

The impacts of four potential bioenergy crops on soil carbon dynamics as shown by biomarker analyses and DRIFT spectroscopy

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Abstract

Perennial bioenergy crops accumulate carbon (C) in soils through minimally disturbing management practices and large root inputs, but the mechanisms of microbial control over C dynamics under bioenergy crops have not been clarified. Root-derived C inputs affect both soil microbial contribution to and degradation of soil organic matter resulting in differing soil organic carbon (SOC) concentrations, storage, and stabilities under different vegetation regimes. Here, we measured biomarker amino sugars and neutral sugars and used diffuse reflectance mid-infrared Fourier transform spectroscopy (DRIFTS) to explore microbial C contributions, degradation ability, and SOC stability, respectively, under four potential bioenergy crops, *M. × giganteus* (*Miscanthus × giganteus*), switchgrass (*Panicum virgatum* L.), a mixed prairie, and a maize (*Zea mays* L.)–maize–soybean (*Glycine max*(L.) Merr.) (MMS) rotation over six growing seasons. Our results showed that SOC concentration (g/kg) increased by 10.6% in mixed prairie over the duration of this experiment and SOC storage (Mg/ha) increased by 17.0% and 15.6% in switchgrass and mixed prairie, respectively. Conversion of row crops to perennial grasses maintained SOC stability and increased bacterial residue contribution to SOC in *M. × giganteus* and switchgrass by 20.0% and 15.0%, respectively, after 6 years. Degradation of microbe-derived labile SOC was increased in *M. × giganteus*, and degradation of both labile and stable SOC increased in MMS rotation. These results demonstrate that microbial communities under perennial grasses maintained SOC quality, while SOC quantity increased under switchgrass and mixed prairie. Annual MMS rotation displayed decreases in aspects of SOC quality without changes in SOC quantity. These findings have implications for understanding microbial control over soil C quantity and quality under land-use shift from annual to perennial bioenergy cropping systems.

KEYWORDS

amino sugars, biomarker, diffuse reflectance mid-infrared Fourier transform spectroscopy, maize–maize–soybean rotation, microbial residue, neutral sugars, perennial bioenergy crops, soil organic carbon decomposition, soil organic carbon stability

1 | INTRODUCTION

Bioenergy production from agriculture is a potential source of renewable energy but will require the conversion of land from current uses to appropriate bioenergy feedstocks (Field, Campbell, & Lobell, 2008; International Energy Agency, 2013). The development of cellulosic biofuels is expected to reduce competition with food production systems, and certain cellulosic bioenergy feedstocks, particularly perennial grasses, may provide a number of ecological benefits (Robertson et al., 2017). For example, the conversion of annual row crops to perennial bioenergy grasses such as *Miscanthus* × *giganteus* (*M.×giganteus*), *Panicum virgatum* (switchgrass), and mixed-species prairie may sequester soil carbon (C) through decreased tillage (Anderson-Teixeira, Davis, Masters, & Delucia, 2009) and a larger contribution of root material than annual crops (Black, Masters, Lebauer, Anderson-Teixeira, & Delucia, 2017).

There is, however, considerable uncertainty whether the stimulation of microbial activity by the addition of root labile C will result in increased degradation and loss of soil organic carbon (SOC). The contemporary understanding of soil organic matter (SOM) formation suggests that the precursors of SOM are microbial products converted from labile plant constituents (Cotrufo, Wallenstein, Boot, Deneff, & Paul, 2013; Miltner, Bombach, Schmidt-Brücken, & Kästner, 2012). The addition of labile plant-derived C can accelerate SOC sequestration by increasing microbial biomass and the resulting microbial necromass, part of which can be relatively recalcitrant and likely to be physically protected (Liang & Balser, 2011; Liang, Cheng, Wixon, & Balser, 2011; Plaza, Courtier-Murias, Fernández, Polo, & Simpson, 2013; Six, Frey, Thiet, & Batten, 2006). Alternatively, stimulating the activity and size of the soil microbial community can increase the rate of decomposition of existing SOM through higher C use efficiency (CUE) or nutrient acquisition from the soil (Chen et al., 2014; Kuzyakov, 2010). To elucidate the mechanisms of microbial control over C dynamics in bioenergy cropping systems, both microbial synthesis that enhances microbe-derived C deposition into SOC pools and microbial degradation that diminishes existing SOC should be simultaneously considered (Liang, Schimel, & Jastrow, 2017).

Litter decomposition, C transformations in the soil, and SOM formation are impacted by the balance between microbial consumption of labile C and competition for nitrogen (N) (Chen et al., 2014; Cotrufo et al., 2013). With abundant labile C, high N availability generally favors fast-growing bacteria to decompose exogenous plant residue, while low N availability has been shown to favor slow-growing fungi and bacteria that consume SOM for N acquisition (Chen et al., 2014; Fontaine, Mariotti, & Abbadie, 2003; Moorhead & Sinsabaugh, 2006). With limited labile C, microbes are forced

to consume existing SOC for survival (Fontaine, Bardoux, Abbadie, & Mariotti, 2004). According to a previous study, root exudates provided the main nutrient source to stimulate microbial growth in a switchgrass bioenergy system (Mao, Li, Smyth, Yannarell, & Mackie, 2014). However, in a maize system, microbes in the rhizosphere mainly degraded relatively recalcitrant C (Li et al., 2014). From these findings, we can speculate that the microbial communities have more access to labile C in perennial grasses than in annual crops. In the N-fertilized maize system, the population of ammonia-oxidizing bacteria (AOB) increased in magnitude and N fixer abundance decreased compared to unfertilized perennials (Mao, Yannarell, & Mackie, 2011), indicating N fertilizer use can lead to differences in microbial community structure and resulting C dynamics in bioenergy systems.

The objective of this research was to explore microbial controls on C dynamics in three potential perennial bioenergy cropping systems, *M.×giganteus*, switchgrass, and a native prairie assemblage, as well as an annual maize–soybean rotation. We measured SOC concentrations and storage over six growing seasons and assessed how bioenergy crops impact microbial residue contribution to SOC and microbial degradation of labile C of different origins using amino sugar and neutral sugar analyses, respectively (Cui et al., 2016; Liang, Duncan, Balser, Tiedje, & Jackson, 2013). We also assessed the degree of existing SOC decomposition as well as SOC stability using diffuse reflectance mid-infrared Fourier transform spectroscopy (DRIFTS) (Demyan et al., 2012; Margenot & Hodson, 2016). Considering the respective root C inputs, fertilization regimes, and management conditions of each bioenergy cropping system, we predict that in the fertilized perennial grass switchgrass, microbial residue C accumulation and microbial degradation of plant-derived C will both increase, ultimately resulting in an increase in SOC stability and magnitude. In the unfertilized perennials *M.×giganteus* and mixed prairie, microbial residue C accumulation and microbial degradation of existing SOM will both increase, ultimately resulting in little change in SOC stability and magnitude. As N availability influences microbial community structure, we also predict changes to the relative dominance of fungal and bacterial residue contributions in SOC in fertilized and unfertilized perennials. In the annual system with regular tillage, we predict there will be less microbial residue C accumulation in soil, and more degradation of existing SOC, resulting in depletion of SOC.

2 | MATERIALS AND METHODS

2.1 | Study site

This research was conducted at the University of Illinois Energy Farm, Urbana, IL, USA, which is 220 m above sea

level (40.06°N, 88.19°W). The site has a mean annual temperature and precipitation of 11.1°C and 1,042 mm, respectively (Zeri et al., 2011). Prior to planting with potential bioenergy feedstocks, the land was managed for over 100 years with maize and soybean, conventional annual row crops. Soils at this site are all Argiudolls, primarily Dana silt loams with some Flanagan and Blackberry silt loams. These soils are characterized as fine, silty, mixed, superactive, mesic Oxyaquic Argiudolls (Dana and Blackberry) and fine, smectitic, mesic Aquic Argiudolls (Flanagan) and are typical of the region.

The field experiment was initiated in May of 2008 in a randomized blocked experimental design with four blocks of 0.7 ha plots and a fifth of 3.8 ha plots [sized to provide adequate fetch for eddy covariance towers as described in Zeri et al. (2011)]. Each block was planted with a potential bioenergy cropping system including *M. × giganteus* (*Miscanthus × giganteus*), switchgrass (*Panicum virgatum* L. “Cave in Rock”), a restored native prairie assemblage of 28 species of grasses and forbs, including a small percentage of N-fixing species [see Zeri et al. (2011) for complete species list], and a MMS (maize–maize–soybean) rotation (*Zea mays* L., *Glycine max* L.). The row crop system received conventional tillage (chisel plow) prior to each maize planting and received 28% UAN as N fertilizer at 168 kg N/ha and 202 kg N/ha in the spring prior to first- and second-year maize crops, respectively. Soybean years were unfertilized, and the soil was prepared with minimal soil disturbance (sunflower cultivator). Row spacing of annuals and initial *M. × giganteus* rhizome planting was 75 cm.

In the early stages of cellulosic bioenergy crop development in the United States, switchgrass was selected as a potential feedstock due to its characteristics as a high-yielding native. At the same time, *M. × giganteus* was under consideration in Europe, where it was higher yielding, though non-native (Heaton, Dohleman, & Long, 2008). The Energy Farm design allows for examination of the two crops side by side, along with maize and soybean, the current leaders in bioenergy feedstock production in the United States. The prairie assemblage was included to represent historic vegetation in central Illinois, a landscape similar to precultivation ecology of the region. Although low yielding compared to the monocultures, prairie was considered for as a feedstock due to interest in bioenergy production from a natural or native ecosystem. All crops presented here were managed according to the best-known agricultural practices in the region, resulting in different management among crops. As large-scale production of these crops represents an important agricultural commodity, this experimental design was established to reflect crops as they would actually be grown, rather than using identical plot management. We acknowledge that this experimental design limits our ability to directly compare differences

between crop types; however, it creates conditions that are closer to reality among field-grown crop ecosystems.

M. × giganteus was initially planted in 2008 and replanted in 2009 in the 0.7 ha plots and in 2010 in the 3.8 ha plots due to poor establishment as described in Smith et al. (2013). Switchgrass received an annual spring application of granular urea at 56 kg N/ha from 2010 onward. *M. × giganteus* and prairie plots were not fertilized for the duration of this experiment. The aboveground tissues were harvested in August and September each year near the time of maximum aboveground biomass (assumed to correspond with maximum leaf area index). Further site information, plot layout, and preplanting soil information (% C, % N, pH, bulk density) can be found in Masters et al. (2016).

2.2 | Soil sampling and processing

Soil was sampled at ten random locations within each 0.7 ha plot in April of 2008, 2010, and 2014. Five cores were taken within each location to a depth of 10 cm and pooled to form a composite sample. From these ten locations, four locations were chosen at random to conduct soil physicochemical measurements. Samples were air-dried and crushed (Dynacrush, Soil Custom Laboratory Equipment) to pass a 2 mm sieve. Bulk samples were then subsampled and ground to a fine powder using coffee grinders (Mr. Coffee, Sunbeam Products Inc.).

2.3 | Soil organic carbon concentration

Total soil carbon (TC) was analyzed using an elemental analyzer (Costech 4010 CHNSO Analyzer, Costech Analytical Technologies Inc. Valencia, California, USA). Soil was combusted with O₂ within a tin capsule, elements were separated using a GC column, and peaks were analyzed using the EA thermal conductivity detector. Acetanilide was used as a quantitative standard, and apple leaves (NIST) were used as quality control. The reported TC values represent the total soil organic carbon (SOC) concentration because no inorganic C was present from 0 to 10 cm at this site.

2.4 | Soil organic carbon storage

Soil organic C storage (SOCS, Mg/ha) in the 0–10 cm soil was calculated by the following formula:

$$\text{SOCS} = \text{SOC} \times \text{BD} \times \text{Dh} \times \text{Aa}$$

SOC is the soil organic carbon concentration for depth interval (g/kg), BD is the bulk density (g/cm), means of which can be found in Table 1, and Dh is the soil thickness interval. Throughout this study, Dh is 10 cm. Aa is the area of studied plot, which is 0.7 ha for the small blocks and 3.8 ha for the large blocks in the study.

TABLE 1 Means of biomarker analysis and DRIFTS results under potential bioenergy crops from 2008 to 2014

Variable	Maize-maize-soybean			<i>M. × giganteus</i>			Switchgrass			Mixed prairie		
	2008	2010	2014	2008	2010	2014	2008	2010	2014	2008	2010	2014
SOCS Mg/ha	15.21a	16.05a	14.72a	14.93a	14.97a	15.83a	15.73b	17.88a	18.40a	15.02b	17.88a	17.36a
SOC g/kg	17.22ab	18.14a	15.92b	17.21a	15.12b	16.23ab	17.92a	18.28a	19.14a	17.38b	18.64a	19.23a
Bulk density g/cm ³	1.27b	1.27b	1.33a	1.25b	1.42a	1.40a	1.25b	1.40a	1.37a	1.23b	1.37a	1.29b
Amino sugar analysis												
GluN mg/kg	1,165.72a	1,205.67a	1,165.60a	1,165.34a	1,156.88a	1,186.72a	1,230.04a	1,229.30a	1,313.39a	1,220.47b	1,291.74b	1,403.41a
MurA mg/kg	33.38a	39.39a	37.01a	33.81b	37.58a	38.39a	34.99b	36.36b	43.56a	37.17b	39.44ab	44.31a
^A Total AS mg/kg	1,617.21a	1,639.34a	1,575.14a	1,610.01a	1,606.97a	1,595.46a	1,710.77a	1,667.98a	1,786.21a	1,698.31b	1,773.45ab	1,861.38a
AS/SOC %	9.40a	9.09a	9.90a	9.32b	10.62a	9.84b	9.60a	9.18a	9.45a	9.85a	9.54a	9.69a
GluN/SOC %	6.78a	6.70a	7.33a	6.74b	7.65a	7.32a	6.89a	6.77a	6.96a	7.07a	6.95a	7.31a
MurA/SOC %	0.19b	0.22ab	0.23a	0.20b	0.25a	0.24a	0.20b	0.20b	0.23a	0.22a	0.21a	0.23a
GluN/MurA	34.98a	31.03a	31.63a	34.55a	31.08a	31.47a	35.37a	34.34a	30.11b	32.85a	32.95a	31.85a
Neutral sugar analysis												
Rha mg/kg	167.85a	158.79a	140.71a	167.04a	135.99a	142.42a	164.80a	170.63a	184.82a	194.65a	178.25a	185.32a
Fuc mg/kg	61.84a	64.00a	53.44a	65.14a	48.37b	68.04a	61.27b	77.47ab	89.04a	76.68a	77.27a	95.76a
Ara mg/kg	440.80ab	488.32a	397.76b	428.73a	375.71a	467.45a	423.80a	469.98a	495.17a	510.27a	508.60a	556.07a
Xyl mg/kg	301.36a	394.44a	313.76a	297.98b	217.26b	458.06a	310.43b	345.62b	454.36a	344.42b	395.47ab	513.35a
deoxyC6/C5	0.31a	0.25b	0.27b	0.32a	0.31a	0.23b	0.31a	0.31a	0.29a	0.32a	0.29a	0.26a
DRIFTS												
rA ₂₉₃₀	15.58b	17.84a	18.67a	17.06a	16.34a	16.02a	18.72a	17.81a	16.71a	16.78a	16.17a	17.59a
rA ₁₆₂₀	75.38a	73.07b	73.31b	72.99b	74.99ab	75.45a	71.94b	73.53ab	75.29a	71.55a	75.51a	73.93a
rA ₁₆₂₀ /rA ₂₉₃₀	4.87a	4.10b	3.96b	4.35a	4.60a	4.72a	3.88a	4.24a	4.54a	4.34a	4.69a	4.23a

Results in this table are compared within each crop over time, and treatments are not compared to each other. Shared letters within a crop are not significantly different, $p > 0.05$. The bold text highlights variables that had significant changes with time. F -ratio and p values can be found in Supporting information Table S1.

A, sum of glucosamine, muramic acid, and galactosamine; Ara, plant-derived arabinose; AS, amino sugar; C5, pentoses (Xyl+Ara); deoxyC6, deoxyhexoses (Fuc+Rha); DRIFTS, diffuse reflectance Fourier transform mid-infrared spectroscopy; Fuc, microbial-derived fucose; GluN, glucosamine; MurA, muramic acid; rA₁₆₂₀, relative peak areas at 1,620 cm⁻¹; rA₂₉₃₀, relative peak areas at 2,930 cm⁻¹; Rha, microbial-derived rhamnose; SOC, soil organic carbon; SOCS, soil organic carbon storage; Xyl, plant-derived xylose.

2.5 | Soil amino sugars

We determined the content of detectable bulk soil amino sugars including glucosamine (GluN), which is predominantly derived from fungal cell walls, muramic acid (MurA), which is unique to bacteria, and galactosamine (GalN), the origin of which is still debated, as described by Zhang and Amelung (1996), to assess microbial residue contribution to SOC accumulation, which are represented by ratios of amino sugars to SOC (Amelung, Miltner, Zhang, & Zech, 2001; Glaser, Turrión, & Alef, 2004; Liang et al., 2013). Soil samples containing approximately 0.3 mg N were hydrolyzed with 6 M HCL for 8 hr at 105°C. An internal standard (100 µg, myo-inositol) was added to the hydrolysate, which was then washed through a fiberglass filter (GF 6, Schleicher and Schuell, Germany), dried using a rotary evaporator, and redissolved in deionized water. After neutralizing with KOH (pH 6.6–6.8), the samples were centrifuged for 10 min at 935 g and the supernatant was freeze-dried, after which amino sugars were washed out from the residues with methanol and centrifuged for 10 min at 935 g to remove the salts. After complete drying, derivatization reagents were added to the dried residue before heating at 75–80°C to transform amino sugars into aldonitrile derivatives, which were then extracted with 1.5 ml dichloromethane from the aqueous solution. Excess anhydride was removed using 1 mol/L HCL and deionized water. The amino sugar derivatives were finally separated on an Agilent 6890A gas chromatograph (GC, Agilent Tech. Co. Ltd., USA) equipped with an HP-5 fused silica column (25 m by 0.32 mm by 0.25 µm) and a flame ionization detector after being redissolved in 200 µl hexane and ethyl acetate solvent (1:1 v/v).

Amino sugars were quantified relative to the internal standard myo-inositol, which was added to the samples before purification and the recovery standard methyl glucamine, which was added before derivatization. Analysis of amino sugars, which showed only a minor bound in living microbial biomass and relative stability after cell death, can provide insights into the fate and sequestration of C and N in microbial residues and also into the long-term shift in SOM dynamics (Amelung et al., 2001; Glaser et al., 2004; Guggenberger, Frey, Six, Paustian, & Elliott, 1999). The total amino sugar concentration was calculated as the sum of the individual amino sugars (Amelung et al., 2001). The proportion of amino sugar in SOC concentration was used to describe the relative contribution of microbial residue to SOC (Liang, Zhang, & Balsler, 2007). The ratio of GluN/MurA was calculated to elucidate relative dominance of fungal to bacterial residues in SOC (Guggenberger et al., 1999; Six et al., 2006).

2.6 | Soil neutral sugars

To determine the origins of soil labile C, we quantified bulk soil neutral sugars, which consist of plant-derived pentoses (C5), including arabinose (Ara), xylose (Xyl), and ribose (Rib), and microbial derived hexoses (C6), including glucose (Glu), mannose (Man), and galactose (Gal), as well as deoxyhexoses (deoxyC6) fucose (Fuc) and rhamnose (Rha) (Cui et al., 2016; Gunina & Kuzyakov, 2015; Jolivet, Angers, Chantigny, Andreux, & Arrouays, 2006), as described by Zhang, He, and Zhang (2007).

Soil samples containing approximately 4 mg organic C were hydrolyzed with 4 M trifluoroacetic acid (TFA) for 4 hr at 105°C. An internal standard (100 µg, adonitol) was added to the hydrolysate, which was then washed through a fiberglass filter (GF 6, Schleicher and Schuell, Germany), dried using a rotary evaporator, and redissolved in deionized water. After neutralizing with KOH (pH 6.6–6.8), the samples were centrifuged for 10 min at 935 g and the supernatant was dried again using a rotary evaporator. The residues were dissolved in 4 ml distilled H₂O, transferred to a 5 ml Reacti-Vial™ and freeze-dried. Derivatization reagents were added to the freeze-dried residue before heating at 75–80°C to transform neutral sugars into aldonitrile derivatives, which were then extracted with 1.5 ml dichloromethane from the aqueous solution. Excess anhydride was removed using 1 mol/L HCL and deionized water. The neutral sugar derivatives were finally separated on an Agilent 6890A gas chromatograph (GC, Agilent Tech. Co. Ltd., USA) equipped with a DM-1 fused silica column (30 m by 0.25 mm by 0.25 µm) and a flame ionization detector after being redissolved in 200 µl hexane and ethyl acetate solvent (1:1 v/v).

Neutral sugars were quantified relative to the internal standard adonitol, which was added to the samples before purification, and the recovery standard myo-inositol, which was added before derivatization. The ratio of (Gal + Man)/(Xyl + Ara) is often used to indicate labile C origins in soil (Oades, 1984; Spielvogel, Priezel, & Kögel-Knabner, 2007). However, some crops and grasses produce Gal and Man, the sugars used to represent microbial sources (Angers & Mehuys, 1990; Sariyildiz & Anderson, 2003; Schädel, Blöchl, Richter, & Hoch, 2010). Therefore, we used the ratio of (Fuc + Rha)/(Xyl + Ara) to prevent the overestimation of microbial sugars within the SOM in this study (Spielvogel et al., 2007).

2.7 | Diffuse reflectance mid-infrared fourier transform spectroscopy (DRIFTS)

Finely ball-milled soil subsamples were diluted with 97% KBr to eliminate scatter light intensity. The mixed samples

(soil:KBr = 1:80) were homogenized by further grinding in an agate mortar and scanned 64 times per spectrum from 4,000 to 400 cm^{-1} with a resolution of 4 cm^{-1} in reflectance mode, using a Fourier transform spectrometer (Nicolet 6700, USA). Relative peak areas of aromatic C=C, ketone and quinone C=O, and/or amide N=H at 1,620 cm^{-1} (rA_{1620}) and aliphatic C-H at 2,930 cm^{-1} (rA_{2930}) were calculated using a tangential baseline method described by Demyan et al. (2012). The ratio of rA_{1620} to rA_{2930} was accepted as humification index (HI) to denote the ratio of stable to labile C fractions in the soil, reflecting the degree of existing SOC decomposition and changing SOC stability (Margenot & Hodson, 2016).

2.8 | Statistical analysis

One-way ANOVA and Duncan's post hoc multiple comparisons were used to compare the measurements in bulk soil. Statistical analyses of measurements within systems over time were performed on plot-level means using R, statistical package version 3.3.1 (R Core Team, 2016). Levene test [car package, see Fox (2011)] and Shapiro–Wilk test [stats package, see R Core Team (2016)] were conducted to test the homogeneity of variance and normality assumption, respectively.

3 | RESULTS

3.1 | Soil organic carbon concentration

Planting prairie on land formerly planted in annual row crops increased SOC concentration. In mixed prairie, SOC increased 10.6% from 17.38 to 19.23 g/kg over six growing seasons (Table 1). From 2008 to 2010, SOC decreased 12.1% in *M. × giganteus*, probably a result of replanting effects, and SOC in *M. × giganteus* showed an increasing trend between 2010 and 2014 (Table 1). Although no statistically significant changes were resolved, there was a trend of decreasing SOC for MMS rotation ($F = 3.68$, $p = 0.09$) over the 6 years of this study. No significant changes in SOC were found in switchgrass during the period of our experiment.

3.2 | Soil organic carbon storage

Conversion from annual row crops to perennial grasses increased SOC storage (SOCS, Mg/ha) in the top 10 cm of the soil profile in two of the three perennial systems. SOCS increased 17.0% from 15.73 to 18.40 Mg/ha in switchgrass and increased 15.6% from 15.02 to 17.36 Mg/ha in mixed prairie over six growing seasons (Table 1). No significant changes in SOCS were found in MMS rotation or *M. × giganteus* from 2008 to 2014.

3.3 | Soil amino sugars

The contribution of bacterial- and fungal-derived residues to SOC varied among crop types. The ratio of MurA to SOC (MurA/SOC), used as an index for bacterial residue contributions to SOC, increased 21.1%, 20.0%, and 15.0% in MMS rotation, *M. × giganteus*, and switchgrass, respectively, without a change in mixed prairie (Table 1). The ratio of GluN to SOC (GluN/SOC), representing fungal residue contributions to SOC, increased 8.6% in *M. × giganteus* without significant changes in the other crops (Table 1). Bacterially produced MurA content in soil (mg/kg) increased 13.5%, 24.5%, and 19.2% in *M. × giganteus*, switchgrass, and mixed prairie, respectively, from 2008 to 2014 with no changes in MMS rotation (Table 1). Fungal-produced GluN increased 15.0%, and total amino sugar (AS) content increased 9.6% in mixed prairie over 6 growing seasons with no changes in the other crops (Table 1). GluN/MurA (fungal/bacterial amino sugar ratio) decreased 14.9% in switchgrass without significant changes in the other crops (Table 1).

3.4 | Soil neutral sugars

The concentration of plant-derived sugars in soil (mg/kg) increased over the period of our experiment in the perennial crops but not in MMS. Plant-derived xylose (Xyl) increased 53.7%, 46.4%, and 49.0% in *M. × giganteus*, switchgrass, and mixed prairie, respectively, from 2008 to 2014 without a change in MMS rotation (Table 1). Microbe-derived fucose (Fuc) increased 45.3% in switchgrass, while remaining unchanged in the other crops from 2008 to 2014 (Table 1). There were no changes in microbe-derived rhamnose (Rha) or plant-derived arabinose (Ara) in any treatments over the 6 years of this experiment (Table 1). In *M. × giganteus*, the ratio of microbe-to plant-derived neutral sugars (deoxyC6/C5), used as an index of soil labile C origin, decreased in MMS rotation and *M. × giganteus* without significant changes in the other crops (Table 1).

3.5 | DRIFT spectroscopy

The degree of SOC decomposition increased in MMS but not in the perennial crops over the 6 years of this study. The humification index (HI) rA_{1620}/rA_{2930} decreased in MMS rotation without changes in the perennials. Soil aliphatic C (rA_{2930}) increased 19.8% in MMS rotation with no changes in the perennials from 2008 to 2014. Soil aromatic C (rA_{1620}) decreased 2.7% in MMS rotation and increased 3.4% and 4.7% in *M. × giganteus* and switchgrass, respectively, from 2008 to 2014 (Table 1). No differences were measured in rA_{1620} in mixed prairie (Table 1).

4 | DISCUSSION

Measurements of soil microbial biomarkers, DRIFTS and SOC, demonstrated that the soil microbial control over carbon dynamics of each bioenergy crop investigated was distinct from the others in a measureable way, with SOC retention occurring in the perennial bioenergy crops. While it is tempting to consider bioenergy crops under umbrella categories such as “perennial monoculture” or “annual row crops,” our measurements show that the accumulations of microbial residues in the soil, the relative dominance of fungal vs bacterial residues in the soil, the microbial uses of soil labile carbon, and the stability of C compounds in the soil vary with each bioenergy crop. Our use of biomarkers and DRIFTS techniques in side by side bioenergy cropping systems yields a more robust understanding of the belowground C cycling processes in these ecosystems. This direct comparison demonstrates that perennial grasses largely increased aspects of SOC storage and stability while the conventional annual row crop did not, and further SOC storage and stability differed between perennial cropping systems.

Microbial residues accumulated under all perennial crops as we hypothesized, but the contribution of bacterial residues to SOC increased only in the monocultures (Table 2). In switchgrass, MurA accumulation outpaced SOC accumulation, increasing MurA/SOC over time (Table 2). The observed increasing trend of bulk SOC in switchgrass was probably due in part to the increase in microbial residues (Jesus et al., 2016). For MMS and *M. × giganteus*, management and disturbance may have

contributed a loss of plant residue to erosion during initial *M. × giganteus* establishment and annual tillage in MMS, resulting in the increased proportions of bacterial residue in SOC (Table 2). However, the increases in MurA/SOC for *M. × giganteus* and MMS likely have different causes: In MMS, the increased results from the observed decrease the ratio denominator with the loss of SOC over time, while SOC decreased in *M. × giganteus* in conjunction with an increase in MurA (Table 2). We found increased bacterial and fungal residue in mixed prairie, which is consistent with the idea that increased aboveground plant diversity could support more robust belowground microbial communities (Liu, Liu, Fu, & Zheng, 2008). However, because SOC also increased, there was no change in the relative contribution of microbial residue to SOC in the prairie. When this information is considered with the increased plant-derived xylose observed in all perennial treatments, it is clear that each microbial community exhibited an individual response to crop choice and management (Table 1). While all three of the perennial crop systems in the study have been previously shown to accumulate plant-derived POM-C over time (Kantola, Masters, & Delucia, 2017), in our study, beneficial microbial residue accumulation in bioenergy cropping systems was greatest in the switchgrass system, where both SOC and MurA increased, with MurA outpacing SOC.

As predicted, the relative dominance of fungal vs bacterial residues differed between fertilized (switchgrass) and unfertilized perennial (*M. × giganteus* and prairie) ecosystems. Based on previous studies, it is reasonable to conclude the added N in this ecosystem accounts for this

TABLE 2 Summary of treatment effects and corresponding measurements under potential bioenergy crops from 2008 to 2014. Arrows indicate the direction of measurements

Treatment effect	Measurement	Maize–maize– soybean	<i>M. × giganteus</i>	Switchgrass	Mixed prairie
Bacterial residue accumulation	MurA	—	↑	↑	↑
Fungal residue accumulation	GluN	—	—	—	↑
Bacterial residue contribution to SOC	MurA/SOC	↑	↑	↑	—
Fungal residue contribution to SOC	GluN/SOC	—	↑	—	—
Relative contribution of fungal versus bacterial residue in SOC	GluN/MurA	—	—	↓	—
Microbial preference of exogenous (C5) or existing (deoxyC6) labile SOC	deoxyC6/C5	↓	↓	—	—
Microbial preference of labile (rA ₂₉₃₀) or stable (rA ₁₆₂₀) SOC	rA ₁₆₂₀ /rA ₂₉₃₀	↓	—	—	—
SOC concentration	SOC	↓	↓	—	↑
SOC storage	SOCS	—	—	↑	↑

Red arrows indicate significant effects ($p \leq 0.05$), gray arrows indicate insignificant trends ($0.05 < p \leq 0.1$), and “—” indicates no change ($p > 0.1$).

C5, pentoses (xylose+arabinose); deoxyC6, deoxyhexoses (fucose+rhamnose); GluN, glucosamine; MurA, muramic acid; rA₁₆₂₀, relative peak areas at 1,620 cm⁻¹; rA₂₉₃₀, relative peak areas at 2,930 cm⁻¹; SOC, soil organic carbon; SOCS, soil organic carbon storage.

bacterial dominance in switchgrass that was not observed in prairie and *M.×giganteus*. Leff et al. (2015) demonstrated a decline in the relative abundance of arbuscular mycorrhizal fungi (AMF) DNA with N additions in numerous grasslands. Oates, Duncan, Sanford, Liang, and Jackson (2016) showed that to meet the N demand for growth, unfertilized switchgrass stimulated more arbuscular mycorrhizal fungi (AMF) and gram-negative (Gm^-) bacterial biomass than fertilized plants. In addition, it has been shown that environments rich in labile C and N favor copiotrophic bacterial growth (Bai et al., 2017; Fierer, Bradford, & Jackson, 2007), potentially at the expense of fungi. It is evident that fertilization regime can influence soil microbial residue dominance in perennial systems, and our comparison between perennial treatments supports this.

Microbes showed different preferences for labile SOC among the perennial ecosystems during our experiment. In general, current understanding of soil dynamics dictates high belowground C inputs from root biomass and cessation of soil disturbance from a reduction in tillage would eventually result in SOC sequestration in perennial crops (Ma, Wood, & Bransby, 2000; Monti & Zatta, 2009), with timescales dependent on site-specific abiotic and biotic variables. This is supported by the increased SOCS observed in prairie and switchgrass. In contrast, despite producing the largest belowground biomass across this experiment (Anderson-Teixeira et al., 2013), we observed a decreasing trend in SOC and no changes in SOCS in *M.×giganteus* (Table 2). The deoxyC6/C5 ratio decreased over time in *M.×giganteus*, indicating increased degradation of microbe-derived labile SOC, without corresponding changes in preference in prairie or switchgrass (Table 2). Management effects in *M.×giganteus* differed from switchgrass and prairie, however, as soils in *M.×giganteus* plots were disturbed by replanting due to poor establishment early in the experiment (Anderson-Teixeira et al., 2013). Additionally, other studies have shown microbial communities will acquire N from microbe-derived labile organic matter when available N becomes limited (Schmidt, Schulz, Michalzik, Buscot, & Gutknecht, 2015), a predictable consequence of long-term agriculture without soil amendment or N-fixing plant species, as in *M.×giganteus*. We observed a similar increase in microbial preference for existing labile SOC in the MMS rotation (decreased deoxyC6/C5), and although the number of variables present does not allow us to attribute this to one treatment characteristic, we can conclude C storage is decreasing in these systems.

The calculated humification index (HI), determined by the ratio of stable aromatic to labile aliphatic C measured via DRIFTS, did not change from 2008 to 2014 for any perennial ecosystem. However, the HI for MMS decreased, indicating increased decomposition of plant-derived stable

SOC and a decrease in SOC stability during the 6 years of this study (Table 2). This supports previous research which showed plant-derived C increasing in perennial crops, but not in the MMS system (Kantola et al., 2017). Further evidence of a decline in SOC stability for MMS can be gathered from the aforementioned decrease of deoxyC6/C5 and the change in microbial preference thus indicated. Although the neutral sugar evidence suggests an increase in degradation of existing SOM in *M.×giganteus* as well, stable aromatic C fractions measured by rA_{1620} increased indicating increasing SOC stability in this treatment (Table 1). Previous studies in these plots have found differences in litter quality (Smith et al., 2013), a variable Spohn et al. (2016) demonstrated influencing microbial CUE. Unlike the perennial monocultures, mixed prairie showed no change in aromatic or aliphatic C compounds. The increase observed in SOC quantity with no change in the type of C compounds present indicates stable belowground C dynamics and sequestration in this diverse ecosystem. Although not universally true for all indices and treatments, such as the aforementioned decrease in the neutral sugar ratio in *M.×giganteus*, the perennial ecosystems in this study primarily showed increases in general indicators of SOC stability, while the annual row crops showed decreases. The stability of existing SOC in the perennials and the increase in bulk SOC storage for switchgrass and mixed prairie suggest that these bioenergy crops have the potential to restore soil C lost to past intensive management of annual row crops. Small increases in bulk SOC over the 6 years of this study show that C accrual in soils is slow, even under fast-growing perennials (Table 1, Kantola et al., 2017).

According to these indications from the biomarker and DRIFTS analyses, we speculate that microbial degradation preference is driven by labile C inputs and available N. If belowground labile C input is relatively low, such as in our annual system, microbes will increase C acquisition from existing SOM, resulting in decreasing SOC stability (Figure 1a). When labile C input is similar between systems, N availability influences microbial substrate preference, exemplified by the different responses observed in *M.×giganteus* and switchgrass. When available N is relatively low, which likely occurred in our unfertilized *M.×giganteus* treatment, microbes will break down microbial residue to satisfy N need (Figure 1b). This could have a negative influence on long-term SOM stability and suggests fertilizer may increase soil C accumulation under these conditions. However, species composition has influence, as was shown by the accumulation of soil C and both bacterial and fungal residues observed in mixed prairie, which contained some N-fixing plant species (Figure 1d). In our fertilized switchgrass treatment where N is available and belowground labile C inputs are high, bacterial and plant residue contributions both increased, increasing long-term

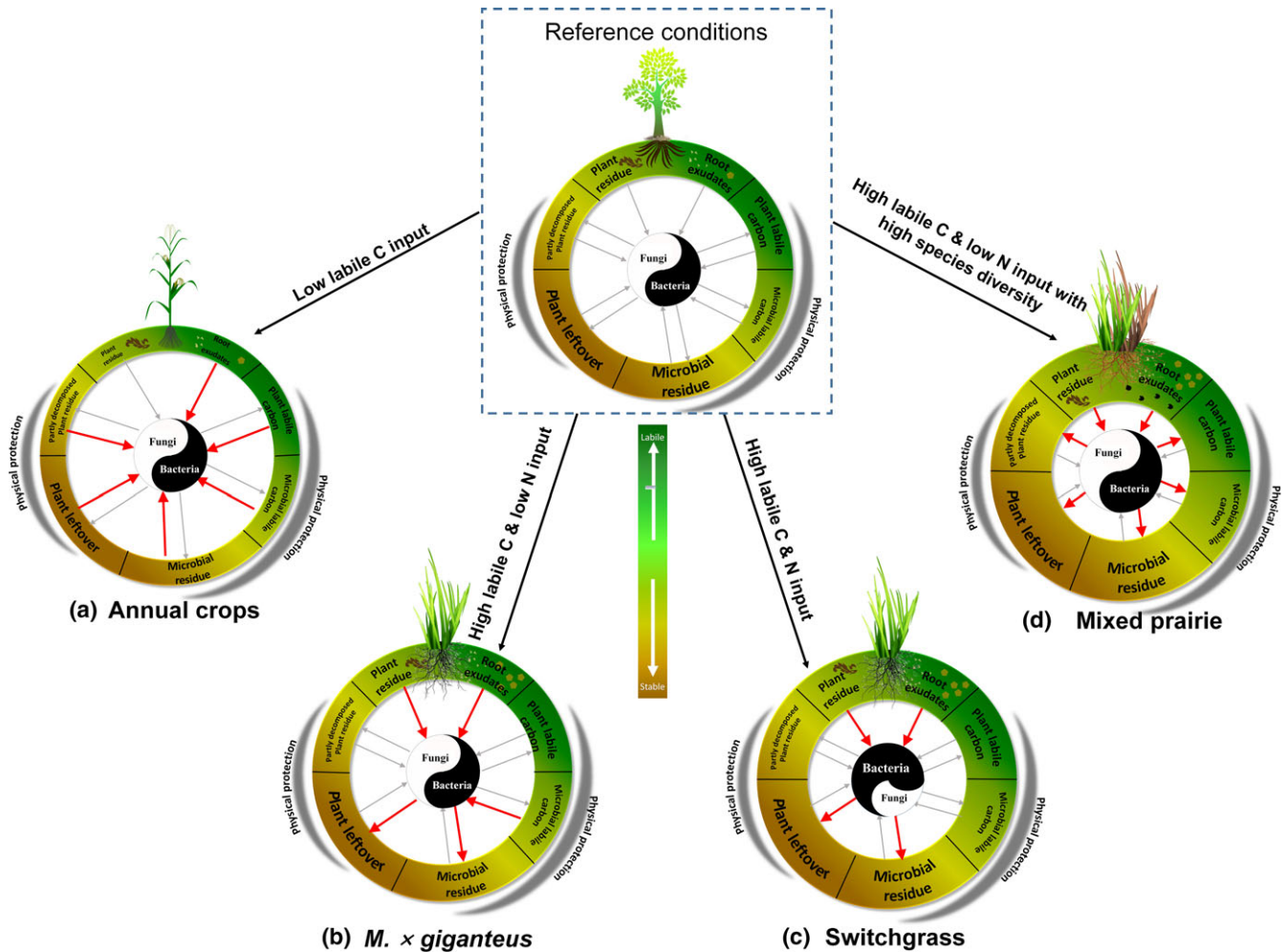


FIGURE 1 Visual speculation of carbon (C) dynamics with different belowground labile C and nitrogen (N) availabilities, analogous to the bioenergy cropping systems in this experiment. (a), conditions under low labile C inputs, high N inputs (maize–maize–soybean); (b), high labile C inputs, low N inputs (*M. x giganteus*); (c), high labile C inputs, high N inputs (switchgrass); and (d), high labile C inputs, low N inputs with high species diversity (mixed prairie). The hollow circle indicates soil organic matter with different forms and stabilities which vary by color. The size of the hollow circle reflects soil organic carbon concentration as a change from the reference conditions. The black and white circles in the center indicate the relative dominance of soil microbial community and its change from the reference conditions. Gray arrows indicate original microbial processes. Red arrows indicate an increase in microbial processes from the reference conditions. The arrows from hollow circle to microbial community circle indicate microbial degradation, and reverse arrows indicate microbial contributions. The gray crescent symbol indicates the components influenced by physical protection

SOC stability and storage (Figure 1c). The results from this study demonstrate that crop type, labile C inputs, and available N interact and dictate SOC accumulation and long-term stability.

Very few agronomic studies have included biomarker amino sugars and neutral sugars as well as DRIFTS techniques simultaneously to assess microbial contributions to SOC. Using these techniques, we have demonstrated that perennial grasses largely maintained aspects of SOC stability, while the conventional annual row crop did not. A maize–maize–soybean rotation and *M. x giganteus* showed increased microbial preference for existing SOM after 6 years, creating the potential for long-term SOC loss. Switchgrass and diverse mixed prairie increased SOC

quantity in this time frame. Combined interpretation of biomarker analyses and DRIFTS techniques demonstrate that the three perennial systems in this study with different management and fertilization regimes likely support microbial communities that differ in behavior affecting belowground C dynamics. From an agricultural production standpoint, the decision to grow perennial bioenergy feedstock is generally made based on climate and yield potential, but our research demonstrates that other factors contribute to changes in SOC quantity and quality and have consequences for long-term soil fertility. Management, species diversity, C inputs, and fertilizer all influence SOC content and stability and are crucial to understanding microbial control over C dynamics.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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