

Factors influencing soil aggregation and particulate organic matter responses to bioenergy crops across a topographic gradient



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ABSTRACT

Bioenergy crops have the potential to enhance soil carbon (C) pools from increased aggregation and the physical protection of organic matter; however, our understanding of the variation in these processes over heterogeneous landscapes is limited. In particular, little is known about the relative importance of soil properties and root characteristics for the physical protection of particulate organic matter (POM). We studied short-term (3-year) changes in aggregation and POM-C pools under three cropping systems (switchgrass, a triticale/sorghum double crop, continuous corn) replicated across five landscape positions along a topographic gradient in Iowa, USA. We isolated POM associated with three aggregate fractions (>2 mm, 0.25–2 mm, and 0.053–0.25 mm) to determine the relative influence of ten soil and three root properties. Aggregation increased in all cropping systems and was greatest under switchgrass; however cropping system effects were not consistent among positions. Total soil organic C stocks did not change, but C within both physically protected (iPOM-C) and unprotected (frPOM) C pools increased. Shifts in iPOM-C were concurrently influenced by soil properties and root traits. Soil texture had the strongest influence (65% relative importance), with finer-textured soils showing greater gains in total iPOM-C, while greater root biomass influenced (35% relative importance) accrual of total iPOM-C. Aggregate fractions varied in their iPOM-C response to soil and root variables, however individual pools similarly showed the importance of soil texture and root biomass and annual root productivity (BNPP). Changes in frPOM-C were strongly correlated with BNPP. Our data suggest that macroaggregate formation drives short-term responses of POM, which are influenced by both soil and root system properties. Crops that maximize root biomass and BNPP will lead to the largest increases in protected soil C stocks. However, C storage rates will vary across landscapes according to soil conditions, with texture as the primary influence.

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1. Introduction

Purported environmental benefits of bioenergy often highlight the increased stabilization and subsequent storage of carbon (C) as soil organic matter (SOM) within agricultural soils (Gelfand et al., 2013; Lemus and Lal, 2005). Maximizing this C storage benefit necessitates understanding how cropping systems that produce bioenergy feedstocks alter belowground C cycling across agroecosystems (Anderson-Teixera et al., 2013; Blanco-Canqui, 2010; Tiemann and Grandy, 2015). Perennial crops, such as switchgrass and short-rotation woody crops, are expected to influence carbon cycling and the overall potential environmental benefits of bioenergy much differently relative to annual crops (Chimento et al., 2014; Robertson et al., 2011), with the realized improvements to ecosystem functioning likely dependent on the location of crops within landscapes (Dale et al., 2011). Sites poorly

sited for annual row crops due to edaphic limitations or susceptibility to erosion and flooding have the potential to produce significant biomass from perennial vegetation (Campbell et al., 2008; Gelfand et al., 2013; Tilman et al., 2006). Still uncertain, however, is the relative capacity for improving soil C storage in annual versus perennial bioenergy crops within both productive and marginal areas that comprise heterogeneous agroecosystems, as well as the duration of time necessary to realize these changes. Understanding the potential of bioenergy cropping systems to stabilize SOM across both productive and marginal locations is necessary for maximizing ecosystem benefits as well as establishing realistic expectations of SOM accrual across diverse agricultural landscapes.

Soil aggregation and particulate organic matter (POM) are key indicators of soil quality and the environmental sustainability of agricultural management practices. Aggregate formation stabilizes organic material within soil microsites, physically protecting POM from microbial decomposition (Golchin et al., 1994; Balesdent et al., 2000) and increasing the mean residence time (MRT) relative to inter-aggregate (unprotected) organic matter (Puget et al., 2000). For example, Liao et al. (2006)

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showed that unprotected POM had shorter average MRTs (30 years) than POM protected within aggregates (60 years) using natural abundance $\delta^{13}\text{C}$ signatures in grasslands invaded by woody shrubs. Because physical protection from microbial activity contributes to the persistence of soil C regardless of the biochemical recalcitrance due to the chemical structure of organic matter (Dungait et al., 2012; Kleber et al., 2011; Torn et al., 2009; Trumbore, 2009), practices that promote the formation of soil aggregates—such as no-till management (Elliott, 1986) and establishment of perennial vegetation (Jastrow, 1996)—ultimately lead to greater soil carbon stocks.

According to the conceptual model of Six et al. (2000), recent inputs of organic matter induce macroaggregate (>250 μm) formation, while the decomposition of SOM within these macroaggregates leads to the formation of stable microaggregates (Gale et al., 2000b) and organo-mineral complexes. Microaggregates (<250 μm) turn over slower relative to macroaggregates from increased stability with smaller size (De Gryze et al., 2005). Consequently, macroaggregate formation leads to longer MRTs of SOM in soil over time through the formation of smaller, more stable soil fractions with increasingly intimate associations between organic matter and mineral surfaces (Martens et al., 2003; Poirier et al., 2005). In aggrading systems, macroaggregate formation may be a good predictor of potential future C stabilization responses due to their importance for protecting recently deposited SOM (Angers and Giroux, 2006; Jastrow et al., 1996) and promoting the formation of stable organo-mineral complexes.

The biophysical drivers of soil aggregation have been a focus within the scientific literature for decades (Six et al., 2004). Early theoretical and experimental work identified the importance of inorganic binding agents, organic residues, soil mineral particles, soil organisms, and the effects of environmental variables (Kemper and Koch, 1966). In particular, the significance of interactions between SOM and silt and clay particles for the formation of highly stable microaggregates was recognized (Edwards and Bremner, 1967). In agricultural soils, soil disturbance from tillage destabilizes aggregates, releasing intra-aggregate organic matter and increasing decomposition (Balesdent et al., 2000; Beare et al., 1994; Blanco-Canqui and Lal, 2007; Cambardella and Elliott, 1992, 1993; Grandy and Robertson, 2006; Six et al., 1999, 2000). Establishment of perennial vegetation following cultivation showed the positive effects of root length, microbial biomass, and mycorrhizae on aggregate formation (Jastrow et al., 1996; Jastrow et al., 1998; Miller and Jastrow, 1990). Based on much of the experimental work done to date, additional biological factors influence aggregation, including the positive influences of microbial biomass and by-products (Zhang et al., 2012), mycorrhizae (Wilson et al., 2009), and soil fauna such as Collembola (Siddiky et al., 2012a,b) and nematodes (Zhang et al., 2013). Recent work has shown strong positive links between root biomass and the abundance of nematodes and several taxa of mesofauna (Eisenhauer et al., 2013), suggesting that changes in root biomass alters the structure of soil food webs, changing belowground C cycling and the mean residence time of different SOC pools (Reid et al., 2012).

With the development of the aggregate hierarchy concept, Tisdall and Oades (1982) suggested that the factors important for aggregate formation differed according to aggregate size. Microaggregates are bound together by persistent binding agents such as humified SOM interactions with clay particles, while macroaggregates are formed from transient (microbial- and plant-derived polysaccharides) and temporary (roots, fungal hyphae) binding agents. Despite the recognition of the importance of multiple factors influencing physical protection, very few studies have considered their interactive effects on aggregation and SOM changes (Six et al., 2004). Particularly important is the need to assess the significance of soil physiochemical and biotic factors simultaneously (Barto et al., 2010) to predict soil C storage in response to changes in management across heterogeneous landscapes (Viaud et al., 2010). It is well understood that SOM levels vary at landscape scales, particularly across topographic gradients (Schimel et al., 1985) in response to soil redistribution (Pennock et al., 1994) and biological

factors affecting C cycling, such as variation in plant C inputs and decomposition (Yoo et al., 2006). Further, topographical influences on soil C can interact with management, resulting in altered responses to management such as tillage (Senthilkumar et al., 2009) and land use (Tan et al., 2004) depending on position in the landscape. Although many of the abiotic and biotic factors important for aggregation and physical protection of POM are known to vary across topographic gradients and in response to land use—and coincide with spatial patterns of soil C stocks—little work has been done to identify the relative influence of these factors and their interactions across heterogeneous landscapes.

In this study, we address the need for understanding both the impacts of topography and bioenergy cropping systems on short-term variation in POM pools, and the relative importance of multiple ecosystems drivers of aggregation in bioenergy cropping systems. We measured changes in aggregation and physically protected SOM among three cropping systems and five landscape positions along a topographic gradient providing variation in numerous soil properties during the initial years following conversion from a conventionally-tilled corn soybean rotation to production of no-till bioenergy cropping systems. The objectives of this study were to (i) assess the interactions between cropping system and landscape position/soil properties on soil aggregation and unprotected (also known as free POM; frPOM) and physically protected (intra-aggregate POM; iPOM) C pools associated with aggregate fractions, and (ii) evaluate the relative importance of multiple cropping system and soil properties on short-term (3 year) changes in unprotected and physically protected SOM.

2. Materials and methods

2.1. Site description and design

This study was conducted between 2009 and 2012 as part of the Landscape Biomass Project (<http://www.nrem.iastate.edu/landscapebiomass/>), located in Boone County, IA, at Iowa State University's Uthe Research and Demonstration Farm. Prior to the establishment of experimental plots, the entire 35 ha site was managed for annual row crop production since the 1970s; all but the floodplain soils have been in continual production since before the 1930s. Soils were managed with conventional tillage practices since at least the 1980s (Lynn Henn, CAD farm manager, personal communication); however, all plots were managed as no-till following establishment in 2008. A complete description of the site including the experimental design, site soil conditions, and cropping systems evaluated can be found in Wilson et al. (2014). Briefly, the three cropping systems included in this study were randomized within three blocks across each of five landscape positions (summit, shoulder, back slope, toe slope, floodplain) situated along a topographic gradient in a randomized complete block design ($n = 3$, 45 plots total). Soils on the site are comprised of two Mollisols consisting of five soil series (see Ontl et al., 2013). All soil series have high cation exchange capacity relative to clay content, consist of mixed mineralogy, and were formed in calcareous glacial till—except the floodplain soils—which were formed in alluvium (Soil Survey Staff, 2013). Three cropping systems were included in the study, 1) switchgrass (*Panicum virgatum* L., cultivar: "Cave-In-Rock"), 2) a double crop system consisting of a winter annual crop (triticale, \times *Triticosecale* Wittm.) seeded in the fall following the harvest of sorghum (*Sorghum bicolor* L. Moench), and 3) continuous corn (*Zea mays* L.). Fertilization rates were determined according to crop needs. Nitrogen was added as urea; annual N addition rates were 134 kg N ha⁻¹ for switchgrass, 168 kg N ha⁻¹ for triticale/sorghum (34 kg N ha⁻¹ prior to triticale, 134 kg N ha⁻¹ prior to sorghum) and 168 kg N ha⁻¹ for continuous corn. In 2011, sorghum received 112 kg urea-N ha⁻¹, while in 2012 sorghum plots were not fertilized due to drought conditions. All plots received 112 kg KCl ha⁻¹ and 56 kg P₂O₅ ha⁻¹ of Triple Super phosphate in 2010 and 2011.

2.2. Soil and cropping system root properties

Soils were sampled to 20 cm depth in all plots on 28 Oct., 2009, and on 7 Nov., 2012. Five cores (32-mm diameter) were randomly collected from each plot and composited into a single sample. Field-moist samples were sieved with an 8-mm sieve by breaking along natural planes of weakness; one half of the sample was further passed through a 2-mm sieve. Roots longer than 10 mm were removed from samples during sieving. Samples were air dried at room temperature to a consistent weight and kept in bags until further processed.

Ten soil properties frequently used as indicators of soil chemical, physical and biological functioning (Doran and Parkin, 1994) were evaluated from soil samples collected in 2009 as predictors of changes to POM from 2009 to 2012. Detailed descriptions of the methods used can be found Ontl et al. (2013). Soil biological properties assessed were total soil organic carbon (SOC) and labile C content estimated as total unprotected POM (McLauchlan and Hobbie, 2004) (see Section 2.4. *Density fractionation of particulate organic matter*). Soil chemical parameters included total soil N content (TruSpec Micro CHNS elemental analyzer, LECO Corporation, St. Joseph, MI), soil P and K (Mehlich-3 extractions analyzed using IRIS Intrepid ICP-OES, Thermo Scientific, Pittsburgh, PA), and pH (1:1 deionized water:soil slurries). Soil physical characteristics included sand and clay contents determined from particle size distribution analysis (Kettler et al. 2001), aggregate geometric mean diameter (GMD) (Kemper and Rosenau, 1986), and bulk density (Blake and Hartge, 1986) determined from the average of three cores per plot.

Measurement of root variables is detailed in Ontl et al. (2013). Annual root production was measured to 20 cm depth using root ingrowth cores ($n = 3/\text{plot}$) during the 2011 growing season. The maximum standing crop root biomass was estimated ($n = 3/\text{plot}$) to a depth of 20 cm at the time of crop flowering. For both ingrowth and root biomass cores, crown nodes, if present, were removed. Only live roots (determined based on root color and consistency) were recovered from root biomass cores. All roots were recovered from root ingrowth cores. All root samples were hand-sorted in deionized water and roots recovered by filtering cleaned samples through 250- μm mesh. Root tissue isolated from ingrowth cores was ground in a ball mill for C analysis to determine C inputs from annual root production (BNPP). Roots from standing crop biomass samples were weighed to estimate the biomass of root standing crop, scanned on a flatbed scanner (400 dpi resolution), and resulting images processed with WinRHIZO 2012b (Regent Instruments Inc., Quebec City, Canada) to determine root lengths. Root length density (RLD) was determined by dividing root lengths by the sample volume.

2.3. Aggregate separations

From the composited soil sample collected within each plot, three 50-g soil subsamples were wet sieved according to methods described in Elliott (1986). Four aggregate size fractions were obtained: (a) large macroaggregates (LM; $>2000 \mu\text{m}$), (b) small macroaggregates (SM; 250–2000 μm), (c) microaggregates (micro; 53–250 μm), and (d) silt and clay sized particles (SC; $<53 \mu\text{m}$). Subsamples were slaked for 5 min by submersion in 2 L of deionized water on top of a 2-mm sieve. During slaking, all roots and other organic debris larger than 2 mm floating on the surface were removed from the sample and discarded. Soils were wet-sieved under water by moving the sieve vertically 50 times in 2 min, carefully breaking the surface of the water with each stroke. Material passing through the sieve was passed onto the next smallest sieve, while material retained on the sieve was backwashed into an aluminum pan. The soil slurry passing through the 53- μm sieve was rinsed into a 4-L glass dish. All size fractions were dried at 60 °C and stored at room temperature in glass vials.

Sand content of aggregate fractions $>53 \mu\text{m}$ was determined for the first set of subsamples from each plot. 10 g of each aggregate fraction

(if sample weight is $<10 \text{ g}$ then the entire fraction was used) was dispersed using 30 mL of 5% (w/v) sodium hexametaphosphate and shaken for 18 h on a reciprocal shaker. Silt and clay particles were washed from dispersed samples by rinsing over a 53- μm sieve to isolate sand and particulate organic matter on top of the sieve, which was backwashed into aluminum tins and dried overnight at 60 °C prior to being weighed. Organic matter of samples was removed from sand by loss-on-ignition prior to determining sand weight (Cambardella et al., 2001). The sand-free weight of each aggregate fraction was determined by subtraction and geometric mean diameter (GMD) of soil samples was estimated (Kemper and Rosenau, 1986).

2.4. Density fractionation of particulate organic matter

Aggregate fractions from the second set of soil subsamples from each plot were used to isolate intra-aggregate (iPOM) as well as free and released (frPOM) POM using densitometric fractionation (Fig. 1). frPOM was isolated from small macroaggregates (coarse frPOM) and microaggregates (fine frPOM); occluded POM was isolated from large macroaggregates (LM-iPOM), small macroaggregates (SM-iPOM), and microaggregates (micro-iPOM). The fractionation methods used were a modification of the sequence described previously by Gale et al. (2000a).

A 10-g subsample from each of the three aggregate size classes was weighed onto a 20- μm nylon filter (55-mm diameter; Tetko, Inc., Briarcliff Manor, NY) overlaid on a glass-fiber filter (55 mm diameter;

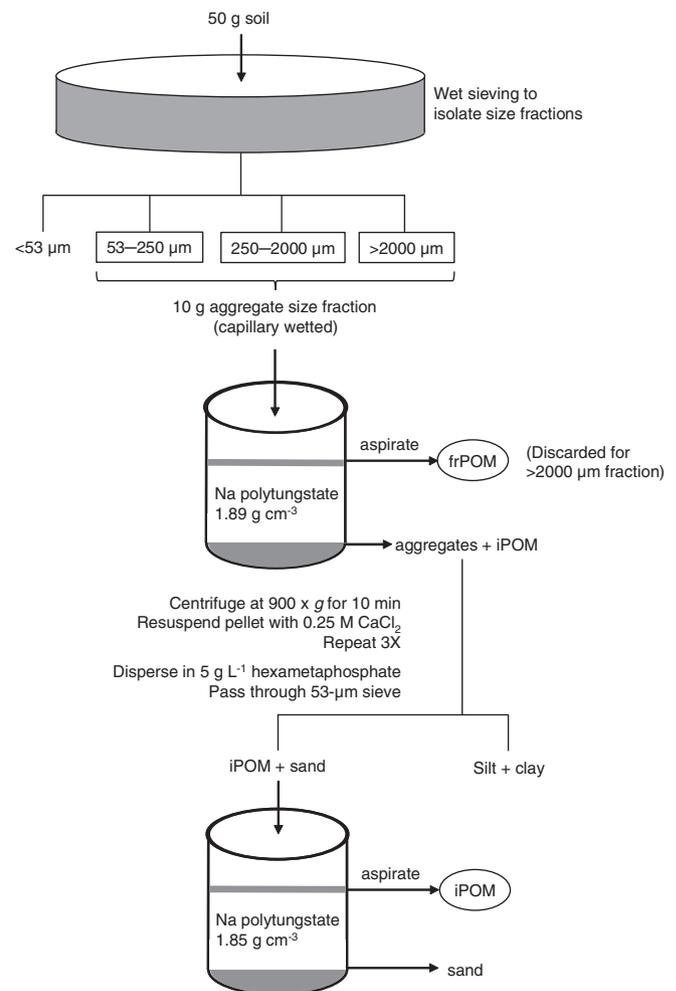


Fig. 1. Density fractionation sequence. Aggregate size fractions (53–250 μm , 250–2000 μm , $>2000 \mu\text{m}$) are shown within boxes. POM pools isolated for each aggregate fraction shown in circles.

Whatman Type GF/A) in an 80-mm plastic Petri dish. If 10 g was not available, the entire fraction was used. Subsamples were wetted on the filters by capillarity to reduce slaking that can occur when dry aggregates are added to liquid during the density fractionation procedure. 5 g of deionized water was added to the >2000- μm & the 250- to 2000- μm fractions, and 4.5 g of water to the 53- to 250- μm fractions. Wetted samples were stored overnight at 4 °C to equilibrate before fractionation began. Samples were rinsed off the nylon filter into a 250-mL beaker using 55 mL of sodium polytungstate (Poly-Gee brand, Geoliquids Inc., Prospect Heights, IL) adjusted to a density of 1.89 g cm⁻³. Previous testing showed that the water within the aggregates would lower the density of polytungstate to a density of 1.85 g cm⁻³ (Gale et al., 2000a). This density was chosen to optimize the balance between isolation of undecomposed organic matter (density ~ <1.6 g cm⁻³) and organo-mineral complexes (density ~ <2.0 g cm⁻³) (Cerli et al., 2012; Elliott and Cambardella, 1991). Samples were gently stirred and allowed to equilibrate overnight. The following day, frPOM floating on the surface was aspirated off onto 20- μm nylon filters, then rinsed into a jar with 100 mL water and allowed to sit at room temperature for 4 h to dissolve any polytungstate remaining in the sample. Water with dissolved polytungstate was removed by rinsing samples onto a 20- μm nylon filter under vacuum followed by washing into aluminum tins. Tins were dried overnight at 60 °C then weighed.

After removal of the frPOM, as much as possible of the remaining polytungstate solution was aspirated without disturbing the aggregates on the bottom of the beaker. Aggregates were rinsed with water into 60-mL polypropylene centrifuge bottles, centrifuged at 900 \times g for 10 min, and the liquid poured off. Aggregates were rinsed two more times by re-suspending the pellet in 0.25 M CaCl₂ and spinning at 900 \times g for 10 min, after which the liquid was poured off. Preliminary tests showed that rinsing the heavy fraction three times adequately removed residual tungstate based on sample dry weights relative to no-tungstate controls. Following the third rinse, the pellets were resuspended in 30 mL of 5% (w/v) sodium hexametaphosphate and shaken for 18 h on a reciprocal shaker to disperse aggregates and release intra-aggregate POM. The dispersed aggregates were passed through a 53- μm sieve and rinsed with water. Material passing through the sieve was rinsed into aluminum pans and dried at 60 °C. Material remaining on top of the sieve was backwashed onto 20- μm nylon filters under vacuum to remove excess water, then rinsed into 250-mL beakers with 60 mL of sodium polytungstate adjusted to a density of 1.85 g cm⁻³. Samples sat undisturbed overnight before aspirating iPOM floating on the surface. Procedures for recovery of iPOM followed those for frPOM. The heavy material remaining at the bottom of the beaker was backwashed over a 20- μm nylon filter under vacuum to remove the remaining polytungstate. All samples were ground to a fine powder and stored in glass vials at room temperature until further analysis.

2.5. Carbon and nitrogen determination

Total C and N were measured on all root samples and soil and POM fractions using a TruSpec Micro CHNS elemental analyzer (LECO Corporation, St. Joseph, MI). Whole soil C was determined from 50-g subsamples of the air-dried 2-mm sieved soil that was ground to pass through a 500 μm sieve. When necessary, total C was corrected for inorganic C using the pressure calcimeter method (Sherrod et al., 2002). 10 g of each aggregate fraction from the third set of subsamples was ground for C and N analysis of individual aggregate fractions. If 10 g was not available, the entire fraction was ground and analyzed. POM fractions isolated from densitometric fractionation within aggregate size classes were analyzed for C content. POM fractions were combined from plots within each landscape position in 2009 due to concerns over limited samples. However, POM isolated from the 2012 samples was analyzed from individual plots.

2.6. Statistical analyses

Cropping system and landscape position effects on changes in whole soil C, soil aggregation, and physically-protected and unprotected POM-C pools were determined using generalized linear mixed models in SAS 9.4 (Proc GLIMMIX, SAS Institute Inc., Cary, NC). Significance of main effects and all interactions was determined based on type III sums of squares. Multiple comparisons of means for cropping system and landscape position were analyzed with Tukey HSD post-hoc tests. Subsamples within plots were as a random effect for analyses of GMD changes. POM-C pools consisted of POM physically protected within large macroaggregates (LM-iPOM-C), small macroaggregates (SM-iPOM-C), and microaggregates (m-iPOM-C); unprotected POM-C pools were the light fraction isolated from the small macroaggregate (coarse frPOM-C) and the microaggregate fractions (fine frPOM-C).

Multiple linear regression analyses and principal component analysis (PCA) using R statistical software (R Core Team, 2013, version 3.0.2) were used to model overall changes to protected and unprotected POM-C fractions from a suite of soil and cropping system root variables. Principal component analysis was performed using the “FactoMineR” package (Lê et al., 2008). Multiple regression used the best subsets selection criteria based on Akaike’s Information Criterion adjusted for small sample sizes (AIC_c). The influences of soil and root properties on protected POM-C was determined for each POM pool (LM-iPOM-C, SM-iPOM-C, m-iPOM-C) and on total unprotected POM by summing frPOM-C from all aggregate fractions. Model parameters were considered significant at $P < 0.05$. The relative importance of factors in multiple regression models was estimated using the “relaimpo” package (Groemping, 2013) in R.

3. Results

3.1. Whole-soil C

Whole soil organic C content did not change between 2009 and 2012 ($P = 0.7916$) indicating that cropping system did not affect total soil organic C stocks over the three years of this study. Following correction for inorganic C, soil C levels in 2009 and 2012 averaged 19.6 and 19.4 g C kg⁻¹ soil, respectively. No effects of either cropping system, landscape position, or their interaction ($P = 0.345$, $P = 0.355$, $P = 0.187$, respectively) were significant for changes in whole soil C over the three years of this study.

3.2. Soil aggregation

Analysis of water-stable aggregates showed that changes in GMD were positive under all cropping systems (switchgrass, $P < 0.0001$; sorghum/triticale, $P = 0.0281$; continuous corn, $P < 0.0001$). Water-stable aggregation increased 34.9% under switchgrass, which was greater than the increase measured under both the triticale/sorghum double crop (19.4% increase, $P < 0.0001$) and continuous corn (18.6% increase, $P = 0.0118$). Although landscape position was not significant for change in GMD when averaged across individual cropping systems, the cropping system by landscape position interaction was significant ($P < 0.0001$), indicating that changes within cropping system were affected differently by landscape positions (Fig. S1).

3.3. POM-C pools

POM-C pools comprised a small proportion of the total soil C pool, yet changed over the short duration of this study. Total POM-C in 2009 averaged 0.44 g C kg⁻¹ soil, comprising 2.8% of total organic C. Unprotected POM-C (frPOM-C) was 0.23 g C kg⁻¹ soil, while the protected POM-C pool (iPOM-C) averaged 0.21 g C kg⁻¹ soil. In 2012, total POM-C increased to 0.75 g C kg⁻¹ soil, or 4.5% of total organic C. The amount of frPOM-C increased by 46% to 0.34 g C kg⁻¹ soil, while iPOM-C increased

by 92% to 0.41 g C kg⁻¹ soil. Analysis of C and N contents of POM fractions indicated that the % C and C:N ratios of fine frPOM were the lowest of all fractions (Table S1). In contrast, C contents and C:N ratios of physically protected POM varied among the different aggregate fractions, with similar % C between iPOM with the large and small macroaggregate fractions while microaggregate iPOM showed lower C contents. However, C:N ratios of SM-iPOM appeared to differ from other physically protected pools due to higher N contents while C contents remained similar.

While protected and unprotected POM-C pools changed during the three years of the study, the effects of cropping system, landscape position, and their interaction were different for each pool. Changes in iPOM-C pools in response to cropping system, landscape position or their interaction were seen for all three aggregate fractions (Table 1). Cropping system significantly affected changes in large macroaggregate iPOM-C (LM-iPOM) ($P < 0.001$). However, changes were greater than zero only for the switchgrass cropping system (Fig. 2A), which increased 0.104 g C kg⁻¹ soil, significantly greater ($P < 0.01$) than the changes under continuous corn (0.012 g C kg⁻¹ soil) or triticale/sorghum ($P < 0.01$, -0.015 g C kg⁻¹ soil). Small macroaggregate iPOM-C (SM-iPOM) had the largest average increase of any POM pool, gaining an average of 0.16 g C kg⁻¹ soil across all cropping systems. In contrast to the large macroaggregates, changes in SM-POM-C were affected by landscape position ($P < 0.01$). Increases were higher in floodplain plots relative to the summit and toe slope; additionally, toe slope was lower compared to the shoulder position (Fig. 2B). Gains in SM-iPOM-C were not impacted by cropping system, although combining POM samples from the beginning of the study (2009 samples) prior to C content analyses may have reduced the sensitivity for detecting cropping

system effects over time. Overall changes to POM protected within microaggregates (m-iPOM-C) did not occur over the duration of the study (-0.003 g C kg⁻¹, $P = 0.479$) and were not impacted by cropping system (Fig. 2C). Although smaller relative to other protected pools, shifts within m-iPOM-C were impacted by landscape position and the landscape position by cropping system interaction ($P = 0.013$ and $P = 0.049$, respectively) with (Table 1).

Changes in coarse frPOM-C isolated with the small macroaggregate fraction (250–2000 μm) were only affected by cropping system ($P < 0.0001$). Switchgrass plots had greater change ($P < 0.0001$) in coarse frPOM-C compared to continuous corn (0.041 g C kg⁻¹ soil) and triticale/sorghum (0.067 g C kg⁻¹ soil) (Table 1, Fig. 3A), with an accrual of 0.174 g C kg⁻¹ soil averaged across all landscape positions. Cropping systems' effects on coarse frPOM-C varied across landscape positions. No differences between cropping systems were apparent on the back slope or toe slope, while switchgrass resulted in significant change compared to continuous corn on the summit, shoulder, and floodplain positions, and larger changes than triticale/sorghum on the shoulder. In contrast, differences in the amount of fine frPOM-C isolated with the microaggregate fraction (53–250 μm) between 2009 and 2012 were not significant ($P < 0.108$), and the effects of cropping system, landscape position, or their interaction were not significant (Table 1, Fig. 3B).

3.4. Influence of soil and root system properties to POM changes

Soil properties varied among plots, particularly in response to landscape position (Ontl et al., 2013), providing a wide variance in soil physical, chemical, and biological conditions. Further, cropping systems

Table 1
POM-C (g C kg⁻¹ soil) fraction change (SE) 2009–2012.

Fraction	Landscape position	Cropping system			Effects		
		Continuous corn	Switchgrass	Triticale/sorghum	CS	LP	CS × LP
LM iPOM-C	Summit	-0.008 (0.013) a	0.084 (0.067) a	-0.008 (0.027) a	###	ns	ns
	Shoulder	0.011 (0.020) a	0.027 (0.021) a	-0.002 (0.007) a			
	Back slope	0.028 (0.028) b	0.105 (0.033) a*	0.023 (0.027) b			
	Toe slope	0.031 (0.021) a	0.083 (0.067) a	-0.069 (0.062) a			
	Floodplain	-0.003 (0.018) a	0.219 (0.246) a	-0.002 (0.069) a			
	Average	0.012 (0.008) B	0.104 (0.032) A*	-0.015 (0.015) B			
SM iPOM-C	Summit	0.089 (0.030) a*	0.151 (0.032) a*	0.172 (0.034) a*	ns	##	ns
	Shoulder	0.182 (0.024) a*	0.179 (0.012) a*	0.182 (0.010) a*			
	Back slope	0.178 (0.020) a*	0.173 (0.050) a*	0.131 (0.010) a*			
	Toe slope	0.111 (0.044) a*	0.109 (0.017) a*	0.124 (0.023) a*			
	Floodplain	0.217 (0.036) a*	0.294 (0.064) a*	0.180 (0.039) a*			
	Average	0.155 (0.024) A*	0.181 (0.031) A*	0.158 (0.013) A*			
m iPOM-C	Summit	-0.045 (0.026) b*	0.010 (0.011) a	-0.007 (0.008) ab	ns	‡	‡
	Shoulder	-0.018 (0.015) a	-0.004 (0.007) a	-0.003 (0.010) a			
	Back slope	0.026 (0.004) a	0.026 (0.004) a*	0.020 (0.013) a			
	Toe slope	0.005 (0.006) a	0.021 (0.008) a	0.001 (0.014) a			
	Floodplain	0.003 (0.011) a	-0.046 (0.023) b*	-0.035 (0.011) b			
	Average	-0.006 (0.012) A	0.002 (0.013) A	-0.005 (0.009) A			
c frPOM-C	Summit	0.062 (0.040) b	0.230 (0.021) a*	0.135 (0.011) ab*	###	ns	ns
	Shoulder	0.082 (0.030) b*	0.166 (0.010) a*	0.037 (0.005) b			
	Back slope	0.042 (0.017) a*	0.091 (0.012) a*	0.039 (0.014) a*			
	Toe slope	0.033 (0.026) b	0.155 (0.031) a*	0.042 (0.033) ab			
	Floodplain	-0.013 (0.082) b	0.230 (0.053) a*	0.080 (0.023) ab			
	Average	0.041 (0.016) B*	0.174 (0.026) A*	0.067 (0.019) B*			
f frPOM-C	Summit	0.008 (0.008) a	0.014 (0.012) a	0.015 (0.004) a	ns	ns	ns
	Shoulder	-0.006 (0.021) a	0.001 (0.013) a	0.006 (0.024) a			
	Back slope	0.007 (0.004) a	0.013 (0.012) a	0.031 (0.010) a			
	Toe slope	0.037 (0.007) a*	0.032 (0.008) a*	0.006 (0.006) a			
	Floodplain	0.010 (0.007) a	-0.001 (0.044) a	0.011 (0.008) a			
	Average	0.011 (0.007) A	0.012 (0.006) A	0.014 (0.005) A			

LM = large macroaggregates, SM = small macroaggregates, m = microaggregates, c = coarse, f = fine. Letters indicate significant difference among cropping systems within landscape position. Capital letters indicate significant differences of means among cropping systems averaged across landscape positions, ns = no significant difference. CS = cropping system, LP = landscape position, CS × LP = interactive effect of CS and LP.

* Indicates that the value is significantly ($P < 0.05$) different than zero.

$P < 0.001$.

$P < 0.01$.

‡ $P < 0.05$.

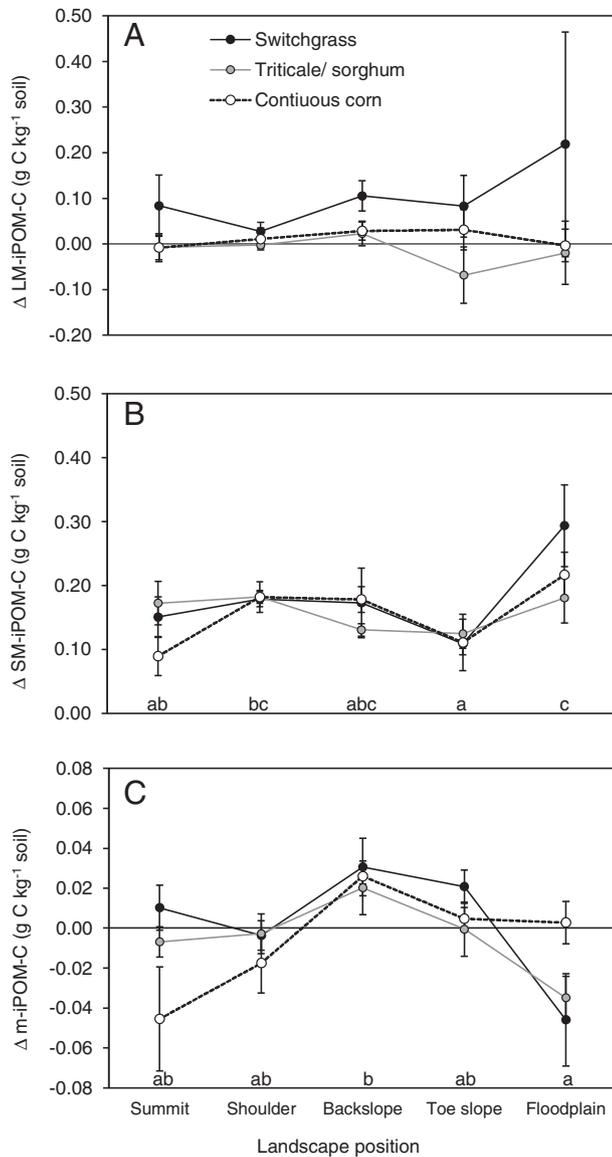


Fig. 2. Protected POM-C fraction changes of cropping systems across landscape positions for (A) large macroaggregate (LM) iPOM-C, (B) small macroaggregate (SM) iPOM-C, and (C) microaggregate (m) iPOM-C. Landscape positions with different letters indicate significant difference ($P < 0.05$) averaged across cropping systems.

differed in root characteristics, with switchgrass showing greater BNPP and root biomass, while triticale/sorghum had largest RLD of the three systems (Fig. 4). Data on root characteristics was only available for one growing season; due to the potential for inter-annual variability in root measurements, these data may best reflect relative differences among cropping systems. Principal component analysis (Fig. S2) showed that many soil properties were strongly correlated with one another. For example, clay, SOC, total N contents were correlated, and were inversely related to sand content and bulk density. Root variables (e.g., root biomass, BNPP, RLD) were additionally correlated. Ordinations suggested that changes in macroaggregate iPOM-C were influenced by both soil and root properties, while shifts in microaggregate iPOM-C were related to root traits and low P and K levels. Course frPOM-C shifts were related to root traits, while fine frPOM-C seemed to be most influenced by soil properties.

Multiple linear regression analyses were used to quantify the relative influence of individual soil and root system properties on short-term changes to total protected and unprotected C pools (Table 2). Main effects significant for changes in physically protected POM pools

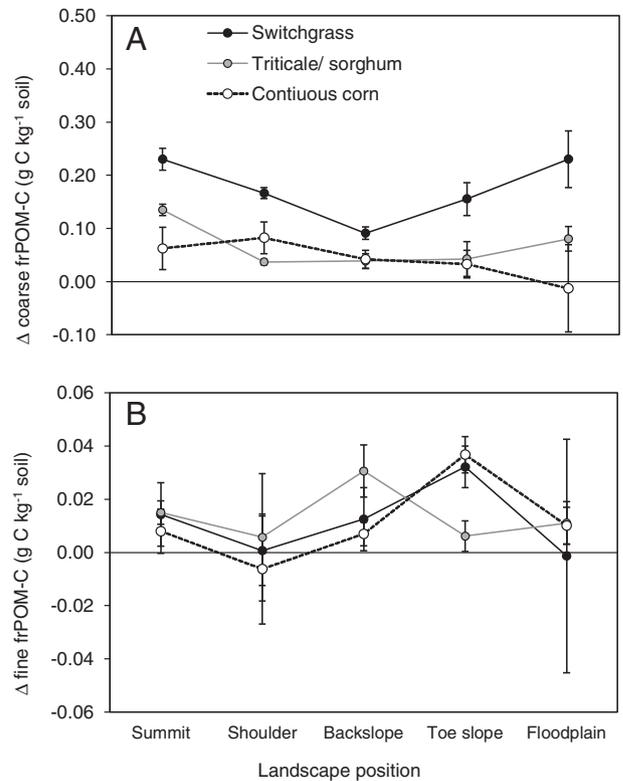


Fig. 3. Differences in cropping systems across landscape positions for unprotected POM-C fraction changes for (A) coarse and (B) fine fr-POM-C.

differed for each aggregate size class, indicating that factors influencing physical protection may differ among fractions. In order to assess individual soil and root property influences on specific POM fractions, multiple regression analyses were performed for each pool individually (Table 2). Shifts in LM-iPOM-C showed that three factors were significant for explaining 31.5% of the variance (adjusted R^2) in iPOM-C change ($F = 6.32$, $P = 0.001$). Of these three factors included in the final model, clay content had the highest relative importance (36.7%), followed by root biomass (31.8%) and labile C content (31.6%) (Fig. 5). Multiple regression modeling showed that changes to SM-iPOM-C were influenced (adjusted $R^2 = 0.325$, $F = 10.1$, $P = 0.0003$) largely by soil sand content (74.2% relative importance), with a smaller contribution from differences in C inputs from BNPP (25.8%). Multiple regression analyses suggested that changes in microaggregate iPOM-C were largely influenced by soil factors (soil phosphorus: 55.8%, $P = 0.002$; bulk density: 18.1%, $P = 0.015$; sand content: 10.4%, $P = 0.032$), with C inputs from root production having a 15.7% relative influence ($P = 0.028$). Overall, the model explained 30.8% of the variance in observed shifts on m-iPOM-C ($F = 4.43$, $P = 0.005$).

Summing the individual iPOM-C pools showed that overall shifts in physically protected C across all aggregate fractions were influenced by two main factors: soil clay content (64.5%, $P < 0.001$) and root biomass (35.5%, $P = 0.005$), which accounted for 33.3% of the variance of the data ($F = 10.47$, $P < 0.001$) (Fig. 5). As changes to fine frPOM-C pools were relatively small and unaffected by crop, landscape position or their interaction, changes to total frPOM-C (course + fine) were modeled. Model selection showed that changes in unprotected POM C contents were influenced solely by C inputs from BNPP (Fig. 6), with 44.2% of the variance explained by the model ($F = 34.02$, $P < 0.0001$).

4. Discussion

Meeting the demands of bioenergy production in the 21st century is expected to occur through the strategic integration of a portfolio of

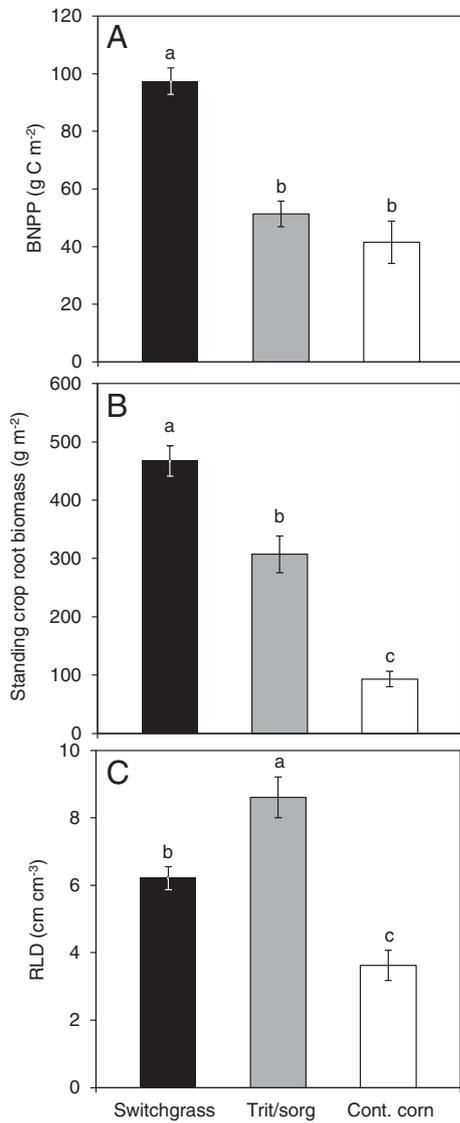


Fig. 4. Cropping system effects on A) belowground root productivity (BNPP), B) maximum standing crop root biomass, and C) root length density (RLD). Letters indicate significant difference ($P < 0.05$). Trit/sorg = triticale/sorghum; Cont. corn = continuous corn.

cropping systems across heterogeneous landscapes in order to balance the optimization of biomass production, economic profitability, and environmental benefits. The choice of cropping systems impacts biomass production rates (Heaton et al., 2013), and tradeoffs are likely between

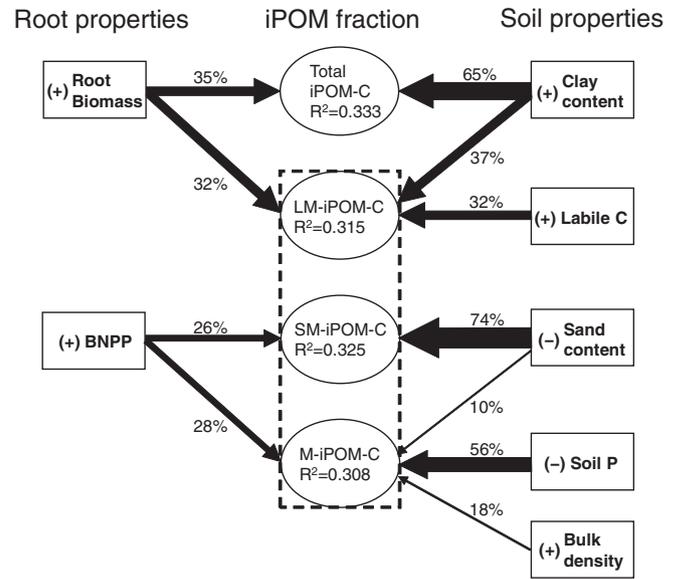


Fig. 5. Results of multiple regression analyses of soil and root property effects on changes in total iPOM-C and individual iPOM-C fractions (circles within dashed box). Variance explained by total model for each fraction is shown within circles. Thickness of arrow indicates the relative importance of factor (next to arrow). Direction of influence is indicated by the +/- next to soil or root property description. LM = large macroaggregates; SM = small macroaggregates; m = microaggregates.

the financial incentives (Manatt et al., 2013) and the impacts to ecosystem functioning derived from carbon cycling processes (Anderson-Teixeira et al., 2013). Our data show that short-term benefits to belowground C cycling—specifically the physical protection of SOM—are apparent within three years following establishment of no-till bioenergy cropping systems. Further, the impacts on physical protection of SOM are dependent on both the cropping system employed—due to differences in root system characteristics—and the location of where those crops are planted, due to site variation in soil properties.

4.1. Soil aggregation

While we found that total soil organic C stocks did not vary over the short duration of the study, changes in belowground C cycling were apparent through impacts on aggregate formation. Aggregation increased over the three years of this study, with switchgrass showing the largest increase. Conversion of croplands to perennial grasslands has shown that aggregation can recover quickly (Jastrow, 1987), as macroaggregate stabilization increases quickly during the first decade following establishment of perennial vegetation (Jastrow, 1996). Studies in restored grasslands have contributed much to our knowledge of the biotic

Table 2
Results of multiple regression models for POM fractions.

Fraction	Factor	Estimate	SE	t value	P-value	Overall model		
						F	P-value	R ²
Total frPOM-C	BNPP	1.813	0.311	5.83	<0.0001	34.02	<0.0001	0.442
	Total iPOM-C	Clay content	1.427	0.368	3.88			
LM-iPOM-C	Root biomass	0.312	0.104	2.99	0.0046	6.32	0.001	0.315
	Clay content	0.879	0.091	2.75	0.0089			
	Labile C	0.129	0.320	2.47	0.0176			
SM-iPOM-C	Root biomass	0.248	0.091	2.73	0.0092	10.12	0.0003	0.325
	BNPP	0.760	0.275	2.76	0.0085			
	Sand content	-0.289	0.069	-4.17	0.0001			
m-iPOM-C	Bulk density	0.113	0.044	2.55	0.0147	4.44	0.0046	0.308
	BNPP	0.311	0.137	2.28	0.0284			
	Soil P	-0.036	0.011	-3.34	0.0018			
	Sand content	-0.117	0.053	-2.23	0.0317			

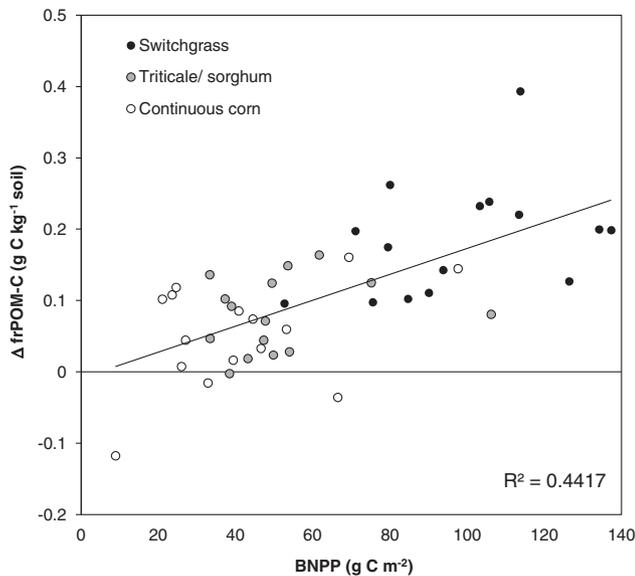


Fig. 6. Regression of annual root C input (BNPP) and changes in total frPOM-C, with cropping systems indicated.

mechanisms impacting aggregation, including the importance of the dense root systems of perennial vegetation and their associated mycorrhizal fungi (Jastrow et al., 1996; Jastrow et al., 1998; Miller and Jastrow, 1990). Similarly, our data show that changes in GMD were highest where both BNPP and standing crop root biomass was highest (Fig. 4). Although cropping system effects on aggregation changes were dependent on position in the landscape, changes were inconsistent between landscape positions. Topography has been shown to impact both soil aggregation in annual cropping systems (Cambardella et al., 2004) and following conversion from annual crops to grassland vegetation (Guzman and Al-Kaisi, 2011). Pierson and Mulla (1990) showed that topographic effects on soil aggregation were due to variation in soil clay content, likely due to the importance of fine mineral surface associations with SOM (Tisdall and Oades, 1982). Comparisons of clay content between landscape positions at our site show that clay contents were higher only on the floodplain soils (data not shown, $P < 0.0001$), suggesting that in addition to clay, other soil or biotic factors were influencing aggregation changes.

4.2. Influences on POM-C pools

Many prior studies have investigated impacts of land cover (John et al., 2005; Leifeld and Kögel-Knabner, 2005; Marquez et al., 1999; Yamashita et al., 2006; Chimento et al., 2014), and topography (Garten and Ashwood, 2002; Cambardella et al., 2004; Hook and Burke, 2000) on POM-C pools. Of these, few have quantified the impacts on functionally different (protected vs. unprotected) POM fractions. Additionally, there is limited information on short-term patterns of POM pools; however studies of temporal patterns from a grassland restoration chronosequence provide evidence of the importance of macroaggregate formation (Jastrow, 1996), particularly the formation of stable microaggregates within macroaggregates for increased C stabilization over time (O'Brien and Jastrow, 2013). This study is unique in that we evaluate, through repeated sampling, the short-term changes in both unprotected and physically protected POM-C pools simultaneously affected by cropping system and topography.

Shifts in physically protected (iPOM) C were concomitantly influenced by soil properties and root traits. The strongest influence on changes to physically protected SOM was through soil texture which had a 65% relative influence on the total iPOM-C change, with a 32% importance to LM-iPOM-C (clay content), a 74% of the relative importance for SM-iPOM-C (silt + clay content), and 10% importance to

microaggregate iPOM-C (silt + clay content). Soil texture influences are probably the best understood factors for physical protection of SOM due to the direct impacts of fine mineral surfaces on SOM interactions and aggregate formation (Pierson and Mulla, 1990; Tisdall and Oades, 1982) and microaggregate stability (Lehmann et al., 2007). Although soil texture had a dominant impact on physical protection, the direct influence of roots as the primary C source to soil (Rasse et al., 2005), and particularly to POM pools (Gale et al., 2000a), is reflected in the significance influence (35% relative importance) of root biomass to changes in total iPOM-C. Likewise, root biomass influenced gains in LM-iPOM-C (32% relative importance), while BNPP was correlated to changes in both SM-iPOM-C (26% relative importance) and microaggregate iPOM-C (28% importance). Root system characteristics differ between annual and perennial species (Eissenstat and Yanai, 1997), influencing both microbial activities (Hargreaves and Hofmockel, 2014) and soil food webs (Eisenhauer et al., 2013). The greater belowground root biomass of the perennial switchgrass likely increased microbial activity, stabilizing aggregates (Jastrow et al., 1998; Wilson et al., 2009; Zhang et al., 2013) through increases in microbially-derived soil binding agents (Oades, 1984) leading to increases in physically protected POM (O'Brien and Jastrow, 2013). However, Tiemann and Grandy (2015) provide evidence that suggests soil texture may modify plant effects on microbial activities through effects of aggregates on microbial enzyme activities. By comparing soil enzyme activities in annual vs. perennial bioenergy systems across sites with differing texture, they showed that microbial activity increased in sandy soils under corn, while in finer-textured soils perennial grasses increased activity (Tiemann and Grandy, 2015).

The gains in POM physically protected within both large and small macroaggregates shown here concur with the results of others that suggest macroaggregates may be good predictors of potential C responses to changes in agroecosystem management (Angers and Giroux, 2006; Grandy and Robertson, 2006; Jastrow et al., 1996). Our results contrast with those of Leifeld and Kögel-Knabner (2005), who saw no clear response in either POM pool 7 years after conversion from both cropland to grassland and grassland to cropland. However, in light of the results presented here, soil textures—particularly the high sand content of the soils in the Leifeld and Kögel-Knabner (2005) study—may have resulted in lower stability of aggregates. For example, Bach et al. (2010) show that increases in aggregation in grassland restoration chronosequences greatly differed between sandy loam and silty clay loam soils. The disparity between our results and those shown in Leifeld and Kögel-Knabner (2005) emphasize the need to consider the importance of multiple factors for influences on physical protection of SOM.

Individual iPOM-C pools were influenced by additional soil properties that did not strongly correlate with texture. For example, LM-iPOM-C increases were related to the amount of labile C in the form of unprotected POM initially in the soil (Table 2; Fig. S2). This could be due to 1) unprotected POM serving as a source of iPOM as aggregates form, or 2) from enhanced microbial enzyme activities under perennial vegetation increasing labile C turnover and contributing microbial- and plant-derived soil binding agents that increase aggregate stability (Golchin et al., 1994). Soil P levels had the largest estimate of relative importance for changes in m-iPOM-C (Fig. 6) which were lowest on the back slope (Ontl et al., 2013) where m-iPOM-C increases were greatest (Fig. 2C). Low P levels can increase abundance of mycorrhizal hyphae within soils to increase the uptake of phosphorus into the host plant under P-limiting conditions (Abbott et al. 1984; Koide and Li, 1990). Tisdall and Oades (1982) predicted enmeshing soil particles by hyphae to be important for macroaggregate stabilization, however it is unclear if these results are due to indirect effects through mycorrhizae affecting microaggregates, or direct impacts of soil phosphorus on microaggregate stabilization. Bulk density had a small but significant positive effect on microaggregate iPOM-C (Table 2; Fig. 6), which may result from reduced pore size in higher density soils that minimize the planes of weakness and increase aggregate stability (Kay, 1990). Overall

changes in m-iPOM-C were small compared to macroaggregate fractions (Fig. 2), likely a result of the greater stability (De Gryze et al., 2005) and longer residence time of C within this aggregate fraction (Rabbi et al., 2013). Additionally, a relatively small response of POM within microaggregate could also be a consequence of the assimilation of free microaggregate structures into newly formed macroaggregates (Six et al., 2002). Soil P and bulk density were not found to be significant for changes to the total iPOM-C pool due to the small change in microaggregate iPOM-C relative to the other pools. However, detection of soil and root effects on microaggregate iPOM-C may have been hindered by reduced sensitivity for estimating changes resulting from combination of iPOM in 2009 samples due to concerns over low sample masses recovered, although changes were more impacted by iPOM mass rather than the C content of iPOM fraction.

Increases in unprotected POM-C pools were observed over the duration of this study in all cropping systems (Fig. 3), indicating the sensitivity of these pools to changes in land use (Chimento et al., 2014). The strong relationship between root productivity and frPOM-C (Fig. 6) emphasizes the importance of freshly derived root biomass as a source for unprotected SOC (Golchin et al., 1994; Gale et al., 2000a). Of the unprotected pools, gains in coarse frPOM-C were the largest (Fig. 3), since this pool represents the least decomposed—and therefore the most recently deposited—SOM pool (Marriott and Wander, 2006). Changes in fine frPOM-C were small by comparison and not found to be significant. The more decomposed state of this C pool (Six et al., 1998), implied by the more fragmented state (Guggenberger et al., 1994) and lower C:N ratios (Table S1) suggests that shifts may be due to reduced turnover from the cessation of tillage-induced disturbance (Kisselle et al., 2001) and not from recent root-derived SOM inputs.

Many of the variables used in this study are often used in process models of C cycling (e.g., CENTURY) and so form a set variables useful for making predicting C cycling responses over heterogeneous landscapes. These variables are often easily obtained from spatial databases of land cover and soil geodatabases. However, other factors not included in this study, but shown to affect aggregation or soil C stocks, may influence physical protection of C. For example, differences among aggregate fractions in iPOM-C changes may be partially a consequence of the effects of the cessation of tillage disturbance on soil biota. Zhang et al. (2013) showed that microbial and nematode communities contribute to C accumulation differently among aggregate size classes in response to tillage practices. In particular, Gram-positive bacteria and plant-parasitic nematodes were linked to C accrual in aggregates < 1 mm in diameter in no-till systems, whereas mycorrhizal fungi were important for C accumulation in aggregates > 1 mm. Gains in iPOM-C, for example, may have resulted from the incorporation of free microaggregate structures and unprotected POM into small macroaggregates through enhanced activity and abundance of bacteria and nematodes, or through impacts on fungal/bacterial ratios (de Vries et al., 2006). Additionally, the 20% lower N fertilization rate in switchgrass from the reduced plant N requirement compared to the annual cropping systems could have impacted soil C stock changes (Jung and Lal, 2011).

The significant influence of root properties for change in physically protected C likely reflects the strong effects of root systems on belowground C cycling processes when comparing annual to perennial bioenergy systems (Anderson-Teixera et al., 2013). However, coincident with those influences, variation in soil characteristics seems to play a dominant role in the regulating short-term changes to POM-C pools. Few studies have addressed the relative influences of these multiple factors affecting aggregation and C protection over landscape to ecosystem scales important for knowledge of drivers of C cycling processes over a variety of scales. This study is unique in investigating the influence of multiple factors on temporal changes in free and occluded POM at landscape scales (10s–1000s m²). Working in arid ecosystems, Chaudhary et al. (2009) showed that at landscape scales the strongest contributions to aggregate stability were from biotic factors, including plant cover and abundance of biological soil crusts and mycorrhizae.

Soil variables such as SOM, N, and P levels were not significant in the model, although soil texture and inorganic C were not included in the analyses. However, in a study comparing the relative importance of soil properties and roots across a regional land-use intensity gradient in Germany, Barto et al. (2010) showed that soil texture and carbonate levels, but not root lengths or fungal hyphae, were the only significant factors for aggregation in soils. Our results suggest that at scales that encompass soil variation within temperate agroecosystems, both soil and root system controls on changes to POM pools are important.

4.3. Conclusions

Our data suggest that increases in aggregation, and unprotected and physically protected POM pools were affected by both cropping system and variation in soil properties across our study site. These results, however, do not imply direct causation, but rather suggest that these factors are a significant link within a causal pathway influencing POM-C that may include soil water dynamics, microbial communities, and soil fauna. Overall, these results suggest that the conversion to perennial switchgrass for bioenergy production, while not having measurable short-term impacts to total soil C stocks, can have important effects on the physical protection of POM, indicating a shift towards increased MRT of soil C and ultimately long-term C storage. Further, our analyses of the influences of soil and root system properties suggest that at landscape scales that include heterogeneity of edaphic conditions, both root and soil factors effect changes in shifts in free and physically protected POM. These results have implications for the expectations of the spatial variation in future C storage resulting in shifts of land use for bioenergy crop production in agroecosystems that include variation in soil conditions between productive and marginal sites. Ultimately, information on effects of multiple drivers of C accrual process is critical for knowledge of pattern–process relationships at intermediate scales (10s–1000s m²) necessary for improving mechanistic models and scaling information to broad spatial extents such as landscapes, regions, and continents (Peters et al., 2007).

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.geoderma.2015.04.016>.

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