Soil Organic Carbon, Aggregation, and Microbial Community Structure in Annual and Perennial Biofuel Crops

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ABSTRACT
The substitution of cellulosic biofuel in place of conventional fuels could reduce greenhouse gas (GHG) emissions from transportation. However, changes in soil organic carbon (SOC) and soil health during biofuel crop production could have a major impact on the GHG balance of biofuels. We assessed temporal changes (10 yr) in SOC stocks to a 90 cm depth in Cumulic Hapludolls from central Kansas under perennial and annual cropping systems. The perennial crops were miscanthus (*Miscanthus sacchariflorus*) and switchgrass (*Panicum virgatum* L.). The annual cropping systems were continuous corn (*Zea mays* L.), and corn, dual-purpose–grain sorghum (*Sorghum bicolor* (L.) Moench), sweet sorghum, and photoperiod-sensitive sorghum (PS) in rotation with soybean (*Glycine max* (L.) Merr.). All standing aboveground biomass was removed at harvest of corn, sorghum, and perennial crops. Stocks of SOC increased in the 0–15 cm depth under switchgrass and miscanthus by 0.8 and 1.3 Mg C ha−1 yr−1, respectively. The SOC stocks did not change at the other depths or at any depth in the annual cropping systems nor throughout the soil profile under any crops. Root biomass measured in the seventh year of the study was 3.7 to 7.8 times greater in perennials than in annual crops. Increases on SOC were correlated with greater root biomass, abundance of arbuscular mycorrhizae and saprophytic fungi, and soil aggregate diameter. These results demonstrate the potential for perennial biofuel crops to enhance C sequestration and improve soil quality while providing feedstock for production of cellulosic biofuel.

Core Ideas
- Removal of corn and sorghum residues did not affect soil carbon under no-tillage.
- Soil carbon (0–15 cm) increased in 10-yr stands of switchgrass and miscanthus.
- Soil carbon increased with root biomass, fungi abundance, and soil aggregate size.
- Perennial crops improved soil health while providing feedstock for biofuels.
declines in SOC during crop production will negatively impact the GHG footprint of biofuel cropping systems (Liska et al., 2014). Perennial grasses such as switchgrass (Panicum virgatum L.) or miscanthus (Miscanthus × giganteus) are attractive alternatives to annual crops because their greater persistent belowground biomass could help maintain SOC while still producing large quantities of biomass.

Crops grown for cellulosic biofuel production should not only be able to maintain SOC stocks, but also be characterized by large biomass production, low input costs, ease of management, and compatibility with current cropping systems. Consequently, there are a variety of bioenergy crops being considered for use in the Great Plains region. In addition to conventional, annual row crops like corn (Zea mays L.) and grain sorghum (Sorghum bicolor (L.) Moench), several annual dedicated bioenergy crops may be grown for cellulosic biofuel. Photoperiod-sensitive sorghums are sorghum cultivars that do not initiate reproductive flowering in most of the United States and produce large amounts of lignocellulosic biomass. Photoperiod-sensitive sorghum commonly yields 20 to 30 Mg ha$^{-1}$ with yields reported as much as 35 Mg ha$^{-1}$ (Propheter et al., 2010; Maughan et al., 2012a). Sweet sorghum cultivars produce quantities of cellulosic biomass similar to photoperiod sensitive cultivars. In addition, they accumulate substantial amounts of juice (9–24% sugar) in the stalks, which can be directly converted into ethanol (Regassa and Wortmann, 2014).

Several perennial crops are considered as potential sources for cellulosic feedstock because of their low input requirements, low management needs, and ability to grow on marginal soils. Switchgrass is a perennial warm-season grass that is native to the prairies of the United States. Average yields in the United States range 8.7 Mg ha$^{-1}$ to 12.9 Mg ha$^{-1}$ (Wullschleger et al., 2010). Miscanthus × giganteus is a sterile hybrid of a perennial warm-season grass originating in Japan. Miscanthus has the potential to produce large amounts of biomass. Yields of 14 to 40 Mg ha$^{-1}$ are common from mature stands in the United States but can be as high as 60 Mg ha$^{-1}$ (Heaton et al., 2008; Maughan et al., 2012b; Arundale et al., 2014). Ethanol production per unit area of perennial grasses (switchgrass) has been shown to be comparable to irrigated and rain-fed corn (Schmer et al., 2008). However, perennial grasses are expected to sustain higher and more stable yields than corn when cultivated in rain-fed marginal areas (Schmer et al., 2008). Thus, the use of perennial grasses may be an option to promote biofuel production from marginal agricultural lands.

Side-by-side studies comparing both perennial and annual bioenergy crops are necessary to gain a better understanding of crop selection on relative yield potential and on SOC dynamics. Such information is necessary to predict the impact of bioenergy cropping systems on the GHG balance of biofuel. The objectives of this study were to assess (i) changes in SOC stocks in long-term (10-yr) biofuel cropping systems including perennial grasses, continuous corn, and annual rotations of corn and different sorghum cultivars with soybean in a Cumulic Hapludoll from central Kansas; and (ii) belowground C inputs, soil microbial communities and physical protection of SOC in annual and perennial biofuel cropping systems.

### Materials and Methods

**Study Site**

This experiment was conducted at the Kansas State University (KSU) Agronomy Research Farm in Manhattan, Kansas (39°11’ N, 96°35’ W). The soil types on the study site were Ivan, Kennebec, and Kahola silt loams (fine-silt, mixed, superactive, mesic Cumulic Hapludolls). Differences among soil series were mostly related to flooding frequency which was rare for Kahola and occasional for the other soils. Nonetheless, Kahola soils represented roughly >80% of the experimental area. The study site was previously planted to wheat and other small grains using conventional tillage practices for at least 60 yr (Nicoloso et al., 2018). The last tillage event was in spring of 2005. Soybean was the prior crop for the entire experimental area in 2007. Soils at the study site were sampled for initial characterization in April 2007 by collecting 15 individual soil cores across the experimental area (Propheter and Staggenborg, 2010). Soils had an average SOC content of 14.5 g kg$^{-1}$, pH of 6.6, and 200 g kg$^{-1}$ of clay, 700 g kg$^{-1}$ of silt, and 100 g kg$^{-1}$ of sand in the 0–15 cm depth (Propheter et al., 2010). In April 2007, seven cropping systems were established in plots measuring 6.1 × 10.7 m and arranged in a randomized complete block design with four replications and seven cropping systems, including two perennial grasses (miscanthus and switchgrass) and five annually planted systems (continuous corn, and corn, photoperiod-sensitive sorghum (PS), sweet sorghum, and dual-purpose–grain sorghum in annual rotations with soybean [Glycine max (L.) Merr.]). Dual-purpose sorghum was used in 2007–2008, and from 2009 onward grain sorghum was used in the dual purpose–grain sorghum treatment. For brevity, the dual purpose–grain sorghum treatment will be referred to as grain sorghum. The crop cultivars, seeding and fertilizer rates used throughout this study are detailed as supplementary information (Supplemental material Tables S1 and S2). The crops in rotation with soybean were duplicated, with both phases of the rotation present each year. All annual crops were established under no-tillage. Further details on plot establishment and crop management practices were reported in Propheter et al. (2010). All standing aboveground biomass was collected at harvest of miscanthus, switchgrass, corn, and sorghum in such way that minimal residue remained in the plots of annual crops. Aboveground residue from senescent foliage was present in miscanthus and switchgrass plots after harvest. Yield results are reported by Roozeboom et al. (2018).

**Soil Organic Carbon Stocks**

For determination of SOC stocks, soil cores (3.8-cm diameter) were taken to a depth of 120 cm using a Giddings hydraulic probe (Giddings Machine Company) in 2009, 2014, and 2017. One core per plot was taken in 2009 and three to four cores per plot were taken in 2014 and 2017 to account for within-plot variability. In 2009 and 2014, samples were taken in March before planting of the annual crops, while in 2017 samples were taken in November after harvest. For annual crops in a 2-yr rotation with soybean, only one of the duplicated plots was sampled. However, the same plots were sampled in every sampling year, and each sampling year came after the corn or sorghum phase of the rotation. All cores were collected from the inter-row of annual systems avoiding areas with evidence of recent machinery traffic and in-between
plants in the perennial systems. Soil cores showing evidence of compaction due to incorrect sampling procedure were discarded. The soil cores were carefully separated into eight depths (0–5, 5–15, 15–30, 30–45, 45–60, 60–75, 75–90, and 90–120 cm) to prevent contamination between separated soil depths. Soil cores collected in 2014 and 2017 were composited by plot and depth. Samples were stored at 4°C. A subsample was taken for soil moisture determination by oven-drying at 105°C. The soil bulk density was calculated using the mass of dry soil per depth, core diameter, and number of cores taken per plot. The remaining sample was air-dried, passed through a 2-mm sieve, and ground with mortar and pestle to pass through a 500-µm sieve. All visible plant material was removed during the sieving process. To remove carbonates, ground samples were treated with 0.1 mL 4 N phosphoric acid and allowed to dry. The acid treatment was repeated until there was no visible effervescence. Preliminary tests found that no significant changes in SOC concentration occurred between samples with and without acid treatment (data not shown). Sample SOC concentration was determined by dry combustion using a C/N elemental analyzer (Flash EA 1112 Series, ThermoScientific, Waltham, MA). The means and standard errors of soil bulk density and SOC concentration for each crop by depth increment are included in Tables S3 and S4.

Soil organic carbon stocks (Mg C ha⁻¹) were calculated using the measured bulk density and SOC concentration by depth and compared in equivalent soil masses (ESM) following the method described by Wendt and Hauser (2013). The ESM calculation method proposed by Wendt and Hauser (2013) corresponds to the original ESM method described by Ellert and Bettany (1995). However, the use of cubic spline functions as proposed by Wendt and Hauser (2013) eliminates laborious calculation and possible errors in the comparison of treatments with multiple sampling times and reference soil masses. Moreover, the cubic spline functions provide a better fit to changes in SOC concentrations across all sampling depths than methods based on linear interpolation (Wendt and Hauser, 2013). Briefly, we used an Excel add-in cubic spline macro function (SRSI Software, Boston, MA) that consisted of a piecewise series of cubic polynomial curves to calculate SOC stocks in the cumulative ESM of 0–700, 0–2100, 0–4200, 0–6300, 0–8400, 0–10,500, and 0–12,600 Mg ha⁻¹ that was approximately the soil mass measured for the 0–5, 0–15, 0–30, 0–45, 0–60, 0–75, and 0–90 cm soil depths across plots as sampled in 2009. The SOC concentration and soil mass measured in the 90–120 cm soil depth were used only for correction of SOC stocks in ESM, and the results were reported only to a 0–12,600 Mg ha⁻¹ or 0–90 cm depth. Soil organic C stocks in ESM, and the results were expressed as soil depths for ease of interpretation. When changes on SOC stocks over time were statistically significant for a given treatment and soil depth, soil C sequestration rates during the experimental period were calculated using SOC stocks as measured in 2009 in the same treatment and soil depth as a baseline (Olson et al., 2014). This procedure accounted for both temporal changes of SOC within treatments and the spatial variability of the experimental area (Olson et al., 2014).

**Root Biomass**

Root biomass of the biofuel crops was measured in fall of 2013. Soil cores (8-cm diameter) were taken from the same plots and depth increments used for determination of SOC stocks with a Giddings hydraulic probe (Giddings Machine Company). Corn (rotated and continuous), sweet sorghum, and grain sorghum were sampled during grain fill (August–September), while miscanthus, switchgrass, and photoperiod sensitive sorghum were sampled at the end of growing season (October). To account for spatial variability of root biomass in rows and inter-rows, three cores were taken from each plot in annual crops: One directly over a plant, one in the inter-row (38 cm from plant row) and one in-between the first two cores (19 cm from the plant row) (Fig. 1). Plant distribution was much different in the perennial grasses than in the annual crops. Switchgrass plants occurred in clumps that were 20 to 70 cm apart. To account for this spatial variability, two soil cores were taken in each plot, one directly over a clump and one in-between clumps. Miscanthus was higher in density and homogeneous such that it was not possible to take a soil core between plants. Thus, only one core was taken in each plot directly over a plant.

After sampling, root biomass was separated from soil by washing the soil core increments in an automated root washer (Benjamin and Nielsen, 2004). Soil samples were inserted into stainless steel mesh cylinders with 300 µm openings. Cylinders were loaded onto the washer, which rotated the samples under a high-pressure water spray. After root washing, the live roots were manually removed from organic debris, coarse soil fragments and rhizomes, rinsed, and oven dried at 50°C. Root density (g cm⁻³) was calculated by dividing dry root mass by soil core segment volume. For plots with annual crops or switchgrass, in which multiple soil cores were taken, the root density was estimated using an area-weighted average to avoid bias from unequal areas represented by different cores in the same plot. The area used to weight each core is represented by A₁, A₂, or A₃ in Fig. 1. Total root stocks (Mg ha⁻¹) were calculated by summing the root densities from 0 to 90 cm.

**Microbial Lipid Analysis**

Soil samples from the 0–30 cm depth (3–4 per plot) were taken with a soil core sampler (1.4-cm diameter) for lipid analysis on September 2013. Soil cores were divided into 0–5, 5–15, and 15–30 cm depths and pooled together by depth to make a composite sample per plot. Samples were frozen, lyophilized, and ground with a mortar and pestle. Phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) were extracted from 5 g of soil using the method of Bligh and Dyer (1959) as modified by White and Ringelberg (1998). Soils were incubated in a 2:1.0:8 methanol/chloroform/phosphate buffer and PLFA and NLFA were isolated using silicic acid chromatography. The phospholipid and neutral lipids were then saponified using KOH and methylated to form fatty acid methyl esters (FAME). The FAME were analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with a DB5-MS column (30 m × 0.25 µm inner diameter × 0.25 µm film thickness; Agilent Technologies, Santa Clara, CA). The FAME peaks were identified by comparison with the bacterial acid methyl esters mix (BAME; Matreya LLC, Pleasant Gap, PA). Tentative assignments of FAME peaks
not present in the BAME mix were made by mass spectral interpretation. Peak concentration was quantified using the internal standard nonadecanoate and converted into nmol PLFA/NLFA g⁻¹ dry soil. PLFA were classified into the following groups: gram-positive bacteria (i15:0, a15:0, i16:0, i17:0, a17:0), gram-negative bacteria (2-OH 10:0, 2-OH 12:0, 3-OH 12:0, 2-OH 14:0, 3-OH 14:0, 16:1ω7c, cy17:0, cy19:0), arbuscular mycorrhizal fungi (AMF) (16:1ω5), and saprophytic fungi (18:2ω6,9c). The NLFA were classified into arbuscular mycorrhizal fungi (16:1ω5) and saprophytic fungi (18:2ω6,9c). Although PLFA 16:1ω5 biomarker has been reported in other organisms, particularly in soil-inhabiting bacteria, the NLFA 16:1ω5 has been found to be a good biomarker to assign AM fungal biomass in soil (Ngosong et al., 2012). The fungal/bacterial ratio (F/B) was calculated by dividing the sum of AMF and saprophytic fungi PLFA by the sum of gram positive and gram negative bacteria PLFA.

**Water Stable Aggregates**

Intact soil samples for water-stable aggregate analysis were taken from the 0–5 and 5–15 cm soil layers using a spade in April 2014. Soil was sieved through a 6-mm sieve and air-dried prior to analysis. Two replicates of 50 g of air-dry soil were wet sieved through 2000-, 250-, 53-, and 20-µm sieves with a wet sieving apparatus as described by Mikha and Rice (2004). Soil was placed on the top of stacked 2000- and 250-µm sieves and slaked by submersion in water for 10 min. The sieves were then oscillated 4-cm lengths at 0.5 Hz for 10 min. Soil that passed through both sieves was poured and gently washed through the 53- and 20-µm sieves. Soil remaining on all four sieves was collected and one of the replicates was dried at 50°C for 2 d until reaching a constant weight. Subsamples were ground with mortar and pestle and analyzed for aggregate-associated C by dry combustion. The soil from the second replicate was dried at 105°C until reaching a constant weight and used for sand correction. Sand correction was performed by adding a fivefold volume of 5 g L⁻¹ sodium hexametaphosphate to 1–5 g of intact aggregates. Aggregates were left overnight and then shaken at 350 rpm for 4 h. Dispersed sand was collected on a 53-µm sieve and dried at 105°C until reaching a constant weight. Distribution of water stable aggregates was reported as the total weight of macroaggregates (>250 µm) and microaggregates (<250 µm), as well as the mean weight diameter (MWD) of treatment, which is calculated as the sum of the aggregate mass remaining on each sieve after sieving, multiplied by the mean aperture of adjacent sieves. Aggregate-associated SOC was reported on a mass basis, expressed as unit mass SOC per unit mass bulk soil (g C kg⁻¹ soil).

**Statistical Analysis**

Root density, PLFA, NLFA, water-stable aggregate distribution, aggregate MWD, and aggregate carbon were analyzed with a two-way factorial design using PROC GLIMMIX (SAS 9.4, SAS Institute Inc., Cary, NC), with crop and depth, and the interaction of these factors as fixed effects, and block as a random effect. Tests for the conditional independence between soil depths were performed using the ‘COVTEST’ option in PROC GLIMMIX. In cases where soil depths were found to be correlated, depth was analyzed as a repeated variable with either unstructured, first-order ante-dependence or first-order autoregressive structure. The covariance structure that minimized the Akaike information criterion was used in the final model. Total root stocks were analyzed using a one-way factorial design with crop as a fixed effect and block as a random effect. All data were checked for normality and homogeneous variance. When the assumption of homogeneous variance was not met, model residual variance was allowed to vary using the ‘GROUP’ option in the ‘RANDOM’ statement of GLIMMIX. Non-normal data were logarithmically transformed before analysis and means converted back to their original scale for presentation. Mean separation was performed using Fisher’s LSD. Pearson correlation coefficients were estimated among SOC, macroaggregates, root density, AMF, and F/B concentrations.
measured in the 0–5, 5–15, and 15–30 cm depths in 2014 by using PROC CORR in SAS.

Soil organic C stocks were analyzed with a two-way factorial design using PROC GLIMMIX with crop, sampling years, and the interaction of these factors as fixed effects, and block as a random effect. All analyses of SOC stocks were performed by soil depth following the recommendation of Kravchenko and Robertson (2011). Sampling years were analyzed as a repeated variable with either unstructured, compound symmetry, or first-order autoregressive structure. The covariance structure that minimized the Akaike information criterion was used in the final model. The LSMEANS statement was used to assess the differences among biofuel crops within sampling years as well as the differences among sampling years within the same biofuel crop treatment. All statistical comparisons were made at the α = 0.05 probability level.

**RESULTS**

**Root Density and Stocks**

The interaction of crop and soil depth was highly significant for root density (Table 1). Differences in root density among annual crops were limited to the top two surface soil layers (Fig. 2A). Miscanthus had significantly greater root density (2.4–18.8 mg dry roots cm⁻³ soil) than all other crops to a depth of 30 cm. Switchgrass had greater root density (1.2–5.7 mg dry roots cm⁻³ soil to 30 cm depth) than rotated corn, grain sorghum, and sweet sorghum at the 0–5 cm and all annual crops at both 5–15 and 15–30 cm soil layers. From 30 to 90 cm, both perennial grasses (0.3–0.8 mg dry roots cm⁻³ soil) had significantly greater root density than the annual crops (0.03–0.13 mg dry roots cm⁻³ soil). Root distribution of the soil profile also varied among crops. More than 80% of sorghum roots and 77% of corn roots occurred within the top 30 cm of soil (Fig. 2B). Switchgrass had only 57% of roots within the 0–30 cm soil layer, and 85% of miscanthus roots occurred in the 0–30 cm of soil. Significant differences occurred for total root stocks in the soil profile (p < 0.0001). Root stocks (0–90 cm) for miscanthus (27.6 Mg ha⁻¹) > switchgrass (12.6 Mg ha⁻¹) > annual crops (Fig. 2B). Differences among annual crops were limited to PS sorghum (4.4 Mg ha⁻¹) being greater than grain sorghum, neither of which differed from the other annual crops (2.9–4.0 Mg ha⁻¹).

**Microbial Lipids**

The interaction between crop and soil depth was not significant for total PLFA, gram positive and gram negative bacteria, fungal PLFA, and NLFA (Table 1). The concentration of all biomarkers decreased with increasing soil depth. The concentration of both gram-positive and gram-negative bacteria was not affected by cropping systems (data not shown), although significant differences were noticed for 18:1ω6,9 fungal PLFA and total PLFA biomarkers (Fig. 3). Total PLFA was greater under miscanthus (27.0 nmol PLFA g⁻¹ dry soil), but no differences were noticed among other cropping systems (13.7–18.0 nmol PLFA g⁻¹ dry soil) (Fig. 3A). The 18:1ω6,9 fungal PLFA biomarker was more sensitive for the tested treatments than total PLFA (Fig. 3B). Miscanthus had the highest concentrations of saprophytic fungi (3.2 nmol 18:1ω6,9 PLFA g⁻¹ dry soil) in comparison with the other crops. There was a positive relationship between the saprophytic fungi and root density (r = 0.574) (Table 2). The soil under switchgrass had concentrations of saprophytic fungi similar to soil under PS sorghum (0.6 and 0.5 nmol 18:1ω6,9 PLFA g⁻¹ dry soil, respectively), but higher than for the other annual crops (0.3–0.4 nmol 18:1ω6,9 PLFA g⁻¹ dry soil) (Fig. 3B). Similar results were found for the concentrations of 18:1ω6,9 NLFA (data not shown).

The concentrations of 16:1ω5 PLFA and NLFA were comparable within our assessment. However, because the 16:1ω5 PLFA biomarker also exists in some bacteria, we focused our study on the 16:1ω5 NLFA biomarker, which is a more reliable indicator for AMF (Frostegard et al., 2011; Ngosong et al., 2012). The interaction between crop and depth was significant for this AMF biomarker (Table 1). The concentration of AMF in the 0–5 cm depth of the soil under miscanthus (80.9 nmol 16:1ω5 NLFA g⁻¹ dry soil) was greater than all other treatments (>20 nmol 16:1ω5 NLFA g⁻¹ dry soil) (Fig. 4A). Nonetheless, the abundance of AMF in the 0–5 cm depth of the soil under miscanthus decreased significantly with soil depth (5–15 and 15–30 cm). The concentration of AMF among soil depths was not different for the other treatments. The lowest concentrations of AMF (0–5 cm) were with PS sorghum and sweet sorghum (3.9 and 2.6 nmol 16:1ω5 NLFA g⁻¹ dry soil, respectively) with the other crops having intermediate results. Variation among treatments was much less for the other soil layers (2.9–20.7 nmol 16:1ω5 NLFA g⁻¹ dry soil).
where the abundance of AMF in the soil under perennial grasses was similar to the soil under corn at 5–15 cm and corn and grain sorghum at 15–30 cm. Photoperiod sensitive and grain sorghum also had the lowest concentrations of AMF biomarker in these soil layers, although the photoperiod sensitive sorghum did not differ from some of the annual crops at the 15–30 cm. As noticed for saprophytic fungi, AMF was also positively correlated with root density (Table 2). The F/B ratio also had a significant interaction between crop and soil depth (Table 1). Miscanthus had higher F/B ratio (0.49–0.56) than all annual crops (0.07–0.24), regardless of soil depth, and switchgrass at the 0–5 and 5–15 cm soil layers (Fig. 4B). Although the F/B ratio remained similar among soil layers under miscanthus, no consistent pattern was observed for the other treatments. Correlation between F/B and root density was not significant (Table 2).

**Water-Stable Aggregate Distribution**

There was a significant interaction of crop and soil depth regarding the distribution of water-stable aggregates (WSA) (Table 1). Differences among cropping systems were limited to the surface soil layer (0–5 cm) where miscanthus and switchgrass had more macroaggregates (>250 µm) than the other crops (Figure S1). In switchgrass and miscanthus soils, macroaggregates were significantly less at 5–15 cm than at 0–5 cm, and macroaggregates in soils under grain sorghum and sweet sorghum were significantly greater at 5–15 cm than at 0–5 cm (data not shown). In contrast, the mean weight diameter of WSA was not affected by soil depth ($P = 0.7394$, Table 1). Nonetheless, the mean weight diameter of WSA averaged over sampling depth in the soil with miscanthus (574 µm) and switchgrass (519 µm) were greater than those in the soil of the annual crops (339–412 µm) (Fig. 5A). Aggregate size (MWD) was positively correlated with root density, AMF, and saprophytic fungi abundance as well.
with F/B ratio only at the 0–5 cm soil layer, but showed significant positive correlation with SOC concentrations across both 0–5 and 5–15 cm sampling depths (Table 2).

Macroaggregate (>250 µm) SOC varied significantly among crops (Table 1). Miscanthus contained significantly greater macroaggregate SOC than all annual crops but was not significantly different from macroaggregate SOC in switchgrass (Fig. 5B). Macroaggregate SOC in switchgrass was significantly greater than in continuous corn, PS sorghum, sweet sorghum, and grain sorghum, but was not significantly different from rotated corn. Averaged across crops, macroaggregate SOC was significantly greater at 0–5 cm than at 5–15 cm (data not shown).

### Soil Organic Carbon Stocks

Cropping system and sampling year had a significant single effect and interaction on SOC stocks to a 15-cm soil depth (Table 3). Tests of effect slices detected increasing differences among cropping systems over time ($P = 0.5999$ in 2009, $P = 0.0025$ in 2014, and $P \leq 0.0001$ in 2017) for SOC stocks measured in the 0–5 cm soil depth (Fig. 6). These differences

**Table 2. Pearson correlation coefficients among soil organic carbon (SOC), root density (Roots), arbuscular mycorrhizae (AMF), saprophytic fungi (Fungi), fungi/bacteria ratio (F/B), and aggregate mean-weight diameter (MWD) as measured in 2014 in the 0–5, 5–15, and 15–30 cm soil depths. Analyses were performed across all crop systems and soil depths unless stated otherwise. Values significant at the 0.05 or 0.001 probability levels are bolded.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>SOC (g kg$^{-1}$)</th>
<th>Roots (mg cm$^{-2}$)</th>
<th>AMF (nmol g$^{-1}$)</th>
<th>Fungi (nmol g$^{-1}$)</th>
<th>F/B</th>
<th>MWD (µm)</th>
</tr>
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<tr>
<td>SOC</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Roots</td>
<td></td>
<td>0.549**</td>
<td>–</td>
<td>0.265*</td>
<td>0.474†</td>
<td>0.474†</td>
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<tr>
<td>AMF</td>
<td></td>
<td>–</td>
<td>0.603***</td>
<td>–</td>
<td>–</td>
<td>0.529**†</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
<td>–</td>
<td>–</td>
<td>0.862**</td>
<td>–</td>
<td>0.464‡</td>
</tr>
<tr>
<td>F/B</td>
<td>–0.141</td>
<td>0.179</td>
<td>0.320*</td>
<td>0.500*</td>
<td>–</td>
<td>0.436‡</td>
</tr>
<tr>
<td>MWD</td>
<td>0.474†‡</td>
<td>0.529**†‡</td>
<td>0.464‡</td>
<td>0.436‡</td>
<td>0.559**‡</td>
<td></td>
</tr>
</tbody>
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*,** Significant at the 0.05 and 0.001 probability levels, respectively.
† Correlation across 0–5 and 5–15 cm depths.
‡ Correlation at 0–5 cm depth only.
were due to SOC accumulation in the miscanthus ($P \leq 0.0001$) and switchgrass ($P = 0.0004$) in the same depth (Table 3). The SOC accumulation rate between 2009 and 2017 in the 0–15 cm soil layer was 0.8 and 1.3 Mg C ha$^{-1}$ yr$^{-1}$ for switchgrass and miscanthus, respectively (Table 4). No differences on SOC stocks were detected for the other soil depths regardless of treatment or sampling year. Root density and the MWD of soil aggregates were strongly correlated ($P < 0.001$) with SOC in 2014 ($r = 0.549$ and 0.474, respectively) (Table 2). A positive relationship ($P < 0.05$) was also found between AMF and SOC ($r = 0.265$) as well as saprophytic fungi and SOC ($r = 0.298$) within the 0–30 cm soil depth.

**DISCUSSION**

The aboveground biomass yields for the entire experimental period are discussed in detail by Roozeboom et al. (2018). The corn and sorghum root stocks as measured in 2013 in our study were within ranges reported elsewhere (Amos and Walters, 2006; Monti and Zatta, 2009; Schittenhelm and Schroetter, 2014). The estimated mean belowground biomass of corn in the reproductive stages from eight studies included in a meta-analysis of Amos and Walters (2006) was 2.1 Mg ha$^{-1}$, with a maximum of 3.2 Mg ha$^{-1}$. Most studies sampled to a 60 or 90 cm soil depth. The root stocks measured in our study were slightly greater with 3.5 and 4 Mg ha$^{-1}$ to a 90 cm depth for corn in rotation with soybean and continuous corn, respectively. Schittenhelm and Schroetter (2014) observed root dry weights in corn and sweet sorghum (3–4.3 Mg ha$^{-1}$; 3.8–4.4 Mg ha$^{-1}$ at 0–30 cm, respectively), which were slightly more than corn and sorghum root stocks at equivalent depth in this study. Monti and Zatta (2009) measured root biomass of 2.1 Mg ha$^{-1}$ in fiber sorghum at 0–120 cm, which was 27–52% less than observed root stocks (0–90 cm) of the different sorghum cultivars used in this study.

The root biomass of switchgrass was within the range reported in other studies (Garten et al., 2010; Ma et al., 2001; Ontl et al., 2013; Wayman et al., 2014; Kibet et al., 2016). In contrast, miscanthus root stocks measured in this study were greater than values reported elsewhere, which varied from 4.8 to 16.8 Mg ha$^{-1}$ (Neukirchen et al., 1999; Monti and Zatta, 2009; Richter et al., 2015; Kibet et al., 2016). The greater root stocks in this study may be due to differences in the methodology of root sampling as well as the age and density of miscanthus. Monti and Zatta (2009) took root samples from the inter-row. Not taking samples directly above the plant may partly explain the lower root stocks observed in this study. Neukirchen et al. (1999) estimated root stocks by sampling both directly under and in-between plants, then calculating an area-weighted average. Plants were distributed in clumps 90–120 cm apart. The greater density of miscanthus plants in the present study, which were evenly spaced with 5–8 cm between individual plants, may have led to larger root stocks. Richter et al. (2015) observed miscanthus clumps spaced 65 cm apart, but their reported values of root stocks measured directly under plants were still lower than those in the present study. The miscanthus stand measured in Richter et al. (2015) was much older at the time of root sampling. The authors noted that yields had been declining, which may have also resulted in lower root stocks.

Similar to other studies, switchgrass tended to have more roots (11% of total root biomass or 2.7 Mg ha$^{-1}$ in the 30–90 cm depth) deeper in the soil than the annual crops (4–7% or < 0.5 Mg ha$^{-1}$) and miscanthus (6% or 2.0 Mg ha$^{-1}$). Garten et al. (2010) found 69% of live roots to be in the top 30 cm of switchgrass. Monti and Zatta (2009) found that 90% of miscanthus root stocks were above 35 cm, while switchgrass only had 35%. Richter et al. (2015) found 78% of miscanthus roots in the top 30 cm. Studies have found that 70–90% of corn roots are in the upper 15–30 cm (Aina and Fapohunda, 1986; Crozier and King, 1993; Osaki et al., 1995; Dwyer et al., 1996). Root biomass, along with dissolved and particulate C is the main C sources for subsoil SOC (Rumpel and Kögel-Knabner, 2011). Although root penetration providing fresh C inputs into subsoil layers may stimulate the decomposition of stable SOC pools (Fontaine et al., 2007; Shahzad et al., 2018), C input from corn roots was found to contribute more to deep SOC than prairie roots (Dietzel et al., 2017). Recent studies also suggested that greater root biomass inputs by switchgrass impacts SOC and...
soil microbial communities throughout the soil profile (Kibet et al., 2016; Roosendaal et al., 2016; Stewart et al., 2017).

We investigated the impacts of bioenergy crops on soil microbial communities with implications on soil health as well as soil carbon storage and stabilization. The perennial grasses (miscanthus and switchgrass) had greater microbial biomass as well as elevated levels of saprophytic fungi and AMF in comparison with the annual crops, though differences among treatments were consistently significant only in miscanthus. Cotton et al. (2013) reported increased soil microbial biomass C and N, 16:1ω5c FAME targeting AMF, and enzyme activity targeting C, N, P, and S cycles 2 yr after the conversion of continuous cotton to sorghum biofuel cropping systems under conventional tillage. Nonetheless, the abundance of 18:2ω6c FAME targeting saprophytic fungi decreased under sorghum biofuel cropping system (Cotton et al., 2013). Jesus et al. (2015) and Liang et al. (2012) measured microbial communities in mixed prairie, switchgrass, and corn. They found greater, but not consistently significant, amounts of total lipids in perennial grasses compared to continuous corn. Both studies observed elevated levels of AMF in perennial systems compared to corn, but only Liang et al. (2012) observed a significant response. Liang et al. (2012) observed a significant increase in gram-negative bacteria under mixed prairie. No other significant differences in bacterial groups were observed in either study.

Stewart et al. (2017) and Roosendaal et al. (2016) observed that different switchgrass cultivars may select specific microbial communities, where rhizodeposition under Kanlow (upland switchgrass cultivar) was selective for gram-negative bacteria (44%), while the preferred group was saprotrophic fungi (48%) for rhizodeposition from Summer (lowland cultivar). The authors concluded that cultivar-specific microbial communities can direct rhizodeposition C flow and SOC accrual in the soil profile.

Schrama et al. (2016) assessed soil biota, microbial communities, aggregate stability, SOC storage, and N mineralization in plots with corn, switchgrass, miscanthus, and willow (Salix fragilis) cultivated for biofuel production in Belgium. Switchgrass had significantly higher proportion of small aggregates (250–800 µm) than corn and miscanthus. The fungi/bacteria ratio was slightly high, but not significantly so, in switchgrass and miscanthus than...
in corn. There were no significant differences in SOC concentration between switchgrass, miscanthus, and corn. The lack differences in SOC concentration, fungi/bacteria ratio, and aggregates observed by Schrama et al. (2016) may be related the cooler, drier climate of the study site and the coarser soil texture (sandy loam), as compared to the present study. These results support the notion that increased fungi and total microbial biomass may favor SOC stabilization within soil aggregates augmenting SOC storage under perennial bioenergy crops, but these relationships may not be consistent across climate and soil conditions (Roosendaal et al., 2016; Stewart et al., 2017; Schrama et al., 2016).

We also observed that perennial grasses, and especially miscanthus, tended to have greater saprophytic and AMF biomass, fungi/bacteria ratio, greater mean weight diameter of soil aggregates, and macroaggregate SOC than the annual crops (Fig. 3, 4, and 5). Both Tiemann and Grandy (2015) and Ontl et al. (2015) observed elevated macroaggregates in switchgrass compared to corn. However, Tiemann and Grandy (2015) observed no significant differences in macroaggregates of miscanthus and corn 4 yr after miscanthus establishment. Dondini et al. (2009) found significantly more macroaggregates in miscanthus compared to conventionally tilled cropland. O’Brien and Jastrow (2013) found macroaggregate levels of restored prairie at levels found in native prairie within 3 yr after conversion to prairie vegetation. Mikha et al. (2010) observed significantly more macroaggregates under perennial grasses compared to various conventional and no-tillage crop rotations.

The SOC stocks increased in the soil surface layers (0–15 cm) with both perennial grasses but no changes were noticed for the annual crops (Table 4). No statistical differences for SOC stocks for the whole soil profile (0–90 cm) were detected for any of the tested treatments. A power analysis of previously published SOC concentration and stock measurements by Kravchenko and Robertson (2011) found that the low number of replicates used in most experiment studies (3–6) is insufficient to detect whole-profile changes in SOC and demonstrated that changes in SOC stocks that were detectable in the surface soil layers were undetectable when considering the whole-profile. Kravchenko and Robertson (2011) recommended the construction of whole-profile changes in SOC stocks should only be based on the results from layers where statistically significant differences are detected. The number of replicates in the present study were likely insufficient to detect whole-profile changes in SOC stocks.

Several characteristics of the perennial grass systems may have contributed to the increase in SOC stocks at the 0–15 cm. Root biomass is considered one of the most important factors predicting potential C sequestration, so soils in perennial grasses tend to have higher SOC stocks compared to annuals (Lemus and Lal, 2005). These differences tend to be greatest in the top 30 cm of the soil where a majority of roots occur, but may also occur deeper in the profile (Lemus and Lal, 2005). Evidence of increased fungal biomass and greater WSA mean weight diameter in the perennial grasses suggest that increased protection of SOC within soil aggregates may be contributing to the increase in SOC as observed in switchgrass and miscanthus treatments from 2009 to 2017. Additionally, indicators of fungal biomass (AM, fungi, and F/B) were positively correlated with aggregate size, as was aggregate size and SOC in 2014. Increased AMF abundance has been found to increase the production of macroaggregates, which can contribute to the stabilization of SOC (Jastrow et al., 2007; Miller and Jastrow, 2000; Six et al., 2002; Wilson et al., 2009).

Many studies have reported elevated SOC stocks under perennials such as miscanthus and switchgrass compared to annual cropping systems (Lemus and Lal, 2005; Dondini et al., 2009; Monti et al., 2012; Cattaneo et al., 2014). In switchgrass, SOC sequestration rates of 0–1 Mg ha⁻¹ yr⁻¹ are common, though rates as high as 4 Mg ha⁻¹ yr⁻¹ have been observed (Monti et al., 2012). We observed a significant SOC sequestration rate of 0.8 Mg ha⁻¹ yr⁻¹ for the 0–15 cm soil layer between 2009 and 2017 with switchgrass (Table 4), within the range.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>0–5</th>
<th>5–15</th>
<th>15–30</th>
<th>30–45</th>
<th>45–60</th>
<th>60–75</th>
<th>75–90</th>
<th>0–15</th>
<th>0–30</th>
<th>0–60</th>
<th>0–90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil mass (Mg ha⁻¹)</td>
<td>700</td>
<td>1,400</td>
<td>2,100</td>
<td>2,100</td>
<td>2,100</td>
<td>2,100</td>
<td>2,100</td>
<td>2–100</td>
<td>0–4200</td>
<td>0–8400</td>
<td>0–12600</td>
</tr>
</tbody>
</table>

Table 3. P-values from the Type III test for fixed effects of crop on root density, and the effects of crop, sampling year and their interaction on SOC stocks in equivalent soil masses. P < 0.05 are bolded.

In corn. There were no significant differences in SOC concentration between switchgrass, miscanthus, and corn. The lack differences in SOC concentration, fungi/bacteria ratio, and aggregates observed by Schrama et al. (2016) may be related the cooler, drier climate of the study site and the coarser soil texture (sandy loam), as compared to the present study. These results support the notion that increased fungi and total microbial biomass may favor SOC stabilization within soil aggregates augmenting SOC storage under perennial bioenergy crops, but these relationships may not be consistent across climate and soil conditions (Roosendaal et al., 2016; Stewart et al., 2017; Schrama et al., 2016).

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Fig. 6. Soil organic carbon stocks in equivalent soil masses as affected by biofuel crops, sampling year, and soil depth. Error bars represent standard errors, and the thick horizontal bar indicate the least significant difference (LSD) values for the comparison of crops within the same year.
Johnson et al. (2014) estimated that roughly 5.7 Mg ha⁻¹ of in conventional and no-tillage corn after 11 yr of residue removal.

Osborne et al. (2014) no significant change in two locations under conventional till-age. Osborne et al., 2014). Villamil et al. (2015) observed decreases in no-tillage systems (Wilhelm et al., 2004; Johnson et al., 2014; stover were found to have a negative impact on SOC stocks, even for bioenergy production. High residue removal rates of corn tillage, but all standing biomass was removed to simulate harvest of sampling depth. In this study, annual crops were under no-tillage, providing high volumes of feedstock for biofuel production.

Stover would be needed to maintain SOC stocks.

In contrast to the perennial crops, no significant changes in SOC were observed for any of the annual crops regardless of sampling depth. In this study, annual crops were under no-tillage, but all standing biomass was removed to simulate harvest for bioenergy production. High residue removal rates of corn stover were found to have a negative impact on SOC stocks, even in no-tillage systems (Wilhelm et al., 2004; Johnson et al., 2014; Osborne et al., 2014). Villamil et al. (2015) observed decreases in SOC after 8 yr of residue removal in no-tillage corn at three locations in Illinois. Kenney et al. (2015) observed lower SOC levels after 2 yr of residue removal in no-tillage corn but observed no significant change in two locations under conventional tillage. Osborne et al. (2014) observed significant decreases in SOM in conventional and no-tillage corn after 11 yr of residue removal. Johnson et al. (2014) estimated that roughly 5.7 Mg ha⁻¹ of stover would be needed to maintain SOC stocks.

However, the amount of aboveground biomass inputs necessary to maintain SOC stocks at steady state levels clearly depends on SOC levels as well on soil tillage, fertilization, residue quality, and other crop management practices (Nicoloso et al., 2016, 2018; Ogle et al., 2012; Poffenbarger et al., 2017; Stewart et al., 2007). Another study performed < 200 m to our plots, found that long-term cultivation (>60 yr) of this experimental area for small grain production under intensive tillage practices decreased original SOC under prairie vegetation by 64% in the 0–30 cm depth (Nicoloso et al., 2018). The SOC stocks found in that area were similar to SOC measured in 2009 in the plots included in our study, and also remained stable with continuous corn under chisel tillage and residues retained during the whole experimental period (25 yr). In contrast, returning of corn residues under no-tillage promoted significant SOC accrual in the 0–5 cm depth (Nicoloso et al., 2018). Thus, the complete removal of crop residues for biofuel production had limited effect on the already depleted SOC levels as noticed in our study. Furthermore, these results suggest that improved harvesting practices, where only a fraction of the aboveground biomass is removed, would result in sufficient stover to protect soils from erosion and SOC loss, while still providing high volumes of feedstock for biofuel production.

**CONCLUSIONS**

Perennial cropping systems with switchgrass and miscanthus used for biofuel production increased SOC at respective rates of 0.8 and 1.3 Mg C ha⁻¹ yr⁻¹ in the 0–15 cm depth between 2009 and 2017. However, no statistical differences on SOC stocks were detected for the whole soil profile (0–90 cm). Although the total removal of aboveground residues was expected to negatively affect SOC in annual cropping systems, even under no-tillage management, no changes on SOC stocks were observed after 10 yr. Thus, our results suggest that the adoption of either improved harvesting practices, where a small percentage of aboveground biomass is left on the field, or the establishment of highly productive cropping systems would result in sufficient stover to protect soils from erosion and SOC loss, while still providing high volumes of feedstock for biofuel production.

### Table 4. Soil organic carbon stocks in equivalent soil masses (cumulative) as affected by biofuel crops, sampling year, and soil profile depth.

<table>
<thead>
<tr>
<th>Year</th>
<th>Corn (cont.)†</th>
<th>Corn (rot.)†</th>
<th>PS sorghum</th>
<th>Sweet sorghum</th>
<th>Grain sorghum</th>
<th>Miscanthus</th>
<th>Switchgrass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg C ha⁻¹</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>0–5 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>9.4 ± 0.9</td>
<td>8.6 ± 0.8</td>
<td>9.7 ± 1.0</td>
<td>10.1 ± 1.3</td>
<td>10.2 ± 0.9</td>
<td>8.5 ± 0.8</td>
<td>9.6 ± 0.9</td>
</tr>
<tr>
<td>2014</td>
<td>9.8 ± 2.0 c‡</td>
<td>10.4 ± 0.6 bc</td>
<td>10.5 ± 0.3 bc</td>
<td>9.4 ± 0.5 c</td>
<td>10.2 ± 0.3 bc</td>
<td>13.7 ± 1.0 aA</td>
<td>12.2 ± 0.7 abA</td>
</tr>
<tr>
<td>2017</td>
<td>9.4 ± 1.2 b</td>
<td>9.6 ± 0.8 b</td>
<td>11.0 ± 0.7 b</td>
<td>10.3 ± 1.0 b</td>
<td>9.8 ± 0.8 b</td>
<td>14.6 ± 1.6 aA</td>
<td>14.2 ± 1.4 aA</td>
</tr>
<tr>
<td>0–15 cm</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>2009</td>
<td>24.0 ± 2.0</td>
<td>22.8 ± 1.1</td>
<td>24.2 ± 1.8</td>
<td>24.3 ± 2.0</td>
<td>24.6 ± 0.9</td>
<td>21.6 ± 1.8 B</td>
<td>24.0 ± 2.1 B</td>
</tr>
<tr>
<td>2014</td>
<td>24.4 ± 4.3 bc</td>
<td>24.5 ± 1.0 bc</td>
<td>24.3 ± 0.7 bc</td>
<td>23.4 ± 0.9 c</td>
<td>23.7 ± 0.5 bc</td>
<td>29.4 ± 2.3 aA</td>
<td>26.9 ± 2.0 abAB</td>
</tr>
<tr>
<td>2017</td>
<td>25.4 ± 3.7 b</td>
<td>24.6 ± 1.5 b</td>
<td>25.7 ± 0.7 b</td>
<td>25.3 ± 1.7 b</td>
<td>24.4 ± 0.6 b</td>
<td>32.2 ± 2.6 aA</td>
<td>30.6 ± 2.4 aA</td>
</tr>
<tr>
<td>0–30 cm</td>
<td></td>
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<tr>
<td>2009</td>
<td>46.5 ± 2.7</td>
<td>44.9 ± 1.4</td>
<td>45.5 ± 4.4</td>
<td>44.8 ± 3.2</td>
<td>43.7 ± 2.2</td>
<td>42.9 ± 3.5</td>
<td>45.8 ± 3.3</td>
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<tr>
<td>2014</td>
<td>46.9 ± 5.6</td>
<td>46.9 ± 1.5</td>
<td>44.8 ± 3.0</td>
<td>45.2 ± 2.3</td>
<td>45.0 ± 1.4</td>
<td>49.2 ± 3.9</td>
<td>48.7 ± 2.8</td>
</tr>
<tr>
<td>2017</td>
<td>50.4 ± 5.0</td>
<td>49.5 ± 1.9</td>
<td>48.2 ± 2.9</td>
<td>49.9 ± 3.0</td>
<td>46.8 ± 1.1</td>
<td>54.9 ± 4.7</td>
<td>53.2 ± 3.5</td>
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<tr>
<td>0–60 cm</td>
<td></td>
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<td></td>
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<tr>
<td>2009</td>
<td>87.7 ± 4.3</td>
<td>91.3 ± 4.6</td>
<td>88.4 ± 10.3</td>
<td>86.1 ± 7.7</td>
<td>82.7 ± 6.6</td>
<td>79.2 ± 10.6</td>
<td>88.1 ± 7.9</td>
</tr>
<tr>
<td>2014</td>
<td>82.8 ± 12.7</td>
<td>88.2 ± 4.7</td>
<td>83.0 ± 9.3</td>
<td>89.5 ± 6.0</td>
<td>76.7 ± 3.4</td>
<td>85.0 ± 11.0</td>
<td>89.4 ± 5.5</td>
</tr>
<tr>
<td>2017</td>
<td>92.9 ± 9.9</td>
<td>95.1 ± 6.5</td>
<td>98.5 ± 6.6</td>
<td>101.4 ± 6.3</td>
<td>79.8 ± 4.4</td>
<td>97.1 ± 12.1</td>
<td>101.1 ± 9.6</td>
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<tr>
<td>0–90 cm</td>
<td></td>
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<tr>
<td>2009</td>
<td>116.2 ± 4.7</td>
<td>117.8 ± 4.7</td>
<td>117.3 ± 14.6</td>
<td>116.1 ± 11.6</td>
<td>106.0 ± 9.0</td>
<td>103.6 ± 16.0</td>
<td>115.7 ± 10.1</td>
</tr>
<tr>
<td>2014</td>
<td>107.4 ± 17.9</td>
<td>109.7 ± 5.9</td>
<td>106.4 ± 11.8</td>
<td>115.9 ± 7.7</td>
<td>94.8 ± 4.5</td>
<td>106.5 ± 14.1</td>
<td>111.5 ± 6.9</td>
</tr>
<tr>
<td>2017</td>
<td>123.0 ± 14.3</td>
<td>119.7 ± 8.2</td>
<td>127.2 ± 7.0</td>
<td>128.6 ± 8.5</td>
<td>99.5 ± 4.9</td>
<td>121.0 ± 15.6</td>
<td>126.8 ± 12.1</td>
</tr>
</tbody>
</table>

† cont., continuous corn; rot., corn in rotation with soybean.
‡ Mean ± standard error (n = 4). Lowercase letters compare biofuel crops within years and uppercase letters compare years within biofuel crops at the same soil depth (LSM P < 0.05)
This study found evidence of several mechanisms that might enhance the stability of SOC in the upper soil layers of perennial crops. Root stocks were 4–8 times larger in the perennial crops, suggesting greater belowground C inputs. Additionally, evidence of elevated AM fungi and increased aggregate size in the perennial crops suggests physical protection of SOC in these soil layers may be enhanced in these systems, especially in miscanthus. The increases in SOC and aggregate size in the perennial systems suggest that these systems have the potential to improve soil health while providing feedstock for biofuel production, which could be important in cases where these crops are utilized on marginal lands.

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