

## ORIGINAL RESEARCH

# Can switchgrass increase carbon accrual in marginal soils? The importance of site selection

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## Abstract

Most soil carbon (C) is in the form of soil organic matter (SOM), the composition of which is controlled by the plant–microbe–soil continuum. The extent to which plant and microbial inputs contribute to persistent SOM has been linked to edaphic properties such as mineralogy and aggregation. However, it is unknown how variation in plant inputs, microbial community structure, and soil physical and chemical attributes interact to influence the chemical classes that comprise SOM pools. We used two long-term biofuel feedstock field experiments to test the influence of cropping systems (corn and switchgrass) and soil characteristics (sandy and silty loams) on microbial selection and SOM chemistry. Cropping system had a strong influence on water-extractable organic C chemistry with perennial switchgrass generally having a higher chemical richness than the annual corn cropping system. Nonetheless, cropping system was a less influential driver of soil microbial community structure and overall C chemistry than soil type. Soil type was especially influential on fungal community structure and the chemical composition of the chloroform-extractable C. Although plant inputs strongly influence the substrates available for decomposition and SOM formation, total C and nitrogen (N) did not differ between cropping systems within either site. We conclude this is likely due to enhanced microbial activity under the perennial cropping system. Silty soils also had a higher activity of phosphate and C liberating enzymes. After 8 years, silty loams still contained twice the total C and N as sandy loams, with no significant response to biofuel cropping system inputs. Together, these results demonstrate that initial site selection is critical to plant–microbe interactions and substantially impacts the potential for long-term C accrual in soils under biofuel feedstock production.

## KEYWORDS

aggregate, agriculture, biofuels, climate change, corn, FTICR-MS, marginal croplands, mortierella, soil, switchgrass

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## 1 | INTRODUCTION

Current estimates suggest that soils contain  $1.5 \times 10^{12}$  tons of carbon (C), or roughly three times more C than the atmosphere, and represent a giant reservoir for potential greenhouse gas emission or sequestration (Scharlemann et al., 2014). The majority of C in most soils is in the form of soil organic matter (SOM). Most new C inputs into soils are derived from plants, providing substrates that influence microbial community composition and activity (Hargreaves et al., 2015; Six et al., 2006). Plant-derived OM is turned over via enzymatic decomposition by soil microorganisms. The decomposition products of plant material and microbial residues both contribute to SOM formation, and estimates suggest that as much as 50% of SOM is of microbial origin (Liang et al., 2019; Ludwig et al., 2015; Simpson et al., 2007). This means a dominant component of SOM is derived from molecules that soil microorganisms release into the environment as a result of active metabolic processes (e.g., extracellular enzymes, biofilms, metabolites) or death (i.e., necromass, including amino sugars, proteins and lipids) (Kallenbach et al., 2016; Ma et al., 2018; Miltner et al., 2012). If microbial metabolism and necromass are dominating SOM formation, it follows that the drivers of SOM chemistry and accrual are similar to those of soil microbial community structure and function. Both microbial metabolic potential and the biomolecular composition of necromass are intimately related to microbial community structure. After all, the majority of SOM is of microbial origin. By linking soil microbial characteristics to SOM chemistry features, we can obtain a better understanding of how the soil microbiome influences belowground C cycling and accrual.

New paradigms are emerging to integrate across the plant–microbe–soil continuum to develop a predictive understanding of OM formation and fate in soils. While plants and soil microorganisms interact to form the bulk SOM pool, consolidated conceptual models suggest the size and stoichiometry of decomposition products interact with the soil environment to determine the persistence of organic C substrates in soil environments (Mikutta et al., 2019; Sanderman et al., 2014; Schmidt et al., 2011). One prevailing hypothesis is that soil particles can become saturated with organic material so that the mineralogical characteristics of a soil type can explain most of the variation in the quantity of refractory SOM (Creamer et al., 2019; Six et al., 2002; Stewart et al., 2008). In addition to soil type, anoxic microsites are implicated as an under-recognized protection mechanism for preserving SOM integrity (Keiluweit et al., 2016, 2017). Aggregate size influences anoxic microsite abundance, with more anoxic space in larger aggregates (Schlüter et al., 2018; Sexstone et al., 1985). By influencing the soil pore space and connectivity, aggregate size distribution regulates microbial activity, soil organic carbon (SOC) mineralization, and physical

protection of decomposition products and microbial residues (Lützow et al., 2006; Six et al., 2004). A substantial fraction of SOM is mineral associated and more resistant to degradation than OM not associated with mineral surfaces (Schmidt et al., 2011; Zhao et al., 2016), so the distribution of mineral surfaces within soil aggregates can also contribute to SOC protection (Castellano et al., 2015; Six et al., 2002). Smaller aggregates have more surface area than larger aggregates and as a result also have greater potential for mineral-associated OM to accumulate.

However, both plant and soil type affect the production and accumulation of microbial biomass (Jesus et al., 2016) which, in turn, impacts the accumulation and formation of C stored in soils (Wilson et al., 2009). Plants can affect microbial community structure (Haichar et al., 2008) and function (Kuiper et al., 2002) by releasing root exudates into the soil. These root exudates can then become stabilized as mineral associated OM via belowground microbial processes in the rhizosphere (Sokol & Bradford, 2019). Differing root structures form unique microbial habitats (Haichar et al., 2008), and the plant impacts on soil microorganisms may accumulate over time (Jesus et al., 2016). Cropping system also impacts soil aggregation with perennial cropping systems containing a higher proportion of large aggregates than annual cropping systems (Bach & Hofmockel, 2016; McGowan et al., 2019; Tiemann & Grandy, 2015). Although plants, microbes, and soils all have a role in SOM cycling, the combined effects of plant inputs and soil aggregation on the microbial community and, in turn, the chemical composition of SOM, has yet to be integrated into empirical studies.

Growing interest in biofuel production has stimulated research on understanding how plant–microbe interactions influence the sustainable production of biofuel feedstocks (Hargreaves & Hofmockel, 2014; Jesus et al., 2016; McGowan et al., 2019). Corn (*Zea mays* L.) is the most abundantly produced biofuel crop in the United States (Center for Sustainable Systems, 2019). Perennial grasses are also being developed for biofuel production because they require less inputs and can grow on marginal soils, thus reserving productive cropland for food and feed crops (Gelfand et al., 2013). In addition, marginal soils have low SOM stocks and represent a potential C reservoir. As a result, identifying how plant, microbe, and soil interactions lead to SOM formation and stabilization in marginal soils could have a profound impact on improving soil health, contributing to food and energy security, and mitigating greenhouse gas accumulation.

We investigated the relative influence of cropping systems and soil selection on the potential for biofuel feedstock production systems to accumulate SOC at the Great Lakes Bioenergy Research Center (GLBRC) Intensive Biofuel Cropping System Experiments (BCSE) in Michigan and Wisconsin, USA. The overarching objective of this research is to identify how plant selection and soil properties

influence the accumulation and persistence of C sources in soil. We performed a sequential solvent extraction to investigate soluble SOM pools and examined both the bioavailable (water-extractable) and more persistent (chloroform-extractable) SOM pools. We hypothesize that (1) soil microbial membership reflects edaphic characteristics, where silty soils and small macroaggregates (SM) support a more diverse and active microbiome due to higher surface area to volume ratios, (2) microbial community activity is strongly influenced by plant inputs, resulting in greater abundance and diversity of soil organic C molecules under perennial (switchgrass (*Panicum virgatum* L.)) compared to annual (corn) plants grown in the same soil, and (3) plant–microbe–soil interactions influence C storage, where perennial cropping systems in silty soils accrue more C than sandy soils with annual cropping systems. To test these hypotheses, we analyzed soil aggregate fractions (large and SM) from two different soil textures (sandy vs. silty loam) under two different cropping systems (corn and switchgrass) that were in place for nearly a decade.

## 2 | MATERIALS AND METHODS

### 2.1 | Soil sampling

Soils were collected from the GLBRC Intensive BCSE, with field sites located at the Arlington Agricultural Research Station (AARS) of the University of Wisconsin–Madison (Arlington, WI, 43°18'N, 89°20'W) and at the W.K. Kellogg Biological Station (KBS) Long-Term Ecological Research Site (Hickory Corners, MI, 42°24'N, 85°24'W). The Wisconsin site receives an average of 833 mm of precipitation per year and has an average annual temperature of 7.4°C, with soils primarily of the Plano series, which are silt loam mesic Typic Argiudolls (Sanford et al., 2016). The Michigan site receives an average of 810 mm of precipitation per year and has an average annual temperature of 9.7°C, with soils predominantly of the Kalamazoo series, which are sandy loam mesic Typic Hapludalfs (Sanford et al., 2016). Both sites have a pH of roughly 6.3.

In 2008, at each field site, 27 × 43 m plots of bioenergy cropping systems were randomly distributed among five blocks. Each block contains one of ten cropping systems, including plots of continuous corn (*Z. mays* L.) and switchgrass (*P. virgatum* L. “Cave-In-Rock”) which we focus on in this study, resulting in five biological replicates of each cropping system at each site. Prior to 2008, KBS was cropped with alfalfa, and at AARS blocks 1–3 were planted with alfalfa, while blocks 4 and 5 were planted in corn. Both systems were managed using no-till practices after preparation and establishment of the plots in 2008. Corn plots receive an average of 167 kg N ha<sup>-1</sup> year<sup>-1</sup>, and P and K

applications based on annual soil tests. Switchgrass receives 56 kg N ha<sup>-1</sup> year<sup>-1</sup> and no P or K fertilization (Sanford et al., 2016). On October 23 and November 28–29, 2016, samples were collected using a slide hammer coring device (0–15 cm deep, ×5.1 cm diameter cores). Intact cores in plastic liners were shipped back to the laboratory overnight on ice packs. Each core was gently sieved to 8 mm to remove roots and rocks and stored at 4°C until aggregate sieving.

### 2.2 | Soil aggregate separation and imaging

An optimal moisture sieving approach, which is low energy and allows soils to separate along their natural fractures, was used for aggregate size fractionation (Bach & Hofmockel, 2014). Soils were dried to 10% moisture content and shaken through ethanol sterilized sieves of different pore sizes, resulting in microaggregates (<250 μm) and small (250–1000 μm), medium (1000–2000 μm), and large macroaggregates (LM; >2000 μm). This approach minimizes laboratory-induced artifacts and allows reproducible disruption of soil aggregates and sampling of microorganisms without disturbing the natural habitat. Subsamples of aggregate fractions were stored at –80°C until further analysis. There was not enough mass from the microaggregate fraction for all analyses, so the SM and LM classes were compared to determine the aggregate size effects on soil microbial communities and soil chemistry. SEM images were collected for aggregates from Michigan and Wisconsin switchgrass. Mineral material was air-dried, then mounted on C tape-covered aluminum SEM stubs (Ted Pella), and sputter-coated with C. The mineral particles were imaged with a FEI Helios NanoLab 600i DualBeam SEM (Thermo Fisher Scientific) at 2 KeV.

### 2.3 | DNA extraction, 16S and ITS sequencing, and analysis

DNA from all four aggregate size classes was extracted using DNeasy PowerSoil Kit (Qiagen) according to the manufacturer's instructions. Extracted DNA from 0.25 g per sample was quantified and checked for purity by spectrophotometry on a Take3 microvolume plate in a Synergy 2 multi-mode plate reader (BioTek Instruments, Inc.). DNA was sequenced targeting the prokaryotic 16S V4/V5 region with the primer pair 515F (5' GTGCCAGCMGCCGCGTAA 3') and 806R (5' GGACTACHVGGGTWTCTAAT 3') by Illumina MiSeq 2 × 151 bp paired-end sequencing, and the fungal ITS-1 region with the primer pair ITS1f (5' CTTGGTCATTTAGAGGAAGTAA 3') and ITS2 (5' GCTGCGTTCTTCATCGATGC 3') by Illumina MiSeq 2 × 250 bp paired-end sequencing according to the Earth Microbiome protocols for 16S and ITS, except for the use of

forward barcoded 16S primers (Bond-Lamberty et al., 2016; Caporaso et al., 2012).

16S and ITS sequences were processed using Hundo version 1.2.8 (Brown et al., 2018). BBDuk2 was used to trim adapter sequences and filter low-quality (Phred score <10) reads (Bushnell, 2018). After quality filtering, VSEARCH was used to merge, precluster (error rate of one) and dereplicate sequence reads (Rognes et al., 2016). VSEARCH was then used to chimera filter the reads and cluster sequences at a 97% similarity cutoff. After clustering, sequences were aligned against CREST curated databases (Silva v128 for 16S and UNITE v7.2 for ITS) using BLAST, and taxonomy was assigned using the CREST least common ancestor method (Lanzén et al., 2012). Sequence data are available on the NCBI-SRA under PRJNA650502.

## 2.4 | Enzyme activities

Potential extracellular enzymatic activity was measured on LM and SM aggregates of both sites and cropping systems using the modified tube incubation protocol (Hargreaves & Hofmockel, 2014) designed to reduce variability in analytical replicates. Briefly, 1 g of soil was homogenized in 125 ml of 100 mM maleate buffer pH 6.3. Soil homogenate was shaken in 5 ml tubes with 4-methylumbelliferone/7-amido-4-methylcoumarin linked substrates at a final concentration of 400  $\mu\text{M}$  for *N*-acetyl- $\beta$ -glucosaminidase (NAG),  $\beta$ -glucosidase (BG), *b*-xylosidase (BX), leucine aminopetidase (Leu) and alanine aminopetidase (Ala), and at 800  $\mu\text{M}$  for acid phosphatase (AP), for 3 h, and then transferred to black microplates before reading on a fluorometer (360 nm excitation and 460 nm emission; BioTek Instruments, Inc.). Saturating substrate concentrations and the linear period of reactions were established during preliminary Km linearity tests. Activities were calculated using standard methods (German et al., 2011, 2012).

## 2.5 | Carbon chemistry of large (LM) and small (SM) aggregates

Total carbon (TC) and total nitrogen (TN) were measured for large and SM as well as in the bulk soil. Samples for TC and TN analysis were oven-dried at 40°C prior to grinding in a ball mill (Retsch MM400) with stainless steel beads before analysis on a Vario EL cube elemental analyzer (elementar Langensfeld). A detailed chemical profile of LM and SM was obtained with Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) on the different chemical pools. Three solvents with different polarities in the order of (1) water, (2) methanol, and (3) chloroform were used to sequentially extract OM from LM and SM aggregates of both

sites and cropping systems (Tfaily et al., 2015, 2017). The use of H<sub>2</sub>O and CHCl<sub>3</sub> extractions enables the investigation of soluble-phase, bioavailable C as measured by water-extracted organic C (WEOC) and the hydrophobic SOM that is captured with the CHCl<sub>3</sub> extraction. The water-extracted pool (WEOC) is considered bioavailable and comprised of molecules that are more kinetically active and likely to undergo biological transformations (Kleber et al., 2007). From now on, the water-extracted pool will be referenced as the kinetic pool. The chloroform-extracted, or hydrophobic pool, represents molecules that are more closely associated with mineral surfaces and therefore relatively less bioavailable than the kinetic C pool. The methanol fraction was non-distinct and contained components of the both the kinetic and hydrophobic pools (Tfaily et al., 2017) and was therefore not reported, as done in related studies (Graham et al., 2017; Li et al., 2019). Although this method does not isolate OM that requires disruption of minerals via dissolution, the chosen extractions target operationally defined pools that may be of recent plant or microbial origin. Therefore, the method addresses our research questions that focus on the abiotic and biotic drivers of SOM chemistry in different biologically relevant C pools.

Samples were prepared and analyzed following the method of Staley et al. (2017) using 1 ml of solvent to 300 mg of soil. To improve electrospray ionization efficiency, the water and chloroform extracts were diluted in methanol at a ratio of 1:2 and 1:1, respectively. The spectrometer and ion trap details for the 21T FTICR-MS are described in Saup et al. (2019). Three stages of differential pumping yielded an ultimate pressure of 1010 Torr in the analyzer stage. ICR signal was acquired using a harmonized ICR cell that utilizes external shimming to approximate an ideal quadrupolar electric field. The inlet capillary temperature was set to 300°C and the electrospray voltage was set to 3.0 kV. Mass spectra generated from the average of 400 transient acquisitions by use of an AGC target of  $3 \times 10^6$  for the *m/z* range 240–1200. Typical mass measurement accuracy was below 50 ppb. Formularity software was used to assign chemical formulas in-house (Tolić et al., 2017), based on the following criteria: signal-to-noise ratio >23, and mass measurement error <0.5 ppm, exclusively considering the presence of C, H, O, N, S, and P. Major biochemical classes were assigned by the ratios of H to C and O to C described in Kim et al. (2003).

## 2.6 | Statistical analyses

### 2.6.1 | Amplicon data

All statistical analyses were conducted using R statistical software (R Core Team, 2019). To account for differences in sequencing depth among samples, cumulative sum scaling

normalization was applied, using the 75th quantile (Paulson et al., 2013). PmartRseq was used to calculate observed richness, Chao1, Simpson evenness, and Inverse Simpson diversity (<https://github.com/pmartR/pmartRseq/>). Beta diversity was calculated using Bray–Curtis distances, using the metaMDS and adonis functions in the vegan package (Oksanen et al., 2017). Aggregate size had no impact on 16S or ITS community structure (based on Bray–Curtis distance) or any of the reported diversity indices ( $p > 0.05$ ) at the time of sampling; thus, the two aggregate classes were combined for downstream community analyses. Indicator species analysis was conducted using the indicpecies package in R with a  $p$  value cutoff of 0.05 and minimum indicator strength value (IndVal) of 0.7, keeping all four site and cropping systems as independent groups (De Cáceres & Legendre, 2009). The indicator analysis is run for every OTU independently and accounts for every possible site and cropping system combination. Therefore, if an OTUs abundance was best explained by a combination of groups, it was reported as such.

### 2.6.2 | Enzymes

Enzymes were log-base-2 transformed for normality and a mixed-effects linear model was used to evaluate treatment effects (Pinheiro et al., 2020). A mixed-effects model allowed for the fact that the different aggregate sizes (large and small) taken from the same block of soil and were not independent of each other. In each case, every interaction term was used (Site, Crop, and Aggregate) and higher-order interaction terms were removed if not significant to increase power to lower-order terms.

### 2.6.3 | FTICR-MS data

FTICR-MS data were subset down to peaks with an  $m/z$  greater than 200 and less than 900. After formula assignment peaks only seen in one out of all samples across the entire dataset were removed to reduce noise and improve confidence in assignments. Compound richness plots and NMDS plots were created by transforming the data into a binary format (presence/absence). The compound richness plots represent the number of compounds in each sample with error bars representing the standard errors from five biological replicates. NMDS plots were plotted by calculating the Jaccard distance using metaMDS in vegan (Oksanen et al., 2017). Ellipses in NMDS plots refer to 95% confidential interval. For these analyses, five replicates from each cropping system and each site were grouped to determine main effects of site or cropping system. A linear model and ANOVA was run to test for differences between sites and cropping systems. A  $p$

value  $< 0.05$  was used to determine the statistical significance (Oksanen et al., 2017).

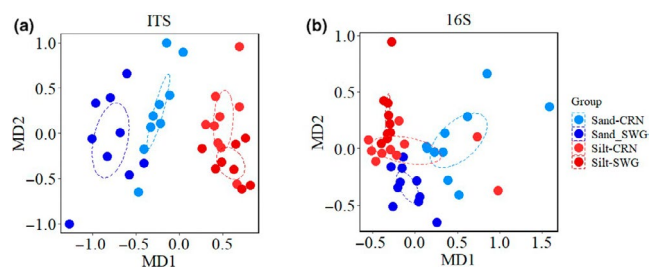
## 3 | RESULTS

### 3.1 | Amplicon sequencing

#### 3.1.1 | Drivers of ITS community composition

After processing the ITS sequences, 1,206,109 sequences were grouped into 1676 operational taxonomic units (OTUs). Major phyla included OTUs identified as Ascomycota (36.6%), Glomeromycota (23.3%), Basidiomycota (16.7%) and Mortierellomycota (13.7%). Aggregate size had no impact on ITS community structure (based on Bray–Curtis distance) or any of the reported diversity indices ( $p > 0.05$ ) at the time of sampling; thus, the two aggregate classes were combined for downstream community analyses. Both cropping system and soil type influenced the ITS community structure (Adonis,  $p < 0.05$ ), and to a greater extent than for 16S. Soil and cropping system interacted to have a small, but significant, effect on ITS composition ( $R^2 = 0.05$ ;  $p = 0.015$ ; Figure 1a). As main effects, soil type had a stronger impact on fungal community structure ( $R^2 = 0.20$ ;  $p < 0.001$ ) than cropping system ( $R^2 = 0.07$ ;  $p < 0.002$ ) (Figure 1a). Soil type had a significant impact on fungal diversity (InvSimp;  $p < 0.001$ ) with silty corn communities being similar to silty switchgrass but harboring significantly more diversity than sandy corn or sandy switchgrass communities ( $p < 0.05$ ). Silty corn communities also had a more even fungal OTU distribution than any other treatment ( $p < 0.05$ ).

The 10 most abundant fungal OTUs represented between 41.5% (silty corn) and 55.4% (silty switchgrass) of the ITS community. *Mortierella* spp. were by far the most dominant fungi in every treatment group (Table 1). Silty soil communities were more similar than sandy soil communities (Figure 1a) with silty soils sharing four of the five most dominant



**FIGURE 1** Ordination of beta diversity calculated using the Bray–Curtis dissimilarity index showing site and crop influences on the fungal (ITS) (a) and bacterial and archaeal (16S) communities (b). Ovals represent 95% confidence intervals for sandy corn (light blue), sandy switchgrass (dark blue), silty corn (light red), and silty switchgrass (dark red) soils

TABLE 1 Dominant taxa in each soil and cropping type based on mean relative abundance

Dominant ITS OTUs				Dominant 16S OTUs			
OTU	Taxonomy	Similarity	Relative abundance (mean ± SD)	OTU	Taxonomy	Similarity	Relative abundance (mean ± SD)
Silty switchgrass				Silty switchgrass			
4	<i>Mortierella hyaline</i>	100%	11.9 ± 14.5%	2	<i>Pseudomonas</i> sp.	100%	10.3 ± 9.1%
1	<i>Mortierella</i> sp.	99%	11.1 ± 3.6%	48	<i>Niabella terrae</i>	97%	1.7 ± 0.8%
151	<i>Mortierella camargensis</i>	98%	6.3 ± 2.5%	23	<i>Chthoniobacter flavus</i>	92%	1.4 ± 0.8%
16	<i>Heydenia alpina</i>	100%	5.6 ± 11.0%	57	<i>Luteitalea pratensis</i>	96%	1.0 ± 0.5%
10	<i>Mortierella</i> sp.	100%	4.2 ± 1.5%	85	<i>Clostridium</i> sp.	100%	0.9 ± 1.3%
Silty corn				Silty corn			
1	<i>Mortierella</i> sp.	99%	7.1 ± 4.4%	2	<i>Pseudomonas</i> sp.	100%	2.1 ± 2.9%
151	<i>Mortierella camargensis</i>	98%	6.4 ± 6.7%	23	<i>Chthoniobacter flavus</i>	92%	1.7 ± 1.1%
21	<i>Solicoccozyma terreus</i>	100%	5.0 ± 2.5%	64	<i>Brevitalea aridisoli</i>	94%	1.6 ± 1.4%
4	<i>Mortierella hyaline</i>	100%	4.4 ± 5.0%	57	<i>Luteitalea pratensis</i>	96%	1.5 ± 1.2%
16	<i>Heydenia alpina</i>	100%	4.2 ± 3.6%	48	<i>Niabella terrae</i>	97%	1.1 ± 0.5%
Sandy switchgrass				Sandy switchgrass			
19	<i>Mortierella exigua</i>	100%	17.0 ± 12.5%	64	<i>Brevitalea aridisoli</i>	94%	2.3 ± 0.9%
12	<i>Mortierella beljakovae</i>	94%	7.4 ± 12.5%	2	<i>Pseudomonas</i> sp.	100%	2.2 ± 1.1%
22	<i>Mortierella beljakovae</i>	95%	5.3 ± 8.0%	67	<i>Brevitalea aridisoli</i>	93%	2.0 ± 0.7%
17	<i>Phallus rugulosus</i>	98%	4.2 ± 9.3%	57	<i>Luteitalea pratensis</i>	96%	1.3 ± 0.7%
14	<i>Mortierella elongata</i>	100%	4.1 ± 4.5	23	<i>Chthoniobacter flavus</i>	92%	1.0 ± 0.5%
Sandy corn				Sandy corn			
14	<i>Mortierella elongata</i>	100%	15.1 ± 16.1%	2	<i>Pseudomonas</i> sp.	100%	4.6 ± 4.3%
19	<i>Mortierella exigua</i>	100%	9.0 ± 6.0%	56	<i>Nitrososphaera viennensis</i>	91%	2.4 ± 4.0%
10	<i>Mortierella</i> sp.	100%	8.5 ± 17.9%	88	<i>Falvobacterium</i> sp.	100%	2.1 ± 1.7%
16	<i>Heydenia alpina</i>	100%	3.8 ± 5.1%	64	<i>Brevitalea aridisoli</i>	94%	1.2 ± 0.7%
84	<i>Exophiala pisciphila</i>	96%	3.6 ± 1.3%	52	<i>Tepidisphaera mucosa</i>	87%	1.1 ± 2.1%

OTUs, while sandy soils shared just two (Table 1). Nearly three times more fungal OTUs were indicative of silty soil communities than sandy soil communities (65 vs. 22) including OTUs 1 and 4, the most dominant OTU in each silty cropping system identified as two different *Mortierella* spp. (Data S1). Crop type had less of an impact on the soil microbiome and corn communities shared just one of the five most dominant fungal OTUs while switchgrass communities shared none. Corn soils had a more consistent unique fungal community composition than switchgrass, and there were over twice as many fungal indicators for corn than switchgrass communities (16 vs. 6) including OTU 84 (Data S1).

### 3.1.2 | Drivers of 16S community composition

After processing of the 16S data, 708,648 sequences were grouped into 11,227 OTUs with 11,153 OTUs identified as

bacteria and 74 OTUs identified as archaea. Dominant bacterial phyla included Proteobacteria (28.6%), Planctomycetes (10.3%), Candidate phylum Parcubacteria (7.9%), Bacteroidetes (7.9%), Acidobacteria (7.2%), Actinobacteria (5.3%) and Chloroflexi (5.2%). Aggregate size had no impact on 16S community structure (based on Bray–Curtis distance) or any of the reported diversity indices ( $p > 0.05$ ) at the time of sampling; thus, the two aggregate classes were combined for downstream community analyses. Crop and soil interacted to significantly influence the community structure ( $R^2 = 0.06$ ;  $p = 0.001$ ) but explained only a small amount of variability (Figure 1b). As main effects, soil type influenced microbial community composition ( $R^2 = 0.13$ ;  $p < 0.001$ ) more than plant type ( $R^2 = 0.08$ ;  $p = 0.001$ ). The 16S communities were similar in richness (Chao1 and observed), evenness (Simpson), and alpha diversity (invSimp;  $p > 0.05$ ) among sites and cropping systems. However, alpha diversity (InvSimp) within site and cropping systems differed

( $p = 0.008$ ) with sandy switchgrass communities being significantly more diverse than sandy corn or silty switchgrass communities (Tukey,  $p < 0.05$ ).

The top 10 16S OTUs comprised 10.7% (silty corn)–19.1% (silty switchgrass) of the community relative abundance in the four cropping system-by-soil groups. Community composition in silty corn soils was most similar to the silty switchgrass soils (Figure 1b), sharing four of the five most abundant OTUs while sandy corn and switchgrass soils shared only two (Table 1). Silty soils also had a more consistent unique community composition than sandy soils with over twice as many OTUs being indicative of silty soil communities (233 vs. 95; Data S1). Between the two sites, switchgrass 16S communities were more similar to each other than were corn communities (Figure 1b); switchgrass communities shared three of the five most abundant OTUs (Table 1). There were 59 OTUs that were indicative of switchgrass soils (Data S1), and although corn communities shared two of the five most dominant OTUs, we found no OTUs that were indicative of corn 16S communities.

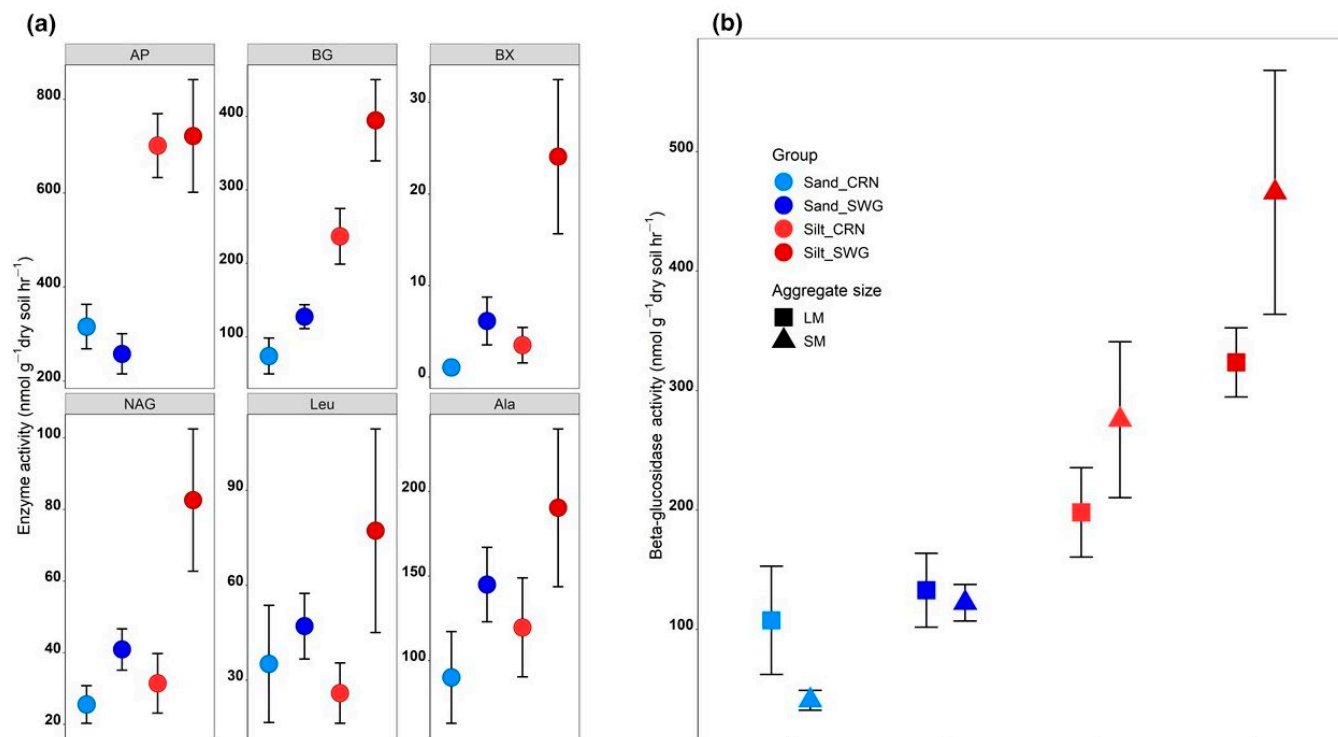
### 3.2 | Enzymes

Soil and plant type had strong and differential effects on extracellular enzyme activity (EEA). Soil type most strongly influenced the two most active enzymes, AP and BG. Silty soils had significantly greater EEA than sandy soils with 2.5

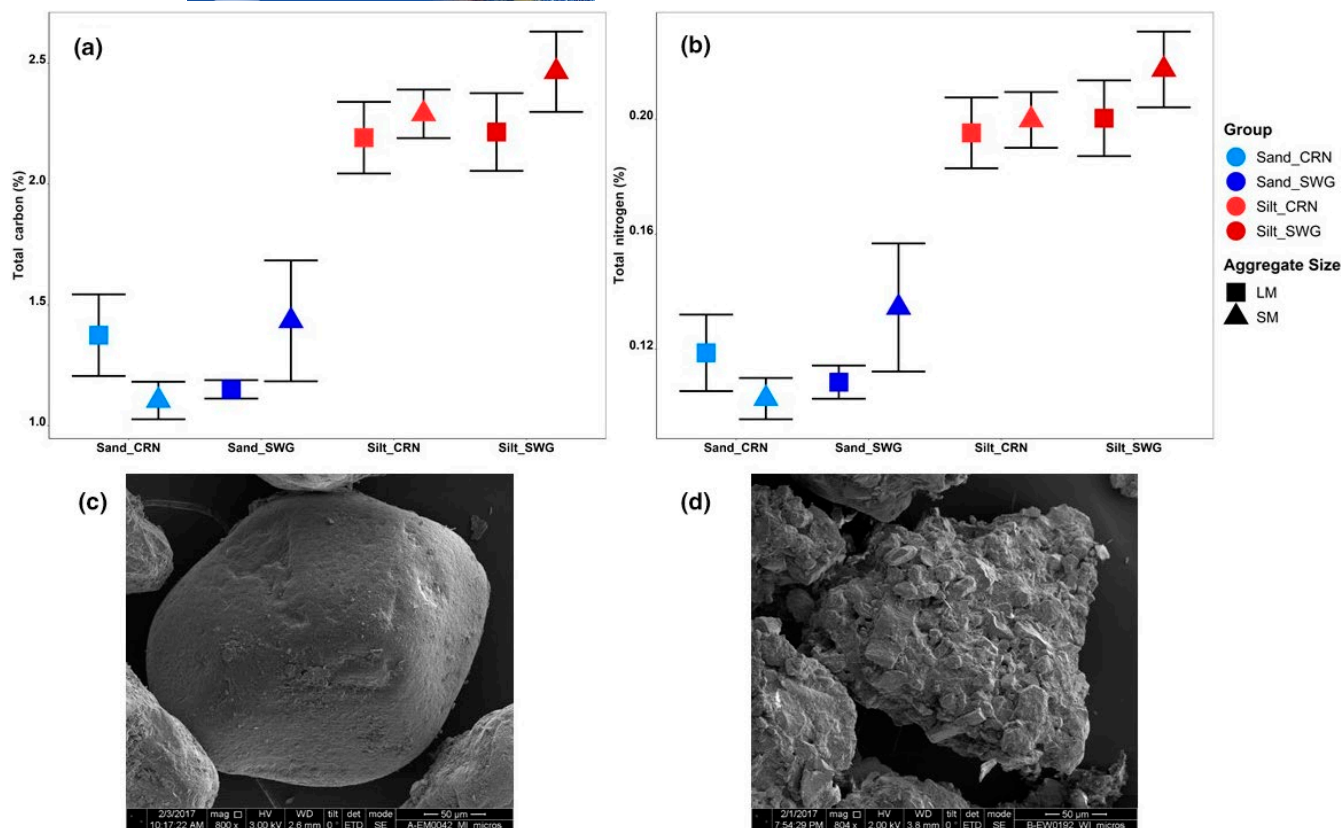
times greater activity for AP, 3.8 times greater activity for BG, and 3.1 for BX (Figure 2). AP had the highest activity of any enzyme measured with 17 times higher activity on average compared to other enzymes. Decreasing sequentially, the next most active enzymes were BG, Ala, and NAG. Cropping system significantly impacted BG, Ala and NAG activity ( $p < 0.05$ ). Switchgrass soils had significantly greater EEA than corn soils with 1.7 times greater activity for BG, 1.6 for Ala, and 2.2 for NAG. Aggregate and soil type interacted to influence BG activity with SMs showing greater activity in silty loams and LMs having a greater activity in silty loams ( $p = 0.02$ ; Figure 2b). There were no significant crop, site, or aggregate effects observed for Leu activity (Table S1).

### 3.3 | Soil properties influence amount of carbon accumulation

Soil type significantly influenced soil TC and TN (Figure 3) with no significant main or interaction effects of cropping system in the bulk soil or macroaggregate fractions. The rough-textured silty soils contained  $2.19 \pm 0.32\%$  TC, which was approximately two times the TC of sandy soils ( $1.05 \pm 0.30\%$ ;  $p < 0.001$ ). Similarly, silty soils contained approximately two times more TN than sandy soils ( $p < 0.001$ ). There was no significant difference in TC or TN between the bulk soil and either aggregate size. The impact of soil type



**FIGURE 2** Soil extracellular enzyme activity averaging large and small aggregates. (a) AP and BX activity is significantly greater in silty loams. NAG and Ala activity is significantly greater in switchgrass compared to corn. BG is the only enzyme that was impacted by site, crop, and aggregate effects so the mean BG activity for large and small macroaggregates is shown (b). Plotted values are mean activity  $\pm$  standard error



**FIGURE 3** Total carbon (a) and total nitrogen (b) in sandy corn (light blue), sandy switchgrass (dark blue) silty corn (light red), and silty switchgrass (dark red) in large (circle) and small (triangle) macroaggregates generated using an optimal moisture sieving approach. Plotted values represent mean percent C or N per g dry soil. The bottom panels are SEM images of small aggregates in sandy (c) and silty (d) soils

on TC and TN concentrations was visible by SEM, with the smooth-surfaced, sandy soil particles being less covered in organic material than the rough-textured silty soil particles (Figure 3c,d). Only slight differences in organic content between cropping systems were visible by SEM, but differences were more prominent in sandy soils (Figure S1a). Crop and soil type interacted to influence soil aggregation ( $p = 0.029$ ), with a greater mean weighted diameter (MWD) of aggregates in the switchgrass plots of the silt compared to sand loams ( $p = 0.013$ ; Figure S1b). In switchgrass soils, differences in aggregate distribution were driven primarily by the significantly higher percentage of LMs in the silty (82.8%) compared to sandy loam (68.5%; Figure S2). Crop type had no main effect on soil aggregation and MWD differences were not detected in corn plots between sites. The impacts of cropping system, soil type, and aggregate size on SOM chemistry were further investigated by characterizing the C compounds within soil pools using high-resolution mass-spectrometry.

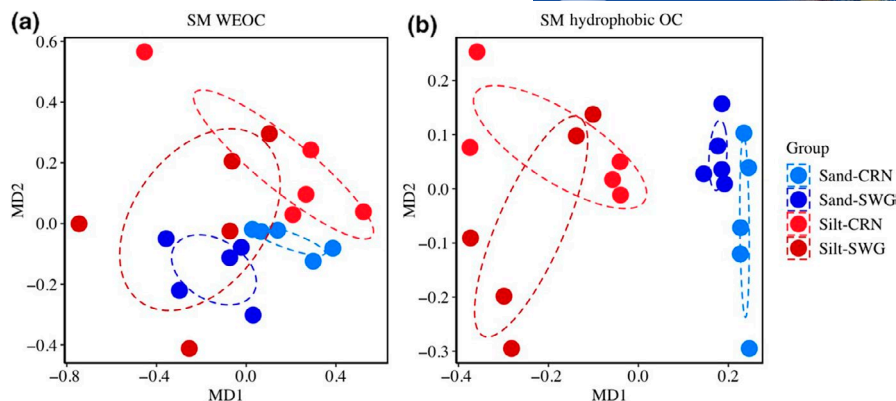
### 3.4 | Soil type is the primary driver of differences in carbon chemistry

Soil type was also the dominant driver of SOM chemistry. To examine the distribution of soil organic C, we characterized

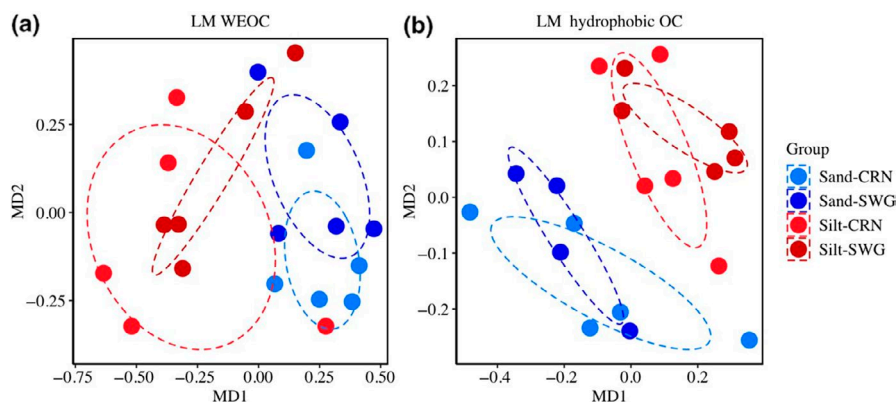
the chemistry of hydrophilic organic compounds extractable with water, and hydrophobic compounds extractable with chloroform. The water-extractable, or kinetic, pool contains more kinetically active C that reflects microbial metabolism while the hydrophobic pool is considered less bioavailable due to stronger associations with organic and mineral components of the soil (Bottos et al., 2018; Sengupta et al., 2019; Zhao et al., 2020). This method does not identify the chemistry of SOM that requires harsh extraction (e.g., high temp, extreme pH) or dissolution of minerals. However, it does allow us to assess biologically relevant C pools that are more accessible to microorganisms and thus, relevant to our research questions.

In three out of the four C pools examined, soil type explained more variability in C chemistry than cropping system ( $p < 0.001$ ). The kinetic pool from SMs was the only pool more strongly influenced by cropping system than soil type (Figure 4a). Soil type explained 28% of the variability in the chemistry of the SM hydrophobic pool (Figure 4b) and 22% for both the LM kinetic pool (Figure 5a) and LM hydrophobic pool (Figure 5b) variability, but just 9% of the SM kinetic pool variability. Cropping system explained 18% of the SM kinetic pool variability, 10% of the LM kinetic pool variability, 9% of the SM hydrophobic pool variability, and only 6% of the LM hydrophobic pool variability.





**FIGURE 4** Ordination of small macroaggregate FTICR-MS composition for the kinetic (a) and hydrophobic (b) carbon pools based on Jaccard dissimilarity. Ovals represent 95% confidence intervals for sandy corn (light blue), sandy switchgrass (dark blue), silty corn (light red), and silty switchgrass (dark red) soils



**FIGURE 5** Ordination of large macroaggregate FTICR-MS composition for the kinetic (a) and hydrophobic (b) carbon pools based on Jaccard dissimilarity. Ovals represent 95% confidence intervals for sandy corn (light blue), sandy switchgrass (dark blue), silty corn (light red), and silty switchgrass (dark red) soils

Richness differences among specific biochemical classes resulted in significantly different chemical compositions between the soil types and cropping systems (Table S2). The chemical richness of compound classes in both the hydrophobic and kinetic pools differed by soil type. Although sandy soils contain less TC and TN than silty soils, they had a greater richness of molecules in every biochemical class that differed between the two soils (Figures S3–S6). Cropping system impacts on SOM chemistry were generally caused by a greater richness of molecules in switchgrass systems. The cropping system effects were much more prominent in the kinetic pool. Between different C pools (kinetic vs hydrophobic), the same compound class often responded differently to soil type and cropping system. For example, in SMs, there was a greater amino sugar and protein-like compound richness in the kinetic pool of switchgrass soils ( $p < 0.01$  for both). However, SM amino sugar and protein-like compound richness in the hydrophobic pool were driven by soil type

with greater compound richness in sandy soils ( $p < 0.01$  for both). Treatment effects on lipid-like compounds also differed between the kinetic and hydrophobic C pools. As expected, the hydrophobic C pool had a greater richness of lipid-like compounds than the kinetic pool for both aggregates, but there was no significant treatment effect on the richness of lipid-like compounds in the hydrophobic pool (Figures S3–S6). Instead, switchgrass cropping systems had a higher richness of lipid-like compounds than corn cropping systems in the SM kinetic pool. Despite subtle treatment effects on the richness of the lipid pools, soil type and cropping system had a large impact on the dissimilarity of the lipidome (Supplemental Figures; Table S3). For both aggregate sizes, the composition of lipids in the kinetic pool was strongly influenced by cropping system (SM  $R^2 = 0.14$ ,  $p = 0.001$ ; LM  $R^2 = 0.17$ ,  $p = 0.008$ ) and the composition of lipids in the hydrophobic pool was strongly influenced by site system (SM  $R^2 = 0.25$ ,  $p = 0.001$ ; LM  $R^2 = 0.30$ ,  $p > 0.001$ ).

## 4 | DISCUSSION

There is a consensus forming among scientists that SOM is in a continuous state of transformation and that microbial processes are largely responsible for the rate and state of decay (Cotrufo et al., 2015; Kallenbach et al., 2015; Lehmann et al., 2020; Ludwig et al., 2015; Paul, 2016; Schimel & Schaeffer, 2012). The basis of this understanding is that plants are the source of most new organic inputs into soils, providing substrates that influence microbial community composition and activity (Hargreaves et al., 2015; Six et al., 2006). The environmental filter of the soil habitat further structures microbial community membership and activity (Bach et al., 2018). Together, plant inputs and the soil environment determine the metabolic activity and turnover of the soil microbiome. The resulting metabolic products and microbial residues interact with soil particles to generate mineral organic associations that contribute to SOM formation and soil fertility (Liang et al., 2019; Miltner et al., 2012). While this general conceptual model is largely accepted, there is much to be discovered about the microorganisms and microbial traits that enhance SOM. We integrate information on microbial community structure and activity with high-resolution SOM chemistry to understand the relative influence of plants and soils on SOC chemistry in biofuel cropping systems.

Soil type was the strongest driver of bacterial/archaeal and fungal community structure and was especially influential on differentiating fungal communities. In support of our first hypothesis, we found that silty loams supported a higher fungal diversity than sandy loams. Notably, a different *Mortierella* sp. was dominant in every treatment group (Table 1). The most abundant fungal OTUs in silty soils were identified as *Mortierella hyaline* and an unclassified *Mortierella* sp., both of which were strongly indicative of silty soils independent of cropping system. *Mortierella elongata* and *Mortierella exigua* were extremely dominant in sandy soils for corn and switchgrass cropping systems respectively (Table 1). The unique biomolecular properties of *Mortierella* and their extreme dominance *in situ* warrant deeper investigation into their potential role in SOM storage.

*Mortierella* is a genus of microscopic, filamentous, saprotrophic fungi that are commonly found in soils, including agroecosystems (Hoffmann et al., 2011; Li et al., 2018; Wagner et al., 2013). *Mortierella* have been associated with the generation of biomolecular compounds that may contribute to persistent fungal necromass, including chitin and melanin (Fernandez & Kennedy, 2018; Li et al., 2018). Chitin is an amino sugar polymer and FTICR-MS analysis of melanin most often identified it as a protein-like compound (Choi et al., 2018). We found that richness of amino sugar and protein-like compounds in both the kinetic and hydrophobic C pools responded similarly to soil type and cropping system treatments, suggesting they may be derived from the same, possibly

fungal, sources. Lipids have also been implicated as being important constituents of persistent SOM, comprising up to 10% of the SOM pool (Haddix et al., 2011; Kallenbach et al., 2016; Keiluweit et al., 2017; Kleber et al., 2007). *Mortierella* spp. are noted to produce large amounts of arachidonic acid, a long-chain unsaturated fatty acid, and some strains have even been selected for use in biofuel research based on this trait (Du et al., 2018; Streekstra, 1997). The lipidome structure in the more persistent hydrophobic C pool was most strongly influenced by soil type. Although our FTICR-MS analysis is inherently not quantitative, we did observe significantly greater richness of lipid-like compounds in the kinetic pools associated with switchgrass SMs than corn SMs, possibly reflecting the turnover of fungal biomass. As fungal necromass interacts with mineral associated OM, lipids become less available for microbial decomposition and may also protect other hydrophilic components from being mineralized (Piccolo et al., 2018). While the contribution of fungal lipids to persistent SOM is not a new idea, the specific organisms and management practices that cultivate these interactions remain unknown. Here we move beyond the general concept of microbial contributions to SOM by identifying a specific and plausible target for necromass accumulation under field conditions. The prevalence of *Mortierella* spp. and their notable chemical signatures merit a quantitative lipidomic analysis with a focus on arachidonic acid to help identify the potential contribution of fungal residues and metabolic byproducts to C stabilization within these biofuel cropping systems.

As well as being a main driver of microbial community structure, soil habitat also influenced community function, with consistently greater enzyme activity in the silty compared to sandy loams. This was particularly pronounced for acid phosphatase activity, which was present in the highest concentrations of all enzymes measured. Because phosphorus is primarily derived from mineral resources, strong site selection for this phenotype is not surprising. Interestingly, *Mortierella* have been noted for their high AP activity. An investigation into the ability of soil fungal isolates to solubilize rock phosphate identified the isolate with the highest activity as a *Mortierella* sp. (Osorio & Habte, 2001). The isolated *Mortierella* sp. was not effective at increasing phosphate concentrations in the plant associate (*Leucaena leucocephala*) on its own, but increased phosphate concentrations in the plant up to 73% when combined with the arbuscular mycorrhizal fungi (AMF) *Glomus aggregatum*. This suggests the existence of a synergistic phosphatase relationship between *Mortierella*, and AMF-associated plants. AMF, which represented 23.3% of the fungal community, are known to strongly associate with both corn and switchgrass (Emery et al., 2018; Sawers et al., 2008) and may be interacting with dominant *Mortierella* sp. to make phosphate bioavailable to these systems. These synergistic relationships may be especially important in marginal soils

**TABLE 2** Bulk soil comparison of total carbon and total nitrogen at KBS and AARS 2 and 8 years after establishment

		%C		%N	
		2 year*	8 year	2 year*	8 year
Sand	Corn	1.23 ± 0.09	1.18 ± 0.38	0.11 ± 0.01	0.09 ± 0.04
	Switchgrass	1.21 ± 0.07	0.92 ± 0.13	0.09 ± 0.01	0.10 ± 0.01
Silt	Corn	2.61 ± 0.12	2.17 ± 0.31	0.27 ± 0.02	0.20 ± 0.03
	Switchgrass	2.30 ± 0.11	2.22 ± 0.37	0.23 ± 0.02	0.22 ± 0.03

Units are in percent dry weight of soil. \* indicates values obtained from Jesus et al. (2016). 8 year samples are reported as mean ± standard deviation.

where resource limitations and importance of AMF associations are more pronounced. It is possible that the dominant organisms, such as *Mortierella* spp. that are indicative of silty soils, are responsible for the high AP activity in those plots, which could elicit positive feedback effects on plant productivity and soil C inputs.

BG activity was more strongly influenced by cropping system than soil type, with greater BG activity in switchgrass compared to corn soils. Enhanced BG activity in switchgrass soils corresponded to a greater richness of water-extractable carbohydrate-like compounds in both soil types. Endoglucanases interact with BG and are important soil enzymes that initiate cellulose degradation by introducing random breaks in a cellulose polymer, but are inhibited by cellobiose accumulation (Dimarogona et al., 2012). BG liberates glucose molecules from cellobiose as the final, and often rate-limiting, step in the deconstruction of cellulose (Cairns & Esen, 2010; Shewale, 1982). Increased BG activity is likely indicative of increased cellulose degradation and endoglucanase activity, resulting in elevated decomposition, and a higher diversity of carbohydrate-like compounds in switchgrass compared to corn cropping systems. *Mortierella* have also been reported to increase BG activity in soils (Hayano & Tubaki, 1985; Li et al., 2018). We found that different *Mortierella* spp. were highly abundant in every treatment group, suggesting there may be environmentally relevant strain-specific differences in potential BG activity within the *Mortierella* genus.

Switchgrass soils also supported higher N mineralization as evidenced by greater pools of enzymes that degrade amino sugars (NAG) and proteins (Ala; Ekenler & Tabatabai, 2004). Increased NAG and Ala activity similarly corresponded to a greater richness of water extractable amino sugar and protein-like compounds in switchgrass soils. Higher activity of these enzymes may reflect the lower fertilization rates in the perennial plots, which require more investment into enzymatic resources to obtain N. However, switchgrass also allocates more resources to belowground biomass generation than corn (Anderson-Teixeira et al., 2013). Therefore, higher NAG and Ala activity in switchgrass systems may also be a result of increased substrate availability. Increasing belowground plant inputs and EEA activity can stimulate the generation of microbial biomass and byproducts (Jackson et al., 2017) and, in turn, result in

increases in soil C stocks under perennial cropping systems (McGowan et al., 2019).

Instead, we found that there was no difference in the TC or TN between switchgrass (perennial) and corn (annual) cropping systems after 8 years of consistent management (Figure 3). Our findings support previous research demonstrating soil type was a stronger driver of microbial community structure, TC and TN values 2 years after establishment (Jesus et al., 2016). We found this was still true 6 years later (Table 2) demonstrating that site characteristics had a stronger control over microbial community composition and SOM content than cropping system. Early drivers of SOC and microbial community composition have persisted 8 years beyond crop establishment, demonstrating a strong and lasting signature of soil habitat. Our results are consistent with recent studies of switchgrass and corn in other marginal soils that found no-till practices enhanced soil C accrual compared to conventional farming, but detected no difference in total C between corn and switchgrass cropping systems (Graham et al., 2019; Ruan & Robertson, 2020).

The re-occurring lack of cropping system impact on TC and TN accrual is somewhat surprising, and in contrast to models that predict preferential C accumulation under switchgrass (Garten & Wulfschleger, 2000; Jin et al., 2019; McGowan et al., 2019; Qin et al., 2012). In fact, previous GLBRC research in these sites after the fourth growing season projected SOC would increase over time (>5 years), especially in the finer textured silty switchgrass soils (Tiemann & Grandy, 2015). However, our data do not support this projection. We report TC/TN values for the top 15 cm of soil, and it is not clear that C is accumulating in deeper soil horizons in these systems. A different experiment at the same LTER site we examined found that switchgrass soils amended with fertilizer reduced both surface and deep C accrual in KBS sandy loams after only 3 years of biofuel production (Valdez et al., 2017). The switchgrass soils in our study were also fertilized, which may be inhibiting SOM accumulation potential of switchgrass throughout the soil profile (Valdez et al., 2017). Although predictions of preferential C accrual under switchgrass cropping systems are not yet realized, the observed differences in C chemistry between cropping systems may lead to more microbial community and C accrual differences over longer time periods, despite switchgrass fertilization.

A C neutral bioeconomy is reliant on soil C retention, which may be impacted by soil management techniques and crop selection. However, there remain uncertainties about the ability to enhance C storage in marginal soils based on crop selection alone. Here we demonstrate that after 8 years in biofuel feedstock production there are significant differences in soil C chemistry, but no detectable differences in total soil C and N concentrations between corn and switchgrass plots in whole soil or optimal moisture aggregate fractions. This supports the idea that biofuel feedstock production can be deployed on marginal soils without significant C loss or gain. Our results further demonstrate the critical role site has over cropping system selection on influencing C chemistry, accumulation, and microbial community dynamics. In the context of previous work, these results support the critical role of no-till agriculture in reducing C loss and reveal the limited impact of annual vs. perennial crops in altering belowground C storage (Graham et al., 2019; Ogle et al., 2019; Plaza et al., 2013; Ruan & Robertson, 2020). It is important to note that calculating the C sequestration potential of a system requires consideration of CO<sub>2</sub> costs associated with fertilizer production and application, as well as seeding for annual cropping systems and other management practices that require energy or generate greenhouse gas equivalents (e.g., N<sub>2</sub>O; Jarchow et al., 2015; Schlesinger, 1999). Field research has shown that corn grown in no-till marginal soils generated more biomass for biofuel production than switchgrass (Ruan & Robertson, 2020), which is consistent with previous reports that switchgrass takes 50%–100% more land to produce the same amount of biofuel as corn (Zhuang et al., 2013). All of the described costs and benefits need to be balanced when selecting a crop for use in biofuel production with the additional intent of offsetting greenhouse gas emissions (Dolan et al., 2020).

## 5 | CONCLUSION

Plant–microbe interactions strongly influence bioavailable C chemistry but the direct effects on persistent SOM are more challenging to detect because edaphic properties seem to define the microbial habitat and regulate the sink capacity. Even though switchgrass allocates more resources belowground (Anderson-Teixeira et al., 2013), no cropping system effects on C accrual were detected in marginal soils after 8 years of management for corn and switchgrass biofuel feedstock production. However, strong differences in potential enzyme activity and the chemical composition of SOM between the cropping systems were evident, suggesting greater decomposition, but not C accumulation in switchgrass plots. This contradicts the proposition that perennial biofuel crops can enhance C accrual in marginal soils over annual crops and, surprisingly, alludes to the possibility of increased DOC leaching to deeper soil horizons or CO<sub>2</sub> emissions from

microbial respiration under switchgrass cropping systems. Our work reveals that biomolecules potentially associated with fungal residues (e.g. amino sugars, proteins, and lipids) were strongly influenced by both soil type (hydrophobic pool) and cropping system (kinetic pool) and highlights the possibilities for harnessing the microbiome for sustainable biofuel feedstock production. Furthermore, this research identifies putative targets (e.g. *Mortierella* spp, arachidonic acid) for moving beyond a conceptual model of bulk biochemical pools to understanding organismal and molecular contributions to SOM formation. *Mortierella* were by far the most dominant fungi in all soil and cropping systems, but their relationship with biofuel cropping systems and role in SOM sequestration remain to be discovered. Integrating a molecular understanding of the plant–microbe–soil continuum holds great promise for developing sustainable biofuel feedstock production systems.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article and through Datahub, a Pacific Northwest National Laboratory data sharing platform.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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