Soil particulate organic matter increases under perennial bioenergy crop agriculture

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ABSTRACT

Annual row crop agriculture contributes to carbon (C) losses from Midwest soils, while the establishment of perennial crops for food and fuel has the potential to increase soil C stocks. Perennial grasses eliminate the need for tillage and increase belowground biomass, both critical to the accumulation and conservation of soil organic matter and to soil C sequestration. The effect of C_4 perennial grasses on particulate organic matter carbon (POM-C), consisting primarily of partially decomposed plant material, was evaluated in Illinois, where native switchgrass (*Panicum virgatum* L.) and a sterile hybrid of the Asian grass *Miscanthus* (*Miscanthus* x *giganteus*) were planted as bioenergy feedstocks at the University of Illinois Energy Farm in 2008. Six years after establishment of perennial crops, POM-C was compared with a maize-maize-soybean (*Zea mays* L., *Glycine max* L.) rotation typical of the area and a 28-species restored prairie. POM-C concentrations increased for all crops between 31 and 71% over 6 years, with the greatest increases in prairie and *M.* x *giganteus* soils. POM-C concentrations were highest at the 0−10 cm depth. Isotopic analyses showed 23−44% of POM-C was new C_4 material under perennial bioenergy crops after 6 years. As soil organic matter is primarily plant-derived, increases in POM-C reflect increased organic matter inputs or decreases in the rate of decomposition from the cessation of tillage. Increases in POM-C under annual row crops may result from the incorporation of aboveground organic matter by tillage, while POM-C increases in untilled perennial crops mirror increases in belowground biomass. As soil aggregation protects POM-C from microbial degradation, untilled soils under long-term perennial crop production increase the residence time for soil C.

1. Introduction

Approximately 70 million hectares of maize (*Zea mays* L.) and soybean (*Glycine max* L.) are grown in the Midwestern United States for human consumption, animal feed, and bioenergy production (USDA, 2012). In recent years, interest has arisen in replacing maize-based grain ethanol with cellulosic ethanol production, particularly the use of perennial grasses for cellulose feedstocks. Perennial crops, for food and fuel, have the potential to improve soil quality through belowground carbon (C) inputs and the cessation of tillage (Jastrow, 1996; Paustian et al., 1997; Allison and Jastrow, 2006; Dou et al., 2013; Chimento et al., 2014; Ontl et al., 2015), to

provide yield security through drought resistance and resiliency (Glover et al., 2010, 2012), and to extend these benefits to marginal lands where conventional agriculture is inadvisable or unprofitable (Davis et al., 2012; Dou et al., 2013; Guzman et al., 2014).

Two C_4 perennial grasses under consideration for bioenergy production in the Midwest, *Miscanthus* x *giganteus* Greef & Deuter ex Hodkinson & Renvoize (*M.* x *giganteus*) and *Panicum virgatum* L. (switchgrass, var. 'Cave in Rock'), can produce higher biomass yields at maturity (3 + years after establishment) compared to maize grain for ethanol production (Heaton et al., 2008; Dohleman and Long, 2009; Anderson-Teixeira et al., 2013), though yields vary by species and location (Heaton et al., 2008; Arundale et al., 2014; Sanford et al., 2016). The perennial life cycles of *M.* x *giganteus* and switchgrass eliminate tillage practices that disturb soil and lead to C losses through erosion and accelerated decomposition (Six et al., 2000; Conant et al., 2001; Glover et al., 2010; Chimento et al.,

2014). Additionally, these crops require less fertilizer, herbicide, and pesticide than conventional maize, and perennial crops allocate a larger portion of their biomass to roots and rhizomes, resulting in higher belowground C inputs (Anderson-Teixeira et al., 2009, 2013; Chimento et al., 2014; Ontl et al., 2015; Masters et al., 2016). Modeled projections indicate that replacing annual row agriculture with perennial grasses that resemble native prairie will restore of a portion of the soil organic carbon (SOC) lost over the last two centuries of agriculture in this region (Davis et al., 2012; Hudiburg et al., 2014; Duval et al., 2015).

The transition from annual to perennial crops may increase soil C stocks in agricultural soils, depending on the mechanism of C stabilization in the soil. Increased organic matter input alone is not enough to ensure increased SOC, as the microbial turnover of soil organic matter (SOM) drives both C loss and the long-term storage of C in the soil (Grandy and Neff, 2008; Kallenbach et al., 2015; Lehmann and Kleber, 2015). Carbon may be retained in soil because organic matter is chemically resistant to microbial consumption or because microbial consumption of plant material and root exudates results in increased soil microbial biomass or partially degraded material that is more stable than fresh litter. Organic matter may be physically protected by aggregation, which shields organic matter from microbial decomposition or creates an anoxic environment in which microbial activity is reduced (Oades and Waters, 1991; Allison and Jastrow, 2006; Cotrufo et al., 2013). Unincorporated organic matter, such as annually produced aboveground litter, is particularly susceptible to depletion through mechanical destruction or removal under land use change (Wander and Traina, 1996; Marriott and Wander, 2006).

Particulate organic matter (POM), defined as fresh or decomposing organic material between 53 and 250 μm in diameter, is a useful index of microbially-important SOM because it consists of recognizable organic matter that can be isolated from mineral soils, and is sensitive to changes in soil management (Franzluebbers, 2000; Wander and Traina, 1996; Willson et al., 2001; Wander, 2004). POM preserved in aggregate structures decomposes on a decadal scale, with unincorporated POM decomposing at approximately twice that rate (Liao et al., 2006.) When soil aggregates are mechanically destroyed during tillage and cultivation, organic matter previously protected within soil aggregate structure is released into the microbially-accessible organic pools (Besnard et al., 1996; Six et al., 2000; Allison and Jastrow, 2006; Grandy and Neff, 2008). The rate of POM turnover also is driven by environmental factors, including moisture, temperature, and pH, affecting microbial activity. Modeled predictions of soil C in the Midwest under maize/soy agriculture show a gradual loss of soil C over time that is reversed under perennial grasses (Davis et al., 2012; Hudiburg et al., 2014; Duval et al., 2015). As POM has been shown to be sensitive to land use change and faster to respond than SOC, measuring POM may allow us to visualize soil C changes and predict patterns of SOC ahead of measureable SOC change.

In this study, SOC and POM-C in soils under potential perennial bioenergy grasses were compared with conventional row crops in a 6-year study to identify the SOC sequestration potential of each crop. A side-by-side replicated field trial of a maize-maize-soybean rotation, M. x giganteus, switchgrass, and a 28-species restored prairie simulating the historic vegetation of the area prior to settlement and cultivation were compared. All plots in this study were managed according to the best-known Midwestern U.S. agricultural practice for each species to accurately represent soils found under future bioenergy production.

The primary objectives of this research were to assess: i) the change in SOC under each crop over a 6-year period; and ii) to determine the relationship between SOC and POM accumulation for these crops. We hypothesize that perennial crops will increase POM-C (representative of litter inputs) and SOC (representing organic matter retained) to a greater extent than annual crops over this 6-year period, and that rates of perennial POM-C accumulation will exceed rates of accrual of whole soil-SOC due to delays between litter inputs and litter consumption by heterotrophs. We also hypothesize that, in contrast with the perennial crops, the rate of change of the isotopic signature of POM under annual crops will exceed the rate of SOC accumulation, due to incorporation of aboveground organic matter by tillage.

2. Materials and methods

2.1. Site description and design

This study was conducted at the University of Illinois Energy Farm (40°3′46″N, 88°11′46″W) in Urbana, IL, USA, established in 2008. Prior to establishment, the Energy Farm produced row crop agriculture, primarily maize and soybean, for more than 100 years. The experiment was a randomized block design, with five blocks each containing one plot of each of the four vegetation types. Four 3.8-ha plots (sized to provide the minimum fetch for eddy covariance towers as described in Zeri et al., 2011) and sixteen 0.7-ha plots were planted in M. x giganteus; switchgrass; a 28-species restored native prairie mixture including grasses, forbs, and several N-fixing species (see Zeri et al., 2011 for complete species list); and a maize-maize-soybean rotation.

Soils on the site are predominately Argiudolls: Dana silt loam (fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls) with some Flanagan silt loam (fine smectitic, mesic, Aquic Argiudolls), Blackberry silt loam (fine-silty, mixed superactive, mesic Oxyaquic Argiudolls), and Drummer silty clay loam (fine-silty, mixed superactive, mesic Typic Endoaquolls) inclusions. Mean annual temperature is 10.9 °C, and mean annual precipitation is 1051 mm (Angel, 2010, 1981−2015 average).

M. x giganteus was propagated by rhizomes, while switchgrass and prairie were broadcast seeded, and maize was planted by drill seeding. Delayed rhizome planting in 2008 and a harsh winter in 2008−2009 resulted in high mortality of juvenile M. x giganteus rhizomes, requiring replanting in 2009 and partial replanting in 2010 (see Smith et al., 2013). Soybean was alternated with maize every third year (2010, 2013) to mimic conventional row crop production in this area. Nitrogen fertilizer was applied prior to maize planting as 28% urea ammonium nitrate at 168 kg N ha^{-1} (2008, 2011, 2014) and 202 kg N ha^{-1} (2009, 2012), and to switchgrass as granular urea, at 56 kg N ha^{-1} from 2010 to 2014. M. x giganteus, soybean, and prairie were not fertilized. Row crop plots were chisel plowed each fall following maize harvest according to the convention of the region, and worked with a field cultivator (Sunflower Mfg., AGCO Corp., USA) in spring prior to planting both maize and soybean. Perennial crops were untilled after planting.

2.2. Biomass measurement

Aboveground and belowground biomass were measured annually at peak biomass (determined by LAI measurements, Zeri et al., 2011), typically in late August or September. Aboveground biomass was sampled at four random locations in each 0.7-ha plot and 8 locations in each 3.8-ha plot from within a 0.20 m^2 quadrat for prairie and switchgrass and a 0.56 m^2 quadrat for maize, soybean, and M. x giganteus. The larger quadrat was sized to encompass only a single row of maize or soybean, to eliminate variation in biomass due to sampling multiple rows. Belowground biomass was collected from within each quadrat by three randomly placed 5.1-cm diameter cores taken to 30 cm. Cores were divided into two depths (0−10 and 10−30 cm), and pooled within a quadrat. Roots

and rhizomes were separated from the soil matrix by elutriation, dried, and weighed.

Aboveground biomass from perennial grass and prairie plots were harvested annually, and the biomass was baled and removed from the site (Case New Holland Global, Burr Ridge IL USA). Maize and soybean grain were harvested via combine (Case New Holland Global, Burr Ridge IL USA), and stover was left on the field. Annual crop harvest occurred in late September or early October, and perennial biomass harvest between November and March, depending on weather conditions.

2.3. Soil sampling

Soil from all plots was collected prior to planting of annual crops in the spring (April/May) at two-year intervals from 2008 to 2014. Five 3.2-cm diameter cores were collected at each of 10 locations in each 0.7-ha plot of each crop, and at each of 40 locations in each 3.8-ha plot using a core extractor (JMC backsaver handle, 38-cm large diameter sampling tube). The cores were sectioned into 0—10 cm and 10—30 cm portions and pooled at each sampling location. Soils were air dried, crushed (Dynacrush DC-5, Custom Laboratory Equipment Inc., Orange City FL USA), and sieved to 2 mm. A subsample from each sampling location was dried at 105 °C to a constant mass and used to determine remaining moisture for bulk density calculations. Subsamples were finely ground using a modified coffee grinder (Mr. Coffee, Rhode Island USA) and analyzed for percentage C and C stable isotope composition (δ^{13}C). Soil carbonate removal was not necessary due to absence of carbonate in the shallow soils.

Soil C concentration was measured by elemental analysis (Costech EA 3010 CHNSO Analyzer, Costech Analytical Technologies, Valencia CA, USA) using apple leaves, acetanilide (National Institute of Science and Technology, Gaithersburg MD, USA), and certified soil reference material (Leco, St. Joseph MI, USA) as standards and quality control. The dried, finely-ground soil was weighed into tin capsules and combusted. Soil organic carbon stock (SOC stock, g m^{-2}) to a depth of 30 cm was computed from the total organic C concentration, bulk density, and depth of each soil layer.

2.4. POM separation and analysis

Particulate organic matter was isolated from whole soil using a procedure modified from Marriott and Wander (2006). Ten grams of soil was weighed into 30-mL containers, a nylon cloth with a pore diameter of 53 μm was stretched across the opening, and a modified cap with an O-ring held it securely in place. Soil was shaken for 1 h in sodium hexametaphosphate (HMP), followed by successively shorter periods of shaking in DI water until no clay or silt particles remained. Sand and POM larger than 53 μm were retained inside the container, while silt and clay particles were removed from the outer containers by repeated rinses. As sand contains no C, the isotopic signature of the POM was measured without separating POM from sand, and the C concentration was derived from the POM-C content of the POM + sand and the ratio of POM + sand to bulk soil. After separation from silt and clay, the POM + sand was dried at 60 °C for 48 h, and then finely ground using a bead beater (Genogrinder 2010; Lebanon NJ, USA). As with whole soil, POM-C was measured by elemental analysis (Costech Analytical Elemental Combustion System 4010, Valencia, CA).

The ^{13}C—^{12}C ratio of the POM-C was measured using an elemental analyzer (Costech 4010, Costech Analytical Technologies, Valencia CA USA) in combination with an isotope ratio mass spectrometer (DeltaV Advantage, Thermo Fisher Scientific, Bremen, Germany) in continuous flow. All ratios were measured relative to laboratory standards, calibrated relative to VPDB. A two-end member-mixing model (Hansen et al., 2004) with 2008 soil as one end member and root material collected from each treatment as the other (maize, *M.* x *giganteus*, and switchgrass roots, and a representative mixed C_3-C_4 sample from prairie) was used to calculate the relative proportions of the POM-C that was associated with C_3 and C_4 crops.

2.5. Statistical analysis

Data were analyzed for each energy crop and each soil depth layer using a one-way ANOVA analysis for a randomized complete block design for each layer separately. Crop type and block were considered fixed factors. Significant differences ($p < 0.05$) were identified using student's *t*-test carried out using JMP (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Organic matter inputs

Litter and turnover of roots and rhizomes represent potential inputs to soil organic matter, which also consists of root exudates and microbial biomass. A six-year average of above- and belowground peak biomass measured from 2009 to 2014 was significantly larger in *M.* x *giganteus* than all other crop types in 2014 (Table 1). Biomass measurements in 2008 were omitted due to low perennial yields and poor *M.* x *giganteus* establishment. Maize was rotated with soybean in 2010 and 2013. Average soybean aboveground biomass was 45% of maize yields between 2009 and 2014. All perennial treatments had 650%—1150% higher belowground biomass than maize/soybean. In contrast with the annual crops, where significant quantities of stover are left on the field surface as litter after mechanical harvest (Table 1), the majority of perennial aboveground material was baled and removed (Anderson-Teixeira et al., 2013), resulting in lower quantities of litter, and a higher ratio of belowground to litter inputs.

3.2. Changes in SOC content

Changes in SOC in surface soil (0—10 cm) over time, while not statistically significant annually within a crop type, contributed to differences among crops after six years. In the surface soil (0—10 cm), soil C under maize/soybean showed an initial positive trend between 2008 and 2010, followed by a decline between 2010 and 2014, resulting in the lowest concentration of SOC of all treatments (Fig. 1A). Conversely, soil C in *M.* x *giganteus* plots initially declined during the establishment phase, followed by an increasing trend between 2010 and 2014. Switchgrass and prairie both showed a positive trend indicating minor soil C accumulation between 2008 and 2014. After six years, SOC in surface soil (0—10 cm) was significantly greater under prairie than under the

Table 1
Average peak growing season aboveground (g m^{-2}) and belowground (g m^{-2}; 30 cm^{-1}) biomass for all crops from 2009 to 2014. Data from 2008 was omitted due to minimal production, cover crops in half the treatments, and a lack of a plot level harvest during the establishment year. Values represent mean ± standard error (n = 6.) Different letters within the same row indicate significant differences between crops, according to student's t.

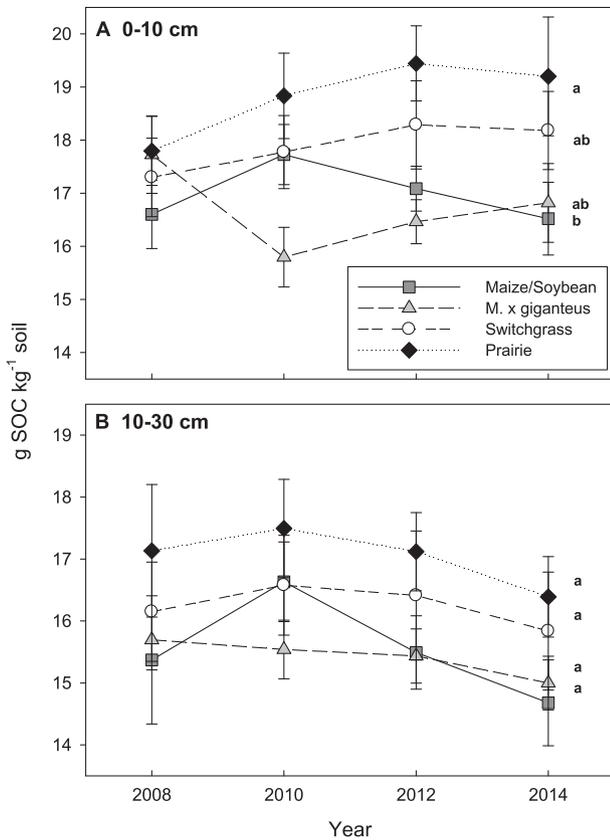|	Maize/Soy	*M.* x *giganteus*	Switchgrass	Prairie
Total biomass	1647 ± 116b	3061 ± 411a	2229 ± 211ab	1521 ± 159b
Aboveground	1557 ± 116ab	2030 ± 372a	1475 ± 154ab	884 ± 113b
Belowground	90 ± 20b	1031 ± 175a	754 ± 144a	637 ± 112a
Litter	472 ± 53a	417 ± 45a	247 ± 29ab	144 ± 35b

Fig. 1. SOC content in the different biofuel crops and prairie soils from 2008 to 2014. Figure 1A shows the 0–10 cm depth, while Figure 1B shows the 10–30 cm depth. Maize was alternated with soybean in 2010 and 2013. Different letters indicate significant differences in SOC between treatments in 2014.

maize/soybean rotation (p < 0.05). Switchgrass and *M.* x *giganteus* had no detectable effect on SOC (Table 2). SOC in deeper soil (10–30 cm) was fairly constant over time and no differences could be resolved among crops (Fig. 1B).

3.3. POM-C pools

POM-C increased significantly for all perennial crop treatments between 2008 and 2014 from 0 to 10 cm (Table 2, Fig. 2). *M.* x *giganteus*, prairie, and switchgrass increased by 68, 50, and 80% respectively over the six-year period. Initial POM-C at 0–10 cm was highest in prairie soil; however this was not significantly different from other crops (1.59–1.79 g POM-C kg^{-1} soil). *M.* x *giganteus*

POM-C decreased 31% from 2008 to 2010, likely due to the establishment problems and subsequent replanting disturbance observed in SOC, but recovered and increased by 171% between 2010 and 2014 (0–10 cm, Fig. 2). This was the fastest rate of POM-C accumulation observed in this study. By 2012 the surface soil (0–10 cm) POM-C was greater in all perennial treatments than in the annual crop rotation, and the difference was even larger in 2014 (Table 2, Fig. 2).

The concentration of POM-C at 10–30 cm depth increased in *M.* x *giganteus* and prairie with time (Table 2). Initial soil POM-C concentrations at 10–30 cm were within a relatively narrow range and not significantly different (0.67–0.97 g POM-C kg^{-1} soil). Between 2008 and 2014 all values increased (p < 0.05), with the smallest increase observed in maize/soybean at 80%, prairie and switchgrass at 120% and 122%, and *M.* x *giganteus* at 183%. *M.* x *giganteus* and prairie each showed significantly greater rates of POM-C accumulation at the deeper depth during the 2012 to 2014 period than maize/soybean and switchgrass (Fig. 2). Though not different from one another, final POM-C at 10–30 cm in 2014 was greater in *M.* x *giganteus* and prairie treatments than in maize/soybean (Table 2).

3.4. POM δ^{13}C

The POM δ^{13}C in surface soil (0–10 cm) increased (became less negative), indicating a net accumulation of material from C$_4$ plants, in all crops between 2008 and 2014 (Fig. 3). The most dramatic change occurred between 2008 and 2010, when the δ^{13}C of all crops increased (p < 0.05). Between 2010 and 2012, *M.* x *giganteus* and switchgrass continued to add C$_4$ organic matter, while the maize/soybean treatment accumulated C$_4$ maize material as well as C$_3$ material from soybean planted in 2010 and 2013. Between 2010 and 2014, the isotopic ratio of the prairie soils did not change significantly.

Changes in POM δ^{13}C were significant (p < 0.05) for the perennial monoculture crops between 2008 and 2012 at 0–30 cm soil depth. *M.* x *giganteus* and switchgrass each initially showed a slow response to establishment, likely a result of soil disturbance; however the accumulation of C$_4$-associated POM-C increased dramatically between 2010 and 2012 for both crops (Fig. 3). Maize/soybean δ^{13}C did not change significantly between 2008 and 2012, a reflection of the C$_4$ and C$_3$ crop rotation. Prairie δ^{13}C became more C$_3$ between 2008 and 2010, did not change between 2010 and 2012, and increased significantly between 2012 and 2014, a pattern which likely reflects the differing rates of establishment of the various C$_3$ and C$_4$ species within the prairie (Fig. 3).

POM-C in surface soil (0–10 cm) increased for all crops from 1.59 to 1.79 g C kg^{-1} soil at the beginning of the experiment to 2.54–3.01 g C kg^{-1} soil after six years, and the mixing model

Table 2
Soil organic carbon (SOC, g kg^{-1} of soil), SOC stock (SOC, g m^{-2}), and particulate organic matter carbon (POM-C, g kg^{-1} soil) in initial (pre-disturbance) soil and under annual maize/soybean agriculture and three potential perennial bioenergy ecosystems in 2014, 6 years after establishment. Values represent mean + standard error (n = 5). Different letters within the same row indicate significant differences between crops (p ≤ 0.05).

|	Initial	Maize/Soy	*M.* x *giganteus*	Switchgrass	Prairie
	(2008)	(2014)	(2014)	(2014)	(2014)
SOC (g kg^{-1}soil)					
0–10 cm	17.4 ± 0.3ab	16.5 ± 0.7b	16.8 ± 0.7b	18.2 ± 1.0ab	19.2 ± 0.6a
10–30 cm	16.1 ± 0.4a	14.7 ± 0.7a	15.0 ± 0.4a	15.8 ± 1.0a	16.4 ± 0.6a
SOC stock (g m^{-2})					
0–10 cm	2090 ± 105b	2180 ± 99b	2340 ± 75ab	2502 ± 135a	2492 ± 53a
10–30 cm	4384 ± 219a	4139 ± 228a	4317 ± 111a	4558 ± 267a	4544 ± 155a
POM-C (g kg^{-1}soil)					
0–10 cm	1.7 ± 0.2c	2.1 ± 0.1bc	2.8 ± 0.3ab	2.5 ± 0.2ab	3.0 ± 0.3a
10–30 cm	0.8 ± 0.1b	1.2 ± 0.1ab	1.9 ± 0.1a	1.6 ± 0.1ab	2.2 ± 0.1a
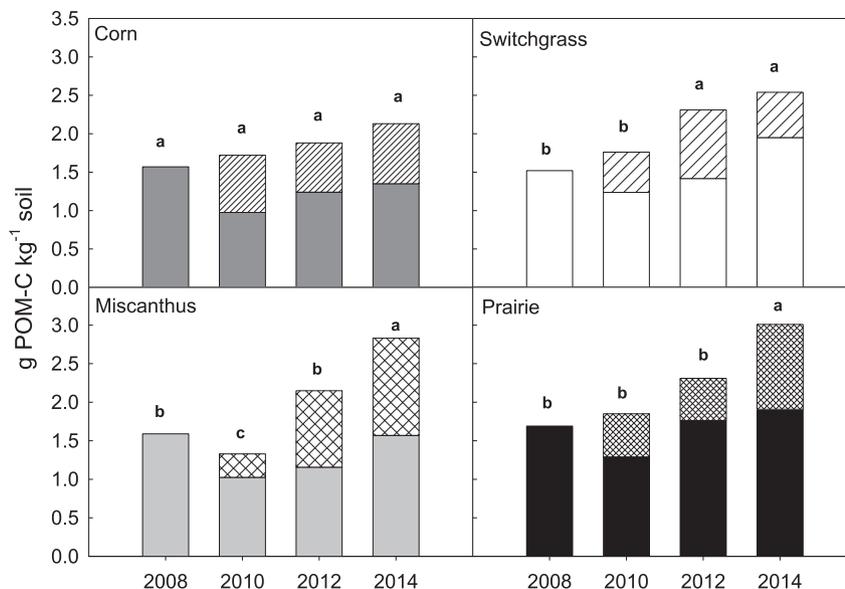
Fig. 2. POM-C content in the different biofuel crops and prairie soils from 2008 to 2014. Patterned portions of each bar represent the proportion of C_4 material derived from the current crop calculated from the δ^{13}C and a two-end member-mixing model. Letters denote within-crop differences in POM between 2008 and 2014 ($p < 0.05$). Maize was alternated with soybean in 2010 and 2013. Different letters within a year indicate significant differences between treatments.
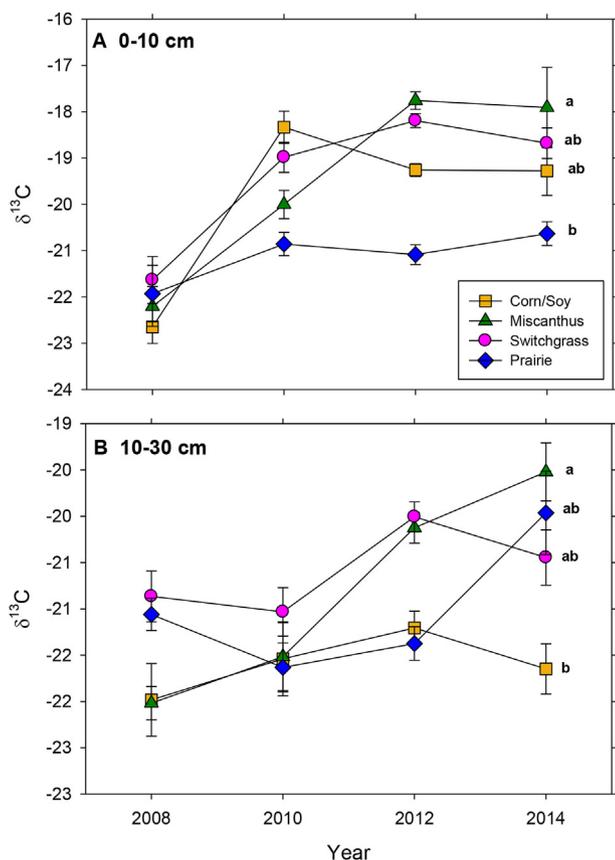

Fig. 3. Change in POM-C δ^{13}C in different treatments for 0—10 cm (A) and 10—30 cm (B) depth from 2008 to 2014. Different letters within a year indicate significant differences between treatments.

revealed that up to 33% (*M.* x *giganteus*) of POM at the end was derived from organic material isotopically-resembling the current crop (Fig. 2). Carbon attributed to post-2008 organic material was detected in POM after two years. In all crops, the combined pre-2008 and "new" POM-C measured in 2014 exceeded pre-treatment POM from 2008. This indicates new organic inputs are not priming the system for a major loss of POM with the establishment of C_4 bioenergy crops, instead POM-C accumulated between 2008 and 2014 supplemented existing POM, resulting in an overall POM increase (Fig. 2).

4. Discussion

4.1. Soil organic matter

Consistent with our hypothesis, six years of perennial and annual agriculture in central Illinois increased soil C in the form of POM-C, and the rate of increase was greater for perennial crops than traditional maize/soybean agriculture. The increase in POM-C was attributed to new organic matter inputs identified by changes in isotopic composition of the POM, which showed increased C_4 organic matter inputs over six years. However, the change in isotopic composition was not more rapid in annual crops than in perennials, despite aboveground litter incorporation by tillage. While POM is an intermediate, partially-decomposed SOC component, it is critical to building long-term soil C storage because it is evidence of substrate to feed microbial biomass and necromass, and for microbes to convert into more stabilized forms of soil C (Janzen et al., 1992; Marriott and Wander, 2006). At least in the prairie and possibly for switchgrass, higher levels of POM-C may have contributed to increased SOC in surface soil. SOC concentrations for all crops in both 2008 and 2014 were low (43—47% of historic SOC) compared to native, undisturbed grasslands in the area (David et al., 2008), indicating that potential exists for further sequestration over time in these systems.

4.2. POM-C pools

While POM is not considered a long-lived component of SOM, cycling on a decadal scale (Jastrow, 1996; Liao et al., 2006), it nonetheless represents organic inputs to a system, the turnover of which controls the rate of C accrual in soils. The annual addition of organic matter in cropped systems offsets annual decomposition. In

areas where organic matter inputs outpace the rate of POM decomposition, POM increases soil aggregate structures and contributes to longer-turnover soil organic fractions (Golchin et al., 1995b). POM consists of labile and recalcitrant components, and soil microbes preferentially metabolize the labile components. Microbial decomposition maintains the continuum of C states in the soil (Lehmann and Kleber, 2015) and alters the quality of organic matter, both releasing a portion of the organic C to the atmosphere and converting organic matter to C compounds that can be sequestered in the soil matrix (Cotrufo et al., 2013). Therefore, POM increase is associated with increased SOC despite the transitional nature of the material: the breakdown of POM by microbes results in the production of mucilages and metabolites that stabilize remaining organic matter into aggregates (Golchin et al., 1997; Ontl et al., 2015).

The physical protection of easily decomposed material increases the overall difficulty of liberating soil C by microbes and contributes to the variety of substrate quality stored in the soils. At this site, increases in POM-C in perennial treatments exceeded annuals by 31−55%, with the highest concentration in the prairie soils. The hypothesis that perennial crops will increase POM-C in excess of annual crops was based on the 650−1150% greater belowground biomass of perennial crops (Table 2), which is expected to contribute both fresh organic material, the source of POM, as well as root exudates to support the microbial community in the rhizosphere, however annual crops also show a small increase despite no change in belowground biomass.

The POM-C increase observed in maize/soybean was unexpected. As a significant portion of POM protection comes from the formation of soil aggregates, one of the driving forces in the liberation of POM from soil is tillage, hence the sensitivity of POM to changes in management (Golchin et al., 1995b; Jastrow, 1996; Conant et al., 2001; Dou et al., 2013; Ontl et al., 2015). In this experiment, the three perennial crops, *M.* x *giganteus*, switchgrass, and prairie, were untilled, while the maize/soybean plots were tilled each fall prior to maize planting. In building our hypotheses, we expected that mechanical tillage in maize/soybean treatments would result in the liberation of POM and subsequent consumption by microbes. However, the annual aboveground litter input is greatest in the maize/soybean system, particularly during maize years, and this material is broken up and incorporated into the soil via tillage. In addition, the entirety of the maize/soybean living belowground biomass dies each year when the aboveground biomass is harvested, while much of the perennial belowground biomass survives the winter and does not enter the SOM. Based upon root/shoot ratios for the annual crops, it is likely that most of the POM found in the maize/soybean plots was derived from aboveground biomass (Table 1), and that repeated disturbance of soil aggregates by tillage results in a larger proportion of unprotected POM, resulting in higher rates of C turnover and decomposition demonstrated at this site by Anderson-Teixeira et al. (2013). Though SOC in *M.* x *giganteus* did not increase over the sampling period, the pattern of POM and SOC accrual in *M.* x *giganteus* (Figs. 1A and 3) mirrored the switchgrass and prairie treatments after 2010, likely a reflection of the 2008/2009 disturbance.

4.3. POM $\delta^{13}C$

The concentration and the $\delta^{13}C$ of POM-C are affected by the rates and isotope signature of the organic matter input, respectively. The two monoculture perennial grasses, *M.* x *giganteus* and switchgrass, are both C_4 species, as are maize and several prairie grass species. The $\delta^{13}C$ of C_4 tissue is less depleted in the ^{13}C isotope than its C_3 counterpart and this can be traced into the SOM.

Prior to Energy Farm establishment in 2008, the soils were maintained in a maize/soy rotation resulting in a $\delta^{13}C$ signature that reflected the mixed C_3-C_4 history of the site in all plots. Using the 2008 soil as the baseline and one end member of the mixing model, and root material from each crop as the other, allows calculation of changes in the isotopic concentration over time. In the case of both maize/soy and prairie, a mix of C_3 and C_4 species contributes to a mixed signal. In whole soils, the effect of C_3/C_4 crops may be apparent shortly after plant establishment, as root exudates would reflect the C_3 or C_4 pathway of the crop. However, POM-C is a measurement of C-containing biological structures preserved in the soil, a longer turnover fraction than exudates.

The amount of POM-C that was derived from the C_4 crops was calculated using a two-end member-mixing model (Golchin et al., 1995a; Hansen et al., 2004), establishing the proportion of the POM-C that was directly attributable to the C inputs from the newly-established crops. As we hypothesized, one of the strongest isotopic signals of C_4 material incorporation into POM-C occurred in maize/soybean (Fig. 3), as a result of the large post-harvest input of aboveground C_4 material tilled into the soil from maize stover. The soil maintains a strong C_4 signature despite triennial rotation of maize with C_3 soybean due to the relatively small biomass inputs from soybean (45% of maize annual average). The $\delta^{13}C$ of maize/soybean POM-C indicates at least 34% of this pool is derived from C_4 parent material after six years (Fig. 2).

For the perennial crops, the proportion of POM-C associated with the establishment of C_4 crops increased over the six-year period. As hypothesized, this effect was greatest in *M.* x *giganteus*, the treatment with the highest belowground biomass. In this system, approximately 45% of the POM-C in 2014 was C_4-derived. The incorporation of C_4 material in switchgrass remained relatively low despite a large belowground biomass and increasing POM-C, which indicates C_4 inputs are rapidly cycling out of the soils system, likely being consumed by microbial decomposers rather than stored in the soil matrix. It is possible the structural composition of this material or an association with a native microbe makes it easier to decompose than tissue derived from *M.* x *giganteus,* which has been shown to be lower in protein and more resistant to insect herbivory than switchgrass (Prasifka et al., 2009), while both were more resistant than maize (Nabity et al., 2011). However, for all treatments, $\delta^{13}C$ isotopes indicate that POM-C is being derived from new plant material while existing C is conserved in these systems, to the greatest extent in perennial monoculture treatments, but with a significant amount in annual crops as well (Fig. 2).

The treatment with the lowest C_4 incorporation into POM-C was prairie, because like maize/soy, the prairie contains a mix of C_3 and C_4 species, resulting in a mixed C_3-C_4 signal that is not appropriate for the two end-member model. Only three of the 28 prairie species use C_4 photosynthesis, though the proportion of biomass contributed by C_4 species is much higher than the species ratio (Feng and Dietze, 2013). Despite the large contribution from these C_4 crops, we would expect organic matter inputs in prairie treatments to have a more negative, C_3-like isotope signature at the plot level.

Measurements of POM-C on the Energy Farm range from 13 to 17% of SOC (Table 1). In other studies, POM-C accounted for up to 38% of SOC under perennial biofuel crops (Chimento et al., 2014) and 39% of SOC in native prairie sod (Cambardella and Elliott, 1992). This disparity shows that there is potential for considerably more accumulation of POM-C in Energy Farm soils, though individual site potential for SOC accumulation differs with soil characteristics (Six et al., 2002). The rate at which SOC at the Energy Farm will approach those numbers is dependent on both the rate at which organic matter is accrued and the rate of microbial activity in the soil, both releasing CO_2 or decomposing plant material into more recalcitrant C forms which will continue to contribute SOC.

Modeled estimates of changes in soil C over time in Midwestern

soils indicate that SOC will increase significantly in the first five years after planting perennials (Anderson-Teixeira et al., 2013; Hudiburg et al., 2014; Duval et al., 2015). Eddy flux data for these treatments are strongly negative, showing large inputs of C to the belowground ecosystem (Zeri et al., 2011; Joo et al., 2016), however these C increases are not reflected in our measurements of SOC. This negates our hypothesis that changes in SOC would be apparent under perennial crops within 6 years. The minimal increase in SOC in these soils is part of the larger conundrum of missing C in these ecosystems (Anderson-Teixeira et al., 2013).

SOC accrual requires both organic matter inputs and retention of C through alteration of the physical or biochemical mechanisms responsible for C loss. Biomass measurements indicate that belowground biomass is increased several orders of magnitude by the establishment of perennial bioenergy crops, while aboveground biomass is comparable between maize and *M.* x *giganteus* and switchgrass (Anderson-Teixeira et al., 2013, Table 1). Despite our inability to resolve differences in bulk SOC over the six-year time period presented here, our results show that POM-C is increasing in all perennial crops at a greater rate than the annual row crop rotation. Isotopic analysis of POM-C indicates significant incorporation of new material into existing SOM, additional evidence of changes to the C cycle. Accumulation of POM-C is an indicator of increases in SOC though the relative transience of POM-C compared to other SOC fractions does not guarantee longevity of C in the soil (Jastrow, 1996; Liao et al., 2006; Chimento et al., 2014). The accumulation of POM-C represents an overall increase in C inputs into the soil, where both labile and recalcitrant C sources are sequestered in soil structure or by chemical bonds that resist degradation. Under perennial crops at other sites, increases in POM have been shown to disproportionally outpace changes in SOC making POM an early and more sensitive indicator of management effects and soil quality than SOC alone (Barrios et al., 1996; Jastrow, 1996; Chimento et al., 2014; Ontl et al., 2015).

Our measurements of POM in agricultural soils in central Illinois show that POM-C increases under perennial grass monoculture as well as restored prairie after conversion from annual crops, outpacing increases in total SOC. Isotopic measurements of $\delta^{13}C$ in POM-C show that C_4 C derived from perennial crop biomass is accruing at a faster rate in the POM-C pool than in whole soil under the perennial grasses. This supports the theory that POM-C is a more rapid and sensitive indicator of the effects of vegetation change and management practices on the soil C environment than whole soil SOC measurements alone. Comparisons with similar crops in similar soils (Blanco-Canqui, 2010; Dou et al., 2013; Chimento et al., 2014) demonstrate the potential of these bioenergy ecosystems to maintain larger POM and SOC pools, and we expect both POM-C and bulk SOC to continue to increase with time in these soils.

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