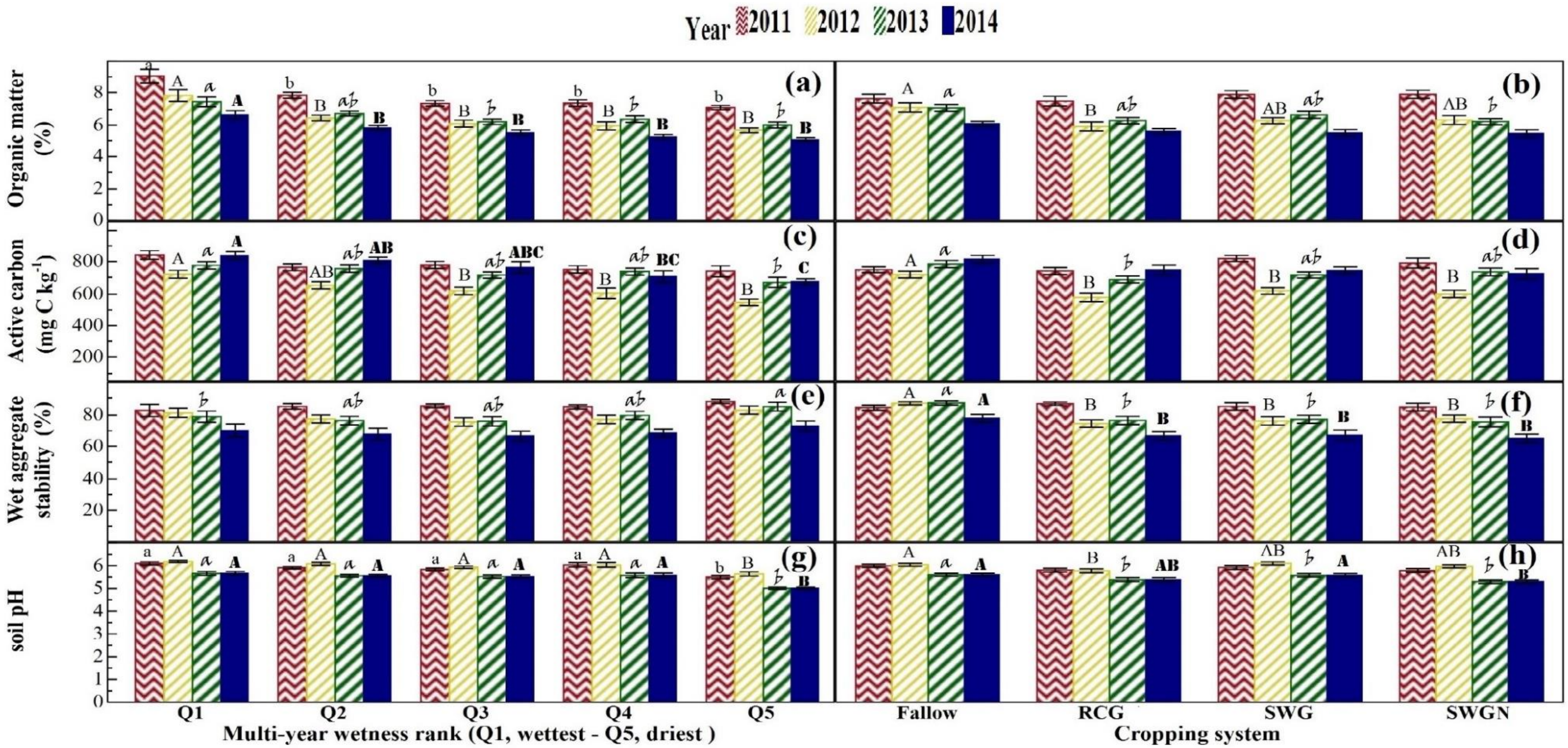

Figure 2.3. Mean values of soil surface (0-15cm) parameters: OM for wetness quintiles (a) and cropping systems (b), active carbon for wetness quintiles (c) and cropping systems (d), wet aggregate stability for wetness quintiles (e) and cropping systems (f) and soil pH for wetness quintiles (g) and cropping systems (h) for the years 2011-2014. Error bars represent standard errors; n varies between 2 and 6 for each wetness quintile and n = 20 for each cropping system from the field set up of 80 subplots with 16 strip plots of quadruplicate cropping system. Different letters in same font above bars indicate significant differences ($p<0.05$) for each parameter in a group for a specific year; no letters indicate no significant differences among treatments. Whenever expressed as wetness quintiles, values are averaged over cropping systems and when represented as cropping systems, are averaged over wetness quintiles.

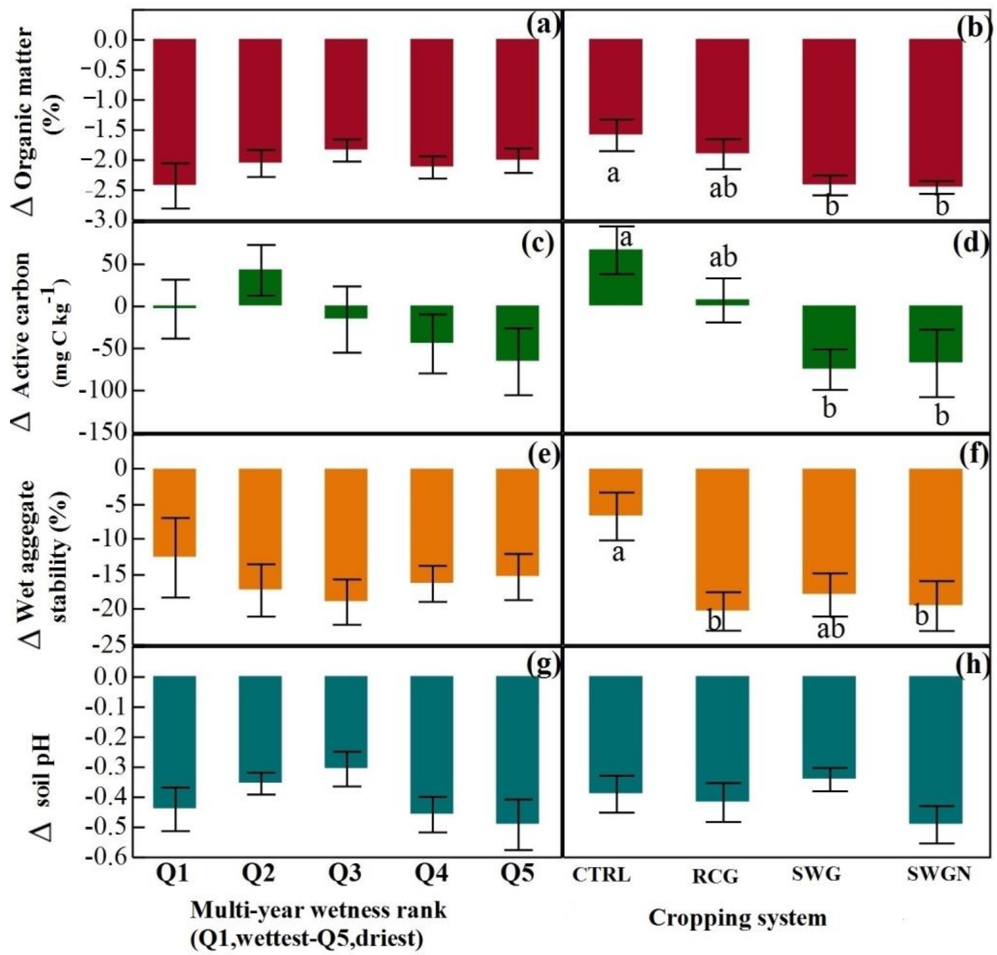

Figure 2.4. Mean values of change of soil surface (0-15cm) parameters from 2011 to 2014: Δ OM for wetness quintiles (a) and cropping systems (b), Δ active carbon for wetness quintiles (c) and cropping systems (d), Δ wet aggregate stability for wetness quintiles (e) and cropping systems (f) and Δ soil pH for wetness quintiles (g) and cropping systems (h). Error bars represent standard errors; n varies between 2 and 6 for each wetness quintile and n = 20 for each cropping system from the field set up of 80 subplots with 16 strip plots of quadruplicate cropping system. Different letters above bars indicate significant differences ($p < 0.05$) among groups for each parameter; no letters indicate no significant differences among treatments. Whenever expressed as wetness quintiles, values are averaged over cropping systems and when represented as cropping systems, are averaged over wetness quintiles.

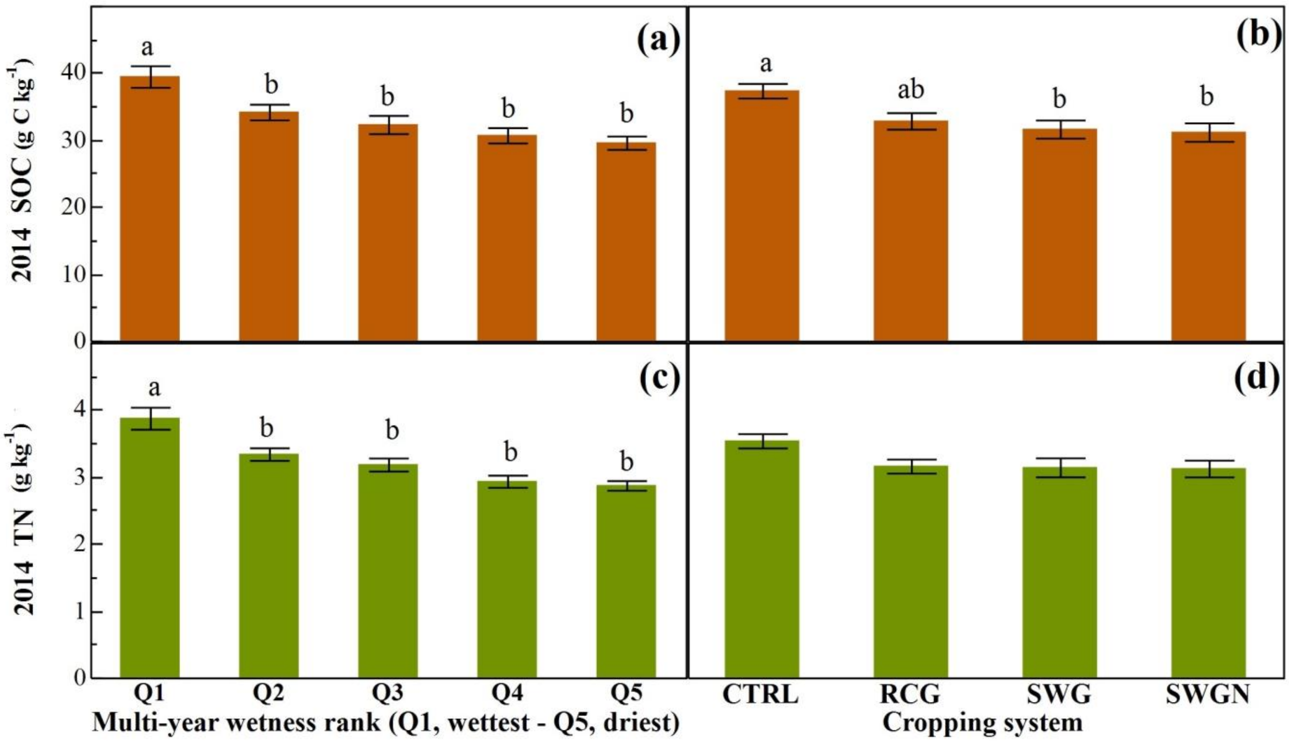

Figure 2.5. Mean values of 2014 soil surface (0-15cm) parameters: 2014 SOC for wetness quintiles (a) and cropping systems (b) and 2014 TN for the wetness quintiles (c) and cropping systems (d). Error bars represent standard errors; n varies between 2 and 6 for each wetness quintile and n = 20 for each cropping system from the field set up of 80 subplots with 16 strip plots of quadruplicate cropping system. Different letters above bars indicate significant differences ($p < 0.05$) among groups for each parameter; no letters indicate no significant differences among treatments. Whenever expressed as wetness quintiles, values are averaged over cropping systems and when represented as cropping systems, are averaged over wetness quintiles.

Table 2.4. Mean soil texture analysis results for surface soils (0-15cm) of 80 subplots grouped by soil wetness quintile. Mean values and corresponding standard errors. Wetness quintiles not sharing the same lowercase letter within a texture class are significantly different at p<0.05.

Wetness Quintile rank	Sand (%)	Silt (%)	Clay (%)
Q1	13.6±0.7, c	68.2±0.7, a	18.2±0.9, a
Q2	15.7±0.8, c	67.4±0.7, ab	17±0.9, a
Q3	16±0.7, bc	67±0.7, ab	17±0.9, a
Q4	19±0.8, ab	66.4±0.8, ab	15±0.9, a
Q5	20±0.7, a	65±0.7, b	15.4±0.9, a

Cumulative harvestable standing biomass The interaction of cropping system and moisture quintile was significant (F= 2.7, p = 0.006), Table 2.7. Cumulative above-ground biomass (Fig. 2.6) across wetness groups for the four cropping systems varied between 7.2±1.2 (SWG, Q2) and 18.8±1.4 Mg ha^{-1} (SWGN, Q5), with above-ground biomass for SWG Q5 significantly greater than SWG, Q2. However, regardless of wetness level, there was no significant difference between the cumulative above-ground biomass of control and RCG (Fig.2.6, Table 2.8).

Relationships between 2014 soil properties and change from baseline in cumulative above-ground and root biomass SOC of soils was positively correlated to root biomass, (r = 0.4, P=0.0008), but was not correlated to cumulative above-ground biomass. It was also positively, but weakly correlated to soil pH (r = 0.3, P = 0.002), silt content (r = 0.2, P=0.05) and clay content (r = 0.2, P = 0.07) and negatively to sand content (r = -0.4, P = 0.001). TN of soils was positively, but weakly correlated to root biomass (r = 0.3, P = 0.02), silt content (r = 0.2, P=0.04) and clay content (r = 0.3, P = 0.02) and soil pH (r = 0.2, P = 0.04) and negatively to sand content (r = -0.4, P <0.000). Active carbon of soils was weakly correlated to root biomass, (r = 0.3, P = 0.008), soil pH (r = 0.4, P = 0.0002), aggregate stability (r=0.3, P=0.004) and strongly to SOC (r = 0.7, P< 0.0001) and TN (r = 0.6, P <0.0001); it was not associated with sand, silt or clay contents or cumulative above-ground biomass. Aggregate stability of soils was correlated to SOC (r = 0.5, P <0.0001), TN (r = 0.4, P <0.0001) and root biomass, (r = 0.3, P = 0.009); it was not associated with soil pH, sand, silt or clay contents.

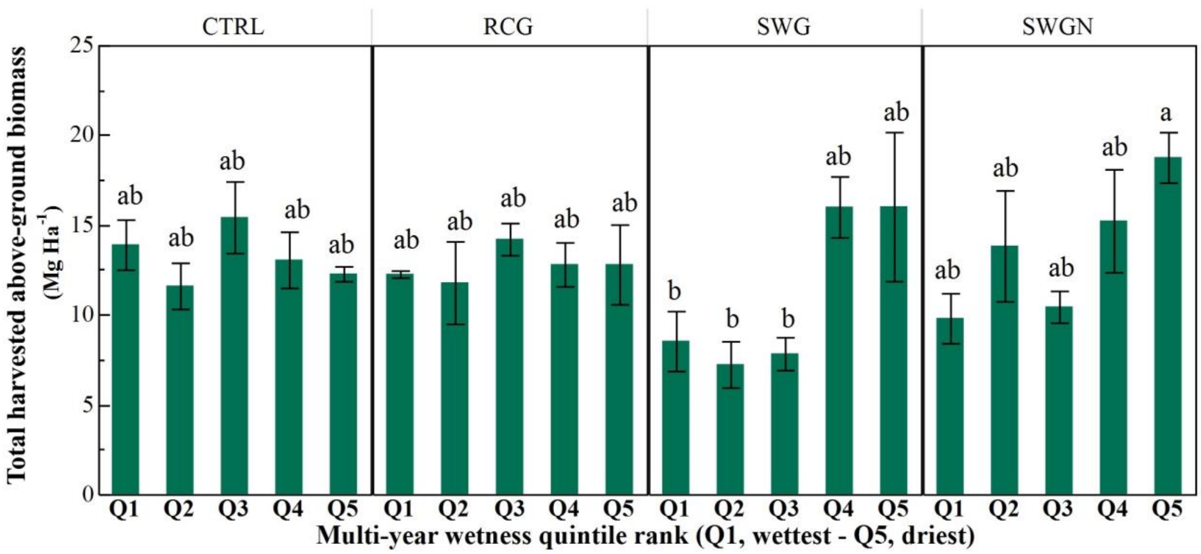

Figure 2.6. Mean values of cumulative harvested above-ground biomass from 2012 to 2014 across all wetness quintiles (Q1 wettest-Q5 driest, water content variation between 0.3 g g^{-1} and 0.5g g^{-1}) of the four cropping systems, fallow-control (CTRL), reed canarygrass + fertilizer 75 kg N ha^{-1} (RCG), switchgrass (SWG) and switchgrass + fertilizer 75 kg N ha^{-1}(SWGN). Error bars represent standard errors; n varies between 2 and 6 for each wetness quintile of the quadruplicate cropping systems design covering 80 subplots from the 16 strip plots. Different letters above bars indicate significant differences (p < 0.05) in above-ground biomass among the moisture-crop groups.

From 2011 to 2014, changes in OM, active carbon and aggregate stability of soils were all correlated {(r = 0.3, P = 0.02), (r = 0.3, P = 0.01), (r = 0.2, P = 0.03), respectively} to cumulative root biomass, but not to cumulative above-ground biomass. Changes in active carbon and aggregate stability were correlated to change in pH (r = 0.2, P = 0.05), (r = -0.3, P = 0.02), respectively} of soils. Scatter plot matrices displaying these relationships are seen in Figure 2.7a and 2.7b.

Table 2.5. Fixed effects for response variables OM (%), active carbon (mg C kg^{-1}), wet aggregate stability (%) and soil pH for the years 2011, 2012, 2013 and 2014

	2011 OM (%)			**2012 OM (%)**			**2013 OM (%)**			**2014 OM (%)**		
Effect	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value
Cropping system	3	0.9	0.4	3	3.4	0.02	3	3.2	0.03	3	1.8	0.02
Moisture rank	4	10	<0.0001	4	11.4	<0.0001	4	6.8	0.0001	4	11	<0.0001
	2011 Active carbon (mg C kg^{-1})			**2012 Active carbon (mg C kg^{-1})**			**2013 Active carbon (mg C kg^{-1})**			**2014 Active carbon (mg C kg^{-1})**		
Effect	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value
Cropping system	3	2.9	0.04	3	7.6	0.0002	3	3.2	0.03	3	1.5	0.2
Moisture rank	4	2.7	0.04	4	6.8	0.0001	4	2.6	0.04	4	5.2	0.001
	2011 Wet aggregate stability (%)			**2012 Wet aggregate stability (%)**			**2013 Wet aggregate stability (%)**			**2014 Wet aggregate stability (%)**		
Effect	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value
Cropping system	3	0.3	0.8	3	7.8	0.0001	3	7	0.0003	3	4.6	0.01
Moisture rank	4	0.8	0.5	4	2	0.1	4	2.8	0.03	4	1	0.5
	2011 soil pH			**2012 soil pH**			**2013 soil pH**			**2014 soil pH**		
Effect	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value
Cropping system	3	1.6	0.2	3	4.2	0.008	3	18	<0.0001	3	4.1	0.01
Moisture rank	4	10	<0.0001	4	7.8	<0.0001	4	10	<0.0001	4	12.5	<0.0001

Table 2.6a. Paired *t*-tests for change in soil properties from 2011 to 2014 (ΔOM, Δ active carbon, Δ wet aggregate stability and Δ soil pH). When expressed as wetness quintiles, values are averaged over cropping systems and when represented as cropping systems, are averaged over wetness quintiles.

	Δ OM (%) (CTRL)	**Δ OM (%) (RCG)**	**Δ OM (%) (SWG)**	**Δ OM (%) SWGN)**	**Δ OM (%) (Q1)**	**Δ OM (%) (Q2)**	**Δ OM (%) (Q3)**	**Δ OM (%) (Q4)**	**Δ OM (%) (Q5)**
Test statistics	-6.03	-7.6	-14.6	-23.2	-6.5	-9.2	-10	-11.5	-9.9
Prob. >|t|	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DF	19	19	19	19	15	15	15	15	15
	Δ Active carbon (mg C kg^{-1}) (CTRL)	**Δ Active carbon (mg C kg^{-1}) (RCG)**	**Δ Active carbon (mg C kg^{-1}) (SWG)**	**Δ Active carbon (mg C kg^{-1}) (SWGN)**	**Δ Active carbon (mg C kg^{-1}) (Q1)**	**Δ Active carbon (mg C kg^{-1}) (Q2)**	**Δ Active carbon (mg C kg^{-1}) (Q3)**	**Δ Active carbon (mg C kg^{-1}) (Q4)**	**Δ Active carbon (mg C kg^{-1}) (Q5)**
Test statistics	2.3	0.3	-3.1	-1.7	-0.1	1.4	-0.4	-1.3	-1.6
Prob. >|t|	0.03	0.8	0.006	0.1	0.9	0.2	0.7	0.2	0.1
DF	19	19	19	19	15	15	15	15	15
	Δ Wet aggregate stability (%) (CTRL)	**Δ Wet aggregate stability (%) (RCG)**	**Δ Wet aggregate stability (%) (SWG)**	**Δ Wet aggregate stability (%) (SWGN)**	**Δ Wet aggregate stability (%) (Q1)**	**Δ Wet aggregate stability (%) (Q2)**	**Δ Wet aggregate stability (%) (Q3)**	**Δ Wet aggregate stability (%) (Q4)**	**Δ Wet aggregate stability (%) (Q5)**
Test statistics	-1.9	-7.3	-5.8	-5.5	-2.2	-4.6	-4.8	-6.3	-4.6
Prob. >|t|	0.1	<0.0001	<0.0001	<0.0001	0.04	0.0008	<0.0001	<0.0001	0.0003
DF	19	19	19	19	15	15	15	15	15
	Δ soil pH (CTRL)	**Δ soil pH (RCG)**	**Δ soil pH (SWG)**	**Δ soil pH (SWGN)**	**Δ soil pH (Q1)**	**Δ soil pH (Q2)**	**Δ soil pH (Q3)**	**Δ soil pH (Q4)**	**Δ soil pH (Q5)**
Test statistics	-6.3	-6.4	-8.8	-7.9	-6	-9.8	-5.3	-7.8	-5.8
Prob. >|t|	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DF	19	19	19	19	15	15	15	15	15

Table 2.6b. Fixed effects for response variables for change in soil properties from 2011 to 2014 (ΔOM, Δ active carbon, Δ wet aggregate stability and Δ soil pH).

	Δ OM (%)			**Δ Active carbon (mg C kg^{-1})**			**Δ Wet aggregate stability (%)**			**Δ soil pH**		
Effect	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value
Cropping system	3	4.1	0.009	3	4.4	0.007	3	3.9	0.01	3	1.3	0.3
Moisture rank	4	0.9	0.5	4	1.1	0.4	4	0.5	0.7	4	1.5	0.2

Table 2.7. Fixed effects and their interaction for the response variables 2014 SOC, (g C kg^{-1}), 2014 TN, (g kg^{-1}), cumulative harvested above-ground biomass (Mg ha^{-1}) from 2012 to 2014.

	2014 SOC (g C kg^{-1})			**2014 TN (g kg^{-1})**			**Total harvested above-ground biomass, 2012-2014 (Mg ha^{-1})**		
Effect	DF	F Ratio	P value	DF	F Ratio	P value	DF	F Ratio	P value
Cropping system	3	5.2	0.003	3	2.4	0.1	3	1.7	0.2
Moisture rank	4	9.4	***<0.0001***	4	13	***<0.0001***	4	3.5	0.01
Cropping system X Moisture rank							12	2.2	**0.02**

Table 2.8. The post-hoc comparisons of least square means for 2014 SOC, (g C kg^{-1}), 2014 TN, (g kg^{-1}), cumulative harvested above-ground biomass (Mg ha^{-1}) from 2012 to 2014, averaged over cropping systems for fallow-control (CTRL), reed canarygrass + fertilizer 75 kg N ha^{-1} (RCG), switchgrass (SWG) and switchgrass + fertilizer 75 kg N ha^{-1}(SWGN) or wetness quintiles (Q1 wettest-Q5 driest, water content variation between 0.3 g g^{-1} and 0.5 g g^{-1}) and their interaction with Tukey adjusted P values. Levels not connected by same letters are significantly different.

2014 SOC (g C kg $^{-1}$) (Cropping system)				**2014 SOC (g C kg $^{-1}$) (Moisture rank)**				**2014 TN (g kg $^{-1}$) (Moisture rank)**				**Total harvested above-ground biomass, 2012-2014 (Mg ha^{-1}) (Cropping system X Moisture rank Interaction)**			
Level		Least Sq. Mean	Std. error	Level		Least Sq. Mean	Std. error	Level		Least Sq. Mean	Std. error	Level		Least Sq. Mean	Std. error
CTRL	A	36.6	1.1	Q1	A	39.2	1.2	Q1	A	3.9	0.1	SWGN, Q5	A	18.8	1.7
RCG	AB	33.5	1.1	Q2	B	33.7	1.2	Q2	B	3.3	0.1	SWG, Q5	AB	16	1.9
SWG	B	31.7	1.1	Q3	B	32.2	1.2	Q3	B	3.2	0.1	SWG, Q4	AB	16	1.7
SWGN	B	31.2	1.1	Q4	B	31.1	1.2	Q4	B	3.0	0.1	CTRL, Q3	AB	15.4	1.9
				Q5	B	30	1.2	Q5	B	3.0	0.1	SWGN, Q4	AB	15.2	2.1
												RCG, Q3	AB	14.2	1.7
												CTRL, Q1	AB	14	1.7
												SWGN, Q2	AB	13.8	1.9
												CTRL, Q4	AB	13	2.6
												RCG, Q4	AB	12.8	1.5
												RCG, Q5	AB	12.8	1.9
												CTRL, Q5	AB	12.3	2.1
												RCG, Q1	AB	12.3	2.1
												RCG, Q2	AB	11.8	2.6
												CTRL, Q2	AB	11.6	1.5
												SWGN, Q3	AB	10.4	1.9
												SWGN, Q1	AB	9.8	1.9
												SWG, Q1	B	8.5	1.9
												SWG, Q3	B	7.8	2.1
												SWG, Q2	B	7.2	1.9

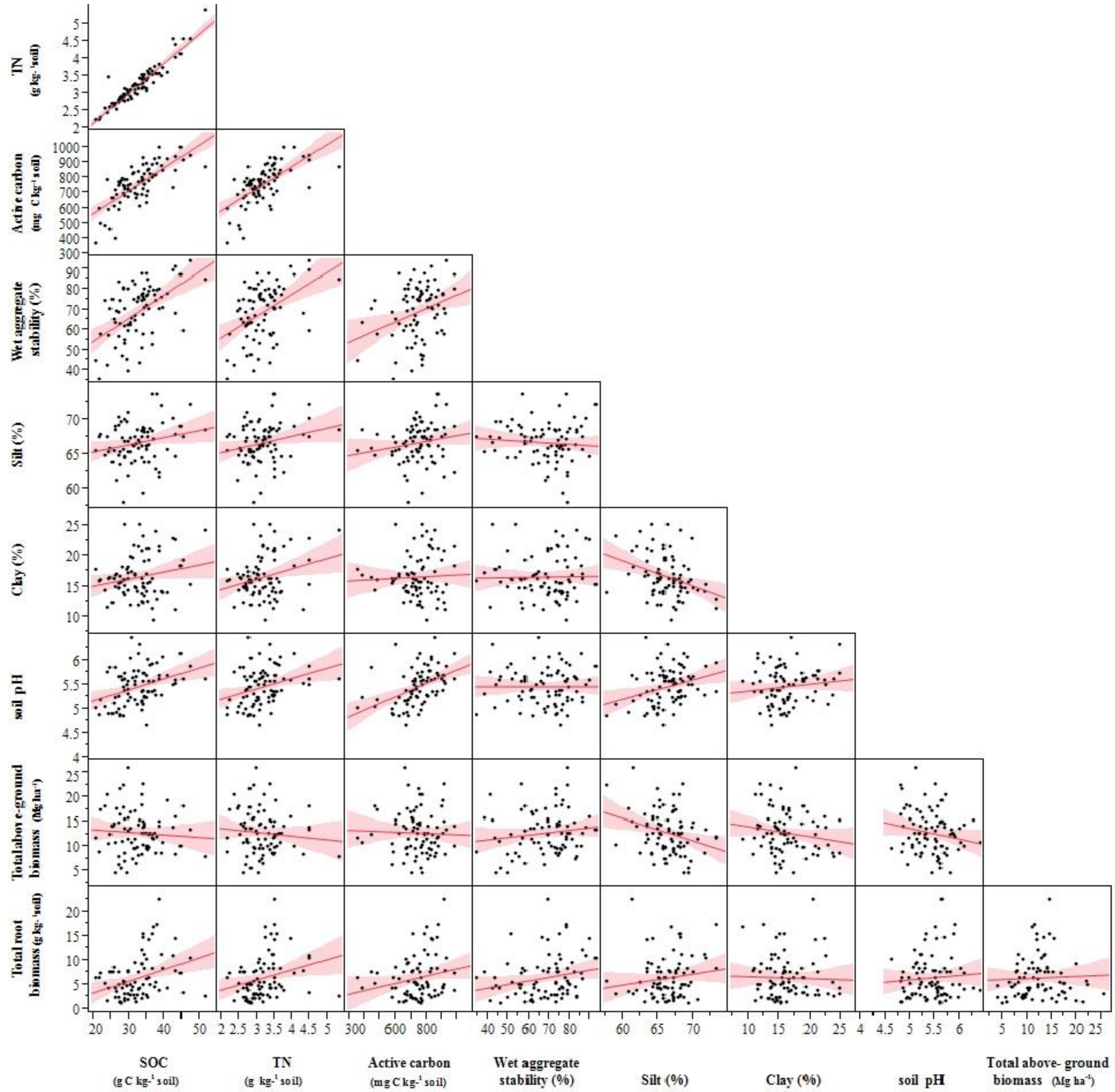

Figure 2.7a. Scatter plot matrices displaying relationships between analyzed variables of 2014, SOC, TN, active carbon, wet aggregate stability, cumulative harvested above-ground biomass and cumulative root biomass from 2012 to 2014 and the edaphic factors, clay contents, silt contents sand contents and soil pH (n=80).

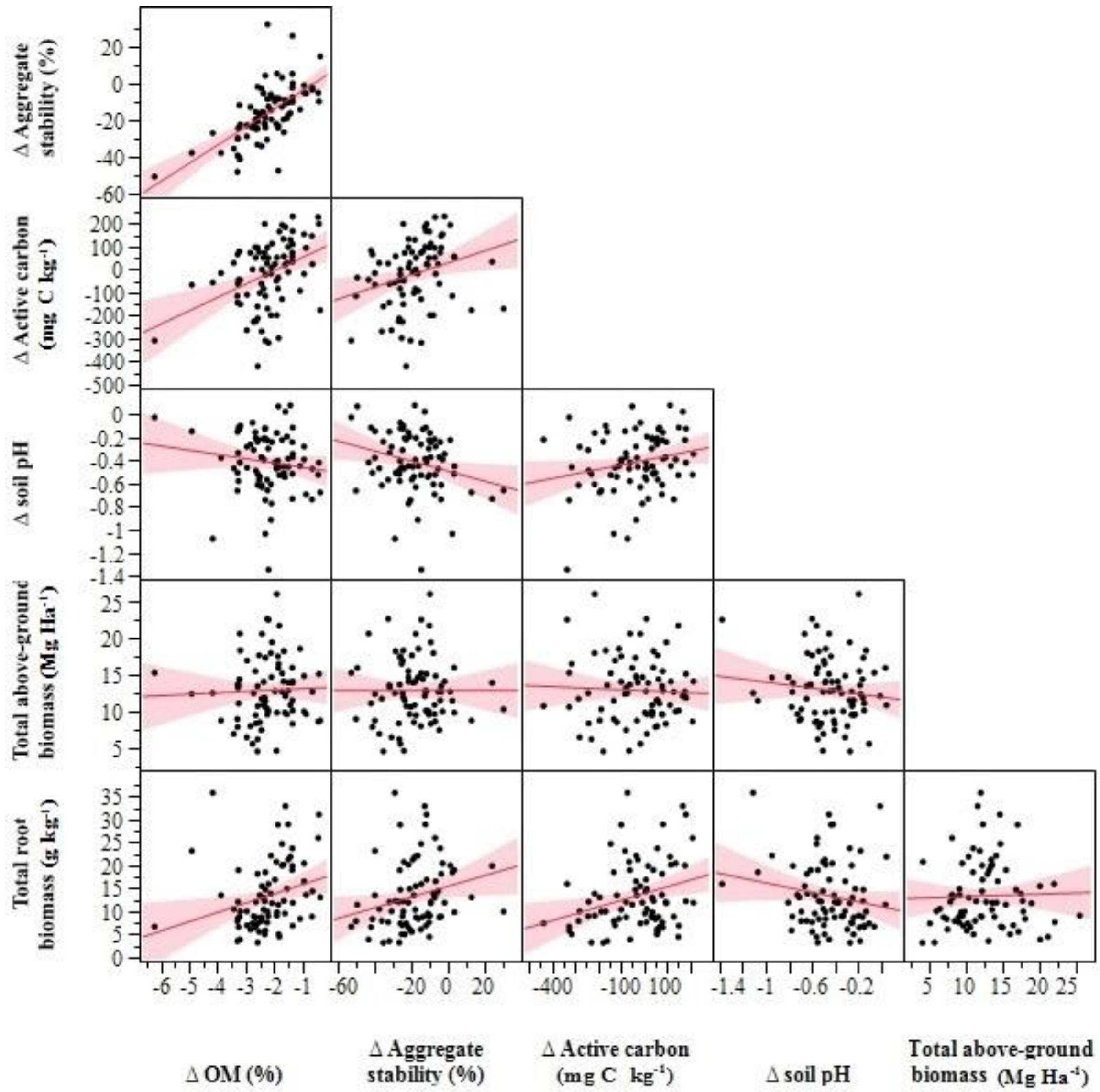

Figure 2.7b. Scatter plot matrices displaying relationships between change in soil properties (ΔOM, Δ active carbon, Δ wet aggregate stability, Δ soil pH) from 2011 to 2014 and cumulative harvested above-ground biomass and cumulative root biomass from 2012 to 2014 (n=80).

Discussion

Influences on OM, SOC and TN It is unsurprising that the wettest Q1 soils displayed significantly greater OM and SOC in comparison to drier (less wet) soils, during any sampling year (Fig.2.3a, Fig.2.5a). Additionally, the driest soils (Q5), displayed the lowest OM/SOC value during any sampled year. Thus, in the moisture range of 0.3g g^{-1} (Q5) to 0.5g g^{-1} (Q1), results from our field indicate that wettest and driest soils were reservoirs of the greatest and smallest OM/SOC pool, respectively, as is well understood (Trumbore 1997; Jobba´gy and Jackson 2000, O'Brien *et al*., 2010) based on how saturated vs. dry conditions affect C mineralization. Conditions of seasonal saturation result in decreased OM mineralization, while increased OM inputs during the growing season (especially during midsummer when moisture is limiting in drier soils, as in Knapp *et al*., 1993, O'Brien *et al*., 2010). As TN is closely associated with SOC and is an important constituent of OM, it displayed similar trends, consistent with other studies (e.g. O'Brien *et al.*, 2010, which showed greater TN accrual in wetter restored prairie soil).

The SOC loss from this 0-15 cm surface soil (Fig. 2.3a, 2.3b, and compared to fallow control in 2014, 5b) associated with reed canarygrass and switchgrass establishment (including mowing, spraying, plowing, disking and harrowing) is consistent with other perennial grass establishment studies, where surface soil SOC values either decreased or remained unchanged (Corre *et al*.,1999; Mehdi *et al*., 1999; Skinner *et al*., 2006; Sanderson, 2008; Miller and Dell, 2012; Jaggard, 2012; Bonin and Lal, 2014; Pryatel *et al*., 2015) during similar or longer timeframe establishment of grasses. Davidson and Ackerman (1993) concluded that up to 40% of the original Ap SOC stocks could be lost within the first five years when uncultivated lands are brought into cultivation. Our study indicates 25-31% OM loss in 3 years for the plots planted to the grasses. Hence, our hypothesis of OM/SOC loss following plowing is supported. In addition to induced direct losses, the low biomass inputs during the seeding year (2011) and during the slow multiyear establishment phase of switchgrass (Corre *et al*., 1999; Paul *et al*., 2002; Anderson-Teixeira *et al*., 2009, Zhang *et al.*, 2010) have probably also contributed to the observed lower OM/SOC levels in switchgrass plots. Furthermore, in an annually harvested bioenergy system, SOC recovery/buildup may require a longer time than an unharvested system where C inputs from above-ground biomass are remain in place, enabling faster recovery (Steinbeiss *et al*., 2008).

However, the notable concurrent decrease in OM levels in the fallow control plots (Fig. 2.3b,2.4b) was surprising (and resulted in repeated rechecking of sampling and analysis records). We subsequently recognized multiple factors that potentially contributed to this observed decline. First, it was recognized that maintaining the control plots in a strictly unmowed fallow condition since 2011 represented a shift from earlier historical treatments in which occasional mowing (e.g. every 1-2 years) took place, which would have stimulated greater biomass production and surface OM deposition in the prior regime. (Historical aerial imagery suggests that annual mowing had actually been discontinued sometime between 2005 and 2009, as 2009 imagery showed decreased vegetative uniformity at the site. Thus, a shift to lower rates of surface deposition occurred prior to the study's onset.) Second, while the subsurface drains installed in 2011 were not extensive in terms of field coverage, their impact by draining the "bottom of the bowl" at the site would have helped bring about drier average conditions somewhat earlier in the spring and later in the fall. OM losses associated with hydrologic modification such as drain installation has been reported as a disadvantage of such systems (Brady and Weil, 2000; Manale, 2002; Williams *et al.*, 2015). Third, annual climatic factors may have also contributed, as the growing seasons of 2011-2014 were drier than the 29-year average (Fig. 2.2a). Net loss of carbon from grasslands during drought months is often observed (Novick *et al*., 2004; Skinner *et al*., 2006). Additionally, the mean annual temperature (especially the non-growing season, October to March) during the study period (Table 2.1, Fig.2.2b) was greater than the 29-year average, a factor that has been observed to contribute to OM losses (Skinner *et al*., 2006). The first two factors (management and drainage) would be expected to result in new lower equilibrium SOC levels, with the warmer and drier conditions in 2011-to 2014 also contributing in the same direction.

The lack of significant difference between C3 reed canarygrass and C4 switchgrass (fertilized and unfertilized) for OM, OM loss from baseline and SOC values, during any sampling year (Fig.2.3b,2.4b,2.5b), suggest that (at least in the short term) plant photosynthetic pathway had little impact on SOC dynamics, consistent with other studies (Corre *et al*., 1999; Mahaney *et al*., 2008). The absence of significant differences between SOC levels of switchgrass and switchgrass+fertilizer indicates that moderate (*i.e.* lower than those used to maximize crop yields, here 75 kg ha^{-1}) N fertilization rates were not (again in this short time frame) either beneficial or detrimental for SOC, as noted by other researchers (Khan *et al*., 2007; Stewart *et al*., 2016). Our hypothesis that there would be no near-term difference in OM or SOC levels as consequences of plant photosynthetic pathway or N fertilization are thus supported. The positive correlation of 2014 SOC levels to both clay and silt contents is consistent with many reports that OM is protected through associations with these particles (Parton *et al*., 1987; Schimel *et al*., 1994; Hassink 1997; Hassink *et al.*, 1997; Jobba´gy and Jackson, 2000; Six *et al*., 2002, Zhao *et al*., 2006, Grandy and Neff 2008; He *et al*., 2009; Baer *et al*., 2010), improving long-term SOC accrual and stabilization.

Influences on active carbon Although moisture regime was not a significant main effect on the percent change in active carbon from baseline levels, the greatest loss in the driest soils was reflective of moisture control of active carbon pools (Fig.2.4c), also evidenced in the wettest soils (Q1) having values that were significantly greater than the driest soils for each sampling year (Fig.2.3c). The effects of the 2011 plowing on active carbon were reflected both in levels in subsequent years (Fig.2.3c,2.3d) and in their change from baseline (Fig.2.4c,2.4d). Rapid recovery to baseline values in the reed canarygrass plots appeared tied to the rapid crop establishment, in contrast to the lag in the slow-to-establish switchgrass treatments (Fig.2.4d). Thus, effects of rapidly established vegetation appeared to be key in offsetting losses initiated by plowing. Increases in active carbon concurrent with decreasing OM in some treatments (especially during 2013 and 2014, Fig.2.3a, 2.3b,2.3c,2.3d) were indicative of a much quicker recovery of active carbon in comparison to SOC. The faster dynamics substantiates its correlation to non-mineral-associated fast-cycling C pools. The strong positive association of active carbon with SOC is as expected and as reported in other studies (Culman *et al*., 2012; Lucas and Weil, 2012).

Influences on wet aggregate stability As aggregates between 0.25 and 2 mm were the basis of stability analysis, trends reported here were indicative of soil quality/soil health changes, as large aggregates of this range are more sensitive to management effects, while smaller aggregates (< 0.25 mm) are related to older and more stable forms of SOC (USDA-NRCS 2008). Thus, decreases in wet aggregate stability values from baseline were primarily reflective of plowing, given that fallow-control soils had significantly greater wet aggregate stability than all other post-plowing treatments during all sampling years (Fig.2.3f). The positive correlation of aggregate stability to SOC is consistent with Grandy and Robertson (2007) and Tiemann and Grandy (2015) in silt-loam soils, in contrast to others who found no association (Carter, 1994; Tiemann and Grandy, 2015). Similar to our results, Kibet *et al*. (2016) found that low (60 and

120 kg N ha^{-1}) rates of N application did not adversely affect aggregate stability, in contrast to decreases in soil macroaggregates under switchgrass at much greater N application rates (202 kg N ha^{-1}, Jung and Lal, 2011).

Influences on soil pH The consistently lower soil pH in the driest soils (Q5) during each sampling year was reflective of the soil series, with these soils most likely to have eroded and been more strongly leached during prior use (Fig.21b, Table 2.4), landscape position being key to the overarching wetness levels (drainage class) and associated soil pH levels. Subsequent pH changes correlated with fertilizer application were evident in 2014, when unfertilized switchgrass soils displayed significantly greater pH than that of fertilized switchgrass soils (Fig.2.3h). The decrease in soil pH with perennial grass plantings (Fig.2.4h) is similar to the modest drop in topsoil pH reported in a 5-year switchgrass study (Schmer *et al.*, 2011).

Influences on cumulative harvested above-ground biomass The initial harvestable biomass yields of reed canarygrass, switchgrass, and switchgrass+N treatments are comparable to other studies in this region (Fick *et al.*, 1994, Hong *et al.*, 2014). The greater yields of switchgrass in the drier quintiles substantiates the better early establishment response of the upland variety of switchgrass, *Shawnee* (with or without N fertilization). At this stage, we found no difference in the cumulative harvested above-ground biomass between switchgrass and switchgrass+N. This is consistent with the findings of Hong *et al.*, 2014, which shows that N response is variable for switchgrass in the NE, with some site showing no yield benefit for added N (at least during establishment), while other, such as the five-year screening trial for perennial grasses in New York state by Fick *et al*. (1994) showing that N fertilization (30-130 kg ha^{-1}) significantly improved yields, especially on poorly drained soils.

Relationships between soil and crop productivity parameters As expected, due to annual harvest removals, there was no correlation between harvested yields and SOC or active carbon. The positive association of SOC, TN, active carbon, wet aggregate stability with cumulative root biomass - and absence of association with cumulative above-ground yields - reinforces the understanding that below-ground biomass is of greater importance for SOC dynamics (Jobbagy and Jackson, 2000; Garten and Wullschleger, 1999; Lemus and Lal, 2005; Rasse *et al.*, 2005; Xiong and Katterer, 2010: Jaggard, 2012). Additionally, higher root biomass correlations to lower OM loss, lower active carbon loss and lower wet aggregate stability loss substantiates it further. However, in addition to the positive influence of root productivity on SOC accretion, attendant counterproductive mechanisms could also result in OM losses. Perennial grasses with more root biomass produce more exudates which increase microbial decomposition of older SOM by the process of positive priming (Kuzyakov, 2002, Dijkstra *et al*., 2006; Kuzyakov, 2006; Bird *et al*., 2011; Tiemann & Grandy, 2015). However, root-mediated OM decomposition is often short-term and root biomass ultimately plays a key role in accrual of SOC in the longer term, offsetting those losses within one growing season (Kuzyakov, 2002) potentially explaining lower OM loss being correlated to greater root biomass.

Conclusions

Overall, soil moisture status (drainage class and associated landscape position) is a key governing variable that needs to be accounted for when assessing soil properties and SOC dynamics resulting from establishment of perennial bioenergy crops. Three years after planting, the loss of soil OM and SOC incurred by conventional moldboard plowing and fitting of formerly fallow had not been repaid under reed canary-grass or slower-to-establish upland switchgrass on wetness-prone marginal land. Use of minimal tillage (such as no-till or zone-till) may be a useful way to avoid incurring this observed SOC debt if resulting crop establishment is satisfactory. Additional time will be needed for SOC recovery at this site, which is being monitored accordingly (substantial biomass inputs in switchgrass treatments since 2014 may help accelerate this recovery). In contrast, the more rapid recovery of the relatively dynamic pool of active carbon suggests that it may be useful as a moisture-sensitive leading indicator of changing SOC dynamics in a shorter time frame. Research quantifying soil respiration and OM turnover through time in long-term experiments is needed to better understand plant-soil interactions affecting soil C dynamics in such seasonally wet soils. Additionally, time-integrated plant input studies and analysis will allow definition of a more complete C balance.

Acknowledgements

This research was supported with funds from United States Department of Agriculture/National Institute for Food and Agriculture Grant No. 2011-67009-20083 and from Federal Capacity (Hatch) Project No. NYC-123486. The work is supported by the yield data from the Section of Plant Breeding and Genetics, Cornell University, who are collaborators for this project. The authors thank them for their extensive help. The authors are also extremely thankful to all project assistants and summer interns for their help and support. The work also could not have been completed without the help of Kevin Packard of the Cornell Statistical Computing Unit. Many thanks to Johannes Lehmann for his comments.

References

Ampleman MD, Crawford KM, Fike DA (2014) Differential soil organic carbon storage at forb and grass-dominated plant communities, 33 years after tallgrass prairie restoration. *Plant and Soil*, 374, 899-913(15).

Anderson-Teixeira KJ, Davis SC, Masters MD, Delucia EH (2009) Changes in soil organic carbon under biofuel crops. *GCB Bioenergy*, 1, 75–96.

Baer SG, Kitchen DJ, Blair JM, Rice, CW (2002) Changes in ecosystem structure and function along a chronosequence of restored grasslands. *Ecological Applications*, 12, 1688–1701.

Baer SG, Meyer CK, Bach EM, Klopf RP, Six J (2010) Contrasting ecosystem recovery on two soil textures: implications for carbon mitigation and grassland conservation. *Ecosphere* 1(1):art5.

Baxter R, Calvert K (2017) Estimating Available Abandoned Cropland in the United States: Possibilities for Energy Crop Production. Annals of the American Association of Geographers. DOI: 10.1080/24694452.2017.1298985

Bills JS (2008) Invasive Reed Canary grass (*Phalaris arundinacea*) and Carbon sequestration in a wetland complex, *Master's Thesis*, Indiana University USA.

Bird JA, Herman DJ, Firestone MK (2011) Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. *Soil Biology and Biochemistry*, 43, 718–725.

Blanco-Canqui H (2010) Energy crops and their implications on soil and environment. *Agronomy Journal*, 102, 403–419.

Bonin CL, Lal R (2014) Aboveground productivity and soil carbon storage of biofuel crops in Ohio. *Global Change Bioenergy*, 6(1), 67-75.

Brady N, Weil R. (2000) The Nature and Properties of Soils, 12th Edition. Prentice Hall. Upper Saddle River, New Jersey.

Burke IC, Lauenroth WK, Coffin DP (1995) Soil organic matter recovery in semiarid grasslands: implications for the conservation reserve program. Ecological Applications 5:793–801.

Camill P, McKone MJ, Sturges ST, Severud WJ, Ellis E, Limmer J, Martin CB, Navratil RT, Purdie AJ, Sandel BS, Talukder S, Trout A (2004) Community and ecosystem-level changes in species-rich tallgrass prairie restoration. *Ecological Applications,* 14, 1680–1694.

Cañasveras JC, Barrón V, Del Campillo MC, Torrent J, Gómez JA (2010) Estimation of aggregate stability indices in Mediterranean soils by diffuse reflectance spectroscopy. *Geoderma.* 15,158(1):78-84

Carter R, Angers DA, Kunelius HT (1994) Soil Structural Form and Stability, and Organic Matter under Cool-Season Perennial Grasses. Soil Science Society of America Journal, 58, 1194-1199.
Cline MG, Bloom AL (1965) Soil survey of Cornell University property and adjacent areas. New York State College of Agriculture at Cornell University, Ithaca.

Corre MD, Schnabel RR, Shaffer JA (1999) Evaluation of soil organic carbon under forests, cool-season and warm-season grasses in the northeastern U.S. *Soil Biology and Biochemistry*, 31, 1531–1539.

Culman, SW, Snapp, SS, Freeman, MA, Schipanski, ME, Beniston, J, Lal, R, Drinkwater, LE, Franzluebbers, AJ, Glover, JD, Stuart Grandy, A, Lee, J, Six, J, Maul, JE, Mirksy, SB, Spargo, JT & Wander, MM (2012) Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management *Soil Science Society of America Journal* 76(2), 494-504. DOI: 10.2136/sssaj2011.0286

Culman SW, Snapp SS Green, JM, Gnetry LE (2013) Short- and long-term labile soil carbon and nitrogen dynamics reflect management and predict corn agronomic performance. *Agronomy Journal* 105, 493.

Davidson EA, Ackerman IA (1993) Changes in Soil Carbon Inventories Following Cultivation of Previously Untilled Soils. *Biogeochemistry*, 20(3), 161-193.

Dijkstra FA, Cheng W, Johnson DW (2006) Plant biomass influences rhizosphere priming effects on soil organic matter in two differently managed soils. *Soil Biology and Biochemistry*, 38, 2519–2526.

Fick, GW, Pfeifer RA, Lathwell DJ (1994) Production patterns of perennial herbaceous biomass crops in the great-lakes region. *Energy Sources* 16, 333–348.

Fissore C, Giardina CP, Kolka RK, Trettin CC (2008) Temperature and vegetation effects on soil organic carbon quality along a forested mean annual temperature gradient in North America. *Global Change Biology*, 14,193–205.

Frankenberger JR, Brooks ES, Walter MT,Walter MF, Steenhuis TS (1999) A GIS-Based variable source area model. *Hydrological Processes*, 13, 804-822.

Garten CT, Wullschleger SD (1999) Soil carbon inventories under a bioenergy crop (switchgrass): measurement limitations. *Journal of Environmental Quality*, 28, 1359-1365.

Geng X, Guillar K, Morris T (2014) Turfgrass growth and color correlated to spring Illinois soil nitrogen test and soil permanganate-oxidizable carbon concentrations. *Crop Science*,54, 383.

Grandy AS, Neff JC (2008) Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. *Science of the Total Environment*, 404, 297–307.

Grandy AS, Robertson GP (2007) Land-use intensity effects on soil organic carbon accumulation rates and mechanisms. *Ecosystems*, 10, 58–73.

Guo LB, Gifford RM (2002) Soil carbon stocks and land use change: a meta-analysis. *Global Change Biology, 8*, 345–360.

Hassink J (1997) The capacity of soils to preserve organic C and N by their association with clay and silt particles. *Plant Soil*, 191, 77–87.

Hassink, J, Whitmore, AP, Kubat J (1997) Size and density fractionation of soil organic matter and the physical capacity of soils to protect organic matter. *European Journal of Agronomy* 7, 189–199.

He N, Wu L, Wang Y, Han X (2009) Changes in carbon and nitrogen in soil particle size fractions along a grassland restoration chronosequence in northern China. *Geoderma,* 150, 308.

Hong CO, Owens VN, Bransby D, Farris R, Fike J, Heaton E, Kim S, Mayton H, Viands D (2014) Switchgrass response to nitrogen fertilizer across diverse environments in the USA: A regional feedstock partnership report. *BioEnergy Research*, 7, 777–788.

Jaggard ELS (2012) Soil carbon dynamics following Switchgrass establishment for /bioenergy production in Southeastern Ontario.MS Thesis Queens's University, Ontario, Canada .

Jastrow JD, Amonette JE, Bailey VL (2007) Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. *Climate Change*, 80, 5-25.

Jelinski NA, Kucharik CJ (2009) Land-use effects on soil carbon and nitrogen on a U.S. midwestern floodplain. *Soil Science Society of America Journal,* 73, 217.

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications,* 10, 423–436.

Jung JY, Lal R (2011) Impacts of nitrogen fertilization on biomass production of switchgrass (Panicum virgatum L.) and changes in soil organic carbon in Ohio. *Geoderma*, 166, 145–152.

Jung JY, Lal R, Jastrow JD, Tyler DD (2011) Nitrogenous fertilizer effects on soil structural properties under switchgrass. *Agriculture, Ecosystems and Environment* 141, 215–220.

Khan SA, Mulvaney RL, Ellsworth TR, Boast CW (2007) The Myth of Nitrogen Fertilization for Carbon Sequestration. *Journal of Environmental Quality*, 36,1821-1832.

Kibet LC, Blanco-Canqui H, Mitchell RB, Schacht WH (2016) Root biomass and soil carbon response to growing perennial grasses for bioenergy *Energy, Sustainability and Society,* 6, 1.

Knapp AK, Fahnestock JT, Hamburg SP, Statland LB, Seastedt TR, Schimel DS (1993) Landscape patterns in soil-plant water relations and primary production in tallgrass prairie. *Ecology*, 74, 549–560.

Knops JMH, Tilman D (2000) Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. *Ecology*, 81, 88–9.

Kucharik CJ, Brye KR, Norman JM, Foley JA, Gower ST, Bundy LG. (2001) Measurements and modeling of carbon and nitrogen cycling in agroecosystems of southern Wisconsin: potential for SOC sequestration during the next 50 years. *Ecosystems*, 4, 237–258.

Kutsch WL, Bahn M, Heinemeyer A (2009) Soil carbon relations:an overview In: *Soil carbon dynamics: an integrated methodology* (eds Kutsch WL, Bahn M, Heinemeyer A), pp. 2-4, Cambridge University Press, Cambridge, UK.

Kuzyakov Y (2002) Review: factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science*, 165, 382–396.

Kuzyakov Y (2006) Sources of CO2 efflux from soil and review of partitioning methods. *Soil Biology and Biochemistry*, 38, 425–448.

Lemus R, Lal R (2005) Bioenergy crops and carbon sequestration. *Critical Reviews in Plant Science*, 24, 1–21.

Linn DM, Doran JW (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Science Society of America Journal*, 48, 1267–1272.

Lucas ST, Weil RR (2012) Can a labile carbon test be used to predict crop responses to improve soil organic matter management? *Agronomy Journal* 104,1160-1170.

Ma, Z, Wood CW, Bransby DI (2000) Impacts of soil management on root characteristics of switchgrass. *Biomass & Bioenergy* 18105-112.

Mahaney WM, Smemo KA, Gross KL (2008) Impacts of C-4 grass introductions on soil carbon and nitrogen cycling in C-3-dominated successional systems. *Oecologia*, 157, 295–305.

Manale AP (2002) Public policy issues in soil carbon trading *in* Agricultural Practices and Policies for Carbon Sequestration in Soil (eds Lal R., Kimble, J. M. and Follett R. F), CRC press, Boca Raton, United States, pp 321-325

Mason CW, Stoof CR, Richards, BK, Rossiter DG, Steenhuis TS (2016) Spring-thaw nitrous oxide emissions from reed canarygrass on wetness-prone marginal soil in New York State. *Soil Science Society of America Journal* 80(2)428-437.

Mason CW, Stoof CR, Richards BK, Das S, Goodale CL, Steenhuis TS (2017) Hotspots of nitrous oxide emission in fertilized and unfertilized perennial grasses on wetness-prone marginal land in New York State. *Soil Science Society Am. J.* DOI: 10.2136/sssaj2016.08.0249

Matamala R, Jastrow DJ, Miller RM, Garten C (2008) Temporal changes in the distribution of C and N stocks in a restored tallgrass prairie in the U.S Midwest. *Ecological Applications*, 18, 1470–1488.

McLaughlin SB, Kszoz LA (2005) Development of switchgrass *(Pancium virgatum*) as a bioenergy feedstock in the United States. Biomass Bioenergy 28:515–535. doi:10.1016/j.biombioe.2004.05.006

McLaughlin SB, Walsh ME (1998) Evaluating environmental consequences of producing herbaceous crops for bioenergy. *Biomass and Bioenergy*, 14, 317-324.

Mehdi B, Zan C, Girouard P *et al* (1999) Soil organic carbon sequestration under two dedicated perennial bioenergy crops. In: Biomass: A Growth Opportunity in Green Energy And Value-Added Products. Proc. 4th Biomass Conference of the Americas, Vol 1. Oakland, California. 29 August-2 September, 1999, pp.17–23. Pergamon, Oxford, UK.

Miller CF, Dell C (2012) Quantifying the role of Native Warm Season Grasses in Sequestering Soil Organic Carbon *,8th Eastern Native Grass Symposium in Charlottesville, VA*

Miller CF, Dickerson JA (1999) The Use of Native Warm Season Grasses for Critical Area Stabilization in the Proceedings of the 2nd Eastern Native Grass Symposium, PP. 222. Baltimore, MD.

Mizin KL (2014) C and N Cycling Dynamics and Microbial Community Composition in High Tunnel Production Systems. PhD Thesis, University of Kentucky, USA.

Moebius-Clune BN, Moebius-Clune DJ, Gugino BK, Idowu OJ, Schindelbeck RR, Ristow AJ, van Es HM, Thies JE, Shayler HA, McBride MB, Wolfe DW, Abawi GS (2016) Comprehensive Assessment of Soil Health. In: *The Cornell Framework Manual: Third Edition,* pp. 51-52, Cornell University, Geneva, United States.

Moyano F, Manzoni S, Chenu C (2013) Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. *Soil Biology and Biochemistry,* 59, 72-85.

Novick KA, Stoy PC, Katul GG, Ellsworth DS, Siqueira MBS; Juang J, Oren R (2004) Carbon dioxide and water vapor exchange in a warm temperate grassland. *Oecologia* 138:259–274.

O'Brien SL, Jastrow JD, Grimley DA, Gonzalez-Meler MA (2010) Moisture and vegetation controls on decadal-scale accrual of soil organic carbon and total nitrogen in restored grasslands. *Global Change Biology,* 16, 2573–2588.

Parrish D J, Fike JH (2005) The Biology and Agronomy of Switchgrass for Biofuels. *Critical Reviews in Plant Sciences* 24(5/6) 423-459.

Paul KI, Polglase PJ, Nyakuengama JG, Khanna PK (2002) Change in soil carbon following afforestation. *Forest Ecology and Management*, 168, 241–257.

Parton WJ, Schimel DS, Cole CV, Ojima D (1987) Analysis of factors controlling soil organic matter levels in Great Plains grasslands. . *Soil Science Society of America Journal*, 51, 1173–1179.

Parton W, Silver WL, Burke IC, Grassens L, Harmon ME,Currie WS, King JY,Dair CE,Brandt LA, Hart SC, Fasth B (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science*, 315, 361-364.

Powlson DS, Brookes PC, Christensen BT (1987) Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biology & Biochemistry*, 19, 159–164.

Propheter JL, Staggenborg SA (2010) Performance of annual and perennial biofuel crops: Nutrient removal during the first two years. *Agronomy Journal*, 102, 798–805.

Pryatel, MJ, Barrett JE, Emrick V (2015) Recovery of soil organic matter stocks from switchgrass plantings in agricultural landscape. *Centennial annual meeting of the Ecological Society of America, Baltimore. COS 9-5.*

Raich JW, Schlesinger WH (1992) The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus Series B-Chemical and Physical Meteorology*, 44, 81-99.

Rasse D P, Rumpel C, Dignac M F (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, *269*, 341-356.

Richards BK, Stoof CR, Mason C, Crawford RV, Das S, Hansen J, Mayton H, Crawford JL, Steenhuis TS, Walter MT, Viands DR (2013) Carbon Sequestration and Gaseous Emissions in Perennial Grass Bioenergy Cropping Systems in the Northeastern US. AAIC Proceedings. Washington DC, United States.

Richards, B.K, Stoof CR, Cary IJ, Woodbury PB (2014) Reporting on Marginal Lands for Bioenergy Feedstock Production - a Modest Proposal. *BioEnergy Research*, 7, 1060-1062. DOI: 10.1007/s12155-014-9408-x

Rodriguez-Iturbe I, Porporato A (2004) Ecohydrology of Water-controlled Ecosystems: Soil Moisture and Plant Dynamics. Cambridge University Press, Princeton University, New Jersey, USA.

Sanderson MA (2008) Upland Switchgrass Yield, Nutritive Value and Soil Carbon Change. Under Grazing and Clipping. *Agronomy Journal*, 100, 510–516.

Sala OE, Parton WJ, Joyce LA, Lauenroth WK (1988) Primary production of the central grassland region of the United States. *Ecology*, 69, 40–45.

Schimel DS, Braswell BH, Holland EA, McKeown R, Ojima DS, Painter TH, Parton WJ, Townsed AR (1994) Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. *Global Biogeochemical Cycles*, 8, 279–293.

Schmer MR, Liebig MA, Vogel KP, Mitchell RB (2011) Field-scale soil property changes under switchgrass managed for bioenergy. *Global Change Biology Bioenergy,* 3, 439–448.

Schnabel RR, Franzluebbers AJ, Stout WL, Sanderson MA, Stuedemann JA (2001) The effects of pasture management practices. In: *The potential of U.S. grazing lands to sequester carbon and mitigate the greenhouse effect* (eds Follet RF, Kimble JM, Lal R), pp. 291–322, CRC Press, Boca Raton, United States.

Sims PL, Singh JS (1978) The structure and function of ten western North American grasslands: III. Net primary production, turnover and efficiencies of energy capture and water use. *The Journal of Ecology,* 66, 573– 597.

Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant and Soil*, 241, 151–176.

Shurpali NJ, Hyvönen, NP Huttunen JT (2008) Bare soil and reed canary grass ecosystem respiration in peat extraction sites in Eastern Finland. *Tellus B*, 60, 200–209

Skinner RH, Sanderson MA, Tracy BF, Sanderson MA, Stuedemann JA (2006) Above- and Below ground Productivity and Soil Carbon Dynamics of Pasture Mixtures. *Agronomy Journal*, 98, 320–326.

Skinner RH, Walter ZL, Schmidt JP (2012) Environmental impacts of switchgrass management for Bioenergy production in A Monti(ed) Switchgrass. Publications from USDA-ARS / UNL Faculty. Paper 1320.

Steenhuis TS, Winchell M, Rossing J, Zollweg JA, Walter MF 1995 (1995) SCS runoff equation revisited for variable source runoff areas. *Journal of Irrigation and Drainage Engineering, 121,* 234-238.

Steinbeiss S, Beßler H, Engels C, Temperton VM, Buchmann N, Roscher C, Krwutziger Y, Baade J, Habekost M, Gleixner G (2008) Plant diversity positively affects short-term soil carbon storage in experimental grasslands. *Global Change Biology,* 14, 2937– 2949.

Stewart CE, Follett RF, Pruessner, Varvel GE, Vogel KP, Mitchell RB (2016) N fertilizer and harvest impacts on bioenergy crop contributions to SOC. *Global Change Biology*

Stockmann, U, Adams, M., Crawford, J., Field, D., Henakaarchchi, N., Jenkins, M., Minasny B, McBratney, A, De Remy De Courcelles V, Singh K, Wheeler I, Abbott L, Angers DA, Baldock J, Michael Birde M, Brookes PC, Chenug C, Jastrow JD, Lal R, Lehmann J, O'Donnell AG, Partonl WJ, Whitehead D, Zimmermannn M (2013) The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems and Environment*, 164, 80-99.

Stoof CR, Richards BK, Woodbury PB, Fabio PB, Brumbach ES, Cherney J, Das S, Geohring L, Hansen J, Hornesky J, Mayton H, Mason C, Ruestow G, Smart LB, Volk TA, Steenhuis TS (2014) Untapped potential: Opportunities and challenges for sustainable bioenergy production from marginal lands in New York and the Northeast. *Bioenergy Research,* 8, 482-501. DOI: 10.1007/s12155-014-9515-8

Tiemann LK and Grandy AS (2015) Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. *Global Change Biology Bioenergy,* 7,161-174.

Trumbore SE (1997) Potential responses of soil organic carbon to global environmental change. *Proceedings of the National Academy of Science of USA,* 94, 8284–8291.

USDA-NRCS 2008 Measuring Water Stable Aggregates is described in the Soil Quality Test Kit Guide, Section I, Chapter 8, pp. 18 - 19. See Section II, Chapter 7, pp. 69 - 71

Wang C, Guo L, Li Y, Wang Z (2012) Systematic comparison of C3 and C4 plants based on metabolic network analysis. *BMC Systems Biology,* 6, S9.

Walter MT, Walter MF, Brooks ES, Steenhuis TS, Boll J, Weiler KR (2000) Hydrologically sensitive areas: Variable source area hydrology implications for water quality risk assessment. *Journal of Soil and Water Conservation,* 3, 277-284.

Weil RR, Islam KR, Stine MA, Gruver JB, Sampson-Liebig SE (2003) Estimating active carbon for soil quality assessment; a simplified method for laboratory and field use. *American Journal of Alternative Agriculture,* 18, 3-17.

Williams J, Ketterings Q, Czymmek K, Russell-Anelli J, Hoepting C, Long E (2015) Conservation of Muck Soils in New York. *Agronomy Fact Sheet Series* Fact Sheet 86 Nutrient Management Spear Program http://nmsp.cals.cornell.edu

Xiong SJ, Katterer T (2010) Carbon-allocation in reed canary grass as affected by soil type and fertilization rates in northern Sweden. *ACTA Agriculturae Scandinavica Section B - Soil and Plant Science,* 60, 24-32.

Zhang K, Dang H, Tan S, Cheng X, Zhang Q (2010) Change in soil organic carbon following the "grain-for-green" programme in China. *Land Degradation and Development*, 21, 13–23

Zollweg JA, Gburek WG, Steenhuis TS (1996) SmoRMod – A GIS-integrated rainfall runoff model. *Transactions of the ASAE,* 39, 1299-1307.

CHAPTER 3

DEPTH DISTRIBUTION OF SOIL ORGANIC MATTER AND SOIL ORGANIC CARBON IN A SEASONALLY-WET, FALLOW FIELD OF THE TEMPERATE REGION[2]

Abstract

In this study, we report the depth profile (in six intervals to 120 cm) of soil organic matter (OM), soil organic carbon (SOC), total nitrogen (TN), carbon-to-nitrogen (C/N) ratio, bulk density and $\delta^{13}C$ of soils from a seasonally-wet fallow (~50 year abandoned aside from occasional mowing) field in New York State, before its conversion to bioenergy cropping. The impact of surface volumetric water content and shallow water table depth on the distribution of these soil properties was also studied. Additionally, accuracy of loss-on-ignition (LOI) assay (using New York State soil testing conversions from LOI to OM and SOC) with respect to dry combustion-elemental analysis for SOC estimations during soil analytical studies was tested. The soil profile displayed characteristic decreases in OM, SOC and TN values with depth; values varied significantly ($p<0.0001$) between 0-5, 5-15, 15-30 cm and beyond 30 cm. SOC for the top 15 cm represented 69% of the SOC in the 120cm profile. SOC stocks for the depth intervals 0-5, 5-10,15-30,30-60,60-90 and 90-120 cm were 22.1±9, 45.1±9, 41±8, 27±2, 37±5 and 39±5 Mg C ha^{-1}, respectively. Surface volumetric water content had a greater influence on soil properties to a depth of 120 cm than did shallow perched water depth. $\delta^{13}C$ values of the Ap horizon soil reflected signature values [-27.9 ±0.3 (‰) and -27.4 ±0.4 for 0-5 and 5-15 cm respectively] consistent with cool-season C3 vegetation dominating the fallow field for decades. LOI derived C (%) vs. combustion elemental analysis C (%) regressions to 60 cm correlated well (R^2=0.99). However, for depths beyond 60 cm at our site, LOI overestimated C (%) values.

[2] Srabani Das, Rachael E. Thomas, Brian K. Richards, Michael F. Walter, Tammo S. Steenhuis (This Chapter is not for current publication.)

Keywords seasonally-wet fallow land, depth profile (120 cm), organic matter, soil organic carbon(SOC), bulk density, $\delta^{13}C$, loss-on-ignition (LOI).

Introduction

Perennial bioenergy crops are a projected option for reducing dependence on non-renewable fossil fuels. In the Northeast (NE) United States, fallow marginal lands are being cited as a resource base for the perennial bioenergy sector, with a potential of improving rural economies. Many soils in this region are unsuitable for row crop agriculture due to seasonal water saturation or near-saturation which primarily results from presence of shallow restrictive layers, rendering about 20% NE soils poorly or very poorly drained (Stout *et al.*, 1994; Stoof *et al.*, 2015). Warm-season (C4) grasses like switchgrass (*Panicum virgatum* L.*)* and giant miscanthus (*Miscanthus X giganteus)* are well known for placing organic matter (OM) in deeper soil layers with their robust rooting systems; OM inputs via grass roots penetrating dense fragipan is also a possibility (Blanco-Cancui, 2010; Mayton personal communication 2012). However, deeper rooting systems could conversely have negative impacts on soil organic carbon (SOC) buildup through distribution of fresh C at depth, stimulating mineralization of ancient buried C by priming effects (Fontaine *et al.*, 2007). Additionally, the deeper rooting hypothesis could be counterintuitive, as root length being a function of water scarcity, could be constrained by wet soils (Dell personal communication 2013).

At the global scale, although SOC typically increases with precipitation and clay content and decreases with temperature, the importance of these controls varies with depth and climate dominating in the shallow layers vs. clay presence dominating in the deeper layers (Jobba´gy and Jackson, 2000). Deeper portions of soil profile have historically been ignored in soil C sequestration studies. Increasing focus on deep rooted perennial grass plantations as part of Global Change mitigation policy and soil conservation strategy necessitates study of the impacts of such vegetation on soil organic carbon (SOC) in deeper soil zones. Plant functional groups control SOC distribution with depth, due to differing root distribution and above-ground/below-ground allocation patterns (Jobba´gy and Jackson 2000). The major processes of OM input into subsoils are root biomass and root exudates, dissolved organic carbon percolating via preferential flow pathways, and particulate OM transport from the surface through bioturbation (Von Lützow 2006; Rumpel and Kögel-Knabner 2011; Schmidt *et al.,* 2011). OM in deeper soil layers constitutes a greater proportion of microbially-derived C than plant-derived C, with decreasing carbon-to nitrogen (C/N) ratio, and increasing stable C and N isotope ratios (Rumpel and Kögel-Knabner 2011; Schmidt *et al*., 2011). It is well known that major photosynthetic pathways (C3 and C4) discriminate against $^{13}CO_2$ to differing degrees which is then reflected in the soil C that accumulates under either C3 or C4 vegetation. As such, land use changes that convert from C3 to C4-based vegetation (or vice versa) provides the opportunity for a natural tracer experiment that is a sensitive method for tracking C sequestration (Balesdent *et al*., 1988; Farquahar *et al*.,1989; Balesdent & Mariotti, 1996; Corre *et al*., 1999; Sanderson 2008). A simple isotopic mixing model can be used to calculate the proportion of new C input to the soil; switchgrass (C4) with a $\delta^{13}C$ of -12‰, often alters the $\delta^{13}C$ of a C3 dominated soil environment (approximately -28‰; Boutton, 1996). Furthermore, the gradient of SOC plotted against $\delta^{13}C$ has often been suggested as a proxy of soil turnover and follows a linear relationship (Acton *et al.,* 2013). Microbes tend to favor the lighter ^{12}C isotope during oxidation of OM, which leads the soil substrate to become enriched in the heavier ^{13}C isotope and ^{13}C atoms being sequestered relative to ^{12}C atoms and smaller OM particles then physically migrate downward by physical mixing processes through time and result in increased $\delta^{13}C$ values at depth. Estimations of C sequestration rates at depth increments is subject to bulk density differences (and imprecision), which can greatly impact determinations of SOC stocks (Ellert and Bettany, 1995). Equivalent soil mass techniques can be used to correct for variation and shifts in soil bulk density (Ellert and Bettany, 1995; Wuest, 2009). While surface volumetric water content (VWC) directly controls OM buildup and C mineralization rates in the Ap zone, shallow/perched water tables are also thought to have an impact on conserving OM stocks (Drösler, 2005).

Loss-on-ignition (LOI) is a widely–used method to estimate organic matter (OM) content of soil, being simple, rapid, and inexpensive. However, the accuracy of the technique and of factors for conversion from LOI values to OM and/or SOC contents have often been questioned (Bernal, 2008; Sutherland, 2010; Pallaser *et al.*, 2013; Lehmann, personal communication 2014). Several inconsistencies as regards to pretreatment, soil heating temperature and time protocols have been reported in literature (Cambardella *et al.,* 2001; Collins and Kuehl 2001; Anderson and Mitsch, 2006). Dry combustion elemental analysis of soils by thermogravimetric methods are used to determine more accurate total C values. However, the scale of sampling may necessitate the use of the LOI assay, hence its suitability and accuracy for assessing SOC merits investigation. Many studies also recommend LOI analyses to be supported by results from standard total carbon analysis or other methods such as thermogravimetric processes that can identify mass changes with differences in temperature in small intervals.

In this study, we analyzed OM in six depth increments to 120 cm in a wetness-prone field maintained for decades in a minimally-managed fallow state before it was plowed and disked in preparation for seeding with perennial grasses. A subset of soil samples were used for SOC, total nitrogen (TN), C/N ratio, bulk density and $\delta^{13}C$ analysis. The objectives were to (i) to determine the profile for preconversion baseline OM and other soil characteristics to a depth of 120 cm of the wet-marginal field before it was planted to perennial grass, (ii) to determine the influence of surface volumetric water content and shallow-water table on the distribution of OM and other soil characteristics to 120 cm, and (iii) to test the suitability of loss-on-ignition (and using the New York State empirical relationships between LOI, OM and organic C) for soil analytical studies by comparing it to dry combustion-elemental analysis. We hypothesized that OM, SOC, TN, C/N ratio of soils would decrease with increasing depth, being significantly low beyond 30 cm. Additionally, we hypothesized that $\delta^{13}C$ and bulk density of soils would increase with depth and $\delta^{13}C$ signature of Ap soil would vary in the range for C3 vegetation. While we expected VWC content in the Ap layer (~0-15cm) to influence OM content for Ap horizon, we expected the shallow-water table to impact the OM content at all depths. Furthermore, we hypothesized that LOI assay technique would be suitable surrogate for SOC for at least the Ap and shallow soil horizons.

Methods

Site description

During the summer of 2011, a seasonally wet, fallow field near Ithaca, NY (N 42° 28.20', W 76° 25.94') was converted to a perennial grass crop field. The presence of restrictive layers (fragipans and/or dense basal till) in combination with slightly undulating topography of the field results in wide a range of soil moisture conditions, with the soil drainage classes varying between moderately well-drained to poorly drained. The predominant drainage catena is comprised of three soil series: small areas of well-drained Canaseraga (coarse-silty, mixed, active, mesic typic Fragiudept, fragipan depth 45-85 cm), somewhat poorly drained Dalton (Coarse-silty, mixed, active, mesic aeric Fragiaquept, fragipan depth 30-55 cm) and poorly drained Madalin (fine, illitic, mesic mollic Endoaqualf) soils. Small areas of associated Rhinebeck (fine, illitic, mesic Aeric Endoaqualfs) and Langford (fine-loamy, mixed, active, mesic Typic Fragiudepts, fragipan depth- 35-70cm) soils are also present. The field topography is undulating, with slopes varying from 0 and 8% along with a small area reaching 15% on the eastern edge. Perched water tables are found seasonally (as well as during excessively wet periods during the growing season), resulting from relatively permeable surface soils atop shallow restrictive layers. In these areas, significant quantities of lateral interflow atop the restrictive layer saturates downslope depressions and concave areas (Steenhuis *et al*., 1995; Zollweg *et al*., 1996; Frankenberger *et al.*, 1999; Walter *et al.*, 2000), a characteristic feature of many wet soils of the region. The soil textures in these profiles are primarily silt-loam. The existing vegetation was dominated by legacy reed canarygrass as well as other cool-season broadleaf forbs and grass species. The field characteristics details can be found in Richards *et al.*, 2013. In preparation for future perennial grass study, the field was divided into 16 adjacent strip plots (each between 0.34-0.44 ha), assigned randomly to quadruplicate perennial grass treatments and fallow controls. A primary goal in the field layout was to capture the natural continuum of soil moisture conditions within each strip plot. Five permanent sampling sub-plots were established along the natural moisture gradients within each stripplot based on an initial intensive survey (June 12, 2011) of surface volumetric water content measurements by time-domain reflectometry (TDR, Campbell Scientific Hydrosense meter using 12 cm sensor rods). Thus, eighty permanent sampling points were established where soil was sampled yearly and periodic TDR and shallow well measurements were made to characterize the relative soil moisture status of each subplot. For each measurement event, a field average VWC of all 80 subplots was calculated, and each specific subplot's value was normalized relative to the field mean (yielding a "relative soil moisture ratio" for that subplot and time point). These relative values were averaged over the entire study period for each subplot, yielding a characteristic relative soil wetness or dryness vis-a-vis the field average. Finally, these multi-years mean values for the 80 subplots were aggregated into "soil wetness quintiles" (VWC Q1, wettest through VWC Q5, driest) for the entire study period. For this study, the dynamic quintile rankings through fall 2013 were used. Perched water table depths were monitored using shallow wells from 2012 onwards. The measuring instrument consisted of a PVC pipe (2 cm diameter, 1.2m long), installed vertically, covered with a loose-fitting (3 cm diameter) PVC cap and used custom depth measurement stick-device that provided audible sensing of the water surface. Flooded subplots were assigned a depth of zero (Mason *et al.*, 2017). The eighty subplots were similarly assigned perched water table depth quintile ranks (PWT Q) Q1 being shallowest (wettest) and Q5 deepest/driest.

Experimental design

For determining baseline OM values, data from all six (0-5, 5-15, 15-30, 30-60, 60-90 and 90-120 cm) depth increments of all 80 subplots (n=480) from 2011 soil corings were used. Three analytical replicates were used for each individual sample (analysis n=480x3=1440).

For determining soil characteristics viz SOC, TN, C/N ratio, bulk density and $\delta^{13}C$ analysis, a subset of 24 subplots were chosen from the 2011 depth samplings and soil samples from six depth increments were utilized (n=144). A full factorial design of four planned cropping systems by two field replicates (present 8 field replicates of fallow lands) by three water content levels (VWC quintiles: Q1(wettest), Q3(mid-wet), and Q5(driest) was utilized.

For LOI-elemental analysis C correlations, 2011 OM data was grouped as per the dynamic wetness quintile rank (through May 2013) and 90 out of 480 samples were chosen for determination of C values by combustion elemental analysis, covering the various depth intervals. A full factorial design of five wetness quintiles (VWC quintiles Q1,Q2 Q3,Q4,Q5) by four field replicates by three depth increments (0-5, 5-15, 15-30 cm) chosen randomly (based on low standard deviation values <0.05) for the top three depth intervals and a full factorial design of five wetness quintiles by two field replicates by three depth increments (30-60, 60-90 and 90-120 cm) chosen randomly (based on low standard deviation values <0.03) for the bottom three intervals, were used. Additionally, LOI-elemental analysis correlations were also done for surface soil samples from 80 subplots sampled in 2014.

Field soil samplings In July 2011, soil was sampled at the eighty subplots to a depth of 120cm using a tractor-mounted soil probe or auger (Giddings Machine Co., Ft Collins), separated by depth increments of 0-5, 5-15, 15-30, 30-60, 60-90 and 90-120cm. Soils were able to be cored only to ~15 cm; due to dense subsoils, all deeper layers had to be extracted via augering. The soil removed was immediately weighed for each depth interval, bagged after hand removal of large stones, re-weighed, subsampled for gravimetric moisture content, and subsequently air-dried and stored for further analysis.

Laboratory analyses Dried soil was passed through a Dynacrush DC-5 Soil Crusher (Custom Laboratory Eqpt, Orange City, Florida) with 10 mesh sieve (2mm openings) three times in succession, and was then sieved with a 1.8 mm sieve, in accordance to the procedure of Garten and Wullschleger (1999). Visible roots were hand-picked during the milling and sieving process and saved. The separated coarse fragments were weighed to help when converting analytical results (which are based on fine soil only) to a realistic areal basis, since said fragments remain present during bulk density determinations. The mineral soil fraction (including any very fine roots not handpicked or sieved) was thus prepared for use in LOI assay and combustion analysis. Elemental C and N analysis was carried out by combustion infrared detection (LECO TruMac

CN, LECO Corp, St. Joseph, MI) using 0.4 g of oven dried (60°C) soil. N precision was 0.1 mg or 0.3% RSD (whichever is greater) and Carbon was 0.01mg or 0.4% RSD (whichever is greater). Bulk density was coarsely estimated using the oven-dry (24 hrs. at 105°C) weight and the nominal volume of sample (based on core diameter and depth interval), corrected for the mass and volume of any large coarse fragments subsequently found in the sampled core.

The isotopic composition ($\delta^{13}C$) and SOC and TN of each sample from the subset of 24 was measured in sterilized tin capsules (containing 15 mg soil for soil intervals above 30 cm and containing 30 mg for soils for below 30 cm depth) using a continuous-flow isotope-ratio mass spectrometer, IRMS (Delta Plus, Finnigan MAT) equipped with Carlo Erba NC 2500 elemental analyzer. The precision of IRMS for the soil (internal standard) material was between 1 and 2% with regard to carbon and <1% for the isotope analysis. The ^{13}C abundance was expressed as delta depletion ($\delta^{13}C$) from the international standard, Pee Dee Belemnite (PDB) as in equation 1:

$$\delta^{13}C\ (‰) = \left[\frac{\text{Rsample} - \text{Rreference}}{\text{Rreference}}\right] \times 1000 \text{---------------------} (1)$$

where R $_{sample}$ is the isotope ratio, $^{13}C/^{12}C$, of the sample and R $_{reference}$ is that of PDB (Balesdent and Mariotti, 1996).

Percent OM was estimated by loss on ignition LOI assay, for which air dried, ground and sieved soil samples (5g) were dried in an oven at 105°C for at least 4 hours, cooled in dessicator (15 mins) weighed and then placed in muffle furnace for 2 hours at 500°C cooled and weighed again. New York State soil testing laboratory empirical relationships between LOI, OM and SOC, OM = 0.7(LOI)-0.23 (R^2= 0.94) and OM = 1.724C were used for conversion to C values (%). (Moebius-Clune *et al*., 2016).

To incorporate a low-cost analysis scheme for 480 samples (also due to not having access to elemental analysis equipment during the first year), LOI assays were used to estimate percent OM (and thus organic C) for 2011 samples. For subsequent years, both elemental C and LOI analyses were performed. Thus, to compare and reconcile the possible discrepancies in carbon (%) estimation from LOI assay, a retroactive elemental analysis (LECO) was done on a subset of 90 (out of 480) samples (representing all depth intervals) from 2011 and all 80 Ap samples (surface depth only) from 2014.

Statistical methods For determining overall differences in OM at different depths, one-way analysis of variance (ANOVA) was performed with depth interval as main effect. Similarly, one-way ANOVA was used to compare soil properties (SOC, TN, bulk density, $\delta^{13}C$) for the different depth intervals.

Additionally, to assess differences of OM between soils of different moisture groups within each depth interval, one-way ANOVA was also used to compare VWC and PWT wetness quintile ranks as main effect. Hierarchical linear models were fit using SOC, TN, C/N, bulk density, $\delta^{13}C$ as response variables and VWC and PWT quintile wetness quintile ranks as main effects for each depth, and subplots were used as random effect. These models assessed the influence of surface volumetric water and perched water table depth on these parameters.

Simple regression analysis and Bland-Altman analysis were done to test the accuracy of LOI assay with respect to dry combustion-elemental C analysis. The LOI-derived values were regressed against the LECO values found for the same samples. Regression analyses were done on sample groupings representing the upper two depth intervals (0-5 and 5-15 cm), upper three depth intervals (0-5, 5-15, and 15-30 cm), lower three depth intervals (30-60, 60-90 and 90-120 cm) and upper four depth intervals (0-5, 5-15, 15-30 and 30-60 cm). Some outliers were removed (from each depth interval, based on standard deviation values). Regressions were also done for all 80 surface soils sampled in 2014. All statistical analyses were carried out using JMP Pro 12 (SAS Inc., Cary, NC).

Results

Depth distribution of OM and soil properties

Overall, the soil profiles displayed characteristic significant ($p<0.0001$) decreases in OM with depth; while values varied significantly between 0-5, 5-15, 15-30 cm and beyond 30 cm, there was no significant difference among values for OM for lower three depth intervals below 30 cm (Fig.3.1). Overall, OM varied between 80.4±13 to 16.6±2.7 g kg^{-1} in the 0-5 and 90-120 cm depth interval, respectively.

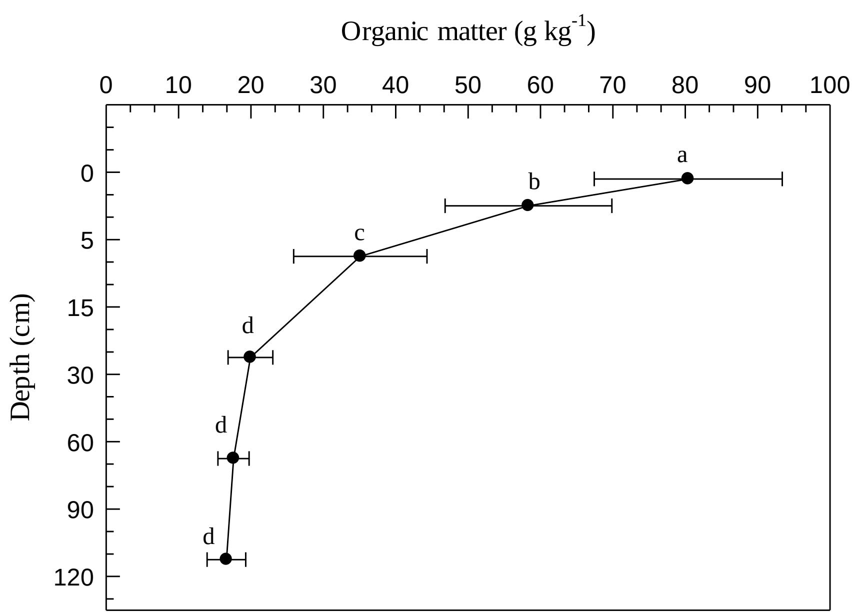

Figure 3.1. Depth distribution of preplow (2011) organic matter (g kg^{-1}) of soil samples from 80 subplots for six depth increments between 0-120 cm (n = 80 for each depth level, total 480)

For the smaller subset of samples, SOC in the first 15 cm represented 69% of the SOC in the upper 120 cm. The mean and standard deviations of soil characteristics for each depth interval is shown in Table 3.1. SOC and TN for soils of the first three depth intervals were significantly

($p<0.0001$) greater than the lower three depth increments (Table 3.1). While C/N ratios of soils from the lowest (90-120cm) depth interval were significantly ($p<0.0001$) greater, those of soils from the depth interval 30-60cm were significantly ($p<0.0001$) lower than all other depths. Bulk density values (estimated from mass and nominal coring volume after subtracting large stone mass and volume) for soils of the 0-5 cm interval were significantly ($p<0.0001$) lower than those of deeper horizons. Bulk density estimations for the depth intervals below 90 cm were judged invalid due to markedly poorer control of extracted soil volume and mass with the auger. The $\delta^{13}C$ of soils from the lowest two depth intervals were significantly ($p<0.0001$) lower than those of soils at 0-60cm.The SOC stocks for the 6 depth increments were 22.1±9, 45.1±9, 41±8, 27±2, 37±5 and 39±5 Mg C ha^{-1} for the depth increments 0-5, 5-15, 15-30, 60-90 and 90-120cm, respectively.

Table 3.1. Mean and standard deviation of soil characteristics for preplow (2011) soils of six depth intervals from 24 subplots (n=24) for each depth level, total 144).

Depth interval (cm)	SOC (g kg^{-1})	TN (g kg^{-1})	C/N ratio	Bulk density (g cm^{-3})	$\delta^{13}C$ (‰)
0-5	55.2 ±8.9 a	4.8 ±0.9 a	11.6±0.2 ab	0.8 ±0.2 d	-27.9 ±0.3 c
5-15	37.6 ±8.7 b	3.5 ±0.9 b	10.7±0.1 b	1.2 ±0.2 c	-27.4 ±0.4 c
15-30	18.2 ±7.3c	1.8 0.7 c	10.2±0.5 b	1.5±0.2 ab	-26 ±3.8 c
30-60	5.6 ±1.5 d	0.8±0.1 d	6.9±0.3 c	1.6 ±0.4 a	-24.9 ±3.1c
60-90	7.2 ±3.4 d	0.7 ±00.1 d	10.4±1.2 b	1.7±0.2 a	-18.9 ±6.8 b
90-120	9.9 ±3.7 d	0.7 ±0.2 d	14.7±1.4 a	NA	-14.3 ±6.3 a

Influence of soil moisture on OM, SOC and soil properties at different depth increments

For the first two depth intervals (0-5 and 5-15 cm) covering the Ap horizon, OM contents of the VWC quintile Q1 soils were significantly greater ($p=0.0003$, $p=0.001$) than drier soil groups. For the 90-120cm interval, the two driest quintile (Q4, Q5) soils had significantly ($p= 0.003$) greater OM contents than those of the wettest soils (Fig.3.2a). When PWT quintile rankings were considered, there was no difference among OM values of the different wetness groups at any depth (Fig.3.2b). The influence of VWC or PWT depth on all soil parameters (subset of 24 soil samples) at each depth interval is seen in Fig. 3.3a and 3.3b, whereas the p values (whenever significant) are depicted in Table 3.2. When considering VWC, the wettest soils had significantly greater SOC in comparison to both the mid-wet soils at 0-5 cm as well as the mid-wet and driest soils at 5-15 cm, whereas mid-wet soils had significantly greater SOC than the wettest soils at depths of 60-90 and 90-120 cm. While the wettest soils also had significantly greater TN compared to the mid-wet and driest soils at 0-5 and 5-15 cm, the mid-wet soils had significantly greater TN than the wettest soils at 60-90 and 90-120cm. C/N ratios of the driest soils were significantly greater than those of the wettest soils at 0-5 and 15-30cm, while the values for the wettest soils were greater than that of the driest soils at 90-120cm. While the bulk density of driest soils was greater than the wettest soils at 5-15 cm, at the 30-60 cm interval that of wettest soils were significantly greater than the driest soils. $\delta^{13}C$ values of soils of driest soils were greater than wettest soils at 0-5cm and that of mid-wet soils were greater than driest soils at 90-120cm.

In terms of the impacts of PWT depth, both $\delta^{13}C$ and bulk density of driest soils were greater than the wettest soils, while at the depth interval of 5-15cm both SOC and TN values were lower than that of the wettest soils. For the rest of the depth intervals, PWT rank only correlated with estimated bulk density (Table 3.2).

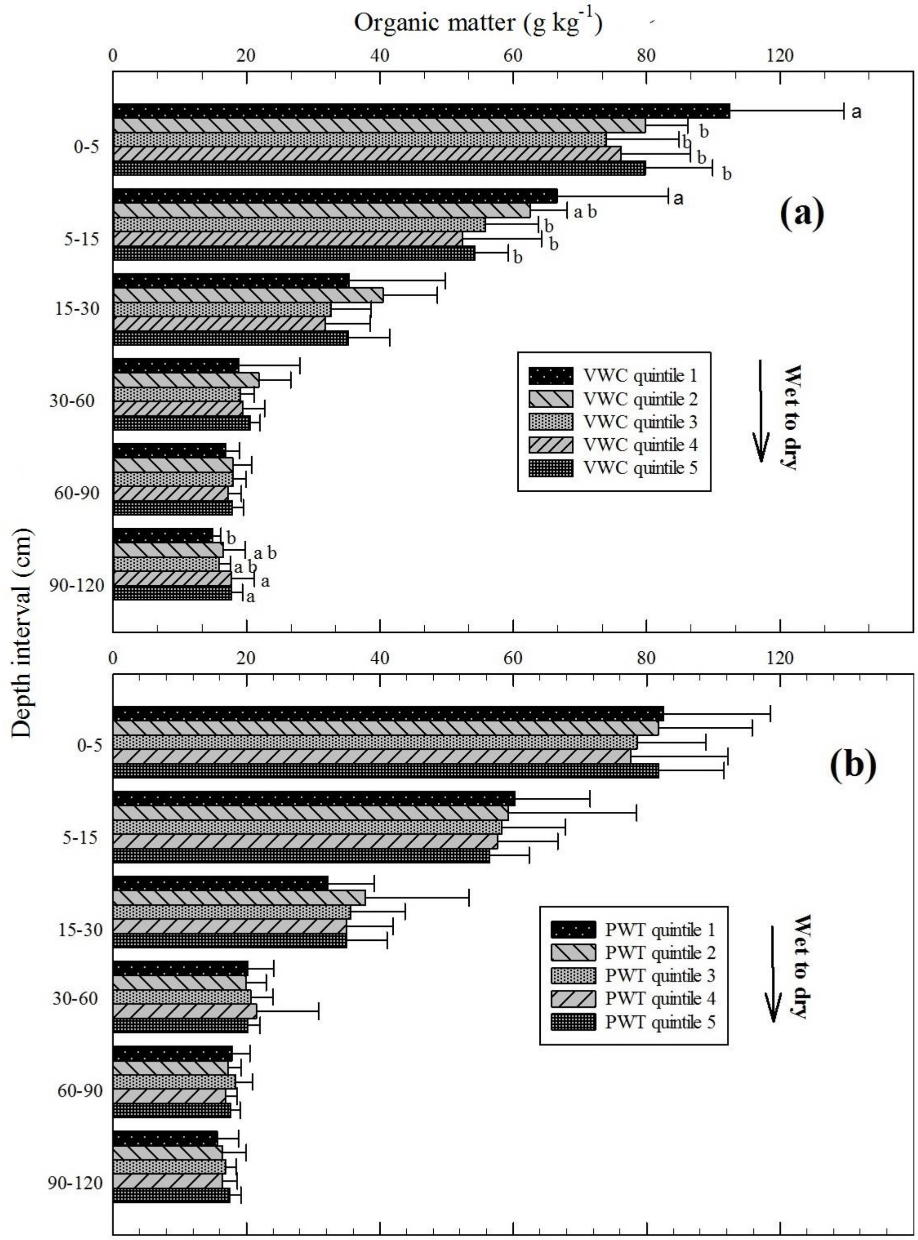

Figure 3.2. Depth distribution of preplow (2011) organic matter (g kg^{-1}) of soil samples from 80 subplots for six depth increments between 0-120 cm (n = 16 for each quintile group of each depth level, total 480) grouped as a function of their (a)volumetric water content (VWC) quintile

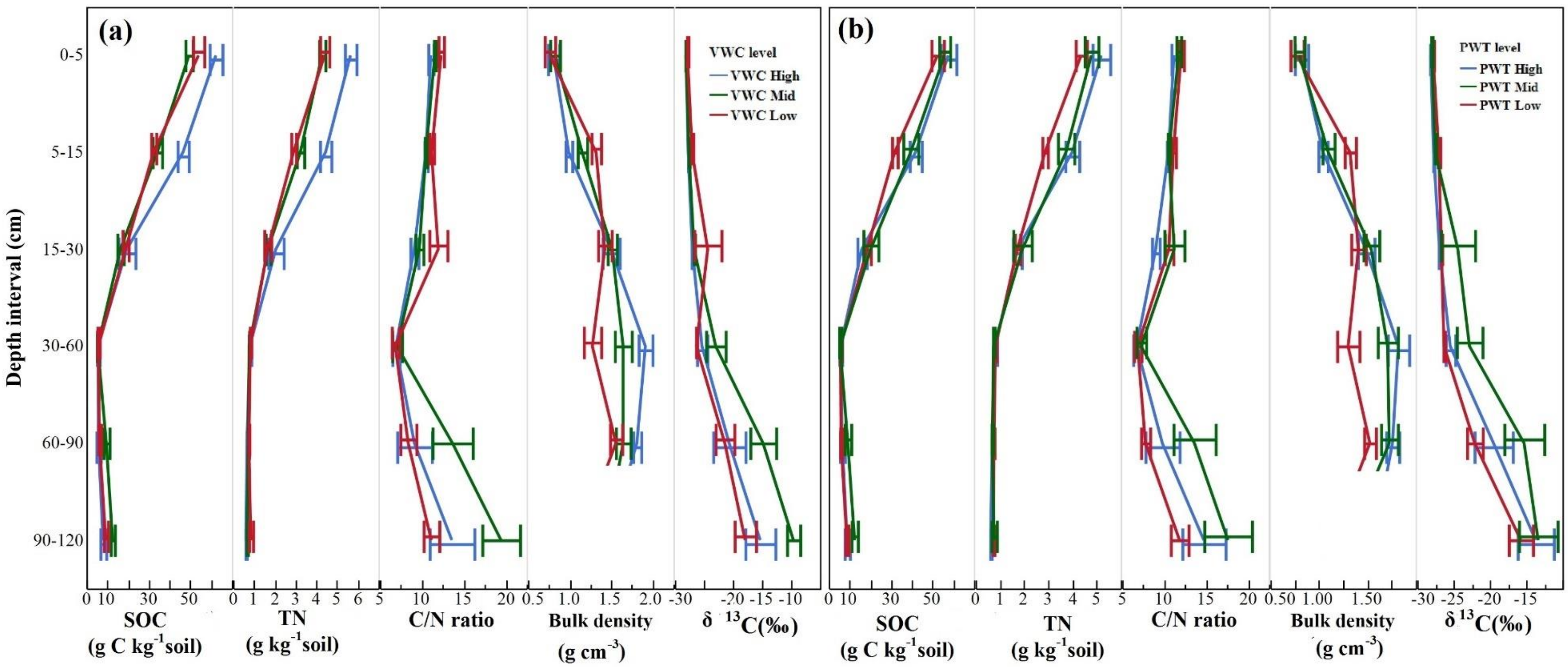

Figure 3.3. Depth profile of preplow soil characteristics from 24 subplots for six depth increments between 0-120 cm (n = 8 for each wetness level for each depth interval, total 144) grouped as a function of their (a) volumetric water content (VWC) quintile rank and (b) as perched water table (PWT) depth quintile rank.

Table 3.2. p values (whenever significant) of the main effects VWC and PWT quintile rank for different soil properties at various depth increments between 0-120 cm. Preplow soils (n = 8 for each wetness level for each depth interval, total 144) grouped as a function of their volumetric water content (VWC) quintile rank and perched water table (PWT) depth quintile rank have been used. Boxes in two shades indicate significance of VWC and PWT in influencing soil parameters at a specific depth.

Depth	SOC		TN		C/N		Bulk density		$\delta^{13}C$ (‰)	
	VWC	PWT	VWC	PWT	VWC	PWT	VWC	PWT	VWC	PWT
0-5	P=0.006		P=0.0004		0.01					
5-15	P=0.0002	P=0.05	P<0.0001	P=0.02			P=0.0009	P=0.005	P=0.0009	P=0.001
15-30					P=0.03					
30-60							P=0.0005	P=0.008		
60-90	P=0.04		P=0.03							
90-120	P=0.03		P=0.03		P=0.03				P=0.02	

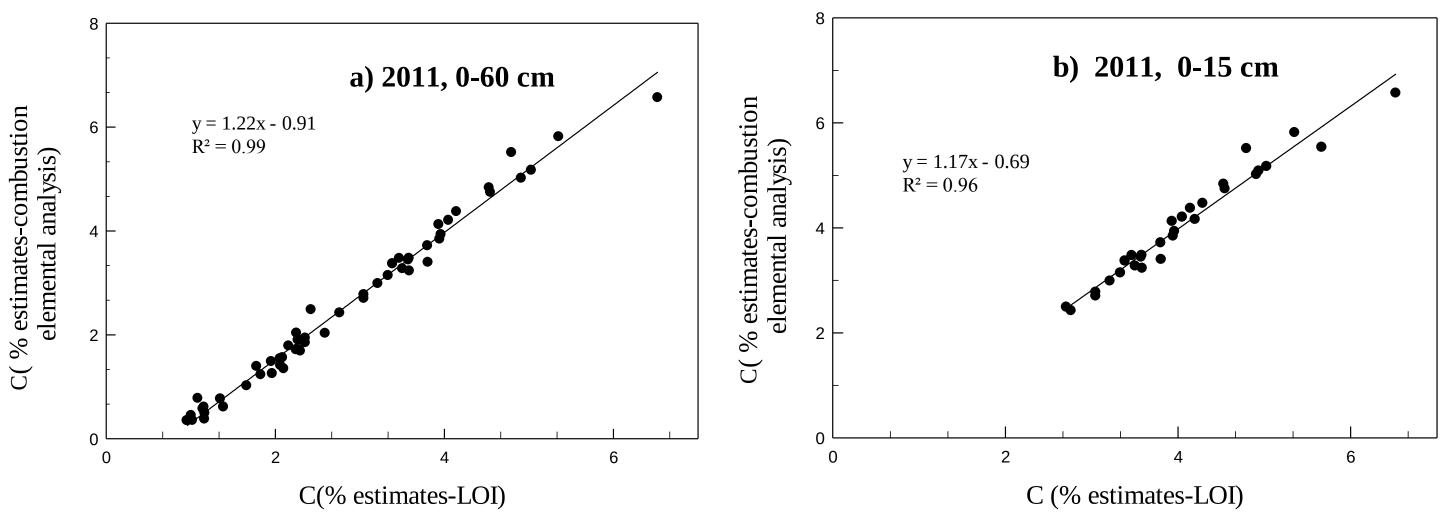

Figure 3.4. Regression analysis of C (% values from combustion elemental analysis) vs C (% values from LOI analysis) of a) 61 soil samples (outliers removed) for depth interval 0-60 cm and b) 31 soil samples (outliers not removed) for depth interval 0-15 cm from the pre-plow 2011 soils.

Comparison of elemental C analysis of 31 surface samples in 2011 and all 80 surface samples in 2014 (for additional testing for post plowing soils) to LOI-based C contents yielded similar results (R^2=0.99). While paired t-tests showed no significant difference during both years [(P= 0.8, t-Ratio = 0.3) for 2011 and (P = 0.1, t-Ratio = -1.6, for 2014] between the two techniques (Table 3.3), Bland-Altman plots (Figure 3.5) provided a visual assessment for the difference between the two processes. The plots for 31 surface soil samples from 2011 and 80 surface soil from 2014 for the two techniques both indicate that as average C values increased, the difference between LOI and elemental analysis became negative and was unevenly scattered around the mean value of the two processes, considered together. Hence, at C values, especially above 3%, LOI assay estimations were lower than elemental C analysis.

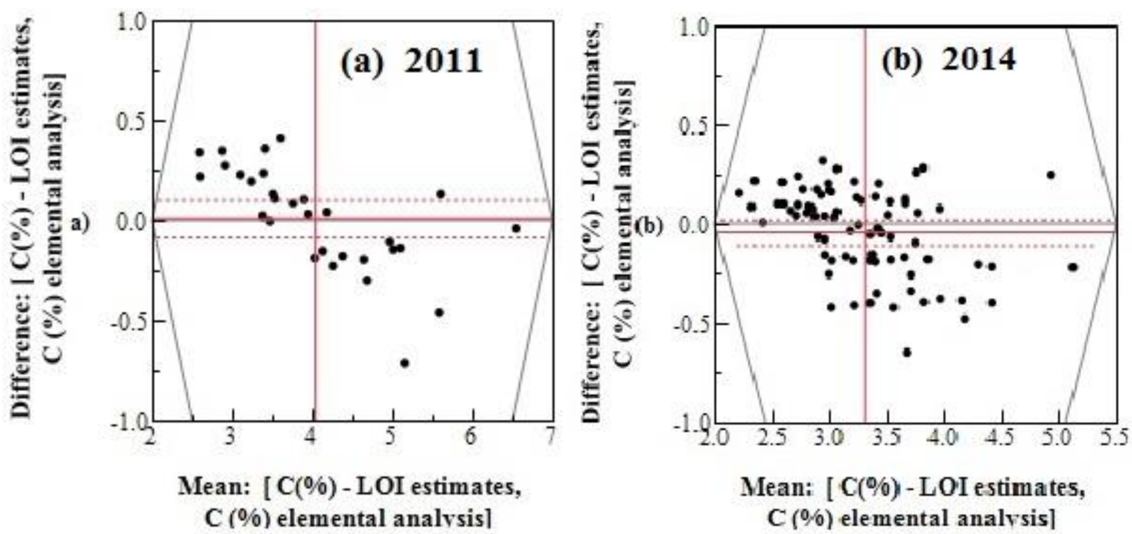

Figure 3.5. Bland-Altman plot for the difference between percent C estimated by the LOI assay (LOI) and the dry combustion elemental analysis method for 31 and 80 surface soil samples (0-15cm, no outliers removed from both sample sets) a) from 2011 and b) 2014. Red bold line indicates the mean difference, the dotted lines in a) indicate 95% upper and lower confidence interval and in b) and 99% upper and lower confidence interval. Soils sampled by coring in 2011 and by shovel in 2014.

Table 3.3. Paired t-tests for the difference between percent C estimated by the LOI assay (LOI) and the dry combustion elemental analysis (LECO) for 31 and 80 surface soils (0-15cm) for 2011 and 2014 respectively.

	n	Mean difference	Std. error	t-Ratio	Df	P value
2011	31	0.01	0.05	0.32	30	0.75
2014	80	-0.04	0.02	-1.61	79	0.11

Discussion

Depth profile of OM, SOC and soil properties

The significant decreases in OM, SOC, TN and increase in bulk density of soils with depth were as expected. With a smaller degree of plant inputs and lower biological activity, lower OM turnover in subsoils in comparison to the Ap horizon is expected. Our observed decreased in SOC, TN and increase in bulk density were comparable to the studies of Corre *et al.*, 1999, Ma *et al.*, 2000, Matamala *et al.*, 2008 and Schmer *et al.*, 2011. The fact that 69% of the profile SOC was found in the top 15 cm is consistent with results of Jobba´gy and Jackson, 2000, who found that on average the 0-15 cm layer contained 42% of the SOC in the 0-100 cm profile in grassland soils, globally. Significant decrease in OM (Fig.3.1, Fig.3.2) SOC and TN beyond the 30-60 cm depth increment (Fig.3.3) could be an indicator of fragipan presence approximately at the same depth. However, the order reversal of SOC (5.6 ±1.5,7.2 ±3.4, 9.9 ±3.7 g kg^{-1}) and C/N ratio (6.9±0.3 ,10.4±1.2, 14.7±1.4) values in the depth intervals 30-60, 60-90 and 90-120 cm (Table 3.1) though puzzling, were probably indicative of carbonate presence beyond depth of 60 cm. Additionally, as such a reverse order was not seen for TN or OM (Fig.3.1), confounding of the elemental C concentrations due to carbonate presence is likely.

The mean SOC (in g kg^{-1}) reported from our study ranged between those of remnant and restored prairie sites in Illinois as reported from the study of Matamala *et al.*, 2008 and were greater than those from the study of Schmer *et al.,* 2011 which reported values from 5yr old switchgrass plantations in Nebraska and from plots planted to perennial grasses in the study of Ma *et al.*, 2000 in Alabama, all at comparable depth intervals. The mean SOC in the first two depth increments (55.2 ±8.9 and 38±8.7 g kg^{-1}, respectively) were close to lower ranges of salt marshes and wetlands (Bernal, 2008; Keller *et al*., 2012) and similar to rangelands and prairies (Davidson and Ackerman, 1993). Being fallow with minimal disturbance for circa 50 years, the site had probably reached a relatively stable equilibrium level of SOC at the time of its conversion in 2011. High initial SOC levels seemed to have limited additional C accumulation by perennial crops in some studies (Tiemann and Grandy, 2015) and this site could similarly result in limited increases in SOC levels with bioenergy cropping in the future years. Low C saturation deficit (difference between the theoretical maximum C storage capacity and the current C content (Six *et al*., 2002) at the field cannot be ruled out. However, on the other hand, changes in management practices at the site, such as introduction of subsurface drains and alteration in mowing regime potentially resulted in OM losses during some instances, indicating room for further SOC accrual with bioenergy cropping.

The $\delta^{13}C$ of the surface soils ~ -27 (‰) was in the range of signature values for C3 vegetation, pointing to the detritus inputs from legacy C3 broadleaf vegetation which historically dominated the field. Increasing values of stable carbon isotope with depth, especially in the 60-90 and 90-120 cm interval suggests absence of deep rooting plant varieties of the legacy C3, beyond 60 cm approximately. The $\delta^{13}C$ signature values at the lower depths indicate primary microbial and abiotic control (Powers and Schlesinger, 2002). Precise fragipan depth estimations, depth-level soil texture and pH characterization are being suggested as a future work areas, which would be help better characterize the soil profile. Acid pretreatment for SOC estimations will also be tested to improve results in the lower depth intervals. We will revisit the site 10 years from establishment, to assess the influence of deep rooted perennial grasses on soil properties at depths and SOC accrual at depth.

Influence of moisture on OM, SOC and soil properties at different depth increments

Contrary to our hypothesis, surface volumetric content displayed a more dominant control on soil properties examined than did the perched water table depth, over the 0 to 120 cm soil depths examined. Although the TDR probes measuring VWC only reach a depth of ~12 cm, the surface VWC was more strongly correlated to depth distribution of the soil properties examined, indicating surface water content being a more useful predictor for the deeper soil characteristics than the perched water table depth. Additionally, as the subset was chosen based on their primary VWC quintile rank, all PWT ranks were close, but not the best representation of their PWT rank. The influence of shallow water table depth was observed from a depth of 5cm and was consistent in its correlation with bulk density value up to 120cm. As both soil wetness indices pointed towards greater OM, SOC, TN and lower C/N ratios, bulk density, $\delta^{13}C$ for the wettest soils, compared to the drier at surface depths, the influence of moisture on OM and SOC accrual is evidenced. As more soil moisture facilitated organic matter inputs, SOC content of soils increased and bulk density decreased. However, the order was reversed from 30-60 cm depth increment, where mid-wet soils displayed greater SOC and TN and driest soils displayed greater C/N, bulk density and $\delta^{13}C$ than wettest soils. This indicated that increased moisture was limiting for SOC build up at greater depth, possibly due to lower soil oxygen availability.

Correlation of LOI to elemental combustion

Significant R^2 values for LOI–elemental analysis regression for surface soils resulting in dependable derived % C values is in line with other work, where clay content of soils was less than 30% (Pallaser *et al.*, 2013) and LOI temperatures close to 500°C (Girard and Klassen, 2001) have been used. The high R^2 value, ~ 0.99 obtained from our analysis can be considered very good in establishing a strong relationship of LOI to a C estimation technique, using the New York State empirical relationships between LOI, OM and SOC. This is consistent with studies that have shown that LOI methods define OM and SOC through calculation within the error range of the elemental analysis methods, but state that the relationship deteriorates at higher analysis temperatures and greater depths. In our study, C values for depth intervals of 0-5, 5-15, 15-30, and 30-60 centimeters were correlated [(elemental analysis-C) = 1.2 (LOI-C) - 0.9] with a R^2 of 0.99 (Fig. 3.4a), showing that the data was linear and with a slope of nearly 1. Below 60 centimeters, values were not well correlated by regression. Low C (%) values and increased occurence of carbonate (which skews elemental C upwardly) presence at lower soil depths seemed to have reduced the correlation. These methods have shown that the greatest mass changes from dry combustion occurred between 200 and 430°C, the range in which the soil organic matter is combusted (Veres 2002; Pallasser *et al*., 2013). Above this temperature range, high percentage clay soils can go through collapse and extra dehydration, leading to inaccurate mass changes. The relatively low percentage of clay (mean 10.7±8.3) in our samples (0-15cm) and burn temperature of 500°C in our work seemed to have been suitable. The Bland-Altman test added another dimension of precision validation, indicating that for C values above 3%, LOI assay underestimated C. Thus, although we hypothesized LOI could be a suitable surrogate for C elemental analysis in our field situation, it was most accurate in the range of 1.5-3 C (%); below 1.5 C (%), the technique overestimated C values and above 3%, it underestimated C values. Though, the two methods for determining C (%) were not interchangeable, LOI assay proved suitable as a simple and cost effective method to estimate OM, which can be used as a surrogate for estimating SOC within a defined soil sample set.

Conclusions

Characteristically decreasing OM, SOC, TN and increasing bulk density and $\delta^{13}C$ with depth were observed in our field, with legacy C3 vegetation dominating the Ap horizon soil $\delta^{13}C$ signature. Elevated SOC values from 60-120 cm and deviations from the LOI vs. C relationship established in upper soil layers indicated likely carbonate presence confounding SOC determinations at that depth interval. Though high initial SOC levels were estimated in the Ap horizon, loss of SOC during subsequent years (in the first study at the same field site), leaves room for future SOC accrual with perennial bioenergy grasses. Furthermore, initial SOC and $\delta^{13}C$ values at depths beyond 30 cm, indicate room also for robust C4 root-mediated SOC accrual at depth, in the future. Surface volumetric water content had a substantial correlation to soil properties up to 120 cm and was more useful than shallow water table depth in this regard. While OM, SOC, and TN of wetter soils were greater, C/N ratios, bulk density, and $\delta^{13}C$ of those wetter soils were lower than those of drier soils, all to depths of 30 cm. Within our narrow sample set, the correlation between LOI-C and combustion elemental analysis-C was very good (R^2=0.99), using the broad-based New York State soil testing correlations between LOI, OM and SOC. Given the continuing use of the simple, inexpensive LOI assay to estimate soil OM, our results suggest that it can be a useful surrogate for SOC and trends within a defined soil sample set.

Acknowledgements

This research was supported with USDA/NIFA Grant No. 2011-67009-20083 and Federal Capacity (Hatch) Project No. NYC-123486. The authors are also extremely thankful to all project assistants and summer interns for their help and support, especially Dhanya Tadipatri and Sarah Maguire. Many thanks to Kim Sparks, Cornell stable Isotope Laboratory, Heather Scott, Plant Sciences, for assistance with sample analyses and Kevin Packard, Cornell Statistical Computing Unit, Cornell University for assistance with the statistical analyses.

References

Acton P, Fox J, Campbell E, Rowe H, Wilkinson M (2013) Carbon isotopes forest for estimating decomposition and physical mixing in well-drained forest soils *Journal of Geophysical Research and Biogeosciences* 118, 1532–1545, doi:10.1002/2013JG002400

Anderson CJ, Mitsch WJ (2006) Sediment, carbon, and nutrient accumulation at two 10-year-old created riverine marshes. *Wetlands* 26, 779-792.

Balesdent J, Mariotti A (1996) Measurement of soil organic matter turnover using 13C natural abundance. *Mass Spectrometry of Soils, 41*(3), 83–111.

Bernal B (2008) Carbon pools and profiles in Wetland soils: The effect of climate and wetland type MS Thesis, Ohio State University.

Blanco-Canqui H (2010) Energy crops and their implications on soil and environment. *Agronomy Journal* 102, 403–419.

Boutton TW (1996) Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. In T. W. Boutton, & S. I. Yamasaki (Eds.), *Mass spectometry of soils*, Marcel Dekker Inc., New York, NY.

Cambardella CA, Gajda AM, Doran JW, Wienhold BJ, Kettler TA (2001) Estimation of particulate and total organic matter by weight loss-on-ignition. Assessment methods for soil carbon, 349-59.

Collins ME, Kuehl RJ (2001) Organic matter accumulation in organic soils. pp. 137-162. In Wetland Soils. Genesis, hydrology, landscapes, and classification. J. L. Richardson and M. J. Vepraskas, Eds. Lewis Publishers, CRC Press. Boca Raton, Florida.

Corre MD, Schnabel RR, Shaffer JA, (1999) Evaluation of soil organic carbon under forests, cool-season and warm-season grasses in the northeastern US. *Soil Biology and Biochemistry* 31, 1531–1539.

Das S, Teuffer K, Stoof C, Walter, MT, Steenhuis, TS, Walter, MF, Richards BK (2017) Impacts of perennial grass bioenergy cropping on soil properties, soil organic carbon and biomass yields in a wetness-prone field of Northeastern United States during initial establishment, in submission, *Bioenergy Research (submitted)*

Dell C (2013) (USDA-ARS-Pasture Systems and Watershed Management Research, University Park, PA). Personal communication.

Drösler M (2005) Trace gas exchange and climatic relevance of bog ecosystems, southern Germany. PhD thesis, Technische Universität München, München.

Ellert BH, Bettany JR (1995) Calculation of organic matter and nutrients stored in soils under contrasting management regimes *Canadian Journal of Soil Science*, 75(4), 529-538, https://doi.org/10.4141/cjss95-075

Farquhar GD, Ehleringer JR, Hubick, K T (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Biology*, *40*(1), 503-537.

Fontaine S, Sebastien B, Pierre B, Bdioui N, Mary B, Rumpel C (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450, 277-281.

Frankenberger JR, Brooks ES, Walter MT,Walter MF, Steenhuis TS (1999) A GIS-Based variable source area model. *Hydrological Processes*, 13, 804-822.

Garten CT, Brice DJ, Castro HF, Graham RL, Mayes MA, Phillips JR, Post WM, Schadt CW, Wullschleger SD, Tyler DD, Jardine PM, Jastrow JD, Matamala R, Miller RM, Moran KK, Vugteveen T, Izaurralde RC, Thomson AM, West TO, Amonette JE, Bailey VL, Metting FB, Smith JL (2011) Response of Alamo switchgrass tissue chemistry and biomass to nitrogen fertilization in West Tennessee, USA. *Agriculture, Ecosystems and Environment*. 140, 289-297. doi:10.1016/j.agee.2010.12.016.

Geological Survey of Canada (2001) A comparison of seven methods for analysis of carbon in soils. In: *Current Research E11* (eds Girard I, Klassen RA), 1-15.

Jobba´gy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* 10, 423–-436.

Ma Z, Wood CW, Bransby DI (2000) Impacts of soil management on root characteristics of switchgrass. *Biomass & Bioenergy* 18,105-112.

Matamala R, Jastrow DJ, Miller RM, Garten C (2008) Temporal changes in the distribution of C and N stocks in a restored tallgrass prairie in the U.S Midwest. *Ecological Applications*, 18, 1470–1488.

Mason CW, Stoof CR, Richards BK, Das S, Goodale CL, Steenhuis TS (2017) Hotspots of nitrous oxide emission in fertilized and unfertilized perennial grasses on wetness-prone marginal land in New York State. *Soil Science Society of America Journal* 81, 452-458. DOI: 10.2136/sssaj2016.08.0249

Moebius-Clune BN, Moebius-Clune DJ, Gugino BK, Idowu OJ, Schindelbeck RR, Ristow AJ, van Es HM, Thies JE, Shayler HA, McBride MB, Wolfe DW, Abawi GS (2016) Comprehensive Assessment of Soil Health. In: *The Cornell Framework Manual: Third Edition,* pp. 51-52, Cornell University, Geneva, United States.

Pallasser R, Minasny B, McBratney AB (2013) Soil carbon determination by thermogravimetrics. *PeerJ*, 1:e6.

Powers, J. S., & Schlesinger, W. H. (2002). Geographic and vertical patterns of stable carbon isotopes in tropical rain forest soils of Costa Rica. *Geoderma, 109*(1-2), 141-160.

Richards BK, Stoof CR, Mason C, Crawford RV, Das S, Hansen J, Mayton H, Crawford JL, Steenhuis TS, Walter MT, Viands DR (2013) Carbon Sequestration and Gaseous Emissions in Perennial Grass Bioenergy Cropping Systems in the Northeastern US. AAIC Proceedings. Washington DC, United States.

Rumpel C, Kögel-Knabner I (2011) Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. *Plant Soil*, 338, 143–158.

Sanderson MA (2008) Upland Switchgrass Yield, nutritive value and soil carbon changes under grazing and clipping, *Agronomy Journal* 100, 510–516

Stout W (1994) Soil drainage influence on biomass and nitrogen accumulation by ryegrass. *Agronomy Journal*, 86, 111-116.

Schmer MR, Liebig MA, Vogel KP, Mitchell RB (2011) Field-scale soil property changes under switchgrass managed for bioenergy. *Global Change Biology Bioenergy* 3(6), 439–448.

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens I, Kleber, M, Kogel-Knabner I, Lehmann J, Manning DAC, Nannipieri, P, Rasse DP, Weiner S, Trumbore S (2011) Persistence of soil organic matter as an ecosystem property. *Nature.* http://dx.doi.org/10.1038/nature10386.

Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant and Soil*, 241, 151–176.

Steenhuis TS, Winchell M, Rossing J, Zollweg JA, Walter MF 1995 (1995) SCS runoff equation revisited for variable source runoff areas. *Journal of Irrigation and Drainage Engineering, 121*, 234-238.

Stoof CR, Richards BK, Woodbury PB, Fabio PB, Brumbach ES, Cherney J, Das S, Geohring L, Hansen J, Hornesky J, Mayton H, Mason C, Ruestow G, Smart LB, Volk TA, Steenhuis TS (2014) Untapped potential: Opportunities and challenges for sustainable bioenergy production from marginal lands in New York and the Northeast. *Bioenergy Research*, 8, 482-501. DOI: 10.1007/s12155-014-9515-8

Tiemann LK, Grandy, AS (2015) Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. *GCB Bioenergy*, *7*(2), 161-174. DOI: 10.1111/gcbb.12126

Veres DS (2002) A comparative study between loss on ignition and total carbon analysis on minerogenic sediments. Quaternary Research Group. Babes-Bolya University, Romania.

von Lützow M, Kögel-Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, Marschner B (2007) SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry* 39, 2183–2207.

Walter, M.T., M.F. Walter, E.S. Brooks, T.S. Steenhuis, J. Boll, and K.R. Weiler. 2000. Hydrologically sensitive areas: Variable source area hydrology implications for water quality risk assessment. *J. Soil and Water Conservation* 3:277-284.

Wuest SB (2009) Correction of bulk density and sampling method biases using soil mass per unit area. *Soil Science Society of America Journal*,73, 312-316.

Zollweg JA, Gburek WG, Steenhuis TS (1996) SmoRMod – A GIS-integrated rainfall runoff model. *Transactions of the ASAE,* 39, 1299-1307.

CHAPTER 4

CARBON MINERALIZATION WITH CHANGING LEGACY WATER AVAILABILITY IN PERENNIAL GRASS BIOENERGY PLANTATIONS[3]

Abstract

To improve our ability to predict soil organic carbon (SOC) mineralizability as affected by legacy soil moisture differences from different bioenergy crops, a 371-day incubation study was conducted at different moisture contents using soils of four cropping systems in New York State. Soils experiencing high (average of 0.5 g g^{-1}), mid (0.4 g g^{-1}) and low (0.3 g g^{-1}) moisture were collected from fields with reed canarygrass+N, switchgrass and switchgrass+N in comparison to a broadleaf-grass fallow. Moisture of the laboratory incubations were adjusted mimicking the three average field moisture levels in a full factorial design. Increasing laboratory moisture in the incubations increased C mineralization (cumulative C mineralization per unit soil) and C mineralizability (cumulative C mineralization per unit SOC) (main effect $p<0.001$), indicating that lower average moisture as found at this site on average limited mineralization but not higher average moisture. C mineralizability was 24-30% lower with high field moisture compared to low field moisture across the cropping systems, regardless of moisture adjustment in the incubation. The mean slow C pool size of soils from high field moisture sites (983.7 ±0.5 CO_2-C g^{-1} C) was 0.8% greater than that of soils from low field moisture sites ($p<0.001$), obtained by fitting a double-exponential model. The mean residence time of the slow mineralizing pool for soils from low field moisture sites was 17.8±0.1 years, in comparison to 23.8±0.1 years for soils from high field moisture sites ($p<0.001$). While permanganate-oxidisable carbon (POXC) per

[3] Manuscript in preparation, Srabani Das, Brian K. Richards, M. Todd Walter, Tammo S. Steenhuis, Kelly L. Hanley, Johannes Lehmann*

unit SOC (r=0.1) was positively correlated to C mineralizability, wet aggregate stability (r=0.3) was negatively correlated to C mineralizability. Above-ground biomass or root biomass did not affect Cmineralizability ($p > 0.05$), after correcting for soil texture variations. Additionally, after correcting for soil texture variations and biomass inputs, C mineralizability significantly decreased with higher field moisture ($p=0.002$), indicating possible stabilization mechanism through mineral interactions of SOC under high water content.

Keywords perennial bioenergy grass cropping system, soil moisture, C mineralization, stabilization mechanism

Introduction

Soil moisture is one of the key environmental controls of plant growth and microbial activity, hence it affects both organic carbon (C) inputs and CO_2 outputs of soil. Globally, soil organic C (SOC) stocks are positively correlated with mean annual precipitation and negatively with mean annual temperature (Trumbore, 1997; Jobbágy and Jackson, 2000). Poorly drained soils usually display higher organic matter contents in comparison to well-drained soils, especially in temperate ecosystems. While initial decomposition rates of plant residues in surface soils correlate with indices of bulk chemical composition of plant materials (e.g., C:N ratio and lignin), it is now understood that long-term stabilization of SOM is essentially an ecosystem property (von Lützow *et al.* 2006; Schmidt *et al.* 2011; Stockmann *et al*. 2013), much under the control of parameters such as soil moisture. While temperature impacts on SOC are better understood, processes relating soil moisture to SOC stabilization are poorly understood and quantified.

Along with moisture, temperature, pH, inorganic nutrients, texture and porosity of soil also impact organic matter decomposition or heterotrophic (microbial) respiration. While soil water content indirectly controls microbial substrate diffusion, aeration, temperature effects on decomposition, microbial movement from one microenvironment to another etc., it directly impacts cell hydration, fluid exchange between microbes and soil and resulting microbial biomass growth. When soil water decreases during dry conditions, water-filled pore space is lowered and water in pores get disconnected, limiting solute supply to microbes and subsequently decreasing microbial activity and viability (Sommers *et al*., 1981; Orchard and Cook 1983; Linn and Doran 1984). On the other hand, when soil water contents increase and soils approach saturation, the proportion of air-filled pores decreases causing lower oxygen availability and hence less metabolic activity of aerobic organisms (Sommers *et al.,* 1981; Davidson, 1993; Franzluebbers, 1999). Subsequently, the microbial community changes from predominantly aerobic organisms to facultative anaerobic organisms and finally to obligate anaerobic organisms (with even less oxygen). Microbial activity has minimums at both moisture extremes and a maximum at a level of moisture, where the balance of water and oxygen availability is optimal (Moyano *et al*., 2013).

Hypotheses such as the 'regulatory gate hypothesis' suggest that the limiting step of C mineralization is controlled by abiotic processes which involve conversion of non-bioavailable SOC to bioavailable forms, regardless of microbial biomass or community composition (Kemmitt *et al.*, 2008), which may be impacted by moisture levels in soil. Soil mineralogy plays an important role in determining the amount of SOC and its residence time, especially the slow cycling pool. Torn *et al*. (1997) showed that the presence of non-crystalline minerals increased along a precipitation gradient and resulted in increased turnover time of stored C. The formation of mineral-organic associations (MOAs) has been recognized as an important mechanism of C stabilization and storage in recent decades (Kleber *et al*. 2015). Newer concepts of SOC stabilization, such as the soil continuum model (SCM) focus on spatial arrangement of soil organic matter and controls of temperature, moisture and soil mineralogy. The spatial arrangement of OM within the mineral matrix, micro-redox environment, microbial ecology and interaction with mineral surfaces, all factors contributing to OM persistence in SCM model are impacted by moisture independently as well as through moisture-temperature interactions (Lehmann and Kleber, 2015).

Several SOC models typically use soil moisture-respiration functions representing the average response of microbial respiration to soil moisture contents; but information about variations in response to different stabilization mechanisms as a result of different moisture contents is lacking. Hence, it is important to understand the impact of legacy soil moisture effects on the respiration-moisture relationship. This may affect C mineralizability that is commonly used in C simulation models (Kirschbaum, 2006).

The objective of this study was to provide mechanistic understanding of the role of moisture on C mineralization for soils that were exposed to different moisture levels. We investigated whether any or all of the following legacy effects from different field moisture levels determine C mineralizability with varying moisture contents: (1) plant above-ground /below-ground biomass inputs and therefore SOC contents; (2) SOC stabilization and therefore the extent of mineral protection of SOC; and (3) SOC accumulation due to lower mineralizability because moisture-mineralization relationships change as a result of different SOC contents or forms.

We hypothesized that those soil moisture contents that result in the lowest mineralization and highest plant growth also generate the greatest SOC accrual.

Methods

Experimental site

The field site was located near Ithaca, New York, USA (42N28.20', 76W25.94') (Fig. S4.1). The mean annual temperature and mean annual precipitation are 10°C and 940 mm, respectively. The soils are comprised of three series: well-drained Canaseraga (coarse-silty, mixed, active, mesic typic Fragiudept), somewhat poorly drained Dalton (Coarse-silty, mixed, active, mesic aeric Fragiaquept) and poorly drained Madalin (fine, illitic, mesic mollic Endoaqualf) soils. The soil texture is primarily silt-loam. The different soil properties are shown in Table 4.1.

Field experiment

A randomized complete block design was used for 16 large strip-plots (denoted A through P, Fig. S4.1) that comprised four replicate plots of four cropping treatments: switchgrass (*Panicum virgatum* L.) switchgrass+ fertilizer N, reed canarygrass (*Phalaris arundinaceae* L.) + fertilizer N and pre-existing fallow control. Where used, the N fertilization rate was 74 kg N ha^{-1}, applied as ammonium sulfate ($(NH_4)_2SO_4$), once annually in the spring starting in 2012 for reed canarygrass and starting in 2013 for switchgrass + N. The soil moisture conditions vary naturally from moderately well-drained to poorly-drained in the strip-plots. Five permanent sampling sub-plots were established along the natural moisture gradients of each strip-plot (Fig. S4.1) based on an initial intensive survey (summer 2011) of surface layer moisture measurements by a time-domain reflectometry (TDR) soil moisture sensor with 0.12 m probes (Hydrosense™, Campbell Scientific Australia Pty. LTD.). The TDR instrument which determines the proportion of volumetric water content (% VWC) used to determine soil moisture from the average of 3 measurements taken at each sub-plot. The subplot approach thus yielded 80 permanent sampling points where frequent periodic water content measurements were used to characterize the relative soil moisture status of each subplot. For each measurement event, a field average volumetric water content of all 80 subplots was calculated, and each subplot's value was normalized relative to the field mean (yielding a "relative soil moisture ratio" for that subplot and time point) (Richards *et al*., 2013). These relative values were averaged over the entire study period for each subplot, and each subplot's characteristic wetness (relative to the field average) was thus established over 40 such measurement events cumulatively representing several thousand readings at the site. The multi-year mean values for the 80 subplots were aggregated into "soil wetness quintiles" for each treatment for the entire study period. The VWC values were converted to the proportion of water filled pore space (WFPS) by scaling the instrument's values based on a linear trend that was established from the average VWC observed for saturated (100% WFPS) and dry (0% WFPS) conditions (Mason *et al*., 2017). Instantaneous values of WFPS at each subplot were converted to ratios of the simultaneous field average. A long-term wetness ranking was established, similar to the VWC ranking technique. The WFPS values corresponding to long-term VWC quintile 1 (highest), quintile 3 (mid-level) and quintile 5 (lowest) were 63%, 50% and 40% respectively.

During soil sampling for the incubation experiment, VWC measurements were undertaken at the 36 designated subplots in August 2014. Also, before starting the experiment, the gravimetric water content of the sampled soils from the 36 subplots was determined as described in the next section. VWC was related to the gravimetric water content (Fig. S4.2a), to compute mean water content values corresponding to the long-term high, mid and low VWC quintiles from the field. The mean gravimetric water content corresponding to quintile 1 (highest), quintile 3 (mid-level) and quintile 5 (lowest) were 0.5, 0.4, and 0.3 g g^{-1} respectively (Table S4.1a).

Field sampling protocol

For the incubation, soils from subplots representing 3 field replicates of the 4 cropping systems belonging to the 3-field moisture content (wetness quintiles) levels were randomly chosen (Table S4.1b). Soils from the surface Ap horizon were sampled in August 2014, after more than a week without rain, and months after N fertilization. A flat shovel was used to dig to a depth of 0.15 m (defined here as the average Ap horizon) at two locations equidistant (1.2 m) from the center of each subplot (as marked by a permanent subplot flag). Approximately 2 kg of soil was dug from each of the two locations, mixed and composited in a bucket, with about 200 g transferred to labeled polyethylene bag in a hand-cooler. Volumetric moisture content at the subplots was also determined by TDR at the time of sampling.

Laboratory analyses of gravimetric water contents, soil properties and crop growth parameters

Gravimetric water contents of field moist soils were determined by weighing 10 g of soil and drying at 105ºC for 24 hr and reweighing (Jarell *et al*. 1999). Elemental C and N analysis of soils was carried out by combustion infrared detection [LECO TruMac CN, LECO Corp, St. Joseph, MI, precision-Nitrogen-0.01 mg or 0.3% RSD (whichever is greater) and Carbon- 0.01mg or 0.4% RSD (whichever is greater)] on oven dried (60°C) 0.4 g of soil. As pH values of all soil samples were below 7, total C was considered equivalent to SOC (Propheter and Staggenborg, 2010; Bonin and Lal 2014), with no carbonate presence assumed, which was further confirmed using 5 M HCl which did not result in any effervescence on a subset of 16 soil samples. Permanganate-oxidisable carbon (POXC) was determined via permanganate oxidation and spectrophotometry (Weil *et al*., 2003, Culman *et al.*, 2012, Moebius-Clune *et al.*, 2016). Wet aggregate stability was measured using a sprinkle infiltrometer that steadily rained on a sieve containing a known weight of soil aggregates between 0.25 mm and 2 mm (Moebius-Clune *et* *al*., 2016); while the unstable aggregates fall apart and pass through the sieve, the fraction of the soil that remains on the sieve is used to calculate the proportion aggregate stability.

Soil pH and texture analyses were performed by a standard technique as outlined in Moebius-Clune *et al*. (2016). Texture for soils from the 36 sub plots was relatively uniform [sand (%)- mean- 16.6, std. dev-4, silt (%) – 67.2, std. dev-3.5, clay (%)- mean-16.2, std.dev-3.3]. There was no significant difference among the soils of the three wetness groups from the different cropping systems (in results section). Nevertheless, to understand the influence of moisture on C mineralization, we used soil texture as a fixed effect, in a model predicting the impact of different variables on C mineralization (in results section).

Above-ground biomass yields from each subplot were determined for the years 2012, 2013 and 2014 using hand-harvesting of replicate 1 m^2 quadrants, weighing and dry matter analysis. Values from the three years were subsequently added to obtain cumulative harvested above-ground biomass (harvestable standing biomass) specific for each subplot. For root biomass, coarse roots were removed by hand picking from dried soil samples (root crowns were not sampled). The roots were then passed through a 2 mm-sieve to remove associated soil particles and then weighed. This procedure (of non-washing and handpicking) recovers ~60% of the root mass typically obtained through more extensive soil washing techniques (Matamala *et al*., 2008). The coarse root biomass estimation from each subplot was thus undertaken for the years 2012, 2013 and 2014 and added towards a cumulative root biomass value for each subplot.

Incubation set-up and C mineralization measurement

Field-moist soils were passed through a 4-mm sieve to remove plant roots and rocks. Sieving was done under a laminar flow hood and subsequently soils were re-stored in the cooler. After sieving, the soil sample was split in two batches, one for use in the incubation experiment and the other for determining gravimetric water content and soil properties. 15 g of field moist soil were transferred into pre-weighed 60-mL glass Qorpak vials for the incubation experiment and they were then air dried for 48 hours at 30°C in a climate controlled incubation chamber, where the samples were kept for the duration of the experiment. Each air-dried soil sample was then adjusted to long-term equivalent levels of high, mid and low (0.5, 0.4 and 0.3 g^{-1} g respectively) field moisture contents, before the incubation. Two technical replicates (duplicates) were set up for each moisture adjustment. Thus, the final incubation set-up consisted of a full factorial design of four cropping systems by three field replicates by three water contents in the field (wet, mid and dry) by three water contents in the lab (wet, mid and dry) in duplicates (technical replicates that were not used in the statistical analysis). The Qorpak vials were transferred to 473-mL wide-mouth Mason jars. A 20-mL scintillation vial containing freshly prepared 15-mL 0.09M KOH was also placed open in the Mason jar and the jar was capped tightly. The KOH solution used to trap CO_2 emitted was prepared with CO_2-free deionized water (DIW) (Whitman *et al,*. 2014). For every 12 samples, 1 blank was used. The blank sample set-up consisted of only a KOH trap in the Mason jar and no soil addition. The incubation was set up in the dark

A staggered sampling schedule for measuring CO_2 emissions was used, due to the presence of a high number of sample vials (216). Three batches of 72 samples consisting of duplicates of each wetness level and six blanks were established for measuring one batch on a single sampling day. On day 1, 3, 7, 14, 21, 28, 42, 105, 210, 294, 371 for each batch, the jars were opened and the electrical conductivity (EC) of the KOH traps was measured at a constant temperature of 30.0 (± 3) °C. Fresh vials of 15 mL 0.09M KOH replaced the previous ones. Mason jars were resealed. With each measurement of electrical conductivity, soil-containing Qorpak vials were weighed and the water content in the soil was readjusted to its wetness level by addition of DIW, whenever needed.

On one of the sampling days, a standard curve was established by sealing KOH traps in Mason jars with rubber septa in their lids and injecting a known volume of CO_2. EC of the traps was measured after 24 hours and linearly correlated with the known CO_2 volumes to create a standard curve (Fig. S4.3). To account for the small amount of CO_2 present in the jar, EC measurements from the 'blank' from each group was subtracted from that of each sample jar. The resulting delta EC value was then converted into total CO_2 released by the sample using the standard curve (Fig. S4.2b). The CO_2 units were converted to gravimetric units of C using the universal gas law equation. The resulting CO_2–C emission was then normalized for per g soil or per g SOC. For the latter, results were divided by the amount of SOC present in the soil at the beginning of the incubation.

Statistical analyses and modeling

SOC mineralization data were fitted to a double exponential model, based on the highest r square values ($r^2 > 0.99$) during pre-analysis. The mean cumulative mineralization value for three field replicates of each field-lab moisture combination was fitted with a first-order, two-pool model (Liang *et al.,* 2008; Zimmerman *et al.,* 2011). Data were fitted in nlsLM (nonlinear regression, Levenberg-Marquardt search), R Studio Team (2015). The Levenberg-Marquardt algorithm estimates the values of the model parameters to minimize the sum of the squared differences between model-calculated and measured values.

The following equation was used:

$$C_{cumulative} = C_1 (1-e^{-k_1 t}) + C_2 (1-e^{-k_2 t})$$

Where, $C_{cumulative}$ = CO_2-C production per unit soil (mg CO_2-C g^{-1} soil), t is time in days, C_1 is the fast pool, C_2 is the slow pool and k_1 and k_2 are the first–order decomposition rate coefficients for fast and slow pools, respectively; the parameter constraints were chosen as follows: $k_1>0$, $k_2>0$ and C_1+C_2 = initial SOC in soil sample in (mg g^{-1} soil). Curve fitting was also done using the same equation for CO_2-C production per unit initial SOC (mg CO_2-C g^{-1} C) with parameter constraints chosen as $k_1>0$, $k_2>0$ and C_1+C_2 = 1000 mg C.

Three-way ANOVA were performed using cumulative C mineralization per unit SOC (from here on called "C mineralizability") and per unit soil (called "C mineralization") at 371 days, with fixed effects of field moisture level, laboratory moisture level and cropping system. ANOVA were also performed for the four parameters estimated by nonlinear regression (double exponential model). The three-way interaction between field moisture level, laboratory moisture level and cropping system and two-way interactions between field moisture level vs laboratory moisture level and laboratory moisture level vs cropping system were not found to be significant (p value > 0.05) in all models. Thus, only a two-way interaction between field moisture level and cropping system (do soils of varying long term wetness mineralize differently when exposed to different cropping systems?) was considered in the final models. Multiple comparison of Least Square Means differences was conducted only when the ANOVA results for the main effects or interactions were significant. Post-hoc comparisons were made using Tukey's HSD method to control for multiple comparisons. Five jars out of 216 were excluded due to suspected leaks (strong outlier, 6-8 standard deviation from all samples).

To explore the primary factors influencing C mineralizability we performed a series of statistical analyses. First, univariate analyses allowed us to establish the soil property variables significantly correlated with C mineralizability. Pearson correlation coefficients were computed between cumulative C mineralizability (mg CO_2-C g^{-1}C) and SOC, total nitrogen (TN), POXC, wet aggregate stability, soil texture, soil pH, cumulative harvested above-ground biomass and cumulative root biomass. To address collinearity among soil properties influencing C mineralizability, we then performed principal components analysis (PCA) for the dataset (adding POXC per unit SOC and excluding soil texture). To tease out the control of soil texture and biomass inputs, if any, on C mineralizability, we performed a mixed effects model analysis with the first two principal components, field and laboratory moisture levels, above-ground and root biomass, sand and clay content as fixed effects and subplots as random effects. Thus, the significance of moisture in influencing C mineralizability, when corrected (controlled) for biomass inputs and soil texture, was tested. Additionally, a principal components analysis for the dataset including C mineralizability and all correlated variables was performed. The bi-plots report the eigenvectors and proportion of variance explained by the first two principal components. The variables included in each analysis were plotted as vectors representative of the strength and direction to which they loaded each component. All statistical analyses were carried out by JMP Pro 12 (SAS Inc., Cary, NC).

Results

C mineralization and C mineralizability

While higher laboratory moisture (averaged over cropping system and field moisture levels) significantly ($p<0.001$, Fig. 4.1a, Table S4.2a) increased cumulative CO_2 evolution per unit soil mass (here called 'C mineralization') at 371 days, field-moisture (averaged over cropping system and laboratory moisture adjustments) did not have a significant effect ($p=0.7$, Fig. 4.1a). Additionally, cropping system (averaged over field moisture levels and laboratory moisture adjustments) had a significant ($p<0.001$, Fig. 4.1a) effect on C mineralization, with C mineralization in fallow-control soils being greater than reed canarygrass and switchgrass soils, and in switchgrass +N being greater than in switchgrass soils (Table S4.2b). The interaction field moisture vs cropping system had a significant effect ($p=0.005$) on cumulative C mineralization (Fig. S 4.3a, Table S4.2a), whereas the interaction field moisture vs lab moisture did not have a significant effect on C mineralization ($p=0.9$). When expressed as cumulative CO_2 evolution per unit SOC (here called ‘C mineralizability’), the values were lower in field-high, than field-mid and field-low moist soils, for all laboratory moisture adjustments of fallow-control, reed canarygrass+N, switchgrass and switchgrass+N (Fig. 4.2a, 4.2b, 4.2c, 4.2d) at 371 days. Furthermore, high field moisture resulted in significantly ($p<0.0001$) lower C mineralizability than both mid and low field moisture levels (Fig. 4.1b, Table S4.3a, S4.3b), when averaged over cropping systems and laboratory adjustments. Higher laboratory moisture (averaged over cropping systems and field moisture levels) significantly ($p<0.0001$, Fig.4.1b) increased C mineralizability, with lab-high being significantly greater than lab-low. This trend was consistent for all sampling time points (Fig. S4.4). The interaction field moisture vs cropping system also had a significant effect ($p=0.001$, Fig. S4.3b Table S4.3a) on C mineralizability. Though the interaction field moisture vs lab moisture did not have a significant effect on C mineralizability ($p=0.9$), the moisture combination, field high-lab low had the lowest value in each of the cropping system soils (Fig. 4.2).

While the size of the fast mineralizing C pool, C_1 varied between 11.3 (±1.5) and 30.6 (± 1.8) mg CO_2-C g^{-1} C (Table 4.3), that of the slow mineralizing C pool, C_2 varied between 969.5 (± 1.8) and 988.7(±1.5) mg CO_2-C g^{-1} C among various moisture-crop combinations (Table 4.3). The interaction between field moisture and cropping system was significant for both C_1 ($p = 0.003$, not shown) and C_2 ($p<0.0001$) (Table S4.4c). Though the interaction field moisture vs laboratory moisture was not significant, the size of C_1 was smallest and concurrently, the size of C_2 was greatest for soils of field high-lab low moisture combination compared to other moisture levels, regardless of the cropping system (Table 4.3). The mineralization rate constants of the slow degrading pool (k_2) (ranging from 0.000112 ±0.000005 to 0.00017 ±0.00001) were three orders of magnitude lower than that of the fast degrading pool (k_1) (ranging from 0.032±0.003 to 0.047±0.008). The mean residence time of the fast mineralizing pool (MRT_1) varied between 21.3 and 34.7 days and that of the slow mineralizing pool (MRT_2), varied between 22.7 and 24.5 years among the different moisture-crop combinations. The interaction between field moisture and cropping system was significant for both MRT_1 ($p=0.02$) and MRT_2 ($p<0.0001$) (Fig. S4.5a & S4.6a, Table S4.5c, S4.6c).

C mineralization and C mineralizability in relation to soil properties and crop growth parameters

No correlations were observed between cumulative C mineralization (mg CO_2-C g^{-1} soil) and soil properties as well as above-ground biomass. C mineralization was positively correlated to root biomass ($r = 0.3$, $n = 108$, $P = 0.004$) (Fig. S4.5a).

Pearson correlations between cumulative C mineralizability (mg CO_2-C g^{-1} C) and soil properties measured at the start of the experiment, before texture corrections, indicated strong negative correlation with SOC ($r = -0.7$, $n = 108$, $p<0.0001$), TN ($r = -0.8$, $n = 108$, $p<0.0001$), POXC ($r = -0.7$, $n = 108$, $p<0.0001$), and weak negative correlation with pH ($r = -0.3$, $n = 108$, $p=0.0002$), wet aggregate stability ($r = -0.3$, $n = 108$, $p=0.0009$) and root biomass ($r = -0.2$, $n = 108$, $p=0.03$). It also indicated weak positive correlations with POXC per unit SOC ($r = 0.12$, $n = 108$, $p=0.2$) and above-ground biomass and mineralizability ($r = 0.2$, $n = 108$, $= 0.04$) (Fig. S4.5b).

Table 4.1. Soil properties from the four cropping system soils belonging to the Low, Mid and High field moisture levels (n= 3 for each moisture level of each cropping system), mean values with standard deviations in parenthesis.

	SOC (g kg^{-1} soil)			TN (g kg^{-1} soil)			Wet aggregate stability (%)			Sand (%)			Silt (%)			Clay (%)			POXC (mg C kg^{-1} soil)			POXC per unit SOC (mg C g^{-1} C)			pH		
	High	Mid	Low	High	Mid	Low	High	Mid	Low	High	Mid	Low	High	Mid	Low	High	Mid	Low	High	Mid	Low	High	Mid	Low	High	Mid	Low
Fallow	40.7 (2.4) A	38.3 (2.3 AB	33.9 (1.4) B	3.9 (0.6) A	3.6 (0.2) A	3.2 (0.2) A	81.8 (6.9) A	76.0 (4.1) A	81.8 (6.4) A	11.4 (4.0) A	14.2 (1.9) A	17.5 (1.5) A	71.9 (1.7) A	66.7 (5) A	65.1 (2.1) A	16.7 (5.5) A	18.9 (4.2) A	17.4 (1.5) A	878 (134) A	926 (67) A	751 (52) A	22 (4.2) A	24.2 (2) A	22.2 (1) A	6.2 (0.1) A	6.2 (0.3) A	6.1 (0.5) A
Reed canarygrass+N	36.9 (0.6) A	36.2 (5.2) A	30.8 (7.4) A	3.6 (0.5) A	3.5 (0.4) A	2.8 (0.5) A	60.4 (8.8) A	62.6 (20.3) A	63.1 (24) A	15.2 (0.3) A	18.4 (2.5) A	18.7 (1.8) A	68.6 (5) A	66.5 (2.5) A	66.6 (0.7) A	16.2 (5) A	15.1 (1.9) A	14.6 (1) A	851 (42) A	829 (103) A	684 (89) A	23 (1.2) A	23 (2) A	23 (4) A	5.6 (0.5) A	5.6 (0.5) A	5.1 (0.1) A
Switchgrass	39.6 (11.5) A	29.8 (4.3) A	27.9 (3.3) A	4.0 (1.2) A	2.9 (0.5) A	2.7 (0.2) A	70.5 (22) A	59.0 (16.5) A	72.3 (9.3) A	11.8 (4.2) A	17.2 (4.6) A	19.6 (1.4) A	68.9 (0.6) A	66.8 (0.7) AB	64.5 (2.5) B	19.7 (4.5) A	16.0 (5.2) A	15.9 (2.1) A	827 (41) A	762 (84) AB	613 (111) B	22 (5) A	26.1 (6) A	22 (2) A	6.2 (0.5) A	5.9 (0.6) A	5.8 (0.5) A
Switchgrass+N	40.5 (9.8) A	26.5 (4.6) A	28.8 (0.6) A	4.0 (0.7) A	2.6 (0.3) B	2.9 (0.1) AB	71.7 (20) A	54.7 (9.2) A	71.2 (9.5) A	15.8 (4.6) A	15.8 (1) A	23.2 (4.2) A	70.0 (2.2) A	68.6 (2.8) AB	62.1 (3.6) B	14.2 (2.7) A	15.6 (2) A	14.7 (1.6) A	876 (119) A	625 (221) A	687 (47) A	22.1 (3) A	23.1 (5.1) A	24 (1.1) A	6.3 (0.2) A	5.8 (0.1) AB	5.7 (0.3) B
P value for cropping system			0.1			0.5			0.05			0.06			0.8			0.2			0.08			0.9			0.007

Table 4.2. Cumulative above-ground biomass and cumulative root biomass from 2012-2014 for the four cropping systems belonging to the High, Mid and Low field moisture levels (n= 3 for each moisture level of each crop), mean values with standard deviations in parenthesis.

	Cumulative above-ground biomass ($Mg\ ha^{-1}$)			**Cumulative root biomass ($g\ kg^{-1}$ soil)**		
	High	Mid	Low	High	Mid	Low
Fallow	2.3 (1.1) B	3.8 (2.4) AB	11.3 (3.8) A	13.1 (3.6) A	5.9 (4.5) B	10.0 (1.9) B
Reed canarygrass+N	5 (3.4) A	6.1 (3.1) A	3.6 (1.8) A	12.7 (3.7) A	6.8 (2.1) B	6.3 (4.1) B
Switchgrass	3 (1.2) B	4 (1.1) AB	6.8 (2.9) A	4.1 (1.3) A	2.0 (0.7) B	3.3 (1.8) B
Switchgrass+N	3.7 (0.7) B	4.3 (1.9) AB	7.7 (2.8) A	7.9 (2.6) A	4.7 (2.2) B	3.0 (2.3) B
P value for cropping system	0.8			<0.0001		

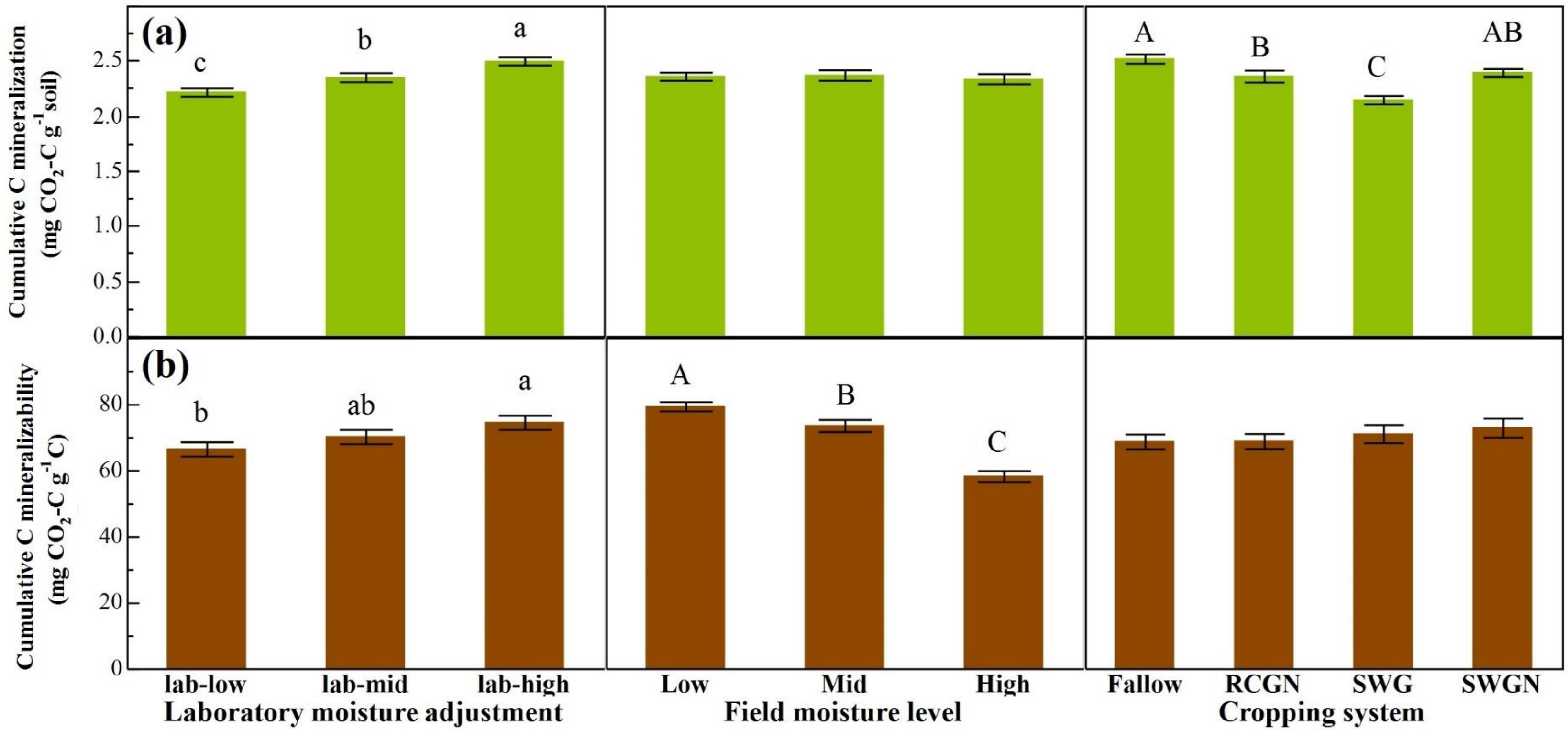

Figure 4.1. Mean cumulative C mineralization and C mineralizability at 371 days. Different letters above bars indicate significant differences within the same figure pane ($p<0.05$). Mean laboratory moisture values [(mean +SE for n=36) (each averaged over 2 technical replicates)] are averaged over field moisture levels and cropping systems, mean field moisture values [(mean +SE for n=36) (each averaged over 2 technical replicates)] are averaged over laboratory moisture levels and cropping systems and mean cropping system values [(mean +SE for n=27(each averaged over 2 technical replicates)] are averaged over field moisture and laboratory moisture levels. The cropping systems were fallow-control (fallow), reed canarygrass + fertilizer 75 kg N ha^{-1} (RCGN), switchgrass (SWG) and switchgrass + fertilizer 75 kg N ha^{-1} (SWGN). The water contents corresponding to field Low, Mid and High and lab-low, lab-mid and lab-high are both 0.3 g g^{-1}, 0.4 g $g^{-1}$and 0.5 g g^{-1}, respectively.

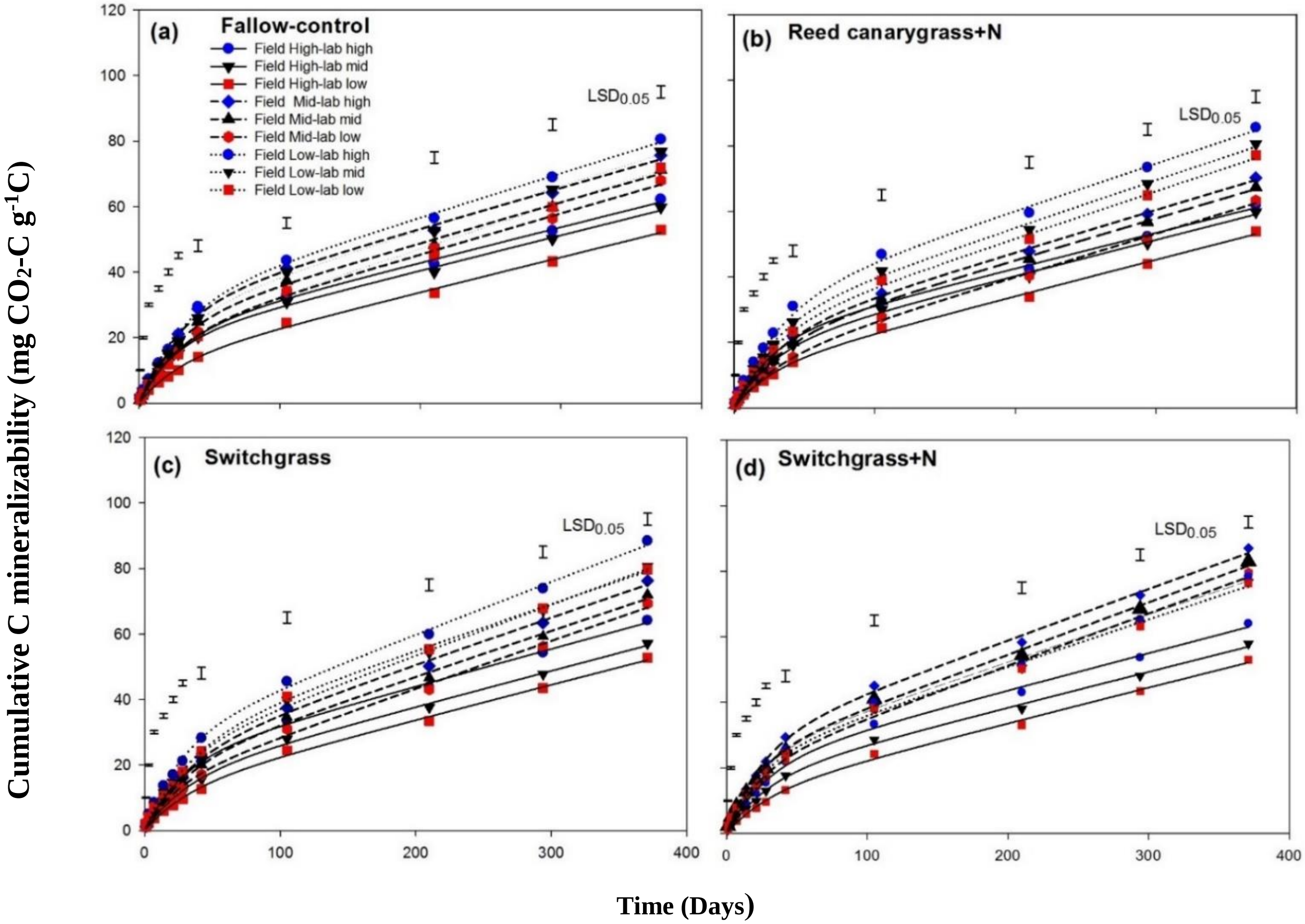

Figure 4.2. Cumulative C mineralizability per unit SOC in soils of the four cropping systems, fallow-control (a), reed canarygrass + fertilizer 75 kg N ha^{-1} (b), switchgrass (c) and switchgrass + fertilizer 75 kg N ha^{-1} (d). Mean +SE for, n = 3, averages of 2 technical replicates each), for the three-similar laboratory moisture level adjustment of three field moisture levels of each cropping system. LSD=least significant differences between all moisture combinations for each cropping systems for each time point at $p< 0.05$; curves fitted by using a double-exponential equation. The water contents corresponding to field Low, Mid and High and lab-low, lab-mid and lab-high are both 0.3 g g^{-1}, 0.4 g g^{-1} and 0.5 g g^{-1}, respectively.

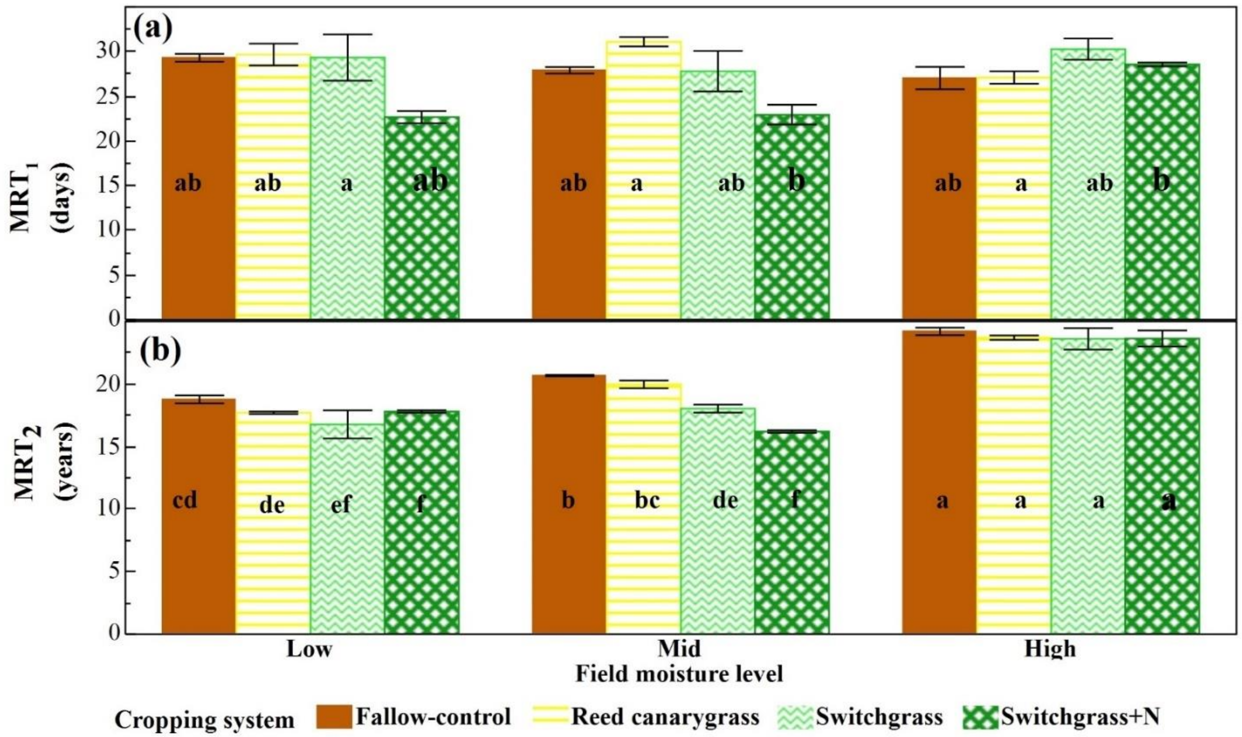

Figure 4.3. Mean residence time of fast mineralizing (MRT_1) and slow mineralizing carbon pool (MRT_2) of the different field moisture-cropping system combinations; curves fitted by using a double-exponential equation. For each cropping system-field moisture combination, mean + SE for n= 3 field representatives, each averaged over 3 laboratory levels, n = 9 (average of 2 technical replicates, each)] Different letters above bars indicate significant differences ($p<0.05$) of the different field moisture-cropping system combinations. The cropping systems were fallow-control, reed canarygrass + fertilizer 75 kg N ha^{-1}, switchgrass and switchgrass + fertilizer 75 kg N ha^{-1} and the laboratory moisture levels were lab-low, lab-mid and lab-high. The water contents corresponding to field Low, Mid and High and lab-low, lab-mid and lab-high are both 0.3 g g^{-1}, 0.4 g g^{-1} and 0.5 g g^{-1}, respectively.

Table 4.3. C mineralization kinetics of soil after 371 days' incubation at 25°C for the different cropping systems. Pool sizes and decay rates of cumulative soil C mineralization using double exponential model $C_{cumulative}= C_1 (1\text{-}\exp(-k_1 x)) + C_2 (1\text{-}\exp(-k_2 x))$, where C_1 is the fast pool, C_2 is the slow pool and k_1 and k_2 are the first–order decomposition rate coefficients for fast and slow pool respectively, the parameter constraints chosen, $k_1>0$, $k_2>0$ and $C_1+C_2=1000$. MRT_1 = mean residence time of fast pool in days ($MRT_1=1/k_1$) and MRT_2 = mean residence time of slow pool in years {$MRT_2=(1/k_2)/365$}. Mean+ SE for n = 3, averages of 2 technical replicates, each), for the three similar laboratory moisture level adjustment of three field moisture levels of each cropping system. The cropping systems were fallow-control, reed canarygrass + fertilizer 75 kg N ha^{-1}, switchgrass and switchgrass + fertilizer 75 kg N ha^{-1}. The water contents corresponding to field Low, Mid and High and lab-low, lab-mid and lab-high are both 0.3 g g^{-1}, 0.4 g g^{-1} and 0.5 g g^{-1}, respectively. Tables S4, S5 and S6 in SI refer to detailed statistics of the parameters.

Cropping system	Moisture level	C_1 (mg CO_2-C g^{-1} C)	k_1 (day^{-1})	C_2 (mg CO_2-C g^{-1} C)	k_2 (day^{-1})	MRT_1 (days)	MRT_2 (years)
Fallow- control	Field high-lab	20.6±1.4	0.034±0.004	979±1	0.000115±0.000005	28.7	23.9
	Field high-lab	18.4±1.18	0.040±0.005	982±1.2	0.000113±0.000005	24.6	24.2
	Field high-lab	12.0 ±1.4	0.036±0.007	988±1.4	0.000112±0.000005	27.8	24.5
	Field mid-lab	27.7±1.9	0.035±0.004	972±1.9	0.00013±0.000001	28.6	20.6
	Field mid-lab	23.3±1.7	0.036±0.005	977±1.7	0.00013±0.00001	27.4	20.7
	Field mid-lab	19.7±1.7	0.036±0.005	980±1.7	0.00013±0.000001	27.7	20.7
	Field low-lab	29.0±1.9	0.033±0.004	971±2	0.00014±0.00001	30.1	19.0
	Field low-lab	24.9±1.5	0.034±0.004	975.±1.5	0.000145±0.000006	29.2	18.9
	Field low-lab	18.2±1.4	0.035±0.004	981.8±1.4	0.00015±0.00000	28.6	18.4
Reed canarygrass	Field high-lab	20.3±1.7	0.037±0.004	980±1.2	0.00012±0.00000	26.4	23.9
	Field high-lab	17.7±1.2	0.035±0.004	982±1.2	0.00012±0.00000	28.5	23.6
	Field high-lab	11.3±1.2	0.037±0.007	989±1.2	0.00012±0.00001	26.4	23.6
	Field mid-lab	21±1.3	0.032±0.003	979±1.3	0.00014±0.00001	30.9	19.9
	Field mid-lab	19±1.1	0.032±0.003	981±1.1	0.00014±0.0000	32.1	20.3
	Field mid-lab	13±1.6	0.033±0.005	987±1.2	0.00014±0.00000	30.3	19.7
	Field low-lab	31±1.9	0.031±0.003	969±1.9	0.00016±0.00001	32.1	17.7
	Field low-lab	25±1.7	0.035±0.004	974±1.7	0.00016±0.00001	28.6	17.6
	Field low-lab	22.1±1.7	0.035±0.005	978±1.7	0.00015±0.00001	28.3	17.8
Switchgrass	Field high-lab	20.5±1.2	0.036±0.004	979±1.3	0.00012±0.00001	27.9	22.7
	Field high-lab	15.2±1.4	0.032±0.005	984±1.4	0.000115±0.000005	31.2	23.7
	Field high-lab	11.7±1.6	0.032±0.007	988±1.6	0.000112±0.000006	31.7	24.4
	Field mid-lab	20.9±1.8	0.032±0.005	979±1.8	0.00015±0.00001	30.9	17.9
	Field mid-lab	18.1±1.9	0.034±0.006	981±1.9	0.00015±0.00001	29.1	18.4
	Field mid-lab	13.3±1.8	0.043±0.011	986±1.8	0.00015±0.00001	23.4	17.8
	Field low-lab	26.6±2.1	0.040±0.005	973±2.1	0.00017±0.00001	26.5	15.8
	Field low-lab	21.4±2.3	0.040±0.007	979±2.3	0.00017±0.00001	27.0	16.5
	Field low-lab	25.5±1.9	0.030±0.004	974±1.9	0.00015±0.00001	34.5	18.0
Switchgrass +N	Field high-lab	20.4±1.8	0.035±0.005	980±1.8	0.00012±0.00001	28.3	22.9
	Field high-lab	16.1±1.5	0.035±0.006	984±1.5	0.000115±0.000006	28.4	23.9
	Field high-lab	11.3±1.5	0.035±0.008	989 ±1.5	0.000114±0.000006	29.0	24.1
	Field mid-lab	26.7±2	0.039±0.005	973±2	0.00017±0.00001	25.1	16.3
	Field mid-lab	21.7±1.9	0.047±0.008	978±1.9	0.00017±0.00001	21.5	16.1
	Field mid-lab	18.5±1.9	0.045±0.009	981±1.9	0.00017±0.00001	22.2	16.2
	Field low-lab	22.5±2	0.043±0.007	978±2	0.00016±0.00001	23.3	17.7
	Field low-lab	21.2±1.9	0.043±0.007	979±1.9	0.00015±0.00001	23.4	17.8
	Field low-lab	21.1±1.9	0.047±0.008	979±1.9	0.00015±0.00001	21.3	17.9

SOC (0.96), TN (0.95), wet aggregate stability (0.7), POXC (0.7), pH (0.58) loaded substantially in the same direction, whereas POXC per unit SOC (-0.46) loaded in the opposite direction for the first principal component (PC1) which explained 58% variability among the soil properties (Fig.4.4, Table S4.8a,4.8b). For the second principal component (PC2) which explained 21% of variability, POXC (0.5) and POXC per unit SOC (0.6) loaded substantially in same direction. PC1 ($p<0.0001$), PC2 ($p=0.002$), field moisture level ($p=0.002$) and lab moisture level ($p<0.0001$) were significant in explaining variations in C mineralizability (Table 4.4). However, cropping system, cumulative harvested above-ground biomass or cumulative root biomass, sand or clay contents were not significant in explaining variations in C mineralizability. Thus, when controlled for soil texture, cumulative above-ground biomass or cumulative root biomass, field moisture was significant in influencing c mineralizability ($p=0.002$, Table 4.3).

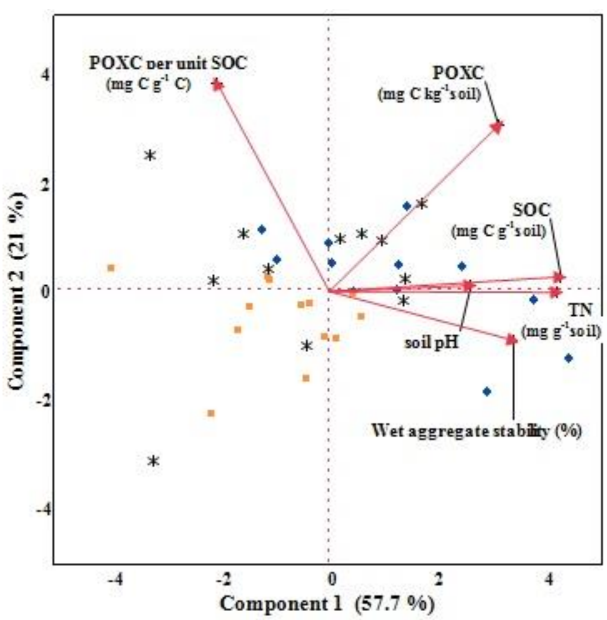

Figure 4.4. Principal Components Analysis biplot of variability among soil properties (excluding soil texture components) related to C mineralizability. Red vectors represent principal component loadings of each variable. The blue, black and orange symbols indicate the dataset for the field high, mid and low field moisture levels, respectively.

Table 4.4. Fixed effects for C mineralizability (mg CO_2-C g^{-1} C) at 1 year (371 days) in model performed to check the influence of field moisture, when controlled for texture components and biomass inputs.

Effect tests	**DF**	**F Ratio**	**P value**
Field moisture level	2	0.4	**0.002**
Laboratory moisture level	2	*0.3*	*<0.0001*
Sand (%)	1	1.5	0.2
Clay (%)	1	1.4	0.2
Cumulative above-ground biomass	1	0.5	0.5
Cumulative root biomass	1	0.9	0.3
PC1	1	21.5	*<0.0001*
PC2	1	4.8	**0.04**

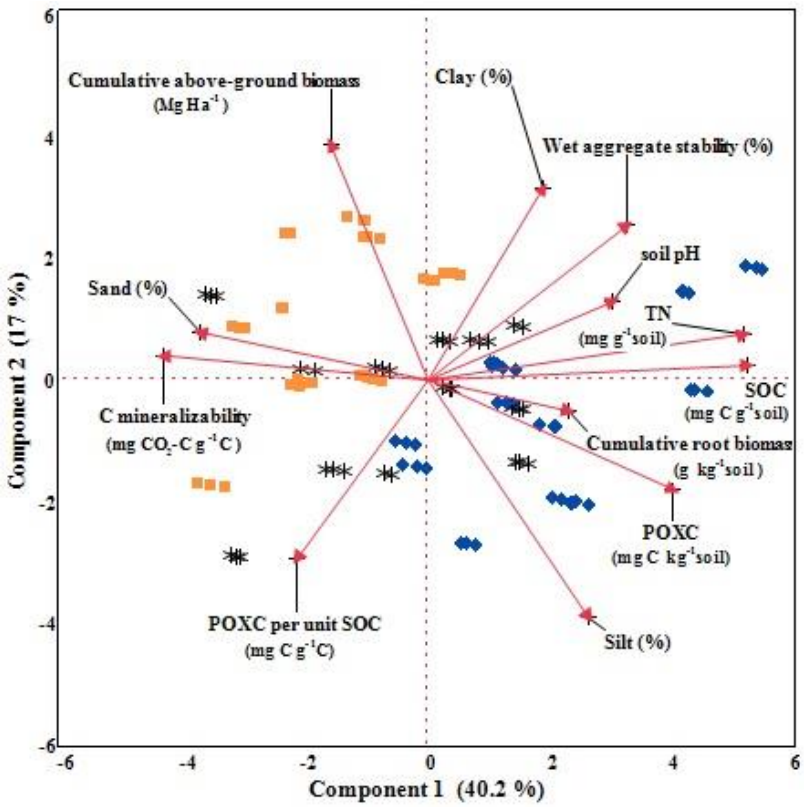

Figure 4.5. Principal Components Analysis biplot of variability among soil properties, crop-growth properties and C mineralizability. Red vectors represent principal component loadings of each variable. The blue, black and orange symbols indicate the dataset for the field high, mid and low field moisture levels, respectively, corresponding to 0.5 g g^{-1}, 0.4 g g^{-1} and 0.3 g g^{-1}, respectively.

For the PCA including C mineralizability and all correlated soil and crop variables (not corrected for soil texture), the eigenvectors and proportion of variance explained by the first two principal components are seen in Fig.4.5 and Tables S4.10 and S4.10b, respectively.

Discussion

Moisture effects on carbon mineralization and soil organic carbon

The wettest soils in this experiment, the field-high moist soils displayed significantly lower C mineralizability, though higher moisture in the laboratory adjusted to the same level as in the field resulted in greater mineralizability. While the former could point towards variation in SOC forms and its stabilization level at higher long-term moisture level in the field, the latter is a more commonly reported observation (Table S4.10). In unsaturated soils, increasing water content enables greater solute diffusion as well as greater microbial viability and movement in microenvironments. Microbial activity is maximized at moisture levels where the balance of water and oxygen availability is optimal (Moyano *et al*., 2013) which is presumably the case at the high moisture levels in our study.

Generally, aerobic microbial activity is optimal at 60% WFPS, with Linn and Doran (1984) reporting highest soil respiration rates between 40 and 70% WFPS and confirmed by other studies (Table S4.10). This indicates that even high (corresponding to 63% WFPS) moisture conditions in our experimental setup were well below the range of any anaerobic limitation, with greater laboratory moisture resulting in increased C mineralization and mineralizability, regardless of the field moisture level chosen (Table 4.3) or averaged over treatments (Fig.4.1). The average WFPS (corresponding to 63% WFPS) in high moisture field sites was exceeded during 46% of the monthly measurements conducted over the four years. The moisture contents of these sites were lower than that corresponding to 70% WFPS during 57% of the moisture measurement events and lower than that corresponding to 80% WFPS during 82% of the moisture measurement events over the four years (unpublished data). Therefore, in water adjustments mimicking high field moisture conditions, SOC mineralization was still favored, and any decrease in C mineralizability at higher field moisture was likely not a result of moisture restrictions to mineralization. The lower mineralizability associated with wetter field soils is therefore most likely related to SOC being stabilized differently under long-term wetter conditions.

The influence of field moisture conditions and laboratory moisture adjustments on C mineralization and C mineralizability are also indicated by the results of the double exponential model. The greatest mean residence time of slow cycling C pools of the wettest soils of each cropping system (Table 4.3, Table S4.7) points to an operative stabilization mechanism for SOC at greater long-term field moisture. Again, the smaller size of the slow pool (C_2) and greater size of the fast pool (C_1) values for the field-high/lab-high moisture soils in comparison to field-high/lab-low for any cropping system (Table 4.3, Table S4.7), points to high field moisture (~ 63% WFPS) not restricting C mineralization or mineralizability.

The wettest soils in the field experiment (the field-high moist soils of all crop groups) had the greatest SOC levels (Table S4.7). Thirty-six out of the 80 field subplots used in this study show a characteristic trend of greater SOC values (ranging between 26-43 mg C g^{-1} soil) with increasing long-term field moisture. When all 80 subplots were considered, the same OM/SOC trend is seen during each sampling year (2011, 2012, 2013, 2014), with the wettest soils displaying significantly greater OM/SOC compared to the drier soils (unpublished data). Generally, low biomass C inputs associated with drier soils in the range of soil moistures observed at the study site and greater C inputs associated with higher soil water conditions are thought to result in decreased and increased SOC stocks, respectively. Though litter inputs measurements had not been included in our study, they can be expected to be proportional to harvested above-ground biomass. As drier soils displayed greater harvested biomass (Table 4.2), litter inputs were expected to also be greatest at low water contents in the range occurring at the study site. Thus, greater above-ground biomass inputs in wetter soils, resulting in higher OM/SOC, in comparison to drier soils, had not occurred in the field. Though coarse root biomass was greater in the wettest soils in comparison to drier soils, for all cropping systems (Table 4.2), OM inputs from root biomass of perennial cropping systems are expected to be low in the early establishment phase (Corre *et al.*, 1999), as is the case for the three cropping systems in the experiment. In such a short time frame they are thought to stimulate active OM decomposition (Wortmann *et al.*, 2017). Additionally, our incubation results indicated that C mineralizability was not correlated to harvested above-ground biomass or coarse root biomass (Table 4.3) and both crop parameters displayed low loading values for principal component analysis with C mineralizability and soil and crop characteristics (Table S4.9b). Thus, it can be concluded that greater biomass inputs were not a primary reason for lower C mineralizability and hence greater SOC in wetter soils in the field. With high soil moisture not restricting C mineralization at the study site (as shown from our incubation) and with biomass inputs not being the reason for greater SOC, the observed lower C mineralizability of soils from wetter fields can only be explained by a specific mechanism of SOC stabilization that is operative in the unsaturated moisture range of the Ap horizon, at neutral/near neutral pH.

Why was C mineralizability lower in soils of higher long-term water content?

As lower C mineralizability at higher field moisture was not a result of moisture restrictions to mineralization or greater biomass inputs, it is most likely related to SOC being stabilized differently under long-term wetter conditions. Low C mineralization may be related to physical protection through aggregation (Six *et al*. 2000; Six *et al.*, 2002; von Lützow *et al*. 2006), which limits diffusion of catabolites and enzymes as well as interaction with mineral surfaces (Oades 1988; Kögel-Knabner *et al.*, 2008; von Lützow and Kögel-Knabner, 2010; Schmidt *et al.* 2011; Kleber *et al.* 2015). Greater physical protection through greater aggregation, though important is less likely the major explanation here, as aggregate stability had a weak negative correlation to C mineralizability and loaded with a value of 0.59, in PC1* (Table S4.9b). The direction and loading value of POXC per unit SOC in PC1* (0.39 Table S4.9b) and its weak positive correlation with C mineralizability indicate that this parameter was also importantly associated to C mineralizability of soils. POXC per unit SOC represents the readily available, biologically active C pool associated with heavier and smaller particulate organic C fractions (Culman *et al.*, 2012). This non-mineral associated fraction is likely used efficiently in heterotrophic respiration and may have been completely depleted in the year-long incubation experiment (the total pool of POXC per unit SOC was less than the cumulative C mineralizability at 371 days for all samples). Thus, the less mineralizable SOC that remained was likely to have been mineral protected (through organo-mineral interactions and/or aggregations) and resulted in lower mineralizability (of wetter soils) in this experiment.

Did mineral attachment lead to lower mineralizability of wetter soils?

Greater soil moisture results in increased organic C contained in the soil aqueous phase, which are smaller sized, solvated, mobile molecular fragments which are highly efficient in forming mineral-organic associations (MOAs) with mineral reactive phases that have large specific surface areas, such as Fe oxides, short-range ordered Al-silicates, permanently charged clay minerals, or metal cations (Al^{3+} and Fe^{3+}) (Eusterhues e*t al.*, 2003; Mikutta *et al.*, 2005; Kalbitz and Kaiser, 2008, Kleber *et al.,* 2015). Increased heterotrophic respiration (and to some extent root respiration) in wetter soils can lead to a drop in soil pH which creates secondary minerals resulting in increased formation of MOAs, through processes of adsorption and coprecipitation (Kleber *et al.,* 2015). While low pH of the soil solution leads to stronger innerspace bonds between metal oxides and clay minerals, weaker outerspace complexation and H-bonds are active at neutral and alkaline pH (Kleber *et al.,* 2015). The low C mineralizability of such soils near neutral pH is an important reason why adsorption is thought to not be the sole reason for long turnover times of OM (Torn *et al.,* 2009). Coprecipitation is a driving mechanism of OM stabilization in wet anaerobic soil systems where reduced Fe (II) in solution is oxidized to Fe (III) upon aeration and then coprecipitates with OM. However, it has been recently recognized that Fe-OM coprecipitate formation is not restricted to aquic soil systems but is also observed in upland soils exposed to partial anaerobic conditions (Fimmen *et al.*, 2007; Thompson *et al.*, 2006; Kleber *et al.,* 2015), especially in the rhizosphere (Collignon *et al.*, 2012; Kleber *et al.,* 2015). For our field soils (pH range of 5.7-6), mechanisms of stabilization that are common to near neutral or mildly acidic pH, and exposed to partial oxygen deficiency conditions during high rainfall events are therefore likely. Additionally, organic matter composition and its effects on sorption or coprecipitation may also dictate MOA formation, with O/N-alkyl C, alkyl C, aromatic C, carboxyl C moieties displaying selective preferences (Kleber *et al.,* 2015). Additionally, as the fact that OM bound via weaker electrostatic forces display greater desorption in comparison to innerspace complexes (Mikutta *et al*., 2007; Wang and Lee, 1993) can also be proposed to be a mechanistic explanation in our experiment, where C stabilization is best explained by outerspace weak bonding in the neutral/near neutral pH range. Therefore, an influence of long-term moisture on the distribution of secondary minerals and any such MOA formation mechanism may be key to answering the cause of decreased C mineralizability of the wetter field soils in our experiment. Furthermore, we cannot rule out variations in long-term moisture causing differences in spatial arrangements of OM within the mineral matrix as in the SCM model (Lehmann and Kleber, 2015), resulting in differential C stabilization and low C mineralizability.

Conclusions

In our experiment, added moisture increased C mineralizability in the tested moisture range (0.3 g g^{-1}, 0.4g g^{-1} and 0.5 g g^{-1}), indicating greater moisture resulted in increased rates of aerobic heterotrophic respiration. However, wetter field soils in the same incubation moisture range displayed decreased C mineralizability, with longer mean residence time of the slow-mineralizing C pools, suggesting a mineral-associated pathway of OM stabilization operative in the wetter soils, in comparison to drier ones. Climate-carbon cycle feedback is intricately related to SOC response to moisture. However, most SOC models, such as CENTURY (Parton *et al.*, 1987) or RothC (Coleman and Jenkinson, 1999), typically assume greater mineralization and do not include greater stabilization with increasing soil moisture within the range examined in this study. Multiple moisture-driven abiotic and biotic factors operative in surface soils are likely to control mineral-related SOC stabilization. Integrating microbial community and extracellular enzyme studies, controlled field respiration studies and analytical approaches characterizing MOAs with such long-term incubation experiments will substantially solidify results and help elucidate the mechanistic processes surrounding moisture- and mineral-associated SOC stabilization.

Acknowledgements

This research was supported with USDA/NIFA Grant No. 2011-67009-20083 and Federal Capacity (Hatch) Project No. NYC-123486. The authors are extremely thankful to all project assistants and summer interns of the Projects for their help and support. Many thanks to Maria Gannet, Leilah Krounbi, Akio Enders for their kind help at different stages of the work. Thanks to everybody at the Lehmann lab. Thanks to Teamrat A. Ghezzei, UC Merced for help with curve fitting procedure with R code. The work also could not be completed without the help of Kevin Packard and Francoise Vermeylen of Cornell Statistical Computing Unit, Cornell University.

APPENDIX FOR CHAPTER 4

Carbon mineralization with changing legacy water availability in perennial grass bioenergy plantations[4]

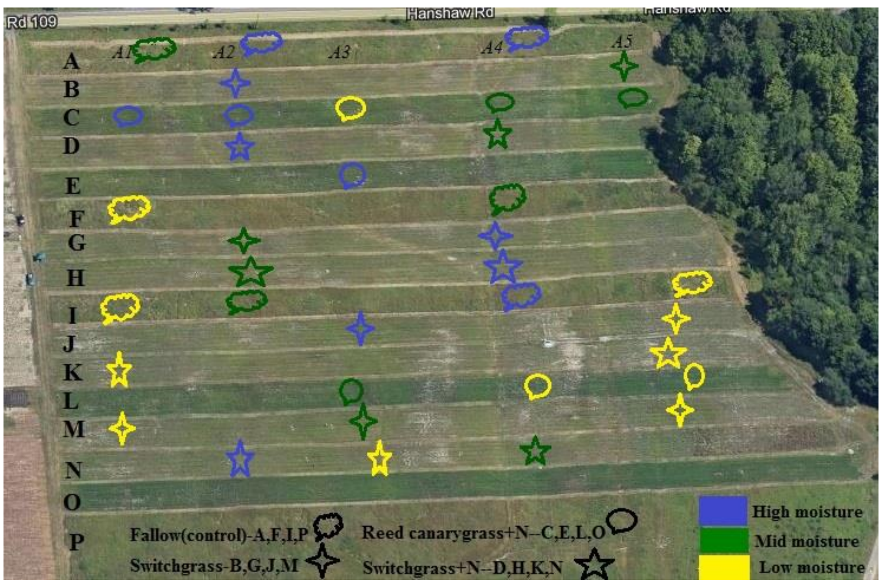

Figure S4.1. Field site layout depicting the experimental subplot selection schematic from the strip plots belonging to the four cropping systems. (drawings on July 2012 Google earth photograph). Sub-plot locations are shown with schematic drawings and subplot ID 's are shown in Table S4.1.

[4] Srabani Das, Brian K. Richards, M. Todd Walter, Tammo S. Steenhuis, Kelly L. Hanley, Johannes Lehmann*

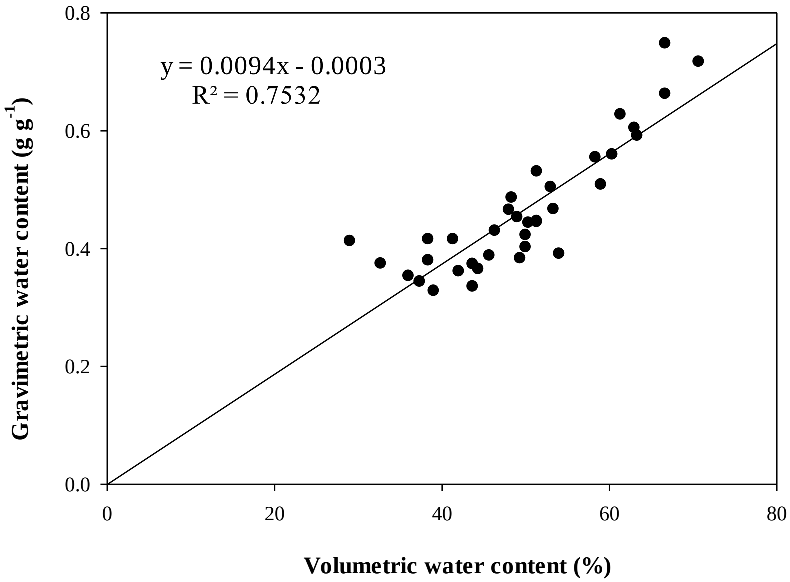

Figure S4.2a. Gravimetric water content as a function of volumetric water content for soils from the subset of chosen subplots (n=36) for the sampling day in Aug 2016. Linear equation used for estimating long-term equivalent gravimetric content of three wetness levels and subsequent laboratory water adjustments.

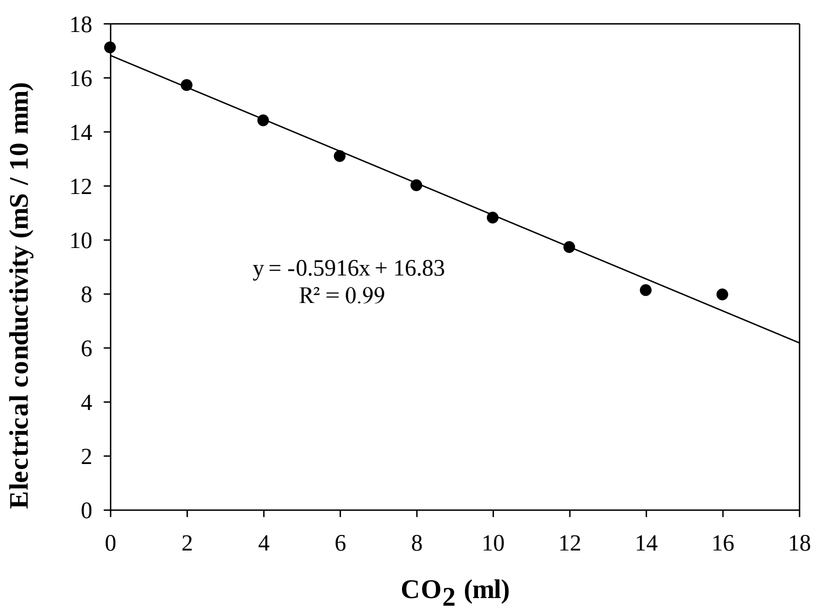

Figure S4.2b. Standard graph regression for determination of electrical conductivity value at known CO_2 volume for use in calibration

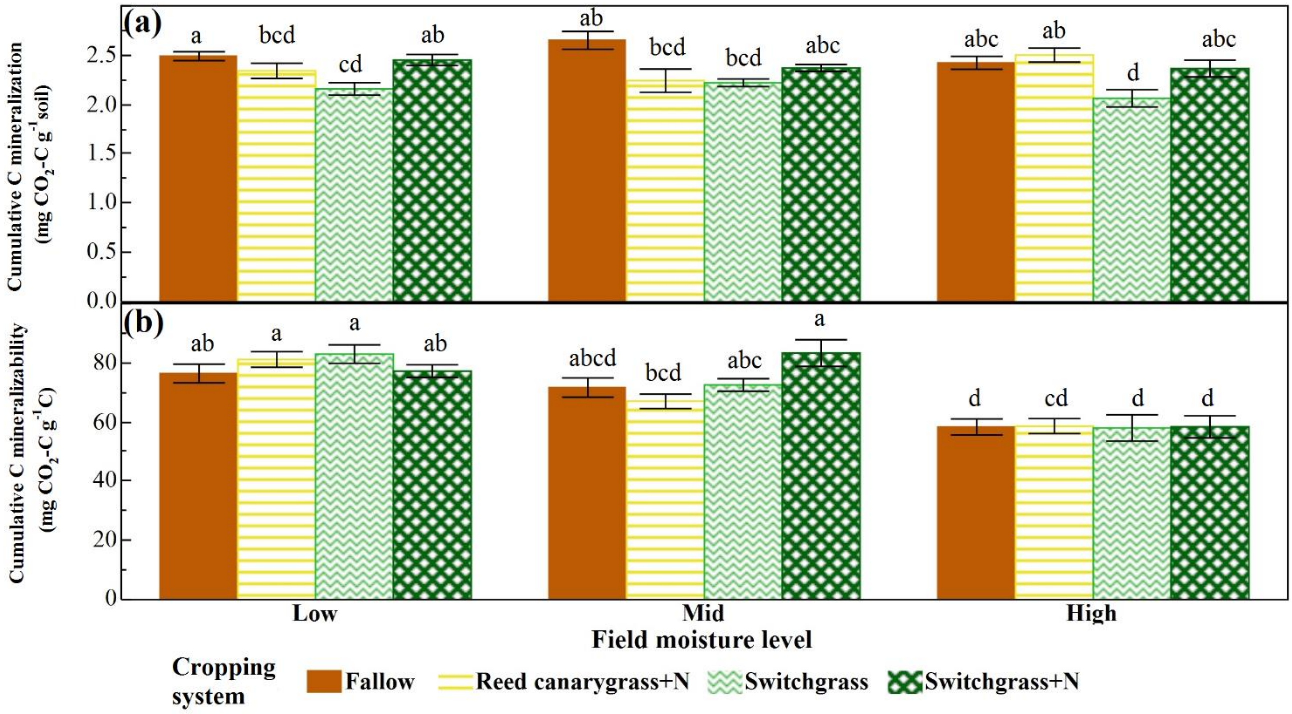

Figure S4.3. Cumulative C mineralization and C mineralizability at the end of 371 days of incubation per unit soil weight (a) and per unit initial SOC (b) for the cropping system-field moisture combinations. For each cropping system-field moisture combination, mean + SE for 3 field representatives each averaged over 3 laboratory levels, n = 9 (average of 2 technical replicates). Different letters above bars indicate significant differences ($p < 0.05$) of the different field moisture-cropping system combinations. The cropping systems were fallow-control, reed canarygrass + fertilizer 75 kg N ha^{-1}, switchgrass and switchgrass + fertilizer 75 kg N ha^{-1} and the laboratory moisture levels were lab-low, lab-mid and lab-high. The water contents corresponding to field Low, Mid and High and lab-low, lab-mid and lab-high are both 0.3 g g^{-1}, 0.4 g $g^{-1}$and 0.5g g^{-1}, respectively.

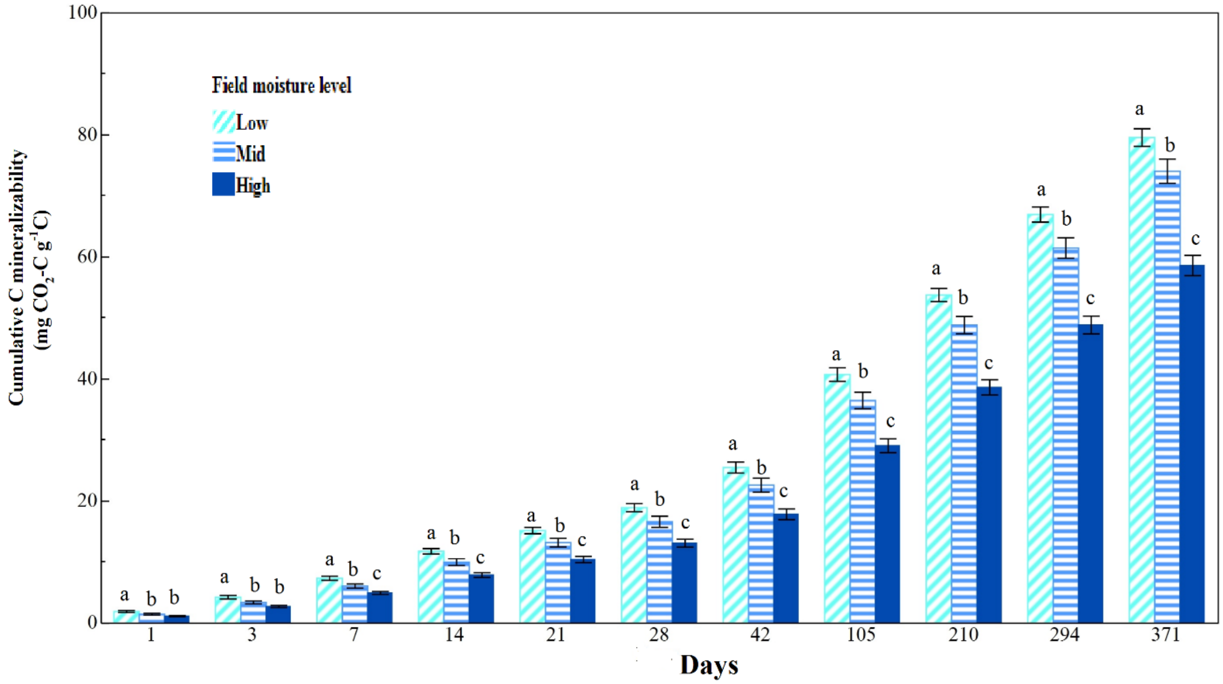

Figure S4.4. Mean C mineralizability at the 11 measurement timepoints. Different letters above bars indicate significant differences at each time point ($p < 0.05$). Mean C mineralizability values for laboratory moisture and cropping system are averaged over the three-field moisture levels: Low, Mid, High. The cropping systems were fallow-control, reed canarygrass + fertilizer 75 kg N ha^{-1}, switchgrass and switchgrass + fertilizer 75 kg N ha^{-1} and the laboratory moisture levels were lab-low, lab-mid and lab-high. The water contents corresponding to field Low, Mid and High and lab-low, lab-mid and lab-high are both 0.3 g g^{-1}, 0.4 g $g^{-1}$and 0.5g g^{-1}, respectively.

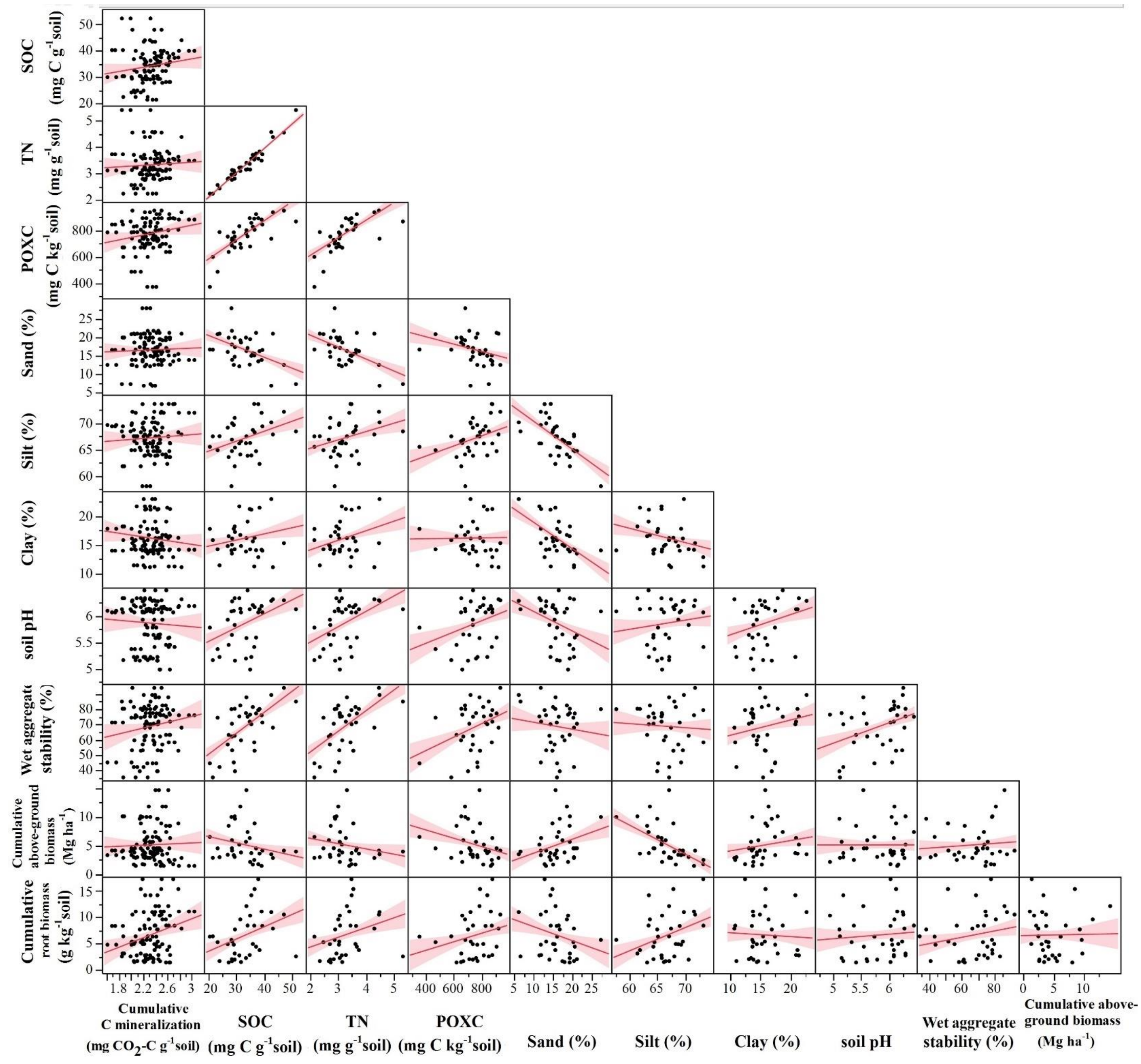

Figure S4.5a. Scatterplot matrix for Cumulative C mineralization (mg C g^{-1} soil) to soil properties and cumulative crop growth parameters.

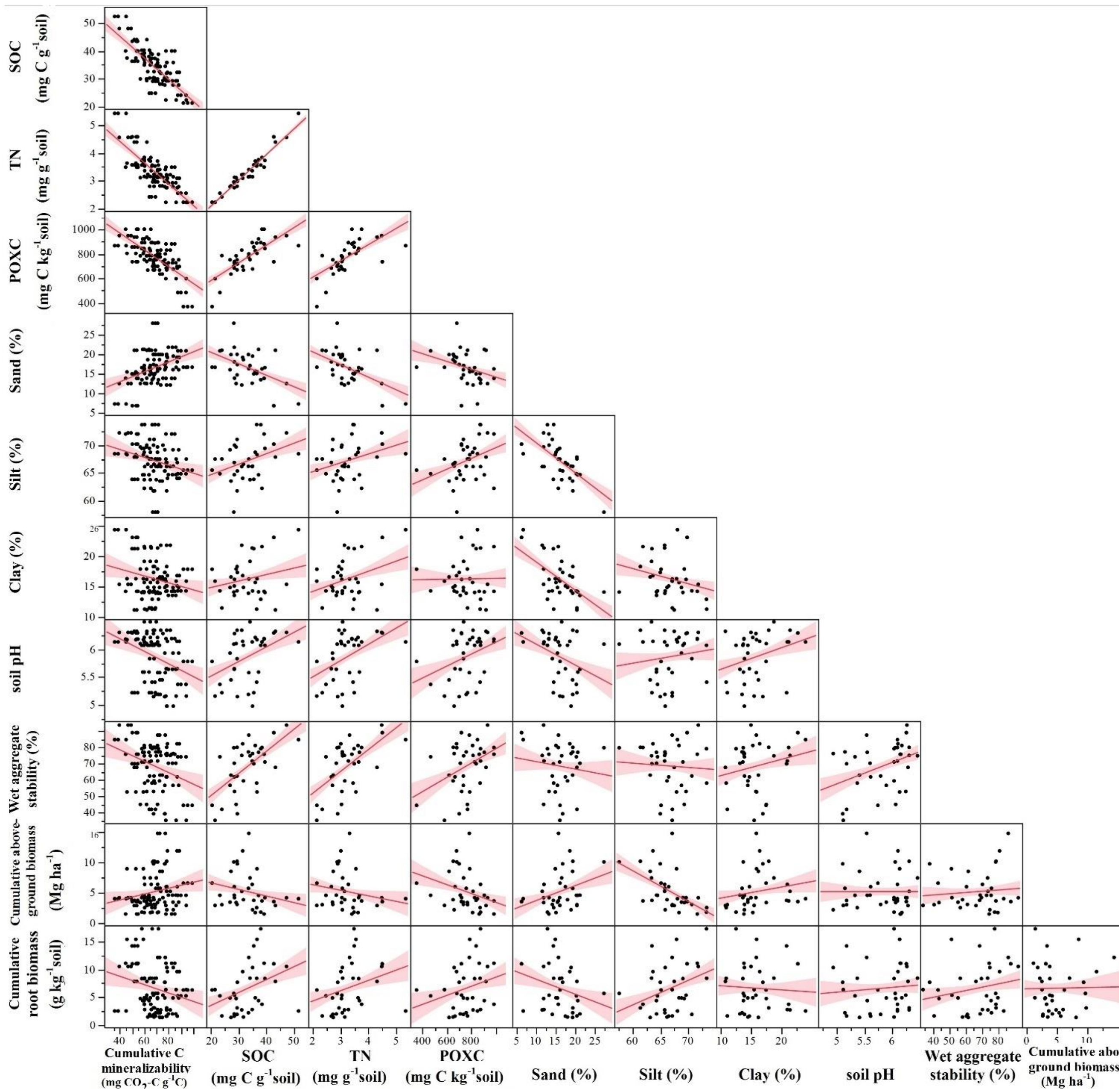

Figure S4.5b. Scatterplot matrix for Cumulative C mineralizability (mg C g^{-1} SOC) to soil properties and cumulative crop growth parameters.

Table S4.1a. Equivalent water content values of long term high, mid and low volumetric wet quintile ranks

Cropping system	Field-moisture level		
	High	**Mid**	**Low**
Fallow-Control	A2	A1	F1
	A4	I2	I1
	I4	F4	I5
RCG	C2	C4	C3
	E3	L3	L5
	C1	C5	L4
SWG	B2	B5	J5
	G4	M3	M5
	J3	G2	M1
SWGN	D2	D4	K1
	H4	N4	K5
	N2	H2	N3

Table S4.1b. Subplot IDs of different moisture and distribution of the four cropping systems

Long term volumetric high value (%)	Long term volumetric mid value (%)	Long term volumetric low value (%)	Long term equivalent high-water content g/g	Long term equivalent mid -water content g/g	Long term equivalent low-water content g/g
53.94	**43.1**	**34.9**	**0.5**	**0.4**	**0.3**

Table S4.2a. Fixed effects and their interaction for the response variable cumulative CO_2 evolution per unit soil (mg CO_2-C g^{-1} soil) at 1 year (371 days)

Effect tests	DF	F Ratio	P value
Field moisture level	2	0.3	0.7
Laboratory moisture level	2	21	*<0.0001*
Cropping system	3	19.1	*<0.0001*
Field moisture level *Cropping system	6	3.4	0.005

Table S4.2b. Post-hoc comparisons of least square means for main effect lab moisture level and cropping system.

Level				**Least Square Mean**
lab-high	A			2.5
lab-mid		B		2.4
lab-low			C	2.2
Level				**Least Square Mean**
Fallow	A			2.5
Switchgrass+N	A	B		2.4
Reed canarygrass+N		B		2.4
Switchgrass			C	2.1

Table S4.2c. Post-hoc comparisons of least square means for the interaction field moisture* cropping system at 371 days for SOC loss expressed in per unit soil, α =0.05 Tukey's HSD used to correct for multiple comparisons

Level					**Least Square Mean**
Mid, Fallow	A				2.7
High, Reed canarygrass+N	A	B			2.5
Low, Fallow	A	B			2.5
Low, Switchgrass+N	A	B			2.5
High,Fallow	A	B	C		2.4
Mid,Switchgrass+N	A	B	C		2.4
High, Switchgrass+N	A	B	C		2.4
Low, Reed canarygrass+N		B	C	D	2.3
Mid, Reed canarygrass+N		B	C	D	2.2
Mid, Switchgrass		B	C	D	2.2
Low, Switchgrass			C	D	2.2
High, Switchgrass				D	2.1

Table S4.3a. Fixed effects and their interaction for the response variable cumulative CO_2 evolution per unit SOC (mg CO_2-C g^{-1} C) at 1 year (371 days)

Effect tests	DF	F Ratio	P value
Field moisture level	2	54	*<0.0001*
Laboratory moisture level	2	7.4	0.001
Cropping system	3	1.4	0.3
Field moisture level	6	2.6	0.02

Table S4.3b. Post-hoc comparisons of least square means of cumulative CO_2 evolution per unit SOC (mg CO_2-C g^{-1} C) for main effect field moisture and lab moisture at 371 days

Level				**Least Square Mean**
lab-high	A			74.5
lab-mid	A	B		70.2
lab-low		B		66.5
Level				**Least Square Mean**
Low	A			79.4
Mid		B		73.6
High			C	58.3

Table S4.3c. Post-hoc comparisons of least square means for the interaction field moisture* cropping system at 371 days for SOC loss expressed in per unit SOC, α =0.05 Tukey's HSD used to correct for multiple comparisons

Level					**Least Square Mean**
Mid, Switchgrass+N	A				83.2
Low, Switchgrass	A				83
Low, Reed canarygrass+N	A				81.1
Low, Switchgrass+N	A	B			77.2
Low, Fallow	A	B			76.4
Mid, Switchgrass	A	B	C		72.5
Mid, Fallow	A	B	C	D	71.6
Mid, Reed canarygrass+N		B	C	D	67
High, Reed canarygrass+N			C	D	58.6
High, Switchgrass+N				D	58.3
High, Fallow				D	58.3
High, Switchgrass				D	58

Table S4.4a. Fixed effects and their interaction for the response variable slow mineralizable pool C_2(mg CO_2-C g^{-1} SOC) at 1 year (371 days)

Effect tests	DF	F Ratio	P value
Field moisture level	2	51.5	*<0.0001*
Laboratory moisture level	2	47.5	*<0.0001*
Cropping system	3	2.4	1
Field moisture level *Cropping system	6	4.7	0.003

Table S4.4b. Post-hoc comparisons of least square means for main effect field moisture and lab moisture level.

Level				**Least Square Mean**
High	A			983.7
Mid		B		979.8
Low			C	975.9
Level				**Least Square Mean**
3	A			983.5
2		B		979.8
1			C	976.1

Table S4.4c. Post-hoc comparisons of least square means for the interaction field moisture* cropping system at 371 days for C_2, α =0.05 Tukey's HSD used to correct for multiple comparisons

Level					**Least Square Mean**
High, Switchgrass	A				984.2
High, Switchgrass +N	A				984.1
High, Reed canarygrass	A	B			983.5
High, Control	A	B	C		980
Mid, Switchgrass	A	B	C		982.6
Mid, Reed canarygrass	A	B	C		982.5
Low, Switchgrass +N		B	C	D	978.4
Mid, Switchgrass +N			C	D	977.7
Mid, Control				D	976.4
Low, Control				D	976
Low, Switchgrass				D	975.4
Low, Reed canarygrass				D	974.1

Table S4.5a. Fixed effects and their interaction for the response variable MRT_1 in days

Effect tests	DF	F Ratio	P value
Field moisture level	2	0.4	0.7
Laboratory moisture level	2	0.3	0.7
Cropping system	3	8	0.0008
Field moisture level *Cropping system	6	3.4	0.02

Table S4.5b. Post-hoc comparisons of least square means for main effect cropping system

Level			Least Square Mean
Reed canarygrass	A		29.3
Switchgrass	A		29.1
Control	A		28.1
Switchgrass +N		B	24.7

Table S4.5c. Post-hoc comparisons of least square means for the interaction field moisture* cropping system MRT_1, α =0.05 Tukey's HSD used to correct for multiple comparisons

Level			Least Square Mean
Mid, Reed canarygrass	A		31.1
High, Switchgrass	A		30.3
Low, Reed canarygrass	A		29.7
Low, Switchgrass	A	B	29.3
Low, Control	A	B	29.3
High, Switchgrass +N	A	B	28.6
Mid, Control	A	B	28
Mid, Switchgrass	A	B	27.8
High, Reed canarygrass	A	B	27
High, Control	A	B	27
Mid, Switchgrass +N		B	23
Low, Switchgrass +N		B	22.7

Table S4.6a. Fixed effects and their interaction for the response variable MRT_2 in years

Effect tests	DF	F Ratio	P value
Field moisture level	2	588.2	*<0.0001*
Laboratory moisture level	2	2.4	0.1
Cropping system	3	35.8	*<0.0001*
Field moisture level *Cropping	6	15.8	*<0.0001*

Table S4.6b. Post-hoc comparisons of least square means for main effect field moisture and cropping system

Level				**Least Square Mean**
High	A			23.8
Mid		B		18.7
Low			C	17.8
Level				**Least Square Mean**
Control	A			21.2
Reed canarygrass		B		20.5
Switchgrass			C	19.5
Switchgrass+N			C	19.2

Table S4.6c. Post-hoc comparisons of least square means for the interaction field moisture* cropping system at 371 days for MRT_2, α =0.05 Tukey's HSD used to correct for multiple comparisons

Level							**Least Square Mean**
High, Control	A						24.2
High, Reed canarygrass	A						23.7
High, Switchgrass+N	A						23.6
High, Switchgrass	A						23.6
Mid, Control		B					20.7
Mid, Reed canarygrass		B	C				19.9
Low, Control			C	D			18.8
Mid, Switchgrass				D	E		18.0
Low, Switchgrass +N				D	E		17.8
Low, Reed canarygrass				D	E		17.7
Low, Switchgrass					E	F	16.8
Mid, Switchgrass +N						F	16.2

Table S4.7. C mineralization kinetics of soil after 371 days incubation at 25°C for the cropping systems: Fallow-control, Reed canarygrass, Switchgrass and Switchgrass +N. Pool sizes and decay rates of soil C mineralization using double exponential model : $C_{umulative} = C_1 (1-\exp(-k_1 x)) + C_2 (1-\exp(-k_2 x))$, where C_1 is the fast pool, C_2 is the slow pool and k_1 and k_2 are the first–order decomposition rate coefficients for fast and slow pool respectively, the parameter constraints chosen, $k_1>0$, $k_2>0$ and C_1+C_2=initial SOC. MRT_1 = mean residence time of fast pool in days ($MRT_1=1/k_1$) and MRT_2 = mean residence time of slow pool in years {$MRT_2=(1/k_2)/365$}. The cropping systems were fallow-control, reed canarygrass + fertilizer 75 kg N ha^{-1}, switchgrass and switchgrass + fertilizer 75 kg N ha^{-1}. The water contents corresponding to field Low, Mid and High and lab-low, lab-mid and lab-high are both 0.3 g g^{-1}, 0.4 g g^{-1}and 0.5g g^{-1}, respectively.

Cropping system	Moisture level	C_1 (mg CO_2-C g^{-1}soil)	k_1 (day^{1})	C_2 (mg CO_2-C g^{-1} soil)	k_2 (day^{-1})	MRT_1 (days)	MRT_2 (years)	Initial SOC (mg C g^{-1} soil)
Fallow- control	Field high-lab	0.85±0.06	0.04±0.00	41.1±0.1	0.00011±0.00001	28.4	24.2	42
	Field high-lab mid	0.77±0.05	0.04±0.01	41.2±0.1	0.00011±0.00001	24.3	24.4	42
	Field high-lab low	0.5±0.06	0.04±0.01	41.5±0.1	0.00011±0.00001	27.8	24.9	42
	Field mid-lab high	1.02±0.07	0.04±0.00	36.2±0.1	0.00013±0.00001	28.6	20.7	37.2
	Field mid-lab mid	0.85±0.06	0.04±0.01	36.3±0.1	0.00013±0.00001	27.5	20.7	37.2
	Field mid-lab low	0.72±0.06	0.04±0.01	36.5±0.1	0.00013±0.00001	27.7	20.7	37.2
	Field low-lab high	0.95±0.06	0.03±0.00	32.1±0.1	0.00014±0.00001	30.0	19.3	33
	Field low-lab mid	0.81±0.05	0.034±0.004	32.2±0.1	0.00014±0.00001	29.0	19.2	33
	Field low-lab low	0.6±0.05	0.04±0.01	32.4±0.1	0.00015±0.00001	28.4	18.6	33
Reed canarygrass	Field high-lab	0.88±0.05	0.04±0.00	42.1±0.1	0.000114±0.000004	26.6	24.1	43
	Field high-lab mid	0.75±0.05	0.03±0.00	42.2±0.1	0.000115±0.000004	28.7	23.8	43
	Field high-lab low	0.5±0.05	0.04±0.01	42.5±0.1	0.000116±0.000005	26.7	23.7	43
	Field mid-lab high	0.71±0.05	0.03±0.00	33.3±0.1	0.000134±0.000005	30.7	20.4	34
	Field mid-lab mid	0.63±0.04	0.03±0.00	33.4±0.0	0.000132±0.000004	31.2	20.8	34
	Field mid-lab low	0.44±0.04	0.03±0.01	33.6±0.1	0.000136±0.000004	29.8	20.1	34
	Field low-lab high	0.86±0.05	0.04±0.00	28.2±0.1	0.000155±0.000007	27.7	17.7	29.1
	Field low-lab mid	0.71±0.05	0.04±0.00	28.4±0.1	0.000156±0.000006	28.3	17.6	29.1
	Field low-lab low	0.63±0.05	0.04±0.01	28.5±0.1	0.000154±0.000006	28.1	17.8	29.1
Switchgrass	Field high-lab	0.71±0.04	0.04±0.00	36.6±0.0	0.00011±0.00000	27.6	23.5	37.3
	Field high-lab mid	0.5±0.05	0.03±0.01	36.8±0.1	0.00011±0.00001	31.5	24.2	37.3
	Field high-lab low	0.37±0.05	0.03±0.01	36.9±0.1	0.00011±0.00001	31.3	25.0	37.3
	Field mid-lab high	0.64±0.05	0.03±0.01	30.4±0.1	0.00015±0.00001	31.0	18.1	31
	Field mid-lab mid	0.55±0.06	0.03±0.01	30.4±0.1	0.00015±0.00001	29.3	18.7	31
	Field mid-lab low	0.41±0.05	0.04±0.01	30.6±0.1	0.00015±0.00001	23.4	18.0	31
	Field low-lab high	0.68±0.05	0.04±0.01	25.5±0.1	0.00017±0.00001	26.7	15.8	26.2
	Field low-lab mid	0.56±0.06	0.04±0.01	25.6±0.1	0.00016±0.00001	27.8	16.7	26.2
	Field low-lab low	0.65±0.04	0.03±0.00	25.5±0.1	0.00015±0.00001	34.7	17.9	26.2
Switchgrass +N	Field high-lab	0.89±0.07	0.03±0.01	40.5±0.1	0.000116±0.000006	28.4	23.7	41.4
	Field high-lab mid	0.65±0.06	0.03±0.01	40.7±0.1	0.000111±0.000005	28.3	24.7	41.4
	Field high-lab low	0.4±0.06	0.03±0.01	40.9±0.1	0.000111±0.000005	29.4	24.7	41.4
	Field mid-lab high	0.7±0.05	0.04±0.01	28.3±0.1	0.000166±0.000006	24.7	16.6	29.1
	Field mid-lab mid	0.62±0.05	0.05±0.01	28.5±0.1	0.000167±0.000007	21.9	16.4	29.1
	Field mid-lab low	0.53±0.05	0.04±0.01	28.6±0.1	0.000166±0.000007	22.5	16.5	29.1
	Field low-lab high	0.72±0.06	0.04±0.01	31.2±0.1	0.000153±0.000007	23.8	17.9	32
	Field low-lab mid	0.67±0.06	0.04±0.01	31.3±0.1	0.000152±0.000007	23.7	18.0	32
	Field low-lab low	0.68±0.06	0.05±0.01	31.3±0.1	0.000152±0.000007	21.8	18.0	32

Table S4.8a. Eigenvector values for principal component analysis showing relation among soil covariates (excluding soil texture components), principal component 1 (PC1) and principal component 2 (PC2)

Eigenvectors	**PC1**	**PC2**
SOC (mg C g^{-1} soil)	0.52	0.05
TN (mg N g^{-1} soil)	0.51	-0.00
Wet aggregate stability (%)	0.41	-0.18
POXC per unit SOC (mg C g^{-1} C)	-0.25	0.76
pH	0.32	0.02
POXC (mg C kg^{-1}soil)	0.38	0.61

Table S4.8b. Loading matrix values for principal component analysis showing relation among soil covariates (excluding soil texture components), principal component 1 (Prin1) and principal component 2 (Prin2)

Loading matrix	**PC1**	**PC2**
SOC (mg C g^{-1} soil)	0.96	0.1
TN (mg g-1soil)	0.95	-0.02
Wet aggregate stability (%)	0.76	-0.3
POXC per unit SOC (mg C g^{-1} C)	-0.46	0.6
pH	0.58	-0.1
POXC (mg C kg^{-1}soil)	0.70	0.5

Table S4.9a. Eigenvector values for principal component analysis with C mineralizability and soil and crop characteristics, principal component 1 (PC1*) and principal component 2 (PC2*)

Eigenvectors	**PC1***	**PC2***
C mineralizability (mg CO_2-C g^{-1} C)	-0.35	0.05
SOC (mg C g^{-1}soil)	0.43	0.03
TN (mg N g^{-1}soil)	0.43	0.09
Sand (%)	-0.30	0.09
Silt (%)	0.21	-0.49
Clay (%)	0.15	0.404
pH	0.24	0.16
Wet aggregate stability (%)	0.27	0.32
POXC (mg C kg^{-1} soil)	0.33	-0.22
POXC per unit SOC (mg C g^{-1} C)	-0.17	-0.37
Above-ground biomass (Mg ha^{-1})	-0.13	0.49
Root biomass (mg g^{-1} soil)	0.19	-0.06

Table S4.9b. Loading matrix values for principal component analysis with C mineralizability and soil and crop characteristics, principal component 1 (PC1*) and principal component 2 (PC2*)

Loading matrix	**PC1***	**PC2***
C mineralizability (mg CO_2-C g^{-1}C)	-0.78	0.07
SOC (mg C g^{-} soil)	0.95	0.04
TN (mg N g^{-1}soil)	0.94	0.14
Sand (%)	-0.68	0.14
Silt (%)	0.48	-0.70
Clay (%)	0.34	0.57
pH	0.58	0.2
Wet aggregate stability (%)	0.59	0.59
POXC (mg C kg^{-1} soil)	0.73	-0.32
POXC per unit SOC (mg C g^{-1} C)	-0.39	-0.53
Above-ground biomass (Mg ha^{-1})	-0.29	0.70
Root biomass (mg g^{-1} soil)	0.42	-0.09

Table S4.10. Cumulative C mineralization in incubation experiments at different moisture levels at constant temperature varying between 25° to 35°C

Study details	**Moisture levels and Cumulative C mineralization**	**Incubation duration (days)**	**Study authors**
High elevation peatlands of Sierra Nevada, USA.	Greatest at the wettest (-0.1bar) and driest (-4 bar) water potential, U shaped pattern.	392	Arnold *et al*. 2014
Forest, grassland and cropland soils of Atlantic humid temperate zone, Spain.		42	Guntiňas *et al.* 2013
Burned and unburned soils of 40-year-old subtropical Chinese Fir forest, China.	Increased with increasing moisture from 25% to 75% WHC.	90	Guo *et al*. 2012
Soils of arid and semiarid ecosystems on the Mongolian plateau.	Increased 23% from 30 to 60% WFPS and by 176% from 60-90%	28	Mi *et al.* 2015
Mediterranean forest soils, Italy	Mineralization increased with increasing moisture content from 20 to 40 to 60 to a maximum at 80% and decreased beyond it, at 100% WHC.	30	Rey *et al*. 2005
Forest soils of Changbai mountain, (temperate region) Northeast China.	Increased with increasing moisture from 20% to 40% to 60% of mass water content (g g^{-1} soil)	42	Qi *et al*. 2011
Soils of humid mid-subtropical forest soils, South China.	More in mid mass water content (33%) in comparison to low (21%) and high (45%) mass water contents	45	Wang *et al*.2015

References

Arnold, CL, T.A. Ghezzehei, and AA Berhe (2014) Decomposition of distinct organic matter pools is regulated by moisture status in structured wetland soils *Soil Biology and Biochemistry* 81,28-37.

Chen X, Wang X, Liebman M, Cavigelli M, Wander M (2014) Influence of residue and nitrogen fertilizer additions on carbon mineralization in soils with different texture and cropping histories. PLoS ONE 9 (7), e103720. doi:10.1371/journal.pone.0103720

Coleman K, Jenkinson DS (1999) *RothC-26.3,* A Model for the Turnover of Carbon in Soil: Model Description and User's Guide, Lawes Agric. Trust, Harpenden, U. K.

Collignon, Ranger J, and Turpault M P (2012) Seasonal dynamics of Al- and Fe-bearing secondary minerals in an acid forest soil: influence of Norway spruce roots (*Picea abies* (L.) Karst.). *European Journal of Soil Science*, 63, 592–602. doi:10.1111/j.1365-2389.2012.01470.x

Corre MD, Schnabel RR, Shaffer JA (1999) Evaluation of soil organic carbon under forests, cool-season and warm-season grasses in the northeastern U.S. *Soil Biology and Biochemistry*, 31, 1531–1539.

Culman, SW, Snapp, SS, Freeman, MA, Schipanski, ME, Beniston, J, Lal, R, Drinkwater, LE, Franzluebbers, AJ, Glover, JD, Stuart Grandy, A, Lee, J, Six, J, Maul, JE, Mirksy, SB, Spargo, JT & Wander, MM (2012) Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management *Soil Science Society of America Journal* 76(2), 494-504. DOI: 10.2136/sssaj2011.0286

Davidson E A (1993) Soil water content and ratio of nitrous oxide and nitric oxide emitted from soil. 369-386, *In* Oremland R (ed), *Biogeochemistry of Global Change: Radiatively Active Trace Gases*. Chapman and Hall, New York.

Eusterhues K, Rumpel C, Kleber M, Kögel-Knabner I (2003) Stabilization of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. *Organic Geochemistry* 34, 1591–1600. http://dx.doi.org/10.1016/j.orggeochem. 2003.08.007

Fimmen RL, Cory RM, Chin Y-P, Trouts TD, McKnight DM (2007) Probing the oxidation–reduction properties of terrestrially and microbially derived dissolved organic matter. *Geochimica et Cosmochimica Acta* 71(12), 3003-3015

Franzluebbers AJ (1999) Microbial activity in response to water-filled pore space of variably eroded southern Piedmont soils. *Applied Soil Ecology*, 11, 91-101.

Guntiñas ME, Gil-Sotres F, Leirós MC, Trasar-Cepeda C (2013) Sensitivity of soil respiration to moisture and temperature *Journal of Soil Science and Plant Nutrition*, 13 (2), 445-46.

Guo X, Drury C F, Yang X, Reynolds WD, Zhang R (2012) Impacts of wet–dry cycles and a range of constant water contents on carbon mineralization in soils under three cropping treatments. *Soil Science Society of America Journal* 76, 485-493.

He D, Ruan H (2014) Long Term Effect of Land Reclamation from Lake on Chemical Composition of Soil Organic Matter and Its Mineralization. *PLoS ONE* 9(6), e99251. doi:10.1371/journal.pone.0099251

Jarrell WM, Armstrong DE, Grigal DF, Kelley EF, Monger HC, Wedin DA (1999) Soil water and temperature status. Pp. 55–73, *In* Robertson GP, Coleman DC, Bledsoe CS, Sollins P (Eds.). Standard Soil Methods for Long-term Ecological Research. Oxford University Press, New York, NY.

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, 10, 423–-436.

Keiluweit M, Bougoure JJ, Pett-Ridge J, Weber PK, Kleber M, (2015) Mineral protection of soil carbon counteracted by root exudates *Nature Climate Change* 5, 588–595

Kemmitt SJ, Lanyon CV, Waite IS, Wen Q, Addiscott TM, Bird NRA, O'Donnell T, Brookes PC (2008) Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass—a new perspective. *Soil Biology & Biochemistry* 40, 61–73.

Kirschbaum MUF (2006) The temperature dependence of organic-matter decomposition — still a topic of debate. *Soil Biology & Biochemistry* 38, 2510–2518.

Kleber M, Eusterhues K, Keiluweit M, Mikutta C, Mikutta R, Nico PS (2015) Mineral-organic associations: formation, properties, and relevance in Soil Environments. *Advances in Agronomy*, 130, 1-140.

Kögel-Knabner I, Guggenberger G, Kleber M, Kandeler E, Kalbitz K, Scheu S, Eusterhues K, Leinweber P (2008) Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science* 171, 61-82 http://dx.doi.org/10.1002/jpln.200700048

Lehmann J, Kleber M (2015) The contentious nature of soil organic matter. *Nature* 528, 60–68.

Liang B, Lehmann J, Solomon D, Sohi S, Thies, JE, Skjemstad JO, Luizao FJ, Engelhard MH, Neves EG, Wirick S (2008) Stability of biomass-derived black carbon in soils. *Geochimica et Cosmochimica Acta* 72, 6069e6078

Linn DM, Doran JW (1984) Aerobic and anaerobic microbial populations in no-till and plowed soils. *Soil Science Society of America Journal* 48, 794–799.

Matamala R, Jastrow DJ, Miller RM, Garten C (2008) Temporal changes in the distribution of C and N stocks in a restored tallgrass prairie in the U.S Midwest. *Ecological Applications*, 18, 1470–1488.

Mason CW, Stoof CR, Richards BK, Das S, Goodale CL, Steenhuis TS (2017) Hotspots of nitrous oxide emission in fertilized and unfertilized perennial grasses on wetness-prone marginal land in New York State. *Soil Science Society of America Journal* 81, 452-458, DOI: 10.2136/sssaj2016.08.0249

Mi J, Li J, Chen D et al. (2015) Predominant control of moisture on soil organic carbon mineralization across a broad range of arid and semiarid ecosystems on the Mongolia plateau. Landscape Ecology 30, 1683. doi:10.1007/s10980-014-0040-0

Mikutta R, Kleber M, Jahn R (2005) Poorly crystalline minerals protect organic carbon in clay subfractions from acid subsoil horizons *Geoderma*, 128, 106–115 http://dx.doi.org/10.1016/j.geoderma.2004.12.018

Mikutta, R., Mikutta C, Kalbitz K, Scheel T, Kaiser K, Jahn R (2007) Biodegradation of forest floor organic matter bound to minerals via different binding mechanisms. *Geochimica et Cosmochimica Acta* 71, 2569-2590.

Moebius-Clune BN, Moebius-Clune DJ, Gugino BK, Idowu OJ, Schindelbeck RR, Ristow AJ, van Es HM, Thies JE, Shayler HA, McBride MB, Wolfe DW, Abawi GS (2016) Comprehensive Assessment of Soil Health. In: *The Cornell Framework Manual: Third Edition*, pp. 51-52, Cornell University, Geneva, United States.

Moyano F, Manzoni S, Chenu C (2013) Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. *Soil Biology and Biochemistry*, 59, 72-85.

Oades J M (1988) The retention of organic matter in soils. *Biogeochemistry* 5, 35–70.

Orchard VA, Cook FJ (1983) Relationship between soil respiration and soil moisture. *Soil Biology and Biochemistry*, 15, 447–453.

Parton WJ, Schimel DS, Cole CV, Ojima DS (1987) Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal.*, 51, 1173–1179, doi:10.2136/sssaj1987.03615995005100050015x.

Propheter JL, Staggenborg SA (2010) Performance of annual and perennial biofuel crops: Nutrient removal during the first two years. *Agronomy Journal*, 102, 798–805.

Qi G, Wang Q, Zhou W, Ding H, Wang X, Qi L, Wang Y, Li S, Dai L (2011) Moisture effect on carbon and nitrogen mineralization in topsoil of Changbai Mountain, Northeast China. *Journal of Forest Science* 57, 340-348.

Rey A, Petsikos C, Jarvis PG, Grace J (2005) Effect of temperature and moisture on rates of carbon mineralization in a Mediterranean oak forest soil under controlled and field conditions. *European Journal of Soil Sci*ence 56, 589–599.

Richards BK, Stoof CR, Mason C, Crawford RV, Das S, Hansen, JL, Mayton HS, Crawford J L, Steenhuis TS, Walter MT and Viands D R (2013) Carbon Sequestration and Gaseous Emissions in Perennial Grass Bioenergy Cropping Systems in the Northeastern US. AAIC Proceedings, Washington DC.

RStudio Team (2015) RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens I, Kleber, M, Kogel-Knabner I, Lehmann J, Manning DAC, Nannipieri, P, Rasse DP, Weiner S, Trumbore S (2011) Persistence of soil organic matter as an ecosystem property. *Nature*. http://dx.doi.org/10.1038/nature10386.

Six J, Elliott ET, Paustian K (2000) Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry*, 32, 2099–2103.

Sommers LE, Gilmour CM, Wildung, RE, Beck, SM (1981) The effect of water potential on decomposition processes in soils, in Parr JF, et al. (eds.): Water Potential Relationships in Soil Microbiology. *Soil Science Society of America Special Publication* No 9, American Society of Agronomy, Madison, WI, 97–117.

Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant and Soil*, 241, 151–176.

Stockmann, U, Adams, M., Crawford, J., Field, D., Henakaarchchi, N., Jenkins, M., Minasny B, McBratney, A, De Remy De Courcelles V, Singh K, Wheeler I, Abbott L, Angers DA, Baldock J, Michael Birde M, Brookes PC, Chenug C, Jastrow JD, Lal R, Lehmann J, O'Donnell AG, Partonl WJ, Whitehead D, Zimmermannn M (2013) The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems and Environment*, 164, 80-99.

Thompson A, Chadwick OA, Rancourt DG, Chorover J (2006) Iron-oxide crystallinity increases during soil redox oscillations. *Geochimica et Cosmochimica Acta* 70(7), 1710-1727.

Torn M, Trumbore SE, Chadwick OE, Vitousek PM, Hendricks DM (1997) Mineral control of soil organic carbon storage and turnover. *Nature*, 389, 170–173.

Trumbore, SE (1997) Potential responses of soil organic carbon to global environmental change. *Proceedings of the National Academy of Sciences U.S.A.* 94, 8284–8291.

von Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions — a review. *European Journal of Soil Science* 57, 426–445.

von Lützow and Kögel-Knabner (2010) Response to the concept paper: "what is recalcitrant soil organic matter?" by Markus Kleber *Environmental Chemistry* 7, 333 - 335 http://dx.doi.org/10.1071/EN10085

Wang XC, Lee C (1993) Adsorption and desorption of aliphatic amines, amino acids and acetate by clay minerals and marine sediments. *Marine Chemistry*, *44*(1), 1-23.

Wang Q, Zeng Z, Zhong M (2016) Soil moisture alters the response of soil organic carbon Mineralization to Litter Addition *Ecosystems* 19, 50. doi:10.1007/s10021-015-9941-2

Weil RR, Islam KR, Stine MA, Gruver JB, Sampson-Liebig SE (2003) Estimating active carbon for soil quality assessment; a simplified method for laboratory and field use. *American Journal of Alternative Agriculture* 18, 3-17.

Wortmann CS, Garcia JP, Shapiro CA, Shaver TM, Ferguson RB, Maharjan B, Blanco H, Ruis S, Little RS (2017) Soil management for increased soil organic matter G2283 Index: Crops, Soil Management, NebGuide, Nebraska Extension, University of Nebraska-Lincoln.

Whitman T, Zhu Z, Lehmann J (2014) Carbon mineralizability determines interactive effects on mineralization of pyrogenic organic matter and soil organic carbon. *Environmental Science Technology*, 48 (23), 3727–13734.

Zimmerman A, Gao B, Ahn MY (2011) Positive and negative mineralization priming effects among a variety of biochar-amended soils *Soil Biology and Biochemistry*, 43, 1169–1179.